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Quantitation of Short-Chain Glycerol-Bound Compounds in Thermoxidized and Used Frying Oils. A Monitoring Study during Thermoxidation of Olive and Sunflower Oils

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Major short-chain glycerol-bound compounds were investigated in olive oil (OO) and conventional sunflower oil (SO) during thermoxidation at 180 °C for 5, 10, and 15 h. These compounds included methyl heptanoate (C7:0), methyl octanoate (C8:0), methyl 8-oxo-octanoate (8-oxo-C8:0), methyl 9-oxononanoate (9-oxo-C9:0), dimethyl octanodiate (C8:0 diester), and dimethyl nonanodiate (C9:0 diester), which were analyzed by GC after derivatization of triacylglycerols to fatty acid methyl esters. An acceptable linear correlation ($r = 0.967$) was found between the total content of these compounds and the total content of polar compounds, suggesting that quantitation of the major short-chain glycerol-bound compounds provides a good indication of the total alteration level of oils heated at frying temperature. Samples with levels of polar compounds around 25% on oil showed total contents within 2−3 mg/g of oil. To determine the content of these compounds in used frying oils, 10 samples from restaurants and fried-food outlets in Spain were analyzed. Results showed total levels between 2.13 and 7.56 mg/g of oil in samples with contents of polar compounds ranging from 18.8 to 55.5% on oil. Samples with levels of polar compounds of ∼25% showed total contents of the short-chain compounds similar to those found in the thermoxidized oils, that is, within 2−3 mg/g of oil.

KEYWORDS: Short-chain fatty acids; core aldehydes; diacids; olive oil; sunflower oil; thermoxidation; used frying oils

INTRODUCTION

During frying of foods, the frying fat is subjected to high temperatures in the presence of air and water, the latter provided by the food, that results in the formation of a number of compounds through thermal, oxidative, and hydrolytic reactions (*1*−*5*). The occurrence of hydroperoxides, the primary oxidation compounds, is very limited due to their great instability at high temperatures (*5*). The hydroperoxides formed decompose rapidly into a wide range of volatile and nonvolatile secondary oxidation products. An important route of hydroperoxide breakdown proceeds by homolytic β-scission of the alkoxyl radicals that arise from allylic hydroperoxides (*6*). Such a cleavage results, on one side, in a volatile compound of low molecular weight and, on the other, in a triacylglycerol molecule containing a short acyl chain. Whereas the former is largely removed from the oil by volatility during heating, the latter remains in the frying oil and is absorbed by the food. In addition, it has been reported that short-chain glycerol-bound compounds, such as core aldehydes, are easily absorbed in the intestinal tract after hydrolysis by pancreatic lipase (*7*, *8*). Furthermore, some reports on 9-oxononanoic acid, the major short-chain glycerol-bound compound in oxidized edible fats and oils, indicate that it could induce hepatic lipid peroxidation (*9*) and affect hepatic metabolism (*10*−*12*).

In a previous paper, we studied the evolution of major short-chain glycerol-bound compounds formed during thermoxidation in model systems consisting of triacylglycerols (TAGs) with a single fatty acid (*13*). Triolein and trilinolein were selected as model compounds because oleic and linoleic acids are the most representative fatty acids that undergo changes during deep-fat frying. Short-chain glycerol-bound compounds were analyzed by gas chromatography (GC) after derivatization of TAGs to fatty acid methyl esters (FAMEs) by base-catalyzed transmethylation. The compounds analyzed were methyl heptanoate (C7:0), methyl octanoate (C8:0), methyl 8-oxo-octanoate (8-oxo-C8:0), methyl 9-oxononanoate (9-oxo-C9:0), dimethyl octanodiate (C8:0 diester), and dimethyl nonanodiate (C9:0 diester). Additional methylation with diazomethane, which reacts only with free carboxylic acid moieties, led to a considerable increase of C8:0 diester and C9:0 diester, indicating the presence of important contents of monoesterified C8:0 and C9:0 diacids. The occurrence of C8:0 diester and C9:0 diester prior to methylation with diazomethane was attributed to the presence of estolides, which are dimeric TAGs joined through an ester linkage. Formation of estolides takes place during thermoxidation and proceeds through esterification of a free carboxylic

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group in a short acyl chain of a TAG molecule with a hydroxy group present in another TAG molecule. By single base-catalyzed transmethylation the esterified carboxyl groups at both extremes of the short chain are converted to methyl ester groups, giving rise to C8:0 diester and C9:0 diester (*14*).

The formation of these six compounds was also studied in the model FAMEs methyl oleate and methyl linoleate, which were thermoxidized under the same conditions as the model TAGs (*13*). Unlike in the case of TAGs, the derivatization step is not required in model FAMEs and the short-chain compounds formed during thermoxidation can be analyzed directly by GC. The derivatization procedure required in TAGs was simulated in the thermoxidized model FAMEs, and results were compared to those obtained before derivatization. The simulation of the derivatization procedure did not lead to formation of artifacts (*14*).

The objective of the present work was to investigate the formation of the major short-chain glycerol-bound compounds during thermoxidation of olive and sunflower oils. In addition, their levels in used frying oils sampled by Food Inspection Services in restaurants and fried-food outlets were also determined in this study.

MATERIALS AND METHODS

Chemicals. Methyl tridecanoate (C13:0) and methyl pentadecanoate (C15:0) were purchased from Nu-Check-Prep (Elysian, MN). *N*-Methyl-*N*-nitroso-*p*-toluenesulfonamide was purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Silica gel 60 for column chromatography (particle size = 0.063−0.100 mm) was acquired from Merck (Darmstadt, Germany). All other chemicals and reagents were of analytical grade and were provided by local suppliers.

Samples. Olive oil (OO) and conventional sunflower oil (SO) were purchased from local outlets and thermoxidized at 180 °C as described below. Used frying oils were sampled by Food Inspection Services in restaurants and fried-food outlets in Andalusia (Spain) and used as received.

Heating Procedure. OO and SO samples (2.00 ± 0.01 g) were weighed into standard glass tubes (10 cm × 12 mm i.d.). The tubes were introduced into Rancimat tubes containing 8 g of glycerol to facilitate the heat transfer and inserted into the heating block of the apparatus heated at 180 ± 1 °C. The tubes were left open to the air during heating (*15*). Samples were heated for 5, 10, and 15 h and kept at −20 °C until analyses.

Fatty Acid Composition. Fatty acid composition was determined by GC after derivatization to FAMEs with 2 N KOH in methanol, according to the IUPAC Standard Method (*16*, *17*). In the case of the thermoxidized oils, quantitative results were approached by taking into account that the content of the saturated fatty acids remains constant during heating due to their high stability. Thereby, the content of the major saturated fatty acid (C16:0) in the nonheated sample was taken as the basis for the recalculation of the content of each of the other fatty acids in the thermoxidized oils (*18*).

Quantitation of Total Polar Compounds and Their Distribution. The content of total polar compounds was determined gravimetrically following the method proposed by the IUPAC (*19*) with slight modifications (*20*). Briefly, the nonpolar and polar fractions were separated from 1 g of sample by silica column chromatography (20 g of silica/H_2O, 95:5, w/w). The nonpolar fraction, which contains the unoxidized TAGs, was eluted with 150 mL of *n*-hexane/diethyl ether (90:10, v/v). A second fraction, which comprises the total polar compounds, was eluted with 150 mL of diethyl ether. The efficiency of the separation was checked by thin-layer chromatography using hexane/diethyl ether/acetic acid (80:20:1, v/v/v) for development of plates and exposure to iodine vapor to reveal the spots. After evaporation of solvents, both fractions were weighed, and the polar fraction was dissolved in diethyl ether (25 mg/mL) for analysis of polar compounds distribution by high-performance size exclusion chromatography (HPSEC). The HPSEC chromatograph was equipped with a Rheodyne 7725i injector with a 10-μL sample loop, a Waters 510 pump (Waters, Milford, MA), an HP 1037 A refractive index detector, and an HP 3392 A integrator (Agilent Technologies, Palo Alto, CA). The separation was performed on two 100 and 500 Å PL gel columns (25 cm × 0.77 cm i.d.) packed with porous, highly cross-linked styrene−divinylbenzene copolymers (5 μm, particle size) (Agilent Technologies) connected in series, with tetrahydrofuran (1 mL/min) as the mobile phase (*21*).

Analysis of Short-Chain Glycerol-Bound Compounds. Short-chain glycerol-bound compounds were analyzed by GC after derivatization of TAGs to FAMEs. Quantitation was carried out using a mixture of methyl tridecanoate (C13:0) and methyl pentadecanoate (C15:0) as internal standards.

Derivatization Procedure. FAMEs were obtained in two consecutive steps: first, direct methylation of carboxylic groups with diazomethane and, second, base-catalyzed transmethylation with sodium methoxide at room temperature.

A standard solution was prepared with a mixture of C13:0 (500 μg/mL) and C15:0 (500 μg/mL) in *tert*-butyl methyl ether (TBME).

Methylation with Diazomethane. Aliquots of 100 mg of oil were accurately weighed into screw-capped centrifuge tubes. A 0.5-mL volume of the standard solution was added, and the oil was dissolved by shaking. The solvent was evaporated under nitrogen, and the oil was dissolved in 2 mL of diethyl ether. Methylation of free carboxylic groups was performed by bubbling diazomethane into the oil solution. Diazomethane was produced by the reaction of sodium hydroxide with *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide using a device consisting of two connected tubes (*22*). The first tube was half-filled with diethyl ether (∼5 mL) and the second one with 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*-toluenesulfonamide was added to the second tube. A stream of nitrogen (5−10 mL/min), saturated in ether vapor as passing through the first tube, was used to carry diazomethane generated in the second tube up to the tube containing the oil dissolved in diethyl ether. Methylation was considered to be complete when the oil solution turned yellow due to an excess of diazomethane. Then, the solvent was evaporated under nitrogen, and the methylated sample was dissolved in 2 mL of TBME.

Transmethylation with Sodium Methoxide at Room Temperature. A 0.5-mL volume of 0.2 M NaOMe solution in methanol was added to the methylated sample dissolved in TBME, and the vial was closed, shaken for 1 min, and left to stand at room temperature for 2 min. For neutralization purposes, a 0.1-mL volume of 0.5 M H_2SO_4 solution was added, and the mixture was shaken for a few seconds. Then, 1.5 mL of water was added, and the mixture was shaken for 10 s and centrifuged. The organic layer was separated for GC analysis.

Gas−Liquid Chromatography. Short-chain FAMEs were analyzed by GC using an HP 6890 series chromatograph (Agilent Technologies). Two microliters from the organic layer (50 mg/mL) was injected into a split−splitless inlet operating in split mode with a 40:1 split ratio at 250 °C. An HP Innowax fused-silica capillary column, 30 m × 0.25 mm i.d., film thickness = 0.25 μm (Agilent Technologies), was used. Analyses were run using hydrogen (1 mL/min) as carrier gas and with the following temperature program: 90 °C held for 2 min, 4 °C/min to 240 °C, held for 20 min. A flame ionization detector was used at 250 °C with hydrogen at 40 mL/min and air at 450 mL/min, and nitrogen at 45 mL/min was used as auxiliary gas.

Correction factors for commercial C8:0, synthesized 9-oxo-C9:0, and commercial C9:0 diester were calculated against the internal standards C13:0 and C15:0. The values found for C8:0 (0.98), 9-oxo-C9:0 (1.80), and C9:0 diester (1.53) were applied for quantitation. Similar weight responses were assumed for their counterparts, C7:0, 8-oxo-C8:0, and C8:0 diester (*14*).

Statistical Analysis. Data for the thermoxidized oils were mean values of triplicate experiments. In the case of the used frying oils, analytical determinations were carried out in duplicate, unless otherwise indicated. Linear regression analysis was performed by using Microsoft Excel 2000 (Microsoft Corp., Redmond, WA). Statistical comparisons between the slopes obtained by linear regression were made by applying Student's *t* test. Significance was defined at $p < 0.05$.

Table 1. Fatty Acid Content (Milligrams per 100 mg of Oil) of Olive Oil and Sunflower Oil: Nonheated Samples and Samples Heated at 180 °C for 5, 10, and 15 h

	fatty acid content[a]				
sample	C16:0	C16:1	C18:0	C18:1	C18:2
OO, 0 h[b]	11.5 ± 0.14[d]	0.9 ± 0.09	2.7 ± 0.08	76.1 ± 0.30	7.8 ± 0.08
OO, 5 h	11.5 ± 0.53	1.0 ± 0.22	2.8 ± 0.17	73.7 ± 1.28	6.6 ± 0.33
OO, 10 h	11.5 ± 0.63	0.8 ± 0.11	2.6 ± 0.22	69.7 ± 1.29	5.6 ± 0.36
OO, 15 h	11.5 ± 0.55	0.8 ± 0.10	2.8 ± 0.25	66.6 ± 1.24	4.8 ± 0.31
SO, 0 h[c]	6.3 ± 0.15	tr[e]	4.7 ± 0.09	27.8 ± 0.18	60.9 ± 0.36
SO, 5 h	6.3 ± 0.55	tr	4.5 ± 0.44	26.5 ± 1.87	56.1 ± 1.73
SO, 10 h	6.3 ± 0.60	tr	4.7 ± 0.35	25.2 ± 1.82	52.2 ± 1.78
SO, 15 h	6.3 ± 0.44	tr	4.5 ± 0.28	24.6 ± 1.61	47.7 ± 1.34

[a] Quantitation based on the major saturated fatty acid (C16:0) present in the nonheated samples. [b] Olive oil and heating time. [c] Sunflower oil and heating time. [d] Mean ± standard deviation ($n = 3$). [e] Traces, <0.05%.

RESULTS AND DISCUSSION

Characterization of Thermoxidized Oils. OO and SO thermoxidized at 180 °C for 5, 10, and 15 h were characterized by quantitative analysis of fatty acids, total polar compounds, and polar compound distribution.

Table 1 shows results of major fatty acids found in thermoxidized OO and SO. For comparative purposes, results for the nonheated oils have also been included. As expected, the level of major unsaturated fatty acids decreased with heating time. The relative loss of linoleic acid was higher than that of oleic acid in both oils, which shows the influence of the degree of unsaturation. Nevertheless, the absolute loss of unsaturated fatty acids clearly depended on their initial contents, as greater absolute losses were found for the most abundant unsaturated fatty acid in each oil. Thus, oleic acid in OO and linoleic acid in SO were the fatty acids that participated to a greater extent in degradation reactions, showing absolute losses as high as 9.5 and 13.2 mg/100 mg of oil, respectively, in samples heated for 15 h.

Table 2 lists the results of the analysis of total polar compounds, which account for the new compounds formed during thermoxidation, and their distribution in triglyceride polymers (TGP), dimers (TGD), oxidized triglyceride monomers (OxTGM), diglycerides (DG), and free fatty acids (FFA), formed through thermal, oxidative, and hydrolytic alteration reactions. For comparative purposes, results for the nonheated oils have also been included. Both initial oils showed low contents of polar compounds, which were within the normal levels for refined oils. At the intermediate heating period assayed (10 h), the total content of polar compounds already reached values of ~25% on oil. This is the level established as the limit for human consumption in most European countries (*23*). For all of the heating periods, significantly higher contents of polar compounds were found for SO, as expected from its higher degree of unsaturation, which favors polymer formation (*24*). In contrast, similar contents of OxTGM were found in both oils at each period of time. DG and FFA remained unchanged during heating, indicating that hydrolytic reactions were not significant, which was expected by the absence of water.

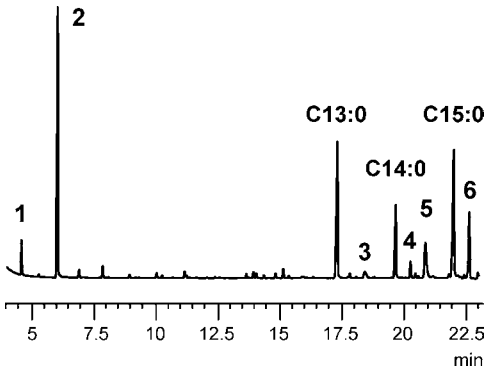

Figure 1. Partial gas chromatogram showing separation of major short-chain oxidation compounds in used frying fats after diazomethane methylation and base-catalyzed transmethylation. Conditions: HP Innowax capillary column (30 m × 0.25 mm i.d.); temperature program, 90 °C (2 min), 4 °C/min, 240 °C (25 min). Peak assignments: C13:0, methyl tridecanoate (internal standard); C14:0, methyl miristate, C15:0, methyl pentadecanoate (internal standard); 1, methyl heptanoate; 2, methyl octanoate; 3, methyl 8-oxooctanoate; 4, dimethyl octanodiate; 5, methyl 9-oxononanoate; 6, dimethyl nonanodiate.

Quantitation of Short-Chain Glycerol-Bound Compounds in Thermoxidized Oils. Short-chain glycerol-bound compounds, including methyl heptanoate (C7:0), methyl octanoate (C8:0), methyl 8-oxo-octanoate (8-oxo-C8:0), methyl 9-oxononanoate (9-oxo-C9:0), dimethyl octanodiate (C8:0 diester), and dimethyl nonanodiate (C9:0 diester), previously identified in model lipid systems (*13*, *14*), were analyzed by GC after derivatization of TAGs to FAMEs. In previous studies, quantitative analysis was performed in two steps that consisted of transmethylation of TAGs to FAMEs followed by direct methylation of free carboxylic acid moieties with diazomethane (*14*). Methylation with diazomethane in a second step led to a significant increase of C8:0 diester and C9:0 diester, indicating the presence of considerable contents of free carboxylic groups corresponding to monoesterifed C8:0 and C9:0 diacids. In the present study we tried to simplify the analytical procedure by determination of these compounds in a single GC analysis after the two consecutive methylation steps. Nevertheless, it was

Table 2. Total Polar Compounds and Their Distribution in Sunflower Oil and Olive Oil Heated at 180 °C for 5, 10, and 15 h

		distribution (wt % on oil)				
sample	total (wt % on oil)	TGP[a]	TGD[b]	OxTGM[c]	DG[d]	FFA[e]
OO, 0 h[f]	4.4 ± 0.14[h]	ND[i]	0.2 ± 0.01	0.7 ± 0.01	3.1 ± 0.02	0.4 ± 0.02
OO, 5 h	14.6 ± 0.14	1.1 ± 0.00	4.4 ± 0.04	5.6 ± 0.08	3.1 ± 0.04	0.4 ± 0.01
OO, 10 h	22.8 ± 0.00	3.2 ± 0.05	7.3 ± 0.02	8.8 ± 0.04	3.1 ± 0.04	0.4 ± 0.06
OO, 15 h	30.8 ± 0.07	6.0 ± 0.04	9.8 ± 0.04	11.5 ± 0.04	3.2 ± 0.02	0.4 ± 0.02
SO, 0 h[g]	3.6 ± 0.14	ND[i]	0.5 ± 0.02	1.1 ± 0.01	1.3 ± 0.02	0.5 ± 0.04
SO, 5 h	17.2 ± 0.35	2.0 ± 0.07	7.6 ± 0.16	5.8 ± 0.12	1.2 ± 0.00	0.5 ± 0.04
SO, 10 h	26.6 ± 0.07	4.9 ± 0.01	11.3 ± 0.06	8.7 ± 0.01	1.2 ± 0.00	0.4 ± 0.01
SO, 15 h	35.4 ± 0.21	8.7 ± 0.09	14.1 ± 0.09	10.9 ± 0.04	1.4 ± 0.04	0.4 ± 0.01

[a] Triglyceride polymers. [b] Triglyceride dimers. [c] Oxidized triglyceride monomers. [d] Diglycerides. [e] Free fatty acids. [f] Olive oil and heating time. [g] Sunflower oil and heating time. [h] Mean ± standard deviation ($n = 3$). [i] Not detected.

Table 3. Concentration of Short-Chain Oxidation Compounds (Milligrams per Gram of Oil) in Sunflower Oil and Olive Oil Heated at 180 °C for 5, 10, and 15 h

sample	C7:0	C8:0	8-oxo-C8:0	C8:0 diester	9-oxo-C9:0	C9:0 diester	total
OO, 5 h[a]	0.08 ± 0.007[c]	0.31 ± 0.014	0.12 ± 0.021	0.04 ± 0.007	0.50 ± 0.078	0.18 ± 0.014	1.23 ± 0.141
OO, 10 h	0.23 ± 0.057	0.78 ± 0.177	0.22 ± 0.049	0.10 ± 0.021	0.85 ± 0.099	0.46 ± 0.064	2.64 ± 0.467
OO, 15 h	0.36 ± 0.021	1.08 ± 0.035	0.26 ± 0.035	0.17 ± 0.021	0.96 ± 0.092	0.71 ± 0.014	3.54 ± 0.218
SO, 5 h[b]	0.02 ± 0.007	0.80 ± 0.148	0.10 ± 0.028	0.02 ± 0.000	0.60 ± 0.141	0.12 ± 0.021	1.66 ± 0.345
SO, 10 h	0.05 ± 0.014	1.36 ± 0.226	0.17 ± 0.028	0.04 ± 0.000	0.98 ± 0.148	0.28 ± 0.000	2.88 ± 0.416
SO, 15 h	0.06 ± 0.007	1.44 ± 0.318	0.22 ± 0.000	0.07 ± 0.007	1.27 ± 0.071	0.43 ± 0.028	3.49 ± 0.431

[a] Olive oil and heating time. [b] Sunflower oil and heating time. [c] Mean ± standard deviation ($n = 3$).

necessary to change the order of the methylation procedures, as losses by volatility of C7:0 and C8:0 were observed during the methylation with diazomethane of the transmethylated sample. Thus, direct methylation of free carboxylic groups with diazomethane was performed on intact samples followed by derivatization of TAGs to FAMEs. As an example, **Figure 1** illustrates a partial gas chromatogram showing separation of major short-chain oxidation compounds after the application of the proposed derivatization procedure.

Quantitative results of short-chain glycerol-bound compounds for thermoxidized OO and SO are given in **Table 3**. Results for the unheated oils are not shown, as the compounds of interest were not detected. Similar total contents of the short-chain compounds were found in both oils at each period of time, ranging between 1.2 and 3.5 mg/g of oil. Of special interest were the contents reached at 10 h of heating, when the samples showed, as mentioned above, alteration levels around the limit established for human consumption. At this point, the total content of the major short-chain glycerol-bound compounds was within 2−3 mg/g of oil for both oils. These contents appeared to be relatively low as compared to the total loss of fatty acids that participated in alteration reactions. As an estimate, the total losses of fatty acids were about 90 and 110 mg/g of oil in samples of OO and SO heated for 10 h, respectively, as deduced from **Table 1**. Taking into account that the molecular weight of short-chain fatty acids is roughly half of the molecular weight of fatty acids with 18 carbon atoms, the levels of fatty acids involved in the formation of the short-chain glycerol-bound compounds constituted only ∼5−6% of the fatty acids lost by thermoxidative reactions in samples with alteration levels around the limit established for human consumption.

In both oils, C8:0 and 9-oxo-C9:0 were the most abundant short-chain compounds, ranging from 0.31 to 1.44 mg/g of oil and from 0.50 to 1.27 mg/g of oil, respectively. In relation to C7:0 and 8-oxo-C8:0, the higher contents of C8:0 and 9-oxo-C9:0 were expected in both oils. C8:0 and 9-oxo-C9:0 arise from the scission of the alkoxyl radical from 9-hydroperoxides, whereas C7:0 and 8-oxo-C8:0 are formed from 8-hydroperoxide. Whereas the formation of 8-hydroperoxide takes place from only oleic acid in significant amounts, the formation of 9-hydroperoxide is significant from both oleic and linoleic acid (*25*). As a result, differences were remarkably greater in the oil containing lower quantities of oleic acid, that is, SO, which showed very low contents of the compounds derived from the 8-hydroperoxide.

The content of 9-oxo-C9:0 was very low in relation to that of C8:0, because formation of the aldehydic compound proceeds directly by the scission of the alkoxyl radical, whereas the formation of C8:0 further requires abstraction of a hydrogen radical after the alkoxyl scission. The same was observed for C7:0 and 8-oxo-C8:0, both from the same hydroperoxide. These results can be attributed to losses of the aldehydic compounds due to participation in further reactions, whereas the saturated short-chain acids accumulate due to higher stability. In fact,

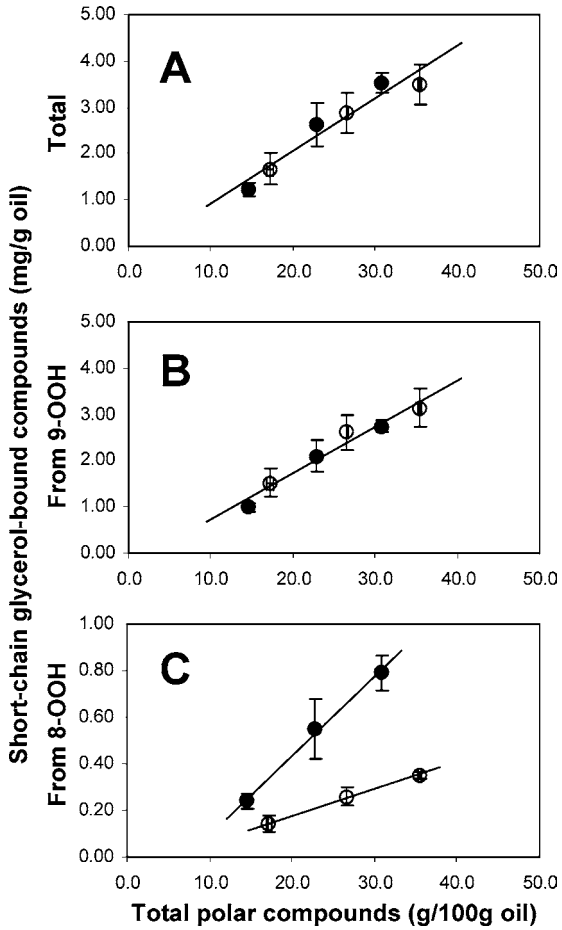

Figure 2. Linear regression analysis between the content of short-chain glycerol-bound compounds and the total content of polar compounds in olive oil (●) and sunflower oil (○) thermoxidized at 180 °C: (**A**) linear regression taking the total content of short-chain compounds [$y = 0.11(\pm 0.015)x$; $r = 0.967$]; (**B**) linear regression taking the content of short-chain compounds formed from 9-hydroperoxide (C8:0 + 9-oxo-C9:0 + C9:0 diester) [$y = 0.100(\pm 0.0099)x$; $r = 0.981$]; (**C**) linear regression taking the content of short-chain compounds formed from 8-hydroperoxide (C7:0 + 8-oxo-C8:0 + C8:0 diester) [olive oil, $y = -0.25(\pm 0.053) + 0.034(\pm 0.0023)x$; $r = 0.998$; sunflower oil, $y = 0.0115(\pm 0.00073)x$; $r = 0.998$]. Error bars express standard deviation ($n = 3$).

further oxidation of the aldehydic groups to carboxylic groups has been proposed to explain the unexpectedly low contents of the aldehydic short-chain compounds in thermoxidized model lipid systems (*14*). Thus, oxidation of 8-oxo-C8:0 and 9-oxo-C9:0 leads to the formation of their acid derivatives, which have also been quantified in the present study as C8:0 diester and C:9 diester.

Only six short-chain glycerol-bound compounds formed from 8- and 9-hydroperoxides were detected in significant amounts.

Table 4. Fatty Acid Composition of Used Frying Oils from Restaurants and Fried-Food Outlets

	fatty acid composition (%)					
sample	C16:0	C16:1	C18:0	C18:1	C18:2	others[a]
1	10.0 ± 0.03[b]	0.8 ± 0.11	3.3 ± 0.10	77.74 ± 0.09	4.6 ± 0.09	3.6 ± 0.23
2	12.6 ± 0.13	1.0 ± 0.13	3.8 ± 0.30	71.7 ± 1.30	6.7 ± 0.23	4.2 ± 1.24
3	10.7 ± 0.11	0.8 ± 0.15	3.4 ± 0.05	75.8 ± 0.10	3.9 ± 0.27	5.3 ± 0.16
4	5.8 ± 0.11	0.4 ± 0.06	4.3 ± 0.36	71.8 ± 1.48	14.3 ± 0.29	3.4 ± 1.36
5	8.3 ± 0.05	0.6 ± 0.06	4.6 ± 0.25	67.2 ± 0.08	15.5 ± 0.26	3.8 ± 0.11
6	7.4 ± 0.08	0.3 ± 0.06	4.5 ± 0.14	31.4 ± 0.24	54.3 ± 0.09	2.2 ± 0.17
7	10.2 ± 0.09	0.8 ± 0.01	3.8 ± 0.04	29.9 ± 0.25	53.2 ± 0.17	2.2 ± 0.02
8	8.4 ± 0.02	0.5 ± 0.03	4.4 ± 0.02	42.2 ± 0.11	42.5 ± 0.17	2.0 ± 0.32
9	9.6 ± 0.00	0.4 ± 0.12	5.7 ± 0.34	35.8 ± 0.68	40.6 ± 0.57	7.9 ± 1.03
10	7.6 ± 0.01	0.2 ± 0.03	5.4 ± 0.18	26.5 ± 0.20	56.1 ± 0.20	4.2 ± 0.27

[a] Results for other polyunsaturated fatty acids were <0.3%. [b] Mean ± standard deviation ($n = 2$).

Table 5. Total Polar Compounds and Polar Compound Distribution in Used Frying Oils from Restaurants and Fried-Food Outlets

		distribution (wt % on oil)				
sample	total (wt % on oil)	TGP[a]	TGD[b]	OxTGM[c]	DG[d]	FFA[e]
1	29.6[f]	6.0	8.0	11.2	3.4	0.9
2	32.8	5.3	10.2	13.6	3.2	0.5
3	42.8	12.3	11.1	14.5	4.4	0.6
4	26.6	4.6	9.0	8.6	3.7	0.7
5	28.0	5.2	9.3	9.9	2.9	0.7
6	18.8	2.7	7.4	7.0	1.2	0.6
7	23.8	5.3	9.1	6.7	2.1	0.6
8	24.9	4.8	8.9	8.5	2.0	0.7
9	27.6	11.9	1.3	9.9	3.8	0.7
10	55.5	25.0	13.8	11.6	2.8	2.3

[a] Triglyceride polymers. [b] Triglyceride dimers. [c] Oxidized triglyceride monomers. [d] Diglycerides. [e] Free fatty acids. [f] Mean ($n = 2$).

However, the formation of unsaturated short-chain glycerol-bound compounds from other hydroperoxides also takes place. Further modifications due to the high reactivity provided by the presence of double bonds could be involved. As an example, oxidation and decomposition of the diunsaturated aldehyde coming from the 13-hydroperoxide of linoleic acid has been proposed as a tentative mechanism for the indirect formation of C7:0 and 8-oxo-C8:0 in model lipid systems consisting only of linoleic acid derivatives (*14*). In the case of the compounds quantified in this study, their high stability due to the absence of double bounds allowed their accumulation in significant amounts. In this respect, the total content of the short-chain compounds increased as degradation proceeded (**Table 3**), which suggests that it could be considered as an indication of the total alteration level in thermoxidized oils. In previous studies, accumulation of the saturated compounds C7:0 and C8:0 during frying has already been proposed as a good indication of the total alteration level (*26*).

The total content of the saturated short-chain glycerol-bound compounds was compared to the total content of polar compounds (**Figure 2A**). Data for both oils seemed to fit to a straight line. Regression analysis showed an acceptable linear correlation ($r = 0.967$) between both determinations. When the six compounds were grouped according to their parent hydroperoxide, significant differences were observed. Although in the case of the compounds derived from 9-hydroperoxide the data points fitted well to a single straight line (**Figure 2B**), different linear equations were found in the case of the compounds formed from 8-hydroperoxide (**Figure 2C**). These differences can be attributed to the formation of 8-hydroperoxide, which depends on the loss of oleic acid. As commented above, whereas the formation of 9-hydroperoxide is significant from both oleic and linoleic acid, 8-hydroperoxide is formed from only oleic acid in significant amounts (*25*). The content of fatty acids showed that oleic acid in OO and linoleic acid in SO were the acids that participated to a greater extent in degradation reactions. Therefore, for the same alteration level, the formation of 8-hydroperoxide is expected to be greater in OO than in SO. With respect to the formation of 9-hydroperoxides, differences between OO and SO are expected to be much lower, because both oleic and linoleic acids are involved. In conclusion, the total content of saturated short-chain glycerol-bound compounds provides a good indication of the total alteration level of oils heated at high temperature.

Characterization of Used Frying Oils. Used frying oils were characterized by determination of fatty acid composition, total polar compounds, and polar compound distribution (**Tables 4** and **5**). The samples were ordered according to increasing levels of the degree of unsaturation. Thus, three groups can be differentiated by means of the oleic-to-linoleic acid ratio, which

Table 6. Concentration of Short-Chain Oxidation Compounds (Milligrams per Gram of Oil) in Used Frying Oils from Restaurants and Fried-Food Outlets

sample	C7:0	C8:0	8-oxo-C8:0	C8:0 diester	9-oxo-C9:0	C9:0 diester	total
1	0.25 ± 0.007[a]	0.81 ± 0.057	0.23 ± 0.007	0.28 ± 0.007	0.61 ± 0.042	0.96 ± 0.035	3.14 ± 0.155
2	0.23 ± 0.057	0.88 ± 0.205	0.22 ± 0.007	0.25 ± 0.014	0.73 ± 0.028	1.02 ± 0.021	3.33 ± 0.332
3	0.44 ± 0.021	1.25 ± 0.064	0.37 ± 0.014	0.56 ± 0.035	0.88 ± 0.064	1.66 ± 0.007	5.16 ± 0.205
4	0.17 ± 0.049	0.82 ± 0.226	0.20 ± 0.007	0.26 ± 0.007	0.83 ± 0.064	0.76 ± 0.028	3.04 ± 0.381
5	0.16 ± 0.000	0.94 ± 0.007	0.18 ± 0.000	0.19 ± 0.014	0.75 ± 0.000	0.76 ± 0.014	2.98 ± 0.035
6	0.04 ± 0.000	1.13 ± 0.085	0.10 ± 0.007	0.05 ± 0.007	0.56 ± 0.007	0.25 ± 0.014	2.13 ± 0.120
7	0.07 ± 0.007	1.38 ± 0.092	0.12 ± 0.007	0.09 ± 0.014	0.62 ± 0.007	0.39 ± 0.021	2.67 ± 0.148
8	0.04 ± 0.000	1.47 ± 0.297	0.14 ± 0.021	0.08 ± 0.021	0.85 ± 0.042	0.33 ± 0.057	2.91 ± 0.438
9	0.11 ± 0.014	2.01 ± 0.148	0.21 ± 0.014	0.11 ± 0.000	1.18 ± 0.028	0.61 ± 0.028	4.23 ± 0.232
10	0.37 ± 0.007	3.36 ± 0.021	0.37 ± 0.035	0.33 ± 0.071	1.61 ± 0.120	1.52 ± 0.233	7.56 ± 0.487

[a] Mean ± standard deviation ($n = 3$).

ranged from 10.7 to 19.3 in samples 1−3, from 4.3 to 5.0 in samples 4 and 5, and from 0.5 to 1.0 in samples 6−10. Within each subgroup, the samples were numbered by order of increasing levels of total polar compounds. Except for sample 6, the content of total polar compounds was close to or well surpassed the limit established for human consumption (25% on oil).

Quantitation of Short-Chain Glycerol-Bound Compounds in Used Frying Oils. Table 6 lists results of major short-chain glycerol-bound compounds found in used frying oils. The total content of short-chain compounds ranged from 2.13 to 7.56 mg/g of oil. Samples with contents of total polar compounds of ∼25% showed total levels of short-chain compounds similar to those found in the thermoxidized oils, that is, within 2−3 mg/g of oil. This fact suggests that the total content of the compounds of interest increases with the total alteration level regardless of the oil, at least up to levels of 25% total polar compounds. In fact, group 3 (samples 6−10) shows a rise of the total content of the short-chain compounds from sample 6 to sample 10, which were numbered by increasing levels of total polar compounds.

As expected, the most abundant compounds were those derived from the 9-hydroperoxide. The content of 9-oxo-9:0 ranged from 0.56 to 1.61 mg/g of oil, which provides valuable information about the levels that can be found in fried foods.

As observed in the thermoxidized oils, the samples containing low levels of oleic acid (samples 6−10) showed low contents of the compounds derived from the 8-hydroperoxide. In this respect, the ratio between the sum of the compounds from 9-hydroperoxide, that is, C8:0, 9-oxo-C9:0, and C9:0 diester, and the sum of those from 8-hydroperoxide, that is, C7:0, 8-oxo-C8:0, and C8:0 diester, clearly provided information about the degree of oil unsaturation irrespective of the total alteration level of the oil. Thus, such a ratio, which can be easily calculated from values in **Table 6**, ranged within 2.8−3.7 for samples 1−3, within 3.8−4.6 for samples 4 and 5, and within 6.1−10.2 for samples 6−10.

From the results obtained in this study it can be deduced that the total content of major short-chain glycerol-bound compounds provides a good indication of the total alteration level of used frying oils. Furthermore, the ratio between the compounds from 9-hydroperoxide and those from 8-hydroperoxide provides a good indication of the degree of unsaturation of the frying oil irrespective of the total alteration level.

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LITERATURE CITED

(1) Chang, S. S.; Peterson, R. J.; Ho, C. T. Chemical reactions involved in deep-fat frying of foods. *J. Am. Oil Chem. Soc.* **1978**, *55*, 718−727.

(2) Dobarganes, M. C.; Márquez-Ruiz, G. Dimeric and higher oligomeric trigicerides. In *Deep Frying: Chemistry, Nutrition and Practical Applications*; Perkins, E. G., Erickson, M. D., Eds.; American Oil Chemists' Society: Champaign, IL, 1996; pp 89−111.

(3) Nawar, W. W. Volatile components of the frying process. *Grasas Aceites* **1998**, *49*, 271−274.

(4) Gasparoli, A. The formation of new compounds. *Grasas Aceites* **1998**, *49*, 303−309.

(5) Frankel, E. N. Frying fats. In *Lipid Oxidation*; Oily Press: Dundee, U.K., 1998; pp 227−248.

(6) Frankel, E. N. Hydroperoxide decomposition. In *Lipid Oxidation*; Oily Press: Dundee, U.K., 1998; pp 55−77.

(7) Kanazawa, K.; Kanazawa, E.; Natake, M. Uptake of secondary autoxidation products of linoleic acid by the rat. *Lipids* **1985**, *20*, 412−419.

(8) Oarada, M.; Miyazama, T.; Kaneda, T. Distribution of C-14 after oral-administration of (U-C-14) labeled methyl linoleate hydroperoxides and their secondary oxidation products in rats. *Lipids* **1986**, *21*, 150−154.

(9) Minamoto, S.; Kanazawa, K.; Ashida, H.; Natake, M. Effect of orally administered 9-oxononanoic acid on lipogenesis in rat liver. *Biochim. Biophys. Acta* **1988**, *958*, 199−204.

(10) Minamoto, S.; Kanazawa, K.; Ashida, H.; Danno, G.; Natake, M. The induction of lipid peroxidation in rat liver by oral intake of 9-oxononanoic acid contained in autoxidation linoleic acid. *Agric. Biol. Chem.* **1985**, *49*, 2747−2751.

(11) Kanazawa, K.; Ashida, H. Target enzymes on hepatic dysfunction caused by dietary products of lipid peroxidation. *Arch. Biochem. Biophys.* **1991**, *288*, 71−78.

(12) Kanazawa, K. Hepatic-dysfunction caused by dietary products of lipid-peroxidation. *Int. J. Tissue React.-Exp. Clin. Asp.* **1991**, *13*, 67−78.

(13) Berdeaux, O.; Velasco, J.; Márquez-Ruiz, G.; Dobarganes, C. Evolution of short-chain glycerol-bound compounds during thermoxidation of FAME and monoacid TAG. *J. Am. Oil Chem. Soc.* **2002**, *79*, 279−285.

(14) Berdeaux, O.; Marquez-Ruiz, G.; Dobarganes, C. Selection of methylation procedures for quantitation of short-chain glycerol-bound compounds formed during thermoxidation. *J. Chromatogr. A* **1999**, *863*, 171−181.

(15) Barrera-Arellano, D.; Márquez-Ruiz, G.; Dobarganes, M. C. A simple procedure to evaluate the performance of fats and oils at frying temperatures. *Grasas Aceites* **1997**, *48*, 231−235.

(16) IUPAC. Preparation of the fatty acid methyl esters 2.301. In *Standard Methods for the Analysis of Oils, Fats and Derivatives*, 7th ed.; International Union of Pure and Applied Chemistry, Blackwell Scientific: Oxford, U.K., 1992.

(17) IUPAC. Gas−liquid chromatography of fatty acid methyl esters 2.302. In *Standard Methods for the Analysis of Oils, Fats and Derivatives*, 7th ed.; International Union of Pure and Applied Chemistry, Blackwell Scientific: Oxford, U.K., 1992.

(18) Dobarganes, M. C.; Pérez-Camino, M. C. Fatty acid composition: a useful tool for the determination of alteration level in heated fats. *Rev. Fr. Corps Gras* **1988**, *35* (2), 67−70.

(19) IUPAC. Determination of polar compounds in frying fats 2.507. In *Standard Methods for the Analysis of Oils, Fats and Derivatives*, 7th ed.; International Union of Pure and Applied Chemistry, Blackwell Scientific: Oxford, U.K., 1992.

(20) Dobarganes, M. C.; Pérez-Camino, M. C. Systematic evaluation of heated fats based on quantitative analytical methods. *J. Am. Oil Chem. Soc.* **1988**, *65*, 101−105.

(21) Dobarganes, M. C.; Pérez-Camino, M. C.; Márquez-Ruiz, G. High performance size exclusion chromatography of polar compounds in heated and non-heated fats. *Fat Sci. Technol.* **1988**, *90*, 308−311.

(22) Cohen, J. D. Convenient apparatus for the generation of small amounts of diazomethane. *J. Chromatogr.* **1984**, *303*, 193−196.

(23) Firestone, D.; Stier, R. F.; Blumenthal, M. M. Regulation of frying fats and oils. *Food Technol.* **1991**, *45*, 90−94.

(24) Márquez-Ruiz, G.; Tasioula-Margari, M.; Dobarganes, M. C. Quantitation and distribution of altered fatty acids in frying fats. *J. Am. Oil Chem. Soc.* **1995**, *72*, 1171−1176.

(25) Frankel, E. N. Hydroperoxide formation. In *Lipid Oxidation*; Oily Press: Dundee, U.K., 1998; pp 23−41.

(26) Márquez-Ruiz, G.; Dobarganes, C. Short-chain fatty acid formation during thermoxidation and frying. *J. Sci. Food Agric.* **1996**, *70*, 120−126.

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