Quantitation and Distribution of Altered Fatty Acids in Frying Fats

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ABSTRACT: The distribution and quantity of polar compounds and altered fatty acids in used frying oils, collected by Food Inspection Services of the Junta de Andalucía in Spain, was measured. Additional samples evaluated were sunflower oil, high-oleic sunflower oil, and palm olein that were subjected to thermoxidation and frying in laboratory experiments. A combination of adsorption and high-performance size-exclusion chromatography was applied to the oil samples both before and after transesterification. Through analysis of fatty acid methyl ester derivatives, differentiation of four groups of altered fatty acids (oxidized monomers, nonpolar dimers, oxidized dimers, and polymers) could be attained. Evaluation of real frying samples with polar compound levels around the limit for fat rejection (21.1–27.6% polar compounds) gave values of total altered fatty acids ranging from 8.1 to 11.3%, and levels higher than 20% were found in the most degradated samples. The results obtained clearly support the need for control and improvement of the quality of used fats in fried-food outlets.
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KEY WORDS: Dimers, frying, HPSEC, oxidized fatty acids, oxidized triglycerides, polar compounds, polymers, thermoxidation.

Frying is one of the most popular procedures for food preparation worldwide. Hence, the use and abuse of frying fats has become of great concern for health administrations and for consumers, especially as the new compounds formed are thought to impair the nutritional value of fats (1–5).

Polar compound determination stands out as the most commonly used methodology to evaluate frying fat alteration and has been included in the regulations of different countries to establish limits of alteration for human consumption (6). Unfortunately, analyses of used frying oils, collected by food inspection services, indicate that a considerable number of samples often surpass the alteration levels permitted (7,8). Increased knowledge on fat degradation under different conditions can be achieved through application of high-performance size-exclusion chromatography (HPSEC) to the complex mixture of compounds included in the polar compound fraction (9). Thus, significant groups of compounds, including oxidized, polymeric, and hydrolytic compounds, can be quantitated.

Methodologies based on the simplest fatty acid derivatives, the fatty acid methyl esters (FAME), enable direct evaluation of the altered fatty acyls included in triglyceride molecules by gravimetric determination (10–12). Further, separation of FAME by size-exclusion chromatography can be carried out directly in the sample (13–15), or else a fraction of higher polarity can be separated first and then analyzed by HPSEC to provide an overall profile of the oxidized and polymerized groups of fatty acids (16). This analytical approach provides better knowledge on fat alteration and is of great value in relation to the nutritional consequences of frying oil consumption as it is focused on the fatty acyl groups that are the products of fat digestion ultimately absorbed. Applications of this methodology to animal studies have permitted evaluation of digestibility of oxidized, dimeric, and polymeric fatty acids, and results generally showed high apparent absorbability of oxidized compounds, particularly for the group of oxidized fatty acid monomers (17). Among the altered fatty acids, *trans* isomers and cyclic monomers cannot be separated by this methodology because they are similar to the nonaltered fatty acids in polarity and have been otherwise quantitated by a different analytical approach (18–20).

The aim of this work was to study the evolution of altered fatty acid level and distribution in oxidized and polymeric structures during frying, and to establish relationships between polar glyceridic compounds and polar fatty acids. Special focus has been placed on those samples with polar compound levels close to the limit for frying fat rejection.

EXPERIMENTAL PROCEDURES

Used frying oils. Sunflower oil (SO), high-oleic sunflower oil (HOSO), and palm olein (PO) were used in laboratory frying experiments that were performed in 1-L commercial electric fryers. Fifteen batches of 100 g potatoes each were fried for 6 min, and 15-min intervals were established between frying operations. Oils were heated over a total period of five hours. Temperature started at 190°C and ranged from 140 to 190°C during frying operations. There was no replenishment with fresh oil during frying. Initial and final surface-to-volume ratios were 0.32 and 0.40 cm^{-1}, respectively.

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Used frying oils from a number of restaurants and fried-food outlets in the south of Spain were supplied by Food Inspection Services of the Junta de Andalucía in Spain. Among them, two sets of samples were selected for the present work, namely, eight samples containing around 25% polar compounds (Group A), and nine samples with alteration levels higher than 30% polar compounds (Group B).

Thermoxidized oils. SO, HOSO, a mixture of SO and HOSO (1:1), and PO were subjected to thermoxidative conditions. Samples were placed in open beakers (surface-to-volume ratio of 1 cm^{-1}) and heated in an oven at 190°C for 10 and 20 h.

Analytical procedures: polar compounds. Total polar compounds were determined in oil samples by silica column chromatography, following the method proposed by the International Union of Pure and Applied Chemists (21), with two slight modifications (9). Distribution of polar compounds was performed by HPSEC in a Konik 500A chromatograph (Konik SA, Barcelona, Spain) with a 10-μL sample loop. A refractive index detector (Hewlett Packard, Pittsburgh, PA) and 100- and 500-Å Ultrastyragel columns (Waters Associates, Milford, MA) connected in series were operated at 35°C. The columns were 25 × 0.77 cm i.d., packed with a porous, highly cross-linked styrenedivinylbenzene copolymer (<10 μm). High-performance liquid chromatography-grade tetrahydrofuran served as the mobile phase with a flow of 1 mL/min, and the sample concentration was between 15 and 20 mg/mL in tetrahydrofuran (9).

Altered fatty acids. FAME were obtained by transesterification of oil samples with sodium methoxide and hydrochloric acid–methanol and subsequent recovery of methyl esters (22). Methyl esters were separated by silica column chromatography, with hexane/diethyl ether (88:12) to elute a nonpolar fraction and diethyl ether and methanol to obtain the polar fraction. Analyses of the nonpolar and polar fractions were performed by HPSEC by using the chromatographic conditions described previously. Quantitation of nonpolar fatty acid dimers (NPFAD) and nonaltered monomers was based on the gravimetric determination of the nonpolar fraction. Oxidized fatty acid monomers (OxFAM), oxidized fatty acid dimers (OxFAD), and fatty acid polymers (FAP) were also quantitated in the polar fraction. The methodology was described in detail, including calibration and reproducibility data, in an earlier publication (16).

Fatty acid composition. Fatty acid composition was determined in starting oils, SO, HOSO, and PO, after transesterification of samples with sodium methoxide and hydrochloric acid–methanol. Methyl esters were analyzed by gas–liquid chromatography in an SP-2380 (Supelco, Bellafonte, PA) fused-silica capillary column, 30 m long and 0.25 mm i.d., at a temperature of 180°C.

RESULTS AND DISCUSSION

Figure 1 shows representative HPSEC of total fatty acids and nonpolar and polar fractions obtained by silica column chromatography. The combined chromatographic analysis used in this study permits quantitation of five groups of compounds that differ in polarity or molecular weight. Fatty acid monomers are separated into nonpolar and polar monomers, the latter originated *via* oxidation, in the first and second fractions, respectively. Otherwise, both fatty acid monomer peaks would overlap due to similar molecular weight. Likewise, nonpolar dimers, representative of thermal alteration as there is no oxygen involved in their structure, are quantitated in the first fraction, independently of oxidative dimers, which are determined in turn in the polar fraction. Finally, polymeric compounds are included and analyzed in this latter fraction.

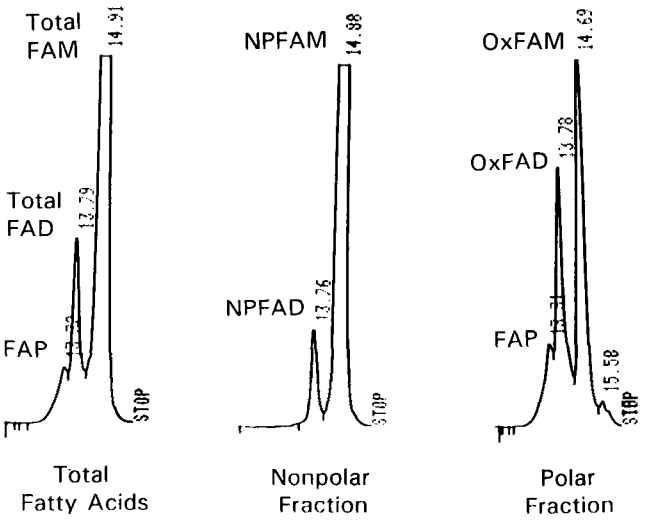

FIG. 1. Significant parts of high-performance size-exclusion chromatograms corresponding to total fatty acids and the nonpolar and polar fractions obtained by silica column chromatography. Abbreviations: FAM, fatty acid monomers; NPFAM, nonpolar fatty acid monomers; OxFAM, oxidized fatty acid monomers; FAD, fatty acid dimers; NPFAD, nonpolar fatty acid dimers; OxFAD, oxidized fatty acid dimers; and FAP, fatty acid polymers.

Table 1 shows polar compound content and distribution, and fatty acid composition (for fatty acids present at >1%) of the oils used in laboratory frying and thermoxidation experiments. Table 2 lists the polar compound content and distribution along with the altered fatty acid content and distribution of the oils after frying. The results selected corresponded to the frying period (5 h) that gave alteration levels around the limit recommended by regulations for discarding frying fats (25–27% polar compounds). Although samples showed similar levels of polar compounds after five hours of frying, distribution data permitted comparisons between oils. Clearly, dimeric and polymeric compounds occurred at the highest level in the most unsaturated oil, SO, whereas, in contrast, PO contained considerable amounts of diglycerides, which were already present in the starting oil (Table 1). Although the level of polar compounds was apparently higher for PO than for HOSO, differences between initial and final oils indicated that alteration had been greater in the latter. As to variations of compounds coming from hydrolysis, neither free fatty acids

TABLE 1
Total Polar Compounds (wt% on oil), Polar Compound Distribution (mg/g oil), and Fatty Acid Composition (wt% on oil) of Oils Used in Laboratory Frying and Thermoxidation

	Polar compounds						Fatty acid composition				
		Distribution[b]									
Sample[a]	Total	TGP	TGD	OxTGM	DG	FFA	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	Others
SO	2.8	—	4.0	8.3	10.6	5.1	6.3	4.6	20.0	66.8	2.3
HOSO	3.1	—	3.4	6.9	16.3	4.4	4.0	4.3	72.3	16.8	2.6
PO	7.7	—	4.6	6.1	65.2	1.1	31.4	4.4	48.6	12.2	3.4

[a]SO, sunflower oil; HOSO, high-oleic sunflower oil; and PO, palm olein.
[b]TGP, triglyceride polymers; TGD, triglyceride dimers; OxTGM, oxidized triglyceride monomers; DG, diglycerides; and FFA, free fatty acids.

nor diglycerides significantly changed during frying. These data give evidence of the small contribution of hydrolytic alteration, in spite of the high moisture content of potatoes.

However, evaluation of polar compounds does not permit differentiation of altered triglycerides with either one or more oxidized fatty acyl groups. In this regard, quantitation of altered fatty acids is of great use as it overcomes this limitation and provides additional information. Thus, after transesterification, contents of altered fatty acids were 11.6, 9.0, and 7.4% for SO, HOSO, and PO, respectively, thereby indicating that thermoxidative alteration depended on oil unsaturation level. It is illustrative to calculate the average number of altered fatty acids per polar glyceride molecule, which was 0.87 for PO vs. around 1.20 in the two sunflower oils (SO and HOSO). PO presented not only the lowest value of altered fatty acids, but also the lowest percentage of altered fatty acids in polar glyceridic compounds. A value below 1 for PO can be explained, as questioned earlier, by the fact that diglycerides form a considerable part of total polar compounds but, after transesterification, result in FAME that are mostly included in the nonpolar fraction.

Apart from the presence of substantial amounts of OxFAD and FAP, the high occurrence of OxFAM (approximately 30 mg/g), given that such compounds can be readily absorbed, presenting digestibility coefficients around 80%, is particularly relevant (17). Oxidized monomers are a complex mixture of oxygenated compounds, including epoxides, ketones, and hydroxyacids, as well as polyoxygenated monomeric compounds (23). In recent years, the literature has focused on the potential biological effects of oxidized lipids, and there is increasing evidence that they may be detrimental to health, particularly in connection with the development of atherosclerosis (24,25), liver damage (26,27), and promotion of intestinal tumors (28,29).

Table 3 shows the results obtained after evaluation of selected used frying oil samples from Food Inspection Services, with polar compound levels close to the limit for discarding the oil. Although the type of oil used, frying conditions, and fried products were variable, the samples included here had in common a similar polar compound content, ranging from 21.1 to 27.6%.

Samples with the same percentage of total polar compounds showed a different pattern of compound distribution. Thus, A6, A7, and A8 had 27.5, 27.6, and 27.6% polar compounds, respectively, but the latter one contained predominantly oxidized triglyceride monomers and diglycerides, whereas the two first ones were primarily comprised of triglyceride dimers and polymers. Such differences are not strange, considering that these samples are of unknown history and may have undergone different treatments, especially in terms of frying temperature, frying periods, and total period of use. These variables are known to exert an important effect on the distribution of polar compounds because oxidized monomers are expected to be major products at low temperature, whereas dimers and polymers would increase greatly at a high temperature. On the other hand, analyses of fatty acid composition (data not shown) clearly indicated that samples A6 and A7 corresponded to conventional SO, with a higher tendency to form polymeric compounds in comparison to sample A8, which presented the fatty acid profile typical of an olive oil. Differences between samples were better reflected in the values of total altered fatty acids, which were

TABLE 2
***Total Polar Compounds* (wt% on oil), Polar Compound Distribution (mg/g oil), Total Altered Fatty Acids (wt% on oil), and Altered Fatty Acid Distribution (mg/g oil) of Laboratory-Used Frying Oils[a]**

	Polar compounds						Altered fatty acids				
		Distribution						Distribution			
Sample	Total	TGP	TGD	OxTGM	DG	FFA	Total	FAP	NPFAD	OxFAD	OxFAM
SO	28.9	50.0	125.1	98.0	11.8	4.1	11.6	10.7	42.1	32.2	31.0
HOSO	23.0	33.4	82.3	91.3	19.3	3.7	9.0	6.1	27.0	24.0	32.9
PO	25.5	26.8	71.1	86.4	69.4	1.3	7.4	5.0	15.5	26.1	27.4

[a]Table 1 for abbreviations; FAP, fatty acid polymer; NPFAD, nonpolar fatty acid dimers; OxFAD, oxidized fatty acid dimers; and OxFAM, oxidized fatty acid monomers.

TABLE 3
Total Polar Compounds (wt% on oil), Polar Compound Distribution (mg/g oil), Total Altered Fatty Acids (wt% on oil), and Altered Fatty Acid Distribution (mg/g oil) in Used Frying Oils from Restaurants and Fried-Food Outlets[a]

	Polar compounds						Altered fatty acids				
		Distribution						Distribution			
Sample	Total	TGP	TGD	OxTGM	DG	FFA	Total	FAP	NPFAD	OxFAD	OxFAM
A1	21.1	37.3	87.8	61.4	18.8	5.7	9.6	8.0	39.4	20.3	28.3
A2	23.1	40.0	92.2	67.9	25.1	5.8	8.1	7.6	19.9	20.9	32.6
A3	25.5	46.8	89.8	85.7	25.8	6.9	10.4	10.8	30.9	28.4	33.9
A4	25.7	62.5	94.3	58.8	35.2	6.2	10.5	12.8	29.6	27.3	35.3
A5	26.4	55.2	88.7	64.4	50.7	5.0	10.8	6.8	38.3	28.8	34.1
A6	27.5	62.2	98.1	76.5	33.8	4.4	10.7	10.9	33.8	27.9	34.4
A7	27.6	65.1	109.4	72.0	23.2	6.3	11.3	10.6	38.2	26.9	37.3
A8	27.6	37.3	72.6	93.8	61.8	10.5	8.7	4.6	16.5	28.2	37.7

[a]Alteration levels close to the rejection limit. See Tables 1 and 2 for abbreviations.

lower for A8 (8.7%) than for A6 and A7 (10.7 and 11.3%, respectively). Clearly, the content of total altered fatty acids is an excellent indicator of the thermoxidative alteration level as it exclusively measures the amount of fatty acyls affected.

In all samples, OxFAM constituted a major fraction among the different groups of altered fatty acids, normally accounting for around 30%. Levels of NPFAD and oxidized dimers were more variable, and polymer content was still low at this stage of alteration. Quantitation of dimers and polymers directly in the oils and after transesterification of the samples revealed some insight into the complexity of the triglyceride polymer structure. The values found for the fatty acid polymers-to-triglyceride polymers ratio were low in contrast to those for fatty acid dimers/triglyceride dimers which, with the exception of sample A2, ranged from 0.6 to 0.8. These data give evidence of the considerable contribution of dimeric linkages to the structures of trimeric and higher oligomeric triglycerides in this group of samples.

Overall, it was surprising that the samples included in Tables 2 and 3, at the limit of rejection, had alteration levels as high as 10% expressed specifically on altered fatty acyls, which means that significant amounts of altered fatty acids can be ingested.

For Table 4, a selection of samples supplied by Food Inspection Services with the highest content of polar compounds was included. It is important to note that, among the 200 samples analyzed, values higher than 50% polar compounds were found, similar to results reported in other European countries (7,8), thus supporting the need for control and improvement of the quality of used fats in fried-food outlets.

In these samples, the process of alteration had already given rise to important amounts of dimers and polymers, as can be observed in the polar compound distribution and, specifically, in the altered fatty acid distribution where FAP constituted 16–25% of total fatty acids, thus showing the importance of fatty acid polymerization. As an additional indication of the high alteration level achieved, it is noteworthy that the calculated number of altered fatty acyls per polar glyceride molecule was over 1.5 in some samples, i.e., a high number of molecules included more than one altered fatty acyl. Finally, amounts of OxFAM ranging from 37.7 to 66.4 mg/g oil were found in these samples.

Data from thermoxidation experiments are listed in Table 5. From comparisons between samples heated during 10 and 20 h, formation of the different groups of compounds can be followed. First of all, in view of the similar patterns of polar compounds and altered fatty acid distribution obtained for thermoxidized oils and used frying oils (Table 2), it would be impossible to differentiate whether a certain sample had been used for frying or had been heated in the absence of food.

TABLE 4
Total Polar Compounds (wt% on oil), Polar Compound Distribution (mg/g oil), Total Altered Fatty Acids (wt% on oil), and Altered Fatty Acid Distribution (mg/g oil) in Used Frying Oils from Restaurants and Fried Food Outlets[a]

	Polar compounds						Altered fatty acids				
		Distribution						Distribution			
Sample	Total	TGP	TGD	OxTGM	DG	FFA	Total	FAP	NPFAD	OxFAD	OxFAM
B1	33.1	64.8	71.9	53.8	123.2	17.3	16.9	34.7	40.1	53.9	40.3
B2	37.3	105.9	143.2	89.6	27.6	6.7	15.6	24.3	45.1	42.5	44.1
B3	39.2	104.7	135.6	98.0	44.7	9.0	15.1	29.2	42.1	42.0	37.7
B4	39.7	78.2	97.7	133.0	75.8	12.3	15.3	25.4	35.4	49.5	42.7
B5	43.5	120.5	139.3	99.6	68.7	6.9	16.3	41.2	50.8	32.0	39.0
B6	47.1	122.9	132.8	151.7	57.0	6.6	21.0	53.0	37.2	60.8	59.0
B7	49.7	210.7	135.2	109.3	34.3	7.5	25.0	56.0	53.6	74.0	66.4
B8	50.2	224.3	141.6	108.9	21.1	6.1	29.1	58.3	85.2	84.3	63.2
B9	53.6	206.4	178.0	123.3	23.0	5.3	23.0	36.5	72.2	61.9	59.4

[a]High alteration levels. See Tables 1 and 2 for abbreviations.

TABLE 5
Total Polar Compounds (wt% on oil), Polar Compound Distribution (mg/g oil), Total Altered Fatty Acids (wt% on oil), and Altered Fatty Acid Distribution (mg/g oil) in Oils Thermoxidized During 10 and 20 h[a]

	Polar compounds						Altered fatty acids				
		Distribution						Distribution			
Sample	Total	TGP	TGD	OxTGM	DG	FFA	Total	FAP	NPFAD	OxFAD	OxFAM
10 h											
SO	33.4	73.5	143.6	100.5	11.4	5.0	14.6	14.6	47.8	41.5	42.1
HOSO	29.9	66.1	103.2	106.1	19.4	4.2	12.9	13.5	39.1	32.1	44.3
SO/HOSO	33.3	80.6	127.2	106.6	14.3	4.3	14.9	15.4	43.1	40.3	50.2
PO	28.8	45.5	94.8	87.5	59.0	1.2	9.0	12.2	25.7	24.3	27.8
20 h											
SO	57.5	213.7	181.5	162.4	11.5	5.9	28.4	66.2	62.4	75.7	79.7
HOSO	46.7	157.6	125.6	153.1	26.0	4.7	22.1	47.2	56.5	53.2	64.1
SO/HOSO	51.6	197.8	148.1	147.0	18.2	4.9	25.5	58.2	62.9	69.5	64.4
PO	44.6	119.6	129.3	133.3	62.3	1.5	20.1	49.0	39.1	50.8	62.1

[a]See Tables 1 and 2 for abbreviations.

However, and consistent with previous studies (30), the alteration level through thermoxidation was comparatively lower than that occurring in discontinuous frying. As can be observed, oils used for frying during 5 h at 190°C at an average surface-to-volume ratio of 0.36 cm^{-1} (Table 2) gave alteration levels close to those found for the same oils heated during twice that much time (10 h) at 190°C at a much higher surface-to-volume ratio (1 cm^{-1}). The distinct behavior could be attributed to differences in the mode of heating. In the oven, the bulk of oil is at the same temperature (190°C) and solubility of oxygen is low, and in discontinuous frying the temperature is much lower in the air-to-oil interface. In this latter situation, when the surface of the oil is not protected by the food, penetration of oxygen would be favored, and hence, oxidative reactions are enhanced.

Thermoxidized samples did not show the clear-cut differences in total alteration levels that could be expected from the different unsaturation degrees of the starting oils, even though alteration was consistently in the order SO > SO/HOSO > HOSO > PO. In general, the triglyceride polymers-to-polar compounds ratio increased with the period of heating and, moreover, a rapid rise of triglyceride polymers/triglyceride dimers could be observed.

Independent of the heating period, the unsaturation degree of the oil used had an important influence on the formation of triglyceride polymers. Thus, SO and SO/HOSO presented the highest levels. Examination of the altered fatty acid profile showed that fatty acid polymers were also the compounds that underwent the largest increase from 10 to 20 h, reaching values that were approximately fourfold higher. Overall, the results obtained showed the importance of polymerization at high alteration levels and the role of oxidized monomers and dimers in the formation of polymeric compounds. At low levels of polar compounds, nonpolar dimers and oxidized monomers would be the main groups among the alteration compounds, both groups deriving from the initial fatty acids. As their levels increase, such compounds also would contribute as intermediates in oxidative polymerization.

In summary, quantitation and distribution of altered fatty acids provide novel and useful information on frying fat alteration and can be combined with the analysis of polar glyceridic compounds to gain some insight into the complex structures of triglyceride polymers. The results presented here, especially those obtained in samples from restaurants and other fried-food outlets, support the view that it is necessary to improve the quality of frying fats and increase knowledge on their nutritional significance. Special attention should be placed on oxidized compounds, in light of their high occurrence in frying fats and enhanced digestibility.

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