Thermoxidative and Hydrolytic Changes in Sunflower Oil Used in Fryings with a Fast Turnover of Fresh Oil

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The modification of a sunflower oil used for 75 repeated deep-fat fryings of potatoes, with a fast turnover of fresh oil during frying, was evaluated by measuring the total polar components isolated by column chromatography. The total polar components increased rapidly during the first 20 fryings from 5.09 ± 0.21 (mean ± SD) mg/100 mg unused oil to 15.99 ± 0.40, followed by minor but also significant changes until the thirtieth frying (17.99 ± 0.41 mg/100 mg oil). The level did not increase further with continued frying. Further, the polar fraction was examined by high-performance size-exclusion chromatography. Triglyceride polymers increased from 0.10 ± 0.01 mg/100 mg unused oil to 1.65 ± 0.13 and 3.44 ± 0.17 mg/100 mg oil at the twentieth and seventy-fifth fryings, respectively. Triglyceride dimers also increased significantly from 0.75 ± 0.12 mg/100 mg unused oil to 6.25 ± 0.28 (mg/100 mg oil) at the twentieth frying and to 7.09 ± 0.31 mg/100 mg oil at the thirtieth frying, with no further significant changes. Oxidized triglycerides also significantly increased, but at the twentieth frying reached a near-steady state of 6.26 mg/100 mg oil. Diglycerides and free fatty acid levels, related to hydrolytic alteration, did not increase with continued fryings. The results indicate that during deep-fat frying of potatoes with fast turnover of fresh sunflower oil, more thermoxidative than hydrolytic processes take place. A dramatic leap of total polar content and a change of compounds related to thermoxidative alteration of the oil were found during the first twenty fryings, followed by minor changes and by a tendency to reach a near-steady state throughout the successive fryings.

KEY WORDS: Column chromatography, HPSEC, oil turnover, polar compounds, sunflower oil.

During deep-fat frying, a wide variety of chemical reactions take place. When a moist food is placed in oil at frying temperatures, air and steam are evolved, initiating a chain of interrelated reactions.

The steam will cause hydrolysis of triglycerides, resulting in the formation of free fatty acids, glycerol and mono- and diglycerides, while the air released into the frying system will initiate a cycle of oxidation reactions involving the formation of free radicals. These oxidation processes will involve fatty acids in intact triglycerides as well as the products of triglyceride hydrolysis. The fatty acid residues of triglycerides formed can react to form polymers and other complex reaction products. Intact or thermally modified triglycerides may be involved in polymerization *via* Diels-Alder reactions. In addition, many of the aforementioned reactions are interrelated, and a complex mixture of products is formed (1,2).

The number and variety of products formed in the frying process are great (as was described above), and the nutritional and toxicological consequences of their consumption are largely unknown. Consequently, there is a need to define the alteration level at which the fats or oils must be discarded. It is claimed that a fat or oil must be discarded when its polar fraction is more than 25% (3–5). The polar content of a fat used for frying is adversely affected by numerous factors, as described in previous studies (6–8).

Shallow or pan frying is accomplished quickly, and repeated re-use of the fat is not a common practice. Therefore, the amount of polar material usually does not reach the critical level of 25%. However, during deep-fat frying in the home or in restaurants, the fat is likely to be kept hot for long periods with only occasional use for frying. There is, therefore, a relatively slow turnover of fat. On the other hand, deep-fat frying is performed with faster turnover of fresh fat to replenish the fryer because so much fat is removed along with the fired food. Most of the debate is about how the slow or fast turnover of fresh fat affects the deterioration of fat used in frying (9). In previous works (6–10), we investigated the alteration of different oils used in frying without turnover of fresh oil. In this report, the deep-fat frying was performed under fast turnover of fresh oil. The alteration of the sunflower oil employed in 75 repeated deep-fat fryings of potatoes was evaluated by measuring the percentage of total polar components by the column chromatographic method of Waltking and Wessels (11). In addition, the polar components were examined by high-performance size-exclusion chromatography (HPSEC) to investigate the thermoxidative and hydrolytic modification. Although many analytical methods have been used for determination of the monomers, dimers and higher polymers of oxidized fats and oils, the technique of HPSEC may be considered as one of the most promising because it increases the possibility of quantitation of all classes of alteration compounds: polymers and dimers of triglycerides, oxidized triglycerides, diglycerides and free fatty acids (12,13).

EXPERIMENTAL PROCEDURES

Performance of frying. Refined sunflower oil (Córdoba, Spain) and potatoes were purchased at a local store. The oil was stored below 15°C in the dark and used as purchased. Fatty acid composition, refractive index and free fatty acid level of unused oil and data regarding the composition of the potatoes are given in Table 1.

Domestic deep-fat fryers with a 3-L aluminum vessel were used for frying. The potatoes were chopped into slices *ca.* 2 mm thick. The proportions of food to frying oil in the repeated fryings were kept at 500 g/3 L. Because so much oil is removed along with the fried potatoes, it is necessary to replenish the fryer bath with unusued oil. Throughout the first twenty fryings, the frying bath volume was replenished with fresh oil every four fryings, following a parallel scheme described in previous works (7,8). After the twentieth frying, the fryer volume was

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TABLE 1

Some Characteristics of Raw Potatoes and Unused Sunflower Oil Employed in Deep-Fat Frying[a]

Potatoes		
Moisture (%)	n = 3	77.3 ± 0.9
Protein (g % fresh matter)	n = 3	2.5 ± 0.2
Fat (g % fresh matter)	n = 3	0.2 ± 0.05
Sunflower oil		
Free fatty acids[b]	n = 3	0.05 ± 0.017
Refractive index at 20°C	n = 3	1.47 ± 0.00
Major fatty acids (% total fatty acids)		
Palmitic	n = 2	6.76 ± 0.25
Stearic	n = 2	3.79 ± 0.15
Oleic	n = 2	32.43 ± 0.15
Linoleic	n = 2	55.52 ± 0.09

[a]Values are means ± SD; n = number of analysis.
[b]Expressed as mg of potassium hydroxide necessary to neutralize the fatty acids contained in 1 g of fat.

made up with unused oil every five fryings to carry out ten fryings per day, and the addition of fresh oil every five fryings instead of every four fryings did not change the aim of this study. A total of 75 fryings were carried out. Figure 1 shows the temperature changes during the frying process. Time required to reach and keep the bath oil at 180°C, before introduction of potatoes, was 20 min. Potatoes were then fried for 8 min. After the end of each frying operation, the oil was again heated to 180°C before starting a new frying. The time required was 10 min. The overall time the oil was heated throughout the whole experiment can be estimated as 25.17 h. The oil loss was about 10% every five fryings, which implies the addition of 4.5 L of new oil throughout the 75 fryings carried out. Aliquots of 50 mL from the unused oil and from the twentieth, thirtieth, fiftieth and seventy-fifth fryings were taken for analysis.

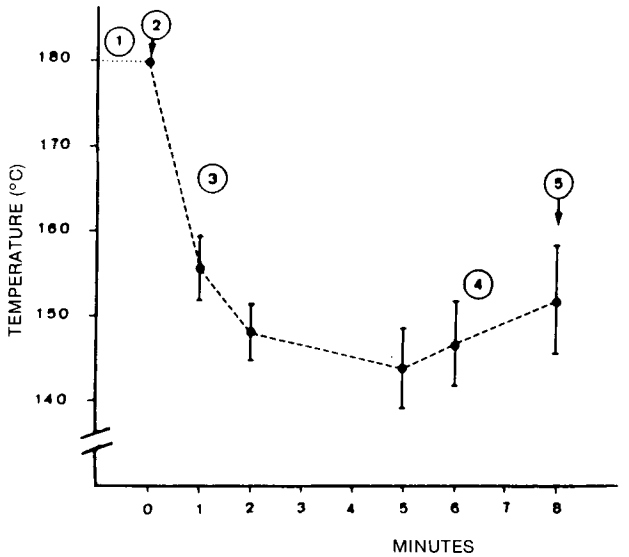

FIG. 1. Average temperature evolution of the frying medium-cooking of fried potatoes: 1, oil held at 180°C; 2, introduction of potatoes; 3, temperature decrease because of water evaporation; 4, temperature increase, possibly because of absorption of oil by food and lower water evaporation; 5, end of the frying operation.

Determination of the percentage of the polar fraction. The polar fraction was evaluated by the column chromatographic method of Waltking and Wessels (11), with a modifying proportion of petroleum ether/diethyl ether used to fill the column and to elute the nonpolar fraction. An accurately weighed sample of 1 ± 0.01 g of sunflower oil was dissolved in 20 mL petroleum ether/diethyl ether, 87:13 (vol/vol) when unused oil was analyzed, and 90:10 (vol/vol) when used oil was analyzed. The sample was then transferred to a silica gel chromatographic column, and the methods of Dobarganes *et al.* (14) and Pérez-Camino (15) were followed. A final elution of the column with chloroform/methanol (1:1, vol/vol) was performed to improve the recovery of the sample.

Two samples each of unused oil and of used oil from the twentieth, thirtieth, fiftieth and seventy-fifth fryings were analyzed.

The separation of the nonpolar and polar fractions was checked by thin-layer chromatography on 0.5 mm-thick 60 F 250 silica gel plates (20 × 20 cm glass). Polar and nonpolar fractions were diluted 50 times (wt/vol) in hexane/diethyl ether (80:20, vol/vol). Samples were applied as 10-μL spots with a 705 Hamilton microsyringe. Plates were developed with hexane/ethyl ether/acetic acid (80:20:1, vol/vol/vol) in a lined tank for *ca.* 25 min (*ca.* 17 cm) and then removed, letting the solvent evaporate. The spots were visualized by coating with iodine vapors.

HPSEC. The polar fraction previously obtained by column chromatography, as described before, was analyzed by HPSEC, following the method of Dobarganes *et al.* (12) to obtain further information about the hydrolytic and/or thermoxidative alterations that occurred in the sunflower oil during frying. Isolated polar fractions were analyzed in a Konic 500 A chromatograph (Barcelona, Spain) with a 10 μL sample loop. A Hewlett-Packard 1037 A refractive index detector (Palo Alto, CA) and two 300 mm × 7.5 mm i.d. (5 μm particle size) 10 μm and 50 μm pL gel (polystyrene-divinylbenzene) columns (Hewlett-Packard), connected in series, were operated at 45°C. High-performance liquid chromatography (HPLC)-grade tetrahydrofuran served as the mobile phase with a flow of 1 mL/min. Sample concentration was 10–15 mg/mL in tetrahydrofuran. All eluents, as well as samples, were precleaned by passing them through a filter (2 μm).

To evaluate the hydrolytic and thermoxidative products, pure fatty acids, diglycerides, triglycerides and total polar fractions at different concentrations were studied. Correlations obtained between the detector response and the weight of different compound groups injected were linear ($r > 0.99$). The response factors for fatty acids, diglycerides, triglycerides and total polar components were similar. Because standards for triglyceride dimers and triglyceride polymers are not available, regression lines relating retention times and molecular weight (MW) of compounds, such as free fatty acids, monoglycerides, diglycerides and triglycerides, were drawn. Then, the retention times of the sample chromatogram peaks were extrapolated on an extension of these regression lines. A MW of ≅1800 and a MW of ≅3000 corresponded to MW of triglyceride dimers and triglyceride trimers, respectively.

Two samples each of unsed oil and of used oil from the twentieth, thirtieth, fiftieth and seventy-fifth fryings were analyzed.

Statistical analysis. The data from the different oils were compared by a one-way analysis of variance (ANOVA) of repeated measures, followed by a multiple-range analysis with a STSC V5.0 statistical packet (STSC Inc., Rockville, MD). Significant differences were assessed at a level of $P < 0.05$. A correlation test was applied to study the relationship between different altered compounds and number of fryings.

RESULTS AND DISCUSSION

Total polar content (mg/100 mg oil) as a representative measurement of the total alteration of the oil and the contents (mg/100 mg oil) of different groups of altered products are given in Table 2. The results were obtained from unused sunflower oil and the corresponding oil used in different fryings.

The results indicate a dramatic leap of total polar content in the oil from 5.09 ± 0.21 (mean ± SD) mg/100 mg unused oil to 15.99 ± 0.40 mg/mg oil when used in twenty repeated and discontinuous fryings, followed by a tendency to reach a near-steady state.

The relationship between the increase of total polar content and total thermoxidative and hydrolytic alterations and the number of fryings showed an adjustment to a cubic equation, with correlation coefficients (R-SQ) of 0.9957, 0.9940 and 0.7194, respectively (Fig. 2).

Cuesta *et al.* (7) have reported an increase of the polar fraction with the number of fryings when analyzing samples from olive oil used in deep-fat frying of potatoes.

Recent data (10) also reported that, during the deep-fat frying of potatoes (in sunflower oil without turnover of fresh oil), a continuous increase of the polar fraction was observed until the sixtieth frying, when an objectionable level of 27.3% of polar material was reached. This is in contrast to the results obtained in the current study where a frequent turnover of fresh oil was made. This comparison is relevant, because most of the debate is about how the slow or frequent turnover of fresh fat affects the deterioration of fat used in frying. As a consequence of the frequent turnover with fresh oil, there are usually fewer problems with fat deterioration, and the objectionable level of 25% of polar material is, probably, rarely reached.

The polar fraction was further examined by HPSEC to investigate the thermoxidative and hydrolytic alterations in the frying oil. The HPSE chromatograms of polar compounds from unused and used sunflower oil samples, which are identified in Figure 3 as A, B, C, D and E, are presented. Table 2 and Figure 2 indicate that the total thermoxidative alteration level changes in a similar way to the total polar level. The amount of triglyceride dimers increased continuously throughout 75 fryings, whereas oxidized trygliceride did not change after the twentieth frying, and triglycerides polymers increased rapidly during the first fifty fryings but did not increase further with continued fryings (Table 2).

Triglycerides initially react to produce triglyceride dimers. The isolation of dimers formed during deep-fat frying has been of great interest. Kupranycz *et al.* (16) found that sunflower oil after both 8 and 16 h of thermal oxidation contained substantially higher amounts of both dimeric and oligomeric triglycerides than did the unheated oil. The rates of dimer accumulation during the first 8 h of heating exceeded the rates of oligomeric triglyceride formation, while the rate of dimer accumulation decreased during the 8–16 h of heating and the amount of oligomeric triglycerides continued to increase at a steady rate throughout the 16-h heating period. The results obtained in this study (Table 2 and Figs. 2 and 3) are in agreement with those found by Kupranycz *et al.* (16), although the levels of these compounds soon reached a near-steady state.

Perrin *et al.* (17) analyzed samples from sunflower oil oxidized by deep-fat frying to a stable foam. They reported the presence of dimers at levels between 12.1 and 12.9% of the oxidized mixtures. Gere (18) also reported the presence of dimeric triglycerides in sunflower oil used in deep-fat frying. Rojo and Perkins (19) described the accumulation of monomeric cyclic fatty acids in a soybean oil heated intermittently for 8 h (8 h/d) of simulated deep-fat frying. Perkins and Pinter (20) carried out studies on

TABLE 2

Distribution of Polar Components into Different Groups of Alteration Compounds in Unused Sunflower Oil and After Being Used in Repeated Fryings of Potatoes[a]

		Number of fryings				
	ANOVA	0	20	30	50	75
Total polar content	⩽0.001	5.09a ± 0.21	15.99b ±0.40	17.99c ±0.41	18.92c ±0.49	19.11c ±0.40
Triglyceride polymers	⩽0.001	0.10a ±0.01	1.65b ±0.13	2.50c ±0.20	3.15d ±0.20	3.44d ±0.17
Triglyceride dimers	⩽0.001	0.75a ±0.12	6.25b ±0.28	7.09bc ±0.31	7.37bc ±0.45	7.51c ±0.34
Oxidized triglycerides	<0.001	2.70a ±0.27	6.26b ±0.30	6.49b ±0.29	6.58b ±0.39	6.26b ±0.30
Diglycerides	NS	1.11a ±0.17	1.33a ±0.06	1.32a ±0.09	1.39a ±0.10	1.41a ±0.02
Free fatty acids	NS	0.43a ±0.10	0.50a ±0.10	0.59b ±0.10	0.43a ±0.06	0.48a ±0.05

[a]Mean of two samples ± SD expressed in mg/100 mg oil. Values in the same row bearing a common letter are not significantly different. NS, not significant; ANOVA, analysis of variance.

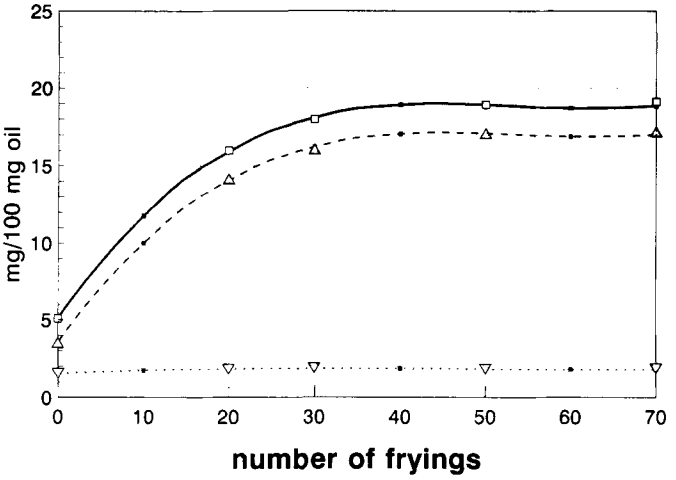

FIG. 2. Compounds related to thermoxidative and hydrolytic modifications. Total polar component (TPC) (solid lune with open box); total thermoxidative modification (TTM) (dashed line with shaded triangle) and total hydrolytic modification (THM) (dotted line with inverted shaded triangle). [Fit functions (line with closed box)]. TPC = 5.102180 + 0.812466F − 0.015524F^2 + 0.000096F^3; TTM = 3.566031 + 0.785211F − 0.014846F^2 + 0.000091F^3; THM = 1.536203 + 0.027329F − 0.000681F^2 + 0.000005F^3. (F = number of fryings).

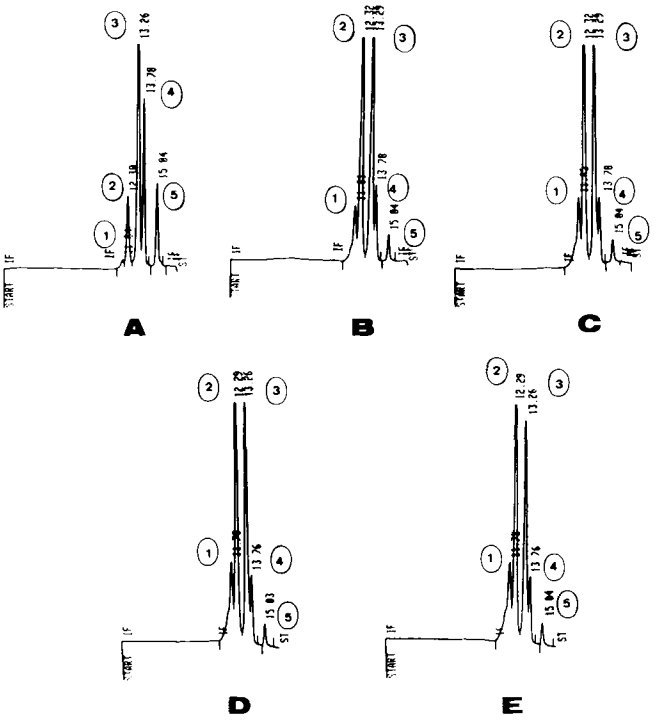

FIG. 3. High-performance size-exclusion chromatograms of unused (A), and used oil samples: B (twentieth), C (thirtieth), D (fiftieth) and E (seventy-fifth) fryings. Peaks 1, 2, 3, 4 and 5 are triglyceride polymers, triglyceride dimers, oxidized triglycerides, diglycerides and free fatty acids, respectively. Conditions: column, series-connected polystyrene-divinylbenzene, 300 mm × 7.5 mm i.d. (5 μm particle size); eluent, tetrahydrofuran at 1 mL/min; 10-μL injection volume; refractive index detection.

the concentrations of oxidized components in abused fat, measuring the levels of these compounds by HPLC and gas chromatography–mass spectrometry (GC–MS), after concentrating altered fatty acid methyl esters of used oil by different separation techniques. The peaks obtained by the HPLC method represented a complex mixture of components and can be utilized to evaluate the quality of used fats by comparison with fresh fats to indicate the degree of deterioration during use. The GC–MS samples showed peaks that were identified as derivatives of stearic acid. Minor component peaks indicated a mixture of cyclic monomers.

The total hydrolytic modifications (diglycerides plus free fatty acids) are presented in Figure 2. The contribution from hydrolytic modifications, as described by Dobarganes *et al.* (12), may be investigated by quantifying diglycerides but not free fatty acids, because the latter are partially lost during frying. As shown in Table 2, diglyceride levels tended to increase during the fryings.

The results also indicate that, during the deep-fat frying of potatoes, more thermoxidative than hydrolytic processes took place. Table 3 shows that the ratio of thermoxidative to hydrolytic compounds in used sunflower oil increased during repeated fryings of potatoes.

In short, repeated fryings of potatoes in sunflower oil, with a fast turnover of fresh oil during the performance of fryings, rapidly increased the level of total polar material in the fryer oil during the first twenty fryings, followed by a tendency of this polar material to reach a near-steady state. Hydrolysis, but mainly thermal oxidation, took place. Data suggest that, with frequent turnover of fresh oil, the critical level of 25% of polar material is rarely reached, and there are fewer problems with fat deterioration.

TABLE 3

Ratios of Nonpolar Compounds to Different Groups of Alteration Compounds, and Ratio of Thermoxidative Alteration Compounds to Hydrolytic Alteration Compounds in Unused Sunflower Oil and After Being Used in Repeated Fryings of Potatoes

	Number of fryings				
	0	20	30	50	75
Nonpolar compounds/polar compounds	18.65	5.25	4.56	4.29	4.23
Nonpolar compounds/thermoxidative alteration compounds	26.74	5.93	5.10	4.74	4.70
Nonpolar compounds/hydrolytic alteration compounds	61.60	45.90	42.94	44.55	42.80
Thermoxidative alteration compounds/hydrolytic alteration compounds	2.31	7.74	8.42	9.40	9.11

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