High-Performance Size-Exclusion Chromatographic Studies on Polar Components Formed in Sunflower Oil Used for Frying

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Thermoxidative and hydrolytic alterations of a sunflower oil used in sixty repeated and discontinuous deep-fat fryings of potatoes were evaluated by column and high-performance size-exclusion (HPSE) chromatography. Successive fryings of potatoes in sunflower oil, without turnover of fresh oil during the performance of fryings, increased the level of total polar components in the oil from 3.75% to 27.28% (w/w). Triglyceride polymers, triglyceride dimers, oxidized triglycerides and diglycerides increased after sixty fryings 89.8, 21.8, 4.9 and 1.7 times, respectively. These increases were well correlated with the number of fryings. However, there was not significant correlation between levels of free fatty acids and the number of fryings. Polar compounds were highly ($r = 0.9691$) and significantly ($P < 0.01$) correlated with triglyceride polymers and also highly ($r = 0.9969$ and $r = 0.9738$) and significantly ($P < 0.01$) with triglyceride dimers and oxidized triglycerides, respectively. Nevertheless polar compounds were not significantly correlated with free fatty acids. Data suggest that an intensive thermoxidative rather than a hydrolytic process takes place in experimental deep-fat frying of potatoes.

KEY WORDS: Column chromatography, deep-fat frying, high-performance size-exclusion chromatography, hydrolysis, polar compounds, sunflower oil, thermoxidation.

The mechanism of thermal degradation of an oil used to fry is complex because it is dominated by several parameters, such as unsaturation of fatty acids, temperature, oxygen absorption, metals in substrates and in the oil, and nature of the food.

During frying, a wide variety of chemical reactions result in the formation of compounds with high molecular weight and polarity. When a moist food is placed in oil at frying temperatures, air and steam are evolved, causing 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 hydroperoxides. The latter will subsequently result in free-radical-mediated reactions. The oxidation processes will involve fatty acids in intact triglycerides as well as the products of triglyceride hydrolysis. The free radicals can react to form polymers and other complex reaction products. Intact or altered triglycerides may be involved in intramolecular or intermolecular polymerization *via* Diels-Alder reactions. In addition, many of the above-mentioned reactions are interrelated, and a complex mixture of products is formed (1,2).

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TABLE 1

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

Potatoes	
Moisture (%)	77.3 ± 0.9
Protein (% fresh matter)	2.5 ± 0.2
Fat (% fresh matter)	0.2 ± 0.05
Sunflower oil	
Free fatty acids[b]	0.27 ± 0.02
Refractive index at 20°C	1.47 ± 0.00
Major fatty acids[c]	
palmitic	7.1 ± 0.06
stearic	4.0 ± 0.05
oleic	31.1 ± 0.28
linoleic	55.6 ± 0.15

[a]Values are means of three analyses ± standard deviation: [b]Expressed as mg of potassium hydroxide necessary to neutralize the fatty acids contained in 1 g of fat; [c]Expressed as % total fatty acids.

In this report, the alteration of a sunflower oil used in sixty 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 (3). To examine further the polar and polymeric materials in the oil, high-performance size-exclusion chromatography (HPSEC) was used. Although many analytical methods have been used for the determination of monomers, dimers and higher polymers in oxidized fats and oils, the technique of HPSEC may be considered one of the most promising (4).

EXPERIMENTAL PROCEDURES

Performance of fryings. 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, refraction index and free fatty acid levels of the unused oil and data regarding the raw 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. Potatoes were fried for 8 min at an initial temperature of 180°C. Time required to reach and keep the oil at 180°C, before introduction of potatoes, was 20 min. After the end of each frying operation, the oil was again heated at 180°C to start a new frying, and the time required was 10 min. A total of sixty fryings, at a rate of ten fryings per day, was carried out. A set of five fryings was carried out before letting the oil cool to room temperature. After 5 h, another set of five fryings was performed before letting the oil cool to room temperature until the following day.

The overall time the oil was heated throughout the

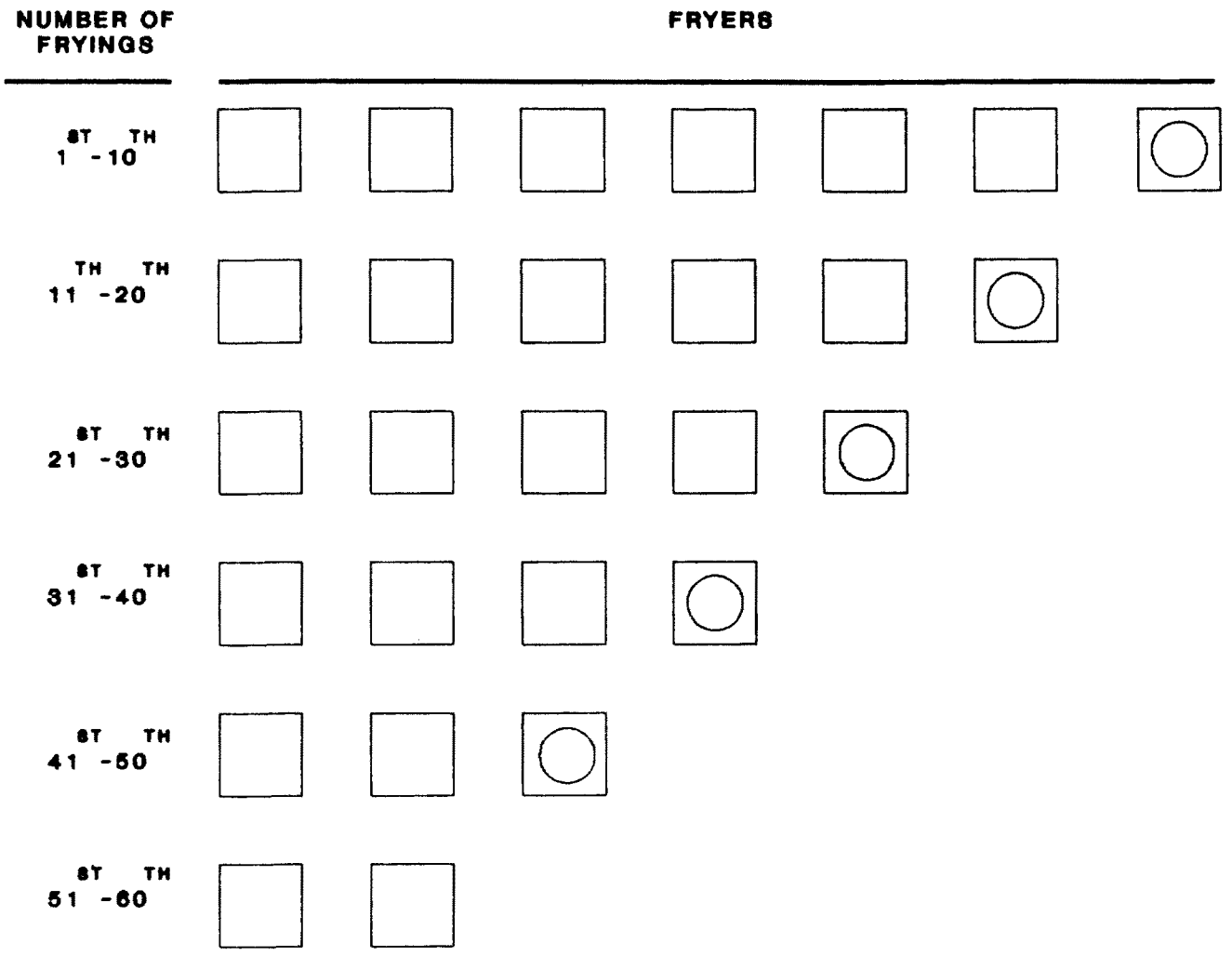

FIG. 1. Frying method. □ Fryers; ⊡ fryer eliminated after each ten fryings by emptying its content to make up the volume of the other fryers to 3 L in order to keep up the proportions of food to frying oil at 500 g/3 L.

whole experiment can be estimated as 20 h. Figure 1 shows a scheme of the frying method. Following this method, the ratio of food to frying oil in the fryers was kept at 500 g/3 L, without addition of fresh oil, by eliminating one fryer after each ten fryings and emptying its contents to make up the volume of the other fryers to 3 L. Figure 2 shows the temperature changes during each frying.

Aliquots of 50 mL from the unused oil and from the tenth, thirtieth, fiftieth and sixtieth fryings were taken for analysis.

Determination of the polar fractions. Polar fractions were evaluated by the slightly modified column chromatographic method of Waltking and Wessels (3). The only modification was the 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 solution was transferred to a silica gel chromatographic column following the methods of Dobarganes *et al.* (5) and Pérez-Camino (6). A final elution of the column with chloroform-methanol (1:1, vol/vol) was performed to improve the recovery of the sample.

Two samples of each oil (unused, and from the tenth, thirtieth, fiftieth and sixtieth fryings), each one taken from a different fryer, were homogeneously mixed and analyzed.

Separation of the nonpolar and polar fractions. The separation of the nonpolar and polar fractions was accomplished by thin-layer chromatography (TLC) on 0.5-mm thick 60 F250 silica gel plates (20 × 20 cm glass) (Merck, Barcelona, Spain). Polar and nonpolar fractions were diluted in hexane/diethyl ether (87:13) 50 times (w/v). Samples were applied as 10-μL spots with a Hamilton 705 microsyringe. Plates were developed with hexane/ethyl ether/acetic acid (80:20:1, vol/vol/vol) in a lined tank. Plates were developed *ca.* 25 min (*ca.* 17 cm) and then removed,

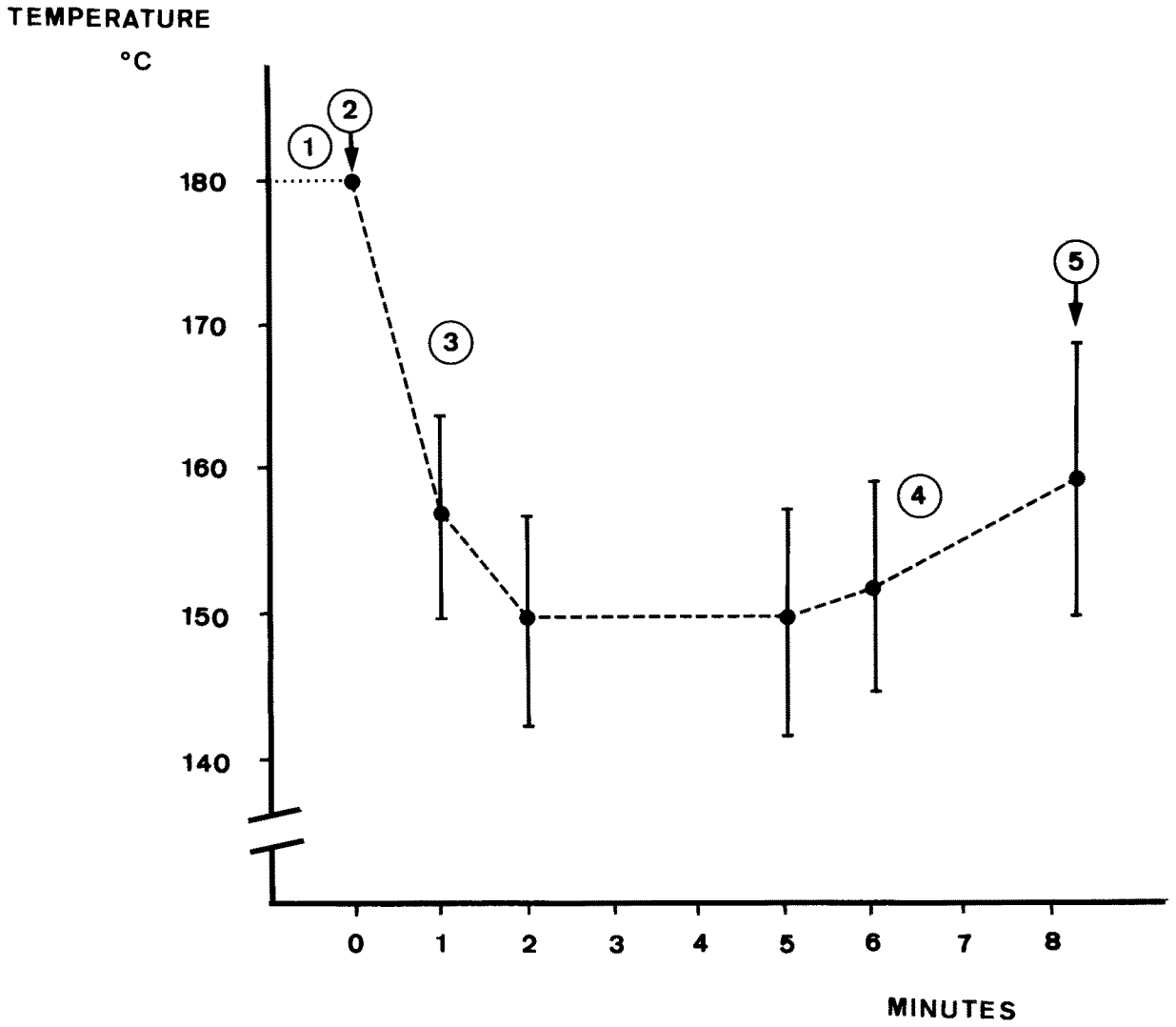

FIG. 2. Average temperature evolution (±SD) of the medium during frying of potatoes. 1. oil held at 180°C; 2. introduction of potatoes; 3. temperature decrease because of water evaporation; 4. temperature increase because of absorption of oil by food and less water evaporation; 5. end of the frying operation.

letting the solvent evaporate. The spots were visualized by spraying them with iodine vapors.

High-performance size-exclusion chromatography (HPSEC). Polar fractions previously obtained by column chromatography as described were analyzed by HPSEC following the Dobarganes *et al.* method (7) to obtain further information about hydrolytic and/or thermoxidative alteration that occurred in the oil during fryings. The isolated polar fractions were analyzed in a Konik 500 A chromatograph (Barcelona, Spain) with a 10-μL sample loop. A Hewlett-Packard 1037A (Palo Alto, CA) refractive index detector and two 25 cm × 0.7 cm i.d. (<5 μm particle size) 100A and 500A Ultrastyragel columns (Waters Associates, Milford, MA) connected in series were operated at 45°C. High-performance liquid chromatography (HPLC)-grade tetrahydrofurane served as the mobile phase with a flow of 1 mL min^{-1}. Sample concentration was 15 to 20 mg mL^{-1} in tetrahydrofurane. All eluents as well as samples were precleaned by passing them through a filter (<2 microns). Alteration products belonging to polar fractions were as follows: triglyceride polymers, triglyceride dimers, oxidized triglyceride, diglycerides and free fatty acids.

Two samples of each oil (unused and from the tenth, thirtieth, fiftieth and sixtieth fryings), each one belonging to a different fryer, were homogeneous mixed and analyzed.

Statistical analysis. The Pearson product-moment linear correlation test was used for statistical evaluation (8).

RESULTS AND DISCUSSION

The HPSE chromatograms of the polar fractions from fresh and used sunflower oil samples are presented in Figure 3. Total polar content (% w/w on oil) as a representative measurement of the total alteration of the oil and the relative (% of polar content) and absolute (% w/w on oil) contents for different groups of alteration products

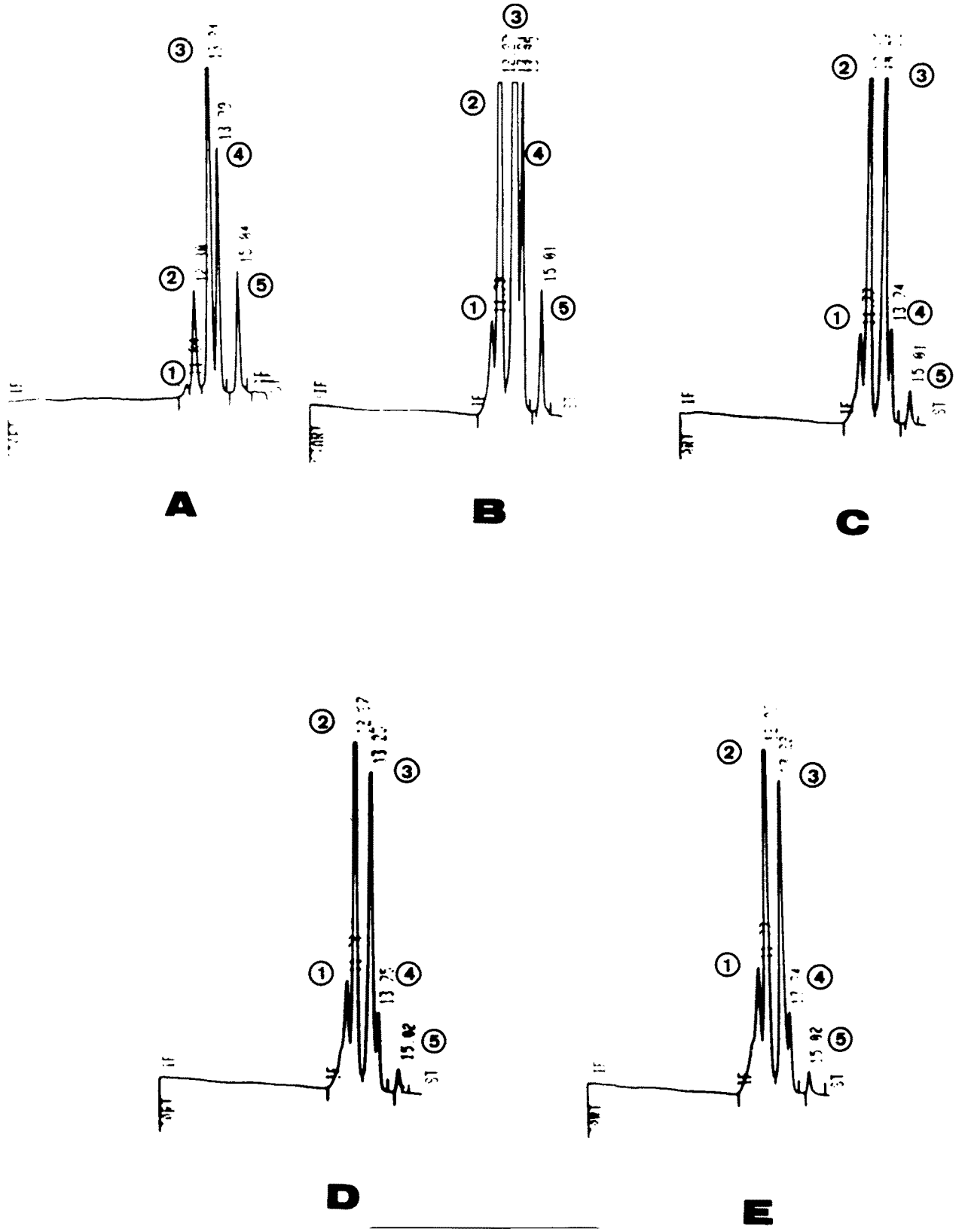

FIG. 3. HPSE chromatograms of unused (A) and used oil samples: B (tenth), C (thirtieth), D (fiftieth) and F (sixtieth fryings). Peaks 1,2,3,4 and 5 are triglyceride polymers, triglyceride dimers, oxidized triglycerides, diglycerides and free fatty acids, respectively. Conditions: Column: series-connected Ultrastyragel, 25 cm × 0.7 cm i.d., <5 μm particle size; eluent: tetrahydrofurane at 1 mL/min, 20 μL injection volume, refractive index detection.

TABLE 2

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

Number of fryings	0	10	30	50	60
Total alteration					
Polar compounds					
% (w/w) on oil	3.75	11.05	17.29	24.13	27.28
Thermoxidative alteration					
Triglyceride polymers					
% of polar fraction	1.62	4.75	11.88	17.19	19.77
% (w/w) on oil	0.06	0.52	2.05	4.15	5.39
Triglyceride dimers					
% of polar fraction	13.40	29.62	40.69	40.42	39.99
% (w/w) on oil	0.50	3.27	7.03	9.75	10.91
Oxidized triglycerides					
% of polar fraction	47.83	50.00	37.77	34.23	32.18
% (w/w) on oil	1.79	5.52	6.53	8.26	8.78
Hydrolytic alteration					
Diglycerides					
% of polar fraction	24.66	11.42	7.25	6.36	6.13
% (w/w) on oil	0.92	1.26	1.25	1.53	1.67
Free fatty acids					
% of polar fraction	12.49	4.21	2.41	1.79	1.93
% (w/w) on oil	0.47	0.46	0.42	0.43	0.53

[a]Data from a single determination of a homogeneous mixture of two oils from their respective fryings.

are given in Table 1. The results were obtained from the unused sunflower oil and the corresponding oil used in different levels of frying. After the tenth, thirtieth, fiftieth and sixtieth fryings, the percentage of the polar fraction of the oil showed an increase from 3.75% (when unused) to 11.05%, 17.29%, 24.13% and 27.28%, respectively (Table 2). These results are also evident when the ratio of nonpolar triglycerides to polar triglycerides is applied (Table 3). Cuesta *et al.* (9) also reported an increase in the polar fraction with the number of fryings when olive oil was used in deep-fat frying of potatoes.

According to Fedelli (10), the speed of degradation is proportional to the temperature and the time of frying. In a previous study (11), the correlation found between the number of fryings in which olive oil is used and the level of nonpolar methyl esters was high and significant ($r = 0.886$; $P < 0.05$).

Deep-fat frying is an important process, which not only involves the lipid part and the intermediate products derived from it, but also allows interaction between these intermediate products and the substrates. The substrates not only may function as catalyst, as was described by Fedelli (10) when frying potatoes, but, in turn, they may be modified by the adsorption of fat and their derivatives (12).

As reported by Cuesta *et al.* (13), the study of degradation compounds produced during frying is not only of physico-chemical interest but also of nutritive importance because it relates to the consumption of polar components of used fats. Distribution of polar components into the different groups of alteration compounds indicates that sunflower oil used in frying shows a tendency for polymerization (Table 2). Quantitation of the different compounds (% w/w on oil) denotes a clear tendency of the oil to increase in triglyceride polymers and triglyceride dimers, which represent a thermoxidative alteration.

As has been described (7), quantitation of diglycerides, but not of fatty acids, allows the determination of the contribution of hydrolytic alteration, because these compounds remain in the fat while fatty acids are partially lost during frying. The results clearly indicate that in deep-fat frying of potatoes thermoxidative reaction takes place more than hydrolytic reactions (Table 2). Repeated fryings of potatoes increased the ratio of thermoxidative alteration compounds to hydrolytic alteration compounds in the used sunflower oil (Table 3).

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 Successive Fryings of Potatoes

Number of fryings	0	10	30	50	60
Nonpolar compounds/ polar compounds	25.6	8.0	4.7	3.1	2.7
Nonpolar compounds/ thermoxidative alteration compounds	40.8	9.5	5.2	3.4	2.9
Nonpolar compounds/ hydrolytic alteration compounds	69.0	51.4	48.6	38.1	32.9
Thermoxidative alteration compounds/hydrolytic alteration compounds	1.7	5.4	9.4	11.3	11.4

The isolation of dimers formed as the result of thermoxidation of fats during deep-fat frying has been of great interest. Kupranycz *et al.* (14) found that sunflower oil after both 8 and 16 h of thermal oxidation contained substantially higher amounts of both dimeric and higher oligomeric triglycerides than unheated oil. These authors indicate that the rates of dimeric triglycerides formation during the first 8 h of heating exceeded the rates of trimeric and higher oligomeric triglycerides formation,

TABLE 4

Pearson Product-Moment Correlations Between Different Groups of Alteration Compounds in the Oil and the Number of Fryings of Potatoes and Between Total Polar Compounds and the Different Groups of Alteration Compounds

Polar compounds/no. of fryings	0.9903[a]	Polar compounds/triglyceride polymers	0.9691[a]
Triglyceride polymers/no. of fryings	0.9919[a]	Polar compounds/triglyceride dimers	0.9969[a]
Triglyceride dimers/no. of fryings	0.9907[a]	Polar compounds/oxidized triglycerides	0.9738[a]
Oxidized triglycerides/no. of fryings	0.9329[b]	Polar compounds/thermoxidative alteration compounds	0.9999[a]
Thermoxidative alteration/no. of fryings	0.9904[a]	Polar compounds/diglycerides	0.9687[a]
Diglycerides/no. of fryings	0.9455[b]	Polar compounds/free fatty acids	0.2200[c]
Free fatty acids/no. of fryings	0.2721[c]	Polar compounds/hydrolytic alteration compounds	0.9449[b]
Hydrolytic alteration/no. of fryings	0.9304[b]		

[a]$P < 0.01$. [b]$P < 0.05$. [c]No significant correlation.

while the rate of dimer formation decreased during the 8–16 h of heating, and the amount of higher oligomeric triglycerides continued to increase at a steady rate throughout the 16-h heating period.

Data obtained in this study, Table 2, indicate that percentages of both triglyceride polymers and triglyceride dimers of the polar fraction increased significantly throughout the first fryings. However, between the thirtieth and the sixtieth frying, triglyceride dimers reach a near-steady state (~40%) while the percentage of triglyceride polymers of the polar fraction increases continuously throughout the experiment. These results were in agreement with those found by Kupranycz *et al.* (14).

However, when the results of the different compounds are given in % (w/w) on oil (Table 2), both triglyceride polymers and triglyceride dimers increased continuously throughout the successive fryings. Thus, triglyceride polymers increased from a basal value of 0.06 to 0.52 at the tenth and to 5.39 at the sixtieth fryings, respectively, while triglyceride dimers increased from a basal value of 0.50 to 3.27 at the tenth and to 10.91 at the sixtieth fryings, respectively. Nevertheless, there was a higher tendency for formation of triglyceride polymers than of triglyceride dimers, because triglyceride polymers content (% w/w on oil) increased by factors of 8.7 and 89.8 after ten and sixty fryings with respect to the basal values, while triglyceride dimers content only increased 6.3 and 21.8 times, respectively.

Perrin *et al.* (15) analyzed samples from sunflower oil oxidized by deep-fat frying to stable foam formation. They reported the presence of dimers at levels between 12.1 and 12.9 of the oxidized mixtures. Gere (16) also reported the presence of dimeric triglycerides in sunflower oil used in deep-fat frying. In addition, Rojo and Perkins (17) reported the formation of monomeric cyclic fatty acids in a soybean oil heated intermittently for 80 h (8 h/day) of simulated deep-fat frying. Perkins and Pinter (18) carried out studies on the concentrations of oxidized components in abused fats, measuring the level of these compounds by HPLC and gas chromatography-mass spectrometry (GC-MS), after concentrating altered fatty acid methyl esters of used oils 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.

Linear correlations between total polar contents or the different polar compounds and the number of fryings are shown in Table 4. Total polar compounds, triglyceride polymers and triglyceride dimers showed a high ($r \geq 0.99$) and significant correlation ($P < 0.01$) with the number of fryings. In addition, diglyceride levels were significantly correlated ($r = 0.9455$; $P < 0.05$), while free fatty acids were not significantly correlated ($r = 0.2721$) with the number of fryings.

In summary, discontinuous and successive fryings of potatoes in sunflower oil, without addition of fresh oil during the performance of fryings, increased the level of total polar compounds in the oil. Hydrolytic alteration took place parallel to the thermoxidative process, as evidenced by the good correlations found for thermoxidative alteration (triglyceride polymers and triglyceride dimers) and for hydrolytic alteration (diglycerides) with the number of fryings.

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