High-Performance Size-Exclusion Chromatographic Studies on a High-Oleic Acid Sunflower Oil During Potato Frying

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ABSTRACT: The behavior of a high-oleic acid 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 content isolated by column chromatography. The total polar content increased in the oil from 3.6 ± 0.1 (mean ± SD) mg/100 mg unused oil to 7.6 ± 0.4 mg/100 mg oil after being used in 20 repeated fryings, followed by a tendency to reach a near-steady state throughout the successive fryings. Further, the polar fraction was examined by high-performance size-exclusion chromatography. Triacylglyceride dimers increased continuously from 0.18 ± 0.01 mg/100 mg unused oil to 2.42 ± 0.12 mg/100 mg oil at the 40th frying with no further significant changes. The amount of triacylglyceride polymers increased from 0.03 ± 0.00 mg/100 mg unused oil to 0.70 ± 0.01 mg/100 mg oil at the 60th frying, but did not increase further with continued frying. Oxidized triacylglycerides also significantly increased from 1.13 ± 0.06 mg/100 mg oil to 3.58 ± 0.09 mg/100 mg oil at the 50th frying to reach a near-steady state in successive fryings. Diacylglycerides and free fatty acids levels, related to hydrolytic alteration, did not change from the starting oil after continued fryings. Data from this study indicated that repeated fryings of potatoes in high-oleic sunflower oil with a frequent turnover of fresh oil throughout the frying slightly increased the level of polar material in the fryer oil during the first fryings, followed by minor changes and a tendency to reach a near-steady state in successive fryings.

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KEY WORDS: Deep-fat frying, high-oleic sunflower oil, HPSE chromatography, polar compounds.

Deep-fat frying can be defined as a process of controlled dehydration and browning with hot oil as the heat transfer medium (1). Many variables are present in the technological operation of frying, which have been described previously (1–6). During frying, many reactions take place, such as hydrolysis, oxidation, polymerization, and interactions between the fat and substrates (5–9).

The selection of the oil plays a significant role in the frying process. One of the most important criteria for the selection of an appropriate oil for frying is its stability, which is related to fatty acid composition. A wide variety of fats are used for frying. Oils with high saturated fatty acid content present unique stability in frying applications. However, these oils are less desirable from a nutritional standpoint and for human health (10–13).

Today, there is a renewed interest in oils rich in monoenic acids because of their stability and health-promoting properties (14). Ninety percent of the world's olive oil consumption takes place in the countries bordering the Mediterranean (15). Recently, in Spain and other countries, a marked increase of alternative new commercial oil sources with a high oleic content for frying has occurred (15). Lower prices are the cause for the increased use of these oils. Traditional sunflower seed oil is an important linoleic-rich oil, about 55–60%, and also contains a high percentage of oleic acid (≈30%). A new sunflower variety has a high oleic acid content of 75–85%, which has improved frying stability. Optimal results for frying in any oil also can be obtained by controlling the frying operations and the oil rejection quality criteria. The thermoxidative and hydrolytic changes can be minimized when there is high "turnover" of the oil in the fryer (6). In addition, one of the best estimates of oil quality is its polar content (16). In many countries, such as France, Germany, and Spain, the limit for polar content is 25% (16,17).

The aim of this study was to establish the behavior of a high-oleic acid sunflower oil in repeated and intermittent deep-fat frying of potatoes with a fast turnover of fresh oil, while controlling the frying process variables. The alteration of this oil was evaluated by measuring the percentage of total polar content by column chromatography. In addition, the polar fraction was examined by high-performance size-exclusion chromatography (HPSEC) to investigate the specific polar compounds related to thermoxidative alteration and those of the polar compounds related to hydrolytic alteration. The technique of HPSEC permits the quantitation of all classes of alteration compounds: polymers and dimers of triacylglycerides, oxidized triacylglycerides, diacylglycerides, monoacylglycerides, and free fatty acids (18).

EXPERIMENTAL PROCEDURES

Performance of frying. Refined sunflower oil, with a high content of oleic acid, trademark VIPA (Andújar, Andalucía,

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Spain), and potatoes (Kennebec variety, Galicia, Spain) were purchased at a local store. Fatty acid composition of the unused oil is given in Table 1. Frying variables, fabrication material and capacity of the fryers, and the frying conditions (temperature, frying frequency, total frying time, oil turnover rate, food type, food/oil rate, and total amount of food fried) are given in Table 2. Because so much oil was removed along with the fried potatoes, it was necessary to replenish the fryer bath with unused oil. Throughout the fryings, the frying bath volume was replenished with fresh oil at the end of each frying operation. Then the oil was again heated to 180°C before starting a new frying. The time required was 10 min. The oil was stored below 15°C in the dark between each heating period. The oil loss throughout the frying process required adding to the original 3 L a total of 4.25 L of fresh high-oleic acid sunflower oil throughout the 75 fryings carried out.

Determination of the percentage of the polar fraction. The polar fraction was evaluated by the column-chromatographic method of Waltking and Wessels (19) with a modified proportion of hexane/diethyl ether to fill the column and to elute the nonpolar fraction. An accurately weighed sample of 1 ± 0.01 g of high-oleic sunflower oil was dissolved in 20 mL hexane/diethyl ether, 87:13 (vol/vol) when unused oil was analyzed, and 90:10 (vol/vol) when used oil was analyzed. A sharper separation of the altered triacylglycerides was obtained by using this latter hexane/diethyl ether proportion (20). The sample was then transferred to a silica gel chromatographic column by following the method of Dobarganes *et al.* (20,21). Two samples each of unused oil and of used oil from the 8th, 20th, 30th, 40th, 50th, 60th, 70th, and 75th 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) (Merck, Darmstadt, Germany). Polar and nonpolar fractions were diluted 50 times (wt/vol) in hexane/diethyl ether, 80:20 (vol/vol). Samples were applied as 20-μL spots with a 705 Hamilton microsyringe (Bonaduz, Switzerland). Plates were developed with hexane/diethyl 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. Each polar fraction previously obtained by column chromatography, as described before, was analyzed in duplicate by HPSEC, following the method of Dobarganes *et al.* (18), to obtain further information about hydrolytic and/or thermoxidative alterations that occur in the high-oleic sunflower oil during frying. Isolated polar fractions were analyzed in a high-performance liquid chromatography system with a Waters 501 pump (Milford, MA) with a 20-μL sample loop. A Waters 410 refractive index detector and two 300 × 7.5 mm i.d. (5 μm particle size) 0.01 and 0.05 μm pL gel (polystyrene divinylbenzene) columns (Hewlett-Packard, Palo Alto, CA), connected in series, were operated at 40°C in an isocratic system. High-performance liquid chromatography-grade tetrahydrofuran (Carlo Erba, Milano, Italy), degassed through helium, served as the mobile phase with a flow rate of 1 mL/min. Sample concentration was 15–20 mg/mL in tetrahydrofuran. All eluents, as well as samples, were precleaned by passing them through a filter (2 μm). The quantity of each polar compound was calculated as described previously (6).

Statistical analysis. One-way analysis of variance and a subsequent Newman-Keuls multiple comparison test were used for statistical comparisons (22).

TABLE 1
Fatty Acid Composition (percentage of chromatographed methyl esters) of Unused High-Oleic Sunflower Oil[a]

Major fatty acids	
Palmitic (16:0)	4.4 ± 0.8
Stearic (18:0)	4.2 ± 0.0
Oleic (18:1)	78.3 ± 0.3
Linoleic (18:2)	10.9 ± 0.0
Arachidic (20:0)	0.3 ± 0.0
Eicosenoic (20:1)	0.2 ± 0.0
Behenic (22:0)	1.0 ± 0.0
Lignoceric (24:0)	0.4 ± 0.0

[a]Values are means ± SD of four samples.

TABLE 2
Variables of Frying Operations

Oil type	Commercial high-oleic sunflower oil[a]
Frying vessel	Domestic electrical fryer with aluminum vessel
Oil quantity (L per batch)	3
Proportion of food to frying oil (g/L per batch)	500:3
Food type	Potatoes[b]
Number of fryings	75
Total oil turnover rate (per entire frying time)	4.25 L
Initial temperature	180°C
Batch time (min)	8
Total frying time (h)	16.5
Frying frequency	Intermittent, 10 fryings/day
Total amount of food fried (kg)	37.5

[a]High-oleic sunflower oil, trademark VIPA, KOIPE Company (Andújar, Andulacía, Spain).
[b]Kennebec variety (Ginzo de Limia, Galicia, Spain).

RESULTS AND DISCUSSION

The transformation of sunflower oil with a high content of oleic acid (78.3%) after repeated fryings of potatoes was followed by monitoring two parameters: the amount of total polar component and the amount of the specific altered compounds (polymeric triacylglycerides, oxidized triacylglycerides, and diacylglycerides).

Table 3 indicates that the total polar content in the starting oil was 3.6 ± 0.1 (mean ± SD) mg/100 mg oil. However, of a total polar content of 3.6 ± 0.1 (mean ± SD) mg/100 mg oil, an amount of 1.9 ± 0.05 mg/100 mg oil was diacylglyceride compounds, which remained stable during frying. The

TABLE 3
Total Polar Content and Different Polar Compounds in Unused High-Oleic Sunflower Oil and After Being Used in Repeated Fryings of Potatoes[a]

	Number of fryings								
	0	8	20	30	40	50	60	70	75
Total polar content	3.6 ± 0.1[a]	6.0 ± 0.4[b]	7.6 ± 0.4[c]	7.9 ± 0.6[c]	8.6 ± 0.4[c]	9.1 ± 0.4[c]	9.5 ± 0.3[c]	9.2 ± 0.0[c]	9.2 ± 0.0[c]
Triacylglyceride polymers	0.03 ± 0.00[a]	0.18 ± 0.06[b]	0.40 ± 0.05[c]	0.44 ± 0.04[d]	0.54 ± 0.03[e]	0.62 ± 0.03[d]	0.70 ± 0.01[g]	0.74 ± 0.02[g]	0.72 ± 0.01[g]
Triacylglycerides dimers	0.18 ± 0.01[a]	1.26 ± 0.29[b]	2.00 ± 0.21[c]	2.15 ± 0.23[c]	2.42 ± 0.12[c]	2.61 ± 0.12[d]	2.75 ± 0.07[d]	2.48 ± 0.11[d]	2.64 ± 0.05[d]
Oxidized triacylglycerides	1.13 ± 0.06[a]	2.26 ± 0.13[b]	2.88 ± 0.12[c]	3.02 ± 0.17[c]	3.31 ± 0.13[d]	3.58 ± 0.09[e]	3.70 ± 0.10[e]	3.58 ± 0.05[e]	3.60 ± 0.05[e]
Diacylglycerides	1.89 ± 0.05[a]	1.91 ± 0.10[a]	1.91 ± 0.02[a]	1.87 ± 0.05[a]	1.90 ± 0.03[a]	1.92 ± 0.05[a]	1.96 ± 0.03[a]	1.98 ± 0.03[a]	1.87 ± 0.03[a]
Monoacylglycerides	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]
Free fatty acids	0.37 ± 0.06[a]	0.41 ± 0.03[a]	0.41 ± 0.01[a]	0.40 ± 0.02[a]	0.39 ± 0.03[a]	0.39 ± 0.05[a]	0.38 ± 0.07[a]	0.42 ± 0.01[a]	0.40 ± 0.01[a]

[a]Mean of two samples ± SD for total polar content, or mean of four samples for the different polar compounds, expressed as mg/100 mg oil. Values in the same row bearing a different letter are significantly different ($P < 0.05$, Newman-Keuls multiple comparison test).

amount of diacylglycerides estimated by HPSEC would contribute to some extent to the starting value of the polar component because diacylglycerides usually elute with the polar components by the column-chromatographic method used in this study. The polar content increased in the fryer's oil from 3.6 ± 0.1 mg/100 mg unused oil to 7.6 ± 0.4 mg/100 mg oil after 20 repeated fryings and tended to reach a near-steady state of approximately 9.2 ± 0.0 mg/100 mg oil throughout the successive fryings (Table 3). Several authors have demonstrated an increase of the polar fraction with the number of fryings (3,4,16,23,24).

Recent data from a study where a typical sunflower oil (55% of linoleic acid) was employed to fry potatoes 75 times and where the performance of fryings also was made with a frequent turnover of fresh oil (6) showed that the amount of polar components increased throughout twenty and thirty fryings, when it reached a near-steady state of 19.1 ± 0.4 mg/100 mg oil of polar content and remained quite stable in later repeated fryings. These results are in agreement with the present study where the polar content did not increase after 20 fryings. The time for polar materials to accumulate to a critical level and mandate oil replacement was much lower when there was a high "turnover" of fresh oil in the fryer (6). In addition, the frying process improved when a frying oil with a high content of monoenic fatty acid was employed instead of a typical high-linoleic sunflower oil.

In the present study, we have taken advantage of the recent development of a sunflower oil with a high content of oleic acid (Table 1). Therefore, investigations were undertaken to determine the amount of oil degradation based on two variables, a high quality of the starting oil for frying purposes (high-oleic sunflower oil) and a high turnover of fresh oil throughout the frying process. These factors explain the lower amount of polar material, 9.2 ± 0.0 mg/100 mg oil, or the high-oleic sunflower oil used in this study as compared to the polar material (19.1 mg/100 mg oil) in a typical linoleic acid-rich sunflower oil that remains after frying (6).

The polar fractions were further examined by HPSEC to investigate the thermoxidative and hydrolytic alterations in the frying oils. The HPSE chromatograms of polar compounds from unused high-oleic sunflower oil and the corresponding used oil are presented in Figure 1. Table 3 also indicates that the amount of triacylglyceride dimers increased continuously throughout the first 40 fryings and did not change further with successive fryings. However, the amount of triacylglyceride polymers and the amount of oxidized triacylglycerides also increased continuously throughout approximately 60 to 70 fryings, followed by a tendency to reach a near-steady state in later successive fryings. The higher contribution of oxidized triacylglycerides with respect to the other compounds altered by frying also should be noted. These results agree with those found in previous work (4–6, 9,25,26) with a traditional linolenic-rich sunflower oil, where triacylglyceride dimers were the major alteration compounds. Data from the present study and those of Perrin *et al.* (27) indicate that triacylglycerides initially react to produce triacylglyceride dimers. Previously (4–6,9), it was found that the rate of dimer accumulation during the first 20 and 30 fryings formation exceeded the rate of oligomeric triacylglyceride formation, but did not increase further with continued fryings. Further, the amount of oligomeric triacylglycerides continued to increase throughout the successive fryings to reach a near-steady state after a large number of fryings. Nevertheless, when using a typical linoleic acid-rich sunflower oil, the amount of triacylglyceride dimers was higher than when using a high-oleic acid sunflower oil (4–6,9).

The hydrolytic modification measured by the amount of diacylglycerides and free fatty acids formed throughout the frying periods is given in Table 3 and in Figure 1. The contribution from hydrolytic modifications, as described by Dobarganes *et al.* (18), may be investigated by quantifying diacylglycerides, but not free fatty acids because the latter are partly lost during frying. As shown in Table 3, the concentration of those compounds remained stable during frying. Also, during the deep-fat frying of potatoes, more thermoxidative than hydrolytic processes took place. Hence, the measurement of free fatty acids may not be the best criterion to test the state of degradation of the oils. As has been described (16), a high level of free fatty acids can exist with low levels of polar material, and the oil should be acceptable for further frying operations.

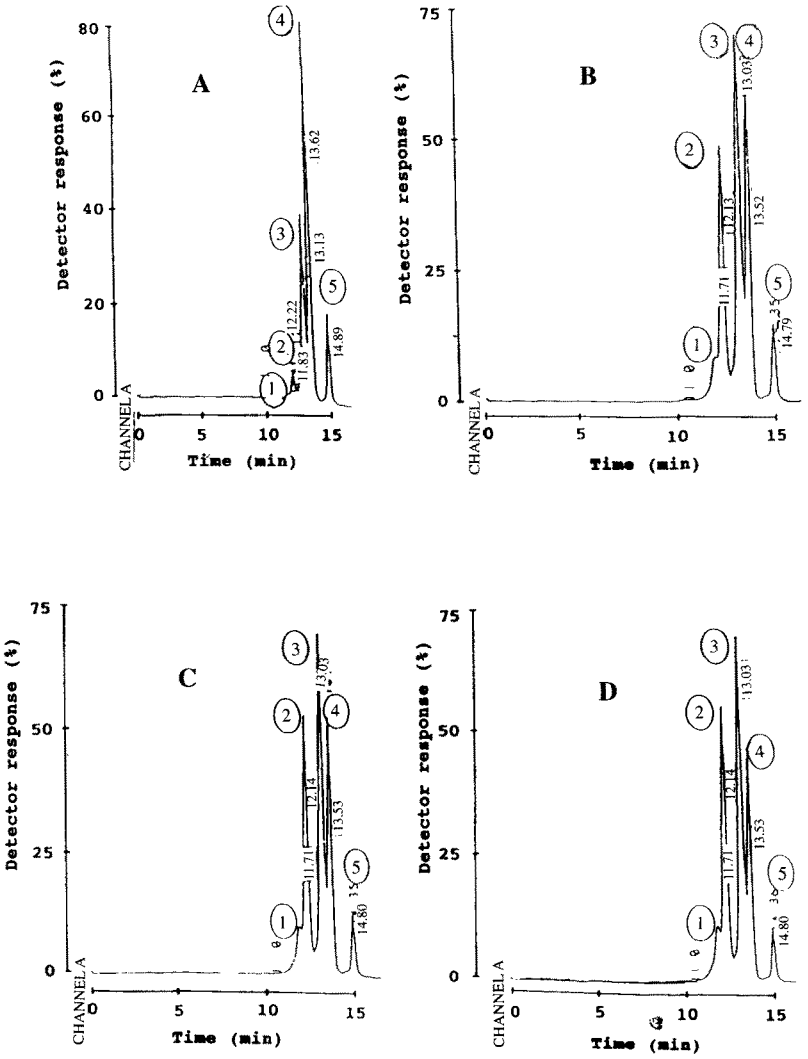

FIG. 1. High-performance size-exclusion chromatograms of unused (A), and used oil samples after 20 (B), 40 (C), and 75 (D) fryings. Peaks 1, 2, 3, 4, and 5 are triacylglyceride polymers, triacylglyceride dimers, oxidized triacylglycerides, diacylglycerides, and free fatty acids, respectively. Conditions: column, series-connected polystyrene-divinylbenzene, 300 × 7.5 mm i.d. (5 µm particle size); eluent, tetrahydrofuran at 1 mL/min; 20-µL injection volume, refractive index detection.

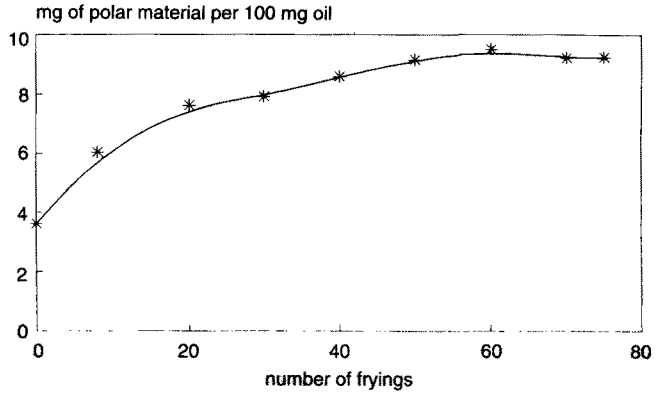

FIG. 2. Total polar content evolution as a function of the number of fryings.

The relationship between the total polar content and the number of fryings showed a parabolic relationship based on Equation 1:

$$P = 4.193 + 0.1830\,F - 0.0016\,F^2 \quad (r = 0.9767) \qquad [1]$$

where P = polar content, F = number of fryings, and r = correlation coefficient (Fig. 2).

Table 4 also shows that the ratio of thermoxidative to hydrolytic compounds in used high-oleic sunflower oil increased during repeated fryings of potatoes from 0.6 in the starting oil to 3.1 after 75 repeated fryings. In a previous report (6), the ratio of thermoxidative-to-hydrolytic alteration compounds increased from 2.3 in unused traditional linoleic

TABLE 4
Ratios of Nonpolar Compounds to Different Groups of Alteration Compounds, and Ratio of Thermoxidative to Hydrolytic Alteration Compounds in Unused High-Oleic Sunflower Oil and After Being Used in Repeated Fryings of Potatoes

	Number of fryings								
	0	8	20	30	40	50	60	70	75
Nonpolar compounds/polar compounds	26.8	15.7	12.2	11.7	10.6	10.0	9.5	9.9	9.9
Nonpolar compounds/thermoxidative alteration compounds	71.9	25.5	17.5	16.4	14.6	13.3	12.7	13.3	13.0
Nonpolar compounds/hydrolytic alteration compounds	42.7	40.5	39.7	40.4	39.7	39.2	38.5	38.8	39.8
Thermoxidative alteration compounds/ hydrolytic alteration compounds	0.6	1.6	2.3	2.5	2.7	2.9	3.0	2.8	3.1

acid-rich sunflower oil to 9.1 after the 75th repeated frying of potatoes. Results clearly suggest that oleic-rich sunflower oil, in comparison with a typical high-linoleic acid sunflower oil, is a better frying oil with increased thermal oxidative stability.

In short, data from this study indicate that repeated fryings of potatoes in high-oleic sunflower oil with a frequent turnover of fresh oil caused a slight increase in the amount of polar material during the first fryings, followed by a tendency to reach a near-steady state. Data also suggest that a sunflower oil rich in oleic acid performs satisfactorily in frying operations and is much better than a typical, high-linoleic acid sunflower oil.

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