Evaluation of Nonpolar Methyl Esters by Column and Gas Chromatography for the Assessment of Used Frying Olive Oils

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The measurement of unaltered methyl esters separated from polar methyl esters by column chromatography was used to evaluate the alteration of an olive oil that had been used 15 times to fry potatoes. Unaltered methyl ester (the nonpolar fraction) decreased significantly (94.9 ± 0.8% *vs* 98.2 + 0.5%; $p < 0.05$), while the polar fraction increased significantly (4.0 ± 0.7% *vs* 2.1 ± 0.7%; $p < 0.05$) after 15 fryings. The unrecoverable fraction also increased. In order to avoid column contamination the gas chromatographic analysis was only done on the nonpolar fractions. Linoleic and oleic acids showed a tendency to decrease while saturated fatty acid tended to increase. The unsaturated/saturated fatty acids ratio decreased from an initial value of 7.05 to 6.40 in the last frying. Quantitative gas chromatographic analysis using both the percentage fatty acid composition and the relative amount of unaltered methyl esters showed a significant oleic acid decrease after 15 fryings (75.8 ± 0.6 *vs* 78.9 ± 0.2 mg/100 mg oil; $p < 0.05$).

KEY WORDS: Column chromatography, fatty acids, gas chromatography, nonpolar methyl esters, polar methyl esters, used frying olive oil.

In deep fat frying the food is completely or almost completely immersed in hot oil or fat in the presence of air (1). Then the fat is exposed to the action of three agents which cause the most drastic changes in its structure—moisture from the foodstuff that involves it in hydrolytic alteration, atmospheric oxygen entering the oil that gives rise to oxidative alteration, and the temperature (180°C) at which the operation takes place accelerates chemical changes (2). All these circumstances make it difficult to find an analytical method which gives complete information on the alteration of the frying fat (2).

Waltking and Wessels (3) described a method for the evaluation of compounds specifically related to frying degradation based on the separation by column chromatography on silica gel of two fractions, one containing the unaltered part of the fat or nonpolar components, which are mostly unaltered triglycerides, while the second concentrates the altered products or polar components of frying fats.

Dobarganes *et al.* (4) proposed a method based on the determination of unaltered methyl esters which gives several advantages with regard to the method of polar triglycerides.

In this report the alteration of an olive oil used in 15 repeated deep fat frying of potatoes was studied, and the relative amounts of unaltered methyl esters and polar methyl esters and the fatty acid composition of the nonpolar fraction were measured.

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EXPERIMENTAL PROCEDURES

Performance of fryings. Pure olive 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.

Domestic deep fat fryers with a 3-L aluminum vessel were used for frying. The potatoes was 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 by eliminating one fryer after each four fryings and emptying its contents to make up the volume of the others fryers to 3 L. A total of 15 fryings were carried out. Potatoes were fried for 8 min at an initial temperature of 180°C. Figure 1 shows the temperature change during the frying. More details of the frying method were described previously (5).

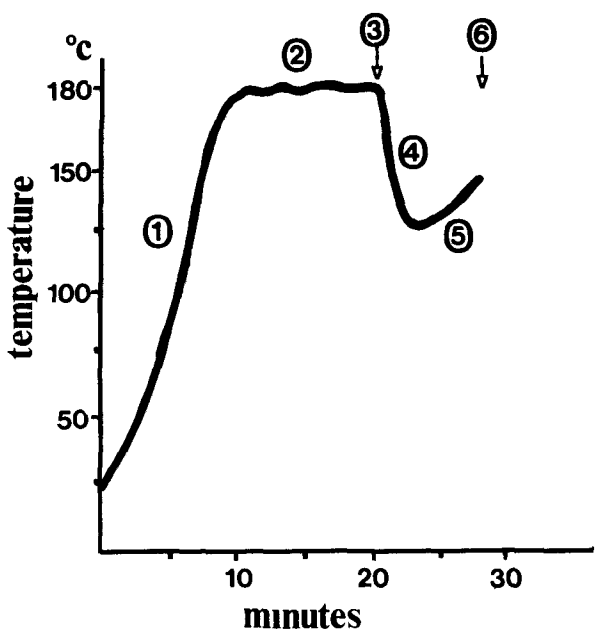

FIG. 1. Temperature evolution of the frying medium-cooking of fried potatoes. 1, Oil heating; 2, oil held at 180°C; 3, introduction of potatoes; 4, temperature decrease because of water evaporation; 5, temperature increase because of absorption of oil by food; and 6, end of the frying operation.

Aliquots of 10 mL from the unused oil and from the fourth, eighth, twelfth and fifteenth fryings were taken for analysis.

Preparation of samples for determination of unaltered methyl esters. Samples of the oil were saponified and methylated according to Metcalfe and Schmitz (6) to achieve complete conversion of oils to methyl esters. Saponification was done for 15 min with 40 mL/g 0.5N NaOH in methanol. Methylation required 15 min with 14% BF3 in methanol. The esters were extracted in hexane, freed of moisture over sodium sulfate and dried under nitrogen gas. The measurement of the methyl esters prepared according to Metcalfe and Schmitz (6) was

more accurate than by transesterification with sodium methylate (7), (99.8% *vs* 97.5%, respectively) as described in a previous work (8). That is why the first method was chosen.

Determination of the percentage of the unalterated methyl esters. An accurately weighed sample of 1 ± 0.01 g (to 0.001 g) of methyl esters was dissolved in 20 mL light petroleum-ethyl ether (95:5, v/v) and transferred to a chromatographic column following the methods of Dobarganes *et al.* (4) and Pérez Camino (9).

Compared to the Waltking and Wessels method (3) the only modifications were the elution of methyl esters instead of triglycerides and the proportion of light petroleum-ethyl ether used to fill the column and to elute the nonpolar fraction.

Control of the separation of the nonpolar and polar fractions. The separation of the nonpolar and polar fractions was done by thin-layer chromatography (TLC) using 0.5 mm thick 60 F250 silica gel plates (20 × 20 cm glass). Polar and nonpolar fractions were diluted in hexane 50 times (w/v). Samples were applied as 10 mL spots using a 705 Hamilton microsyringe. Plates were developed with hexane/ethyl ether/acetic acid (80:20:1, v/v/v) in a lined tank. Plates were developed *ca.* 25 min (*ca.* 17 cm). Plates were removed, letting the solvent evaporate. The spots were visualized by spraying them with iodine vapors.

Gas chromatographic analysis of fatty acid esters. A Hewlett-Packard 5710 chromatograph (Palo Alto, CA) equipped with flame detection and oven temperature programming was used. The oven temperature was held 8 min at 170°C and then was programmed from 170°C to 240°C at 2°C/min. The upper limit oven temperature was held 4 min prior to recycling it. The injector and the detector blocks were set at 250°C and 300°C, respectively. Carrier gas (nitrogen) flow rate was set at 30 mL/min. The oven was fitted with stainless steel columns packed with 10% Supelcoport 2330 (Supelco, Inc., Bellefonte, PA) on 100-120 Chromosorb WAW (6 feet, 1/8 inch). Sample size was 0.5 μL. The methyl esters were identified by comparing their relative and absolute retention times with those of commercial standards. Peak areas were measured using a Perkin Elmer Minigrator M-2 7123A integrator (Norwalk, CT). Peaks were quantitated in mg/100 mg sample according to Pérez Camino (9) and Pérez Camino *et al.* (10).

The quantity of each fatty acid (A) was calculated as follows: A= % of the fatty acid A in the nonpolar methyl ester fraction × % nonpolar methyl ester fraction/100.

According to Pérez Camino (9) the values obtained by this procedure were similar to those obtained using heptadecanoic acid as internal standard. Data are given in mg/100 mg oil, because the Metcalfe and Schmitz method (6) achieves complete conversion of oils to methyl esters.

Statistical analysis. Student's *t*-test was used for statistical comparisons (11).

RESULTS AND DISCUSSION

Measurements of nonpolar and polar methyl ester fractions are given in Table 1. The results were obtained from the unused olive oil and the corresponding oil after different fryings.

The basal values obtained for nonpolar, polar and unrecoverable fractions were similar to those found in other studies (4,12). From the several unused oils examined so far, the olive oil average nonpolar fraction was always higher than for that other oils (4,12,13).

TABLE 1

Relative Percentages of Polar and Nonpolar Fractions of Methyl Esters by Column Chromatography

Number of frying	Nonpolar fraction	Polar fraction	Unrecoverable fraction
0	98.2 ± 0.5[a]	2.1 ± 0.7[a,b]	-0.3 ± 1.2
4	97.6 ± 0.6[a,b]	1.9 ± 0.3[a]	0.5 ± 1.2
8	96.2 ± 0.3[b]	3.0 ± 0.3[a,b]	0.8 ± 0.6
12	96.9 ± 0.0[b]	3.1 ± 0.0[b]	0.0 ± 0.0
15	94.9 ± 0.8[c]	4.0 ± 0.7[c]	1.1 ± 1.5

[a,b,c]Values (mean of three samples ± standard deviations) for the same fraction bearing different letters are significantly ($p < 0.05$ Student *t*-test) different. The unrecoverable fraction variations were not statistically studied.

After 15 fryings the nonpolar fraction of the oil showed a significant decrease (94.9 ± 0.8% *vs* 98.2 ± 0.5%), while the polar fraction increased (4.0 ± 0.7% *vs* 2.1 ± 0.7%). The unrecoverable fraction also showed a trend towards increasing (Table 1).

According to Fedeli (14) the speed of degradation is proportional to the temperature and the time of frying. In previous work (8), the correlation found between the number of fryings the olive oil is used and the level of nonpolar methyl esters was high and significant ($r=0.886$, $p < 0.05$).

The fatty acid composition of olive oil (Table 2) differs from that of the majority of other edible oils used for frying because of its high oleic acid content (80.3% in this study), low saturated fatty acid content (palmitic and stearic acids, 9.6% and 2.8%, respectively, in this study) and a modest presence of polyunsaturated fatty acids, of which linoleic acid is the only representative (7.1% in this study).

Deep fat frying is an important process which not only involves the lipidic part and the intermediate products derived from it, but also allows interaction between the same and the substrates. The latter not only may function as catalysts, as was described by Fedeli (14) when frying potatoes but, in their turn, may be modified by the adsorption of fat and their derivatives by the potatoes (15).

The variation of percentage composition in the olive oil fatty acid esters analyzed in "fresh" and after a different number of fryings is shown in Table 2. The percentage of unsaturated fatty acids tended to decrease (linoleic acid decreased nonsignificantly from 7.1 ± 0.2% to 6.5 ± 0.6%, and the oleic acid decreased nonsignificantly and lightly from 80.3 ± 0.2% to 79.9 ± 0.6%). The results are in agreement with those found by different authors (15-19). Percentage of saturated fatty acids, such as palmitic and stearic acids, increased lightly, probably due to linoleic and oleic acid degradation (18,19). However, the fatty acid modifications occurring during repeated fryings are not only related to the thermooxidative conditions but also to the absorption of some fatty acids by the potatoes (8). The unsaturated/saturated acids ratio (calculated by considering the percentages of oleic acid plus linoleic acids divided by the sum of percentages of palmitic and

TABLE 2

Fatty Acid Composition of Unused Olive Oil and After Being Used in Successive Fryings of Potatoes

Number of frying	$C_{16:0}$		$C_{18:0}$		$C_{18:1}$		$C_{18:2}$	
	%*	mg/100 mg oil**	%*	mg/100 mg oil**	%*	mg/100 mg oil**	%*	mg/100 mg oil
0	9.6 ± 0.5^a	9.4 ± 0.5^a	2.8 ± 0.4^a	$2.7 \pm 0.4^{a,b}$	80.3 ± 0.2^a	78.9 ± 0.2^a	7.1 ± 0.2^a	7.0 ± 0.2^a
4	10.1 ± 0.5^a	9.9 ± 0.5^a	2.6 ± 0.2^a	2.5 ± 0.2^a	80.3 ± 0.2^a	78.4 ± 0.2^a	6.8 ± 0.5^a	6.6 ± 0.5^a
8	10.2 ± 0.7^a	9.8 ± 0.7^a	3.3 ± 0.2^a	3.7 ± 0.2^b	79.5 ± 0.1^b	76.5 ± 0.1^b	6.8 ± 0.5^a	6.5 ± 0.5^a
12	10.0 ± 0.6^a	9.7 ± 0.6^a	3.3 ± 0.2^a	$3.2 \pm 0.2^{a,b}$	79.9 ± 0.0^a	77.4 ± 0.0^c	6.6 ± 0.4^a	6.4 ± 0.4^a
15	10.1 ± 0.2^a	9.6 ± 0.2^a	3.4 ± 0.2^a	$3.2 \pm 0.2^{a,b}$	$79.9 \pm 0.6^{a,b}$	$75.8 \pm 0.6^{b,c}$	6.5 ± 0.6^a	6.2 ± 0.6^a

^{a,b,c}Values (mean of three samples ± standard deviations) for the same fatty acid bearing different letters are significantly ($p < 0.05$ Student *t*-test) different.

*Percentage in nonpolar methyl ester fraction.

**Quantitative measurements of major fatty acids in mg/100 mg oil is based in the fatty acid percentage composition of the nonpolar methyl ester fraction and in the relative percentage of nonpolar methyl ester fraction.

stearic acids) decreased from an initial value of 7.05 to 6.4 in the fifteenth frying.

Also given in Table 2 are quantitative measurements of individual fatty acid methyl esters in mg/100 mg oil sample in the unused olive oil and after different fryings. Similar results were obtained with regard to the percentage evaluation. However, oleic acid concentration decreased significantly ($p < 0.05$) after the eighth frying. This concurs with another study done by Figueroa (19).

Even when the linoleic acid concentration did not change significantly, the relative decrease (nonused oil amount minus fifteenth frying oil amount/nonused oil amount × 100) observed for linoleic acid was triple that the oleic acid (11.4% *vs* 3.9%). The changes observed in the fourth and eighth fryings in the stearic acid concentration are not easy to explain, but they could be due (as it has been already indicated) to the different kinetics of penetration of the fatty acids into the potatoes during frying, as described previously (5).

Vigneron *et al.* (16), Guillaumin *et al.* (17) and Causeret *et al.* (20), pointed out that heat treatment of fats induces modifications of fatty acids with double and triple bonds, which produces polar, high molecular weight compounds.

The low level of alterations seen after 15 fryings in the oil is in agreement with Dobarganes *et al.* (4), who indicated that the percentage of alterations of used frying fats is related to the unsaturated fatty acid content and the unsaturation degree of the fats, as well as the heat treatment itself.

In short, successive fryings of potatoes increased the level of polar fatty acid esters in the olive oil remaining in the fryer, decreasing the unsaturation of fat slightly but progressively.

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