Cyclic Fatty Acid Monomers and Thermoxidative Alteration Compounds Formed During Frying of Frozen Foods in Extra Virgin Olive Oil

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ABSTRACT: The measurement of polar content and specific polar compound distribution was used to evaluate the alteration of an extra virgin olive oil (EVO) used 20 times to fry frozen foods employing two methods of frying (with or without oil replenishment during frying). In addition, cyclic fatty acid monomers (CFAM) were quantified and identified throughout the 20 frying operations. Total polar content and specific polar compounds increased in the used oil (with or without replenishment). Nevertheless, frequent replenishment (FR) permits a higher number of fryings because of a dilution effect that helps maintain lower amounts of polar and specific alteration compounds than when null replenishment (NR) was used. CFAM were absent in the unused EVO, but appeared in the oil bath as a consequence of oil heating. The total CFAM concentration was higher when the oil was used with the NR method. Cyclopentyl fatty acids were more abundant than cyclohexyl ones. The data suggested that the FR frying is more appropriate to maintain the quality of the oil during frozen food frying.

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KEY WORDS: Cyclic fatty acids, deep-frying, extra virgin olive oil, oil replenishment, thermoxidation.

Deep-fat frying is widely used in food preparation because it provides appealing characteristics. The fat or oil acts as a heat transfer medium and as an important ingredient of the fried food. As a consequence fried foods are considered a significant part of the diet. In addition, consumption of fried frozen prefried foods has greatly increased during the last decade (1). During the frying process various chemical reactions occur, such as oxidation, hydrolysis, polymerization, isomerization, and cyclization (2,3). Thus, chemical and physicochemical characteristics of the oil are affected. This thermal degradation should be studied not only for technological reasons (production of fried foods with acceptable qualities) but also for safety and nutrition because it is now known that some polar compounds from frying fats can present a certain toxicity (4).

In addition, numerous cyclic fatty acids are formed in vegetable oils at temperatures ≥200°C. Their structures, occurrence, and biological effects have been reviewed (5). These compounds are potentially toxic and have been detected at low, but varying, levels (0.01–0.7%) in commercial frying oil. The levels required to produce a physiological effect are unknown.

Most of the studies on heated fats have been carried out on oil heated, without food fried in it, in the laboratory, and information is scarce on what happens when oils are used to fry foods (6). The traditional source of dietary monounsaturated fatty acids in Mediterranean countries has been olive oil. However, several types of olive oils are commercially available. At present a commercial mix of refining olive oil plus virgin olive oil is consumed in greatest quantity. Nevertheless, due to the high stability and nutritional properties of extra virgin olive oil (EVO), its use for frying purposes has increased in Spain in recent years (7). During deep-fat frying at home or in restaurants, oil is likely to be kept hot for long periods with only some occasional use for frying. There is therefore a relatively slow or null turnover of oil in frying.

The present study represents a real approach to what happens in frying oil when frozen foods are fried in it. In addition, this is the first study that quantifies and identifies cyclic fatty acid monomers (CFAM) formed when EVO is used for frying.

Thus, the aim of this study was to establish the deterioration of oil by measuring the total polar material of an EVO used in 20 discontinuous deep-fat fryings of frozen foods by two methods: null replenishment (NR) and frequent replenishment (FR) of oil. Furthermore, this polar fraction was examined by high-performance size-exclusion chromatography (HPSEC) to investigate the thermoxidative and hydrolytic changes. In addition CFAM formed were analyzed by gas chromatography–mass spectrometry (GC–MS), the method of choice for identifying their structures in heated fats and oils.

MATERIALS AND METHODS

Materials. EVO (Mora, Toledo, Spain) and frozen prefried potatoes (McCain Alimentaire S.A., Harnes, France), croquettes, tuna pasties, fish fingers, battered squid, and breaded fish (Pescanova, Redondela, Galicia, Spain), spring rolls (Oetker, Bielefeld, Germany), fritters (La Cocinera, Torrejón de Ardoz, Madrid, Spain), and breaded ham and cheese

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TABLE 1
Frying Sequence of Frozen Foods

Frying order	Type of frozen food	Batch time (min)	Food weight (g wet matter)
1, 5, 8, 12, 16, 20	Frozen prefried potatoes	8	495
2	Fish fingers	4	415
3	Fritters	6	305
4, 11	Tuna pastries	5	440
6, 14, 19	Croquettes	3	365
7, 13	Breaded ham and cheese	4	335
9, 17	Battered squid	3	400
10, 18	Breaded hake	3	400
15	Spring rolls	5	460

(Findus-Nestlé España S.A., Esplugues de Llobregat, Barcelona, Spain) were purchased at a local store. The frozen foods used in the study (Table 1) were selected based on a survey performed on a sample of university students in the Madrid Autonomy Community (8).

Frying procedure. Domestic deep fat fryers with 3-L vessels were used for frying. The amounts of frozen foods in the successive fryings were 305 to 495 g (Table 1). In total, 20 fryings were carried out by employing the NR and the FR methods. The NR method was performed by avoiding replenishment of the bath oil, whereas the FR method was carried out with replenishment of the bath oil with fresh oil after each frying. Two fryings were carried out per day, one in the morning and the other in the afternoon. The 20 fryings took place over 10 consecutive days. The frying sequence of frozen food is shown in Table 1. The time required to bring the bath oil to 180°C before introduction of frozen food was ~12 min. Frozen foods were then fried for 3 to 8 min (Table 1). The overall time that the oil was heated throughout the whole experiment can be estimated as ~100 min.

Oil loss throughout the 20 fryings required the addition of ~1.2 L and was carried out in the FR modality. Samples of frying oils were packaged under nitrogen and stored at –20°C.

Lipid extractions. Lipids from the prefried frozen potatoes were extracted four times with hexane at a 1:50 (wt/vol) ratio in each extraction operation (3).

Polar content. Total polar fraction of the oil was determined by silica column chromatography (9). Two samples each of unused EVO and of used EVO from the first, eighth, twelfth, and twentieth fryings were analyzed. Separation of nonpolar and polar fractions was checked by thin-layer chromatography as previously indicated (9).

HPSEC. To obtain further information about hydrolytic and/or thermoxidative changes occurring during frying, polar fractions of EVO, previously obtained by column chromatography, were analyzed by HPSEC (10,11). Two determinations of each polar fraction of both fresh and used EVO were performed.

Hydrogenation of CFAM. Methyl ester derivatives were hydrogenated in 10 mL of a mixture of chloroform/methanol (2:1 vol/vol) with hydrogen and platinum oxide catalyst under about 3–4 bars of hydrogen atmosphere for 4 h in order to ensure complete hydrogenation (12). The catalyst was removed by filtration and the hydrogenated methyl esters were extracted with chloroform after addition of water. Ethyl palmitate (1.5 μg) was added as an internal standard before esterification and hydrogenation.

High-performance liquid chromatography (HPLC). The hydrogenated CFAM obtained were isolated by HPLC following the method of Sébédio *et al.* (13). HPLC analyses were carried out on a reversed-phase column (Lichrosorb; Merck, Darmstadt, Germany) C18 (7 mm i.d. × 25 cm in length, 5 μm particle size). A Waters 410 refractive index detector (Milford, MA) was used. A mixture of acetone/acetonitrile of 90:10 served as the mobile phase with a flow of 4 mL/min. The sample concentration was 20 mg of hydrogenated fatty acid methyl esters in 100 μL of acetone.

GC. The fraction isolated by HPLC was further analyzed on a Hewlett-Packard Model 5890 Series II gas chromatograph (Palo Alto, CA). The instrument was fitted with a split/splitless injector (a split ratio of 50:1 was used) and equipped with a fused-silica capillary column (0.33 mm i.d. × 50 m in length) BPX70, film thickness 0.25 μm. The oven was temperature-programmed from 60 to 190°C at 20°C/min. Helium was the carrier gas. The total content of CFAM in the sample was calculated relative to the internal standard (ethyl palmitate).

GC–MS. To establish the carbon skeleton of the hydrogenated CFAM, GC–MS analyses were performed using a DB-Wax column (J&W Scientific, Folsom, CA) (30 m × 0.25 mm i.d., film thickness, 0.5 μm) and a Hewlett-Packard 5970 Mass Selective Detector coupled with a Hewlett-Packard gas chromatograph (Model 5890). The temperature of the column was programmed from 50 to 200°C at 20°C/min, held at 200°C for 25 min, then programmed from 200 to 220°C and held at 220°C until completion of the analyses. Splitless injection was used, and the injection port was maintained at 240°C.

Statistical analysis. Linear adjustment between total CFAM content, total polar content, diacylglycerol content and thermoxidative alteration in EVO, and the number of frying operations performed with both frying methods were established by analysis of variance. The comparison between linear equation adjustments of both NR and FR was performed by a two-way analysis of covariance.

TABLE 2
Total Polar Content[a], Thermoxidative Alteration[b], Hydrolytic Alteration[b] and the Thermoxidative to Hydrolytic Alteration Ratio in Frozen Prefried Potatoes and in Extra Virgin Olive Oil (EVO) Before and After Frying of Frozen Foods

	Prefried	Fresh	Null replenishment frying[c]				Frequent replenishment frying[c]			
Number of frying	potatoes	EVO	1	8	12	20	1	8	12	20
PC[a] % (wt/wt) on oil	13.8 ± 0.28	3.0 ± 0.01	3.6 ± 0.42	8.1 ± 0.10	11.7 ± 0.00	17.3 ± 0.17	3.5 ± 0.04	7.1 ± 0.31	8.1 ± 0.01	13.5 ± 0.09
TA[b] % (w/w) on oil	6.0 ± 0.21	0.9 ± 0.00	1.3 ± 0.12	5.7 ± 0.13	9.4 ± 0.02	14.8 ± 0.14	1.3 ± 0.02	4.8 ± 0.19	6.0 ± 0.01	11.0 ± 0.06
HA[b] % (w/w) on oil	7.8 ± 0.07	2.1 ± 0.06	2.3 ± 0.30	2.3 ± 0.75	2.4 ± 0.03	2.5 ± 0.22	2.2 ± 0.02	2.3 ± 0.12	2.1 ± 0.22	2.5 ± 0.03
T/H ratio	0.76	0.42	0.54	2.45	3.93	5.83	0.57	2.08	2.81	4.41

[a]Mean ± standard deviation of two samples.
[b]Mean ± standard deviation of four samples.
[c]Samples of frying and analyzed after the number of fryings indicated. Abbreviations: HA, hydrolytic alteration; PC, polar content; TA, thermoxidative alteration; T/H ratio, the thermoxidative alteration/hydrolytic alteration ratio.

RESULTS AND DISCUSSION

Thermoxidation. Total polar content as a representative measurement of the total alteration of the oil and the content of different groups of altered compounds (oligomeric triacylglycerols and oxidized triacylglycerols, related to thermoxidative alteration; and diacylglycerols and free fatty acids, related to hydrolytic alteration) were monitored. The results obtained from unused EVO and the corresponding oil used in the different frying operations are given in Tables 2–4.

Table 2 shows that the total polar content in the starting oil was 3.0 ± 0.01 (mean ± SD) mg/100 mg oil. Discontinuous frying increased this polar content in the used oils for both frying methods. Changes in oils were adjusted significantly different to a linear equation in FR than in NR, showing the FR a lower slope (Table 4).

In a previous study, in which fresh potatoes were fried in sunflower oil, no differences in total polar content due to the frying method (NR or FR), up to the 30th frying, were found (14). Nevertheless, the polar content was higher at the 50th frying in NR than in FR (24.1 vs. 18.9%). Further, our group found that discontinuous frying of frozen foods in sunflower oil (SO) and high-oleic acid sunflower oil (HOSO) employing both methods, frequent replenishment (FR) or null-replenishment (NR) of oil, increased the polar content in all of the used oils (3).

In the present study a lower thermoxidation level was found employing the FR modality rather than the NR modality (Table 2) because of the dilution effect caused by the frequent replenishment of fresh oil. On the other hand, the polar content did not exceed 25%, which some countries suggest as a criterion for discarding oil used in frying (15).

Table 3 shows that diacylglycerols were the major compounds of the polar fraction in fresh EVO at (1.6 ± 0.01 w/w on oil), while the amount of oxidized triacylglycerols was 0.8 ± 0.01 w/w on oil, and triacylglycerol polymers were not present. Only low amounts of triacylglycerol dimers were also present in the fresh EVO employed in the current study. These results are in accordance with those of others (16).

During frying, dimers, polymers, and oxidized triacylglycerols were the major compounds formed in both frying methods (NR and FR). The greatest increase was observed for dimers. Thus, after 20 repeated uses of EVO for frying frozen foods, the higher amounts of oligomers (dimers plus polymers) were found in NR modality and accounted for about 7.6 mg/100 mg oil. This amount was 5.4 mg/100 mg oil in the FR modality. During the first eight fryings (NR and FR), and after 20 fryings (FR), oxidized triacylglycerols were the major

TABLE 3
Distribution of Polar Compounds[a] in Frozen Prefried Potatoes and in EVO Before and After Frying of Frozen Foods

	Prefried	Fresh	Null replenishment frying				Frequent replenishment frying			
Number of frying	potatoes	EVO	1	8	12	20	1	8	12	20
PTG % (wt/wt on oil)	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.4 ± 0.01	0.9 ± 0.01	1.8 ± 0.01	0.0 ± 0.00	0.4 ± 0.02	0.5 ± 0.01	1.2 ± 0.03
DTG % (wt/wt) on oil	3.3 ± 0.11	0.1 ± 0.01	0.3 ± 0.03	2.2 ± 0.01	3.8 ± 0.03	5.8 ± 0.03	0.4 ± 0.00	1.9 ± 0.08	2.4 ± 0.02	4.3 ± 0.05
OTG % (wt/wt) on oil	2.7 ± 0.10	0.8 ± 0.01	0.9 ± 0.09	3.1 ± 0.12	4.7 ± 0.00	7.2 ± 0.10	0.9 ± 0.02	2.6 ± 0.08	3.1 ± 0.01	5.6 ± 0.02
DAG % (wt/wt) on oil	7.6 ± 0.11	1.6 ± 0.01	1.8 ± 0.21	1.9 ± 0.01	2.0 ± 0.02	2.2 ± 0.01	1.7 ± 0.01	1.9 ± 0.01	1.7 ± 0.02	2.1 ± 0.02
FFA % (wt/wt) on oil	0.2 ± 0.04	0.5 ± 0.01	0.5 ± 0.08	0.4 ± 0.01	0.4 ± 0.01	0.4 ± 0.00	0.5 ± 0.01	0.4 ± 0.02	0.4 ± 0.01	0.4 ± 0.01

[a]Mean ± standard deviation of four samples. Abbreviations: PTG, triacylglycerol polymers; DTG, triacylglycerol dimers; OTG, oxidized triacylglycerols; DAG, diacylglycerols; FFA, free fatty acids. For other abbreviation see Table 2.

TABLE 4
Linear Adjustments of Polar Content, Thermoxidative Alteration, and Total CFAM Content in EVO and the Number of Frying Operations[a]

	Frying method	*r*	*A*	*B*	*P*	FR vs. NR
Total polar content	FR	0.9874	2.66	0.54	<0.001	
	NR	0.9943	2.25	0.75	<0.001	<0.001
Thermoxidative alteration	FR	0.9925	0.63	0.52	<0.001	
	NR	0.9950	0.32	0.71	<0.001	<0.001
Diacylglycerol content	FR	0.9384	1.54	0.03	<0.001	
	NR	0.9719	1.57	0.04	<0.001	<0.1
Total CFAM content	FR	0.9923	−7.46	29.9	<0.001	
	NR	0.9992	−3.09	34.2	<0.001	<0.001

[a]Abbreviations: CFAM, cyclic fatty acid monomers; FR, frequent replenishment; NR, null replenishment; *r*, regression coefficient; *A*, intercept; *B*, slope.

compounds in the used oil (Table 3). In a previous paper (14), polymers and dimers of triacylglycerols, oxidized triacylglycerols, and diacylglycerols were found to be higher in NR than in FR after 50 fryings when using SO to fry potatoes.

Total thermoxidative alteration levels changed parallel to the polar content (similar slopes) for both frying methods (Tables 2 and 4). After 20 fryings, the total thermoxidative alteration was 14.8 (NR) and 11.0 (FR) mg/100 mg oil (Table 2), which shows that FR is safer than NR.

Diacylglycerol content increased significantly during fryings in both NR and FR (Table 3). Nevertheless, in previous work we found that diacylglycerols remained quite stable during fresh potato frying (2,17). In the present study we observed a high amount of diacylglycerols in frozen prefried potatoes (Table 3). Thus, these compounds increased during frying as a consequence of an exchange between frozen foods and frying oil. These results agree with those previously reported when frozen foods were fried with both SO and HOSO (3).

The thermoxidative alteration/hydrolytic alteration ratio was higher in NR than in FR (Table 2), in agreement with the results found in a previous work when deep-fat frying of frozen foods was performed with SO or HOSO (3).

Measurement of CFAM. Although nonnegligible quantities of these compounds are found in commercially available oils due to the deodorization process, CFAM were not found in the fresh EVO because it was not submitted to previous heat treatment.

During frying in EVO, total CFAM increased with the number of fryings (Fig. 1). Nevertheless, the formation of CFAM in FR modality was 12% lower than in the NR method (Table 4, Fig. 1). Because experimental conditions were controlled (initial temperature for frying, food/oil ratio, frying-time, order, and sequence of food fried), it can be accepted that CFAM differences between FR and NR first should be attributed to the frequent addition of fresh oil and thus to a dilution effect. Further, replenishment with fresh oil would favor the addition of antioxidant vitamins such as tocopherols and other phenolic antioxidants, which may protect the cyclization of the fatty acids of EVO (18). Burton and Ingold (19) indicated that phenolic antioxidants such as tocopherol inhibit the cyclization of hydroperoxides because they are donors of hydrogen.

Previous works (5,20) reported that structures of formed CFAM depend very little on the temperature used in the range of 200–275°C. Under low or high temperature, the same major CFAM were formed. Differences were observed only in the relative proportions and amounts of these compounds in the oil related to the increase in frying time.

In the current study, after 20 fryings of frozen foods the amount of isolated CFAM found (Fig. 1) was at the lower level of 0.01–0.66% reported in 93 frying oil samples (5).

Present CFAM data concur with those described in six samples from heated polyunsaturated oils (21). However, the conditions were different because in our study EVO was highly monounsaturated and was involved in frying operations. Oils with high amounts of CFAM probably derive from oils with high amounts of linoleic acid that have been overheated, and they probably contain the objectionable level of 25% of polar material when an oil used in frying must be discarded. According to the present linear adjustments (Table 4), and following the same experimental conditions, the EVO used should contain about 0.10–0.11% of CFAM when its level of polar material is 25%.

Structural analysis and identification of CFAM. The easi-

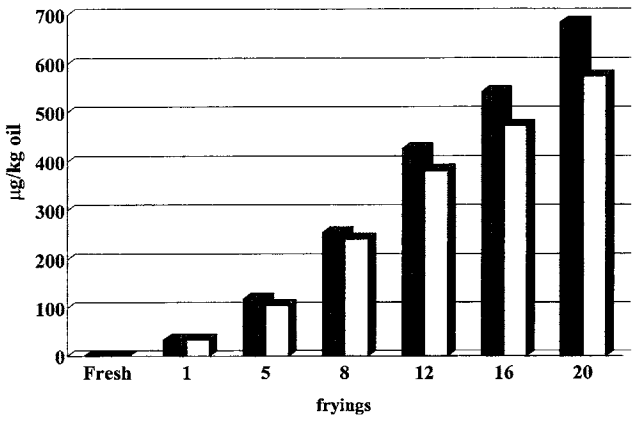

FIG. 1. Quantitation of the total cyclic fatty acid monomers (µg/kg oil) in an extra virgin olive oil used for frying frozen foods. Cross-hatched bar: null replenishment of the oil in the frying bath. Open bar: frequent

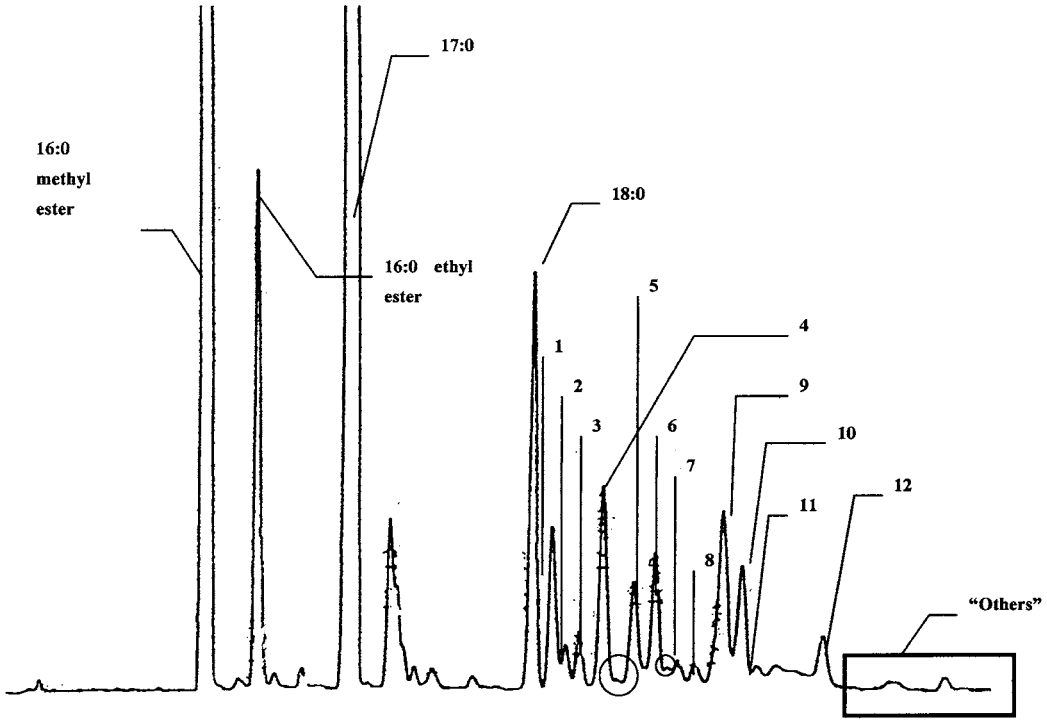

FIG. 2. Gas chromatogram of the isolated cyclic fatty acid monomer fraction from an extra virgin olive oil used in 16 fryings of various frozen foods with frequent replenishment of the oil bath. All the numbered peaks correspond to monocyclic fatty acid monomers (see Table 5). The 16:0 ethyl ester was used as the internal standard. Peaks that are circled are unidentified. "Others" refers to bicyclic fatty acids.

est and most commonly used method to determine the skeleton of the molecule of the CFAM is total catalytic hydrogenation, because it eliminates positional and geometrical isomers and simplifies complex mixtures of unsaturated compounds into a simpler mixture of saturated ones. However, interpretation of mass spectra is often very difficult, especially when cyclopentyl isomers are involved.

The determining factor for the structures of CFAM formed is the type of fatty acids present in the original oil. Monocyclic monoenoic and dienoic fatty acids are derived from linolenic and linoleic acids, respectively (22), but cyclization of oleic acid gives saturated fatty acids (23). Because linoleic and linolenic acids were present in relatively low and very low amounts, respectively, in EVO, originally only a few unsaturated CFAM would be originated from this oil.

The GC profile of the isolated CFAM fraction showed 12 peaks corresponding to monocyclic fatty acids (Fig. 2). According to the mass spectra obtained, it was possible to identify their basic structures (Table 5). Others in Figure 2 includes those peaks eluting after peak 12; according to their retention times, these probably correspond to bicyclic fatty acids (24).

The mass spectra of peaks 10 and 12 were similar and presented the characteristic ions proceeding from the typical fragmentation of the methyl 9-(2′-propyl-cyclohexyl)-nonanoate (23). In taking into account that *trans* isomers elute before *cis* isomers (25), peak 10 should correspond to the *trans* isomer and peak 12 to the *cis* one (Table 5).

In the mass spectra of peaks 4 and 9, more than one fragmentation was observed in the alkyl moiety (Table 5). This aspect has been widely described for the cyclopentyl CFAM (25,26). Ions at m/z = 239, 171, 125, and 57 revealed the presence of the two geometrical isomers of the methyl-9-(2′-butyl-cyclopentyl)-nonanoate (23).

Methyl 7-(2′-hexylcyclopentyl)-heptanoate cannot be formed by cyclization of oleic acid (21). Nevertheless, the mass spectrum of peak 2 indicated the presence of the *trans* isomer of this compound (Table 5). This fact is due to the small amount of linoleic acid present in EVO. Although we did not find the *cis* isomer, it is possible that it coelutes with another compound in peak 7.

Peaks 1 and 6 were identified as the isomers of the methyl 4-(2′-nonyl-cyclopentyl)-butanoate (23), as their mass spectra contained a very intense ion at m/z = 220 and another abundant ion at m/z = 134 (Table 5). A similar fragmentation for this compound has been described (5).

The mass spectrum of peak 3 was characterized by the presence of the molecular ion (M^+ = 296) and an abundant ion at m/z = 164. A base ion at m/z = 164 in both heated triolein and trilinolein has also been reported (21). We cannot make a hypothesis about the structure of this compound because the fragmentation mechanism is not typical.

In the mass spectrum of peak 5 there was observed a particularly intense ion at m/z = 223. This compound cannot be identified considering the typical fragmentation of CFAM,

TABLE 5
GC—MS Fragmentation [ion fragment: *m/z* (% relative intensity)] of Some Hydrogenated Cyclic Fatty Acid Monomers Isolated from an Extra Virgin Olive Oil Used in 20 Discontinuous Frying Operations of Various Frozen Foods

Peak number[a]	M^+	M – 31	M – 32	D[b]	D – 32	D – 32 – 18	B + 1	B	C	A	Base
1	296 (2)	265 (2)	264 (5)	169 (2)	137 (9)	119 (12)	172 (1)	171 (0)	195 (0)	127 (0)	220 (100)
2	296 (11)	265 (8)	264 (12)	211 (3)	179 (16)	161 (9)	144 (5)	143 (44)	153 (7)	85 (4)	55 (100)
				224 (0)	193 (6)	175 (6)					
4	296 (10)	264 (4)	264 (10)	239 (4)	207 (8)	189 (12)	172 (4)	171 (4)	125 (13)	57 (19)	55 (100)
				253 (7)	221 (2)	203 (2)					
				267 (2)	235 (1)	217 (1)					
5	296 (25)	265 (7)	264 (5)	169 (3)	137 (18)	119 (15)				127 (5)	87 (100)
6	296 (2)	265 (0)	264 (5)	169 (3)	137 (14)	119 (13)	172 (0)	171 (0)	195 (1)	127 (3)	220 (100)
9	296 (8)	265 (3)	264 (8)	239 (2)	207 (5)	189 (6)	172 (3)	171 (3)	125 (12)	57 (19)	55 (100)
				253 (3)	221 (2)	203 (1)					
				267 (1)	235 (2)	217 (0)					
10	296 (7)	265 (3)	264 (2)	253 (20)	221 (15)	203 (12)	172 (14)	171 (3)	125 (28)	43 (25)	55 (100)
12	296 (9)	265 (4)	264 (3)	253 (16)	221 (15)	203 (11)	172 (12)	171 (1)	125 (30)	43 (40)	55 (100)

[a]Peak identification: **1**, *trans* methyl 4-(2′-nonyl-cyclopentyl)-butanoate; **2**, *trans* methyl 7-(2′-hexyl-cyclopentyl)-heptanoate; **4**, *trans* methyl 9-(2′-butyl-cyclopentyl)-nonanoate; **5**, *trans* methyl 3-(2′-nonyl-cyclohexyl)-propanoate; **6**, *cis* methyl 4-(2′-nonyl-cyclopentyl)-butanoate; **9**, *cis* methyl 9-(2′-butyl-cyclopentyl)- nonanoate; **10**, *trans* methyl 9-(2′-propyl-cyclohexyl)-nonanoate; **12**, *cis* methyl 9-(2′-propyl cyclohexyl) nonanoate. Abbreviation: GS–MS, gas chromatography–mass spectrometry.
[b]Successive cleavage of the alkyl moiety for the cyclopentyl derivatives.

but the retention time is similar to that of *trans* methyl 3-(2′-nonyl-cyclohexyl)-propanoate (23), which has been described in HOSO (22). Nevertheless, the structure of this compound should be confirmed by looking at the dimethyloxazoline derivatives.

Peak 9 was identified as *cis*-methyl 9-(2′-butyl-cyclopentyl) nonanoate. Dobson *et al.* (23) described a co-elution of *cis* methyl 3-(2′-nonyl-cyclohexyl)-propanoate and *cis* methyl 9-(2′-butyl-cyclopentyl)-nonanoate employing the BPX70 column. Our findings agree with these results and could explain why we did not detect the *cis* isomer of methyl 3-(2′-nonyl-cyclohexyl)-propanoate.

There were several unidentified peaks ("Others," in Fig. 2), most likely corresponding to bicyclic fatty acids derived from polyunsaturated fatty acids. These compounds have been described in SO (22,27) and in HOSO (24).

In EVO, these bicyclic compounds accounted for about 5% of the total cyclic fatty acids (Table 6). Dobson *et al.* (23) found 4 and 13% of bicyclic fatty acids in a HOSO or a SO, respectively, after 30 frying operations of potatoes.

In the present study, cyclopentyl structures constituted a greater proportion than the cyclohexyl structures (Table 5). Thus, in the EVO used during 20 fryings of frozen foods, the greater amount of the total cyclic fatty acids was represented by *cis* and *trans* isomers of the methyl 9-(2′-butyl-cyclopentyl)-nonanoate (peaks 9 and 4, respectively), and methyl 4-(2′-nonyl-cyclopentyl)-butanoate (peaks 6 and 1, respectively), methyl 3-(2′-nonyl-cyclohexyl)-propanoate (peak 5), and the *trans* isomer of the methyl 9-(2′-propyl-cyclohexyl)-nonanoate (peak 10). These results are in agreement with those found for the monocyclic saturated fatty acids formed from oleic acid in heated HOSO and conventional SO (23).

As a result of the total catalytic hydrogenation employed, the four basic structures formed in EVO used for frying frozen foods can originate from cyclization of oleic or linoleic acid. However, most monocyclic saturated fatty acids in EVO may be formed from oleic acid due to the much greater amounts of this fatty acid in this oil, even if the identified methyl 7-(2′-hexyl-cyclopentyl)-heptanoate was exclusively formed from the linoleic acid (21).

Ribot (21) described that isomers of the methyl 9-(2′-propyl-cyclohexyl)-nonanoate are formed from both oleic and linoleic acids. This author also described small amounts of both methyl 6-(2′-hexyl-cyclohexyl)-hexanoate and methyl 8-(2′-butyl-cyclohexyl)-octanoate compounds after heating trilinolein. However, the temperature used by Ribot (22) was much higher than in the present study. This might explain the absence of isomers of methyl 4-(2′-octyl-cyclohexyl)-butanoate and *trans* methyl 8-(2′-butyl-cyclohexyl) octanoate in the frying olive oil.

The results suggest that replenishment of oil during frying is not a determining factor that influences the structures of CFAM because differences were observed only in their rela-

TABLE 6
Distribution of CFAM (% chromatographed peaks) in an EVO Used in 20 Frozen Food Fryings with FR and with NR of the Oil Bath

Peak number[a]	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	Others
FR-frying	10.9	3.7	3.5	16.0	8.8	12.4	2.9	2.4	18.2	10.7	2.5	4.0	3.1
NR-frying	11.9	2.4	2.1	15.9	8.6	11.5	2.1	1.2	17.3	15.8	1.2	4.4	4.9

[a]For identification of peaks **1**,**2**,**4–6**,**9**,**10**,**12**, and "Others" see Table 5; **3**, a not well-identified structure (for more details, see text); **7**, tentatively identified as *cis*-methyl 7-(2′-hexyl-cyclopentyl)-heptanoate; **8** and **11**, not identified. For abbreviations see Tables 2 and 4.

tive proportions and amounts. In the present study lower amounts of total CFAM were found when fryings were performed with oil replenishment (Table 5).

In short, the FR frying modality shows lower thermoxidative alteration and CFAM levels than the NR frying modality. Moreover, FR helps maintain the initial composition of EVO used in frying and effectively permits one to fry frozen prefried foods a greater number of times without discarding the oil.

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