Cyclic FA Monomers in High-Oleic Acid Sunflower Oil and Extra Virgin Olive Oil Used in Repeated Frying of Fresh Potatoes

A. Romero[a], C. Cuesta[b], and F.J. Sánchez-Muniz[a,*]

[a]Departamento de Nutrición y Bromatología I (Nutrición), Sección Lípidos, [b]Instituto de Nutrición y Bromatología (Consejo Superior de Investigaciónes Científicas-Universidad Complutense Madrid), and [a,b]Facultad de Farmacia, Universidad Complutense, 28040 Madrid, Spain

ABSTRACT: The measurement of FA profile, polar material, oligomers, oxidized triacylglycerols (OTG), total polyphenols, and cyclic FA monomers (CFAM) was used to evaluate the alteration of a high-oleic sunflower oil (HOSO) and an extra virgin olive oil (EVOO) used in 75 domestic fryings of fresh potatoes with frequent replenishment (FR) of unused oil. CFAM were absent in the unused EVOO but appeared in small amounts in the unused HOSO. Although polar material, oligomers, OTG, and CFAM contents increased and linoleic acid and polyphenols content decreased in both oils during repeated frying, the changes produced should be considered small and related to the use of very stable oils and FR. Throughout the 75 fryings, the total CFAM concentration was higher in HOSO than in EVOO. OTG increased more quickly in EVOO, whereas oligomers increased more quickly in HOSO. Polar material and oligomer content appear significantly correlated ($r = 0.9678$ and $r = 0.9739$, respectively; for both, $P < 0.001$) with the CFAM content. A 25% polar material and 12% oligomer content would correspond to about 1 mg·kg^{-1} oil of CFAM. Data suggest that both oils, particularly EVOO, perform very well in frying, with a low production of oligomers, polar materials, and CFAM.

Paper no. J10289 in *JAOCS 80*, 437–442 (May 2003).

KEY WORDS: Cyclic fatty acids, deep-frying, extra virgin olive oil, high-oleic sunflower oil, oil replenishment, polyphenols, thermal oxidation.

Deep-fat frying is a widely used culinary technology. However, in choosing an oil for frying purposes, different factors such as oil stability, price, and nutritive value should be considered. During the frying process, oxidation, hydrolysis, polymerization, isomerization, and cyclization occur (1,2), with the intensity of these reactions being highly dependent of the type and quality of the oil used (1). Because some new compounds, such as oligomers and cyclic FA that have been identified as forming during frying (2–4) show potential toxicity (4–6), thermal degradation should be studied, not only for technological reasons (production of fried foods with acceptable qualities) but also for safety and nutrition. Monounsaturated oils show unique properties in frying, permitting the frequent replenishment of these oils to extend their shelf life considerably (7). The traditional source of dietary monounsaturated FA in Mediterranean countries has been olive oil. However, several types of olive oils are commercially available. In addition, new monoenoic oils have also appeared and are extensively used (1,7,8). On the other hand, during deep-fat frying in bars and restaurants, oil is likely to be kept hot for long periods with only intermittent use for frying. Under these circumstances of slow or no turnover of oil in frying, there is a buildup of undesirable compounds. However, our group has repeatedly found benefits in frying with a frequent replenishment of fresh oil (1,7).

Most studies on heated fats have been carried out on oils heated without food fried in it, in the laboratory. Thus, only partial information is available on what happens when frying oils are used to fry foods. Previously, our group found that frying frozen foods with extra virgin olive oil (EVOO) increased the content of cyclic FA monomers (CFAM) to 0.58–0.69 mg·kg^{-1} oil after 20 fryings of frozen foods (3). This amount was much lower than the 100–7000 mg·kg^{-1} detected in commercial frying oils (4). Moreover, in comparison with infrequent replenishment with unused oil, frequent replenishment (FR) with unused oil maintains oil quality during frying because of the addition of antioxidants and the dilution of the alteration compounds formed (3).

Taking into account all these facts, the present study aims (i) to be a real approach to what is happening in the frying oil when fresh potatoes are fried in it; (ii) to establish and compare the deterioration of two monoenoic oils—EVOO and high-oleic acid sunflower oil (HOSO)—in 75 discontinuous deep-fat fryings using FR of oil by measuring the FA profile and the total polar material, the oligomer, the oxidized triacylglycerol (OTG), CFAM, and polyphenol contents.

MATERIALS AND METHODS

Materials. EVOO (Patrimonio Comunal Olivarero, Mora, Toledo, Spain), refined HOSO (VIPA, Andujar, Jaén, Spain), and fresh potatoes (Kennebec variety, Xinzo de Limia, Galicia, Spain) were purchased at a local store.

Frying procedure. Domestic deep-fat fryers with 3-L vessels were used for frying. The amount of fresh potatoes used in the successive fryings was 500 g. In total, 75 fryings were carried out with FR, adding unused oil after each frying to maintain the initial volume of 3 L of oil during the 75 fryings. Fifteen batches of five frying operations were conducted. Ten fryings were performed every day, five in the morning and

*To whom correspondence should be addressed.
E-mail: frasan@farm.ucm.es

five in the afternoon. The oil cooled to room temperature between batches. More extensive details of the frying method have been described previously (7). Replenishment of the oil lost after each frying throughout the 75 frying operations implied the total addition of 4 L of unused EVOO and 4.25 L of unused HOSO. These amounts comprised the oil absorbed into the potatoes/vaporized/splattered and the amount taken for analyses (about 150 mL in total for each oil). The average oil amount added after each frying was 53.3 mL of EVOO and 56.7 mL of HOSO. The whole procedure was performed in duplicate by using two fryers for each oil.

Polar material. Total polar material of the oils was determined by silica column chromatography (1,7). Separation of nonpolar and polar fractions was checked by TLC as previously described (1,7). Samples from the unused oils and from those belonging to frying numbers 1, 4, 8, 12, 16, 20, 30, 40, 50, 60, 70, and 75 were analyzed.

High-performance size-exclusion chromatography (HPSEC). To obtain further information about thermal oxidative changes occurring during frying, polar fractions of EVOO and HOSO, previously obtained by column chromatography, were analyzed by HPSEC (2). Two determinations on each polar fraction of both fresh and used EVOO and HOSO were performed. Samples from the unused oils and from those belonging to frying numbers 1, 4, 8, 12, 16, 20, 30, 40, 50, 60, 70, and 75 were analyzed.

FA determination. Analysis of FA was carried out by GC. Samples of the oils were saponified for 30 min at 60°C with 40 mL·g^{-1} 0.5 M NaOH in methanol and then methylated with boron trifluoride/methanol complex to achieve complete conversion to methyl esters. The esters were extracted into hexane, freed of moisture over anhydrous sodium sulfate, and dried under nitrogen. The FA content of the oil was analyzed by a Hewlet-Packard 5890 Series II gas chromatograph (Palo Alto, CA) equipped with a 1/8 in. 2-m SP 2330 (Supelco, Barcelona, Spain) packed column. Samples from the unused oils and from those belonging to frying numbers 1, 4, 8, 12, 16, 20, 30, 40, 50, 60, 70, and 75 were analyzed.

Hydrogenation of cyclic FA monomers (CFAM). Methyl ester derivatives were hydrogenated in 10 mL of a mixture of chloroform/methanol (2:1 vol/vol) by using platinum oxide as catalyst under about 3–4 bar of hydrogen atmosphere for 4 h in order to ensure a complete hydrogenation (9). 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 internal standard before hydrogenation. Samples (100 mg) from the unused oils and from those belonging to frying numbers 20, 50, and 75 were analyzed.

HPLC of CFAM. The hydrogenated CFAM were isolated by HPLC following the method of Sébédio *et al.* (10). HPLC analyses were carried out on a reversed-phase C18 column (Lichrosorb; Merck, Darmstadt, Germany; 7 mm i.d. × 25 cm in length, 5 μm particle size). A Waters 410 refractive index detector was used. A mixture of acetone/acetonitrile of 90:10 served as the mobile phase with a flow of 4 mL·min^{-1}. Sample concentration was 20 mg of the hydrogenated FAME in 100 μL of acetone.

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

Polyphenol content. The polyphenol content was determined as caffeic acid (11) in unused EVOO and in EVOO that had been used in 75 fryings.

Statistical analysis. Linear adjustments between the content of total CFAM, total polar material, oligomers, and OTG from EVOO and HOSO were established by ANOVA test. The comparison between linear equation adjustments of both oils was performed by a two-way ANCOVA. The polar material and the oligomer content were correlated with the CFAM content by using Pearson product-moment correlations.

RESULTS AND DISCUSSION

FA and total polyphenol changes. Table 1 shows that the two fresh, unused oils had similar oleic acid contents, but EVOO had lower linoleic acid and higher palmitic and linolenic acid contents. After 75 frying operations, small changes in the FA profile were found in both oils. Changes in FA were linearly related to the number of fryings for all FA except 18:1 in EVOO (Table 1). The small changes found show the high stability of both oils and the benefits of FR with unused oil in the frying of potatoes. However, the higher slope found for linoleic acid in HOSO suggests that EVOO was relatively more stable than HOSO. This fact should be related to the higher amount of linoleic acid initially found in HOSO that is preferentially oxidized in frying. However, the antioxidant effects of minor compounds, such as polyphenols, tocopherols, and phytosterols should be considered.

Table 1 shows that the total amount of polyphenols was relatively high in EVOO and markedly decreased (about 70% in EVOO) after 75 fryings. According to Boskou (12), polyphenols are present in relatively high amounts in olive oil, with hydroxytyrosol being a very efficient antioxidant compound in frying. Polyphenols tend to disappear during frying (12). Thus, polyphenols, tocopherols, and phytosterols may efficiently protect linoleic and linolenic acids from oxidations in EVOO, whereas tocopherols and phytosterols protect linoleic acid from oxidation in HOSO (13).

Thermal oxidation. Total polar material contents in the unused oils were similar and low, corresponding to good-quality oils. Frying increased this polar material in both oils (Table 1). Changes in EVOO and HOSO were significantly different, showing that EVOO has a lower intercept than the HOSO. Moreover, the slope of OTG was 9.9% higher in EVOO, whereas that of oligomers was 26% higher in HOSO

TABLE 1
Linear Adjustments of Changes in Major FA (mg/100 mg total FA) in Total Polyphenols (mg/kg oil), Polar Material, Oligomer Content, and Oxidized TG Content (mg/100 mg oil) in EVOO and HOSO with the Number of Frying[a]

	EVOO						HOSO						EVOO vs. HOSO
	Fresh	Frying #75	*a*	*b*	*r*	*P*	Fresh	Frying #75	*a*	*b*	*r*	*P*	
Palmitic acid	9.81 ± 0.07	9.96 ± 0.17	9.79	0.0023	0.3133	*	4.38 ± 0.08	4.19 ± 0.25	4.33	–0.0009	0.3871	**	***
Stearic acid	3.69 ± 0.07	3.83 ± 0.04	3.76	0.0009	0.2759	*	4.20 ± 0.02	4.32 ± 0.08	4.22	0.0013	0.6701	***	*
Oleic acid (18:1)	80.06 ± 0.26	80.16 ± 0.34	80.06	0.0013	0.1326	NS	78.46 ± 0.10	78.89 ± 0.43	78.34	0.0073	0.6608	***	*
Linoleic acid	4.54 ± 0.12	4.24 ± 0.11	4.58	–0.0038	0.6461	***	10.99 ± 0.04	10.03 ± 0.42	10.66	–0.0084	0.7241	***	***
Linolenic acid	0.55 ± 0.12	0.43 ± 0.05	0.511	–0.0009	0.3871	**	Tr	Tr	ND	ND	ND	ND	ND
Total polyphenols	153 ± 0.71	42 ± 2.83	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Polar material	2.76 ± 0.01	8.01 ± 0.00	3.686	0.0672	0.9427	***	3.60 ± 0.13	9.25 ± 0.05	5.155	0.0686	0.8943	***	***
Oligomers	0.03 ± 0.00	2.55 ± 0.05	0.5518	0.0303	0.9390	***	0.021 ± 0.00	3.36 ± 0.04	1.0334	0.0382	0.8890	***	***
Oxidized TG	0.56 ± 0.05	2.99 ± 0.07	0.9485	0.0321	0.9456	***	1.10 ± 0.02	3.60 ± 0.05	1.8580	0.0292	0.8923	***	***

[a]Data are the mean ± SD of two determinations. *a*, Intercept; *b*, slope; *r*, linear correlation; $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; NS, not significant; ND, not determined; Tr, trace (<0.06%); EVOO, extra virgin olive oil; HOSO, high-oleic sunflower oil.

(Table 1). This clearly suggests that the two oils behave differently, with preferential oxidation in EVOO but polymerization in HOSO. Dobarganes *et al.* (14) indicated that oils with low linoleic acid and high oleic acid contents tend to oxidize more than oils with higher linoleic acid and lower oleic acid contents, which preferentially polymerize. This fact is of nutritional relevance because oligomers are potentially toxic (5,6) and actively absorbed (15).

Measurement of CFAM. As found previously (3), CFAM were present in fresh HOSO in small quantities (Table 2) as a consequence of the deodorization process, but they were absent in fresh EVOO because it was not submitted to previous heat treatment. Total CFAM increased after repeated fryings, and concentration changes were linearly related to the number of fryings in both oils. Nevertheless, the slopes show a 42.9% greater formation of CFAM in HOSO (Table 2). As experimental conditions were exactly the same, differences between both oils must be attributed to the higher content of linoleic acid in HOSO, which is more susceptible to forming CFAM than oleic acid (16).

However, we noted a greater formation of CFAM during the first 20 frying operations than with the next 55 fryings (data not shown). Thus, the high turnover rate and the addition of new antioxidant compounds imply a protection against cyclization for these monoenoic oils. Burton and Ingold (17) indicated that antioxidants such as tocopherol inhibit the cyclization mechanism because they are hydrogen donors.

In the current study the total amount of CFAM found after 75 frying operations of fresh sliced potatoes was very small in comparison with those reported in 93 samples of frying oils (4). Moreover, the levels found were also lower than those found in frying of frozen foods with EVOO with FR or null replenishment of fresh oil (3). Again, frying fresh potatoes turned out to be less deleterious to the oil than frying frozen foods.

Structural identification of CFAM. In a previous work (3), we determined by GC–MS the structures of the hydrogenated CFAM molecules from an EVOO used for frying (3). EVOO and HOSO showed similar profiles and the same number of peaks (Figs. 1A and 1B, respectively). In Table 2, the monocyclic CFAM structures for peaks 1, 2, 4, 5, 6, 8, 9, 10, and 11 were identified according to their corresponding retention times of the chromatographed profiles described previously (3,19). "Others" in Figures 1 and 2 includes those peaks eluting after peak 11 and presumably corresponding to bicyclic FA (18). In both monoenoic oils, bicyclic compounds accounted for less than 3% of the total CFAM. Oleic acid is the major FA in EVOO and HOSO (80 vs. 78.5%, respectively), yielding saturated CFAM (19). Linoleic acid (4.5% in EVOO vs. 11% in HOSO) gives rise to monocyclic monoenoic FA (16). Because HOSO contains linolenic acid in trace amounts, only EVOO will contain monocyclic dienoic FA, which are formed from linolenic acid cyclization.

After 75 frying operations, cyclopentyl structures appeared in both oils in a greater proportion (62.9% for EVOO

TABLE 2
Linear Adjustments of Changes in Major Cyclic FA Monomers (CFAM) (μg/kg oil) in EVOO and HOSO with the Number of Frying of Fresh Potatoes[a]

	EVOO						HOSO						EVOO vs. HOSO
	Fresh	Frying #75	*a*	*b*	*r*	*P*	Fresh	Frying #75	*a*	*b*	*r*	*P*	
trans-Methyl-4-(2′-nonyl-cyclopentyl)-butanoate	0.0 ± 0.0	26.0 ± 6.9	6.41	0.3249	0.7824	*	3.7 ± 0.2	41.4 ± 3.1	9.76	0.4332	0.8988	**	NS
trans-Methyl-7-(2′-hexyl-cyclopentyl)-heptanoate	0.0 ± 0.0	3.4 ± 0.3	1.02	0.0360	0.6874	+	3.8 ± 0.4	16.4 ± 0.1	5.78	0.1388	0.8729	**	***
trans-Methyl-9-(2′-butyl-cyclopentyl)-nonanoate	0.0 ± 0.0	36.8 ± 3.9	8.01	0.4407	0.8478	**	11.0 ± 0.1	65.0 ± 2.9	16.4	0.6760	0.9690	***	**
trans-Methyl-3-(2′-nonyl-cyclohexyl)-propanoate	0.0 ± 0.0	17.9 ± 0.6	3.37	0.2265	0.8824	**	3.1 ± 0.0	27.7 ± 0.7	6.08	0.3132	0.9559	***	*
cis-Methyl-4-(2′-nonyl-cyclopentyl)-butanoate	0.0 ± 0.0	22.9 ± 1.3	5.13	0.2965	0.8229	*	6.0 ± 0.6	37.6 ± 2.9	9.79	0.4020	0.9568	***	*
cis-Methyl-3-(2′-nonyl-cyclohexenyl)-propanoate	0.0 ± 0.0	2.2 ± 1.9	−0.06	0.0243	0.5295	NS	1.73 ± 0.02	4.7 ± 6.6	3.14	0.0537	0.3262	NS	ND
cis-Methyl-9-(2′-butyl-cyclopentyl)-nonanoate	0.0 ± 0.0	35.3 ± 2.1	5.90	0.4234	0.8976	**	9.4 ± 0.9	61.6 ± 7.1	17.7	0.6348	0.9199	**	**
trans-Methyl-9-(2´-propyl-cyclohexyl)-nonanoate	0.0 ± 0.0	24.3 ± 0.8	4.35	0.2915	0.8759	**	4.7 ± 1.0	29.7 ± 2.1	9.8	0.3030	0.8786	**	+
cis-Methyl-9-(propyl-cyclohexyl)-nonanoate	0.0 ± 0.0	9.2 ± 0.5	1.16	0.1065	0.9316	***	4.3 ± 2.1	14.8 ± 5.0	5.1	0.1341	0.7993	*	**
Total CFAM	0.0 ± 0.0	195.0 ± 10.5	39.32	2.3775	0.8697	**	64.0 ± 7.0	334.0 ± 36.0	101	3.3977`	0.9413	***	**

[a]Data are the mean ± SD of two determinations. *a*, Intercept; *b*, slope; *r*, linear correlation; $^{+}P < 0.1$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; for other abbreviations see Table 1.

and 60.3% for HOSO) than cyclohexyl structures (27.3% for EVOO and 26.3% for HOSO) (Table 2). The distribution of CFAM originated in both oils was quite similar. Thus, in EVOO and HOSO, the higher amount of CFAM 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). Similar results have been found in studying saturated CFAM from oleic acid (19). These four basic structures can be formed by cyclization of both oleic and linoleic acids. In a previous paper peak 2 was identified as methyl 7-(2′-hexyl-cyclopentyl)-heptanoate (3). This peak is exclusively formed from the linoleic acid (16). This explains the significantly higher formation of this CFAM in HOSO vs. EVOO (Table 2).

Relationship between polar material, thermal oxidation, and CFAM contents. As described above, the polar material, oligomers, and OTG increased in both oils during the 75 frying operations. Figure 2 shows linear regressions for the content of polar material and of oligomers vs. the CFAM contents.

These linear correlations with CFAM are as follows:

$$\text{CFAM} = -107.06 + 43.128 * \text{polar material};\quad r = 0.9678,\ P < 0.001 \qquad [1]$$

$$\text{CFAM} = 19.532 + 83.854 * \text{oligomers};\ r = 0.9739,\ P < 0.001 \qquad [2]$$

where CFAM is expressed in μg/kg oil and polar material and oligomers are both expressed as g/100 g oil.

It has been suggested that an oil should be discarded when it contains 12% oligomer content or 24% polar material (20). However, the cutoff points of 10% oligomers and/or 25% polar content are extensively used. Employing these equations, one can calculate that the cutoff points of 10 or 12% oligomers would correspond to 859 or 1027 μg CFAM/kg oil, respectively, and 24 or 25% polar material would correspond to 928 or 971 μg CFAM/kg oil, respectively. Nevertheless, these calculations should be carefully interpreted because the present study was finished at frying number 75 with polar material and oligomer contents lower than 10 and 4%, respectively. Moreover, total CFAM in EVOO appears to reach a steady state of ~200 $\mu g \cdot kg^{-1}$ (Fig. 2), whereas in HOSO this tendency does not occur, thus suggesting a lower tendency for EVOO than for HOSO to form CFAM during repeated frying of fresh potatoes. These results are related to the already-noted lower linoleic acid content of EVOO in comparison to HOSO. These data also suggest the benefits of frying potatoes with monoenoic oils, because much higher amounts of CFAM have been found with other oils (4).

In short, the 75 fryings of potatoes with both monoenoic oils produced a low thermal oxidative alteration and CFAM formation. Moreover, EVOO seems to present better characteristics than HOSO, taking into account the lower losses of linoleic acid and the lower production of polar material, oligomers, and CFAM.

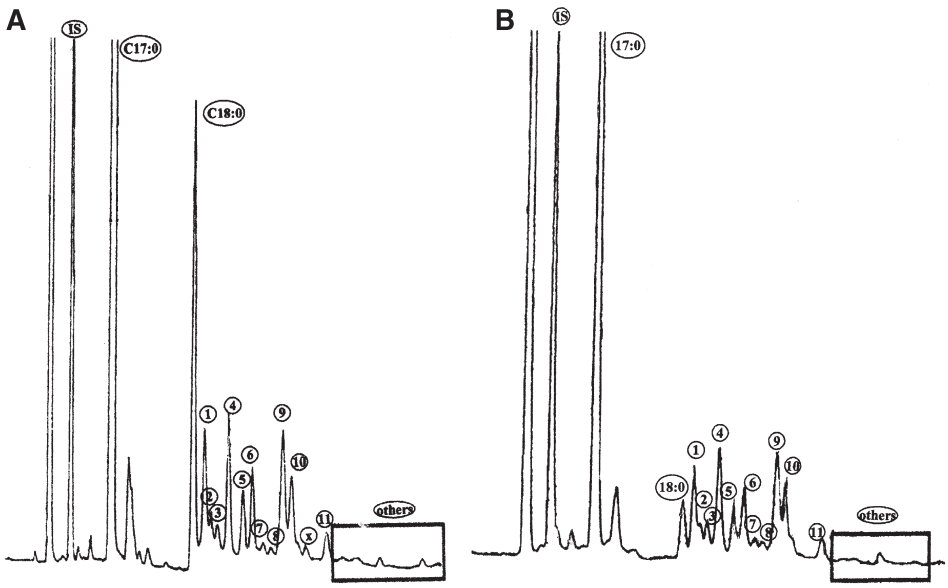

FIG. 1. Gas chromatograms of the isolated cyclic FA monomer fraction from monoenoic oils used in frying of fresh potatoes with frequent replenishment of the oil bath. (A) Extra virgin olive oil. (B) High-oleic acid sunflower oil. All the numbered peaks correspond to monocyclic FA monomers. **IS** corresponds to 16:0 ethyl ester, which was used as the internal standard. 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, **8**. *cis*-methyl-3-(2′-nonyl-cyclohexyl)-propanoate, **9**. *cis*-methyl-9-(2′-butyl-cyclopentyl)-nonanoate, **10**. *trans*-methyl-9-(2′-propyl-cyclohexyl)-nonanoate, and **11**. *cis*-methyl-9-(propyl-cyclohexyl)-nonanoate, **X**. Unidentified peak. "Others" refers to bicyclic FA.

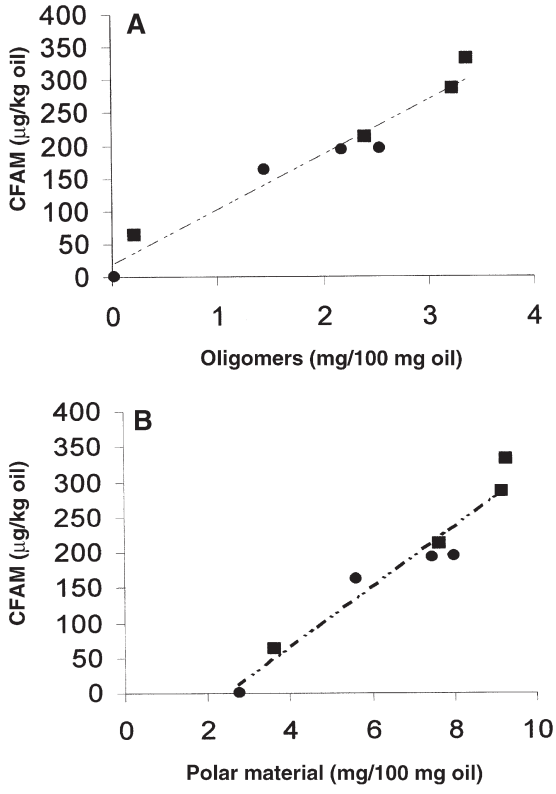

FIG. 2. Pearson product-moment correlations (A) between the cyclic FA monomer (CFAM) content (μg/kg oil) and the oligomer content (mg/100 mg oil) ($r = 0.9739$, $P < 0.001$) and (B) between the CFAM content (μg/kg oil) and the polar material content (mg/100 mg oil) ($r = 0.9678$, $P < 0.001$). ●, Extra virgin olive oil; ■, high-oleic acid sunflower oil.

ACKNOWLEDGMENTS

This study was supported by Spanish Comisión Interministerial de Ciencia y Tecnología (CICYT) Project ALI-92-0289-CO2-01. Thanks are due for the fellowship granted to Antonio Romero by the Consejería de Educación y Cultura de la Comunidad de Madrid (Spain). The authors are indebted to Frank Julliard and Jean-Louis Sébédio for their valuable help.

REFERENCES

1. Dobarganes, M.C., M.C. Pérez-Camino, and G. Márquez-Ruiz, High-Performance Size-Exclusion Chromatography of Polar Compounds in Heated and Nonheated Fat, *Fat Sci. Technol. 90*: 308–311 (1988).
2. Romero, A., C. Cuesta, and F.J. Sánchez-Muniz, Effect of Oil Replenishment During Deep-Fat Frying of Frozen Foods in Sunflower Oil and High-Oleic Acid Sunflower Oil, *J. Am. Oil Chem. Soc. 75*:161–167 (1998).
3. Romero, A., C. Cuesta, and F.J. Sánchez-Muniz, Cyclic Fatty Acid Monomers and Thermoxidative Alteration Compounds Formed During Frying of Frozen Foods in Extra Virgin Olive Oil, *Ibid. 77*:1169–1175 (2000).
4. Sebedio, J.L., and A. Grandgirard, Cyclic Fatty Acids: Natural Sources, Formation During Heat Treatment, Synthesis and Biological Properties, *Prog. Lipid Res. 28*:303–336 (1989).
5. López-Varela, S., F.J. Sánchez-Muniz, and C. Cuesta, Decreased Food Efficiency Ratio, Growth Retardation and Changes in Liver Fatty Acid Composition in Rats Consuming Thermally Oxidized and Polymerized Sunflower Oil Used for Frying, *Food Chem. Toxical. 33*:181–189 (1995).
6. Márquez-Ruíz, G., and M.C. Dobarganes, Nutritional and Physiological Effects of Used Frying Fats, in *Deep Frying: Nutritional and Practical Applications*, edited by E.G. Perkins and M.C. Erickson, AOCS Press, Champaign, 1996, pp. 160–182.

7. Romero, A., C. Cuesta, and F.J. Sánchez-Muniz, Does Frequent Replenishment with Fresh Monoenoic Oils Permit the Frying of Potatoes Indefinitely? *J. Agric. Food Chem. 47*:1168–1173 (1999).
8. Sebedio, J.L., M.C. Dobarganes, G. Márquez, I. Wester, W.W. Christie, G. Dobson, F. Zwobaba, J.M. Chardigny, T. Mairot, and R. Lahtinen, Industrial Production of Crisps and Prefried French Fries Using Sunflower Oils, *Grasas Aceites 47*:5–13 (1996).
9. Christie, W.W., The Preparation of Derivatives of Fatty Acids, in *Gas Chromatography and Lipids: A Practical Guide*, edited by W.W. Christie, The Oily Press, Ayr, Scotland, 1989, p. 83.
10. Sebedio, J.L., J. Prevost, E. Ribot, and A. Grandgirard, Utilization of High-Performance Liquid Chromatography as an Enrichment Step for the Determination of Cyclic Fatty Acid Monomers in Heated Fats and Biological Samples, *J. Chromatogr. 659*: 101–109 (1994).
11. Singleton, S.L., and J.A. Rossi, Colorimetry of Total Phenols with Phosphomolybdic Phosphotungstic Acid Reagents, *Am. J. Enol. Viticul. 16*:144–158 (1965).
12. Boskou, D., Non-Nutrient Antioxidants and Stability of Frying Oils, in *Frying of Food: Oxidation, Nutrient and Non-Nutrient Antioxidants, Biologically Active Compounds and High Temperatures*, edited by D. Boskou, and I. Elmadfa, Technomic Publishing, Lancaster, PA, 1999, pp. 183–204.
13. Blekas, G., and D. Boskou, Phytosterols and Stability of Frying Oils, in *Ibid.*, pp. 205–221.
14. Dobarganes, M.C., M.C. Pérez-Camino, G. Márquez-Ruiz, and M.V. Ruiz-Méndez, New Analytical Possibilities in Quality Evaluation of Refined Oils, in *Edible Fats and Oils Proceedings: Basic Principles and Modern Practices*, edited by D.R. Erickson, American Oil Chemist's Society, Champaign, 1990, pp. 427–429.
15. Sánchez-Muniz, F.J., S. Bastida, and M.J. González-Muñoz, Column and High-Performance Size Exclusion Chromatography Applications to the *in vivo* Digestibility Study of a Thermoxidized and Polymerized Olive Oil, *Lipids 34*:1187–1192 (1999).
16. Sebedio, J.L., J. Prevost, E. Ribot, and A. Grandgirard, Utilization of High-Performance Liquid Chromatography as an Enrichment Step for the Determination of Cyclic Fatty Acid Monomers in Heated Fats and Bilogical Samples, *J. Chromatogr. A 659*:101–109 (1994).
17. Burton, G.W., and K.U. Ingold, Vitamin E: Application of the Principles of Physical Organic Chemistry to the Exploration of Its Structure and Function, *Acc. Chem. Res, 19*:194–199 (1986).
18. Dobson, G., W.W. Christie, and J.L. Sebedio, Saturated Bicyclic Fatty Acids Formed in Heated Sunflower Oil, *Chem. Phys. Lipids, 87*:137–147 (1997).
19. Dobson, G., W.W. Christie, and J.L. Sebedio, Monocyclic Saturated Fatty Acids Formed from Oleic Acid in Heated Sunflower Oils, *Ibid. 82*:101–110 (1996).
20. Firestone, D., Regulation in the United States, in *Frying: Improving Quality*, edited by J.B. Rossell, CRC Press, Woodhead Publishing, Boca Raton, FL, 2001, pp. 49–58.

[Received April 3, 2002; accepted January 9, 2003]