Deep-Frying of Sardines in Different Culinary Fats. Changes in the Fatty Acid Composition of Sardines and Frying Fats

Francisco J. Sánchez-Muniz,* Jesús M. Viejo, and Rafaela Medina

Departamento de Nutrición y Bromatología I (Nutrición), Facultad de Farmacia, Universidad Complutense, E-28040 Madrid, Spain

The goal of this study is to determine how deep-fat-frying in olive oil, sunflower oil, or lard affects the fatty acid composition of sardines. Absolute saturated fatty acid content (grams per 100 g of dry matter fish) increased with frying in the case of lard. As for monounsaturated fatty acid, an increase of 4.2 times was noted in the fish fried in sunflower oil and 8 times with lard, while a 10 times increase was noted in the fish fried in olive oil. Polyunsaturated fatty acids (PUFA) n-6 content rose 4 times with olive oil, 6.3 times with lard, and 19.9 times with sunflower oil. The PUFA n-3 fell 3.3 times in the case of sunflower oil and 2.2 times with olive oil, with no changes with lard. Small quantities of eicosapentaenoic and docosahexaenoic acids appeared in the olive and sunflower oils used; only traces were found in the case of lard. The content of polar methyl esters significantly increased in lard after the first frying of sardines but remained unmodified in olive oil and sunflower oil. Sardine cholesterol content (milligrams per 100 g of dry matter) significantly decreased after frying. According to these results frying produced an exchange between the fat in the sardines and the frying media, which caused significant changes in the fatty acid composition and in the n-6/n-3 ratio of the oily fish.

INTRODUCTION

Most of the polyunsaturated fatty acid (PUFA) n-3 studies have been carried out on fish oil concentrates and on fatty fish (Schouten and Beynen, 1986; Flaten et al., 1990; Kinsella et al., 1990), but those studies do not usually take into account that the qualitative and quantitative fat composition of fatty fish can be greatly affected by seasonality, fishing grounds, and industrial and culinary processing (Moreiras-Varela et al., 1988; Nawar et al., 1990; Varela et al., 1990). Moreover, there is scarcely any information trying to relate health or disease treatments and consumption of processed fish, e.g., fried fish (Moreiras-Varela et al., 1988; Sánchez-Muniz et al., 1991).

According to the Spanish National Food Survey (Instituto Nacional de Estadística and Instituto de Nutrición (CSIC) (1985)], Spain and Portugal rank first in fish consumption among the EEC countries: 72 g/day, 11 g/day being fatty fish and 6.6 g/day of sardines in particular. In Spain, and mainly in Andalucia, frying is a typical method of fish processing, and a large percentage of the fish consumed is fried. Sánchez-Corcoles et al. (1990) have indicated in their study fish consumption of 1 kg of fish per week among some people of Malaga.

During frying, interactions among components of food and the culinary fat used take place (Gall et al., 1983; May et al., 1975; Nawar, 1984; Nawar et al., 1990; Sims and Fiorty, 1975; Varela et al., 1990). These exchanges and interactions would imply that the concentrations of some specific fatty acids in the fish, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), deeply change.

The purposes of this paper are to study how deep-fat-frying of sardines in different culinary fats (olive oil, sunflower oil, and lard) affected (1) the fatty acid composition of the fish, with special remarks to the n-6/n-3 ratio changes, and (2) the fatty acid composition of these three frying fats.

MATERIALS AND METHODS

Materials and Reagents. Olive oil with a 0.4° acid value (UNE 55-011-73, 1973), refined sunflower oil, and lard were purchased at a local store. The choice of such fats was conditioned by their fat composition: very high in monounsaturated fatty acids (MUFA) in olive oil, high in PUFA in sunflower oil, and high in saturated fatty acids (SFA) in lard (Table II). Sardines (*Sardina pilchardus* WALB.) were also purchased at a local store.

Boron trifluoride–methanol complex (20% solution in methanol), hexane, chloroform, methanol, sodium chloride, diethyl ether, petroleum ether, and acetic acid were from E. Merck AG, Darmstadt, FRG. Chromatography standards were obtained from Sigma, St. Louis, MO. Gas chromatography columns were obtained from Supelco, Barcelona, Spain. Silical gel 60F 250 plates (20 × 20 cm glass) and silica gel 60 (0.063–0.200 nm) for column chromatography were obtained from E. Merck.

Methods. *Performance of Frying.* Fried sardines were prepared as follows: sardines (600–700 g), head, scales, viscera, and backbone removed, were opened into a fan shape, floured in wheat flour (~8% w/w) and fried in one of the three culinary fats at 180 °C. Domestic fryers with a capacity of 3 L were used as cooking receptacles. In this study culinary fats were used once. Once fried, the sardines were freeze-dried and kept at -20 °C under nitrogen atmosphere until analyses were made.
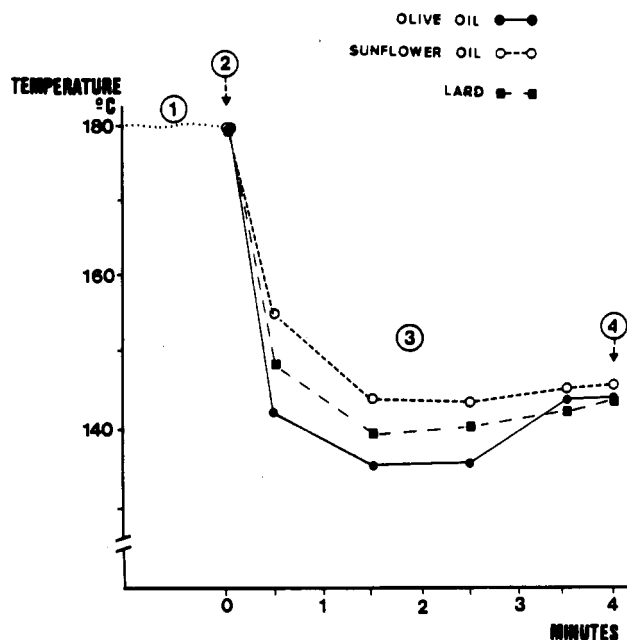
Figure 1 shows that during the first 0.5 min, the temperature of the frying fat undergoes a deep change, later decreasing more slowly until stabilization at 1.5 min with frying complete at 4 min. The culinary fat temperature remained below 150 °C almost all of the time.

Fat Extraction and Fatty Acid Analyses. Fish fat was extracted according to the method of Bligh and Dyer (1959), saponified with 0.5 N of sodium hydroxide in methanol, and then methylated following the method of Metcalfe et al. (1966).

The fatty acid methyl esters of olive oil, sunflower oil, and lard and the fish fats were analyzed by gas chromatography. A Hewlett-Packard 5710 chromatograph with a steel column packed with 10% Supelcoport 2330 on 100/120 Chromosorb W AW, 6 ft × 1/8 in., was used. The temperature of the column was held for 8 min at 170 °C and then increased to 240 °C at 2 °C/min. The temperature of the injector was 250 °C and that of the detector 300 °C. Sample size was 0.5 μL. The peak areas were measured using a Perkin-Elmer Minigrator M-2 integrator.

The fatty acids were identified by comparing their relative and absolute retention times with those of commercial standards. The different fatty acid contents were calculated on the basis of

---

* Author to whom correspondence should be addressed.

Fatty Acid Changes in Frying of Sardines

J. Agric. Food Chem., Vol. 40, No. 11, 1992 **2253**

OLIVE OIL ●—●

SUNFLOWER OIL ○--○

LARD ■- -■

Figure 1. Changes in the culinary fat temperature during frying of sardines. Phase 1: Fat held at 180 °C. Phase 2: Addition of 600–700 g of "clean", opened into a fat shape, floured sardines to 3000 mL of culinary fat. Phase 3: Change of temperature during frying. Phase 4: End of frying process.

percentage in the fat and the proportion of fat in the fish, using the conversion factor for fish suggested by Paul and Southgate (1979). To assure the value of this conversion factor under our experimental conditions, samples with and without pentadecanoic acid as internal standard were studied.

Peroxide Value. Peroxide values of frying fats and of fat extracted from both raw and fried sardines were calculated according to UNE 55-023-73 (1973).

Polar Methyl Fatty Acid Ester. Polar methyl fatty acid content was evaluated using the silica gel column chromatography method of Dobarganes et al. (1984) and Cuesta et al. (1991).

An accurately weighed sample of 1 ± 0.01 g of fat or oil was dissolved in 20 mL of petroleum ether–diethyl ether 96:4 (v/v) and transferred to a silica gel chromatographic column. A final elution of the column with diethyl ether was performed to check separation of polar and nonpolar fraction by thin-layer chromatography on 0.5-mm-thick silica gel plates. Samples were applied as 10-μL spots using a Hamilton 705 microsyringe. Plates were developed with hexane–ethyl ether–acetic acid 80:20:1 (v/v/v) in a lined tank for ca. 25 min (ca. 17 cm) and removed, letting the solvent evaporate. The spots were visualized after exposure to iodine vapor. Spots corresponding to polar and nonpolar fractions were clearly separated, proving the success of the separation by column chromatography.

Cholesterol Content, Cholesterol–Saturated Fat Index, and Cholesterol Index. The cholesterol content of frying fats and of raw and fried sardines was calculated according to the method of Cabrera (1988), taking into account the moisture content of sardines and the cholesterol content of culinary fats (null in olive oil and sunflower oil and 70 mg/100 g in lard). The hypercholesterolemic–atherogenic potential of sardines was evaluated using the cholesterol–saturated fat index (CSFI) suggested by Connor et al. (1986), where CSFI = (1.01 × g of saturated fat) + (0.05 × mg of cholesterol). The cholesterol index (CI) of sardines was calculated according to the method of Zilversmit (1979), where CI = 1.01 (g of saturated fat – 0.5 × g of polyunsaturated fat) + (0.05 × mg of cholesterol).

Statistical Analyses. The similarity of variance was tested using the F_{max} of Snedecor (Domenech, 1982). Comparisons among the different culinary fats used and the kinds of sardines were made using the Anova one-way test of Newman–Keuls. Differences were studied at the $p \leq 0.5$ level (Domenech, 1982).

RESULTS AND DISCUSSION

In studies on frying conditions Varela (1977) and Varela et al. (1983) found that the temperature of the fat at which the food was fried had relatively little bearing on the thermal damage done to the food, provided that the food had a high water content. The reason that the temperature inside the food does not rise above 100 °C is due to water evaporation. The fat does not begin to penetrate the food until a substantial part of the water it contains has evaporated. As a result, the hot fat acts on the food for a very short time. Moreover, as shown in Figure 1, during sardine frying, the temperature of the *media* remains below 150 °C during almost the whole process.

According to Blumenthal (1991) wetting of the heater surfaces ultimately leads to complete carbonization of an oil layer and the formation of on insulating blanket around the heater elements. Insulation leads to higher temperature on the heater surfaces as the controllers call for more sensible heat from the sources. The higher water loss produced when sardines are fried in sunflower oil or lard (Table I) would produce higher wetting of the heater surfaces and explain why frying of sardines with those culinary fats was done at higher temperature than with olive oil (Figure 1).

Table I shows moisture and fat contents of raw and fried sardines. Moisture decreased approximately 70%, whereas fat increased several times in the three kinds of studied sardines. Data concur with those found by Ruiz-Roso (1983).

According to the data of Table I, lard-fried sardines had more fat and sunflower-fried sardines less than the olive-oil-fried ones. In a previous work, Sánchez-Muniz et al. (1990), studying the efficiency of three culinary fats used in repeated fryings of sardines, lard in the fryer showed the highest loss in volume during the first fryings, which in turn would explain the higher fat content of lard-fried sardines seen in the current study. Also, the higher polar methyl ester content found in lard than in olive oil or sunflower oil (Table I) would influence the higher fat content seen in lard-fried sardines (Table I), because according to Blumenthal (1991) the more altered the culinary fat is, the higher the mass transfer and surfactant production are, increasing therefore the fried food fat content. Looking to the lower polar methyl ester content prior to and after sardine frying (Table I), olive oil seems to be more stable than the other two fats when sardines are fried.

Peroxide indices of culinary fats and sardines prior and after frying (Table I and II) were rather low and do not seem to be related with the possible fat autooxidation. However, as has been pointed out, peroxides are unstable and break down to other secondary products (Frankell, 1991).

The fat content of raw sardines determines the fat exchanges and interactions between the culinary fat and that of the fish when frying. May et al. (1975) found in a study on freshwater fish that the higher the fat content of the fish was, the fewer lipid changes during frying were produced. In contrast, fish that have a low fat content absorb more fat.

Nawar et al. (1990) indicated that batter coating appeared to protect the fish fillets against loss of moisture, absorption of cooking oil, and dilution or loss of flavor volatiles. When pollock was deep-fat-fried without batter, extensive absorption of the frying oil occurred.

Varela (Varela, 1977; Varela et al., 1988) indicated that olive oil forms a crust that protects the food against

Table I. Moisture and Fat Contents and Peroxide Value of Raw and Fried Sardines[a]

	raw sardines	olive-oil-fried sardines	sunflower-oil-fried sardines	lard-fried sardines
moisture content, g/100 g of fish	62.0 ± 1.5[a]	17.9 ± 0.9[b]	16.3 ± 1.1[c]	16.3 ± 0.9[c]
fat content, g/100 g of dry matter	10.0 ± 4.6[a]	39.1 ± 2.7[b]	34.8 ± 4.3[b]	46.0 ± 1.7[c]
peroxide index	3.5 ± 0.3[a]	4.4 ± 1.2[a]	4.7 ± 0.9[a]	6.6 ± 1.9[a]

[a] Values are means ± standard deviations of six determinations. Values in the same raw bearing different letters are significantly different.

Table II. Major Fatty Acid Composition, Peroxide Index, and Polar Methyl Ester Content of Olive Oil, Sunflower Oil, and Lard Prior to and after Frying Sardines[a]

fatty acid, % w/w of total fatty acids	olive oil		sunflower oil		lard	
	unused	used	unused	used	unused	used
C16:0	9.7 ± 0.5	10.3 ± 0.5	7.8 ± 0.4	7.9 ± 0.4	24.9 ± 0.5	25.4 ± 0.5
C18:0	3.4 ± 0.2	3.8 ± 0.2	4.8 ± 0.3	5.1 ± 0.4	13.2 ± 0.3	14.7 ± 0.5
C18:1	77.9 ± 1.0	76.8 ± 0.7	29.8 ± 0.7	30.5 ± 0.6	44.4 ± 0.6	42.8 ± 0.6
C18:2	7.4 ± 0.3	7.0 ± 0.5	56.0 ± 0.8	55.6 ± 1.0	9.7 ± 0.5	9.4 ± 0.5
peroxide index	5.3 ± 0.2	3.1 ± 1.7	5.1 ± 0.1	6.1 ± 0.5	3.1 ± 0.2	3.1 ± 0.9
polar methyl ester (mg/100 mg)	1.5 ± 0.5	3.0 ± 0.4	4.1 ± 0.4	6.4 ± 0.5	11.5 ± 0.8	16.5 ± 0.6

[a] Values are the mean ± SD of two determinations. No significant differences were found between comparisons for the same fat, except for polar methyl esters content in lard.

Table III. Major Fatty Acid Content of Raw and Fried Sardines[a]

fatty acid[b]	raw sardines	olive-oil-fried sardines	sunflower-oil-fried sardines	lard-fried sardines
C14:0	3.7 ± 0.2[a]	1.4 ± 0.02[b]	0.8 ± 0.1[c]	1.8 ± 0.1[b]
C16:0	27.6 ± 0.7[a]	14.1 ± 0.2[b]	11.1 ± 1.5[b]	23.7 ± 0.3[a]
C18:0	9.6 ± 1.0[a]	4.2 ± 0.2[b]	5.2 ± 0.7[b]	12.5 ± 0.4[b]
total saturated	42.0 ± 1.4[a]	20.6 ± 0.1[b]	17.7 ± 2.2[b]	38.4 ± 0.8[a]
C16:1	2.6 ± 0.1[a]	1.4 ± 0.1[b]	0.7 ± 0.1[c]	2.4 ± 0.1[d]
C18:1	20.5 ± 0.4[a]	65.8 ± 0.4[b]	30.5 ± 2.8[c]	42.1 ± 0.5[d]
total monosaturated	27.3 ± 0.8[a]	68.1 ± 0.6[b]	32.1 ± 3.0[a]	46.0 ± 0.3[c]
C18:2	3.9 ± 0.3[a]	7.2 ± 0.3[a]	46.1 ± 5.0[b]	8.5 ± 0.2[a]
C18:3	2.1 ± 0.1[a]	0.7 ± 0.03[b]	0.5 ± 0.01[b]	1.1 ± 0.1[c]
C20:5	4.7 ± 0.00[a]	1.0 ± 0.1[b]	0.7 ± 0.2[b]	1.1 ± 0.02[b]
C22:6	16.2 ± 0.4[a]	1.2 ± 0.4[b]	1.1 ± 0.2[b]	3.0 ± 0.7[c]
total polyunsaturated	30.7 ± 0.6[a]	11.3 ± 0.6[b]	50.2 ± 5.2[c]	15.7 ± 1.0[b]

[a] Values are means ± standard deviations of two determinations in homogenized samples of sardines. Values in the same row bearing different letters are significantly different. [b] Calculated on % (w/w) of total fatty acids.

absorption of oils, whereas other fats do not form such a defined crust and the food contains more fat after fried.

Morton [1974, quoted by Varela (1988)] found that the extent to which a fat penetrates a fried food is heavily dependent on whether the food is fried in batter or not and, if it is, on the thickness and other features of the batter. Fish that have been dredged with a fine coating of flour do not fry as fish-fingers, which are covered with a thick batter. In the latter case the food is cooked rather than fried since the fat does not penetrate the food but remains on the outside coating.

In the current study fan-shape sardines were dredged with flour as normally is done at home and in bars in Spain and were not covered with a batter. These experimental conditions would explain the extensive absorption of frying fat by the sardines.

Table III shows the fatty acid composition of raw and fried sardines. As indicated above, frying involves an exchange of fatty acids between the fat in the sardines and the culinary fat used, which caused significant changes in the fatty acid composition of the oily fish. Table III indicates that the relative SFA content of the sardines decreased when fried in olive and sunflower oils. Palmitic acid was the major SFA, being about twice higher in lard-fried sardines than in the other kinds. Oleic acid increased about 50% when fried in sunflower oil, 100% when in lard, and 200% in olive oil. Linoleic acid increased about 12 times with sunflower oil. The levels of PUFA n-3 represented by EPA and DHA decreased in all cases. However, in the fats used for frying (Table II) only small changes were detected. Small quantities of EPA (0.03–0.05%) and DHA (0.12–0.16%) were detected in olive and sunflower oils, but only traces of these fatty acids were

found in the case of lard. This means that roughly 0.9–0.15 g of EPA and 3.6–4.8 g of DHA are released from 600–700 g of sardines when frying in olive or sunflower oil.

Data of Tables II and III could be explained by the existence of fatty acid gradients from the frying media to the sardines and from the fish to the fryer, which in turn dilute or increase the fatty acid content of both culinary fats and sardines. However, not all of the fatty acid exchanges were produced in the same proportion. For example, sardines fried in sunflower oil increased their oleic and stearic acid contents to almost the same level of the fryer oil, whereas other fatty acids change to an intermediate level. The existence of different kinetics for the various fatty acids has been previously suggested by our group in potato fryings on the basis of fatty acid concentration of oils and potatoes (Sánchez-Muniz et al., 1989).

All of these results indicate that the fat composition of the fried sardines tends to be similar to that of the frying culinary fat (Tables II and III). May et al. (1975) indicated that fish with a low fat content tend to end up with a fatty acid composition similar to that of the fat used for frying. In the present study raw sardines contained 10.0 ± 4.6 g/100 g of dry fresh matter of fat, which explains the similarity between the fatty acid composition of the culinary fat and those of the fried sardines.

Gall et al. (1983) found that frying with soybean oil produced a considerable decrease in the SFA content of all the fish studied. PUFA levels represented by arachidonic acid and DHA fell in all fishes. In every case the unsaturated fatty acid/SFA ratio increased when the food was fried.

The absolute contents (grams per 100 g of dry matter)

Fatty Acid Changes in Frying of Sardines

J. Agric. Food Chem., Vol. 40, No. 11, 1992 **2255**

Table IV. Fatty Acid Content, Polyunsaturated/Saturated Fatty Acid (P/S) Ratio, n–6/n–3 Ratio, Cholesterol Content, Cholesterol Index (CI), and Cholesterol–Saturated Fat Index (CSFI) in Raw and Fried Sardines[a]

	raw sardines	olive-oil-fried sardines	sunflower-oil-fried sardines	lard-fried sardines
SFA[b,c]	3.8 ± 0.2^a	7.5 ± 0.1^a	5.9 ± 0.7^a	16.8 ± 3.6^b
MUFA[b,c]	2.5 ± 0.2^a	25.0 ± 0.4^b	10.6 ± 1.0^c	20.1 ± 0.1^d
PUFA n–6[b,c]	0.8 ± 0.03^a	3.2 ± 0.2^b	15.9 ± 1.6^c	5.0 ± 0.5^b
PUFA n–3[b,c]	2.0 ± 0.1^a	0.9 ± 0.04^b	0.6 ± 0.1^b	1.9 ± 0.1^a
total PUFA[b,c]	2.8 ± 0.1^a	4.1 ± 0.2^a	16.5 ± 1.7^b	6.9 ± 0.5^a
P/S ratio[b]	0.7 ± 0.04^a	0.6 ± 0.03^a	2.9 ± 0.7^a	0.4 ± 0.03^a
n–6/n–3 ratio[b]	0.4 ± 0.00^a	3.6 ± 0.3^a	27.3 ± 4.2^b	2.6 ± 0.4^a
cholesterol content[b]	210.5 ± 5.1^a	142.5 ± 1.4^b	129.3 ± 6.1^b	155.8 ± 3.7^b
CI[d]	4.9 ± 0.1^a	10.4 ± 0.1^b	3.5 ± 1.3^a	17.8 ± 0.5^d
CSFI[d]	5.5 ± 0.1^a	12.1 ± 0.0^b	10.4 ± 0.6^b	20.6 ± 0.3^c

[a] Values are means ± standard deviations of two determinations. Values in the same row bearing different letters are significantly different. For more details see Materials and Methods. [b] Calculated on g/100 g of dry matter. [c] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. [d] Calculated on 100 g of fresh matter.

of groups of fatty acids are shown in Table IV. Total SFA content of sardines increased about 350% in lard-fried sardines. MUFA increased 10, 4.2, and 8.0 times in olive-oil-, sunflower-oil-, and lard-fried sardines, respectively. PUFA increased 5.9 times in sunflower-oil-fried sardines; however, PUFA n–3 and n–6 were not equally affected. PUFA n–3 decreased significantly in olive-oil- and sunflower-oil-fried sardines but not in lard-fried sardines, whereas PUFA n–6 increased 4.0, 19.9, and 6.3 times in olive-oil-, sunflower-oil-, and lard-fried sardines, respectively.

The PUFA to SFA (P/S) ratio rose more than 3 times (but not significantly) with sunflower oil and decreased by 47% (not significantly with lard (Table IV). These results are due to the entrance of large quantities of linoleic acid into the sardines when frying with sunflower oil and SFA when frying with lard. Frying with olive oil increased in a similar way both SFA and PUFA percentages.

The cholesterol contents of the different kinds of fried sardines were similar, being significantly decreased, mainly due to culinary fat absorption, with respect to the cholesterol content of raw sardines (Table IV).

Table IV also shows that the CSFI of sardines increases with frying and appears to be much higher in lard-fried sardines. CSFI of raw sardines was similar to CSFI values indicated for salmon and shellfish by Connor et al. (1986). Frying sardines in lard increased their SFA, which in turn influenced the CSFI and the CI. CSFI of lard-fried sardines was 3.7 times higher than that of raw sardines and about 1.5 times those of olive-oil- and sunflower-oil-fried sardines. CI increased in olive-oil- and lard-fried sardines.

It has been suggested that there may be a balance between n–6 and n–3 fatty acids in membrane phospholipids (Gudjarnason et al., 1991). The levels of n–6 and n–3 fatty acids in membrane cells are rapidly modified by stress or dietary fat (Gudjarnason et al., 1991; Sánchez-Muniz, 1987). However, the n–6/n–3 *optimum* ratio has not yet been established and a *desirable dietary ratio* has not been universally agreed upon; these numbers are necessary for further research to determine the *optimum ratio*.

Nevertheless, Budowski and Crawford (1985) suggested that there may be a *desirable ratio* of dietary n–6 to n–3 fatty acid of about 5; meanwhile, Kinsella (1987) indicated that the *desirable ratio* would be 1. According to these suggestions, we would consider that n–6/n–3 ratios of sardines fried in olive oil or lard had a more *desirable ratio* than did sardines fried in sunflower oil. However, it must be also taken into account that lard-fried sardines were richer in SFA and PUFA n–3 and have higher CI and CSFI than the other two kinds of fried sardines, whereas olive-oil-fried sardines contained higher proportions of

MUFA (oleic acid) and sunflower-oil-fried sardines are richer in PUFA (mainly PUFA n–6).

In conclusion, the data of the current work indicate that the fat composition of sardines and thereby its n–6/n–3 ratio can be deeply changed when sardines are fried. That fact may be of special relevance for some people who usually eat large quantities of fried fish.

LITERATURE CITED

Bligh, A. C.; Dyer, W. J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Phys.* **1959**, *37*, 911–917.

Blumenthal, M. M. A new look at the chemistry and physics of deep-fat frying. *Food Technol.* **1991**, *45*, 68–94.

Budowski, P.; Crawford, M. A. α-Linoleic acid as a regulator of the metabolism of arachidonic acid: dietary implications of the ratio, n–6:n–3 fatty acids. *Proc. Nutr. Soc.* **1985**, *44*, 221–229.

Cabrera, L. Nutritional quality of the fat intake in the Spanish population. Ph.D. Dissertation, Universidad Complutense of Madrid at Facultad de Farmacia, 1988.

Connor, S. L.; Gustafson, J. R.; Artaud-Wild, S. M.; Flavell, D. P.; Classick-Kohn, C. J.; Hatcher, L. F.; Connor, W. E. The cholesterol/saturated-fat Index: an Indication of the Hypercholesterolaemic and Atherogenic Potential of Food. *Lancet* **1986**, *1*, 1229–1232.

Cuesta, C.; Sánchez-Muniz, F. J.; Hernández, I. Evaluation of nonpolar methyl esters by column and gas chromatography for the assessment of used frying olive oils. *J. Am. Oil Chem. Soc.* **1991**, *68*, 443–445.

Dobarganes, M. C.; Pérez Camino, M. C.; Gutiérrez González-Quijano, R. Applied analytical methods in heated fats. *Grasas Aceites* **1984**, *35*, 172–177.

Domenech, J. M. In *Bioestadística. Métodos estadísticos para investigadores*, 4th ed.; Herder: Barcelona, 1982; Chapters 9 and 10.

Flaten, H.; Hostmark, A. T.; Kierulf, P.; Lystard, E.; Trygg, K.; Bjerkedad, T.; Osland, A. Fish-oil concentrate: effects on variables related to cardiovascular disease. *Am. J. Clin. Nutr.* **1990**, *52*, 300–306.

Frankell, E. N. Review. Recent advances in lipid oxidation. *J. Sci. Food Agric.* **1991**, *54*, 495–511.

Gall, K. L.; Otwell, W. S.; Kobverger, J. A.; Appledorf, H. Effects of four cooking methods on the proximate, mineral and fatty acid composition of fish fillets. *J. Food Sci.* **1983**, *48*, 1068–1078.

Gudjarnason, S.; Benediktsdóttir, V. E.; Gudmundsdóttir, E. Balance between ω3 and ω6 fatty acids in heart muscle in relation to diet, stress and ageing. *World Rev. Nutr. Diet.* **1991**, *66*, 292–305.

Instituto Nacional de Estadística and Instituto de Nutrición (CSIC). In *Encuesta de presupuestos familiares, 1980–1981. Estudios sobre la nutrición*; INE, Artes Gráficas: Madrid, 1985; pp 155–109.

Kinsella, J. E. In *Seafoods and fish oils in human health and disease*; Dekker: New York, 1987; pp 239–300.

Kinsella, J. E.; Lokesh, B.; Stone, R. A. Dietary n–3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. *Am. J. Clin. Nutr.* **1990**, *52*, 1–28.

May, J.; Shimp, J.; Weihrauch, I.; Kinsella, J. E. Lipids of fish fillet: Changes following cooking by different methods. *J. Food Sci.* **1975**, *42*, 1669–1674.

Metcalfe, L. V.; Schmitz, A. A.; Pelka, J. R. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Anal. Chem.* **1966**, *38*, 514–515.

Moreiras-Varela, O.; Ruiz-Roso, B.; Varela, G. Effects of frying on the nutritive value of food. In *Frying of food. Principles, changes, new approaches*; Varela, G., Bender, A. E., Morton, I. D., Eds.; Ellis Horwood: Chichester, U.K., 1988; pp 93–102.

Nawar, W. W. Chemical changes in lipids produced by thermal processing. *J. Chem. Educ.* **1984**, *61*, 229–302.

Nawar, W.; Hultin, H.; Li, Y. J.; Xing, Y. H.; Kelleher, S.; Wilhem, C. Lipid oxidation in seafoods under conventional conditions. (Review). *Food Rev. Int.* **1990**, *6*, 647–660.

Paul, A. A.; Southgate, D. A. T. In *McCance and Widdowson's the composition of foods*, 4th revised ed.; Medical Research Council: London, 1979; p 17.

Ruiz-Roso, B. Influence of the Cooking–freezing and reheating (CFR) system in the nutritive quality of protein of some animal foods. Ph.D. Dissertation, Universidad Complutense of Madrid at Facultad de Farmacia, 1983.

Sánchez-Corcoles, E.; Ros, A.; Escolar, J. L.; Valdivielso, P.; García, C.; González-Santos, P. Fish consumption and cardiovascular risk factors with special reference to plasma lipids. *Nutr. Clin.* **1990**, *10*, 170–174.

Sánchez-Muniz, F. J. Dietary prevention and longevity. Importance of fish consumption. *Rev. Clin. Esp.* **1987**, *180*, 43–47.

Sánchez-Muniz, F. J.; Hérnandez, I.; Cuesta, C. Quality of the extracted oil from fried potatoes in olive oil. *Grasas Aceites* **1989**, *40*, 399–405.

Sánchez-Muniz, F. J.; Medina, R.; Higón, E.; Viejo, J. M. Efficiency and stability of olive oil, sunflower oil, and lard used in repeated frying of sardines. *Grasas Aceites* **1990**, *41*, 256–262.

Sánchez-Muniz, F. J.; Higón, E.; Cava, F.; Viejo, J. M. Acceptability of diets containing olive oil-fried sardines in the prevention of dietary hypercholesterolemia in rats. *J. Sci. Food Agric.* **1991**, *56*, 155–165.

Schouten, I. A.; Beynen, A. C. Fish oils, omega-3 fatty acids and serum lipids. In *Nutritional effects on cholesterol metabolism*; Beynen, A. C., Ed.; Transmondial, Voorthuizen, Den Haag, 1986; pp 67–79.

Sims, R. J.; Fioriti, J. A. High temperature reactions of fats with aminoacids. *J. Am. Oil Chem. Soc.* **1975**, *52*, 144–147.

UNE 55-011-73. *Determinación de la acidez libre*; Instituto Nacional de Racionalización del Trabajo (IRANOR): Madrid, 1973.

UNE 55-023-73 *Materias grasas. Indice de peróxidos*; Instituto de Racionalización y Normalización (IRANOR): Madrid, 1973.

Varela, G. Heated fats: contribution to the study of frying of foods. *Bibl. Nutr. Dieta* **1977**, *25*, 112–121.

Varela, G. Current facts about the frying of food. In *Frying of food. Principles, changes, new approaches*; Varela, G., Bender, A. E., Morton, I. D., Eds.; Ellis Horwood: Chichester, U.K., 1988; Chapter 1.

Varela, G.; Moreiras-Varela, O.; Ruiz-Roso, B. Utilization of some oils in repeated frying. Changes in fats and sensorial analysis of fried foods. *Grasas Aceites* **1983**, *34*, 101–107.

Varela, G.; Pérez, M.; Ruiz-Roso, B. Changes in the quantitative and culinary processing. *Bibl. Nutr. Dieta* **1990**, *46*, 104–109.

Zilversmit, D. B. Cholesterol index of foods. *J. Am. Diet. Assoc.* **1979**, *74*, 562–565.