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Cooking–freezing–reheating (CFR) of sardine (*Sardina pilchardus*) fillets. Effect of different cooking and reheating procedures on the proximate and fatty acid compositions

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Abstract

The sequential cooking–freezing–reheating (CFR) method was studied. Sardine (*Sardina pilchardus* Walb) fillets were cooked by frying, oven-baking or grilling, frozen, and then reheated using conventional or microwave ovens in order to study changes occurring in the proximate and fatty acid compositions. Both cooking and freezing–reheating affected ($P<0.001$) the proximate composition. Frying produced the highest water loss and fat gain, followed by grilling, and then by oven-baking. Microwave oven-reheating (MR) induced higher dehydration than conventional oven-reheating (OR), with grilled–frozen–MR samples also loosing fat and ash. Frying significantly ($P<0.001$) affects the fatty acid composition of sardine, increasing oleic and linoleic acids and decreasing eicosapentaenoic and docosahexaenoic acids. Oven-baking and grilling minimally affected the fatty acid content. Freezing–reheating significantly affected ($P<0.001$) the fatty acid composition with the content of oleic acid increasing and those of the ω-3 fatty acids, decreasing more in MR than in OR. Thus, according to the positive effect attributed to ω-3 fatty acids, cooked samples with no further treatment would be preferred to their respective CFR counterparts. However, OR should be used instead of MR when the CFR system is performed.

Keywords: Baking; Cooking–freezing–reheating (CFR); Fatty acid composition; Frying; Grilling; Microwave-oven; Proximate composition; Sardine

1. Introduction

Cooking–freezing–reheating (CFR) has become an alternative system of handling foods in catering where the prepared food is either frozen or chilled before the reheating procedure is carried out shortly prior to eating (Skjöldebrand, Ohlsson, O' Sullivan, & Turner, 1984). The production of the food will take place either in the food production premises or in specially designed production kitchens related to catering. Moreover, when it comes to findings ways to save time, CFR is used more at home nowadays (Creed, 2001).

Heat (boiling, baking, roasting, frying and grilling) is applied to food in different ways to improve its hygienic quality by inactivation of pathogenic microorganisms and to enhance its flavour and taste, and increase shelf life (Bognar, 1998; Pokorny, 1999). The reactions involved are often interrelated, depending on time/temperature treatment and water activity (Skjöldebrand, 1984). In most cooking procedures, when the surface temperature has reached 100 °C, the evaporation zone starts to move towards the centre of the product, and a crust starts to form which decreases the heat conductivity by acting as insulation (Quaglia & Bucarelli, 2001). On the other hand, the use of the microwave oven for defrosting or cooking has increased considerably during the past few decades (Sumnu, 2001). Microwave ovens change regular electricity into high-frequency microwaves that water, fat and sugar can

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absorb, causing food particle vibration, and thus the heating of the foodstuff.

According to the American Heart Association (Krauss et al., 2000) at least two servings of fish per week are recommended to confer cardioprotective effects. However, although the beneficial effect of fish has been mainly ascribed to its particular fatty acid composition (Mataix & Gil, 2002), some studies have shown that the fish protein also plays a role in this benefit (Vázquez & Sánchez-Muniz, 1994). Fish is rarely eaten raw but is usually cooked in different ways before consumption. During cooking, chemical and physical reactions take place that improve or impair the food nutritional value (e.g. digestibility is increased due to protein denaturation in food but the content of thermolabile compounds, fat-soluble vitamins or polyunsaturated fatty acids is often reduced) (Bognar, 1998; Finot, 1997). Cooking induces water loss in the food, that in turn increases its lipid content in most cases and only some fat is lost in the case of the oiliest fish. Moreover, this effect is also dependent on the type of cooking (Gall, Otwell, Koburger, & Appledorf, 1983; Ohta, Shinozaki, Sasaki, Ikuma, & Kamimura, 1988). The deleterious effects of frozen storage, followed by slow defrosting at 4 °C in the refrigerator, on the nutritional value of raw sardine have been reported by Castrillón, Álvarez-Pontes, García-Arias, and Navarro (1996). Candela, Astiasaran, and Bello (1997) have studied the effect of maintaining the food in a hot state on the fatty acid and cholesterol contents of different fish. Thus, it would be worthwhile to determine whether freezing, followed by reheating, modifies the changes observed during the cooking of fish. The present study aims to study the effect of CFR on proximate and fatty acid compositions of sardine fillets using three different ways of cooking (frying, oven-baking and grilling) and two reheating systems (conventional and microwave ovens).

2. Material and methods

2.1. Sample preparation

Ten kilograms of sardines (*Sardina pilchardus* Walb) from 21 to 23 cm long and from 84.7 to 105.1 g in weight were purchased at a local store in Madrid to where they had previously been transported in refrigerated trucks from the harbour of Vigo, Spain after captive. The lapse of time between catching and arrival at the laboratory was less than 30 h. Head, scales, viscera, backbone, skin and tail were removed, and two fillets were obtained from the resulting sardine-carcass. Fillets were subsequently randomly divided into 10 homogeneous groups of ~0.5 kg each. One group was kept fresh-raw and used as reference (R). The other nine were cooked, three were fried (F samples), three oven-baked (B samples) and the other three grilled (G samples). After cooking, one group from each cooking method was analysed (F1, B1, and G1 samples) while the other two of each cooking modality were frozen at −30 °C and stored at −20 °C for 4 months. After this period of time, frozen–cooked sardine fillets were reheated in a conventional oven (F2, B2, and G2 samples) or in a microwave oven (F3, B3, and G3 samples) and analysed.

2.2. Frying

Sardine fillets were fried for 4 min in olive oil (palmitic acid 10.5%, stearic acid 3.3%, oleic acid 77.9% and linoleic acid 6.6%, acidity value 1°, Koipe, Andujar, Jaén, Spain) at an initial temperature of 180 °C in domestic pans of 2 l-capacity. Oil was used once with the food/oil ratio being 250 g/l. Once fried, fillets were gently drained for about 2 min.

2.3. Oven-baking

Baking of sardine fillets was performed in a conventional oven (Balay, Zaragoza, Spain) at 200 °C for 22 min. The process finished when the inner fillet temperature, measured with a quartz electronic thermometer (Huger, Oregon Scientific Trade Mark, Germany), ranged from 60 to 70 °C.

2.4. Grilling

Grilling of fillets was performed for 3 min on a stainless steel grill (Balay, Zaragoza, Spain) with its thermostat set at 350 °C. To keep the fish from sticking, the grill was slightly greased, before cooking, with a spread of 5–10 ml of olive oil.

2.5. Conventional oven- and microwave oven-reheating

Reheating in a conventional oven (Balay, Zaragoza, Spain) was performed initially at 50 °C for 19 min, followed by an increase of 3 °C/min rate to 80 °C. Microwave reheating of fillets was carried out at a frequency of 2450 MHz, 500 W power for 6 min in a Goldstar ER 435 OE oven (Newport, UK).

2.6. Analytical techniques

Freeze-dried fillets from the different groups were used for analyses. Protein was determined by the Kjeldahl procedure (AOAC, 1993). The moisture was determined by oven-drying at 100 °C to constant weight (AOAC, 1993). Total fat was extracted with petroleum ether (BP 40–60 °C) using the Soxlab U-6 (Kilab, Sweden). Ash was gravimetrically determined using a muffle

furnace by heating at 500 °C to constant weight (AOAC, 1993).

Food-fat was extracted according to the Bligh and Dyer (1959) method, saponified with 0.5 N sodium hydroxide, and then methylated, following the method of Metcalfe, Schmitz, and Pelka (1966). The fatty acid methyl esters were analysed by gas chromatography, as described by Sánchez-Muniz, Viejo, and Medina (1992).

The C22:6/C16:0 ratio was employed as a thermal oxidation index of sardine fat (Álvarez-Pontes, Viejo, Sánchez-Muniz, & Castrillón, 1994; Beltran & Moral, 1990) in order to study the effect of freezing–reheating on the quality of fatty acid sardine fillets.

2.7. Statistical analysis

The data were analysed using the analysis of variance test (ANOVA). The cooking and freezing–reheating effects and their interactions were evaluated by two way ANOVA. The Bonferroni test was used to compare means when a significant variation was highlighted by the ANOVA one-way and Brown–Forsythe tests. The significance of results was established at the $P<0.05$ level. The software used was SPSS, release 11.0.1. for windows.

3. Results and discussion

3.1. Proximate composition

The compositions of the different kinds of raw, cooked, frozen and reheated samples are shown in Table 1. R samples presented a high fat content (~15%), showing that the sardine used in the current study belongs to the oiliest species of the genus *Sardina* (Moreiras-Varela, 1966). Moisture of the R samples was lower than the values usually present in other oily fish (Bognar, 1998). The water-fat content has been described in different fish and is highly dependent on the catch season (Varela, Pérez, & Ruíz-Roso, 1990). The protein content was also rather high and in agreement with that described by others (Waters, 1988; Moreiras, Carvajal, & Cabrera, 1998).

After frying (F1 samples), moisture content decreased significantly while fat increased. These results are in agreement with those of Castrillón, Navarro, and Álvarez-Pontes (1997), Sánchez-Muniz et al. (1992) and Gall et al. (1983). However, when data were expressed on a dry matter basis, frying decreased the fillet fat content. According to Cuesta and Sánchez-Muniz (2001) and Varela and Ruiz-Roso (1992), during frying, bath oil penetrates the food after water is partially lost by evaporation. However, this oil–water exchange is less in oily foods and exchange between the food-fat and the bath oil also occurs. Fat content data, expressed on a dry matter basis (Table 1) suggest that the balance of this exchange was negative, with more fat being released from the sardines than being adsorbed by them. This effect seems to be specific for the fat because protein and ash increased.

During oven-baking, sardine fillets (O1 samples) lost water and fat and gained protein and ash (Table 1). However, dehydration was lower than during frying. These changes were similar to those found by Mai, Shimp, Weihrauch, and Kinsella (1978) in trout, by Gall et al. (1983) in red grouper and by Mustafa and Medeiros (1985) in catfish. Dry matter data (Table 1) show that oven-baking implies losses of fat greater than 6g/100 g of edible portion. Similar results have been found by Gall et al. (1983) in mackerel and other oily fish, while in white fish, such as red grouper, a large increase of fat content took place. Grilling (G1 samples) produced higher water losses than oven-baking (O1 samples) but lower than frying (F1 samples). These modifications appear to be related to the rate of food temperature change (quicker in frying), and the process temperature (higher in grilling than in oven-baking). The fat content (on a dry matter basis) of G1 decreased similarly to that of O1 with respect to the R sample.

The behaviour of F samples was different after reheating them in conventional or microwave ovens. Thus, F2 samples showed minor changes in moisture content while it was highly significant in F3 samples

Table 1
Proximate composition (g/100 g wet matter and g/100 g dry matter) for raw, fried, oven-baked, and grilled sardine fillets

	Raw	Fried	Oven-baked	Grilled	ANOVA
Moisture g/100 g wet matter	60.68a±0.28	43.12b±0.81	55.74c±0.44	50.81d±0.93	***
Protein g/100 g wet matter	20.7a±0.62	32.3b±0.54	26.0c±0.59	30.0d±0.08	***
Fat g/100 g wet matter	15.44a±0.12	21.23b±0.13	14.60c±0.25	16.40d±0.17	***
Ash g/100 g wet matter	3.26a±0.08	5.39b±0.09	4.22c±0.24	4.31c±0.17	***
Protein g/100 g dry matter	52.6a±0.16	56.8b±0.95	58.7bc±1.34	59.5c±0.17	***
Fat g/100 g dry matter	39.25a±0.33	37.33b±0.22	32.99c±0.56	33.35c±0.34	***
Ash g/100 g dry matter	8.30a±0.17	9.48b±0.15	9.53b±0.53	8.76ab±0.35	**

Values are means±standard deviation of six samples. Values bearing different letters are significantly different (ANOVA one-way and Brown–Forsythe tests, *** $P<0.001$. ** $P<0.01$).

Table 2
Effect of cooking–freezing–reheating (CFR) on proximate composition of sardine fillets

	Non-frozen and non-reheated			CFR (frozen-reheated in conventional oven)			CFR (frozen-reheated in microwave oven)			ANOVA		
	F1	O1	G1	F2	O2	G2	F3	O3	G3	Cooking effect	Freezing–reheating effect	Interaction
Moisture g/100 g wet matter	43.12Aa ± 0.81	55.74Ba ± 0.44	50.81Ca ± 0.93	41.05Ab ± 0.15	57.20Bb ± 0.06	48.24Cb ± 0.07	28.20Ac ± 0.11	49.14Bc ± 0.26	47.64Cbc ± 0.14	***	***	***
Protein g/100 g wet matter	32.3Aa ± 0.54	26.0Ba ± 0.59	30.0Ca ± 0.08	33.2Aa ± 0.34	25.3Ba ± 0.11	28.9Cb ± 0.11	41.6Ab ± 0.38	30.6Bb ± 0.45	31.4Cc ± 0.12	***	***	***
Fat g/100 g wet matter	21.23Aa ± 0.13	14.60Ba ± 0.25	16.40Ca ± 0.17	20.43Ab ± 0.19	14.03Bb ± 0.02	18.66Cb ± 0.05	25.68Ac ± 0.06	16.59Bc ± 0.07	15.95Cc ± 0.17	***	***	***
Ash g/100 g wet matter	5.39Aa ± 0.09	4.22Ba ± 0.24	4.31Ba ± 0.17	5.34Aa ± 0.12	3.34Bb ± 0.08	4.14Ca ± 0.07	6.21Ab ± 0.11	3.95Ba ± 0.04	4.16Ca ± 0.04	***	***	***
Protein g/100 g dry matter	56.8Aa ± 0.95	58.7Aa ± 1.34	59.5Aa ± 0.17	56.4Aa ± 0.58	59.1Ba ± 0.25	55.9Ab ± 0.22	57.9Aa ± 0.53	60.1Ba ± 0.88	60.1Ba ± 0.23	***	***	***
Fat g/100 g dry matter	37.33Aa ± 0.22	32.99Ba ± 0.56	33.35Ba ± 0.34	34.65Ab ± 0.33	32.79Ba ± 0.05	36.06Cb ± 0.11	35.77Ac ± 0.08	32.62Ba ± 0.13	31.47Cc ± 0.33	***	***	***
Ash g/100 g dry matter	9.48Aa ± 0.15	9.53Aa ± 0.53	8.76Aa ± 0.35	9.05Aab ± 0.21	7.81Bb ± 0.19	8.00Bb ± 0.14	8.65Ab ± 0.16	7.77Bb ± 0.05	7.94Bb ± 0.07	***	***	***

Cooking processes studied were frying (F), oven-baking (O) and grilling (G). 1,2 and 3 correspond to non-frozen and non-reheated, conventional oven-reheated, and microwave oven-reheated samples, respectively. Values are means ± standard deviation of six samples. *** $P < 0.001$ ANOVA two-ways. Values bearing different capital letters mean significant differences between culinary processes for the same frozen-reheated group (ANOVA one-way and Brown–Forsythe tests). Values bearing different small letters mean significant differences between reheating methods for the same cooking group (ANOVA one-way and Brown–Forsythe tests).

(Table 2). Thus, microwave-reheating produced a marked dehydration in samples that had been previously fried. It can be hypothesized that water evaporation in fried samples would be very much increased later during microwave-reheating because of the porosity of the fried sample. According to Quaglia and Bucarelli (2001), during frying, fat interchanges with water by using the pores opened in the food during the water evaporation. Moreover, when a frozen food is reheated in a conventional oven, the iced surface thaws forming a water surface that reduces the conduction velocity of heat throughout the foodstuff (Fellows, 1988). This does not happen in microwave ovens where the thermal energy transference is 10–20 times higher than in a conventional oven (Sumnu, 2001). Although F3 fillets contain more fat than F2 fillets, calculation on a dry matter basis indicates that, during reheating, about 2% of fat was lost. The large increase in the contents of protein and ash of F3 samples with respect to F1 seems to be clearly related to the dehydration produced (Table 2).

Samples G2 and G3 contain less moisture and contain more fat than their O2 or O3 counterparts, an effect that can be related to the higher temperature of grilling. Microwave oven-reheating (O3 and G3) induced more dehydration than in conventional oven-reheating (O2 and G2). Calculations on a dry matter basis for the oven-baked samples (O2 and O3) indicate that losses are only of water because the fat content did not change. However, dehydration in G3 samples was also accompanied by fat and ash losses. Ash data for conventional- or microwave oven-reheating (calculated on a dry matter basis) suggest agreement with the finding of García-Arias, Álvarez-Pontes, García-Linares, García-Fernández, and Sánchez-Muniz (in press) that water loss is accompanied in these samples by ash losses caused by leaching (Table 2).

3.2. Changes in the fatty acid composition

Raw sardine fillets showed high contents of palmitic, oleic, eicosapentaenoic and docosahexaenoic acids (Table 3). These findings are in agreement with those obtained by others in oily fish (Gall et al., 1983; García Arias, 1989; Mai et al., 1978; Varela et al., 1990). The polyunsaturated fatty acid (PUFA) content almost doubled that of saturated fatty acids (SFA) while the -3 fatty acid content was four times higher than that of ω-6 fatty acids. These results emphasize the high quality of sardine fillet-fat from a cardiovascular point of view (Kinsella, Lokesh, & Stone 1990; Mataix & Gil, 2002; Sánchez-Muniz et al., 1992). R samples presented a 22:6/16:0 ratio of 0.86 (data not shown) that ranged between those obtained by Mai et al. (1978) in trout, Gall et al. (1983) in mackerel, and by Beltrán and Moral (1990) in sardines.

Table 3
Fatty acid compositions of raw, fried, oven-baked and grilled sardine fillets (g/100 g wet weight)

	Raw	Fried	Oven-baked	Grilled	ANOVA
C14:0	0.94a±0.01	0.49b±0.01	0.85c±0.01	0.89ca±0.04	***
C16:0	3.03a±0.03	3.01a±0.06	2.89b±0.01	3.18c±0.07	***
C16:1 n-7	1.05a±0.01	0.60b±0.07	1.04a±0.00	1.09a±0.04	***
C17:0	0.19a±0.01	0.10b±0.00	0.17a±0.00	0.16a±0.02	***
C18:0	0.61a±0.01	0.76b±0.02	0.65a±0.01	0.61a±0.02	***
C18:1 n-9	1.66a±0.00	10.84b±0.13	1.62a±0.00	1.72a±0.05	***
C18:1 n-7	0.22a±0.00	0.08b±0.03	0.23a±0.00	0.21a±0.02	***
C18:2 n-6	0.21a±0.00	0.89b±0.02	0.20a±0.00	0.22a±0.04	***
C20:0	0.24a±0.00	0.17b±0.01	0.24a±0.00	0.20b±0.02	***
C18:3 n-3	0.77a±0.00	0.48b±0.01	0.62c±0.00	0.91d±0.05	***
C20:1 n-9	0.50a±0.01	0.24b±0.01	0.49a±0.00	0.48a±0.03	***
C18:4 n-3	0.07a±0.01	0.05a±0.00	0.00b±0.00	0.09a±0.03	***
C22:1 n-9	1.12a±0.01	0.69b±0.01	0.91c±0.01	1.39d±0.09	***
C20:5 n-3	1.92a±0.01	1.01b±0.01	1.98c±0.00	1.99c±0.01	***
C22:5 n-3	0.25a±0.00	0.17b±0.02	0.25a±0.00	0.27a±0.01	***
C22:6 n-3	2.61a±0.01	1.70b±0.01	2.42c±0.01	2.84d±0.02	***

Values are means±standard deviation of six samples. Values bearing different letters are significantly different (ANOVA one-way and Brown–Forsythe tests, *** $P<0.001$).

Frying significantly changed the fatty acid composition of sardine fillets (Table 3). Some time ago our group (Sánchez-Muniz et al., 1992) proposed that the fatty acid change in a foodstuff during frying was a consequence of fatty acid gradients. Present results show that frying in olive oil with a modest content of linoleic acid markedly increases the content of oleic and linoleic acids that in turn dilutes the concentration of most of the other fatty acids. Moreover, as previously reported, in oily fish, an exchange of fat between the food and the frying oil takes place, thus increasing the losses of some specific fish fatty acids, such as eicosapentaenoic and docosahexaenoic acids (Sánchez-Muniz et al., 1992; Varela & Ruiz-Roso, 1992). Thus, SFA and PUFA content decreased while monounsaturated fatty acids (MUFA) and the ω-6/ω-3 fatty acid ratio increased (Table 4).

Oven-baking minimally affected the sardine fillet fatty acid content (Table 3). This effect must be primarily a consequence of the low fat loss produced by this process. However, the observed changes were not homogeneous for the different fatty acids because some fatty acids decreased, some increased and others did not change (Table 3). Oily fish accumulates fat mainly as triglycerides in red and white muscle (Polvi, 1989). Thus fatty acid changes have to be a consequence of the action of heat, mainly, on these muscle triglycerides, favouring the loss of the more accessible fatty acids.

To assist in the understanding of changes induced by the CFR system, the analytical data (g of major fatty acids/100 g total fatty acids) were compared with those of the cooked samples that had been frozen and reheated (Fig. 1). Samples reheated in a conventional oven (F2, O2, and G2) showed that CFR produced thermal oxidation, preferentially affecting eicosapentaenoic and docosahexaenoic acids and increasing the oleic and palmitic acids. However, microwave-reheating, as a consequence of the high dehydration produced, increased the content of oleic acid in F3 and O3 samples.

Table 4
Fatty acid groups in raw, fried, oven-baked and grilled sardine fillets (g/100 g wet weight)

	Raw	Fried	Oven-baked	Grilled	ANOVA
Total SFA	5.01a±0.02	4.53b±0.04	4.80c±0.01	5.04a±0.12	***
Total MUFA	4.55a±0.02	12.5b±0.10	4.29a±0.00	4.89c±0.04	***
Total PUFA n-6	0.21a±0.00	0.89b±0.02	0.20a±0.00	0.22a±0.04	***
Total PUFA n-3	5.62a±0.02	3.41b±0.03	5.27c±0.01	6.10d±0.16	***
Total PUFA	5.82a±0.03	4.30b±0.02	5.47c±0.01	6.30d±0.06	***
SFA/PUFA	0.86a±0.01	1.05b±0.01	0.88a±0.01	0.80c±0.01	***
ω-6/ω-3	0.04a±0.00	0.26b±0.01	0.04a±0.00	0.04a±0.00	***

Values are means±standard deviation of six samples. Values bearing different letters are significantly different (*** $P<0.001$, ANOVA one-way and Brown–Forsythe tests).

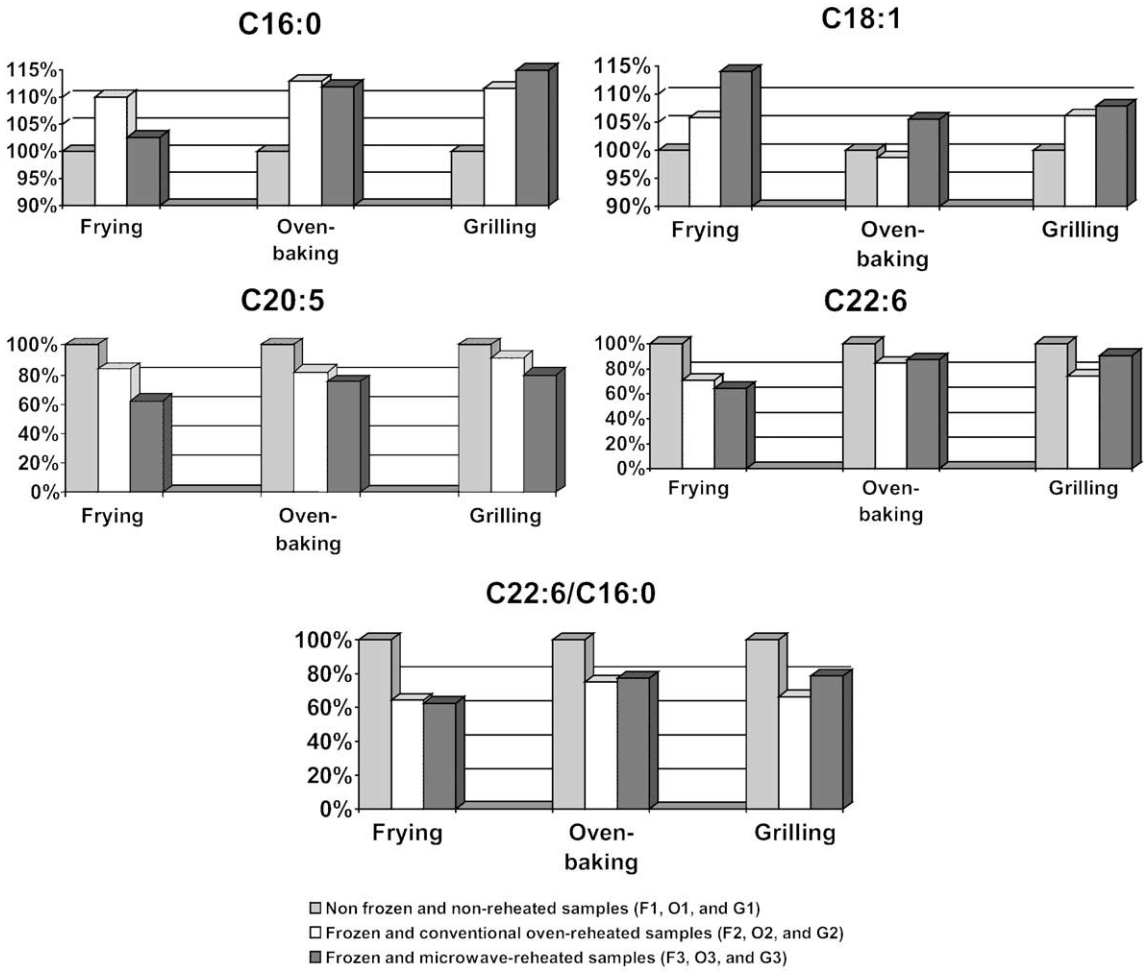

Fig. 1. Changes in the contents of major fatty acids (g fatty acids/100 g total fatty acids) and in the ratio of docosahexaenoic acid to palmitic acid in sardine fillets prepared by CFR. Values for the non frozen–non reheated samples of each culinary modality (frying: F, oven-baking: O, and grilling: G) were used as reference values (100%).

As already noted, grilling (G1) induced lower fat losses than oven-baking (O1). However, the shorter time and higher temperature of grilling with respect to oven-baking would explain the differences in the fatty acid contents found after the two cooking processes. Nonetheless, these changes can be considered small and irrelevant (Table 2). Grilled samples frozen and reheated (G2, and G3 samples) showed important modifications in the fatty acid compositions (Tables 5 and 6). Thus, all PUFA, except linoleic acid, decreased after microwave oven-reheating. Oleic acid increased and ω-3 fatty acids decreased more in microwave oven-reheating than in conventional oven-reheating. All these data suggest that, although the heating time is lower in the microwave oven, the induced molecular disruption would be higher than in the conventional oven. García-Arias et al. (2002) found that thawing frozen non-cooked sardines with a microwave oven induced more fatty acid modifications than thawing with a conventional oven.

The 22:6/16:0 ratio decreased in F2, F3, G2, and G3 samples with respect to F1, O1, and G1 samples, suggesting that thermal oxidation is produced in both reheating treatments. Moreover, according to the positive health effect attributed to docosahexaenoic acid (Mataix & Gil, 2002), R, F1, O1 and G1 samples would be preferred to their respective CFR counterparts.

Nevertheless, the results for total PUFA or total ω-3 fatty acids differ from those found for docosahexaenoic acid because G3 sardines contain more C22:6 and less PUFA than the G2 samples (Tables 5 and 6). At present we have no hypothesis explaining such differences but sn-location in triglyceride or phospholipid molecules (Polvi, 1989) has to be involved in the thermal damage of the different PUFA.

Future research is necessary to study which lipidic molecules, present in fish and in other fatty foodstuffs, are most affected by these three culinary processes performed in the CFR system

Table 5
Fatty acid contents of fried (F), oven-baked (O) and grilled (G) sardine fillets. Effect of freezing, followed by reheating in conventional oven (2) or freezing, followed by reheating in microwave oven (3) (g/100 g wet weight)

	Non-frozen and non-reheated			CFR (frozen and reheated in conventional oven)			CFR (frozen and reheated in microwave oven)			ANOVA		
	F1	O1	G1	F2	O2	G2	F3	O3	G3	Cooking effect	Freezing–reheating effect	Interaction
C14:0	0.49Aa±0.01	0.85Ba±0.01	0.89Ba±0.04	0.48Aa±0.01	0.89Ba±0.02	1.20Cb±0.02	0.49Aa±0.01	1.23Bb±0.01	1.03Cc±0.03	***	***	***
C16:0	3.01Aa±0.06	2.89Aa±0.01	3.18Ba±0.07	3.19Ab±0.01	3.13Ab±0.04	4.04Bb±0.10	3.74Ac±0.00	4.12Bc±0.04	3.55Cc±0.06	***	***	***
C16:1 n-7	0.60Aa±0.07	1.04Ba±0.00	1.09Ba±0.04	0.60Aa±0.00	1.14Bb±0.01	1.34Cb±0.02	0.68Aa±0.00	1.40Bc±0.01	1.16Ca±0.01	***	***	***
C17:0	0.10Aa±0.00	0.17Ba±0.00	0.16Ba±0.02	0.05Ab±0.00	0.14Ba±0.00	0.18Ca±0.02	0.05Ab±0.00	0.19Ba±0.03	0.14Ba±0.03	***	**	***
C18:0	0.76Aa±0.02	0.65Ba±0.01	0.61Ba±0.02	0.71Ab±0.01	0.66Ba±0.01	0.79Cb±0.01	0.88Ac±0.00	0.78Bb±0.01	0.64Ca±0.02	***	***	***
C18:1 n-9	10.8Aa±0.13	1.62Ba±0.00	1.72Ba±0.05	11.0Aa±0.04	1.54Bb±0.01	2.08Cb±0.04	15.0Ab±0.01	2.18Bc±0.01	1.81Ca±0.01	***	***	***
C18:1 n-7	0.08Aa±0.03	0.23Ba±0.00	0.21Ba±0.02	0.00Ab±0.00	0.20Ba±0.04	0.23Ba±0.03	0.00Ab±0.00	0.20Ba±0.01	0.14Cb±0.01	***	***	***
C18:2 n-6	0.89Aa±0.02	0.20Ba±0.00	0.22Ba±0.04	0.81Ab±0.01	0.22Ba±0.01	0.27Ca±0.01	1.13Ac±0.01	0.30Bb±0.01	0.25Ca±0.02	***	***	***
C20:0	0.17Aa±0.01	0.24Ba±0.00	0.20Aa±0.02	0.13Ab±0.00	0.16Ab±0.02	0.24Ba±0.01	0.16Ab±0.00	0.27Bc±0.01	0.23Ca±0.01	***	***	***
C18:3 n-3	0.48Aa±0.01	0.62Ba±0.00	0.91Ca±0.05	0.45Aa±0.00	0.66Bb±0.01	1.10Cb±0.03	0.48Aa±0.00	0.96Bc±0.01	0.79Cc±0.01	***	***	***
C20:1 n-9	0.24Aa±0.01	0.49Ba±0.00	0.48Bab±0.03	0.21Ab±0.01	0.41Bb±0.03	0.52Ca±0.01	0.20Ab±0.00	0.52Ba±0.00	0.42Cb±0.00	***	***	***
C18:4 n-3	0.05Aa±0.00	0.00Ba±0.00	0.09Aa±0.03	0.05Aa±0.00	0.05Ab±0.01	0.08Ba±0.00	0.05Aa±0.00	0.08Bc±0.00	0.06Ca±0.00	***	***	***
C22:1n-9	0.69Aa±0.01	0.91Ba±0.01	1.39Ca±0.09	0.58Ab±0.03	0.99Bb±0.01	1.61Cb±0.05	0.60Ab±0.00	1.37Bc±0.02	1.27Cc±0.02	***	***	***
C20:5 n-3	1.01Aa±0.01	1.98Ba±0.00	1.99Ba±0.01	0.81Ab±0.07	1.55Bb±0.01	2.07Ca±0.10	0.75Ab±0.00	1.88Bc±0.0	1.55Cc±0.03	***	***	***
C22:5 n-3	0.17Aa±0.02	0.25Ba±0.00	0.27Ba±0.01	0.09Ab±0.00	0.17Bb±0.00	0.22Cb±0.01	0.10Ab±0.00	0.22Bc±0.00	0.19Cb±0.01	***	***	***
C22:6 n-3	1.70Aa±0.01	2.42Ba±0.01	2.84Ca±0.02	1.16Ab±0.00	1.96Bb±0.02	2.40Cb±0.07	1.33Ac±0.00	2.68Bc±0.03	2.50Cc±0.04	***	***	***

1, 2, and 3 correspond to non-frozen and non-reheated, conventional oven-reheated, and microwave oven-reheated samples, respectively. Values are means±standard deviation of six samples. *** $P<0.001$ ANOVA two-ways. Values bearing different capital letters mean significant differences between culinary processes for the same frozen-reheated group (ANOVA one-way and Brown–Forsythe tests). Values bearing different small letters mean significant differences between reheating methods for the same cooking group (ANOVA one-way and Brown–Forsythe tests).

Table 6
Effect of cooking–freezing–reheating (CFR) on the contents of fatty acid groups (g/100 g of wet weight)

	Non-frozen and non-reheated			CFR (frozen and reheated in conventional oven)			CFR (frozen and reheated in microwave oven)			ANOVA		
	F1	O1	G1	F2	O2	G2	F3	O3	G3	Cooking effect	Freezing-reheating effect	Interaction
Total SFA	4.53Aa±0.04	4.80Ba±0.01	5.04Ca±0.12	4.56Aa±0.02	4.98Bb±0.04	6.45Cb±0.08	5.32Ab±0.01	6.59Bc±0.02	5.59Ac±0.03	***	***	***
Total MUFA	12.5Aa±0.10	4.29Ba±0.00	4.89Ca±0.04	12.4Aa±0.19	4.28Ba±0.05	5.78Cb±0.06	16.4Ab±0.01	5.67Bb±0.02	4.80Ca±0.02	***	***	***
Total PUFA n-6	0.89Aa±0.02	0.20Ba±0.00	0.22Ba±0.04	0.81Ab±0.01	0.22Ba±0.01	0.27Ca±0.01	1.13Ac±0.01	0.30Bb±0.01	0.25Ba±0.02	***	***	***
Total PUFA n-3	3.41Aa±0.03	5.27Ba±0.01	6.10Ca±0.16	2.56Ab±0.07	4.39Bb±0.02	5.87Ca±0.10	2.71Ab±0.01	5.82Bc±0.07	5.09Cb±0.09	***	***	***
Total PUFA	4.30Aa±0.02	5.47Ba±0.01	6.30Ca±0.06	3.37Ab±0.06	4.60Bb±0.03	6.13Ca±0.17	3.84Ac±0.01	6.11Bc±0.03	5.33Cb±0.29	***	***	***
SFA/PUFA	1.05Aa±0.01	0.88Ba±0.01	0.80Ca±0.01	1.35Ab±0.02	1.08Bb±0.01	1.05Bb±0.01	1.39Ab±0.01	1.08Bb±0.01	1.05Bb±0.02	***	***	***
ω-6/ω-3	0.26Aa±0.01	0.04Ba±0.00	0.04Ba±0.00	0.32Ab±0.01	0.05Bb±0.00	0.05Ba±0.00	0.42Ac±0.01	0.05Bb±0.00	0.05Ba±0.00	***	***	***

Cooking processes studied were frying (F), oven-baking (O) and grilling (G). 1,2 and 3 correspond to non-frozen and non-reheated, conventional oven-reheated, and microwave oven-reheated samples, respectively. Values are means±standard deviation of six samples. *** $P<0.001$ ANOVA two-ways. Values bearing different capital letters mean significant differences between culinary processes for the same frozen-reheated group (ANOVA one-way and Brown–Forsythe tests). Values bearing different small letters mean significant differences between reheating methods for the same cooking group (ANOVA one-way and Brown–Forsythe tests).

References

AOAC. (1993). Methods of analysis for nutrition labelling. In D. M. Sullivan, & D. E. Carpenter. Virginia, USA: AOAC.

Álvarez-Pontes, M. E., Viejo, J. M., Sánchez-Muniz, F. J., & Castrillón, A.Mª. (1994). Fritura de filetes de sardinas congeladas en aceite de oliva. Influencia de diferentes métodos de descongelación sobre el contenido graso y la composición en ácidos grasos. *Grasas y Aceites*, *45*, 119–125.

Beltrán, A., & Moral, A. (1990). Gas chromatographic estimation of oxidative deterioration in sardines during frozen storage. *Lebensmittel Wissenchaft und Technologie*, *23*, 499–504.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*, 911–917.

Bognàr, A. (1998). Comparative study of frying to other cooking techniques. *Influence on the nutritive value*. *Grasas y Aceites*, *49*, 250–260.

Candela, M., Astiasarán, I., & Bello, J. (1997). Effects of frying and warmholding on fatty acids and cholesterol of sole (*Solea solea*), codfish (*Gadus morhua*) and hake (*Merluccius merluccius*). *Food Chemistry*, *58*, 227–231.

Castrillón, A. M., Álvarez-Pontes, E., García-Arias, M. T., & Navarro, P. (1996). Influence of frozen storage and defrosting on the chemical and nutritional quality of sardine (*Clupea pilchardus*). *Journal of Science of Food & Agriculture*, *70*, 29–34.

Castrillón, A. M., Navarro, P., & Álvarez-Pontes, E. (1997). Changes in chemical composition and nutritional quality of fried sardine (*Clupea pilchardus*) produced by frozen storage and microwave reheating. *Journal of Science of Food & Agriculture*, *75*, 125–132.

Creed, P. G. (2001). The potential of foodservice systems for satisfying consumer needs. *Innovative Food Science & Emerging Technologies*, *2*, 219–227.

Cuesta, C., & Sanchez-Muniz, F. J. (2001). La fritura de los alimentos. Fritura en aceite de oliva y oliva virgen extra. In J. Mataix (Ed.), *Aceite de oliva virgen extra. Nuestro patrimonio alimentario Vol. 1* (pp. 173–209). Granada: Universidad de Granada y Puleva Food.

Fellows, P. (1988). *Food processing technology: principles and practice*. Chichester (England): Ellis Horwood Ltd.

Finot, P. A. (1997). Effect of processing and storage on the nutritional value of food protein. In S. Damodaran, & A. Paraf (Eds.), *Food protein and their applications* (pp. 551–576). New York: Marcel Dekker.

Gall, K. L., Otwell, W. S., Koburger, J. A., & Appledorf, H. (1983). Effects of four cooking methods on the proximate, mineral and fatty acid composition of fish fillets. *Journal of Food Science*, *48*, 1068–1074.

García-Arias, M. T. (1989). *Influencia del modo de cocción y tiempo de esterilización y almacenamiento en el valor nutritivo de las conservas de atún blanco*. PhD dissertation, Universidad Complutense de Madrid at Facultad de Farmacia.

Garcia-Arias, M. T., Álvarez-Pontes, M. E., García-Linares, M. C., García-Fernández, M. C., & Sánchez-Muniz, F. J. Effects of grilling on the protein composition of fresh, frozen and refrigerator or microwave defrosted sardine fillet (*Clupea pilchardus*). *Lebensmittel-Wissenschaft und Technologie* (in press).

Kinsella, J. E., Lokesh, B., & Stone, R. A. (1990). Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. *American Journal of Clinical Nutrition*, *52*, 1–28.

Krauss, R. M., Eckel, H. R., Howard, B., Appel, L. J., Daniels, S. R., Deckelbaum, R. J., Erdman, J. W., Kris-Etherton, P., Goldberg, I. J., Kotchen, T. A., Lichtenstein, A. H., Mitch, W. E., Mullis, R., Robinson, K., Wylie-Rosett, J., Jeor, S. S., Suttie, J., Tribble, D. L., & Bazzarre, T. L. (2000). AHA dietary guidelines. Revision 2000: a statement for healthcare professionals from the Nutrition Committee of the American Heart Association. *Circulation*, *102*, 2284–2299.

Mai, J., Shimp, J., Weihrauch, & Kinsella, J. E. (1978). Lipids of fish fillets: changes following cooking by different methods. *Journal of Food Science*, *43*, 1669–1674.

Mataix, J., Gil, A. (Eds.). (2002). *Libro blanco de los omega-3*. Granada: Editorial Puleva Food.

Metcalfe, L. V., Schmitz, A. A., & Pelka, J. R. (1966). Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Analytical Chemistry*, *38*, 514–515.

Moreiras, O., Carvajal, A., Cabrera, M. L. (Eds.). (1998). *Tablas de composición de los alimentos*. Madrid: Ediciones Pirámide.

Moreiras-Varela, O. (1966). Variaciones estacionales en la composición cuali-cuantitativa de la porción comestible y de los residuos de algunos pescados de consumo en España. *Anales de Bromatología*, *18*, 161–234.

Mustafa, F. A., & Medeiros, D. M. (1985). Proximate composition, mineral content and fatty acid of catfish (*Ictalurus punetatus rafinesque*) in different seasons and cooking methods. *Journal of Food Science*, *50*, 585–587.

Ohta, S., Shinozaki, R., Sasaki, K., Ikuma, T., & Kamimura, S. (1988). Changes in fatty acid composition during the cooking of coho salmon. *Yukagaku*, *37*, 663–667.

Pokorný, J. (1999). Changes of nutrients at frying temperatures. In D. Boskou, & I. Elmadfa (Eds.), *Frying of food. Oxidation, nutrient and non-nutrient antioxidants, biologically active compounds and high temperatures* (pp. 69–103). Lancaster: Technomic Publishing CO. Inc.

Polvi, S. M. (1989). *Diet and availability of omega-3 fatty acids in salmonids*. MSc thesis, Technical University of Nova Scotia, Halifax.

Quaglia, G. B., & Bucarelli, F. M. (2001). Effective process control in frying. In J. B. Rossell (Ed.), *Frying. Improving quality* (pp. 236–265). Boca Raton: CRC Press.

Sánchez-Muniz, F. J., Viejo, J. M., & Medina, R. (1992). Deep frying of sardines in different culinary fats. Changes in the fatty acid composition of sardines and frying fats. *Journal of Agricultural and Food Chemistry*, *40*, 2252–2256.

Skjöldebrand, C., Ohlsson, Th., O' Sullivan, K., & Turner, M. (1984). Reheating of food in catering. In G. Varela (Ed.), *Thermal processing and quality of foods* (pp. 425–431). New York, USA: Elsevier Applied Science Publishers Ltd.

Skjöldebrand, C. (1984). Introduction to process group A (Frying, Grilling, Boiling). In G. Varela (Ed.), *Thermal processing and quality of foods* (pp. 313–317). New York, USA: Elsevier Applied Science Publishers Ltd.

SPSS for windows, release 11.0.1. (1989–2001). SPSS Inc.

Sumnu, G. (2001). A review on microwave baking of foods. *International Journal of Food Science and Technology*, *6*, 117–127.

Varela, G., & Ruiz-Roso, B. (1992). Some effects of deep frying on the dietary fat intake. *Nutrition Reviews*, *50*, 256–262.

Varela, G., Pérez, M., & Ruiz-Roso, B. (1990). Changes in the quantitative and qualitative composition of fat from fish, due to seasonality and industrial and culinary processing. *Bibliotheca Nutritio and Dieta, Karger, Basel*, *46*, 104–109.

Vázquez, J., & Sánchez-Muniz, F. J. (1994). Proteína de pescado y metabolismo de colesterol. *Revista Española de Ciencia y Tecnología de los Alimentos*, *34*, 589–608.

Waters, M. E. (1988). Chemical composition of frozen storage of weakfish (Cynoscium regalis). *Marine Fish Reviews*, *50*, 27–33.