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Effects of frying and warmholding on fatty acids and cholesterol of sole (*Solea solea*), codfish (*Gadus morrhua*) and hake (*Merluccius merluccius*)

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Fatty acids and cholesterol contents of various fish dishes made from sole (*Solea solea*), hake (*Merluccius merluccius*) and codfish (*Gadus morrhua*) were analysed to determine the effect of deep-fat frying and 'warmholding' (internal temperature 65°C, 3 h). The results showed an increase in the ratio *cis*-polyunsaturated/saturated fatty acids (PUFAs/SFAs) due to the absorption of the oil used in the culinary process. Furthermore, an increase in the ratio ω-6/ω-3 PUFAs was observed, giving rise to a negative effect on the benefits related to the intake of eicosapentaenoic and docosahexaenoic acids. Cooking did not impart a significant variation in the cholesterol supply. Warmholding showed some differences in the content of fatty acids as compared to immediately cooked fish. The ratio ω-6/ω-3 PUFAs slightly increased with warmholding for codfish and sole, and decreased for hake. Cholesterol content was slightly affected by warmholding. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Epidemiological studies carried out in the 1970s on the incidence of cardiovascular disease in the Eskimo population suggested a beneficial role of ω-3 polyunsaturated fatty acids (PUFAs) in the prevention of cardiovascular risk (Dyerberg & Bamg, 1979; Kromhout *et al.*, 1985). Other data support the notion that ω-3 PUFAs have beneficial effects in hypertension, inflammation, arrhythmias, psoriasis, other autoimmune disorders and cancer. As a result of these investigations the importance of the ω-6/ω-3 balance has been established. Thus, the ω-6 fatty acid content of the diet needs to be reduced and the ω-3 fatty acid content increased (Simopoulos, 1994). An increase in the consumption of fish is therefore recommended.

Studies of nutrient intake in relation to health are frequently carried out with data obtained from raw food, without taking into account that cooking and other processes can give rise to major changes in composition. Nevertheless, the effects of different cooking methods on the fatty acids of different fish have been studied, in particular with frying (Mai *et al.*, 1978; Gall *et al.*, 1983; Ågren & Hänninen, 1993; Toth-Markus & Sass-Kiss, 1993; Sánchez-Muñiz *et al.*, 1992; Ohgaki *et al.*, 1994).

In addition, food is sometimes subject to processes such as 'warmholding' and 'coldholding', which are used in the catering industry. Maintaining food for some hours above 65°C ('warmholding') will maintain its microbiological quality but will accelerate the deterioration of heat-labile nutrients and may have marked effects on sensory quality (Glew *et al.*, 1987). Although there is no evidence of changes to the lipid fraction during warmholding, time and temperature conditions may affect it, leading, at least partially, to changes in the sensory quality. Thus, it would be interesting to determine whether or not the stability of this food fraction changes with the applied combination of temperature/time.

The present research was therefore undertaken to study the effects of deep-frying (one of the most important single operations in the catering and food-processing

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industries) on the fatty acid and cholesterol contents of three marine fish and the stability of these components during warmholding.

MATERIALS AND METHODS

Materials

Three different species of fish—sole (*Solea solea*), codfish (*Gadus morrhua*) and hake (*Merluccius merluccius*)—were studied. The fish were purchased at the market. They had been recently caught and stored on chopped ice. Fish samples (six helpings of each fish) were cooked by a catering firm following the usual process. They were rolled in wheat flour and egg and introduced into an industrial deep-fryer for 5 min with cooking oil (sunflower oil) at 180°C. After draining, a quantity corresponding to three helpings was homogenized and immediately analysed. The remaining samples were placed in a thermos container used by the company for distribution. The internal temperature of the food was 65°C. After 3 h, the samples were homogenized and analysed. Raw samples were analysed in the same way. The study was carried out in four batches (four different days) for each dish.

The sunflower oil used for frying (0.2° acid value) was also analysed.

Analytical procedures

The method of Folch *et al.* (1957) was used for the extraction of lipids. Fatty acid composition was determined by gas chromatograhy. Boron trifluoride/methanol was used for the preparation of fatty acid methyl esters (AOAC, 1990). A Perkin-Elmer Autosystem gas chromatograph fitted with a capillary column SP-2380 (60 m×0.25 mm) of fused silica (Supelco, Bellefonte, PA) and flame ionization detection was used. The temperature of both the injection port and the detector was 250°C. The oven temperature was programmed to increase from 170°C to 200°C at a rate of 2°C min^{-1}. The carrier gas was hydrogen, 13 psi. The sample size was 0.5 μl. The quantification of individual fatty acids was based on nonadecanoic acid methyl ester (Sigma, St. Louis, MO, USA) as internal standard. Sunflower oil was analysed (by gas chromatography) under the same conditions.

The cholesterol content was calculated by GC with previous extraction according to Kovacs *et al.* (1979). A Perkin–Elmer Sigma 300 gas chromatograph equipped with an SP-2250 column (2 m×6 mm×2 mm) packed with Supelcoport (Supelco) was used. The oven temperature was 260°C. The temperature of both the injection port and detector was 285°C. The sample size was 0.5 μl. Cholesterol was identified by com- paring its relative and absolute retention times with those of cholestane (Sigma) as an internal standard. A Perkin-Elmer Turbochrom programme was used for quantification.

Statistical analysis

Data analysis was carried out with a one-way ANOVA and a Tukey's posteriori test (STATGRAPHICS 4.0). Differences were studied at the $P \leqslant 0.01$ level.

RESULTS AND DISCUSSION

Tables 1–3 show the levels of fatty acids and cholesterol in raw, cooked and warmheld samples for each of the three fishes analysed. Data were referred to dry matter to avoid the differences due to the possible moisture loss that can take place during the processes. Several studies

Table 1. Fatty acid and cholesterol contents of codfish

Fatty acid	Fatty acid content (g per 100 g)		
	Raw codfish	Fried codfish	Warmheld codfish
Myristic 14:0	—	—	—
Palmitic 16:0	0.91 ± 0.00^a	2.15 ± 0.01^b	2.10 ± 0.01^b
Stearic 18:0	0.24 ± 0.00^a	1.10 ± 0.00^b	1.12 ± 0.00^c
Palmitoleic *cis*-16:1 ω-7	0.07 ± 0.00^a	0.13 ± 0.00^b	0.13 ± 0.00^b
Palmitelaidic *trans*-16:1 ω-7	—	0.04 ± 0.00^b	0.05 ± 0.00^c
Oleic *cis*-18:1 ω-9	1.07 ± 0.00^a	4.92 ± 0.01^b	5.04 ± 0.01^c
Erucic *cis*-22:1 ω-9	0.03 ± 0.00	—	—
Brassidic *trans*-22:1 ω-9	—	0.03 ± 0.00^b	0.04 ± 0.00^b
Linoleic *cis*-18:2 ω-6	0.53 ± 0.00^a	11.20 ± 0.04^b	11.53 ± 0.03^c
Linolelaidic *trans*-18:2 ω-6	—	0.04 ± 0.00^a	0.05 ± 0.00^a
Eicosapentaenoic *cis*-20:5 ω-3	0.24 ± 0.00^a	0.26 ± 0.00^b	0.19 ± 0.00^c
Docosahexaenoic *cis*-22:6 ω-3	0.95 ± 0.00^a	0.76 ± 0.00^b	0.73 ± 0.01^b
Cholesterol (mg per 100 g)	$365.04^a \pm 2.75$	$312.76^b \pm 10.22$	$276.85^b \pm 4.48$

All values are referred to dry matter (mean ± standard deviation). Values in the same row bearing different letters are significantly different ($P \leqslant 0.01$).

have established that the concentrations of fatty acids are influenced by the type of fish, their size and lipid content. The analysed fishes were low-fat species. The results show that cooking affects every fatty acid in a similar way in all three fish types.

Frying with a coating causes an extensive absorption of fat (Ohgaki *et al.*, 1994). Thus the fat composition of fried fish tends to be similar to culinary fat (Sánchez-Muñiz *et al.*, 1992). The fatty acid profile of the sunflower oil that was used for frying (Fig. 1) shows that it was especially rich in oleic and linoleic acids, and also contained significant concentrations of palmitic acid at lower levels and traces of brassidic acid. In fact, there were higher levels of many of the fatty acids in the cooked fish than in the raw samples, but the most significant increases were of palmitic, oleic and linoleic acids.

Trans fatty acids were not uniformly affected by cooking. These unsaturated fatty acids do not have the same nutritional value as *cis*-unsaturated fatty acids, having no beneficial effects in the prevention of disease. Raw sole showed the highest level of brassidic acid (22:1 ω-9), which increased with cooking. This *trans* fatty acid is the only one present in sunflower oil.

Eicosapentaenoic (EPA, 20:5 ω-3) and docosahexaenoic (DHA, 22:6 ω-3) fatty acids are typical of fish fat and are not present in vegetable oils. Hake showed the highest level of these ω-3 fatty acids. Their content is

Table 2. Fatty acid and cholesterol content of hake

Fatty acid	Fatty acid content (g per 100 g)		
	Raw	Fried	Warmheld
Myristic 14:0	0.49 ± 0.00^{a}	0.44 ± 0.02^{a}	0.68 ± 0.01^{b}
Palmitic 16:0	2.82 ± 0.03^{a}	3.60 ± 0.1^{b}	4.32 ± 0.06^{c}
Stearic 18:0	0.38 ± 0.00^{a}	1.09 ± 0.01^{b}	1.09 ± 0.00^{b}
Arachidic 20:0	0.01 ± 0.00^{a}	0.05 ± 0.00^{b}	0.05 ± 0.00^{b}
Palmitoleic *cis*-16:1 ω-7	0.71 ± 0.01^{a}	0.59 ± 0.02^{b}	1.02 ± 0.01^{c}
Palmitelaidic *trans*-16:1 ω-7	0.05 ± 0.00^{a}	0.03 ± 0.00^{b}	0.06 ± 0.00^{c}
Oleic *cis*-18:1 ω-9	2.10 ± 0.01^{a}	5.47 ± 0.07^{b}	5.96 ± 0.04^{c}
Elaidic *trans*-18:1 ω-9	0.02 ± 0.00^{a}	—	0.03 ± 0.00^{b}
Erucic *cis*-22:1 ω-9	0.12 ± 0.00^{ab}	0.11 ± 0.00^{a}	0.13 ± 0.00^{b}
Brassidic *trans*-22:1 ω-9	0.10 ± 0.00^{a}	0.07 ± 0.00^{b}	0.22 ± 0.00^{c}
Linoleic *cis*-18:2 ω-6	0.68 ± 0.00^{a}	11.37 ± 0.15^{b}	11.12 ± 0.07^{b}
Linolelaidic *trans*-18:2 ω-6	—	0.06 ± 0.00^{a}	0.06 ± 0.00^{a}
Linolenic *cis*-18:3 ω-3	0.17 ± 0.00^{a}	0.15 ± 0.00^{a}	0.28 ± 0.02^{b}
Eicosapentaenoic *cis*-20:5 ω-3	1.56 ± 0.00^{a}	1.32 ± 0.00^{b}	1.94 ± 0.00^{c}
Docosahexaenoic *cis*-22:6 ω-3	2.23 ± 0.03^{a}	1.81 ± 0.03^{b}	2.28 ± 0.03^{a}
Cholesterol (mg per 100 g)	$294.92^{a} \pm 9.79$	$231.05^{b} \pm 1.01$	$196.61^{b} \pm 3.28$

All values are referred to dry matter (mean ± standard deviations). Values in the same row bearing different letters are significantly different ($P \leq 0.01$).

Table 3. Fatty acid and cholesterol content of sole

	Fatty acid content (g per 100 g)		
	Raw	Fried	Warmheld
Myristic 14:0	0.42 ± 0.01^{a}	1.11 ± 0.01^{b}	1.54 ± 0.02^{c}
Palmitic 16:0	1.03 ± 0.01^{a}	3.62 ± 0.02^{b}	4.20 ± 0.5^{c}
Stearic 18:0	0.16 ± 0.00^{a}	1.05 ± 0.00^{b}	1.14 ± 0.01^{c}
Palmitoleic *cis*-16:1 ω-7	0.96 ± 0.01^{a}	2.89 ± 0.05^{b}	3.44 ± 0.05^{c}
Palmitelaidic *trans*-16:1 ω-7	0.02 ± 0.00^{a}	0.07 ± 0.00^{b}	0.08 ± 0.00^{c}
Oleic *cis*-18:1 ω-9	0.84 ± 0.01^{a}	6.97 ± 0.03^{b}	7.94 ± 0.06^{c}
Elaidic *trans*-18:1 ω-9	0.03 ± 0.00^{a}	0.06 ± 0.00^{b}	0.08 ± 0.00^{b}
Erucic *cis*-22:1 ω-9	0.16 ± 0.00^{a}	0.55 ± 0.00^{b}	0.74 ± 0.02^{c}
Brassidic *trans*-22:1 ω-9	0.93 ± 0.02^{a}	3.02 ± 0.01^{b}	4.24 ± 0.07^{c}
Linoleic *cis*-18:2 ω-6	0.2 ± 0.01^{a}	10.19 ± 0.04^{b}	11.55 ± 0.11^{c}
Linolelaidic *trans*-18:2 ω-6	—	0.05 ± 0.00^{a}	0.14 ± 0.00^{b}
Linolenic *cis*-18:3 ω-3	0.08 ± 0.00^{a}	0.27 ± 0.00^{b}	0.39 ± 0.00^{c}
Eicosapentaenoic *cis*-20:5 ω-3	0.73 ± 0.00^{a}	1.69 ± 0.00^{b}	1.35 ± 0.00^{c}
Docosahexaenoic *cis*-22:6 ω-3	0.59 ± 0.01^{a}	0.95 ± 0.00^{b}	0.73 ± 0.1^{c}
Cholesterol (mg per 100 g)	$267.13^{a} \pm 5.70$	$206.92^{b} \pm 1.10$	$211.40^{b} \pm 5.56$

All values are referred to dry matter (mean ± standard deviation). Values in the same row bearing different letters are significantly different ($P \leq 0.01$).

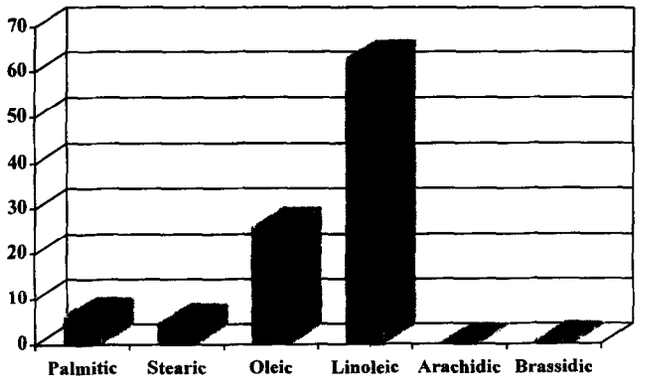

Fig. 1. Relative percentages of fatty acids in sunflower oil (g per 100 g fat).

negatively affected by cooking in all cases except for sole. These compositional changes gave rise to modifications in the contribution of the different lipid fractions in fried fish (referred to the raw fish). This modification could be important in relation to some aspects of health.

Tables 4–6 show the intake of different lipid fractions for 100 g of an edible portion. Frying increased the contribution of saturated, monounsaturated and *cis*-polyunsaturated fractions. It seems that stearic acid (18:0) does not behave like a saturated fatty acid with regard to effects on cholesterol levels (Kritchevsky *et al.*, 1982; Bonanone *et al.*, 1992; Tholstrup *et al.*, 1994), so it should be excluded from the saturated fraction (Zapelena *et al.*, 1995). The ratio *cis*-PUFAs/(SFAs–stearic acid) increased with cooking 1.84 times in sole, 2.66 times in hake and 3.01 times in codfish. Sánchez-Muñiz *et al.* (1992) found that the PFAs/SFAs ratio increased 4.14 times for sardines fried in sunflower oil. Gall *et al.* (1983) found that PUFAs/SFAs increased 1.56 times for Spanish mackerel fried in soybean oil. Although the increase of the ratio PUFAs/(SFAs–stearic acid) should be beneficial, the change of the ratio ω-6/ω-3 PUFAs which takes place at the same time does not seem to be adequate. Several studies with animals have shown that a high dietary fat intake, especially of ω-6 fatty acids, is related to an increased incidence of breast, prostate and colon cancer (Carroll & Khor, 1971; Reddy, 1986), whereas diets high in ω-3 fatty acids (EPA and DHA) have beneficial effects against several types of malignant tumors (Cave, 1991). Epidemiological studies have also shown that ω-3 fatty acid intake is inversely related to cancer prevalence (Kaizer *et al.*, 1989; Willett *et al.*, 1990; Dolecek & Grandits, 1991). Because of this, polyunsaturated fatty acids should be considered separately as ω-6 and ω-3 fatty acids.

The effect on EPA and DHA contents depends on the method of frying. Ohgaki *et al.* (1994) observed that suage (frying without coating) caused a decrease of both of these ω-3 PUFAs; however, tempura frying and frying with coating had little or no effect on the quantities of fatty acids. They found that the ω-6/ω-3 PUFA ratio increased from 0.11 in raw fish to 0.76, 1.14 and 1.40 after tempura, suage and frying with coating, respectively. Ågren & Hänninen (1993) established that vegetable oils rich in ω-6 PUFAs should be avoided in pan and deep-fat frying if an increase of ω-3 PUFA intake at the expense of ω-6 PUFAs is desired. In our study, ω-6/ω-3 ratios increased with cooking from 0.13 to 3.49 in sole, 0.16 to 3.48 in hake and 0.44 to 11.43 in codfish.

A significant decrease in the cholesterol content was found after frying in all cases when the results were referred to dry matter (Tables 1–3). Other authors have

Table 4. Lipid fractions in 100 g of edible portion of codfish

	Raw	Fried	Warmheld
ΣSFA (g)	0.24	0.92	0.95
ΣSFA–18:0 (g)	0.19	0.61	0.62
ΣMUFA (g)	0.24	1.44	1.52
Σ*cis*-PUFA (g)	0.36	3.48	3.66
Σ*cis*-PUFA/(ΣSFA–18:0)	1.89	5.70	5.90
ω-6/ω-3	0.44	11.43	12.73
Cholesterol (mg)	76.51[a]	92.34[b]	82.11[a]

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Values in the same row bearing different letters are significantly different ($P \leqslant 0.01$).

Table 5. Lipid fractions in 100 g of edible portion of hake

	Raw	Fried	Warmheld
ΣSFA (g)	0.90	1.77	2.13
ΣSFA–18:0 (g)	0.81	1.39	1.75
ΣMUFA (g)	0.72	2.14	2.47
Σ*cis*-PUFA (g)	1.12	5.11	5.44
Σ*cis*-PUFA/(ΣSFA–18:0)	1.38	3.67	3.10
ω-6/ω-3	0.16	3.48	2.47
Cholesterol (mg)	72.64[a,b]	80.74[a]	68.71[b]

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Values in the same row bearing different letters are significantly different ($P \leqslant 0.01$).

Table 6. Lipid fractions in 100 g of edible portion of sole

	Raw	Fried	Warmheld
ΣSFA (g)	0.42	2	2.31
ΣSFA–18:0 (g)	0.28	1.64	1.93
ΣMUFA (g)	0.51	3.60	4.08
Σ*cis*-PUFA (g)	0.42	4.54	4.69
Σ*cis*-PUFA/(ΣSFA–18:0)	1.50	2.76	2.03
ω-6/ω-3	0.13	3.49	4.72
Cholesterol (mg)	71.94[a]	71.83[a]	70.92[a]

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Values in the same row bearing different letters are significantly different ($P \leqslant 0.01$).

shown similar effects. Sánchez-Muñiz *et al.* (1992) showed that sardine cholesterol content significantly decreased after frying, mainly due to absorption of culinary fat. They also found that this change was more marked when sardines were fried in sunflower oil than in other oils. Mai *et al.* (1978) found a significant decrease in the cholesterol content of all species following cooking. However, in our study when the results are referred to an edible portion (Tables 4–6), no significant differences were observed in sole and hake after cooking.

Warmholding dishes show some differences in fatty acid content compared to immediately cooked fish (Tables 1–3). Oleic acid increased significantly in all fishes whereas linoleic and stearic acids only increased in sole and codfish. Some differences can also be observed in the ω-3 PUFA concentrations. The ω-6/ω-3 ratio increased slightly for codfish and sole and decreased for hake (Tables 4–6). However, the differences produced by warmholding were not as relevant as those produced by cooking. In relation to the cholesterol content, no significant effects were observed during warmholding for sole. For codfish and hake there were statistical differences but they do not imply significant variations in their supply.

In conclusion, deep-fat frying of fish leads not only to an increase in the total amount of fat (an aspect that was not analysed in this work) but also to an increase in the ω-6/ω-3 PUFA ratio, limiting the positive effects of the high ω-3 PUFA level of raw fish. This technology hardly affects the cholesterol level. Warmholding (65°C, 3 h) gives rise to some changes in the lipid fraction. Thus, it should be of interest to analyse the effect of different time/temperature combinations.

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