Food Chemistry **42** (1991) 99–109

Changes in Fatty Acid Composition of Fresh and Frozen Sardine (*Sardina pilchardus* W.) During Smoking

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(Received 20 September 1989; revised version received and accepted 1 November 1990)

ABSTRACT

Changes in fatty acid composition in both fresh and frozen sardine during mixed smoking (2 h at 30°C and 45 min at 75°C) were studied. After extraction, the lipids from unsmoked and smoked sardine were separated into the three main fractions (triglycerides, phospholipids, and free fatty acids) by thin-layer chromatography; the fatty acids were then analyzed using gas chromatography. Oxidation during smoking brought about losses in the proportion of the long-chain n-$3C_{20}$ *and* C_{22} *fatty acids, as determined by increases in the peroxide value and in the 2-thiobarbituric acid index. On the other hand, the above polyunsaturated long-chain fatty acids in some conditions increased in frozen sardine, because of extraction-related effects caused by weakening of the protein–lipid linkages.*

INTRODUCTION

The large catches of sardine are underutilized in Spain at the present time. Smoking is, therefore, seen as a potentially simple and attractive means of augmenting consumption (Beltrán, 1988).

The lipid content is usually high in this species, although the chemical composition is subject to seasonal variations. The lipid fraction contains a high proportion of long-chain polyunsaturated fatty acids (PUFA) of the family *n*-3 (Jiménez-Colmenero, 1979). Such PUFA's are extremely important both technologically, given their marked vulnerability to oxidation, and nutritionally, in view of their role in the prevention and management of cardiovascular disease (Driss & Darcet, 1988).

Food Chemistry 0308-8146/91/$03·50

While some work has dealt with the effect of thermal processing on the fatty acid composition of fish (Tsubouchi *et al.*, 1985; Bhuiyan *et al.*, 1986), no data referring to sardine were found in the literature.

The object of the present study, thus, was to examine variations in the fatty acid composition of the total lipids, triglycerides (TG), phospholipids (PL), and free fatty acids (FFA) in smoked fresh and frozen sardine.

MATERIALS AND METHODS

Preparation and smoking of samples

The sardines (*Sardina pilchardus* W.) used in this study were caught in the Mediterranean Sea off Castellón, Spain, in the month of June. The fish were boxed in ice and transported to the Instituto del Frío for processing. One lot of fish was frozen in a plate freezer at −40°C and stored for 3 months at −18°C before smoking. Another lot was smoked fresh within 32 h of capture. Processing consisted of filleting the fish; the fillets were then brined (16% NaCl) for 3 min and afterwards air-dried in a forced-ventilation cold store at 0°C for 24 h. Smoking took place in an AFOS Torry-type kiln for 2 h at 30°C, followed by 45 min at 75°C.

Methods of analysis

All measurements were taken as the means of two replications on each of three separate sample groups. Samples from within each group were homogenized before measurement.

The lipid content in the sardine was determined according to AOAC methods (AOAC, 1975). The peroxide value (POV) as per UNE standard (UNE, 1973) and the 2-thiobarbituric acid (TBA) index according to the method of Lemon (1975) were used as measures of rancidity. The method recommended by AOCS (1955) was employed in the free fatty acid (FFA) determinations.

Lipid extraction was in accordance with the method of Bligh and Dyer (1959). The lipid fractions (triglycerides, phospholipids, and free fatty acids) were separated by thin-layer chromatography (TLC) using BHT as described by Acebal (1975). The fatty acids of the total lipids and the lipid fractions were then methylated as per Morrison and Smith (1964), with addition of an internal standard (nonadecanoic acid). The methyl esters of the fatty acids so obtained were analyzed by gas chromatography in a Perkin-Elmer 3920 gas chromatograph equipped with a flame ionization detector. A 25-m long, 0·25 mm i.d. WCOT fused silica (Supelcowax 10)

capillary column was employed. Working conditions were: injector and detector temperatures 250°C; initial oven temperature 185°C for 32 min, with a programmed increase to 200°C at a rate of 2°C/min, remaining at 200°C until completion of elution; carrier gas helium, at a flow rate of 2 ml/min. Chromatographic peaks of fatty acid esters were identified by a comparison of their retention times with those of available standards as well as by log-plots of retention times against the number of carbon atoms in the chain. Quantitative determination of the lipid fractions (triglycerides, phospholipids, and free fatty acids) was performed using the method of Christie *et al.* (1970).

Analysis of variance was carried out using BMDP PV programs on a CDC CYBER 180/185 computer.

RESULTS AND DISCUSSION

The lipid content in the sardine used in this study was 10·9%; hence it was termed 'fatty sardine' to distinguish it from sardine caught in the month of March, the lipid content of which was considerably lower (Beltrán, 1988).

Table 1 lists the 42 fatty acids identified in the chromatograms (Fig. 1). The sixteen most important acids, quantitatively, were subsequently used in the statistical analyses. The percentage composition of the fatty acids in the total

TABLE 1
List of Fatty Acids in Fig. 1[a]

1—14:0	16—16:4 (*n*-3)	31—20:3 (*n*-6)
2—14:1 (*n*-9)	17—18:0	32—20:4 (*n*-6)
3—Iso 15:0	18—18:1 (*n*-9)	33—20:3 (*n*-3)
4—Ante-iso 15:0	19—18:1 (*n*-7)	34—20:4 (*n*-3)
5—15:0	20—18:1 (*n*-5)	35—20:5 (*n*-3)
6—Iso 16:0	21—18:2 (*n*-6)	36—22:0
7—16:0	22—18:3 (*n*-6)	37—22:1 (*n*-11)
8—16:1 (*n*-7)	23—19:1 (*n*-8)	38—22:1 (*n*-9)
9—16:1 (*n*-5)	24—18:3 (*n*-3)	39—21:5 (*n*-3)
10—Iso 17:0	25—18:4 (*n*-3)	40—22:5 (*n*-6)
11—Ante-iso 17:0	26—18:4 (*n*-1)	41—22:5 (*n*-3)
12—16:2 (*n*-4)	27—20:0	42—22:6 (*n*-3)
13—17:0	28—20:1 (*n*-9)	D—Solvent
14—17:1 (*n*-8)	29—20:1 (*n*-7)	NI—Unidentified
15—Iso 18:0	30—20:3 (*n*-9)	PI—Internal standard (19:0)

[a] In this fatty acid notation system, the first number indicates chain length; the number after the colon indicates the number of methylene-interrupted double bonds; and the number following the letter *n* indicates the number of carbon atoms beyond the terminal double bond.

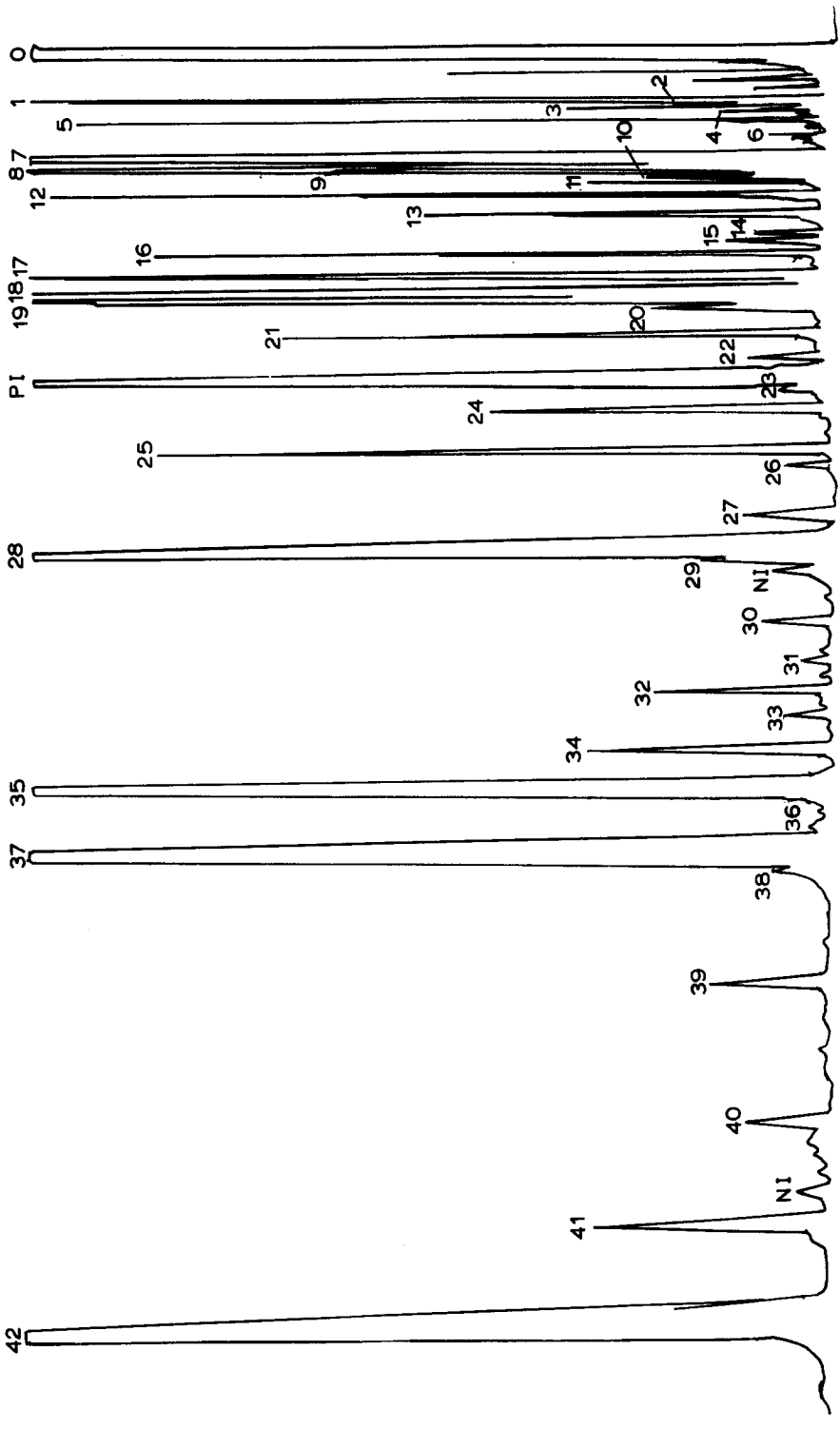

Fig. 1. Chromatogram of the fatty acid methyl esters of the total lipids from sardine, including the internal standard (19:0)(peak identifications listed in Table 1).

TABLE 2
Fatty Acid Composition (mean % weight) of the Total Lipids in Unsmoked and Smoked Sardine (fresh and frozen)[a]

Fatty acid[b]	*Fresh sardine*		*Sardine frozen 3 months*	
	Unsmoked	*Smoked*	*Unsmoked*	*Smoked*
14:0	8·83*a*	8·50*a*	8·56*a*	8.49*a*
16:0	18·34*a*	19·43*b*	18·83*a*	22·11*c*
16:1 (*n*-7)	12·05*a*	12·70*a*	8·37*b*	8·22*b*
16:2 (*n*-4)	1·11*a*	0·92*a*	0·97*a*	0·89*a*
16:4 (*n*-3)	0·51*a*	0·59*a*	0·65*a*	0·70*a*
18:0	4·65*a*	4·85*a*	4·27*a*	6·52*b*
18:1 (*n*-9)	6·32*a*	6·35*a*	5·92*a*	5·89*a*
18:1 (*n*-7)	2·43*a*	2·49*a*	2·52*a*	2·58*a*
18:3 (*n*-3)	0·73*a*	0·70*a*	0·76*a*	0·99*b*
18:4 (*n*-3)	2·08*a*	1·62*b*	2·22*a*	2·02*a*
20:1 (*n*-9)	2·65*a*	2·66*a*	3·33*b*	3·38*b*
20:4 (*n*-3)	0·82*a*	0·61*b*	0·79*a*	0·58*b*
20:5 (*n*-3)	9·44*a*	8·72*b*	12·37*c*	11·40*d*
22:1 (*n*-11)	3·11*a*	5·94*b*	5·04*c*	6·87*d*
22:5 (*n*-3)	1·74*a*	1·24*b*	1·30*b*	1·22*b*
22:6 (*n*-3)	11·30*a*	10·49*b*	16·24*c*	15·22*d*

[a] Different letters in the same row indicate significant differences at $p < 0.05$.
[b] In this fatty acid notation system, the first number indicates chain length; the number after the colon indicates the number of methylene-interrupted double bonds; and the number following the letter *n* indicates the number of carbon atoms beyond the terminal double bond.

lipids in fresh and frozen sardine is set out in Table 2. The fatty acid composition in the fresh sardine used in this study was similar to that reported by other workers for sardine with similar lipid content levels (Herzberg, 1987). The *n*-3 fatty acids C_{20} and $C_{22:6}$ in the frozen sardine were significantly higher than in the fresh sardine, a result of extraction-associated effects due to weakening of the protein–lipid linkages (Jiménez-Colmenero, 1979).

Smoked fresh and frozen sardine showed three distinct trends with regard to alterations in fatty acid composition (Table 2): first, the percentage share of most of the fatty acids studied remained constant; secondly, the percentage concentration of another group of fatty acids, e.g. $C_{16:0}$ and $C_{22:1}$ (*n*-11), rose after smoking, in both fresh and frozen sardine; lastly, smoking brought about a decrease in the percentage share of such *n*-3 polyunsaturated fatty acids as $C_{20:4}$, $C_{20:5}$, and $C_{22:6}$. The losses in PUFA percentage composition were recorded for both fresh and frozen sardine.

These results agreed with the fatty acid composition in smoked herring reported by Meizies and Reichwald (1973), who observed an increase in acid $C_{22:1}$ (*n*-11) and decreases in the acids $C_{20:5}$ (*n*-3) and $C_{22:6}$ (*n*-3) after

TABLE 3
Effect of Smoking on Lipid Oxidation and Hydrolysis in Fresh and Frozen Sardine Fillets[a]

	POV (*meq/1000 g*)		*TBA* (*μmoles MA/100 g*)		*FFA* (*% oleic acid*)	
	Fresh	*Frozen*	*Fresh*	*Frozen*	*Fresh*	*Frozen*
Unsmoked	$0{\cdot}77_1$	$4{\cdot}81_1$	$0{\cdot}35_1$	$2{\cdot}17_1$	$0{\cdot}76_1$	$4{\cdot}45_1$
Smoked	$2{\cdot}73_2$	$4{\cdot}92_1$	$1{\cdot}29_2$	$4{\cdot}94_2$	$2{\cdot}43_2$	$5{\cdot}92_2$

[a] Different subscripts in the same column indicate significant differences at $p < 0{\cdot}05$.

smoking. Other investigators have also reported decreases in the percentage composition of polyunsaturated fatty acids associated with smoking (Ostyakova & Skupova, 1983). Conversely, certain authors recorded practically no change in the fatty acid composition of fish after smoking (Bhuiyan *et al.*, 1986).

Fatty acid losses (chiefly $C_{20:5}$ and $C_{22:6}$) have often been related to lipid autoxidation (Bhuiyan *et al.*, 1986). These fatty acids are highly susceptible to oxidative phenomena, because the oxidation rate increases nearly geometrically with the number of double bonds (Enser, 1974). This is demonstrated quite clearly in the present instance by the POV and TBA determination values given in Table 3.

Smoked fresh sardine exhibited a substantial increase in the POV, due to the autoxidation of certain unsaturated acids, mainly $C_{18:4}$, $C_{20:4}$, $C_{20:5}$, $C_{22:5}$ and $C_{22:6}$ (Table 2). The higher TBA values in the smoked fresh sardine as compared to the unsmoked fresh sardine, representing the detection of oxidation breakdown products (chiefly malonaldehyde), are also indicative of oxidation.

The POV and TBA values were higher in the frozen raw material than in the fresh sardine (Table 3), and thus while smoking intensified peroxide formation, it also contributed to peroxide breakdown, which may explain why smoking had no effect on the POV in the frozen sardine (4·81 meq/100 g and 4·92 meq/100 g before and after smoking, respectively). The high TBA index values recorded for the smoked frozen samples (2·17 μmoles MA/100 g in the unsmoked frozen sardine and 4·94 μmoles MA/100 g in the case of the smoked frozen sardine), reflecting the higher levels of breakdown products (mainly malonaldehyde) detected in the medium due to the degradation of precursors (i.e. peroxides), bear this out.

Table 3 shows the effect of smoking on lipid hydrolysis. In the case of iced and frozen stored fish the various factors involved in smoking resulted in a higher content of free fatty acids. In this respect, heating the fillets at 30°C for 2 h seemed to accelerate the hydrolysis reactions and activate the enzyme

TABLE 4
Effect of Smoking on the Percentage Composition of Triglycerides, Phospholipids, and Free Fatty Acids in Fresh and Frozen Sardine Fillets[a]

	% *TG*		% *PL*		% *FFA*	
	Fresh	*Frozen*	*Fresh*	*Frozen*	*Fresh*	*Frozen*
Unsmoked	$94{\cdot}11_1$	$92{\cdot}17_1$	$5{\cdot}43_1$	$3{\cdot}97_1$	$0{\cdot}46_1$	$3{\cdot}86_1$
Smoked	$93{\cdot}75_2$	$92{\cdot}49_1$	$4{\cdot}38_2$	$3{\cdot}20_2$	$1{\cdot}87_2$	$4{\cdot}31_2$

[a] Different subscripts in the same column indicate significant differences at $p < 0{\cdot}05$.

systems. Increased fatty acid production in smoked fish has been recorded in a number of papers (Shevchenko & Lapshin, 1975; Hanumanthappa & Chandrasekhar, 1987).

It therefore follows that frozen storage of the raw material (for 3 months) might also be expected to give rise to losses in the proportion of polyunsaturated acids like $C_{20:5}$ (*n*-3) and $C_{22:6}$ (*n*-3), through the oxidation

TABLE 5
Fatty Acid Composition (mean % weight) of the Triglyceride Fraction in Unsmoked and Smoked Sardine (fresh and frozen)[a]

Fatty acid[b]	*Fresh sardine*		*Sardine frozen 3 months*	
	Unsmoked	*Smoked*	*Unsmoked*	*Smoked*
14:0	8·51*a*	8·77*a*	8·70*a*	8·60*a*
16:0	18·27*a*	19·36*b*	18·08*a*	20·17*c*
16:1 (*n*-7)	12·22*a*	9·78*b*	8·32*c*	7·46*d*
16:2 (*n*-4)	1·39*a*	1·50*a*	0·89*b*	0·81*b*
16:4 (*n*-3)	0·50*a*	0·66*a*	0·60*a*	0·55*a*
18:0	4·09*a*	4·85*b*	3·91*a*	5·62*c*
18:1 (*n*-9)	6·33*a*	5·91*a*	5·16*b*	5·06*b*
18:1 (*n*-7)	2·60*a*	2·38*a*	2·56*a*	2·40*a*
18:3 (*n*-3)	0·90*a*	0·93*a*	0·71*b*	0·99*a*
18:4 (*n*-3)	2·31*a*	2·22*a*	2·10*a*	2·00*a*
20:1 (*n*-9)	2·87*a*	3·12*a*	5·34*b*	4·92*b*
20:4 (*n*-3)	0·55*a*	0·61*a*	0·50*a*	0·36*b*
20:5 (*n*-3)	9·35*a*	9·54*a*	10·95*b*	10·02*a*
22:1 (*n*-11)	3·21*a*	5·94*b*	5·01*c*	5·67*d*
22:5 (*n*-3)	1·26*a*	1·37*a*	1·21*a*	1·32*a*
22:6 (*n*-3)	10·64*a*	9·87*b*	12·36*c*	11·49*d*

[a] Different letters in the same row indicate significant difference at $p < 0{\cdot}05$.
[b] In this fatty acid notation system, the first number indicates chain length; the number after the colon indicates the number of methylene-interrupted double bonds; and the number following the letter *n* indicates the number of carbon atoms beyond the terminal double bond.

TABLE 6
Fatty Acid Composition (mean % weight) of the Phospholipid Fraction in Unsmoked and Smoked Sardine (fresh and frozen)[a]

Fatty acid[b]	*Fresh sardine*		*Sardine frozen 3 months*	
	Unsmoked	*Smoked*	*Unsmoked*	*Smoked*
14:0	1·53*a*	1·87*b*	1·63*a*	2·21*c*
16:0	21·68*a*	25·30*b*	27·40*c*	27·29*c*
16:1 (*n*-7)	2·20*a*	1·99*a*	2·25*a*	3·20*b*
16:2 (*n*-4)	0·39*a*	0·40*a*	0·67*b*	0·70*b*
16:4 (*n*-3)	0·36*a*	0·28*a*	0·34*a*	0·29*a*
18:0	4·30*a*	4·92*b*	3·90*a*	4·82*b*
18:1 (*n*-9)	2·39*a*	2·50*a*	2·60*a*	2·48*a*
18:1 (*n*-7)	1·77*a*	2·72*b*	1·82*a*	2·66*b*
18:3 (*n*-3)	0·27*a*	0·28*a*	0·32*a*	0·36*a*
18:4 (*n*-3)	0·53*a*	0·61*a*	0·48*a*	0·57*a*
20:1 (*n*-9)	0·80*a*	1·54*b*	1·48*b*	1·89*c*
20:4 (*n*-3)	0·22*a*	0·42*b*	0·22*a*	0·55*c*
20:5 (*n*-3)	9·25*a*	8·33*b*	8·13*b*	7·39*c*
22:1 (*n*-11)	1·18*a*	2·23*b*	2·02*b*	2·75*c*
22:2 (*n*-3)	1·30*a*	1·04*b*	0·94*b*	0·90*b*
22:6 (*n*-3)	42·76*a*	37·85*b*	34·21*c*	30·20*d*

[a] Different letters in the same row indicate significant differences at $p < 0{\cdot}05$.
[b] In this fatty acid notation system, the first number indicates chain length; the number after the colon indicates the number of methylene-interrupted double bonds; and the number following the letter *n* indicates the number of carbon atoms beyond the terminal double bond.

attested to by the POV and the TBA index (Table 3). However, Table 2 shows that the percentage concentration of these two acids rose after 3 months at −18°C. Such seemingly anomalous behaviour has been described by other workers reporting on the alterations taking place in the fatty acid composition of frozen fish (Markina *et al.*, 1977; Jiménez-Colmenero, 1979). The increases in these fatty acids were explained by these researchers by virtue of their increased susceptibility to extraction by solvents as a result of the weakening of protein–lipid linkages during frozen storage.

On fractionation of the sardine lipids, the sum of TG's, PL's, and FFA's accounted for 99% of the total lipids, and, consequently, for calculation purposes, these three fractions combined were regarded as representing 100% (Table 4).

The oxidation and hydrolysis phenomena mentioned above led to a rise in the proportion of FFA's after smoking, accompanied by decreases in the proportion of TG's and, in particular, of PL's (Table 4). Analogous behaviour during smoking has been reported for similar species

TABLE 7
Fatty Acid Composition (mean % weight) of the Free Fatty Acid Fraction in Unsmoked and Smoked Sardine (fresh and frozen)[a]

Fatty acid[b]	*Fresh sardine*		*Sardine frozen 3 months*	
	Unsmoked	*Smoked*	*Unsmoked*	*Smoked*
14:0	6·45*a*	7·18*b*	6·98*b*	6·35*a*
16:0	15·39*a*	16·64*b*	17·86*c*	17·71*c*
16:1 (*n*-7)	8·01*a*	8·53*a*	8·47*a*	6·63*b*
16:2 (*n*-4)	1·92*a*	1·87*a*	1·99*a*	1·82*a*
16:4 (*n*-3)	0·70*a*	0·87*a*	0·70*a*	0·75*a*
18:0	4·96*a*	5·61*b*	6·03*b*	5·98*b*
18:1 (*n*-9)	6·57*a*	4·59*b*	6·17*a*	5·21*c*
18:1 (*n*-7)	2·56*a*	2·57*a*	2·60*a*	2·54*a*
18:3 (*n*-3)	1·01*a*	0·63*b*	0·92*a*	1·07*a*
18:4 (*n*-3)	0·85*a*	0·55*b*	0·79*a*	0·35*c*
20:1 (*n*-9)	2·53*a*	4·25*b*	2·62*a*	3·75*c*
20:4 (*n*-3)	0·53*a*	0·56*a*	0·54*a*	0·61*a*
20:5 (*n*-3)	10·64*a*	9·44*b*	11·09*a*	9·88*b*
22:1 (*n*-11)	3·09*a*	5·61*b*	3·94*c*	5·41*b*
22:5 (*n*-3)	1·62*a*	1·31*b*	1·60*a*	1·38*b*
22:6 (*n*-3)	13·45*a*	12·08*b*	16·91*c*	23·64*d*

[a] Different letters in the same row indicate significant difference at $p < 0.05$.
[b] In this fatty acid notation system, the first number indicates chain length; the number after the colon indicates the number of methylene-interrupted double bonds; and the number following the letter *n* indicates the number of carbon atoms beyond the terminal double bond.

(Schevchenko & Lapshin, 1975; Tsubouchi *et al.*, 1985). The decrease in the PL fraction was most pronounced, as pointed out by Markina and Lapshin (1981). However, given that the TG fraction, as is frequently the case in species of this kind, made up over 90% of the total lipids, slight decreases in the percentage proportion of this fraction represent substantial contributions to FFA formation.

Changes in the fatty acid composition of the TG fraction with smoking (Table 5) were similar to those recorded for the total lipids (Table 2). Again, this was to be expected, since the TG's were the major fraction.

Marked changes were recorded in the percentage composition of the fatty acids in the PL fraction with smoking (Table 6), perhaps because of this fraction's greater vulnerability to rancidity (Markina & Lapshin, 1981). Table 7 shows higher variations for the fatty acids in the FFA fraction than for the fatty acids in the TG fraction, possibly because the free forms are more susceptible to oxidation than the esterified forms (Jiménez-Colmenero, 1979). Acid $C_{22:6}$ (*n*-3) in the FFA fraction underwent an increase in the

smoked frozen sardine, again indicative of extraction-related effects in the frozen samples. This could have been a consequence both of naturally occurring alterations in the protein–lipid linkages in the fish and of linkages between proteins and the free fatty acids forming due to lipid hydrolysis during storage. Smoking did not appear to have any effect on alterations in the protein–lipid linkages in the present experiment, because the manifestation of such effects was similar to that in the raw material; however, no similarity between the mechanisms involved could be established.

Thus, the alterations occurring in the fatty acid composition of sardine during smoking are dependent upon the balance between autoxidation, lipolysis, and protein–lipid linkages. Decreases in PUFA levels are regulated by the oxidative phenomena taking place through exposure of the fish to atmospheric oxygen and to temperatures attained during smoking, as attested to by the POV's and TBA index values. The weakening of protein–lipid linkages would seem to be the determining factor causing the increase recorded for the acids $C_{20:5}$ (*n*-3) and $C_{22:6}$ (*n*-3) in frozen sardine.

REFERENCES

Acebal, C. (1975). Metabolismo y composición de glicéridos y ácidos grasos en tejidos adultos y fetales de conejo. PhD Thesis, Universidad Complutense de Madrid, Facultad de Ciencias, Sección Químicas.

AOAC (1975). *Official Methods of Analysis.* (12th edn), *Anal. Chem.* Washington, DC.

AOCS (1955). *Official and Tentative Methods of the American Oil Chemists' Society.* (2nd edn), ed. Mehlenbacher, U.C. Free Fatty Acids. Ca. 5a–40.

Beltrán, A. (1988). Caracteristicas y conservación por el frío de sardina (*Sardina pilchardus* W.) ahumada: influencia de la estacionalidad y del tratamiento frigorífico de la materia prima. Estudio de los ácidos grasos. PhD Thesis, Universidad Complutense de Madrid, Facultad de Veterinaria.

Bhuiyan, A. K. M. A., Ratnayake, W. M. N. & Ackman, R. G. (1986). Stability of lipids and polyunsaturated fatty acids during smoking of Atlantic mackerel (*Scomber scombris* L.) *J. Am. Oil Chem. Soc.*, **63**, 324.

Bligh, E. G. & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Can. J. Bioch. Phys.*, **37**, 911.

Christie, W. W., Noble, R. C. & Moore, J. H. (1970). Determination of lipid classes by a gas-chromatographic procedure. *Analyst (London)*, **95**, 940.

Driss, F. & Darcet, P. (1988). Effets des huiles de poisson riches en acides gras *n*-3 sur les facteurs de risque des maladies cardiovasculaires. *Rev. Franç. Corps Gras*, **1**, 7.

Enser, M. B. (1974). Factors affecting the development of oxidative rancidity in frozen meat. *Meat Freezing. Why and How.* Meat Research Institute Symposium. Bristol.

Hanumanthappa, B. & Chandrasekhar, C. (1987). Preparation and keeping quality of hot smoked mackerel. *Fish. Technol.*, **24**, 112.

Herzberg, A. M. (1987). Polyunsaturated fatty acids in fish from the Eastern Mediterranean and the Lake of Ballilee. In *Third Technical Consultation on the Utilization of Small Pelagic Species in the Mediterranean.* Amalfi, Italy.

Jiménez-Colmenero, F. (1979). Elaboración y conservación al estado congelado de pastas de jurel (*Trachurus trachurus*, L.). PhD Thesis, Universidad Complutense de Madrid, Facultad de Ciencias Químicas.

Lemon, D. W. (1975). An improved TBA test for rancidity. Environment Canada. Fisheries and Marine Service Halifax Laboratory. *New Series Circular*, **51**.

Markina, E. V. & Lapshin, I. I. (1981). Utilization of the MINKH smoking solution for preserving frozen fish. *Tovarovedenie*, **13**, 39.

Markina, E. V., Kuznetsov, D. I., Lapshin, I. I. & Shevchenko, A. D. (1977). Effect of brining liquid on changes in fatty acid composition of bream during storage. *Tovarovedenie*, **10**, 58.

Meizies, A. & Reichwald, I. (1973). Lipids in the meat and role of fresh and smoked fish. *Z. Ernährungswiss.*, **12**, 248.

Morrison, W. R. & Smith, L. L. (1964). Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *J. Lipid Res.*, **5**, 600.

Ostyakova, E. B. & Skupova, I. P. (1983). Physicochemical changes in canned Caspian sprats during storage. *Rybn. Khoz.*, **8**, 69.

Shevchenko, M. G. & Lapshin, I. I. (1975). The effect of smoking dried fish products on the stability of lipids to oxidation. *Rybn. Khoz.*, **11**, 74.

Tsubouchi, T., Matsui, K., Kusaka, H., Otha, S. & Kaminura, S. (1985). Changes in fatty acid composition during the drying and smoking of Pacific saury. *J. Jap. Oil Chem. Soc.*, **34**, 563.

UNE (1973). Standard No. 55-023-73. *Materias grasas.*Indice de peróxidos.