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Long chain polyunsaturated fatty acids in smoked Atlantic mackerel and Baltic sprats

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Abstract

Atlantic mackerel and Baltic sprats are rich sources of $n-3$ long chain polyunsaturated fatty acids (LC PUFA). Literature data point to an influence of the properties of the raw material, storage conditions, and processing parameters of hot- and cold-smoking on the stability of these acids. The effects of industrial smoking in an automatic smokehouse in controlled, mild conditions at core temperature below 60 °C, as well as of cold storage, on the fatty acids (FA) in mackerel and sprats have been investigated. The FA were determined by gas chromatography (GC) according to the AOCS Ce 1b-89 method, in lipids extracted from the meat of several batches of defrosted and smoked fish early after smoking and during storage at 2 °C for up to 2 weeks. The contents of eicosapentaenoic acid C20:5 $n-3$ (EPA) and docosahexaenoic acid C22:6 $n-3$ (DHA) in different assortments of smoked mackerel meat were from 50 to 55 and from 67 to 100 mg/g of lipids, respectively while, in hot-smoked sprats, they were from 48 to 68 and from 73 to 128 mg/g of lipids. The results show that the variability of the FA composition of the frozen raw material was larger than the changes induced by smoking or by storage within the period of high quality life of the smoked product.

Keywords: Smoked fish; Mackerel; Sprat; Long chain polyunsaturated fatty acids; Eicosapentaenoic acid; Docosahexaenoic acid

1. Introduction

In Europe, particularly in Germany, Poland and the UK, there is a high market demand for smoked fish,such as Bückling, eel, halibut, herring, mackerel, salmon and sprats. According to the FAO (2003), the total world productions of smoked herring and salmon are about 38,000 and 86,000 tons, respectively. The consumer preference for these products resulted not only from their traditionally desirable smoky flavour, but also from their high contents of LC PUFA of the $n-3$ family in fish lipids. These FA decrease the contents of triacylglycerols, cholesterol, and low density lipoproteins in the human serum, and inhibit the aggregation of blood platelets and the damage to blood vessels (Lands, 1986).

Fish oils containing LC PUFA with up to 6 double bonds, such as EPA and DHA, are very susceptible to oxidation. The educated consumer, who in selecting his food takes into consideration also the content of these nutritionally desirable $n-3$ PUFA, should be informed, not so much about their contents in the fresh fish, but rather in the ready-to-eat commodities, e.g. baked, fried, canned or smoked.

The composition of lipids in smoked products depends primarily on their contents and state in the fish used for smoking. The conditions and time of chilling and frozen storage of fish affect the rate of oxidation (Kołakowska, Macur, Pankiewicz, & Szczygielski, 1998). Further factors influencing the state of lipids

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are the preparation of the raw material for smoking, the smoking itself, and storage of the products. Brining, drying, heating, and the reactivity of smoke components may have an impact on the rate of lipid changes by affecting the tissue enzymes involved in oxidation reactions, as well as by generating and changing the stability of radicals. During brining,the fish meat takes up the required quantity of salt, but also some cations present as impurities, that may have prooxidative activity. The reaction rate of peroxidation of FA may also increase due to drying and heating in the initial stages of the process, when the concentrations of phenolic smoke antioxidants in the meat are still low. The extent of loss of moisture and the duration and temperature of heating may also be important. The effects of various factors influencing the oxidation of lipids in fish tissues have been recently reviewed by Kołakowska, Olley, and Dunstan (2002).

The antioxidant effect of smoking has long been recognised, since lipids in smoked fish and meats were known not to undergo rapid oxidation. The role of smoke components in retarding oxidation was investigated by Watts in the early fifties of the last century (Watts & Faulkner, 1954). Later, it was shown that, among the several hundred known wood smoke components certain phenols had the highest antioxidative activity (Kurko, 1969). The phenol fraction of wood smoke is a mixture of about 240 compounds; one third of this number have been positively identified (Tóth & Potthast, 1984). The composition of this fraction, containing mono- di- and trihydroxyphenols, as well as derivatives with additional functional groups in the substituting chains, e.g. hydroxyl, carbonyl, carboxyl groups and ester bonds, depends primarily on the temperature of smoke generation, but also on the kind of wood and the access of air to the smouldering material. Thus, the effectiveness of the antioxidant action of smoking depends much on the composition of the smoke and the chemical character and quantity of phenols deposited on the smoked food. The antioxidant activity of some wood smoke phenols is higher than that of various known commercial antioxidants. The most active are pyrogallol, resorcinol, 4-methylguaiacol, 4-vinylguaiacol, and 4-*trans*-propenylsyringol. Less active as antioxidants are guaiacol, syringol, 4-methylsyringol, and 4-vinylsyringol (Kurko, 1969; Miler & Sikorski, 1990).

Since heating, applied during smoking, may affect the state of fish lipids, data on lipid oxidation in dried fishery products should also be considered. According to Tabara et al. (1998), drying of horse mackerel, frog flounder, Japanese whiting, hard clam, arkshell and scallop, under various conditions, did not induce significant changes in the contents of EPA and DHA in the lipids, regardless of the differences in the fat contents of these commodities. Deep-fat frying of Atlantic mackerel steaks in canola oil at 180 °C for 7 min resulted in about 20% decrease in the concentration of EPA and DHA in the lipids of the fish meat and about 40% in the lipids of the skin. This loss, however, was caused rather by exchange of the lipids between the fried mackerel and the frying medium than by thermal changes in the fatty acids (Sebedio, Ratnayake, Ackman, & Prevost, 1993).

The literature contains contradictory information regarding the stability of PUFA in smoked fish. Most of the earlier publications regarding this subject have been reviewed by Kołakowska et al. (2002).

The aim of this investigation was to determine the effect of industrial smoking in an automatic smokehouse in mild conditions, as well as of refrigerated storage, on the FA, particularly on EPA and DHA, in fish lipids. The results were expected to answer the question, whether smoking, under these conditions, prevents significant oxidation of LC PUFA, and particularly EPA and DHA in these fatty fish during storage within the period of shelf life.

2. Materials and methods

2.1. Smoking and sampling

In the experiments, the effects of hot smoking on the lipids of gutted Atlantic mackerel, fillets of mackerel and whole Baltic sprats were investigated. The experiments were conducted in cooperation with a fish processing plant, PRORYB, Rumia, in Poland.

Whole mackerel (*Scomber scombrus*), frozen on board, imported from Norway, were stored in the plant at −30 °C for 1–4 months. After thawing in a water/steam atmosphere at 21 °C the fish were gutted, brined in a 20% salt solution, 1:1, for 2.5–3 h at 7 °C. In experiment 1, the gutted mackerel was smoked in an automatic kiln according to the procedure presented in Fig. 1. The smoke was produced from a mixture of oak and beech shavings in an external smouldering-type generator. The contents of FA in the meat of thawed fish prior to brining and in the smoked fish were compared. In experiment 2, mackerel fillets were smoked in a similar process. However, the fillets were brined only for 3 min and after spraying, dripping, and surface drying on mesh, they were heated to an internal temperature of 47 °C for 1.5 h and smoked at 45 °C for 1.5 h. In this experiment, one fillet of each pair was left raw for refrigerated storage and analysis, while the other one was smoked and the product was stored at the same temperature as the controls. The results of corresponding fillets in each pair were compared.

In experiment 3, mackerel fillets were cold-smoked by pre-drying at 28 °C for 3–3.5 h and smoking at 26 °C for 1–1.5 h.

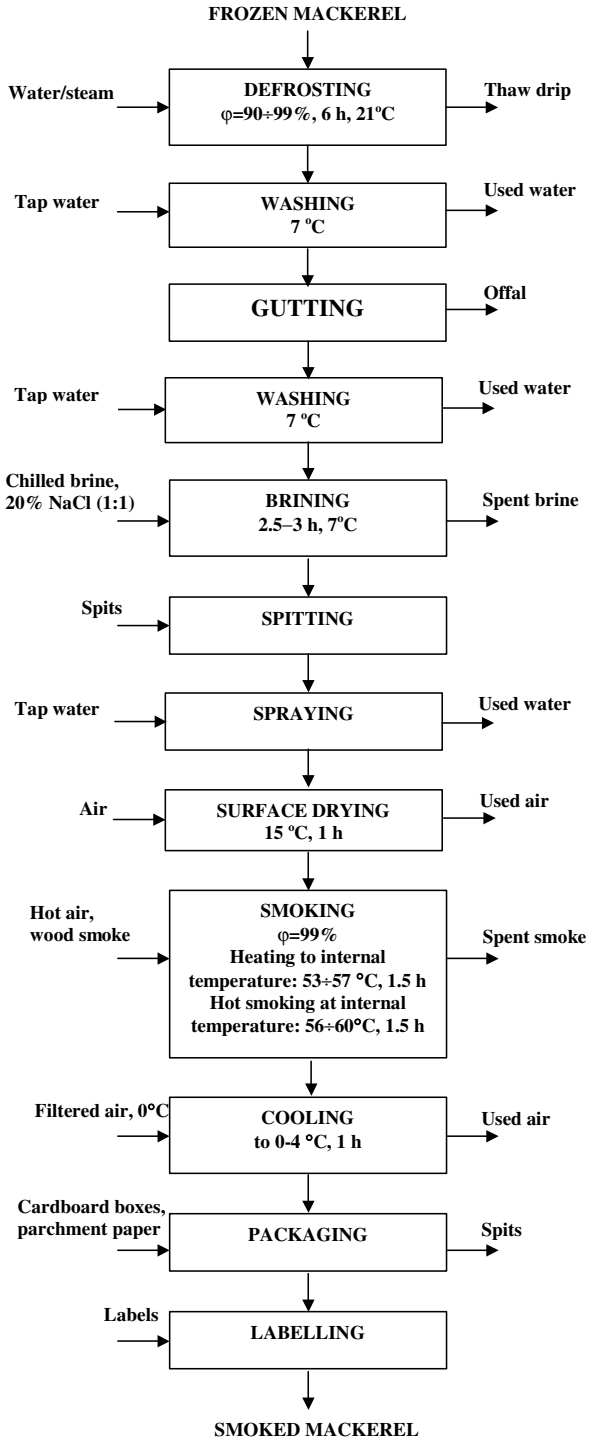

Fig. 1. The flow sheet of hot-smoking of Atlantic mackerel in mild conditions.

In experiment 4 Baltic sprats (*Sprattus sprattus*) were used, that had been caught in March 2003 and stored for 9–10 months at −30 °C. After defrosting and washing, the sprats were brined in a 20% salt solution, 1:1, for 5 min at 7 °C, spitted, and surface-dried at 15 °C for 60 min, heated in the same smokehouse at 53 °C for 20 min, and smoked at 51 °C for 50 min. After chilling to 0–4 °C they were taken to the laboratory for analysis.

In experiment 5, the winter Baltic sprats, kept in ice one day after capture, were smoked according to a conventional hot-smoking procedure in a traditional kiln. The smoking lasted about 2.5 h. The smoked product was immediately taken to the laboratory for analysis.

In the storage experiments the smoked fish were kept in closed cardboard boxes at 2 °C for up to 2 weeks.

2.2. Sample preparation

For analysis, the meats separated from three mackerels, the skinless fillets of each mackerel, or of about 60–70 sprats were minced in a meat grinder, using a plate with 2 mm orifice, and mixed thoroughly to prepare each homogeneous sample. The lipids were extracted from 1 g of the lyophilized mince according to Folch, Lees, and Stanley (1957) using, for the first extraction, 60 ml and, for the second extraction, 30 ml of chloroform:methanol 2:1 (v:v). After adding 18 ml of water to the pooled chloroform:methanol extract, the lipids dissolved in the chloroform phase were dried under nitrogen. Methyl esters of FA (FAME) were prepared according to the AOCS Ce 1b-89 method.

2.3. Fatty acid analysis

GC analysis of FAME was carried out in a Hewlett–Packard model 6890 instrument equipped with a split/splitless injector, FID and a column, Rtx 2330 (Restek USA), 105 m, 0.25 mm ID, d_f 0.2 μm. The initial temperature of the column was 175 °C, injection port temperature 235 °C, detector temperature 250 °C, initial time 30 min, temperature increment 1.5 °C/min, final temperature 210 °C, and the total time of analysis 90 min. For identification, the reference sample of 36 FAME, Supelco 37 was used. The results were recorded and processed using HP-Chem software. The quantification of EPA and DHA was done according to AOCS Ce 1b-89(1)

The results were evaluated statistically by analysis of variance (one-way procedure) or Students *t*-test.

3. Results and discussion

3.1. FA in hot smoked mackerel

All quantitative estimations of the FA concentration in the analysed samples were done on the basis of GC separations, as shown in the chromatogram presented in Fig. 2.

The FA compositions (% of total FA) in the lipids extracted from two batches of thawed mackerel and from three batches of smoked mackerel did not differ

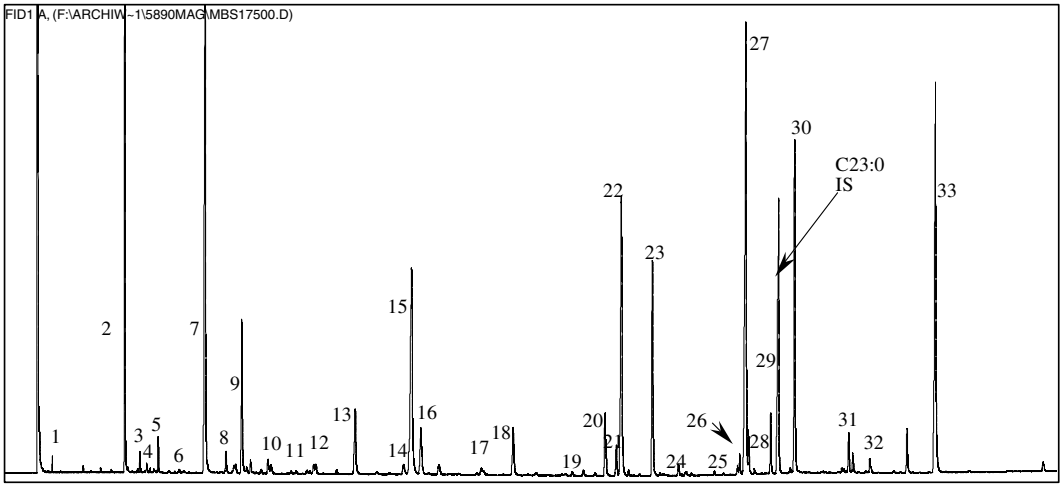

Fig. 2. A typical chromatogram of the methyl esters of FA isolated from the samples of mackerel with the internal standard (IS). Conditions of separation as described under Section 2. Identification of FAME represented by peaks: 1 – C8:0, 2 – C14:0, 3 – C14:1 t, 4 – C14:1 c, 5 – C15:0, 6 – C15:1, 7 – C16:0, 8 – C16:1 t, 9 – C16:1, $n-9$, 10 – C17:0, 11 – C17:1 t, 12 – C17:1 c, 13 – C18:0, 14 – C18:1 t, 15 – C18:1, $n-9$, 16 – C18:1, $n-7$, 17 – C18:2 t, 18 – C18:2, $n-6$, 19 – C20:0, 20 – C18:3, $n-3$, 21 – C20:1, $n-11$, 22 – C20:1, $n-9$, 23 – C18:4, $n-3$, 24 – C20:2, 25 – C22:0, 26 – C20:3, $n-6$, 27 – C22:1, $n-11 + n-13$ + C20:3, $n-3$, 28 – C20:4, $n-6$, 29 – C22:1, $n-9$, 30 – C20:5, $n-3$, 31 – C24:1 c, 32 – C22:5, $n-6$, 33 – C22:6, $n-3$.

Table 1
The FA present in highest concentrations in the lipids extracted from the meat of thawed mackerel and hot-smoked mackerel stored at 0–2 °C[a] (experiment 1)

Fatty acid	Peak area[a] of the respective FA in % of total peak area			
	Thawed fish	Time of storage of smoked fish (days)		
		1	7	14
C14:0	6.4 ± 0.9	6.0 ± 0.9	6.1 ± 0.7	6.4 ± 1.3
C15:0	0.5 ± 0.1	0.5 ± 0.0	0.6 ± 0.1	0.5 ± 0.1
C16:0	12.9 ± 1.7	11.1 ± 0.9	11.4 ± 1. 3	13.0 ± 1.9
C17:0	0.4 ± 0.2	0.4 ± 0.0	0.4 ± 0.1	0.4 ± 0.1
C18:0	2.5 ± 0.2	2.1 ± 0.2	1.8 ± 0.1	2.3 ± 0.6
C16:1 t	0.5 ± 0.2	0.4 ± 0.0	0.5 ± 0.1	0.6 ± 0.0
C16:1 $n-9$	3.7 ± 0.2	2.7 ± 0.3	3.0 ± 0.2	3.5 ± 0.4
C18:1 t	0.6 ± 0.1	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.1
C18:1 $n-9$	10.9 ± 0.7	8.8 ± 1.0	7.6 ± 0.0	10.8 ± 3.8
C18:1 $n-7$	2.1 ± 0.2	1.5 ± 0.2	1.4 ± 0.1	2.0 ± 0.7
C20:1 $n-9$	11.4 ± 0.2	11.5 ± 1.2	10.9 ± 0.5	9.8 ± 1.2
C18:2 $n-6$	1.5 ± 0.1	1.6 ± 0.1	1.7 ± 0.2	1.5 ± 0.2
C18:4 $n-3$	3.3 ± 0.2	4.4 ± 0.5	4.4 ± 0.4	3.8 ± 1.3
C20:5 $n-3$	5.8 ± 1.6	6.3 ± 0.5	6.0 ± 0.9	6.0 ± 1.5
C22:6 $n-3$	7.7 ± 1.9	10.6 ± 2.8	10.3 ± 1.5	9.5 ± 1.0

[a] Mean value ± SD characterizing samples from two batches of thawed fish and three batches of smoked fish.

Table 2
The contents of EPA and DHA in the lipids extracted from corresponding fillets from thawed and hot-smoked mackerel[a] (experiment 2)

Fish number	EPA (mg/g extracted lipids)		DHA (mg/g extracted lipids)	
	Thawed	Smoked	Thawed	Smoked
0	47.0	54.5	69.5	84.2
1	46.9	46.0	75.0	72.4
2	48.1	49.5	79.0	81.0
3	46.5	42.8	76.5	77.2
4	49.1	46.3	106.1	86.0
5	54.8	60.1	87.7	93.1
6	50.6	50.7	95.1	89.8
7	50.3	45.2	92.2	74.9
8	53.5	58.4	78.1	85.3
All samples	49.7 ± 2.94	50.4 ± 6.10	84.4 ± 11.72	82.7 ± 6.87

[a] All data are mean values of two determinations, each obtained from a separate extract from the same mince (fillet). In the last row – mean values ± SD.

significantly ($p > 0.05$) (Table 1). This conclusion has been validated also by the results on EPA and DHA in thawed mackerel and the corresponding smoked fillets of the same mackerel (Table 2). The lack of statistically significant changes in the concentrations of FA in the thawed and smoked mackerel was possibly due to the mild conditions of smoking. According to Beltran and Moral (1989) hot smoking of sardine fillets, iced 30–32 h after capture, containing 5.1% lipids, brined, surface-dried for 12 h and smoked for 2 h at 30 °C plus 45 min at 75 °C in the kiln, resulted in small, although statistically significant decreases in the contents of EPA (by 11.5%) and DHA (by 12.9%) in the total lipids. These losses were accompanied by an increase in the concentration of the C16:0 acid by 7.3% and of C22:1 ($n-11$) by 10.3%. The cited authors indicated also, that smoking caused significant increases in the peroxide value, thiobarbituric acid index (TBA), and concentration of free FA. Because of the lack of other information, it must be assumed that the changes described by the authors, as caused by smoking, are the effect of all treatments of the fish, namely brining, surface-drying, and smoking.

Neither a consistent pattern nor generally statistically significant changes in the contents of the FA in lipids extracted from thawed and from smoked mackerel, after up to 14 days of refrigerated storage, were observed (Table 1 and Table 3). The concentration of EPA decreased slightly in the smoked samples for up to 14 days at 2 °C (Table 4). However, the loss was not statistically significant in one-way analysis of variance ($p > 0.05$). In the experiments of Cha et al. (2001), no significant changes in the concentration of EPA and DHA were found in stored seasoned-dried Pacific saury treated with liquid smoke. The fillets of saury, containing 28.1–30% of fat, seasoned with a mixture of sugar, salt, monosodium glutamate, and sorbitol, soaked for 10 s in 5% liquid smoke Scansmoke PB 2110, and dried for 40 h at 40 °C, were compared with seasoned and dried controls. The products were packaged in polyethylene film and stored at about 19 °C. After 15 and 60 days, the concentrations of total PUFA, EPA and DHA, in the lipids extracted from the samples containing liquid smoke and from the controls, did not differ significantly. However, the content of C22:1 ($n - 11$) increased in the samples containing Scansmoke, as compared to that in the controls, by 5.5% and 17% after 15 and 60 days, respectively. The TBA and the peroxide value, in the stored product containing liquid smoke, were significantly lower than in the control samples, which has been attributed by the cited authors to the antioxidant activity of Scansmoke.

To investigate the effect of storage of the smoked product, avoiding the impact of the variability of the samples, the contents of FA were determined in corresponding smoked fillets of the same mackerel after 0 and 14 days at 2 °C (Table 5). The results indicate that there was a tendency toward slight, although statistically significant ($p < 0.02$) decrease of about 8%, in the contents of EPA in the lipids extracted from hot-smoked mackerel stored for 14 days. The changes in the contents of DHA were of the same order. However, due to the high variability in the concentration of this acid in the minces prepared from each fillet of the same fish, they were not statistically significant.

The variability of the contents of different FA in the individual frozen fish, taken from the same batch imported as first quality raw material, was considerably high (Table 6). The contents of the saturated, monoenoic, and polyenoic FA in the extracted lipids, calculated on the basis of nine fishes, were in the range 21–28%, 26–33%, and 32–43%, respectively. There were no statistically significant differences in the contents of these groups of lipids extracted from the thawed and smoked mackerel fillets (Table 6).

Table 3
The fatty acids present in highest concentrations in the lipids extracted from the meat of thawed mackerel stored at 0–2 °C (experiment 1)

Fatty acid	Peak area[a] of the respective FA in % of total peak area			
	Time of storage (days)			
	0	1[b]	7	14
C14:0	6.4 ± 1.0	7.1	6.3 ± 1.0	6.3 ± 0.9
C15:0	0.5 ± 0.1	0.6	0.5 ± 0.1	0.6 ± 0.0
C16:0	12.9 ± 1.7	12.3	11.3 ± 1.1	13.6 ± 3.0
C17:0	0.4 ± 0.2	0.4	0.4 ± 0.1	0.4 ± 0.1
C18:0	2.5 ± 0.2	2.2	2.1 ± 0.0	2.5 ± 0.7
C16:1 t	0.5 ± 0.2	0.4	0.5 ± 0.0	0.7 ± 0.3
C16:1 $n - 9$	3.7 ± 0.2	3.1	3.0 ± 0.3	3.3 ± 0.2
C18:1 t	0.6 ± 0.1	0.5	0.6 ± 0.1	0.5 ± 0.1
C18:1 $n - 9$	10.9 ± 0.7	9.9	9.1 ± 0.3	10.7 ± 5.9
C18:1 $n - 7$	2.1 ± 0.2	1.7	1.8 ± 0.0	2.1 ± 0.9
C20:1 $n - 9$	11.4 ± 0.2	13.0	11.9 ± 0.6	10.5 ± 2.8
C20:5 $n - 3$	5.8 ± 1.6	5.1	5.5 ± 0.2	5.1 ± 0.4
C22:6 $n - 3$	7.7 ± 1.9	6.8	8.7 ± 0.4	8.5 ± 0.5

[a] Mean value ± SD characterizing samples from two batches of fish.
[b] Data from one batch of fish.

Table 4
The contents of EPA and DHA in the lipids extracted from thawed and hot-smoked mackerel meat stored at 2 °C[a] (experiment 1)

Fish	Time of storage (days)	EPA (mg/g extracted lipids)	DHA (mg/g extracted lipids)
Thawed	0	46.1–52.5	64.8–88.8
		49.3 ± 3.17	74.2 ± 12.77
	1	50.3–54.8	66.9–99.5
		51.9 ± 2.52	86.7 ± 17.39
Smoked	7	45.4–53.7	76.3–94.3
		48.9 ± 4.31	87.1 ± 9.52
	14	37.9–55.7	76.4–94.5
		46.6 ± 8.88	87.2 ± 9.53

[a] Range of variability and mean value ± standard deviation characterizing samples from three batches of fish.

Table 5
The content [a] of EPA in the lipids extracted from corresponding fillets from hot-smoked mackerel, immediately after smoking and after storage for 14 days at 2 °C[a] (experiment 2)

Fish number	Days of storage			
	EPA (mg/g extracted lipids)		DHA (mg/g extracted lipids)	
	0	14	0	14
1	60.7	51.1	101	81.3
2	42.4	40.2	124	122
3	52.5	49.3	91.5	90.7
4	63.5	54.6	94.6	86.6
5	53.0	50.9	59.3	57.2
6	47.5	48.2	91.2	92.4
7	47.1	41.8	77.7	80.7
8	55.1	51.4	91.6	84.4
All samples	52.7 ± 7.1	48.4 ± 5.0	91.4 ± 18.5	86.9 ± 17.9

[a] All data are mean values of two determinations, each obtained from a separate extract from the same mince (fillet). In the last row – mean values ± SD.

Table 6
The contents[a] of groups of FA in lipids extracted from thawed and hot-smoked mackerel meat (experiment 2)

Fish number	FA (% of total FA)					
	Saturated		Monoenoic		Polyenoic	
	Thawed	Smoked	Thawed	Smoked	Thawed	Smoked
1	23.4	21.9	26.4	31.4	38.6	39.4
2	21.3	21.7	30.5	30.9	43.2	42.6
3	24.4	24.2	31.9	31.6	36.2	37.9
4	22.8	22.9	29.0	28.8	42.0	42.4
5	27.7	27.1	33.2	32.7	32.5	33.7
6	24.4	24.6	30.1	29.4	39.6	40.2
7	24.4	25.1	28.2	28.4	43.0	42.1
8	27.9	28.6	32.8	33.0	33.7	32.6
9	24.1	22.7	26.1	22.9	41.0	43.2
All samples	24.5 ± 2.2	24.3 ± 2.4	29.8 ± 2.6	29.9 ± 3.1	38.9 ± 4.0	39.4 ± 3.9

[a] All data are mean values of two determinations, each obtained from a separate extract from the same mince (fillet). In the last row – mean values ± SD.

3.2. FA in cold-smoked mackerel

The effect of cold-smoking was investigated by determining the contents of EPA and DHA in the lipids extracted from the corresponding thawed and cold-smoked mackerel fillets (Table 7). There was no statistically significant difference ($p > 0.05$) in the contents the two acids in the lipids from the thawed and cold-smoked fish. This is an evidence that the state of the lipids in frozen, stored mackerel is more important than the slight changes induced in the contents of these FA due to cold-smoking under the described conditions.

3.3. FA in smoked sprats

The effect of smoking on the lipids of Baltic sprats was investigated in two experiments. In No 4 experiment 3 batches of thawed fish, and of corresponding sprats smoked in the automatic smokehouse, as described in Section 2, were analysed. In experiment No. 5, the lipids from fresh sprats and from corresponding fish smoked traditionally were compared. The results regarding fish smoked in the automatic smokehouse indicate (Table 8), that there was no statistically significant difference ($p > 0.05$) in the contents of EPA and DHA in the lipids extracted from the raw and smoked fish. Furthermore, even traditional smoking did not decrease the contents of either LC PUFA (Table 8). The large differences in the concentrations of EPA and DHA in the sprats used for each experiment could be attributed to seasonal changes and method of preservation. The sprats smoked in the automatic smokehouse were caught in 2003 and frozen, while the samples smoked in the traditional kiln were fresh and caught in 2004.

Table 7
The content[a] of EPA and DHA in the lipids extracted from corresponding fillets from thawed and cold-smoked mackerel (experiment 3)

Fish number	EPA (mg/g extracted lipids)		DHA (mg/g extracted lipids)	
	Thawed	Smoked	Thawed	Smoked
1	47.9	63.5	91.6	92.3
2	47.7	58.8	86.4	102
3	53.9	46.1	92.7	114
4	65.1	49.8	105	91.2
5	55.7	45.1	103	82.2
6	62.7	55.5	104	99.1
7	53.2	61.2	99.4	110
8	66.2	56.5	113.7	99.1
9	49.4	60.0	84.4	97.5
All samples	55.8 ± 7.3	55.2 ± 6.7	97.8 ± 9.7	98.6 ± 9.7

[a] All data are mean values of two determinations, each obtained from a separate extract from the same mince (fillet). In the last row – mean values ± SD.

Table 8
The contents of EPA and DHA in the lipids extracted from the mince prepared from thawed and from hot-smoked sprats

Fish	EPA (mg/g extracted lipids)	DHA (mg/g extracted lipids)
(a) in an automatic smokehouse[a] (experiment 4)		
Thawed	46.5–54.9	68.2–78.7
	51.1 ± 2.9	74.1 ± 3.6
Smoked	44.8–54.6	66.5–82.9
	50.9 ± 3.8	75.1 ± 5.9
	EPA[c] (mg/g extracted lipids)	DHA[c] (mg/g extracted lipids)
(b) in a traditional kiln[b] (experiment 5)		
Thawed	63.2 ± 1.9	113.2 ± 6.7
Smoked	68.1 ± 1.0	128.1 ± 4.6

[a] Range of variability and mean value ± SD characterizing samples from three batches of fish.
[b] Data supplied by Zdzisław Domiszewski.
[c] Mean value ± SD from five separate extracts of lipids from one batch of fish.

4. Conclusions

The results presented above have shown that the FA composition of the lipids extracted from freshly smoked mackerel, and from smoked mackerel stored for up to 14 days at 2 °C, was primarily affected by the high biological variability of the raw fish composition. Furthermore, in determining the FA composition in such a complex matrix as fish muscle tissues, large variability of results, caused by differences in the yields of separate extractions of various lipids from samples of the same mince and uneven distribution of the lipids in the mince prepared for analysis, should be considered. Some published results indicate that the extractability, and thus the FA composition, of the extracted lipids may be affected by thermal changes of protein–lipid complexes in the tissues.

Mild hot-smoking at a core temperature in the meat not exceeding 60 °C, in an automatic smokehouse equipped with a smoke generator, did not cause significant diminution of the content of LC PUFA, including EPA and DHA, in the meat of high quality mackerel. Thus such smoked fish is a valuable dietary source of $n-3$ PUFA.

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References

Beltran, A., & Moral, A. (1989). Effect of smoking on lipid stability in sardine (*Sardina pilchardus* W.). *Zeitschrift für Lebensmittel-Unterschung und-Forschung A, 189*, 317–321.

Cha, Y. J., Park, S. Y., Kim, H., Jeong, E. J., Chung, Y. J., & Kim, J. S. (2001). Oxidative stability of seasoned-dried Pacific saury (imported product) treated with liquid smoke. *Journal of Food Science and Nutrition, 6*(4), 201–205.

FAO (2003). *FAO yearbook of fishery statistics 2001* (Vol. 93). Rome: Food and Agriculture Organization of the United Nations.

Folch, J., Lees, M., & Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 497–509.

Kołakowska, A., Olley, J., & Dunstan, G. A. (2002). Fish lipids. In Z. E. Sikorski & A. Kołakowska (Eds.), *Chemical and functional properties of food lipids* (pp. 221–264). Boca Raton: CRC Press.

Kołakowska, A., Macur, I., Pankiewicz, A., & Szczygielski, M. (1998). Mackerel as a source of $n-3$ polyenoic fatty acids. In A. Stołyhwo (Ed.). *Evening primrose oil and other oils containing $n-6$ and $n-3$ fatty acids in prophylaxis and treatment. Proceedings of the 3rd Symposium* (pp. 71–77). Sulejów (in Polish).

Kurko, V. I. (1969). *Khimia kopchenya*. Moskva: Izdat Pishchevaya Promyshlennost (in Russian).

Lands, W. E. M. (1986). *Fish in human health*. Orlando, Fl: Academic Press.

Miler, K. M. B., & Sikorski, Z. E. (1990). Smoking. In Z. E. Sikorski (Ed.), *Seafood: resources, nutritional composition, and preservation* (pp. 163–180). Boca Raton, Florida: CRC Press.

Sebedio, J. L., Ratnayake, W. M. N., Ackman, R. G., & Prevost, J. (1993). Stability of polyunsaturated $\omega-3$ fatty acids during deep fat frying of Atlantic mackerel (*Scomber scombrus* L.). *Food Research International, 26*, 163–172.

Tabara, Y., Ueki, S., Ito, M., Watanabe, T., Kan, T., Hiraoka, Y., et al. (1998). Changes in EPA and DHA levels during manufacture of dried fish and shellfish. *Nippon Shokuhin Kagaku Kaishi, 45*(2), 93–99.

Tóth, L., & Potthast, K. (1984). Chemical aspects of the smoking of meat and meat products. In C. O. Chichester, E. M. K. Mrak, & B. S. Schweigert (Eds.). *Advances in food research* (Vol. 29, pp. 87–158). New York: Academic Press.

Watts, B. M., & Faulkner, M. (1954). Antioxidative effects of liquid smokes. *Food Technology, 8*, 158–161.