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Stability of Lipids and Polyunsaturated Fatty Acids During Smoking of Atlantic Mackerel (*Scomber scombrus* L.)

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Fall Atlantic mackerel (*Scomber scombrus* L.), nonsmoked and hot smoked according to the method of Torry (Aberdeen, Scotland) Advisory Note #82, in an AFOS-Torry Mini Kiln, were used to study changes in oxidative rancidity and composition of major lipid classes and fatty acids. After smoking there was an increase in thiobarbituric acid (TBAM) value and peroxide (PO) value, but the values were still indicative of acceptable quality. The percentages of triglycerides (TG) and phospholipid (PL) did not change significantly, and free fatty acids could barely be detected. The overall fatty acid composition remained virtually unchanged after the smoking process. This included the longer chain C_{20} and C_{22} n-3 fatty acids, now regarded as potentially essential fatty acids for humans.

Smoking currently is an important process in Canada for mackerel (1). New developments in the role of fish in cardiovascular-oriented diets, including smoked canned mackerel (2–4), make it important to evaluate the smoking process for effects on the quality and nutritional benefits of the product, particularly in respect to the labile longer-chain (C_{20}, C_{22}) n-3 polyunsaturated fatty acids of the lipids (5,6).

Smoking is a process that combines the effects of brining, heating, drying and, finally, of the smoke itself (7). Not all fish are suitable for smoking purposes (8). Depending on their chemical composition, different fish react to the smoking process in various ways (9). The Atlantic mackerel is being used in considerable quantity for smoking (10), but there are few reports on the effects of smoking on any aspect of this fish. The few data that are available deal only with smoked fish without direct comparison to the unsmoked fish from the same batch, although Deng et al. (10) reported the use of the same batch of Spanish mackerel (a different species and family, *Scomberomorus maculatus*) as a source of both raw and smoked fish.

Not much information is available on the effect of smoke on individual lipids. We have investigated the effect of smoking on the lipids of Atlantic mackerel.

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MATERIALS AND METHODS

The fish used for the study were fall Atlantic mackerel (*Scomber scombrus* L.) caught on October 5, 1982. Details about the fish, including their proximate composition and the method of hot smoking in a Torry AFOS Mini-Kiln and pooling of muscle (skinned fillet) samples (usually n = 8) for analysis, were given in a separate part of this study (12). In Nova Scotia fall fish examined in this study, males and females contained equally high TG percentages in their lipids; once the fish were gutted, no distinction by sex was made in further processing. Lipids from the fish fillets were extracted by the Bligh and Dyer procedure (13), and the extracts were stored at −80 C under nitrogen until used. The oxidative condition of the mackerel lipids was assessed by the 2-thiobarbituric acid (TBAM) test (14,15) and by the peroxide value (PO) (16) (AOCS Official Method Cd 8-53).

The major lipid classes of mackerel were quantitatively analyzed by TLC/FID using silica gel Chromarods-SII and an Iatroscan TH-10 Mark III Analyzer (17,18) (Iatron Laboratories Inc., Tokyo, Japan, world distributor Newman-Howells Assoc., Ltd., Winchester, United Kingdom). This was used with a Hitachi Stereo Cassette Tape Deck, Model D-E55, and Spectra-Physics Model 4200 integrating recorder.

Aliquots of fish lipids and standards were applied to Chromarods-SII (Iatron Laboratories, Tokyo, Japan) as dilute solutions in chloroform. 3-Hexadecanone was used as the internal standard which was spotted on each rod,

always at the same concentration, along with the standard or samples. The rods were developed for 40 min in hexane:diethyl ether:formic acid (97:3:1.2, v/v/v) and scanned on the FID of the Iatroscan. The operating conditions were: H_2 flow rate, 160 ml/min; airflow rate, 2000 ml/min; chart speed, 16 cm/min, and detector voltage, 8 mv full scale.

Preparative TLC was performed on 20 cm × 20 cm Adsorbosil-5 Prekotes (Applied Science Laboratories, State College, Pennsylvania). Development was in hexane:diethyl ether:acetic acid (85:15:1, v/v/v) (19,20). The separated bands were visualized under UV light after being sprayed with 2′7′-dichlorofluorescein.

Analytical gas liquid chromatography of fatty acid methyl esters (FAME) prepared with 7% BF_3-methanol was carried out on a Perkin-Elmer Model 990 Gas Chromatograph fitted with a flame ionization detector. A wall-coated, open-tubular column (stainless steel, 47 m in length and 0.25 mm I.D.), coated with SILAR-5CP (Perkin-Elmer, Norwalk, Connecticut) was used. The operating conditions were: injection port and split assemblies, 250 C; manifold assemblies, 250 C; column temperature, 185 C, and carrier gas pressure, 60 psig. Areas of peaks were recorded and integrated by a Fisher Recordall, Series 5000, 1 mv recorder. The weight-percent data were corrected for FID response by a computer program as described by Ackman and Eaton (21). Parts of all ester samples were hydrogenated, and the resulting saturated esters were used to verify accurate determination of unsaturated acids by chain length and to determine minor saturated compounds. In addition to obtaining calculated iodine value (IV) from the computer program used to obtain the weight-percentage data of fatty acids, an IV also was determined chemically by the AOCS Wijs method (16) (AOCS Official Method Cd 1-25).

RESULTS AND DISCUSSION

After salting and smoking, the TBAM value was almost double the original value (Table 1). The TBAM value in untreated mackerel fillet without skin, measured after 40 days of storage at −30 C in a vacuum-packed (oxygen-impermeable) bag, was 4.0. Ke et al. (22) reported similar TBAM values of 2.1 and 3.3 for non-smoked mackerel vacuum packed and frozen at −26 C for 1 and 2 mo, respectively.

During the smoking process fish are subject to heating, smoking and atmospheric oxygen. All of these factors can accelerate the oxidative deterioration of the fish. Salt is another factor to be considered. Prior to smoking, the fish were brined for 17 hr and their salt content increased from 0.38 to 3.78%. At the lower concentration, salt can act as a prooxidant (23). Hobbs (24) stated that the antioxidative effect of smoke in smoked fish can be outweighed by the prooxidant effect of salt used during brining. However, there is other evidence to suggest that salt may have a protective action against the oxidation of lipid (23). Heating undoubtedly results in oxidation of lipids in fish (25). Investigations by Barylko-Pikielna (26) and others (7) have shown that a phenolic fraction, deposited on the fish surface during smoking, is mainly responsible for the antioxidative activity, whereas other smoke fractions, e.g. hydrocarbons or organic bases, may have prooxidative activity.

TABLE 1

Effect of Smoking Process on Oxidation in Fillets of Fall Atlantic Mackerel

	TBAM (μmoleMA/kg fish)	PO (meq/kg fat)
Non-smoked	3.98 ± 0.2	1.30 ± 0.1
Smoked	6.86 ± 0.2	8.96 ± 0.5

TBAM and PO values for smoked sample are significantly different from non-smoked sample as tested by paired difference 't' test (≤0.05).

The TBAM value of the smoked fish is still relatively low, although the fish were kept in brine at room temperature for as long as 17 hr. It is concluded that the brine itself worked as a protective barrier against atmospheric oxygen during that period, and thus the oxidation process did not proceed as fast as might be expected. In the brine there is little mechanical damage which would expose fresh surfaces, and free oil which may be exuded, possibly as a result of slight contraction of tissue, tends to float free.

The TBAM value of hot-smoked mackerel is still well within the safe limits recommended by Ke et al. (27). There was a moderate increase in PO in the smoked fish, similar to the increase in the TBAM values (Table 1). This probably was due to increased conversion of some unsaturated fatty acids on the surface into peroxides. The PO values also support the conclusion that the smoked mackerel were in very good condition. Chandrasekhar et al. (28) reported that in smoked oil sardine a PO value below 20 was quite acceptable. Peroxide values as high as 184.6 meq/kg lipid have been reported for edible salted sun-dried Indian mackerel *(Rastrelliger kenagarta)* (29). For all practical purposes, mackerel remain safely edible after the smoking process. The fish were readily consumed by both lay persons and experienced fishery scientists in demonstrations and consumer-oriented displays.

Major lipid classes were measured using an internal standard in the Iatroscan TLC-FID system (30,31). The area of each standard or sample originating from each rod was divided by the corresponding 3-ketone area. This result was then averaged from nine rods. The FID response measured in this way was linear both for TG and PL in the concentration ranges from 0.76 to 9.98 μg and 0.49 to 9.99 μg, respectively. R^2 values were 0.986 and 0.998, respectively, and both were significant at a 5% level of confidence. For determination of lipid composition of the smoked and non-smoked mackerel, three lots of lipid extracts for each category of fish (smoked and non-smoked) were used. Each of these extracts was analyzed on nine Chromarods. Thus, the values given in Table 2 are an average of 27 Chromarod analyses.

The smoking process had no effect on the proportions of TG and PL in edible fillet (Table 2). The level of TG (96%) was higher than other literature values. Ackman and Eaton (30) reported the TG content in lipids of Atlantic mackerel (fall) to be 89.5 and 74.2% for light and dark flesh, respectively. Hardy and Keay (33) found that fall male fish contained 89.4% of TG as compared to the 78.7% in spring males. Kaitaranta and Ke (34) found

88.9% TG in Atlantic mackerel meat. Compared to these values, the present value (96.1%) is reasonable.

The PL content (3.5%) of lipid extracted from non-smoked muscle was also found to be very close to literature values. Ackman and Eaton (32) found 4.7% PL in light flesh and 4.3% in dark flesh. Hardy and Keay (33) found 3.5 and 4.6% PL in male and female lipid, respectively. Almost all fish contain approximately 0.7% PL in the muscle tissue (5,6), and in the present study the percentage was 0.73% of the fillet.

Free fatty acids (FFA) could not be detected by the TLC-FID system on the Chromarods, indicating that there was less than 0.1% FFA and suggesting that the overall quality of both non-smoked and smoked fish was retained. In one previous study, no hydrolysis of lipids occurred when fish tissue (muscle and liver) was heated at 100 C for periods up to 90 min, yet heating cod for 30 min at 100 C hardly affected phospholipase activity (35). In this study, FFA in lipids of smoked fall mackerel could just be detected on TLC plates using a very high loading of lipids, but the low levels of FFA precluded recovery and measurement. Thus, in mackerel slowly cooked during smoking no adverse enzymic hydrolysis of lipids could be detected. From their study of minced fish flesh stored at 20 C, Takama et al. (36) concluded that lipid degradation could be due to both hydrolysis and oxidation. However, smoke itself is regarded as having profound antioxidative action (7,24,37,38). Thus, in this study the overall result of mild salting and heating during smoking was that of an insignificant change in the two major muscle lipid classes TG and PL.

References to the hydrocarbon content of mackerel oil could not be found in the literature. Kaitaranta and Ke (34) showed that it must be less than 0.06% of lipid in mackerel meat lipid. Herring oil, which may be roughly comparable to mackerel oil, contains from 0.05 to 0.33% hydrocarbons (39,40). Due to the very low concentration, natural hydrocarbon could not be detected in the present study on TLC plates. There was no evidence of an increase in hydrocarbons, especially of the polyaromatic type (7), in the lipids of smoked mackerel as evidenced by inspection of TLC plates under ultraviolet light.

Fatty acids of mackerel oils from various species, including *Scomber japonicus*, have been studied in varying degrees of detail (32,33,36,41). The percentage distribution of the different fatty acids in the present study is representative of values in these reports (Tables 3–5). The rationale for the insignificant changes in TG and PL content due to the smoking process also can be extended to the fatty acids. From the results in Tables 3–5, it is obvious that there were no significant changes in overall fatty acid composition as a result of the smoking process. Although some individual fatty acids show differences (Table 3), these could be due partly to errors in GLC calculations for small peaks and partly to diet and other natural factors affecting one or more of the fish in the lot analyzed. The insignificant change in iodine value (Table 5) indicates the lack of effect of the smoking process. In theory, heat and oxygen could polymerize and oxidize polyunsaturated fatty acids in the smoked fish, but neither the GLC data nor the Wijs iodine values show such changes.

Mild heating during smoking induces slow moisture loss which may reduce surface exposure to oxygen, but if properly handled, the fish muscle does not "gape" to

TABLE 2

Effect of the Smoking Process on the Proportions of Triglyceride and Phospholipid of Fall Atlantic Mackerel (Fillet) Lipids[a]

Sample and lipid content	% Triglyceride	% Phospholipid
Non-smoked (20.6%)	96.1 ± 2.76	3.9 ± 0.45
Smoked (21.2%)	96.3 ± 3.60	3.7 ± 0.32

[a]Averaged from 9 TLC/FID analyses on 3 lots of extracts.

TABLE 3

Fatty Acid Composition (Weight %) Derived from Total Lipids of Edible Parts of Non-smoked and Smoked Atlantic Mackerel

Fatty acid[a]	Non-smoked	Smoked
10:0	0.13	0.16
12:0	0.26	0.24
13:0	0.06	0.05
I 14	0.03	0.04
14:0	6.03	6.79
14:1n-7	0.06	0.12
I 15	0.33	0.41
AI 15	0.21	0.20
15:0	0.57	0.70
I 16	0.07	0.09
16:0	15.07	15.35
16:1n-9	0.38	0.24
16:1n-7	3.93	4.52
16:1n-5	0.84	0.81
16:2n-4	0.62	0.72
16:3n-4	0.42	0.57
16:3n-3	0.15	0.21
16:4n-3	0.36	0.18
7 ME	0.33	0.24
I 17	0.34	0.02
AI 17	0.46	0.16
17:0	0.19	0.05
I 18	0.11	0.13
18:0	2.34	2.28
18:1n-9	9.80	9.40
18:1n-7	4.00	3.47
18:1n-5	0.75	1.17
18:2n-6	1.08	0.99
18:2n-4	0.23	0.19
18:3n-6	0.04	0.04
18:3n-3	1.39	1.46
18:4n-3	3.33	3.12
19:0	0.09	0.33
20:0	0.20	0.16
20:1n-9+n-11	8.40	7.28
20:1n-7	1.72	2.01
20:1n-5	0.35	0.42
20:2n-6	0.23	0.25
20:3n-6	0.04	0.04
20:3n-3	0.23	0.24
20:4n-6	0.45	0.48
20:4n-3	0.82	0.82
20:5n-3	7.05	6.93
21:5n-3	0.31	0.15
22:1n-11+n-13	10.56	8.49
22:1n-9	2.53	3.66
22:1n-7	0.71	0.77
22:5n-3	0.64	0.72
22:6n-3	10.49	10.87
24:0	0.19	0.26
24:1	1.07	1.58

[a]Shorthand notation for chain length, number of double bonds and position relative to methyl group. I = iso, AI = anteiso.

TABLE 4

Nutritionally Important Fatty Acids of Edible Parts of Non-smoked and Smoked Fall Atlantic Mackerel Summarized by Type (in Weight %)

Fatty acid class	Non-smoked	Smoked
Saturated	27	28
Monounsaturated	44	42
Polyunsaturated n-6	2	2
Polyunsaturated n-3	25	25

TABLE 5

Iodine Values of Non-smoked and Smoked Fall Atlantic Mackerel (Fillet) Lipid

	Iodine value	
Method of determination	Non-smoked	Smoked
Calculated (from FAME)	141.6	141.3
Wijs method	146.1	143.2

expose inner surfaces. The point that wood smoke itself has antioxidant properties has been reviewed at length recently (7).

Atlantic mackerel generally is known for its high fat content, although in the spring season the fat content can be as low as 2.0% (20). However, the high fat content (15–20%) of the fall fish poses some problems in effective marketing for the fish processors (22). Fall mackerel, in general, are more suitable for smoking purposes than the low fat spring mackerel. Lately there has been amelioration of concern about possible toxic components in smoked fish (7,42–45), further increasing the appeal of smoked fish products for varied healthful diets (46).

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Tocopherols and Tocotrienols in Finnish Foods: Oils and Fats

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The tocopherols and tocotrienols of vegetable oils, cod liver oil, margarines, butter and Voimariini dairy spread were analyzed by HPLC. The total tocopherol content varied from 4 (coconut oil) to 242 mg/100 g (wheat germ oil). α-tocopherol equivalents varied from 2 (coconut oil) to 225 mg/100 g (wheat germ oil). Semisoft and soft margarines had an average total tocopherol of 53 and 61 mg, and an average α-tocopherol equivalent of 17 and 27 mg/100 g, respectively. Hard margarines averaged 29 mg total tocopherol and 9 mg α-tocopherol equivalent/ 100 g. The average tocopherol content of butter and Voimariini was 2 and 15 mg/100 g, respectively, and the average α-tocopherol equivalent 2 and 6 mg/100 g.

The study reported here is part of a research project to survey in detail the vitamin E content of Finnish foods, including complete diets (1,2), human milk and baby foods (3), milk products and egg (4), fish and fish products (5), meat (6) and cereals (7). In this study the tocopherol levels in vegetable oils, cod liver oil, margarines and butter available in Finland were measured and the α-tocopherol/polyunsaturated fatty acid ratios derived.

EXPERIMENTAL

The tocopherols and tocotrienols were determined by high-performance liquid chromatography (Hewlett Packard 1084B), a LiChrosorb Si60 column (5 μm, 25 × 0.4 cm, Merck) and a di-isopropyl ether gradient of 8 to 17% in hexane. The oil (0.2, 1 and 5 g) or fat (1 and 5 g) was dissolved in 100 ml n-hexane; a clear solution was obtained after a small amount of insoluble material had settled. The solution was filtered through a Millipore 0.45 μm FH membrane and injected directly into the column. The column temperature was 30 C (45 C for the separation of γ-tocopherol and β-tocotrienol). A Perkin-Elmer M3000 fluorescence spectrometer was the detector. The excitation and emission wavelengths were 290 and 325 nm. Calibration was made with purified tocopherols as described before (5). The recovery of α-, β-, γ- and δ-tocopherols added to oils was essentially quantitative (mean values 98%, 96%, 98% and 98%). The individual tocopherol and tocotrienol values were converted to α-tocopherol equivalents (mg/100 g) (8).

The polyunsaturated fatty acid contents were calculated from fatty acid compositions determined in this laboratory by gas chromatographic methods (9).

RESULTS AND DISCUSSION

The proportions of the individual tocopherols (α-T, β-T, γ-T and δ-T) and tocotrienols (α-T3, β-T3, γ-T3 and δ-T3) and the total tocopherol contents of the oils are given in Table 1. The results are the mean values (6-10 determinations) of each oil purchased from 3 to 5 different manufacturers. All tocopherols and tocotrienols were found, although tocotrienols were not detected in every oil. Tocotrienols might have been present, but the determination of tocopherols and tocotrienols together was difficult because of very large differences in concentration. Differences between crude and refined rapeseed, soybean, sunflower and palm oils were determined. The refining losses were about 10-33% of the α-tocopherol, 20-33% of the other tocopherols and 43-48% of the tocotrienols.

The contents of the tocopherols and tocotrienols in margarines (representing the most popular brands from all five manufacturers in Finland) are given in Table 2. The tocopherol content and composition of the margarines varied greatly, in accordance with the oils and fats used in their manufacture. On the basis of their tocopherol content the margarines can be divided into three groups. The first group comprises the hard margarines containing α-, β-, γ- and δ-tocopherols at about 7, 0, 17 and 3 mg/100 g, respectively. The second and third groups contain the semisoft and soft margarines with corresponding tocopherol figures of 13, 0, 32 and 8 mg and 24, 1, 27 and 9 mg/100 g. With the exception of β-tocotrienol, small amounts of tocotrienols were found; none were detected at those sample concentrations used for the measurement of the tocopherols.

The butter samples contained α-tocopherol (2 mg/100 g in summer and 1 mg/100 g in winter) and small amounts of α-tocotrienol (4). In Voimariini, a butterfat-vegetable oil mixture, the tocopherol level reflected the proportion of the oil ingredient used, being higher in winter than in summer (Table 2).

The α-tocopherol (and α-tocopherol equivalent)/PUFA ratio (Table 1) was low in linseed, peanut and soybean oil: 0 (0.1), 0.3 (0.3) and 0.2 (0.3) mg/g, respectively. All the margarines whose PUFA content had been determined were found to have, with one exception, α-tocopherol equivalent/PUFA ratios higher than the value of 0.6 mg/g (11). The α-tocopherol/PUFA ratio was less than 0.6 mg/g in three brands only. For butter and Voimariini average ratios of 0.8 and 0.6 mg/g, respectively, were found.

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