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Lipids and Fatty Acids of a Whitefish (*Coregonus albula*) Flesh and Roe

Jukka K. Kaitaranta

Technical Research Centre of Finland, Laboratory for Food Research and Technology, 02150 Espoo 15, Finland and University of Turku, Department of Chemistry and Biochemistry, 20500 Turku 50, Finland

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The total lipids of whitefish flesh and roe averaged 3.0 and 9.8% fresh weight, respectively. Although neutral lipids dominated in both tissues the polar lipid content of fresh roe was unexceptionally high (2.5%). The fatty acid patterns of the total lipids from flesh and roe and of neutral lipids were similar to each other. Polyenoic acids constituted about half of the total fatty acids and monoenoic and saturated components existed in equal amounts. The calculated iodine values for the total lipids from flesh and roe indicated a slightly higher degree of unsaturation for roe fatty acids. The polar lipids were enriched in polyenoic and saturated fatty acids. Similarities in the composition and content of fatty acids from flesh and roe suggest that storage problems, apparent as a rancidity of roe products, may result from the high content of polar lipids readily hydrolysed during storage.

1. Introduction

Whitefish is a common name for a group of *Coregonous* spp. which belong to the genus *Salmonidae*. Whitefish are widely distributed over North America, East Asia and Europe and are generally highly regarded as a food fish. In addition to fillet products, whitefish roe salted as caviar is consumed in Finland where its utilisation amounts to about 250 t year^{-1}.[1] A lucrative whitefish roe industry has recently developed in Canada based mainly on exports to Japan.[2] The Saskatchewan lakes accounted for over half (about 2.5 t) of whitefish caviar processed by the inland fisheries in 1978. This annual prairie caviar production could be increased to approximately 10 t in Saskatchewan.[2]

The caviar-type roe products are available in Finland during the spawning time of the fish and for some weeks after spawning. Rapid deteriorative enzymic and chemical processes take place in salted cold-stored roe products, in particular the hydrolysis and oxidation of lipids.[3] Moreover, lipid hydrolysis in fish is generally accompanied by protein denaturation,[4] hence lipids are of practical importance to the overall quality of these fish products.

Fatty acids of two whitefish, *Coregonus artedii*[5,6] and *Coregonus albula*,[7] have been described previously. The composition of mature roe,[8] and changes in the lipid classes during maturation,[9] of *C. albula* have also been studied. In this study the composition of whitefish roe lipids has been studied by thin-layer chromatography–flame ionisation detection (t.l.c.–f.i.d.) methods. The fatty acid composition of roe was determined by glass capillary gas–liquid chromatography (g.l.c.) and is compared to the composition of whitefish flesh fatty acids.

2. Experimental

Fish samples consisting of about 100 fully matured specimens were collected during the spawning period of *C. albula* in the autumn of 1972, 1973, 1975 and 1976. Fillet samples of mature female

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whitefish were taken in 1975 and 1976. The roe samples were pooled annually and these and the fillet samples were stored at −18°C for further analysis. Under these conditions the samples could be stored for a few months without any measurable oxidation of lipids.[3]

Lipids were extracted by the method described by Bligh and Dyer[10] followed by the separation of neutral and polar lipids on a silicic acid column. The fatty acid composition of each fraction was determined by g.l.c. and g.l.c.–m.s. as described previously.[7] The peak areas from the g.l.c. analyses were converted to weight percentages using appropriate conversion factors.[11]

2.1. Lipid class determinations

For quantitative lipid class determinations Chromarod-S quartz rods with a coating of silica gel were used in an Iatroscan TH-10 Analyzer (Iatron Laboratories Inc., Japan, distribution by Newman-Howells Assoc. Ltd, UK). The rods were stored in 4.5M H_2SO_4 and were rinsed with distilled water immediately before use. The activation of the rods was accomplished by passing them through the flame of the detector.

Standard mixtures and the samples (1–10 μg lipid) were spotted on rods using disposable micro pipettes (Drummond Microcap, Drummond Scientific Co., USA). The rods were developed using a two-step method in paper-lined glass tanks. The first development (17.5 min) was performed using a mixture of petroleum ether–benzene–formic acid (92 : 17 : 1). After drying for about 5 min, the rods were developed again for 17.5 min in a mixture of petroleum ether–diethyl ether–formic acid (97 : 4 : 1). After drying, the lipid classes were determined on the Iatroscan Analyzer which was connected to a Linear Instruments Co. (Irvine, USA) Model 252 A recorder. The combination of the instruments and the technical conditions for the analyses were as described by Sipos and Ackman.[12] The peak areas were changed to weight percentages using appropriate conversion factors established with standard compounds.

2.2. Column chromatography

Silicic acid column chromatography was applied to separate neutral and polar lipids. Silicic acid (Mallinckrodt Chemical Works, USA) was screened and the fraction from the 100–200 mesh was collected. The adsorbent was washed several times with distilled water and any floating material removed by decantation. The washed silicic acid was filtered and activated for 20 h at 115°C. This acid had a weight loss of 4.0–6.1% when heated for 30 min at 500°C.

The chromatographic columns (14 mm i.d., 20 cm high) were prepared and the lipids fractionated according to the method described by Rouser *et al.*[13] The maximum load of 15 mg lipid g^{-1} silicic acid was applied. The column chromatographic separation was monitored by t.l.c. on silica gel G (E. Merck AG, Germany) developed in a mixture of hexane–diethyl ether–acetic acid (85 : 15 : 1). The plates were sprayed with H_2SO_4 and then charred in order to locate the different bands.

3. Results

The lipid content of whitefish roe and fillets ranged from 9.1 to 11.4% (9.8% on average) and from 1.8 to 4.3% (3.0% on average), respectively, as calculated on a wet weight basis. No significant variations were found between samples of successive years. The Iatroscan chromatograms for standard compounds and for roe lipids are illustrated in Figure 1. Quantitative results from t.l.c. and column chromatographic determinations are presented in Table 1. The fatty acid compositions and the calculated iodine values of various lipid fractions are presented and compared in Table 2.

4. Discussion

4.1. Lipid composition

The roe of whitefish, like those of salmon and other *Salmonid* fishes,[8,14–16] contain a large proportion of fat which consists mainly of neutral lipids. These together form the major lipid class in fillets (Table 1). However, if calculated on a wet weight basis the polar lipids in fillets are found to

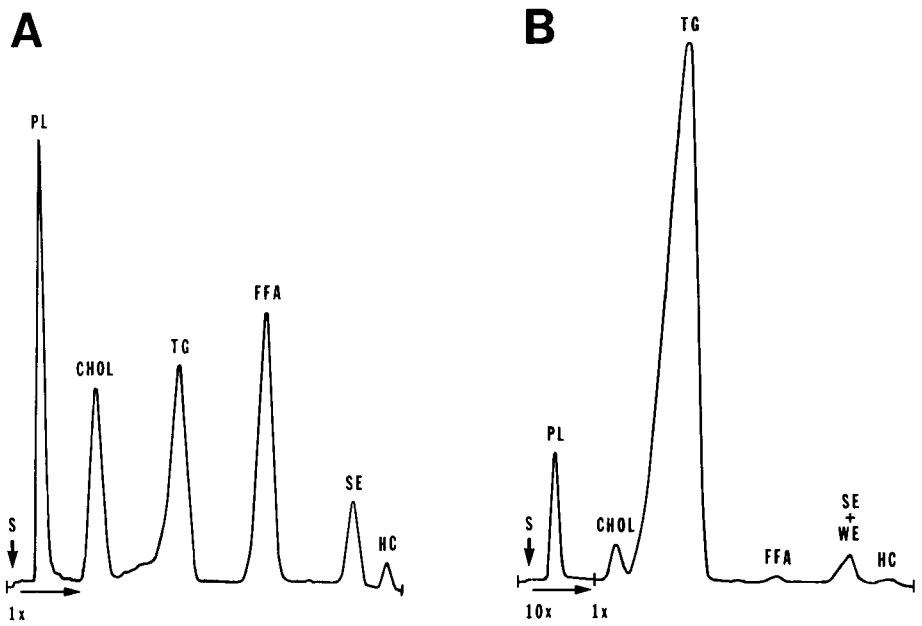

Figure 1. Separation of standard lipid compounds (A) and whitefish roe lipids (B) with an Iatroscan Analyzer. Analytical methods are presented in the text. Note attenuation change from 10× to 1× in the part B after polar lipid position. S= start of scan; PL=polar lipids; CHOL=cholesterol; TG=triglyceride; FFA=free fatty acid; SE=sterol ester; WE=wax ester; and HC=hydrocarbon.

Table 1. Lipid class composition of whitefish flesh and roe. The determinations were made using the column chromatography on silicic acid and the quantitative thin-layer chromatography with an Iatroscan Analyzer. The average values and ranges are expressed as weight percentages of the total lipids

	Flesh	Roe	
Lipid group	SiO_2-chromatography	SiO_2-chromatography	Iatroscan
Polar lipids	33.7 28.7–38.8	26.1 22.2–29.9	31.7 28.4–36.9
Neutral lipids	66.3 61.2–71.3	73.9 70.1–77.8	68.3[a]
Triglycerides			64.9 59.3–68.7
Cholesterol			1.4 1.1–1.8
Free fatty acids			0.02 0–0.10
Sterol and wax esters			1.8 1.1–2.6
Hydrocarbons			0.23 0.08–0.44
Number of samples	2	8	7

[a] Calculated as the sum of the neutral lipid subfractions.

Table 2. Fatty acid composition of the total lipids and the neutral and polar lipid fractions from whitefish flesh and roe

Fatty acid[a]	Flesh[b]			Roe[b]		
	TL	NL	PL	TL	NL	PL
12:0	0.16	0.26	0.03	0.04	0.04	ND
13:0	0.05	0.07	ND	0.01	0.02	ND
i-14:0	0.14	0.22	ND	0.08	0.10	ND
14:0	6.1	8.6	2.5	4.6	4.9	2.9
i-15:0	0.66	1.1	0.05	0.58	0.64	0.31
ai-15:0	0.30	0.61	0.02	0.22	0.27	0.07
15:0	0.47	0.53	0.38	0.61	0.58	0.73
i-16:0	0.16	0.19	0.01	0.24	0.24	0.19
16:0	15.9	12.3	21.8	15.6	14.1	26.0
i-17:0	0.38	0.41	0.10	0.53	0.61	0.57
ai-17:0	0.66	0.73	0.11	0.70	0.77	0.53
17:0	0.63	0.52	0.32	0.51	0.54	0.47
i-18:0	0.18	0.19	0.02	0.14	0.15	0.15
18:0	3.9	3.6	3.3	2.1	2.5	2.0
19:0	0.17	0.13	0.02	0.09	0.12	0.01
Σ	29.9	29.5	28.7	26.1	25.6	33.9
16:1ω9	0.65	0.75	0.32	0.80	0.81	0.76
16:1ω7	4.5	5.5	1.1	4.6	5.3	1.5
16:1ω5	0.52	0.47	0.51	0.59	0.63	0.66
17:1	0.34	0.36	0.04	0.62	0.67	0.36
18:1ω9	10.3	11.9	4.5	10.3	11.5	6.9
18:1ω7	3.0	3.2	1.9	3.0	3.5	2.0
18:1ω5	0.18	0.23	0.06	0.34	0.45	0.21
20:1ω9	0.68	0.88	0.25	0.16	0.22	0.09
20:1ω7	0.14	0.26	0.05	0.18	0.21	0.05
7-Me-16:1[c]	0.46	0.41	0.12	0.36	0.41	0.41
Σ	20.8	24.0	8.9	21.0	23.7	12.9
16:2ω4	0.50	0.43	0.04	0.42	0.38	0.45
18:2ω6	4.4	4.6	1.2	3.4	3.8	1.5
20:2ω6	0.39	0.51	0.26	0.34	0.34	0.20
Σ	5.3	5.5	1.5	4.2	4.5	2.2
18:3ω6	0.53	0.55	0.05	0.46	0.53	0.12
18:3ω3	4.6	5.1	1.7	4.9	5.5	1.2
20:3ω6	0.31	0.38	0.12	0.37	0.32	0.21
20:3ω3	0.35	0.41	0.19	0.54	0.75	0.24
Σ	5.8	6.4	2.1	6.3	7.1	1.8
16:4ω1	0.12	0.14	0.01	0.11	0.12	0.02
18:4ω3	3.9	5.1	0.75	5.1	5.5	0.96
18:4ω1	0.26	0.39	0.05	0.07	0.12	ND
20:4ω6	4.9	4.6	7.0	5.3	5.1	6.3
20:4ω3	1.7	2.0	1.0	2.1	2.5	0.85
22:4ω6	0.55	0.64	0.84	0.20	0.30	0.25
Σ	11.4	12.9	9.7	12.9	13.6	8.4
20:5ω3	8.7	8.0	11.2	10.6	9.8	9.8
21:5ω3	0.25	0.38	ND	0.26	0.30	0.13
22:5ω6	2.0	2.0	3.4	1.7	1.6	2.6
22:5ω3	3.1	3.5	2.4	2.5	2.3	3.1
Σ	14.1	13.9	17.0	15.1	14.0	15.6
22:6ω3	13.4	8.4	32.6	14.9	12.0	25.7
Calculated i.v.	194	180	257	210	200	221

[a] Shorthand notation implies chain length: number of double bonds. The addition of 'ω' gives the position of double bond closest to the terminal methyl group. The notations i- and ai- imply the branched-chain *iso*- and ante*iso*-structures, respectively.

[b] TL = total lipids, NL = neutral lipids, PL = polar lipids.

[c] 7-Methylhexadecenoic acid, the percentages calculated from hydrogenated samples.

ND = not detected.

be about 1% (33.7% of the total lipids), and the comparable value for roe exceeds 2.5%. For normal metabolic functions 0.5–1% phospholipids, on a wet weight basis, are needed in fish tissues.[17,18] Thus roe is extremely rich in polar lipids. In coho salmon (*Oncorhynchus kisutch*) Braddock and Dugan[19] have found that the increase in the amount of free fatty acids during frozen storage of muscle was primarily due to phospholipid hydrolysis. The large proportion of polar lipids and their high susceptibility to lipid hydrolysis may be one reason for the storage problems encountered with whitefish roe products.[3]

Iatroscan analysis revealed that triglycerides comprised about 65% of the total lipids in roe (Table 1). Lizenko *et al.*[9] reported that the proportion of triglycerides in the lipids (10.9% wet weight) of mature whitefish roe was 29%, whereas phospholipids were the major lipid class (55%). For lipid extractions they used a mixture of chloroform and methanol [2 : 1 (v/v)] but omitted the subsequent washing of the extract with water. Thus, the lipid extracts apparently included some non-lipid material which later interfered with the lipid class analyses and decreased the reliability of the determinations.

Cholesterol amounted to 1.4% of lipids which corresponds to 0.13% in the fresh roe (Table 1). This amount of cholesterol is approximately twice the value usually found in fish muscle, but it is only one-quarter of the value for a hen egg (0.07 and 0.55%, respectively, US Department of Agriculture, Handbook No. 8, 1963). Sterol and wax esters which were not separated by the Iatroscan analysis constituted 1.8% of the total lipids.

4.2. Fatty acid composition

In a previous study the total fatty acid composition of whitefish flesh lipids was discussed.[7] With some exceptions the fatty acid compositions of neutral lipids in flesh and roe are very similar (Table 2). Indeed, since neutral lipids are the dominant components, the total fatty acids in flesh and roe are also similar. However, the percentage of myristic acid (14 : 0) in flesh neutral lipids is higher than that in roe lipids. However, the proportions of 20 : 5ω3 and 22 : 6ω3 are higher in roe neutral lipids. These differences are also reflected in the calculated iodine values which are 16–20 units higher for roe fatty acids.

The polar lipids of whitefish roe and flesh are rich in saturated and polyenoic fatty acids; monoenoic acids are present only in small proportions. Approximately 75% of the saturated fatty acids are composed of palmitic acid (Table 2), which is usually one of the major fatty acids in fish phospholipids. As presented in Table 2, monoenes in neutral lipids of flesh and roe constitute about one-quarter of the fatty acids as compared to only 8.9 and 12.9% in the flesh and roe polar lipids, respectively. The major polyenoic acid in the polar lipids is docosahexaenoic acid (22 : 6ω3) which averaged 32.6 and 25.7% in the flesh and roe, respectively. Differences are also found in the iodine values of the polar lipids from flesh and roe, which were 257 and 221, respectively.

5. Conclusions

The approximate composition of roe has been shown to vary according to the fish species.[8] This is also the case with the lipid and fatty acid compositions of roe which are not necessarily related to the composition of depot fat in fish. Differences between the mesenteric and roe lipid fatty acids have been shown for Pacific sardine (*Sardinops caerulea*),[20] whereas the component fatty acids of cod (*Gadus morhua*) roe have been reported to resemble the flesh lipid fatty acids to a considerable degree.[21] Mullet (*Mugil cephalus*) deposit wax esters as the major lipid component in roe although the other tissues have triglycerides as the major lipids.[16]

Whitefish roe contain more polar lipids, on a wet weight basis, than does the flesh. However, the profiles of fatty acids for polar lipids in each case are very similar, and thus it may be assumed that these are functional lipids, primarily associated with membranes.

It is known that some fish mobilise depot fat at the time of egg maturation, probably partly for the formation of roe lipids and partly for energy purposes.[22–24] Analyses carried out on Finnish whitefish show that there is no extreme depletion of neutral lipids in the muscle and the fish has ample reserves of fatty acids for the production of roe lipids. Moreover, the fatty acid similarities

in flesh and roe suggest that the storage quality problem in the roe may result from the high content of polar lipids, known to be subject to autolytic and oxidative processes, rather than to differences in the fatty acid compositions.

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