Effects of Season and Processing on Oil Content and Fatty Acids of Baltic Herring (*Clupea harengus membras*)

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Fatty acid composition, oil content, free fatty acid content, and peroxide value of Baltic herring (*Clupea harengus membras*) and two processed products (fried fillets and fish burgers) were investigated. The highest oil content of the fillets was found in autumn (10%), at the time when the free fatty acids had their minimum (1.4%). The main fatty acids were oleic (18–23%), palmitic (17%), palmitoleic (8–12%), and docosahexaeneoic (8–10%) acids. The proportion of saturated fatty acids was a constant 23% all year around, whereas mono- and polyunsaturated acids varied from 34 to 39% and 33 to 37%, respectively. During processing the oil content doubled and the fatty acid composition changed to the pattern of the rapeseed oil used for frying. Oleic acid was a major fatty acid in the products comprising over 40% of the total fatty acids. The proportion of n-3 acids decreased during processing but the total amount of polyunsaturated acids remained fairly constant.

INTRODUCTION

Baltic herring (*Clupea harengus membras*), a subspecies of the Atlantic herring, is an important raw material for the Finnish fish processing industry. In 1997, about 20 000 tons of Baltic herring was processed, being more than half of all fish used in the industry. The main products were fresh fillets and frozen fillets that constituted 87% of all Baltic herring products processed. About 6% was processed as smoked Baltic herring, whereas the amount of other ready-to-eat products was low (Finnish Game and Fisheries Institute, 1999).

Baltic herring is a fatty fish, the lipid content of which varies from 2% to 11% (Linko, 1967; Linko et al., 1985; Kallio et al., 1998) being lower still than that in Atlantic herring (Henderson and Almatar, 1989). About 65% of Baltic herring flesh lipids are composed of triacylglycerols, and about one-third is phospholipids. The proportions of diacylglycerols, cholesterol, and free fatty acids are negligible. The fat content and lipid composition show seasonal variation which is obviously related to spawning time and the plankton feed available (Linko et al., 1985; Rajasilta, 1992a). According to the spawning time, Baltic herrings can be divided into two groups: spring and autumn spawners. In Finnish coastal areas the majority of herrings spawn between May and July, and the proportion of autumn spawners is low. The time is dependent on feeding conditions, water temperature and other environmental factors (Hahtonen and Joensuu, 1984; Oulasvirta et al., 1985; Rajasilta, 1992b). Seasonal variation in lipid content and fatty acid composition has also been noticed in other species of the genus *Clupeidae* (Bandarra et al., 1997; Krynowek et al., 1992).

Seasonal variation of many freshwater and marine fish has been studied intensively (Ågren et al., 1993; Fidanza et al., 1992; Gallagher et al., 1991; Mazzotta

et al., 1993; Schwalme et al., 1993) but little attention has been paid to the use of those fish species which show seasonal variation as a raw material in the fish processing industry. A major question is whether it is possible to keep the content of fish products constant when the lipid composition of raw material fluctuates according to season.

The aim of this study was to investigate the total lipid content and fatty acid composition of Baltic herring and two ready-to-eat products made of it. The quality of lipids in raw materials and products was investigated by analyzing the content of free fatty acids and peroxide value. Special attention was paid to the effect of industrial processing on lipids. Also, the seasonal variation in the lipid composition of Baltic herring and its effects on the industrial products were investigated.

MATERIALS AND METHODS

Sample Collection and Preparation. The Baltic herrings (*Clupea harengus membras*) were caught between March 1996 and September 1998 in the northern area of the Baltic Sea (the Archipelago Sea and the Gulf of Bothnia). The fish were caught by trawling or trap nets by professional fishermen. In some cases, part of the fish was manually filleted immediately after catching. After landing, the herrings were sorted and mechanically headed and gutted in a fish processing plant (size class 0: 18–24 fish/kg). When being prepared as fillets they were also deboned (size class 00: 12–17 fish/kg). Filleted and gutted fish were packed in plastic containers under ice and transported to the food factory.

Two commercial Baltic herring products were processed in a local food factory (Lännen Tehtaat Ltd., Turku). One product was a fried double fillet with rye breading, salt, and spices. The other was a burger of minced fish, breadcrumbs, salt, spices, and wheat breading. The fillets were dipped in rapeseed oil (about 57% oleic acid, 22% linoleic acid, and 11% α-linolenic acid) before frying in a tunnel oven, whereas the burgers were deep fried. The frying time was about 2 min and the temperatures were about 250 °C for fillets and 170 °C for burgers. Both products were frozen in a belt freezer.

The study was carried out on 43 batches of fried fillets and 12 batches of burgers. The samples were collected at several consecutive stages of the process. One batch of fried fillets

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included five subsamples in total: fish fillets, which were manually filleted immediately after catching (1A, number of subsamples $n = 13$); mechanically filleted fish from the processing plant (1B, $n = 30$); mechanically filleted fish transported to the food factory (1C, $n = 43$); raw fish fillets with rye breading, salt, spice, and rapeseed oil (1D, $n = 34$); and fried, frozen fillets (1E, $n = 43$). One batch of burger included six subsamples in total: gutted and headed fish from the processing plant (2A, $n = 11$); gutted and headed fish transported to the food factory (2B, $n = 12$); minced, deboned, and skinned fish (2C, $n = 12$); minced fish with breadcrumbs, salt, and spice, (2D, $n = 12$); formulated, coated product before frying (2E, $n = 12$); and deep fried, frozen burger (2F, $n = 12$). Seasonal variation was studied using fillets from the fish processing plant (1B).

All samples (about 2 kg) were packed in low-density polyethylene (LDPE) bags and transported to the laboratory in insulated containers together with solid carbon dioxide. The samples were stored at -70 °C until analyzed. Before analysis, thawed samples were homogenized using a Robot Coupe R8 (Robot Coupe S. A., Bourgogne, France) homogenizer.

Extraction of Lipids for the Analysis of Peroxide Value and Free Fatty Acid Content. Lipids were extracted according to the modified method of Bligh and Dyer (1959). About 60 g of the sample in 120 mL of chloroform/methanol (1:2, v/v) was homogenized by an Omni-Mixer Homogenizer (OMNI International, Waterbury, CT) for 2 min, 60 mL of chloroform was added, and homogenizing continued for 1 min. Then 60 mL of distilled water was added, the mixing continued for 1 min, and the mixture was filtered in a vacuum. The residue was homogenized with 60 mL of chloroform, 60 mL of methanol, and 54 mL of distilled water for 2 min, then followed by vacuum filtration. The supernatants were combined in a separatory funnel and stored at 4 °C for at least 2 h to separate into two phases. The lower phase was isolated and dried with Na_2SO_4 for 1 h. The solvent was removed in a rotary evaporator in a vacuum. The lipid fraction was stored at -70 °C in a dark vial, the headspace volume of which was replaced with nitrogen. Each sample was extracted once, and the oil was used for the determination of peroxide value and the free fatty acid content.

Extraction of Lipids for the Analysis of Lipid Content and Fatty Acids. Lipids were extracted by the Folch method (1957). About 2 g of the sample in 20 mL of methanol was homogenated by an Ultra-Turrax T25 (IKA, Janke and Kunkel, Staufen, Germany) for 1 min at 8000 rpm, 40 mL of chloroform was added, and the mixing continued for 2 min. The mixture was filtered in a vacuum, and the extraction procedure for the residue was repeated. The supernatants were combined. The residue was washed with 20 mL of methanol and 40 mL of chloroform, which were then combined with the supernatants. The extract was washed with 45 mL of 0.88% potassium chloride (aqueous) solution and then with 45 mL of water/methanol (1:1, v/v). The solvents were evaporated using a rotary evaporator, and the lipid fraction was dissolved in hexane containing 0.02% butylated hydroxytoluene. The headspace volume of the sample vials was replaced with nitrogen, and the vials were stored at -20 °C for esterification. Two subsamples of each sample were extracted.

Fatty Acid Analysis. Fatty acid methyl esters were prepared by a base-catalyzed transesterification according to Christie (1982). They were analyzed using a Perkin-Elmer AutoSystem gas chromatograph (Norwalk, CT) equipped with a flame ionization detector, a split injector, and a fused silica capillary column (NB-351, 25 m × 0.32 mm i.d., 0.2 μm film thickness, Nordion, Helsinki, Finland). The temperature program was 2 min at 120 °C, 3 °C/min to 240 °C, and 20 min at 240 °C. The temperatures of the injector and detector were 225 and 240 °C, respectively. Helium was used as a carrier gas with a split ratio of about 40:1. All fatty acid methyl esters were analyzed in duplicate. Fatty acids were identified using a mixture of commercial fatty acid methyl esters 68 D (Nu Chek Prep, Elysian, MN).

Peroxide Value. The peroxide value was determined using a microdetermination method of Asakawa and Matsushita

Table 1. Oil Content (w/w, %) and Peroxide Value (meq/kg) and Free Fatty Acid Content (%) in the Oil of Baltic Herring Fillets in Different Seasons (Mean ± Standard Deviation).

season	number of samples	oil content	peroxide value	free fatty acid content
Spring (Mar, Apr, May)	9	6.3 ± 1.5^a	2.7 ± 0.9^a	3.2 ± 1.1^a
Summer (Jun, Jul, Aug)	11	5.2 ± 0.6^b	2.6 ± 1.5^a	3.7 ± 0.6^a
Autumn (Sep, Oct, Nov)	7	10.4 ± 1.3^c	2.6 ± 1.3^a	1.4 ± 0.1^b
Winter (Dec, Jan, Feb)	3	9.4 ± 0.8^c	2.8 ± 0.8^a	1.7 ± 0.3^b

$^{a-c}$ Different characters in the same column are significantly different ($p < 0.05$).

(1980). Hydroperoxides reacted with potassium iodide in the presence of aluminum chloride as an acid catalyst. Liberated iodine was measured using an Ultrospec 4050 spectrophotometer (LKB Biochrom, Cambridge, UK) at 560 nm after the addition of starch in 0.01 M hydrochloric acid. The sample weight used in the analysis was about 200 mg. Each sample was analyzed in triplicate.

Free Fatty Acid Content. The free fatty acid content was analyzed by titration of approximately 0.5 g of oil, dissolved in a mixture of 150 mL of ethanol and diethyl ether (1:1,v/v), with 0.01 M potassium hydroxide. Phenolphthalein was the indicator. The results were expressed as percentage of oleic acid. All samples were analyzed in duplicate.

Statistical Analysis. Statistical analysis was performed using SAS software, version 6.11 and SPSS software, version 7.5. The comparisons of fat content, fatty acid compositions, peroxide value, and free fatty acid content between different seasons and between processing stages were tested using an ANOVA test. The relation of fat content and fatty acid composition in raw materials to that in the products were also subjected to an ANOVA test. For comparisons, the fried fillets were placed in three groups and the burgers were placed in two groups according to the contents in the corresponding raw material. The groups were formed by taking into consideration the mean contents in raw materials but also the number of samples in each group. In the figures, an outlier means cases with values between 1.5 and 3 box lengths from the upper or lower edge of the box, and an "extreme" means cases with values more than 3 box lengths from the upper or lower edge of the box. The box length is the interquartile range.

RESULTS AND DISCUSSION

The results of oil content, peroxide value, free fatty acid content, and fatty acid composition are grouped according to the four seasons in Tables 1 and 2. The corresponding results concerning the various steps of production are listed in Tables 3−6. The only problem in quantification was the overlapping of docosahexaenoic (DHA, 22:6n-3) acid and tetracosaenoic (24:1n-9) acid in the chromatograms. Random analyses with a capillary column coated with polyethylene glycol (HP-INNO-Wax) showed that in Baltic herring. DHA comprised the majority, quite constantly about 90% of the peak.

Seasonal Variation. The oil content of the Baltic herring fillets varied from 4 to 11% (of wet weight). Fish caught in autumn and winter were significantly more fatty than those caught in spring or summer (Table 1). In summer the oil content decreased to its lowest level, being related to spawning and feeding activity. The majority of Finnish Baltic herring spawn between May and July (Hahtonen and Joensuu, 1984; Oulasvirta et al., 1985; Rajasilta, 1992b), and during this time they

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Table 2. Fatty Acids (w/w % of Total Fatty Acids) of Baltic Herring Fillets in Different Seasons (Mean ± Standard Deviation). Number of Samples Analyzed in Parentheses.

fatty acid	Spring (9) (Mar, Apr, May)	Summer (11) (Jun, Jul, Aug)	Autumn (7) (Sep, Oct, Nov)	Winter (3) (Dec, Jan, Feb)
14:0	3.45 ± 0.16[a]	3.60 ± 0.18[b]	3.78 ± 0.21[c]	3.65 ± 0.05[bc]
16:0	17.12 ± 0.88[a]	16.84 ± 0.25[a]	17.17 ± 1.07[a]	16.63 ± 0.49[a]
18:0	1.27 ± 0.04[a]	1.31 ± 0.07[a]	1.02 ± 0.06[b]	1.06 ± 0.05[b]
others	1.58 ± 0.15	1.62 ± 0.11	1.69 ± 0.14	1.63 ± 0.28
Σ saturated	23.41 ± 0.97[a]	23.36 ± 0.30[a]	23.67 ± 1.06[a]	22.97 ± 0.24[a]
16:1n-7	8.41 ± 1.38[a]	8.58 ± 0.78[a]	9.51 ± 1.38[b]	11.87 ± 1.07[c]
18:1n-9	23.13 ± 1.25[a]	22.39 ± 1.21[a]	17.79 ± 1.62[b]	19.50 ± 0.62[c]
18:1n-7	3.26 ± 0.42[a]	3.38 ± 0.36[a]	3.51 ± 0.33[a]	3.82 ± 0.06[b]
20:1n-9	2.29 ± 0.34[a]	2.24 ± 0.29[a]	1.88 ± 0.15[b]	1.64 ± 0.14[c]
others	1.93 ± 0.20	1.87 ± 0.16	1.95 ± 0.16	1.64 ± 0.11
Σ monounsaturated	39.01 ± 1.22[a]	38.46 ± 0.92[b]	34.64 ± 2.22[c]	38.47 ± 1.37[ab]
18:3n-3	1.26 ± 0.22[a]	1.14 ± 0.14[b]	1.71 ± 0.20[c]	1.53 ± 0.08[d]
18:4n-3	1.04 ± 0.18[a]	1.06 ± 0.25[a]	1.99 ± 0.11[b]	1.85 ± 0.10[c]
20:3n-3	1.00 ± 0.14[a]	0.81 ± 0.10[b]	1.47 ± 0.28[c]	1.19 ± 0.14[d]
20:4n-3	1.21 ± 0.14[a]	1.12 ± 0.09[a]	1.82 ± 0.16[b]	1.60 ± 0.19[c]
20:5n-3	4.49 ± 0.51[a]	4.74 ± 0.40[a]	5.41 ± 0.47[b]	5.92 ± 0.28[c]
22:4n-3	1.57 ± 0.15[a]	1.42 ± 0.11[b]	1.90 ± 0.22[c]	1.56 ± 0.20[ab]
22:5n-3	0.90 ± 0.05[a]	0.89 ± 0.03[a]	1.01 ± 0.09[b]	0.92 ± 0.06[a]
22:6n-3+24:1n-9	8.26 ± 1.04[a]	9.69 ± 1.12[b]	7.71 ± 0.95[a]	7.81 ± 0.53[a]
24:4n-3	1.87 ± 0.23[a]	1.59 ± 0.32[ac]	2.10 ± 0.36[b]	1.60 ± 0.21[c]
24:5n-3	1.04 ± 0.12[a]	0.93 ± 0.17[a]	1.05 ± 0.16[a]	0.85 ± 0.10[b]
others	2.20 ± 0.29	2.11 ± 0.55	2.44 ± 0.53	1.71 ± 0.33
Σ n-3	24.84 ± 0.87[a]	25.49 ± 0.94[b]	28.62 ± 1.43[c]	26.54 ± 0.94[ab]
18:2n-6	4.88 ± 0.55[ac]	4.42 ± 0.24[b]	4.96 ± 0.48[c]	4.54 ± 0.25[ab]
20:2n-6	2.25 ± 0.27[a]	1.96 ± 0.27[b]	2.34 ± 0.34[ac]	1.95 ± 0.23[b]
22:2n-6	1.48 ± 0.26[a]	1.28 ± 0.31[ab]	1.41 ± 0.28[a]	1.10 ± 0.19[b]
others	0.48 ± 0.07	0.57 ± 0.07	0.42 ± 0.06	0.50 ± 0.03
Σ n-6	9.09 ± 0.96[ac]	8.24 ± 0.69[bd]	9.12 ± 1.01[bc]	8.09 ± 0.64[d]
Σ polyunsaturated	33.93 ± 0.92[a]	33.73 ± 0.77[a]	37.74 ± 2.14[b]	34.64 ± 1.22[a]
unidentified	3.66 ± 1.22	4.45 ± 0.21	3.95 ± 0.33	3.93 ± 0.32

[a–d] Different characters in the same row are significantly different ($p < 0.05$).

Table 3. Oil Content (w/w, %), Peroxide Value (meq/kg), and Free Fatty Acid Content (%) in the Processing of Fried Fillets (Mean ± Standard Deviation). Number of Samples Analyzed in Parentheses.

stage of process	oil content	peroxide value	free fatty acid content
1A. fillets after catching	6.6 ± 1.8[a] (13)	3.3 ± 0.8[a] (13)	2.6 ± 0.6[a] (13)
1B. fillets in fish processing plant	7.2 ± 2.4[a] (30)	2.6 ± 1.4[b] (30)	2.8 ± 1.2[a] (30)
1C. fillets transported to food factory	6.4 ± 2.3[a] (43)	2.7 ± 1.7[bd] (25)	3.6 ± 1.0[b] (25)
1D. fillets with rye breading, salt, spice, and rapeseed oil	15.5 ± 1.5[b] (34)	1.9 ± 1.2[c] (25)	1.4 ± 0.3[c] (25)
1E. fried fillets	11.9 ± 1.3[c] (43)	2.2 ± 1.3[cd] (43)	1.4 ± 0.2[c] (43)

[a–d] Different characters in the same column are significantly different ($p < 0.05$).

Table 4. Oil Content (w/w, %), Peroxide Value (meq/kg), and Free Fatty Acid Content (%) in the Processing of Fish Burger (Mean ± Standard Deviation). Number of Samples Analyzed in Parentheses.

stage of process	oil content	peroxide value	free fatty acid content
2A. gutted fish in fish processing plant	6.0 ± 1.2[a] (11)	2.9 ± 1.3[a] (11)	2.9 ± 0.7[a] (11)
2B. gutted fish transported to food factory	6.2 ± 1.4[a] (12)	2.4 ± 2.1[ac] (7)	3.8 ± 0.9[b] (7)
2C. minced fish	5.4 ± 1.2[a] (12)	3.2 ± 2.5[ac] (7)	4.3 ± 1.2[b] (7)
2D. minced fish with breadcrumbs, salt, and spice	5.1 ± 1.0[a] (12)	2.4 ± 1.7[ac] (7)	4.6 ± 1.3[b] (7)
2E. formed, coated product	4.1 ± 0.8[b] (12)	2.6 ± 2.0[ac] (7)	3.9 ± 1.1[ab] (7)
2F. fried burger	12.1 ± 1.2[c] (12)	1.8 ± 0.9[c] (12)	1.4 ± 0.5[c] (12)

[a–c] Different characters in the same column are significantly different ($p < 0.05$).

consume very little feed. Late summer and autumn are the periods of active feeding (Arrhenius and Hansson, 1993). The seasonal variation in oil content was analogous to that reported earlier for Baltic herring (Linko, 1967; Linko and Karinkanta 1970; Linko et al., 1985). However, in earlier studies the total oil content varied from 2.1 to 7.2%, which was less than in the present study. It should be noted that in our investigation the herrings were collected mainly for industrial purposes and thus the smallest fish were removed during sorting. In addition, the fish were caught in a rather large area of the Northern Baltic and, despite sorting, there

appeared to be a variation in size and oil content according to the catching ground. The fish from the Gulf of Bothnia seemed to be larger and fattier than the fish caught in the Archipelago Sea.

The free fatty acid content of the fillet oil was significantly lower in autumn and winter compared to spring and summer. The values varied between 1.1 and 5.1%. The lower levels in autumn and winter can be related to low seawater temperature in those seasons. No significant differences could be noticed in peroxide values between different seasons. The values ranged from 1.1 to 5.0 meq/kg.

Table 5. Fatty Acids (w/w % of Total Fatty Acids) in the Processing of Fried Baltic Herring Fillets (Mean ± Standard Deviation). Number of Samples Analyzed in Parentheses.

fatty acid	1C. fillets (43)	1D. fillets with rye breading, salt, spice, and oil (34)	1E. fried fillets (43)
		stage of process	
14:0	3.52 ± 0.34^a	1.31 ± 0.38^b	1.51 ± 0.42^c
16:0	16.91 ± 0.63^a	8.54 ± 1.38^b	9.65 ± 1.38^c
18:0	1.27 ± 0.18^a	1.46 ± 0.09^b	1.44 ± 0.09^b
others	1.51 ± 0.28	1.00 ± 0.16	1.05 ± 0.14
Σ saturated	23.21 ± 0.81^a	12.30 ± 1.70^b	13.66 ± 1.76^c
16:1n-7	9.31 ± 1.65^a	3.46 ± 1.33^b	4.08 ± 1.34^c
18:1n-9	21.68 ± 2.35^a	44.24 ± 4.35^b	41.59 ± 4.51^c
18:1n-7	3.48 ± 0.37^a	3.00 ± 0.26^b	3.15 ± 0.30^c
20:1n-9	2.00 ± 0.39^a	1.45 ± 0.14^b	1.54 ± 0.12^c
others	1.80 ± 0.25	0.88 ± 0.20	1.02 ± 0.20
Σ monounsaturated	38.27 ± 2.24^a	53.03 ± 2.92^b	51.38 ± 3.31^c
18:3n-3	1.29 ± 0.28^a	7.40 ± 0.86^b	6.67 ± 0.84^c
18:4n-3	1.30 ± 0.45^a	0.52 ± 0.33^b	0.58 ± 0.34^c
20:3n-3	0.97 ± 0.31^a	0.41 ± 0.22^b	0.46 ± 0.25^c
20:4n-3	1.23 ± 0.37^a	0.50 ± 0.25^b	0.56 ± 0.28^c
20:5n-3	5.18 ± 0.79^a	1.80 ± 0.72^b	2.22 ± 0.71^c
22:4n-3	1.46 ± 0.31^a	0.58 ± 0.23^b	0.65 ± 0.28^c
22:5n-3	0.91 ± 0.08^a	0.33 ± 0.12^b	0.39 ± 0.13^c
22:6n-3+24:1n-9	9.36 ± 2.07^a	3.09 ± 0.63^b	4.00 ± 0.65^c
24:4n-3	1.70 ± 0.42^a	0.66 ± 0.25^b	0.75 ± 0.31^c
24:5n-3	0.93 ± 0.18^a	0.35 ± 0.11^b	0.40 ± 0.14^c
Others	1.93 ± 0.57	0.61 ± 0.33	0.71 ± 0.40
Σ n-3	26.26 ± 1.96^a	16.24 ± 2.16^b	17.40 ± 2.28^c
18:2n-6	4.54 ± 0.46^a	15.72 ± 1.54^b	14.43 ± 1.57^c
20:2n-6	1.97 ± 0.41^a	0.83 ± 0.27^b	0.94 ± 0.32^c
22:2n-6	1.26 ± 0.33^a	0.49 ± 0.17^b	0.56 ± 0.22^b
others	0.54 ± 0.15	0.17 ± 0.04	0.23 ± 0.04
Σ n-6	8.31 ± 0.98^a	17.20 ± 1.21^b	16.15 ± 1.19^c
Σ polyunsaturated	34.57 ± 2.17^a	33.44 ± 1.26^b	33.55 ± 1.44^b
n-6/n-3 ratio	0.32	1.06	0.93
unidentified	3.95 ± 0.76	1.23 ± 0.43	1.41 ± 0.50

[a-c] Different characters in the same row are significantly different ($p < 0.05$).

The most abundant saturated and monounsaturated fatty acids in the fillets were oleic (18:1n-9), palmitic (16:0), and palmitoleic (16:1n-7) acids (Table 2). The major polyunsaturated fatty acids were eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), a characteristic feature of Baltic herring. Results were similar to those reported earlier for Baltic herring (Linko et al., 1985; Kallio et al., 1991; Kallio et al., 1998). The total amount of saturated fatty acids remained constant over all seasons, being about 23%, whereas the proportions of monounsaturated and polyunsaturated acids appeared to change seasonally. Monounsaturated acids composed the main group (about 39%) in all seasons except autumn when their level decreased significantly. The level of monounsaturates was highly dependent on the content of oleic acid, the major compound in the group. Its content was the lowest in autumn and reached the maximum in spring.

The level of unsaturation of the fillet oil increased from spring to autumn, mainly because of the changes in n-3 family fatty acids, the sum of which rose from 25 to 29% followed by a 2% units drop in winter. This was in contrast to the investigations of Linko et al. (1985), who reported the n-3 fatty acids to show a decreasing trend from May to October. They also recognized in their six-month investigations that the seasonal variation in Baltic herring lipid composition was related to changes in the plankton lipids.

Increases were seen in the proportions of most of the n-3 fatty acids (Table 2). The intermediates of the EPA cascade (18:3 → 18:4 → 20:4 → 20:5) increased from spring to autumn, whereas the profiles of 24:5 and 22:6 were more irregular. Also 20:3n-3, the elongation product of α-linolenic acid, was highest in autumn. The annual profiles of EPA and DHA were clearly out of phase, which is also an indication of their independent biosyntheses and existence in the plankton feed. Among the n-6 fatty acids, the trend outlined in the n-3 family did not exist.

Processing. Peroxide values were quite low in both the raw fish and the products. The free fatty acid content increased slightly during transport to the factory but decreased again during processing due to the dilution by rapeseed oil (Tables 3 and 4). Fish fillets and gutted fish were stored under ice until prepared. The storage time was 3 days maximum. In herring fillets, a significant increase in oxidation products has been detected after 2–3 days in ice storage (Undeland et al., 1999). The rate of oxidation is dependent on the layer of the fillet and occurs most rapidly in the tissue right under the skin. A storage time of 2 days has been defined to be the maximum for well-iced sardines in order to preserve the quality (Nunes et al., 1992). The peroxide value in iced sardines increased rapidly over the first storage days even when no changes were detected in the sensory evaluation. In contrast, Smith et al. (1980) noticed that the peroxide value in herring stored in ice as whole fish can remain acceptable even for more than 10 days. Mincing of raw fish can greatly affect the storage time by increasing the amount of oxygen to which the fish flesh is exposed (Hultin, 1994). However, in the processing of burger the minced fish

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Table 6. Fatty Acids (w/w % of Total Fatty Acids) in the Processing of Baltic Herring Burger (Mean ± Standard Deviation). Number of Samples Analyzed in Parentheses.

fatty acid	2B. gutted fish (10)	2C. minced fish (12)	2D. minced fish with breadcrumbs, salt and spice (12)	2E. formed, coated product (12)	2F. burger (12)
14:0	3.70 ± 0.34^a	3.69 ± 0.37^a	3.67 ± 0.37^a	3.48 ± 0.36^a	1.18 ± 0.26^b
16:0	17.34 ± 0.55^a	17.48 ± 0.50^a	17.52 ± 0.50^a	17.41 ± 0.52^a	8.43 ± 0.90^b
18:0	1.39 ± 0.15^a	1.39 ± 0.15^a	1.39 ± 0.13^a	1.42 ± 0.13^a	1.65 ± 0.05^b
others	1.25 ± 0.28	1.26 ± 0.27	1.25 ± 0.24	1.16 ± 0.27	0.89 ± 0.11
∑ saturated	23.69 ± 0.54^a	23.82 ± 0.62^a	23.83 ± 0.63^a	23.47 ± 0.59^a	12.15 ± 1.19^b
16:1n-7	8.35 ± 1.64^a	7.99 ± 1.56^a	8.19 ± 1.59^a	7.32 ± 1.28^a	2.50 ± 0.58^b
18:1n-9	19.06 ± 2.58^a	18.72 ± 2.20^a	18.69 ± 2.29^a	18.72 ± 1.59^a	45.91 ± 2.41^b
18:1n-7	3.36 ± 0.36^{ab}	3.35 ± 0.35^{ab}	3.34 ± 0.34^{ab}	3.16 ± 0.32^b	2.98 ± 0.19^c
20:1n-9	1.44 ± 0.26^a	1.44 ± 0.28^a	1.47 ± 0.22^a	1.52 ± 0.26^a	1.39 ± 0.30^a
others	1.68 ± 0.24	1.68 ± 0.26	1.63 ± 0.23	1.92 ± 0.31	0.80 ± 0.21
∑ monounsaturated	33.90 ± 2.24^a	33.18 ± 2.01^a	33.32 ± 2.16^a	32.64 ± 1.50^a	53.57 ± 1.65^b
18:3n-3	1.92 ± 0.32^{ab}	1.91 ± 0.31^{ab}	1.92 ± 0.25^a	2.11 ± 0.27^b	7.07 ± 0.65^c
18:4n-3	2.21 ± 0.55^a	2.15 ± 0.49^a	2.09 ± 0.50^a	1.96 ± 0.47^a	0.61 ± 0.22^b
20:3n-3	0.94 ± 0.32^a	0.94 ± 0.30^a	0.92 ± 0.24^a	0.86 ± 0.22^a	0.29 ± 0.12^b
20:4n-3	1.38 ± 0.33^a	1.41 ± 0.35^a	1.38 ± 0.31^a	1.30 ± 0.30^a	0.42 ± 0.15^b
20:5n-3	6.82 ± 0.84^a	6.78 ± 0.90^{ab}	6.53 ± 0.85^{ab}	6.19 ± 0.82^b	1.97 ± 0.53^c
22:4n-3	1.12 ± 0.37^a	1.17 ± 0.41^a	1.16 ± 0.33^a	1.10 ± 0.33^a	0.37 ± 0.14^b
22:5n-3	0.84 ± 0.11^a	0.86 ± 0.10^a	0.84 ± 0.08^a	0.80 ± 0.08^a	0.26 ± 0.06^b
22:6n-3+24:1n-9	12.17 ± 1.12^{ab}	12.64 ± 1.34^a	11.88 ± 1.18^{ab}	11.76 ± 1.12^b	3.78 ± 0.68^c
24:4n-3	1.10 ± 0.50^a	1.15 ± 0.57^a	1.13 ± 0.47^a	1.08 ± 0.48^a	0.36 ± 0.18^b
24:5n-3	0.58 ± 0.21^a	0.60 ± 0.24^a	0.59 ± 0.23^a	0.56 ± 0.19^a	0.15 ± 0.11^b
others	1.33 ± 0.62	1.40 ± 0.66	1.35 ± 0.57	1.24 ± 0.56	0.28 ± 0.19
∑ n-3	30.42 ± 1.74^a	31.00 ± 1.50^a	29.80 ± 1.90^{ab}	28.97 ± 1.67^b	15.56 ± 1.31^c
18:2n-6	4.85 ± 0.54^a	4.85 ± 0.57^a	5.97 ± 0.64^b	8.36 ± 0.94^c	16.62 ± 1.23^d
20:2n-6	1.63 ± 0.33^a	1.46 ± 0.57^a	1.44 ± 0.53^a	1.36 ± 0.51^a	0.50 ± 0.22^b
22:2n-6	0.80 ± 0.27^a	0.81 ± 0.28^a	0.80 ± 0.23^a	0.76 ± 0.23^a	0.26 ± 0.10^b
others	0.60 ± 0.09	0.60 ± 0.10	0.59 ± 0.08	0.56 ± 0.09	0.16 ± 0.01
∑ n-6	7.87 ± 0.90^a	7.72 ± 0.99^a	8.80 ± 1.11^b	11.05 ± 1.29^c	17.54 ± 1.19^d
∑ polyunsaturated	38.30 ± 2.13^a	38.73 ± 1.85^a	38.60 ± 2.05^a	40.01 ± 1.68^b	33.10 ± 0.99^c
n-6/n-3 ratio	0.26	0.25	0.30	0.38	1.13
unidentified	4.12 ± 0.41	4.28 ± 0.78	4.26 ± 0.73	3.88 ± 0.79	1.18 ± 0.54

[a–d] Different characters in the same row are significantly different ($p < 0.05$).

was not stored for long periods before preparation and thus the time for oxidation to occur was minimized.

The final oil content in processed products was twice that of the raw Baltic herring material. In the processing of burger the oil content decreased when the product was coated but during deep-frying rapeseed oil was absorbed until the oil content tripled (Table 4). On fillets the oil was added before frying and the oil content reached the maximum at this stage followed by a significant decrease during contact frying (Table 3).

The dominant fatty acids in rapeseed oil were oleic (57%), linoleic (22%), and α-linolenic (11%) acids and their relative amounts in the fish samples increased markedly during both processes. Accordingly, the fatty acids characteristic of Baltic herring, (palmitic acid, palmitoleic acid, EPA, and DHA) decreased. However, the total proportion of EPA and DHA in the products was still about 6%. In processing the fried fillets, the main difference occurred at stage 1D, where the fillets were spiced, breaded, and dipped in rapeseed oil (Table 5). As mentioned above, the frying step reduced the oil content while at the same time the levels of oleic, linoleic, and α-linolenic acids decreased. Thus, the oil released was mainly rapeseed oil from the surface of the fillets. The fatty acid composition remained quite stable throughout the burger process until the product was deep-fried (Table 6).

In both products, the total amounts of saturated fatty acids were lower than in the raw material whereas the amounts of monounsaturates were higher because of the

oleic acid of rapeseed oil. The total amount of the polyunsaturated acids was almost the same in both the fried and raw fillets but in the burger the amount was slightly lower than in the corresponding raw material, gutted fish. The original level of polyunsaturates was higher in the gutted fish compared to the fillets. One possibility is that the thin belly tissue which was removed from the fillets was rich in polyunsaturates. The levels of n-3 acids decreased and n-6 acids increased significantly during both processes. The rapeseed oil contained rather a high amount of α-linolenic acid and thus the level of n-3 fatty acids remained high, 17% in fried fillets and 15% in burger. Despite different ingredients and frying methods, the fatty acid composition of both ready-to-eat products was quite similar: on average 51−53% monounsaturated, 33% polyunsaturated, and 13% saturated fatty acids. The ratio of n-6/n-3 was 0.93 in fried fillet and 1.13 in burger. There are many opinions concerning the optimum ratio of n-6 to n-3 acids in diet. According to Budowski and Crawford (1985) the desirable ratio may be less than 5. Simopoulos (1999) stated that in the western diet the ratio is between 10 and 20:1, while during evolution it was 1:1 or less.

Deep frying and surface frying with oil affect the lipid and moisture content as well as the fatty acid composition of the fish products. The amount of oil absorbed is highly dependent on the original lipid content of the fish (Candela et al., 1998; Gall et al., 1983). Lean fish absorbs a lot of oil from the cooking medium, whereas
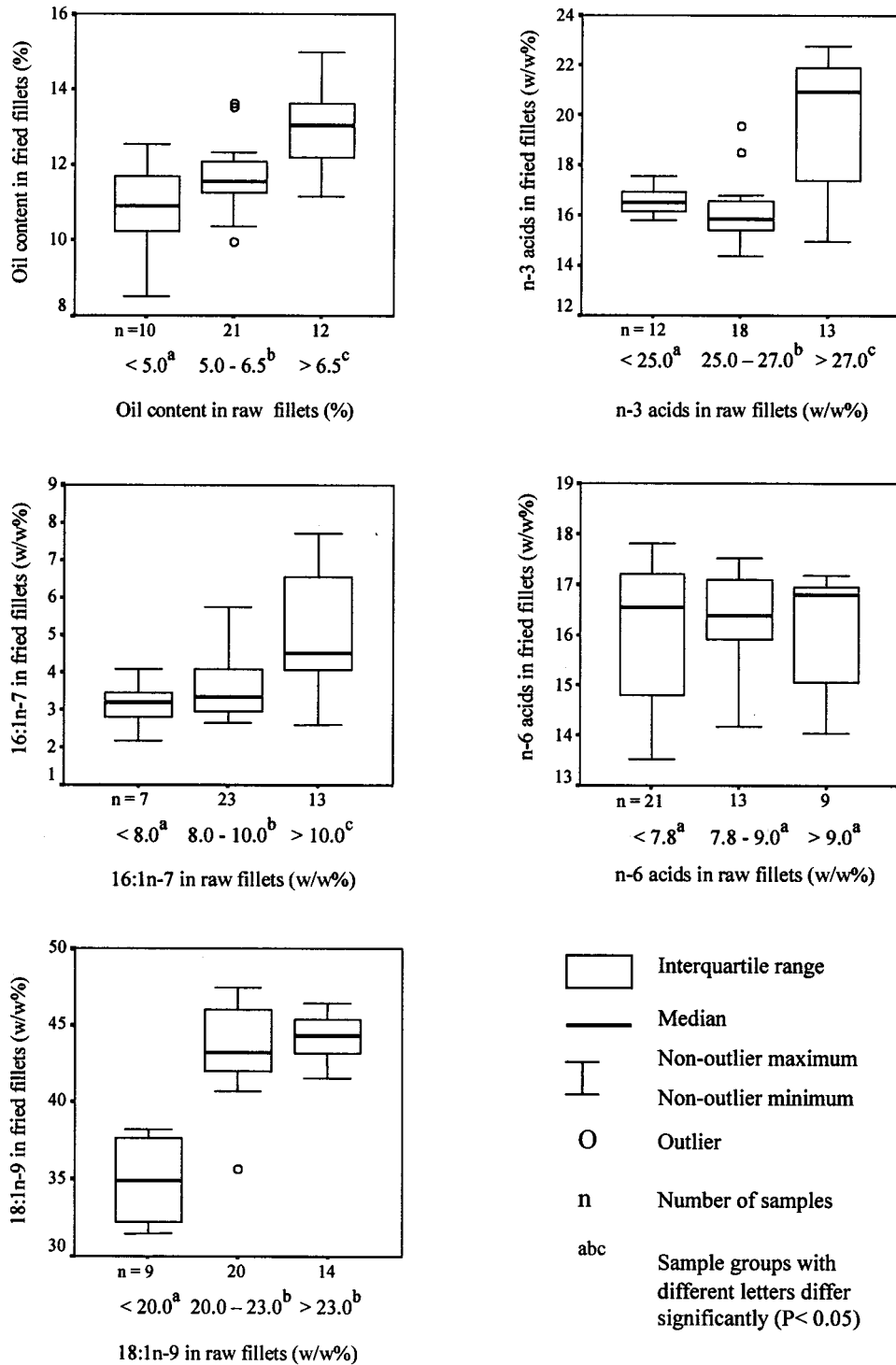
Figure 1. Variation in the oil and fatty acid contents of fried fillets according to contents in raw material (raw fillets).

the fat content of fatty fish can even slightly decrease during deep-frying. Absorption of oil modifies the fatty acid composition of the product from that of fish toward the pattern of frying oil (Sanches-Muniz et al., 1992), which was also noticed in this study. Thus, the selection of frying oil is an important factor from a nutritional point of view. The interactions between fish and frying oil are dependent also on many other factors such as frying time, the particle size of the fish, and the composition of the coating material (Makinson et al., 1987; Ågren and Hänninen, 1993). Coating protects fish fillets against moisture loss and oil absorption but, on the other hand, the coating itself can absorb oil causing

an increase in the total fat content (Mai et al., 1978; Nawar et al., 1990). Makinson et al. (1987) tested different coatings and found that batters form a better barrier against oil absorption than breading does.

The fillets and herring burgers absorbed significant amounts of rapeseed oil. Both products were breaded, so most of the oil was possibly absorbed by the breading material. Thus, the absolute amounts of nutritionally important fatty acids in Baltic herring may have remained almost constant during frying even though the relative proportions changed considerably. Sebedio et al. (1993) reported that the content of long-chain n-3 polyunsaturated fatty acids is not affected by deep
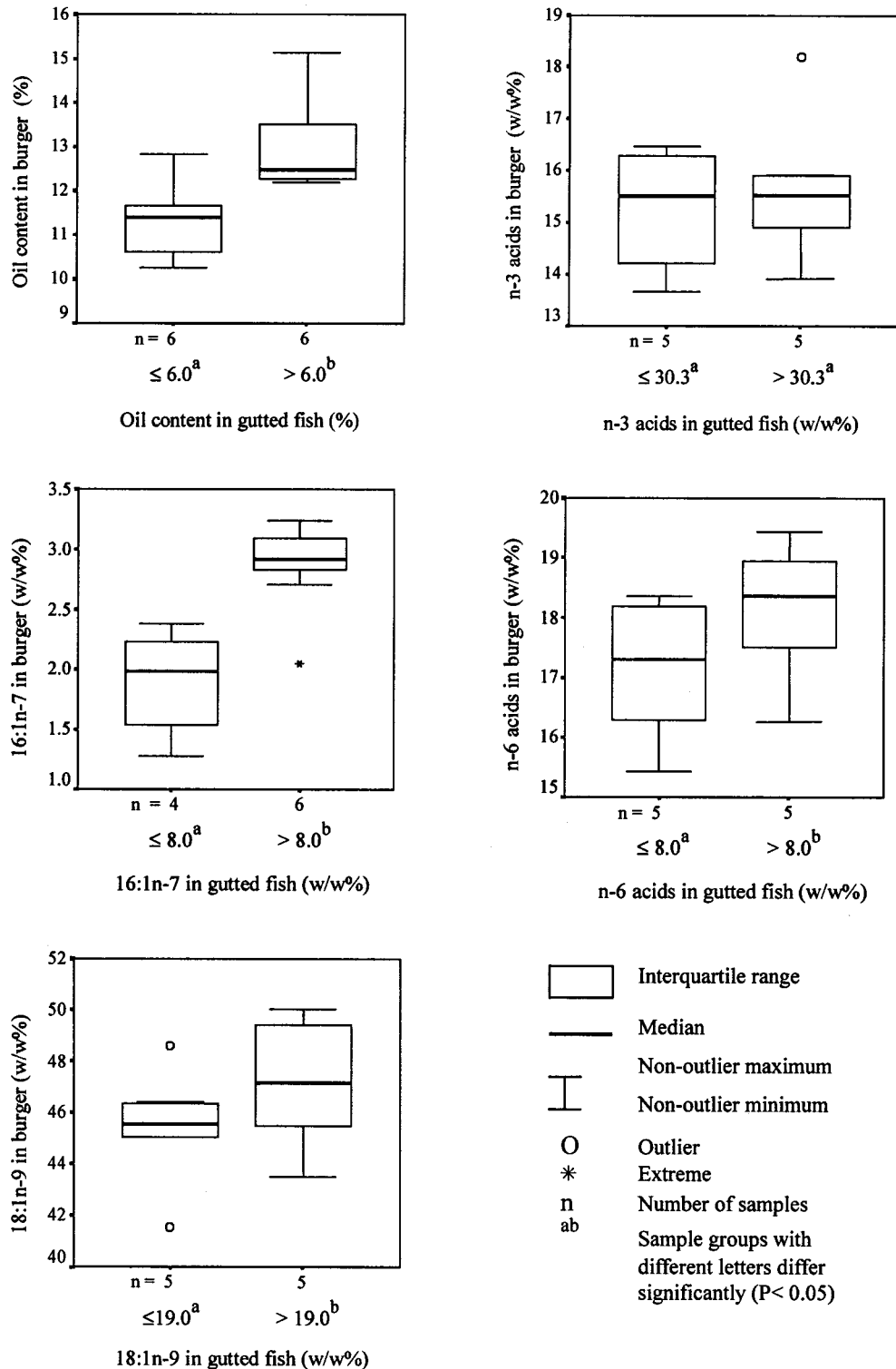
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Figure 2. Variation in the oil and fatty acid contents of fish burger according to contents in raw material (gutted fish).

frying of mackerel. In contrast, Candela et al. (1998) noticed that the EPA and DHA contents in mackerel and sardine decreased, whereas in salmon these acids remained quite stable. It seems evident that the changes in EPA and DHA levels can be related to the initial amounts of these fatty acids in raw fish. The higher the initial content of EPA and DHA, the larger the decrease during frying (Candela et al., 1997; Candela et al., 1998)

The oil content and fatty acid compositions of raw materials compared to the ready-to-eat products are shown in Figures 1 and 2. Certain fatty acids or groups

that showed seasonal variation were selected for statistical analysis. Fried fillets were divided into three groups and burgers into two groups according to content in the corresponding raw material. The oil content and the levels of oleic and palmitoleic acids in both products seemed to be related to the original content in the raw fish. The higher the contents in the raw material, the higher were the contents also in the products. However, the oleic acid in the fried fillets appeared to reach the maximum level when the content in the raw fillets exceeded 20%. As can be seen in Figure 1, a remarkable

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difference was found between the groups of raw fillets containing oleic acid of less than 20% and those containing more than 20%. In the fried fillets the total amount of n-3 acids increased when the content in the raw material was over 27%, but in the burgers the amount of n-3 acids remained constant despite variations in the raw material. Rapeseed oil increased the amount of linoleic acid so that the small variation in n-6 acids disappeared during both processes. These results indicated that fried fillets manufactured in autumn and winter were more fatty and contained more palmitoleic acid and less oleic acid than fillets made in spring or summer. The total amount of n-3 acids reached the highest level in fillets processed in autumn (Figure 1, Tables 1 and 2).

In conclusion, the current work shows that the fat and fatty acid composition of Baltic herring varies seasonally, and some of these differences remain throughout industrial processing. The oil content of the two Baltic herring products investigated doubled during the processes. In addition, processing changed the fatty acid compositions to the pattern of the frying oil. However, both products still contained quite large amounts of nutritionally important n-3 acids, especially EPA and DHA. The rapeseed oil used for frying also increased the proportion of α-linolenic acid to a level higher than in the raw material.

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