FATTY ACID PROFILE OF THE FAT IN SELECTED SMOKED MARINE FISH

Bożena Regulska-Ilow[1]*, Rafał Ilow[2], Klaudia Konikowska[1], Anna Kawicka[1], Dorota Różańska[1], Agnieszka Bochińska[2]

[1]Department of Dietetics, Wrocław Medical Univeristy, Wrocław, Poland
[2]Department of Food Science and Dietetics, Wrocław Medical University, Wrocław, Poland

ABSTRACT

Background. Fish and marine animals fat is a source of unique long chain polyunsaturated fatty acids (LC-PUFA): eicosapentaenoic (EPA), docosahexaenoic (DHA) and dipicolinic (DPA). These compounds have a beneficial influence on blood lipid profile and they reduce the risk of cardiovascular diseases, atherosclerosis and disorders of central nervous system. The proper ratio of n-6/n-3 fatty acids in diet is necessary to maintain a balance between the effects of eicosanoids synthesized from these acids in the body.

Objective. The aim of this study was the evaluation of total fat and cholesterol content and percentage of fatty acids in selected commercial smoked marine fish.

Material and methods. The studied samples were smoked marine fish such as: halibut, mackerel, bloater and sprat. The percentage total fat content in edible muscles was evaluated *via* the *Folch* modified method. The fat was extracted *via* the *Bligh–Dyer* modified method. The enzymatic hydrolysis was used to assesses cholesterol content in samples. The content of fatty acids, expressed as methyl esters, was evaluated with gas chromatography.

Results. The average content of total fat in 100 g of fillet of halibut, mackerel, bloater and sprat amounted respectively to: 14.5 g, 25.7 g, 13.9 g and 13.9 g. The average content of cholesterol in 100 g of halibut, mackerel, bloater and sprat was respectively: 54.5 mg, 51.5 mg, 57.5 mg and 130.9 mg. The amount of saturated fatty acids (SFA) was about ¼ of total fatty acids in the analyzed samples. The oleic acid (C18:1 n-9) was the major compound among monounsaturated fatty acids (MUFA) and amounted to 44% of these fatty acids. The percentage of polyunsaturated fatty acids (PUFA) in halibut, mackerel, bloater and sprat was respectively: 31.9%, 45.4%, 40.8% and 37.0%. The percentage of n-3 PUFA in mackerel and bloater was 30.1% and 30.2%, while in halibut and sprat was lower and amounted to 22.5% and 25.6%, respectively.

Conclusions. In terms of nutritional magnitude the meat of mackerel and herring, compared to the meat of sprat and halibut has a much better n-3 PUFA content, while relatively low content of cholesterol.

Key words: *marine fish, fat, cholesterol, fatty acids, diet*

STRESZCZENIE

Wprowadzenie. Tłuszcz pochodzący z ryb i zwierząt morskich zawiera unikalne długołańcuchowe wielonienasycone kwasy tłuszczowe (ang.: long chain polyunsaturated fatty acids, LC-PUFA), tj. kwas eikozapentaenowy (EPA) oraz kwas dokozaheksaenowy (DHA). Wpływają one korzystnie na profil lipidowy krwi, zmniejszają ryzyko wystąpienia chorób sercowo-naczyniowych, miażdżycy, nowotworów oraz zaburzeń ośrodkowego układu nerwowego. Odpowiednia proporcja kwasów tłuszczowych n-6/n-3 w diecie jest niezbędna dla zachowania równowagi pomiędzy skutkami działania eikozanoidów, powstających z tych kwasów w organizmie.

Cel. Celem badania była ocena zawartości tłuszczu ogółem i cholesterolu oraz procentowego udziału kwasów tłuszczowych w wybranych wędzonych rybach morskich, dostępnych w handlu detalicznym.

Materiał i metody. Materiał do badań stanowiły morskie ryby wędzone tj.: halibut, makrela, pikling i szproty. Zawartość procentową tłuszczu całkowitego w tkance mięsnej oznaczono zmodyfikowaną metodą *Folch'a.* Tłuszcz rybi wyekstrahowano za pomocą zmodyfikowanej metody *Bligh'a–Dyer'a.* Zawartość cholesterolu oznaczono metodą enzymatycznej hydrolizy. Skład kwasów tłuszczowych w postaci estrów metylowych oznaczono metodą chromatografii gazowej.

Wyniki. Średnia zawartość tłuszczu w 100 g filetów z halibuta, makreli, śledzia-piklinga i szprotach wynosiła odpowiednio: 14,5 g, 25,7 g, 13,9 g i 13,9 g. Średnia zawartość cholesterolu w 100 g halibuta, makreli, piklinga i szprotach wynosiła odpowiednio: 54,5 mg, 51,5 mg, 57,5 mg i 130,9 mg. Kwasy tłuszczowe nasycone (NKT) stanowiły ¼ sumy wszystkich kwasów tłuszczowych (KT). Dominującym KT jednonienasyconym (JNKT) był kwas oleinowy (C18:1 n-9) i stanowił około

*Corresponding author: Bożena Regulska-Ilow, Department of Dietetics, Wrocław Medical University, Parkowa Street 34, 51-616 Wrocław, Poland, phone: +48 71 34825 70,
e-mail: bozena.regulska-ilow@umed.wroc.pl

44% udziału wszystkich JNKT. Udział wielonienasyconych kwasów tłuszczowych (WNKT) w halibucie, makreli, piklingu i szprotach wynosił odpowiednio: 31,9%, 45,4%, 40,8% i 37,0%. Udział WNKT n-3 w makreli i piklingu był zbliżony i wynosił odpowiednio 30,1% i 30,2%, natomiast w halibucie i szprotach był niższy i wynosił odpowiednio 22,5% i 25,6%. **Wnioski.** Pod względem dietetycznym mięso makreli i śledzia, w porównaniu do mięsa halibuta i szprota charakteryzuje się znacznie lepszym udziałem WNKT n-3, przy jednoczesnej stosunkowo niewielkiej zawartości cholesterolu.

Słowa kluczowe*: ryby morskie, tłuszcz, cholesterol, kwasy tłuszczowe, dieta*

INTRODUCTION

Fish and marine animals fat is a source of an important for human health polyunsaturated fatty acids (PUFA). It has a unique nutritional value because it is the only source of n-3 long chain polyunsaturated fatty acids (LC-PUFA): eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and dipicolinic acid (EPA).

Linoleic (LA, C18:2 n-6) and α-linolenic (ALA, C18:3 n-3) acids are converted in the human body to PUFA through controlled bioconversion by a result of elongases and desaturases activeness [18]. The effectiveness of these reactions may be differ. It was observed that dependent on the source and proportion of PUFA n-6/n-3 in diet, the bioconversion of ALA to PUFA may account about 6-20% for EPA and 0.5-9% for DHA [3, 4]. It was also found that in a group of women in reproductive age the effectiveness of ALA to n-3 PUFA conversion was 2.5-fold higher than in a group of men. In diet rich in n-6 PUFA a bioconversion of ALA to n-3 PUFA may be reduced on approximately 40-50% [1, 40].

Intake of oily marine fish at least twice a week may play a significant role in prevention of cardiovascular diseases [21]. In a lot of studies it was observed that supplementation of ≥ 3 g/day EPA and DHA caused a reduction of hypertriglyceridemia with no effect on hypercholesterolemia, what was associated with significant cardioprotective benefits [5, 30, 43, 46]. Other authors pointed out the differences in EPA and DHA influence on LDL and HDL cholesterol level in blood. EPA stimulates synthesis of eicosanoids, which affect the cardiovascular system, while DHA has an important role both in human proper growth and functioning of nervous system [19, 29, 38].

In diet of our ancestors n-6/n-3 ratio was as 1:1, while nowadays in Western diet this proportion is significantly impaired and amount even to 15-20:1 [11, 39]. The proper n-6/n-3 ratio is essential to maintain a balance between antioxidative and oxidative processes in organism. The increase intake of n-6 PUFA and increased proportion of n-6/n-3 caused the increased risk of many diseases, such as cardiovascular diseases and cancers [25, 39].

The main sources of n-6 fatty acids in diet are vegetable oils, such as: sunflower, soybean, corn, safflower, grape seed, wheat germ and margarines produced from these products [24]. According to the world organizations intake of oily marine fish, like: hearing, mackerel, sprat, halibut, salmon, tuna, sardines, food fortified in n-3 PUFA should be increased or n-3 PUFA diet supplements should be considered to get n-6/n-3 ratio lower than 4:1 [13, 21].

The aim of this study was to evaluate total fat and cholesterol content and percentage content of fatty acids in selected commercial smoked marine fish.

MATERIAL AND METHODS

Materials

The material for the study were commercial smoked marine fish: halibut, mackerel, herring-bloater and sprats. Ten samples of each fish species were bought. Fish meat was examined after removal of inedible elements, comminuted the meat and mixed it in order to harmonized the sample. The content of total fat, cholesterol and fatty acids profile was assessed in the samples.

Assay of total fat content

The total fat content in fish meat tissue was assessed *via* the *Folch* method [9] in 40 samples (10 samples from 4 fish species) duplicate. This method involves hydrolysis with hydrochloric acid (HCl) in order to release all the fatty substances present in the sample. This allows efficient and accurate extraction of fat, however, owing to the hydrolysis of the fat is not suitable for the determination of cholesterol content in them.

2 g of the fish muscle tissue was weighted, then 20 cm^3 of chloroform and methanol mixture (1:1) and 0.5 cm^3 of concentrated HCl were added. The prepared samples were centrifuged for 10 min at 4000 rpm. The solution from over sediment was carefully removed to the *Erlenmeyer* flask with ground-glass joint. The procedure was repeated. First and second solutions were mixed together and then 20 cm^3 of 5% HCl was added. After separation of the layers the chloroform layer was collected and buret was replenish by anhydrous chloroform to 20 cm^3. After accurate mixing, 10 cm^3 of the chloroform layer was taken to the weighing vessel previously weighed to constant weight. Chloroform was evaporated in a water bath until the solvent smell

had completely disappeared. To removed the remainder from the solvent the vessel was put in a drying oven for 15 min at 105°C. The weighing vessel was weighed. The mass of extracted fat was computed by subtracting the mass of vessel without fat from the mass of vessel with fat.

Extraction of fish fat

The fat from analyzed fish was extracted *via* the *Bligh–Dyer* method [37]. At the beginning the fat product was homogenized with a chloroform and methanol mixture. Subsequently, the chloroform layer containing fat and methanol-water layer, in which nonfatty substances can be found, were separated. This method provides a rapid and efficient extraction of the fat and is conducted under conditions that protect against oxidation and other changes that may occur in the fat.

Weighted sample of fish (100 g) was homogenized for 2 min with 100 cm^3 chloroform and 200 cm^3 methanol. After that 100 cm^3 chloroform was added to the mixture and further homogenization was carried out for 30 s. The homogenate was filtered. After separation of the layers, the water-alcohol layer was removed. The chloroform solution was filtrated into a separatory funnel with anhydrous sodium sulphate. Then, the obtained clear chloroform extract was dried over anhydrous sodium sulphate for 24 h in a dark place. The dehydrated extract was filtrated into a volumetric flask and replenish with chloroform to 250 cm^3. The extract in amount of 25 ml was transferred to a round bottom flask and the solvent was evaporated in a vacuum evaporator, in a nitrogen atmosphere. The fat obtained during extraction was used to assessed cholesterol content.

Saponification of fish fat

The extracted fish fat (0.3 g of fat fish) from analyzed samples was saponificated with methanolic KOH (9 cm^3 methanol, 1 cm^3 10 M aqueous KOH) in order to obtain a clear solution. 10 cm^3 methanolic KOH was added to 0.3 g of fish fat and then the mixture was heated for 25 min under the reflux condenser in a water bath, stirring. To the supernatant 2-propanol was added to replenish the content to 25 cm^3. The mixture was stirred and left for 24h. Cholesterol was a part of the nonsoapy fraction of fat.

Enzymatic hydrolysis

The total cholesterol content was assessed in 40 samples (10 samples from 4 fish species) in the fat extracted from muscle tissue of fish *via* enzymatic hydrolysis, using the *Boehringer* Mannheim test (Cat. No. 139 050). Free cholesterol is oxidized by cholesterol oxidase and in numerous changes gave a yellow dye - lutidine. The lutidine concentration is directly proportional to the amount of cholesterol in the sample.

The increase in the content of cholesterol in the sample caused an increase of light absorbance in the visible spectrum at a wavelength of 405 nm.

The precision of the method was determined by computed the coefficient of variation of 10 samples, which amounted to 6.2. The accuracy of the method was verified by assessing of the recovery of 5 mg of cholesterol added to the tested material. During the study 6 recoveries were carried out and the average of recovery was 89.6 %. The value of the recovery obtained in each case was taken into account in the calculations.

Assay of fatty acid composition via gas chromatography

The fatty acid composition in the form of methyl esters was determined *via* gas chromatography using chromatograph type HP Agilent 6890N with a glass capillary column of 100 m length and 0.25 mm diameter with a CP-Sil 88 stationary phase. Helium at the pressure 0.24 MPa was used as a carrier gas. The division was conducted at programmed temperature from 165°C/min. The fatty acids identification was carried out by comparing their retention times with the standards. The content of each fatty acids and their isomers were expressed as percentage of total fatty acids.

Separation of the nonsoapy fraction

Using a Pasteur pipette, 10 drops of fat were measured into an esterification test tube. After that 1 ml of 2 M KOH solution in 75% methanol was added. The test tube was sealed shut and heated at a temperature of 70°C for 60 min. After cooling, 1 ml of n-hexane was added, and next the obtained mixture was shaken for 5 min. Subsequently, the hexane layer was removed precisely and discarded, than it was repeated. After that 1 ml of a 2 M aqueous solution of HCl was added to the remainder, to receive an acidic pH. The obtained mixture was heated for 30 min at 70°C. After cooling, once again 1 ml of n-hexane was added along with a saturated NaCl solution at such a volume that the hexane layer would be in the narrowing of the esterification test tube, and then the obtained mixture was shaken for 5 min. Subsequently, the hexane layer was transferred to vials containing anhydrous sodium sulphate, and this step was repeated. In the next stage the hexane layers were combined, and the whole lot was sealed shut and left in a cool dark place for 24 h.

Esterification of the fatty acids

The hexane extract, obtained in the previous stage, was transferred to esterification test tubes and then evaporated in a glycerine bath. Subsequently, 1 ml of 0.5 M KOH in anhydrous methanol was added to the dry residue. The tubes were sealed and heated for 30 min at 70°C and cooled. After that, 1 ml of 1.25 M HCl in anhydrous methanol was added and it was heated

for 30 min at 70°C, then cooled once again. In the next step 1 ml of n-hexane and saturated NaCl was added, and the whole obtained mixture was shaken for 5 min. Subsequently, the hexane layer was transferred to vials containing anhydrous sodium sulphate, and this stage was repeated. The combined whole was thickened by evaporating the hexane. After that a gas chromatography was performed. Both steps of the esterification were carried out for two parallel samples of the same fat sample.

Calculation of fatty acids n-6/n-3 ratio and P/S ratio

In the analyzed fish PUFA n-6 (C18:2+C20:4) to n-3 (C18:3+C20:5+C22:6) was calculated. According to the Polish Cardiac Society recommendations n-6/n-3 ratio should be at most 4:1, while preferably 1:1 [21].

The P/S ratio, which define the content of PUFA in relation to SFA in product, was also calculated. It is a measure of the food or diet atherogenicity. As an optimal value for P/S ratio was assumed 1.25, where PUFA amounted to 10% of total energy in product and SFA amounted to 8%.

Statistical analysis

The obtained results were summarized by average and standard deviation (SD). The statistical analysis was performed using Statistica v 10.0 PL software, StatSoft. Inc. (USA). Level of statistical significance was set at $p < 0.05$. To compare data between groups the non-parametric *Kruskal-Wallis* test was used.

RESULTS AND DISCUSSION

Depending on the fat content fish are classified as: lean (below 2% of fat), medium fat (2-7% of fat), oily (7-15% of fat) and very oily (above 15% of fat) [33]. The total fat content in fish is associated mainly with the fish species, their nutritional status, fishing seasons, the life cycle of the fish, and the technological processes used. Moreover, fish that live in the wild has a higher n-3 fatty acids and a lower n-6 fatty acids content compared with fish breeding [15, 27].

The smoked fish analyzed in this study has different total fat and cholesterol content, dependent on the species, what was presented in Table 1. In bloater, sprat and halibut the average total fat content was similar. In mackerel significantly higher fat content than in other analyzed samples was observed. Halibut, bloater and sprat were classified as a oily fish, while mackerel to a very oily, because of the fat content exceeded 15%.

In the study of *Usydus* et al. [45] a fat content in smoked sprats was approximately 13.9 g/100 g, what was similar to the results obtained in the presented study. The fat content in smoked herring assessed by *Usydus* et al. [45] was however lower than in this study and amounted to 9.0 g /100 g. According to the "Food Compositions Tables" [24] total fat content in herring "Bloater" is 14.3 g/100 g, what is similar to the value obtained in the present study (13.9 g/100g). The fat in meat of halibut is not evenly distributed and especially high concentration occurs around the dorsal and ventral fins [22]. Similar content of total fat in smoked halibut (14%) compared with the present study was assessed by *Karl* et al. [20].

Usydus et al. [45] observed a high content of fat in smoked mackerel (20.8 g/100g), what was similar to the results obtained in the present study (25.7 g/100g). Based on the "Food Compositions Tables" [24] the fat content in smoked mackerel (15.5 g/100g) is much lower than observed in this study. *Balasa* et al. [2] also found lower fat content in mackerel (11.6%) than in the present study.

The total fat content in smoked herring is higher than in fresh fish, what was observed by other authors [28, 34]. The reason of these differences is probably technological process – smoking, which causes a loss of water in the tissue of the fish meat and increased accumulation of nutrients, including fat and cholesterol [44].

The average cholesterol content in 100 g of the analyzed smoked fish fillets ranged 51.5 mg – 130.9 mg. The average cholesterol content in the fat of analyzed fish ranged from 202.6 mg/100 g to 948.6 mg/100 g. According to the "Food Compositions Tables" [24] the cholesterol content in smoked mackerel (70 mg/100g) and bloater (90 mg/100 g) is higher than observed in this study (51.5 mg and 57.5 mg/100g, respectively).

No significant differences were observed between average cholesterol content in the fat of halibut and

Table 1. Content of total fat and cholesterol in analyzed smoked marine fish

Parameter	Halibut (A) n=10	Mackerel (B) n=10	Herring-Bloater (C) n=10	Sprat (D) n=10	Statistically significant differences between samples (p<0.05)
	$X \pm SD$	$X \pm SD$	$X \pm SD$	$X \pm SD$	
Total fat content [g/100 g]	14.5 ± 2.9	25.7 ± 4.0	13.9 ± 0.7	13.9 ± 1.1	A vs B; B vs C; B vs D
Cholesterol content in fish fat [mg/100 g]	390.7 ± 100.4	202.6 ± 40.0	412.2 ± 84.7	948.6 ± 141.1	A vs B; A vs D; B vs C; B vs D; C vs D
Cholesterol content in fish meat [mg/100 g]	54.5 ± 8.1	51.5 ± 9.6	57.5 ± 11.0	130.9 ± 18.7	A vs D; B vs D; C vs D;

*X- average; SD- standard deviation, n– number of samples

bloater. Significantly lower cholesterol per 100 g of fat was found in mackerel in comparison with other analyzed fish. In the fat of sprats the cholesterol content was significantly higher than in other analyzed samples and average amounted to 948.6 mg/100 g. High content of cholesterol in analyzed sprats was caused by using to the assay an average fat content of the meat tissue with liver, which is rich in this component. This procedure is justified due to the widespread habit of consumption the sprats including the liver, due to the small size of the fish.

Polish Forum for Prevention of Cardiovascular Diseases recommends to healthy people limiting cholesterol intake below 300 mg per day [21]. However, in the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) to reduce the occurrence of cardiovascular disease basis on atherosclerosis the multifactoral lifestyle changes including i.a. reducing dietary cholesterol intake to less than 200 mg per day are recommended [7]. In order not to exceed the recommended daily intake of dietary cholesterol the consumption of products being sources of this component in diet should be taken into account especially by those with abnormal lipid profiles. Based on the present study 10 sprats (140 g) contain about 183 mg of cholesterol, while one average mackerel (180 g), without skin and bones (125 g), contains about 64 mg of this component. The average portion of halibut (200 g) and bloater (150 g) after removal of inedible parts (170 g and 130 g) contain about 93 mg and 75 mg of cholesterol, respectively.

The fatty acid profile in the fat of analyzed smoked marine fish was shown in Table 2. The average percentage of SFA in the fat of analyzed samples was comparable and amounted to about ¼ of the assessed total fatty acids. A dominant SFA in all the examined fish was palmitic acid (C16:0), what was consistent with the results obtained by other authors [2, 14, 24, 35]. The highest percentage of palmitic acid was assessed in sprats, while the lowest in halibut. *Usydus* et al. [45] also observed that palmitic acid had the largest share of SFA in the fat of smoked sprats (19.7%).

The highest percentage of monounsaturated fatty acids (MUFA) was observed in halibut (39.8% of total fatty acids), while in mackerel, bloater and sprats was comparable and amounted respectively to: 26%, 31.1% and 30.4% of total fatty acids. Among MUFA dominated oleic acid. Its content in sprats, halibut, bloater and mackerel was respectively: 59.3%, 41.9%, 40.3% and 37.4% of all MUFA. The largest share among assessed MUFA in halibut and sprats had oleic acid and amounted to 16.7% and 18%, respectively. In addition, in smoked marine fish a large share of palmitoleic acid

(*cis*-C16:1 n-7) were observed. Palmitoleic acid is produced by endogenous fat synthesis and is linked to both beneficial and deleterious metabolic effects, potentially confounded by diverse determinants and tissue sources of endogenous production [31]. The largest amount of C16:1 amounting to 15.1%, was determined in halibut. In the remaining part of the analyzed fish the amount of palmitoleic acid was similar and amounting from 4.7% to 6.6%.

The percentage of PUFA in halibut, mackerel, bloater and sprats was respectively: 31.9%, 45.4%, 40.8% and 37% of total fatty acids. The percentage of n-3 PUFA was similar in analyzed samples and ranged 22.5% - 30.2% of total fatty acids. *Grela* and *Dudek* [14] observed that the content of n-3 PUFA in fresh cod and salmon amounted to about 36.5% and 22.1%, respectively. However, *Usydus* et al. [45] observed lower content of n-3 PUFA in smoked fish compared with this study, which was respectively: 10.6% in herring, 19.2% in mackerel, 22.3% in sprats.

The highest percentage of ALA, which amounted to 8.2% was assessed in halibut, while the lowest (about 1%) in sprats. Among n-3 PUFA predominated DHA, which percentage was as follows: in halibut – 6.8%, in mackerel – 14.6%, in bloater – 13% and in sprats – 14.6%. The percentage of EPA was comparable in mackerel, bloater and sprats and amounted respectively to: 8%, 8.6% and 8.8% of total fatty acids. Lower percentage of EPA, compared with other samples was in halibut (6.1% of total fatty acids). Results obtained in the present study were confirmed by other authors [2, 14]. *Usydus* et al. [45] observed that among n-3 PUFA in smoked mackerel, herrings and sprats predominated LC-PUFA: DHA (8.8%, 4.3%, 10.5%, respectively) and EPA (5.3%, 3.6%, 7%, respectively).

Taking into account the sum of LC-PUFAs the highest percentage of these fatty acids was observed in sprats and mackerel (24.6% and 24.1% of total fatty acids, respectively). Although the percentage of n-3 PUFA was comparable the mackerel was more preferable than sprats because it contained less than half cholesterol. In the present study the percentage of the sum of LC-PUFAs in bloater was 22.7%, while in the study of *Usydus et al.* [45] was lower and amounted to 8.4%.

EPA and DHA have a beneficial influence on the circulatory system, because of the inhibiting the development of atherosclerosis and preventing the occurrence of thromboembolic events [23]. Many authors proved that fish consumption and diet supplementation in LC-PUFA may protect against the development of certain types of cancer. The most convincing data indicate the beneficial effects of LC-PUFA in relation to colon and skin cancer [6, 32, 36]. Moreover, it has been shown that the intake of several grams of LC-PUFA daily gives beneficial health effects for certain inflammatory diseases inclu-

Table 2. The percentage of individual fatty acids (% of total fatty acids) in fat of the analyzed smoked marine fish

Fatty acids	Halibut n=10	Mackerel n=10	Herring-Bloater n=10	Sprat n=10
	$X \pm SD$	$X \pm SD$	$X \pm SD$	$X \pm SD$
Saturated fatty acids (SFA)				
C 12:0	0.39 ± 0.37	0.19 ± 0.07	0.22 ± 0.08	0.15 ± 0.05
C 14:0	8.06 ± 1.96	7.40 ± 1.31	7.63 ± 1.52	5.61 ± 0.69
C 15:0	0.90 ± 0.18	1.04 ± 0.29	0.87 ± 0.15	1.22 ± 0.12
C 16:0	10.07 ± 1.08	11.17 ± 0.85	12.41 ± 1.41	15.07 ± 1.09
C 17:0	0.71 ± 0.18	0.85 ± 0.18	0.75 ± 0.14	0.87 ± 0.11
C 18:0	2.96 ± 0.30	3.00 ± 0.41	2.27 ± 0.50	3.13 ± 0.44
C 19:0	0.35 ± 0.33	0.28 ± 0.07	0.10 ±0.04	0.16 ± 0.05
C 24:0	0.73 ± 0.24	0.78 ± 0.26	0.78 ± 0.45	1.80 ± 0.58
Σ SFA	24.2	24.7	25.0	28.0
Monounsaturated fatty acids (MUFA)				
C 14:1 n-5	0.50 ± 0.16	0.30 ± 0.33	0.19 ± 0.06	0.14 ± 0.04
C 16:1 n-7	15.13 ± 5.84	4.71 ± 0.50	5.70 ± 0.84	6.61 ± 0.38
C 17:1 n-7	0.93 ± 0.17	1.32 ± 0.28	0.83 ± 0.19	1.52 ± 0.08
C 18:1 n-9	16.67 ± 2.22	9.72 ± 0.56	12.54 ± 1.97	18.03 ± 2.30
C 20:1 n-9	1.50 ± 0.63	2.5 ± 10.78	1.59 ± 0.55	3.59 ± 0.44
C 22:1 n-9	5.07 ± 1.48	7.44 ± 2.01	10.21 ± 3.69	0.54 ± 0.19
Σ MUFA	39.8	26.0	31.1	30.4
Polyunsaturated fatty acids (PUFA)				
C 18:2 n-6	2.62 ± 0.42	3.12 ± 0.28	2.56 ± 0.54	3.37 ± 0.23
C 18:3 n-3	8.23 ± 1.87	5.94 ± 0.98	7.53 ± 1.96	0.99 ± 0.32
C 20:2 n-9	3.17 ± 0.90	7.33 ± 1.05	5.16 ± 1.34	4.17 ± 0.53
C 20:3 n-6	0.99 ± 0.38	0.66 ± 0.26	0.51 ± 0.23	0.57 ± 0.32
C 20:4 n-6	1.12 ± 0.36	0.85 ± 0.23	0.63 ± 0.21	1.21 ± 0.15
C 20:5 n-3 (EPA)	6.08 ± 0.65	8.04 ± 0.72	8.63 ± 2.12	8.75 ± 1.10
C 22:2	0.82 ± 0.20	1.95 ± 0.33	0.93 ± 0.41	1.15 ± 0.17
C 22:3	0.41 ± 0.20	0.74 ± 0.32	0.45 ± 0.14	0.34 ± 0.18
C 22:4 n-6	0.26 ± 0.15	0.73 ± 0.17	0.38 ± 0.11	0.57 ± 0.10
C 22:5 n-3 (DPA)	1.35 ± 0.92	1.52 ± 0.27	1.06 ± 0.71	1.26 ± 0.84
C 22:6 n-3 (DHA)	6.82 ± 2.82	14.55 ± 2.51	12.99 ± 3.52	14.58 ± 2.17
Σ PUFA	31.9	45.4	40.8	37.0
Σ LC-PUFA (EPA + DHA +DPA)	14.3	24.1	22.7	24.6
Σ n-3	22.5	30.1	30.2	25.6
Σ n-6	5.0	11.3	4.1	5.7
Σ n-6/ Σ n-3	0.2	0.4	0.1	0.2
P/S ratio	1.3	1.8	1.6	1.3
Trans fatty acids mono- and polyunsaturated				
C 16:1 *trans*	1.14 ± 0.34	1.44 ± 0.22	1.11 ± 0.19	1.57 ± 0.12
C 18:1 *trans*	0.34 ± 0.16	0.14 ± 0.05	0.10 ± 0.04	0.11 ± 0.06
C 22:1 *trans*	0.18 ± 0.04	0.26 ± 0.16	0.23 ± 0.17	0.16 ± 0.07
C 18:2 *trans*	0.33 ± 0.11	0.60 ± 0.16	0.10 ± 0.10	0.58 ± 0.15
C 18:3 *trans*	0.48 ± 0.14	0.41 ± 0.07	0.34 ± 0.08	0.46 ± 0.06
Σ *trans*	2.5	2.9	1.9	2.9
Unidentified fatty acids				
Σ	1.3	1.0	0.9	1.8

* X- average; SD- standard deviation, n– number of samples; Σ- sum; P/S- polyunsaturated fatty acids [%]/saturated fatty acids [%]

ding rheumatoid arthritis [12]. Whereas other studies results suggested, that n-3 PUFA supplementation has a positive impact on the central nervous system [8, 10]. The beneficial effects of fish consumption or EPA and DHA supplementation during pregnancy on the proper development of the fetus was also documented. The authors of several studies suggested that supplementation of LC-PUFA helps pregnant women slightly longer duration of pregnancy, increased infant birth weight and reducing the risk of premature birth [16, 41, 42]. Moreover, it is recommended to fortified blends for infant feeding in LC-PUFA, because of the significant role of DHA in proper development and functioning of the central nervous system and retina [26].

The percentage of n-6 PUFA in analyzed samples was significantly lower than n-3 PUFA. In all analyzed fish the LA was predominant among n-6 fatty acids. *Usydus et al.* [45] also found that LA was predominant among n-6 PUFA in smoked fish. Similar results in fresh herring and salmon were obtained by *Balas et al.* [2]. Whereas in fresh mackerel a predominant of n-6 fatty acids was arachidonic acid (AA, C20:4) [2].

The ratio of n-6/n-3 PUFA in analyzed fish was low and ranged from 0.1/1 to 0.4/1. Prophylactic meaning of

low n-6/n-3 PUFA ratio is caused by a difference in the physiological activity of eicosanoids synthesized from these acids in the human body [1, 13]. The recommendations of Polish Forum for Prevention of Cardiovascular Diseases indicated that the n-6/n-3 ratio should be at most 4-5/1 and therefore it is justified to increased intake of products that are sources of n-3 PUFA in the daily food ration [21].

Atherogenicity of the analyzed smoked fish was assessed by P/S ratio. An optimal value of P/S ratio for food should be ≥ 1.0, because such product is more valuable in terms of the content of PUFA and may be helpful in reducing the risk of atherosclerosis and coronary heart disease. The P/S ratio in analyzed smoked fish was as follows: 1.3 in halibut, 1.8 in mackerel, 1.3 in sprats and 1.6 in bloater.

Analyzed smoked marine fish had a comparable content of *trans* fatty acids, which ranged from 1.9% to 2.9% of total fatty acids. The highest percentage of total *trans* fatty acids was found in sprats (2.9%) and mackerel (2.9%), while the lowest in bloater (1.9%). Lower content of *trans* fatty acids in marine fish, compared with the present study, was assessed by *Regulska-Ilow and Ilow* [35], in whose study these acids in fresh herrings and in herrings after thermal processes (cooking, grilling, traditional frying and microwave frying) ranged from 0.6% to 0.8% of total fatty acids. Among the total *trans* fatty acids the palimitoleic acid (*trans*-C16:1 n-7) was dominated. The highest level of *trans* C16:1 was observed in sprats (1,57%) and in mackerel (1,44%). *Trans* palmitoleic acid is mainly obtained from exogenous sources [31]. As an adverse for human health are considered these *trans* fatty acids, that arose as a result of technological processes and are found in processed and fried foods [17].

CONCLUSIONS

1. The fat of smoked marine fish is a very good source of an essential n-3 long chain polyunsaturated fatty acids. The content of total fat, cholesterol and individual fatty acids in smoked marine fish depends on their species.
2. In terms of nutritional magnitude the meat of mackerel and herring, compared to the meat of sprat and halibut has a much better n-3 PUFA content, while relatively low content of cholesterol.

Aknowledgement

Conflict of interest
The authors declare no conflict of interest.

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