Evaluation of the balance of oils from fish by-products

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Abstract. Fish by-products is a promising raw material for fish oil extraction, which is a source of polyunsaturated fatty acids, including omega-3 and omega-6 classes. Gas chromatography method was used to study the fatty acid composition of oils extracted from six types of fish by-products including heads, ridges and internal organs of Atlantic mackerel, Baltic herring, fresh Baltic sprat, heads and ridges of Atlantic salmon and heads of fresh herring and smoked sprat. The highest content of omega-3 polyunsaturated fatty acids was found in mackerel and herring processing by-products – 43.6 and 49.6 %, respectively. The coefficients of oil balance are calculated. The coefficient of rationality of the fatty acid composition, which reflects the degree of compliance of oil with the "ideal" lipid for human nutrition was the highest for sprat oil (0.87 units fraction). Sprat oil is characterized by the most favorable ratio of saturated and polyunsaturated fatty acids, since the coefficient of biological efficiency is as close as possible to 1. The coefficients of rationality of the fatty acid composition of other oils are reduced due to the significant predominance of omega-3 fatty acids. It is promising to use the studied oils to optimize the fatty acid composition of products based on animal fats and vegetable oils, which are characterized by a high content of omega-6 fatty acids.

1. Introduction
Fat is an integral part of human nutrition. According to the FAO/WHO recommendation fat should account for 15 to 35 % of the energy value of the daily diet. The main criterion for the balance of dietary fat is the quantity and quality of unsaturated fatty acids. It has been shown that replacing some of the saturated fatty acids with monounsaturated and polyunsaturated ones reduces the level of cholesterol in blood and the risk of insulin resistance developing. The optimal content of saturated, monounsaturated and polyunsaturated fatty acids in the human diet is 1 : 1 : 1 [1–4].

Omega-3 and omega-6 fatty acids have the highest biological significance. Polyunsaturated fatty acids (PUFAs) of these classes have the ability to change the composition of plasma membranes, regulate gene transcription, and modulate cellular signals. They are precursors of various lipid mediators, including eicosanoids. The preventive function of omega-3 fatty acids in relation to cardiovascular diseases is well known, which is realized due to eicosanoids formed in the corresponding metabolic pathways. In addition to anti-atherogenic and cardioprotective effects, recent studies have established anti-carcinogenic, anti-inflammatory, antimicrobial, and immunomodulatory effects of such eicosanoids. Omega-6 fatty acids, on the contrary, are precursors in the synthesis of biological regulators that have an atherogenic, inflammatory effect. Since omega-3 and omega-6 acids are biological competitors in the synthesis of various eicosanoids, it is necessary to maintain an optimal ratio of these fatty acids in order to ensure a healthy human diet [4–8]. The ratio of omega-3 and omega-6 fatty acids consumed by people should be at least 1:10 for a healthy person and at least
1:5 for people with diseases of the cardiovascular system [2, 4]. The consumption of such important omega-3 polyunsaturated fatty acids as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and alpha-linolenic acid (ALA) is separately normalized. It is recommended to consume daily from 0.2 to 0.5 g of eicosapentaenoic and docosahexaenoic acids and 1 g of alpha-linolenic acid [4].

The main source of polyunsaturated fatty acids, in particular, omega-3 class, are marine pelagic fish, in which they enter the body through the food chain from plankton [4]. Muscle tissue of these fish, as a rule, is characterized by high nutritional and biological value, so its use for oil production is not appropriate. Fish by-products or waste generated during the processing of fish for food also contain valuable fish oil, which is not inferior in quality to oil from muscle [9–11]. The prospects for using the protein component of fish by-products in the production of food hydrolysates and various protein additives are widely covered in the scientific literature [12], but the potential for producing fat products has not been sufficiently studied. On the territory of the Kaliningrad region, as a result of the activities of fish processing enterprises, up to 10 tons of fish by-products, or about 1 ton of fish lipids, are generated daily. At the same time, almost half of these by-products (4–5 tons) are heads of smoked sprat, which are by-products of sprat production [13]. It is relevant to study the fatty acid composition of lipids of these by-products in order to assess their balance in biologically important acids and determine the direction of the use for human nutrition.

2. Objects and methods of research

The objects of the study were oils extracted from fish by-products formed during fish processing in the Kaliningrad region. For oil extraction, following fish by-products were used: Atlantic mackerel (Scomber scombrus), fresh and smoked Baltic sprat (Sprattus sprattus), Atlantic herring (Clupea harengus), Baltic herring (Clupea harengus membras) and Atlantic salmon (Salmo salar). Fish by-products of mackerel, sprat, and Atlantic herring included heads, ridges, and internal organs of fish. Salmon processing by-products were represented by heads and ridges, herring and smoked sprat by-products included heads.

For oil extraction raw material was crushed in a grinder with a hole diameter grid 5 mm, and then was added water at temperature of 60 °C at a weight ratio of water: raw material equal to 1 : 1. Fat was separated by centrifugation of the mixture for 15 minutes at a rotation frequency of 4,000 rpm. After centrifugation the liquid fraction was filtered through 260 μm nylon filter and fractionated into protein hydrolysate and fish oil. After this operation fish oil was filtered through 125 μm nylon fine particle filter to separate fine impurities.

The content of individual fatty acids was determined in the oil purified from impurities. Sample preparation consisted of saponification of fatty acids and further esterification using methanol. The obtained fatty acid esters were analyzed on a TRAXE GC 2000 Ultra FINNIGAN gas chromatograph with flame ionization detection under the following conditions: the column was quartz capillary SPTM-2560 (100 m × 0.25 mm); the injector temperature, the initial and final temperatures of the column thermostat were maintained at 260, 100 and 240 °C, respectively. The volume of the injected sample was 1 µl. The peaks were identified by comparing their retention times with those of authentic reference compounds (Sigma-Aldrich, St. Louis, Missouri, USA). Baltic herring and smoked Baltic sprat oils were analyzed using gas chromatograph GC 2010 from Shimadzu (Kyoto, Japan). Transesterification was carried out by DGF standard procedure using TMSH (trimethyl-sulfoniumhydroxide) in tert-butyl-methylether. Quantification was done by reference standard mixtures from Supelco. Analytical system was GC 2010 from Shimadzu (Kyoto, Japan) equipped with flame ionization detection and computer system. Quantification was done by response corrected total area principal. Separation phase was SP2380 from Supelco, 0.2 µm film thickness, 0.5 mm diameter, 25 m. detector and injector at 250 °C, temperature program starting at 75 °C with 5 K/min to 125 °C, followed by 2 K/min to 225 °C. Equilibration after run at 225 °C was done for 5 minutes.

Fatty acids were grouped into categories depending on their saturation level, i.e. the number of double bonds. Thus, the amount of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) containing one double bond, and polyunsaturated fatty acids (PUFAs) containing two or
more double bonds was calculated. Polynsaturated fatty acids were also classified by the location of the first double bond into omega-6 and omega-3 groups.

The coefficient of biological efficiency of lipids was determined as the ratio of the total amount of PUFA to the total amount of SFA (1) [3].

$$BE = \frac{\Sigma PUFA}{\Sigma SFA}$$

(1)

where $BE$ – coefficient of biological efficiency, units fraction;
\(\Sigma PUFA\) – total content of polyunsaturated fatty acids in lipids, %,
\(\Sigma SFA\) – total content of saturated fatty acids, %.

The adequacy of the composition and the ratio of fatty acids to the standard was evaluated by the coefficient of rationality of the fatty acid composition (2) [1].

$$R_l = \left[ \prod_{i=1}^{n} \left( \frac{L_i}{L_{si}} \right)^{sign(1-L_i/L_{si})} \right]^{1/n}$$

(2)

where, $R_l$ – coefficient of rationality of the fatty acid composition, units fraction;
$L_i$ – mass fraction of the i-th fatty acid in the raw material or product, g/100 g of fat;
$L_{si}$ – mass fraction of the i-th fatty acid corresponding to the physiologically necessary norm (standard), g/100 g of fat;

$i = 1$ corresponds to the amount of SFAs, $i = 2$ – amount of MUFAs, $i = 3$ – amount of PUFAs;

For evaluation of the fatty acid balance, in addition to the coefficients, such criteria as the content of fatty acids in each fraction and their ratio, the ratio of omega-3 and omega-6 polyunsaturated fatty acids, the degree of satisfaction of the daily human need for eicosapentaenoic fatty acid (EPA), docosahexaenoic fatty acid (DHA) and alpha-linoleic acid (ALA) were used (Table 1).

**Table 1. Criteria for evaluating the fatty acid balance of oil [1–4].**

| Indicator                              | Value               |
|----------------------------------------|---------------------|
| SFAs:MUFAs:PUFAs                      | 1:1:1               |
| omega-3:omega-6                       | 1:10:1:5            |
| The daily need of an adult person in a given fatty acid: |                     |
| EPA + DHA                              | 0.5-1.0 g           |
| ALA                                    | 1.0 g               |
| Coefficient of biological efficiency of lipids | $BE = 1$          |
| Coefficient of rationality of the fatty acid composition | $R_l \rightarrow 1$ |
3. Results
Table 2 shows the results of the study of the fatty acid composition of oils extracted from different fish processing by-products.

| Table 2. Fatty acid composition of fish processing by-products. |
|----------------------------------|--------|--------|-------|--------|--------|--------|
| C:D                             | Name   | Mackerel| Fresh sprat | Herring | Salmon | Smoked sprat | Baltic herring |
|-------|--------|--------|--------|--------|--------|--------|--------|
| SFA   |        |        |        |        |        |        |        |
| 8:0   | Caprylic acid     | –      | 0.2    | –      | –      | –      | –      |
| 10:0  | Capryc acid       | 1.4    | 0.2    | 0.4    | –      | –      | –      |
| 11:0  | Undecylic acid    | –      | 0.15   | –      | –      | –      | –      |
| 12:0  | Lauric acid       | –      | –      | 0.1    | –      | –      | –      |
| 14:0  | Myristic acid     | 8.6    | 4.2    | 8.3    | 2.5    | 4.6    | 4.6    |
| 15:0  | Pentadecanoic acid| 0.45   | 0.5    | 0.4    | 0.2    | 0.7    | 0.7    |
| 16:0  | Palmitic acid     | 13.9   | 18.0   | 11.1   | 10.1   | 22.6   | 23.6   |
| 17:0  | Margaric acid     | 0.3    | 0.9    | 0.3    | 0.1    | 0.3    | 0.4    |
| 18:0  | Stearic acid      | 2.1    | 2.3    | 0.8    | 2.7    | 3.1    | 3.2    |
| 20:0  | Arachidic acid    | 0.2    | –      | 0.3    | 0.3    | 0.3    | –      |
| 21:0  | Heneicosylic acid | –      | –      | –      | 0.05   | 1.4    | 2.0    |
| 22:0  | Behenic acid      | –      | –      | –      | 0.1    | –      | –      |
| 23:0  | Tricosylic acid   | 1.1    | 0.3    | 0.4    | 0.75   | –      | –      |
| Total SFA |       | 28.05  | 26.75  | 22.1   | 16.8   | 33.0   | 34.5   |
| MUFA  |        |        |        |        |        |        |        |
| 14:1  | Myristoleic acid  | 0.2    | 0.4    | 0.1    | 0.1    | 0.8    | 0.5    |
| 15:1  | Pentadecanoic acid| –      | 0.2    | 0.1    | –      | –      | –      |
| 16:1  | Palmitoleic acid  | 3.4    | 5.3    | 4.65   | 0.1    | 4.9    | 5.0    |
| 17:1  | Margaroleic acid  | 0.2    | 0.45   | 0.4    | 0.3    | 0.7    | 0.7    |
| 18:1n9 | Oleic acid       | 10.7   | 26.6   | 8.65   | 37.6   | 30.9   | 28.6   |
| 20:1  | Gondoic acid      | 1.0    | 0.5    | 1.5    | 2.6    | 0.7    | 0.5    |
| 22:1n9 | Erucic acid      | –      | 0.5    | 0.1    | –      | –      | –      |
| 24:1  | Nervonic acid     | –      | –      | –      | 2.5    | 1.8    | –      |
| Total MUFA |       | 15.5   | 33.95  | 15.5   | 40.7   | 40.5   | 37.1   |
| PUFA  |        |        |        |        |        |        |        |
| 18:2n6t | Octadecadienoic | 0.1    | –      | –      | 0.05   | 3.2    | 1.8    |
| 18:2n6c | Linoleic acid    | 1.8    | 3.3    | 1.7    | 13.3   | 3.2    | 2.4    |
| 18:3n3 | Alpha-linolenic acid | 11.3  | 2.8   | 14.7   | 5.4    | 2.5    | 2.5    |
| 18:3n6 | Gamma-linolenic acid | 0.1    | –      | 0.1    | 0.05   | –      | –      |
| 20:2  | Eicosadienoic acid| 7.1    | 0.4    | 0.3    | 1.3    | 0.6    | 0.5    |
| 20:3n3 | Eicosatrienoic acid | 14.4  | –      | 23.0   | 0.5    | 0.4    | –      |
|       | Dihomo-gamma-linolenic acid | –    | –     | –      | 0.15   | –      | –      |
| 20:4n6 | Arachidonic acid  | –      | –      | 0.2    | 0.3    | 0.8    | 0.8    |
| 20:5n3 | Eicosapentaenoic acid | 8.85  | 7.2   | 5.4    | 4.2    | 4.4    | 6.9    |
| 22:2  | Docosadienoic acid| –      | –      | –      | –      | 0.4    | 0.4    |
| 22:5n3 | Docosapentaenoic acid | –    | –     | –      | 0.6    | 0.7    | –      |
| 22:6n3 | Docosahexaenoic acid | 9.1   | 12.4  | 6.6    | 8.3    | 7.7    | 10.5   |
| Total PUFA |       | 52.75  | 26.1   | 52.0   | 33.6   | 23.8   | 26.5   |

Using chromatographic analysis, 21 fatty acids were identified in oils extracted from mackerel, fresh sprat and herring by-products, 23 fatty acids were identified in oils extracted from smoked sprat fatty acids were identified in oils extracted from, and 24 fatty acids were identified in oils extracted
from herring and salmon oil. Fish oils are characterized by low degree of saturation. The content of saturated acids does not exceed 35% of the total amount of fatty acids and is especially low in the oil of Atlantic salmon which is 16.8%. The main fatty acid of the saturated fraction of fish oils is palmitic acid (16:0). Among monounsaturated fatty acids, oleic acid prevails.

The largest number of biologically valuable polyunsaturated fatty acids contain the oils extracted from mackerel and herring processing by-products with the share of 52% of total fatty acids. Salmon oil contains 33.6% polyunsaturated fatty acids. The oils extracted from herring processing by-products, fresh sprat and sprat smoked heads have approximately the same content of polyunsaturated fatty acids making up 23-26%. The fraction of omega-3 fatty acids in fish oils is represented by alpha-linolenic, eicosatrienoic, eicosapentaenoic and docosahexaenoic acids. Among the omega-6 acids, linoleic acid prevails, while the content of gamma-linolenic, digomo-gamma-linolenic and arachidonic acids is insignificant.

The total content and ratio of individual fractions of fatty acids as well as the coefficients that characterize the fatty acid balance are presented in Table 3.

Table 3. Main indicators characterizing the fatty acid balance of fish oils extracted from fish processing by-products.

| Indicator                              | Mackerel | Fresh sprat | Herring | Salmon | Smoked sprat | Baltic herring |
|----------------------------------------|----------|-------------|---------|--------|--------------|----------------|
| Total SFAs                             | 28.05    | 26.75       | 22.1    | 16.8   | 33.0         | 34.5           |
| Total MUFAs                            | 15.5     | 33.95       | 15.5    | 40.7   | 40.5         | 37.1           |
| Total PUFAs                            | 52.75    | 26.1        | 52.0    | 33.6   | 23.8         | 26.5           |
| SFAs:MUFAs:PUFAs                       | 1.8:1:3.4| 1:1.3:1     | 1.4:1:3.3| 1:2:1.5| 1:4:1.7:1    | 1:3:1:4:1      |
| Total omega-3                          | 43.6     | 22.4        | 49.6    | 18.4   | 15.6         | 20.6           |
| Total omega-6                          | 2.1      | 3.3         | 2.0     | 13.9   | 7.2          | 5.0            |
| omega-3 : omega-6                      | 20.7:1   | 6.8:1       | 24.8:1  | 1.3:1  | 2.2:1        | 4.1:1          |
| EPA+DHA                                | 17.95    | 19.6        | 11.9    | 12.5   | 12.1         | 17.4           |
| ALA                                    | 11.3     | 2.8         | 14.7    | 5.4    | 2.5          | 2.5            |
| BE                                     | 1.88     | 0.98        | 2.36    | 1.99   | 0.72         | 0.77           |
| \( R_l \)                              | 0.63     | 0.85        | 0.58    | 0.74   | 0.71         | 0.69           |

Table 3 shows that the content of omega-3 polyunsaturated fatty acids exceeds the content of omega-6 fatty acids several times in the oils of mackerel, sprat and herring. The content of omega-3 and omega-6 fatty acids in the oil extracted from salmon processing by-products is at the same level. The predominance of omega-3 fatty acids causes high values of the ratio of omega-3 : omega-6, significantly different from the recommended 1 : 10 – 1 : 5.

From the point of view of balance in the ratio of saturated, monounsaturated and polyunsaturated fatty acids, the oil from fresh sprat is the closest one to the “ideal”, as evidenced by the close to one coefficient of biological efficiency (0.98) and the coefficient of rationality of the fatty acid composition (0.85). Low \( R_l \) values for mackerel (0.63) and herring (0.58) oils are associated with a large amount of polyunsaturated fatty acids in their composition. These oils can be successfully used to correct the diet of a person who has a pronounced deficiency of omega-3 polyunsaturated fatty acids.

All oils are rich sources of eicosapentaenoic and docosahexaenoic acids. The minimum daily dose of eicosapentaenoic and docosahexaenoic fatty acids recommended for a person is 2–3 g of oil extracted from mackerel, sprat or herring processing by-products and 4 g of herring or salmon oil. It is
advisable to consider mackerel and herring oils as sources of alpha-linolenic acid. The daily value of this polyunsaturated fatty acid for an adult is contained in 9 g of mackerel oil and in 7 g of herring oil.

4. Conclusions
This research allows us to make conclusions about the high biological potential of the fat component of fish processing by-products of the Kaliningrad region, the possibility and rationality of using this oils in the human diet as sources of polyunsaturated fatty acids, including omega-3 eicosapentaenoic and docosahexaenoic fatty acids. The calculation of the biological efficiency and rationality of the fatty acid composition showed the feasibility of using fish oils in combination with vegetable and animal fats rich in omega-6 fatty acids for the design of fat compositions balanced by omega-3 and omega-6 fatty acids.

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