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Obtention of omega-3-fatty acids cryoconcentrated fish oil from by-products of preserves industry

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Abstract. The technology for obtaining and cryoconcentration of high-quality fish oil from collagen-containing wastes of slightly salted herring under gentle conditions using electrochemically obtained catholytes has been developed. Physicochemical analysis of raw materials was carried out and the yields of products from raw materials at all stages of processing were determined. Concentration of omega-3 fatty acids in oil was carried out using the cryo method. The main phase transitions in oil with decreasing temperature have been determined. The mass yields were determined and the biochemical composition of the cryoconcentrated fish oil fractions was investigated. It was defined the temperature of -14° C at which a phase transition is observed, providing an increase in the concentration of omega-3 fatty acids in oil by 3 times. The usage of cryoconcentrated fish oil allows to produce biologically active food supplement or raw materials for a functional food.

Key words: cryoconcentration, omega-3 fatty acids, by-products, fish oil, salted herring preserves, biologically active substances, hydrolyzate, functional food, extraction.

INTRODUCTION

It is known that unsaturated fatty acids are an indispensable factor in nutrition, affect many biochemical processes, ensure normal growth, development and determine the physical state of the body as a whole (Freitas et al., 2017; Calder et al., 2020).

Omega-3 fatty acids are the most important nutrient, a structural element of cell membranes, providing vascular permeability, a regulator of nervous, endocrine, and metabolic processes in the body (Vorslov, 2017; Sacca et al., 2018; Radzikowska et al., 2019). Currently, there is a deficiency of omega-3unsaturated fatty acids in the daily human diet, since their main food sources, such as sea fish, seafood (Mohanty et al., 2019) are not consumed in the required amount, which is due to the nutritional traditions of the population of many European countries (Sioen et al., 2017).

Omega-3 deficiency is experienced by about 80% of the inhabitants of all developed countries, which might provoke cardiovascular diseases, leading to heart attacks or strokes, which occupy a leading place in mortality. Also, their lack in the diet causes a decrease in mental activity, a violation of mental states, etc. (Jahangard et al., 2018).

The richest sources of omega-3 polyunsaturated fatty acids (PUFA) are seafood, in particular oils from marine fish and other aquatic organisms (Durmuş, 2018). Also, these valuable components are found in large quantities in the waste from their cutting (Repnikov, 2017; Suresh et al., 2018). A particularly valuable source of omega-3 acids are fatty fish of the Herring family, the capture of which accounts for more than a third of the world catch of aquatic organisms (Kurkotilo & Vasilyeva, 2017; Bazarnova et al., 2020; Egerton et al., 2020,).

The most popular product of these fish in the northwest region of Europe is lightly salted herring preserves (Neuimin, 2017). In the production of preserves, more than 30% of secondary waste is generated, among which the ratio of skin during skinning of fillets accounts for about 10% (Kazakova & Zemlyakova, 2020). Therefore, investigation on the production of essential fatty acids and fish oil obtained from collagen-containing waste from the production of preserves of slightly salted herring is very promising.

It is known that wastes from cutting Atlantic herring, like fish itself, are characterized by a valuable chemical composition and high fat content (Table 1), which indicates the expediency of using them as a raw material source for obtaining biologically active substances (BAS) of lipoid nature (Kosman et al., 2016; Alfio, et al., 2021). Collagen-containing waste from lightly salted herring is one of the most promising sources of fish oil and protein hydrolysates, since due to the popularity of this type of product, the amount of this waste is large and they are not used in agriculture due to the presence of salt in them. Also, the promising use of these wastes is due to the partial hydrolysis of raw materials during the salting process, which facilitates the processes of extracting nutrients from the protein matrix (Mayta-Apaza et al., 2021). If in oil isolated from the carcass of fresh fish, amine nitrogen is almost completely absent, then in the oil isolated from the carcasses of salted fish, amine nitrogen is found in a rather significant amount. Its accumulation proceeds in proportion to the time, which suggests the formation of compounds from the breakdown products of proteins and oil and their dissolution in oil. Control over ripening (loss of raw taste and smell by fish, acquisition of the appropriate taste and aroma – 'bouquet') can be carried out by observing the distribution of oil in fish flesh (Porotikova et al., 2015). In fresh and salted immature fish, oil is either in the cells of muscle tissue, or in the subcutaneous tissue in the form of isolated drops; in fish, in which the processes of protein breakdown and the structure of muscle tissue have changed, oil permeates its entire mass - a solid film of fat is visible on the cut. This indicator is very characteristic of ripened salted fish.

The most widespread technologies are the enzymatic and chemical hydrolysis of wastes (Mukatova et al., 2018, Abuine et al., 2019, Varygina & Davydova, 2021, Zakharchuk et al., 2021), but they are expensive and do not always ensure the quality of the lipids obtained. The method of electrochemical hydrolysis is devoid of these drawbacks (Zakharchuk et al., 1995), which makes it possible to obtain hydrolysates with a given degree of hydrolysis at low energy consumption and does not require the use of acids and alkalis.

In the process of electrochemical treatment, proteins, polypeptides, lipids go into solution in the form of an emulsion, and the mineral precipitate deposits (Chikisheva et al., 2020).

The chosen method has several advantages:

– 95–97% of lipids from raw materials go into solution;

– the released lipids are of high quality due to the gentle conditions and processing modes;

– when extracting oil, it is refined, which is especially important when processing herring skin, which, as it's known, undergoes unfavorable oxidative changes during salting and fermentation.

It is known that in fish of the Herring family, most of the oil is located in the subcutaneous layer and goes to the waste at the skinning stage (Baydalinova et al., 2011; Kosman et al., 2016). Obviously, it is advisable to use skin to obtain fish oil enriched with unsaturated fatty acids, which can be used either as biologically active food additives (BAA) or as part of functional food products (FFP) (Kuprina et al., 2019).

Research aim. Developing a technology for obtaining and cryoconcentration of fish oil from skin of slightly salted herring, investigating biochemical, physicochemical and fatty acid composition of cryoconcentrated fractions of fish oil and developing recommendations for its use.

MATERIALS AND METHODS

Raw materials

The main industrial semi-finished product was used - Atlantic herring fillet on the skin. Producer - Faroe Islands, plant FO229, catch area FAO227, size range 4–7 pcs kg^{-1}, catch period - October 2020.

Sampling

Research objects are wastes of skin obtained by cutting slightly salted herring in the process of producing frozen preserves from Atlantic herring were selected. The herring salting was carried out in the traditional way - wet salting in accordance with the technological scheme (Fig. 1).

The collection of herring skin was carried out mechanically by means of a vacuum system. To the skinning machine is mounted a branch pipe of the vacuum pipeline, into which the removed skin enters and then moves to the place of waste collection - plastic vat containers.

Protein hydrolysate obtention

The processing of waste from the production of preserves from herring fillets was carried out electrochemically, followed by the release of lipids from protein solutions (Kuprina et al., 2019. For complete protein dissolution, the optimal parameters of the raw material processing were selected: voltage, current, hydronic modulus, time and temperature of suspension heating in a stirred reactor.

Fermented herring skin was dispersed to a particle size of 5×10^{-3} m, mixed with catholyte in stirred laboratory reactor LR 1000 (CZL, Russia). The catholyte was a weak saline solution obtained in the cathode chamber of a diaphragm electrolyzer, pH not less than 12.2, Eh less than - 860 mV in a ratio of 1: 3. The mixture was thermostated in the

stirred reactor for 40 minutes at 85 ± 5 °C. After holding in the reactor, the mixture was centrifuged at 4,000 rpm for 15 minutes to obtain oil.

To obtain protein-lipid hydrolyzate from herring skin wastes remaining in the production of preserves, with the subsequent isolation of oil, the raw material was electro-chemically hydrolyzed in catholyte medium, obtained electrochemically and possessing alkaline and reducing properties (pH not less than 12.0, Eh not more than -860 mV. The catholyte was obtained in industrial electrolyzers on STEL-20 installation (Rostekhnologiya, Russia).

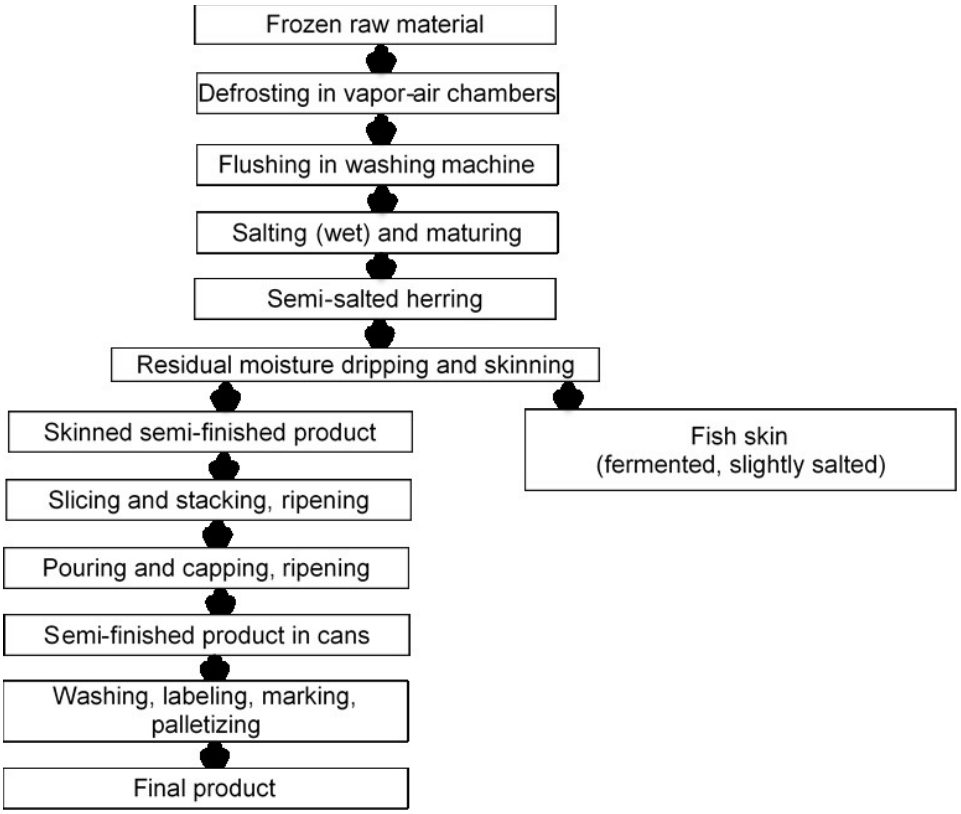

Figure 1. General technological scheme for the production of preserves from herring fillets.

Physico-chemical analysis

The content of protein, fat, ash of raw materials, as well as the iodine and acid value of lipids were determined in accordance with GOST 7636-85 (Fish, marine mammals, invertebrates and products of their processing. Methods of analysis).

Determination of the Fatty acid composition of lipids

The oil was treated with a KOH solution in methanol. As a result, methyl esters of fatty acids were formed, which were extracted with hexane. The hexane solution was used for gas chromatography-mass spectrometric analysis.

The analysis of the fatty acid composition of the obtained fish oil samples was carried out on gas chromatograph (GCMC-TQ 8040 Shimadzu, Japan) in the full ion current mode and in the scanning mode for individual ions (SIM). The temperature of the ion source was 200 °C, the interface was 250 °C, and the mass scanning was in the range $m\,z^{-1} = 45–500$. In the analysis of fatty acids, was used their precolumn derivatization with KOH solution in methanol to obtain methyl esters of carboxylic

acids. Separation of the resulting derivatives was carried out on Rxi-5SiMs capillary chromatographic column (30 m × 0.25 mm × 0.25 μm). The carrier gas was helium, the gas flow rate was 1.03 mL min^{-1}. Split / splitless mode was used (splitless 1 min, then split 10:1). Injector temperature was 220 °C. Temperature programming mode: the initial isothermal section was 50 °C for 1 min, then the column temperature rises to 250 °C (10 °C min^{-1}), the final isothermal section was 250 °C for 10 minutes. The total chromatography time was 35 min.

Fractional division of lipids by cryoconcentration

In order to concentrate unsaturated fatty acids in fish oil, lipid processing was carried out by the cryoconcentration method. 4 glass tubes (2×14 cm) with fish oil with a volume of 50 mL were placed in a container with a 28% aqueous solution of calcium chloride cooled by low-temperature refrigeration unit. The temperature was measured inside the sample and in a cooling medium using electronic thermometers 'Vapan' (TZ 003 X VP, Russia) with a standard deviation of 0.14. Phase transitions in oil were recorded at temperatures of 4, -7, -14 and -37 °C. Then the oil was mixed with acetone in a ratio of 1: 8 (v v^{-1}) and the cooling was repeated from 20 to - 40 °C. The average speed of cooling and freezing is 0.3 °C min^{-1} (Kuprina et al., 2019). After each phase transition, the oil was separated from the liquid fraction by filtration. For this purpose, synthetic filter with a pore diameter in the range of 0.3–0.5 mm was used. Filtration was carried out in a cooling unit.

The necessary and sufficient level of data reliability ($p < 0.05$) was obtained by repeating the experiment three times. Statistical data processing was performed using standard methods for evaluating test results for small samples using Microsoft Excel 2010.

RESULTS AND DISCUSSION

We studied the material balance of the skinning process of herring after salting and determined the chemical composition of Atlantic herring before and after salting.

It was found that the oil content of fresh Atlantic herring is higher than that of medium-salted Atlantic herring by 4%, and the moisture content is 1.7% lower. The protein content in herring before and after salting is approximately the same (Table 1).

Table 1. Chemical composition of fresh and slightly salted Atlantic herring

Sample	Moisture, %	Fat, %	Acid value of fat, mg KOH g^{-1}	Protein, %	Ash, %	Energy value, kcal
Fresh Atlantic herring	61.3 ± 2.0	19.5 ± 0.3	1.8	17.7 ± 0.2	1.5 ± 0.1	248
Atlantic herring, medium salted	63.0 ± 2.0	16.3 ± 0.3	2.6	17.0 ± 0.2	11.5 ± 1.0	215

It is also known that during the salting process, partial lipid hydrolysis occurs, leading to an increase in the acid number (Table 1). This process involves the tissue's own enzymes - lipases, while the quality of the fatty acid composition deteriorates. In addition, when interacting with atmospheric oxygen, lipids begin to oxidize (Ghnimi et al., 2017; Hematyar et al., 2019). However, since the process of obtaining lightly salted

herring under production conditions is optimized and reduced to seven days, these undesirable processes are not implemented.

The final output of the dispersed skin waste part is on average 6.5% of the mass of fillets skin-on taken for processing. In terms of physicochemical parameters, the semi-finished product of the waste part is close to the indicators of a similar initial semi-finished product - herring fillet on the skin:

Salt content - 3.8–4.2%;

Fat content - 27–28%

Acidity - 0.17–0.23% (according to vinegar essence70%);

pH = 5.8–6.1%.

The dispersed waste was processed in stirred reactor with in the environment of the catholyte obtained by the electrochemical method.

Unlike the previously developed electrochemical method for obtaining fish oil (Chikisheva et al., 2020), the dispersed raw material is not processed in the interelectrode space of the electrolyzer, which eliminates the need for frequent equipment washing.

At the same time, a complete dissolution of tissues was achieved, including bone tissues (fins), this effect was obtained in gentle conditions without processing the raw material in an electric field inside the electrolyzer space and a significantly lower hydromodule 1: 3, and not 1: 6, as was done earlier (Kuprina et al., 2019). This effect was probably achieved due to partial hydrolysis of tissues during salting.

Even if we assume the possibility of some oxidative deterioration of oil during salting, due to the presence of reducing properties in the electrochemically obtained catholyte, restoration of the quality of the original oil is ensured (Kuprina et al., 2019, Kuprina et al., 2020).

The mass yield of oil from the hydrolyzate of the skin of slightly salted herring after centrifugation was significant - 22–25% of the mass of the sample skin.

It was revealed that fish oil, obtained electrochemically from wastes of fermented salted herring (Table 2, Fig. 2) has an acid number of ≤ 0.15 mg KOH g^{-1}, contains more than 20% omega-3 polyunsaturated fatty acids from the total fatty acids, but this amount is not enough to meet the daily human needs (according to methodological recommendations MP 2.3.1.2432–08) (Rospotrebnadzor). Thus, the development of a technology for the concentration of omega-3 polyunsaturated fatty acids is relevant.

Table 2. Fatty acid composition of salted herring skin oil

No.	Acid name	Content, mg g^{-1}	Content, %
1	Lauric $C_{12}H_{24}O_2$	0.7	0.07
2	9-tetradecene $C_{14}H_{28}O$	0.6	0.06
3	Myristic $C_{14}H_{28}O_2$	52	5.2
4	Pentadecane $C_{15}H_{32}$	3	0.3
5	9-hexadecene $C_{16}H_{30}O$	41	4.1
6	Hexadecanoic (palmitic) $C_{16}H_{32}O_2$	85	8.5
7	Linoleic $C_{18}H_{32}O_2$	31	3.1
8	Linolenic $C_{18}H_{30}O_2$	4	4
9	9-octadecene (oleic) $C_{16}H_{36}$	53	5.3
10	Stearic $C_{18}H_{36}O_2$	5	0.5
11	Cis-5,8,11,14,17-Eicosaenoic $C_{21}H_{32}O_2$	43	4.3
12	Cis-4,7,10,13,16,19-Docosahexaenoic $C_{22}H_{32}O_2$	33	3.3
13	Docosanoic $C_{22}H_{44}O_2$	47	4.7

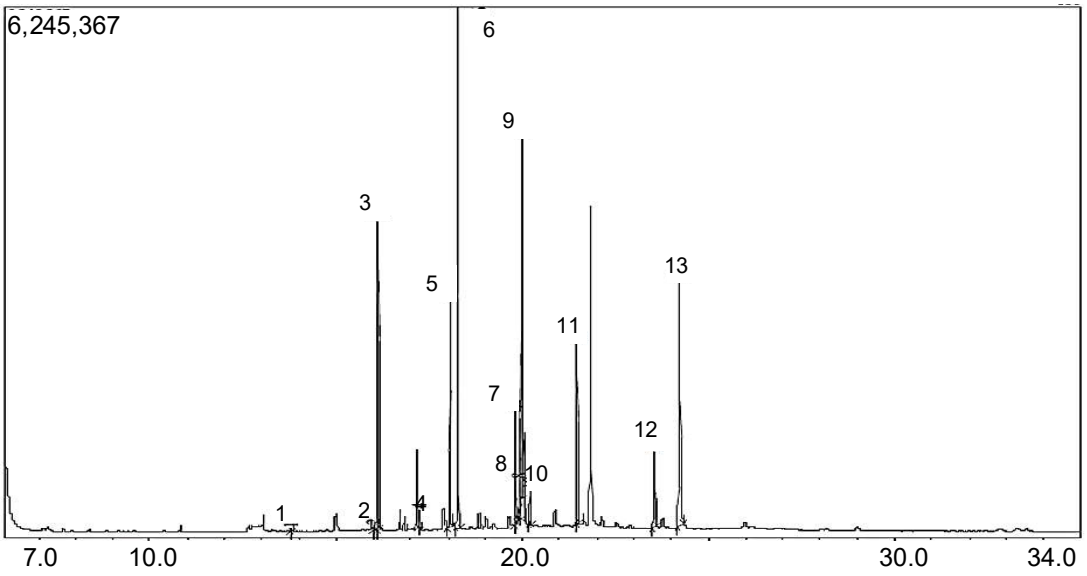

Figure 2. Chromatogram of the fatty acid composition of slightly salted herring lipids before cryoconcentration.

It was found that phase transitions in oil occur at temperatures: 4 °C, -7 °C, -14 °C and -37 °C. Phase transitions are accompanied by the precipitation of lipid fractions less saturated with double bonds. After filtration the contents of the tubes on a nylon filter with a pore diameter of ≤ 03 mm in the cooling unit, the lipid supernatant fraction enriched in unsaturated fatty acids was further cooled (Table 3).

Table 3. Fatty acid composition of oil from the skin of slightly salted herring after cryoconcentration at a temperature up to −14 °C (Sample 'Raw Material')

No.	Acid name	Content, mg g^{-1}	Content, %
1	Lauric $C_{12}H_{24}O_2$	0,5	0,05
2	9-tetradecene $C_{14}H_{28}O$	0,6	0,06
3	Myristic $C_{14}H_{28}O_2$	35	3,5
4	Pentadecane $C_{15}H_{32}$	2	0,2
5	9-hexadecene $C_{16}H_{30}O$	38	3,8
6	Hexadecanoic (palmitic) $C_{16}H_{32}O_2$	54	5,4
7	Linoleic $C_{18}H_{32}O_2$	35	3,5
8	Linolenic $C_{18}H_{30}O_2$	9	0,9
9	9-octadecene (oleic) $C_{16}H_{36}$	41	4,1
10	Stearic $C_{18}H_{36}O_2$	3	0,3
11	Cis-5,8,11,14,17-Eicosaenoic $C_{21}H_{32}O_2$	135	13,5
12	Cis-4,7,10,13,16,19-Docosahexaenoic $C_{22}H_{32}O_2$	106	10,6
13	Docosanoic $C_{22}H_{44}O_2$	46	4,6

In the course of the experiment of cooling the oil obtained from the skin of slightly salted herring, a temperature versus time curve was obtained (Fig. 3) with areas of phase transitions and fractionation, which corresponds to the data of patent (Zakharchuk et al., 1995).

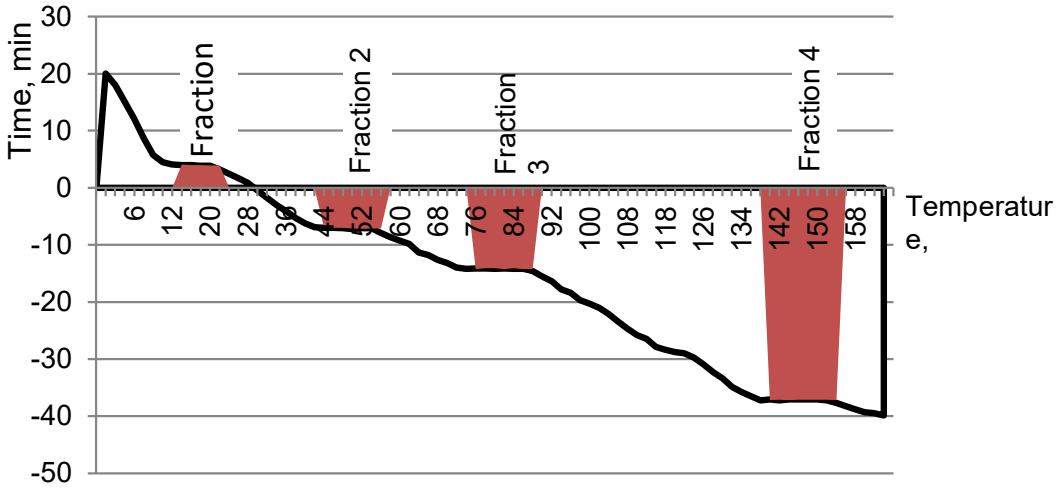

Figure 3. Dependence of phase transitions of fish oil on temperature and time of cooling and freezing.

Fractional analysis results are presented in Table 3, 4 and Fig. 4.

Table 4. Dependence of the yield of solid fraction of lipids from the skin of Atlantic herring on temperature

Temperature, °C		+4	-7	-14	-37
Separated fraction, %	Experiment 1	29.6	38.4	51.3	55.2
	Experiment 2	27.8	38.2	51.8	56

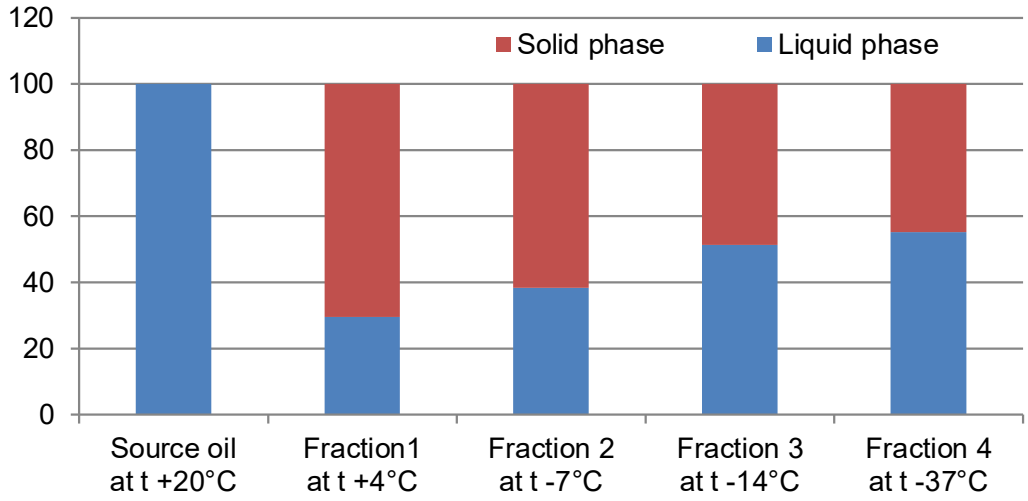

Figure 4. The phase composition of lipids, obtained from skin wastes from slightly salted herring.

With a decrease in temperature from 20 to -14 °C, an increase in the concentration of omega-3 fatty acids in oil is observed by 3 times. At the same time, the ratio of eicosapentaenoic and docosopentaenoic acids remains constant. When the oil temperature reached -14 °C, the concentration of omega-3 fatty acids increased to 24.1%, which was sufficient for the use of the obtained fish oil as a dietary supplement for food or as part of the FFP. Further temperature reduction is impractical, since the

next fraction is released at the temperature of -37 °C (Kuprina et al., 2019) and its achievement is not economically viable in industrial conditions, since it requires high energy consumption.

Table 5 presents biochemical properties and mass yields of omega-3 fatty acids of fish oil before and after cryoconcentration at different stages of phase transition.

Table 5. Biochemical properties and mass yields of cryoconcentrated lipid fractions isolated from skin hydrolyzate of Atlantic herring fillets

Indicator	Oil before cryoconcentration at t +20 °C	Solid fraction of oil released at t +4 °C (Fraction 1)	Solid fraction of oil released at t -7°C (Fraction 2)	Solid fraction of oil released at t -14 °C (Fraction 3)	Liquid fraction of oil released at t -14 °C (Fraction 4)
Acid value, mg KOH*g^{-1}	1.2	1.3	1.2	1.3	1.9
Iodinenumber, g 100 g^{-1}	96.03	101.05	104.6	103.29	201.52
Eicosapentaenoic acid, % in oil	4.3	1.3	1.4	3.6	13.5
Docosahexaenoic acid, % in oil	3.3	0.9	1.2	2.6	10.6
EPA:DHA ratio	1.3	1.4	1.2	1.4	1.3
Total content of ω-3,% in the skin oil, %	7.6	2.2	2.6	4.4	24.1
Mass of cryoconcentrated oil fractions, g	8	2.37	2.16	1.78	1.69
Yield of fractions obtained from the skin oil, %	100	29.6	27	22.3	21.1

CONCLUSIONS

1. Fish oil obtained by the electrochemical method from fish skin was characterized by satisfactory quality characteristics due to the presence of reducing properties in the extractant: Eh not more than 860 mV, which made it possible to reduce the acid number of fat contained in the initial raw material - slightly salted herring from 2.6 mg KOH g^{-1} to 0.5 mg KOH g^{-1}.

2. The technology of cryoconcentration of oil from hydrolysates of fish skin made it possible to enrich it with omega-3 fatty acids.

3. Chemical analysis of raw materials was carried out and the yields of the raw material product were determined at all stages of processing.

4. The composition of the fractions of cryoconcentrated oil was investigated and the expediency of lowering the temperature to -14 °C was established, at which a phase transition was observed, providing a significant concentration of omega-3 fatty acids. Their concentration increased from 7.6 to 24% in oil.

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