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Technology development of obtaining essential fatty acids from hydrobionts hydrolyzates

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Abstract. ω-3, 6-fatty acids from hydrobiontsare a minor component in the nutrition of European countries population. This causes a number of diseases, such as cardiovascular ones, cancer etc. There is a task of concentrating these acids in oil due to the fact that to meet their daily needs it is problematic to use large quantities of fish oil-from 15 to 20 g. Particularly rich in ω-3, 6-acids are wastes from the cutting of hydrobionts, containing muscle tissue and skin.
Protein hydrolysates were obtained from rainbow trout (*Oncorhynchusmykiss*) and Atlantic herring (*Clupeaharengus*) wastes by the electrochemical method using electrolyzers of the original design which are allowed to be used in food industry. A technological scheme of separating of lipids from protein hydrolyzates has been developed and experimental batches of oil samples have been developed. To concentrate the fatty acids the cryoconcentration method was used. The phase transitions of the obtained lipids were studied after their cryoconcentration in the temperature range from + 15 °C to minus 40 °C in the environment of calcium chloride using a low-temperature refrigeration unit. To analyze phase transitions the plant was used, which is a container with a solution of calcium chloride cooled by a low-temperature refrigeration machine. The properties of 5 fractions of lipids formed at the time of lipid phase transitions have been identified and studied (the fractional composition, acid, iodine numbers, the content of polyunsaturated fatty acids (PUFAs), vitamin D_3 and A).
It was established that as cryoconcentration increases the concentration of PUFAs, reaching values close to 90%, which allows the resulting product to be attributed to biologically active food additives (BAA). By calculation, it was shown that to create functional food products on fish base from fish of the Gadidae family it is enough to inject 4 grams of BAA to 100 grams of the product. Organoleptic properties of food products from low-fat fish species were improved.

Key words: fish oil, omega-3-fatty acids, omega-6-fatty acids, functional food, cryoconcentration, biologically active food additives.

INTRODUCTION

Currently, there is an acute shortage of ω-3 and ω-6-unsaturated fatty acids in the daily ration of most European countries, since their main dietary sources – fatty sea fish, seafood (Lee et al., 2009, Ryckebosch et al., 2012) – are not common food for people in mainland countries.

Structural components of lipids prevent the deposition of low-density lipoproteins and cholesterol in the blood vessels walls, prevent the aggregation of blood cells and the formation of blood clots, relieve inflammations, etc. (Caterina et al., 2006). In the absence of these essential nutritional factors, severe cardiovascular diseases, heart attacks, strokes occur, and the life expectancy of the population is reduced.ω-3alsohave been used in the management of diabetes and arthritis (Weylandt & Kang, 2005; Hurst et al., 2010; Adeyemi & Olayaki, 2018). The consumption of polyunsaturated fatty acids (PUFA) as an advice of dietary guideline recommendations replaces saturated fatty acids for the prevention of cardiovascular disease (Chowdhury & Steur, 2015). Importantly, the richest sources of omega-3 PUFA (ω-3 FA) are marine oils, which consumption have been linked to cardiovascular protection (Nogueira, et.al., 2018). Besides, it was shown that ω-3 FA from fish oil is more potent then plant ω-3 FA to inhibit mammary tumors, the problem which is world-spread nowadays (Liu et al., 2018).

Numerous studies show that in addition to hydrobionts themselves, a large amount of essential fatty acids is also contained in the wastes from their cutting. Therefore, it is advisable to use them to obtain BAA in the form of concentrated solutions of unsaturated fatty acids. To develop the technology, the biochemical composition of the salmon and herring was studied and the feasibility of their use was shown.

It is known that to obtain lipid-derived BAA from oily fish raw materials saturated with omega-3 and omega-6 acids, vitamins and phospholipids, technologies based on the hydrolysis of protein components of the raw material, followed by the release of oil components from the protein-lipid emulsion, have the advantage; for example, technology of producing a vitamin A concentrate (Honold et al., 2016; Ghelichi et al., 2017; Najm et al., 2017).

Among the existing technologies of the hydrolysis of the waste from the processing of hydrobionts, namely, acid-alkaline, enzymatic, etc., was selected alkaline hydrolysis technology, based on the synthesis of alkali in the process of electrochemical treatment of raw materials (Gajanan, 2016; Hleap-Zapata & Gutiérrez-Castañeda, 2017). The electrochemical processing parameters were developed by us earlier for the isolation of protein from crustaceans (Kuprina et al., 2015). The technology makes it possible to combine usage of a non-reactionary solution with the direct effect of a constant electric field on raw materials, which ensures the rapid achievement of the process of dissolution and hydrolysis of dispersed raw materials at low concentrations of hydroxyl ions and preserving the quality of nutrients. Lipids are separated from the protein solution by separation using standard equipment.

The fatty acid composition of the isolated lipids was investigated and the presence of a large number of ω-3 fatty acids in them was shown. The special value of lipids isolated from protein hydrolysates is due to the fact that lipids of muscle tissues (in particular, waste from cutting of hydrobionts), unlike lipids contained in the internal organs of hydrobionts, contain substances that are highly biologically valuable (phospholipids, ω-3,6-fatty acids, vitamins), while in the internal organs triglycerides prevail. Despite the difficulties in the separation of ω-3,6-fatty acids (due to the connection with proteins in the form of lipoprotein complexes), we conducted studies on their preparation from lipids as the target product in the framework of the electrochemical technology for processing raw materials (Jacobsen, 2012; García-Morenoet al., 2013).

Concentration of the resulting oil components is promising due to the problematic intake of large amounts (15–20 g per day) of traditional dietary supplements-fish oil, due to its fatty and specific consistency (Drusch, 2012). In this regard, particular interest is in the development of technology for the extraction of essential fatty acids from fish oil, in particular by the method of cryoconcentration, which allows preserving the quality of fat thanks to the use of low-temperature regimes, which significantly slow down the oxidation processes.

MATERIALS AND METHODS

Sampling

The following raw materials were chosen as objects of research for the production of protein-fat emulsion (hydrolyzate): rainbow trout (*Oncorhynchus mykiss*) and Atlantic herring (*Clupea harengus*). As a sample for cryoconcentration was taken the fat obtained by separation from the emulsion.

Raw fish was obtained in chilled (trout) and frozen (herring) form. The trout was transported in hermetically packed plastic containers. Storage was carried out in a refrigerating chamber at a temperature of + 5 °C. Frozen herring was delivered in blocks, storage was carried out in a freezer at a temperature of minus 18 °C.

Electrochemical method of obtaining protein hydrolyzate

The process of electrochemical processing includes certain stages: swelling, extraction of water-soluble components (albumin, carbohydrates, etc.) and extraction of difficult-to-dissolve components (myofibrillary and other proteins, protein-lipid, protein-glycoside, and other complexes). The process completes by the transition of proteins, polypeptides, lipids into a solution in the form of an emulsion and precipitation of bone or crustaceous tissues. The selected method has several advantages:

– high yield (95–98%) of lipids from raw materials;

– carrying out simultaneously the extraction of fat and its refining (due to processing in the cathode chamber of the electrolyzer at pH values ≥ 12.2);

– preservation of high quality of the obtained lipids due to gentle processing modes (since they are not exposed to long-lasting high temperatures, pressure (as in the press-drying technology) or solvents.

Due to the fact that the fat, isolated from protein solutions, is characterized by high values of pH, a stage of its neutralization is necessary. The first stage of hydration is washing with 10% sodium chloride solution and hot water (temperature 90–100 °C) at a ratio of 1:1 until complete removal of alkali and soap. The number of washes ranges from 3 to 5. Each washing is completed by mixing the mass for 10–15 minutes, settling for 1–2 hours and draining the bottom of the sludge. The top is washed again.

To remove remaining moisture the washed fat is dried at a temperature of about 140 °C and in vacuum of at least 79.98 kPa during non-stop work of the stirrer. After drying, the fat should not contain more than 0.6% moisture. Then the fat is sent to the separation.

Physico-chemical analysis

Physico-chemical properties of lipids were determined according to GOST 7636-85, namely, iodide and acid numbers.

Determination of the Fatty acid composition of lipids

The fatty acid composition was investigated by gas chromatography method (with preliminary methylation of the samples). The obtained samples of fish oil were investigated by chromatographic method (Godoy & Rodriguez-Amaya, 1993). Analysis of the qualitative composition of fish oil was carried out on gas chromatographic mass spectrometer (Shimadzu GCMS-TQ8040). The collection and processing of data was carried out using the software of the indicated device. When establishing the calibration characteristics and performing measurements of the mass fraction of fatty acids, certain conditions were observed.

Fractional division of lipids by cryoconcentration

Fish oil was cooled in glass tubes with a volume of 50 mL, placed in a container with a 28% solution of calcium chloride, cooled by low-temperature refrigeration unit. The temperature was measured inside the sample and in a cooling medium with electronic thermometers of the 'Vapan' brand. After fixing the phase transitions in the oil at temperatures of +4, minus 6, minus 14 and minus 37 °C, the fat was mixed with acetone in a ratio of 1:8 and then repeated cooling from +20 to minus 40 °C. After each phase transition the oil was separated into solid and liquid fractions by filtration.

The reliability of the data was achieved by planning the number of experiments necessary and sufficient to achieve a confidence level in scientific experiments $P = 0.95$. Statistical data processing was performed using standard methods for evaluating test results for small samples using Microsoft Excel 2010.

RESULTS AND DISCUSSION

It is known that fish raw materials contain a number of valuable biologically active substances of lipoid nature, namely: essential polyunsaturated omega-3 and omega-6 fatty acids, vitamins A, D and E, phospholipids.

Currently, there is a tendency to obtain biologically active food supplements based on fish oils from the fatty fish processing waste. The waste composition from fish cutting is presented in Table 1.

Table 1. Chemical waste composition from cutting of Atlantic herring *Clupea harengus* and rainbow trout *Oncorhynchus mykiss*. Differences between herring and trout samples are statistically significant

Component	Moisture, %	Fat, %	Protein, %	Ash, %	Energyvalue, kcal
	Atlantic herring				
Meat with skin	69.0 ± 0.70	7.3 ± 0.39	22.6 ± 0.37	1.8 ± 0.03	161.0
Bones	58.6 ± 0.89	10.6 ± 0.33	18.8 ± 0.27	9.2 ± 0.38	175.7
Fins	65.4 ± 0.95	9.4 ± 0.37	16.7 ± 0.31	10.0 ± 0.21	156.0
Head	69.5 ± 0.98	9.7 ± 0.08	13.2 ± 0.20	4.7 ± 0.40	139.0
	Rainbow trout				
Meat with skin	71.3 ± 0.92	5.3 ± 0.26	22.0 ± 0.88	1.5 ± 0.10	139.0
Bones	62.4 ± 1.13	11.04 ± 0.72	18.2 ± 0.70	9.0 ± 0.40	176.0
Fins	66.4 ± 0.53	6.6 ± 0.44	16.9 ± 0.70	9.75 ± 0.76	130.7
Head	70.8 ± 0.64	10.2 ± 0.70	14.8 ± 0.40	3.4 ± 0.28	156.0

To achieve a confidence probability of measurements of $P = 0.95$ with the number of experiments repeated $n = 3$, the mean square deviation of the arithmetic mean was S = 0.01, the average deviation of the measurements was Sm = 0.0058, with a Student coefficient tst = 4.3, the confidence interval of the arithmetic mean $\Delta x = 0.02$.

From the data of Table 1 it can be seen that waste from fish cutting, like fish themselves, are characterized by valuable chemical composition, high fat content, which indicates the feasibility of using them as a raw material source for obtaining lipid-type biologically active substances. There was no significant difference in the biochemical composition of the skin, bones, fins and heads of the Atlantic herring and the Rainbow trout. Therefore, to extract fat and other dietary supplements, it is possible to use all the waste products listed in Table 1.

To obtain biologically active substances saturated with omega-3 and omega-6 acids, vitamins and phospholipids, from fatty fish raw materials, a technology was chosen based on the electrochemical hydrolysis of protein components of the raw material with subsequent release of fat components from the protein-lipid emulsion (Kirillov & Kuprina, 2014).

The electrochemical method of influencing the biological raw materials includes a direct effect of the electric field on raw materials and the aquatic environment, which allows for fine control of processes, simplifies their automation, and also reduces energy costs. When processing raw materials in an electric field, the processes of diffusion and extraction are accelerated, and the intensity and extent of chemical and physical processes increase. Thanks to all this, there is an intensification of the raw materialsprocessing.

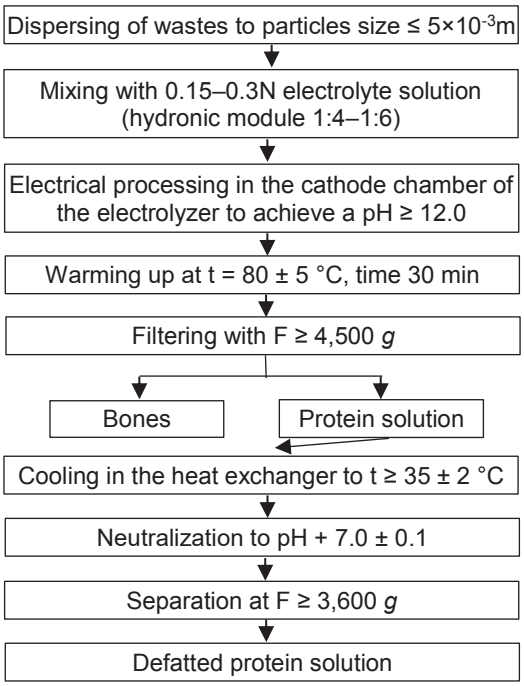

Figure 1. Technological scheme of complex waste processing from fish cutting by electrochemical method with obtaining lipids from protein solutions.

For electrochemical processing, electrolyzers with a plane-parallel arrangement of electrodes separated by an ion-selective membrane were used. The optimal processing parameters of the dispersed raw material were selected, which ensured the complete dissolution of the protein fraction: current, voltage, processing time of the suspension in the electrolyzer, time for heating the suspension after the electrolyzer in a stirred reactor. The insoluble bone residue was separated by centrifugation. Lipids from the protein solution were isolated by separation. The technological scheme of complex waste processing (pegs, fins, bones, scales, skin) from salmon and herring cutting by electrochemical method with obtaining lipids from protein solutions is shown in Fig. 1.

The yield of oil from trout and herring waste cutting during their processing by electrochemical method was 7 and 8%, respectively, which is close to 90% of the theoretical.

This technology was developed with the aim of preserving the quality of biologically active substances of lipoid nature, due to the gentle conditions of exposure to raw materials in the process of extraction of the first. As the existing technologies based on the use of organic solvents, high temperatures, chemical reagents, are distinguished by the 'hardness' of the impact on the raw materials, which in turn leads to oxidative deterioration of biologically active substances from lipids and proteins.

The fatty acid composition of the obtained fat was investigated by gas-liquid chromatography and is presented in Table 2.

Table 2. Fatty acid composition of fat samples of herring and trout, % of the amount of fatty acids. Differences between samples are statistically significant

Acid name	Fatty acid index	Average sample herring	Herring protein hydrolyzate fat	Average sample trout
Caprylic	10:0	0.12	0.02	0.02
Lauric	12:0	0.28	0.58	0.25
Myristic	14:0	2.18	5.98	1.15
Myristoleic	14:1	0.06	0.06	0.07
Isopentadecanoic	15:0	0.05	0.16	0.20
Anteiso-pentadecanoic	15:0	0.05	0.05	0.09
Pentadecanoic	15:0	0.26	0.31	0.42
Pentadecenic	15:1	0.05	0.08	0.04
Palmitic	16:0	13.48	10.65	13.15
Hexadecenoic	16:1	0.14	0.12	0.23
Palmitoleic9-cis	16:1	2.55	4.31	3.43
Heptadecylic	17:0	0.22	0.40	0.62
Heptadecenoic	17:1	0.40	0.29	0.49
Stearic	18:0	4.1	3.81	4.84
Elaidic 9-trans	18:1	0.88	0.81	1.13
Oleic 9-cis	18:1	16.80	18.33	20.12
Vaccenic 11-trans	18:1	2.01	1.59	2.05
Octadecenoic 11-cis	18:1	0.09	0.22	0.14
Linoleic ω-6	18:2	3.59	1.08	1.97
γ-linolenic ω-6	18:3	0.17	0.12	0.06
α-linolenic ω-3	18:3	5.80	0.86	0.67
Arachidic	20:0	0.11	0.16	0.21
Gadoleic (∑ isomers)	20:1	6.90	14.31	10.83
Eicosadienoic	20:2	0.33	0.39	0.51
Eicosatrienoic 8, 11, 14-trans	20:3	0.21	0.15	0.23
Arachidonic ω-6	20:4	0.87	0.41	1.08
Eicosapentaenoic ω-3	20:5	6.25	2.3	5.45
Behenic	22:0	0.07	0.06	0.07
Erucic (∑ isomers)	22:1	5.99	15.99	11.23
Docosadienoic	22:2	0.05	0.09	0.13
Docosapentaenoic ω-6	22:5	3.66	3.71	3.54
Docosahexaenoic ω-3	22:6	22.30	7.81	12.03
Lignoceric	24:0	0.04	0.10	0.04
Nervonic	24:1	0.78	0.77	1.24

From the data of Table 2 it follows that the fish oil obtained by the electrochemical method from trout and herring wastes contains a significant amount of omega-3 polyunsaturated fatty acids (about 30% of the total fatty acids), but in quantities insufficient to meet the daily human need (according to MR 2.3.1.2432–08), therefore it was necessary to develop the technology of their concentrating, since the task was to obtain fish oil with a high content of omega-3 acids for subsequent encapsulation.

The fat from the secondary fish raw material was filtered until a transparent, viscous mass without inclusions was obtained and stored at +4 °C. To concentrate fat containing omega-3 acids, the subject of study was cooled. The fat was placed in glass tubes. The process of cooling the fat in test tubes was carried out on the installation, which is a container with a solution of calcium chloride cooled by a low-temperature refrigeration installation. The average rate of cooling and freezing is 0.3 °C s^{-1}. The temperature was fixed inside the sample and in the cooling medium by thermocouples (Drusch, 2012; Ghelichi et al., 2017).

It has been established that phase transitions in fat intensively occur at temperatures: -6 °C, -14 °C, -37 °C. Phase transitions are accompanied by precipitation of lipid fractions that are less saturated with double bonds. After centrifugation, the supernatant lipid fraction enriched in unsaturated fatty acids was further cooled. Fig. 2 shows the photographs of the fractions released during cryoconcentration.

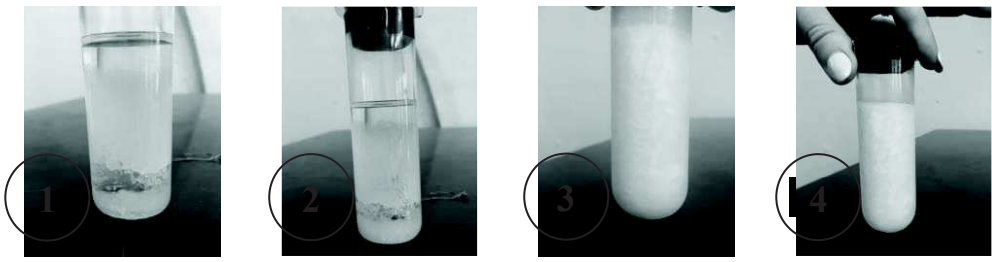

Figure 2. Fat fractions obtained by cryoconcentration of biologically active substances of lipoid nature from secondary fish raw materials. 1 – the photo of the fat sample at 4 °C; 2 – the photo of the liquid fat fraction separated from sample1 at -6 °C; 3 – the photo of the liquid fat fraction separated from sample 2 at -14 °C; 4 – the photo of the liquid fat fraction separated from sample 3 at -37 °C.

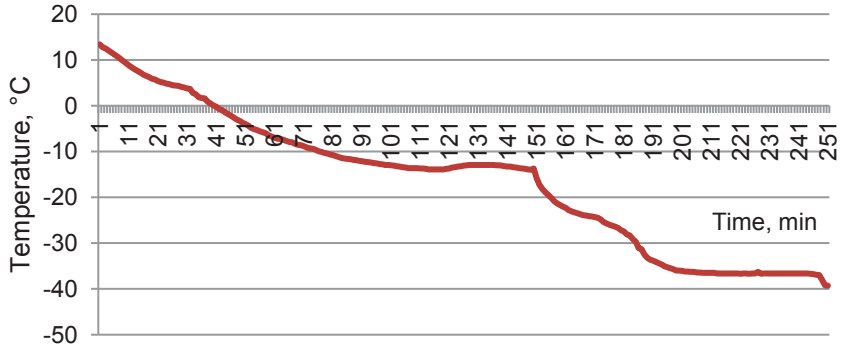

Figure 3. Dependence of temperature in fish oil from the time of its cooling and freezing.

The experiment on cooling and freezing was repeated by mixing fat with acetone in a ratio of 1:8 to allow the quantitative separation of fractions during freezing.

In the course of the experiment of cooling a lipid-containing biologically active substance from a secondary fish raw material, a temperature-time dependence curve was obtained (Fig. 3), where phase transitions and separation of fractions were observed, which corresponds to the data of RF patent No. 2031923.

Temperature measurements were made on a Vapan thermometer with a standard deviation of 0.14. After each phase transition, the precipitated lipoid precipitate was separated and quantified. The results of the fractional analysis are presented in Table 3 and Fig. 4.

Table 4 presents data on the biochemical characteristics of fat and its sediment obtained at different temperatures.

Table 3. Dependence of the yield of solid fraction of biologically active substances of lipoid nature from secondary fish raw materials on temperature

Temperature, °C		+4	-14	-37
Separated fraction, %	Experiment 1	6.0	92.5	55.2
	Experiment2	5.1	93.0	56.0

From the data of Table 4 it follows that due to the process of cryoconcentration, it was possible to increase the content of fat fractions, including omega-3 fatty acids, approximately three times. According to MP 2.3.1.2432-08, the average daily requirement for omega-3 acids is 7.3 g per day. The calculation was carried out taking into account the fact that the daily requirement should correspond to 1–2% of the daily caloric intake (for example, 2,400 kcal for group II of the population, including people under 30 years old with an activity coefficient of 1.6). Considering that Atlantic herring selected for the study contains 1.64 g 100 g^{-1} of omega-3, and trout contains 0.97 g 100 g^{-1}, to meet the daily need for omega-3 acids, herring and trout products need additional enriching with these acids, which can be achieved by introducing into them 22 g of fish oil in the composition of functional food product.

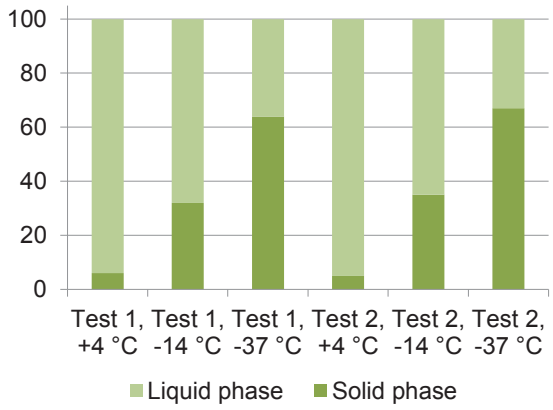

Figure 4. Diagram of the fractional composition of biologically active substances of lipoid nature, obtained from rainbow trout waste cutting.

Table 4. Biochemical properties of cryoconcentrated fat isolated from rainbow trout waste hydrolysate cutting

Indicator	BAS at temperature +4 °C	BAS at temperature -14 °C	BAS at temperature 14 °C	BAS at temperature -37°C
Acid number, mg KOH*g^{-1}	1.6	1.7	2.3	2.5
Iodinenumber, g100 g^{-1}	109.03	298.29	207.52	341.38
Eicosapentaenoic acid, % in fat	0.75	10.00	-	31.3
Docosahexaenoic acid, % in fat	1.80	24.07	-	62.7

However, it is technologically difficult to introduce such a quantity of lipids into functional food products, especially for non-minced products, this drawback can be eliminated by the developed technology of cryoconcentration of lipids. Taking into account the fact that during cryoconcentration the concentration of omega-3 acids increases by 3 times, it is enough to introduce 6 g of fat per 100 g of herring product and 6.6 g of fat per 100 g of trout to meet the daily consumption of omega-3 acids. To meet 30% of the daily consumption of omega-3 acids, the required amount of injected cryoconcentrated fat is up to 1.8 and 1.98 g, respectively, which is technologically easy to implement.

According to FAO and WHO, a food product belongs to the functional food product group, if its consumed portion (100 g) provides a 30% daily consumption rate of the target component.

CONCLUSIONS

Thus, the technology has been developed for obtaining biologically active substances of lipoid nature, enriched with omega-3 acids, from hydrobiont processing waste by the method of electrochemical hydrolysis and cryoconcentration. A comparative analysis of the waste composition from cutting of herring and trout is carried out and the expediency of using them to obtain lipid-type biologically active substances is shown. A process operational diagram has been developed and fat outputs have been determined when it is obtained from fish waste by electrochemical method. The fatty acid composition of the fat obtained by the electrochemical method was determined. It has been established that cryoconcentrated fat obtained from waste from cutting trout and herring using an electrochemical method has a significantly higher content of omega-3 acids and, accordingly, biological value in comparison with edible and medical fish oil from the liver of the Cod family.

It is shown that in order to meet 30% of the recommended daily intake of omega-3 acids in the development of functional food products based on rainbow trout and Atlantic herring, it is necessary to introduce 1.98 g and 1.8 g of cryoconcentrated fish oil. The resulting product is suitable for the enrichment of any fish products with omega-3 acids, and its required amount is determined by calculation, based on the fat content of raw materials. After encapsulation in nanocapsules, the medicine will be suitable for enriching omega-3 acids of any food, which is the subject of further research.

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