Development of electro-biochemical technology for processing secondary fish raw materials and new types of products based on minced fish

M.E. Chikisheva[1,*], E.A. Kuprina[1], *A.N.* Manuilov[1], *A.N.* Yakkola[1]. *I.A.* Naumov[1], and *O.* Kabysh[1]

[1]ITMO University 9, Lomonosov str., St. Petersburg, 191002, Russia

Abstract. A new complex low-waste electro-biochemical technology has been developed for processing waste from cutting fish raw materials in the North-Western region (Atlantic cod, mackerel, herring and artificially grown trout) to produce nutrients with high yield and low degree of destruction - protein hydrolysates, as well as mineral precipitates, fat. The composition of the waste from cutting fish used for complex processing by the electro-biochemical method was investigated. The influence of the fishing season on the parameters of processing waste obtained from it was determined. The fractional composition of protein hydrolysates was studied. A comparative assessment of traditional enzyme preparations and preparations obtained by the electro-biochemical method as protein hydrolyzing components has been carried out. The process of obtaining an enzyme preparation from the insides of fish using catholyte and the process of enzymatic hydrolysis of secondary fish raw materials upon receipt of a protein hydrolyzate are investigated. The characteristics of waste processing equipment are given. The properties of nutrients were investigated and the feasibility of their use in the composition of fish products based on minced fish and mixtures for injecting fish fillets was shown. A formulation was developed and the organoleptic evaluation of minced products using the obtained protein hydrolysates was carried out.

1 Introduction

The growing interest of consumers in obtaining a sufficient amount of high-quality animal protein, including fish, as well as the high consumer demand for fast and healthy nutrition dictate for manufacturers ways to develop a direction of integrated waste-free technologies for processing raw materials. At the same time, many environmental problems are solved [1], [2], [3].

Many Russian scientists made a significant contribution to the development of deep fish processing, including Maslova G.V., Kuprina E.E., TelishevskayaL.Y., Neklyudov A.D., Novikov V.Y., etc. For the production of protein hydrolysates, two methods of proteolysis are mainly used: chemical (acid or alkaline) and enzymatic hydrolysis. It has also been proposed to use the electrochemical method to isolate the most valuable substances from fish waste: proteins, fats, and minerals (Maslova et al., 1991; Kuprina et al., 2003; Nyanikova et al., 2003). The efficiency of separating the protein fraction with its further drying for the purpose of long-term storage was shown. Slutskaya, Kozyreva (1999), Ivankin, Neklyudov, Kudryashov (2001) and others were involved in the enzymatic hydrolysis of fish waste. Biologically valuable substances – essential fatty and amino acids, vitamins – were separated for the purpose of their subsequent usage in pharmaceuticals (Telishevskaya, 2000; Mukhin, Novikov , 2001). Drying after electrochemical treatment provides long-term storage and ease of use of protein hydrolyzate, but there is a significant loss of biologically active substances. At the same time, energy costs for the process increase and additional production areas and equipment are required.

For the processing of waste in this study, it is proposed to use enzymatic and electrochemical methods of exposure at various stages of the processing of raw materials. The latter are methods of direct and indirect effects of the electric field on raw materials and the aquatic environment, which in turn allows for fine-tuning of processes, simplifying their automation and reducing production costs. The enzymatic treatment of waste accelerates the process of hydrolysis at the stage of electrochemical processing. The advantages of this technology also include the complexity of the approach and resource efficiency, obtaining new products from secondary raw materials, the high quality of the obtained bioactive components, the ease of introducing the technology in small and medium-sized enterprises. The article presents the physicochemical characteristics of protein hydrolysates [4], [15], obtained by the electro-biochemical method from different types of fish raw materials. The advantages of combining electrochemical and enzymatic processing are shown.

The method has been developed for producing an enzyme preparation from protein serum of the viscera of

* Corresponding author: mechikisheva@gmail.com

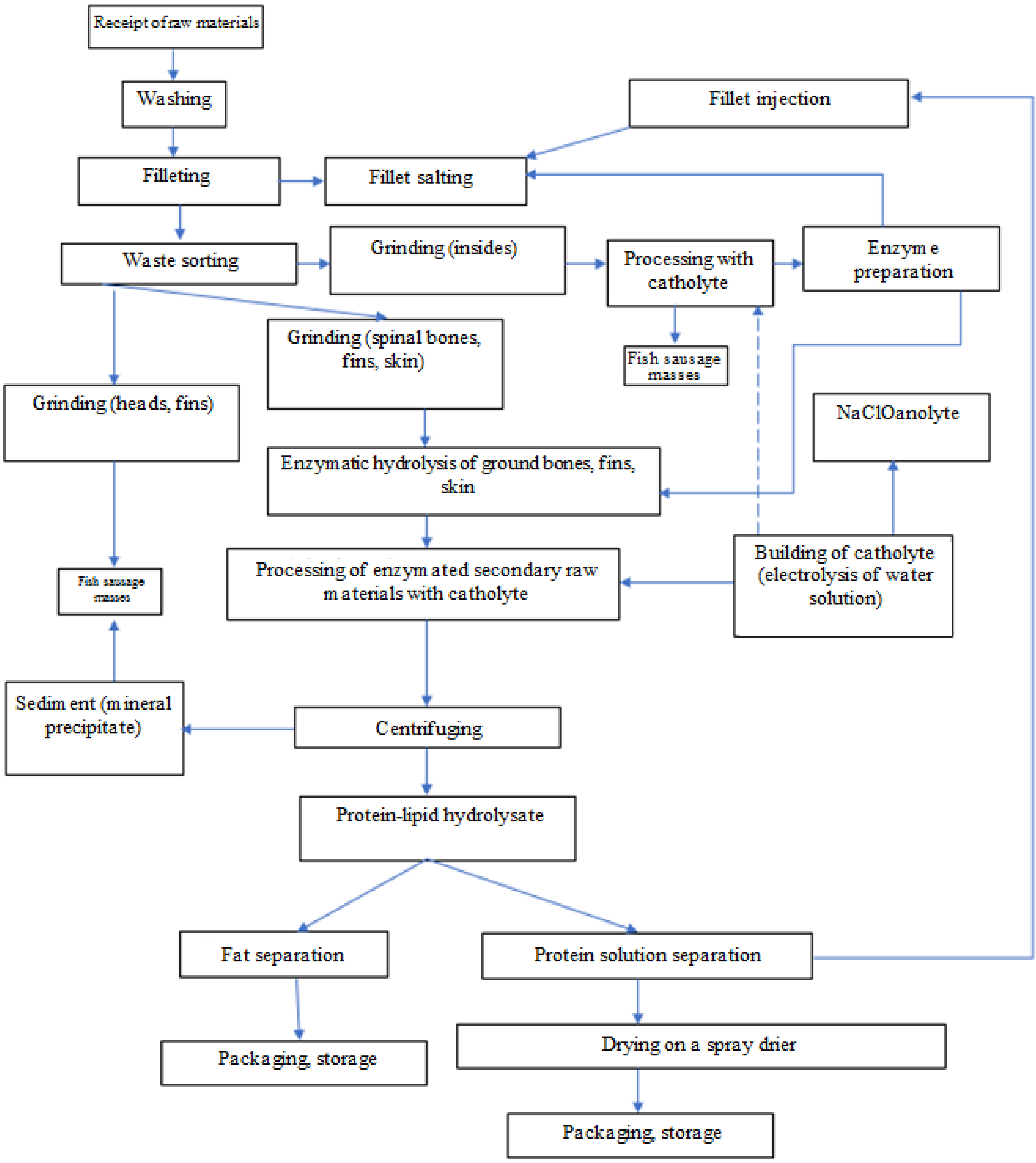

Fig. 1. Technological scheme of integrated waste-free processing of fish raw materials using primary and secondary raw materials.

various species of fish caught in different periods. The activity of protein whey and, accordingly, enzymes during the hydrolysis of secondary fish raw materials was studied depending on its duration, the final content of the lipid - protein and protein fractions in the resulting protein hydrolyzate.

The purpose and objectives of this study were:

– creation of a fundamentally new technological scheme of complex processing of secondary fish raw materials, including its electrochemical and enzymatic processing;
– development of a technology for producing an enzyme preparation from the intestines of different fish species using catholytes;
– the choice of enzymes that ensure the greatest environmental cleanliness of processes and the selection

Table 1. Wastewater parameters before and after treatment with lime.

Waste	Moisture content, %	Content of basic components in % to wet stock			
		Ash	Total nitrogen	Protein	Lipids
Spinal bones of cod with excess flesh	64.8±0.5	11.0 ±0.4	3.8±0.5	18±3	1±0.5
Skin of cod with excess flesh	52.2±0.5	5.0±0.4	4.0±0.5	22±3	1±0.5
Spinal bones of mackerel with excess flesh	58.4±0.5	8.0 ±0.4	2.9±0.5	16±3	12.9±0.5
Skin of mackerel with excess flesh	57.2±0.5	4.0±0.4	3.0±0.5	20±3	15.3±0.5
Spinal bones of herring with excess flesh	59.3±0.5	7.8±0.4	3.7±0.5	13±3	10.4±0.5
Skin of herring with excess flesh	56.4±0.5	3.8±0.4	3.9±0.5	15±3	15.7±0.5
Spinal bones of trout with excess flesh	48.7±0.5	10.5±0.4	2.9±0.5	14±3	8.3±0.5
Skin of trout with excess flesh	53.4±0.5	4.7±0.4	3.1±0.5	17±3	11.5±0.5

of technological modes of electrolysis and enzymatic hydrolysis in order to maximize nutrient output;
– increase the yield of the edible part of fish raw materials due to the isolation of valuable nutrients from waste and their inclusion in food products (in minced products or by injection in the form of a solution in fish fillet);
– recommendations for the efficient and rational use of all types of products obtained from waste by the electro-biochemical method from fish waste (fish oil, protein hydrolyzate, mineral precipitate, collagen).

2 Objects and methods

As the objects in this study,secondary raw materials from processing the main commercial fish species for the Northwest region were selected: Atlantic cod (Gadusmorhua), Atlantic herring (Clupeaharengus), Atlantic mackerel (Scomberscombrus), Rainbow trout (Oncorhynchus mykiss) and, more precisely, the secondary resources obtained during their deep cutting (skin, bones with cuts of meat, viscera) [5], [13], [16].
We have studied samples of the listed fish species in the spring and autumn fishing seasons, which differ in the activity of the enzyme system.

The content of crude protein, lipids and ash was determined by standard methods – according to GOST-7636. In the protein hydrolyzate, the crude protein content was determined by the Kjeldahl method. Protein content was determined on VelpScientifica installation - UDK 159. Lipids were determined using Soxhlet extractor [6], [17], [18]. The method for determining ash is based on the removal of organic substances from a sample of the analyzed product by burning and determination of ash by weighing.

3 Research results and discussion

The technology developed during the study is presented in Figure 1. This technology is compiled in accordance with all requirements of the fish processing industry regarding the inclusion of secondary raw materials (SRM) in the production process of primary and auxiliary products.

The biological potential of SRM fish processing is confirmed by the chemical analysis data presented in table 1.

The analysis of the chemical composition of the waste from cutting fish correlates with the data of the scientific literature [7], [12].

From table 1 it follows that the waste has a fairly high biological value in view of the high protein content on average up to 24%, as well as minerals (up to 10%) and lipids (up to 16%).

After the stage of fish filleting, three types of waste were obtained - heads, entrails, spinal bones and fins. The waste was dispersed. The heads were sent for drying to obtain fish tankage, and the insides in the form of a homogeneous mass were subjected to electrochemical treatment with catholyte in order to obtain their own enzyme preparation, which was then used to enzymatically treat skin, bones, fins, or to accelerate the maturation of fish with fillet salting. A photograph of the experimental electrochemical plant is shown in Fig. 2.

Fig. 2. The experimental electrochemical installation for producing protein hydrolyzate from SRM.

The electrolyze of SRM was conducted in the installation that consisted of 3 electrolyzers, the rectifier, the salt solution preparation reactor, enzyme and electrochemical hydrolyzate accumulators [8].

To carry out the electrochemical treatment, catholyte was produced during electrolysis of water under the influence of direct current up to PH 12.2. In the storage reactor with a stirrer, the viscera were processed by mixing them with catholyte at a given hydraulic module, time, temperature. The obtained enzyme preparation was mixed in another reactor with waste products (heads, entrails, spinal bones and fins), which were also hydrolyzed.

To evaluate the effectiveness of using electrochemically obtained enzyme preparations, it was compared with other enzyme preparations.

As enzyme preparations, proteolytic enzymes were used - beef pepsin (manufacturer of ZEF CJSC (activity of at least 100,000 conv units / g), collalysin collagenase (manufacturer of research institutes of vaccines and serums (activity of at least 600 units), as well as complex enzyme preparations obtained from the internal organs of fresh-bream river fish, chilled rainbow trout, Atlantic frozen mackerel, electrochemically or without electrolysis (Table 2). Since the fishing season affects the enzyme activity [9], [10], the trends of fish in the spring and fall seasons of the catch.

Table 2. Symbols of enzyme preparations used for the treatment of SRM.

Name	**Origin**
Pepsin	Beef proteolytic enzyme
Collalysin	Collagenase
BSWE	The enzyme preparation from the bream of spring catch without electro-chemical processing
BSE	The enzyme preparation from the bream of spring catch with electro-chemical processing
TSWE	The enzyme preparation from the trout of spring catch without electro-chemical processing
MFE	The enzyme preparation from mackerel of autumn catch with electro-chemical processing

Tables 3 and 4 present the results of studies of the chemical composition of some samples of fish protein hydrolysates that may be of interest for processing fish to produce food protein products and additives. From the data in tables 3 and 4 it follows that the treatment of SRM with enzyme preparations obtained from the intestines of fish by the electrochemical method showed the greatest efficiency.

Promising for food processing samples is characterized by high protein content, more than 35%. The fat content in the samples ranges from 2% (Cod / collagenase) to 6.5% (Mackerel / collagenase). The protein component of all samples is represented mainly by the water and salt-soluble fraction. The total amount

Table 3. Chemical composition of specimens of hydrolyzed protein from cod, processed with various enzymes.

Name of specimen raw material/enzyme preparation	**Crude protein (N□6.25), % per a.d.w.**	**Fractional structure of proteins, % of total protein**				**Fat, % per a.d.w.**
		Water-soluble	Salt-soluble	Acid-soluble	Insoluble residue	
Cod/pepsin	39.69	48.46	35.59	4.16	11.79	3.19
Cod/collagenase	41.37	50.22	36.70	7.19	10.91	2.70
Cod/ “ BSE ”	45.21	47.6	34.10	3.40	4.42	3.24
Cod/ “BSWE”	45.01	47.96	34.12	3.50	4.11	3.20
Cod/ “ BSEA”	44.81	40.62	38.52	3.20	4.82	3.13
Cod/ “BSWEA”	45.05	45.42	38.52	3.20	4.82	3.23
Cod/ “TSWES”	43.91	42.63	34.12	3.50	4.42	3.34
Cod/ “TSES”	43.85	41.80	34.12	3.50	4.42	3.50
Cod/ “ TSWESA”	42.78	42.45	34.12	3.50	4.42	3.46
Cod/ “TSESA”	42.67	43.36	34.12	3.50	4.42	3.48
Cod/ “MFES”	41.95	45.98	34.12	3.50	4.42	3.80
Cod/ “MFEWS”	41.78	46.23	34.12	3.50	4.42	3.75
Cod/ “MFEO”	41.67	46.50	34.12	3.50	4.42	3.95
Cod/ “MFEWO”	41.50	45.90	34.12	3.50	4.42	3.90

Table 4. Chemical composition of the specimens of hydrolyzed protein from mackerel, processed with various enzymes.

Name of specimen raw material/enzyme preparation	**Crude protein (N□6.25), % per a.d.w.**	**Fractional structure of proteins, % of total protein**				**Fat, % per a.d.w.**
		Water-soluble	Salt-soluble	Acid-soluble	Insoluble residue	
Mackerel/pepsin	32.24	38.20	46.02	4.87	10.93	5.56
Mackerel/collagenase	33.23	43.12	48.79	6.21	6.87	5.86
Mackerel / “ BSE ”	39.60	47.96	33.12	3.40	4.22	4.95
Mackerel / “BSWE”	39.25	47.90	34.12	3.50	4.42	4.98
Mackerel / “ BSEA”	38.92	46.96	34.30	3.50	4.40	4.92
Mackerel / “BSWEA”	38.80	47.56	34.72	3.55	4.45	4.84
Mackerel / “TSWES”	37.21	47.95	34.05	3.50	4.42	4.20
Mackerel / “TSES”	37.35	47.96	34.10	3.95	4.40	4.43
Mackerel / “TSWESA”	37.86	47.80	34.32	3.50	4.80	4.35
Mackerel / “TSESA”	37.70	46.90	35.55	3.60	4.40	4.24
Mackerel / “MFES”	34.52	46.80	35.60	3.51	4.42	4.90
Mackerel / “MFEWS”	34.21	45.78	34.12	3.50	4.41	5.00
Mackerel / “MFEO”	33.98	47.95	34.80	3.45	4.45	5.15
Mackerel / “MFEWO”	33.87	45.96	37.12	3.20	4.55	6.04

of soluble proteins in the samples is at a high level and varies from 78% (Mackerel / FP EL TR / SC) to 93% (Mackerel / collagenase).

The study of the effect of the duration of the electrochemical hydrolysis of SRM from different fish species on the crude protein content in hydrolysates is presented in Fig. 3.

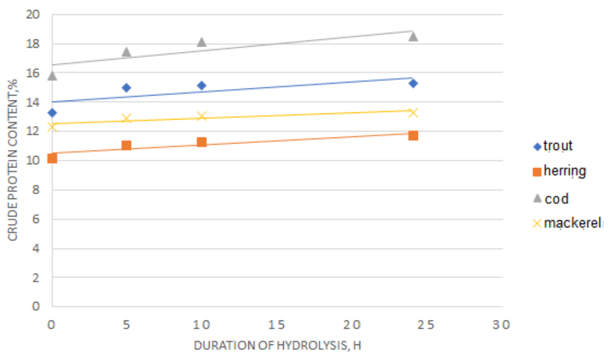

Fig. 3. Dependency of crude protein content on prehydrolysis in the protein solution obtained after electrolysis, %.

The “zero point” was taken as the percentage of crude protein in samples that were subjected to electrochemical processing without preliminary enzymatic treatment.

From the data in Fig. 3 follows that the recommended time for the electrochemical processing of SRM is not more than 10 hours.

The resulting protein solution is proposed to be partially returned to production as a biologically valuable component at the stage of fillet salting, and more precisely at the stage of fillet preparation. During the experiment, positive results were obtained by injecting fish fillet with the protein solution in combination with the enzyme preparations obtained from pyloric systems of processed fish. The list of products obtained from secondary production resources is presented in table 5

Table 5. Products obtained from secondary production resources.

Name of product	**Sphere of implementation / utilization**
Hydrolyzed protein	Injected fillet
	Sale in dry condition
Enzyme preparation	Hydrolysis of protein-lipoprotein solution at a manufacturing facility
	Acceleration of maturing in fish fillet salting
	Injected fillet
Mineral precipitate	Enrichment of the microelement composition of stuff products
	Enrichment of agricultural feed stuffs
Fat	Enrichment of agricultural feed stuffs

The resulting protein hydrolyzate was used as components in the production of minced meat and the development of formulations that satisfy various requirements. Assessment of organoleptic characteristics of minced fish included an assessment of appearance,

Table 6. Organoleptic characteristics of minced meat.

Name of product, grades (points)	Product characteristics				
	Appearance	**Color**	**Odor**	**Taste**	**Consistency**
Trout staff 60/10/30/10	Fatty, complies with this type	Complies with this type of product	Fresh odor, but odor characteristic of fatty raw material is felt	Slight oxidation in aftertaste	Tender consistency, flaking fat
Grades (points)	9	9	7	6	8
Cod stuff 60/30/10	Lean, complies with this type	Light gray, beige	Complies with this type	In aftertaste taste cod	Loose consistency, pressing leaves dents
Grades (points)	6	6	5	5	5
Mackerel stuff 60/30/10	Fatty, complies with this type	Complies with this type of product	Sufficiently fresh, mackerel	Taste of mackerel after cooking	A little dry, dense
Grades (points)	5	8	6	7	7
Mixed stuff	Moderately fatty	Mixed	pleasant	A slightly pungent flavor	Resilient
Grades (points)	9	9	7	7	8

color, texture, smell and taste on a nine-point scale [11], [14].

The results of the organoleptic evaluation of minced fish are presented in table 6.

The best organoleptic characteristics were observed in the stuffing composition based on trout mince after neopress with the following recipe: trout mince (60%) / protein hydrolyzate (30%) / dry water and fat-binding components (10%) (egg powder, plant isolate, salt).

4 Conclusion

Based on theoretical and experimental studies, a comprehensive resource-saving electro-biochemical technology for the deep processing of secondary fish raw materials from cod, trout, herring and mackerel using electrochemical and enzymatic methods for its processing has been developed.

The process of obtaining an enzyme preparation from the insides of fish using catholyte and the process of enzymatic hydrolysis of secondary fish raw materials in the preparation of the protein hydrolyzate of cod and mackerel are studied.

It has been established that this technology makes it possible to obtain protein hydrolyzate, proteolytic enzymes, a lipid fraction and high-quality mineral precipitate from waste with a satisfactory yield.

The expediency of using a protein hydrolyzate obtained by the electro-biochemical method in the composition of minced fish products and as the basis of solutions for injecting fish fillet is shown.

References

[1] On Consumption of Fish and Fish Products (data of the research by the All-Russian Public Opinion Research Center) [Electronic resource]. Available at: http://csef.ru/ru/politica-i-geopolitica/510/opotreble-nii-ryby-i-rybnyh-produktov-dannye-issledovaniya-vcziom-8616.

[2] Federal Agency for Fisheries [Electronic resource]. Available at: http://www.fish.gov.ru/.

[3] O.Y. Mezenova, V.V. Volkov, S.V. Agafonova, N.Y. Mezenova, Evaluation of the Potential of Secondary Protein-Containing Raw Materials at Enterprise of Kaliningrad Region of Russia, Bulletin of Science and Education of the North-West Russia, **3**, 4 (2017).

[4] M.D. Mukatova, N.A. Kirichko, R.R. Uteushev, Enzymes, Means of Obtaining Enzymes and Methods of Determining, Their Activity: Method, Directions to Laboratory Practicum, Astrakhan: Astrakhan State Technological University, 62 (2006).

[5] M.E. Kleimenova, Development of the Technology for Secondary Processing of Products on the basis of Aquatic Organisms with Subsequent Implementation at Fish Processing Enterprises of the North-West Federal District,International Research Magazine,**48**, 6-5, 171-174(2016).

[6] A.O. Vartminskaya, M.E. Kleimenova, E.A. Ovsyuk, Revisiting the Use of Enzyme Preparations to Enhance Quality and Resource Efficiency in the Processing of Fish Raw Materials, VIII International Scientific-Technical Conference "Low-temperature and Food Technologies in the 21st century", Materials of the conference, Saint Petersburg, 411-412 (15-17 November 2017).

[7] L.S. Baidalinova, L.V. Gorodnichenko, Dependency of the Degree of Separation of Components of Secondary Fish Raw Materials (Fish Heads) on the Conditions of Enzymolysis and Thermolysis,"News of Kaliningrad State Technological University", **496** (2018).

[8] O.Y. Mezenova,*Physical Methods of Processing Aquatic Organisms*, Monograph, Kaliningrad: Publishers of the Federal State Budget Educational Institution of Higher Professional Education Kaliningrad State Technological University, 255 (2010).
[9] A.O. Vartminskaya, M.E. Kleimenova, E.A. Ovsyuk, Revisiting the Use of Enzyme Preparations to Enhance Quality and Resource Efficiency in the Processing of Fish Raw Materials, VIII International Scientific-Technical Conference "Low-temperature and Food Technologies in the 21st century", Materials of the conference, Saint Petersburg, 411-412 (15-17 November 2017).
[10] L.S. Baidalinova, L.V. Gorodnichenko, Dependency of the Degree of Separation of Components of Secondary Fish Raw Materials (Fish Heads) on the Conditions of Enzymolysis and Thermolysis, News of Kaliningrad State Technological University, **496** (2018).
[11] M.V. Matkovskaya, Comprehensive Processing of Secondary Fish Resources for Functional Products, Relevant Problems of Developing Biological Resources of the World Ocean: 2nd International Scientific Conference, Vladivostok, 81-868 (2012).
[12] V.V. Volkov, T. Grimm, A. Helig, O.Ya. Mezenova, A study of Various Means of Hydrolysis of Secondary Raw Materials of Pacific Salmons on an Example of Redfish Heads, News of Kaliningrad State Technological University, **45**, 136-147 (2017).
[13] O.Ya. Mezenova, V.V. Volkov, S.V. Agafonova, N.Yu. Mezenova, Comparative Evaluation of Means of Hydrolysis in Obtaining Protein Products from Collagen-containing Fish Raw Materials and Evaluation of Their Quality, Scientific magazine "News of Kaliningrad State Technological University", 49 (2018).
[14] O.R. Mnuskina, M.E. Kleimenova, E.A. Ovsyuk, Revisiting Optimization of Technologies of Stuff Products from Secondary Raw Materials Based on Aquatic Organisms, VIII International Scientific-Technical Conference "Low-temperature and Food Technologies in the 21st century",Materials of the conference,Saint Petersburg, 393-396 (15-17 November 2017).
[15] N.V. Yartseva, N.V. Dolganova, A Study of Organoleptic and Technological Properties of Cutlets from Fish Stuffs with Addition of Lactulose, Bulletin of Astrakhan State Technological University, Series: Fisheries, 125-129 (2010).
[16] M.V. Novikova,T.V. Besedina, Yu.I.Chimirov,Biologically Active Additives of Aquatic Organisms, Your Nutrition, M., **2**, 34 (2001).
[17] Á. Gudmundsdóttir, H. Hilmarsson, B. Stefansson, Potential Use of Atlantic Cod Trypsin in Biomedicine, Biomed Res Int., 749078 (2013).
[18] W.B. Jensen, The Origin of the Soxhlet Extractor (Engl.), J. Chem. Educ., **84**, 12, 1913-1914 (2007).
1. [19] F. Soxhlet, Diegewichts analytische, Bestimmungdes Milchfettes, Dingler's Polytechnisches Journal, **232**, 461-465 (1879) [Electronic resource]. Available at: http://www.cyberlipid.org/extract/soxhlet.PDF.