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THE USE OF SECONDARY FISH RAW MATERIALS FROM SILVER CARP IN THE TECHNOLOGY OF STRUCTURING AGENTS

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ABSTRACT

The expediency and prospects of using secondary fish raw materials from silver carp in the structuring agents technology have been substantiated. The combination of secondary fish raw materials and seaweed with different mechanisms of gelation has a targeted effect on the organoleptic, structural-mechanical, and physicochemical properties of food products. Accordingly, the expediency of adding the Black Sea alga cystoseira (2%) to optimize the mineral composition of the structuring agents and increase their jelly-making properties was established. Using experimental studies and multicriteria optimization, the optimal ratio of secondary fish raw materials – 40%; water – 60% and rational heat treatment – 150 min, at a temperature of 85 – 100 °C has been determined. An analysis and comparison of various preservation methods have confirmed the expediency of using the freeze-drying method of drying fish broths for the production of structuring agents, which allows to preserve of the original properties of the product, biologically active substances, reduce the mass of the dried product and increase the shelf life of the structuring agents. The optimal storage conditions have been determined – the ready-made structuring agents were packed in three-layer paper kraft bags and stored in a cool dry place at a temperature of 22 ±2 °C and relative humidity of no more than 70% for 12 months. It was found that the physical and chemical indicators of the structuring agents depend on the features of the structure, amino acid composition of fish collagen. The presence of the overwhelming number of high-molecular-weight molecules in the composition of the structuring agents and the preservation of the native structure determine high indicators of dynamic viscosity, dissolution time, and strength. As a result of the research, it was revealed that the high protein content largely depends on the amount of collagen in the secondary fish raw material, which has a positive effect on the gelling properties of the product being created.

Keywords: secondary raw material; silver carp; structuring agent; physical indicator; chemical indicator; biological value

INTRODUCTION

The natural and climatic conditions and significant resource potential of Ukraine contribute to the development of fisheries in their freshwater reservoirs. The production of commercial fish in domestic aquaculture farms is 23 thousand tons, of which 60% is silver carp **(State Statistics Service of Ukraine, 2020)**.

Accordingly, an important task of fish processing enterprises in Ukraine is to ensure and increase the efficiency of using the resource potential of the domestic fishery complex, possibly under the condition of rational use of fish from own water reservoirs of the country **(Makarenko et al., 2021)**.

One of the main ways of efficient use of fish resources is the processing of secondary fish raw materials left after their processing. About a third of secondary fish raw materials from silver carp are heads, bones, and fins, which are a valuable source of proteins, primarily collagen, and are widely used in the food industry **(Mushtruk et al., 2020)**.

A promising direction in the processing of collagen-containing secondary fish raw materials from silver carp is the production of structuring agents. Monitoring of modern trends in the market for structuring agents testifies to their limited assortment based on domestic raw materials, the most common of which is gelatin. However, due to massive cases of cattle diseases, the use of collagen of animal origin is dangerous **(Suchenko et al., 2017)**.

A great contribution to the solution of the problem of complex fish processing and production of structuring agents were made by scientists **Kaklyugin and Belousova (2017)**, **Nikiforova (2020)**, **Antipova et al. (2019)**,

Andreev and Morozov (2020), **Kao et al. (2014)**, **Makarov et al. (2019)** and **Postnov et al. (2018)**.

However, there are no conceptual developments in the direction of the study of consumer properties of structuring agents based on secondary fish raw materials from the most common object of aquaculture in Ukraine – silver carp, which determines the relevance and practical importance of scientific research.

Scientific Hypothesis

The hypothesis of the research work was based on the assumption that the combination of protein (heads, bones, fins of silver carp) and polysaccharide (cystoseira alga) structuring agents improves the organoleptic characteristics of food products, increases the water-holding capacity, and allows to create products with a predictable complex of consumer properties. According to the results obtained, it was confirmed that the combination of structuring agents of various natures makes it possible to more accurately regulate the consistency of the product, purposefully affects the structural-mechanical and physicochemical properties of food products through the use of raw materials with various mechanisms of gelation.

MATERIAL AND METHODOLOGY

Samples

Research of structuring agents for control and experimental samples (packed edible gelatin, grade P-7, manufactured by the Lysychansk Gelatin Plant; structuring agents based on secondary fish raw materials; structuring agents based on secondary fish raw materials with the addition of the Black Sea algae cystoseira) were carried out using the following raw materials: heads, bones, and fins of freshwater herbivorous fish – silver carp (*Arictichthys nobolis Rich*, two-year-old, autumn catch, weight 2 – 2,5 kg), grown in the Irkliivskyi herbivorous fish hatchery according to **DSTU 2284 (2010) (**Figure 1); algae cystoseira, which is sold in the form of a dry powder following **GOST 31413 (2010)**; drinking water following **DSTU 7525 (2014)**.

Chemicals

Calcium chloride, $CaCl_2$. (AGROZONE Ukraine).

Hydrochloric acid, HCl (grade A, analytical grade, LLC “Khimlaborreaktyv” Ukraine).

Sodium hydroxide, NaOH (grade A, analytical grade, LLC “Khimlaborreaktyv” Ukraine).

Sulfuric acid, H_2SO_4 (grade A, chemically pure, LLC “Khimlaborreaktyv” Ukraine).

Animals and Biological Material

The studies used variegated silver carp weighing 2 – 2.5 kg, 50 – 60 cm long, autumn catch, which was grown at the Irkliivskyi fish farm of herbivorous fish. Conducted studies of the structural and morphological composition of experimental groups (heads, internal organs, carcasses, fillets, scales, fins, bones), calculated the proportion of edible and inedible parts.

Instruments

Drying cabinet (SNOL, LLC “Labimpex LTD” Ukraine).

Muffle furnace (SNOL, LLC “Labimpex LTD” Ukraine).

Automatic amino acid analyzer (T 339 produced by “Mikrotekhna” Czech Republic).

Atomic absorption spectrophotometer (Shimadzu AA-6200, LLC “SPEKTROLAB” Ukraine).

Fat analyzer (SOX 406, LLC “Khimlaborreaktyv” China).

Gas chromatograph (“Kupol-55”, “Shimadzu Corporation” Japan).

Program viscometer (Brookfield LVDV-II+PRO USA), pH meter HI8314 HANNA (“SPECTRO LAB” Ukraine).

Laboratory Methods

The study of the chemical composition of the structuring agents was carried out according to the following methods: mass fraction of moisture by drying a sample of the product to constant mass at a temperature of 100 – 105 °C according to **DSTU 8029 (2015)**; mass fraction of ash – by the gravimetric method, after mineralization of the sample weight in a muffle furnace at a temperature of 500 – 600 °C following **DSTU 8718 (2017)**; mass fraction of lipids by the Soxhlet method, which consists in the fact that fat is weighed after its extraction with a solvent from a dry sample weight in a Soxhlet apparatus, based on the determination of the change in the mass of the sample after extraction of fat with a solvent following **DSTU 8718 (2017)**; mass fraction of protein by determination of total nitrogen by the Kjeldahl method, based on the ability of the organic material of the product sample to be oxidized with concentrated sulfuric acid in the presence of a catalyst following **DSTU 8030 (2015)**.

Figure 1 Silver carp.

Figure 2 Structuring agents.

The amino acid composition of proteins was determined by ion-exchange liquid column chromatography on an automatic amino acid analyzer T 339 using an LG ANB cation exchange resin **(Sorochan and Shtemenko, 2005)**.

The mineral composition was determined by inductive plasma atomic emission spectrometry according to **DSTU ISO 11885 (2005)**.

The study of physical and chemical indicators was carried out according to the following methods: transparency – according to own patented method **(Patent No. 73282, 2012)** on a universal computing device; strength – by the method of establishing the maximum load required for the destruction of the gelatin surface following **GOST 11293 (2017)**; melting temperature by defining the temperature at which the gelatin turns into a liquid state following **GOST 11293 (2017)**; dissolution time by the method of establishing the duration of complete dissolution according to **GOST 25183.3 (1982)**; dynamic viscosity by the method of determining the viscosity of a solution at certain temperatures and a decrease in viscosity after determining the holding time on a capillary viscometer according to **GOST 25183.4 (1982)**; active acidity by determining the pH of a solution of a certain concentration at a certain temperature with using a pH meter according to **GOST 11293 (2017)**.

Description of the Experiment

Sample preparation: Fish raw materials (heads, bones, fins) of silver carp were washed in clean running water at a temperature not exceeding 15 – 20 °C; while mucus, blood, and other residues were removed simultaneously with washing. Washing was carried out until the water was clear. The quality of washing was monitored visually. The washed fish raw material was crushed and subjected to heat treatment. Next, the fish raw material is fed to the shredder "Volgar 5 U", where it is ground to a homogeneous mass. In the process of mixing, all the raw materials are thoroughly mixed for further loading into the boiler cooker of the required portion. The experimentally established optimal ratio of fish waste and water was 1:1.5; heat treatment was 2.5 hours at a temperature of 85 – 100 °C. At the initial stage of heat treatment, cystoseira was added in the form of a dry powder (2%). To reduce the mass fraction of moisture, the fish broth was subjected to freeze-drying at a temperature of (50 – 70 °C). The ready-structuring agents were packed in three-layer paper kraft bags and stored in a cool dry place at a temperature of 22 ±2 °C and relative humidity of no more than 70% for 12 months. A control sample, structuring agents based on secondary fish raw materials were sent for analysis; structuring agents based on secondary fish raw materials and cystoseira. Then, an average sample was taken from each unit, which was characterized by its general chemical composition, mineral content, the amino acid composition of proteins, and physicochemical parameters.

Number of samples analyzed: Three types of structuring agents were used in the study of the samples: control – gelatin of animal origin and two samples based on secondary fish raw materials of silver carp and addition of cystoseira.

Number of repeated analyses: The study was repeated 5 times, and the experimental data were processed using the methods of mathematical statistics.

Number of experiment replication: Each study was carried out five times, the number of samples was three, resulting in fifteen repeated analyzes (Figure 2).

Statistical Analysis

The statistical analysis data were produced by Microsoft excel and Statistica 15. The accuracy of the obtained experimental data was determined using the Student's test for a confidence probability of ≤0.05 based on the number of parallel determinations at least 5. Linear programming problems were solved using the MS Excel spreadsheet processor "Search for a solution" setting (Excel Solver).

RESULTS AND DISCUSSION

Structuring agents experimental samples were evaluated according to the following physicochemical parameters: dissolution time, active acidity, dynamic viscosity, gelatin strength, gelatin melting point, solution transparency (Table 1). Previous studies have established the effectiveness of the use of fish collagen in comparison with collagen of animal origin in terms of the main physicochemical parameters **(Mezenova, 2017; Hanani, 2016)**. Research shows that dissolution time is one of the important quality criteria for structuring agents **(Andreev and Morozov, 2020; Kao, 2020)**. It is known that the degree of solubility of the structuring agents depends on many factors: the size of the granules, the type of raw materials used, temperature, etc.

Table 1 Physical and chemical quality indicators of structuring agents.

Indicators	Control	Structuring agents based on	
		secondary fish raw materials	**secondary fish raw materials and cystoseira**
Dissolution time, min.	12 ±0.6	5 ±0.2	6 ±0.3
Dynamic viscosity, mPa·s^{-1}	16 ±0.8	17 ±0.7	18 ±0.9
Gelatine strength, H	10 ±0.5	10 ±0.5	11 ±0.5
Solution transparency,%	28 ±1.4	32 ±1.6	30 ±1.5
Melting temperature of gelatine, ºC	27 ±1.3	30 ±1.5	31 ±1.5
Active acidity, pH	5.5 ±0.2	6 ±0.3	6 ±0.3

Note: (n = 5, $p \leq 0.05$).

However, first of all, dissolution is associated with the hydration of proteins, that is, the binding of water molecules to proteins. Scientists associate this phenomenon with the structural features of collagen in various fish species **(Shokina et al., 2020; Bredikhina and Zarubin, 2019)**. Protein, like any hydrophilic high molecular weight compound, swells, and then the protein molecules begin to gradually transfer into solution.

When swelling, water molecules penetrate the protein and bind to its polar groups. A dense network of polypeptide chains is delimited. Further absorption of water leads to the separation of protein molecules from the total mass and dissolution. This is the process of electrostatic binding of water molecules to the polar groups of side radicals of acidic amino acids with a negative charge and basic amino acids with a positive charge. However, swelling does not always lead to rapid dissolution, for example, animal collagen, unlike fish collagen, can remain in a swollen form, having absorbed a large amount of water. Similar statements were highlighted in the works of scientists **(Mezenova et al., 2018; Zarubin, 2018; Palamarchuk et al., 2020)**. This can explain almost twice as fast the dissolution time of research structuring agents based on secondary fish raw materials – 5 minutes and cystoseira – 6 minutes, compared to the control – 12 minutes. The solubility of animal collagen is hampered by structural features – cross-links between polypeptide chains. A paradoxical phenomenon is observed: the protein contains many anionic or cationic groups, and its solubility in water is relatively low **(Vorobiev and Nizhnikova, 2017; Dzyuba and Zemlyakova, 2019)**. It was noted that the melting point of the control sample is the lowest (27 °C), of the structuring agents based on secondary fish raw materials (30 °C), and structuring agents based on secondary fish raw materials and cystoseira (31 °C). Scientists have proven that the lowered melting temperature of fish gelatin-based on scales of various fish species (by 4 °C compared to the minimum normalized value for gelatin of animal origin) is due to the peculiarities of the amino acid composition of the raw material **(Yakubova and Bekesheva, 2018a; Abdelhedi et al., 2019; Palamarchuk et al., 2019)**.

The transparency of the structuring agent solution characterizes the degree of their contamination with mechanical impurities. It was found that the value of the transparency index of new structuring agents is higher than that of control. During storage, the transparency of all samples ranged from 27 – 30%. Accordingly, high transparency indicates their high quality. The predecessors **(Yakubova and Bekesheva, 2018b)** noted that the transparency index of a 5% solution of fish gelatin exceeds the traditional standard values by more than 17.0%, which confirms its high organoleptic properties as a food consistency regulator.

Dynamic viscosity is one of the main characteristics of structuring agents. According to this indicator, all product samples are within acceptable limits (16 – 18 mPa.s^{-1}). Scientists noted the higher values of viscosity indicators of fish structuring agents (by more than 18%), compared with animal gelatin **(Bekesheva, 2019)**. However, it is worth noting that the results of the prototypes of Structuring agents based on secondary fish raw materials are the most optimal compared to the control and Structuring agents based on secondary fish raw materials and cystoseira. It has been proven that in the manufacture of any product with a gelatin structure, it is advisable to use structuring agents with medium viscosity, since structure formers with high viscosity can render the created product too rigid and elastic consistency, respectively, structuring agents with low viscosity, on the contrary, do not provide the required strength of the product.

In works **(Nikolaev et al., 2016; Zarubin and Bredikhina, 2016)**, the effectiveness of combining protein-polysaccharide structuring agents to obtain the required structure of a food product is confirmed.

An indicator of the quality of the structuring agents is the strength of the gelatin, which directly depends on the pH of the medium. The greatest strength is observed in the range from 5 to 7 pH.

The nutritional value of the structuring agents was examined by their chemical composition, namely, the content of proteins, fats, and minerals (Table 2).

So, the high content of proteins (82%) and minerals characterizes the degree of the biological value of natural structuring agents. It should be noted that the structuring agents based on secondary fish raw materials and cystoseira contain a greater amount of minerals (1.6%) compared to the structuring agents without cystoseira (1.3%). Accordingly, the addition of cystoseira made it possible to rationalize the mineral composition of the product, since cystoseira contains a complete complex of mineral elements.

Table 2 Chemical composition of structuring agents.

Content, %	Control (gelatine)	Structuring agents based on	
		secondary fish raw materials	secondary fish raw materials and cystoseira
Moisture	14.0 ±0.7	15.0 ±0.7	15.0 ±0.7
Protein	83.0 ±4.1	82.0 ±4.1	82.0 ±4.1
Fat	1.5 ±0.07	1.4 ±0.07	1.1 ±0.05
Minerals	1.4 ±0.07	1.3 ±0.06	1.6 ±0.09

Note: (n = 5, $p \leq 0.05$).

Table 3 Amino acid rate of protein of structuring agents.

Amino acid, %	Amino acid content according to the FAO/WHO scale	Control	Structuring agents based on	
			secondary fish raw materials	secondary fish raw materials and cystoseira
Valine	50	45	47	49
Isoleucine	40	29	32	34
Leucine	70	50	71	74
Lysine	55	72	101	105
Methionine + Cystine	35	34	78	83
Threonine	40	50	93	87
Phenylalanine + Tyrosine	60	38	70	71

Table 4 Mineral composition of structuring agents, mg.100g^{-1}.

Mineral element	Control	Structuring agents based on	
		secondary fish raw materials	secondary fish raw materials and cystoseira
Potassium	7.94 ±0.31	98.0 ±4.1	90.45 ±4.2
Calcium	343.0 ±10.61	68.1 ±3.32	69.3 ±3.46
Iron	0.91 ±0.04	1.09 ±0.05	1.02 ±0.05
Chlorine	454.0 ±22.49	545.7 ±27.16	475.6 ±23.70
Selenium	-	-	6.24 ± 0.28
Bromine	2.78 ±0.13	3.37 ±0.16	5.01 ±0.24
Phosphorus	300 ±10.12	120 ±5.6	170 ±8.1
Zirconium	0.10 ±0.004	0.05 ±0.002	0.16 ±0.008
Sulfur	2.1 ±0.10	2.8 ±0.14	4.98 ±0.24

Note: (n = 5, $p \leq 0.05$).

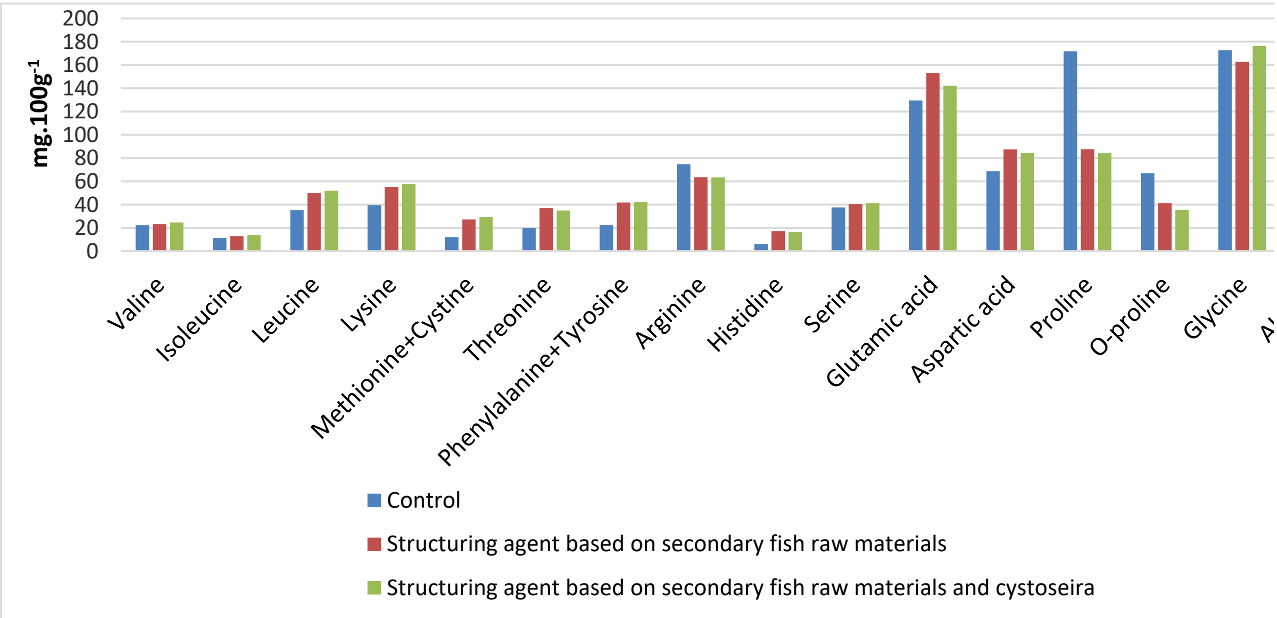

Figure 3 Analysis of the amino acid composition of protein structuring agents.

In the works **(Kao, 2017; Baidalinova and Lyapustina, 2018; Zheplinska et al., 2019)** it is indicated that the amount of protein in the research fish gelatin is within the range (87.36 – 91%), which confirms the rather high biological value of the product.

One of the important quality criteria for structuring agents is their biological value, which is largely determined by the content and balance of amino acids, especially essential ones **(Holembovska et al., 2021; Nesterenko et al., 2020)**. Characteristics of the amino acid composition are shown in Figure 3.

During studies of the amino acid composition of proteins, the structuring agents, the highest content of such amino acids was noted: glycine, alanine, proline, aspartic and glutamic acids. These amino acids form a repetitive sequence in the polypeptide chain, resulting in the helical structure of collagen. Accordingly, the high content of glycine 162.7 – 176.4 $mg.100g^{-1}$ and glutamic acid 142.1 – 153.1 $mg.100g^{-1}$ in research structuring agents is a characteristic feature of the amino acid composition of fish collagen. Amino acids such as histidine, isoleucine, valine are found in a smaller amount, which is due to the smaller number of cross-links. Experimental studies of the amino acid composition of fish gelatin **(Antipova et al., 2019)** showed that it is almost identical to the animal. However, animal gelatin lacks tyrosine and cystine.

It was also found that fish gelatin has a lower content of hydroxyproline, but almost 2.5 times more proline **(Antipova and Storublevtsev, 2016)**. An objective assessment of the biological value of proteins was determined by a set of indicators: amino acid rate, KRAS, utilitarian coefficient. Amino acid rate of structuring agent proteins was determined following the FAO/WHO scale (Table 3). The biological value of protein compositions depends on the content of essential amino acids in them and their ratio in the product and the idealized model **(Karpenko, 2019)**. So, the dominant amino acids are lysine, threonine, phenylalanine, and tyrosine, the limiting ones are valine and isoleucine.

To assess the degree of protein utilization, the coefficient of difference of the amino acid rate was calculated. KRAS of the control sample is 16.4%, of the research ones is 37.8 – 38.2%.

The biological value of the reference protein is 100%. In experimental samples, biological value is 61.8% (structuring agents based on secondary fish raw materials) and 62.2% (structuring agents based on secondary fish raw materials and cystoseira), control ones – 83.6%. The value of the utilitarian coefficient of the amino acid composition of the experimental samples (0.618 – 0.836) indicates a high balance of amino acids relative to the standard.

Thus, the qualitative composition of structuring agent proteins based on secondary fish raw materials from silver carp indicates the expediency of their effective use in the food industry.

One of the important indicators of the nutritional value of structuring agents is the content of the main macro- and microelements in their composition (Table 4).

Analyzing the quantitative composition of macro- and microelements of research structuring agents in comparison with the control, an increase in the level of potassium, iron, sulfur, bromine, chlorine should be noted. Experimental samples of structuring agents, in comparison with the control, contain a significant amount of potassium necessary for the human body, which ensures the normal activity of the cardiovascular system. The potassium content in structuring agents based on secondary fish raw materials is 98.0 $mg.100g^{-1}$, in the sample from cystoseira – 90.45 $mg.100g^{-1}$, which exceeds the value of the control sample by 113.94%.

An increased amount of iron 1.2 times in experimental samples compared to the control will contribute to a positive effect on the protective functions of the body, since iron is involved in respiration, hematopoiesis, immunobiological and redox reactions, and a sufficient amount of calcium (68.1 – 69.3 $mg.100g^{-1}$) will improve its assimilation by the human body.

In terms of sulfur content, the dominant is structuring agents sample based on secondary fish raw materials and cystoseira – 4.98 $mg.100g^{-1}$, because sulfur is used in the human body to neutralize many toxic products formed during metabolism.

A distinctive feature of the studied samples of the structuring agents with cystoseira is a significant bromine content, which is 5.01 $mg.100g^{-1}$ which is almost twice as much as in the control – 2.78 $mg.100g^{-1}$, and selenium – 6.24 $mg.100g^{-1}$. The enrichment of the mineral composition of the structuring agents with bromine and selenium can be explained by their sufficient content in cystoseira.

The results of the study show that the addition of seaweed – cystoseira to the structuring agents contributes to the optimization and enrichment of their mineral composition in comparison with the structuring agents without cystoseira and the control sample.

CONCLUSIONS

It was found that structuring agents based on secondary fish raw materials are characterized by a high protein content (82%), which has a positive effect on the jelly-making properties of the structuring agents. The dominant amino acids are glycine, proline, alanine, aspartic and glutamic acids. The value of the utilitarian coefficient of the amino acid composition of the new structure-formers is within the range (0.61 – 0.83), which indicates a high degree of assimilation of their proteins by the human body.

It has been proven that the addition of cystoseira to the structuring agents based on secondary fish raw materials increases the content of Bromine (5.01 $mg.100g^{-1}$) compared to the control (2.78 $mg.100g^{-1}$) and Selenium (6.24 $mg.100g^{-1}$).

Confirmed a high degree of jelly-making properties of structuring agents, characterized by a complex of indicators of viscosity (16 – 18 $mPa.s^{-1}$), strength (10 – 11 H), melting point (27 – 31 °C), and solubility (5 – 6 minutes).

It was found that the addition of cystoseira to the structure-forming agents promotes optimization and enrichment of their mineral composition in comparison with the structure-forming agents without cystoseira and the control sample.

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