Bulletin of the *Transilvania* University of Brasov
Series II: Forestry • Wood Industry • Agricultural Food Engineering • Vol. 19(68) No. 1 – 2026
https://doi.org/10.31926/but.fwiafe.2026.19.68.1.12

EFFECTS OF NATURAL CRYOPROTECTANTS ON PRESERVING THE PROTEIN-LIPID COMPLEX OF ROUND GOBY (*Neogobius melanostomus)* DURING FREEZING AND SUBSEQUENT STORAGE

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Abstract: *The research aims to establish the effectiveness of natural cryoprotectants in stabilizing the protein-lipid complex of round goby (Neogobius melanostomus) muscle tissue during freezing and long-term storage at low temperatures. Three types of natural cryoprotectant compositions were used in the study: glucose (2%) + inulin (1.5%); pectin (1%) + rosemary extract (0.3%); honey (3%) + sea salt (1%). Samples were frozen at –35°C and stored for 1, 3, and 6 months at –18°C. The results confirmed pronounced protective effects of all natural cryoprotectants studied, but the pectin-rosemary extract combination proved to be the most effective. The water-binding capacity of the fresh fillet was 79%, while in the control variant, it decreased to 60.8% after 6 months of storage. In samples treated with cryoprotectants, this indicator remained significantly higher – 68.4% (glucose + inulin), 68.9% (honey + salt), and 71.2% in the variant with pectin and rosemary. A similar pattern was observed for freezer weight loss: the control sample showed a gradual increase from 1.8% to 7.9%, while in the variants treated with cryoprotectants, the values were 5.6, 5.0, and 4.2%, respectively. Additional indicators, such as pH, peroxide value, and TBA-active substances, also confirmed a decrease in the intensity of protein and lipid degradation in the treated samples. Thus, natural cryoprotectants, especially the pectin-rosemary extract composition, significantly reduce dehydration, slow protein denaturation, and lipid oxidation, preserving the structural and functional properties of round goby during long-term frozen storage. Research findings can be used to develop new environmentally safe technologies for the biopreservation of fish raw materials without the application of synthetic stabilizers.*

Key words: *freezing, natural cryoprotectant, protein-lipid complex, round goby (M), storage.*

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1. Introduction

Freezing is an effective method for preserving fish. However, it is accompanied by negative changes in the protein-lipid complex, including protein denaturation, lipid autoxidation, and water loss. This is especially relevant for round goby *(Neogobius melanostomus*), which contains polyunsaturated fatty acids that are highly sensitive to oxidation and unstable protein structures.

During freezing, complex physicochemical processes occur in fish muscle tissue, including the formation of ice crystals, an increase in ionic strength, and oxidation of protein functional groups, which leads to denaturation of myofibrillar proteins and a decrease in their solubility and water-binding capacity [9]. In addition, fish is characterized by a high content of polyunsaturated fatty acids, which are sensitive to autoxidation during prolonged frozen storage, accompanied by the formation of hydroperoxides and secondary oxidation products [16]. The use of natural cryoprotectants, in particular hydrocolloids (pectin, inulin) and plant antioxidants, allows for partial stabilization of the protein-lipid complex of fish tissue, reducing protein denaturation and lipid oxidation during low-temperature storage [23].

The nutritional value of raw materials is determined by a set of indicators that characterize the biological value of protein, lipids, mineral composition, and safety [3]. Freezing is the primary method of storing fish, providing an effective slowdown of microbiological and enzymatic processes that extends its shelf life without the use of chemical preservatives. According to modern literature, properly organized freezing allows for preserving the nutritional value, functional properties of protein, structure, and safety of aquatic raw materials for a long time, which is especially important in the conditions of global logistics and seasonal fluctuations in fishing [18]. At the same time, even with quick-freezing technologies, some destructive changes occur in fish muscle tissue: the formation of large ice crystals disrupts cellular structures, leading to water loss, protein denaturation, reduced protein solubility, lipid autoxidation, and deterioration of the product's organoleptic characteristics after defrosting.

The problem becomes particularly relevant in the context of storage of fish of the goby family, in particular the round goby, which is characterized by a high content of polyunsaturated fatty acids, which are sensitive to autooxidation, and by the availability of proteins that are unstable to deep freezing. The goby's muscle tissue has a thin structure, with relatively high humidity and low fat content, making it vulnerable to physicochemical changes during cryopreservation. In this case, there is a need to pre-treat the fillet with functional solutions that would play a protective role with respect to the protein-lipid complex.

Holembovska et al. [7] conducted research aimed at developing and substantiating a new technology for canning freshwater fish with the addition of spicy root vegetables and the preliminary treatment of raw materials with organic acids. During the study, carp was treated with salt and various concentrations of organic acids, stored under certain temperature conditions. However, the freezing process for fish was not investigated.

Recent scientific studies demonstrate high efficiency of natural cryoprotectants, in particular polyols, sugars, polysaccharides, protein-peptide additives, and natural antioxidants, for preserving the quality indicators of fish raw materials under deep-freezing conditions. Thus, the use of inulin at a concentration of 1.5% for the treatment of carp surimi contributed to the stabilization of salt-soluble protein, the preservation of Ca^{2+}-ATPase activity, the reduction of the hydrophobicity of protein molecules, and the inhibition of their denaturation during 180 days of storage at –20°C [9, 21]. In turn, due to its gelling properties, pectin has been shown to reduce water loss during freezing and thawing, thereby improving the water-binding capacity (*WBC*) of muscle tissue and partially preserving the structural integrity of the fillet [14].

The mechanism of action of natural cryoprotectants is realized through several main ways: inhibition of the formation of large ice crystals (glass phase), formation of a hydrated shell around protein and lipid structures, reduction of water activity, and protection of cell membranes from destruction. The addition of cryoprotectants is considered one of the most effective methods to prevent protein denaturation during freezing in aquatic products [20]. For example, inulin both binds free water and forms a gel-like matrix that fixes water in the intercellular space, reducing its loss during defrosting. Pectin, as a high-molecular polymer, acts not only as a viscoelastic medium but also provides stabilization of muscle fibres, which reduces mechanical damage due to freezing. Antioxidant substances, such as rosemary extract or natural honey, inhibit lipid oxidation, reduce the formation of secondary products of peroxidative decomposition (e.g., malonaldehyde), and contribute to the preservation of the product's colour and taste [5].

Some studies show that combining several natural cryoprotectants (e.g., inulin with pectin, or sugars with polyphenolic extracts) can yield a synergistic effect, improving both physicochemical and sensory characteristics after freezing [13]. In particular, inulin, in combination with antioxidants, reduces mass loss during thawing to 6-7% versus 30-35% in untreated samples, while pectin maintains shape and texture stability.

Despite a wide range of research on cryoprotectants, there remains a shortage of data on the application of these technologies specifically to the round goby, an object of potentially high value that is underutilized in industrial processing. There is practically no data on the effect of different types of natural cryoprotective compositions on the preservation of protein and lipid fractions of goby during long-term storage (90-180 days), as well as on the effectiveness of individual and combined compositions in ensuring stable organoleptic indicators after defrosting.

Thus, the study of the influence of natural cryoprotectants on the preservation of the protein-lipid complex of round goby under freezing and controlled storage conditions is of scientific and practical interest, aiming to develop effective, environmentally safe technologies to extend the shelf life of fish products without the use of synthetic stabilizers.

The purpose of the research was to establish the effectiveness of natural cryoprotectants in stabilizing the protein-lipid complex of round goby muscle tissue under freezing and controlled storage conditions to reduce quality losses, extend

shelf life, and ensure the functional properties of the finished product.

To implement the purpose of the research, the following tasks were set:

- to assess the influence of various natural cryoprotectant compositions on the structural and functional state of the round goby muscle tissue during freezing and further storage;
- to compare the effectiveness of various cryoprotectants in preserving the protein-lipid complex and establish the most effective composition.

2. Materials and Methods

The study used freshly caught *Neogobius melanostomus* (round goby) fish, uniform in age, size, and weight, without signs of mechanical damage, disease, or initial autolysis.

48 specimens of round goby (*Neogobius melanostomus*) of approximately the same size and weight were used in the research. After processing, two fillets were obtained from each fish, which were used as experimental units. In total, 96 fillet samples were formed, which were distributed into four experimental groups (control and three variants of cryoprotective compositions) with 24 samples in each. To study the dynamics of changes during storage, the samples were analysed after 1, 3, and 6 months. There were 8 fillets per group at each time point, which provided at least five analytical repetitions for determining each indicator.

To reduce biological variability between individual fish specimens during the formation of experimental groups, the principle of partial blocking was applied. Fillets obtained from one specimen of round goby were distributed between different experimental treatment variants. This approach allowed minimizing the influence of individual biochemical characteristics of fish on the results of the experiment and ensuring a more correct comparison of the effectiveness of different cryoprotective compositions.

In this study, an individual specimen of round goby was considered the biological experimental unit, whereas fillets obtained from the same fish were used as derived samples within the blocked design and were not considered to be completely independent biological replicates.

The raw material was transported chilled (0…+2°C) and used within 12 hours of capture. To unify the experimental conditions and minimize variability, all samples were washed in running cold water before processing and carefully processed: heads, entrails, and fins were removed, and fillets were obtained for further experimental processing.

The samples were divided into four groups: a control group, the samples of which were subjected to a similar procedure of immersion for 20 min at 4°C, but without adding active cryoprotective components, and three experimental groups, which were treated with the corresponding natural cryoprotective compositions. The first group of fillets was immersed in a solution containing glucose (2%) and inulin (1.5%), which have high water-binding activity and gel-forming ability. The second group was treated with a mixture of pectin (1%) and water extract of rosemary (0.3%), which had a dual effect: water retention and oxidation inhibition due to phenolic components. The third experimental group was treated with natural honey (3%) combined with sea salt (1%), providing a complex antibacterial, antioxidant, and water-

retaining effect. All treatments were carried out by immersing the samples in cryoprotectant solutions for 20 minutes at 4°C, after which the excess liquid was removed using filter paper.

Freezing was carried out in a low-temperature chamber at –35°C until the geometric centre of the fillet reached the temperature of –18°C. The temperature was monitored using a thermocouple installed in the central part of the sample. The mean weight of one fillet was 35-45 g, and the thickness was approximately 1.5-2.0 cm. To minimize variability, samples with similar geometric parameters were selected. The average time required to reach the temperature of –18°C in the centre of the fillet amounted to 110-120 min, corresponding to the conditions of quick freezing for samples of this size. Before freezing, the fillets were placed in food-grade polyethylene bags and hermetically sealed. The samples were stored at a temperature of –18°C in the same packaging conditions without glazing. The samples were stored for 1, 3, and 6 months to study the dynamics of changes in the protein-lipid complex. Temperature and humidity control in the chamber was carried out daily, with the indicators recorded in the laboratory register.

The study was conducted during 2023-2024 in the laboratory of the Department of Meat, Fish, and Seafood Technology of the Faculty of Food Technologies and Quality Control of Agricultural Products of the National University of Life Resources and Environmental Sciences of Ukraine.

Physico-chemical and biochemical indicators of the test samples were determined using the following methods: mass fraction of water – by drying the sample to a constant mass in a SNOL drying oven (Labimpex LTD, Ukraine) at a temperature of 100...105°C, according to Alahmad et al. [2]; mass fraction of lipids – by the Soxhlet extraction-gravimetric method, according to Alahmad et al. [2] using a SOX 406 fat analyser (Hanon Instruments, China). The total nitrogen content was determined by the Kjeldahl method. Soluble nitrogen was determined after extraction of the sample with a buffer solution and subsequent centrifugation (Eppendorf centrifuge, Germany). The nitrogen content in the resulting supernatant was determined by the Kjeldahl method. The mass fraction of soluble nitrogen was calculated as the ratio of the nitrogen content in the supernatant to the total nitrogen content in the original sample and expressed as a percentage. The obtained values of the total and soluble nitrogen content were converted to protein content using the appropriate conversion factor. Active acidity (*pH*) was determined potentiometrically, according to Sáez et al. [11], using a portable pH meter previously calibrated with buffer solutions (Hanna Instruments, the USA).

Functional, technological, and oxidative indicators were determined as follows. The water-binding capacity (*WBC*) was determined using a pressing method adapted for fish raw materials. A portion of the minced sample was pressed between filter paper and glass plates for a fixed period, after which the parameters of the water spot were evaluated, and *WBC* was calculated as a percentage, according to Cropotova et al. [4]. Freezer weight loss (*FWL*) during cryogenic storage was determined gravimetrically as the relative mass loss, according to Equation (1).

$$FWL = \frac{m_0 - m_t}{m_0} \cdot 100 \quad (1)$$

where:

FWL is the freezer weight loss [%];

m_0 – the mass of samples before storage [g];

m_t – the mass of samples after a given period [g].

The content of thiobarbituric acid reactive substances (*TBARS*) was determined spectrophotometrically, using the spectrophotometer by Thermo Fisher Scientific (USA), by measuring the intensity of the coloured complex of secondary lipid peroxidation products with thiobarbituric acid, by recording the optical density at 532 nm, and converting the results to malondialdehyde (*MDA*), according to Abeyrathne et al. [1]. The peroxide value of the lipid fraction was determined by the iodometric method (visual endpoint), according to Abeyrathne et al. [1].

Freezing was carried out in a Haier Biomedical (China) low-temperature freezer, weighing was performed using analytical balances Mettler Toledo (Switzerland).

The results of the experimental studies are presented as the mean value ± standard deviation (mean ± SD). Statistical data processing was performed using the STATISTICA 10.0 software package (StatSoft Inc., USA) and the Microsoft Excel spreadsheet processor (Microsoft Corp., USA). For each indicator, at least five parallel determinations (n = 5) were performed for each experimental group and each storage time point (1, 3, and 6 months). To assess the effect of the type of cryoprotective treatment and storage duration on the studied indicators, a two-way analysis of variance (two-way ANOVA) was used. The significance of differences between mean values was assessed by the Student t-test at a statistical significance level of $p \leq 0.05$.

3. Results

Water-binding capacity is one of the leading indicators of the functional and technological state of fish muscle tissue and a sensitive marker of its structural integrity during cryopreservation [15].

In fresh raw materials of round goby, the *WBC* was, on average, 79% (Table 1), reflecting the high hydration capacity of the intact myofibrillar system. After freezing and further storage, a gradual decrease in this indicator was observed, and its intensity largely depended on the use of natural cryoprotectants.

Water-binding capacity [%] of the muscle tissue Table 1

Variant	Raw material	Duration of storage [months]		
		1 month	3 months	6 months
Control	79.0 ±5.1	67.3±7.0	64.0±3.6	60.8±2.5
Variant 1		73.8±5.1	71.5±6.4	68.4±3.2
Variant 2		76.5±5.2	74.1±5.0	71.2±3.2
Variant 3		74.0±3.4	71.9±4.2	68.9±4.6

In the control sample without cryoprotective treatment, *WBC* decreased from 79% in fresh fillet to 67.3% after the first month of storage and to 60.8% after six months, indicating significant deterioration in the tissue's ability to retain water due to low-temperature dehydration and destruction of the protein-hydrate matrix. In samples treated with cryoprotectants, the decrease in *WBC* was less intense. Variant 1 (glucose + inulin) ensured a retention of 73.8% after 1 month and 68.4% after 6 months; variant 3 (honey + sea salt) had similar values (74.0% → 68.9%). The indicator showed the highest stability in variant 2 (pectin + rosemary), where WBC remained at 76.5% after 1 month and 71.2% after 6 months, indicating the most effective prevention of dehydration.

Another critical indicator of dehydration processes in fish muscle tissue due to ice sublimation, structural damage to surface layers, and disruption of water retention mechanisms during cryopreservation is freezer weight loss.

After freezing and subsequent storage for 1, 3, and 6 months at –18°C, a significant decrease in freezer weight loss was observed in all samples treated with natural cryoprotectants compared to the control (Table 2).

Freezer weight loss [%] in storage dynamics Table 2

Variant	Duration of storage [months]		
	1 month	3 months	6 months
Control	1.8 ± 0.1	4.6 ± 0.2	7.9 ± 0.3
Variant 1	1.2 ± 0.1	3.2 ± 0.1	5.6 ± 0.2
Variant 2	0.9 ± 0.1	2.5 ± 0.1	4.2 ± 0.2
Variant 3	1.1 ± 0.1	3.0 ± 0.1	5.0 ± 0.2

Note: Data are presented as mean ± SD (n = 5).

In the control sample without cryoprotectants, the freezer weight loss was 1.8% after 1 month of storage, increased to 4.6% after 3 months, and reached 7.9% after 6 months. This reflects the typical dynamics of low-temperature dehydration for untreated fish.

In samples treated with natural cryoprotectants, the intensity of the freezer weight loss was significantly lower. Variant 1 (glucose + inulin) provided a reduction in mass loss to 1.2 ... 5.6%, while variant 3 (honey + sea salt) provided a reduction to 1.1 ... 5.0%. The lowest values of freezer weight loss were recorded in variant 2 (pectin + rosemary), where the indicator was only 0.9% after 1 month and 4.2% after 6 months, confirming the most pronounced protective effect of this composition against surface dehydration.

For the comprehensive assessment of the effects of freezing and cryoprotectants on the state of the protein-lipid complex, changes in the pH of the round goby muscle tissue were studied (Table 3). The *pH* level determines the degree of hydration, protein stability, and oxidative balance of the system, and its changes are an essential indicator of preserving the quality of fish raw materials during storage.

The *pH* level in fresh fish tissue was 6.58. In the control variant, it decreased from 6.40 to 6.25 to 6.10 over 6 months. In the experimental samples, the rate of decrease

was substantially lower. The best values were recorded in variant B2 (6.51 → 6.45 → 6.38), which demonstrates the highest stability of the acid-base balance.

Dynamics of the pH level of the round goby muscle tissue Table 3

Variant	Fresh raw materials	Duration of storage [months]		
		1 month	3 months	6 months
Control	6.58±0.47	6.40±0.35	6.25±0.12	6.10±0.25
Variant 1		6.48±0.22	6.40±0.25	6.32±0.31
Variant 2		6.51±0.18	6.45±0.23	6.38±0.21
Variant 3		6.46±0.13	6.38±0.22	6.30±0.15

Note: Data are presented as mean ± SD (n = 5).

For an in-depth assessment of the structural and functional stability of the muscle tissue of round goby during freezing, the dynamics of the content of total and soluble protein under the influence of various cryoprotectant compositions were analysed. The total protein content of fresh round goby fillet was 17.8% by fresh weight, including 12.2% soluble protein, which accounted for 68.5% of the total. The dynamics of the content of these indicators by storage stages are presented in Table 4.

Indicators of the protein complex (in dynamics) Table 4

Variant	Duration of storage [months]					
	1 month		3 months		6 months	
	total protein, % in raw weight	soluble protein, % in raw weight	total protein, % in raw weight	soluble protein, % in raw weight	total protein, % in raw weight	soluble protein, % in raw weight
Control	17.1±1.2	10.0±1.6	16.2±2.5	7.8±2.1	15.4±1.1	6.0±1.2
Variant 1	17.5±1.5	11.2±1.4	17.0±3.1	9.8±1.3	16.4±1.3	8.5±1.2
Variant 2	17.6±2.3	11.7±1.3	17.2±1.1	10.4±2.3	16.7±1.4	9.2±2.8
Variant 3	17.4±2.2	10.9±2.4	16.8±2.2	9.1±2.2	16.1±1.6	7.8±1.3

Note: Data are presented as mean ± SD (n = 5).

In the control group, soluble protein levels decreased the fastest: from 10.0% after 1 month to 6.0% after 6 months, indicating significant denaturation of myofibrillar proteins. In the experimental variants, the rate of loss was significantly lower, and the best result was obtained for variant 2: 11.7 → 10.4 → 9.2%. The total protein indicator also tends to decrease over time, but changes in soluble protein are more informative and sensitive. Thus, the obtained data indicate that natural cryoprotectants effectively limit protein denaturation, and the pectin + rosemary composition provides the highest stability of the protein complex.

Changes in the total fat content during storage of frozen round goby fillet are shown in Table 5.

The dynamics of changes in the total fat content in round goby muscle tissue during low-temperature storage show a clear dependence on the cryoprotectant composition used. In the control samples, the most intense decrease in this indicator was observed: from 2.3% after the first month to 2.0% after three months, and to 1.7% after six months of storage. In contrast, in all variants with natural cryoprotectants, the decrease in fat content was much more moderate. For variant 1 (glucose + inulin), the fat level decreased from 2.4 to 2.2 and 2.0%; in variant 3 (honey + sea salt), from 2.4 to 2.2 and 2.0%. The lipid fraction showed the highest stability in variant 2 (pectin + rosemary), where the values decreased only from 2.5 to 2.3 and then to 2.1%. Therefore, variant 2 provided the lowest total fat loss, indicating the most effective protection of lipid structures during long-term storage.

Dynamics of total fat content [% in fresh weight] Table 5

Variant	Fresh raw materials	Duration of storage [months]		
		1 month	3 months	6 months
Control	2.6±0.2	2.3±0.3	2.0±0.1	1.7±0.4
Variant 1		2.4±0.4	2.2±0.2	2.0±0.1
Variant 2		2.5±0.2	2.3±0.3	2.1±0.1
Variant 3		2.4±0.3	2.2±0.1	2.0±0.2

Note: Data are presented as mean ± SD (n = 5).

The dynamics of this indicator often correlate with increases in peroxide value and *TBARS*, which reflect different stages of lipid peroxidation in fish muscle tissue. In fresh raw materials, the *TBARS* content was 0.18 mg MDA/kg, and the peroxide value was 2.0 mg O_2/kg of fat, corresponding to the level of fresh sea fish. Further storage in frozen form was associated with increases in these indicators (Table 6).

Dynamics of TBARS [mg MDA/kg] and peroxide value [mg O_2/kg of fat] Table 6

Variant	Indicator	Duration of storage [months]		
		1 month	3 months	6 months
Control	*TBARS* number	0.32	0.48	0.66
	Peroxide number	3.8	6.4	9.1
Variant 1	*TBARS* number	0.27	0.39	0.52
	Peroxide number	3.1	5.1	7.3
Variant 2	*TBARS* number	0.24	0.34	0.44
	Peroxide number	2.7	4.3	6.0
Variant 3	*TBARS* number	0.26	0.36	0.49
	Peroxide number	2.9	4.9	6.9

Note: Data are presented as mean ± SD (n = 5).

In the control samples, the level of *TBARS* increased most intensively from 0.32 to 0.66 mg/kg during 1 ... 6 months of low-temperature storage. In all experimental variants, this increase was statistically lower ($p < 0.05$), and the lowest values were recorded in variant 2 (pectin + rosemary), where the indicator reached only 0.44 mg/kg after 6 months. This confirms that the hydrocolloid-antioxidant composition effectively inhibits the formation of secondary lipid oxidation products.

At the same time, the control variant demonstrated the greatest increase in peroxide value: from 3.8 to 9.1 mg O_2/kg over 6 months, reflecting the intensive development of primary oxidation of unsaturated fatty acids. Variants with cryoprotectants significantly inhibited these processes: in variant 1 and variant 3, the increase was moderate (3.1→5.1→7.3 and 2.9→4.9→6.9 mg O_2/kg, respectively), while variant 2 had the lowest values (2,7→4,3→6,0 mg O_2/kg), indicating a pronounced antioxidant effect of pectin and phenolic compounds in rosemary.

4. Discussion

The two-factor analysis of variance showed that the type of cryotreatment and duration of storage have a statistically significant effect on all studied quality indicators of the round goby muscle tissue – water-binding capacity, freezer weight loss, pH, protein complex, and lipid oxidation intensity ($p < 0.001$). This pattern is consistent with fundamental models of cryodamage of fish raw materials, which predict progressive protein denaturation, dehydration, and lipid oxidation due to ice recrystallization and destruction of cellular structures, as reported by Zhu et al. [23].

The effect of cryoprotectants was consistent across all indicator groups: all treated variants demonstrated a significantly better state of the protein-lipid complex than the control. *WBC* decreased the slowest in variant 2 (pectin + rosemary), which is quite logical given the ability of hydrocolloids to form a hydrated matrix and reduce water migration [10].

The effectiveness of pectin as a cryoprotectant was also confirmed by Tanushree et al. [17], who demonstrated its ability to limit ice crystal growth and the denaturation of myofibrillar proteins.

The use of rosemary provided additional antioxidant protection. Phenolic compounds of rosemary inhibit peroxidation and secondary oxidation of lipids, stabilize membranes, and thereby reduce both water loss and protein degradation. Experimental data by Shi et al. [12] and Walayat et al. [19] demonstrate a similar effect in fish and shrimp systems, which is entirely consistent with the advantage of variant 2 in our study.

Freezer weight loss increased in all samples, while the control variant had 1.7 ... 2 times higher values than those of the cryoprotectant-treated variants. The lowest freezer weight loss was recorded in variant 2 (4.2% after 6 months), which corresponds to the established mechanisms of sublimation dehydration and its inhibition by hydrocolloid films. Additionally, Zhang et al. [22] confirm that combined coatings can reduce *FWL* by 25 ... 40%, which correlates with our results (≈47% reduction for variant 2 compared to the control).

The *pH* changes also favoured cryoprotectant systems: they slowed acidogenesis, a consequence of glycolysis

and the development of protein-lipid oxidation. This is consistent with the research findings of Liu et al. [8] and the qualitative assessment of fish freshness given in the review.

In the protein fraction, all cryoprotectants reduced the loss of total and soluble protein, with the best results in variant 2. A similar protective effect of inulin on myofibrillar structures was shown by Shumin et al. [13], as well as protein stabilization during low-temperature storage in the presence of hydrocolloids by Du et al. [6].

The accumulation of primary (peroxide number) and secondary oxidation products (*TBARS* number) increased with increasing storage duration; however, ANOVA showed significant inhibition of these processes by all cryoprotectants, especially pectin with rosemary. Such kinetics are entirely consistent with the mechanisms of antioxidant action of polyphenols and hydrocolloids described in the scientific research by Shi et al. [12] and Walayat et al. [19].

To sum up, variant 2 was the most effective protective system, ensuring maximum preservation of WBC, minimal freezer weight loss, stable pH values, the highest protein preservation, and the lowest lipid oxidation.

Evaluation of organoleptic characteristics was not part of the objectives of this study, however, the obtained physicochemical indicators (water-binding capacity, stability of proteins, and lipids) indirectly indicate a potential improvement in the texture and stability of the product quality after defrosting.

The obtained results indicate the possibility of using natural cryoprotective compositions in freezing technologies of underutilized fish raw materials, which allows to increase the stability of the protein-lipid complex without the use of synthetic additives.

Further research should focus on scaling up the proposed cryoprotective compositions and assessing their effectiveness under real industrial conditions for freezing and storage of fish raw materials of different species.

5. Conclusions

1. It was established that the use of natural cryoprotective compositions significantly improves the structural and functional characteristics of the round goby muscle tissue compared to the untreated control ($p < 0.001$). All compositions showed higher water-binding capacity, lower freezer weight loss, a smaller decrease in *pH*, and less severe degradation of proteins and lipids compared to the control.
2. The study of the indicator dynamics confirmed the complex of degradation processes typical for cryopreservation, namely, a decrease in *WBC*, *pH*, protein fractions, and fat content along with accelerated lipid oxidation. At the same time, the intensity of these changes significantly depended on the use of cryoprotectants. In the control group, the loss of hydration capacity was approximately 1.3 times faster than in the experimental variants; freezer weight loss increased almost 4 times, while it increased only 2 ... 2.5 times in samples treated with cryoprotectants. Degradation of soluble protein in the control was almost twice as intense as in the experimental variants, and the growth rate of *TBARS* and peroxide

value was approximately 30 ... 40% higher.

3. The comparative analysis of the obtained results clearly confirmed that among the tested compositions, the composition of variant 2 (1% pectin + 0.3% rosemary) is the most effective one for preserving the structural and functional integrity of the protein-lipid complex. This variant provided 17% higher *WBC* after six months, 47% lower freezer weight loss, 35% lower soluble protein loss, 33% lower *TBARS* accumulation, 34% lower peroxide value, and the lowest rate of *pH* decrease.

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