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Optimization of Soluble Collagen Technology from Trout Skin

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Abstract. The aim of this work was to develop and optimize the technology of soluble collagen derived from secondary fish raw materials – waste from the processing of aquaculture products from the Murmansk region (Russia). The raw material used was the skin of lightly salted trout fillets. Standard physicochemical research methods were employed for the study; the organoleptic evaluation of collagen samples was carried out using a 20-point product quality scale developed by the authors, and the results were processed using methods of mathematical statistics. The research included an analysis of the chemical composition of the raw material, which confirmed a high content of protein substances (about 60%) – potential sources of collagen. Due to the high content of fat (8%) and sodium chloride (up to 10%) in the raw material, additional technological steps (fleshing and soaking of the raw material) were introduced to remove them. Special attention was paid to optimizing the aqueous extraction stage: it was found that the yield of the finished product is influenced by the extraction duration, the pH of the extractant, and the temperature conditions of the operation. It was established that conducting extraction in a slightly acidic zone at pH 5.5–5.6 leads to a threefold increase in product yield with the same process duration; increasing the extraction duration from 4 to 8 hours further increases the yield by four times. The research revealed the influence of temperature parameters on the technological indicators of the production process (total collagen yield) and the extractability of protein substances (protein yield). As a result of mathematical data processing, the collagen extraction temperature close to the optimal value was determined to be (44.3 ± 1) °C. The results of the experiments allowed the final technological scheme for the production of dry soluble collagen to be established. Organoleptic and chemical studies determined the quality of the dry soluble collagen sample obtained using the refined technological scheme: the mass fractions of protein, water, fat, and mineral substances were 90.7 %, 6.2 %, 1.9 %, and 1.2 %, respectively, and the product quality level was 94 %. The research results demonstrated the potential of the technological solution for incorporating waste from mariculture product processing into the production of soluble collagen.

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1 Introduction

The development of new technologies for the harvesting of aquatic biological resources, as well as for the deep and integrated processing of raw materials, is one of the tasks requiring a solution for the fishing industry. Increasing the production rate and output volumes of fish products is inextricably linked to the improvement and development of technologies for the integrated use of secondary raw material resources (SRMR), such as waste from the processing of aquatic organisms, which makes it possible to obtain high-value-added products from them [1]. From the perspective of processing potential, fish skin is one of the most promising types of fish waste [2, 3], with the quantity of this type of secondary product accounting for 2–6% of the mass of the processed fish.

At the current stage, one of the successfully developing areas of the fishing industry is the production and processing of aquaculture products. Aquaculture is a knowledge-intensive sector that has significant potential as a supplier not only of food products but also of highly liquid raw materials for other industries. At the end of 2020, global aquaculture production volumes amounted to 123 million tons [4]. It is worth noting that the production of farmed Atlantic salmon is highly concentrated. The main representatives of salmonid mariculture are Norway, Chile, the United Kingdom, Canada, and the Faroe Islands. However, Norway is the absolute leader in global Atlantic salmon production. In 2020, production volume reached 1.23 million tons. This accounts for nearly half of the world's Atlantic salmon production. In addition, Norway's production of salmon trout amounted to 93111 tons in 2020. [5]. In 2016, Atlantic salmon fillet production in Norway amounted to 814172 tons [6]. One of the cost-effective types of processed products derived from this raw material is deboned, lightly salted salmon fillet, the production waste of which is salted fish skin. The annual quantity of this type of waste can amount to tens of thousands of tons. For example, in 2010, imports of fresh Atlantic salmon into Poland totaled approximately 106 thousand tons. As a result of fish processing into fillets, skin with subcutaneous fat tissue amounted to 6 thousand tons, among other waste products [7]. The crude protein content in Atlantic salmon skin exceeds 20% [8].
Currently, numerous methods for producing collagen from various raw material sources are well known. In industry, skin, bones, tendons, and animal cartilage are used to produce collagen. Natural type I collagen is obtained from bovine bone tissue [9]. One source of hydrolyzed collagen is pig skin [10]. However, the methods cited typically use either low (below 5) or high (above 10) pH values [11]. Therefore, in most cases, researchers obtain not native collagen, which is particularly valuable due to its physicochemical properties, but rather its fractions [12]. Isolating collagen from secondary fish raw materials in the form of a molecularly dispersed aqueous solution is a relevant task.

2 Materials and methods

Objective of the study – to develop and optimize a technology for producing soluble collagen from skin waste generated during the production of lightly salted rainbow trout fillet farmed in aquaculture facilities.

To achieve this objective, the following tasks were required to be solved:
- Investigate the chemical composition of the raw material (salted trout skin);
- Conduct preliminary preparation of the raw material (removal of fat and salt);
- Optimize the collagen extraction stage;
- Develop an evaluation scale for the sensory assessment of collagen quality;
- Investigate the quality of the obtained product based on a range of organoleptic, physical, and chemical indicators.

The main experiments were carried out on waste from the processing of lightly salted rainbow trout farmed at mariculture enterprises in the Murmansk region and processed by salting at a number of fish processing plants in the city of Murmansk. The study used fish skin (hide) separated during processing (filleting) and portioning of salted trout, as well as after its fleshing (cleaning from residual muscle tissue and fatty trimmings); samples of the obtained dry soluble collagen were also used.

The mass fraction of water, lipids, protein and mineral substances, sodium chloride, as well as active acidity (pH), were determined by standard methods according to GOST 7636-85 [13]: protein substances – by determining total nitrogen using the Kjeldahl method with Selecta Bloc Digest and Selecta Pro-Nitro A equipment (Spain), using a conversion factor of 5.55 for calculating the protein content [14]; fat content – by the Soxhlet method using a "Det-gras N" extractor (Spain).

The mass fraction of trout skin after fleshing and the total product yield (collagen) were determined by the gravimetric method. The yield of protein substances (protein yield) was calculated based on the protein content in the raw material and the obtained collagen (respectively), taking into account the total product yield.

The organoleptic quality assessment of collagen samples was carried out based on appearance, consistency, taste, odor, and overall acceptability using a specially developed 20-point scale (Table 1), which included significance coefficients, where the highest values were assigned to the most characteristic organoleptic indicators: appearance, taste, and odor (aroma) [15] with the following quality ratings: excellent quality 18.1–20 points, good 16.1–18 points, satisfactory 14.1–16 points, and unsatisfactory quality below 14 points.

Table 1. Organoleptic quality assessment of dry soluble collagen.

Quality indicators	Score	Significance coefficient of indicators	Score taking into account significance
Appearance	1 – 5	1.0	1.0 – 5.0
Consistency	1 – 5	0.7	0.7 – 3.5
Taste – overall impression	1 – 5	1.0	1.0 – 5.0
Odor	1 – 5	0.8	0.8 – 4.0
Overall acceptability	1 – 5	0.5	0.5 – 2.5
Total score			4.0 – 20.0

The point scores were recalculated into the quality level according to the formula:

$$QL = (S / Sm) \cdot 100\ (\%) \tag{1}$$

where QL is the quality level of the sample, S is the score taking into account the significance coefficient, and Sm is the maximum possible score.

Thus, excellent product quality in terms of its level was characterized by a score of more than 90%, good quality from 80 to 90 %, and so on.

Primary processing of experimental data and construction of graphical dependencies were carried out using Microsoft Excel software.

3 Results

It is known that collagen can be isolated from raw materials not only in fibrous form but also in the form of a molecularly dispersed aqueous solution of tropocollagen. The main techniques for isolating collagen from skin are as follows: aqueous extraction of collagen-containing raw materials, filtration, and drying.

At the first stage of the research, the collection of rainbow trout skin after light salting and fillet separation was carried out. Since undetached fragments of muscle tissue and fat remained on the inner side of the skin, they were removed mechanically, i.e., by so-called fleshing.

After fleshing, the yield of cleaned skin was 43.1%. The trimmings separated during cleaning were returned to production as recyclable waste.

The chemical parameters of cleaned trout skin (protein substances, lipids, water, mineral substances, sodium chloride) used as raw material in further studies were investigated (Table 2).

Table 2. Chemical composition of cleaned salted trout skin.

No.	Protein substances, %	Lipids, %	Water, %	Mineral substances, %	Sodium chloride, %
1	59.91	8.09	15.1	14.16	10.24
2	58.95	8.92	16.51	14.71	9.16
3	59.43	8.51	15.98	14.44	9.70

The results presented in Table 2 indicate that protein substances predominate in salted trout skin (about 60%), while the ash content exceeds 14% due to the high content of sodium chloride (about 10%) used for fish salting.

In order to remove sodium chloride, preliminary desalting of the raw material (cleaned skin) was carried out by soaking in water (skin-to-water ratio of 1:10) at a temperature of 4–6 °C. The low-temperature soaking regime was chosen to prevent undesirable enzymatic and microbiological processes leading to collagen loss at this processing stage, on the one hand, and, on the other hand, to prevent its microbiological spoilage. The duration of desalting was determined based on the results of sodium chloride content measurement in the soaked skin. The optimal duration of the soaking stage was experimentally established to be 24 hours (under the selected conditions, the salt content in the fish skin is reduced to trace amounts).

For more complete and efficient collagen extraction, the prepared trout skin was ground (cut) into fragments with approximate dimensions of 3 to 5 mm (the specific surface area – the ratio of the total surface area of the sample to its volume – was 3200–4222 m^2/m^3).

The desalted ground semi-finished product was sent directly for water-soluble collagen extraction by water extraction at a hydromodulus of 4 (raw material-to-water ratio of 1:4).

The efficiency of water-soluble protein extraction is influenced by the process duration, medium acidity, and temperature conditions.

In accordance with the developed experimental plan, at the first stage of research on process optimization, the effect of extraction duration and medium acidity on product yield was clarified. Water extraction of collagen was carried out at a constant temperature (25 °C) with stirring, varying the process duration and the pH value of the extraction mixture.

According to the experimental plan and conditions, in variant 1_1 (control), extraction was carried out for 8 hours at normal pH. In the second variant (2_1), with the same process duration (8 hours), extraction was carried out at a reduced acidity of the mixture (pH 5.5–5.6) (using 0.1 M acetic acid solution for acidification). In the third variant (3_1), the extraction duration was reduced to 4 hours, and extraction was carried out at a reduced pH. The conditions of the first stage of experiments are presented in Table 3.

Table 3. Variants and variable conditions of collagen extraction.

Variant No.	**Extraction duration, h**	**pH of the medi**
1_1	8	6.9–7.0
2_1	8	5.5–5.6
3_1	4	5.5–5.6

Upon completion of the process, the extract was separated by centrifugation using a sedimentation centrifuge at a rotor speed of 8000 rpm for 30 minutes.

The separated centrifugate (water-soluble collagen extract) from each variant was dehydrated by freeze-drying using a laboratory freeze-drying unit at a temperature of –50 °C for 15 hours. After completion of freeze-drying, the total collagen yield, as well as the mass fraction of water and protein substances in the obtained collagen, were determined for each variant, and the protein yield (as an indicator of protein extractability) was calculated.

The results of the studies on the obtained collagen samples are presented in Table 4, 5.

Table 4. Protein substance content and product yield (total and protein-based) depending on extraction conditions, %.

Product name	**Variant No.**	**Mass fraction of protein substances, %**	**Product mass, g**	**Protein mass, g**	**Product yield, %**	
					total	protein-based
Salted trout skin	1_1	59.43	7.34	4.36	-	-
	2_1	58.95	7.01	4.13	-	-
	3_1	59.91	4.85	2.91	-	-
Collagen	1_1	81.86	0.050	0.041	0.69	0.94
	2_1	79.37	0.152	0.121	2.17	2.93
	3_1	80.99	0.026	0.021	0.54	0.72

It was found that increasing the temperature increases the extractability of protein substances and the total collagen yield by 5–7 times.

It was found that carrying out extraction in a slightly acidic zone at the same process duration and pH (variants 1_1 and 2_1) leads to a threefold increase in product yield; reducing the extraction duration from 8 to 4 hours during collagen extraction in a slightly acidic medium (comparison variants 2_1 and 3_1) reduces the collagen yield by 4 times. Assessing the dynamics of protein substance extraction, it was established that the protein yield is also more greatly influenced by the extraction duration (the yield of protein substances increased by 4 times), whereas acidification of the extraction mass increases protein extractability by 3 times.

The most acceptable technological regime for obtaining dry collagen was determined at a process temperature of (21±1) °C (variant 2_1: collagen extraction duration of 8 hours, pH of the medium 5.5–5.6). Under these conditions, both the total product yield and the protein yield were maximal (2.17% and 2.93%, respectively).

Therefore, at the next stage, in the second series of experiments to identify the effect of temperature conditions on collagen yield, it was decided to take the technological parameters of variant 2_1 as a basis, and in each subsequent experiment to increase the temperature of the extraction mixture by 10 °C, i.e., maintain it at 31 °C (variant 2_2), 41 °C (variant 2_3), and 51 °C (variant 2_4).

Experiments on collagen production using the same trout skin and the same technological operations, instruments, and equipment were carried out in duplicate at each temperature. The (averaged) experimental results are presented in Table 5, 6.

Table 5. Protein substance content in raw material and product (collagen), %.

Variant No.	Mixture temperature, °C	Mass fraction of protein substances, %	
		in skin	in collagen
2_1	21±1	58.95	79.37
2_2	31±1	58.95	81.14
2_3	41±1	58.95	90.66
2_4	51±1	58.95	89.51

Table 6. Product yield depending on extraction temperature (total and protein-based), %.

Variant No.	Material balance data for collagen production					
	mass of raw material (skin), g	mass of protein in raw material, g	mass of product (collagen), g	mass of protein in product (collagen), g	Product yield, %	
					total (*Y*)	protein-based (*P*)
2_1	7.010	4.130	0.152	0.121	2.17	2.93
2_2	6.470	3.814	0.653	0.530	10.09	13.90
2_3	6.540	3.860	1.107	1.004	16.93	26.01
2_4	6.210	3.660	1.050	0.940	16.91	25.68

The experimental results presented in Table 5 for the total yield of dry soluble collagen (Y) and protein extractability (P – protein-based yield) as a function of extraction temperature (X) allow for mathematical processing of the data to find an interpolation polynomial. As a result of processing the obtained data using the Lagrange interpolation polynomial, equations (2, 3) were found for the influence of factor X (extraction process temperature) on the optimization parameter Y (yield of dry soluble collagen) and P (protein-based yield – protein extractability):

$$Y=0.0001758666\,X^4-0.0268846\,X^3+1.4580533\,X^2-32.156133\,X+249.77 \quad (2)$$
$$P=-0.00096333\,X^3+0.0813\,X^2-1.44267\,X+6.21 \quad (3)$$

Graphical interpretations of the obtained dependencies are presented in Figures 1 and 2.

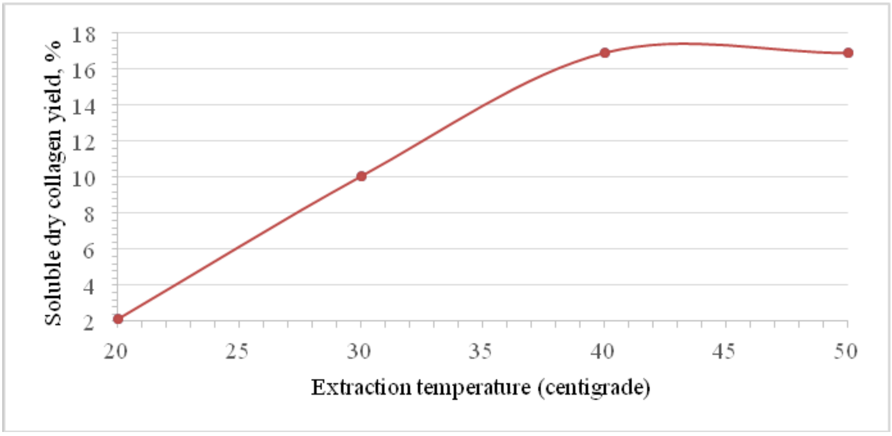

Fig. 1. Graphical interpretation of the interpolation equation for establishing the optimal extraction temperature (X) influencing the product yield (Y) in the production of dry soluble collagen.

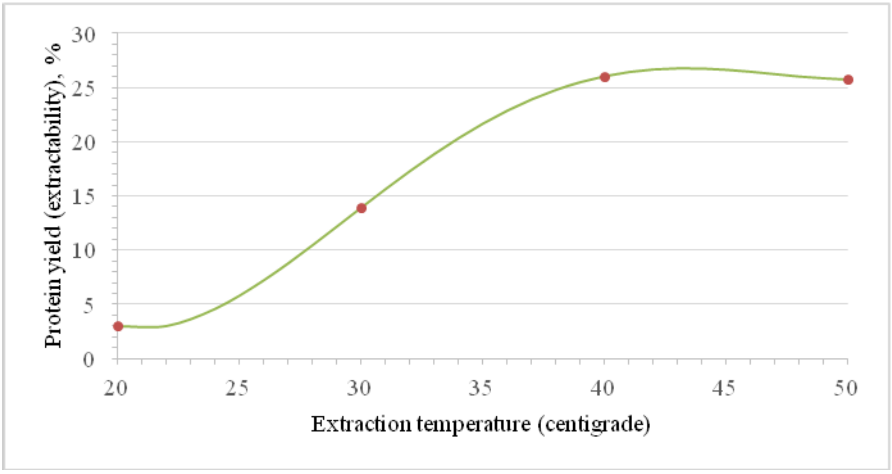

Fig. 2. Graphical interpretation of the interpolation equation for establishing the optimal extraction temperature (X) influencing the yield (extractability) of protein substances (P) in dry soluble collagen.

To find the optimal factor X (extraction temperature), mathematical processing methods (differentiation) were applied. The optimal factor value according to equation (2) was 45.2 °C with a maximum total product yield of 18.16 %; according to equation (3), the optimal factor value was 43.3 °C with the highest yield of protein substances of 26.70 %. Taking into account the obtained optimization results (differentiation of equations 2 and 3), a temperature for the collagen extraction stage close to optimal was found to be (44.3±1.0) °C, at which the total product yield is 18.10% and protein extractability is 26.69 %.

Using the values obtained from the optimization results, a dry soluble collagen was produced. The chemical quality indicators of the obtained product are presented in Table 7.

Table 7. Chemical quality indicators of dry soluble collagen.

Chemical indicators, %			
Proteins	**Water**	**Lipids**	**Mineral substances**
90.66 ± 0.21	6.24 ± 0.11	1.920 ± 0.086	1.2 ± 0.16

As a result of the organoleptic evaluation of the produced sample in accordance with the developed scoring scale, the quality level of the obtained collagen was calculated to be 94%, which corresponds to excellent product quality. In terms of the set of chemical indicators, the water-soluble collagen from salted trout skin also meets the generally accepted requirements for this type of product. The research is planned to be continued.

4 Discussion

The results of the conducted research on the development of a technology for producing soluble dry collagen from waste (skin) generated during the filleting of salted rainbow trout are presented. It was found that the protein substance content in rainbow trout skin is at the level of 60%. This unequivocally indicates the possibility of using this secondary raw material for the extraction of soluble collagen.

Based on the conducted research, a refined technology for producing soluble dry collagen is proposed. The introduction of additional stages (fleshing and desalting) made it possible to remove undesirable components (lipids and sodium chloride) before the extraction stage. The use of water as an extractant (in contrast to the use of enzymatic or alkaline hydrolysis) allowed for the isolation of collagen in the form of a molecularly dispersed aqueous solution (soluble collagen), while the use of freeze-drying guaranteed the preservation of the collagen structure and, consequently, all of its valuable physicochemical properties. Carrying out extraction in a slightly acidic zone at pH 5.5±0.1 at a moderate temperature (44.3±1) °C for 8 hours made it possible to significantly increase the extractability of protein substances and the total product yield, as well as to prevent its microbiological spoilage during the technological process. The quality indicators of the dry collagen sample obtained according to the refined technological scheme were studied: the mass fractions of protein, water, fat, and mineral substances were 90.7%, 6.2%, 1.9%, and 1.2%, respectively. The product quality level was 94%.

The results of the conducted research established that, along with animal hide raw materials, which are currently widely used for collagen production, salted trout skin can be used as a secondary raw material for producing soluble collagen.

5 Conclusion

Based on the results obtained during the research, a technology for producing collagen from processing waste of mariculture products farmed in the Arctic region of Russia has been developed. The chemical composition of lightly salted rainbow trout skin, which is a waste product generated during the production of lightly salted fillets of this fish, has been determined. As a result of the conducted research, the technology for producing water-soluble collagen from trout skin has been refined through the introduction of additional stages (fleshing and desalting). Its chemical and technological regimes and parameters close to optimal have been established. The yield of dry soluble collagen from the studied raw material, as well as the yield of protein substances, has been experimentally determined. The quality of dry soluble collagen obtained from salted trout skins has been investigated. Thus, the possibility of using waste from the production of lightly salted trout fillets as a raw material for the production of water-soluble collagen has been established, which will allow solving the problem of involving secondary raw material resources in the production of highly profitable products. The research is planned to be continued in the direction of studying the structural-mechanical and biochemical properties of collagen from trout skin, developing products based on it, as well as creating a comprehensive technology for producing soluble collagen using recyclable waste and by-products generated during its manufacture.

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