 doi:10.20944/preprints202107.0412.v1

Article

Application of the *Gadidae* Fish Processing Waste for Food Grade Gelatin Production

Nikita Yu. Zarubin [1], Elena N. Kharenko [1], Olga V. Bredikhina [1] , Leonid O. Arkhipov [1], Konstantin V. Zolotarev [2,*], Anton N. Mikhailov [2] ,Valeriya I. Nakhod [2] and Marina V. Mikhailova [2]

[1] Russian Federal Research Institute of Fisheries and Oceanography, 17 Verkhnyaya Krasnoselskaya str., Moscow, 107140 Russia; vniro@vniro.ru
[2] Institute of Biomedical Chemistry, 10 Pogodiskaya str., Moscow, 119121 Russia; inst@ibmc.msk.ru
* Correspondence: fireaxe@mail.ru; Phone: +7 916 433 17 90

Abstract: Waste from fish cutting (heads, swim bladders, fins, skin, bones) is a high-value technological raw material for obtaining substances and products based on them with a wide range of properties. The possibility of using waste from cutting fish of the *Gadidae* family: the Alaska pollock (*Gadus chalcogrammus*) and the Pacific cod (*Gadus macrocephalus*), processed in the coastal zone, is scientifically substantiated. In this work, a technology has been developed for processing accumulated waste from fish cutting in order to obtain fish gelatin, which is characterized by high protein content (more than 80.0%) and a full set of essential and nonessential amino acids. We studied the quality of fish gelatin obtained from wastes from cutting the fish of the *Gadidae* family. The possibility of using fish gelatin as a component of fish products is shown; the dose of its introduction into the fish products is substantiated. The data obtained made it possible to recommend the use of fish processing waste products as a gelling component and a source of amino acids in multicomponent food systems.

Keywords: fish processing waste; Alaska pollock; Pacific cod; fish gelatin; gelling component.

1. Introduction

Fish processing industry produces large amounts of by-products (heads, bones, fins, skin, etc.) worldwide. Some of those products are nutritionally valuable, so it is reasonable to evaluate the applicability of various fish by-products for different purposes including human nutrition [1]. Fish collagen and fish gelatin (partially hydrolyzed collagen) can be derived from heads (initial collagen content 9.2–33.0%), fins (0.8–8.0%), skin (2.0–12.6%), scales (0.8–6.0%), bones (9.0–19.0%) and dead fish bodies [2]. Fish collagen can be used for various purposes including wound healing, tissue engineering, drug delivery, cell differentiation in cell culture, skin care, or as a source of amino acids [3].

Gelatin is used as a gelling component for cooking. Mammalian gelatin is derived from porcine and bovine by-products, so there are some religious restrictions in its application [4]. Fish gelatin is a potential alternative to the mammalian gelatin for application in Kosher and Halal foods. Besides that, fish gelatin can be produced for domestic use in local coastal communities earning their lives with fishing, so fish gelatin may help relieve local malnutrition there [5].

Some of the *Gadidae* fish species are among the most captured ones. Alaska pollock was the second most captured in 2018 (3.4 million tonnes) [6]. Pacific cod has been one of the most captured fish species in Russia for the last several years [7,8]. Generally, only 30–50% of the fish wet weight is used in the production of fish fillets [1]. Thus, it is reasonable to evaluate the applicability of Alaska pollock and Pacific cod by-products for food grade gelatin production. Besides that, if the mixture of the by-products is applicable as well as separated components, it will increase the economic valuability of the whole process because a tedious stage of sorting would not be necessary. Unfortunately, fish gelatin is applicable only for fish meals cooking due to fish odor [4]. Fish gelatin may

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be used for fish galantine or fish mince production, so it is necessary to study the chemical composition of the gelatin samples and some physicochemical properties of a gel prepared using the gelatin (in comparison with a mammalian gelatin). In addition, it is necessary to study the amino acid content of gelatin samples for their nutritional value estimation.

2. Results

2.1. Chemical analysis of by-products

All the types of by-products of both fish species were analyzed for total protein, total fat, collagen, ash and water content (Table 1).

Table 1. Chemical parameters of by-products of the Alaska pollock and the Pacific cod. Content values are expressed as % by wet weight (mean ± SD).

By-product type	Water content		Total protein content		Collagen content		Total fat content		Ash content	
	pollock	cod	pollock	cod	pollock	cod	pollock	cod	pollock	cod
Heads	78.55 ± 1.44	78.21 ± 1.43	16.41 ± 0.40	15.71 ± 0.39	11.37 ± 0.28	10.02 ± 0.24	1.34 ± 0.03	0.77 ± 0.01	3.70 ± 0.09	5.37 ± 0.14
Skin + scales	76.32 ± 1.41	75.79 ± 1.39	19.49 ± 0.48	18.61 ± 0.46	15.23 ± 0.38	14.38 ± 0.34	1.23 ± 0.03	1.03 ± 0.02	2.97 ± 0.06	3.56 ± 0.08
Tails and fins	77.67 ± 1.42	79.55 ± 1.45	16.71 ± 0.41	14.38 ± 0.36	13.56 ± 0.33	11.52 ± 0.27	1.28 ± 0.03	0.53 ± 0.01	4.37 ± 0.12	5.54 ± 0.14
Spinal bones	79.34 ± 1.46	74.32 ± 1.36	15.68 ± 0.38	18.25 ± 0.45	11.21 ± 0.28	13.51 ± 0.32	0.88 ± 0.01	0.75 ± 0.01	4.10 ± 0.12	6.68 ± 0.15
Viscera (without liver)	75.32 ± 1.38	74.47 ± 1.36	19.10 ± 0.47	19.96 ± 1.36	15.31 ± 0.38	14.98 ± 0.35	2.35 ± 0.06	2.58 ± 0.06	3.23 ± 0.08	2.99 ± 0.06
Mixture sample	74.41 ± 1.36		21.23 ± 0.52		17.07 ± 0.42		1.31 ± 0.03		3.05 ± 0.10	

According to the data in Table 1, it can be concluded that the chemical parameters do not have strong differences. All types of waste are high in total protein content protein (over 15%) and collagen content (over 10%) in contrast to total fat and ash content. In this regard, the mixing of waste will not lead to a deterioration in the chemical composition, namely the content of total protein and collagen, which is confirmed by mixture sample parameters. Collagen content is about 80% of the total protein in the mixture sample, which is positive for obtaining fish gelatin.

Thus, in further work, we used a mixture of by-products from pollock and cod in equal proportions, which is reasonable, since these fish species belong to the same family and have a similar chemical composition. In addition, pollock and cod are most widely used at fish processing plants in Russia for the production of fish products of various types, which implies their complete cutting to fillets, with subsequent waste collection [9].

2.2. Fish gelatin quality parameters assessment

Fish gelatin samples were prepared from the mixtures of by-products; their quality parameters were compared with the parameters of the K-10 standardized mammalin gelatin derived from porcine and bovine by-products according to [10] (Table 2).

Table 2. Quality parameters of fish gelatin derived from a mixture of fish by-products (Alaska pollock + Pacific cod) in comparison with a standardized mammalin gelatin.

Parameter	Fish gelatin	K-10 mammalin gelatin standardized by [10]

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Appearance	Powder	Granules, grains, plates, powder
Color	Smoky white	From light yellow to yellow
Odor	Fishy (weak)	None
Flavor	Insipid	Insipid
Max. particle size, mm	3	5
Max. small particle fraction, %	None found	30
Max. dissolution time, min	18	25
pH of 1% solution	5.4	5–7
Min. viscosity of 10% solution, mPa·s	38.5	18.5
Min. melting point of galantine with 10% of gelatin, °C	26	30
Min. transparency of 5% solution, %	73–76	50
Min. gel strength of galantine with 10% of gelatin, g	1800	1000
Max. water content, %	5.2–6.3	16
Max. ash content, %	1.4–1.8	2
Total protein content	90.8–92.6	Not standardized
Collagen fraction of total protein, %	72.3–74.1	Not standardized
Total fat content, %	0.8–1.1	Not standardized
Impurities content, %	None	None allowed

According to the data in Table 2, it can be concluded that fish gelatin meets the standardized requirements. There were observed differences in color and odor of the product due to the organoleptic properties of the fish by-products. Fish gelatin solution is more transparent in comparison with the K-10 gelatin solution. The melting point of the galantine was lower than the standardized value, which is explained by the fact that fish collagen is sensitive to heating due to unstable cross-links, compared to mammalian collagen. Collagen and gelatin derived from cold water fish by-products has a lower content of hydroxyproline and has lower thermal stability than collagen and gelatin from warm water fish by-products. This phenomenon is caused by the invovlvemen of hydroxyproline in the formation of a chain of hydrogen bonds that stabilizes the triple helical structure of collagen [11–16]. At the same time, the technological features of obtaining fish gelatin also affect the melting point. Due to the lower content of proline and hydroxyproline, which are involved in the stabilization of the triple superstructure of collagen, the gelation process is slower, and the fish gelatin-based gel melts at a lower temperature, in contrast to gel with gelatin derived from another type of raw material. It should be noted that fish gelatin dissolves faster than gelatin derived from porcine and bovine by-products because fish collagen has lower molecular fractions [11,17].

Thus, dry fish gelatin contains a significant amount of protein (up to 92.6%), including collagen accounting for 74.1% of the total protein, and therefore it can be recommended for use as an independent food supplement (a source of collagen proteins) and also as a structurant.

2.3. Fish gelatin amino acid analysis

Amino acid analysis of the fish gelatin was performed (Table 3).

Table 3. Amino acid content of fish gelatin derived from a mixture of fish by-products (Alaska pollock + Pacific cod) (mean ± SD).

Amino acid	Content, % of total protein

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Lys	5.66 ± 0.12
His	1.61 ± 0.07
Arg	6.08 ± 0.14
Asp	7.29 ± 0.11
Thr	2.53 ± 0.09
Ser	4.23 ± 0.21
Glu	12.22 ± 3.23
Pro	9.79 ± 0.19
Cys	0.10 ± 0.02
Gly	33.20 ± 0.44
Ala	6.59 ± 0.61
Val	2.94 ± 0.25
Met	0.03 ± 0.007
Ile	2.57 ± 0.11
Leu	4.64 ± 0.41
Tyr	0.03 ± 0.004
Phe	2.51 ± 0.13

The amino acid composition of fish gelatin (Table 3) is characterized by the presence of proline and hydroxyproline (9.76 and 8.51%), which is a feature of the connective tissue of fish. At the same time, the content of glycine in is high (33.2%). Glycine, proline and hydroxyproline are the most important amino acids in collagen, accounting for 50% of the total amino acids in the protein. The content of proline and hydroxyproline is especially important for the gelling effect and stabilization of the collagen triple helix due to its ability to bind hydrogen through the OH group, which also affects the technological properties of fish gelatin.

2.4. Fish gelatin molecular weight analysis

The result of the analysis indicates that the fish gelatin consists of fragments of collagen fibers (102.2 kDa on average). This results also shows structural changes that have occurred in the polypeptide chains of collagen during enzymatic hydrolysis, and indicates the presence of polypeptide chains of protein, which contribute to the formation of dense galantine gels.

2.5. Assessment of quality parameters of fish mince prepared using fish gelatin

Fish mince samples without gelatin (control) and with added 2%, 4%, 6%, 8% and 10% gelatin were prepared. Some quality parameters of the samples were estimated (Table 4).

Table 4. Quality parameters of fish mince samples with or without fish gelatin (mean ± SD).

Parameter		**Fish gelatin content, %**					
		0	2	4	6	8	10
Before heat treatment	MAC [1], %	64.46 ± 1.05	68.34 ± 1.22	73.53 ± 1.44	79.48 ± 1.67	83.72 ± 1.78	84.23 ± 1.89
	USS [2], MPa	0.66 ± 0.02	0.84 ± 0.02	0.96 ± 0.02	1.34 ± 0.02	1.58 ± 0.02	1.67 ± 0.02
After heat	WHC [3], %	71.34 ± 1.34	74.35 ± 1.51	76.81 ± 1.62	78.32 ± 1.66	79.65 ± 1.73	79.44 ± 1.67

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treat-ment	FAC [4], %	55.23 ± 0.71	57.83 ± 0.80	59.55 ± 0.88	61.23 ± 0.95	63.25 ± 0.99	62.97 ± 0.97

[1] Moisture absorption capacity

[2] Ultimate shear stress

[3] Water-holding capacity

[4] Fat-absorption capacity

Alaska pollock fillets were used as a core component for the preparation of fish mince samples because they have unsufficient functional, technological and structural quality values. The introduction of fish gelatin could potentially improve these properties and structure of fish mince.

One of the most important indicators of mince is the moisture absorption capacity (MAC). During heat treatment, physicochemical and colloidal changes occur; some water and fat from raw fish mince is lost. But some water is held in the mince, the amount of which is characterized by water-holding capacity (WHC). At the same time, WHC characterizes the water content in the mince and the amount of water separated during the heat treatment. This parameter is associated with the output of finished products [12].

Analysis of the MAC dynamics showed that it grows with gelatin content increase. The highest MAC value was reached at a 10% dosage of fish gelatin. A similar dynamics was observed, the sample with 8% gelatin had a maximum WHC value. It is explained by clasterization and gelatinization of the gelatin components during heat treatment.

The ultimate shear stress (USS) value gradually increased with an increase in the dose of fish gelatin and reached a minimum at a 10% dosage, where fish mince had the densest structure. It is explained by increase in collagen content in the fish mince, which led to improvement in its hydrophilic properties. As a result of the presence of dispersed collagen fibers, water absorption enhanced and USS value increased. Thus, the presence of fish gelatin promotes structure formation and an increase in the density of fish mince.

Fat-absorption capacity (FAC) dynamics with an increase in the content of fish gelatin was similar to the dynamics of WHC.

2.6. Sensory evaluation of fish mince prepared using fish gelatin

Fish mince samples without gelatin (control) and with added 2%, 4%, 6%, 8% and 10% gelatin were evaluated (Figure 1).

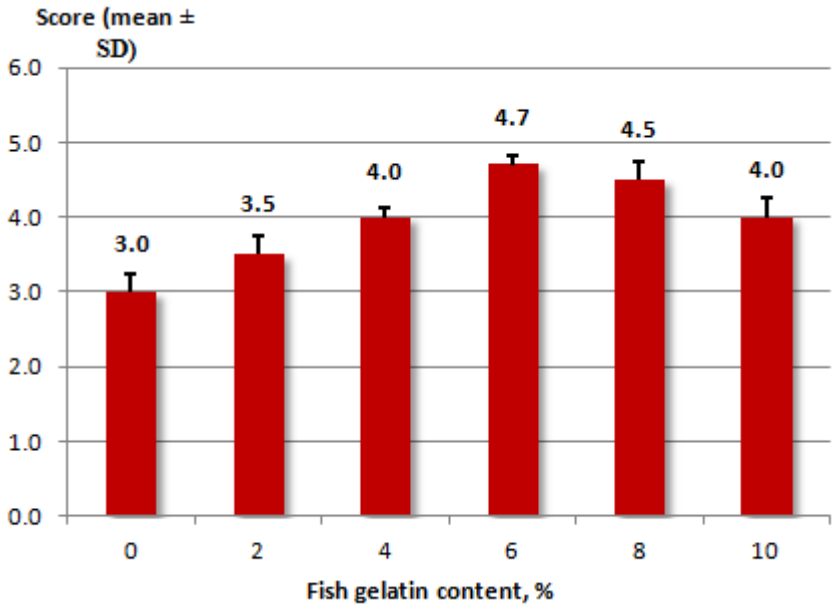

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Figure 1. Results of sensory evaluation of fish mince with or without fish gelatin.

The samples with added 6% and 8% gelatin had the highest score on a 5-point scale (Figure 1). All the samples had a gray color, moderately salty and pleasant aroma, the taste of finished product, typical of the fish used. The control sample (0% gelatin) had a looser consistency. Samples with added 6 and 8% fish gelatin had soft and juicy consistency. The sample with added 10% fish gelatin was rated 4 points, had a lighter color and firm consistency, which increased rigidity compared to other samples.

3. Discussion

As a result of this study, the possibility of solving the problems of processing waste from cutting fish of the *Gadidae* family was substantiated. The approach makes it possible to produce fish gelatin with high protein content (> 90%) and quality parameters not inferior to those of food grade gelatin derived from porcine and bovine by-products. Differences with mammalian gelatin in smell, melting point and solubility were noted; they are associated with the organoleptic characteristics of fish collagen-containing raw materials and the structure of collagen.

The fish gelatin derived from *Gadidae* fish processing by-products contains a full set of essential and nonessential amino acids. The amino acid composition of the gelatin derived from a mixture of Alaska pollock and Pacific cod was close to the previously published composition of Alaska pollock skin [18] and Atlantic cod skin [19]. Thus, our fish gelatin can be recommended for use as an independent food supplement (a source of amino acids).

The cost of producing gelatin from porcine and bovine bone raw materials in Russia is about 2350–2650 US$ per 1 ton of product, including the cost of raw materials, equipment and labor [20]. The cost of fish gelatin is lower and ranges from 1200 to 1300 US$ per 1 ton of product, which is achieved due to the lower cost of raw materials and a more simplified production line.

The possibility of using fish gelatin in the production of fish culinary products based on fish mince has been studied. According to the results of this study, the recommended content of fish gelatin in the fish mince is 6–8%. The appropriate samples showed high values of MAC, WHC, FAC and USS, which is a positive factor in the formation of the consistency of fish products. A higher dosage of fish gelatin is not recommended, since in this case, despite the high functional and technological properties, there is deterioration in the organoleptic characteristics of the finished product.

Thus, fish gelatin derived from the *Gadidae* fish processing waste can be rationally used as natural gelling component and an amino acid source.

4. Materials and Methods

4.1. Samples of by-products

The frozen fish samples of the Gadidae family (the Alaska pollock (*Gadus chalcogrammus*) and the Pacific cod (*Gadus macrocephalus*)) were obtained from local fish dealers in Russia. The mixture for gelatin production was prepared from fish by-products in equal proportions by weight: skin with scales + tails and fins + spinal bones + viscera (without liver), each by-product from both species in equal proportion. 5 samples of the mixture were prepared for comparison.

4.2. Total protein content determination

Total protein content was determined using the Kjeldahl method with a Kjeltec 1002 System Distilling Unit (Tecator AB, Höganäs, Sweden); total protein content was calculated as 6.25 × total N content.

4.3. Total fat content determination

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A sample (5–10 g) was grinded in a mortar with Na_2SO_4 (1:3 sample to salt ratio) for dehydration. Next, the dehydrated sample was placed into boiling flask of a Soxhlet extractor and diethyl ether was added; the ether volume was about 1.5 × of the flask volume. Next, the condenser was adjusted, cooling water was let through and the flask was gently heated in a water bath for 12 hours.

After extraction, the ether was distilled off and the fat collected in a small preliminarily weighed flask was dried at 105 °C to constant weight and weighed.

4.4. Ash content determination

A sample (about 4 g) was digested in a muffle furnace at 500–700 °C to constant weight; the ash was collected and weighed.

4.5. Water content determination

The water content (moisture) of a sample was approximately estimated as 100% - (total protein content, %) – (total fat content, %) – (ash content, %).

4.6. Collagen content determination

A homogenized sample (about 50 mg) was hydrolyzed by autoclaving with 1.0 mL of 6 M HCl in sealed tubes for 3 hours at 3.5 bar pressure. One milliliter of 0.01 M $CuSO_4$, 1 mL of 2.5 M NaOH and 1 mL of 6% H_2O_2 were added into the sample tube and a blank tube with 1 mL of distilled water. The solutions were mixed and shaken occasionally during a period of 5 minutes and placed in a water bath at 80°C for 5 minutes with frequent intensive shaking. Next, the tubes were cooled in ice water bath and 4 mL of of 3.0 N (1.5 M) H_2SO_4 was added with shaking, then 2 mL of p-dimethylaminobenzaldehyde solution in n-propanol was added with thorough shaking. The tubes were heated at 70°C for 16 minutes and cooled in tap water. Absorption at 540 nm of the prepared solutions was measured with a spectrophotometer. Hydroxyproline content was calculated using a previously made calibration with hydroxyproline standard (Sigma-Aldrich, St. Louis, MO). Collagen content was calculated as 7.4 × hydroxyproline content [21].

4.7. Fish gelatin preparation

The samples of by-products were preliminarily three times treated with a 10% aqueous solution of citric acid for 60 min with constant stirring. After washing, the samples were frozen at -25 °C for 2 hours and later crushed. After crushing, the samples were preliminarily heated at 80–90 °C for 20–25 minutes in water (1:2 sample to water ratio) and cooled to 35–40 °C. Next, the resulting mass was hydrolyzed with enzyme preparations “Food collagenase” (Endocrine Enzymes Plant, Moscow, Russia) and “Protepsin” (Bioprogress, Shchyolkovo, Russia) at 40 °C for 4 hours; enzyme to sample ratio was 1:1000 by weight. Next, the enzymes were inactivated by heating at 70 °C for 15 min, and the broth was decanted.

To reduce energy consumption for drying, the obtained gelatin broth was preliminarily thickened by ultrafiltration using UF-401/402 pilot filtration system (BioTechno Group, Moscow, Russia) at 48–52 °C to a dry matter content of 25–30%. Membranes of the UPM type made of aromatic polysulfonamide were used for ultrafiltration; the retention capacity of the membrane is 20–100 kDa.

The gelatin concentrate was dried in a SD-1000 spray dryer (EYELA, Japan) at 50–60 °C by spraying through 0.7 mm nozzles. The yield of dry fish gelatin powder was 18%.

4.8. Odor and flavor estimation

Ten grams of gelatin was dissolved in 90 ml of distilled water in a plugged flask and heated at 40–50 °C for 1 hour. Odor was estimated organoleptically after unplugging the flask. Next, the solution was cooled to 17–19 °C in an open glass. Flavor of the cooled solution was estimated organoleptically.

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4.9. Particle size and small particle fraction determination

Determination of these parameters was based on sieve analysis with two parallel sieves (10 and 0.5 mm mesh size) with a receiver tray beneath. One hundred grams of gelatin was sifted through a sieve system with 1 shake per second shaking rate. The biggest gelatin particles remaining on the upper sieve were selected and measured manually with a caliper. The gelatin collected in a tray was weighed for the small particle fraction calculation.

4.10. Dissolution time determination

Ten grams of gelatin was mixed with 100 ml of 15–18 °C distilled water and left for swelling at this temperature for 30 min. Then the mixture was thermostated at 40 °C until the gelatin was fully dissolved. Dissolution time was measured as time from actual reaching 40 °C by the mixture until its full dissolution.

4.11. Preparation of gelatin solution and galantine

A portion of gelatin (1.03 × calculated mass) was placed in a flask; the calculated amount of water was added; the mixture was gently stirred to homogeneity, covered and kept at room temperature with periodical stirring for 1.5 h for gelatin swelling. The flask with the swollen gelatin was thermostated at 55 °C for 30–40 minutes with careful stirring for gelatin dissolution. Then the solution was filtered through 4 layers of gauze and cooled to 41–43 °C. When 10% gelatin solution is cooled to room temperature it turns into galantine.

4.12. Determination of pH

A SevenExcellence pH meter with InLab Expert Go-5m-ISM electrode (Mettler Toledo, Greifensee, Switzerland) was used to determine pH of the gelatin solution.

4.13. Viscosity determination

Viscosity of the gelatin solution was determined using a U-tube viscometer. A portion of gelatin (20 g dry weight, the actual weight was calculated using the results of water content determination) was placed in a flask, the calculated amount of water was added, the flask was closed with a rubber plug with a narrow hole (for air outlet) and the solution was kept at room temperature with periodical stirring for 1 hour for gelatin swelling. The flask with the swollen gelatin was thermostated at 65 °C for 30 minutes with careful stirring for gelatin dissolution. Then the solution was cooled to 41–43 °C. Determination of viscosity was carried out no later than 30 minutes after cooling the solution.

The solution was filtered through a glass filter, poured into a viscometer and thermostated at 4.0 °C for 10–15 minutes and the outflow time was measured. Viscosity was calculated using the values of the outflow time and the viscometer constant.

4.14. Melting point determination

Melting point of the galantine was determined using the Cambon's fusiometer consisting of a brass crucible and a brass rod with a hole for hanging. The rod was placed onto the center of the crucible bottom. Next, the crucible was filled to the top with a 10% gelatin solution.

The filled crucible was first kept for 30 min at room temperature, then for 1 h at 11 °C for gelling. Next, the crucible was placed in a glass of 20 °C water and the rod was hung up so that the crucible edge was at the water surface level in the glass.

At the same time, a thermometer was attached at 0.5 cm from the crucible, deepening its ball to the level of the crucible bottom. The glass with the installed system was placed in a water bath and was uniformly heated, increasing the bath temperature by 1 °C for 3 minutes until the crucible was separated from the rod. The melting point of gel-

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atin-based galantine was the temperature of the water at which the crucible was separated from the rod and fell down to the glass bottom.

4.15. Transparency determination

The 5% solution was prepared and cooled to 40 °C. Its transparency was measured as optical transmission using a colorimeter with a blue filter and distilled water as a blank sample.

4.16. Gel strength determination

Gel strength was determined with a Valent's device. Fifty milliliters of the gelatin solution was poured into the device vessel, and then the vessel was cooled at room temperature for gelling and later cooled to 8 °C for 18 hours. Next, the vessel was warmed to 15 °C in a cool water bath for 2 hours and gel strength was immediately measured. The load mass was increased with 10–12 g per second rate until the galantine crushed. The load mass at the crushing time was considered as gel strength.

4.17. Impurities content determination

One liter of 10% gelatin solution was prepared and filtered through a sieve. Next, the sieve was washed by hot water (65 °C) for 5 min. The sediment was collected, dried to constant weight and weighed.

4.18. Amino acid analysis

The amino acid analysis was performed using Agilent 1200 series chromatographic system equipped with fluorescent detector and ZORBAX Eclipse AAA (5μm; 4.6 x 150 mm) column (Agilent Technologies, Santa Clara, CA). The mobile phases were 40 mM pH 7.8 phosphate buffer solution (Solution A) and 80% water solution of acetonitrile (Solution B). Borate buffer with pH 10.2 and o-phtalaldehyde were used for amino acid derivatization. See [22] for details.

4.19. Molecular weight analysis

Average molecular weight of gelatin components was estimated using viscometric method with a U-tube viscometer. The solution from the analysis by Kjeldahl method (see subsection 4.2) was used for this procedure. A set of four serially diluted solutions was made from that one. Viscosity of all those solutions and 1.0 M KCl as a standard was determined as described above (subsection 4.13). The value of intrinsic viscosity was determined graphically as

$$[\eta] = \lim_{c \to 0} \frac{1}{c} \ln \frac{\eta}{\eta_0}, \quad (1)$$

where c is calculated as 100/(total N content in a solution) in g/mL;
η and η_0 is the viscosity of a solution and 1.0 M KCl respectively.
The average molecular weight (in Da) was calculated using the Mark-Houwink equation:

$$M = \left(\frac{[\eta]}{k}\right)^{1/a}, \quad (2)$$

where k and a are constants for each type of biopolymer. For gelatin at room temperature, k = 0.1681 mL/g and a = 0.9211 [23].

4.20. Preparation of fish mince samples

Alaska pollock fillets were minced with a meat grinder; NaCl was added to the mince (1% of mince weight) and it was mixed. Fish gelatin was added in an appropriate amount (0%, 2%, 4%, 6%, 8% or 10% of mince weight): it was diluted in water (1:3 ratio) and heated to 67 °C in a water bath, and then gelatin solution was incorporated into the mince. The mince was mixed and kept for 30 min for stabilization. Some quality parameters were determined before heat treatment.

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For heat treatment, the samples were placed into 250 ml glass jars with hermetically sealed lids, the lids were sealed and the samples were heated at 80–85 °C for 85 min. Some quality parameters were determined after heat treatment.

4.21. Moisture absorption capacity (MAC) determination

MAC was determined before heat treatment. A preliminarily weighed portion of a mince sample was placed onto a piece of filter paper and gently pressed with a glass stick until water release ended. The area of the water stain was measured and the volume of water was calculated using the preliminarily made calibration. MAC was calculated as a ratio of water volume to mince portion.

4.22. Ultimate shear stress (USS) determination

USS was determined before heat treatment. USS was measured using APN-360MG4 automatic penetrometer (SKB Stroypribor, Chelyabinsk, Russia). A portion of a mince sample was put into a glass. The time of cone hold after crush was set (5 seconds), the sample was lifted until it touched the cone and the measurement procedure was initiated. The USS value was calculated (in Pa) using Rehbinder equation:

$$\Theta_0 = \frac{km}{h^2}, \quad (3)$$

where k = 2.1 N/kg is a Rehbinder's constant for the cone used with 60° apex angle;
m = 0.05069 kg is the cone + holding bar weight;
h is the cone immersion depth being measured (in meters).

4.23. Water-holding capacity (WHC) determination

WHC was determined after heat treatment. A preliminarily weighed portion of a mince sample (30–250 g) was placed into a preliminarily weighed glass jar with hermetically sealed lid, the lid was sealed and the sample was heated until the temperature in the core of the portion reached 70 °C. Next, the sample was cooled, the broth was decanted and the glass with the sediment was weighed. WHC was calculated as relative loss of the sample weight.

4.24. Fat-absorption capacity (FAC) determination

FAC was determined after heat treatment. A preliminarily weighed portion of a mince sample (30–250 g) was placed into a glass jar with hermetically sealed lid. Vegetable oil (30% of sample weight) was added to the jar, the lid was sealed and the mixture was heated until the temperature in the core of the portion reached 70 °C. Next, the mixture was cooled, and the broth was decanted. The broth was heated at 60 °C for 20 min in a water bath. Fat fraction of the broth was collected and weighed. FAC was calculated as (fat fraction weight - initial oil weight) / mince sample portion weight.

4.25. Sensory evaluation

The fish mince samples were evaluated organoleptically by overall appearance, color, odor, flavor and consistency by 10 adult people. Each person gave an integer score of 5-point scale (from 1 to 5) to each mince sample and described his/her impressions verbally.

Author Contributions: Conceptualization, E.K. and M.M.; methodology, N.Z. and L.A.; software, K.Z.; validation, N.Z., L.A. and A.M.; formal analysis, O.B.; resources, O.B. and V.N.; data curation, A.M. and M.M.; writing—original draft preparation, K.Z. and V.N.; writing—review and editing, N.Z., E.K., O.B., L.A., A.M. and M.M.; supervision, M.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Acknowledgments: The work was done in the Framework of the Russian Federation Fundamental Research Program for the long-term period for 2021–2030.

Conflicts of Interest: The authors declare no conflict of interest.

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