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Technology and Physico-chemical Properties of Gelatin from Atlantic Cod Skin

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Abstract

In this work, the technology of fish gelatin from the skin of Atlantic cod from the Barents Sea was developed. The influence of enzyme type, extraction time, and pH of extraction on properties of fish gelatin was studied. The usage of proteolytic enzymes increases the yield of gelatin in comparison with acidic treatment, but the molecular weight of gelatin and viscosity of its solution decrease. The results observed related to the cleavage of peptide bonds of collagen during the enzymolysis process. The increase the time of extraction leads to an increase in molecular weight and quality of gelatin obtained. It was shown that gelatin obtained can be successfully used in the production of functional food products based on fish raw materials. The results obtained are of great importance for the fish-processing industry. They allow transforming existing technologies into non-waste processes and thereby enhance the effectiveness of enterprises and create better environmental conditions.

Keywords: fish gelatin, food technology, gelatin extraction

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1. Introduction

Gelatin is a natural biopolymer derived from fibrillar collagen which widely used in the food industry due to its unique capacity to thermoreversible structure formation [1, 2]. Traditionally, the commercial gelatin is derived from animal collagen [3]. However, the usage of such gelatin is limited due to some cultural and religious aspects, as well as the development of bovine spongiform encephalopathy [4]. In addition, fish gelatin is safer and hypoallergenic, unlike gelatin of animal origin. Fish gelatin can replace the gelatin of animal origin. Thus, collagen-contained marine fish materials are ones of the most promising sources of gelatin. Large quantities of co-products (skin, cartilages, etc.) from commercially valuable marine fish species, such as Atlantic cod, are produced during the fish processing. These waste products contain a large amount of collagen and thus can be used as a prospective source of fish gelatin [5].

Fish gelatin produced by acid or alkaline treatment of collagen-contained raw materials [3]. However, the usage of such methods provides a rather low yield of the final

product. The yield of gelatin can be increased by enzymatic treatment of collagenous raw materials, which provides deep destruction of native collagen and increase the bioavailability of gelatin obtained [6, 7]. Proteolytic enzymes of fish origin have high specificity for fish protein, which makes them the most promising in the gelatin production [6, 7].

So that, this work is aimed to develop the technology of fish gelatin from Atlantic cod skin by using the extraction methods and proteolytic enzymes, which helps to transform the existing technologies into wasteless and environmentally friendly.

2. Materials and Methods

2.1. Materials

The skin of Atlantic cod (*Gadus Morhua*) was used as a raw material for obtaining gelatin. The skin was rinsed in cold tap water to remove small amounts of muscular tissues and impurities and then frozen. The chemical composition of cod skin is presented in Table 1.

Table 1: Chemical composition of cod skin.

Component	Moisture	Fat	Total protein	Mineral
Content, %	69.5	0.4	27.4	3.0

Glacial acetic acid, 10% solution of sodium hydroxide and distilled water were used to control the pH of gelatin extraction.

The proteolytic enzymes Protosubtilin and Hepatopancreatin from digestive gland of Kamchatka crab were used for the enzymatic treatment of cod skin (Table 2).

Table 2: Optimal conditions of proteolytic enzymes.

Enzyme	Protosubtilin	Hepatopancreatin
pH	6.5 -- 7.5	7 -- 9
T, °C	45 -- 55	50 -- 55

Gelatin from bovine skin and gelatin from cold water fish skin by Sigma Aldrich were used as reference samples.

2.2. Methods

2.2.1. Gelatin extraction

Cod skin was preliminary defrosted and then cut into the pieces of 5 mm. Then, suspension of cod skin in water was prepared by addition of distilled water to the skin in 3:1 ratio at room temperature. After that, gelatin was extracted it two ways:

a) pH of skin in water suspension was adjusted to 5 by glacial acetic acid. Then, the suspension was heated to 50±1 °C, after that gelatin was extracting for 0.5, 1, 2 and 4 hours at 50±1 °C and constant mixing;

б) pH of skin in water suspension was adjusted to the value of optimum enzyme conditions (pH 6-9) by sodium hydroxide. Then, an enzyme was added in the amount of 0.25 % based on the mass of cod skin. Then, the suspension was heated to 50±1 °C and held at this temperature for 0.5 -- 1 hour at constant mixing. After enzymatic treatment, the suspension was quickly heated to 60 °C to deactivate enzyme and cooled again to 50 °C. pH of skin in water suspension was adjusted to 4-5 by glacial acetic acid, and then gelatin was extracting for 1-1.5 hours at 50±1 °C and constant mixing.

At the end of the extraction, the gelatin-containing solution was filtered through the filter paper at a temperature no lower than 30±1 °C. After filtration, gelatin was dried in the drying oven at 50±5 °C or in the vacuum freeze dryer.

The yield of gelatin was calculated by using the following equation:

$$Y = \frac{m}{M} \cdot 100\% \quad (1)$$

where m -- mass of dry gelatin, g; M -- mass of dry skin, g.

2.2.2. Preparation of gelatin solutions

Gelatin aqueous solutions were prepared by preliminary swelling in distilled water at room temperature and the following dissolution at 40 °C.

2.2.3. Moisture content

The moisture content in gelatin samples was measured by drying to constant weight at 105±2 °C for 18 h:

$$X = \frac{(m_1 - m_2)}{m_1 - m} \cdot 100\% \quad (2)$$

m -- tare weight, g;

m_1 -- weight of tare with sample before drying, g;

m_2 -- weight of tare with sample after drying, g.

2.2.4. Dynamic viscosity

The dynamic viscosity (η) of 10% solution of gelatin was determined by using capillary viscosimeter at 40,0 ± 0,1 ˚C:

$$\eta = k \cdot \tau \cdot \rho \tag{3}$$

where k -- viscosimeter constant, mm^2/s^2;

τ -- flow time, s;

ρ -- density of 10% solution of gelatin at 40.0 ˚C and shall be taken to equal 1.025 g/cm^3.

2.2.5. Clarity of gelatin solutions

The clarity of 5% solution of gelatin was measured by using spectrophotometer Unico S-1201E (USA) at wavelength λ = 440 nm.

2.2.6. Viscosity-average molecular weight

The viscosity-average molecular weight of gelatin was determined by using capillary viscosimeter at 35±1 ˚C. The pH of gelatin solutions was adjusted to the pH of isoelectric point by the addition of a buffer solution. Each sample was preliminarily placed into the thermostat at hold at 35 ˚C for 20 minutes. The flow time of water (t_0) and gelatin solution (t) was determined five times.

The relative viscosity was calculated using the following equation:

$$\eta_{rel} = \frac{\eta - \eta_0}{\eta_0} = \frac{t - t_0}{t_0} \tag{4}$$

where η, η_0 -- viscosity of gelatin solution and water respectively; t, t_0 -- flow time of gelatin solution and water respectively.

The characteristic viscosity was calculated by using the graphical method in coordinates of $\eta_{rel}/C = f(C)$, where C -- concentration of gelatin solution. The characteristic viscosity [η] was defined as the intercept of the Y-axis. The viscosity-average molecular weight of gelatin was calculated by using the Kuhn--Mark--Houwink equation:

$$[\eta] = K \cdot M^{\alpha} \tag{5}$$

where [η] -- characteristic viscosity; K, α -- empirical constant ($K = 1.66 \cdot 10^{-3}$, α = 0,885); M -- viscosity-average molecular weight of gelatin.

2.2.7. Weight-average molecular weight

High-effective liquid chromatography was used to determine the weight-average molecular weight of gelatin by using high-effective liquid chromatograph Shimadzu CTO-20A/20AC (Japan) mounted with column Tosoh Bioscience TSKgel G3000SWxl with a pore diameter of 5 mcm. The high-temperature beam-splitting detector ELSD-LT II was used. 0.5 M solution of acidic acid was used as an eluent at a flow rate of 0.8 ml/min. Narrow-disperse dextran samples (Fluca) with a molecular weight of 1000 to 410000 Da were used for calibration.

2.2.8. Isoelectric point

Turbidimetry was used to determine the isoelectric point (pI) of gelatin. Spectrophotometer Unico S-1201E (USA) was used to measure the absorbance of gelatin solutions with concentration of 0.05 to 1 wt.% in the range of pH values from 4 to 9. pI was determined as a maximum on the curve τ = f(pH):

$$\tau = \frac{2,303 \cdot A}{l}$$

where τ -- turbidity of gelatin solution, m^{-1}; A -- absorbance; l -- optical path length, m.

Viscosimetry was also used to determine pI of gelatin. The flow time of water and gelatin solutions with concentration of 0.05 to 1 wt.% was determined for three times. The average flow times was determined. Kinematic viscosity was calculated as:

$$\nu = \nu_0 \frac{\tau_{cp}}{\tau_0} = \frac{\eta_0 \cdot \tau_{cp}}{\rho_0 \cdot \tau_0}$$

where ν -- kinematic viscosity, mm^2/s;

ν_0 -- kinematic viscosity of water;

τ_0 -- dynamic viscosity of water at 25 °C;

ρ_0 -- density of water at 25 °C.

pI was determined as a minimum on the curve ν= f(pH).

2.2.9. Infrared spectroscopy

Infrared spectra of gelatin samples were obtained by using the Fourier transform IR spectrometer IRTracer-100 (Shimadzu Corp., Japan) at the wavelength of 2000 to 500 cm^{-1}. First, gelatin samples were ground by micro grinder Pulverisette 7 (FRITSCH GMBH, Germany) for 20 min and then mixed with KBr at mass ratio 1: 120 for the

preparation of tablets. Tablets with a mass of 250 g and a diameter of 13 mm were extruded under the pressure of 63.7 MPa for 1 min at room temperature.

3. Results and Discussion

3.1. Characterization of fish gelatin

The yield of gelatin samples from Atlantic cod skin obtained at various conditions was determined (Table 3). The use of enzymes increases the yield of gelatin by 13-19 % in comparison with simple acidic treatment because the enzyme leads to the deeper cleavage of peptide bonds of collagen during the enzymolysis. The extraction time and pH do not significantly affect the yield of the gelatin.

Table 3: Yield of fish gelatin.

Sample	pH of extraction	Extraction time, h	Enzyme	Drying regime	Yield, %
1	4	1	Protosubtilin	DO*	49.2
2	5	1	Protosubtilin	DO	52.3
3	5	1	Hepatopancreatin	DO	45.9
4	5	0.5	-	DO	32.7
5	5	1	-	DO	36.1
6	5	2	-	FD**	36.9
7	5	4	-	FD	39.3

*DO -- drying oven at 50±5 °C

**FD -- freeze dryer

To assess the compliance of gelatin samples with State standard specification for food-grade gelatin GOST 11293 -- 89 [8], basic indicators of the quality of the gelatin were determined. The analogical investigations were conducted for the reference sample of fish gelatin Sigma. The results of the studies are presented in Table 4.

Gelatin samples 1-3 obtained by using proteolytic enzymes do not conform to GOST 11293 -- 89 and cannot be used in the food production, since their solutions have low viscosity, high turbidity and, also, have a salty taste, which sharply limits their application. Samples 4 - 7, in the production of which the stage of enzyme treatment was excluded, have higher consumer qualities. The use of more rigid processing conditions, for example, low pH or using enzymes with high substrate specificity, therefore causes deep destruction of collagen and gelatin molecules, leading to a decrease in consumer qualities of the final product.

Table 4: Physicochemical characteristics of gelatin samples.

Parameter	Food-grade gelatin	1	2	3	4	5	6	7	Sigma
Physical form	Granules, plates, powder	Plates			Powder		Plates		Granules
Color	From light yellow to yellow	Light yellow			Pale yellow		Light yellow		
Smell	Odorless	Fishy odor							Odorless
Taste	Blank taste	Salty			Tasteless				
pH of 1% solution	5-7	5							
Moisture content, %	16	8	8	7	8	8	3	3	14
Dynamic viscosity 10% solution, mПa·s	14.4	12.6	14.4	10.6	9.4	10.1	25.5	24.3	57.7
Clarity of 5% solution	25	23	25	21	20	21	25	25	87

3.2. Molecular weight distribution

Molecular weight distributions of gelatin samples obtained by using proteolytic enzymes are presented in Figure 1. The distribution for these samples is polymodal because of the presence of several fractions with different molecular weights. The fractions with molecular weight of 1 kDa are presented in samples. The enzymatic treatment has a destructive effect on the structure of collagen and leads to the partial destruction of the gelatin molecules themselves. In addition, the obtained samples may contain impurities of various origins and varying degrees of dispersion: peptides, colloids, residues of small tissues, pigmenting substances, calcium salts, protein particles, etc. Impurities impart turbidity to the gelatin solution and reduce other quality indicators. Fish gelatin Sigma is characterized by a narrow monomodal distribution and do not contain low molecular weight components and impurities.

The numerical values of the viscosity-average molecular weights ($\overline{M}_{\eta}$) and weight-average molecular weight ($\overline{M}_{\omega}$) of gelatin samples are given in Table 5. The use of enzymes in the processing of soft collagen-containing fish raw materials leads to the destruction of collagen and gelatin molecules, leading to the formation of low molecular weight fractions. Low molecular weight components negatively affect the organoleptic and physicochemical properties of the final product. Thus, a decrease in molecular weight reduces the viscosity of the solution and increases its turbidity. In the absence of the enzymatic treatment, samples with higher molecular weights were obtained, and an increase in the extraction time leads to a significant increase in this characteristic. So, the maximum value of the average molecular weight corresponds to the sample,

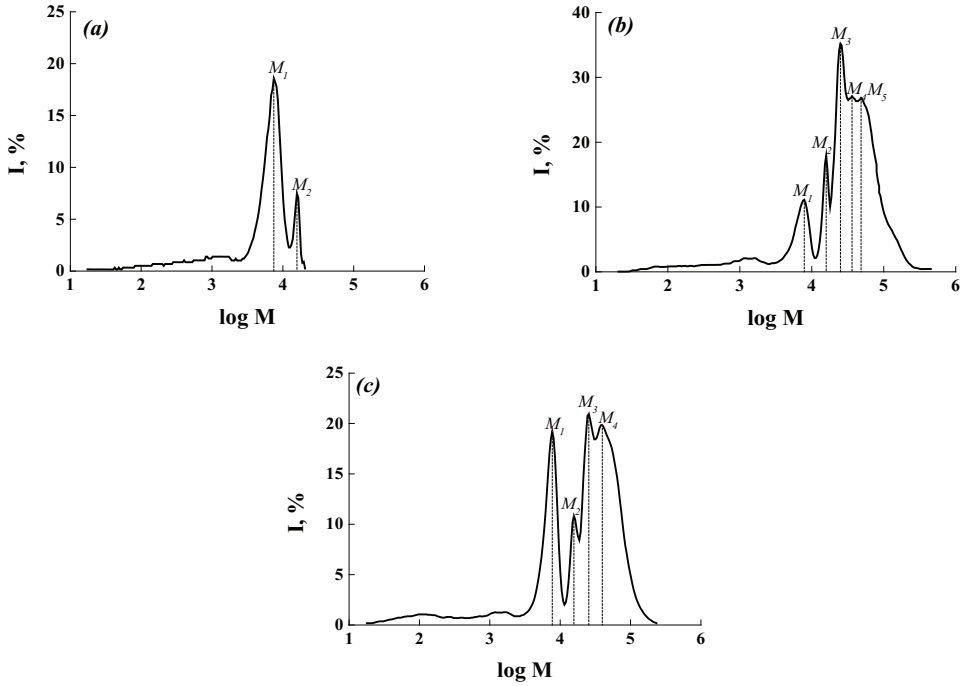

Figure 1: Molecular weight distribution of gelatin samples 1 (a), 2 (b), 3 (c).

the extraction time for which was 2 hours. It should be noted that gelatins obtained without enzymatic treatment conform to GOST 11293 -- 89 for food-grade gelatin.

TABLE 5: Average molecular weight of gelatin samples.

Sample	1	2	3	4	5	6	7	Fish gelatin Sigma
$\overline{M}_{\eta}$, kDa	7.4	31.4	29.6	13.6	32.9	67.8	64.6	90.4
$\overline{M}_{\omega}$, kDa	7.5	37.8	29.4	--	--	--	--	91.3

3.3. Isoelectric point

The values of isoelectric point (pI) of gelatin samples are presented in Table 6. pI of obtained gelatins is within the value of 7. pI of fish gelatin Sigma is equal to 4.8 as this sample was obtained by alkaline treatment.

3.4. IR spectra

The samples of gelatin from cod skin were identified in comparison with the IR spectrum for fish gelatin Sigma (Figure 2). The main absorption bands of the functional groups of gelatins are given in Table 7. The IR spectra of proteins and their decay products

Table 6: Isoelectric point of gelatin samples.

Sample	1	2	3	4	5	6	7	Fish gelatin Sigma
pI*	6.7	6.8	6.8	6.6	6.8	7.1	7.4	4.8
pI**	6.7	6.9	6.8	-	-	-	-	4.8

* -- determined by viscosimetry

** -- determined by turbidimetry

(peptides) are characterized by two main absorption bands: amide I and amide II, due to stretching vibrations of the C = O bond and planar deformation vibrations of the NH bond. An absorption band at 3330 cm^{-1} also characterizes collagen or gelatin [9].

Table 7: Main absorption bands of gelatin functional groups [9, 10].

Functional group	Wavelength, cm^{-1}
AmideA (NH)	3320 -- 3370
Amide I (CO, CN)	1650 -- 1680
Amide II (CH, NH)	1530 -- 1550
Amide III (CN, NH)	1240

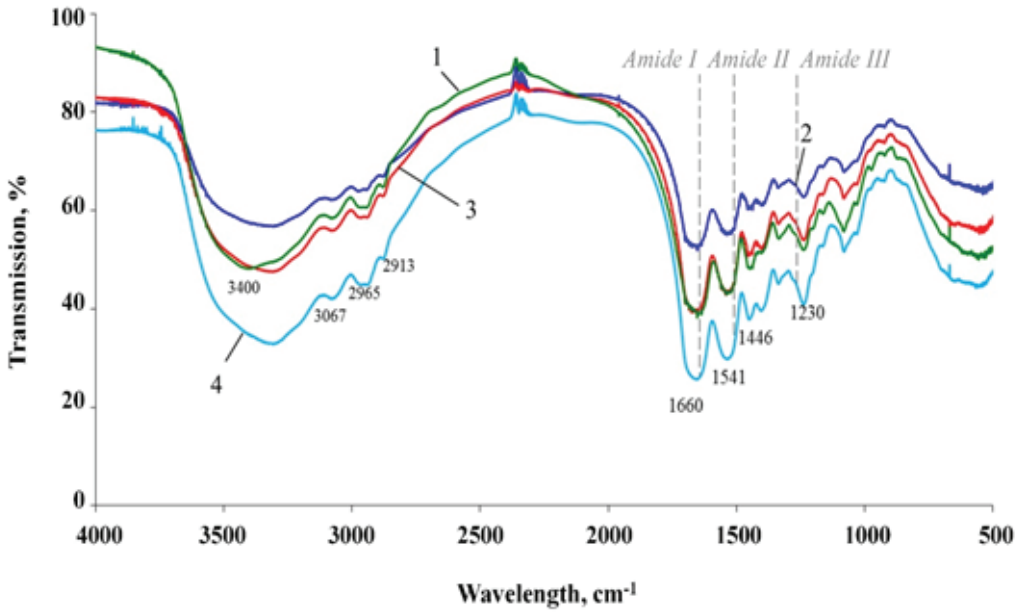

Figure 2: Infrared spectra of gelatin samples: 1 -- Fish gelatin Sigma; 2 -- gelatin from cod skin (extraction time 1 h.), 3 -- gelatin from cod (extraction time 2 h.), 4 -- gelatin from cod (extraction time 4 h.).

4. Conclusion

The technology of fish gelatin was developed. It was shown that the usage of proteolytic enzymes increases the yield of gelatin in comparison with acidic treatment, but the molecular weight of gelatin and viscosity of its solution decrease. The molecular weight distribution shows that the molecular weight of gelatin obtained depends on the extraction conditions. It was shown that the mild regimes of gelatin extraction ensure the fullest destruction of fish collagen and make it possible to produce high-quality fish gelatin. Gelatin obtained can be used to produce functional food products.

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Conflict of Interest

The authors have no conflict of interest to declare.

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