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TECHNOLOGY OF ENZYMATIC-ACID HYDROLYSIS OF BONE RAW MATERIALS IN PRODUCTION OF GELATIN

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Keywords: *gelatin, enzymes, production, hydrolysis, quality indicators*

Abstract

Bone gelatin is an important and irreplaceable item widely used in the food industry and pharmaceutical production; it is also widely used in tissue engineering and other spheres. Due to widespread use of gelatin it is necessary to search for new safe and effective technologies for bone gelatin production. This research represents the results of enzymatic-acid hydrolysis of raw material in the process of gelatin production. The article presents the results of hydrolysis analyzes, the results of the main quality parameters of the obtained gelatin samples; and the major technological scheme for gelatin production is proposed here. As result of developed technology of enzymatic-acid hydrolysis of bone raw material with the ratio of raw material mass to the volume of solvent (HCl 1M and pepsin with an enzymatic activity of 40 units) as 1:9, duration of exposure: 180 minutes (3 hours), at the stage of demineralization, liming and de-ashing, we obtained samples of gelatin at yield rate of 12.1% from the initial mass of raw materials, which is 6.9% higher in comparison with the lowest yield of gelatin according to the proposed schemes. It is shown that the samples have a high protein mass fraction 91.4%, and a low fat mass fraction 0.4%, the obtained results indicate the high technological qualities of the obtained gelatin sample, this is also confirmed by high strength of gel according to Bloom scale, which value varies within the range of 290 ± 0.7 units.

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Introduction

Gelatin is a water-soluble protein with a molecular weight of 20 to 250 kDa. The main raw material for its production is the skin and bones of farm animals [1, 2]. This type of protein has found wide and significant application in various industries. In the modern market of food products and food components gelatin is a popular product, while a great demand for this product is observed in the market for medical and cosmetic products. According to the data of Grand View Research, in 2018 the gelatin market volume amounted to 2.91 billion USD [3]. The gelatin market will grow at rate of 8% per annum, and by 2025 the volume of market will reach 5 billion USD in monetary terms [4,5]. In the territory of the Russian Federation the production of gelatin still hasn't grown; so due to this situation a big import dependence on this type of raw material is observed. First of all, this underachievement is caused by complicated multistage processes in the production of gelatin, where large amount of acids, alkali and water are used. The application of a large amount of chemical substances leads to voluminous formation of wastewater. If this wastewater is not properly treated and neutralized, the untreated wastewater from gelatin production can cause serious environmental problems [6,7,8]. The second problem in gelatin production by the classical scheme is the long duration of certain technological stages of the production process, for example: demineralization, liming and de-ashing of bone raw materials can take up to 30–40 days, and therefore there is a need for vast industrial areas for continuous technological process of gelatin production [9,10].

In the classical scheme of gelatin production, demineralization, liming and de-ashing are the main preparatory technological stages before the extraction of gelatin. These processes take quite a long time, for example, demineralization lasts from 7 to 14 days, liming takes from 3 to 8 weeks, de-ashing takes from 1 to 2 days, while each of these preparatory technological stages requires a lot of chemical reagents, such as HCl and Ca $(OH)_2$. Modern highly efficient production cannot afford these long-term processes in its technological line. Therefore there is an urgent need for new technological solutions that will help optimize this stage of production [11,12,13].

One of the optimal solutions to accelerate the process of demineralization of fat-free bone raw materials is to expose the materials to hydrolysis with enzymes or enzyme preparations.

Bone gelatin is an important and indispensable product of food and pharmaceutical industries, but it is also widely used in tissue engineering and other spheres. Due to the widespread use of gelatin, it is necessary to search for new safe and efficient technologies for production of bone gelatin [14,15,16].

There is a modern, environmentally friendly and safer method that can be applied at certain stages of the technological production of gelatin — that is hydrolysis [17,18]. During hydrolysis proteolytic enzymes are widely used; they are increasingly used in enzymatic hydrolysis in pro-

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duction of gelatin from collagenous (gelatinous) structures of fish waste and animal skins. Enzymatic hydrolysis of raw materials with high collagen content makes it possible to obtain gelatin in a shorter time with less waste [19,20].

When using bone raw materials in the production of gelatin, enzymatic hydrolysis with pepsin is ineffective, since the bone matrix is not hydrolyzed by enzymes due to dense mineral intermolecular adhesion and interlacing, while the collagen structure inside the bone matrix is susceptible to the action of the enzyme pepsin to a high degree [21,22]. For enzymatic-acid hydrolysis of bone raw materials, it is necessary to seek a balance between acidic and enzymatic effects on the surface and internal structure of the bone matrix — on the mineral calcium phosphate, of which the bone matrix consists.

Thus, the objectives of the study were to determine the effect of the duration of hydrolysis according to the developed schemes on the yield of gelatin after extraction; to determine the main indicators of the quality of gelatin samples, which had the highest yield from the mass of the original bone raw material; to develop a major technological scheme for the production of gelatin from bone raw materials.

The aim of the work was to develop a technology for enzymatic-acid hydrolysis of bone raw materials in production of gelatin.

Objects and methods

To run the experimental part of the work, preliminarily defatted bovine bone raw material was used. The raw material was finely crushed to a size of 3 ± 0.5 mm. For experiment we used tubular bones of cows obtained from a farm located in the Kemerovo region — Kuzbass. For the experiment 3 kilograms of tubular bovine bones were crushed in the laboratory chain crusher. Further, the obtained bone raw material was subjected to hydrolysis by enzyme. Pepsin of microbial origin with an enzymatic activity of 300,000 units was used. The characteristics of the used enzyme are presented below in the Table 1.

Table 1. Characteristics of the applied enzyme

Parameter	Characteristics
Composition	Pepsin based on Rhyzomucor miehei (CAS:9001–92–7)
Origin	Microbial
Assumed decomposition	Phe [1] + Val, Gln [4] + His, Glu [13] + Ala, Ala [14] + Leu, Leu [15] + Tyr, Tyr [16] + Leu, Gly [23] + Phe, Phe [24] + Phe и Phe [25]
Appearance, color	White powder
Activity in 1 g, unit	Not less than 300 000
Activation temperature, °C	30 ± 2
Manufacturer	"Meito Sangyo Co., Ltd.", Japan

The enzyme pepsin is activated by interaction with hydrochloric acid (HCl) and during interaction pepsin shows high proteolytic properties, while HCl promotes the destruction of the bone matrix. Hydrolysis with microbial enzyme pepsin and hydrochloric acid (HCl 1M) was performed according to the schemes presented below in the Table 2.

Table 2. Schemes of preliminary treatment of bone raw materials

Scheme, No.	Enzyme concentration, unit	Bone raw material to solvent ratio, (weight / volume)	Duration of exposure, min.	pH, unit	Temperature of exposure, °C
1	30	1m HCl 1:7	60	1.5–2.0	27.0 ± 2 °C
			120		
			180		
			240		
		1m HCl 1:8	60		
			120		
			180		
			240		
		1m HCl 1:9	60		
			120		
			180		
			240		
		1m HCl 1:10	60		
			120		
			180		
			240		
2	35	1m HCl 1:7	60	1.5–2.0	27.0 ± 2 °C
			120		
			180		
			240		
		1m HCl 1:8	60		
			120		
			180		
			240		
		1m HCl 1:9	60		
			120		
			180		
			240		
		1m HCl 1:10	60		
			120		
			180		
			240		
3	40	1m HCl 1:7	60	1.5–2.0	27.0 ± 2 °C
			120		
			180		
			240		
		1m HCl 1:8	60		
			120		
			180		
			240		
		1m HCl 1:9	60		
			120		
			180		
			240		
		1m HCl 1:10	60		
			120		
			180		
			240		
4	45	1m HCl 1:7	60	1.5–2.0	27.0 ± 2 °C
			120		
			180		
			240		
		1m HCl 1:8	60		
			120		
			180		
			240		
		1m HCl 1:9	60		
			120		
			180		
			240		
		1m HCl 1:10	60		
			120		
			180		
			240		

The crushed bone raw material was submerged into solution of hydrochloric acid (HCl 1M), where the microbial enzyme was added to according to the submitted schemes. The raw material was exposed to hydrolysis at a temperature of 27 ± 2 °C for 60 to 240 minutes. pH of the medium varied within the range of 1.5–2.0 units. For uniform treatment of bone raw materials with a solution during the entire duration of the experiment, a magnetic laboratory stirrer "MM-5" (Russia) was used. The bone raw material was stirred at 100 rpm at the temperature up to 27 ± 2 °C. After hydrolysis, the bone raw material was subjected to centrifugation, which facilitated the separation of mineral sediment from ossein. The bone raw material was centrifuged on a high-speed centrifuge "Avanti J-26S — Beckman" (Beckman Coulter, USA). The technical characteristics of the centrifuge are presented below in the Table 3.

Table 3. Technical characteristics of the high-speed centrifuge "Avanti J-26S — Beckman"

Speed range for angle rotors, rpm	100–26 000
Maximum speed for bucket rotors, rpm	13 000
Maximum acceleration for angle rotors (x g)	81 800
Maximum acceleration for bucket rotors (x g)	26 500
Maximum volume for angle rotors, ml	6 000 ml (6 x 1 000 ml)
Maximum volume for bucket rotors, ml	4 000 ml (4 x 1 000 ml)
Volume of the tested sample in one capsule	1,5 ml — 1 000 ml
Time range	Up to 180 min., Hold mode
Engine's type	valve-inductor brushless motor
Number of acceleration / deceleration modes	2 acceleration / 3 deceleration
Friction reduction system	Yes
Temperature range	From –10 °C to +40 °C (in increments of °C)
Temperature control	± 2 °C of the setpoint
Heat dissipation (kW)	2
Noise level, dB	<57
Width x depth x height (cm)	71.0 × 86.0 × 86.0
Weight, kg)	290
Type of installation	Floor installation

After that the obtained ossein was washed with demineralized water. After removal of the mineral residue, the ossein was transferred to the stage of gelatin extraction. In order to save resources and ensure the environmental friendliness of production, gelatin was extracted with water at a temperature of 60 °C. The obtained gelatin broths were dried by a laboratory drying sprayer, model "Mini Spray Dryer B-290" (Buchi, Sweden) at a temperature of 95 °C, the feed rate of the broths into the spray chamber amounted to 3.0–3.2 ml/min. The mass fraction of protein was determined on a thermal digestor "FOSS Tecator Digestor 2520" (produced by Foss Tecator, Sweden). The strength of the gel according to Blum scale was determined on a texture analyzer "Structurometer ST-2" completed with an indenter "Bloom". The technical characteristics of the texture analyzer are presented below in the Table 4. The materials were prepared for analysis according to the following procedure: 7.5 g of gelatin was placed in a glass of cold water (105 ml of water) and this mixture was kept for 180 min at a temperature not over 22 °C. Then the swollen gelatin was heated in a water bath to a temperature of 60 °C and stirred for 15 min until complete dissolution. The prepared gelatin solution (with concentration of 6.67%) was poured into a special calibrated vessel and kept for 17 hours at a temperature of 10 ± 0.1 °C.

Table 4. Technical characteristics obtained by the texture analyzer "Structurometer ST-2"

Indenter speed (indenter penetration rate or rate of medium deformation), mm / s	0 … 4,5
Indenter stroke range, mm	0 … 220 ± 1
Reduced relative load measurement error,%, no more	1
Discreteness of setting the loading speed (increase of load), g / s	0,1
Device weight, kg	30
Frequency of the power voltage, Hz	50/60
Overall dimensions, mm	280 × 440 × 680

Experimental researches were run in the Department of Food Technology of Animal Origin and the Research Institute of Biotechnology, Kemerovo State University.

The experimental results were processed using the software *Statistica 10.0* (StatSoft Inc., 2007, USA).

Results and discussion

The yield of gelatin from bone raw materials is influenced by many production factors. The main of which are the method of processing bone raw materials and the method of its demineralization in order to obtain ossein [23,24,25]. In this regard, at the first stage of research we analyzed the influence of enzymatic-acid hydrolysis duration according to the proposed schemes (Table 2) on yield of gelatin during subsequent extraction of gelatin.

To determine this influence, after the extraction of gelatin from gelatin broths, the obtained samples were spray dried on a laboratory spray drier of model "Mini Spray Dryer B-290" (Buchi, Sweden). The influence of hydrolysis duration on gelatin yield during its extraction are shown below in the Figure 1.

The yield of gelatin after extraction ranged depending on the scheme of preliminary processing. According to the data presented in Figure 1, it can be seen that the minimum yield of gelatin among all the presented hydrolysis schemes, is 5.2%, the maximum is 12.1%, which is 6.9% higher in comparison with the least yield. At the same time, each of the schemes proves that pepsin efficiently dissolves collagen in the bone, breaking down the peptide bonds and providing a higher yield of gelatin, depending on enzymatic activity of applied enzyme and the volume of solvent in ratio to the mass of the initial raw material.

Figure 2 shows the results of the comparative yield of gelatin depending on the scheme of hydrolysis.

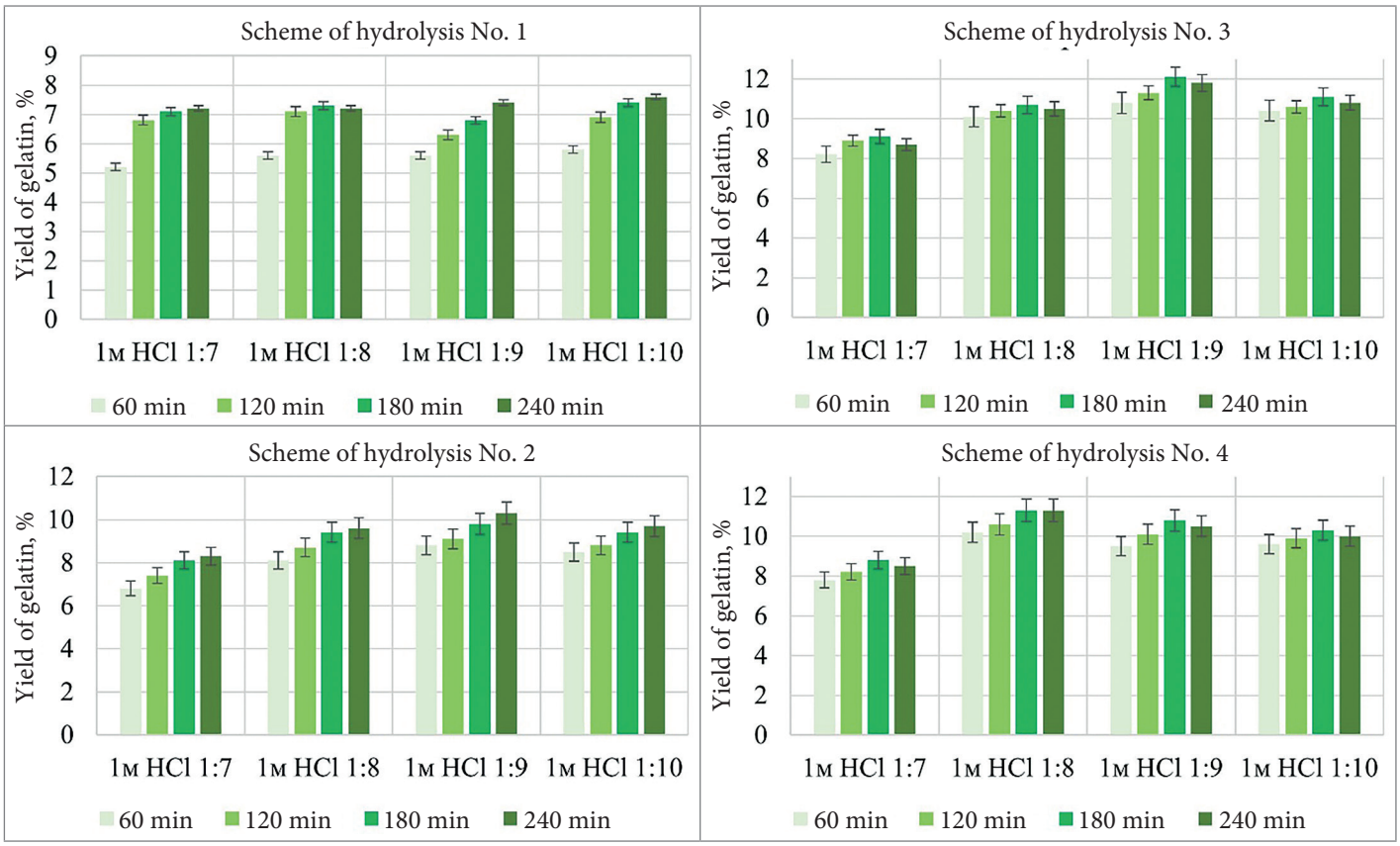

Figure 1. Influence of hydrolysis duration on yield of gelatin during its extraction

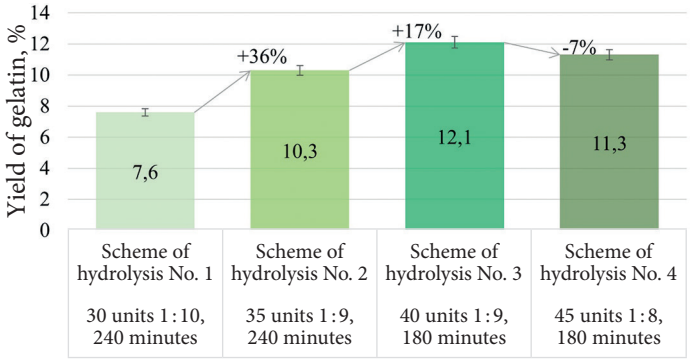

Figure 2. Comparative yield of gelatin

Basing on assessment of influence of hydrolysis duration on yield of gelatin during extraction, it can be concluded that the highest yield of gelatin is achieved during hydrolysis in the scheme 3 with a ratio of raw material to the volume of solvent 1:9 for 180 minutes (3 hours). It can also be noted that hydrolysis in the scheme 4 with a ratio of raw material to the volume of solvent 1:8 for 120 minutes also has a high yield of gelatin after its extraction; the yield amounts to 11.3% of the initial raw bone material.

Further, the main indicators of gelatin samples quality were determined, which had the highest yield from the mass of the initial raw bone material. The results are presented below in the Table 5.

If to compare the results of quality indicators of the obtained gelatin samples, with the standardized indicators GOST 11293–2017 "Gelatin. Specifications" for gelatin, the proposed hydrolysis technology allows obtaining gelatin of high quality. Some results of analysis of quality indicators of the obtained gelatin samples surpass the data obtained by other researchers. Thus, the mass fraction of protein in the obtained samples is 3–5% higher in average, the mass fraction of fat is also higher 0.2% in average [26,27,28,29].

Table 5. Main indicators of the obtained gelatin quality

Parameter	Sample obtained by hydrolysis scheme No. 3	Sample obtained by hydrolysis scheme No. 4	Normative value by GOST 11293-20171
Mass fraction of protein, %	91.4 ± 0.2	90.7 ± 0.1	—
Mass fraction of fat, %	0.4 ± 0.06	0.6 ± 0.03	—
Mass fraction of moisture, %	7.8 ± 0.2	8.1 ± 0.1	Not more than 16,0
Mass fraction of ash, %	0.4 ± 0.03	0.6 ± 0.04	Not more than 2,0
Gel strength by Bloom, unit	290 ± 0,7	275 ± 0.5	from 100 to 300

The obtained data confirm the applicability of enzymatic-acid hydrolysis of bone raw materials in order to obtain a high-quality gelatin, which makes it possible to develop new resource-saving methods for gelatin production and to launch a new branch of the industry in whole [30, 31].

At the final stage of scientific research, a schematic diagram of technology of gelatin production by enzymatic-acid hydrolysis of bone raw materials was developed according to the scheme No. 3. The developed scheme is shown below in the Figure 3.

[1] GOST 11293–2017 "Gelatin. Specifications". Moscow: Standartinform, 2020. — 35 p.

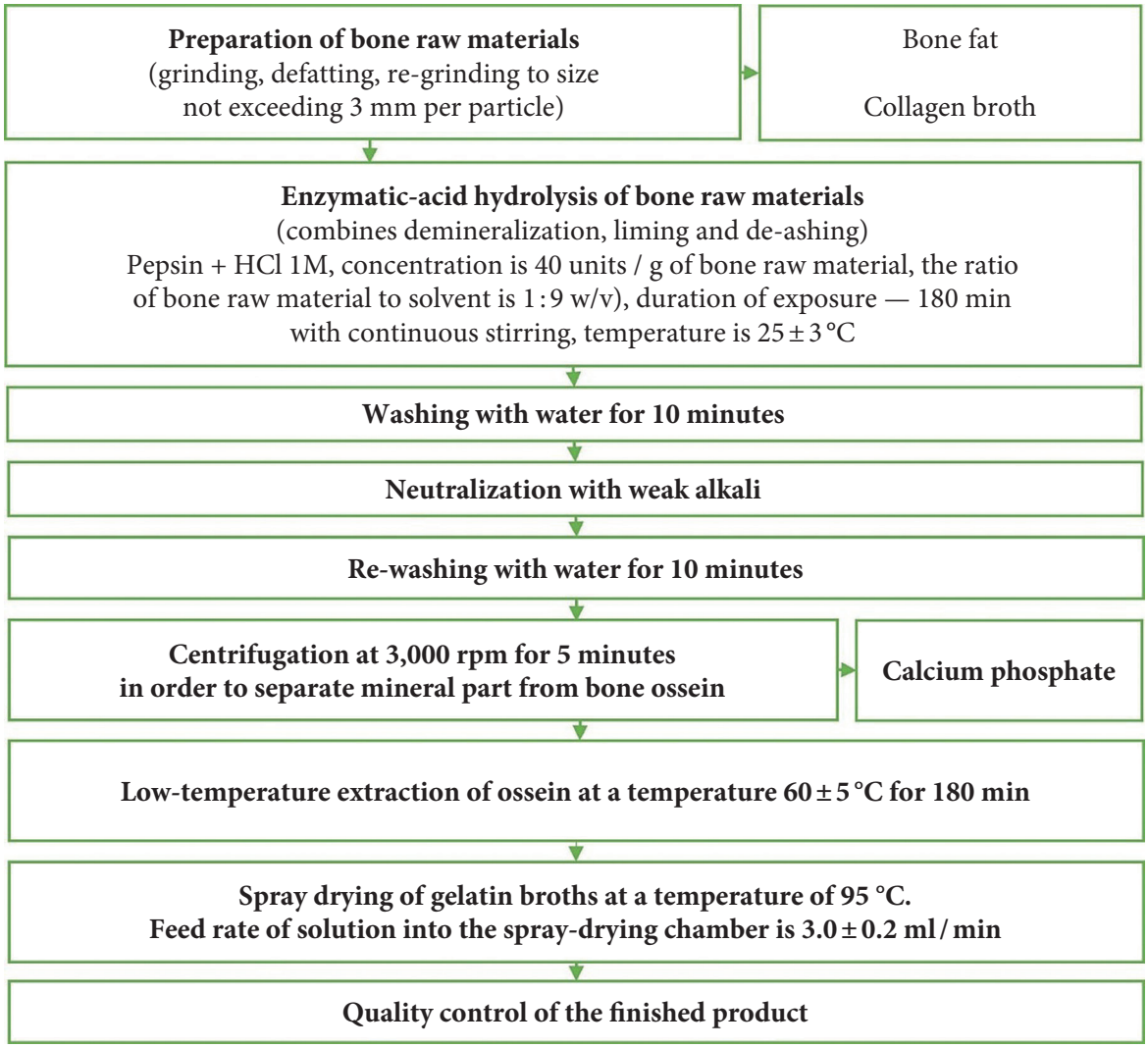

Figure 3. Basic technological scheme for production of gelatin from bone raw materials

On the basis of the performed studies, it is proposed to apply enzymatic-acid hydrolysis of bone raw materials at stages of demineralization, liming and de-ashing during the production of edible gelatin. The proposed technology is capable to shorten the technological process of gelatin production and to achieve sufficiently high yield of the final product in reference to the initial raw material.

Conclusion

The developed technology of enzymatic-acid hydrolysis of bone raw material with ratio of raw material mass to the volume of solvent 1:9 (HCl 1M and pepsin with enzymatic activity of 40 unit) for 180 minutes (3 hours) at stage of demineralization, liming and de-ashing allowed obtaining gelatin samples with a yield of 12.1% of the initial mass of raw materials, which is 6.9% higher than the lowest yield of gelatin in the proposed schemes. According to results of analysis of main indicators of quality, the obtained gelatin meets or exceeds the standards of GOST 11293–2017 as the obtained samples feature high mass fraction of protein — 91.4%, and low mass fraction of fat — 0.4%. The obtained results indicate high technological qualities of obtained sample of gelatin. This is also confirmed by the high strength of gel according to Bloom scale, which value varied within the range of 290 ± 0.7 units.

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Completely prepared the manuscript and is responsible for plagiarism.

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