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# DETERMINATION OF OPTIMAL CONDITIONS FOR THE EXTRACTION OF GELATIN FROM SECONDARY LOCAL RAW MATERIALS


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**Abstract**: This article presents the experimental results of extracting gelatin from protein-rich secondary local raw cattle skin by alkaline hydrolysis and determining the dependence of gelatin yield on the concentration of the hydrolyzing solution, temperature, and duration of hydrolysis. Also, the effect of hydrolysis conditions on the molecular weight of gelatin has been studied.

**Keywords:** gelatin, secondary raw materials, hydrolysis, protein content.

**Introduction.** Gelatin is widely used in medicine due to its biocompatibility, biodegradability, and toxic effects. Gelatin dissolves easily in the digestive system at 35°C and it is used in the pharmaceutical industry to produce hard and soft capsules due to its easy release of bioactive compounds, nutrients and drugs. It also stimulates various functions such as regeneration of injured tissues, healing of wounds and strengthening of bone formation [1-3].

Gelatin is a water-soluble polypeptide obtained by partial hydrolysis of collagen, a natural protein substance. Collagen protein is the main component of the connective tissue of vertebrates. In particular, it is found in large quantities in connective tissues such as bone, skin, cornea, tendon, blood vessels and dentin, and performs an important task of maintaining their integrity. Nowadays, gelatin is mainly extracted from pig skin (46%), cattle skin (29.4%), pig and cattle bones (23.1%) and fish (1.5%) [4-6].

Two different types of gelatin are obtained from the connective tissues of mammals, i.e. pig skin (type A) and cattle skin (type B). While gelatin with a molecular weight of 10 kDa to 400 kDa is obtained from both of these raw materials, the difference lies in different isoelectric points, solubility values, and average molecular weight of gelatin.

Fish gelatin is extracted from the skin and bones of fish. Fish processing waste contains up to 30% collagen [7].

The productivity and quality of gelatin are affected not only by the type of raw material, but also by other factors while it is being extracted. For instance, the concentration of hydrolyzing reagents, pH, temperature and time directly influence on this process [8].

For the extraction of gelatin, collagen-containing raw materials such as purified bone and skin are widely used. 93% of all proteins in bone are collagen. Also, in addition to collagen protein, the protein part of the bone contains elastin, reticulin, albumin, globulin and mucopolysaccharides - mucins and mucoids. Since the skin is the main raw material that stores collagen, it is the most valuable raw material for the production of gelatin. The most valuable raw materials for obtaining high-purity gelatin are pieces of cattle skin. Skin raw materials contain collagen that is resistant to various substances, insoluble in cold water, salts, weak acid and alkaline solutions, organic solvents, and turns into gelatin when heated with water. There are three types of the separation of gelatin from collagen protein: physical, chemical and enzymatic. The isoelectric point of gelatin, that is obtained by the hydrolyzing the skin using a solution of sulfate or hydrochloric acid, is equal to 9.0. The isoelectric point of gelatin hydrolyzed using an alkali (NaOH) solution is 5.0 [9-12].

**The purpose of the study.** It consists in determining the optimal hydrolysis conditions for the extraction of gelatin from local secondary cattle skin raw materials.

**Materials and methods.** Cattle skin was chosen as a research object, and reagents of chemical purity and pure brand for analysis were used in the experiments.

Extraction of gelatin from cattle skin. Skin pieces (1x1 cm) were hydrolyzed in alkaline solution for 12-48 hours. The effect of alkali concentration, temperature and duration of hydrolysis on the yield of gelatin during hydrolysis was studied.

Neutralization. After hydrolyzing the skin, it was neutralized with 1% HCl solution.

Determination of humidity. In order to determine the humidity of the skin samples, they were weighed and dried in a drying oven at a temperature of 105°C for 2 hours to a constant weight and weighed.

Determination of gelatin yield by nitrogen. The amount of nitrogen in dry samples of gelatin was determined using the K'eldal method using a semi-automatic device (VELP Scientifica). In this method, nitrogenous organic matter is decomposed under the influence of concentrated sulfuric acid in the presence of catalysts (salts of heavy metals), nitrogen is converted into ammonium sulfate, and ammonia released from ammonium sulfate under the influence of alkali is titrated with an acid solution [13]. The coefficient of conversion of nitrogen to gelatin is 5.62.

Determination of the molecular weight of gelatin. Molecular weights of gelatin samples hydrolyzed under different conditions were determined by the Laemmli method in a 10% polyacrylamide gel [14].

**Results and their discussion.** There are secondary raw materials suitable for extracting gelatin in the skin processing plants. Chemical processing of these raw

materials is effective not only economically but also ecologically. Keeping this in mind, we used local cattle skin raw material in our research.

In our experiments, initially, gelatin was extracted by hydrolyzing skin with aqueous solution of 0.5; 1; 1.5; 2 and 2.5% NaOH for 12, 24, 36 and 48 hours.

Also, in the experiments, we studied the influence of the concentration of the hydrolyzing reagent and the length of the hydrolysis time on the yield of gelatin extraction. The results of the experiment are presented in Figure 1.

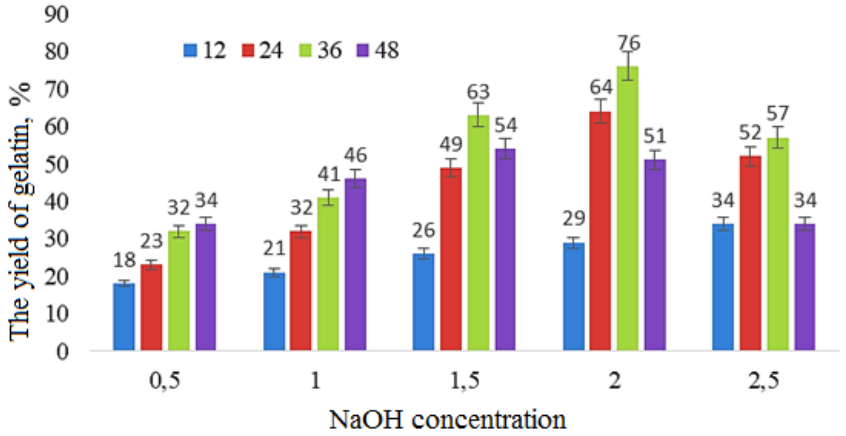


**Figure 1.** The influence of the alkali concentration and the length of the hydrolysis time on the yield of gelatin.

From the results presented in Figure 1, it can be seen that when hydrolyzing the skin with 0.5 and 1% NaOH solution from 12 to 48 hours, the gelatin yield increased to 34 and 46%, respectively. When hydrolysis with 1.5% NaOH solution was carried out from 12 to 36 hours, it was found that the yield of gelatin first increased to 63% and then decreased to 54% in 48 hours. When hydrolysis with 2% NaOH solution was carried out from 12 to 36 hours, the yield of gelatin initially increased to 76%, and when the duration of hydrolysis was 48 hours, the yield decreased to 54%. In 2.5% NaOH solution, the best yield was 57% in 36 hours when chemically processing skin.

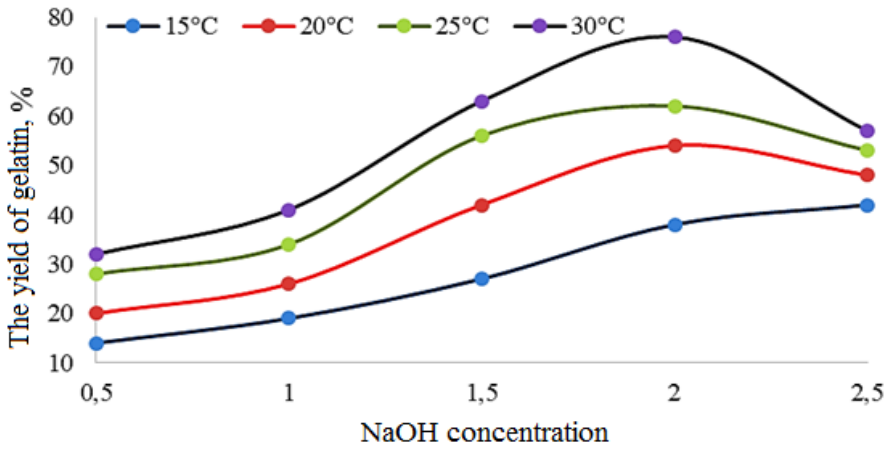


**Figure 2.** The influence of the alkali concentration and the temperature on the yield of gelatin (the length of the hydrolysis time – 36 hours).

In our next experiments, the influence of alkali concentration and temperature on gelatin yield was studied. The results of the experiment are presented in Figure 2.

The results of the experiment presented in Figure 2 showed that the yield of gelatin extraction increased from 14% to 42% depending on the alkali concentration under hydrolysis conditions at 15°C. At a temperature of 20°C, gelatin yield increased from 20% to 54% in accordance with alkali concentration (0.5-2%). When the concentration of alkali is 2.5%, it was found that the yield of gelatin decreased to 48%. This situation was also observed at temperatures of 25 and 30°C. For example, when the concentration of alkali increased from 0.5% to 2% at a temperature of 25°C, the yield of gelatin increased from 28% to 62%, and when the concentration of alkali was 2.5%, the yield decreased to 53%. At a temperature of 30°C, when the concentration of NaOH increases from 0.5% to 2%, the yield of gelatin increases from 32% to 76%, and when the concentration of NaOH is 2.5%, the yield of gelatin decreases to 57%.

There are several methods for determining the molecular mass of proteins. Among them, the most effective and widely used method is the electrophoresis method. Gelatin samples extracted under different hydrolysis conditions were electrophoresed in a 10% polyacrylamide gel (Figure 3).

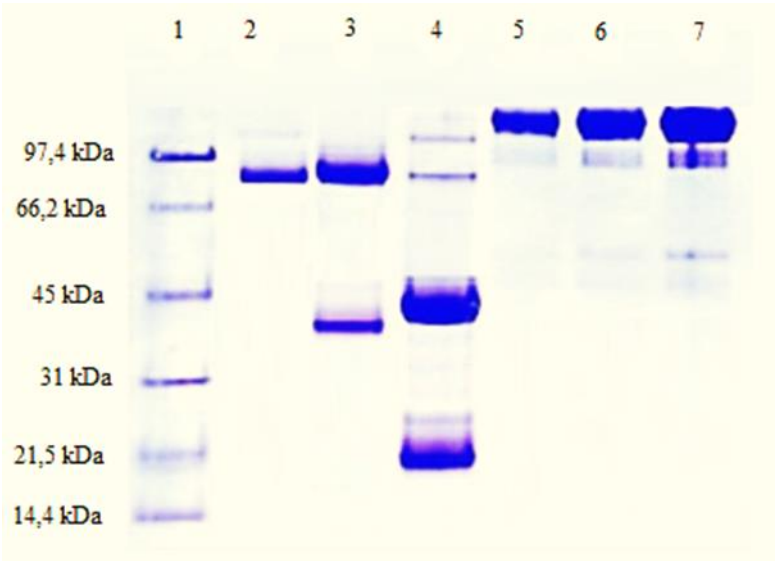


**Figure 3. The results of polyacrylamide gel electrophoresis of gelatin samples:** standard 1 (97.4; 66.2; 45; 31; 21.5; 14.4 kDa); 2- NaOH 2%, 36 hours; 3- NaOH 2.5%, 36 hours; 4- NaOH 2%, 48 hours; 5- NaOH 0.5%, 36 hours;, 6- NaOH 1%, 36 hours; 7– NaOH 1.5%, 36 hours.

According to the results of electrophoresis, it was determined that the molecular weight of gelatin (2), which was hydrolyzed with 2% NaOH solution for 36 hours, was 80 kDa. Under these conditions, gelatin with a molecular mass of 21, 42, and 80 kDa was observed when the hydrolysis time was extended to 48 h (4). In the gelatin sample hydrolyzed for 36 hours using 2.5% NaOH solution, it was found that it consists of two different molecular weights of 80 kDa and 38 kDa. Due to the incomplete hydrolysis at

NaOH concentration of 0.5 (5), 1 (6) and 1.5% (7), hydrolyzate with relatively high molecular weight and low water solubility was obtained.

**Conclusion.** During the research, gelatin samples were obtained from local secondary skin raw materials by hydrolyzing with different concentrations of NaOH solution. Hydrolysis of cattle skin at 30°C using 2% NaOH solution for 36 hours was chosen as optimal conditions for gelatin extraction. Under these conditions, the yield of gelatin was 76%, and according to the results of electrophoresis, its molecular weight was determined to be 80 kDa.

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