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QUALITATIVE AND QUANTITATIVE ANALYSIS OF COLLAGEN PROTEIN

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Abstract

This research mainly presents the results of the analysis of the quality and quantity of proteins extracted from cattle hide. The study focuses on the processing of cattle hide, which is considered an industrial waste, and the proper organization of collagen extraction. Additionally, the composition and purity of the extracted protein are assessed. The total nitrogen content in the sample was measured using the Kjeldahl method, and the total percentage of protein was calculated using a conversion factor (usually 6.25, or a factor adjusted to the research conditions). Furthermore, the amount of water-soluble proteins in the mixture was analyzed using the Lowry method at a wavelength of 570 nm with a UV–Vis 1900i instrument. The individual amino acids in the mixture were quantitatively and qualitatively analyzed using HPLC at a wavelength of 269.4 nm.

Keywords: *hayvon terisi, oqsil, kollagen, aminokislota, Kjeldahl method, Lowry method, spektroskopiya, xromatografiya*

Introductıon

Protein deficiency remains a serious global 0issue among the world's population. Regional and socio-economic disparities are expressed in significantly varying indicators. This is influenced by people's lifestyles and ethnographic environments, ranging from protein biosynthesis in the body to the protein content of consumed products, as well as the consumption of protein-rich foods to prevent protein deficiency in humans. The quality of proteins and their bioavailability remain crucial factors. Proteins are not only produced as preparations but also used in enriching the composition of products, especially food products, improving their quality indicators, which has become one of the most relevant issues today.

Looking at global indicators and trends, in 2019, the global prevalence of protein deficiency was estimated to affect approximately 147 million people, resulting in about 212,000 deaths. Between 1990 and 2021, the components of food products related to protein deficiency had a significant impact on age-related disease rates (54.9%) and mortality rates (72.2%). During this period, the rate of protecting consumers' rights (DALY) decreased to 51.9%. Regional disparities and threats of protein deficiency are particularly evident in areas with low socio-demographic indexes (SDI), especially in many countries of South Asia and Africa. In 2021, South Asia had the highest intensity of people suffering from protein deficiency. The reasons and impacts include food shortages, unbalanced diets, economic constraints, and insufficient consumption of animal protein products. The negative consequences include slowed growth (especially in children), weakened immune systems, rapid muscle mass loss in the elderly, lower quality of life, and the development of various blood, bone, and skin diseases.

Currently, scientific research is being conducted to address these issues. Over the past three years, global trends have been moving in a downward direction, but the protein problem persists in low-income regions. In 2024–2025, the situation is expected to worsen due to rising food prices and climate change. In the future, to meet protein needs, there is an increasing focus on alternative protein sources such as animal tissues, plant samples, microbial biomass, and protein-rich substitutes (e.g., insects, microalgae, synthetic proteins).

Literature Review

Collagen is an important structural component in the extracellular matrix and is one of the most widely distributed proteins in the animal kingdom. It is crucial for the shape, structure, and mechanical properties of tissues that aid in structural formation. Collagen has been known to be used in various fields such as food, medicine, and others since ancient times. A prominent example is "catgut", sutures made from the cut and cleaned submucosa of sheep intestines, which are fully absorbable by the body (British Standard 757: 1975). Despite the possibility of allergic reactions like unwanted immune responses to xenotransplants, collagen-based biomaterials have been processed into different morphologies and are currently widely used as barrier or filler materials in reconstructive surgery (Buljan, J., Reich, G. & Ludvik J., 2000).

Animal by-products, particularly those from meat supply chains and the residual materials such as skin, bones, hooves, intestines, and other animal organs, are rich in collagen proteins and are widely used in food industry product manufacturing (Dubois, M., 1992; Ericson, C.M., 1993). However, processing these by-products can be a complex technological task and might lead to low-quality protein samples, such as collagen with low nutritional value (Gault, N. F. S. & Lawrie, R. A., 1980).

The largest volume of waste in skin processing comes from bovine hides. This large base of raw material is sufficient for collagen extraction. However, from an environmental standpoint, such production poses challenges, as the residues left after protein extraction can lead to ecological problems. According to scientific literature, 40–60% of the protein content may remain in these residues, which is difficult to process and extract in industry (Dubois, M., 1992; Eduard Roether, Darmstadt, Germany. Hwang, K. T. & Regenstein, J. M., 1993).

Collagen extraction through partial degradation (hydrolysis) results in fewer protein residues. This process is also suitable for processing small amounts of collagen waste such as skin scraps, short and long fibers, bones, and others. Protein extraction from by-products of the meat industry (heart, kidneys, liver, lungs, stomach, and intestines) can be achieved by dissolving at temperatures not exceeding 60 °C under mild alkaline conditions. Protein hydrolysates are prepared through enzymatic (pepsin, papain, neutralase, or alcalase) treatment of degreased tissues at 50 °C. The yield of soluble hydrolysates is very high (up to 85%), and the resulting protein hydrolysates show no significant loss of amino acids (Gault, N. F. S. & Lawrie, R. A., 1980; Langmaier, F., Mladek, M., Kolomaznik, K. & Maly, A., 2002 a).

Under enzymatic reaction conditions, hydrolysis leads to partial breakdown of collagen, resulting in low-solubility collagen with an average molecular mass of 300 kDa. This protein mixture can be used as a component in cosmetics (moisturizing films regulating the water content in skin). Collagen preparations, collagen fibers, or hydrolysates can also be successfully used as carriers for food or cosmetic supplements (British Standard 757: 1975; Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J., 2011).

Deep processing of collagen is typical in gelatin production, where the protein molecule is broken down to a molecular mass of 220 kDa. This collagen is not only used in gelatin production but is also increasingly added to food products as a thickening agent or "flavor extender" and is used in pharmaceuticals, food supplements, and as a biologically degradable material for capsule coating in cosmetics (Langmaier, F., Mladek, M., Kolomaznik, K. & Maly, A., 2002a; Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J., 2011).

The main goal of this article is to analyze the composition of collagen from bovine skin using modern methods and to organize scientific research aimed at improving its industrial applications

Methods

The Lowry method is the most widely used and highly sensitive technique among those used to determine protein content. It is based on two reactions: the Biuret reaction and the colorimetric reaction with Folin's reagent, which specifically interacts with tyrosine and cysteine residues.

After preparing 5 series of standard solutions from the water extracts of collagen protein samples, 1 ml of each solution is taken and 4 ml of solution A and B mixture is added. The liquids are mixed thoroughly using a vortex shaker (Vortex-Dlab-MX-S) and left at room temperature for 10 minutes. Then, 0.4 ml of Folin's reagent is added using an automatic pipette, and the mixture is allowed to react for 20–30 minutes to form the color. Afterward, the optical density is measured at a wavelength of 670 nm using a UV–Vis 1900i spectrophotometer.

Determination of Total Protein Content. The method involves determining nitrogen content by Kjeldahl's method, followed by conversion to protein content. The essence of the method consists of digesting the organic substance of the sample with boiling concentrated sulfuric acid to form ammonium salts, converting ammonia into ammonium, distilling it into an acid solution, quantitatively determining ammonia using a titrimetric method, and calculating the nitrogen content in the examined material.

A precise sample of the ground homogenized sample for analysis was weighed in a test tube with an error of no more than 0.1%. The sample was quantitatively transferred into a Kjeldahl flask. The subsequent experiments were conducted according to the methodological instructions (Methods of control. 2004).

Processing of Results: The mass fraction of nitrogen (X) in the sample was calculated as a percentage of its mass during the distillation of ammonia into sulfuric acid using the formula.

$$X = \frac{(V_1 - V_0) \times K \times 0.0014 \times 100}{M}$$

Volume V_0 – the volume of the 0.1 mol/L sodium hydroxide solution used to titrate the 0.05 mol/L sulfuric acid in the control experiment, in milliliters (ml).

Volume V_1 – the volume of the 0.1 mol/L sodium hydroxide solution used to titrate the sulfuric acid in the sample solution, in milliliters (ml).

K – correction factor for the titration of the 0.1 mol/L sodium hydroxide solution.

0.0014 – the amount of nitrogen equivalent to 1 ml of 0.05 mol/L sulfuric acid solution.

M – the mass of the sample in grams (Steven A., Cohen Daviel J., 1988).

Free Amino Acid Isolation. Precipitation of proteins and peptides from the aqueous extract of the samples was conducted in centrifuge tubes. For this, 1 ml of the sample was mixed with 1 ml (accurate volume) of 20% trichloroacetic acid (TCA). After 10 minutes, the precipitate was separated by centrifugation at 8000 rpm for 15 minutes. Then, 0.1 ml of the supernatant was lyophilized. The hydrolysate was concentrated, and the dry residue was dissolved in a mixture of triethylamine-acetonitrile-water (1:7:1) and dried. This operation was repeated twice for neutralizing the acid. The phenylthiocarbamyl derivatives (PTC) of amino acids were obtained by reacting with phenylthiocyanate according to the method of Steven A. and Cohen Daviel. Identification of the amino acid derivatives was performed using HPLC.

HPLC conditions: Chromatograph Agilent Technologies 1200 with a DAD detector, 75x4.6 mm Discovery HS C18 column. Solvent A: 0.14 M sodium acetate + 0.05% TEA, pH 6.4; Solvent B: CH3CN. Flow rate: 1.2 ml/min, absorption at 269 nm. Gradient %B/min: 1–6%/0–2.5 min; 6–30%/2.5–40 min; 30–60%/40.1–45 min; 60–60%/45.1–50 min; 60–0%/50.1–55 min

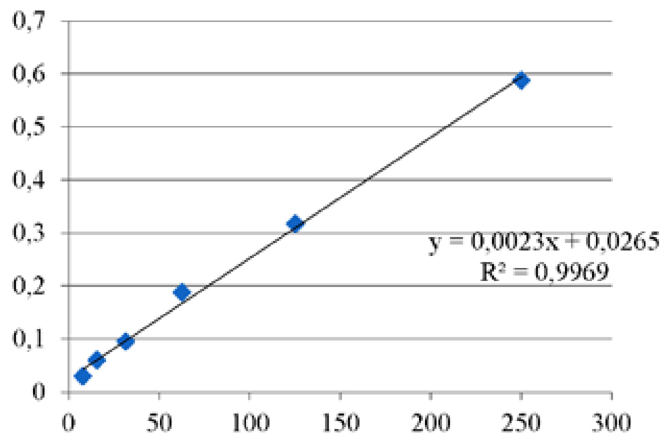
Results and Discussion

The text you're referring to describes the method for determining soluble protein content, specifically focusing on a calibration curve for albumin in the range of 0.025 mg to 0.3 mg. This curve is used to calculate the amount of soluble proteins in a sample based on the obtained formula. This method is commonly used for protein analysis, where the albumin standard helps quantify proteins in complex mixtures such as the collagen extracted from animal tissues, as described in your document.

Figure 1. *UB – Spectroscopic analysis process*

A graph was created after performing the Lowry reaction with 5 series of standard protein solutions. The optical density was plotted on the ordinate axis, and the protein concentration was plotted on the abscissa axis, with points marked according to the FEK indicator. The standard used was egg albumin that had been recrystallized four times (Methods of control. 2004).

Figure 2. *Calibration curve for determining the amount of water-soluble proteins in the fruit of Maclura*



To analyze the amount of water-soluble proteins in the sample extracted from the bovine hide, Brazilian-produced collagen protein was selected for comparative study. The spectral average light absorption errors of the protein quantities were determined, and the following formula was used to calculate the amount of water-soluble protein in the composition:

For the Uzbekistan sample, the protein calculation in Sample 1 is based on the light absorption coefficient.

$$y = 0{,}0023x + 0{,}0265$$
$$R^2 = 0{,}9969$$

"Overall average in the sample (average result of 3 repeated measurements):" 0,516

Calculation: 0,516 = 0,0023x + 0,0265

0,516–0,0265 + 0,4895

0,4895=0,0023x

$$x = \frac{0{,}4895}{0{,}0023} = 212{,}8\,mkg\,/\,mg$$

Taking into account that the initial obtained amount is 250 µg, the final calculation is performed as follows:

212,8 * 4 = 851,2

"The amount of water-soluble protein in the first sample was determined to be 851.2 µg/ml in the protein solution with a volume of 1 ml.

The amount of water-soluble protein in the Brazilian sample, Sample 2, was determined as follows:

Protein calculation based on the light absorption coefficient.":

$$y = 0{,}0023x + 0{,}0265$$
$$R^2 = 0{,}9969$$

"Average in the sample (average result of 3 repeated measurements).: 0,408

Calculation: 0,483 = 0,0023x + 0,0265

0,408–0,0265 + 0,3815

0,4565=0,0023x

$$x = \frac{0{,}4565}{0{,}0023} = 198{,}47\,mkg\,/\,mg$$

"Considering that the initial amount was 250 µg, the final calculation is as follows:

198,47 * 4 = 793,91

The amount of water-soluble protein in the second sample was calculated to be 793.91 µg/ml in a 1 ml solution.

According to the protein content measured by the Lowry method and analyzed using UV-spectroscopy, the water-soluble protein amounts in both samples were found to be similar. Considering that both solutions were prepared in a 1:1 ratio, it was determined that the first sample contained 851.2 µg/ml, while the second sample contained 793.91 µg/ml of water-soluble protein.

In numerous literature sources, the water-soluble portion of collagen protein is reported to range from 0.8 to 1.3 mg/ml. The amounts of water-soluble proteins in both analyzed samples are consistent with the data presented in the literature, and the variation in protein amounts can be attributed to the method of isolation and the quality of the sample.

The total protein content was analyzed using the Kjeldahl method. The final test result was calculated as the arithmetic average of five parallel test results. The obtained results were analyzed up to three decimal places, with two decimals used for rounding

$$X_3 = \frac{X_1 \times 100}{100 - W}$$

X_1 – the mass fraction of nitrogen in the test sample, %;

W – the moisture content of the test sample, %.

The mass fraction of protein (Y) was calculated in percent using the following formula:

$$Y = K\,CHX$$

"The nitrogen-to-protein conversion coefficient: with the average lipid content, is 6.38."

Table 1. *Nitrogen Content and Protein Percentage in Sample Compositions (%)*

No.	Samples	N (%)	protein (%)
1.	Uzbekistan	12.2	77.68
2.	Brazil	14.73	93.82

The difference in nitrogen and protein content in the samples can be clearly seen through the following diagram based on the table data.

The total protein content in the sample extracted from cattle hide was analyzed, using collagen protein produced in Brazil as a reference standard for comparison.

Both samples extracted from cattle hide were analyzed in the same way, with the nitrogen content of the Uzbekistan sample determined to be 12.2%. It was calculated that the total protein content in this sample was

77.68%. In comparison, the nitrogen content of the Brazil sample was found to be 14.73%, and the total protein content in this sample was calculated to be 93.82%. Chromatographic analysis was conducted to determine the amount of free amino acids in the extracted protein from the cattle hide. The resulting chromatogram was compared to a standard chromatogram developed for amino acids, and the peaks observed in the chromatogram corresponded to specific amino acids at specific time intervals, confirming the consistency of the analysis.

Figure 3. *Results of protein analysis using the Kjeldahl method*

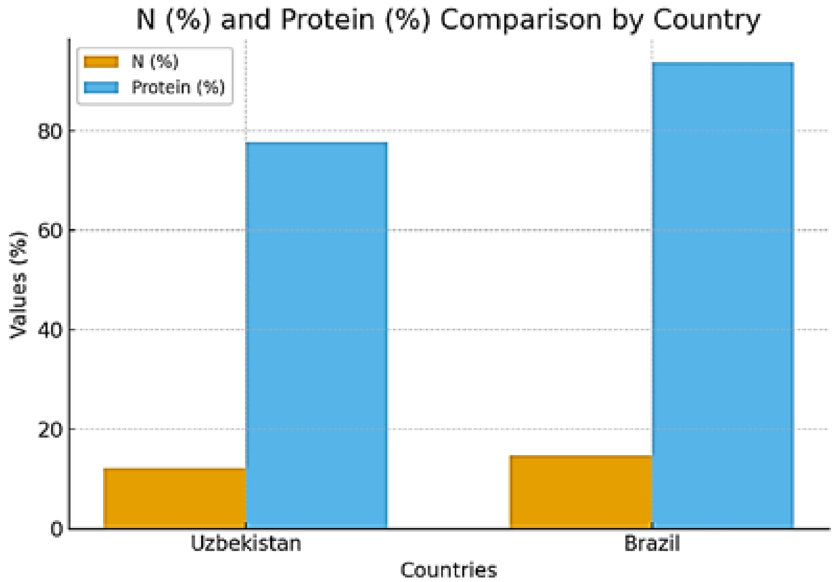
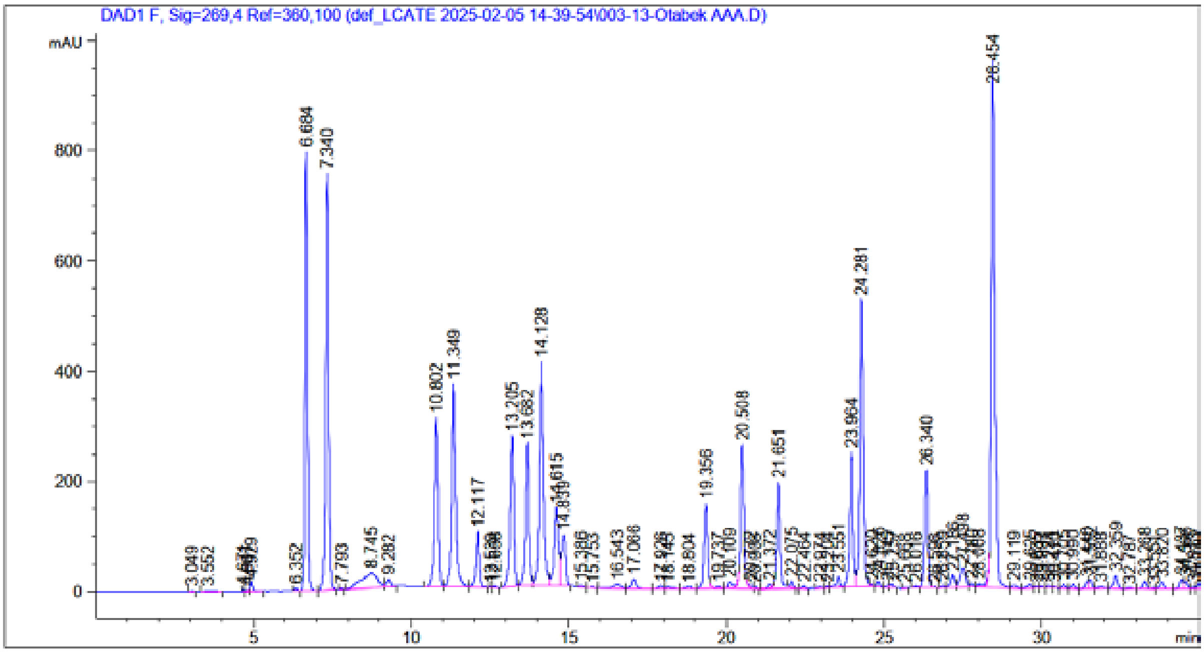


Figure 4. *Chromatogram of free amino acids in the collagen proteins extracted from bovine hide (HPLC analysis)*



This research work, which focuses on the quality and quantity analysis of amino acids in collagen proteins, is a result of using High-Performance Liquid Chromatography (HPLC). The exact quantities of amino acids are provided in Table 2.

If we pay attention to the data in the table, we can see that there is a distinct difference in the amino acid content between the two samples. This can be explained by the fact that the Brazilian sample was processed using a two-stage enzymatic hydrolysis method. As for the cause of the high level of asparagine acid in the Uzbekistan sample (107.537 mg/g), this can be attributed to the acidic environment generated during the process. Similarly, a significant difference in cysteine content is observed, where the Brazilian sample contains a considerably higher amount (258.3738 mg/g). Other statistical results from the study can be seen in the table above. The final conclusion of this research suggests that enzymatic approaches lead to an increase in the amount of free amino acids in the collagen protein.

Table 2. *Amino acid content in the samples*

Names of Amino Acids	Uzbekistan	Brazil
	Concentration mg/g	
Asparagine	107.537	26.33114
Glutamine	77.273	23.2169
Serine	16.329	41.8192
Glycine	35.172	14.02153
Asparagine	0	0
Glutamine	0	0
Cysteine	23.959	258.3738
Threonine	19.699	8.884653
Arginine	12.842	13.41166
Alanine	15.097	39.24491
Proline	13.558	60.50574
Tyrosine	9.695	15.05598
Valine	22.622	57.44512
Methionine	23.564	3.748216
Histidine	7.464	14.10037
Isoleucine	21.685	26.37163
Leucine	52.709	49.4889
Tryptophan	0	0
Phenylalanine	13.477	16.94842
Lysine	48.553	10.16152
Total	521.235	679.1297

Conclusion:

Through the extraction of collagen protein and its subsequent physical, chemical, and biochemical analyses, it is possible to study the full bio-functions of the protein, which can improve its significance in the production of food and other industrial products. Based on the results of the study, it can be concluded that the enzymatic approach in collagen extraction increases the quantity of free amino acids in the protein.

In the analysis of water-soluble protein content using the Lowry method, considering that each solution was prepared in a 1:1 ratio, the amount of water-soluble protein in sample 1 was found to be 851.2 μg/mg, and in sample 2 it was 793.91 μg/mg. Several literature sources show varying amounts of soluble collagen protein in the range of 0.8–1.3 mg/ml. The obtained results for water-soluble proteins correspond with those values found in the literature, and also demonstrated the changes in the protein's properties depending on the extraction method and the quality of the sample.

Both samples extracted from bovine skin were analyzed in the same manner, with the Uzbekistan sample showing a nitrogen content of 12.2%, and the total protein content in the sample being calculated at 77.68%. The nitrogen content in the Brazilian sample was found to be 14.73%, with the total protein content calculated to be 93.82%.

Using High-Performance Liquid Chromatography (HPLC), this study also determined the quality and quantity of amino acids in the collagen protein. Based on the findings, it can be concluded that the enzymatic approach in collagen extraction increases the amount of free amino acids within the collagen protein.

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