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Waste-free technology for the production of hydrolyzed beef collagen from leather production waste

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ABSTRACT
The problem of both the processing and the rational use of leather production waste in recent years has become relevant throughout the world. This is explained by the fact that a large amount of waste (30-50% of the mass of raw materials) is generated in manufacturing leather. It contains up to 50% of protein substances and many other by-products. It is proposed that cattle hides be used as the primary raw material to obtain collagen proteins used in the food industry. The research aims to develop a technology for processing raw split leather. The development makes recycling up to 50% of leather production waste possible. Split leather waste isolates collagen materials suitable for the food industry. Protein obtained from split leather due to enzymolysis by proteolytic enzymes is subsequently subjected to mechanical and thermal processing. When preparing raw materials, cowhides are treated with a solution of hydrogen peroxide at a concentration of 1.0-3.0% and alkali at a concentration of 1.5-3.0% at a temperature of 20 ± 5 °C for 10-20 hours. Studies have been conducted to confirm that hydrolyzed beef collagen meets the requirements of regulatory and technological norms of food safety. The object of the study is the technology for producing hydrolyzed beef collagen obtained by enzymatically processing the cowhide split. For the enzymatic degradation of collagen tissues, collagenase and alkaline protease enzymes were used.

Keywords: Collagen, Hydrolyzed, Enzymes, Split leather.
Article type: Note.

INTRODUCTION
As part of the creation of a waste-free, environmentally friendly technology for processing collagen-containing raw materials, solving the problem of both the processing and the rational use of collagen-containing waste from the meat and leather industry is an urgent task today (Kurchaeva 2018; Mezenova *et al.* 2020; Dunchenko *et al.* 2021). The new technologies for producing collagen involve studying the structural features of collagens of the dermal layer in various topographic areas of the cowhide (Ahmad *et al.* 2023). The cowhide contains about 50% of all collagen in the animal's body (Gómez-Guillén *et al.* 2011). The mass fraction of collagen in the cowhide is 63.7-80% of dry matter. The most significant proportion of collagen, 85-95% of the dry matter weight occurs in the middle layers of the dermis, 57-65% in the upper part of the papillary layer, and 85-96% in the lower part of the reticular layer. To use collagen in food production, fermentation must first convert it into a soluble state (Shukurlu *et al.* 2022). The use of hydrolytic decomposition of collagen-containing raw materials due to the influence of proteolytic enzymes of collagenase specificity is promising (Anokhova *et al.* 2020).

MATERIALS AND METHODS
The aim of the study is the technology for producing hydrolyzed beef collagen obtained by enzymatically processing the split of cowhide. For the enzymatic degradation of collagen tissues, collagenase and alkaline protease were used. The prepared mixture was heated to 160 °C, cooled, and then fermented for 80 minutes. At the end of enzymolysis, the mixture was heated to a temperature of 100 ± 2 °C, and the obtained broth was filtered,

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evaporated, and dried. Organoleptic and physicochemical parameters were determined in the resulting hydrolyzate. Safety studies have also been conducted for animal connective tissue proteins.

RESULTS AND DISCUSSION

Developing new technologies based on the maximum and rational use of collagen requires a more detailed and in-depth study of the structural features of collagens in the dermis of various topographic areas of the cowhide. Especially those that go to waste during the primary processing of raw hides and scraps of hides and leather formed in the tanning industry (Kurchaeva *et al.* 2018). To minimize the amount of waste, it is necessary to determine the source and volume of waste generated, its physicochemical properties, environmental friendliness or toxicity, and the possibility of recycling (Trukhachev *et al.* 2016; Shukurlu *et al.* 2020).

According to the existing classification, all waste can be divided into:

- untanned;
- tanned;
- fat-containing;
- other.

The most valuable are untanned wastes, which can be used to produce sausage casings, gelatin, feed additives, glue, medicines, and cosmetic products (Trukhachev *et al.* 2016; Suurs *et al.* 2020; Dunchenko *et al.* 2021). The production volume and range of these products are constantly expanding. Untanned waste includes hide split, split cuttings, flesh, and so forth, which can be used in various industries (Table 1).

Table 1. Waste and possibilities for its processing and disposal (own research).

No.	Type of waste	Compound	Directions for use/processing/disposal
1	Waste after contouring raw materials	Collagen, hair, fat	Glue, feed additives/heat treatment, biogas/landfill
2	Hair after dehairing	Keratins, fat	Feed additives, lanolin/composting, heat treatment, biogas/landfill
3	Flesh	Fat	Protein hydrolysates, fat/composting, biogas/landfill
4	Hide split	Collagen	Split leather, gelatin and glue, protein hydrolysates, sausage casings
5	Spent solutions after degreasing	Solvents, surfactants, fats	Solvent recycling, fats/heat treatment
6	Spent solutions after tanning	Chromium compounds	Recycling of chromium compounds
7	Split tanned	Collagen, chromium compounds	Split leather production, fiber materials, protein hydrolysates, fertilizers/composting, heat treatment/landfill
8	Chrome shavings and cuttings	Collagen, chromium compounds	Fiber materials, protein hydrolysates, fertilizers/composting, heat treatment/landfill
9	Leather scrap, cuttings	Collagen, chromium compounds, and other chemicals	Fiber materials, decorative materials/heat treatment/polygon
10	Wastewater treatment plant sludge	-	Fertilizers/composting, biogas, heat treatment/landfill

For the processing and food industries, methods for obtaining collagen proteins from processing waste of cowhides, primarily split, split cuttings, and flesh, are essential (Rasulova *et al.* 2024). Pork skins and fish waste are used to a lesser extent as collagen-containing raw materials (Yankovskaya *et al.* 2021; Dunchenko *et al.* 2022). First, the traditional technology of processing raw cowhides is aimed at producing tanned leather and is focused on obtaining materials from the top layers of the hides. This involves several operations involving removing non-commercial components from the feedstock: hair, split shavings and cuttings, flesh, and fat. At the same time, split leather and cuttings are considered valuable raw materials for producing collagen used in food. Flesh is used for these purposes to a lesser extent. (Antipova *et al.* 2015; Khudoiberdiev *et al.* 2023). Characteristics of the general chemical composition of the studied leather splits are given in Table 2.

Table 2. Characteristics of the general chemical composition of the studied leather splits (Antipova 2015; Khudoiberdiev 2023).

Raw material	Mass fraction of components (%) by weight of raw materials			
	Protein	Fat	Ash	Moisture content
Cattle leather split	23.4	1.10	0.90	74.0

When obtaining collagen materials for food purposes, the degree of the grinding of raw materials can be any and is limited only by technological feasibility. This versatility opens up a world of possibilities for innovative food products. Collagen, the foremost valuable raw material obtained from processing leather materials for food purposes, is truly a marvel of nature. Its triple helical structure, consisting of alpha-helical chains forming a stable protein form, provides strength and elasticity to connective tissues. This unique building element, with its high resistance to various physical and chemical influences, is an indispensable component for maintaining the body's structural integrity. Currently, 19 types of collagen have been characterized, each of which is present in different body tissues and performs unique functions (Ahmad *et al.* 2023). Type I collagen is the most common type of protein studied and makes up the bulk of fibrous tissues. It provides high strength and elasticity. Type I collagen is predominantly present in cartilage tissue and plays an important role in the elasticity and flexibility of joints. This type of collagen is also found in the eye's vitreous humor. The triple strand of type I collagen molecules are constructed from polypeptide chains, each including a repeating sequence G-X-Y, in which G is glycine, and X and Y are most often proline or hydroxyproline. The diameter of collagen fibers ranges from 50 to 200 nm. Fibrils located together form fibrous bundles with a diameter of 500 to 3000 nm (Rocha-Mendoza *et al.* 2007). The integrity of the collagen triple helix as the main secondary structure of various types of fibrous collagen can be determined by the relationship between the optical densities at 1235 and 1450 cm^{-1}. The ratio values for the denatured collagen approach are 0.5, and for intact structures, 1. It is assumed that this feature determines collagen's biological and mechanical properties (Gómez-Guillén *et al.* 2011). The three-dimensional structure of the collagen network varies significantly depending on its location in the body and its biological function (Litvinova *et al.* 2022; Yankovskaya *et al.* 2023). To determine the prospects for using collagens of various origins in food production, it is crucial to study and analyze the amino acid composition of various types of collagen-containing raw materials. This research is not just informative, but it also paves the way for future advancements in food science. The study conducted a comparative analysis of the amino acid composition of various collagen-containing raw materials and cattle split leather (Table 3).

Table 3. Amino acid composition of various types of collagen-containing raw materials (Litvinova 2022).

№	Raw material	Amino acid composition (%)								
		isoleucine	leucine	lysine	methionine	cysteine	phenylalanine	tyrosine	threonine	valine
1	Cattle split leather	2.01	3.99	3.52	0.80	0.82	1.75	0.98	2.06	2.36
2	Beef ears	2.1	4.2	4.2	0.3	0.4	1.5	1.3	2.2	3.4
3	Beef lips	3	5.7	6.4	0.7	0.5	1.6	0.6	3	3.6
4	Pork skin	2.4	4.7	5.9	0.5	0.7	0.4	0.6	1.78	1.9
5	Beef tendons	2.4	4.6	2.2	0.3	0.3	1.58	0.54	2.01	4.3
6	Bird skin	2	3.3	3.3	0.4	0.2	1.01	0.47	2.01	1.5
7	Bird heads	2.3	4.3	4.7	0.3	0.5	1.02	0.7	2.8	2.1
8	Bird legs	2	3.5	3.7	0.4	0.3	1.01	0.7	1.99	1.7
9	Bird comb	1.8	3.5	4.1	0.3	0.27	1.03	0.8	1.87	1.7

As shown in Table 3, collagen substances of different origins have the same amino acid composition but are characterized by different contents of particular amino acids. The amino acid composition of cattle split leather is superior to that of other raw materials in terms of phenylalanine, cysteine, and methionine. The chemical properties of collagen can also vary depending on the source. Hydrolyzed beef collagen is a protein formed by enzymatically processing the split of cowhide, followed by mechanical and heat treatment (Rebezov *et al.* 2011). This non-gelling product dissolves quickly in cold and hot water to form a clear solution. In addition, the composition and sequence of amino acids in collagen are unique: about 35% are glycine residues, and approximately 11% are alanine residues, which is unusually high for most known proteins. Another characteristic feature of hydrolyzed collagen is the high proportion of proline and 4-hydroxyproline, an amino acid rarely found in other polymers except collagen and elastin. Thus, hydrolyzed beef collagen is a unique protein that can be used for food purposes (Mofieed 2020; León-López *et al.* 2020; Kıyak *et al.* 2024). The authors proposed a technology for producing hydrolyzed collagen by enzymolysis. This process is a complex system that includes advanced technologies and biochemical methods. It aims to obtain high-quality products with unique properties with a wide range of applications in various sectors of the economy. The technological scheme for producing hydrolyzed beef collagen includes several stages, from preparing raw materials to packaging and selling the finished product (Fig. 1). The production parameters of the technology for producing hydrolyzed beef collagen are as follows. When preparing raw materials, cowhides are treated with a solution of hydrogen peroxide at a concentration of 1.0-3.0% and alkali at a concentration of 1.5-3.0% at a temperature of 20 ± 5 °C for 10-20 hours. Upon completion, they are neutralized with acid to pH 5.5-6.

Preparation of raw materials (peroxide-alkaline pretreatment, washing with water)

Consistent grinding of raw materials

Adding water, heating the mixture to 100 ± 5 °C

Heat treatment at 160 ± 5 °C

Filtration and cooling of the mixture to 60 ± 5 °C

Fermentation (80 min)

Heating and separation of the mixture 100 ± 5 °C

Broth filtration

Evaporation and drying

Packaging, storage

Fig. 1. Processing flow diagram for producing hydrolyzed beef collagen (Current study).

Then, they follow two stages of grinding the raw materials, first on a grinder with a 25 and 15 mm grid diameter, then on a grinder with 10 and 6 mm. Water is added to the resulting mixture and heated to a temperature of 100 ± 5 °C, followed by thermization in digesters at 160 ± 5 °C. The heated mixture is separated in separators and cooled to 60 ± 5 °C. At the next step, the enzymes are added to the container with the cooled liquid fraction, kept for 80 minutes, and heated in a tubular heat exchanger to 100 ± 5 °C. The hot mixture undergoes another step of separation through a separator, after which the resulting broth is filtered and subjected to evaporation and drying. The finished product is packaged in plastic bags and sent for storage. Hydrolyzed beef collagen must meet regulatory food safety requirements. The organoleptic and physicochemical parameters of beef collagen were determined. Hydrolyzed beef collagen has a powdery texture. To assess quality indicators, 100 mL water at 20 ± 2 °C was added per 1 gram of product and allowed to stand for 1 minute. The results are presented in Table 4. Hydrolyzed beef collagen complies with the standards established in TR CU 021/2011 “On the safety of food products” (Table 5) regarding microbiological and hygienic indicators. Collagen must be stored in specific conditions to maintain its quality. These conditions include a dry, ventilated, darkened room with a temperature not exceeding 25 °C and a relative humidity of less than 70%. When stored correctly, collagen can maintain its quality for up to 36 months.

Table 4. Quality and safety requirements for hydrolyzed beef collagen (Current study).

Indicators	**Research results**
Appearance	Homogeneous crumbly mass without dense lumps that do not crumble when pressed
Color	White to light yellow
Taste and smell	Corresponds to the raw materials from which they are made, without any foreign taste or smell
Mass fraction of protein in dry matter (%), not less	90.0
Mass fraction of fat (%), not more	2.0
Mass fraction of moisture (%), not more	6.0
Mass fraction of collagen to total protein mass (%), not less	25.0

Table 5. Microbiological and hygienic indicators of hydrolyzed beef collagen (Current study).

Indicators	**Research results**
KMAFAnM (CFU/g)	Not more than 2.5×10^4
Coliform bacteria	Not allowed in product weight of 1.0
Pathogenic, including *Salmonella*	Not allowed in product weight of 25
Molds	Not more than 100
Listeria monocytogenes	Not allowed in product weight of 25
Toxic elements including lead, arsenic, cadmium, mercury (mg kg^{-1}), not more	In terms of the original product
HCH (isomers; mg kg^{-1}), not more	0.1
DDT and its metabolites (mg kg^{-1}), not more	0.1
Levomycetin (chloramphenicol; mg kg^{-1}), not more	Not allowed (< 0.0003 mg kg^{-1})
Tetracycline group (mg kg^{-1}), not more	Not allowed (< 0.01 mg kg^{-1})
Bacitracin (mg kg^{-1}), not more	Not allowed (< 0.02 mg kg^{-1})
Cesium-137 (Bq kg^{-1}), not more	200

The materials used, including auxiliary materials, consumer and transport packaging, are accompanied by documentation certifying their safety. Packaging materials must comply with the requirements of TR CU 005/2011 “On the safety of food packaging” and be:

- allowed for contact with food;
- ensure the safety of products during storage, transportation, and sale during the established shelf life of the product, subject to the specified conditions;
- do not change the quality indicators and organoleptic properties of the product.

When transporting collagen products, vehicles must be dry, clean, and free of foreign odors and grain pests. This precaution helps to prevent any potential contamination and ensures the quality of the products.

CONCLUSION

The studies conducted show that collagenase and alkaline protease can be used in the enzymolysis of F-collagen-containing raw materials (cattle split leather) with a high mass fraction of connective tissue proteins. This technology makes it possible to isolate purified collagen fractions from by-products and tannery waste. As a result of the use of proteolytic enzymes, hydrolyzed beef collagen was obtained in the form of a powder with a homogeneous, elastic texture and neutral odor, which can be used for food purposes.

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