ТЕХНІЧНІ НАУКИ

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BIOTECHNOLOGICAL BASICS AND PRACTICAL METHODS OF PROCESSING COLLAGEN-CONTAINING RAW MATERIALS FROM POULTRY

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Subject. *The resources of collagen-containing raw materials are significant. These also include processed poultry products, which are used only for feed purposes or are generally disposed of. Therefore, it is necessary to use special methods for processing these collagen-containing raw materials, in particular chicken legs, to obtain a protein product with appropriate functional and technological parameters. Today there are many ways to influence collagen-containing raw materials and obtain collagen preparations for use in the food industry, cosmetology, medicine, etc.* ***Purpose****. The purpose of the work is to scientifically substantiate and experimentally confirm the possibility of obtaining high-quality protein products from collagen-containing raw materials (including chicken legs) by using various processing* methods. ***Methods****. Comprehensive standard and recognized methods for determining the physical, chemical and mechanical properties of meat products.* ***Results.*** A study was *carried out of the chemical composition of chicken legs in comparison with mechanically deboned poultry meat and grade II beef. The results of the study indicate the possibility of using this raw material as an integral part of the formulations of meat products. Studies were carried out on two groups of samples – collagen-containing ground meat and collagen-containing gel; the results of the collagen content in the samples for different periods of cooking time practically do not change, which confirms the possibility of using collagen-containing products as stabilizers of meat systems in the production of meat products. Collagen-containing ground meat and collagen-containing gel in an amount of 20% in minced chicken meat influences the quality parameters of the finished products. This is confirmed by the results of measuring shear force, critical shear stress, elasticity and consistency of the products. It can be concluded that the use of chicken legs for the production of meat products is possible only with their preliminary processing, namely the separation of flesh tissues from the bones. The method of obtaining these products determines the directions for further their use.* ***Scope of results.*** *Enterprises for processing poultry and producing meat products.*

Key words: *collagen-containing products, biotechnological processing, secondary poultry raw materials, poultry meat and bone by-products, oxyproline, collagen.*

БІОТЕХНОЛОГІЧНІ ОСНОВИ ТА ПРАКТИЧНІ СПОСОБИ ОБРОБЛЕННЯ КОЛАГЕНОВМІСНОЇ СИРОВИНИ З ПТИЦІ

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Предмет*. Ресурси колагеновмісної сировини доволі значні. До них відносяться і продукти вторинної переробки птиці, які використовуються тільки на кормові цілі або взагалі утилізуються. Тому необхідно застосовувати спеціальні методи обробки цієї колагенвмісної сировини, зокрема ніг курей, для отримання білкового продукту з належними функціонально-технологічними характеристиками. Сьогодні існує багато способів впливу на колагенвмісну сировину та отримання колагенових препаратів для використання в харчовій промисловості, косметології, медицині тощо.* ***Мета****. Метою роботи є наукове обґрунтування та експериментальне підтвердження можливості отримання високоякісних білкових продуктів з колагенвмісної сировини (зокрема з ніг курей) шляхом залучення різних способів її обробки.* ***Методи.*** *Сучасні стандартні та загальновизнані методи визначання фізико-хімічних та структурно-механічних властивостей м'ясних продуктів.* ***Результати.*** *Проведене дослідження хімічного складу вихідної сировини (ноги курей) в порівнянні з м'ясом птиці механічного обвалювання та яловичини ІІ сорту. Результати дослідження вказують на можливість використана даної сировини у якості складової частини рецептур м'ясних виробів. Проведено дослідження двох груп зразків – колагенвмісного фаршу та колагенвмісного гелю, результати вмісту колагену в зразках на протязі різних проміжків часуваріння практично не змінюються, що підтверджує можливість використання колагенвмісних продуктів у якості стабілізаторів м'ясних систем при виробництві м'ясних виробів. Колагенвмісний фарш і колагенвмісний гель у кількості 20% у складі фаршу з м'яса курей впливає на якісні характеристики готових продуктів. Це підтверджують результати вимірювання показників зусилля зрізу, граничної напруги зсуву, еластичності та консистенції виробів. Можна зробити висновки, що використання ніг курей для виробництва м'ясних виробів можливо тільки за умови їх попередньої обробки, а саме відділення м'якушевих тканин від кісток. Спосіб отримання цих продуктів визначає напрями їх подальшого використання.* ***Сфера застосування результатів.*** *Підприємства з переробки птиці та виробництва м'ясних продуктів.*

Ключові слова: *колагенвмісні продукти, біотехнологічне оброблення, вторинна сировина з птиці, м'ясокісткові субпродукти птиці, оксипролін, колаген.*

Formulation of the problem. Proteins are the most abundant organic macromolecules in living cells, and in humans. They serve as structural components, enzymes, hormones, messengers, transporters and components of the immune system, among others. They are built from the same set of 20 amino acids, of which nine cannot be synthesized in the body, which is why they are considered essential amino acids [1]. Adequate protein intake is crucial for the optimal functioning of the body. Depending on their source, proteins may be of animal or plant origin. The former are an excellent source of zinc, heme iron, B vitamins and essential amino acids, are more easily digested and provide all essential amino acids [2].

Currently the term "collagen" is used to classify a family of 27 proteins that are facially found in the proportions of the body's connective tissues. It is the most important compound in skin tissue and is an important structural element in multicellular organisms. Collagen is a fibrous protein found throughout the animal kingdom, where it contains peptide chains of the amino acids glycine, proline, lysine, hydroxylysine, hydroxyproline and alanine [3, 4]. Theoretically, collagen has about 30% glycine, 12% proline, 11% allinine, 10% hydroxyproline, 1% hydroxylysine and small amounts of polar and charged amino acids [4, 5]. Its chain is organized parallel to an axis, developing collagen fibers, which can provide resistance and elasticity to the present structure. The main function of the substance is to contribute to the structural integrity of the extracellular matrix or to help fix cells in the matrix. [4, 6].

The effective use of collagen protein depends on its quality characteristics, which change under the influence of various factors [7, 8]. Unfortunately, the lack of systematic scientifically substantiated data regarding the influence of various methods of processing collagen-containing raw materials on improving the functional and technological properties of collagen protein hinders the increase in the volume of procurement of such raw materials on an industrial scale and its rational use. The very study of the important properties of secondary raw materials and the influence of different processing methods on it constitutes the content of this work.

The composition of individual types of connective tissue is approximately the same: water, proteins (including collagen, elastin), lipids, minerals, mucopolysaccharides, etc. However, in quantitative terms, the content of these substances in specific types of connective tissue is different. There are especially many minerals in bones, mucopolysaccharides in cartilage, and collagen in rough connective tissue. Collagen is the most abundant protein. Using electron microscopy, it was established that collagen fibers are built from fibrils, which in turn consist of collagen macromolecules – tropocollagen, containing three polypeptide chains that create a helix [9, 10].

Collagen fibers are characterized by significant strength. The insolubility and stability of collagen is explained by the presence of cross-links in its molecule, occurring both in tropocalagenes (intramolecular bonds) and between individual tropocollagen units (intermolecular bonds) (Fig. 1.).

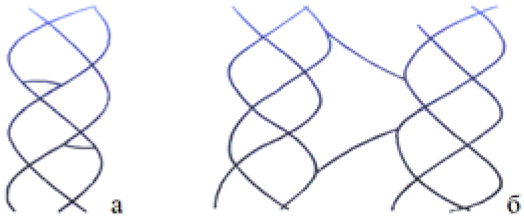

Fig. 1. Cross-links in a collagen molecule:
a – intramolecular; b – intermolecular

Hydrogen bonds are created between the CO groups of peptide bonds and the OH groups of hydroxyproline; in addition, other types of bonds can arise.

Collagen in water can swell greatly, its mass increases by 1.5–2.0 times, that is, it absorbs about 200 g of water per 100 g of dry protein, of which about 70 g is hydration water. Part of the hydration water is bound by the polar groups of the collagen side chains. The attachment of water molecules to these hydration centers leads to a weakening of the bonds between the polypeptide chains contained in the structure of the collagen fiber precisely as a result of the interaction of the side chains. However, these bonds do not completely disappear due to the fact that the hydrated groups remain fixed relative to each other [11].

With further content of hydrated collagen in water, additional water absorption and swelling of the collagen occurs. The thickness of collagen fibers increases by an average of 25%. The total volume of the collagen sample increases 2–3 times.

As a result of the weakening of the bonds between the main chains in the three-dimensional structure of collagen caused by the penetration of water into it, the strength of collagen fibers decreases by 3–4 times. As the temperature rises, weakened ligaments begin to break due to increased mobility of polypeptide chains – this is the process of peptization. Thus, watering collagen fibers promotes their disintegration into individual polypeptide chains upon further heating [11, 12]. Next, the structural ligaments collagen protein are completely destroyed. After heat treatment at denaturation temperature, collagen is transformed with the formation of compounds of lower molecular weight: gelatin, gelatin, glutin, which after cooling form strong gels [13–15].

The transition of collagen to glutin is accelerated not only by heating, but also by an acidic environment. The functional and technological properties of collagen-containing proteins significantly depend on the pH of their aqueous solutions. It is known that the isoelectric point of muscle proteins is at a pH of 5.0 and below (acidic environment), while the isoelectric point of collagen-containing proteins is in the range from 6.0 to 6.75. A shift in pH to the alkaline or acidic side leads to a change in the functional properties of the protein. For example, at pH=3, the denaturation temperature (Td) of collagen decreases to (35-40)°C; at pH=1, Td is 30°C [16, 17]. The pH level also affects the solubility of collagen in water. It was proven that the percentage of soluble collagen was lower at pH=7.4 than at pH=5.6. Of course, it is not only the pH level that affects the performance of gels, but also other ingredients included in the collagen protein. Therefore, it is advisable to consider the influence of some impurities on the functional and technological characteristics of collagen-containing proteins [16–18].

Collagen has the ability to react with both acids and alkalis. In animal proteins, the carriers of acidic functions are amino acid residues containing carboxyl groups; carriers of alkaline functions are amino acid residues with amino groups. The study of the interaction of acids and alkalis with collagen-containing protein is individual in nature due to the fact that the protein structure contains different functional groups [12]. In addition to acids and alkalis, salts have a certain effect on collagen protein. In salt solutions, it undergoes modifications, depending mainly on the concentration and type of anion and cation.

It has been established that collagen absorbs predominantly chlorine ions from a sodium chloride solution due to the fact that most proteins are characterized by pronounced acidic properties. Sodium chloride reduces the strength of collagen protein gels due to the fact that it is able to disable hydrophobic and hydrogen bonds [9, 16].

The degree of exposure to salts can be divided into 3 groups:

- the first group includes rhodanates, iodides, chlorates, barium, calcium, magnesium and lithium salts – compounds that cause severe swelling of collagen at any concentration;
- the second group includes sodium chloride and other salts that do not significantly affect the hydration properties of collagen;
- the third group includes salts with a great dehydrating effect. These are, for example, sulfates, thiosulfates, acetates and carbonates.

In the case of the action of salts of the first group, the collagen fibers swell, and the denaturation temperature decreases sharply; collagen is able to denature at low temperatures. When collagen interacts with salts of the second group, slight swelling occurs at low salt concentrations; in the case of using high concentrations, slight dehydration occurs [17, 18].

To isolate collagen and obtain protein products with increased availability of collagen from collagen-containing raw materials, various processing methods are used – mechanical, physical, chemical, etc. Each of these methods allows you to obtain both a soluble form of collagen and denatured collagen. Each method has its advantages and disadvantages. During hydrothermal treatment, collagen denatures with disruption of the specific configuration of polypeptide chains and intra- and external molecular bonds [19]. Complete hydrolysis of collagen occurs at 126ºC for 3 hours. A promising way to disrupt the supramolecular structure of collagen is to dissolve it. The transition of tissues to a dissolved state is usually carried out through a combination of mechanical and chemical processes. There are several ways to disrupt the supermolecular structure of collagen while maintaining the structure and properties of tropocollagen. Among them is the method of alkali-salt treatment.

The method of alkali-salt treatment is the process of destruction of the supramolecular structure of collagen at high osmotic pressure of a solution of such a salt, the diffusion of which into the tissue is difficult due to the fact that osmotic pressure prevents the dispersion of collagen and its complete alkaline hydrolysis. Collagen does not pass into the alkaline salt solution, but is in a coagulated state. Solutions of sodium hydroxide, sodium chloride, sodium sulfate can be used [20–23].

Treatment of category II by-products with chemical compounds helps remove non-collagenous proteins and carbohydrates (mucopolysaccharides) from the raw material and loosen the structure. For processing, inorganic compounds approved for use in the food industry (NaOH and NaCl) are used. The addition of other neutral salts, for example sodium sulfate, during alkali-salt processing, and for neutralizing alkalis of other acids, for example sulfuric or acetic, is impractical due to the fact that protein preparations will be enriched with inedible salt during neutralization. At the same time, neutralization of NaOH with hydrochloric acid leads to the formation of only NaCl and water: $NaOH + HCl = NaCl + H_2O$

The NaCl salt distributes evenly throughout the protein preparation and thus provides a bacteriostatic and preservative effect.

With the use of collagen as an analogue of dietary fiber in the meat industry, it has become possible to rationally use secondary raw materials, when the percentage of collagen-containing tissues introduced into the formulation without deteriorating quality indicators reaches 25–30 [24–26].

Consequently, an analysis of literary sources indicates that the special properties of collagen make it possible to create protein preparations based on it.

Materials and methods. The objects of research were meat and bone by-products of poultry – chicken legs, collagen-containing raw materials obtained from legs, collagen-containing products – collagen-containing minced meat (CM), collagen-containing gel (CG) and meat sausages using CM or CG.

The sequence of experiments, objects and stages of research are shown in Fig. 2.

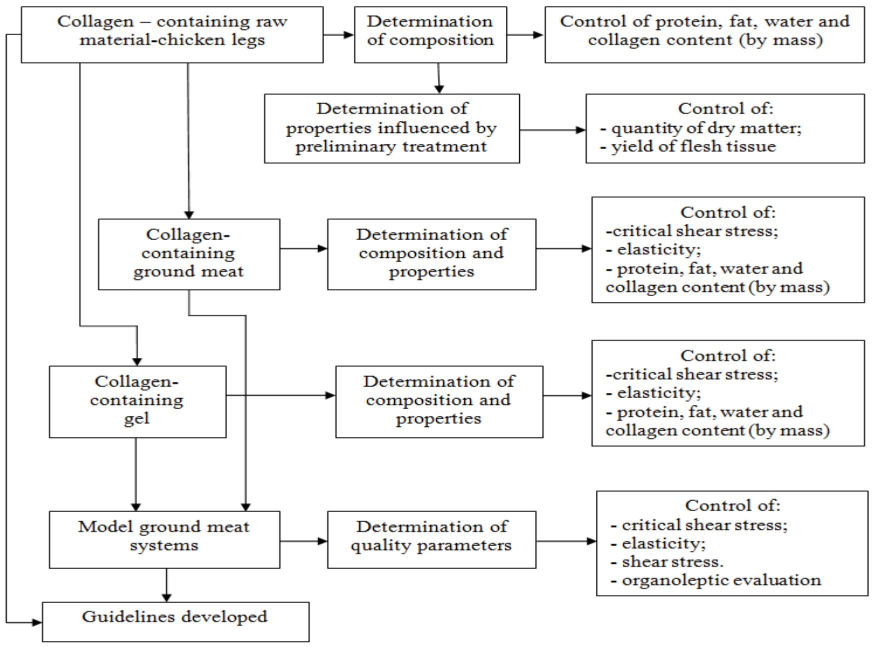

Fig. 2. Scheme of experimental studies

The research was carried out according to the following plan.

The raw materials were boiled in water at a temperature of (95+5)°C at a liquid ratio of 1:2 for 30; 60; 90 minutes.

The target product, collagen-containing minced meat, was obtained by manually chopping and chopping the boiled flesh.

The second target product, a collagen-containing gel, was obtained by mixing collagen minced meat with broth in a 1:1 ratio.

Before starting the research, we made a reasonable choice and mastered the necessary research methods, namely:

- the amount of dry substances passing into the broth during cooking – drying and weighing the dry residue;
- yield of flesh tissues – by weighing before and after deboning;
- texture parameters: critical shear stress, elasticity, shear stress – with the universal testing machine SANS CMT 2503 manufactured by Shenzhen SANS Testing Co. Ltd (China);
- content of protein (by mass) – according to DSTU ISO 937:2005 "Meat and meat products. Determination of nitrogen content (Reference method)" (ISO 937:1978, IDT) [27];
- content of fat (by mass) – according to DSTU 8380:2015 "Meat and meat products. Method for measurement the mass fraction of fat" [28];

- content of water (by mass) – according to DSTU ISO 1442:2005 "Meat and meat products. Method for determining moisture content (reference method)" (ISO 1442:1997, IDT) [29];
- content of collagen (by mass) – using a standard method using a standard preparation of hydroxyproline;
- sensorial assessment – according to a 5-point system.

Consequently, one of the main tasks in developing technology for using chicken legs is to substantiate an effective method of preliminary preparation, which should provide the necessary degree of destruction of this specific raw material.

Analysis of scientific and technical information suggests that pre-treatment of collagen-containing raw materials can be performed in different ways, causing greater or lesser changes in the native structure. Treatment can be done by physical means through mechanical dispersion [30]; chemically by treatment with acidic and/or alkaline reagents to disrupt the supramolecular structures of collagen, its partial hydrolysis, which causes an increase in protein solubility and, accordingly, a change in functional properties, or enzymatically using drugs with a general proteolytic effect or collagenase activity. The listed methods of preliminary preparation are in most cases suitable for collagen-containing raw materials with a uniform morphological structure, namely pork skin, beef lips, tendon, etc.

Therefore, preliminary treatment of the legs, in addition to softening the flesh tissues, should include the operation of separating them from the bone tissue. In this regard, it is necessary to provide a two-stage scheme for the preparation of raw materials – boiling in water, followed by mechanical separation of flesh tissues and their simultaneous grinding. A significant part of the protein passes into the broth during cooking. Therefore, it is advisable to explore the possibility of using two options for obtaining a protein product from boiled and crushed raw materials in water: the first – without broth; the second – mixed with broth (proportion 1:1) [31].

It should be noted that chicken legs show significant variability in composition – within the range from 15 to 24%.

Results and discussion. A study was carried out of the chemical composition of the starting raw material (chicken legs) taken from three different batches (Table 1).

The data obtained indicate that the raw material can be classified as high-protein: the mass fraction of protein in muscle tissue is higher than in the mechanically deboned poultry meat (MDM).

Table 1

Chemical composition of raw materials (average values)

Сировина	Content, % (by mass)				Ratio fat : protein
	protein	water	fat	ash	
Muscle tissue from thermally treated chicken legs	21.59	67.94	8.94	1.53	0.36
Mechanically deboned poultry meat	18.43	70.64	9.88	1.06	0.54
Beef of the 2nd category	19.8	69.4	9.8	1.0	0.49

The value of the quality index (fat-protein ratio) of muscle tissue does not exceed the quantitative value of this indication (0.49), characteristic of grade II beef. That is, muscle tissue obtained from the legs of chickens can reasonably be used as an integral part of meat product recipes, for example, to replace grade II beef.

Two groups of samples were prepared for the research [31]:

- Group 1 – raw materials that are cooked for 30; 60; 90 min and subjected to manual deboning and grinding (samples 1–3). These are samples of collagen-containing ground meat;
- Group 2 – similarly prepared samples, but additionally mixed with broth in a 1:1 ratio (samples 4-6). These are samples of collagen-containing gel.

Based on these groups, an analysis was carried out to determine the content of collagen (by mass), the results of which are shown in Table. 2.

Table 2

Collagen content in the samples of collagen-containing ground meat and collagen-containing gel [31]

Boiling time of chicken legs, min	Content, % (by mass)	
	oxyproline	collagen
Collagen-containing ground meat		
30	0.652	5.26
60	0.649	5.24
90	0.648	5.23
Collagen-containing gel		
30	0.559	4.51
60	0.523	4.22
90	0.476	3.84

It should be noted that the duration of cooking influences the yield of flesh tissues separated during manual deboning of chicken legs. Within the involved cooking interval, it changes from 55.34 to 70.30%, which is probably due to the loosening of the connective tissue structure and, accordingly, a more complete separation of the flesh tissues from the bones. And the content of collagen (by mass) practically does not change depending on the duration of cooking. That is, those changes in the structure of collagen protein that occurred during cooking did not affect its quantity. The results obtained showed the possibility of using collagen-containing products (CM and CG) as stabilizers of meat systems in the production of meat products. To study the effect of adding CM and CG on the characteristics of ground meat obtained by two-stage processing of chicken legs, the protein product was evaluated from the point of view of compatibility and feasibility of its use. To do this, 20% CM or CG was added to ground chicken meat. In addition, 20% water was added to each sample. Next, samples of the ground meat were filled into the sausage casing, formed as sausages and boiled in water at (95±5) ºC for 30 minutes. After cooling, the quality parameters of the samples were evaluated. The assessment results are presented in Table 3.

Table 3

Quality parameters of the samples of ready products

Зразок	Parameter			
	Specific shear force, kN/m^2	Critical shear stress, kN/m^2	Elasticity, kN/m^2	Sensorial value of consistency, points
Control	20.65	86.80	100.21	3.0
Experiment with collagen-containing ground meat	21.98	101.24	185.73	5.0
Experiment with collagen-containing gel	12.82	95.03	152.35	5.0

It was found that the sample using CM does not differ significantly from the control in terms of the shear force. At the same time, the indicators of critical shear stress (CSS) and elasticity have higher values. This means that the addition of CM thickens the product, which indicates its influence on the formation of one of the main consumer characteristics of sausages with a dense structure, for example, semi-smoked ones.

The sample using CG is characterized by lower values of the shear force and CSS than the previous two samples, but the elasticity parameter is quite high. That is, it can be expected that the addition of CG to the composition of ground meat will contribute to the formation of an elastic structure of the product, which is important for products such as pates, brawn, etc.

The addition of CM or CG to the ground meat improves the moisture-holding and fat-holding capacity, which affects its stability. This assumption is confirmed by an sensorial assessment of the consistency of the products. In the prototypes it was elastic and strong. The control sample had a loose consistency; there were deposits of fat and broth under the shell.

The improvement in the consistency of the test samples compared to the control is due to further disruption of the bonds in the collagen molecule and an increase in the amount of gelling substances involved in the formation of a strong protein frame during heat treatment of the samples.

After research, a two-stage scheme for processing raw materials was proposed in order to obtain a collagen-containing product in 2 versions: ground meat and gel.

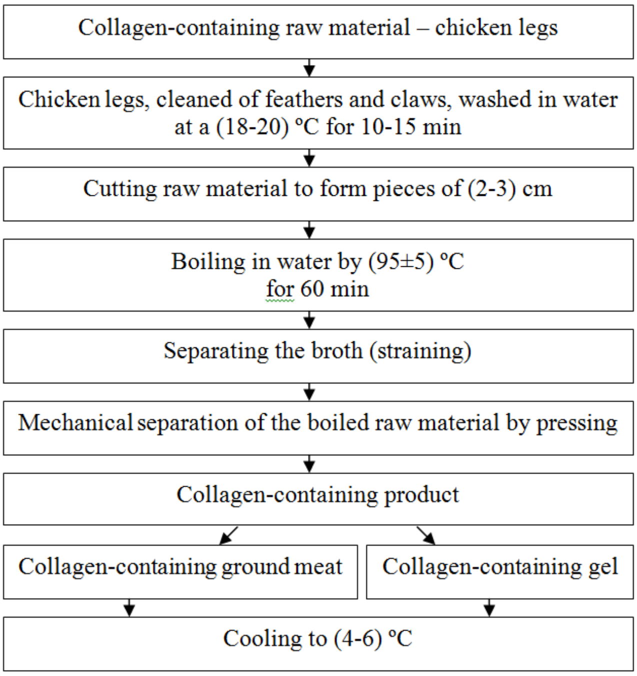

Fig. 4. Scheme for processing collagen-containing raw materials

A collagen-containing gel was obtained by mixing collagen-containing minced meat with broth in a ratio of 1:1.

Conclusion. In general, it can be stated that the use of chicken legs for the production of meat products is possible only if they are pre-processed, which involves separating the flesh tissue from the bones. It should be noted that the method of obtaining protein products determines the direction of their further use.

Using the obtained raw materials, two types of protein products were created: collagen-containing ground meat and collagen-containing gel, differing in chemical composition, collagen content, structural and mechanical characteristics as well as appearance.

The influence of CM or CG in an amount of 20% in the composition of minced chicken meat on the quality characteristics of finished products was studied. The results of determining the indicators of shear force, boundary shear stress, elasticity, and consistency of products indicate the positive effect of CM and CG on the consistency of meat products.

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