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THE INFLUENCE OF BROOD CHICKENS BY-PRODUCTS PROCESSING WITH PROBIOTIC CULTURE STARTER ON CHANGE OF THEIR FUNCTIONAL AND TECHNOLOGICAL PARAMETERS

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Keywords: *enzymatic hydrolysis, poultry wastes, bioresource, chicken by-products, gizzard, comb*

Abstract
By-products are the potential source of animal protein obtained from brood chickens and egg-laying hens. Certain by-products like gizzards and combs are quite tough and possess low nutritional and biological value due to their high content of connective tissue. Biotechnological processing improves the quality parameters of collagen-containing by-products. In this article a probiotic starter culture of propionic acid bacteria, which have high proteolytic activity, was used to treat the gizzards and combs of brood chickens. Before processing of by-products with starter culture, physical and chemical parameters and the yield of by-products in relation to poultry live weight were analyzed and recorded. 5%, 10% and 15% starter culture were added to the tested samples of chopped by-products, the samples were kept at a temperature of 30 °C, and every 4 hours the following functional and technological parameters were monitored: moisture binding capacity, water holding capacity (MBC and WHC) and yield of the product after heat treatment. The results proved that increase of starter culture amount and longer exposure of by-products to hydrolysis led to decrease of functional and technological parameters values, but for the combs those parameters remained at a sufficiently high level compared to the gizzards, as the gizzards were exposed to more intense hydrolysis than combs. The decrease in the pH value correlated with the dynamics of MBC and WHC changes; and dynamics of the product yield after the heat treatment. Also the stained histological preparations were studied in order to assess the influence of biotechnological processing on by-products microstructure, where significant differences were found in the morphological structure of muscle and collagen fibers of hydrolysates of combs and gizzards exposed to action of bacterial concentrate. The results of rheological studies showed that hydrolyzed chicken combs differed from gizzards; the combs were denser and featured more elastic structure due to a lower degree of hydrolysis by bacterial enzymes. In general, the properties of collagen-containing by-products (muscular gizzards and combs) change significantly after being exposed to enzymes of propionic acid bacteria.

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Introduction

Poultry farming is the most dynamically developing branch of the agro-industrial complex all over the world. The poultry processing products are important resource for the country's food security. The active development of this industry is facilitated not only by the high consumer properties of the poultry processing products, but also by their market availability in comparison with other types of raw meat food products [1].

Because of raising intensity of poultry industrial farming and its processing, it becomes necessary to search for new ways of rational use of secondary products and by-products still rich in proteins, but not only in proteins but also in other biologically important components.

All over the world the poultry processing plants generate a large volume of by-products and offals: heads, legs, bones, viscera and feathers [2,3]. This waste is often recycled into feed for farm animals and house pets, used for soil fertilization, or just disposed of. It is necessary to solve the problem of utilization of secondary poultry processing products taking into account the intensity and dynamism of the development of poultry farming and poultry processing in Russia. Irrational utilization of these wastes leads to environmental pollution; spread of contagious diseases, as well as it is just a loss of useful biological production resources like protein, enzymes and lipids [4].

Transformation methods, involving the use of these components for production of bioproducts with added value, can be a promising direction in reducing the concentration of unprocessed waste in the environment. The problems of studying secondary resources as sources of protein hydrolysates, enzymes, polyunsaturated fatty acids, are being solved now by both Russian and foreign researchers [4,5].

Poultry by-products mainly consist of collagen proteins, for which extraction various types of hydrolysis are widely used: hydrothermal, acidic, alkaline and enzymatic.

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The characteristics of each type of hydrolysis are shown further in Figure 1 [6].

Comparing different types of hydrolysis of secondary raw materials, it can be concluded that the enzymatic treatment is more physiological, because it runs both with the help of proteolytic enzymes and with the use of live bacterial producer cultures [7]. The processing of by-products with enzymes and microorganisms allows almost complete preservation of all essential amino acids [6, 8]. The enzymatic method is predominantly chosen to obtain functional and bioactive hydrolysates [9,10]. However, the use of enzyme preparations in industrial volumes is currently not widely used due to their high costs. An alternative is to introduce into emulsified collagen-containing by-products substrates the living culture of microorganisms, which allows reducing the cost of processing of the collagen-containing raw materials.

Application of biotechnological methods to the by-products to increase the stability of agricultural production, to obtain high-quality and environmentally friendly food, as well as to process the wastes and solve food security issues, can be referred now to the priorities of development of the agricultural and industrial complex [11].

Processing of secondary collagen-containing raw materials with bacterial concentrates allows reducing the toughness of raw materials, increasing their nutritional and biological value due to the production of lactic acid and other metabolites by bacteria, as well as due to their proteolytic activity [8].

Under the influence of microorganisms the functional, technological, physical & chemical and morphological characteristics of raw materials change, as well as the nutritional and biological value of the finished product which changes too. Positive changes in food substrates during microbial fermentation are shown in Figure 2. Partially hydrolyzed protein products feature improved functional properties like solubility, fat absorption, foam stability and emulsifying properties [12]. Propionic acid bacteria have the same characteristics in reference to food substrates, which properties are promising for processing of secondary poultry products. Scientists [13] proved the high biochemical activity of propionic acid bacteria in meat mass, which contributes to formation of optimal functional and technological properties of raw meat materials. Also, a high proteolytic activity has been found, due to which the processes of protein chains breakdown are accelerated. In the process of metabolism of propionic acid bacteria, there is an intense accumulation of volatile fatty acids and amine nitrogen, which are involved in formation of the peculiar taste and smell of meat products. The positive changes in structural and mechanical, microbiological parameters and biological value of food substrates based on meat raw materials, indicated in Figure 2, were also found during treatment of meat by-products with propionic acid bacteria [13].

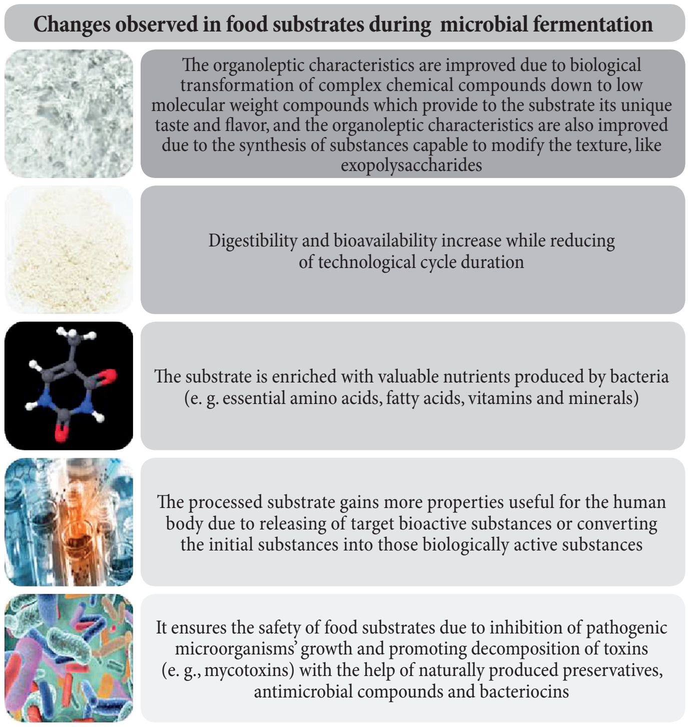


Figure 2. The influence of microbial fermentation on changes of food substrates properties

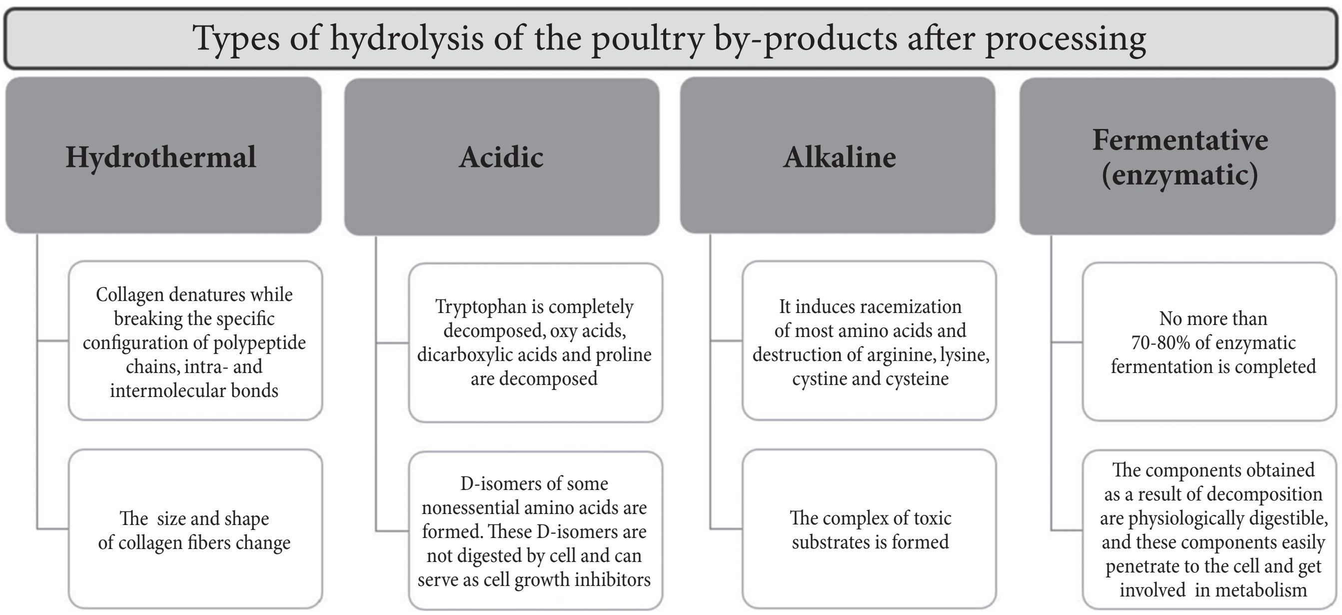


Figure 1. Characteristics of different types of hydrolysis of secondary poultry processing products.

The goal of this research is to study the influence of biotechnological processing on functional and technological parameters and microstructure of secondary products of brood chickens by-products — gizzards and combs.

Objects and methods

The object of the study is the gizzards and combs of brood chickens, which were later used in the preparation of collagen emulsions. The brood chickens were slaughtered at age of 11 months, the average live weight was 4 kg, the total number of slaughtered brood chickens was 300 pcs.

The yield of gizzards, heads and combs separately was defined as a percentage of the processed raw materials. Also, in the raw material the following physical and chemical parameters were determined according to generally accepted methods: the mass fraction of protein[1], of moisture[2], fat[3] and ash[4].

To improve the functional and technological parameters of the gizzards and combs of brood chickens, they were treated with the probiotic starter culture "Propionix" (LLC "Propionix", Moscow), which is a concentrated microbial mass of the *Propionibacterium freudenreichii subsp. shermanii* — KM 186 with an activity of 10^{10}–10^{11} CFU / cm^3.

For biotechnological treatment gizzards and combs were chopped in the chopper MFP 076 at second speed (Binatone, China) until an emulsion was obtained. 5%, 10% and 15% probiotic starter culture was added to the chopped by-products, mixed thoroughly and kept in a thermostat at a temperature of 30 °C for 16 hours with monitoring of parameters every 4 hours. As a control sample we used the chopped by-products without adding a probiotic starter culture. The following designations were assigned to the samples: C–C, G-C — control samples of chicken combs and chicken gizzards, respectively; C-Pro-5, C-Pro-10, C-Pro-15 — experimental samples of chicken combs with 5%, 10% and 15% probiotic starter culture, respectively; G-Pro-5, G-Pro-10, G-Pro-15 — experimental samples of chicken gizzards with 5%, 10% and 15% probiotic starter culture, respectively. The following parameters were monitored: pH level, moisture-binding capacity, water-holding capacity and yield after heat treatment.

The moisture-binding capacity (MBC) was determined by pressing, and the water-holding capacity (WHC) was determined thermogravimetrically, assessing the amount of moisture released from the sample during heat treatment. The yield of the product after heat treatment was determined by the difference in the mass of the samples before and after cooking at a temperature of 75 °C.

The pH values were measured in water extracts prepared by mixing 10 g of by-products samples with 40 ml of distilled water for 2 min. pH was measured with the digital pH meter model 710 A + with a measurement range from 0 to 14 pH units, measurement error of 0.02 pH units (Orion Research, Inc., USA).

To assess the influence of propionic acid bacteria on the structural components of raw materials, microstructural studies were conducted on the sliced sections of samples after their staining with hemotoxylin-eosin and picrofuchsin by the Van Gieson method. Histological preparations were studied with LEICA DMRXA microscope (Germany). The images of slices were taken with a digital video camera LEICA DFC290 (Germany) in the format of TIFF graphic files in RGB color space, which images served as objects of morphometric studies. For morphometric studies, the ImageScope M image analysis program (Germany) was used. The following parameters were analyzed: specific area of collagen fibers (%), which was defined as the ratio of the absolute area of all tissues of the histological preparation to the area of collagen (connective) tissue; average thickness of collagen fiber bundles (μm); average thickness of myocytes (μm).

The experiment on animals, including their housing, welfare, care and all manipulations, were run in compliance with the Decree No. 267 of the Ministry of Health of the Russian Federation of June 19, 2003 "On Approval of laboratory practice regulations", as well as the Council Directive 86/609/EEC of November 24, 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes.

All measurements were taken in triplicate. The results were processed statistically in Excel software (Office Microsoft: 42936865). The results were considered reliable at a significance level of $P \leq 0.05$.

Results and discussion

Physical and chemical parameters and the yield of raw materials to the live weight of poultry are presented in Table 1.

Table 1. Parameters of brood chickens by-products

Parameters	Gizzard of brood chickens	Combs of brood chickens
Yield, % of live weight	**0.59 ± 0.03**	**0.11 ± 0.01**
Mass fraction of protein, %	**17.88 ± 0.02**	**14.81 ± 0.03**
Mass fraction of fat, %	**4.38 ± 0.01**	**10.47 ± 0.01**
Mass fraction of moisture, %	**75.08 ± 0.03**	**71.82 ± 0.03**
Mass fraction of ash, %	**1.76 ± 0.01**	**1.26 ± 0.01**

The data on the chemical composition of poultry gizzards found in the scientific literature showed their significant variation depending on conditions of poultry method of farming and feeding and other factors also. For example, Zhumanova et al. found the protein content in chicken gizzards at the level of 20 g per 100 g of the product, which is

[1] GOST 25011–2017 "Meat and meat products. Protein determination methods". Moscow: Standartinform, 2018. — 14 p.

[2] GOST R51479–1999 "Meat and meat products. Method for determination of moisture content". Moscow: Standartinform, 2006. — 4 p.

[3] GOST 23042–2015 "Meat and meat products. Methods of fat determination". Moscow: Standartinform, 2019. — 9 p.

[4] GOST 31727–2012 "Meat and meat products. Determination of total ash". Moscow: Standartinform, 2019. — 8 p.

slightly higher than the values we obtained [14]. In terms of protein and moisture content, the results obtained by Abdullah and Buchtova (2016) are closer to the values we obtained: protein content — 17.34%, fat content — 0.76%, moisture content — 78.60%, ash content — 0.97% [15].

The results of pH change, presented below in Figure 3 and Figure 4, show that the acidity of the mass decreases due to the vigorous activity of propionic acid bacteria on the gizzards of brood chickens, and the combs apparently are less susceptible to hydrolysis due to the weak activity of bacteria on this substrate. This fact can be explained by the specificity of the combs structure and chemical composition, where collagen predominates. Changes in titratable acidity showed the similar dynamics; lactic acid accumulates faster in the gizzard-based substrate. That fact confirms the activity of propionic acid bacteria. At the same time the combs-based substrate based happened to be stable in terms of the lactic acid content; its amount increased only insignificantly by the end of the experiment. Mejri et al. (2017) explain the decrease in pH by the accumulation of acidic metabolic products of bacteria when processing raw materials with starter cultures [16].

The results of determining the moisture-binding capacity (MBC) of brood chickens gizzards samples are shown below in Figure 5, and MBC of combs are shown in Figure 6. A decrease in the MBC values was noted along with hydrolysis of by-products and with increase in the amount of starter culture. This process correlates with pH decrease, which was also noted by group of Gorlov et al. [17]. The MBC values varied from 76.2% in the experimental sample of broiler gizzards with 15% probiotic culture, up to 99.4% in the control sample during the initial period of hydrolysis (Figure 5). Gorlov et al. defined the level of MBC for chicken gizzards as 89.07%, which is slightly lower than the values we obtained [17]. The decrease of MBC along with increase in amount of bacterial starter culture can be explained by the fact that the starter culture is used in liquid form — the whey contains the active propionic acid bacteria. When the amount of starter culture is increased, the content of additional moisture introduced into

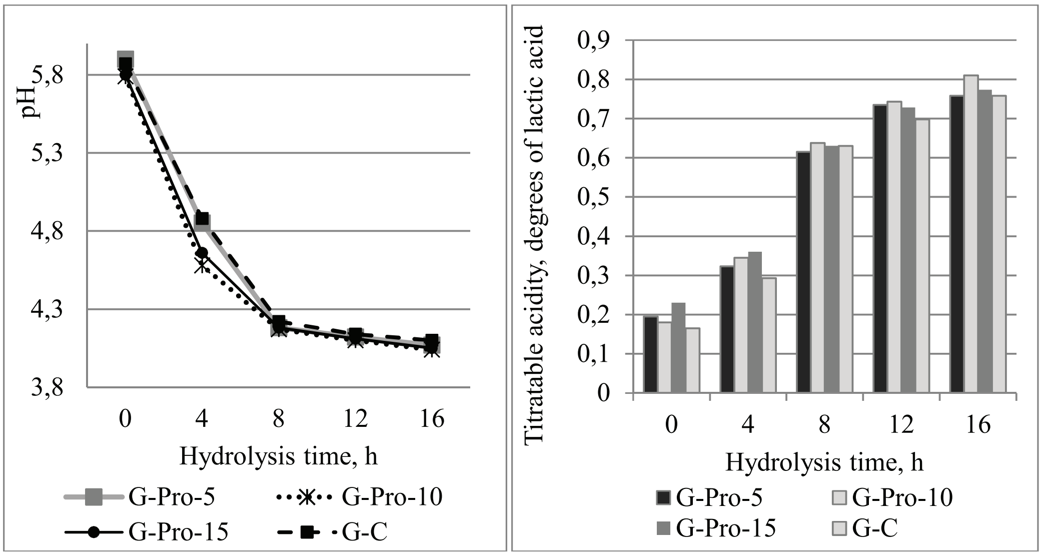


Figure 3. Changes in pH values and titratable acidity during hydrolysis of brood chickens gizzards

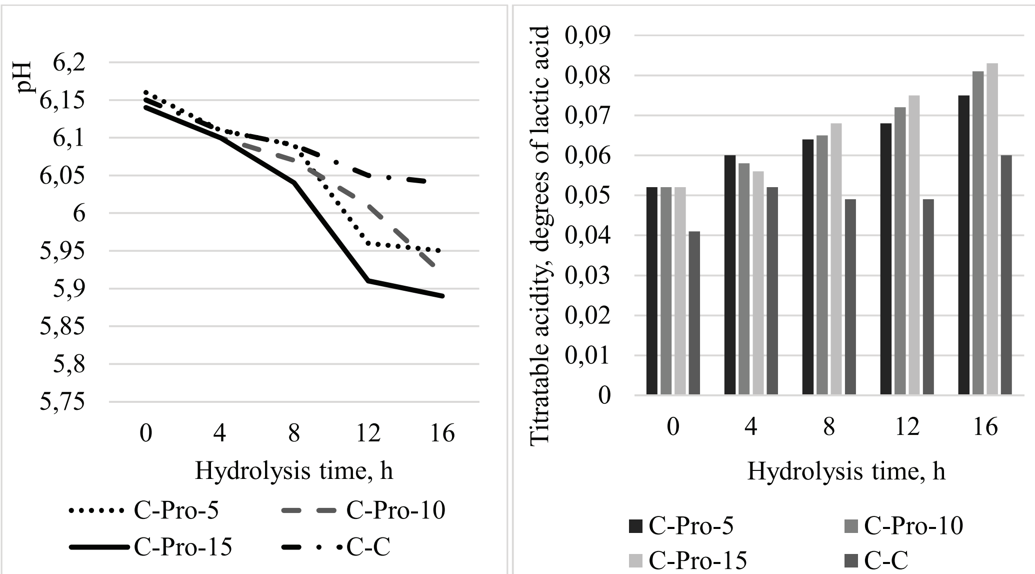


Figure 4. Changes in pH and titratable acidity values during hydrolysis of chicken combs

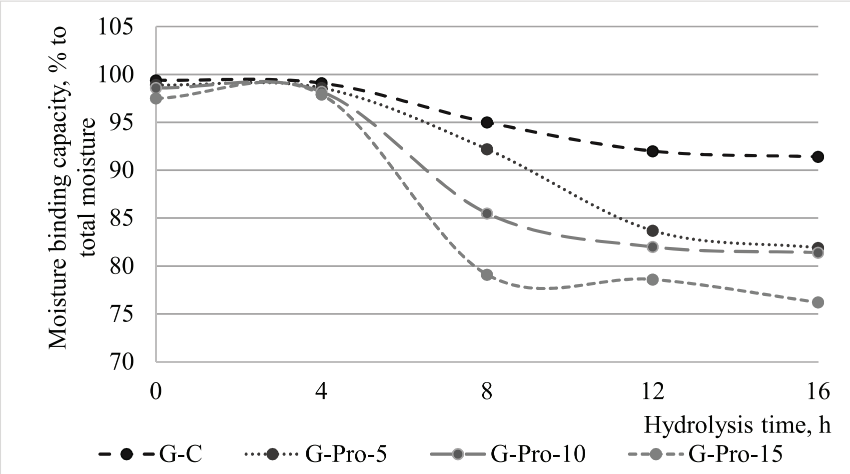


Figure 5. Changes in MBC values during hydrolysis of brood chickens gizzards

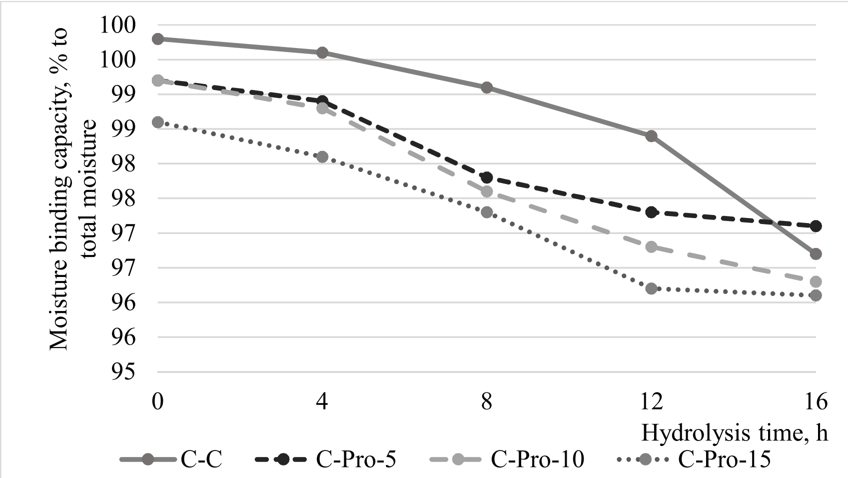


Figure 6. Changes in MBC values during hydrolysis of the gizzards of brood chickens

the by-product mass also increases. In samples of by-products, the intensity of hydrolysis increases with addition of starter culture, which is clearly seen in gizzard samples. The peptides formed during hydrolysis are not able to bind significant amount of excessive moisture. This is also confirmed by the results obtained for determination of WHC and losses during heat treatment of samples of by-products (Figure 7 and Figure 8).

Gombozhapova et al. [18] explain the decrease in MBC and WHC of raw meat during its prolonged mechanical processing by partial denaturation of proteins [18]. The capacity of meat to bind water is a complex characteristic influenced by structural and biochemical changes that occur to proteins [19,20]. During the fermentation of raw meat material its pH decreases, and similar changes occur in proteins structure. Tavdidishvili et al. [21] underline the importance of the WHC parameter, because the moisture loss during heat treatment and the sensory parameters of the product depend on its level [21].

When analyzing the dynamics of the structural and mechanical properties of hydrolysates (refer to the Figure 9 and Figure 10 below), a trend was found for increase in the flexibility properties of both control and experimental samples of chicken combs and gizzards during the fermentation period of 16 hours long; the plasticity increases due to destruction of native protein macromolecules and, as a consequence, it causes their functional properties decrease. It has been proven that hydrolyzed gizzards of brood chickens feature softer, more flexible structure in samples fermented with a concentrate of propionic acid bacteria — while during 16 hours of hydrolysis, the total deformation increased by 18.9–38.5%, and elastic deformation decreased by 22.5–38.4% in comparison with the control samples. Hydrolyzed chicken combs differed from hydrolyzed chicken gizzards by their denser and more elastic structure, which is explained by lower degree of hydrolysis under the action of bacterial enzymes.

Histological examination of microstructure in the hydrolyzed muscular gizzards of brood chickens of the control group found the fragments of the muscle wall with small areas of the mucous membrane (Figure 11a). The bundles of smooth muscle fibers are located in mutually perpendicular directions. Collagen fibers of the connective tissue matrix are grouped into compact bundles and stained with picrofuchsin to get crimson shades (Figure 11c). In the preparations treated with the bacterial concentrate, a fragmented muscle layer is visualized, in comparison with the control group the stain was absorbed quite weakly (Figure 11b). Thin collagen fibers in the preparation are discomplexed, weakly stained with picrofuchsin to shades of pink (Figure 11d).

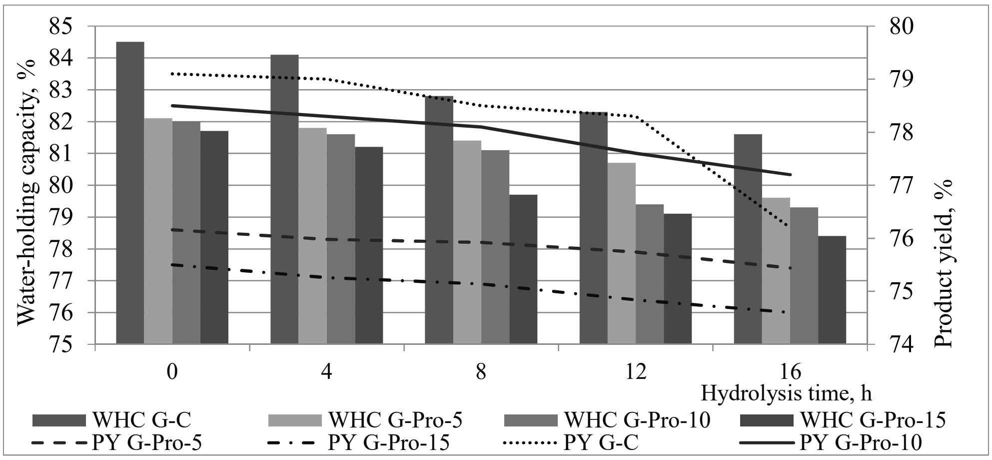


Figure 7. Water-holding capacity (WHC) and product yield after heat treatment of chicken gizzards

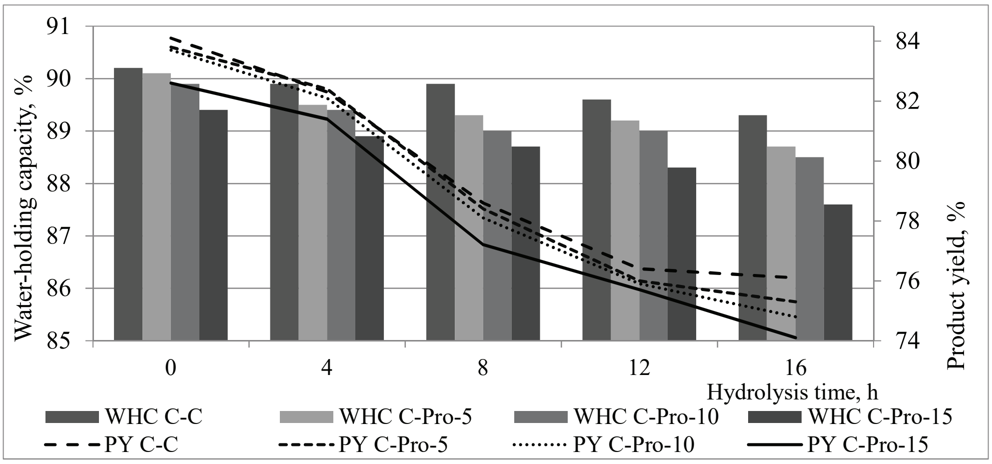


Figure 8. Water-holding capacity (WHC) and product yield after heat treatment of chicken combs

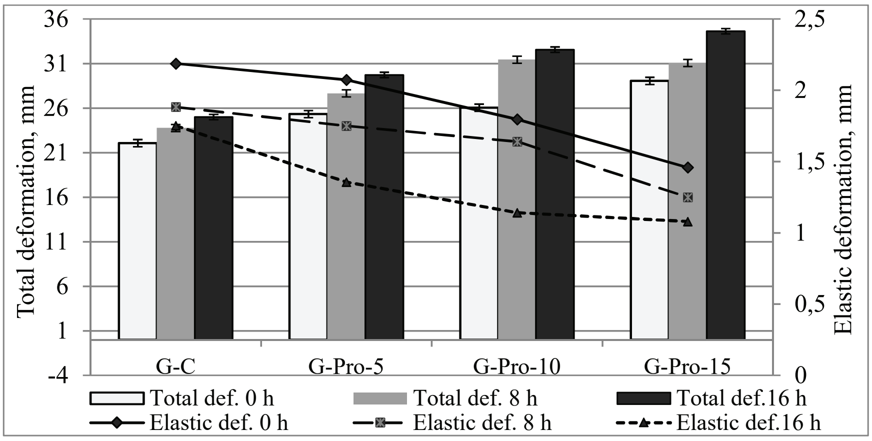


Figure 9. Dynamics of deformation characteristics during heat treatment of brood chickens gizzards

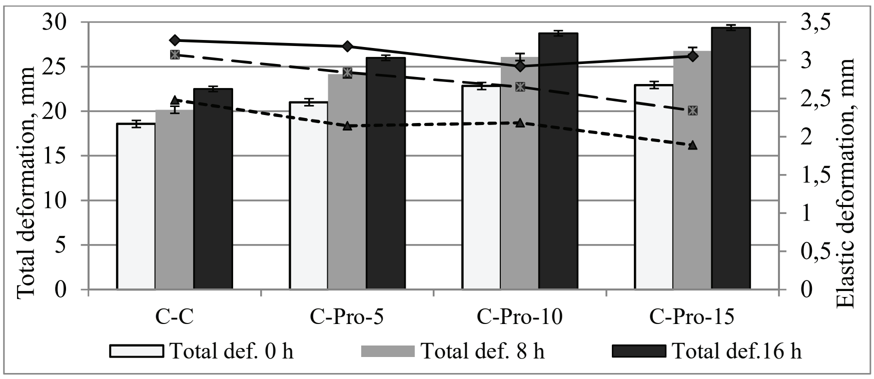
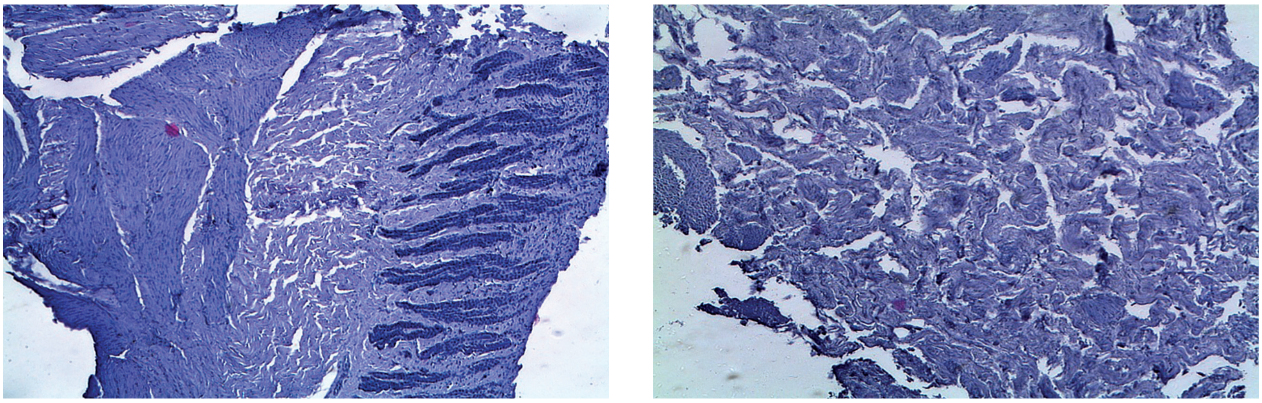


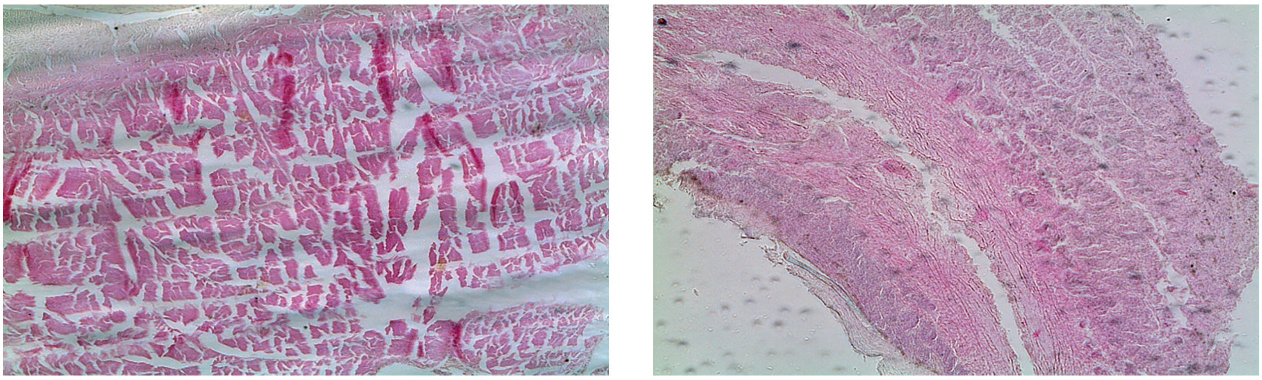
Figure 10. Dynamics of deformation characteristics during heat treatment of chicken combs

The gizzards of brood chickens. Hematoxylin-eosin staining



a b

The gizzards of brood chickens. Van Gieson staining



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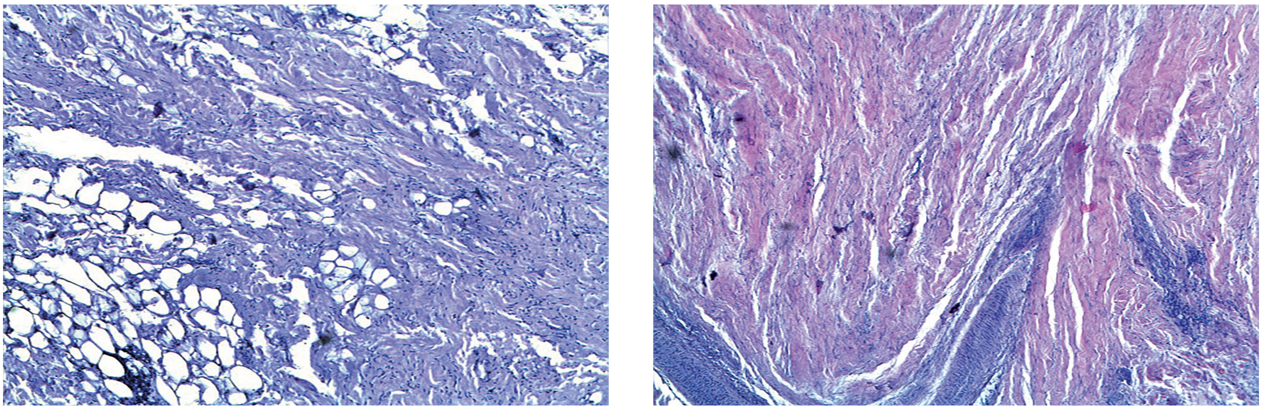
Combs of brood chickens. Hematoxylin-eosin staining



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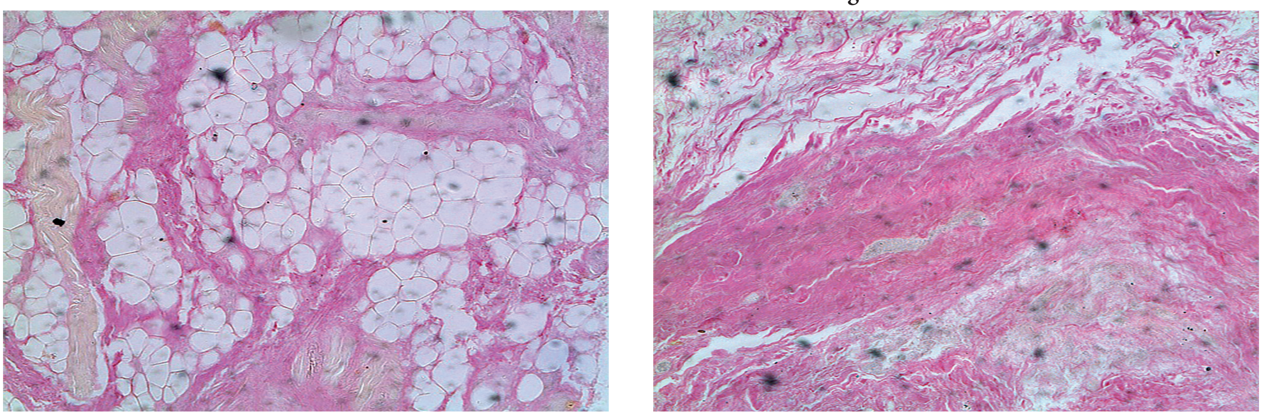
Combs of brood chickens. Van Gieson staining



g h

Figure 11. Results of histological examination of gizzards and combs of brood chickens: gizzards of brood chickens stained with hematoxylin-eosin: a) G-C, b) G-Pro-15; gizzards of brood chickens stained by Van Gieson method: c) G-C, d) G-Pro-15; combs of brood chickens stained with hematoxylin-eosin: e) C–C, f) C-Pro-15; combs of brood chickens stained by Van Gieson method: g) C–C, h) C-Pro-15

Histological preparations of the combs in the control sample are represented by fragments of folded skin with a thin *stratum corneum*. Cells and their nuclei are clearly visible, with good absorption of histological stains (Figure 11e). The extracellular matrix is represented by randomly located collagen fibers. In the deep layers, the layers of fat tissue are visible, which are separated by thick connective tissue septa, consisting mainly of compactly grouped collagen fibers. The areas free of collagen fibers (bundles of smooth muscle fibers) are colored yellow with picric acid (Figure 11g).

In the samples of combs hydrolyzed with propionic acid bacteria, in comparison with the control sample, the attention is drawn to the reduced absorption of histological dyes, as well as the “blurring” of the connective matrix and the boundaries of cellular elements (Figure 11f). When stained with picrofuchsin according to Van Gieson method, the test samples show a diffuse pinkish-crimson staining in all fields of view (Figure 11h).

As a result of a morphometric study, the regularity was established for a decrease in both the specific area of connective tissue and the diameter of collagen fiber bundles for samples of combs and gizzards hydrolyzed by enzymes of the bacterial concentrate. A decrease in muscle fiber thickness was also noted in gizzard samples (Table 2). The data obtained are due to the proteolytic activity of enzymes of propionic acid bacteria, which are capable of hydrolyzing both muscle and connective tissue proteins. Also, the change in the structure of muscle and collagen fibers is influenced by lactic and propionic acids, which are the products of the metabolism of propionic acid bacteria. As noted by authors Aktas & Kaya (2001), lactic acid is harmful to animal tissues [22].

Conclusion

When the by-products of brood chickens are processed with propionic acid bacteria, this enzymatic processing caused significant changes in the structural and mechanical, functional and technological parameters, as well as in microstructure of the samples. The values of moisture-binding capacity, water-holding capacity were significantly decreased, while the flexibility and general deformation of fermented gizzards and combs of brood chickens increased due to intensive hydrolysis of proteins chains being exposed to action of microbial enzymes. These regularities must be taken into account when using the biotechnological method of preliminary enzymatic processing of raw meat materials during production of various meat foods.

Table 2. Results of morphometric analysis of gizzards and combs of brood chickens

Parameters	G-C	G-Pro-15	C–C	C-Pro-15
Specific area of connective tissue (%)	61.43 ± 1.922	33.80 ± 1.981	59.22 ± 3.067	43.77 ± 1.742
Average thickness of collagen fiber bundles (μm)	15.36 ± 3.056	6.65 ± 0.953	19.90 ± 3.006	7.18 ± 2.492
Average thickness of myocytes (μm)	15.59 ± 1.856	9.98 ± 1.395	—	—

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