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IMPROVING THE FUNCTIONAL AND TECHNOLOGICAL PROPERTIES OF MINCED PORK USING A PROTEOLYTIC ENZYME

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Keywords: *pork, minced pork, curing, enzymatic treatment, chymotrypsin, electrophoresis, water-binding capacity, pH, cooking loss*

Abstract
The use of pork obtained from intensively growing hybrid animals as the main raw material in meat processing, in particular sausage production, is accompanied by undesirable variability of consumer characteristics of sausage products and a decrease in the economic indicators of enterprise performance. The main technological disadvantages of processed pork are reduced water-binding capacity and significant loss of meat fluid, which in practice is usually compensated by the increased use of food additives and non-meat ingredients. The aim of the study was to assess the possibility of targeted improvement of the technological properties of minced pork using a proteolytic enzyme of animal origin. Model samples of minced meat were developed and cured for 24 hours at a temperature of 4 °C. The following control samples were used: minced meat with the addition of 2% edible salt and minced meat with the addition of 2% edible salt and 0.2% sodium bicarbonate. Moreover, 0.0001% chymotrypsin was added to the test samples containing similar curing ingredients. During electrophoretic study, in samples with the enzyme, an increase in low-molecular fractions (20 kDa, 15 kDa and lower) was observed, which indicated the manifestation of proteolytic activity of chymotrypsin in model systems. Enzymatic treatment led to an increase in pH and water-binding capacity. Cooking loss was reduced by 3 to 6 times, compared to Control 1. After cooking, histological studies of model systems showed that the test samples subjected to enzymatic treatment were characterized by a denser arrangement of structural elements, less pronounced cellular components of muscle tissue and the presence of glutin formed as a result of protein breakdown, filling the microcapillaries. Thus, the use of an enzyme preparation provides an opportunity for targeted improvement of the technological properties of pork obtained from intensively growing hybrid animals.

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Introduction

The use of proteolytic enzymes is one of biotechnological methods of raw material processing and has great potential in food production, including meat industry [1].

Currently, enzymes in meat technology are considered in two main applications. On the one hand, the action of endogenous tissue enzymes (endoenzymes) during meat aging after slaughter is being studied in order to create the best conditions and optimal duration for converting animal muscles into meat with high consumer value [2]. This transformation is of great importance for the meat industry. Due to endoenzymes, complex processes of "cell death" occur in meat, which form new functional and technological properties of muscle tissue, improving tenderness, juiciness, taste, flavor, color and texture of meat [3]. At the same time, a special role in this transformation is given to the multicatalytical proteinase complex consisting of calpains, lysosomal cathepsins and proteasomes [4]. On the other hand, an increasing number of studies are devoted to the use of exogenous proteases of non-meat origin, i. e. plant proteases [1,5,6], bacterial proteases [6,7] and fungal proteases [7], including new preparations whose properties and safety require careful study [8]. Unfortunately, in recent years, the interest of researchers in the use of proteolytic enzymes of animal origin (pepsin, trypsin, pancreatin, chymotrypsin), even as objects of comparison with enzymes obtained from other sources, has somewhat decreased.

Obtaining enzymes of animal origin is associated with the slaughter of animals. The meat industry has large resources of by-product raw materials [9], including those for the production of enzymes. The production and use of enzymes of animal origin simultaneously solves the problems of increasing the demand for low-value types of by-products, expanding the possibilities for their use, creating the additional value [10] and, consequently, reducing the environmental pressure from meat processing plants.

Recent studies show that the use of proteases in meat processing has the following main goals: improving the texture and tenderness of meat [5,6]; obtaining biologically active peptides, including from low-value by-products [10], reducing the allergenicity of introduced proteins or meat's own proteins in the manufacture of meat products [11,12]. At the same time, a completely justified goal of using proteolytic enzymes may also be improving the functional and technological properties of meat raw materials [13]. Currently, this goal is especially relevant for pork processing.

Pork is the most consumed meat in Asia and Europe, and in European countries up to 75% of pork is consumed in the form of processed meat products, which implies a high need for enterprises to stabilize the quality of this meat raw material [14]. At the same time, the widespread breeding of intensively growing hybrid animals in pig farming leads to undesirable variability in consumer characteristics of both raw materials and meat products, and is accompanied by a decrease in the economic indicators of enterprise performance.

The muscle tissue of intensively growing pigs is characterized by a high content of white muscle fibers capable of forming areas of "hypercontraction". This feature, which ensures intensive growth during the life of the animal, leads to the production of meat with reduced water-binding capacity, characterized by significant loss of meat fluid [15]. Subsequently, the processing of such meat is accompanied by a decrease in the yield of finished products. Pork obtained from intensively growing animals is characterized by such defects as PSE (pale, soft, exudative) and RSE (red soft exudative), as well as "destructured" meat [16]. In order to stabilize the quality and yield of sausages and other pork products, enterprises are forced to widely use non-meat ingredients and food additives in recipes [17]. However, the use of the latter causes constant concern among consumers about the composition and health benefits of such food products [18].

The main reason for the decrease in the functional and technological properties of pork is denaturation of muscle proteins [16,19]. For the production of high-quality finished products, the condition of muscle proteins (especially myofibrillar proteins), their ability to bind water and form new protein structures are of exceptional importance. In the presence of pork defects (PSE, RSE, destructured meat, muscle fiber damage), the ability of protein structures to bind water and interact decreases [16]. Their condition is close to the condition of proteins during thermal denaturation and may be aggravated by a decrease in salt in the product, a reduction in the curing duration, the absence of phosphates, protein oxidation and other factors. Already the initial stages of processing pork with quality defects are characterized by significant loss of water and salts dissolved in it, which affects the activity of endoenzymes in meat [16]. A decrease in the intensity of proteolysis as a result of protein denaturation may be an important reason for the insufficient quality of sausages and other finished products. However, excessive proteolysis of muscle proteins may also lead to excessive softness and an unpleasant taste of the meat product [13,16].

The study was based on the hypothesis of the possibility to use proteases during pork curing and minced meat formulation. In this regard, the choice of an enzyme of animal origin obtained from meat by-products seemed to be of the most interest. Thus, the purpose of this work was to assess the possibility of targeted improvement in the technological properties of pork obtained from intensively growing hybrid animals through the use of a proteolytic enzyme of animal origin, i. e. chymotrypsin, a preparation made in Russia.

Objects and methods

Research objects

The objects of the study were model systems based on minced meat made from pork with a mass fraction of adipose tissue of no more than 10%, treated and untreated with the enzyme, not subjected to heat treatment and after heat treatment.

To obtain the model systems, chilled pork was minced in a laboratory meat grinder through a grid with 5 mm hole diameter. Then the minced meat was weighed in portions of 500 ± 1 g followed by the introduction of pre-weighed and prepared food ingredients and additives, i. e. edible salt (extra grade boiled, Russol LLC, Russia), acidity regulator sodium bicarbonate (E500, $NaHCO_3$, Bashkir Soda Company JSC, Russia), chymotrypsin enzyme preparation (Samson-Med LLC, Russia). Then the minced meat was mixed to evenly distribute the components.

The pH value of pork before curing was 5.58 ± 0.02. According to literature [20,21], chymotrypsin effectively acts in an alkaline environment with a pH of 7.0 to 8.5 with an optimum at pH of 7.8 to 8.0. In this regard, to increase the pH value in the model system, the acidity regulator E500 was used.

The enzyme was pre-diluted with saline in the following ratio: 2 ml of saline per 0.01 g of the preparation. The enzyme was not added to the control samples, but the same amount of saline was added instead.

In total, two control samples and two test samples containing the following components in the composition of minced meat were prepared as model systems:

- Control 1–2% edible salt;
- Control 2–2% edible salt and 0.2% sodium bicarbonate;
- Test sample 1–2% edible salt and 0.0001% chymotrypsin;
- Test sample 2–2% edible salt, 0.2% sodium bicarbonate and 0.0001% chymotrypsin.

Model systems were stored in a refrigerator for 24 hours at a temperature of 4 ± 2 °C. After that, samples were taken for research of cured minced meat, including determination of protein fractional composition, as well as the functional and technological characteristics of minced meat, i. e. pH and water-binding capacity.

To obtain heat-treated model systems, samples of 100 ± 1 g were taken. The samples were packed in polyethylene-propylene bags on Webomatic Easy-pack vacuum packaging machine (Webomatic Maschinenfabrik GmbH, Germany) and subjected to heat treatment (cooking) in PE4310 laboratory water bath (Ekroskhim LLC, Russia) with a water temperature of 95 ± 1 °C. The cooking duration was 15 minutes. After cooking, losses during heat treatment were determined and the samples were sent for histological studies.

Research methods

The molecular weight distribution of protein fractions was analyzed by one-dimensional electrophoresis [22]. Sample preparation was performed as follows. 50 mg of each minced meat sample was taken and homogenized in 1000 µl of lysis solution (9 M urea (PanReac, Germany), 5% β-mercaptoethanol (PanReac, Germany), 2% Triton X-100 (Helicon, Russia), 2% ampholine pH 3–10 (Serva, Germany)) using Stegler S10 homogenizer (STEGLER, China). The resulting homogenate was clarified by centrifugation using Eppendorf 5427 R centrifuge (Eppendorf, Germany) at 14,000 rpm for 20 minutes. After that, 50 µl of the supernatant were collected in Eppendorf tubes and 50 µl of protein buffer (1 ml of 10% sodium dodecyl sulfate (SDS, PanReac, Spain), 250 µl of concentrated β-mercaptoethanol (PanReac, Germany), 625 µl of 0.5 M Tris-HCl (PanReac, Germany), 1.5 g of urea (PanReac, Germany) were added. Then bromophenol blue (Helicon, Russia) was added until a dark color and a volume was adjusted to 5 ml with distilled water. The resulting samples were heated in a boiling water bath for 5 minutes. For one-dimensional electrophoresis, VE-10 chamber (Helicon, Russia) filled with 12.5% polyacrylamide gel was used. Visualization and analysis of the images were carried out by staining proteins with Coomassie G-250 solution, consisting of 10% acetic acid (Komponent-Reaktiv; Russia), 25% isopropanol (PanReac, Germany), 0.05% Coomassie G-250 (Helicon, Russia). To remove unbound dye, 10% acetic acid (Komponent-Reaktiv, Russia) was used. Computer densitometry of the one-dimensional electropherogram, which was in a wet state, was performed using Bio-5000 Plus scanner (Serva, Germany) in 600 ppi 2D-RGB mode.

pH was measured by the potentiometric method using Testo 205 laboratory pH meter (Testo SE and Co., Germany).

The mass fraction of moisture was determined in accordance with GOST 9793–2016[1] by drying to a constant mass in a drying cabinet at a temperature of 103 ± 2 °C;

The water-binding capacity of the samples was determined by the Hamm and Grau method (pressing method) modified by Volovinskaya [23].

Cooking loss was determined by the gravimetric method: after heat treatment, the bags with samples were removed from the water bath and cooled to room temperature, then the liquid formed in the bag was drained, and a piece of minced meat was placed on filter paper to drain. Cooking loss in% was determined by the formula:

$$\text{Loss} = ((M_1 - M_2)/M_1) \times 100 \quad (1)$$

where M_1 is the weight of the sample before cooking;

M_2 is the weight of the sample after cooking and draining the liquid.

Histological studies were carried out in accordance with GOST 31796–2012[2] with sample fixation in accordance with GOST 31479–2012[3]. The pieces were placed in a 10% aqueous solution of neutral formalin and kept for 72 hours at room temperature. Then they were washed with cold running water for 12 hours. The washed material was first impregnated with a 12.5% gelatin solution, and then with a 25% gelatin solution in a thermostat at 37 °C for 12 and 24 hours, respectively. To make histological sections, pieces of 15 × 15 × 4 mm were cut from the fixed samples and placed in the freezer of MIKROM-NM525 microtome-cryostat (Carl Zeiss, Germany) for freezing to a temperature of minus 20 ± 3 °C. Frozen pieces were cut into 10 to 30 µm thick sections using a microtome knife. The sections were transferred to a glass slide and stained with Ehrlich's hematoxylin for 3 to 4 minutes. The sections were then rinsed with water to remove excess dye, immersed in a 1% hydrochloric acid solution until a pink color appeared, then in ammonia water until a blue color appeared, and rinsed again with water for 2 minutes. After this, the sections were stained with a 1% aqueous eosin solution for 1 minute and rinsed with water. The sections were then placed under cover slides. The prepared histological preparations were studied and photographed using AxioImaiger A1 light microscope (Carl Zeiss, Germany) with a magnification of x340 using AxioCam MRc 5 video camera. The images were processed using AxioVision 4.7.1.0 computer image analysis system adapted for histological studies.

Statistical analysis

All indicators were determined in 3 to 5 replicates. The obtained data were processed statistically with the determination of the mean value and deviation (M ± m). When pairwise comparing samples, the significance of differences was determined using the Student's t-test.

Results and discussion

After formulation (adding all components and mixing), the control samples and test samples did not differ in appearance, color, and texture (Figure 1).

[1] GOST 9793–2016 "Meat and meat products. Methods for determination of moisture content" Moscow: Standartinform, 2018. Retrieved from https://docs.cntd.ru/document/1200144231 Accessed April 16, 2024

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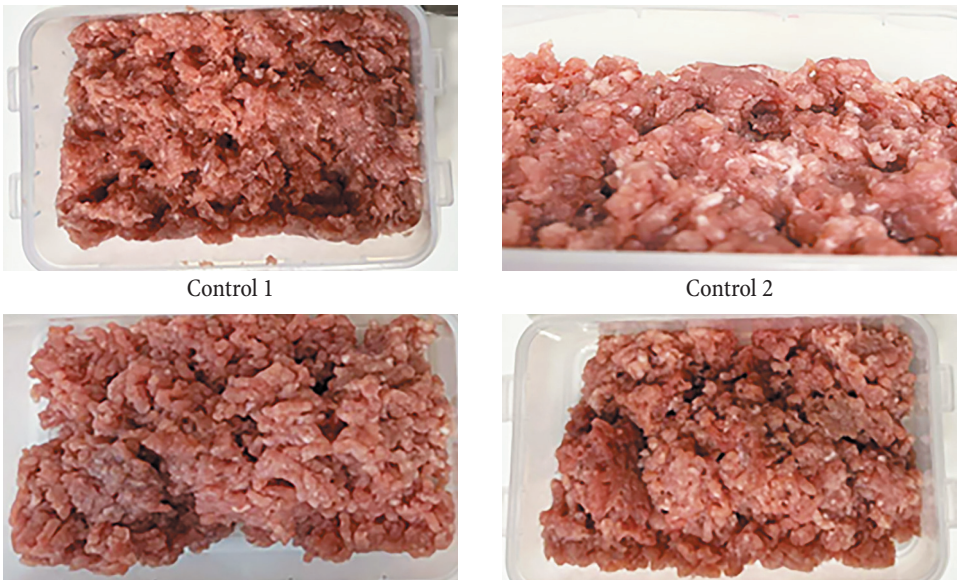


Figure 1. Appearance of model systems — control samples and test samples after formulation

In the test samples formulated with the addition of the enzyme preparation, a change in the texture of minced meat was observed after just 30 minutes. Minced meat became more viscous and stickier than in the control samples. Thus, the effect of chymotrypsin was observed already at the very beginning of the curing process. An increase in stickiness during the treatment of meat with proteases, e. g. proteases of microbial origin, was also noted by other authors [13].

Electrophoretic studies of the samples showed a change in the fractional composition of meat proteins as a result of exposure to the enzyme preparation (Figure 2).

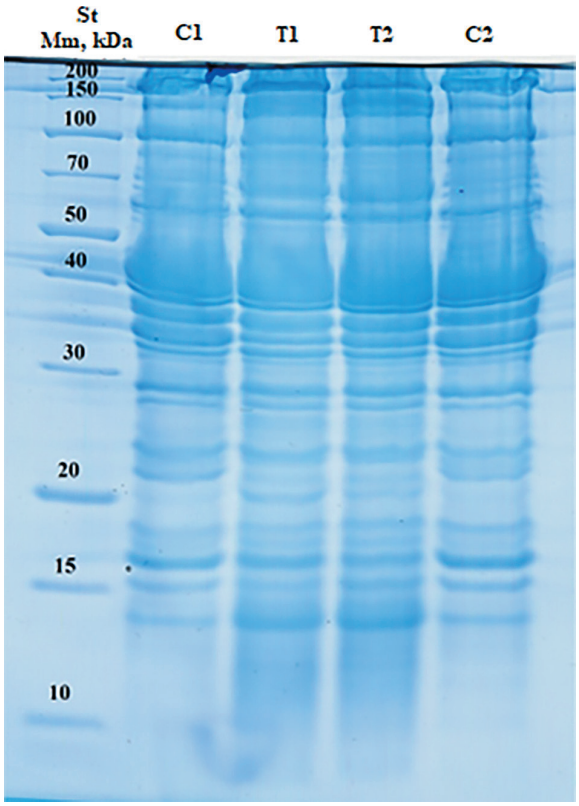


Figure 2. One-dimensional electropherogram of control samples and test samples. St — standard; C1 — Control 1; T1 — Test sample 1, T2 — Test sample 2, C2 — Control 2

Electropherogram at Figure 2 shows that the enzyme-treated Test sample 1 and Test sample 2 contained more low-molecular proteins with a molecular weight of 15 kDa and lower. Also, more intense bands on the electropherogram were observed in the region of about 20 kDa. On the contrary, in the region of 100 kDa, the intensity of protein bands in the test samples was reduced. This indicated the manifestation of the proteolytic activity of chymotrypsin in model systems, both without an acidity regulator (Test sample 1) and with an acidity regulator (Test sample 2), where the pH value of the system was increased.

The decrease in the manifestation of bands, as well as the presence of a protein background in the test samples, was due to the fact that the proteolytic activity of chymotrypsin degrades most protein fragments to low-molecular peptides. A similar formation of low-molecular peptides is observed during prolonged meat aging [24].

Among the decreased bands in the region from 30 to 50 kDa, there may presumably be protein fractions of myosin heavy chains (36.0 kDa) and actin heavy chains (41.7 kDa). The protein structure of myosin is sensitive to the action of chymotrypsin. During the degradation of myosin, degradation of the actin-myosin complex occurs simultaneously, which was noted in studies on the effect of enzymatic treatment on meat digestibility [25,26].

The study of pH, water-binding capacity (WBC) and cooking loss (Table 1) showed that control samples and test samples differed in functional and technological parameters.

Table 1. Functional and technological indicators of minced meat

Model systems (samples)	pH	WBC, %	Weight loss during cooking, % of initial weight
Control 1	5.79 ± 0.01^{b}	86.1 ± 3.7	12.2 ± 2.8^{b}
Control 2	6.05 ± 0.03^{a}	100.0 ± 0.0	4.1 ± 2.8^{a}
Test sample 1	5.83 ± 0.02^{ab}	100.0 ± 0.0	2.0 ± 1.4^{a}
Test sample 2	6.06 ± 0.07^{a}	100.0 ± 0.0	3.5 ± 0.7^{a}

Note: a — differences with Control 1 are statistically significant ($p < 0.05$), b — differences with Control 2 are statistically significant ($p < 0.05$)

The pH value in the control and test samples of minced meat after curing was higher than in the original meat raw material. The highest pH values were observed in Control 2 and Test sample 2, to both of which the acidity regulator was added. However, these samples did not have significant differences in pH.

In the model systems that did not contain the acidity regulator, the pH values were lower, but the differences between Control 1 and Test sample 1 were statistically significant ($p < 0.05$). This means that the action of the enzyme preparation contributed to an increase in the pH of minced meat.

Thus, the introduction of the enzyme preparation into the meat system during curing (Test sample 1) led to an insignificant increase in pH compared to the sample without the enzyme preparation (Control 1). However, the effect of the enzyme increasing the pH of minced meat was not observed compared to the addition of the acidity regulator.

After curing and aging for 24 hours, the WBC of three model systems, i. e. Test sample 1, Test sample 2 and Control 2, reached the maximum value of 100%. Cured meat did not release moisture during pressing for 10 minutes. Only Control 1 had a reduced WBC value of 86.1%. It should be noted that no differences were found in the mass fraction of moisture among the model systems. The mass fraction of moisture in the samples was 64.8 ± 3.7%.

The data obtained allowed to conclude that the introduction of the enzyme into the system led to an increase in the WBC of the cured meat to maximum values, as did the addition of the acidity regulator. This was consistent with the previously obtained results (using meat by-products), indicating that enzymatic treatment is accompanied by swelling of muscle fibers and an increase in WBC [13].

An important property of meat systems is their ability to retain moisture during heat treatment. Evaluation of loss during cooking of samples showed that the highest losses were typical for the Control 1 model system. In other samples, the losses during cooking were 3 to 6 times lower. This indicator also significantly differed in Control 1 compared to all other samples. This indicated that enzyme treatment and/or the introduction of an acidity regulator made it possible to reduce cooking loss.

Thus, the study of pH, WBC and cooking loss of model systems showed that enzymatic treatment with chymotrypsin combined with curing is able to improve the functional and technological properties of pork.

The results of the histological examination of the samples subjected to cooking showed the following.

The microstructure of Control 1 and Test sample 1 (Figure 3) was represented by fragments of muscle, connective and adipose tissues, and also contained a fine-grained protein mass formed as a result of mechanical destruction of raw meat. In Control 1, the muscle tissue fragments included non-swollen muscle fibers. The boundaries between them were well defined. The transverse striation of the fibers was clearly defined, the fiber nuclei were homogeneous. In Test sample 1, the muscle tissue fragments contained swollen round muscle fibers that were tightly adjacent to each other. The boundaries between them were poorly distinguishable, the transverse striation was not defined, the nuclei were shadow-like, and the swollen myofibrils were disintegrated.

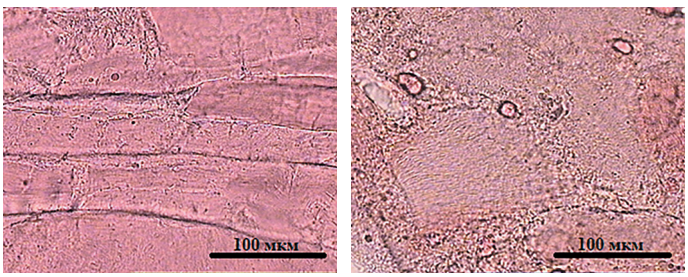
In Control 1, the average fiber diameter was 52.7 μm, while in Test sample 1 it was 75.0 μm. Test sample 1 differed from Control 1 in pronounced destructive changes in the form of multiple microcracks.

In Control 1, the connective tissue layers of the perimysium were characterized by dense bundles of collagen fibers, and the cell nuclei were clearly visible. In Test sample 1, the connective tissue layers of the perimysium were swollen and/or loosened, and the cell nuclei were poorly distinguishable.

In Control 1, the adipose tissue fragments included adipose cells containing fat droplets. The fat was also distributed in the form of 10–30 μm droplets in a fine-grained protein mass. The membranes of the adipose cells were not damaged. On the contrary, in Test sample 1, the membranes of the adipose cells were partly destroyed, due to which the fat was distributed in the fine-grained protein mass in the form of small 2–10 μm droplets.

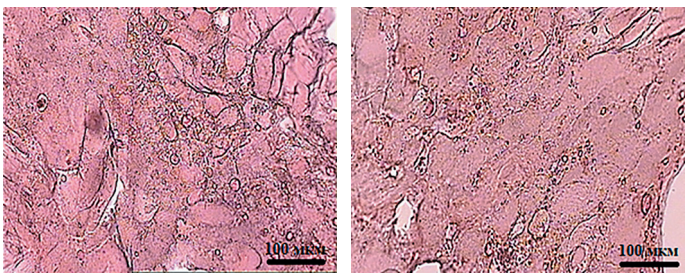
The microstructure of Control 2 was characterized by a denser arrangement of structural elements compared to Control 1. At the same time, as a result of the destruction of muscle fiber fragments to a fine-grained protein mass under the action of the enzyme preparation, Test sample 2 had even denser arrangement of meat structural elements (fragments of muscle, connective, and adipose tissue) (Figure 4).

In Control 2, the muscle fibers were swollen, the boundaries between them were clearly visible. The transverse striation of the muscle fibers that retained their integrity was expressed in most of the fibers. Destructive changes in the muscle fibers were in the form of transverse cracks with the formation of a fine-grained protein mass in the destruc-



a) b)

Figure 3. Muscle tissue fragments:
a) Control 1; b) Test sample 1. (Magnification × 340)



a) b)

Figure 4. Microstructure of the minced meat:
a) Control 2; b) Test sample 2. (Magnification × 340)

tion areas. In Test sample 2, more pronounced swelling of the muscle fibers was observed. In some areas, the fibers merged with each other, and the boundaries between them were not distinguishable. Transverse striation, on the contrary, was not expressed, the fiber nuclei were shadow-like, destructive changes in the form of multiple microcracks and transverse cracks were pronounced with the formation of a fine-grained protein mass in the destruction areas.

Different conditions of the sarcolemma of the muscle fibers were noted. In Control 2, the integrity of the sarcolemma was preserved, while in Test sample 2 it was damaged; in some areas, small fragments were destroyed to a fine-grained protein mass, which enhanced the interconnection of the structural elements that had preserved their integrity.

It was noted that in Test sample 2 sample, the preserved muscle fibers were also more tightly adjacent to each other. The average diameter of muscle fibers in this sample was 88.0 μm, while in Control 2 it was 73.8 μm.

In Control 2, swollen or partly loosened connective tissue layers of the perimysium were observed. In some areas, a mass of glutin was formed, filling the microcapillaries. In Test sample 2, the loosening of the connective tissue layers and the formation of a homogeneous mass of glutin was more pronounced. Glutin was located in the microcapillaries in the form of a homogeneous structureless mass stained with basic dyes.

Differences in the condition of adipose cells were also noted. In Control 2, the adipose cell membranes were not damaged, and the fat was distributed in the fine-grained protein mass in the form of small 5–10 μm droplets. In contrast, in the Test sample 2, the adipose cell membranes were destroyed, and the fat was distributed in the form of 1–3 μm droplets in the fine-grained protein mass.

Thus, the test samples treated with the enzyme were characterized by a denser arrangement of structural elements, a less pronounced cellular components of muscle tissue, and the presence of glutin, a nitrogenous gelatinous substance that filled the microcapillaries and was formed as a result of protein degradation.

In our study, chymotrypsin was selected as the enzyme preparation. Chymotrypsin is not included in the list of enzyme preparations permitted according to TR CU029/2012 "Safety requirements for food additives, flavorings and processing aids". However, the current international practice of assessing the safety of enzyme preparations shows that pancreatic enzymes do not raise concerns about their safety under the expected conditions of use for food purposes on the basis that they originate from edible parts of animals [27].

Chymotrypsin is a serine protease (endopeptidase) and is found in the pancreas of animals. Chymotrypsin has one polypeptide chain of 245 amino acid residues and a molecular weight of 25.7 kDa [28]. This enzyme is currently considered one of the most significant proteolytic enzymes, which is widely used in the food industry and medicine [29]. The mechanism of chymotrypsin action is that it acts on a non-reactive carbonyl (–C=O) using a nucleophile. This enzyme does not exhibit allosteric effects, i. e. it does not have an active center that affects the conformational state of the enzyme [30]. Chymotrypsin exhibits its specificity by catalyzing the hydrolysis of peptide bonds at the C-terminal side of tryptophan, tyrosine, phenylalanine and leucine (the latter to a lesser extent), releasing polypeptides. In foreign practice, chymotrypsin is permitted in protein processing to obtain hydrolysates for use as ingredients in formulas for infants and young children [27].

All the results obtained, i. e. electrophoresis data, functional and technological indicators and histological examination data, confirmed the positive effect of enzymatic treatment. Previous studies also showed that treatment with proteases led to an improvement in the functional and technological characteristics of pork [13]. This result is consistent and may be explained by the fact that water holding capacity by myofibrils improves after enzymatic cleavage of denatured protein structures due to the formation of new hydrophilic centers and a change in the charge of molecules [31]. However, there is another opinion that denatured proteins, in particular sarcoplasmic proteins, contribute to the retention of moisture in muscle tissue [32], which may cast a doubt on the effectiveness of pork enzymatic treatment. Nevertheless, the most important functional proteins of meat are myofibrillar proteins, accordingly, their condition and transformation are most responsible for the quality of the final product [33].

In our study, parallel control samples and test samples were presented, differing only in the presence an acidity regulator. Analysis of the results showed that the presence of E500 food additive in the model systems was not mandatory. The use of enzyme treatment made it possible to achieve the same technological effect as the introduction of an acidity regulator. This clearly confirms the validity of the opinion by a number of authors that the use of proteases as an environmentally friendly material has not only economic advantages, but also far-reaching positive consequences in achieving sustainability [29].

We also noted that the desired technological effect on improving the functional properties of minced pork was achieved at an aging temperature of 4±2 °C (corresponds to the temperature conditions in the meat curing chamber), although most exogenously used proteolytic enzymes of plant origin have an optimal temperature of 50 to 70 °C, which corresponds to the temperatures of heat treatment of meat products [4]. With regard to chymotrypsin, there is evidence that its optimal activity begins at 30 °C [27]. Our results show that enzymatic treatment of meat with chymotrypsin can be easily integrated into pork processing.

However, other conditions and other proteolytic enzymes may obviously be selected for specific technological solutions. Many authors studying various preparations emphasize the importance of the functional state of proteins and the positive role of enzymes in achieving the required quality indicators [4,26,34]. Thus, it was reported that papain treatment had a positive effect on the functional,

rheological and physicochemical properties of myofibrillar proteins. Compared with the control samples, the fermented samples of myofibrillar proteins showed better functionality. Moreover, papain treatment led to an increase in hydrophobic groups on the surface of proteins and a decrease in the number of α-helix and β-sheet structures, which contributed to a change in the conformation of proteins, improving their solubility and emulsifying properties [34].

Thus, in the future, the use of proteolytic enzymes in the production of meat products may be considered in the context of creating new effective technological approaches to improve the functional and technological properties of meat raw materials.

Conclusion

The results of the studies on model systems showed that the minced pork treatment with the enzyme preparation improves its functional and technological properties, contributes to an increase in WBC, reduces the product weight loss during cooking, and increases the density of the microstructural components of minced meat. At the same time, the improvement of the functional and technological properties of pork is accompanied by a partial degradation of muscle proteins and formation of low-molecular protein fractions of 20 kDa and below. The results obtained convincingly proved the targeted improvement of the technological properties of pork obtained from intensively growing hybrid animals through the use of a proteolytic enzyme of animal origin. Nevertheless, for the practical use of proteases in industrial pork processing, further studies are needed to select the most economically acceptable enzyme preparation, as well as to determine the optimal duration and conditions for enzyme treatment, including depending on the technology and type of the final product.

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