Functional and technological indicators of fermented minced meat

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Abstract. Enzymatic processing of raw meat is a promising technique for balancing its properties. The paper presents the results of a study of the functional and technological parameters of minced meat subjected to enzymatic treatment with transglutaminase and sequential treatment with pepsin and transglutaminase. The object of the study is beef with a connective tissue content of 20%. The highest values of moisture-binding, water-holding abilities and product yield after heat treatment were established for a test sample of minced meat with sequential treatment with pepsin and 0.3% transglutaminase. According to the results of the studies, it can be concluded that low-grade beef should be treated first with a proteolytic enzyme to soften the connective tissue, and then with transglutaminase for better binding and retention of moisture.

1. Introduction

Currently, there is a significant shortage of raw meat. This restriction predetermined the need to solve the problem of a more complete use of available raw materials, including those with a high content of connective tissue and low-quality indicators. Various methods and approaches are widely used to adjust the properties of meat at enterprises, such as massaging and tumbler, soaking in solutions with chemical additives, exposure to ultrasound and electric current, etc. [1-9]. However, the application of these methods does not guarantee a product that is not inferior in quality indicators to products derived from high-quality raw materials. One way to solve the existing problem of adjusting the properties of raw meat is the use of enzymatic processing [10].

In the meat processing industry, enzyme preparations of animal origin are most often used as the most physiological, showing high activity to connective tissue proteins. Moreover, according to the effect on meat proteins, various enzymes differ significantly from each other. Proteolytic enzymes are used for the tenderizing of meat with a high content of connective tissue, and an enzyme such as transglutaminase (TG), which belongs to the class of transferases, is used to make the product monolithic by gluing together particles of raw materials [11, 12].
TG catalyses the formation of cross-links both inside the protein molecule and between the molecules of different proteins. Such a feature of this enzyme affects changes in the functional capabilities of the protein, viscosity, heat resistance, emulsifying ability, foaming and solubility [13]. The most favourable temperature for the catalytic activity of TG is 40 °C; however, it was found that at a temperature of about 0 °C the enzyme retains its activity [14].

Pepsin breaks down various animal and vegetable proteins, including casein, collagen, gluten, elastin, keratin, and histone.

Enzymatic hydrolysis of raw meat proteins is accompanied by destructive changes in muscle fibres, loosening of connective tissue layers, which positively affects the quality of finished products. Surface proteolysis, in which partial destruction of protein molecules occurs, leads to an increase in the content of free amino acids and an improvement in the consistency of meat, while fully preserving it.

The aim of the study is to establish the effect of enzyme processing on the functional and technological properties of low-grade beef.

2. Materials and methods

2.1. Test samples production

The object of the study was minced beef of the 2nd grade with a connective tissue content of 20%, obtained by grinding in a meat grinder with a particle size of 2 - 3 mm. For enzymatic processing, pork pepsin (RUE Belmetpreparaty) and transglutaminase BioBond TG-EB3 (Flora Ingredients) were used. As a control sample prepared minced meat without enzyme treatment was used, the experimental minced meat samples were subjected to sequential treatment with pepsin and TG. Control sample preparation scheme of prototypes is shown in figure 1.

![Diagram](image-url)
2.2 Research Methods

Functional and technological indicators were determined in the control and experimental samples: water-binding capacity, water-holding capacity, and product yield.

2.2.1 Water-binding capacity. Water-binding capacity (WBC) was determined by using a paper filter according to the method of Grau & Hamm. This method is based on measuring with a planimeter the area of the wet spot separated by a paper filter after lightly pressing a sample of minced meat. The mass fraction of bound moisture in the sample of meat was calculated by the following formula (%):

$$X = \frac{(A - 8.4 \times B)}{A} \times 100\%$$

where X is the mass fraction of bound moisture in the meat sample (of the total moisture content in the sample);
A is the total mass of moisture in the sample (mg);
B is the wet spot area (mm$^2$).

2.2.2 Water-holding capacity. Water-holding capacity (WHC) was determined by the gravimetric method proposed by Zhang et al [15].

WHC was calculated by the following formula (%):

$$\text{WHC} = \left[\frac{(M_2 - M)}{(M_1 - M)}\right] \times 100\%$$

where M is the mass of the sample of meat, g; $M_1$ is the mass of the sample of meat after heating and removal of the released moisture, g; $M_2$ is the mass of the sample of minced meat after centrifugation and removal of the obtained moisture, g.

2.2.3 Product yield. Product yield was determined by the gravimetric method based on measuring the mass of the sample of minced meat before and after heat treatment. To conduct the study, meatballs weighing 12 g were formed from prepared minced meat and subjected to heat treatment in a steam-air medium at a temperature of 75 °C for 10 min. Product yield (%) after heat treatment was determined by the following formula:

$$W = 100 \times \frac{m_2}{m_1}$$

where $m_1$ is the mass of the raw sample of minced meat, g; $m_2$ is the mass of the sample of minced meat after heat treatment, gr.

3. Results and discussion

The results of the WBC determination are presented in figure 2.
The results of the research show that with an increase in the content of TG, WBC increases, a similar trend was established for samples treated sequentially with pepsin and TG, but with a more pronounced effect. Zeng et al. note that myofibril breakdown as a result of proteolytic action of enzymes leads to better moisture binding [16].

The results of the WHC determination (figure 3) also show a positive dynamics of its change during the processing of samples with enzymes as compared with the control ones, however, the best result - 78.72% was shown for the sample with the introduction of pepsin and 0.3% TG. The results of determining the yield of the product after heat treatment (figure 3) are consistent with the results of determining WHC, an increase in the content of enzymes contributes to an increase in the yield of the product, and sequential treatment with pepsin and TG is more effective. Aaslyng et al., Pietrasik et al. also noted in their studies a correlation between WHC and losses during heat treatment of pork [17, 18].

The results obtained in our study are consistent with the data of many researchers. Kang et al. found that the addition of various levels of microbial TG increases the stability of the emulsion, cooking yield and texture properties of sausages [19]. Atilgan and Kilic note that the use of microbial transglutaminase in the production of meat products can be beneficial due to the reduction of losses during heat treatment, in addition, the textural properties of the products are improved [20].

The results of sequential enzymatic treatment with pepsin and TG of sunflower protein, obtained by Ivanova et al., showed that there is an improvement in solubility, foaming ability and thermal stability [21].

4. Conclusion
The results of the studies of minced meat show that an increase in the content of the enzyme TG with the addition of pepsin helps to improve the functional and technological indicators. The best result was shown with a pepsin-added sample followed by 0.3% transglutaminase. The yield of the sample increased by 9.5% compared with the control sample, the WBC of the sample is 88.2%, and the WHC is of 78.72%.

According to the results of the research, it can be concluded that it is advisable to treat low-grade meat formula with a high collagen content first with a proteolytic enzyme to soften the connective tissue, and then use TG to bind and retain moisture, which helps to increase the yield.
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