Enzyme preparations in the technology of whole muscle turkey products

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Abstract. The article presents a study of the effects of the enzyme preparations of propepsin, bromelain, and therizine on the quality characteristics of whole muscle turkey products. A technology has been developed and the enzyme preparations used in the production of whole muscle turkey products have been substantiated. Based on sensory and physicochemical studies, the optimal recipe composition of the product has been proposed. The quality regularities of whole muscle turkey products with respect to the enzyme preparation have been determined. The comparative study of technological parameters, affecting the enzymatic activity, has found that the Propepsin enzyme preparation was optimal for the production of whole muscle turkey products. There have been established proper ripening time for the raw material (12 hours) and the concentration of the enzyme preparation in an amount of 0.2% of the process weight, which made it possible to reduce the technological time of manufacturing whole muscle products by 20%, increase the yield of the finished product by 13%, and reduce the production costs by 13.9%.

1. Introduction

Today, the meat production is developing towards its acceptable level of profitability while maintaining the quality of products and safety requirements for the human body. The effectiveness of the means and methods applied to achieve these goals comes to the fore. This predetermines the need for a research on functional food products with new properties and characteristics that comply with requirements of resource saving, nutritional and biological values, and storage. In the total volume of whole muscle meat products in the world, turkey raw material is used in much smaller volumes in comparison, for example, with pork or beef. This is due to its specific technological properties and tissue composition. To correct the properties of the raw material that meets the requirements of the technology of whole muscle products, industry experts widely use enzyme preparations of various origins that create conditions for the intensification of autolysis of meat raw materials, penetration of curing substances into the product, and enhancement of biochemical processes [1].
It has been long established that technological changes in meat raw material are predetermined by enzymatic processes. The study of these processes opens up new opportunities for improving the technologies for manufacturing whole muscle meat products, allows active and direct control over the enzymatic catalysis of muscle proteins, and thereby increase the nutritional value of meat products. Currently, enzyme preparations used in the technology of whole muscle meat products are still limited due to the lack of experience and knowledge in the meat industry. Of great scientific and practical interest are the enzyme preparations of plant, animal, and microbial origins in whole muscle turkey products to change their technological properties and increase their biological value, which is a serious challenge for science and practice [2].

2. Problem statement
In the world, new enzymes and their properties are investigated for further use in the meat processing industry. The fermentation of raw meat is a promising area of the meat processing industry [3]. Enzyme preparations are actively used in the production of meat products, they affect the yield of the finished product, its quality, and sensory characteristics. Researchers are interested in the issue of the choice between enzymes of plant, animal, and microbial origins and their influence on the physicochemical parameters and technological properties of whole muscle meat products. All this requires careful study [4].

3. Research questions
In studying the influence of enzyme preparations—protepsin, bromelain, and therizine—in terms of the qualitative characteristics of whole muscle turkey products, the following questions were raised:

- How do these enzymes affect the physicochemical and sensory characteristics of finished products?
- How do the functional and technological characteristics of the raw material treated with the Protepsin enzyme change during cooking with respect to the temperature?
- How does the quantitative composition of microflora change at different stages of the production technology after the treatment of the raw material with Protepsin?
- How to determine the optimal modes of processing raw materials in each operation of the technological chain for the production of whole muscle turkey products, taking into account the characteristics of the Protepsin enzyme preparation?

4. Purpose of the study
The purpose of our research was to develop a technology for whole muscle turkey products, using enzyme preparations, and determine the most effective enzyme preparation for the production.

5. Methods
The studies were performed on the basis of the Rostov Reference Center of the Rosselkhoznadzor, Rostov-on-Don, Russia and in the conditions of OOO Meat Processing Enterprise TEMP, Novoshakhtinsk, Rostov Region, Russia. The objects of the study were enzyme preparations bromelain (a preparation of plant origin that is a proteinase from pineapple fruit, is thermostable, has a high temperature optimum, and exhibits high collagenase and elastase activities), protepsin (a preparation of animal origin that contains a complex of acid proteinases and is a synergist of cathepsins—intracellular enzymes of the meat system), and therizine (a preparation of microbial origin that was isolated by chemical methods from the waste products of fungi and microbes of special types); model samples of whole muscle turkey products prepared from chilled fillets, the autolysis period of 24 hours after slaughter at a temperature of 0-2 °C [5].

Based on the current experience in this field, we identified a list of enzyme preparations intended for injection into the raw material, selected their optimal concentrations, injected the turkey meat with brine that contained 0.1%, 0.2%, and 0.3% of enzyme preparations, respectively, and placed the meat into a
solution of sodium chloride at a ratio of 2:1 (water to turkey). The exposure was carried out at a temperature of 4 °C for 12-24 hours. The control samples were not fermented. We also studied changes in the structure of the raw material and noted unequal levels of swelling of the fibers. The Protepsin preparation was found to provide a higher level of swelling due to the higher fermentation rate of the raw material. When ripening was over, the physicochemical and rheological parameters of whole muscle turkey products were determined. We studied the redistribution of water in the turkey fillet meat under the action of fermentation by determining the water activity value and calculating the binding energy of moisture in the product. To determine the optimal modes of technological processing of the product, we studied the thermograms of the heat treatment mode, established the tissue digestibility level, its biological value, as well as changes in the functional and technological characteristics [6].

The effects of pH, temperature, and sodium chloride concentration on the proteolytic activity of various enzyme preparations were studied, and the results are given below (table 1).

| Parameter                  | Enzyme preparation |
|----------------------------|--------------------|
|                            | Therizine | Protepsin | Bromelain |
| Proteolytic activity, PE/mg| 95±3      | 490±3     | 370±4     |
| pH optimum                 | 6.5±0.05 | 5.5±0.02  | 6±0.05    |
| T inactivation limit °C     | 40±2     | 65±1.5    | 70±1.5    |
| NaCl concentration, %      |          |          |           |
|                           | 2        | 1        | 1         |
|                           | inhibits | activates | activates |
|                           | >2       | inhibits | inhibits  |

It was found that, the Protepsin preparation, compared with therizine and bromelain, showed enzymatic activity that was higher by 395 and 120 PE/mg, respectively, although its temperature optimum was slightly lower than bromelain’s one by 5 °C. The therizine’s temperature optimum of 40 °C did not correspond to the conditions of the effective heat treatment of whole muscle turkey products. Therizine also showed low activity at the sodium chloride concentrations in brine of above 2%. The only advantage of the plant enzyme preparation—therizine—was a high pH optimum, when it showed the maximum enzymatic activity.

Based on these data and taking into account the cost of all three enzyme preparations, we opted for Protepsin to be used in the production technology of whole muscle turkey products. Then we determined the optimal concentration of the enzyme preparation in the raw material [7].

The parameters and modes of technological operations for the production of whole muscle cooked turkey products with the Protepsin enzyme preparation were determined with respect to conventional technologies for poultry meat, taking into account the specifics of the raw material (composition and properties), the equipment applied, and required sensory characteristics of the product [8].

The turkey meat was injected according to the standard procedure with curing agents, containing 0.1-0.3% of Protepsin and a sodium chloride concentration of 2%. According to the calculations, the volume of injection brine was 12% of the process weight. After injection, the raw material was being ripened at a temperature of 4 °C for 12-24 hours. At the end of maturation, the effect of the enzyme preparation on the physicochemical and rheological characteristics of the turkey was determined. The comparative assessment of the main physicochemical characteristics (table 2) of the model turkey samples showed that different concentrations of the enzyme preparation had different effects on the pH, the water-binding capacity (WBC), and thereby the moisture content change. Compared indices of four samples made it possible to consider the most acceptable concentration of Protepsin at the level of 0.2% to the process weight.
Table 2. Physical and chemical characteristics of turkey meat during ripening.

| No. | Sample                          | WBC*, % to the total moisture | pH          | Moisture content, % |
|-----|---------------------------------|------------------------------|-------------|---------------------|
|     |                                 | 24 hours | 12 hours | 24 hours | 12 hours | 24 hours | 12 hours |
| 1   | Control (no Protepsin)          | 56.5 ± 1.3 | 60.8±1.2 | 5.65±0.01 | 5.83±0.04 | 68.3±0.3 | 73.2 ± 0.3 |
| 2   | Test I (0.1 % of Protepsin)     | 70.1±1.2 | 78.4±1.5 | 5.74±0.03 | 5.95±0.05 | 72.5 ± 0.25 | 75.5 ± 0.2 |
| 3   | Test II (0.2 % of Protepsin)    | 73.9±1.3 | 82.9±1.2 | 5.66±0.02 | 5.65±0.02 | 72.9 ± 0.4 | 77.6 ± 0.5 |
| 4   | Test III (0.3 % of Protepsin)   | 72.7±1.4 | 81.1±1.3 | 5.54±0.02 | 5.88±0.03 | 72.5±0.55 | 76.4 ± 0.3 |

Rheological studies of processed meat raw material gave an idea of further changes in the consistency of the product, sensory indicators (effort when chewing the product), and the possible assimilation level of the product by the human body. The consistency of the final product is one of the important sensory characteristics of meat products (table 3).

Table 3. Rheological parameters of the samples of whole muscle turkey products, depending on the concentration of the Protepsin enzyme preparation.

| Sample                          | Penetration coefficient at h = 25 mm | Yield value  | Shear stress | Cutting operation |
|---------------------------------|--------------------------------------|--------------|--------------|-------------------|
|                                 | 12 hours | 24 hours | 12 hours | 24 hours | 12 hours | 24 hours | 12 hours | 24 hours |
| 1. Control (no Protepsin)       | 11.60 ± 0.10 | 9.75 ± 0.20 | 5.689 ± 0.1 | 8.456 ± 0.2 | 1.55 ± 0.2 | 2.05 ± 0.1 | 6.35 ± 0.2 | 6.58 ± 0.1 |
| Test I (0.1 % of Protepsin)     | 13.77 ± 0.1 | 13.24 ± 0.2 | 4.156 ± 0.1 | 4.369 ± 0.1 | 1.88 ± 0.3 | 1.85 ± 0.1 | 6.12 ± 0.2 | 6.15 ± 0.2 |
| Test II (0.2 % of Protepsin)    | 14.45 ± 0.1 | 13.88 ± 0.1 | 3.655 ± 0.1 | 4.358 ± 0.2 | 1.15 ± 0.2 | 1.35 ± 0.1 | 4.35 ± 0.2 | 4.55 ± 0.2 |
| Test III (0.3 % of Protepsin)   | 14.25 ± 0.1 | 14.48 ± 0.1 | 3.456 ± 0.1 | 3.756 ± 0.1 | 1.77 ± 0.1 | 1.85 ± 0.1 | 3.85 ± 0.3 | 4.25 ± 0.1 |

The research results presented in table 3 show that the samples of raw materials treated with Protepsin were distinguished from the control sample by better quality indicators, with 0.1% Protein concentration having a slight effect on the properties of turkey meat and 0.2% concentration causing considerable changes in some parameters under study. This was due to the severely destroyed structure of muscle fibers under the action of the enzyme preparation.

These data indicated the Protepsin concentrations of 0.1% and 0.3% being inexpedient to be used. When comparing the rheological parameters of the raw material after 12 and 24 hours of exposure in the brine, insignificant changes in the values were noted in all test samples, which was apparently caused by a slowdown in the protein decomposition after a day of maturation.

At the next stage of the research, we established the water activity (Aw) and the moisture binding energy (E u, T) in the model samples in order to have an idea of the loosely bound moisture that allows...
putrefactive microflora to develop in the product. The obtained experimental data (table 4) indicated that the indices of the water activity for all four samples were in the range of \( Aw = 0.486 \text{ to } 0.812 \), with the 0.2% Protepsin sample having the lowest water activity and an increased proportion of the binding energy of moisture. In comparison with the other samples, especially with the Control, this sample of the whole muscle turkey product had a higher resistance to putrefactive microorganisms, so it had the best results [9].

Table 4. Water activity changed during maturation of turkey meat with Protepsin, \( Aw \).

| No. | Sample                        | 6 hours | 12 hours | 24 hours |
|-----|-------------------------------|---------|----------|----------|
| 1.  | Control (no Protepsin)        | 0.812   | 0.691    | 0.645    |
| 2.  | Test I (0.1 % of Protepsin)   | 0.716   | 0.486    | 0.675    |
| 3.  | Test II (0.2 % of Protepsin)  | 0.645   | 0.517    | 0.642    |
| 4.  | Test III (0.3 % of Protepsin) | 0.769   | 0.602    | 0.786    |

The water activity value made it possible to establish proper technological modes of processing raw meat and production of finished products. The water activity value is closely related to the binding energy of moisture in the product, which was calculated by the formula

\[
E(u, T) = -RT\ln(Aw), [1]
\]

where \( E(u, T) \) is the binding energy of moisture with a substance at a temperature \( T \) and moisture content \( u \), J/kg;

\( T \) is the absolute temperature of the wet substance and gaseous medium in equilibrium, °K; and

\( Aw(u, T) \) is the relative humidity of the gaseous medium in equilibrium with the wet substance [10].

The data on the binding energy of moisture of the test samples are given in table 5.

Table 5. Moisture binding energies in samples of whole muscle turkey products with Protepsin, kJ/kg.

| No. | Sample                        | 6 hours | 12 hours | 24 hours |
|-----|-------------------------------|---------|----------|----------|
| 1   | Control (no Protepsin)        | 12.38   | 27.12    | 22.72    |
| 2   | Test I (0.1 % of Protepsin)   | 24.37   | 43.23    | 29.56    |
| 3   | Test II (0.2 % of Protepsin)  | 34.66   | 59.19    | 35.56    |
| 4   | Test III (0.3 % of Protepsin) | 15.65   | 43.56    | 21.50    |

Based on the indices of the water activity and the binding energy of moisture in the samples of whole muscle turkey products with Protepsin, we established the optimal parameters of enzymatic processing raw material, i.e. aging in brine at a temperature of 4 °C and the Protepsin concentration of 0.2% to the process weight for 12 hours.

At the further stage of the research, we produced control samples of whole muscle meat turkey products according to the conventional process design that included the preparation of raw material, injecting with brine in an amount of 12% to the process weight, holding for 3 days, placing in a container, cooking at 80-82 °C for 40 minutes, cooling, and packaging. The process weight yield was 75%.

Test samples were produced, using Protepsin in the composition. In the course of the research, the mode of heat treatment of whole muscle turkey products with an enzyme preparation was divided into 3 stages with respect to the Protepsin’s effect on the raw material (this is the optimum temperature of inactivation of the enzyme preparation, temperature of 65 °C), i.e. Stage 1—temperature 55 °C for 10 minutes; Stage 2—temperature 65 °C for 10 minutes; and Stage 3—temperature 85 °C for 10 minutes, to a temperature in the center 72 ± 2 °C. Due to this heat treatment mode, the time spent on this operation was reduced by 25%, from 40 to 30 minutes. According to the technology developed for the production of whole muscle turkey products with Protepsin, a batch of control and prototype samples was produced on the basis of OOO TEMP meat processing enterprise, Novoshakhtinsk, Rostov region, Russia. For
this production, chilled turkey meat was used with an autolysis period of 24 hours after slaughter at a temperature of 0-2 °C. The meat was injected with 15% concentration brine, containing 0.2% sugar, 0.0075% sodium nitrite, and 0.2% Protepsin in an amount of 12% by weight of the raw material. After injection, the raw material was brinen for 12 hours at t=4 °C, the amount of brine was 50% to the process weight. Spices were added at the last stage of the production. Matured raw material was molded and heat-treated. The finished product was cooled to a temperature of not higher than 4 °C in the center. At the end of the production process, the yield of prototypes was determined and made 88%, which was by 13% higher than the control sample (75%). The products were packed according to the standard in polymer atmosphere packagings (the gas composed of 40% CO₂ and 60% N₂). The recommended storage time for the finished products was established after the laboratory observation and made up to 60 days at 4 °C and humidity of 75% [11].

At the next stage of the research, a sensory assessment of the whole muscle turkey products was performed on a 5-point scale that adequately characterized all the advantages of the prototype over the control; according to the average score, this advantage was 0.4 points. The prototype had a softer texture and was juicier (table 6).

| Sample | Quality parameter, points |
|--------|---------------------------|
|        | Appearance | Colour | Flavour | Consistency | Taste | Juiciness | Average |
| Control | 4.2±0.2 | 4.2±0.2 | 4.1±0.2 | 3.9±0.2 | 4.1±0.2 | 3.8±0.2 | 4.05±0.2 |
| Prototype (0.2 % of Protepsin) | 4.3±0.2 | 4.4±0.2 | 4.4±0.2 | 4.6±0.2 | 4.5±0.2 | 4.5±0.2 | 4.45±0.2 |

After the sensory evaluation, bacteriological studies were conducted according to generally accepted procedures. The total microbial contamination (sanitary indicative E. coli and Proteus bacteria, Clostridium, and Salmonella) in 1 gram of the product was determined. The microbiological indices met the sanitary requirements for whole-muscle cooked poultry products; the total number of microorganisms changed during the technological process from 2.2x10³ before the heat treatment mode to 1.9x10³ after the heat treatment of the control sample; in the prototype these indices were 4.7x10³ and 1.7x10³, respectively; lactic acid was the only residual microflora present in the samples; Clostridium bacteria were not detected [12]. The economic efficiency of the production of whole muscle turkey products with Protepsin was also calculated. The total cost of 1 ton of the control sample (75% yield) was 2264.8 dollars (178,926 rubles); the prototype’s yield of 88% of the process weight made 1956.8 dollars (154,589 rubles) of the total cost. The difference was $ 307.9 (RUB 24,337) or 13.6%.

6. Results
The comparative studies of the effects of various parameters on the enzymatic activities of the enzyme preparations during the raw material ripening enabled establishing that the Protepsin enzyme preparation is optimal in terms of aggregate characteristics for manufacturing whole muscle turkey products.

There was determined the optimal Protepsin concentration in the brine composition in an amount of 0.2% to the process weight, which made it possible to reduce the technological operation time of the heat treatment by 25% and the entire process of manufacturing whole muscle meat turkey products by 20%, increase the yield of the finished product to the process weight by 13%, increase the sensory properties of the finished product by 0.4 points, and reduce the production costs by 13.9%.

7. Conclusions
The specialists and technologists of the meat industry are faced with an important task to provide the increasing population with high-quality and relatively inexpensive meat products. The implementation of the technology for the production of whole muscle turkey products with the Protepsin enzyme
preparation allows expanding the range of meat products for enterprises, reduce the production cost these products, and increase their production [13-15].

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References
[1] Solodova S V, Slozhenkina M I, Fedotova A M, Mosolova E A and Knyazhechenko O A 2020 Statistics of food quality as a factor in the dynamics of development of nutritionally dependent diseases in russia IOP Conf Ser.: Earth Environ. Sci. 548 82033
[2] Titov E I, Tikhomirova N A, Nguyen B C, Slozhenkina M I and Mosolova N I 2020 Research of lactose hydrolysis depending on the type of the enzyme IOP Conf Ser.: Earth Environ. Sci. 548 82040
[3] Cheret R, Delbarre-Ladrat C, Lamballerie-Anton D M and Verrez-Bagnis V 2007 Calpain and cathepsin activities in post mortem fish and meat muscles Food Chemistry 101(4) 1474-9
[4] Qihe C, Guoqing H, Yingchun J and Hui N 2006 Effects of elastase from a Bacillus strain on the tenderization of beef meat Food Chemistry 98(4) 624-9
[5] Benito M J, Rodriguez M, Acosta R and Cordoba J J 2003 Effect of the fungal extracellular protease EPg222 on texture of whole pieces of pork loin Meat Science 65(2) 877-84
[6] Fedonin M Y 2000 Development of molded beef products using an enzyme preparation (Moscow) p 145
[7] Nalinanon S, Benjakul S, Visessanguan W and Kishimura H 2008 Improvement of gelatin extraction from bigeye snapper skin using pepsin-aided process in combination with protease inhibitor Food Hydrocolloids 22(4) 615-22
[8] Tantamacharik T, Carne A, Agyei D, Birch E and Bekhit A 2018 Use of Plant Proteolytic Enzymes for Meat Processing DOI: 10.1007/978-3-319-97132-2_3
[9] Arshad M S, Kwon J-H, Imran M A, Aslam A and Nawaz I 2016 Plant and bacterial proteases: A key towards improving meat tenderization, a mini review Cogent Food And Agricult 2 1-10
[10] Moon S S 2018 Effect of Proteolytic Enzymes and Ginger Extract on Tenderization of M. pectoralis profundus from Holstein Steer Korean J. for food sci. of animal res 38(1) 143-51 DOI: 10.5851/kosfa.2018.38.1.143
[11] Marques A Y, Marostica M R and Pastore G M 2010 Some nutritional, technological and environmental advances in the use of enzymes in meat products Enzyme res 480-923 DOI: 10.4061/2010/480923
[12] Doneva M, Nacheva I, Dyankova S, Metodieva P and Miteva D 2018 Application of plant proteolytic enzymes for tenderization of rabbit meat Biotechnol in Animal Husbandry 34 DOI: 10.2298/BAH1802229D
[13] Whitaker J 2018 The Proteolytic Enzymes DOI: 10.1201/9780203742136-19
[14] Maqsood S et al. 2018 Degradation of myofibrillar, sarcoplasmic and connective tissue proteins by plant proteolytic enzymes and their impact on camel meat tenderness J. of food sci. and technol. 55(9) 3427-38 DOI: 10.1007/s13197-018-3251-6
[15] Antipova L V, Shamhanov Ch Yu and Danyliv MM 2004 Enzymic hydrolysis waterand salt-soluble fraction of proteins of category 2 lamb News of univer. Food Technol.