The prospects for the use of proteinase from Bacillus pumilus for processing raw meat

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Abstract. The purpose of the work was to assess the prospects of using the microbial proteinase from Bacillus pumilus for processing meat raw materials with a high content of connective tissue fibers. This proteinase has broad substrate specificity and is capable of deep hydrolysis of proteins. The effect of protease from Bacillus pumilus on meat raw materials was studied with the prospect of using in the production technology. Proteinase is very effective at the structural elements of meat raw materials, in particular water-soluble and salt-soluble protein complexes, as confirmed by spectrophotometric studies. Enzymatic treatment with proteinase from B. pumilus destructively affects cellular structures, leads to swelling and loosening of collagen fibers. The accumulation of free amino acids was recorded during processing, the degree of hydrolysis was estimated, which amounted to an average of 15-20% depending on the type of protein fraction. The positive effect of the use of proteinase to improve the functional and technological properties of raw meat was established, which resulted in an increase in its water-binding and water-holding capacity, as well as the yield of finished products after heat treatment. Based on the results of the work, it was concluded that proteinase from B. Pumilus is promising for the processing of meat raw materials with reduced functional and technological properties, which is unsuitable for the production of meat products without additional processing.

1. Introduction

The quality of meat and meat products is determined by a combination of characteristics, including chemical composition, technological properties and organoleptic characteristics. One of the most important indicators that determine the consumer properties of a food product is its structure, which in relation to meat products depends on the content of connective tissue fibers, the degree of grinding, as well as the severity of autolytic changes in meat [1, 2].

The consistency of meat is associated with specific myofibrillar proteins, changes in which lead to the degradation of peptide molecules with the formation of amino acids and peptides with a small molecular weight. These processes occur due to natural lysosomal and proteasome systems, the activation of which leads to the destruction of muscle tissue proteins and formation of the flavor and aroma of raw meat; the term meat aging describes these processes [3]. They mainly affect sarcoplasmic and myofibrillar structures and, to a lesser extent, connective tissue proteins represented by collagen and elastin [4].

In some types of raw meat, the amount of connective tissue, represented mainly by collagen, can reach 20%. The specific amino acid composition of collagen, including the amino acids proline,
oxyproline and oxylysine, and the structure in the form of a triple helix determine its low functional and technological properties, as well as the stiffness of products containing this protein in large quantities. At the same time, collagen does not contain a number of essential amino acids, which reduces its nutritional and biological value. Nevertheless, the inclusion of collagen in the composition of meat products is advisable from both a physiological and a technological point of view, since the proteins of the connective tissue after heat treatment are able to form a three-dimensional skeleton that increases the water-binding capacity of the raw material. Currently, the range of using collagen in the meat industry is limited by a number of food products, the preparation technology of which is associated with prolonged heat treatment, during which it undergoes thermal degradation with the formation of gelatin. Thus, the targeted use of collagen in animal tissues, based on their special properties, remains an urgent task [3].

The use of exogenous proteolytic enzymes for processing raw meat allows accelerating the processes of its aging and tendering, which is especially important when processing hard meat with a high content of connective tissue obtained from such parts of the carcass as shank, flank, and back-chest part [5]. The most promising is the use of enzymes of microbial origin due to their availability, short growth cycle, and the possibility of synthesis of the enzyme in a nutrient medium, which facilitates subsequent isolation and makes it possible to obtain a preparation with a high degree of purification [4].

Among the variety of microorganisms that are relevant to the possibility of synthesizing biologically active substances, including enzymes, the bacteria Bacillus pumilus can be distinguished [6, 7]. These bacteria synthesize a number of extracellular enzymes that can be used in various industries. In particular, B. pumilus produce alkaline serine proteinase [8], which has proved to be effective as a feed additive in poultry farming to reduce the negative effect of digestive inhibitors [9].

Proteinase, which by the nature of the hydrolysis of specific chromogenic substrates and the type of inhibition related to subtilisin-like serine proteinases, was separated from Bacillus pumilus using ion exchange chromatography by employees of the Department of Microbiology of Kazan Federal University [4]. The enzyme breaks the bonds formed by carboxyl groups, has a temperature stability range from 0 to 40 °C, has maximum activity in a slightly acidic environment (pH 5.5) and retains significant activity in the pH range of 6-6.5, which allows it to be used for processing meat raw materials. It was established that this proteinase has broad substrate specificity and is capable of deep hydrolysis of proteins. The enzyme is non-toxic, which is confirmed by experiments on animals in a 10-day test [10].

The purpose of the work was to study the prospects of using proteinase from B. pumilus for processing meat raw materials, including those with a high content of connective tissue fibers.

2. Materials and methods

Beef with a connective tissue content of 9% according to GOST R 55445-2013 was used as object of research [11]. Sampling was carried out in accordance with GOST R 51447-99 [12].

The processing of raw meat was carried out by proteinase from B. pumilus according to the following scheme. Model minced systems were obtained by grinding meat in a laboratory meat grinder with grinder plates of 4 mm hole diameter. Proteinase was introduced in a dosage of 0.03-0.09%. As a control sample was used raw meat, not subjected to enzymatic treatment. Samples were kept at a temperature of 2-10 °C for 0-6 hours. The selected processing conditions are dictated by the need to comply with the regimes adopted in meat processing plants, as well as previously conducted studies [13]. Technochemical studies of the samples were carried out according to the procedure [14].

3. Results and discussion

At the initial stage of the study, the effect of proteinase from B. pumilus on water- and salt-soluble protein fractions of raw meat obtained by extraction was studied [13]. The absorption spectra of various meat chromophores were evaluated, such as the heme group, hydroxy, deoxy, and metmyoglobin [15]. The observed changes in the chromophores were extreme in nature with signified maxima at 60-180 minutes of the experiment. The accumulation of free amino acids in extracts in the wavelength range of
210-280 nm was found. The degree of hydrolysis of various protein fractions was estimated, which averaged 18-20% for the water-soluble protein fraction by 6 hours of the experiment and the concentration of the introduced enzyme was 0.06% by weight of the feed. For the salt-soluble protein fraction, the degree of hydrolysis was within 12-15% under the same experimental conditions. The use of protease in a dosage of 0.09% by weight of raw materials led to the achievement of similar action by 6 hours of the experiment and the form of clusters. It is retained in the product by hydrogen bonds and organized in the microcapillary network, which was confirmed by histological studies [13].

Histological studies that enable to assess the effectiveness of enzymatic processing at a structural level have confirmed that the introduction of protease from B. pumilus into meat raw materials leads to significant destructive changes in muscle fibers, expressed in their swelling, membrane damage, and loosening of intramuscular connective tissue and cellular structures. Collagen fiber swelling was also noted [13].

To assess the prospects for the industrial use of protease from B. pumilus in meat technology, it seemed advisable to study the effect of enzymatic processing on the main functional and technological properties of raw meat. The most important characteristics that determine the quality of the finished product are the ability of the raw materials to hold water during the heat treatment, the associated indicator of the yield of the finished product, as well as the moisture content in the product [5]. The results of the studies are presented in table 1.

Control samples of meat raw materials contained on average 70% moisture, had a water-holding capacity of 62%, and the product yield from this raw material was 96%. These indicators did not depend on the duration of sampling exposure.

The introduction of the enzyme in the composition of the minced meat led to an increase in the water-binding capacity of the meat, and the maximum values were noted at 3 hours of processing, and then decreased to control values. This indicator characterizes the presence of free moisture associated with the product by hydrogen bonds and organized in the form of clusters. It is retained in the product by capillary interaction forces and represents the moisture that is removed from the product with the broth during heat treatment and as part of the meat juice during defrosting. A decrease in this indicator during the enzymatic treatment is associated with the destruction of cell structures and a violation of the microcapillary network, which was confirmed by histological studies [13].

Table 1. Functional and technological properties of meat raw materials treated with protease from Bacillus pumilus.

| Sample | Water binding capacity, % | Water holding capacity, % | Moisture content, % | Yield, % |
|--------|---------------------------|---------------------------|--------------------|---------|
| Control | 59.00±0.3 | 62.00±0.4 | 70.00±0.5 | 96.00±0.5 |

Processing time 3 h
| Sample | Water binding capacity, % | Water holding capacity, % | Moisture content, % | Yield, % |
|--------|---------------------------|---------------------------|--------------------|---------|
| 0.03 % | 61.50±0.3 | 60.20±0.4 | 70.00±0.5 | 96.00±0.5 |
| 0.06 % | 62.00±0.3 | 71.50±0.4 | 76.50±0.5 | 97.00±0.5 |
| 0.09 % | 62.60±0.3 | 64.00±0.4 | 73.00±0.5 | 94.00±0.5 |

Processing time 6 h
| Sample | Water binding capacity, % | Water holding capacity, % | Moisture content, % | Yield, % |
|--------|---------------------------|---------------------------|--------------------|---------|
| 0.03 % | 60.50±0.3 | 63.00±0.4 | 75.00±0.5 | 97.00±0.5 |
| 0.06 % | 60.70±0.3 | 69.50±0.4 | 75.00±0.5 | 98.00±0.5 |
| 0.09 % | 60.00±0.3 | 65.50±0.4 | 74.50±0.5 | 96.00±0.5 |

The water-holding capacity is due to the presence of tightly bound moisture in meat raw materials, including adsorption moisture, as well as hydration water having the structure of hydrogen bridging compounds [5]. Such moisture is retained in meat due to the adsorption of water dipole proteins by hydrophilic centers. Enzymatic treatment affects the structural elements of the protein, increasing the number of active centers, and thereby contributes to an increase in water-holding capacity. The
maximum amount of retained moisture was observed in samples subjected to enzymatic treatment at a proteinase concentration of 0.06% and a holding time of 3-6 hours. An increase in the dosage of the enzyme led to a decrease in the considered indicator as a result of the development of destructive processes in raw meat and a decrease in the number of active centers because of hydrolysis.

A similar trend was observed when evaluating technological indicators, such as product yield and moisture content. The maximum yield of products obtained from meat raw materials that underwent enzymatic treatment was observed at a dosage of the enzyme preparation of 0.06% and a processing time of 6 hours.

Organoleptic evaluation was carried out on prepared samples of meat products, according to which the appearance, taste, aroma and texture were evaluated. As control, meat products obtained according to a similar recipe from raw materials that did not undergo enzymatic processing were used. Prototypes of products were distinguished by better consistency and appearance relative to control ones and received a higher rating in terms of the totality of results.

The results obtained allow the authors to positively assess the prospect of using proteinase from Bacillus pumilus for processing meat raw materials, including those with a high content of connective tissue. It should be emphasized that the use of enzymatic processing will expand the raw material base of the meat processing industry by involving sources of animal protein with reduced functional and technological properties that are unsuitable for producing high-quality meat products without additional processing.

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