SAT–LCR-P-2-BFT(R)-11

OPTIMIZATION OF MEAT-CONTAINING SEMI-FINISHED PRODUCTS FORMULATIONS WITH THE MICROBIOLOGICAL DERIVED PROTEASES APPLICATION

Prof. Vasyl Pasychnyi, DSc
Head of the Department of meat and meat products,
National University of food technologies, Kyiv
E-mail: pasww1@ukr.net

Dmytro Shvediuk, postgraduate
Problematic scientific research labaratory,
National University of food technologies, Kyiv
E-mail: shvedyuk.d@ukr.net

Abstract: *The article is devoted to the influence of microbiological derived proteases on the functional and technological characteristics of meat-based semi-finished products, which included in their recipes the fillets of broiler chickens, chickpeas and lentils. Optimal modes of hydration and pre-treatment of plant raw materials have been established for further enzymatic treatment with Aspergillus niger proteases (1). Thus, the experimental samples presented semi-finished products with different content of vegetable and meat raw materials, which resulted in the determination of the optimal formulation and its comparison with the data obtained by modeling the formulation by chemical composition and nutritional value (2,3). According to the data obtained, the maximum value of water holding capacity (78.6%) was recorded in the semi-finished product using boiled chickpeas in combination with step-by-step enzymatic treatment with the introduction of 35 mg of enzyme raw material per gram of substrate. Limit formulations of meat-based semi-finished products are also modeled, based on organoleptic characteristics and minimal value of water holding capacity during heat treatment (frying). The change of the herbal component in the composition of the formulation, as well as its combination, had a significant effect on the consistency and organoleptic characteristics of the finished product, but had little effect on the pH and yield of the studied products (4). The further aim of the research should be to determine the shelf life of meat-containing semi-finished products in all thermal states (under freezing conditions at -40 and -18 °C and at + 4-6 °C), as well as to adjust the organoleptic parameters of finished semi-finished products.*

Keywords: *meat, semi-finished products, microbiological derived proteases, plant stuff.*

INTRODUCTION

In modern conditions, the development of combined formulations of semi-finished products based on meat and vegetable raw materials is relevant due to several factors. First, the introduction into the formulations of meat semi-finished products of vegetable raw materials allows to enrich the finished product with such a set of amino acids, which would complement those present in the meat according to Mitchell's rule (6). Secondly, such a technological technique facilitates the regulation of the consistency of the finished product, such as vegetable raw materials can affect the textural properties of the product in a wide range (7). When selecting vegetable raw materials, the form of introduction of this raw material into meat products - native raw material, texture, concentrate or isolate - is important. Accordingly, the choice between these forms of ingredient introduction allows you to adjust the amount of protein and, accordingly, the amount of bound moisture in the recipe of the product. Thirdly, the production of semi-finished products with a combined composition of raw materials, including vegetable products, makes it possible to significantly reduce the cost of the finished product, given the lower costs of producing the same specific volume of raw materials (8). However, the production of combined products creates some complications when introduced into industrial conditions. There are two main problems in this area - the complication at the stage of preparation of raw materials and the negative impact of vegetable raw materials on the organoleptic characteristics (in particular taste) of the finished product. One of the possible solutions to eliminate these problems is the use of proteases of microbiological origin.

Microbial proteases are one of the most important hydrolytic enzymes from the whole set of widely studied proteases under current conditions. Proteases from microorganisms have attracted a great deal of attention in the last decade because of their biotechnological potential in various industrial processes, including industries such as detergent, textile, leather, dairy and pharmaceutical industries. Given these factors, proteolytic enzymes of microorganisms are the preferred source in the industrial use of enzymes because of their technical and economic advantage. This enzyme group is one of the largest industrial enzyme groups and accounts for approximately 60% of the world's total enzyme sales. Fungal proteases are an important raw material of the meat processing industry, including the fact that fungi can grow on low cost substrates and release large amounts of enzymes into the culture medium, which can facilitate processing and selection of new protease strains (8, 9).

The use of the microbiological protease produced by the Aspergillus niger strain is a relatively poorly researched aspect in meat technology, but has a proven positive effect on animal protein. Resource-saving processes are becoming increasingly important in industry as they are crucial to maintaining the environment and sustainability of ecosystems. Aspergillus niger enzymes, a natural catalyst for many types of proteolytic action, play an important role in the food industry as they are a convenient tool in many stages of food processing. Enzymatic transformations are unique in that they require less energy and are highly specific. Amylase, cellulase, protease, pectinase, lipase and the like are all common enzymatic choices for catalyzing the various stages of processing in the food industry. The search for new enzymes and their sources is essential to support environmental trends in the sector. Asparagine proteases and Aspergillus niger proteases, which play a major role in the degradation of proteinaceous materials, contain a small group of enzymes, including cathepsin, renin and pepsin. Enzymes contain two residues of aspartate in its active center and act in combination with a bound molecule of water in acidic pH. They are highly specific for the dipeptide with hydrophobic residues and the beta-methylene group. Their use has proven itself well in the processing and production of both traditional and new foods. They are widely used for refining drinks, making cheese, as well as for preserving wine. Detailed knowledge of the mechanism of action affecting the factors and structure of the enzyme will certainly bring further significant use of this enzyme in the food industry (10).

Mitofibrillar viscosity is caused by the onset of mortality in slaughterhouse meat, and the enzymatic breakdown of contractile proteins in meat after slaughtering causes softening of the meat. In older animals, the formation of stronger and more complex collagen bonds in connective tissue is increased; proteolytic enzymes can degrade 80% collagen connective tissue for meat.

Meat meat is the most important attribute that regulates consumer eligibility, consumer satisfaction and periodic purchasing trends and the market value of meat and meat products. Factors that affect the strength of the meat also contribute to the tenderness of the meat. Meat tenderness depends on the type of muscle factors, pre- and post-slaughter factors, as well as the pH and temperature after death. The chemical composition, structure and amount of connective tissue, usually dependent on the age of the animal and specific muscle types, also affect meat tenderness.

The use of exogenous proteases for tenderizing meat is a relatively progressive method of improving meat quality. According to most government licensing services, there are currently five exogenous proteolytic enzymes, including plant proteases (papain, bromelain and ficin) and proteases from Aspergillus oryzae and Bacillus subtilis, which are generally considered safe for use in m ' clear industries and agriculture. These proteolytic enzymes are mixed with meat to break down muscle proteins and hydrolysis of collagen and elastin, which helps in softening the meat. The use of enzymes reduces the amount of connective tissue and does not break down myofibrillar proteins. Papain and bromelain are the most commonly used vegetable enzymes for tenderizing meat. Proteolytic enzymes as meat tenderizers are best suited for the degradation of collagen and elastin in connective tissue at relatively low pH and low temperature.

Thus, considering as proteases to improve the functional and technological characteristics of the Aspergillus niger protease, we can conclude that this type of enzymes requires more detailed studies. Previously, a positive effect of proteolytic enzymes (in particular, the studied protease of microbiological origin) on functional and technological properties and autolytic processes in both axial raw meat (broiler chicken meat and semi-fat pork) and in vegetable raw materials (nutritional raw material)) (1,2). The use of lentils and chickpeas is a rational approach in the field of formulation of combined semi-finished products using vegetable raw materials. However, refinement requires the proper procedure for carrying out heat treatment and enzymatic processes in the production of this type of semi-finished products. When brought to a state of thermal readiness, the advantage is the possibility of final inactivation of the enzyme introduced, which makes it impossible to further alter the organoleptic properties and consistency of the product. However, for the improvement of organoleptic and functional parameters it is rational to use vegetable raw materials with pre-heat treatment already at the stage of forcing.

EXPOSITION

According to the design of the experiment, three groups of samples of semi-finished products were developed, which differed in several parameters. The first group of samples provided for a completely separate enzymatic treatment. Vegetable raw materials (chickpeas and lentils) were treated by crushing into 3-4 mm pieces, then mixed with the liquid form of the Aspergillus niger protease in relation to 35 mg of enzyme for each kg of raw material and kept for the fermentation process for 2 days at 4-6 ℃. Raw meat (meat of the thighs of broiler chickens) was obtained in chilled form and ground to pieces weighing 50-55 g, after which it was mixed with 2.5% salt and introduced aspergillus protease in liquid form in the calculation similar to that adopted for plant material, and kept for 36 h at -6 ° C. The processed raw materials of both types were mixed in a ratio of 60% of meat raw material and 40% of vegetable, after which secondary grinding of minced meat was made to visible homogeneity. After preparation of the minced meat, semi-finished products (cutlets) with a mass of 100 g were formed and subjected to freezing at -8 ° C for 6 hours. Thawed at 18-20 ° C for 2 h, the semi-finished products were fried for 25 min (12.5 min on each side) until full thermal readiness (temperature at the center of the product was 72 ° C with 5 min exposure).

The second group of samples provided for separate enzymatic treatment, but differed from the first group by pre-heat treatment of vegetable raw materials. Vegetable raw materials (chickpeas and lentils) were ground into 3-4 mm pieces, after which 150% water by weight of the raw material was added and cooked for 1 hour at 95-98 ℃. After cooling to 30 ° C, the feedstock was mixed with the Aspergillus niger liquid protease with a ratio of 35 mg of enzyme per kg of feedstock and kept for the fermentation process for 2 days at 4-6 ° C. Raw meat (meat of the thighs of broiler chickens) was also obtained in chilled form and ground to pieces weighing 50-55 g, after which it was mixed with 2.5% salt and introduced aspergillus protease in liquid form in the calculation similar to the accepted one. for vegetable raw materials, and kept for 36 h at -6 ° C. The processed raw materials of both types were mixed in a ratio of 50% of meat raw material and 50% of vegetable, after which secondary grinding of minced meat was made to visible homogeneity. After preparation of minced meat, it was formed semi-finished products (cutlets) weighing 100 g and subjected to freezing at -8 ℃ for 8 hours. Thawed at 18-20 ° C for 2 h, the semi-finished products were fried for 20 min (10 min on each side) until full thermal readiness (temperature at the center of the product was 72 ° C with 5 min exposure). The difference in the durations of some types of processing and the percentage of input of vegetable raw materials is due to the higher moisture content and heat capacity of the raw material previously subjected to cooking.

The samples of the third group provided for simultaneous processing of meat and vegetable raw materials. Vegetable (chickpeas and lentils) and meat (meat of thighs of broiler chickens) raw materials were crushed into 5 mm pieces, after which additional 120% of water (by weight of vegetable) was added, 2.5% of table salt (calculated by weight of both raw materials) and 35 mg of enzyme per gram of raw material (minus water input). After incubation for 36 h at 4-6 ° C, the raw material was ground to visible homogeneity and the absence of visible inclusions of the vegetable grain. After preparation of the minced meat, semi-finished products (cutlets) with a mass of 100 g were formed and subjected to freezing at -8 ° C for 6 hours. Thawed at 18-20 ° C for 2 h, the semi-finished products were fried for 28 min (14 min on each side) until full thermal readiness (temperature at the center of the product was 72 ° C with 8 min exposure). The increase in the duration of heat treatment is due to the coarser consistency of the stuffing in the product.

Each sample group provided samples of three species, depending on the input vegetable. Samples under No. 1 made only chickpeas (40 or 50% depending on the series), samples under No. 2 had lentils (40 or 50% depending on the series), and samples with No. 3 had both raw materials (20 or 25 each). % depending on the series).

Among the studied parameters were selected - the pH value of the water extract of the finished product, the moisture content of the finished product, wettability (VZZ) and ductility of the finished product. The product yield and the amount of separated broth were determined by the arbitration method. The pH was measured using a potentiometric laboratory pH meter. Extraction of the extract from the finished product was carried out on the basis of distilled water in an amount of 10 parts relative to the sample weight of the finished product for 30 min. The moisture content was determined after pre-drying the sample (3-5 g) of product at 120-130 ºC to constant weight. The values of OHS were established by the method of pressing the sample (0.28-0.32 g) of the product.

Table 1

Ingredient,%	Group 1			Groups 2, 3		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Meat of poultry tights	60	60	60	50	50	50
Chickpea	40		20	50		25
Lentil		40	20		50	25
Total	100	100	100	100	100	100

Determination of physico-chemical parameters of finished products

Indicators of finished products are given in table.2. From the presented research data, it can be concluded that all the types of treatment used allow to obtain a product with high for this type of products physico-chemical parameters and functional characteristics. The moisture content of the finished products was in the range of 68-72% for all samples of the combined semi-finished products, which indicates a rational selection of processing modes, which compensated for fluctuations in the amount of moisture introduced during the preparation of raw materials. Group 1 samples had slightly lower moisture content of the finished product (68.05-69.1%), which is due to the time of addition of additional moisture - even with the introduction of water during the preparation of forcemeat, even pre-enzymatically processed vegetable raw materials are not able to bind sufficient moisture and to ensure a tight consistency of the finished product. If there is a need for the development of products, the pre-fermentation of raw materials for which must occur at low temperatures in the raw form, the rational step is to introduce hydrocolloids or reduce the proportion of vegetable raw materials in favor of high-grade meat raw materials. The highest moisture content was recorded in Group 2 samples (70.2-72.0%). This is due to the fact that during cooking vegetable raw material absorbs a much higher percentage of moisture than the similar percentage absorbed during the preparation of minced meat. Also a significant role is played by the change of heat treatment mode - minimization of heat treatment is possible due to less dense consistency of both vegetable raw materials (and its pre-heat treatment) and meat (compared to traditional technology). The thermal treatment of these types of products can essentially be reduced to a minimum (reaching 68-70 in the middle of the product without exposure), but the limiting factor here is the microbiological safety of the finished product. This area needs further research.

The pH values were in the same range for all products, without clearly observable trends and the ability to track the impact of a particular method of processing or preparation of raw materials. The pH value in all finished products was in the range of 6.75-6.85. The discrepancy in performance may be due to the standard deviation or measurement error.

Table 2 Indicators of finished products

Properies	Group 1			Group 2			Group 3		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
pH	6,8	6,75	6,8	6,85	6,75	6,75	6,8	6,85	6,85
Moisture content,%	68,05	68,4	69,1	70,2	70,65	72	68,34	68,6	68,92
WHC, %	67,94	69,9	70,38	72,68	76,45	75,3	70,05	67,15	71,3
Mass of ready-to-eat product (product yeld), %	74,5	77,2	78,6	84,3	80,8	86,1	70,5	74,4	74,9

Determination of functional and technological indicators of finished products

According to the distribution of the values of OHS and the output of the finished product, it can be clearly concluded that the heat treatment of vegetable raw materials has a positive effect on the functional and technological characteristics of the prepared cutlets, but the values of OES in groups 1 and 3 are also quite high (67,94-71 , 3%). The output of the finished product in groups 1 and 3 is also significantly lower than the similar indicator of group 2 (maximum values of 78.6 for group 1 versus 86.1 for group 2). Thus, heat treatment of vegetable raw materials not only binds more moisture, but also preserves it at the expense of less rigid heat treatment modes.

CONCLUSIONS

According to the results obtained, it can be concluded that the development of semi-finished products based on vegetable and meat raw materials using chickpeas and lentils, as well as the meat of the thighs of broiler chickens, the introduction of microbiological protease Asperagillus niger allows to achieve high functional and technological parameters and improve the consistency of the finished product. Pre-treatment of vegetable raw materials is best done by combining enzymatic treatment with pre-cooking of raw materials in the presence of additional moisture. This technology allows you to optimize the cost of resources and time for heat treatment, which can be reduced to minimum values, the only limiting factors for which are microbiological safety and organoleptic characteristics of the finished product.

When applying moisture to vegetable raw materials during pre-cooking, it is necessary to anticipate changes in the modes not only of the heat treatment, but also in the freezing and thawing of products, which may be longer due to the greater part of the bound moisture in the product. The best results were achieved by combining chickpeas and lentils in equal quantities. Further research requires microbiological safety and product storage times, as well as the introduction of additional flavoring ingredients.

REFERENCES

Shvediuk D & Pasichnyi V. (2018) *Application of the target fermentation in the technology of extended shelf-life meat-based products* - Bulletin of the National Technical University "KhPI". Series: New solutions in modern technology, № 16 (1292), 184-190.

Shvedyuk, D. & Pasichnyi, V. & Moroz, O. & Heredchuk, A.. (2018). The influence of a microbiological derived protease on the autolysis process in meat of broiler chickens. Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. 20. 32-35. 10.32718/nvlvet9007.

Da Silva, R. R. (2017). Bacterial and fungal proteolytic enzymes: production, catalysis and potential applications. Applied biochemistry and biotechnology, 183(1), 1-19.

Souza, P. M. D., Bittencourt, M. L. D. A., Caprara, C. C., Freitas, M. D., Almeida, R. P. C. D., Silveira, D., ... & Magalhães, P. O. (2015). A biotechnology perspective of fungal proteases. Brazilian Journal of Microbiology, 46(2), 337-346.

Brandelli, A., Sala, L., & Kalil, S. J. (2015). Microbial enzymes for bioconversion of poultry waste into added-value products. Food research international, 73, 3-12.

Kumar, P., Chatli, M. K., Mehta, N., Singh, P., Malav, O. P., & Verma, A. K. (2017). Meat analogues: Health promising sustainable meat substitutes. Critical reviews in food science and nutrition, 57(5), 923-932.

Arshad, M. S., Kwon, J. H., Imran, M., Sohaib, M., Aslam, A., Nawaz, I., ... & Javed, M. (2016). Plant and bacterial proteases: A key towards improving meat tenderization, a mini review. Cogent Food & Agriculture, 2(1), 1261780.

Sun, Q., Chen, F., Geng, F., Luo, Y., Gong, S., & Jiang, Z. (2018). A novel aspartic protease from Rhizomucor miehei expressed in Pichia pastoris and its application on meat tenderization and preparation of turtle peptides. Food chemistry, 245, 570-577.

Manea, I., Manea, L., & Marinescu, V. (2016). Study Concerning The Proteolytic Activity Of Vegetable Enzymes On Cured Meat Quality. Annals. Food Science and Technology, 421-425.