STUDY OF M4 PEPTIDASE FAMILY'S ACTION IN THE ENZYMATIC TREATMENT OF RAW MEAT AND COLLAGEN-CONTAINING RAW MATERIALS

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Abstract – This article analyzes the principles and characteristics of different peptidase families' action on collagen molecule. A comparative evaluation of the action of M4 peptidase family on raw meat and collagen-containing raw materials was conducted; new approaches for the selection of proteolytic enzymes for the enzymatic treatment of raw meat and collagen-containing raw materials were described with the aim of tenderization. In order to study the impact of peptidase family M4 on the raw meat it was carried out development of model samples of horse meat, injected with supernatant culture fluid obtained from Aeromonas salmonicida. In order to study the impact of peptidase family M4 on collagen- offal was held to develop model samples boiled scars injected prior to the heat treatment of the culture supernatant liquid obtained from Aeromonas salmonicida. Different options of these enzymes in the processing of raw meat and collagen-containing raw materials have been proposed based on the studied properties. After analyzing the results, we concluded that the peptidase family M4, having a wide range of substrate activities would be useful in the tenderizing of raw meat and the tenderizing of collagen raw material.

Key Words – Aeromonas salmonicida, collagen-containing and meat raw materials, M4 peptidase families, propionic acid bacteria, tenderization of collagen-containing and meat raw materials.

I. INTRODUCTION

Processing technology for raw materials of animal origin is accompanied by high yield of raw materials that are needed to be tenderized before sale. Such raw materials include tough raw meat and collagen-containing raw materials.

Meat tenderness is known to be determined by the structural characteristics of muscle tissue (actin-myosin effect), connective tissue (background effect) and fat tissue (marbling). Actin-myosin effect is associated with sarcomere length and diameter of muscle fibers; background effect is associated with the total content and distribution of the connective tissue between muscle fibers [1, 2].

Meat mainly consists of muscle protein. Muscles are fascicles of protein fibers that are assembled into blocks surrounded by connective tissue. Connective tissue also contains structural proteins such as collagen and elastin. Collagen is insoluble protein; it forms white fibers that are tough and inelastic. Meat can be tenderized by preliminary breakdown of some connective tissue proteins and some muscle fibers [3].

Given the structural features that influence the tenderness of meat, it is necessary to look for a comprehensive approach regarding action on muscle and connective tissue.

The main technological problems with collagen-containing raw materials processing (in particular collagen-containing byproducts) are the energy consumption and process duration. Manufacturing technology of liver sausages, brawn, jellies provides long-term heat treatment of collagen-containing byproducts, and the cooking time primarily depends on the type of raw material. The basis of collagen-containing products is native collagen. Therefore, it is necessary to look for new ways of connective tissue processing, while taking into account the depth of hydrolysis, which will influence the degree of raw material tenderization before heat treatment.

The search for new methods of raw meat and collagen-containing raw materials tenderization is relevant and necessary to create the products that will satisfy the consumer needs regarding flavor profile.

In meat industry, enzyme preparations of animal, plant and microbial origin are widely used for

tenderization of collagen-containing and meat raw materials. However, there are number of problems in their implementation.

The main criterion for such enzymes usage in food industry should be the compliance with safety requirements - GRAS recommendations (Generally Recognized As Safe). The list of GRAS enzymes is generally represented by proteases of plant origin (bromelain, ficin, papain) that are extremely expensive to produce, because of which are not widely used.

Besides, optimal action of the enzymes used should correlate with the main process parameters of meat production (meat pH and temperature when exposed to salting and heat treatment, concentration of enzyme preparations added to product, enzyme treatment duration, presence of activators and inhibitors of the enzymes used). Changing even one of the parameters can affect the performance of the enzymes used and dramatically change the desired result.

Moreover, specificity of enzyme preparations has an important role. Since the raw meat is a multicomponent object, the ratio of muscle and connective tissue of which is unstable and depends on meat type, physiological and anatomical features of the animal, from which it was obtained, there is a need for enzymes of multi-directional action depending on the target substrate of raw meat, whether it is muscle or connective tissue.

In the processing of collagen-containing raw materials, particularly collagen-containing byproducts, which are mainly consist of native collagen, the need to use the highly specific peptidase of targeted action and to hardly "cut" the collagen molecule is dropped away. It is important to partially tenderize raw material before heat treatment. Collagen is the main component of connective tissue; it is insoluble and most common protein in mammals, ranging from 25% to 35% of the total amount of protein. This is fibrillar protein providing strength and elasticity of connective tissue. Thus, it is hardly digestible protein and one of the factors affecting the meat toughness.

It is known that specific sequence of collagen chain is «G-X-Y», where often «X» is proline; «Y» is hydroxyproline or one of hydrophobic acids. Of particular interest are the peptidases from M4 and M9 families that are able to specifically act on collagen molecule. However, «G-Pro» link is cut only by metalloproteases from M9 family [4].

After analysis of possible hydrolysis sites for M9 and M4 peptidase families, it was noted that the peptidases from M4 family were able to extensively break down muscle proteins with a depth of hydrolysis up to low molecular weight peptides. Thus, peptidases from M9 family will not hydrolyze proteins of muscle tissue due to lack of recognition sites. The opposite pattern was observed when analyzing the action of these proteases on the collagen chain: in this case, only peptidases from M9 family are specific with regard to collagen sequences.

However, peptidases from thermolysin family possessing good overall proteolytic activity could partially tenderize native collagen.

II. MATERIALS AND METHODS

Model samples of horse meat injected with supernatant of culture fluid obtained from Aeromonas salmonicida (representative of bacteria producing M4 peptidase family) were developed to study the effects of M4 peptidase family on raw meat.

Model samples of tanked tripe injected prior to the heat treatment with supernatant of culture fluid obtained from Aeromonas salmonicida (representative of bacteria producing M4 peptidase family) were developed to study the effects of M4 peptidase family on collagen-containing byproducts.

The culture fluid from Aeromonas salmonicida has been obtained after submerged cultivation on peptone media supplemented with collagen hydrolyzate, which served as an inductor of collagenase production.

Microbial cells were precipitated by centrifugation. Sample of horse meat was injected with supernatant of culture fluid obtained from Aeromonas salmonicida. Fermentation time of injected samples was 72 hours; fermentation temperature was 4-6 °C. After fermentation, the meat products were cut into bars. Structural and mechanical tests of experimental samples were carried out on Instron 3342 instrument.

Beef tripes were cleaned, skinned from mucosa, washed out in flowing cold water and cut into two strips 10 to 15 cm wide. Thereafter, they were immersed into hot water (t = 80 °C) and held for

15 to 20 minutes to delay the development of undesirable microflora on the surface of byproduct. One of the strips was held in supernatant of culture fluid obtained from Aeromonas salmonicida; the second one served as a control and was held in normal saline. After 24 hours of exposure, strips were formed into rolls, which were immersed in cold water, boiled and cooked on low heat for 3 to 4 hours. After preparation, rolls were allowed to cool down. Structural and mechanical tests of experimental samples were carried out on Instron 3342 instrument.

Sample identification	Max. shear force, (N/m^2)	Average load, (kgf)	Standard shear force, (N/m2)	Mean value of the average load, (kgf)
C *	622376.71	1.59	405535.20	1.7
C	700792.12	1.47	375060.97	
C	846776.81	2.04	520150.00	
E *	513513.89	1.15	293746.51	1.07
E	412361.16	1.08	275809.69	
E	518189.76	1.00	255231.05	

Sample identification	Max. shear force, (N/m^2)	Average load, (kgf)	Standard shear force, (N/m^2)	Mean value of the average load, (kgf)
C *	603545.85	1.40	355812.14	1.47
C *	597072.07	1.19	304232.88	
C *	848721.89	1.82	462893.46	
E *	550466.42	1.16	295375.52	1.24
E *	488935.10	1.37	350440.06	
E *	612638.89	1.19	303188.39	

III. RESULTS AND DISCUSSION

Table 1 shows that the experimental sample was 15% softer compared to control, which confirms the pronounced action of peptidase from M4 family on raw meat.

Table 1. Structural and mechanical evaluation of horse meat samples

Notes: The studies were performed in triplicate.
C* - control sample of horse meat treated with normal saline.
E* - sample of horse meat treated with supernatant of culture fluid obtained from Aeromonas salmonicida.

The results of structural and mechanical evaluation of tripe rolls are presented in Tables 2 and 3.

Table 2. Structural and mechanical evaluation of beef tripe rolls

Notes: The studies were performed in triplicate.
C* - control - beef tripe roll treated before cooking with normal saline.
E* - experiment - beef tripe roll treated before cooking with supernatant of culture fluid obtained from Aeromonas salmonicida.

Table 3. Structural and mechanical evaluation of beef tripe rolls in expanded form.

Sample identification	Max. shear force, (N/m^2)	Average load, (kgf)	Standard shear force, (N/m^2)	Mean value of the average load, (kgf)
C *	355734.88	0.73	185173.74	1.91
C	379897.56	0.88	224004.41	
C	383017.77	0.92	233680.94	
E *	327058.00	0,82	209539.65	1.69
E	301480.64	0,74	187734.16	
E	199764.66	0,39	100568.24	

Notes: C* - control - beef tripe strip treated before cooking with normal saline.
E* - experiment - beef tripe strip treated before cooking with supernatant of culture fluid obtained from Aeromonas salmonicida.

Tables 2 and 3 show that the experimental samples treated with culture fluid obtained from Aeromonas salmonicida were softer compared to controls (average load factor of the instrument was 37% lower (Table 1) and 12% lower (Table 2)).

After analysis of the results, we concluded that M4 peptidase family possessing a wide range of substrate activities would be useful in tenderization of raw meat and collagen-containing raw materials.

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