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Study of lysate activity to modificate collagene raw materials to use in sausage mixture

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Abstract: In the current conditions of import substitution, the effective use of secondary raw materials in the meat industry is a relevant issue. A significant source of animal proteins is by-products, the yield of which is about 10% of livestock weight. Some by-products, including beef rumen, contain collagen-containing tissues which require modification for tenderization and deodorization. In order to modify rumen tissues, the biotechnological method of treatment with an enzyme solution, lysate, obtained from a whole bovine abomasum was preferred to the known method where enzyme solution is prepared from an abomasal mucosa. The purpose of this project was to study the activity of lysate from a whole bovine abomasum for the modification of rumen tissue to use it in cooked sausage formulations. We have suggested the method of obtaining enzyme solution based on infusing the minced abomasum in a reaction mixture – water, chlorohydric acid, and sodium tripolyphosphate – followed by filtering. The dependence of proteolytic and collagenase activities of the solution obtained from phosphate dose introduced have been studied; it have been revealed that 1.5% of tripolyphosphate is the optimal dose for efficient extraction of enzymes from the whole abomasum. Besides, an effect of the enzyme solution on functional and technological properties of a heat-treated rumen has been studied, and the improvement of hydro- and lipophilic characteristics has been revealed. Paste with modified rumen has been developed and found that the maximum possible dose of rumen for use in cooked sausage from horsemeat is 15%. The color on the cut of sausage developed was identical to that of beef sausage. Thus, paste made on the basis of modified rumen contributes to the formation of functional and technological properties, the stabilization of the color characteristics of the final product, as well as the effective use of basic meat raw materials and the expansion of the range of economy class high-protein sausage production.

Keywords: Modified rumen, cooked sausage, formulation, functional and technological properties, quality

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INTRODUCTION

In the current conditions of import substitution, the effective use of secondary raw materials in the meat industry to create natural, protein-containing products of high quality using innovative technological methods is a relevant issue.

A significant source of animal proteins is by-products [1–4], the yield of which is about 10% of livestock weight. Most of by-products are characterized by rather low fat content and increased mass fraction of connective tissue; the latter brings about reduced biological value.

Collagen-containing by-products contain a large amount of connective tissue, which is difficult to digest in humans. However, modern scientific research on processing of collagen-containing raw materials has shown that the use of innovative technologies to modify rumen allows the degree of collagen assimilation to be increased. In addition, collagen, being hard to digest, can act as dietary fiber, improving gastrointestinal tract peristalsis [1, 3, 5, 6].

One of factors limiting the possible effectiveness of by-products use is the specificity of their morphological composition: internal organs are composed of muscular, connective, fatty, and parenchymal tissues. Since various types of raw materials differ in their composition and structure, it requires the use of individual methods for their preliminary processing, which predetermines final product quality as a whole and, in particular, organoleptic indicators and functional and technological properties. Most of the traditional technologies in sausage-canning manufacture, however, include the grouping of secondary protein-containing raw materials in accordance with external morphological

characteristics (fleshy, meat-and-bone, and mucous), heat treatment under high temperatures, and the homogenization of raw materials to destruct connective and cartilaginous tissues, whereas taking into consideration the chemical composition features, organoleptic characteristics, and morphological structure of certain types of secondary raw materials would provide great opportunities for making fundamentally new types of meat products with high quality characteristics.

The low efficiency of using collagen-containing by-products is mainly due to the specificity of chemical and morphological composition and the need for a variety of technological methods aimed both at improving organoleptic characteristics and modifying functional and technological properties, such as water-binding capacity, swelling capacity, water and fat absorbing ability, emulsifying ability, etc.; each type of raw material requires individual methods for their preliminary processing.

The efficiency of using both food and biotechnological potential of collagen-containing by-products is the subject of many studies. Researchers suggest using collagen-containing by-products in formulations of protein-oil emulsions [7], gelatin [8], preparations for zootechnics [9], and meat products [10–12].

Local and foreign researchers suggest various ways of technological processing by-products: physical, chemical, and biotechnological. Rumens, abomasums, stomachs are deodorized by single or multiple cooking in water, broths, spice solutions, milk, whey, weak solutions of organic acids (acetic, ascorbic), or hydrogen peroxide solution; this allows both structural-mechanical and functional-technological properties to be improved.

There are many studies on modification of collagen-containing raw materials by the biotechnological method of processing [13–18]. This method is the most effective but the use of ready-made enzyme preparations in the meat industry is limited due to high cost. Moreover, it is difficult to select an appropriate enzyme which would be exposed to structural proteins of both muscle and connective tissues.

Pepsin, the enzyme preparation isolated from mucous membrane of young calves' abomasums, has proteolytic effect and can be used to soften proteic materials, including collagen-containing ones. The manufacturing process of this enzyme preparation, however, is laborious, since it requires separation of mucous membrane first and then treatment to isolate pepsin. We have suggested a technique for obtaining the enzyme solution from the whole bovine abomasum which can be used to tenderize collagen-containing raw materials.

In connection with the above mentioned, the purpose of the project was to study the activity of lysate from the whole bovine abomasum for modification of rumen tissue to use it in cooked sausage formulations.

STUDY OBJECTS AND METHODS

Experimental research was carried out at the Technology of Meat and Canned Products Department Laboratory of the East-Siberian State University of Technology and Management (Ulan Ude, Russia).

The subjects of the research were bovine abomasum, rumen, sausage mixture, and cooked sausage.

In the previous study we suggested [19] a method of obtaining an enzymatic solution, lysate, from a whole bovine abomasum which contains a mixture of proteolytic enzymes. The lysate was obtained by infusing minced abomasum in a reaction mixture containing water, chlorohydric acid, and sodium polyphosphate. In order to create optimal conditions for activating pepsinogen by adjusting a dose of hydrochloric acid, the reaction mixture acidity was maintained as large as 2.0. The pepsin solution was prepared according to the traditional technique for pepsin isolation from mucous membranes, but whole rumen was used instead of mucous membrane due to difficulty in separating it. This technique is presented in Fig. 1.

The enzyme solution for rumen fermentation must be used within six hours at the storage temperature 2–4°C.

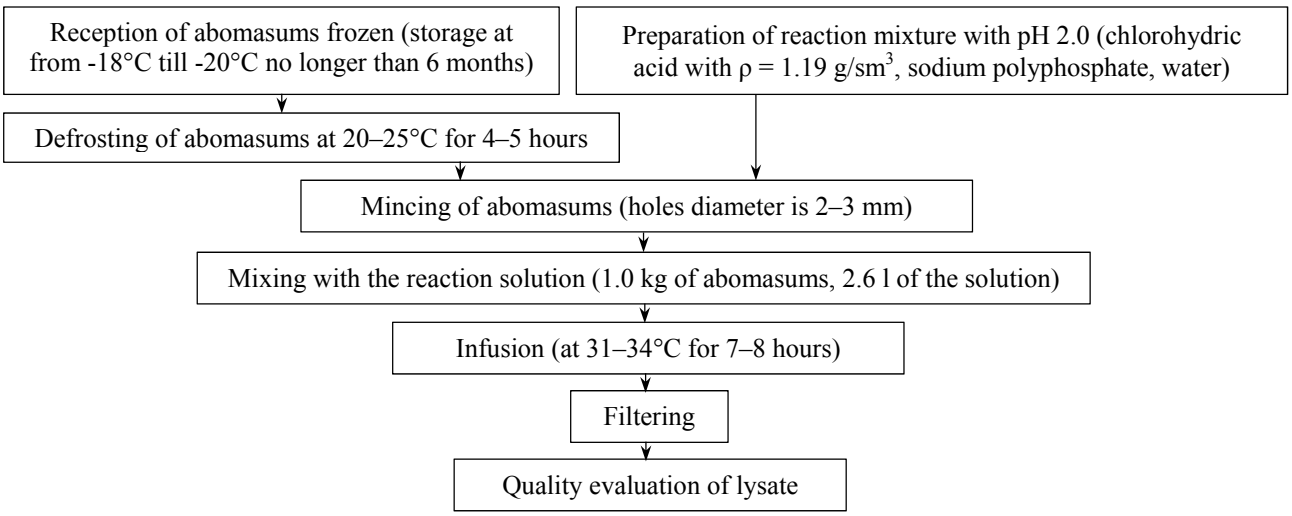

Fig. 1. Technological scheme of pepsin solution preparation from whole bovine abomasums.

In the course of the experiments, organoleptic indicators were determined by organoleptic tasting, protein mass fraction by the Kjeldahl method, fat mass fraction by the Soxhlet method, water content by drying to constant weight, and mineral substances content by ashing. Functional and technological properties, such as water-holding capacity (WHC), fat-holding capacity (FHC), and thermal stability, were determined by the VNIMMP method [20], moisture binding capacity by Grau-Hamm method, and critical shear stress with a penetrometer. The number of pigments was studied with a photoelectric colorimeter based on the optical density of colored solutions. The mass fraction of common salt was determined in water extract by Mohr's method, residual sodium nitrite content by the photometric method, which is based on the measuring of color intensity formed as a result of interaction between nitrite with sulfonylamide and N-(1-naphthyl)-ethylenediamine dihydrochloride in a protein-free filtrate. Collagen content was determined from the amount of oxyproline (GOST R 50207-92) multiplied by a factor of 8.07 [21].

In order to study lysate proteolytic activity, the method of Ganguly and Bhaler with some modification was used: casein agar was substituted by myosin one as a substrate [22]. The substrate was poured into Petri dishes, holes with a diameter of 12 mm were made in the solidified medium, after that the enzyme solution was poured for incubating. The diameter of the precipitate zone determined solution activity (in mm).

The collagenase activity of the lysate was studied by spectrophotometric method [23] where collagen was used as a substrate. Collagen was obtained from the Achilles tendon of cattle by removal of extraneous proteins by precipitation, further repeated centrifugation, washing, and drying the collagen resulted. Collagenase activity was determined as follows: 5 ml of the lysate was added to 20 mg of collagen and incubated at 37°C for 18 hours. In addition, two control samples were prepared: in the first one, the substrate was incubated with the lysate heated at 60°C for 2 minutes to inactivate enzymes, while the second sample was prepared as the experimental one, but without incubation. The samples were cooled, filtered, and an equal volume of biuret reagent was introduced into. The experimental and the first control samples were measured with the green filter of a colorimeter against the second control sample. The collagenase activity was determined from an extinction value (E); to convert it to percentage of protein dissolved, the standard data presented in Table 1 were used.

Denaturation temperature was measured by using a device with accordance to GOST 32078-2013 [24]. The amino acid composition of proteins was determined with an amino acid analyzer.

Experiments were carried out in three replications.

RESULTS AND DISCUSSION

Researchers working in this field are developing food with modified collagen from hide, splits, connective tissue, etc. It has been established that collagen has properties of dietary fiber and exerts favorable effect on intestinal tract peristalsis and beneficial microflora function.

We have conducted research on modification of collagen-containing rumen properties by the biotechnological method using enzyme solution obtained from a whole abomasum.

The technological scheme of preparing the enzyme solution is represented in Fig. 1. We used sodium polyphosphate (SP) to improve an efficiency of pepsinogen extraction. In order to prevent the process of enzymes extraction from an influence of the medium pH, the latter had the constant value (2.0) due to introduction of chlorohydric acid solution. When adding 0.5% of sodium polyphosphate to the reaction mixture volume, pH was 2.02, and 2% of sodium polyphosphate led to pH = 2.1.

Sodium tripolyphosphate promotes the loosening of the epithelial layer of the abomasum, thereby assisting enzymes release into the solution, since the epithelium is a loose connective tissue with a touch of reticular one.

In the meat industry, introduction of sodium tripolyphosphate in amount of 1–2% is sufficient to increase hydrophilic activity of muscle proteins. Proteolytic and collagenase activities of enzyme solutions without SP and with 1.0, 1.5, and 2.0% of SP were investigated. The enzyme solution obtained from mucous membrane of bovine abomasum was used as a control sample, while experimental samples were prepared from whole abomasum. This method of obtaining the enzyme solution eliminates the difficult process of mucous membrane separation. Fig. 2 shows the change in the proteolytic activity of the solution as a function of a SP dose.

The data in Fig. 2 indicate that addition of sodium polyphosphate increases the proteolytic activity of the solution, for instance, 1.0% of SP raises the proteolytic activity by 18.5% however its level remains below the control value. Introduction of SP in amounts of 1.5 and 2.0% increases the proteolytic activity up to 18.4–18.9 mm that is 4.0–6.0% higher than in the control sample. This must have been due to an increase in pepsinogen concentration, which indirectly confirms that SP improves enzyme extraction.

Further, the collagenase activity of the enzyme solution was analyzed (Fig. 3).

According to the data in Fig.3, SP increases both proteolytic and collagenase activities of the lysate. Introduction of SP in the amount of 1.5 increases the collagenase activity of the pepsin solution up to 29.93%, which is close to that for the control sample (30.16%). A further increase in SP dose up to 2.0% increases the lysate collagenase activity slightly. Probably, SP facilitates a breakage of bonds between polypeptide chains and fibers separation in the abomasum structure, which assist the process of pepsinogen molecules release.

Table 1. Dependence of extinction value on protein cleavage degree

Indicators	Values				
Extinction value, E	0.174	0.115	0.088	0.062	0.029
Collagen dissolved, %	54.51	36.00	27.81	23.71	11.60

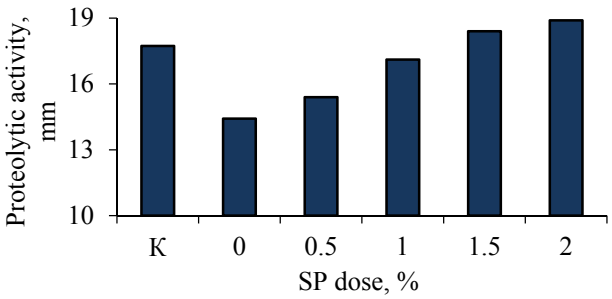

Fig. 2. Change in proteolytic activity of enzyme solution containing SP.

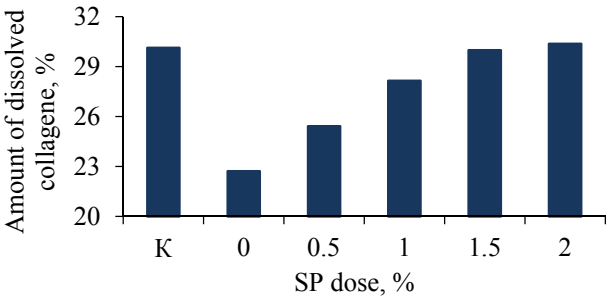

Fig. 3. Change of collagenase activity of enzyme solution containing SP.

Based on the data obtained, the optimal SP dose was accepted to be 1.5%, since it ensures high proteolytic and collagenase activities of the solution. Table 2 shows qualitative indicators of the lysate with SP obtained by using the technological scheme suggested.

The findings from Table 1 show that the pepsin solution obtained from the whole abomasum is similar in appearance to that obtained from the mucous membrane and has high enzyme activity.

On the next stage, experiments on modification of rumen properties with the enzyme solution obtained were carried out. As known, rumen tissue contains about 7% of collagen and 0.5% of elastin. Collagen and elastin fibers are able to stabilize rumen connective tissue and to give it high mechanical properties. For this experiment, we used prepared rumen meeting the requirements of the documentary standard. Chemical composition and technological properties of bovine rumen were analyzed (Table 3).

The data indicate that the total protein content in rumen is as high as 15.2% which is almost equal to that in muscle tissue; the protein fraction of rumen, however, contains about 65% of collagen.

According to the results of rumen technological parameters analysis, rumen has low water-binding capacity (55.4%) and high shearing force value (6.8×10^2 Pa); this is due to high mechanical characteristics of collagen and elastic fibers, which impart an increased hardness to rumen tissue.

Denaturation temperature is an important indicator of collagen which characterizes its structural changes; for bovine rumen its value is 66.3°C. At this temperature, collagen bundles diminish to the maximum, bend, and intra- and intermolecular interactions in collagen structure tend to weaken. This made it necessary to study the modification process of rumen tissue after a short-term heat treatment, which retards the contamination process.

For modification, rumen was held in the enzyme solution prepared from the bovine abomasum. For this, 30% of the enzyme solution was added to minced rumen and the fermentation process was carried out at 35–36°C for 12–14 hours. This duration was chosen based on the fact that pepsin hydrolyzes proteins and peptides far slower than other proteinases do [23]. In addition, pH was reduced to 5.3–5.5, since at pH 5.6 and higher an inhibitor inactivates enzymes; moreover, acidic conditions contribute to microorganism growth inhibition.

Table 2. Qualitative indicators of enzyme solution

Indicators	Enzyme solution (lysate) obtained from	
	Mucous membrane of bovine abomasum (control)	Whole bovine abomasum (experimental)
Color	Light-beige	Beige
Texture	Slime	Slime
Aroma	Specific	Specific
Proteolytic activity, mm	17.81	18.42
Collagenase activity(amount of dissolved collagen, %)	30.21	29.81

Table 3. Chemical composition and technological properties of bovine rumen

Organoleptic indicators	Characteristics	Chemical composition	Value	Technological properties	Value
Appearance	Fat-free, cut up, separated from mucous membrane, washed, with no dark spots	Mass fraction of protein, % – collagen, %	15.2 ± 1.0 9.6 ± 0.5	Water-binding capacity, %	55.4 ± 1.0
Color	Yellowish-white, with grayish tint	Mass fraction of fats, %	4.9 ± 0.4	Shearing force, $\times 10^2$ Pa	6.8 ± 0.2
Aroma	Typical of good by-products, typical of rumen, without foreign smell	Mass fraction of mineral substances, %	1.4 ± 0.1	Denaturation temperature, °C	66.3 ± 2.0

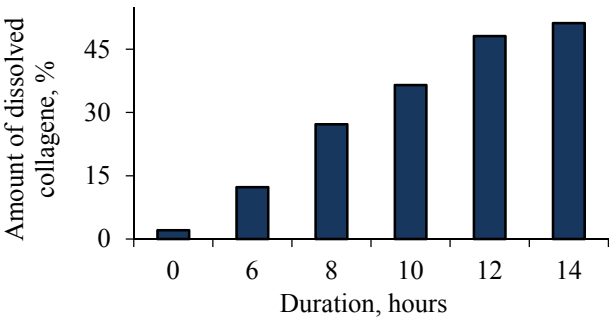

Fig. 4. Effect of holding duration of rumen samples in enzyme solution on amount of collagen dissolved.

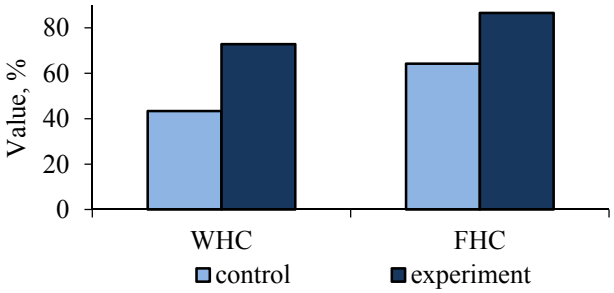

Fig. 5. Functional and technological indicators of rumen.

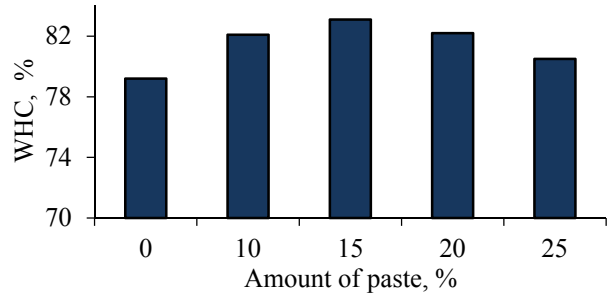

Fig. 6. Effect of paste on water-holding capacity of horse mince.

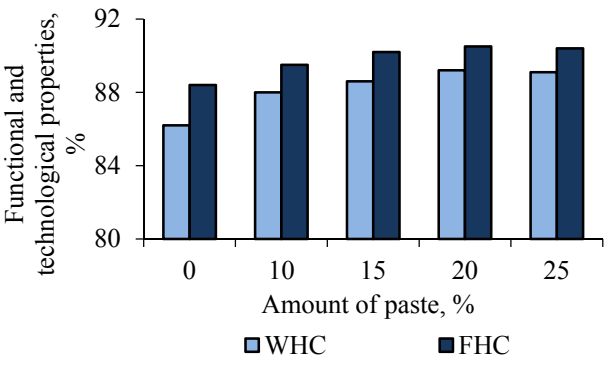

Fig. 7. Water-holding capacity change as a function of amount of paste.

In order to analyze the modification process, an effect of the enzyme solution obtained on the amount of collagen dissolved at various holding duration of rumen samples in the enzyme solution was studied (Fig. 4).

Table 4. Characteristics of paste based on modified rumen

Organoleptic indicators	Value	Physical and chemical indicators	Values
Appearance	Grey-white color	Mass fraction of water, %	79.8 ± 1.4
Texture	Pasty	Mass fraction of proteins, %	13.4 ± 0.7
Aroma	Flavorless	Mass fraction of ash, %	1.2 ± 0.1
Taste	Tasteless	Mass fraction of fats, %	5.4 ± 0.2

The results in Fig. 4 demonstrate a considerable increase of dissolved collagen amount with an increase of the holding duration of the substrate in the enzyme solution, which can indicate the increase of collagen fragmentation degree. Since after 12 hours of holding changes in the studied indicator was insignificant, the optimal time was 12 hours. It should be noted that pepsin is able to destroy hydrogen and disulfide bonds between polypeptide chains that leads to formation of "open" forms of protein molecules. As a result of modification, morphological changes in muscle, collagen and elastin fibers can occur which contribute to improve rumen properties. In order to prove this, functional and technological characteristics of rumen tissue modified were studied (Fig. 5).

The results demonstrated in Fig. 5 show a favorable effect of holding rumen in the enzyme solution on improvement of water- and fat-holding capacities which speaks for modification of collagen-containing rumen properties by treatment with pepsin solution obtained from the whole bovine abomasum. Undoubtedly, the factors that contribute to improvement of functional and technological properties of rumen are changes in the structure of collagen and unstriped and cross-striped muscle fibers of rumen with formation of active functional hydro- and lipophilic groups.

Modified rumen was then finely minced and diffused until paste was obtained. Table 4 represents qualitative characteristics of the paste based on modified bovine rumen.

The next stage was to find a possibility to include the resulting paste in the formulation of horse cooked sausage. In order to determine the optimal amount of the paste, the functional and technological properties of horse mince with 10, 15, 20, and 25% of the paste were analyzed. Fig. 6 illustrates the effect of the paste amount on the water-holding capacity of the mince.

The data in Fig. 4 show that 15% of the paste increases meat hydrophility by 3–4%. Further increase of the paste amount makes the mince water-logged therefore WHC declines.

The indicators of water- and fat-holding capacities of the mince after heat treatment were now studied (Fig. 7).

The results indicate improvement of functional and technological properties of the heat-treated mince with increasing amount of the paste. The paste in amount of 25% increases WHC and FHC by 3% and 2%,

respectively. Denaturation of modified collagen fibers also improves the functional and technological properties, since gelling agents are able hold additional water molecules. Collagen play a critical part in this process, but the role of unstriped and cross-striped muscle fibers, which contribute to active functional hydro- and lipophilic groups, is also significant.

Further, critical shear stress, a parameter which characterizes rheological properties of the system, was studied (Fig. 8).

According to the results from Fig. 8, the critical shear stress of the mince is 450 Pa. Introduction of the paste in amounts of 10% and 15% causes the decrease in critical share stress by 2.3% and 4.9%, respectively; with the increase of the paste amount, the magnitude decreases markedly (by 11–13%) due to the mince watering.

Further, sausages with spices and various amounts of the paste were made according to the formulation of 2nd grade cooked horse sausage; the content of nitroso pigments, which provide for forming the color and appearance of the product, was studied. The reason for using horse sausage was the fact that the paste contains a small amount of muscle tissue which is able to react with sodium nitrite to form nitroso pigments, and horse meat contains more the pigments than beef and pork do. Fig. 9 illustrates the change in nitroso pigments content in horse sausage samples with the paste in amounts of 10, 15, 20, and 25% in comparison with that in control samples of pork, beef, and horse sausages.

The data in Fig. 9 indicate that the paste affects nitroso pigments content, since the rate and the efficiency of their formation depend on myoglobin and oxymyoglobin content in the mince. Myoglobin content is 72.8% in pork sausage, which is light pink in color, 79.3% in beef sausage, and 84.4% in horse sausage, which is dark red in color. The values obtained are consistent with pigments content in these meats. Introduction of the paste caused discoloration of the product as a result of depigmentation. In the experimental sample containing the paste in amount of 15%, color is identical to that of beef sausage, and color of the sample with 20% of the paste is similar to that of pork sausage.

Based on the research conducted, the amount of the paste was established to provide optimal functional and technological properties and color of the product: the paste in amount of 15% was used in the formulation of the cooked sausage instead of 10% of horse meat and 5% of fat. The formulation of the cooked sausage named “Zaigraevskaya” with 15% of the paste based on modified rumen was developed (Table 5).

As seen from Figure 10, organoleptic parameters of the experimental sample are similar to those of the control one.

Quality parameters of the cooked horse sausage with the paste are represented in Table 6.

Since cooked sausages are a mass-consumption product, a priority target on their manufacturing is to keep the balance between proteins and fats, as well as between water and dry matter contents.

Immediately after manufacturing, quality inspection by all parameters of control and experimental samples was performed.

Seven experts participated in sensory analysis of sausages developed; their evaluations were entered in a tasting sheet. The tasting evaluation data were recorded in the form of scores (using nine-point scale method) and description of each indicator by members of the taste panel.

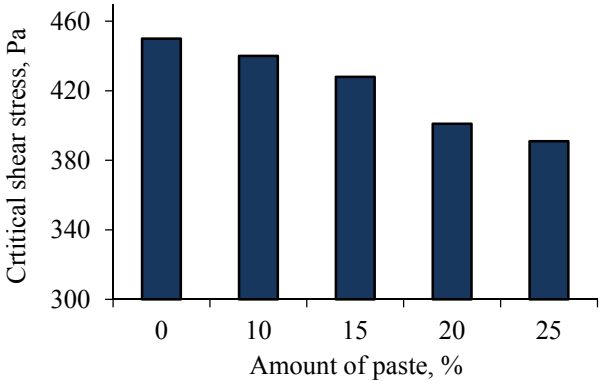

Fig. 8. Effect of amount of paste based on modified rumen on critical share stress.

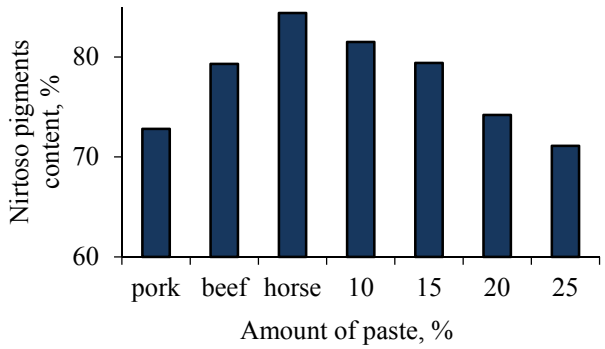

Fig. 9. Nitroso pigments content in cooked horse sausage samples with paste based on modified rumen.

Table 5. Formulation of cooked sausage “Zaigraevskaya”

Raw materials	Amount of raw, kg (per 100 kg of raw)	
	Horse cooked sausage control	Cooked sausage “Zaigraevskaya” experiment
1st grade trimmed horse meat	83.0	73.0
Fat	15.0	10.0
Starch	2.0	–
Paste based on modified rumen	–	15.0
Soya protein	–	2.0
Total amount	100.0	100.0
Salt	2.5	2.5
Sodium nitrite	0.005	0.005
Sugar	0.1	0.1
Black pepper	0.05	0.05

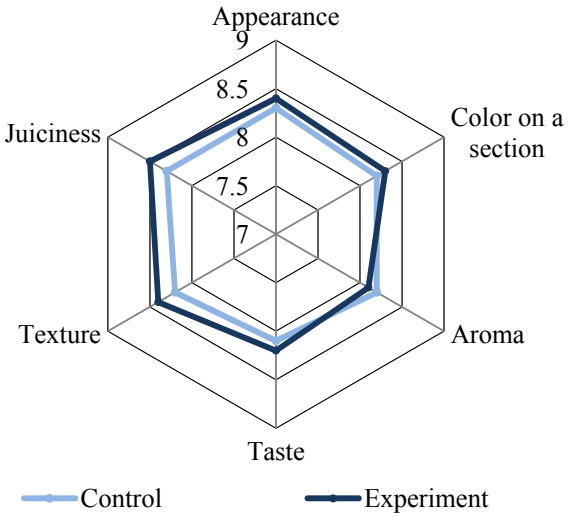

Fig. 10. Profilogram of organoleptic evaluation of cooked horse sausage "Zaigraevskaya" with paste based on modified rumen.

Table 6. Quality parameters of cooked horse sausage "Zaigraevskaya" with paste based on modified rumen

Parameter	Characteristics	Physical and chemical parameters	Value
Appearance	Clean dry surface	Mass fraction of fat, %	16.2 ± 0.5
Texture	Elastic	Mass fraction of protein, %	17.5 ± 0.6
Color and appearance on a cut	Pink, mince is mixed uniformly	Mass fraction of salt, %	2.4 ± 0.3
Aroma and taste	Typical of this product, no foreign taste and odor, with spicy flavor, properly salted	Mass fraction of sodium nitrite, %	0.002 ± 0.0001

Fig. 10 demonstrates tasting sheets data.

The data in Table 10 show that the chemical composition and organoleptic parameters of experimental samples conform to requirements GOST R 52196-2003 for cooked sausages of grades A and B according to which the mass fraction of fat is no more than 20–30%, protein – no less than 10–12%, salt – up to 2.4%, and sodium nitrite – up to 0.005%.

CONCLUSION

As a result of the research, a beneficial impact of the enzyme solution obtained from a whole bovine abomasums with added sodium tripolyphosphate on tough rumen tissue has been established. It has been determined that the optimal time for holding rumen in the pepsin solution is 12 hours. Biotechnological treatment of rumen tissue by holding it in the solution for 12 hours at the temperature of 35–36°C facilitates the improvement of functional and technological characteristics and the increase of collagene fragmentation degree. Based on fine rumen tissue, paste with high organoleptic and technological properties was developed. The paste was used in the formulation of cooked horse sausage. The maximum possible amount of the paste from modified rumen which contributes to form hydrophilic and structural and mechanical characteristics of the mince has been established (15%) to use it in the formulation. It has been revealed that horse sausage with 15% of the paste is similar in color to beef sausage. Thus introduction of the paste based on modified rumen into horse sausage composition provides the formation of functional and technological properties and the stabilization of organoleptic parameters of the final product as well as the efficient use of basic meat raw materials and the expansion of the range of the economy-class high-protein sausage production.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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