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# Biomodification Of Meat For Improving Functional-Technological Properties Of Minced Meat.

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**ABSTRACT**

To date, the actual creation of technologies that allow reducing the cost of production of meat products, while guaranteeing the consumer the preservation of the specified quality standards. With the development of biotechnology, it became possible to develop and introduce new technologies aimed at intensifying the set of complex biochemical transformations that occur in meat raw materials in the production of sausages. The paper presents the results of a study of a complex of lactic acid bacteria. As a result of the research, positive results were obtained to increase the moisture-binding, moisture-retaining and fat-retaining abilities of model minced meat after biomodification, and a slight decrease in pH was shown. As a result of biomodification of meat raw materials by starter cultures, digestibility of model minced meat by digestive enzymes of the gastrointestinal tract increases.
**Keywords**: functional and technological properties, starter cultures, meat raw materials, microflora

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## INTRODUCTION

In recent years much attention has been paid to the production of meat products from non-traditional raw materials. To intensify the existing technology, it is advisable to use strains of lactic acid and denitrofication bacteria. In this regard, many experts carried out research to determine the targeted impact on low grade meat of a mixture of cultures of microorganisms of specified qualitative and quantitative compositions. It was revealed that the acceleration of proteolytic processes and ripening of minced meat of raw cured meat and cold-smoked sausages is accompanied by an increase in the biological value of finished products [1,2].

With the development of biotechnology, it became possible to produce new types of meat products of general, special and therapeutic and prophylactic purposes affecting the natural intestinal microflora, to improve methods of enzymatic processing of meat raw materials with the aim of improving its functional and technological properties [3].

The normal microflora that inhabits the human intestine is important for regulating the optimal level of metabolic processes occurring in the body, as well as for creating high colonization resistance of the intestinal tract to opportunistic microorganisms [2]. The variety of functions performed by saprophytic microorganisms determines their extremely important role in maintaining normal human vital activity. However, in recent years there has been a trend towards an increase in pathological conditions, accompanied by a violation of the microecological balance of the intestine, which in almost all cases requires pharmacological correction. Over a number of years, the search has been made for optimal means aimed at preventing the emergence of dysbacteriosis, and increasing the resistance of the organism to unfavorable environmental factors [4,5]. For this purpose there appear attempts to use sour-milk products fermented with the help of bacteria, which are now an important component of human nutrition. However, microorganisms contained in these products are usually transient and do not colonize in the intestine. Continuous improvement of technologies and recipes, manufactured products led to the creation of biologically active food additives based on eubiotics, which in modern conditions take a leading place in the prevention and complex therapy of a number of diseases. [6,7,8]

All this shows that food products (meat, dairy, etc.) containing lactic acid bacteria and bifidobacteria should be considered not only as food products of increased biological value, providing the body with plastic and energy substances, but also as the most valuable preventive and therapeutic means .

In this regard, the purpose of the work is to select strains of microorganisms that are capable of biomodifying collagen-containing meat raw materials in the technology of producing cold smoked sausages.

## MATERIALS AND METHODS

In accordance with the tasks assigned, a selection of objects, methods of investigation was carried out, and a scheme for setting up the experiment was developed. Based on the obtained data on the study of starter cultures for smoked sausages [9,10,11], we selected starter cultures of Lactobacillus plantarum, Bifidobacterium siccum, Staphylococcus carnosus. Experimental studies were carried out in the laboratories of the departments: the technology of storage and processing of livestock products; biotechnology, biochemistry, biophysics of the Kuban State Agrarian University named after I.T. Trubilin (KubSAU), the “Argus” test center.

Determination of pH was carried out on a universal ionometer pH-150M according to GOST (all-Union State Standard) R 51478-99 [12].

Water-binding capacity (WBC) of the raw material was determined by the Grau-Hamm method [13].

The number of mesophilic aerobic and facultative-anaerobic microorganisms was determined in accordance with GOST (all-Union State Standard) 10444.15-94 [14].

Mass changes in the raw material were determined by weighing on scales and in a ratio in % to the mass of the feedstock. The mass fraction of moisture was determined in accordance with GOST (all-Union State Standard) 9793-74 by drying the sample to constant weight at a temperature of 105 ° C [15, 16]. The moisture retaining capacity (MRC) of minced meat was calculated as the difference between the mass fraction of moisture in minced meat and the amount of moisture that was separated during heat treatment [13]. The stickiness was determined by the Sokolov-Bolshakov method [13]. The fractional composition of proteins was determined on the basis of their solubility [13].

Mathematical processing of data was carried out in the STATISTICA program in modules: descriptive statistics, correlation analysis and experiment planning.

To determine digestibility, an enzymatic method for determining the biological value of meat in vitro was used. The basis of the method is enzymatic hydrolysis under conditions in which the availability of attacked peptide bonds is determined not only by the properties of the protein, but also by additional factors associated with the structure and chemical composition of the food product [13]. The method consists in sequential action on the protein substances of the test product with a system of proteinases consisting of pepsin and trypsin with continuous stirring and removal of the products of hydrolysis by dialysis from the reaction sphere. This allows avoiding the inhibition of digestive enzymes by low molecular peptides and free amino acids.

## RESULTS AND DISCUSSION

The basis of the effectiveness of any biotechnology is the knowledge of all the regularities of changes in the properties of the raw materials used in the course of the technological process. In the technology of meat products, the most important parameters are the so-called functional-technological indicators: water-binding, water-retaining, the ability of meat raw materials, its stickiness (especially in sausage technology) [17, 18]. When choosing the optimal regimes of enzymatic treatment, one should take into account the change in each of these parameters, and, in addition, the structural and mechanical parameters, the main one being the shear force. All these indicators to some extent are the guarantor of the success of technological processes and directly or indirectly determine the quality characteristics of the finished products.

Water-binding capacity (WBC) characterizes the ability of meat raw materials to absorb and retain water during salting and massaging. This phenomenon is due to the ability of meat proteins to form hydrate shells, due to the retention of water molecules by hydrogen bonds and electrostatic interactions [16, 19]. Increase of the level of WBC is facilitated by the salting process itself, namely the action of table salt, in addition, for the same purpose food phosphates are used, as well as various water-binding agents (of protein or polysaccharide nature). Of the physical factors, it is worth noting the effect of pH. Since the isoelectric points of the meat proteins are in the "acidic" pH range (5.3), an increase in the concentration of hydrogen ions leads to a decrease in WBC.

For the experiment, the formed combination of bacteria was used for processing minced meat from beef muscle tissue, minced meat from beef flank, and minced meat from horse meat.

One of the tasks facing the starter cultures is the modification of collagen. According to the amino acid composition, collagen does not contain tryptophan, therefore it is referred to as inferior proteins. The naturally occurring collagen is insoluble in water, but swells in it. Slowly digested with pepsin and almost not digested with trypsin and pancreatic juice. Heating the collagen to 60-70$^oC$ and careful mechanical destruction increases the digestive action of pepsin.

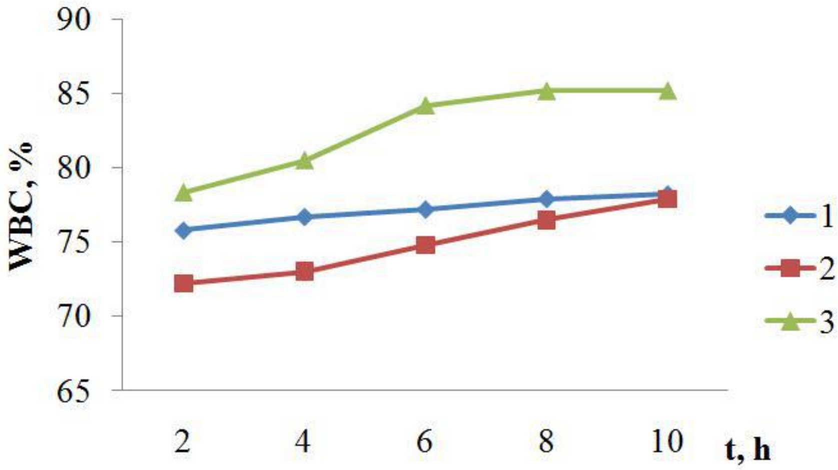
Thus, collagen is relatively slowly absorbed by the body. Therefore, the consumption of a product containing more than 15-20% of this defective protein is not recommended.

In moderate doses, collagen retains high-grade proteins in the food supplying amino acids to the body, that in significant quantities are contained in it, especially hydroxyproline - an essential component of the connective tissues of the body.

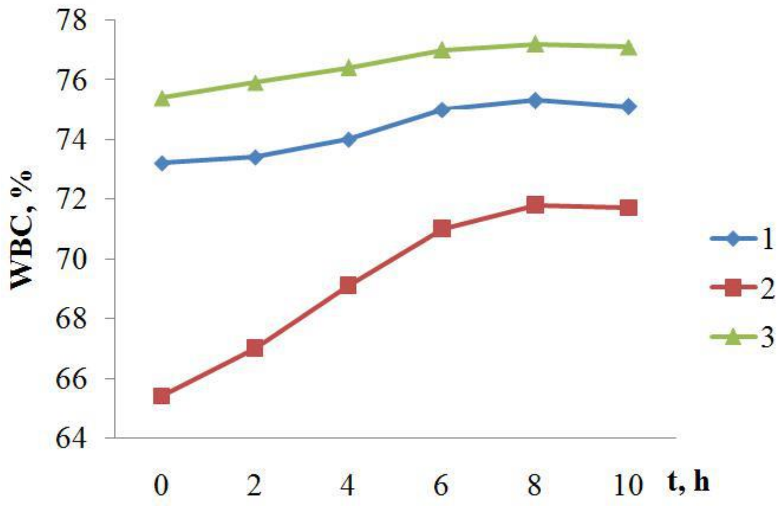
For preparation of samples, lactic acid and bifidobacteria were activated in sterile meat broth with lactose under sterile conditions in a thermostat for 12 hours, after that they were added to the model minced meat together with salt in a volume of 1 ml / 100 g, control samples were made, also meat was chopped and salt added.

At the end of the experiment WBC was determined in the samples by the pressing method.

In the process of traditional salting, a gradual increase in WBC occurs, the level of which, with the passage of time, stabilizes. The study of the effect of the created consortium of microorganisms showed (Fig.1a, b) that its application in the salting process leads to insignificant (3-8%) and stable growth of WBC throughout the salting for all three types of model minced meat.




**a) With lactic acid bacteria**




**b) Without lactic acid bacteria**

**1 – minced flank; 2 – minced horse meat; 3 - minced beef**

**Figure 1: Effect of salting time to water-binding capacity of minced meat**

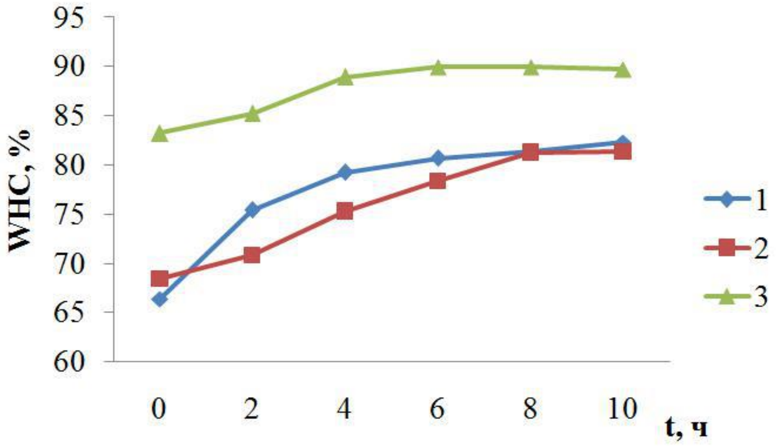
So for mince meat from beef flank, the maximum value of WBC with the addition of the created consortium was 78.2% versus 75.1% with traditional salting, for minced meat from horse meat and minced meat from muscle tissue was 77.9% versus 71.7% and 78.2% against 77.1%, respectively. In the traditional salting, the nature of the dependence can be explained by the fact that during the initial stages of hydrolysis protein molecules (proteinase activity) are formed, which have a large number of easily accessible charged groups that can hold water. With further hydrolysis, oligopeptides and free amino acids accumulate, which, as is known, are not capable of efficient water binding. In addition, the resulting amino acids, reducing the pH of the medium, further contribute to the fall of WBC.

The results obtained with the addition of microorganisms are evidently related to the increased intensity of the action of microorganisms on the connective tissue proteins of the minced meat raw material, which obviously accounts for the accumulation of a large number of easily accessible charged groups, and lactic acid bacteria assimilate the amino acids that are formed in the process of vital activity.

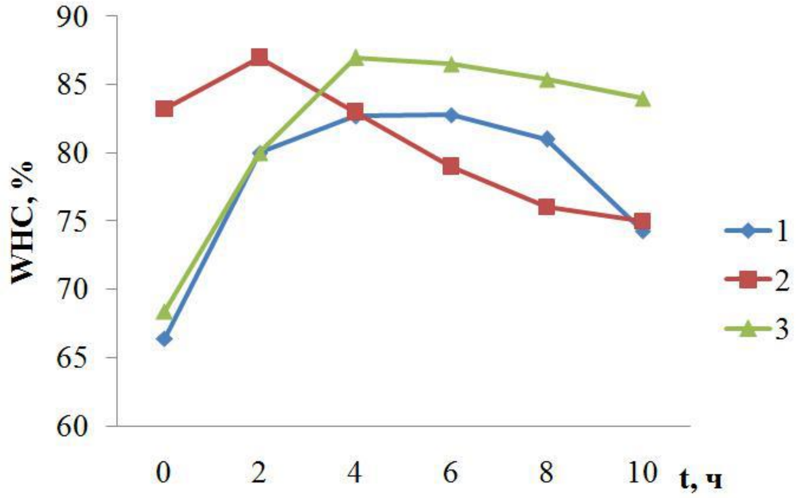
The water retaining capacity (WRC) of the raw materials is the most important indicator for meat products undergoing heat treatment. This indicator demonstrates the ability of the raw material to retain moisture during heating, which primarily affects the finished product. It should be noted that the mechanism of formation of the WRC is associated with the formation of hydrocolloids, such as gels. The collagen protein plays an important role, which during the heat treatment is converted into gelatin, capable of forming a gel. Consequently, the destruction of collagen may adversely affect the level of the WRC.

Preparation and processing of samples was carried out in a manner similar to the definition of WBC.

Studies have shown that during traditional salting, there is a sharp increase in the first hours. Maximum values of the WRC are achieved after two hours of treatment for minced meat from horse meat and beef muscle tissue, four hours for minced beef, then the WRC indicators decrease (Fig. 2a, b).




**a) With lactic acid bacteria**




**b) Without lactic acid bacteria**

**1 – minced flank; 2 – minced horse meat; 3 - minced beef**

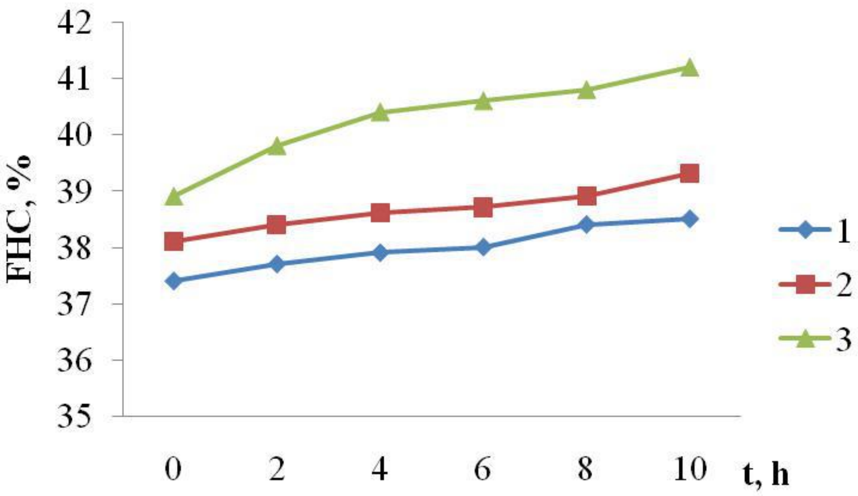
**Figure 2: Effect of salting time to water-holding capacity of minced meat**

When co-salting with microbial treatment more smooth increase of WRC during first 4 - 6 hours takes place. Further, a slight decrease in the WRC, with finite values for salting combined model for all types of minced much higher than traditional salting without the addition of a consortium of microorganisms.

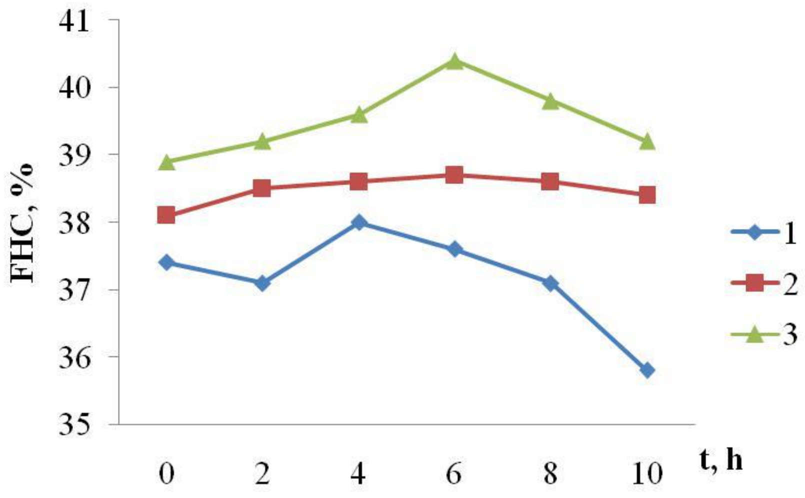
Such results indicate the synergy (mutual reinforcement) of the action of a consortium of microorganisms and table salt in the process of salting.

For the determination of the fat-retaining capacity (FRC) the mass of the sample was found after determination of the WRC. Then it was quantitatively transferred to the sample bottles, and then dried to constant weight at the temperature of 150 ° C. Then a 2.0 g sample was placed in a porcelain mortar, 2.5 g of calcined quartz sand and 6.0 g of α-monobromonaphthalene were added and thoroughly ground for 5 minutes. At the end of the time, the mixture was filtered through a paper filter and the refractive index was determined in a clear filtrate. A similar determination was made for each sample of minced meat without heat treatment (before determining the WRC). Further, the FRC was calculated.

The parameters of the FRC (Fig. 3a, b) showed that the FRC of model minced meat with the addition of a consortium of microorganisms is slightly higher in comparison with the control samples, evidently this is due to the high fat-holding properties of the connective tissue protein, primarily collagen.




**a) With lactic acid bacteria**



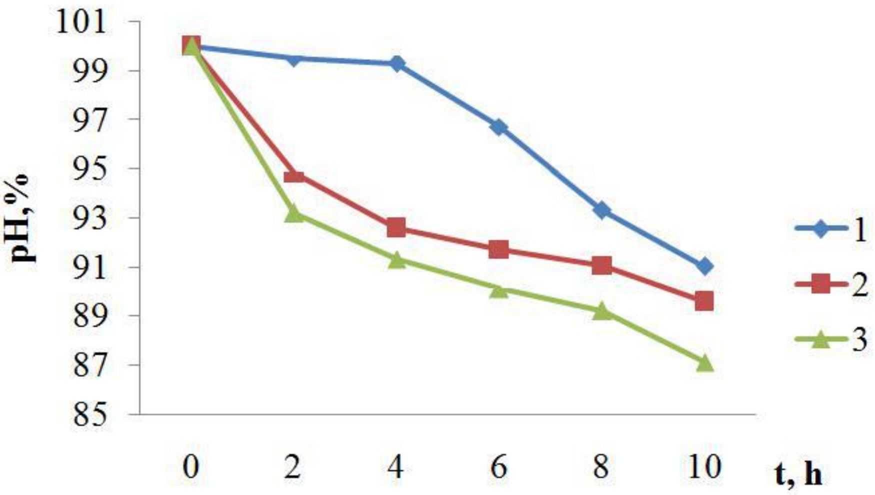

**b) Without lactic acid bacteria**
**1 – minced flank; 2 – minced horse meat; 3 - minced beef**
**Figure 3: Effect of salting time to fat-holding capacity of minced meat**

The pH of the medium in the production of meat products is also one of the important indicators. Since the isoelectric points of the meat proteins are in the "acidic" pH range (4.8-5.1), increase of the concentration of hydrogen ions leads to a decrease in WBC. According to literature sources and our studies [9,10] it is known that lactic acid and bifidobacteria reduce the pH of the medium due to the formation of lactic acid.

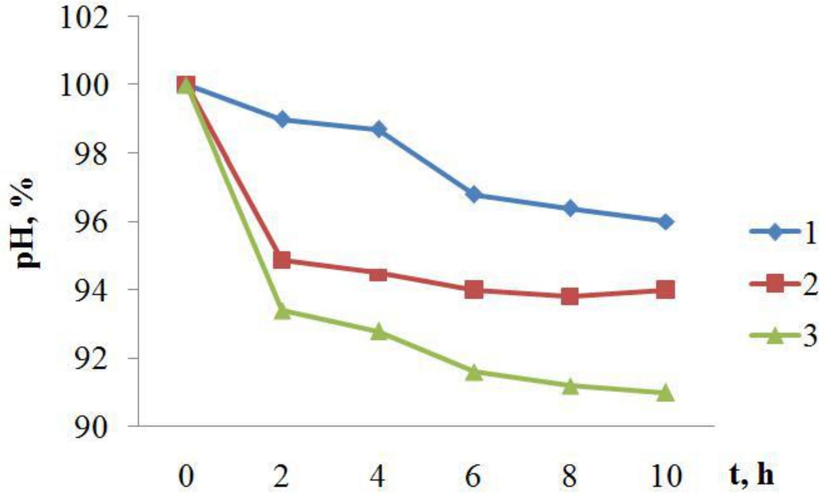
To determine the pH of the meat, an aqueous extract was prepared in a ratio of 1:10, for which a meat sample weighing 10 grams was thoroughly grinded, placed in a 100 cm3 beaker and extracted with distilled water for 30 minute at ambient temperature and intermixing with a glass rod. The resulting extract was filtered through a folded filter and used to determine the pH.

The pH of solutions of hydrolysates was determined by the potentiometric method on a universal ionometer pH-150M.

Interpreting the obtained pH results shown in Fig. 4a and b, it can be said that the pH of model minced meat decreased significantly in the course of the experiment in comparison with the controls.




**a) With lactic acid bacteria**



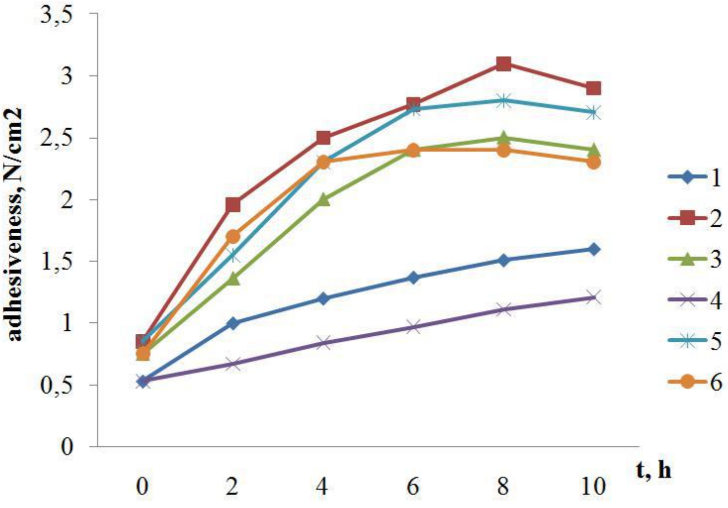

**b) Without lactic acid bacteria**
**1 – minced flank; 2 – minced horse meat; 3 - minced beef**

**Figure 4: Effect of salting time to pH of minced meat**

As a result of the breakdown of proteins and their transition to a dissolved state during ripening and salting of meat raw materials, unstable thixotropic structures can form, forming highly plastic, gelatinous masses of high viscosity, with strong adhesion properties [20, 21]. This effect affects the stickiness index. Stickiness plays an important role in the molding process and characterizes the ability to form a monolithic structure during the heat treatment, which is especially important for chopped (minced) meat products: sausages, hams, etc.

Preparation of minced meat and processing of minced meat was carried out in a manner similar to WBC. To determine the stickiness (adhesion), the sample of the minced meat was uniformly applied to a polished metal plate with a uniform layer of 3 mm thick and pressed against stop on top by a second polished metal plate with a projection of 2 mm high. Thus, between the plates an even 2 mm layer of minced meat was created. A 1 kg weight was placed on the top plate and then connected to a dynamometer. By increasing the force applied to the dynamometer, the upper plate pursued to be stripped from the surface of the forcemeat. At the moment of detachment, the dynamometer readings were recorded.

The results of experimental studies have shown that the effect of microorganisms significantly increases the stickiness of all three types of minced systems (Figure 5). In the presence of a consortium of microorganisms, the growth of adhesiveness occurs somewhat faster, and higher maximum stick values (2.8-3.1 N / cm2, depending on the type of minced meat) are achieved. The results obtained are evidently associated with a decrease in pH to 5.4-5.6 during which collagen swelling, hydrolysis of low molecular weight bonds, and activation of cellular enzymes occur. An increase in the duration of exposure (over 8 hours) resulted in a slight decrease in stickiness, which, apparently, is due to the formation of low molecular weight proteolysis products that do not possess high adhesion.



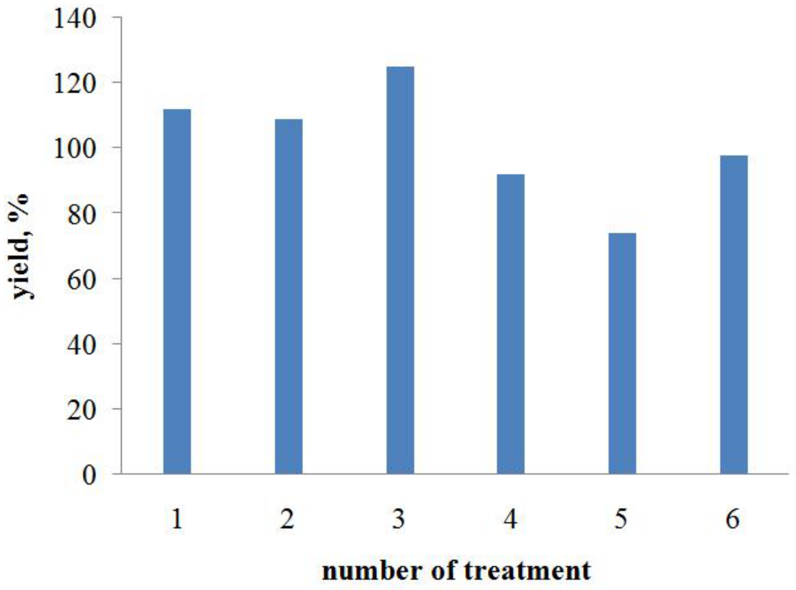

1 – minced flank with lactic acid bacteria;
2 – minced horse meat with lactic acid bacteria;
3 – minced beef with lactic acid bacteria;
4 – minced flank without lactic acid bacteria;
5 – minced horse meat without lactic acid bacteria;
6 – minced beef with lactic acid bacteria.

**Figure 5: Effect of salting time to adhesiveness of minced meat**

The yield of the product during heat treatment is one of the main indicators characterizing the economical and manufacturability of the adopted solution. In this connection, the effect of heat treatment on the yield of the product was studied.

Samples of model minced meat were prepared similarly to the WBC definition. The prepared samples were kept at a temperature of 0-4 ° C. After the set time, the samples were heat-treated in a microwave oven for 15 minutes at a power of 100 W, after which they were re-weighed. The controls were samples subjected to salting without microbial treatment for 12 hours.

The obtained results indicate a certain increase in yield (Fig. 6).




1 – minced flank with lactic acid bacteria;
2 – minced horse meat with lactic acid bacteria;
3 – minced beef with lactic acid bacteria;
4 – minced flank without lactic acid bacteria;
5 – minced horse meat without lactic acid bacteria;
6 – minced beef with lactic acid bacteria.

**Figure 6: Yield of minced meat after thermal treatment**

Analyzing the obtained results we can say that the addition of our complex of lactic acid bacteria to the flank, to horse meat, and to beef muscle tissue leads to an increase in the indices of functional and technological properties such as WBC, WRC, FRC, yield, stickiness, and also to lowering the pH of the medium, which is important in the production of meat and sausage products.

It is necessary to recognize the processing of meat raw materials with lactic and bifidobacteria effective and economically expedient, since during the addition of lactic acid and bifidobacterium, the salting terms are halved.

The nature of the action of the consortium of microorganisms makes it possible to recommend it for use in order to soften the muscle tissue containing a large amount of collagen, improve the quality of raw materials in the technology of a wide range of meat products with different ratios of muscle and connective tissue.

To determine digestibility, an enzymatic method for determining the biological value of meat in vitro was used.

Hydrolysis is carried out in a special device that provides continuous mixing and dialysis of low molecular weight hydrolysis proteins.

When carrying out experiments on digestibility, the results shown in Table 1 and in Fig. 7 were obtained.

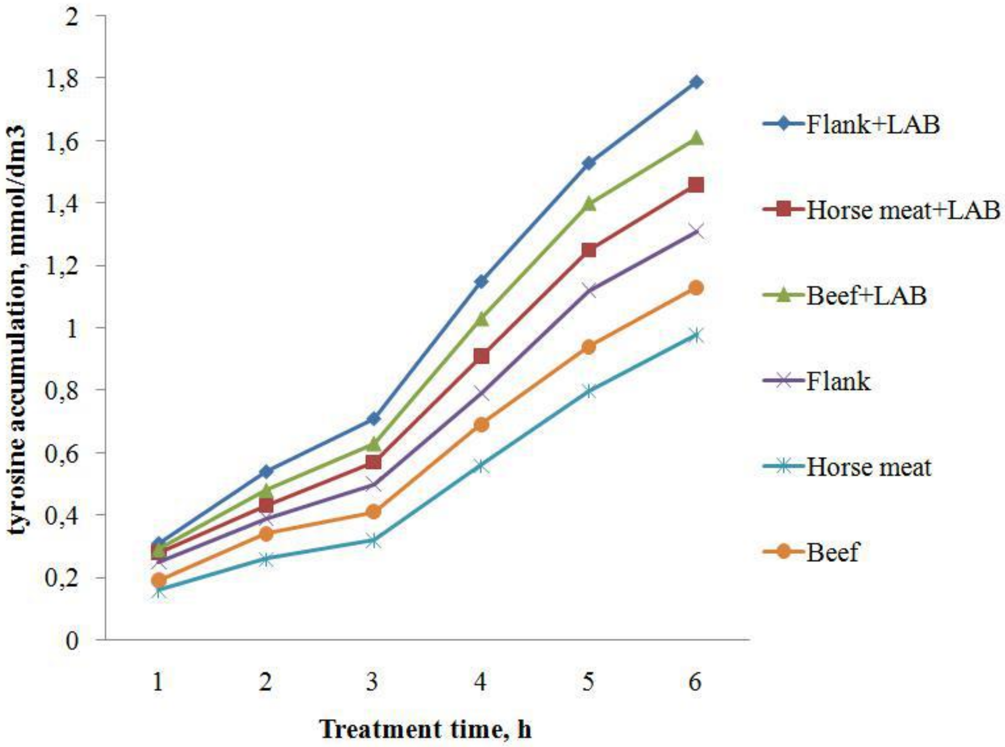



**Figure 7: In vitro digestibility of minced meat**

**Table 1 - Results of in vitro digestibility studies**

| Brief description of the product | Accumulation of products of enzymatic hydrolysis (mmol / dm$^3$) with the duration of hydrolysis, h | | | | | |
|---|---|---|---|---|---|---|
| | By pepsin | | | By trypsin | | |
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Flank +LAB | 0,31 | 0,54 | 0,71 | 1,15 | 1,53 | 1,79 |
| Horse meat+LAB | 0,29 | 0,43 | 0,57 | 0,91 | 1,25 | 1,46 |
| Beef +LAB | 0,29 | 0,48 | 0,63 | 1,03 | 1,4 | 1,61 |
| Flank | 0,25 | 0,39 | 0,5 | 0,79 | 1,12 | 1,31 |
| Horse meat | 0,16 | 0,26 | 0,32 | 0,56 | 0,8 | 0,98 |
| Beef | 0,19 | 0,34 | 0,41 | 0,69 | 0,94 | 1,13 |

Analyzing the results obtained, it can be said that the degree of hydrolysis of proteins in samples with the addition of a complex of lactic acid bacteria was higher than in samples of pure minced meat without the use of lactic acid bacteria.

When adding our complex of lactic acid bacteria, an increase in the digestibility of the initial products is observed.

In the course of the work, consortiums of microorganisms have been studied that have an effect on the functional and technological properties of model minced meat. Application at the salting stage of the microflora studied leads to an increase in the WBC of model minced meat from 3 to 8%, and to an increase and stabilization of the WRC and the FRC of model minced meat in comparison with the control. The pH of the model reduces from 3 to 5% in comparison with the control and is in the range from pH 5.45 to 5.6. The results of experimental studies have shown that the effect of microorganisms significantly increases the stickiness of model minced meat from 2.8 to 3.1 N / cm$^2$. As a result of biomodification of meat raw materials by starter cultures, digestibility of model minced meat by digestive enzymes of the gastrointestinal tract increases.

**ACKNOWLEDGMENTS**

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