CRIAÇÃO E USO DO CONSÓRCIO DE MICROORGANISMOS NA PRODUÇÃO DE CARNES

CREATION AND USE OF MICROORGANISM CONSORTIUM IN MEAT PRODUCTION

СОЗДАНИЕ И ИСПОЛЬЗОВАНИЕ КОНСОРЦИУМА МИКРООРГАНИЗМОВ ПРИ ПРОИЗВОДСТВЕ МЯСНЫХ ПРОДУКТОВ

GIZATOV, Albert^{1*}; GIZATOVA, Natalia²; MIRONOVA, Irina³; GAZEEV, Igor⁴; NIGMATYANOV, Azat⁵

^{1,2,3} Bashkir State Agrarian University, Department of technology of meat, dairy products, and chemistry. Russian Federation

⁴ Bashkir State Agrarian University, Faculty of food technologies, Department of life safety, and technological equipment. Russian Federation

⁵ Ufa State Petroleum Technological University, Department of special chemical technologies. Russian Federation

* Corresponding author
e-mail: albertgizatov@yahoo.com

Received 07 March 2020; received in revised form 22 May 2020; accepted 18 June 2020

RESUMO

O objetivo desta pesquisa é desenvolver produtos funcionais a partir de subprodutos biomodificados de baixa qualidade e resíduos usando consórcios de microrganismos. Para criar condições para uma dieta equilibrada e melhorar a saúde da população, foi proposta a técnica de utilização de métodos biotecnológicos direcionados à atração de recursos não tradicionais. O objetivo do estudo foram subprodutos secundários da carne e resíduos da indústria de processamento de carne. Os produtos cárneos e resíduos foram processados com bactérias lácticas como *Lactobacillus bulgaricus*, *Bifidumbacterium siccum* e *Staphylococcus carnosus*. As bactérias do ácido láctico foram cultivadas passo a passo em diferentes combinações: *Staphylococcus carnosus* e *Lactobacillus bulgaricus*; *Bifidumbacterium siccum* e *Staphylococcus carnosus*; *Lactobacillus bulgaricus* e *Bifidumbacterium siccum*. Mudanças na atividade de crescimento das espécies selecionadas de bactérias lácticas em várias combinações foram realizadas em vários meios nutricionais. Ao cultivar as cepas estudadas em um meio de agar de carne e peptona, sua identificação completa foi realizada. A etapa final foi a análise da qualidade dos objetos reais: carne de cavalo, flank e tecido muscular padrão da carne bovina. A dinâmica das mudanças nos indicadores de propriedades funcionais, tecnológicas e organolépticas da carne provou um efeito positivo do uso de bactérias do ácido láctico. As raças desenvolvidas demonstraram a capacidade de suprimir ativamente a microflora prejudicial nos produtos à base de carne. Além disso, amoleceram a carne secundária para processamento e provaram positivamente melhorar as características organolépticas do produto acabado. O processamento de recursos secundários de subprodutos e resíduos permitirá o uso mais econômico e racional da reserva alimentar mais essencial de carne e derivados, contendo colágeno. A cobertura insuficiente desta questão sobre o uso de uma cepa combinada dessas espécies de microrganismos para a biomodificação de subprodutos secundários contendo colágeno e resíduos na indústria de carne indica a relevância do tópico escolhido.

Palavras-chave: biomodificação; biotecnologia; subprodutos e resíduos contendo colágeno, bactérias do ácido láctico.

ABSTRACT

This research purpose is to develop functional products from biomodified low-grade by-products and waste using microorganism consortia. To create conditions for a balanced diet and improve the health of the population, the technique of using biotechnological methods directed at attracting non-traditional resources was proposed. The object of the study was secondary meat by-products and waste from the meat processing industry. The meat products and waste were processed with such lactic-acid bacteria as *Lactobacillus bulgaricus*, *Bifidumbacterium siccum*, and *Staphylococcus carnosus*. Lactic-acid bacteria were cultured step-

by-step in different combinations: *Staphylococcus carnosus* and *Lactobacillus bulgaricus*; *Bifidobacterium siccum* and *Staphylococcus carnosus*; *Lactobacillus bulgaricus* and *Bifidobacterium siccum*. Changes in the growth activity of selected species of lactic-acid bacteria in various combinations were carried out in various nutrient media. When growing the studied strains in a medium of meat-and-peptone agar, their complete identification was carried out. The final stage was the analysis of the quality of real objects: horse meat, flank, and standard beef muscle tissue. The dynamics of changes in indicators of functional and technological and organoleptic meat properties proved a positive effect from the use of lactic acid bacteria. Developed races have shown the ability to suppress harmful microflora in meat products actively. Moreover, they softened the secondary meat for processing and positively proved to improve the finished product's organoleptic characteristics. Processing of secondary resources of by-products and waste will allow more economical and rational use of the most essential collagen-containing food reserve of meat and meat products. The insufficient coverage of this issue on the use of a combined strain of these species of microorganisms for the biomodification of secondary collagen-containing by-products and waste in the meat industry indicates the relevance of the chosen topic.

Keywords: *biomodification; biotechnology; collagen-containing by-products and waste, lactic-acid bacteria.*

АННОТАЦИЯ

Цель исследования - разработка функциональных продуктов из биомодифицированного низкосортного сырья с использованием консорциумов микроорганизмов. Для создания условий для рационального питания и улучшения здоровья населения нами предлагается использовать прием, направленный на привлечение нетрадиционных ресурсов с использованием методов биотехнологии. В качестве объекта исследования служило вторичное мясное сырьё мясоперерабатывающей промышленности: мяса конины, пашины и говяжьей мышечной ткани второго сорта. Объектом исследования выступили *Lactobacillus bulgaricus*, *Bifidobacterium siccum*, *Staphylococcus carnosus*. Культивация молочнокислых бактерий осуществлялась поэтапно в разных сочетаниях: *Staphylococcus carnosus* и *Lactobacillus bulgaricus*; *Bifidobacterium siccum* и *Staphylococcus carnosus*; *Lactobacillus bulgaricus* и *Bifidobacterium siccum*. Активность роста молочнокислых бактерий изучали на различных питательных средах (мясопептонный агар и мясопептонный желатин). Для этого суспензию культур культивировали в стерильных условиях на питательных средах, являющейся оптимальной для их роста. В процессе выращивания исследуемых штаммов на среде с применением мясопептонного агара была проведена полная их идентификация. Для оценки скорости роста тестируемых штаммов использовали специфические питательные среды. На завершающем этапе был проведен анализ качества реальных объектов: мяса конины, пашины и говяжьей мышечной ткани второго сорта по показателям функционально-технологических свойств и был установлен положительный эффект от их применения, поскольку выведенные расы проявили способность активно подавлять вредную микрофлору, развивающуюся в мясных продуктах. Кроме того, переработки вторичных сырьевых ресурсов позволит более экономно и рационально использовать важнейшие пищевой коллагенсодержащий резерв мяса и мясных продуктов. Недостаточная освещенность данного вопроса по применению комбинированного штамма данных видов микроорганизмов для биомодификации вторичного коллагенсодержащее сырьё мясной промышленности свидетельствует об актуальности выбранной темы.

Ключевые слова: *биомодификация, биотехнология, коллагенсодержащее и вторичное сырьё, молочнокислые бактерии.*

1. INTRODUCTION:

Currently, it is possible to produce new types of meat by-products and waste for general, special and therapeutic purposes, improve methods of enzymatic processing of meat raw materials in order to improve its functional and technological properties, produce food and feed hydrolysates, synthesize flavorings, dyes, biologically active substances, to synthesize feed proteins, and in the future, proteins for human nutrition based on biotechnology in the meat industry (Antipova, 2001; Antipova *et al.*, 2011; Armuzzi *et al.*, 2001; Audenaert *et al.*, 2010;

Dušková *et al.*, 2016; Wanget *et al.*, 2013).

Products containing consortia of lactic acid and bifidobacteria play an essential role in the nutrition of people, especially children, the elderly, and patients. First of all, the dietary properties of such products are in that they improve metabolism, stimulate the secretion of gastric juice, and stimulate the appetite. Being in food products and making up most of the microflora of the human gastrointestinal tract, taking root in the intestines, they actively suppress putrefactive microflora and lead to inhibition of putrefactive processes and stop the formation of toxic decay products of proteins

entering the human blood. Along with this, microorganisms are active producers of useful substances that are capable of transforming natural or chemically synthesized compounds into substances valuable to humans. Their sources are inexpensive and practically inexhaustible. Many of them must synthesize heterogeneous systems of enzymes that convert proteins. This suggests the effectiveness of their use for biotransformation of protein systems of low-grade meat by-products and waste by microbial fermentation with the selection of specific consortia (Armuzzi *et al.*, 2001; Dušková *et al.*, 2016).

Some American factories use a pure culture of *Pediococcus cerevisiae* to manufacture summer types of sausages such as cervelat and salami. When sugar is added, it promotes the formation of lactic acid and gives the sausages a specific, characteristic flavor. When using this culture, the sausage manufacturing process is reduced to 48 hours while usually it is kept at (7-10) °C for 3-7 days, and then smoked at (27-44) °C for 2 -3 days (Antipova *et al.*, 2016; Gavrilova *et al.*, 2019).

From the point of view of aroma formation, the starting culture of *Moraxella phenylpyruvica*, the Danish Meat Institute's development product, is of interest. This is a psychrophilic culture – an optional anaerobic which allows it to develop in the thickness of the product actively and, as studies have shown, to produce substances that are precursors of aroma. Along with traditional bacteria, such as *Lactobacillus* and *Pediococcus*, American technologists include *Micrococcus* in the starter cultures, which can reduce nitrates to nitrites while improving the taste and color of finished sausages (de Souza Barbosa *et al.*, 2015).

In Italy, strains of *Micrococcus* sp., *Lactobacillus bulgaricus* were tested to study the organoleptic properties of dry sausages as starter cultures. A new bacterial culture, *Lactobacillus pentosus*, was tested in the laboratory of R. Muller (Germany) in the production of dry sausages. Several other cultures were used to compare the technological effect: *Petrostreptococcus parvulus*, *Lactobacillus bulgaricus*, *Pediococcus acidilactici*, and their combination with *Streptococcus carnosus* MIII. In all variants of testing microorganisms, the best results were obtained with *Lactobacillus pentosus*. The effect was expressed in a rapid decrease in pH by obtaining sausages of attractive color, soft-sour taste, and pronounced meat aroma (Cocolin *et al.*, 2009).

In the meat industry, biotechnology is used in raw meat processing with enzyme preparations to soften its stiffness and increase the nutritional value, making it possible to use low-grade raw materials to produce high-quality meat products. Experts consider the enzymatic method of softening meat one of the most effective (de Souza Barbosa *et al.*, 2015; Doulerakiet *et al.*, 2012; Ilina *et al.*, 2017).

The All-Russian Scientific Research Institute of the Meat Industry (VNIIMPom) conducted studies on the use of microbial preparations for the complete separation of meat from bones after deboning and sending the resulting mass to prepare meat pastes, emulsions, hydrolysates (Antipova and Uspenskaj, 2016; Gavrilova *et al.*, 2019). Studies were conducted at the same institute on the use of brewer yeast biomass as polyezyme preparations for the hydrolysis of blood proteins in slaughtered animals, resulting in an increase in the biological efficiency of blood proteins by 10-30%. Hydrolyzed by yeast, blood is recommended to be used as an additive to meat products to give them a good taste.

One of the biotechnology applications to obtain additional protein sources is the use of unicellular bacteria, fungi, yeast, and algae for the enrichment of meat products. As shown by studies conducted by institutes of the Russian Academy of Sciences, the Russian Academy of Medical Sciences (Antipova *et al.*, 2016), VNIIMPom, this method allows getting diet meat products. The Kemerovo Technological Institute of the Food Industry researched the use of cooked sausages in technology to replace soy protein brewing yeast, which is the secondary raw material for brewing (Antipova *et al.*, 2015; Gavrilova *et al.*, 2019). One of the new biotechnology areas in sausage production is the improvement of the taste, color, and aroma of meat products of high-quality flavorings, various flavoring substances of microbial origin (Armuzzi *et al.*, 2001; Cocolin *et al.*, 2009). Another promising area of application of the principles of biotechnology is the fortification of meat products and the solution of issues related to the development of this problem. Scientists of VNIIMP, KemTIIP, and others are enriching foods with vitamins (Antipova *et al.*, 2011; Audenaert *et al.*, 2010). For example, KemTIIP experts in collaboration with scientists of the Institute of Nutrition RAMS developed a technology to enrich meat products with vitamins C, B1, B2, and PP, which allows saving almost half of their activity (Gabitov *et al.*, 2019; Wang *et al.*, 2013). In the

neighboring countries, research is also being carried out to introduce biotechnological processes in the meat industry, which in their direction coincide with the Russian ones. In Belarus, technology has been developed for two bacterial preparations: Acidolact and Lactobact (liquid and dry) used as part of multi-component brine for extrusion of beef products (Bonomo *et al.*, 2008).

In Kazakhstan, the Semipalatinsk Institute of Meat and Dairy Industry created a composition for producing a protein fortifier used in sausage production (de Souza Barbosa *et al.*, 2015; Dušková *et al.*, 2016). In the Almaty branch of the Dzhambul Technological Institute of Light and Food Industries, a biological product of decolorized sedimentary yeast used to enrich sausages was obtained (Gabitov *et al.*, 2018; Konashova *et al.*, 2018).

Much attention is paid to the application of biotechnological techniques in the meat industry in far abroad countries. The directions there are mainly focused on improving the quality of meat products, intensifying production, creating new types of products based on meat balanced in chemical composition, environmentally friendly directed actions, including therapeutic and prophylactic purposes (Buchanan and Gibbons, 1994).

The main areas of application of biotechnology in sausage production are the use of biomass of microorganisms and preparations based on them as a substitute for meat raw materials, a source of vitamins, micro and macro elements for the production of sausages, increased nutritional value, producer of enzymes, amino acids, flavorings, and colorings in order to improve technological processes, create fundamentally new technologies, improve genetic safety, lengthen the shelf life, improve the taste, aroma and other values of product quality; the use of immobilized enzymes, the advantage of which is the possibility of their repeated use, the increased stability, and duration of enzymatic activity, the possibility of use in continuous technological processes, the relatively short exposure time to the substrate, the possibility of creating multi-enzyme systems that are highly effective and, finally, in hygienic safety (Holko *et al.*, 2013; Nesterenko *et al.*, 2018).

In recent years, in Romania, Italy, Bulgaria, Switzerland, and other countries, the use of molds for producing smoked sausages has increased. It was noted that sausages with a dense coating of white or gray mold on the shell

are better than without it. Swedish scientists have developed a method for the production of minced meat with improved properties. According to this method, the meat is treated with a heat-sensitive proteinase (Gizatova *et al.*, 2014). In the UK, it is recommended to use two groups of enzymes in the food industry. The first group includes enzymes of animal and vegetable origin, and the second – enzymes of microbial origin – temporarily used (Ammor and Mayo, 2007). A new direction is being planned in the fermentation of meat. For improving the quality of meat, enzyme preparations (papain) are introduced into the blood of slaughtered animals immediately before slaughter, which significantly improves the meat grade. A mixture of papain and bromelain can be used for salting beef (Casquete *et al.*, 2011; Gizatov and Gizatova, 2015; Nesterenko *et al.*, 2016; Rakhimov *et al.*, 2018).

The problem of obtaining proteins from non-traditional types of raw materials (bacteria, fungi, yeast) to use them in meat products is intensively worked in the USA, Great Britain, Italy (Iliina *et al.*, 2017; Juntunen *et al.*, 2001; Knolet *et al.*, 2001). In the USA, dietary sausages are being produced with the protein product Muco protein, which is made from a filamentous fungus that can double its volume in 2.5 hours (Dušková *et al.*, 2015). In Italy, yeast extract is used in the production of cooked, liveried, semi-smoked, and cooked smoked sausages. In Yugoslavia, studies are being conducted on the use of brewer yeast preparations as substitutes for sodium caseinate and soy isolate in meat products (Argyriet *et al.*, 2015).

For the production of dry sausages in the hot season, foreign enterprises of Germany and Italy use pure culture *Pediococcus cerevisiae* as part of the recipe. According to the technology for the preparation of sausages, a certain amount of sugar is introduced, favorably affecting the formation of a taste and aroma specific to this type of product. The introduction of *Pediococcus cerevisiae* reduces the production time of sausages to 48 hours against 3-7 days required for precipitation and 2-3 days for smoking (Sydykova *et al.*, 2019; Vladimirovna and Borisovich, 2016; Zinina and Rebezov, 2016).

From the point of view of aroma formation, the Danish Meat Institute's development, the *Moraxella phenylpyruvica* culture, is of interest. It is a psychrophilic culture – an optional anaerobic that allows growing exponentially in the thickness of the product and, as studies have shown, producing substances that are precursors of aroma. Along with native bacteria, such as

Lactobacillus and Pediococcus, American technologists include Micrococcus in starter cultures, which can restore nitrates to nitrites, improving the taste and color of finished sausages (Karam *et al.*, 2013). In Italy, when studying sensory properties, strains of *Micrococcus* sp., *Lactobacillus bulgaricus* were used as test starter cultures (Adams and Mitchell, 2002). During the development of uncooked smoked sausages, a new bacterial culture of *Lactobacillus pentosus* was tested in the research laboratory of R. Muller (Germany). Many other cultures were used to compare the effect of the technology: *Petrostreptococcus parvulus*, *Lactobacillus bulgaricus*, *Pediococcus acidilactici*, as well as their combination with *Streptococcus carnosus*. Of all the microorganisms tested, the best results were obtained with *Lactobacillus pentosus*. There was a rapid decrease in pH, which resulted in an attractive color, soft-sour taste, and a pronounced meat aroma of the obtained sausages (Martynov *et al.*, 2018). The aim of this study is to combine the above microorganism strains to soften the secondary collagen-containing by-products and waste of the meat industry.

2. MATERIALS AND METHODS:

Meat and meat products are an excellent source for the development of lactic acid bacteria. They get everything they need from meat: sources of carbon, nitrogen, vitamins, and mineral salts. The meat's pH and humidity also contribute to growth (Martynov *et al.*, 2018). The objects of study were: – fresh and frozen beef of the highest, first and second grades according to GOST 779 (Ilin *et al.*, 2017), model minced meat, sausages obtained on its basis with and without biotechnological processing methods; – sturdy and low-grade collagen-containing raw materials of the meat industry (beef flank, beef veins, horse meat). Beef and horsemeat samples for research were taken under GOST R 51447, GOST 9792 (Antipova, 2001; Dušková *et al.*, 2015; Kno *et al.*, 2001) after which they made a combined sample and, denoting the name of the sample, were wrapped in parchment. Processing model minced meat with an ingredient such as salt does not adversely affect the growth of lactic acid bacteria, as most strains can withstand salt concentration. The temperature has a definite effect on the salt tolerance of lactic acid bacteria. At optimal growth of temperatures, they resist a salt concentration of up to 12%. Certain doses of

salt even stimulate growth (Martynov *et al.*, 2018).

An essential parameter of the starter culture quality is the suitability of the product, which should be confirmed in the study. When compiling a consortium of crops, it is necessary to take into account the characteristics of the product being produced, the temperature regime of the product (Bulat *et al.*, 2012; Ivanov *et al.*, 2018; Konashova *et al.*, 2018). The temperature should be maintained at an optimum level without violating the boundaries. The most commonly used thermophilic cultures are *Str. thermophilus*, *lac. bulgaricus*, *Las. lactis*, *Lbm. helveticus* and *Lbm. acidophilus*. In a broader sense, the *Bifidus* group (the *Bifidobacterium* *Atinomyetaceae* family) can be assigned to this group of microorganisms (Bulat *et al.*, 2012).

Like mesophilic organisms, thermophilic biomass culture has antimicrobial properties due to the inhibition of nutrients, low pH, the formation of antibiotics, and other factors that have a deterrent effect. When choosing, a number of requirements were taken into account, including those that are not harmful to the human body, a high growth rate, and, therefore, cell reproduction.

During the experiment, the growth activity of lactic acid bacteria on various nutrient media (meat peptone agar and meat peptone gelatin) was studied in order to select strains with specific properties and create consortia based on them taking into account physiological and biochemical characteristics. The suspension of cultures was cultivated under sterile conditions in a box, introduced into nutrient media in meat peptone agar and meat peptone gelatin, and grown in a thermostat at an optimum temperature of + 28°C for growth.

The next stage of our research was to study the growth of selected strains of lactic acid bacteria on specific nutrient media: gelatin and collagen gels, which were prepared by hydration of gelatin and animal protein with meat broth in a ratio of 1:5. During cultivation, pH, acidity, and mass fraction of protein were determined by the biuret method in a nutrient medium for 8 hours. The macro morphological properties of the consortium of lactic acid bacteria were studied in order to search for optimal combinations and concentrations of lactic acid bacteria in association with each other and their effective influence on the meat systems. The bacteria *Staphylococcus carnosus*, *Lactobacillus bulgaricus*, and *Bifidobacterium siccum* were cultivated on a gelatin gel. When sowing on

gelatin gel, the number of bacteria was taken into account. The number of bacteria was not less than $1 \cdot 10^7$ CFU/g. Before sowing on gelatin gel, nutrient media were preliminarily prepared, and then lactic acid bacteria were cultured in stages in different combinations: *Staphylococcus carnosus* and *Lactobacillus bulgaricus*; *Bifidumbacterium siccum* and *Staphylococcus carnosus*; *Lactobacillus bulgaricus* and *Bifidumbacterium siccum* in milk to identify the nature of the biological interaction of our crops and their growth rate.

Next, we studied the change in the functional and technological properties (moisture-binding capacity (MBC), water-holding capacity (WHC), fat-holding ability (FHC), yield, emulsion stability (ES) of minced meat from flank and horsemeat upon salting with the addition of a complex of lactic acid bacteria. All changes are functional; technological properties were investigated according to established methods.

Moisture binding ability (MBC) was evaluated by the pressing method. A sample of muscle tissue of (0.30 ± 0.01) g was weighed on an analytical balance on a mug of polyethylene with a diameter of 15-20 mm. It was then transferred to an anesthetized filter with a diameter of 9-11 cm, placed on a glass or Plexiglass plate so that the hitch was under a plastic circle. The hinge was covered with a plate of the same size as the bottom. A load of 1 kg was placed on it for 10 minutes. Then the filter with a hitch was freed from the load and the lower plate. A contour of the spot around the compressed meat was outlined with a pencil. The contour of the wet spot appears when the filter paper dries in the air. The area of the spot formed by adsorbed moisture was calculated by the difference between the total area of the spot and the area of the spot formed by the meat. The area of spots formed by compressed meat and adsorbed moisture was measured with a planimeter. It was experimentally established that 1 cm^2 of the filter's wet spot area corresponds to 8.4 mg of water (Martynov *et al.*, 2018).

For determining the mass fraction of total moisture, a sample of tissue weighing (2.00 ± 0.01) g was placed in a paper bag with a filter paper insert and evenly distributed. Then it was placed in a Chizhova apparatus (a high-frequency device), preheated to (160°C) , and dried for 3-5 minutes. After drying, the bag was cooled and weighed with an accuracy of ± 0.1 g. The contents of the bag were released, and the bag was weighed. The results were recorded and used in calculating the SCD of muscle tissue

samples.

Determination of water holding capacity (WHC). A sample weighing (5.00 ± 0.01) g was uniformly applied with a glass rod on the inner surface of a wide part of the milk butyrometer. The butyrometer was tightly closed with a stopper and placed in a water bath at the boiling point with a narrow part down for 15 minutes. The calculated paths determined the mass of released moisture by the number of divisions on the butyrometer scale (Martynov *et al.*, 2018).

When determining the WHC, the mass of meat remaining in the butyrometer was found with an accuracy of ± 0.0001 g. The meat was placed in a bottle and dried to a constant mass at a temperature of 150°C for 1.5 hours. After drying, a weight of (2.0000 ± 0.0002) g was placed in a porcelain mortar, where 2.5 g of fine calcined sand and 6 g of α -monobromonaphthalene were added. The contents of the mortar were thoroughly triturated for 4 minutes and filtered through a folded paper filter. 3-4 drops of the test solution were uniformly applied with a glass rod to the refractometer's lower prism. The prism was closed, fastened with a screw. A beam of light was directed using a mirror onto the refractometer's prism, setting the telescope so that intersecting filaments were clearly visible. The pointer is moved until the boundary between the illuminated and dark parts coincides with the point of intersection of the threads, and the refractive index is counted. At the same time, the refractive index of monobromo-naphthalene was determined. The determinations were repeated several times using average data in the calculation (Konashova *et al.*, 2018).

For determining the stickiness (adhesion), the minced meat sample was uniformly applied on a polished metal plate with a uniform thickness of 3 mm and pressed against the top from the top with a second polished metal plate with a 2 mm protrusion. Thus, an even layer of minced meat with a thickness of 2 mm was created between the plates. A load weighing 1 kg was placed on the upper plate and connected to a dynamometer. By increasing the force applied to the dynamometer, they achieved separation of the upper plate from the stuffing surface. At the time of separation, the dynamometer readings were recorded. The stickiness calculation ρ , (N/cm^2) was carried out according to the formula (Martynov *et al.*, 2018).

For determining the yield, samples of model minced meat were prepared similarly to the

determination of BCC. The prepared samples were kept at a temperature from 0 to 4°C. After a predetermined time, the samples were heat-treated in a microwave oven for 15 minutes at a 100 W power, after which they were re-weighed. The control was samples subjected to salting without microbial treatment for 12 hours.

The pH of solutions and meat systems was determined using the potentiometric method on a universal pH-121 ionometer. A portion of each meat sample weighing (10.00 ± 0.02) g was extracted with distilled water in a ratio of 1:10 for 30 min at $(20 \pm 5)^\circ\text{C}$, mixed and filtered through a folded paper filter.

The determination of the mass fraction of proteins in muscle tissue was carried out according to the Kjeldahl method. The test samples were ground. 0.2 g of collagen gel was added to a Kjeldahl flask with a capacity of 50 cm³. Using a piece of glass, the sample was lowered to the bottom of the flask. 1-2 cm³ of concentrated sulfuric acid, 1 g of a mixture of copper sulfate, and potassium sulfate as a catalyst were added. The contents of the flask were heated in a fume hood. When the mixture turned brown, the flask was removed from the heat, cooled at room temperature. 2-3 cm³ of hydrogen peroxide solution with a mass fraction of 30% was added, and heating continued until a colorless mineralate was obtained. The latter is used for the quantitative determination of protein (Ammor and Mayo, 2007; Zinina *et al.*, 2016).

The mineralate was cooled, quantitatively transferred into a volumetric flask with a capacity of 250 cm³. The volume was adjusted to the mark with distilled water. The contents were mixed. 5 cm³ of the obtained mineralizate solution was added to a volumetric flask with a capacity of 100 cm³. The volume was re-adjusted to the mark with distilled water. For the color reaction, 1 cm³ of the re-diluted mineralizate was introduced into the tube, and 5 cm³ of reagent 1 and 5 cm³ of reagent 2 were added sequentially. The contents of the tube were mixed. At the same time, a control solution was prepared using a control mineralizate (sample using distilled water). After 30 minutes, the optical density of the solutions was determined on a red color filter photo electro colorimeter. The measurement was carried out in comparison with a control solution. The determination of lactic acid content was carried out by color reaction with para-oxydiphenyl. Sample preparation: 10 cm³ of trichloroacetic acid solution with a mass fraction of 10% and 2-4 g of minced meat were introduced into the mortar and ground for 10 minutes. The resulting suspension

was transferred into a volumetric flask with a capacity of 50 cm³, first using 20 cm³ of a trichloroacetic acid solution with a mass fraction of 10%, and then a few cubic centimeters of distilled water. The flask was left for 30 min at room temperature, shaking every 10 min, then the volume was adjusted to the mark with distilled water, the flask was closed with a stopper, the contents were well mixed, transferred to centrifuge tubes, centrifuged at a speed of 50 s⁻¹ for 10-15 min. The centrifuge was poured into a dry flask, 25 cm³ of a clear liquid was taken, transferred to a volumetric flask with a capacity of 100 cm³, and the volume was adjusted to the mark with distilled water. Analysis: For carbohydrates' precipitation, 1 cm³ of a copper sulfate solution with a mass fraction of 20% was added to 2 cm³ of the diluted centrifuge. Distilled water was adjusted to 10 cm³ with water, pipetted with water, or from a burette. 1 g of powdered calcium hydroxide was added, shaken vigorously and left resting for 30 minutes, shaken from time to time, and then centrifuged. The centrifuge was poured into a flask. For the color reaction, 1 cm³ of the centrifugate was transferred to a flask of about 25x200 mm in size, 1 drop of a solution of copper sulfate was added with a mass fraction of 4%, and placed in ice water. With stirring, 6 cm³ of concentrated sulfuric acid was added from the micro burette. The tube was placed for 5 minutes in a water bath at the boiling temperature, and then it was cooled in cold water to 20 °C. A vapor solution—oxydiphenyl (0.1 cm³) was added to the tube, mixed very carefully, after which the tubes were placed for 30 minutes in a water bath at 30 °C, occasionally slightly shaking. After this period, the tube was placed in a strongly boiling water bath for 90 s, then it was cooled in cold water, and the color intensity was measured using a spectrophotometer. The measurement was carried out at a wavelength of 560 nm. The thickness of the cuvette is 1 cm. A control experiment with reagents was carried out starting from the moment of carbohydrate precipitation. Instead of 2 cm³ of the centrifugate of our sample, we used 0.3 cm³ of a solution of trichloroacetic acid and 1.7 cm³ of distilled water. We built a calibration graph. According to the calibration graph, the concentration of lactic acid in the volume of the solution taken for the color reaction was found (Sarbatova *et al.*, 2018).

Determination of shear forces. To do this, turn on the PM-3 device with the VK toggle switch. The handle leads the arrow of the device to zero, and the holes in the plate of the working body and the shifting clamp are combined. The prepared meat sample is carefully placed in the

formed cylindrical hole, and pressure plates with a knife surface at the end are inserted into the guides to cut off the excess meat and fix the sample. By pressing the “Start” button, the drive of the working body is set in motion, the shifting plate of which produces a slice of the sample. The force required to cut the sample is transmitted to the strain gauge and, through the strain gauge, is fixed in the form of a peak on the tape of the potentiometer (Omarovet *et al.*, 2018).

3. RESULTS AND DISCUSSION:

In the process of cultivation on meat and peptone agar, we studied morphology and carried out a complete identification of the selected strains of microorganisms. The choice was limited to three species: *Lactobacillus bulgaricus*, *Bifidumbacterinumsiccum*, *Staphylococcus carnosus*. *Lactobacillus bulgaricus* and *Staphylococcus carnosus* were selected because of the fast growth during cultivation on different nutrient media. Bifidobacteria are present in large amounts in the intestinal microflora. Therefore, *Bifidumbacterinumsiccum* was the third type of bacteria to be selected.

When cultivated on meat peptone gelatin, biochemical properties were studied by determining microorganisms' proteolytic activity in thinning the gelatin medium. It was found that *Lactobacillus bulgaricus* and *Staphylococcus carnosus* have greater proteolytic activity since they liquefied gelatin on the 4th day of cultivation. Bifidumbacterinumsiccum enzyme systems were less active and thinned dense media only for 6 days at room temperature.

During the cultivation of *Lactobacillus bulgaricus*, the pH of the collagen gel decreased from 6.54 at the beginning of cultivation to 5.8 eight hours later after the beginning of cultivation. The acidity accordingly increased from 35°T to 82°T, which indicates the intensive growth and metabolism of the bacteria *Lactobacillus bulgaricus*.

The growth and development of *Bifidumbacterinumsiccum* were less active: acidity increased from 36°T to 74°T, the mass fraction of protein decreased from 1.51 to 1.09 mg/cm³, and the pH from 6.83 to 6.09. After interpreting the obtained data, it can be concluded that the biosynthetic activity of *Bifidumbacterinumsiccum* and *Lactobacillus bulgaricus* is slightly different and has special features.

During the cultivation of *Staphylococcus carnosus*, the pH decreased from 6.61 to 5.9, and the acidity increased from 65.3°T to 98°T. In contrast to other lactic acid bacteria, the proportion of protein increased from 1.5 to 1.62, indicating a lower degree of protein hydrolysis in the presence of *Staphylococcus carnosus*. The data obtained are shown in figures 1, 2, 3.

Figure 1. pH values during the growth of lactic acid bacteria on collagen gel

Figure 2. Acidity values of the medium during the growth of lactic acid bacteria on collagen gel

Figure 3. Values of the protein fraction of the medium during the growth of lactic acid bacteria on collagen gel

During cultivation on a gelatin gel, the pH and mass fraction of protein was determined by the biuret method, every two hours during the first 10 hours of cultivation. Simultaneously, 2 cm³ of an alkaline protein extract was taken for analysis and mixed with 15 cm³ of a biuret solution. A control experiment was prepared similarly.

It was found that over this period, the pH value decreased from 6.19 at the beginning of cultivation to 5.8 at the end of cultivation. There was also a decrease in the proportion of protein from 0.45 mg/cm³ to 0.26 mg/cm³. The control when measuring pH was an extract from the minced meat without adding a consortium of microorganisms.

The minced meat's functional and technological properties, which were obtained, are presented in Figures 4, 5.

Figure 4. Minced flank meat with the addition of lactic acid bacteria complex

Figure 5. Stuffed horse meat with the addition of a complexo-flactic-acid bacteria

The research results indicate a positive symbiosis of the selected strains with the preservation of all biochemical properties. The formed consortium of lactic acid bacteria was used in the processing of real objects: horse meat, flank, and beef muscle tissue of the second grade.

Connective tissue, which is connected with muscle tissue and is organically part of the meat, reduces its nutritional value: the utilization rate in anabolism for connective tissue is three times less than for meat. Besides, it increases its rigidity. Therefore, the quality of meat depends not only on the amount of connective tissue but also on the ratio of elastin and collagen fibers, their structure, and thickness.

Many researchers use one single pure culture as a culture of microorganisms. Some cultures are very rarely used in the consortium. Microorganism cultures are mostly used only as starter cultures to accelerate the production of raw smoked sausages (Adams and Mitchell, 2002; Argyriet al., 2015; Audenaert et al., 2010; Bonomo et al., 2008; Dušková et al., 2016; Gizatov and Gizatova, 2015; Gizatova et al., 2014; Holko et al., 2013). In this regard, the superiority of these studies is precisely the use of microorganism cultures in the form of a consortium and their use in the production of any kind of sausages as not an accelerator, but a softener of low-value meat raw materials. Cocolinet al. (2009), De Souza Barbosa et al. (2015), Juntunen et al. (2001), Dušková et al. (2016) were developing the use of starter cultures as additives to fermentable sausages. Selected starter cultures (SAS) (i.e., *Lactobacillus sakei* 8416, *Lactobacillus sakei* 4413, and *L. sakei* 8426, *L. Plantarum* 7423 and *L. curvatus* 8427) were used as starter cultures in addition to the control

treatment in the production of fermented sausages. SAS cultures had rapid growth, and the randomness of the LAB population prevailed throughout fermentation and maturation. A process of improvement in sensory properties was observed compared to the control sample. Besides the treatment obtained with *L. sakei* 8416, all other SAS cultures prevented lipid oxidation to below 1 mg malondialdehyde/kg.

The number of Micrococcaceae and the redness of the sausages were not affected. The acidification during the fermentation in the treatment is performed with *L. sakei* 8416 and *L. sakei* 4413. The values for *L. sakei* 4413 were the lowest (* P <0.05), the content of all biogenic amines compared with control, the decrease in tyramine was 13%, tryptamine 55%, cadaverine 60%, and putrescine 72%. Sausages made with SAS cultures of *L. sakei* 4413 and *L. sakei* 8416 had the highest ratings for all sensory properties. The results showed that the SAS culture of *L. sakei* 4413 is the best starting culture for fermented sausages (Andreeva *et al.*, 2018; Omarov *et al.*, 2018). Several researchers are involved in the use of starter crops in sausage production (Adams and Mitchell, 2002; Cocolinet *et al.*, 2009; Doulgeraki *et al.*, 2012; Dušková *et al.*, 2015; Iliina *et al.*, 2017; Juntunen *et al.*, 2001; Knolet *et al.*, 2001). To improve the food safety of Chinese fermented sausages, starter cultures of *Lactobacillus sakei* were added to sausages, and the effects on sausage quality were studied. The results clearly showed that due to *L. sakei* inoculation, lactic acid bacteria quickly dominated. In total, the microflora and growth of foodborne pathogens such as *E. coli* and enterobacteria was completely eradicated in fermentable sausages. The pH of sausages fermented through *L. sakei* significantly decreased from 6.31 to 4.52, while spontaneous fermentation decreased from 6.41 to 5.42. In addition, the nitrite content of sausages fermented by *L. sakei* quickly fell from 100 ppm to 9.6 ppm. Accordingly, spontaneous fermentation, the nitrite content, slowly fell from 100 parts per million to 32.1 ppm. After a sensory evaluation, sausages fermented through *L. sakei* are accepted with pleasure and are in high demand with consumers. *L. sakei* vaccination was beneficial for microbiological quality against the growth of foodborne pathogens and contributed to the depletion of nitrite and improved sensory characteristics (Ivanov *et al.*, 2018).

In the cultivation process on GMK-1 nutrient medium, we studied morphology and carried out a complete identification of the selected

microorganism strains (*Lactobacillus bulgaricus*, *Bifidobacterium sicum*, *Staphylococcus carnosus*).

When identifying, it was noted the differentiating features characteristic of these types of microorganisms:

- for staphylococci, the cells are spherical, gram-positive, motionless, the arrangement of cells in the form of irregular clusters

- bifidobacteria are characterized by straight or branched bacilli, bifurcated Y – or V – forms, club-shaped or lap-shaped, gram-positive, motionless.

- for lactobacilli long and thin to short, straight, or curved gram-positive sticks.

During the microscopic examination of cultures under a light microscope, microphotography of microorganisms' strains at a magnification of 7x90 was carried out. It was also revealed that during cultivation in the consortium of the selected types of bacteria, growth inhibition does not occur, which indicates the synergism of microorganisms in relation to each other.

The number of cells of microorganisms was also calculated. Counting was carried out from the same crops. The number of microorganisms was: *Lactobacillus bulgaricus* 394.0 10⁵ CFU/g, *Bifidobacterium sicum* 393.0 10⁵ CFU/g, *Staphylococcus carnosus* 396.5 10⁵ CFU/g.

When cultivating *Lactobacillus bulgaricus*, the pH of collagen gel decreased by 19% compared to the initial value 24 hours after the beginning of cultivation. The amount of accumulated lactic acid was 27 mg%; the degree of protein hydrolysis was 17% of the initial value. During the cultivation of *Bifidobacterium sicum*, the pH decreased by 14%, the amount of lactic acid was 20 mg%, the degree of hydrolysis of proteins was 13% of the initial value. For *Staphylococcus carnosus*, respectively, these data were pH decreased by 15.8%, the amount of lactic acid 30 mg%, the degree of hydrolysis of proteins 19% to the initial, respectively.

The data obtained indicate that the selected microorganism strains grow on a collagen gel, as evidenced by the accumulation of lactic acid and a decrease in pH, as well as the breakdown of the protein of the collagen connective tissue, which makes the meat stiff. As a result, there is an accumulation of free amino acids and polypeptides. At the same time, the selected bacteria were cultured on a collagen gel in various combinations: 1) *Lactobacillus bulgaricus* + *Bifidobacterium sicum*; 2) *Staphylococcus*

carnosus + *Lactobacillus bulgaricus*; 3) *Staphylococcus carnosus* + *Bifidobacterium sicum*. During cultivation, the same values were determined as in the cultivation of each species separately.

The research results showed that the selected microorganism strains grow well on a collagen gel with the intensive accumulation of lactic acid with a decrease in pH, which indicates the absence of antagonism between the selected microorganism strains. In this case, gel proteins are actively cleaved, which is ensured by the presence of a high level of proteolytic activity.

An important factor in the application of the created consortium of microorganisms is the resistance of these types of bacteria to the action of various temperature parameters used in the production of meat products. For conducting the study, a consortium of microorganisms was activated for 12 hours in skim milk under sterile conditions at an optimum microorganism growth temperature of 30 °C in a thermostat. After activation, a consortium was added to ground beef at a temperature of + 4 °C, + 12 °C, + 20 °C, + 40 °C, + 80 °C. The temperature parameters were chosen in accordance with the sausages used in the technology. + 4 °C – temperature for salting and ripening meat, + 12 °C – drying temperature, + 20 °C – smoking temperature, + 40 °C – hot smoking temperature, 80 °C – cooking temperature. The growth of microorganisms was monitored by the dynamics of titratable acidity of ground beef meat for 24 hours. During the study, it was found that at all variants of the selected temperatures, except for a temperature of 80 °C, titratable acidity of ground beef increases, which indicates the development and consortium of microorganisms. At 80 °C, changes in acid formation were practically not observed, which indicates the death of microorganisms.

Assessment of the growth ability and manifestations of the biochemical activity of the created consortium of lactic acid bacteria on real objects to the most significant functional and technological properties of meat systems: moisture-binding, the moisture-holding ability of meat raw materials, its stickiness, especially in sausage technology. It was interesting to study the effect of raw meat's fermentation on the numerical values of these values. In the process of traditional salting, a gradual increase in MBC occurs, which stabilizes over time. A study of the influence of a consortium of microorganisms showed that their use in the salting process leads to insignificant (3-8%) and stable growth of MBC

during salting time for all three types of minced meat. When using a traditional way of salting, the nature of the dependence can be explained by the fact that during the initial stages of hydrolysis, the formation of hydrolysis products of protein molecules occurs due to the proteolytic activity of microorganisms included in the consortium, which leads to an increase in the number of readily available charged groups that can hold water. With deeper hydrolysis, oligopeptides, and free amino acids accumulate, which are not known to be capable of efficiently binding water. The results obtained during salting with the addition of microorganisms are obviously associated with an increased intensity of the action of microorganisms on the connective tissue proteins of minced meat raw materials. Obviously, these results in the accumulation of a large number of readily available charged groups, and lactic acid bacteria assimilate the formed amino acids during the life process. The water-holding capacity (WHC) of raw materials is the most critical indicator for meat products subjected to heat treatment with traditional salting. A sharp increase in WHC in the first hours of salting occurs.

The maximum WHC values are achieved after 2 hours of processing for minced meat from horse meat and muscle tissue of beef, 4 hours – for minced meat from the beef flank, after which the WHC values decrease. When using consortia, a smooth increase in the WHC occurs during the first 4-6 hours, and then a slight decrease in the WHC occurs, and the final values when using a consortium of microorganisms for all types of minced meat are significantly higher than with traditional salting without adding a consortium of microorganisms. Such results indicate the synergistic (mutual reinforcement) action of a consortium of microorganisms and salt in the process of salting.

In scientific studies, Adams and Mitchell (2002), Bonomo *et al.* (2008), Coccolinet *et al.* (2009), it was revealed that after three days of product storage, the number of *thermophilic streptococcus* was $1.3 \cdot 10^7$ CFU/g. When storing the product, the total number of microorganisms with different microbial contamination of the meat component remained at the same level within seven days, $1 \cdot 10^2$ CFU/g. According to the results of these studies, three days after development, the number of lactic acid and bifidobacteria cells was $1 \cdot 10^8$ CFU/g, on the seventh day of storage, it was $1.4 \cdot 10^6$ CFU/g and remained at that level.

The use of our complex of lactic acid

bacteria both to the flank and to horse meat and beef muscle tissue leads to an increase in the values of functional and technological properties such as MBC, WHC, as well as to a decrease in the pH of the environment, which is important when producing meat and sausage products. It should be recognized that the processing of raw meat with lactic acid and bifidobacteria is effective and economically feasible since salting time is halved during the addition of lactic acid and bifidobacteria. The nature of the action of the consortium of microorganisms allows us to recommend it for use with the goal of softening, improving the quality of raw materials in the technology of a wide range of meat products with different ratios of muscle and connective tissue.

The developed product, enriched with consortiums of microorganisms, will have a positive effect on human health. It will contribute to economically and rationally use the most important food resources. Using selection methods opens up the possibility of obtaining products with valuable characteristics (original taste, aroma, right color consistency). It is assumed that the resulting race can severely suppress the harmful microflora that grows in meat products.

5. CONCLUSIONS

The macro morphological and biochemical properties of microorganisms were studied during the research: *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Staphylococcus carnosus*, as well as their synergism on various nutrient media, including collagen gel. The laws of growth and changes in the biochemical properties of strains were established. The selection of strains for creating a consortium was justified. The consortium's influence on the functional and technological properties of the biomodified model minced meat from horse meat, flank, and beef grade II was studied.

The analysis of the obtained results proves that, against the background of a positive symbiosis of the selected strains, it was possible to create a consortium of microorganisms with preserving all biochemical properties. The addition of the studied consortium of wet organisms to both flank and horse meat increased the functional and technological properties such as MBC, WHC, FHC, Yield, ES, as well as to lower the pH of the medium, which is not unimportant in the production of meat and

sausages. The results of these studies, compared with analogs, are in a more advantageous position since the use of the obtained consortium in the production of meat products allows not only accelerating the maturation of minced systems but also makes it possible to use low-value meat raw materials in production by increasing the grade of low-grade raw materials in the fermentation process.

Sausages produced using this technology have functional properties as they are rich in mineral matters (calcium 98.75 mg, sodium 9.07 mg, magnesium 33.85 mg, iron 1.27 mg, respectively) B vitamins 2.8 mg%. The economic effect of the use of a consortium of microorganisms in the technology of sausage production consists of a reduction in terms of salting and replacement of the main raw materials and amounts to 3875.26 rubles per ton of the finished product.

5. ACKNOWLEDGMENT:

The authors thank the universities (Bashkir State Agrarian University and Ufa State Petroleum Technological University) for the study's support.

6. REFERENCES:

1. Adams, M., and Mitchell, R. (2002). Fermentation and pathogen control: a risk assessment approach. *International Journal of Food Microbiology*, 79(1-2), 75-83.
2. Ammor, M. S., and Mayo, B. (2007). Selection criteria for lactic acid bacteria to be used as functional starter cultures in dry sausage production: An update. *Meat science*, 76(1), 138-146.
3. Andreeva, A. V., Nikolaeva, O. N., Ismagilova, E. R., Tuktarov, V. R., Fazlaev, R. G., Ivanov, A. I., ... and Khakimova, A. Z. (2018). Effect of probiotic preparations on the intestinal microbiome. *Journal of Engineering and Applied Sciences*, 13(S8), 6467-6472.
4. Antipova, L. V. (2001). *Methods used to study meat and meat products*. Moscow: Kolos.
5. Antipova, L. V., and Uspenskaj, M. E. (2016). Bio-modification of plasma protein complex blood of farm animals in preventive technologies and products for special purposes. In *Collection of articles: Modern state and development prospects*

- of dairy farming and agrifood industry. *Proceedings of International Scientific and Practical conference* (pp. 167-171). Omsk State Agrarian University named after P.A. Stolypin. Institute of International Education.
6. Antipova, L. V., Glotova, I. A., Storublevtsev, S. A., Boltyhov, J. V., Vtorushina, I. V., Ilina, N. M., and Galina, J. F. (2011). Realization of biopotential minor collagen raw materials in processing branches of agrarian and industrial complex on the basis of biotechnological methods. In *Biotechnology and the Ecology of Big Cities* (pp. 159-170).
 7. Antipova, L. V., Gorbunkov, M. Y., and Storublevtsev, S. A. (2015). The experience of enzyme preparations application in the processing of animal origin raw materials. *European Journal of Natural History*, 2, 42-43.
 8. Antipova, L. V., Gorbunkov, M. Y., and Storublevtsev, S. A. (2016). New biotechnologies of raw animal materials. In *Collection of articles: Immediate problems of chemistry, biology, and biotechnology. Proceedings of the 10th All-Russian Scientific conference* (pp. 296-297).
 9. Argyri, A. A., Mallouchos, A., Panagou, E. Z., and Nychas, G. J. E. (2015). The dynamics of the HS/SPME–GC/MS as a tool to assess the spoilage of minced beef stored under different packaging and temperature conditions. *International journal of food microbiology*, 193, 51-58.
 10. Armuzzi, A., Cremonini, F., Ojetto, V., Bartolozzi, F., Canducci, F., Candelli, M., ... and Gasbarrini, G. (2001). Effect of Lactobacillus GG supplementation on antibiotic-associated gastrointestinal side effects during Helicobacter pylori eradication therapy: a pilot study. *Digestion*, 63(1), 1-7.
 11. Audenaert, K., D'Haene, K., Messens, K., Ruysen, T., Vandamme, P., and Huys, G. (2010). Diversity of lactic acid bacteria from modified atmosphere packaged sliced cooked meat products at sell-by date assessed by PCR-denaturing gradient gel electrophoresis. *Food Microbiology*, 27(1), 12-18.
 12. Bonomo, M. G., Ricciardi, A., Zotta, T., Parente, E., and Salzano, G. (2008). Molecular and technological characterization of lactic acid bacteria from traditional fermented sausages of Basilicata region (Southern Italy). *Meat Science*, 80(4), 1238-1248.
 13. Buchanan, R. E., and Gibbons, N. E. (1994). *Manual of Determinative Bacteriology*. Bergey's 8 ed. Baltimore: The Williams and Wilkins Company.
 14. Bulat, P. V., Zasuhin, O. N., and Uskov, V. N. (2012). On classification of flow regimes in a channel with sudden expansion. *Thermophysics and Aeromechanics*, 19(2), 233-246.
 15. Casquete, R., Benito, M. J., Martín, A., Ruiz-Moyano, S., Hernández, A., and Córdoba, M. G. (2011). Effect of autochthonous starter cultures in the production of "salchichón", a traditional Iberian dry-fermented sausage, with different ripening processes. *LWT-Food Science and Technology*, 44(7), 1562-1571.
 16. Cocolin, L., Dolci, P., Rantsiou, K., Urso, R., Cantoni, C., and Comi, G. (2009). Lactic acid bacteria ecology of three traditional fermented sausages produced in the North of Italy as determined by molecular methods. *Meat Science*, 82(1), 125-132.
 17. de Souza Barbosa, M., Todorov, S. D., Ivanova, I., Chobert, J. M., Haertlé, T., and de Melo Franco, B. D. G. (2015). Improving safety of salami by application of bacteriocins produced by an autochthonous Lactobacillus curvatus isolate. *Food microbiology*, 46, 254-262.
 18. Doulgeraki, A. I., Ercolini, D., Villani, F., and Nychas, G. J. E. (2012). Spoilage microbiota associated to the storage of raw meat in different conditions. *International journal of food microbiology*, 157(2), 130-141.
 19. Dušková, M., Kameník, J., Lačanin, I., Šedo, O., and Zdráhal, Z. (2016). Lactic acid bacteria in cooked hams—Sources of contamination and chances of survival in the product. *Food Control*, 61, 1-5.
 20. Dušková, M., Kameník, J., Šedo, O., Zdráhal, Z., Saláková, A., Karpíšková, R., and Lačanin, I. (2015). Survival and growth of lactic acid bacteria in hot smoked dry sausages (non-fermented salami) with and without sensory deviations. *Food Control*, 50, 804-808.
 21. Gabitov, I. I., Mudarisov, S. G., Gafurov, I. D., Ableeva, A. M., Negovora, A. V., Davletshin, M. M., ... and Yukhin, G. P. (2018). Evaluation of the efficiency of

- mechanized technological processes of agricultural production. *Journal of Engineering and Applied Sciences*, 13(S10), 8338-8345.
22. Gabitov, I. I., Negovora, A. V., Khasanov, E. R., Galiullin, R. R., Farhshatov, M. N., Khamaletdinov, R. R., ... and Razyapov, M. M. (2019). Risk reduction of thermal damages of units in machinery heat preparation for load acceptance. *Journal of Engineering and Applied Sciences*, 14(3), 709-716.
 23. Gavrilova, N., Chernopolskaya, N., Rebezov, M., Moisejkina, D., Dolmatova, I., Mironova, I., ... and Derkho, M. (2019). Advanced biotechnology of specialized fermented milk products. *International Journal of Recent Technology and Engineering*, 8(2), 2718-2722.
 24. Gizatov, A. Y., and Gizatova, N. V. (2015). Biotechnological aspects of bifidobacteria usage to obtain products of animal origin with the desired properties. In *Innovative Processes in AgroIndustrial Complex* (pp. 105-106).
 25. Gizatova, N. V., Gizatov, A. Ia., and Mironova, I. V. (2014). Reason in selecting microorganism species for collagen containing raw materials. In *Collection of articles: Prospects of innovative development of Agri-Industrial complex. Proceedings of International Scientific and Practical conference within the framework of the 24th International Specialized Exhibition "Agrocomplex-2014"* (pp. 19-24).
 26. Holko, I., Hrabě, J., Šalaková, A., and Rada, V. (2013). The substitution of a traditional starter culture in mutton fermented sausages by *Lactobacillus acidophilus* and *Bifidobacterium animalis*. *Meat Science*, 94(3), 275-279.
 27. Ilina, N. M., Kutsova, A. E., Builenko, Iu. S., and Fomina, T. (2017). Application of biotechnological methods in meat production. *Bulletin of the South Ural State University Vestnik. Series of papers: Food Technologies*, 5(3), 21-28.
 28. Ivanov, A. I., Andreeva, A. V., Skovorodin, E. N., Shaimukhametov, M. A., Altynbekov, O. M., Sultangazin, G. M., ... and Nikolaeva, O. N. (2018). Anaerobic microflora impact on pathomorphogenesis of swine dysentery. *Journal of Engineering and Applied Sciences*, 13(S11), 8796-8802.
 29. Juntunen, M., Kirjavainen, P. V., Ouwehand, A. C., Salminen, S. J., and Isolauri, E. (2001). Adherence of probiotic bacteria to human intestinal mucus in healthy infants and during rotavirus infection. *Clinical and Diagnostic Laboratory Immunology*, 8(2), 293-296.
 30. Karam, L., Jama, C., Dhulster, P., and Chihib, N. E. (2013). Study of surface interactions between peptides, materials and bacteria for setting up antimicrobial surfaces and active food packaging. *Journal of Materials and Environmental Science*, 4(5), 798-821.
 31. Knol, J., Poelwijk, E. S., Van der Linde, E. G. M., Wells, J. C. K., Bronstrup, A., Kohlschmidt, N., ... and Fusch, C. (2001). Stimulation of endogenous bifidobacteria in term infants by an infant formula containing prebiotics. *Journal of Pediatric Gastroenterology and Nutrition*, 32, 399.
 32. Konashova, S. I., Sultanova, R. R., Khairtdinov, A. F., Gabdrakhimov, K. M., Konovalov, V. F., Rakhmatullin, Z. Z., ... and Muftakhova, S. I. (2018). Forestry and ecological aspects of the broad-leaved forest formation. *Journal of Engineering and Applied Sciences*, 13(S11), 8789-8795.
 33. Martynov, V. M., Gabitov, I. I., Karimov, K. T., Masalimov, I. K., Permyakov, V. N., Ganeev, I. R., ... and Saitov, B. (2018). Reasoning barley grain drying modes for vacuum-infrared drying machines. *Journal of Engineering and Applied Sciences*, 13(S11), 8803-8811.
 34. Nesterenko, A. A., Keniiz, N. V., Ilina, N. M., and Korshunova, Y. M. (2016). Studying the properties of starter cultures in meat raw materials. In *Collection of articles: Modern problems of fundamental and applied sciences development* (pp. 18-24). Printing house "Maestro".
 35. Nesterenko, A., Koshchaev, A., Keniiz, N., Akopyan, K., Rebezov, M., and Okuskhanova, E. (2018). Biomodification of Meat for Improving Functional-Technological Properties of Minced Meat. *Research Journal of Pharmaceutical Biological and Chemical Sciences*, 9(6), 95-105.
 36. Omarov, R. S., Antipova, L. V., Konieva, O. N., Meshcheryakov, V. A., and Shlykov, S. N. (2018). Biotechnological aspects in the development of functional food products.

37. Rakhimov, Z., Mudarisov, S., Rakhimov, I., Farkhutdinov, I., Mukhametdinov, A., Gareev, R., ... and Tarkhova, L. (2018). Reasoning a construction diagram and parameters of tillers for primary cultivation. *Journal of Engineering and Applied Sciences*, 13(S11), 8812-8818.
38. Sarbatova, N. Y., Frolov, V. Y., Omarov, R. S., Sycheva, O. V., and Antipova, L. V. (2018). Study of biotechnological potential of blood agricultural animals and poultry for the development of new balanced food products. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 9(2), 555-562.
39. Sydykova, M., Nurymkhan, G., Gaptar, S., Rebezov, Y. M., Khayrullin, M., Nesterenko, A., and Gazeev, I. (2019). Using of lactic-acid bacteria in the production of sausage products: modern conditions and perspectives. *International Journal of Pharmaceutical Research*, 11(1), 1073-1083.
40. Wang, X. H., Ren, H. Y., Liu, D. Y., Zhu, W. Y., and Wang, W. (2013). Effects of inoculating *Lactobacillus sakei* starter cultures on the microbiological quality and nitrite depletion of Chinese fermented sausages. *Food Control*, 32(2), 591-596.
41. Zinina, O. V., and Rebezov, M. B. (2016). A Biotechnological processing of collagen containing by-products of bovine animals. *Research Journal of Pharmaceutical Biological and Chemical Sciences*, 7(1), 1530-1534.
42. Zinina, O. V., Rebezov, M. B., and Vaiscrobova, E. S. (2016). A microstructure of the modelling systems on the basis of the ferment raw material with a high collagen content. *Pakistan Journal of Nutrition*, 15(3), 249-254.