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Justification and microbiota compositions development for the fermentation of raw meat

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ABSTRACT

In the production of fermented meat products, microorganisms of various taxonomic groups play an extremely important role, namely in the formation of specific taste, aroma, colour, and consistency. Both fermentative and spontaneous microflora take part in the components' transformation of meat raw materials during the maturation of such products, and the course of this process depends on the metabolic activity of the strains. In accordance, this article's purpose is to select microbiota compositions (lactic acid bacteria and coagulase-negative cocci) for the fermentation of meat raw materials. So, as a result of the research, 4 compositions were selected, two of which are lactic acid bacteria with micrococci (No. 2, 3) and two lactic acid bacteria with staphylococci (No. 1, 6). They were characterized by the high productivity of each of the components of the leavening composition, in particular, it was established that the number of MKB increased – by 4.3-6.5 times, and micrococci and staphylococci – by 7.7-28.6 times, respectively. For these compositions, mutual stimulation of the components was observed, contributing to the active microorganisms' development and their biochemical activity. Fermentation compositions No. 1, 6, 4, and 6 had the highest nitrite-reducing activity, and a high level of proteolysis characterized compositions No. 1, 2, 3, and 4. According to the results of determining the antagonistic activity against opportunistic and pathogenic microorganisms, it was established that the investigated compositions exhibit antagonistic activity against both gram-negative and gram-positive microorganisms.

Keywords: microbiota, fermentation, raw meat, leaven cultures, nitrite-reducing activity

INTRODUCTION

Traditional fermented meat products are very popular and have been consumed for many years because of their unique taste **[1]**. These products are usually fermented by spontaneous microbiota, usually do not pose a health hazard, and are currently carried out under controlled conditions with the addition of starter cultures. It is known that meat starters consist of a combination of lactic acid bacteria and coagulase-negative staphylococci. Thanks to such combinations, the technological efficiency increases and the range of desired properties of bacterial preparations expands **[2]**. The participation of lactic acid bacteria is related to acid and aroma formation, under the action of their proteolytic activity, proteins are split into free amino acids which are important components, forming a pleasant taste and aroma of sausages **[3]**. They are responsible for color formation and hygienic safety of meat products **[4]**. An important role is also given to micrococci or staphylococci, which provide a stable color, reduce the content of residual sodium nitrite, slow down the oxidation and rancidity of fats and lead to the formation of aromatic compounds **[5]**, **[6]**. To create leaven for the production of dry sausages, the authors **[7]** recommend using *Lactobacillus sakei*, *Pediococcus acidilactici*, and **[8]** such types of staphylococci

Staphylococcus equorum, Staphylococcus saprophyticus Staphylococcus xylosus and *Staphylococcus carnosus.* Landeta et al. **[12]** recommend two strains of *Staphylococcus carnosus* and *Staphylococcus equorum* with the highest nitrate reductase and proteolytic activity to create potential leaven for the production of meat products **[9]**. A coagulase-negative *Kocuria rhizophila* culture was selected from Nuodeng ham, which is recommended as a potential leaven culture for faster and safer meat fermentation **[10]**. Researchers **[11]** showed the effectiveness of using microbiota *Lactobacillus plantarum* MSZ2 and *Staphylococcus xylosus* YCC3 to improve the taste, quality and duration of storage of fermented sausages. To suppress the formation of biogenic amines, for example, *Lactobacillus* spp. which reduces nitrite residues and suppresses the accumulation of biogenic amines. It is shown that the L. curvatus strain demonstrates optimal performance in suppressing the formation of N-nitrosamine in the production of Harbin dry sausages **[12]**. Lipolytic catalase-negative cocci play an important role in releasing fatty acid precursors and improving the sensory profile of meat **[13]**. The authors **[14]** confirm that the use of a mixture of the *Lactobacillus sakei* strain and the *Staphylococcus equorum, Staphylococcus epidermidis* or *Staphylococcus saprophyticus* strain in the production of sausages makes it possible to obtain homogeneous products without giving up the desired typical characteristics obtained in non-industrial production. During meat fermentation, leaven cultures, mainly lactic acid bacteria and coagulase-negative staphylococci, are often used to standardize product properties, reduce ripening time, and improve product safety nitrate reductase activity of catalazoneative cocci is responsible for the typical stabilized color of dried meat due to the formation of nitroso myoglobin. The activity of nitrate reductase and catalase of cocci also provides protection against severe oxidation of lipids and proteins, which leads to the deterioration of color, texture, taste and nutritional value of meat products **[15]**. Alfaia and others showed the prospects of using selected isolates of coagulase-negative staphylococci *Staphylococcus xylosus*, *Staphylococcus equorum* and *Staphylococcus carnosus*, and lactic acid bacteria *Lactobacillus curvatus*, *Lactobacillus plantarum* and *Lactobacillus sakei*, based on their production, to avoid the formation of biogenic amines in meat products, as well as to ensure special organoleptic characteristics of meat products and bioprotection against pathogen **[16]**. *K. varians (Micrococcus varians)* is used as a leaven to improve the sensory profile of fermented meat and reduce the formation of biogenic amines **[17]**. The relationship between the presence of interaction between beneficial strains of *Lactobacillus sakei* and coagulase-negative cocci *Staphylococcus xylosus* and *Kocuria varians* and the strength of technical characteristics such as proteolysis has been determined. In work **[18]**, it was found that proteolytic *K. varians* affects the amino acid profile, thus potentially enhancing the sensory properties of the meat product, and the mixture of *K. varians* and *Lactobacillus acidophilus* strains brought desirable changes in the amino acid profile and sensory characteristics.

Therefore, the use of leaven cultures in the production of fermented meat products brings desirable biochemical changes, resulting in improved sensory properties through flavour development and softening of texture, as well as proteolysis and lipolysis of added cultures, causing a significant improvement in the organoleptic qualities of fermented meat products. To improve the organoleptic, high-quality colour-forming characteristics, lactic acid bacteria alone are not enough, and the contribution of nitrate/nitrite-reducing organisms becomes significant. The natural leavens development is very promising, as it allows to obtain meat products with high sanitary and sensory qualities.

Scientific Hypothesis

The basis of bacterial preparations for the fermentation of meat raw materials is technologically promising strains that have high productivity and nitrite-reducing activity, possess antagonism towards pathogenic and opportunistic microorganisms, and form a significant amount of aromatic compounds. It is recommended two leavening compositions are based on productivity, nitrite-reducing, proteolytic and antagonistic activities, which include high-tech strains of *L. rhamnosus Kocuria rosea L. casei* and *L. plantarum.*

MATERIAL AND METHODOLOGY

Samples

The test cultures of *E. coli* HISK 240111, *S. aureus* HISK 049065, *P. vulgaris* HISK 160209, *P. aeruginosa* ATCC 27853, and *L. monocytogenes* NCTC 5105 were used in the work.

Chemicals

Distilled water, H_2O (TOV Novokhim, Ukraine).
Sodium chloride, NaCl (TOV Khimlaborreaktiv, Ukraine).
Hydrochloric acid, HCl (TOV Khimlaborreaktiv, Ukraine).
N-(1-naphthyl)-ethylenediamine-dihydrochloride (TOV Khimlaborreaktiv, Ukraine).
Nitrous oxide, NO_2 (AT ZPD, Denmark).
Sodium nitrite, $NaNO_2$ (ATK Ukraine, Ukraine).

Animals, Plants and Biological Materials

From meat products of non-commercial production, strains of the cocci form were extracted and identified, which belonged to the species *S. simulans, S. carnosus, S. xylosus, M. varians, M. roseus* and lactic acid bacilli of the species *L. casei, L. plantarum, L. rhamnosus, L. sakei and L. curvatus*.

Instruments

Ph meter MP 512 ("Ulab").
Petri dishes.
Unico S 2100 spectrophotometer.
Bunsen beaker, (TOV SkyLab).
Conical flask, (TOV SkyLab).
Glass rods, (TOV SkyLab).

Laboratory Methods

The number of cells was determined by the Koch plate method – counting colonies after sowing appropriate dilutions on Petri dishes for catalase-positive cocci with MPA with 6.5% sodium chloride and growing for 72 ±2 h at a temperature of 30 ±1 °C; of lactic acid bacteria from MRS – 72 ±2 h at a temperature of 30 ±1 °C.

Active acidity (pH) was measured potentiometrically using a pH meter MP 512 ("Ulab", Nitrite-reducing activity of the compositions was assessed by the intensity of the color, which was formed by the interaction of nitrite with sulfonamide and N-(1-naphthyl)-ethylenediamine-dihydrochloride in the protein-free filtrate.

The antagonistic activity of the strains was studied by the method of wells on a solid nutrient medium and by co-cultivation with test cultures.

Description of the Experiment

Sample preparation: The most promising strains for starters are those isolated from the local microbiota. At the preliminary stages of the work, from 29 types of meat products of non-commercial production, strains of the cocci form were extracted and identified, which belonged to the species *S. simulans, S. carnosus, S. xylosus, M. varians, M. roseus* and lactic acid bacilli of the species *L. casei, L. plantarum, L. rhamnosus, L. sakei* and *L. Curvatus.*

From these strains, 7 compositions were composed, in particular, 3 compositions with micrococci and 4 with staphylococci (Table 1).

Number of samples analyzed: 7 compositions were composed, in particular, 3 compositions with micrococci and 4 with staphylococci.

Number of repeated analyses: All measurements were performed 3 times.

Number of experiment replication: The number of replicates of each experiment to determine one value was 5 times.

Design of the experiment: Cultures of staphylococci and micrococci were maintained on mysopeptone agar (MPA), and lactic acid bacteria on MRS medium, kept at a temperature of 4 ±2 °C between cultures. Before the experiments, the cultures were activated by several successive transplants on appropriate nutrient media (MPA, MPB, MRS), with incubation at optimal temperatures for 14-18 hours.

The ability to grow together in the compositions was checked by the indicator of the accumulation of viable cells of each of the composition components under the conditions of cultivation in meat-peptone broth with the addition of 1% glucose (pH 7.0). The amount of introduced inoculum was 5% of the volume of the nutrient medium. The fermentation composition "Lakmik", which included a four-component combination of strains *L. rhamnosus, L. casei, L. plantarum,* and *M. varians*, was taken as a control (Cl). The decomposition of sodium nitrite dynamics by the created compositions in the medium of MPB with the addition of 1% glucose, 3% NaCl and with the initial salt content of $NaNO_2$ (Merck) 60 mg.100 cm^{-3} was studied. A 5% inoculum of microorganisms was added to the medium and cultivated for 17 days at a temperature of 30 °C. Measurements were performed on the 1st, 3rd, 4th, 6th, 7th, 10th, and 17th day of the study.

It was determined by taking a 0.5 cm^3 aliquot of the medium with the composition and treating this sample with 0.5 cm^3 (10 $g.dm^{-3}$) of sulfonamide in 3 $mol.dm^{-3}$ HCl and 0.5 cm^3 (0.2 $g.dm^{-3}$) N-(1-naphthyl)-ethylenediamine dihydrochloride. After 20 minutes, the solution was diluted to 4.5 cm^3 with deionized water and the absorbance (540 nm) was measured using a Unico S 2100 spectrophotometer. To calculate the amount of NO_2- contained in the sample, the standard curve was prepared in the same way as for the sample, but using 0.5 cm^3 aliquots of $NaNO_2$ standard solutions (containing 0 to 140 $mmol.dm^{-3}$ NO_2).

The control was MPB medium with 1% glucose, 3% NaCl and 60 mg.100 cm^{-3} $NaNO_2$ (K). The proteolytic activity of the compositions was evaluated by the increase of free amino acids in the culture medium according to the modified ninhydrin method 5% of the inoculum was added to the meat peptone broth with 1% glucose and 3% NaCl. After incubation at 30 °C for 4 and 7 days, the compositions were centrifuged to remove bacterial cells before analysis. The total amount of free amino acids were measured. Amino acid content was determined by the

ninhydrin colourimetric method using a Unico S 2100 spectrophotometer, using glutamic acid as a standard. The medium without the addition of bacteria was used as a control.

Table 1 Characteristics of the created leavening compositions.

			The relationship between the strains	Active acidity, units pH
1	*L. coryniformis* 3401+ *L. casei* 3322+ *L. plantarum* 3201+ *S. saprophyticus* 5302	4	1:1:1:1	3.0 ±0.07
2	*L. casei* 3302+ *L. rhamnosus* 3303+ *L. rhamnosus* 3305+ *M. roseus* 5401	5	1:1:1:1	3.80 ±0.05
3	*L. casei* 3321+ *L. casei* 3322+ *L. plantarum* 3201+ *K. roseus* 5400	6	1:1:1:1	3.55 ±0.07
4	*L. coryniformis* 3401+ *L. casei* 3322+ *S. saprophyticus* 5302	7	1:1:1	3.60 ±0.05
5	*L. rhamnosus* 3308+ *L. tolerans* 3340+ *L. rhamnosus* 3305+ *S. simulans* 5301	8	1:1:1:1	3.60 ±0.05
6	*L. rhamnosus* 3308+ *L. tolerans* 3340+ *S. simulans* 5301	9	1:1:1	3.75 ±0.06
7	*L. rhamnosus* 3308+ *L. tolerans* 3340+ *L. plantarum* 3201+ *K. varians* 5200	10	1:1:1:1	3.80 ±0.07
Kl	*L. casei* 3302+ *L. rhamnosus* 3303+ *L. plantarum* 3200+ *M. varians* 5200	14	1:1:1:1	3.80 ±0.06

Statistical Analysis

The STATISTICA Microsoft Excel editor processed experimental data using mathematical statistics methods. The accuracyof the obtained experimental data was determined using the Student'st-test with confidence coefficient $p \leq 0.05$ with many parallel definitions of at least 5 (confidence probability $p = 0.95$). Linear programming problems were solved using the MS Excel table processor's'Search for a solution'setting(Excel Solver).

RESULTS AND DISCUSSION

The main criteria for evaluating the prospects of leavening compositions for fermentation of meat raw materials were the number of cells of each of the components of the composition, nitrite-reducing and proteolytic activity. It was established that after 18 hours of cultivation, all created leavening compositions reduced active acidity by 45-50% relative to the medium's initial value (pH 7.0) (Table 1). An active decrease in acidity to pH 3.55-3.60 in the culture liquid negatively affected the viability of micrococci (MC), while staphylococci (ST) were insensitive to this factor and had an increase in numbers in the range of 8.9-10.9 times (Figure 1).

In all created compositions, lactobacilli increased for 24 hours of growth, and at the end of cultivation in MPB, the increase continued in compositions No. 1, 2, 3, 7, by 4.3-6.5 times. Composition No. 5 was characterized by a decrease in the number of lactic acid bacteria by 1.3 times the initial content. In composition No. 2, despite the low pH level, an increase of micrococci by 3.7-28.6 times the initial content was observed. The growth of micrococci for 72 hours of cultivation in the remaining compositions decreased by 1.2-2 times.

In compositions with staphylococci #5, 6 their increase by 3 times to the initial content was observed. And in compositions No. 1, and 7, suppression of staphylococci by lactobacilli was observed, their number remained at the level of the initial number or decreased by 1.5 times (Figure 1).

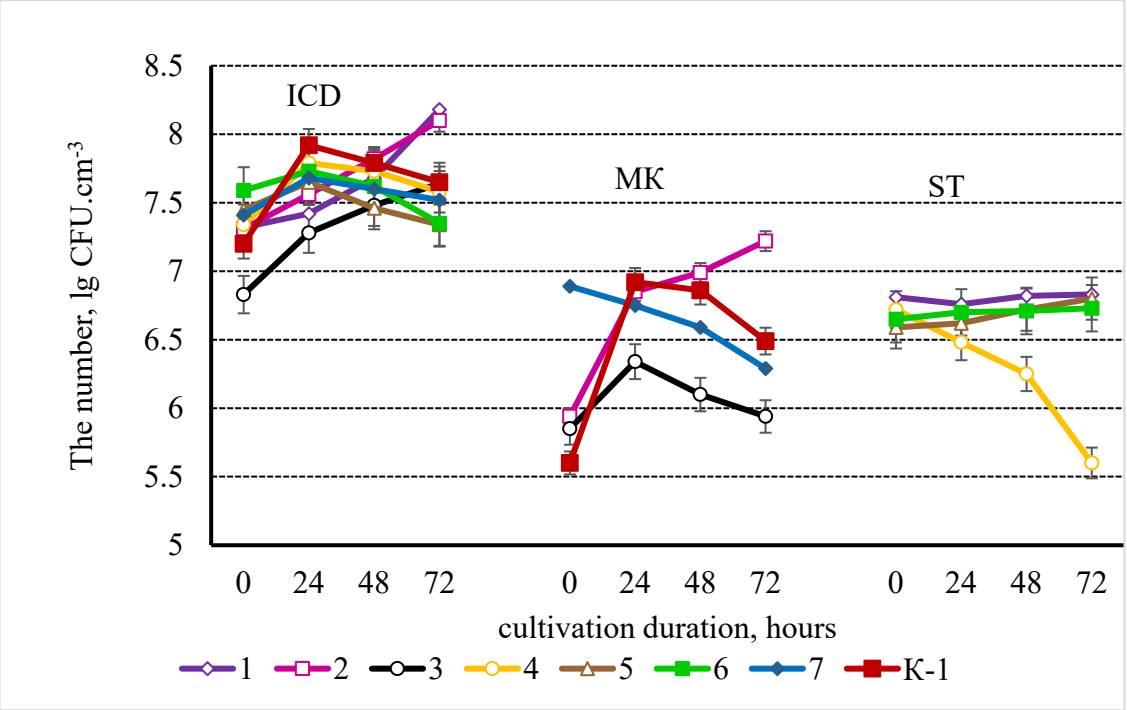
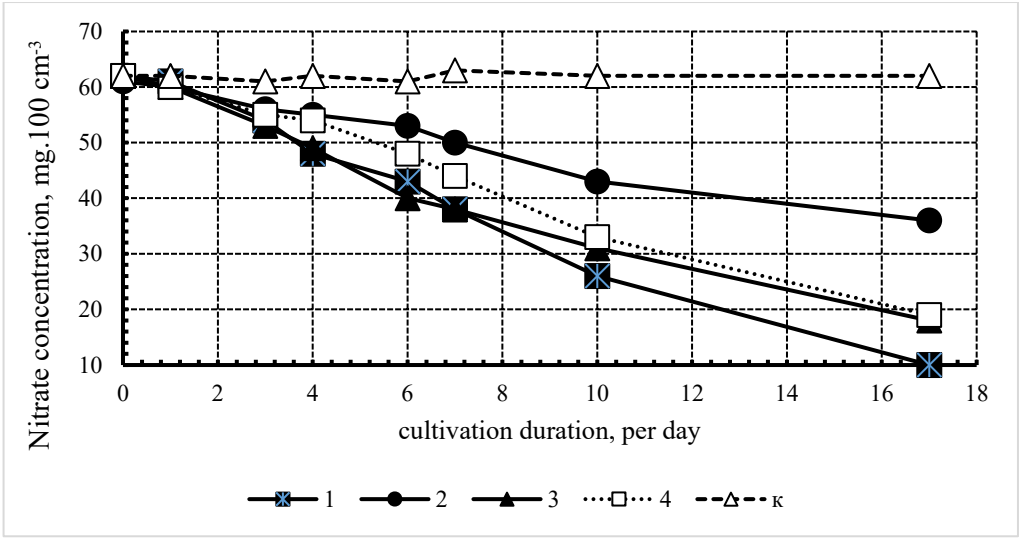


Figure 1 Dynamics of the number of leavening compositions during joint cultivation (ICD – lactic acid bacteria, MK – micrococci, ST – staphylococci).

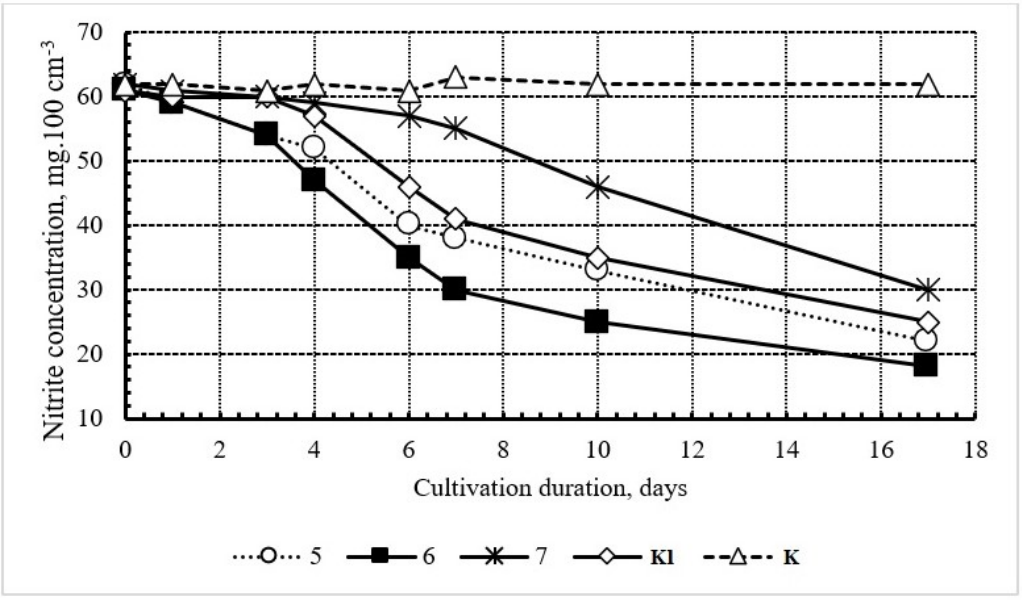
Thus, the compositions of lactic acid bacteria with micrococci (No. 2, 3,) and with staphylococci (No. 1, 6) were the most stable in terms of number, in which the number of microorganisms increased: ICD – by 4.3-6.5 times and micrococci and staphylococci – by 7.7-28.6 times. For these compositions, mutual stimulation of the components was observed, which contributed to the active microorganisms' development. Similar results were obtained and described in many subsequent scientific works, the use of different types of compositions of lactic bacteria **[19]**, research on the development of microorganisms **[20]** and the use of different compositions of lactic acid bacteria **[21]**.

Nitrite-reducing activity: The dynamics of the decomposition of sodium nitrite by the created compositions were studied. It was established that the studied compositions actively reduced the content of nitrites in the culture medium – by 41-83% from the initial. The most active of them were compositions No. 1-6 (Figure 2 a, b). Compositions of lactic acid bacteria with staphylococci (No. 1, 4, 5, 6) more intensively reduced the nitrite content by 67-83%, and compositions with micrococci (No. 2, 3) by 70-71% compared to control K1 (60%). Fermenting composition No. 7 – by 50% was characterized by the lowest nitrite-reducing activity.

In the control, the nitrite content remained constant throughout the experiment (curve K). At the end of fermentation, the residual concentration of sodium nitrite in the culture liquid inoculated with the compositions was 10.5-36.3) mg.100 cm^{-3}.



a)



b)

Figure 2 Dynamics of changes in the content of sodium nitrite during the cultivation of leavening compositions No. 1-4 (a), No. 5-7 (b). K is a medium with sodium nitrite.

The composition of the compositions is given in Table 1. Fermentation compositions No. 1, 3, 4, and 6, which reduced nitrite in the culture medium by 70-83%, are promising for the fermentation of meat raw materials.

Proteolytic activity: The proteolytic activity of the compositions was assessed by the level of increase of free amino acids in the culture medium (Figures 3 and 4). On the 4th day of cultivation in the presence of leavening compositions No. 1, 3, and 4, the dynamics of an increase in the level of cyclic amino acids (C) by 0.6-9.6% compared to the control, at an initial level of 486.1 μg.cm^{-3}, can be observed in the rest of the compositions the consumption of these amino acids is up to 4.2%. Further, on the 7th day of fermentation, an intense decrease in cyclic amino acids was observed in all compositions ranging from 3.5 to 26.1%, except for No. 7, where there was an increase of 22% compared to the initial content of cyclic amino acids (Figure. 3).

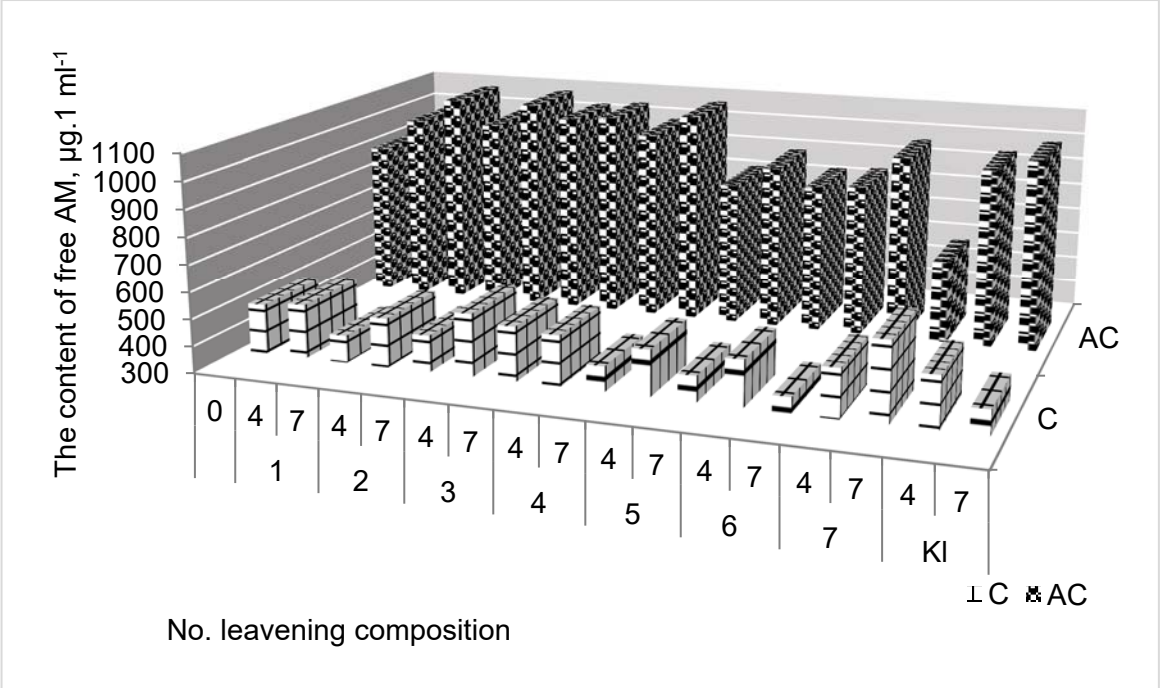


Figure 3 Dynamics of the content of free amino acids during the cultivation of leavening compositions No. 1-7 and Kl. The composition of the compositions is indicated in Table 1. 0, 4, 7 – duration of cultivation, day; C – cyclic amino acids, AC — acyclic amino acids; K is the initial amount of free amino acids.

At the same time, in the environment in the presence of compositions No. 1-5, the dynamics of the accumulation of acyclic amino acids (AC) by 4.2-29.7% was observed throughout the entire fermentation period, compared to the initial content AC = 882.2 µg.cm^{-3}.

On the 4th day, in the medium with all compositions, there was an increase in acyclic amino acids from 9.8 to 19.6%, except for No. 5 and 6, where there was a decrease in AC amino acids by 4.5% and 2.8%, respectively. Whereas on the 7th day in the medium with compositions No. 6, and 7, these amino acids decreased more by 2.35-29.7%, compared to the 4th day of cultivation (Figure 3).

Among the leavening compositions, No. 1-4 were characterized by a high level of proteolysis compared to control KL. The total amount of free amino acids increased the most in variants with micrococci (No. 2, 3) by 15%. In the rest of the compositions, this indicator ranged from 8 to 10% (see Figure 4).

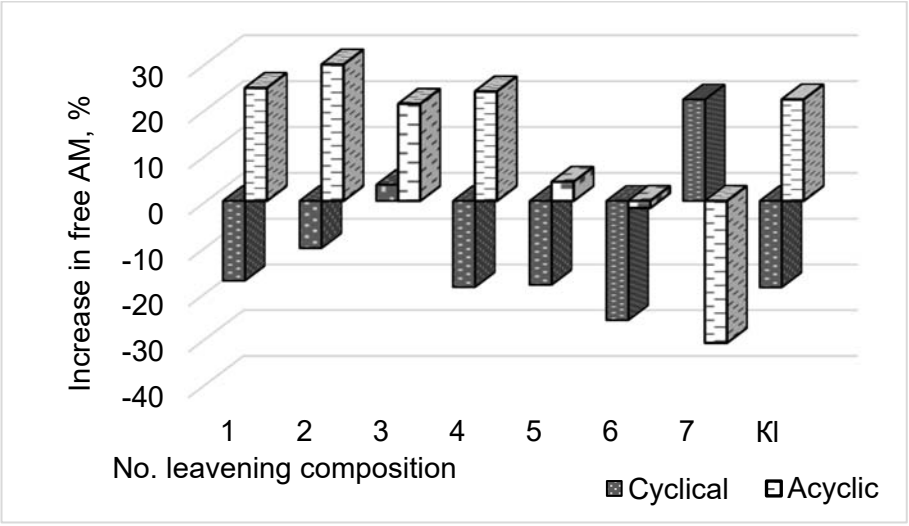


Figure 4 Increase in the content of free amino acids (cyclic (C) and acyclic (AC)) during cultivation of leavening compositions. The numbers of the compositions are indicated in the Table. 1.

Therefore, on the 7th day of cultivation, the composition (No. 2) reduced the level of cyclic amino acids (C) by 10%, relative to the control. Also, in the medium inoculated with this composition, the accumulation of acyclic

amino acids (AC) was 30% higher compared to the control. In the presence of composition No. 3, the amount of both cyclic and acyclic amino acids increased by 3.5% and 22%, respectively. For composition No. 7, the level of acyclic amino acids decreased by 31%, while cyclic amino acids increased by 22% (Figure 4).

So, as a result of the research, 4 compositions were selected, two of which are lactic acid bacteria with micrococci (No. 2, 3) and two lactic acid bacteria with staphylococci (No. 1, 6). They were characterized by the high productivity of each of the components of the leavening composition, in particular, it was established that the number of MKB increased – by 4.3-6.5 times, and micrococci and staphylococci – by 7.7-28.6 times, respectively. For these compositions, mutual stimulation of the components was observed, which contributed to the active microorganism development and the manifestation of their biochemical activity. Fermentation compositions No. 1, 6, 4, and 6 had the highest nitrite-reducing activity, and compositions No. 1, 2, 3, and 4 were characterized by a high level of proteolysis.

Antagonistic activity of compositions: The study of the antagonistic activity of compositions of strains of lactic acid bacteria and cocci about the spontaneous microbiota of meat is a mandatory condition for the selection of cultures of bacterial preparations for the production of fermented meat products, as evidenced by the results described in scientific works **[22]**, **[23]**. The results of the study of antagonistic activity against pathogenic and opportunistic microbiota are presented in Table 2.

Table 2 The composition of antagonistic activity is based on microorganisms of different taxonomic groups.

No. of composition	Types of microorganisms in the composition	Test-cultures				
		P. vulgaris **HISK 160209**	***E. coli*** **HISK 240111**	***P. aeruginosa*** **ATCC 27853**	***L. monocytogenes*** **NCTC 5105**	***S. aureus*** **HISK 049065**
1	*L. coryniformis* 3401+ *L. casei* 3322+ *L. plantarum* 3201 + *S. saprophyticus* 5302	14 ±2	10 ±2	5 ±1	12±1	0
2	*L. casei* 3302+ *L. rhamnosus* 3303+ *L. rhamnosus* 3305+ *M. roseus* 5401	11 ±1	9 ±2	11 ±2	0	12 ±2
3	*L. casei* 3321+ *L. casei 3322*+ *L. plantarum* 3201+ *K. roseus* 5400	16 ±1	14 ±1	9 ±1	0	18 ±2
4	*L. coryniformis* 3401+ *L. casei* 3322+ *S. saprophyticus* 5302	12 ±1	0	0	2 ±1	14 ±2
5	*L. rhamnosus* 3308+ *L. tolerans* 3340 + *L. rhamnosus* 3305+ *S. simulans* 5301	14 ±2	0	12 ±1	18 ±1	0
6	*L. rhamnosus* 3308+ *L. tolerans* 3340+ *S. simulans* 5301	25 ±1	12 ±2	14 ±1	0	16 ±1
7	*L. rhamnosus* 3308+ *L. tolerans* 3340 + *L. plantarum* 3201+ *M. varians* 5200	10 ±1	12 ±1	16 ±1	12 ±1	0
Kl	*L. casei* 3302+ *L. rhamnosus* 3303+ *L. plantarum* 3200+ *M. varians* 5200	11 ±2	13 ±1	5 ±1	0	12 ±2

The investigated compositions have a different degree of antagonistic activity, as evidenced by the size of the growth retardation zones of the test cultures, the size of which varied from 2 to 25 mm.

Of the 7 fermentation compositions studied, antagonistic activity against *P. vulgaris* HISK 160209 was found in all created compositions, *S. aureus* HISK 049065 – 58%, *E. coli* HISK 240111 – 72%, *P. aeruginosa* ATCC 27853 – 86%, *Listeria monocytogenes* NCTC 5105 – 29%.

For compositions No. 1, 3, 5, 7, the maximum growth retardation zone for *L. monocytogenes* reached 12-18 mm, *P. vulgaris* 10-14 mm, *E. coli* 12-14 mm, *S. aureus* 14-18 mm, and *P. aeruginosa* 5-16 mm.

Thus, according to technological parameters the productivity, nitrite-reducing, proteolytic and antagonistic activities, of two leavening compositions No. 3 and No. 7 were selected as promising for fermentation of meat raw materials, which include strains of microorganisms of the species *Kocuria rosea, K. varians, Lactobacillus rhamnosus*, *L. casei, L. plantarum* and *L. tolerans.*

In the production of fermented meat products, microorganisms of various taxonomic groups play an extremely important role, namely in the formation of specific taste, aroma, color, and consistency **[24]**. Both fermentative and spontaneous microflora take part in the components' transformation of meat raw materials during the maturation of such products, and the course of this process depends on the metabolic activity of the strains **[25]**, **[26]**.

According to their composition, leavening preparations can be single- or multi-component. The latter contains several strains of one or more genera, particularly *Lactobacillus, Staphylococcus, Pediococcus, Kocuria*, etc.

A necessary condition for creating stable symbiotic compositions is the combination of lactic acid bacteria, which produce organic acids and bacteriocins, and coagulase-negative cocci, which form specific taste-aromatic compounds and shape the color of the finished product **[27]**.

The studied compositions were characterized by high productivity of each of the components of the leavening composition, in particular, it was established that the number of ICD increased by 4.3-6.5 times, and micrococci and staphylococci by 7.7-28.6 times, respectively. For these compositions, mutual stimulation of the components was observed, which contributed to the microorganism's active development and the manifestation of their biochemical activity.

Expressed nitrite-reducing activity is characteristic of certain strains of staphylococci. Nitrites and nitrates have long been used in producing fermented meat products: on the one hand, they have a positive effect on color, taste and aroma, stability during storage, on the other – in an acidic environment, they can be precursors to the formation of nitrosamines. The lack of substances that are functionally able to replace the use of these compounds prompts the search for cultures with high nitrite reductase activity **[28]**, **[29]**, **[30]**.

The dynamics of the decomposition of sodium nitrite at its initial content of 60 mg.100 cm^{-3} was studied. The concentration of sodium nitrite used in this study was 6 times higher than recommended by the recipe for fermented sausages.

Under such conditions, high nitrite-reducing activity was characteristic of leavening compositions No. 1, 4, and 6, on the 17^{th} day of cultivation, the compositions actively reduced the content of nitrites in the culture medium – by 41-83% from the initial. These compositions had higher nitrite-reducing activity than is known from the literature data **[31]**, **[32]**, **[33]**. The microorganism's participation in the process of forming an aromatic bouquet is associated with the formation of certain amino acids, volatile fatty acids, and aromatic compounds during their vital activity **[34]**, **[35]**, **[36]**.

Evaluation of proteolytic activity made it possible to select the most active compositions, which were tested by the level of increase in free amino acids after 7 days of cultivation in MPB enriched with glucose and salt **[37]**, **[38]**. It was determined that compositions No. 1, 2, 3, and 4 were characterized by a high level of proteolysis, in the medium, there was an increase in the content of acyclic amino acids and an intensive decrease in cyclic amino acids, respectively, by 4.2-29.7% and 3.5 to 26.1%. Undoubtedly, antagonistic activity against opportunistic and pathogenic microorganisms is a desirable trait for the selection of cultures for the fermentation of meat raw materials **[39]**, **[40]**. *L. plantarum* synthesizes some compounds: 3-hydroxy-fatty acids, antifungal cyclic peptides, phenyl-lactic acid, and a mixture of substances with a low molecular weight similar to lactic acid. Most of these substances are active against moulds, and yeasts, and some also against bacteria, including genera *Listeria* and *Salmonella* **[41]**, **[42]**. According to the results of determining the antagonistic activity against opportunistic and pathogenic microorganisms, it was established that the investigated compositions exhibit antagonistic activity against both gram-negative and gram-positive microorganisms. Thus, all compositions suppressed the growth of test cultures *P. vulgaris*, *E. coli*, *S. aureus*, *P. aeruginosa*, and compositions No. were also characterized by the ability to suppress listeria: the zone of inhibition of *L. monocytogenes* growth was (12 ±1) mm. Our data are consistent with the publications of other researchers **[43]**, **[44]**.

CONCLUSION

Fermentation compositions No. 1, 3, 4, and 6, which reduced nitrite in the culture medium by 70-83%, are promising for the fermentation of meat raw materials. So, as a result of the research, 4 compositions were selected, two of which are lactic acid bacteria with micrococci (No. 2, 3) and two lactic acid bacteria with staphylococci (No. 1, 6). They were characterized by the high productivity of each of the components of the leavening composition, in particular, it was established that the number of MKB increased – by 4.3-6.5 times, and micrococci and staphylococci – by 7.7-28.6 times, respectively. For these compositions, mutual stimulation of the components was observed, contributing to the active microorganisms' development and their biochemical activity. Fermentation compositions No. 1, 6, 4, and 6 had the highest nitrite-reducing activity, and a high level of proteolysis characterized compositions No. 1, 2, 3, and 4. Thus, according to technological parameters the productivity, nitrite-reducing, proteolytic and antagonistic activities, of two leavening compositions No. 3 and No. 7 were selected as promising for fermentation of meat raw materials, which include strains of microorganisms of the species *Kocuria rosea, K. varians, Lactobacillus rhamnosus*, *L. casei, L. plantarum* and *L. tolerans.*

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