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Influence of starter cultures on microbiological and physical-chemical parameters of dry-cured products

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ABSTRACT

Using the antagonistic competitive interaction of the microbiological cultures has become one of the potential and modern ways to improve the quality of dry-cured meat products. These studies aim to substantiate the use of the starter cultures for producing fermented pork meat products. The studies' physicochemical, microbiological, organoleptic, and statistical methods were used for their implementation. Two starter cultures were used on the basis of *Lactobacillus plantarum*, *L. rhamnosus* and *Kocuria rosea* (SC 1); *Staphylococcus carnosus*, *L. plantarum*, *L. Rhamnosus* and *L. paracasei* (SC 2). The dynamics of microbiota development, the dynamics of acidity, the content of sodium nitrite, parameters of water activity, the dynamics of moisture changes, the content of nitrogen-containing substances, the accumulation of free cyclic and acyclic amino acids, as well as the organoleptic characteristics were studied. It had been established that the undesirable microflora is suppressed due to the active development of the starter cultures in fermented meat products. The study results confirm that using the starter cultures while producing the dry-cured meat contributes to their dehydration and reduction of the parameters of water activity. Based on the tasting results, the samples with the addition of the compositions of the starter cultures received a total score higher than the control sample, in particular, they had a beautiful appearance, a cut of red color, an elastic consistency, a delectable flavor with a characteristic sour after-taste. The use of the starter culture based on the combination of *Staphylococcus carnosus*, *L. plantarum*, *L. rhamnosus*, and *L. paracasei* (SC 2) for the dry-cured meat products to be produced activates the microbiological processes, which improves the structural-mechanical properties of the meat product, increases the water-binding power and, as a whole, positively influences on the quality of the finished product. It has been shown that the use of the drugs SC 2 and SC 1, when the dry-cured pork balyks are produced, decreases the duration of the technological cycle by 3-4 days, as well as ensures a high degree of sanitary and epidemic safety of the finished product: the absence of pathogenic and opportunistic microorganisms, the low residual content of sodium nitrite of up to 0.003%.

Keywords: fermented meat products, starter cultures, lactic bacteria, staphylococci, product quality

INTRODUCTION

Fermentation is a technology, which is used to increase the safety and nutritional value of food products, reduce production time, extend their shelf life, as well as create unique food tastes. Fermented meat is a typical and popular traditional meat product [1]. One of the modern ways for solving the technology development problem of meat products is related to the biotechnological modification principle of the meat raw materials and is aimed at regulating the biotechnological, physical-chemical, and microbiological processes, as a result of which the

structure, color and taste-aromatic characteristics of the finished product are formed [2]. The primary function of the starter cultures is the acidulation process by converting sugars, which were added to the meat mixture, into an acid. The acid, which is produced during this fermentation (primarily lactic acid), contributes to the spiciness (sour taste) of the product and contributes to the release of water as the pH value is decreased to the isoelectric point of the meat proteins for desired final water activity (a_w) to be obtained, protects against food-borne pathogens or enterotoxin production, contributes to the final texture by modifying the meat proteins, as well as has a part in fixing the red color of the meat. The acid-producing starter cultures, which are commonly used, belong to the genera *Pediococcus* and *Lactobacillus* [3]. Only those species that are relatively stable to salt while fermenting the meat are used [4]. The role of lactic acid bacteria in the formation of nitrosamine and their indirect or direct influence on the reduction of nitrosamines due to inhibiting development of bacteria, which are produced by precursors such as biogenic amines, has been discussed in the paper of Sallan et al. [5].

The behaviour of combined starter cultures based on *Penicillium chrysogenum*, *Penicillium digitatum*, *Penicillium Sangiovese*, *Debaryomyces hansenii*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Pediococcus pentosaceus*, *Micrococcus varians* and *L. plantarum*, *L. acidophilus*, *P. pentosaceus*, *M. varians* and their influence on the microbiological and physicochemical characteristics of the cured ham had been evaluated by Toledano, A.M. et al. It has been proven that using the selected starter cultures did not influence the cured ham's main characteristics and increased the non-protein nitrogen content [6].

Staphylococcus xylosus, *Staphylococcus equorum*, *Staphylococcus saprophyticus*, and *Staphylococcus carnosus* are the most commonly used among catalase-negative staphylococci [7]. Usually, *Micrococcaceae* and staphylococci fulfill the denitrifying and aroma-producing function, as well as have a slight influence on the pH value. For these reasons, it is advisable to use them together with acid-producing microorganisms – lactic acid bacteria [8].

Having studied the functioning of compositions made of *L. sakei*, *S. equorum*, and *S. success* in the production process, Montel, M.-C. et al. concluded that the use of autochthonous "starter" cultures contributes not only to an increase in hygienic safety but also guarantees to obtain products with traditional aroma and taste [9]. This was recorded during the production of "Napoli" salami with the use of cultures *S. xylosus* and *L. curvatus* [10], Turkish dry-cured sausage "Sujuk" – with strains of *L. plantarum* GM 77 and *S. xylosus* GM 92 [11], as well as during spontaneous fermentation of Chinese Sichuan sausages [12].

While ripening, the protein components of the meat raw materials undergo great changes: the activity of the tissue enzymes of the meat and microorganisms is activated, destroying the cellular structure of the muscle tissue and proteolysis, alongside the content of free amino acids can double [13]. Irish researchers observed a higher proteolysis degree in salami made with the composition of cultures *L. sakei* LAD and *S. carnosus* MC compared to the control sample. Thus, the total content of free amino acids in the finished product was 1292 mg per 100 g of dry matter, which is 1.7 times more than in the control sample; accordingly, more intensive hydrolysis of the myofibrillar proteins, especially myosin and actin, was occurred [14]. Japanese scientists, having investigated the functioning of commercial bacterial compositions in fermented sausages, had noticed a higher proteolysis degree in the variants made with cultures *L. sakei*, *S. carnosus*, *P. pentosaceus*, and *S. xylosus*. All samples were similar in the content of free amino acids at the beginning of the fermentation. Still, the finished products differed significantly due to the starter strains' specific properties [15].

Staphylococci are critical for product lipid oxidation to be prevented due to the production of superoxide dismutase [16], a lesser activity was found in *L. sakei* [17].

Peptidase activity is the most developed in micrococci, especially in strains *M. ristinae* and *M. varians*, however, strains *S. xylosus* and *S. carnosus* are the pronounced producers of aroma precursors, in particular, 3-methyl butanol, and from representatives of lactic-acid bacteria – *L. casei* and *L. plantarum* [18].

The species and qualitative composition of commercial bacterial drugs are quite diversified and depend on the technological direction [19]. Well-matched cultures have a multifunctional action and can provide the accumulation of the required amount of lactic acid in a short period, intensify the formation process of carbonyl compounds and volatile fatty acids, reduce the proportion of residual sodium nitrite, and, most importantly, prevent the development of foreign microflora, in particular, pathogenic and sanitary-indicative ones [20].

Sausage flavor is made due to salt, spices, and acid, formed by fermentation, various secondary fermentation metabolites of carbohydrates, and by-products of proteolytic and lipolytic activity of the starter culture and additional flora. Lactic-acid bacteria, used as starter cultures, have relatively weak proteolytic and lipolytic/osteolytic activity, while micrococci and staphylococci have more pronounced proteolytic and lipolytic enzymes. Peptides and amino acids, obtained due to the metabolism of lactic acid bacteria, can be further converted into aromatic carbonyls, alcohols, and esters by micrococci and staphylococci. This pool of aroma-contributing components is complemented by the proteolytic and lipolytic activity of micrococci and/or staphylococci [21].

Nitrite-reducing microorganisms *Micrococcus*, which are used in the composition of the bacterial drug "Lakmik," provide a bright coloring of the product and guarantee a low residual content of sodium nitrite up to 0.003%. It has been proven that the sausage, which is produced with the use of this bacterial drug based on *L. casei*, *L. rhamnosus*, *L. plantarum*, and *M. variant*, have a high content of free amino acids and flavour-aromatic compounds [22].

It has been proven by Burtseva G.V. et al. that the microbiota of the bacterial drug "MKS" based on *L. rhamnosus*, *L. plantarum*, and *S. simulans*, which are adapted to be developed in various types of meat raw materials, contribute to the reduction of the ripening period of the dry-cured whole-muscle products by 3-4 days, provide the formation of the traditional organoleptic characteristics, as well as guarantees a low content of residual nitrites. The strains of the above-mentioned compositions perform several metabolites, which can suppress the development of the undesirable microbiota (lactic acid, ammonia, volatile acids, ethers, alcohol, acetone, diacetyl) and specific antibacterial substances – bacteriocins [23].

It has been proven [24], [25] that the typical taste of the cured product is due to the enzymatic activity of the microbiota of the starter cultures. Product safety is also preserved due to the suppression of spoilage microorganisms, and pathogenic and opportunistic pathogen microorganisms, improving sensory qualities and stability during storage.

All these prerequisites arouse interest in testing the starter cultures based on LAB and CNC. Therefore, this scientific work aims to check the behavior and dynamics of the selected combined starter cultures and their influence on the characteristic parameters of fermented meat during its production.

Scientific Hypothesis

Microsoft Excel editor processed experimental data using mathematical statistics methods. The accuracy of the obtained experimental data was determined using the Student's t-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).

MATERIAL AND METHODOLOGY

Samples

The study was conducted with three samples:

- control sample obtained by classical technology;
- experimental sample with starter culture SC 1;
- experimental sample with starter culture SC 2.

Chemicals

Sodium chloride, NaCl (TOV Khimlaborreaktiv, Ukraine).

Distilled water, H₂O (TOV Novokhim, Ukraine).

Selective medium (Oxoid, Basingstoke, UK).

Hydrochloric acid, hcl (TOV Khimlaborreaktiv, Ukraine).

Agar de Man-Rogosa-Sharpa, (Conda, Ukraine).

Mannitol-salt agar (himedia Laboratories Pvt. Ltd.).

Egg yolk emulsion (himedia Laboratories Pvt. Ltd.).

Agar Saburo ("Pharmaktiv" LLC, Ukraine).

Chloramphenicol ("Farmaktiv" LLC, Ukraine).

The Slanets-Bartli environment (Conda, Ukraine).

Bile-glucose agar (himedia Laboratories Pvt. Ltd.).

Pseudomonas Agar Base with the addition of C-F-C (himedia Laboratories Pvt. Ltd.).

Tryptone soy agar (Merck, Germany).

Sulfadiazine agar (Merck, Germany).

N-(1-naphthyl)-ethylenediamine-dihydrochloride (TOV Khimlaborreaktiv, Ukraine).

Concentrated hydrochloric acid H₂SO₄ (Shostka Chemical Reagents Plant, Ukraine).

Trichloroacetic acid ccl₃cooh (TOV Khimlaborreaktiv, Ukraine).

Chemically pure reagents were used.

Animals, Plants and Biological Materials

Bacterial preparation 1: (Institute of Food Resources NAAS of Ukraine, Ukraine) containing *Lactobacillus rhamnosus*, *L. plantarum*, and *Kocuria rosea*. In 1 g, the number of viable lactic acid bacteria and micrococci was 3.5×10^{10} CFU/g and 2.9×10^8 CFU/g, respectively Bacterial preparation.

Bacterial preparation 2: (Institute of Food Resources NAAS of Ukraine, Ukraine) containing *Staphylococcus carnosus*, *L. plantarum*, *L. rhamnosus*, *L. paracasei* in 1 g, the number of viable lactic acid bacteria and

staphylococci was 5.1×10^{10} CFU/g and 3.3×10^8 CFU/g back muscle *Longissimus dorsi* of the Great White pig (SE "DG Stepne", Institute of Pig Breeding and Agro-Industrial Production of the National Academy of Sciences, Poltava Region)

Bacterial preparations are manufactured under industrial conditions of the Institute of Food Resources of the National Academy of Sciences of Ukraine in accordance with TU U 15.5-00419880-101-2010. Bacterial preparations for the production of fermented meat products. Specifications.

Instruments

Lab Blender Stomacher (Seward Medical, London, United Kingdom)

Ph meter MP 512 ("Ulab").

Petri dishes (LLC Ukragrotest).

Thermostat TSO-80 (LLC Ukragrotest).

Gas Chromatograph (GE Lifesciences BPG 100/500, Germany).

Drying Scarf SNOL 60/300 LSN 11(Lithuania).

Unico S 2100 spectrophotometer (LLC Ukragrotest).

Analytical balances (Thermoengineering LLC, Ukraine).

Kjeldahl apparatus UDK 149 Velp Scientifica™.

AquaLab 3TE Series (CIHA).

LC-2000 Biotronic (Germany).

"SANS" of the SMT 2000 series, model 2503 (Shenzhen SANS Testing Co. Ltd.) with Warner-Blatzler nozzle.

Laboratory Methods

The following microbiological parameters were determined:

- ▶ the amount of *Staphylococcus carnosus* CFU in 1 g of the product – on salt broth with mannitol at 30 ± 1 °C for 72 ± 2 hours [26];
- ▶ the number of CFU lactic acid bacteria in 1 g of product on de Man, Rogoza, and Sharp (MRS) agar (Oxoid CM359) at 30 °C for 72 ± 2 hours;
- ▶ the total number of microorganisms, CFU in 1 g of product – on tryptone-soy agar during incubation at a temperature of 30 °C for 72 hours;
- ▶ the number of lactic acid bacteria – on MRS-agar during incubation at a temperature of 30 °C for 5 days [27];
- ▶ the amount of mold and yeast, CFU in 1 g of product – on Sabouraud's medium with 200 mg/l chloramphenicol during incubation at 24 °C for 5 days [28];
- ▶ the total number of *Enterobacteriaceae* was checked on violet-red bile-glucose agar and incubated at 35-37 °C for 24 hours. Results were expressed as log CFU/g;
- ▶ to detect *Salmonella* and *L. monocytogenes*, 25 g of each sample was aseptically taken. DSTU ISO 6579-1:2017 method [29] was used to determine *Salmonella*. In the case of *L. monocytogenes*, the DSTU EN ISO 11290-2:2017 method was used [30];
- ▶ *Clostridium spp.* was counted on sulfite polymyxin-sulfadiazine agar at 45 °C for 48 hours under anaerobic conditions. Results were expressed as log CFU/g;
- ▶ fifty grams of each sample was taken and crushed to obtain a homogeneous sample and placed in clean and dry containers at 4 °C until analysis. Non-protein nitrogen (NPN) was analyzed using the procedure described by Bandeira et al. [31];
- ▶ the mass fraction of moisture was determined gravimetrically by drying a weight of the product in a drying cabinet at a temperature of 105 °C to a constant mass [32];
- ▶ the content of sodium nitrite was determined by the reaction with N-1-naphthyl ethylenediamine dihydrochloride in an acidic environment with the formation of diazo compounds, the color intensity of which was measured photometrically. the mass fraction of protein – by the content of total nitrogen by the Kjeldahl method with the subsequent distillation of ammonia [33];
- ▶ the water activity (aw) of the meat samples was measured using an Aqua Lab Series 3 TE METER Group Inc. USA according to ISO 21807:2004 [34];
- ▶ the pH value was measured with an MP 512 pH meter ("Ulab", China) after mixing 10 g of the sample with 90 ml of distilled water ISO 2917:1999 [35];
- ▶ the amino acid composition of proteins was studied after hydrolysis of product samples with a mixture of 6 n. hydrochloric and 4% thioglycolic acids at temperatures of 105-110 °C for 48 hours in a CO₂ environment and subsequent evaporation under vacuum at a temperature of 45 °C. Identification of amino acids was performed after grinding the samples, removing fat, and precipitation of protein compounds with 10% trichloroacetic acid [36]. For the identification of free amino acids, computer chromatographic processing using the Kodak Digital Science ID software package was used;

- structural and mechanical studies were carried out on the universal mechanical test machine "SANS" of the CMT series using special nozzles: Warner-Bretzler for determining the shear force. Calculation of indicators was carried out using the Power Test_DOOE software;
- the organoleptic evaluation of fermented meat products was carried out by a trained group of nine experts, according to [37]. Previously, the samples were kept at 25 ± 1 °C in the laboratory. Samples were evaluated by participants in random order.

Description of the Experiment

Sample preparation: The pig skinned-off back muscle *Longissimus dorsi* with a layer of fat no more than 0.5 cm thick, no more than 45 cm long, and about 10 cm wide was used for the balyk to be produced.

3 samples were produced: a control sample – without bacterial drug and experimental samples with starter cultures – SC 1, SC 2. 3 samples were produced: control – without tank preparation and experimental samples with starter cultures - SK 1, SK 2. Starter cultures were added to the brine of the experimental samples in the amount of 0.05% (to the mass of raw materials). Water in the amount of 0.05% (to the mass of raw materials) was added to the control sample instead of starter cultures. From each batch replication, the samples were taken for further analysis right after the salting, after the salting process, and after the ripening and drying in 6 or 9 days.

The starter cultures were added to the brine of experimental samples at 0.05% (to the mass of raw meat materials). The technological parameters of the balyk production are as follows:

- injection of the brine into the meat in the amount of 30% of its weight;
- massaging of the meat for 4 hours;
- brine aging of the meat at a temperature of 5-10 °C for 72 hours;
- incomplete drying of the meat at a temperature of 18-20 °C for 1 hour, $\varphi = 55\%$;
- smoking of the meat at a temperature of 40 °C for 1 hour;
- incomplete drying of the meat at a temperature of 18-20 °C for 1 hour, $\varphi = 55\%$;
- drying of the products in a climate chamber according to the following modes:

the first day: $t = 20-22$ °C, $\varphi = 88\%$;

the second day: $t = 18-20$ °C, $\varphi = 84\%$;

the third day: $t = 16-18$ °C, $\varphi = 80\%$;

the fourth day: $t = 14-16$ °C, $\varphi = 78\%$;

the fifth day: $t = 12-14$ °C, $\varphi = 76\%$;

from the sixth to the ninth day: $t = 11 \pm 1$ °C, $\varphi = 76-74\%$.

Number of samples analyzed: 54.

Number of repeated analyses: 3.

Number of experiment replication: 3.

Description of the Experiment

Two starter cultures were used for the studies, namely: (starter culture 1) *Lactobacillus plantarum*, *L. rhamnosus*, and *Kocuria rosea* (SC 1) and (starter culture 2) *Staphylococcus carnosus*, *L. plantarum*, *L. rhamnosus*, *L. paracasei* (SC 2). All strains were recovered from domestic dry-cured and raw-smoked meat products produced according to traditional technology. In the products, these strains belonged to the dominant bacteria. The number of viable lactic acid bacteria and micrococci was 3.5×10^{10} CFU/g and 2.9×10^8 CFU/g, respectively, per 1 g of the starter culture 1. The number of viable lactic acid bacteria and staphylococci was 5.1×10^{10} CFU/g and 3.3×10^8 CFU/g, respectively, per 1 g of the starter culture 2.

According to traditional technology, three different batches of the balyk were produced in triplicate. These batches were developed according to the added starter culture: C batch (control sample without any starter culture), SC 1 batch (inoculated with *Lactobacillus rhamnosus*, *L. plantarum*, and *Kocuria rosea*), and SC 2 batch (*Staphylococcus carnosus*, *L. plantarum*, *L. rhamnosus*, *L. paracasei*).

After the aseptic skinning, an approximately 10-g piece of the sample was diluted with 90 mL of 0.9% (w/v) sterile NaCl solution in the stomach bag and homogenized in a Lab Blender Stomacher (Seward Medical, London, UK) for 2 min. Decimal dilutions were subsequently prepared and plated on a selective medium (Oxoid, Basingstoke, UK) for specific microbial counting: LAB was counted on de Man–Rogosa–Sharpe agar, and CNC was counted on mannitol-salt agar with added egg yolk emulsion after an incubation at 30 °C for 48 hours; yeast on Sabouraud dextrose agar with 200 mg/l chloramphenicol, after incubation at 30 °C for 72 hours; enterococci were incubated on Slanetz and Bartley medium at 42 °C for 24 hours; *Enterobacteriaceae* on violet-red bile-glucose agar were incubated at 37 °C for 24 hours; *Pseudomonas* were controlled based on Pseudomonas Agar Base with added C-F-C additives and incubated at 30 °C for 48 hours. The analyses were carried out in triplicate at the initial stage (0 days), as well as after 4 and 30 days.

All strains were isolated from domestic raw-cured and raw-smoked meat products produced according to traditional technology. In these products, these strains were among the dominant bacteria. 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.

Physico-chemical studies: Fifty grams of each sample were taken, ground until smooth, and placed in clean, dry containers at 4 °C until the analysis was carried out. Non-protein nitrogen (NPN) was analyzed using the procedure described. The mass fraction of moisture was gravimetrically determined by drying a batch weight of the product in a drying cabinet at 105 °C to a constant weight. The sodium nitrite content was determined by the reaction with N-1-naphthyl ethylenediamine dihydrochloride in an acidic environment with the formation of diazo compounds, the color intensity of which was photometrically measured. The total nitrogen content determined the mass fraction of protein according to the Kjeldahl method followed by ammonia stripping. The water activity (a_w) of the meat samples was measured using a device "aqua lab" of series 3 of the meter group Inc. USA according. The pH value was measured with a pH-meter MR 512 ("Ulab", China) after mixing 10 g of the sample with 90 ml of distilled water. The amino-acid composition of proteins was studied after hydrolysis of the product samples with a mixture of 6 n. hydrochloric and 4% thioglycolic acids at 105-110 °C for 48 hours in a CO₂ environment and subsequent vacuum evaporation at 45 °C. The amino acids were identified with the use of an automatic amino acid analyzer LC-2000 Biotronik (Germany) after grinding the samples, removing the fat, as well as precipitating the protein compounds with 10% trichloroacetic or sulfosalicylic acids. The computer processing with a chromatograph using the Kodak Digital Science ID software package was used for free amino acids to be identified.

Structural-mechanical studies: The structural-mechanical studies were carried out using a universal mechanical test machine "SANS" of series CMT using special Warner-Bratzler nozzles for shear force to be determined. The parameters were calculated with the use of Power Test_DOOE software. The fermented meat products were organoleptically evaluated by a trained nine experts according to DSTU 4823.2:2007. Sample temperature – 18 ±2 °C, laboratory temperature – 21 ±2 °C, strain gauge – cone height – 10 mm, cone diameter – 10 mm, penetration speed – 20 mm/min, penetration depth – 2 cm. Participants evaluated the samples at random.

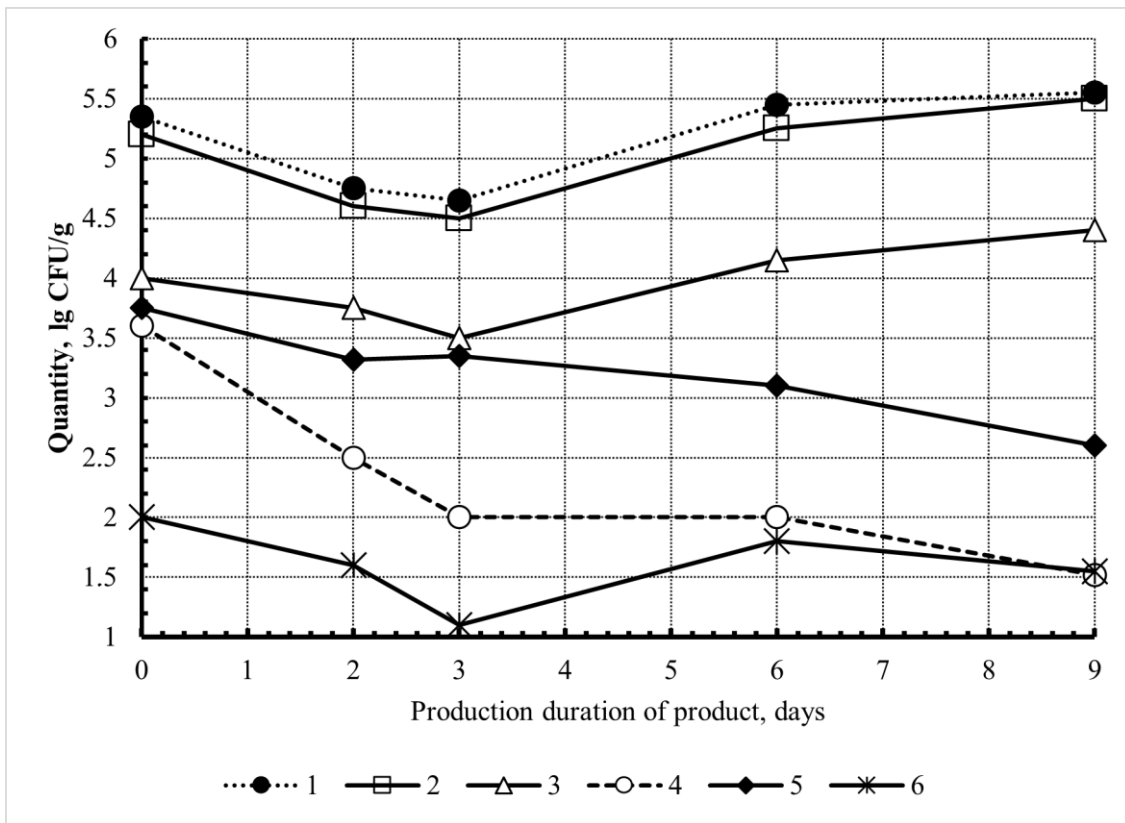
Statistical Analysis

All studies were carried out in triplicate. The data were statistically processed in Excel MS Office 2010 according to the standard methods. The figures show average statistically reliable data for 95% probability.

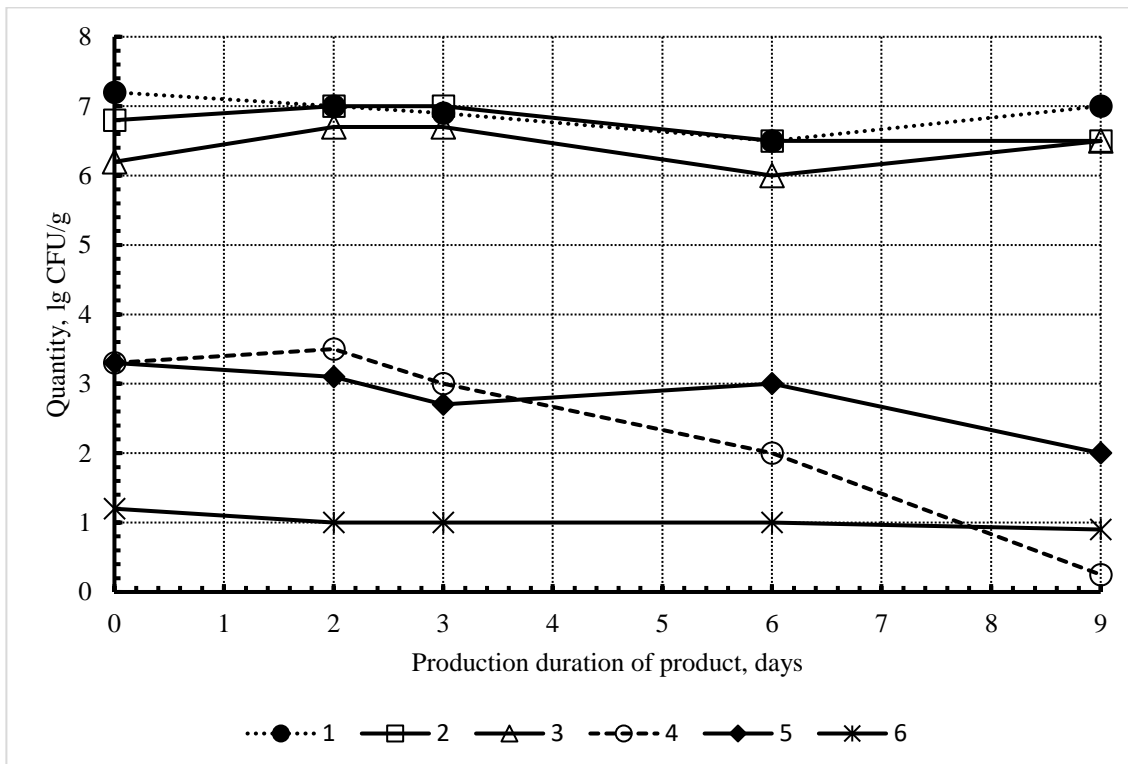
RESULTS AND DISCUSSION

For the guaranteed dominance of the starter cultures to be provided, the inoculant level should be 6.0-7.0 log CFU/g, which is usually two orders of magnitude higher than the level of autochthonous microflora [38].

The number dynamics of lactic acid bacteria, the number of micrococci, the number of yeasts and molds, and *Enterobacteriaceae* were studied at all stages of the technological process - after the salting, salting process, ripening and drying in 6, 9 days (Figure 1).



a)



b)

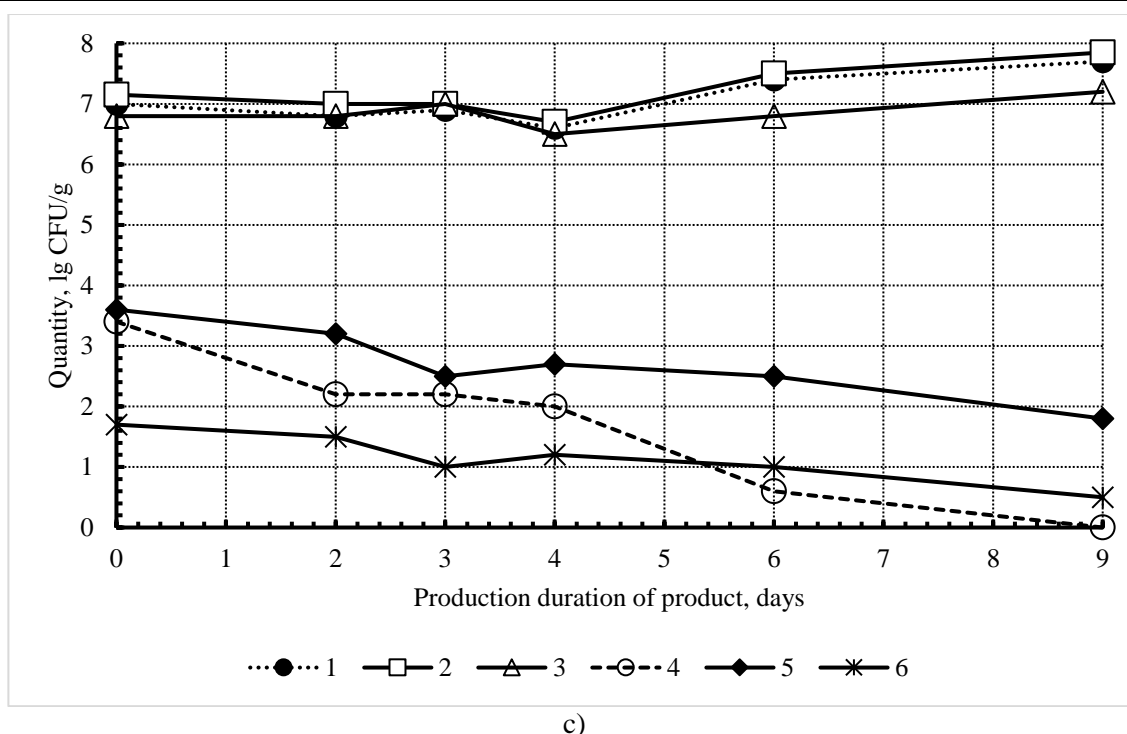


Figure 1 Development dynamics of the microbiota of dry-cured balyk during its maturation.

Note: a) the control sample is the product, which is produced without the use of the composition; b) the product with the use of the SC 1 composition; c) the product with the use of the SC 2 composition; 1 is the total number of microorganisms; 2 is the lactic-acid bacteria (LAB); 3 is the micrococci (MC); 4 is the *Enterobacteriaceae*, 5 is the yeast (YE), 6 is the mold (MO).

At the beginning of maturation, the number of spontaneous lactic acid bacteria in the control sample was 1.5×10^5 CFU/g, which is higher than reported in the papers [39], and at the end of the fermentation, it was increased by 1.74 times to the initial content of cells. The content of micrococci was increased more intensively by 3.5 times compared to the initial amount (1.0×10^4 CFU/g). The yeast was present at the beginning (7.1×10^3 CFU/g) and at the end of the process. Their content during the study was decreased by 18.6 times, and the amount of mold was decreased by 1.6 times compared to the initial amount (1.0×10^2 CFU/g) (Figure 1 a).

It should be noted here that during the entire technological process, the coagulase-positive *Staphylococcus* ssp. was absent in all variants of the minced meat, indicating that the meat raw materials are of high quality, as well as *Salmonella*, *L. monocytogenes*, and *Clostridium perfringens* were not found either during the processing of the studied samples or in any of the batches [40].

The number of *Enterobacteriaceae* equal to 4.5×10^3 CFU/g was detected at the beginning of the process. These indicators were gradually decreased; at the end of the experiment, they were decreased by 112.2 times. Similar results were obtained by authors of scientific works [41].

After the 3rd day of the salting, the fermentation microbiota in the studied variants was decreased only by 1.2-1.5 times, and the spontaneous microbiota was decreased by 3.2 times, respectively; this shows the adaptability of the studied compositions to sodium chloride [42].

When the development of the composition microbiota on the 9th day of the fermentation is compared with the 4th day, the greatest increase in viable cells of lactic acid bacteria was observed in the SC 2 composition – by 12.6 times. This indicator in the SC 1 product was increased by 2.5 times. The intensive development of lactic acid bacteria in SC 2 can be explained by the adaptability of the composition to this raw meat material (Figure 1 b, c).

In the studied variants SC 1, and SC 2, the intensive development of micrococci and staphylococci especially occurred during the last 5 days. At the same time, the increase in the number of these microorganisms was recorded by 2.58 and 5.62 times, respectively, compared to their concentration on the 9th day of the drying. These results are consistent with those obtained by authors of scientific works [43].

According to the results of the conducted studies, the SC 2 composition was the best among the studied starter cultures in terms of functioning in pork meat [44].

It should be noted here that the SC 2 and SC 1 compositions had a more intensive influence on the death of *Enterobacteriaceae*, which disappeared on the 9th day of the fermentation. In the control sample, their presence was still observed on this day in the amount of 0.4×10^2 CFU/g. The most intense death occurred in the SC 2

composition (Figure 1). Such results are consistent with those obtained by authors of scientific works [45] and confirm the additional effectiveness of the hygiene starter cultures.

At the end of the fermentation, all studied composition variants inhibited the development of yeasts by 30.2-63.1 times more intensively compared to the control sample (by 18.6 times). Still, this process occurred most actively in the variant SC 2. Regarding molds, they died intensively in all studied variants by 3.2-19.9 times compared to the control sample. In the control sample, their number was decreased by only 1.6 times according to the initial concentration of cells. It shows the advisability of the starter-culture compositions for the purity of the fermented meat products to be provided [46].

Therefore, the undesirable foreign microflora in the balyks with the bacterial drug is suppressed earlier, primarily due to the active development of the starter cultures, especially in the first 3-5 days. The active growth of the starter-culture microflora correlates with a more intense increase in the acidity in the balyks with the starter cultures compared to the control samples [47].

The formation of acids, particularly lactic acid, when the meat raw materials are fermented not only prevents the development of putrefactive and pathogenic microflora, thereby increasing the finished product's safety, but also positively influences the technological parameters.

During the first 5 drying days, the acidity increase occurred 2.4-2.5 times more intensively in the variants with the bacterial drugs than without them. During the entire ripening process, the acidity in the balyks with the bacterial drug was higher than in the control sample (Figure 2). At the end of the ripening, it was 5.0-5.2 pH units in the samples SC 1 and SC 2. Pathogenic and opportunistic bacteria of food products do not practically develop at pH values below 4.6 [48], [49].

For the formation process of nitric oxide pigments, the optimum acidity of the medium is 5.4-5.1 pH units; that is, the range close to the optimum bacterial denitrification is approx. 5.5 pH units.

It is known that nitrite is added both for coloring and for protecting the meat products against toxins being formed, which is produced by the anaerobic bacterium *Clostridium botulinum*, as well as against the development of salmonella and staphylococci when the meat products are ripened [50].

The World Health Organization (WHO) recommends adding sodium nitrite in an amount that does not influence the human body – up to 20 g per 100 kg of the raw meat materials to be salted, but technologically justified dosages are twice as low. In the European Union, sodium nitrite can be used only as an additive to table salt of 0.5 to 0.9% [51].

The concentration of free nitrite is decreased when reducing substances and microorganisms are applied and continues when the finished product is stored at a rate that depends on pH, duration, and storage temperature.

The authors of scientific works [52] believe that the optimal concentration of hydrogen ions for nitrite to be bonded is within the range of 5.0-6.2 pH, and the lower the pH, the faster the nitrosation processes, the less residual nitrite remains: 5% of nitrite from its initial concentration is stored in the product at 5.05 pH; 21% – at 5.75 pH; 60% – at 6.2 pH and above.

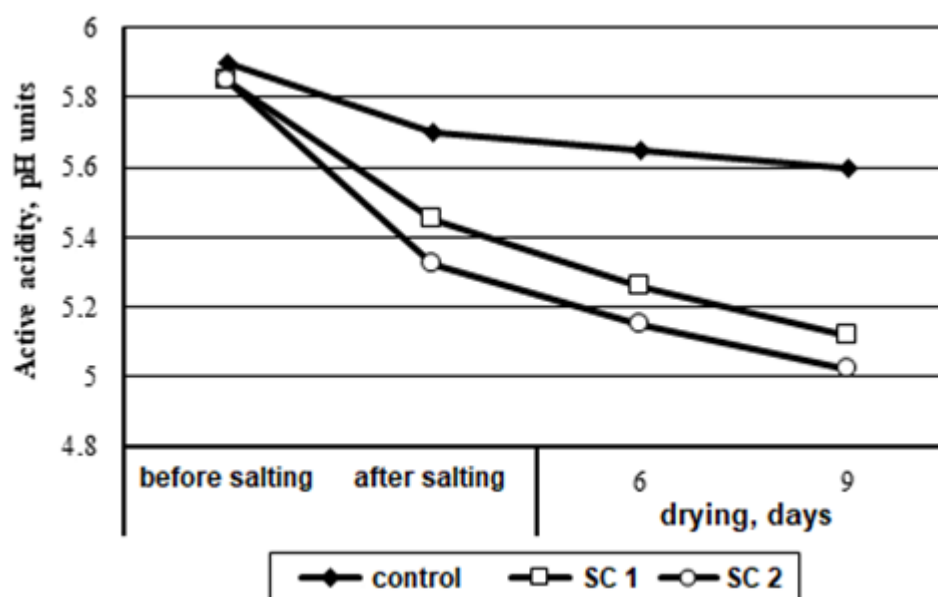


Figure 2 Acidity dynamics when dry-cured balyk is ripened.

Taking the potential danger of sodium nitrite and the regulation complexity of the formation reactions of nitric oxide pigments into consideration, the content of sodium nitrite was studied when dry-cured balyk was ripened (Figure 3).

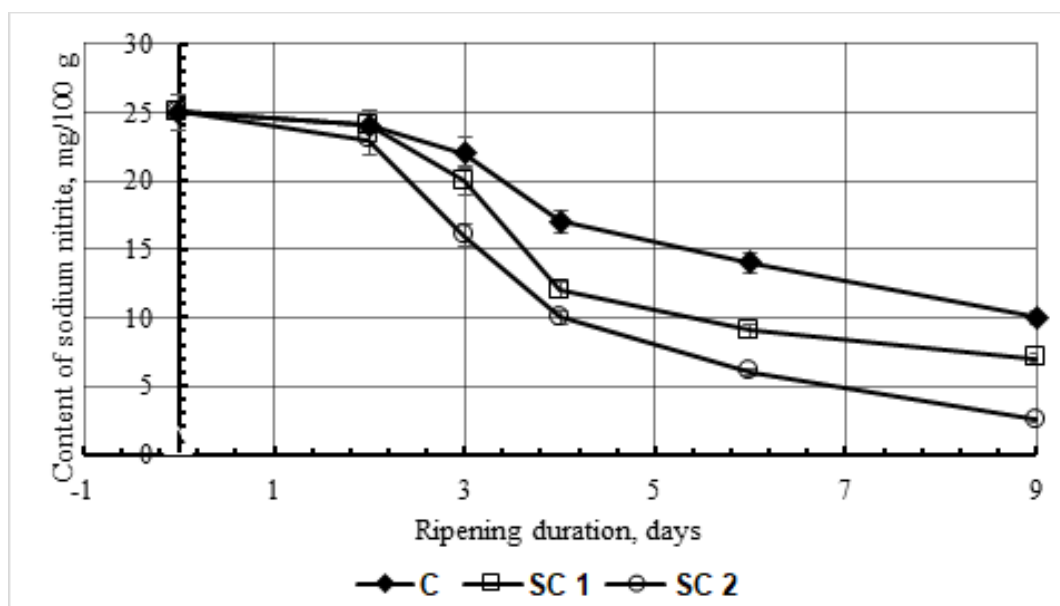


Figure 3 Content of sodium nitrite when dry-cured balyk is ripened.

During the first 3 salting days, the content of sodium nitrite slowly decreased - within the range of 20.5-32.5% in the products that were fermented with the starter-culture compositions. In the control sample, it was 11.3% compared to the initial content of salt, which was 25 mg/100 g of the raw meat materials, which corresponds to the production recipe of the pork balyk [53].

It had been established that in the studied variants, the content of nitrites actively decreased on the 9th ripening day by 70-88% from the initial level. The most active were the compositions SC 1, and SC 2 compared to the control sample (60%) (Figure 3).

The obtained results were consistent with the literature data, which also established the positive role of the bacterial drugs, which include nitrite-reducing microorganisms, for a stable color of the fermented sausages to be formed [54], [55].

The parameter of water activity is of particular importance for the fermented products to be dried, which makes it possible to establish the connection between the state of weakly bound moisture in the product and the possibility of microorganisms developed in it since, as is well-known, microorganisms can consume only active moisture part for their vital activity (Table 1).

Table 1 Parameter of water activity of fermented pork products during their production.

Sample	Meat before salting	Meat after salting	Drying, days	
			4	9
C	0.986	0.978	0.966	0.958
SC 1	0.986	0.978	0.969	0.957
SC 2	0.986	0.976	0.962	0.956

Having analyzed the results according to the quality parameter of water activity, it had been established that the observed dynamics did not show any differences in the starter cultures. The behavior of a_w occurred due to the weight loss, which had no significant differences [56].

The provided study results make it possible to conclude that the use of SC 2 activates the microbiological processes during the salting and drying of the dry-cured meat products and contributes to their dehydration and a sharp decrease of the parameter of water activity in such products.

It is known that the closer the pH value is to the isoelectric point of meat proteins (5.4 units), the lower its ability to bind moisture and, accordingly, the higher the drying speed.

In parallel with the decrease in pH, the content of total moisture decreased (Figure 4) in all samples by an average of 13.65% by the end of the technological process.

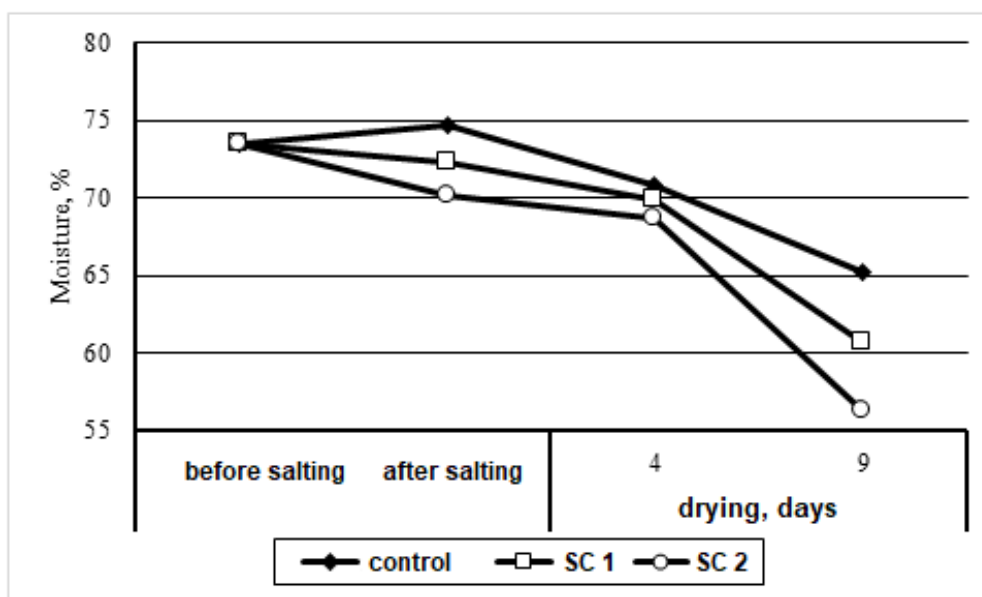


Figure 4 Dynamics of moisture changes of fermented whole-muscle pork products during production.

During the ripening, the protein components of the raw meat materials are substantially changed due to the activity of the tissue enzymes of the meat and microorganisms, which determines the course of biochemical transformations. Free amino acids and other metabolites, associated with the vital activity of microorganisms, have a significant role in the taste-aroma bouquet of the fermented products to be formed.

Since experiments proved that during the fermentation and ripening, the composite cultures dominated in the variants with the bacterial drugs, it can be assumed that it was the starter cultures that determined the specific direction of the biochemical transformation of the proteins of the raw meat materials and the formation of the taste and aroma range of the finished product [57].

Nitrogen-containing substances are transformed when the dry-cured meat products are ripened (Table 2).

Table 2 Content of nitrogen-containing substances in dry-cured meat products.

Nitrogen-containing substances by fractions	Content of nitrogen on a dry matter basis, %			
	Im	C	SC 1	SC 2
	0 days	9 days		
Total nitrogen	12.9*	11.6	11.8	12.0
Non-protein nitrogen	1.1	1.5	1.6	1.7
Protein nitrogen	11.3	10.1	10.3	10.4

Note: *Measurement error does not exceed 0.1 %.

At the beginning of the fermentation, the level of non-protein nitrogen for the raw meat material was 1.1 % of dry matter. On the 9th ripening day, non-protein nitrogen was increased for all samples: SC 1 – by 14.5%, SC 2 – by 15.4%, and control sample – by 13.6%. The protein nitrogen content decreased on the 9th day: SC 1 by 8.8%, SC 2 by 8%. The parameter was slightly higher in the control sample – 10.6%.

Proteolysis greatly influences the quality characteristics of the fermented product, as it is an important source of taste compounds, such as free amino acids [58].

When the dry-cured balyk is ripened, the level of free amino acids is increased, and their spectrum is different in the studied variants with various compositions (Figure 5).

On the 9th fermentation day of the raw meat materials, the concentration of free cyclic amino acids was increased in the studied variants by 1.41-1.57 times and in the control samples – by 1.32 times compared to their initial content (160.28 mg/100 g of dry matter). Regarding the concentration change of acyclic amino acids, in these products, their content was increased by 5.54-7.17 times, in the control sample – only by 4.41 times, compared to the initial raw meat materials. Their initial content was 348.98 mg/100 g of dry matter.

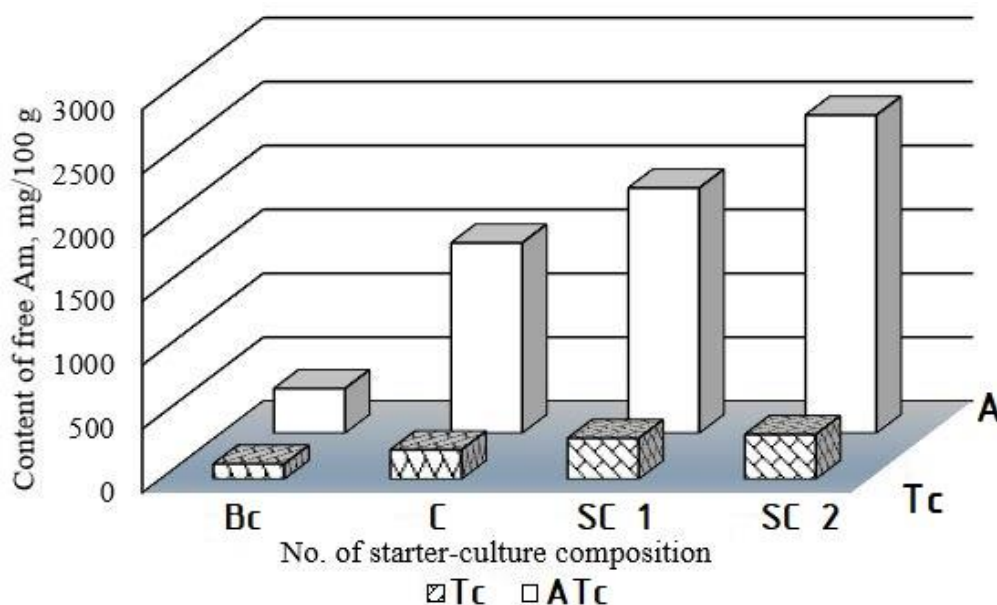


Figure 5 Accumulation of free cyclic and acyclic amino acids in finished dry-cured balyk: Im – initial raw meat materials; C – control sample, produced without the starter culture; SC 1 and SC 2 are the fermented products.

In the products inoculated with the compositions SC 1 and SC 2, the content of acyclic amino acids was increased by 112.4% and 276.0%, respectively, and the content of cyclic amino acids was increased by 8.8% and 24.7%, respectively, compared to their level in the finished control variant (Figure 5).

Having compared the influence of the various starter-culture compositions on pork proteolysis, it had been established that the proteolytic processes occurred more actively in the studied variant SC 2. Casaburi et al. proved that the protein degradation in the sausage inoculated with *Lactobacillus* spp. and *Staphylococcus xylosus* occurs faster during the ripening [59], [60].

The specific sensory quality of fermented meat products is mainly the smell and taste [61]. The addition of the bacterial drugs influenced the general aroma and taste of the products (Figure 6).

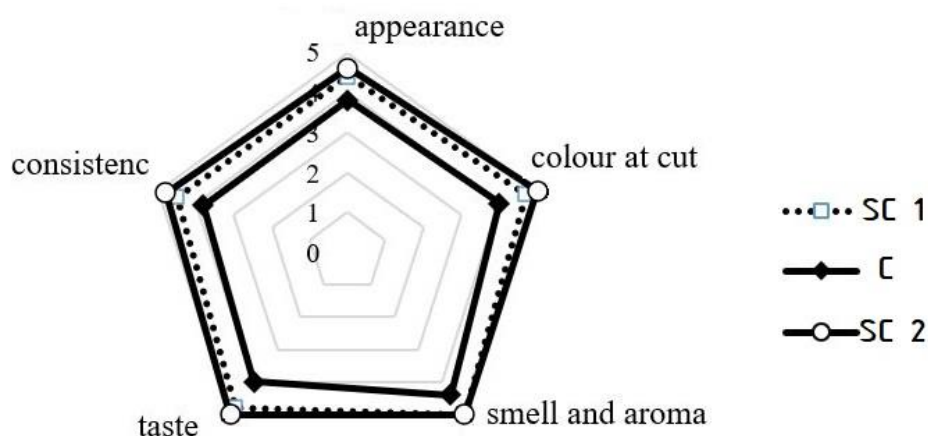


Figure 6 Profile diagram for quality parameters of control and studied (SC 1 and SC 2) product samples.

The sensory properties of fermented meat products (consistency, color, and aroma) depend on many compounds, which are formed during the chemical or biochemical transformations of the raw meat materials, [62].

The differences in the organoleptic characteristics of the dry-cured products with various starter-culture compositions were determined according to the quality evaluation results of the finished product. The finished dry-cured meat products of the variants SC 1 and SC 2 had a beautiful appearance, a cut of red color, an elastic consistency, and a delectable flavor with a characteristic sour after-taste, and for the SC 2. T

here was also a pronounced aroma of dry-curing. Variant C 1 had a good appearance but a slightly darker color and less pronounced taste and aroma. The control variant had a dark-red color, somewhat rubbery consistency, unpronounced taste and aroma, and after-taste of old fat. The aroma and taste of the meat products with the various

bacterial drugs did not differ significantly. Based on the tasting results, the product samples with the addition of the compositions SC 1 and SC 2 received a total score higher than the control sample.

The conducted scientific studies of the influence of starter cultures on the microbiological and physicochemical parameters of dried meat products are an interesting and important topic, therefore, further directions of research can develop in the following directions: research:

Study the influence of different starter cultures on dried products' microbiological composition.

We are optimizing the proportions of different types of bacteria to achieve the best results in production.

Study microbiota dynamics in the production and storage process of dried products.

Analysis of the impact of starter cultures on product shelf life and consumption safety.

Study the effect of starter cultures on textural characteristics and color of dried meat products.

Determination of physicochemical parameters such as moisture, pH, and salt concentration in products depending on the starter cultures used.

Considering the influence of the growing environment of starter cultures on the quality and characteristics of dried products.

Study the influence of seasonal changes and geographical aspects on the microflora structure and product quality.

Development of recommendations for manufacturers regarding optimal production conditions, including temperature, humidity, and process duration.

Study the influence of starter cultures on the organoleptic characteristics of dried products.

Evaluating consumer properties and product taste qualities depends on using different starter cultures.

Therefore, the proposed research directions can be aimed at a detailed study of the influence of starter cultures on various aspects of the quality and safety of various meat products.

CONCLUSION

The SC 1 starter cultures were tested based on *Lactobacillus plantarum*, *L. rhamnosus*, and *Kocuria rosea*, and SC 2 based on *Staphylococcus carnosus*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus* and *Lactobacillus paracasei*. The influence of the created drugs SC 1 and SC 2 on the main physico-chemical and biochemical parameters of the dry-cured meat products was studied during their production. It had been established that in the studied variants, the content of nitrites actively decreased on the 9th ripening day by 70-88% from the initial level. The most active were the compositions SC 1, and SC 2 compared to the control sample (60%). On the 9th fermentation day of the raw meat materials, the concentration of free cyclic amino acids was increased in the studied variants by 1.41-1.57 times and in the control samples – by 1.32 times compared to their initial content (160.28 mg/100 g of dry matter). The characteristic differences in the biochemical processes in the dry-cured meat products produced without/with the bacterial drug were determined. It had been established that the fermented products have better quality, caused by the action of the starter cultures. The product with the created drug SC 2 had the highest quality parameters, and the death of Enterobacteriaceae occurred on the 9th fermentation day. At the same time, this process in the control sample lasted until the 12th day. It has been shown that the use of the drugs SC 2 and SC 1, when the dry-cured pork balyks are produced, decreases the duration of the technological cycle by 3-4 days, as well as ensures a high degree of sanitary and epidemic safety of the finished product: the absence of pathogenic and opportunistic microorganisms, the low residual content of sodium nitrite of up to 0.003%. The use of the selected starter culture based on the combination of *Staphylococcus carnosus*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, and *Lactobacillus paracasei* demonstrates a potential interest for its use when the fermented pork product is produced.

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