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Improvement of the quality of pork meat during salting due to the use of starter bacterial cultures

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ABSTRACT

The influence of the starter cultures, such as *Lactobacillus rhamnosus, L. plantarum, Kocuria rosea, Staphylococcus carnosus, L. plantarum, L. rhamnosus* and *L. paracasei,* on the functional-technological and physicochemical characteristics of the pork meat during the salting *is* investigated in this paper. It has been proven that the use of these starter cultures in the technology of raw ba-lik products makes it possible to obtain finished products with improved quality indicators, which is promising in the food industry. It had been shown that in the pork meat samples with the starter cultures, the acidity from 5.74 pH units is more intensively decreased – to 5.52 pH units compared to the control sample (up to 5.64 pH units). Using the starter cultures based on nitrite-reducing microorganisms for the salting in 72 hours positively influenced the formation of the required colour characteristics. It had been established that the moisture-binding capacity and plasticity of the pork meat samples with the starter cultures are characterised by the increased indicators compared with the control sample – by 4.73% and 7.73% and by 2.19 cm^2.g, respectively. The difference in the volatile fatty acids content in the pork meat samples with the starter cultures compared with the control sample is 22 and 33%, respectively, in 72 hours of salting. The obtained results can be used in the enterprises of the meat processing industry to produce fermented meat products, particularly raw dried logs.

Keywords: salting, starter culture, meat fermentation, lactic-acid bacteria, coagulase-negative staphylococci

INTRODUCTION

Lactic acid bacteria and coagulase-negative staphylococci or micrococci are used in the composition of the starter cultures for the fermentation process in the technology of the meat products. The most common types of lactic-acid bacteria during the fermentation process, when the sausages are produced, are *Lactobacillus sakei*, *Lactobacillus curvatus*, *Lactobacillus plantarum* and *Lactobacillus casei* **[1]**, **[2]**, which, due to their fermentative, proteolytic and lipolytic activity, will improve the structures and consistencies of the meat products, the recovery of nitrates to nitrites, the formation of nitrosomyoglobin, the dehydration and inhibition of lipid oxidation **[3]**, **[4]**.

A traditional pleasant taste of fermented meat products is achieved due to a large content of lactic bacteria. Researchers associate the participation of catalase-positive cocci in the aroma development process with the high biochemical activity of these microorganisms **[5]**.

Volatile low-molecular fatty acids contribute to forming a pronounced taste and are formed under lipolytic activity **[6]**, **[7]**. Some scientists studied the technological criteria that must be included in selecting lactic acid bacteria for producing fermented meat **[8]**.

The aroma development in fermented sausages mainly occurs throughout the ripening process, and the influence of biochemical reactions on the aroma depends on microbial diversity, which is strongly influenced by the production conditions **[9]**.

During fermentation, the metabolites of lactic bacteria perform antibacterial and antioxidant functions and improve the physical and chemical qualities of the fermented meat products **[10]**.

Culler et al. **[11]** proved that using starter cultures based on *L. curvatus* and *S. xylosus* makes it possible to produce salami with a lower fat and salt content while providing satisfactory product quality.

Rodríguez-González et al. **[12]** studied the influence of the addition of two different autochthonous starter cultures, including the strain of *Lactobacillus sakei* and *Staphylococcus equorum* or *Staphylococcus saprophyticus* on the biochemical changes, which are occurred during the production of Galician chorizo sausage. They confirmed the influence of the quality improvement and safety of such products.

When the meat is salted, the salting substances are penetrated, distributed, and accumulated in the meat, as well as the chemical and fermentative processes are developed with the formation of the taste and aromatic substances. Adding starter cultures to the brine can increase the safety of fermented meat products due to the rapid acidulation of the matrix or the production of antimicrobial substances such as bacteriocins.

Therefore, the use of the starter cultures from the strains, which were recovered from spontaneous meat products, their influence on the microbiological, and physicochemical properties, and the safety of various types of fermented meat products is the highly topical issue of the study.

Scientific Hypothesis

The scientific hypothesis lies in the fact that the technology of the fermented meat products can be optimized by salting the meat raw materials with the addition of the starter cultures within 72 hours. Such changes will increase the functional-technological characteristics after the end of the complete fermentation process.

MATERIAL AND METHODOLOGY

Samples

The pork meat of Poltava meat breed - according to DSTU 7158:2010 **[13]**, the starter cultures: *Lactobacillus rhamnosus* (LLC "Chr. Hansen", Denmark), *L. plantarum*, *L. paracasei* ("MKS", Ukraine), *Staphylococcus carnosus* ("Van Hees", Germany), edible salt according to DSTU 3583 **[14]**, white sugar according to DSTU 4623, sodium nitrite.

The pork back muscle of Poltava meat breed aged 7 months (supplier "Agro Plus", Cherkasy region, Ukraine) was used for the salting.

Chemicals

Potassium hydroxide, KOH (brand A, analytic grade, LLC "Khimlaborreaktiv", Ukraine), acetone (brand A, LLC "Khimlaborreaktiv", Ukraine).

Animals, Plants and Biological Materials

The pork meat of Poltava meat breed, the starter cultures: *Lactobacillus rhamnosus* (LLC "Chr. Hansen", Denmark), *L. plantarum, L. paracasei* ("MKS", Ukraine), *Staphylococcus carnosus* ("Van Hees", Germany).

Instruments

pH-meter (MP 512 manufacturer (LLC Ulab", China), analyzer of water activity "Aqua Lab", series TE METER ("Group Inc", USA), spectrophotometer Unico S 2100 ("United products &instruments", USA), steam-distillation apparatus PSD 1 (LLC "Chimlaborreaktiv", Ukraine), Digital laboratory thermometer TH310 Milwaukee (LLC "SPECTRO LAB", Ukraine), laboratory scales AXIS BDM 3 (LLC "SPECTRO LAB", Ukraine), conical flask (CF-100, CF-150, CF-200, CF-250, CF-500, producer (Laboratory equipment) Limited Liability Company, Ukraine), burette for titration (producer (Laboratory equipment) Limited Liability Company, Ukraine), filters (producer (Laboratory equipment) Limited Liability Company, Ukraine).

Laboratory Methods

The pH value was measured by the potentiometric method (DSTU ISO 2917) **[15]** after mixing 10 g of the sample with 90 ml of distilled water. The meat samples' water activity (aw) was measured using the method **[16]**. The pressing method determined the moisture-binding capacity, which consists of extracting water from the test sample during pressing, sorbing the extracted water with filter paper, and determining the amount of separated moisture by the size of the spot left on the filter paper. Plasticity was determined by the area of the minced meat spot formed on an ashless filter under the action of a static load of 1 kg for 10 minutes **[17]**.

The amount of nitric oxide pigments was determined by extraction with the use of the aqueous solution of acetone; the color stability was determined based on the difference in the optical density of the extracts of nitric oxide pigments before and after exposure under light source at a wavelength of 540 nm **[16]**.

Volatile fatty acids were determined by recovering such volatile fatty acids that accumulated in the pork meat during its salting and by determining its amount by titrating the distillate with potassium hydroxide. Volatile fatty

acids were recovered with the use of a steam distillation device. The number of volatile fatty acids (X) in milligrams of potassium hydroxide per 100 g of the meat is calculated by the following formula:

$$X=(Y-Y_0)\times K\times 5.61\times 100/M$$

Where:
Y and Y_0 amount is 0.1 n. of potassium hydroxide solution, which was used for the titration of 200 ml of the distillate from the meat and the control sample, respectively, in ml; K is the correction of titer of 0.1 n. of potassium hydroxide solution; 5.61 is the amount of potassium hydroxide, which is contained in 1 ml of 0.1 n. of solution in ml; M is the weight of the weight in g.

The temperature of the studied brines was measured using a digital needle thermometer TH310 Milwaukee. The samples were weighed using laboratory technical scales AXIS BDM 3.

Description of the Experiment

Sample preparation: The balyk samples, produced according to the traditional technology, but with three different recipes of the injected brines, were used for the studies. The longest muscle from the dorsal and lumbar parts was isolated along the line of placement of the spinous processes of the spine from the fifth rib to the first sacral vertebra.
The studies were conducted at the biotechnology department of the Institute of Food Resources of National Academy of Agricultural Sciences (Ukraine).

Number of samples analyzed: The analyzed samples were developed according to the added starter culture: control sample without any starter culture, SC 1 sample (inoculated with *Lactobacillus rhamnosus*, *L. plantarum,* and *Kocuria rosea*), and SC 2 sample (*Staphylococcus carnosus, L. plantarum, L. rhamnosus, L. Paracasei)*.

Number of repeated analyses: The studies were carried out in triplicate, and the mathematical statistics methods processed the experimental data.

Number of experiment replication: Each study was carried out three times, the samples were six, consequently fifty-four repeated analyzes were carried out.

Design of the experiment: All strains were recovered from domestic dry-cured and raw-smoked meat products produced according to traditional technology. In these 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 SC 1, and 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 SC 2.

The technological scheme for the production of raw-dried beam included the following processes: dividing half-carcasses, deboning the meat, veining the meat with the removal of rough films and tendons, injecting with brine (30% to the weight of the meat), massaging according to the described program, sedimentation in the chamber (temperature 8 – 10 °C, duration 72 hours), drying in a chamber until the moisture content reaches 28-38%.

To prepare the brine, dissolve the required amount of table salt and glucose, and, by the brine recipe, the starter culture in 1 litre of water, stirring until the components are completely dissolved (Table 1). The temperature of the brine should be from minus 2 °C to plus 2 °C. Pork meat was injected with previously prepared brine (Table 1) using an injector of 30% of the weight of the meat.
This meat was massaged within 4 hours according to the program: 15 min – rotation (3-4 rpm), 15 min – pause. The vacuum depth in the massager was at least 90%. After massaging, the meat was kept in a chamber at a temperature of 8 – 10 °C for 72 hours. Then, the meat was moved to the drying chamber; the drying process continued until the moisture content in the product reached 28-38%.

The use of starter cultures in the production of raw balyks, which were injected into the meat during injection as part of the brine, ensured not only high organoleptic characteristics of the finished product, such as taste, aroma, and stable colour, but also increased functional and technological properties - moisture-binding capacity and plasticity, which was also reflected in the improved performance of the finished product.

For further analysis at 0 (right after the salting), 4, 14, 24, 48, and 72 hours after the salting, the samples were taken from each replication of each batch.
It should be noted that the classic production technology of raw-smoked balyk includes the following processes: preparation of raw materials, injection with brine, and ageing in a refrigerator at a temperature of 2 ±2 °C for 5-6 days, smoking for 24-36 hours, drying at a temperature of 11 ±1 °C. The developed raw-dried log technology differs from the existing technology of smoked products by the shorter duration of the heat treatment of the log,

namely, the shorter period of exposure in the refrigerating chamber (72 hours instead of 5-6 days), the absence of the smoking process, the shorter duration of the technological process of manufacturing this product due to the used starter cultures to accelerate fermentation and ensure high organoleptic and functional-technological indicators.

Table 1 Recipe compositions of brines (per 1 liter) CFU/ml of bacteria in the brines samples.

Components	Sample		
	Control sample	SC 1	SC 2
Edible salt, g	100		
Glucose, g	15		
Lactobacillus rhamnosus, *L. plantarum* and *Kocuria rosea*, g	-	2.5	
Staphylococcus carnosus, L. plantarum, *L. rhamnosus, L. Paracasei*, g	-	-	2.5

Statistical Analysis

The STATISTICA Microsoft Excel editor processed experimental data using mathematical statistics methods. The accuracy of 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

Study of pH-value

The pH unit was more intensively decreased in the samples with the starter cultures than in the control sample (Figure 1). Thus, the pH-unit was decreased from 5.74 to 5.64 in the control sample in 72 hours, and the pH-unit was decreased from 5.73 to 5.61 in the sample SC 1 in 48 hours, and at the end of the study (in 72 hours) – 5.5. It was indicated that the pH-unit was slowly decreased for the sample SC 2 in the first 24 hours, and in 72 hours of the salting, it had been almost equaled with SC 1 and was 5.52 **[18]**, **[19]**. This course of the biochemical changes in the meat raw materials is explained by the activity of lactic-acid microbiota, which, in the process of vital action, ferments the meat carbohydrates with the formation of acids, including lactic ones, which leads to the decrease in the pH-unit of the meat medium **[20]**.

It is known that the pH range of the meat raw materials of 5.5-5.8 is the most desirable because of the partial denaturation of the proteins, the tenderization of the muscle tissue, and the formation of the substances that are responsible for the taste and aroma of the "mature" meat are occurred at such acidity **[21]**.

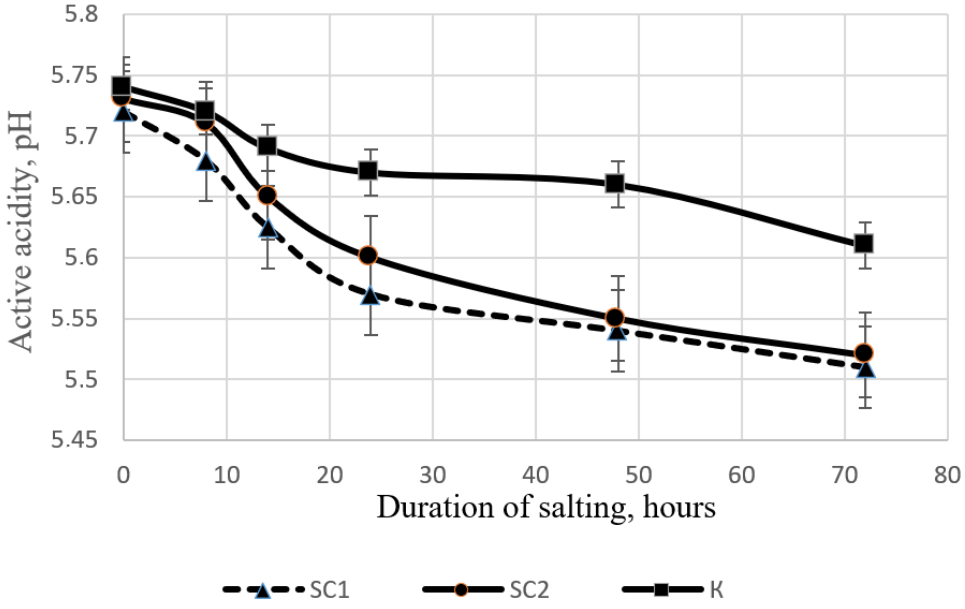

Figure 1 Dynamics of pH-unit changes in control and studied samples with bacterial drugs for salting.

The formation of lactic acid, particularly when the meat raw materials are fermented, prevents the development of putrefactive and pathogenic microbiota, thereby increasing the safety of the finished product **[22]**, **[23]**. It is known that the closer the pH value is to the isoelectric point of meat proteins (5.4 pH units), the lower its ability to bind moisture and, accordingly, the higher the drying speed **[24]**, **[25]**. A similar trend was observed when the bacterial drug "MKS" was used **[12]**.

Study of water activity

The content of free and weakly-bound water in the medium is an important factor for developing microorganisms. The water content available for microorganisms, and therefore, the stability of the product, can be estimated according to the parameter of water activity **[26]**, **[27]**.

A close relationship between the active acidity and water activity indicators was established for the samples SC 1 and SC 2 (Figure 2). It had been established that the reduction of a_w in the samples with the starter cultures more intensively than in the control sample, which may be a consequence of its active consumption during the active development of the starter-culture microbiota. In the first 24 hours, all samples' water activity was intensively decreased. The indicator for the sample SC 2 was 0.975 the control sample was 0.974, and the sample SC 1 was 0.972. Thus, at the 72nd hour of the salting, the water activity in the samples SC 1 and SC 2 was 0.965 and 0.963, respectively.

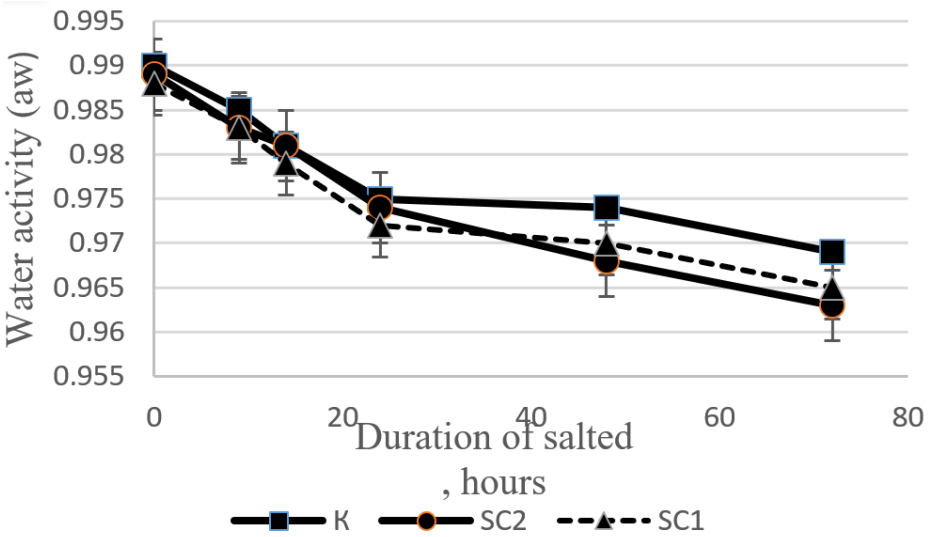

Figure 2 Change in water activity when control and studied samples are salted.

Therefore, adding the created compositions leads to the salting intensification of the meat products, which is desirable in production **[28]**.

Influence of culture starters on changes in the amount of nitric oxide pigments and colour stability of meat

In the production of meat products, one of the main food additives is sodium nitrite, which, when the meat products are salted, influences color formation and aroma development while providing preservative and antioxidant effects **[29]**, **[30], [31]**.

Improved color characteristics characterize the studied variants in comparison with the control ones; this is due to nitrite reductase, the producer of which is *Kocuria rosea*, as well as lactic bacteria, which actively reduced the pH-unit of the medium **[32]**, **[33]**. At 72 hours of the salting, the color stability of the studied samples exceeded the control ones by approximately 19%. Using the starter cultures, the studied samples are characterized by more stable and active color formation than the control ones, by 8-12% at 72 hours of the salting. The content of nitric oxide pigments in sample SC 1 was 6% higher than in SC 2, and 18% higher than in the control sample (Figure 3).

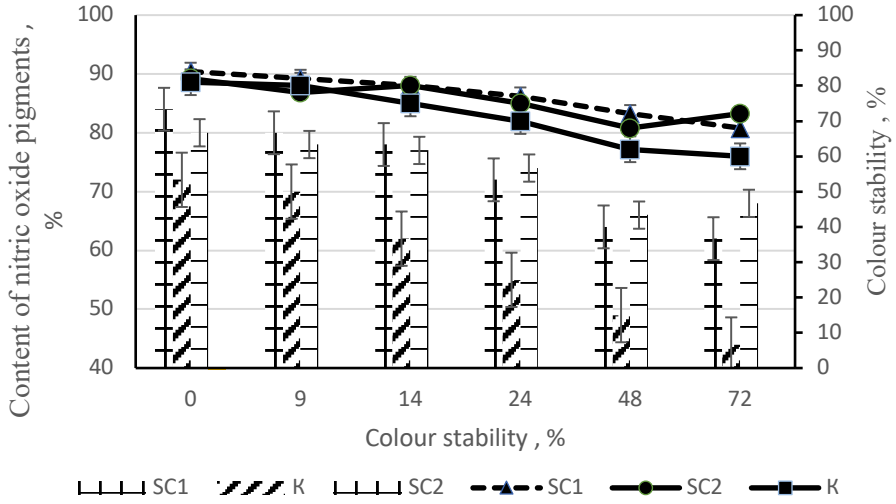

Figure 3 Dynamics of changes in the amount of nitric oxide pigments and color stability of control and studied samples while salting.

Thus, using bacterial drugs based on nitrite-reducing microorganisms for the salting positively influenced the formation of the required color characteristics.

Study of moisture-binding capacity and plasticity

An increase in the moisture-binding capacity of the meat raw materials is observed in the studied samples with the starter cultures (Figure 4). The studied variant, with the use of the starter cultures, was characterized by higher values of the moisture-binding capacity than the control sample and was 84-87 % at 72 hours and 84-87% at 72 hours of the salting.

At the end of the salting, this indicator for the control sample was 79.27%, 7.73% lower than for SC 2.

This influence is due to rapid glycolysis and the accumulation of the sour products of the metabolism of lactic bacteria, which more actively reduce the pH level, bringing it closer to the isoelectric point of the protein substances, which leads to structural changes in the protein **[34]**, **[35]**.

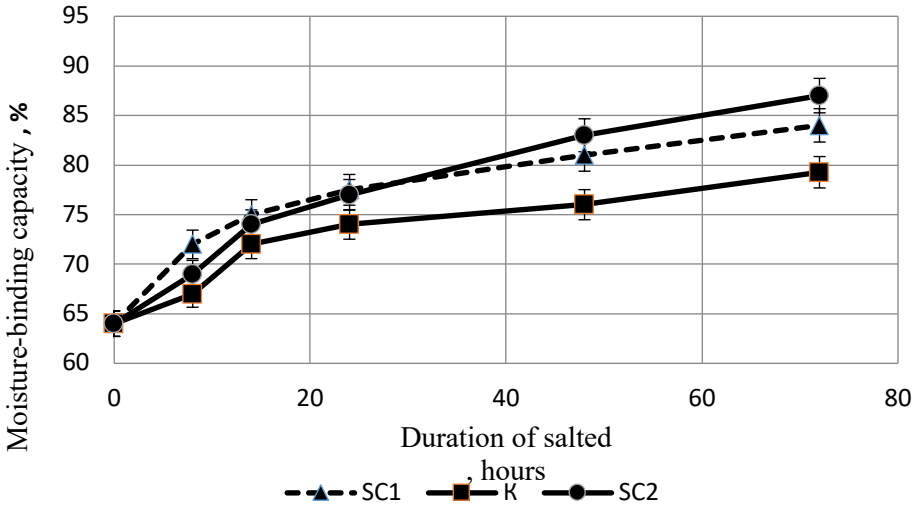

Figure 4 Dynamics of changes in the moisture-binding capacity of control and studied samples of meat raw materials while salting.

The plasticity of the studied samples is increased in direct proportion to the brine aging time (Figure 5). The plasticity of SC 1 varies from 7.78 cm^2.g to 17.23 cm^2.g, SC 2 – from 7.76 cm^2.g to 17.45 cm^2.g, and C – from 7.76 cm^2.g to 15.11 cm^2.g. This indicator for the studied samples SC 1 and SC 2 were at the same level and exceeded the control ones by 2.19 cm^2.g. Such a course of the biochemical changes of the studied samples can be explained by the proteolytic activity of lactic acid bacteria **[36]**, **[37]**.

An increase in plasticity can be considered a characteristic of the tenderization process of meat raw materials. An improvement in the plasticity of the studied samples is a positive result since the tenderness and, to a certain extent, the juiciness of the finished products depend on the plasticity of the meat raw materials **[38]**, **[39]**.

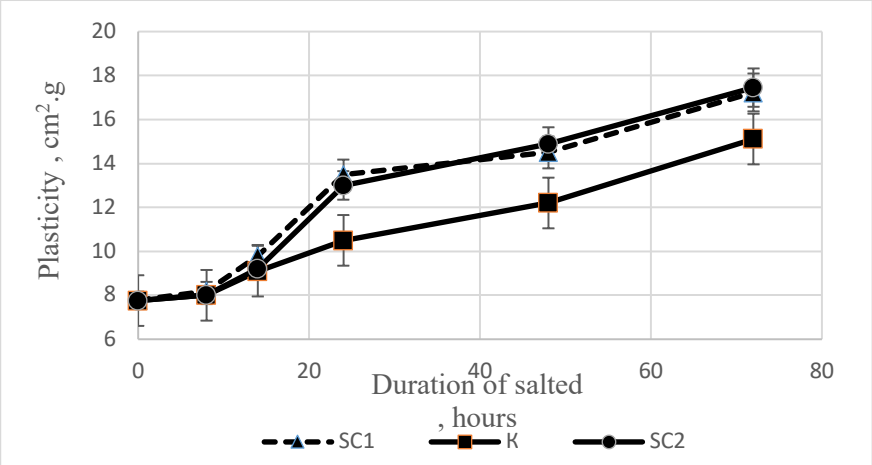

Figure 5 Dynamics of changes in control plasticity and studied samples of meat raw materials while salting.

Specific biochemical transformations occur when the meat's raw materials are salted, which determines the required organoleptic characteristics of the finished product **[40]**. Bacteria of the genus *Lactobacillus* are the pronounced producers of aroma and taste precursors and contribute to forming the specific organoleptic characteristics of the finished products **[41]**, **[42]**.

In the studied variants SC 1 and SC 2, a more intense accumulation of volatile fatty acids is observed compared to the control sample (Figure 6). Thus, the difference in the content of volatile fatty acids between SC 1 and the control sample was 22% at 72 hours of the salting. Because the formation process of volatile fatty acids is fermentative, this tendency is probably explained by the fact that the lipid hydrolysis has occurred under the action of not only tissue enzymes of cathepsins (as in the control ones) but also lipases, which were formed as a result of the vital activity of the bacterial compositions.

The content of volatile fatty acids is one of the most informative indicators for the taste-aromatic properties of the meat raw materials to be formed **[43]**.

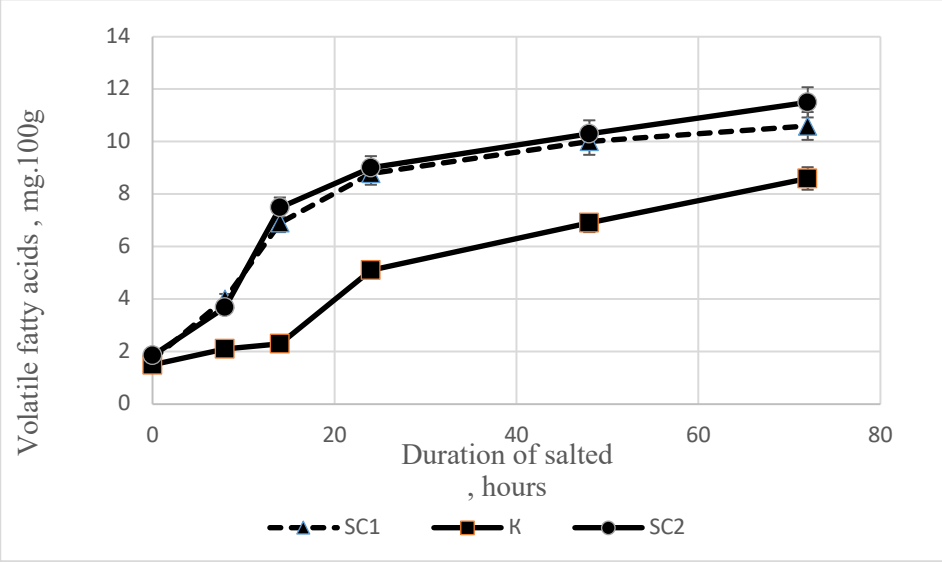

Figure 6 Accumulation dynamics of volatile fatty acids in studied variants while meat raw materials are salted.

The results of the final products of dry-cured balyks are shown in Figure 7.

Figure 7 Dry-cured balyks.

It should also be noted that the increase in the proteolytic activity of the enzymes depends on the medium acidity, which should be in the range of 5.4-5.6 pH units. As established earlier (Figure 1), the medium pH unit in the studied samples reaches the optimal values at 72 hours of the salting.

Thus, the results of our studies indicate that it is possible to use the starter cultures, which were created based on symbiotic compositions of strains of SC 1 *Lactobacillus rhamnosus*, *L. plantarum,* and Kocuria rosea and SC 2 *Staphylococcus carnosus, L. plantarum***,** *L. rhamnosus, L. paracasei* for the fermented meat products – dry-cured balyks to be produced.

CONCLUSION

The starter cultures of *Lactobacillus rhamnosus*, *L. plantarum Kocuria rosea,* and *Staphylococcus carnosus, L. plantarum***,** *L. rhamnosus, L. paracasei* were tested. At the same time, the pork meat is salted in the production of the dry-cured balyks. It had been established that in the samples with the starter cultures the acidity was more intensively decreased in 72 hours (from 5.74 pH units to 5.52 pH units) than in the control ones (from 5.74 pH units to 5.64 pH units). A close relationship was established between indicators of active acidity and water activity in SC 1 and SC 2 samples. At the 72nd hour of pickling, water activity in samples SC 1 and SC 2 was 0.965 and 0.963, respectively. At 72 hours of the salting, the color stability of the studied samples exceeded the control ones by approximately 19%. The moisture-binding capacity and plasticity of the pork meat samples with the starter cultures are characterised by the increased indicators compared to the control sample – by 4.73 and 7.73% and by 2.19 cm^2.g, respectively. The difference in the content of volatile fatty acids between SC 1, SC 2, and the control sample was 22% and 33%, respectively, at the 72 hours of the salting. The use of the starter cultures, such as *Lactobacillus rhamnosus*, *L. plantarum,* and *Kocuria rosea,* and *Staphylococcus carnosus, L. plantarum***,** *L. rhamnosus, L. paracasei*, when the pork meat is salted in the technology of the meat products makes it possible to obtain the finished products with the improved quality indicators, which is advanced in the food-processing industry.

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