International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 23s, 2025
https://theaspd.com/index.php

Prospects of Using Lactobacillus Sakei Strain in the Production of Fermented Sausages

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Abstract: *The search and selection of strains of lactic acid microorganisms that ensure high quality of fermented meat products is an important and urgent task. Meat raw materials are a complex protein system and the use of lactic acid bacteria strains with high proteolytic potential will make it possible to purposefully influence the structural elements of muscle proteins to accelerate maturation and form an attractive sensory profile. In this work, the biotechnological potential of a promising strain of lactic acid bacteria Lactobacillus sakei LSK-104 was evaluated in order to use it as a starter culture for the production of fermented meat products. The strain being studied is promising for use as a starter culture. A distinctive feature of the strain is its ability to form proteases that hydrolyze protein fractions of muscle proteins and possess a universal proteolytic complex that is active in a wide range of pH values. Samples of fermented meat products were obtained and their microbiological and technological parameters were evaluated. The population of the L. sakei strain is well adapted to meat raw materials, intensively accumulates lactic acid and reduces the pH of the environment. Fermented sausages using the L. sakei LSK 104 strain received a high sensory evaluation. They had a pronounced pungent taste and aroma, distinct texture, and dense consistency. Based on the totality of the results, it was concluded that the L. sakei strain LSK 104 (B-8936) has a high potential for use as a starter culture in the production of fermented sausages.*
Keywords: *fermented sausages, proteolytic potential, lactobacilli, starter cultures, sourdoughs*

1. INTRODUCTION

The production of fermented meat products is one of the oldest methods of processing meat raw materials in order to increase its shelf life. Currently, fermented sausages are widely represented in many regions of the world, are in steady demand among consumers and are perceived as a delicacy product. [1].

The production of fermented meat products, in particular dry and dry-cured sausages, is a complex process of interrelated physico-chemical, biochemical and microbiological processes occurring in meat raw materials during maturation. The key role in the formation of the quality characteristics of the finished product, as well as its safety, is given to lactic acid bacteria, in particular representatives of the genera Lactobacillus, Lactococcus, Leuconostoc, Pediococcus and Streptococcus.

The main functional contribution of lactic acid bacteria (LAB) in the production of fermented meat products is the formation of lactic acid, leading to a decrease in the pH of the environment. [2].

From a technological point of view, the use of lactic acid microorganisms as starter cultures due to their ability to rapid and predictable acid formation, developed proteolytic and lipolytic activities, as well as the ability to produce a wide range of antimicrobial metabolites ensures product safety, stability during shelf life, formation of texture, as well as taste and aroma of the finished product [3].

One of the promising areas of use of starter cultures in the production of fermented sausages is the ability of lactobacilli to form antimicrobial compounds - bacteriocins. These are protein molecules that are able to control the growth of pathogenic microorganisms. Bacteriocins have great potential as bioconservants [4]. An example of bacteriocins synthesized by lactic acid microorganisms is nisin, a polypeptide lantibiotic produced by Lactococcus Lactis, whose use in the food industry is permitted in many countries [5].

One of the most promising types of lactic acid bacteria with the ability to synthesize bacteriocins is Lactobacillus sakei bacteria. These bacterial strains effectively inhibit the growth of L. monocytogenes, and are also capable of producing various antimicrobial compounds, including sacacin [6]. Various strains of Lactobacillus sakei are used in the production of fermented sausages, as they are not only active producers of lactic acid, but also resistant to table salt and low temperatures [7]. A feature of Lactobacillus sakei is the presence of heme-dependent catalase, that participates in the maintenance of cellular redox homeostasis. These bacteria are able to absorb iron and heme, that increases their survival rate. Given that the sacroplasmic proteins of meat are rich in heme iron, the choice of Lactobacillus sakei as a starter culture in the production of fermented meat products is preferable [8].

The ability of lactic acid microorganisms to produce proteolytic enzymes is of considerable interest in the

production of fermented meat products. In lactic acid bacteria (LAB), proteolysis is a key physiological feature that ensures the growth and formation of sensory properties of fermented foods. The classical proteolytic LAB system includes three functional units - extracellular cell wall proteases (CEP) - serine proteases that initialize protein hydrolysis to oligopeptides, membrane peptide transporters, and intracellular peptidases of diverse specificity (endo- and amino-, di-, tripeptidases, as well as proline-specific peptidases) [9].

However, most of the existing industrial strains of lactic acid bacteria used as starter cultures have limited proteolytic activity, which does not allow them to fully realize their biotechnological potential for effective action on muscle proteins, taste formation and acceleration of maturation. Moreover, many industrial LAB strains were selected based on the criteria of rapid acidification and resistance to salt and nitrites, but without taking into account their ability to perform deep proteolysis [10].

In this context, screening of new LAB strains, especially those isolated from traditional fermented foods or natural sources, is a promising direction. Such strains can carry unique genes encoding proteases and peptidases with high activity in a meat matrix. In addition, it is important to take into account the synergetic interaction between different strains in starter cultures, since the combined use of proteolytically active and acid-forming cultures can ensure a balanced and efficient fermentation process [11].

Despite significant progress in understanding the physiology and metabolism of LAB, there is an urgent need to find and develop new strains that would combine high proteolytic potential with optimal technological properties: rapid growth, resistance to fermentation conditions, safety and the ability to form the desired aroma and texture. Modern approaches, including genomic sequencing, metagenomic analysis, proteomics, and metabolomics, make it possible to purposefully select and characterize promising strains, as well as modify them using genetic engineering to enhance the desired traits [12, 13].

Many of the lactobacilli, such as L. sakei, L. curvatus, L. plantarum, and L. casei, are capable of hydrolyzing myofibrillary and sarcoplasmic muscle proteins, which has been experimentally confirmed. Protein profiles of pork sarcoplasmic proteins after exposure to L. sakei cells were analyzed by denaturing electrophoresis (SDS-PAGE) and capillary electrophoresis. It was found that L. sakei preferably hydrolyze individual sarcoplasmic proteins in the early stages of fermentation [14].

Reverse-phase high-performance liquid chromatography (RP-HPLC) also showed that L. sakei and L. curvatus cultures efficiently hydrolyze a range of soluble muscle proteins, but the extent and degree of hydrolysis depended on the strain and type of enzyme source: cell extracts and supernatants often provided more noticeable degradation compared to whole cells. Proteolytic activity was detected for individual protein fractions in L. curvatus, and a specific feature of free amino acid release was also established for L. sakei [15, 16].

Thus, the search and selection of strains with high proteolytic activity, as well as optimization of incubation conditions, can purposefully enhance the cleavage of sarcoplasmic proteins, improving the development of taste and maturation of fermented meat products. Of particular interest in the search for starter cultures with an effective proteolytic profile are L. sakei strains [17].

L. sakei strains have high biotechnological potential, are functionally adaptable to the meat environment, are able to grow in conditions of carbon deficiency, are also resistant to stress factors, and have a developed antioxidant system. L. sakei has the ability to form biofilms, which gives an advantage over other microorganisms, and also does not produce biogenic amines such as tyramine, histamine, what is typical for such LAB strains as L. curvatus, L. brevis [18, 19].

The purpose of this research was to study the biotechnological potential of a promising strain of lactic acid bacteria Lactobacillus sakei LSK-104 in order to use it as a starter culture for the production of fermented meat products.

2. MATERIALS AND METHODS

2.1. The Studied Strains of Lactic Acid Bacteria

A promising strain of Lactobacillus sakei LSK-104 (Registration number of the VKPM collection: B-8936), provided from the collection of the BRC VKPM of the Kurchatov Institute National Research Center, was chosen as the object of research. The cells of the Lactobacillus sakei LSK-104 strain are short rods arranged singly, capable of forming short chains. The strain forms opaque colonies with a smooth, shiny surface, rounded shape with a pronounced zone of sterility. The strain under study is characterized by an optimal growth temperature of 37 °C, produces lactic acid and bacteriocins, is antagonistic to Escherichia coli, Proteus vulgaris, Salmonella typhimurium, has aroma-forming properties and heme-dependent

catalase activity [20].

An industrial starter culture based on L. plantarum was used as a comparison strain in the production of experimental batches of fermented sausages. MEATFERM SLP 1U starter culture (Biochem s.r.l., Italy) is a starter culture with medium oxidative activity, recommended for all types of fermented sausages. The bioprotective culture of L. plantarum, which is part of the starter culture, produces the bacteriocin plantaricin, which inhibits the growth of pathogenic microflora and L. monocytogenes. Starter cultures based on L. plantarum are widely used in industry in the production of fermented sausages to form the texture of the product, improve taste and aroma, and extend the shelf life of sausages [21].

2.2. Preparing Fermented Sausages

Experimental samples of dry fermented sausages were obtained in accordance with the recipe presented in Table 1.

Table 1: Recipe of meat product

Name of raw materials and supplies	**Ingredients quantity, kg per 100 kg**
Beef without visible connective tissue inclusions	35
Pork with 30% fat content	65
Table salt	3
Sodium nitrite	0.005
Black pepper	0.1
Nutmeg	0.03
Dried garlic	0.03

The preparation of fermented sausages was carried out according to the following scheme. The meat raw material was ground in a meat grinder with 2-3 mm grid holes. Then the mince was made according to the recipe with the addition of salt, spices and sodium nitrite.

The studied starter cultures were inoculated in minced meat in an amount of 0.05%. The following samples were obtained: Sample 1 with L. sakei and sample 2 with an industrial starter culture containing L. plantarum. A sample produced without the use of starter cultures was used as a control. Minced meat was stuffed into a fibrotic casing. The obtained samples of fermented sausages were placed in a drying chamber and kept for 20 days at the following temperature and humidity: 1 day at a temperature of 18 ± 0.1 ° C and a relative humidity of 80 ± 0.1%, 1 day at a temperature of 16 ± 0.1 ° C and a relative humidity of 82 ± 0.1%, 3 days at at a temperature of 14 ± 1 ° C and a relative humidity of 76 ± 0.1%, further processing took place at a temperature of 12 ± 1 ° C and a relative humidity of 70 ± 0.1%. Sampling was carried out on the 1st, 3rd, 6th, 9th, 15th and 20th days of treatment. Chemical and microbiological parameters were evaluated in the samples. Upon reaching the adjusted humidity of 40±2%, a sensory evaluation of the products was performed.

2.3. Determination of Proteolytic Activity

To determine the activity of caseinase, Eijkman milk agar was used, where skimmed milk was used as a substrate. The ability to form proteolytic enzymes was determined by transparent halos around the bacterial growth zone.

The ability of lactic acid bacteria to hydrolyze sarcoplasmic and myofibrillar proteins was assessed by the diameter of the transparent zone on a dense nutrient medium enriched with sterile extracts of sarcoplasmic or myofibrillar proteins. The corresponding protein fractions were obtained by extraction in an amount of 1 mg/ml. [22, 23].

The degree of effect of microbial proteases on protein substrates (casein, albumin) was assessed by changes in the content of proteins, peptides, and free amino acids spectophotometrically according to the method [24-26].

The degree of protein hydrolysis was determined as the ratio of amino nitrogen to total nitrogen [27], the content of amino nitrogen in the solution was determined by the formol titration method.

The activity of proteases was evaluated by the modified Anson method [28], proteolytic activity was determined by the amount of released tyrosine accumulated in the substrate for 1 hour under the action of proteases secreted into the culture fluid by lactobacilli after 12 hours and 24 hours of cultivation in MRS liquid nutrient medium. A 2% solution of hemoglobin A 2% solution of hemoglobin in a universal buffer (acetic, orthophosphoric and boric acids with a concentration of 0.1 mol/dm^3 in equal

proportions) with pH values of 5.0, 6.5 and 8.0 was used as a substrate to determine the proteolytic activity of slightly acidic, neutral and alkaline proteases of lactic acid bacteria, respectively.

2.4. Determination of Technological Indicators

The determination of protein, moisture, and fat content was carried out by near-infrared spectroscopy using the InfraLUM FT-12 analyzer according to the recommendations of the M 04-89-2019 methodology (FR.1.31.2019.34465) of LUMEX company [29]. The pH was determined in the water extract of the product at different drying periods using a pH meter [30]. The amount of lactic acid was determined spectrophotometrically according to the modified method [31].

NaCl content determination was established by Volhard method [32]. Microbiological analysis of samples was performed according to the method [33].

2.5 Sensory Evaluation

Sensory characteristics of fermented sausages were evaluated on the 20th day of the experiment by a group of 9 experts. A 9-point system was used to evaluate the sensory characteristics. The final result represents the average value [34]. The validity of the sensory analysis results was confirmed by a priori factor ranking method [35, 36].

2.6 Statistical Analysis

All analyses were performed in three repetitions. The obtained research results were statistically processed using the Microsoft Excel application. Statistical significance of the results was determined at the $P < 0.05$ level.

3. RESULTS AND DISCUSSION

The study examined the proteolytic potential of the L. sakei LSK-104 strain. Using molecular genetic analysis methods using the PCR method [37], genes responsible for proteolytic activity (PrtP, PrtM, PrtB, PrtH, and PrtR) were found in Lactobacillus microorganisms, and the results showed that the maximum number of proteinase genes was present in the L. sakei strain. However, possession of a set of genes does not guarantee high proteolytic activity, and selection of strains based on this criterion requires an integrated approach [38]. The results of the study of the proteolytic activity of the L. sakei LSK 104 strain on various protein substrates are presented in Table 1.

Table 1: Proteolytic activity of L. sakei strain LSK 104

Parameters	L. sakei LSK 104	
Ability to hydrolyze proteins of animal origin		
Protein substrate	The diameter of the transparent zone, mm	
Milk protein	10.5±0.1	
Sarcoplasmic proteins of meat	20.4±0.3	
Myofibrillar proteins of meat	15.9±0.1	
Changes in fractions of soluble nitrogenous compounds		
Protein fractions, mg/100 ml	Investigated substrates	
	casein	albumin
Proteins	184.4±13.4	705.6±28.2
Peptides	713.9±22.7	247.3±16.3
Amino acids	25.8±4.1	16.7±4.1
Degree of hydrolysis of protein substrates		
Substrate	Degree of hydrolysis, %	
casein	39.7±0.5	
Albumin	32.5±0.5	
Sarcoplasmic proteins of meat	65.4±0.6	
Myofibrillar proteins of meat	58.5±0.6	
Proteolytic activity of strains at different pH levels, μg tyrosine/(ml·min)		
pH	Duration of fermentation	
	12 h	24 h
5.0	18.84±0.96	16.56±0.82
6.5	24.91±1.22	16.59±0.82

8.0	47.92±2.40	20.69±1.06

Studies have shown that the L. sakei LSK 104 strain forms transparent halos of casein peptization on an agarized medium with the addition of skimmed milk. That indicates the ability of the strains to form proteolytic enzymes that hydrolyze milk protein. The diameter of the transparent zone, indicating peptization of the milk protein in the L. sakei LSK 104 strain, measured 10.5±0.1mm. At the same time, it was found that strain L. sakei hydrolyzes sarcoplasmic and myofibrillary proteins more efficiently, the diameter of the transparent zone of hydrolyzed sarcoplasmic proteins is 20.4±0.1 mm, and myofibrillary proteins is 15.9±0.1 mm. It should be noted that the strain under study hydrolyzes animal proteins more effectively, in particular sarcoplasmic proteins. The data obtained are consistent with the literature data [8].

To identify the activity of extracellular proteolytic enzymes of the L. sakei strain, the content of soluble nitrogenous compounds in model substrates (1% solutions of casein and whey albumin) after fermentation was evaluated. It has been found that the accumulation of peptides and amino acids occurs during fermentation. Assessing the degree of hydrolysis of model substrates, as well as protein fractions obtained by extraction from muscle proteins, it was found that strain L. sakei is highly active in the hydrolysis of sarcoplasmic and myofibrillary proteins, which is in good agreement with the data obtained by estimating the diameters of the halos of illumination on a dense nutrient medium. The lowest activity of the L. sakei strain was observed during albumin hydrolysis.

It can be concluded that the activity of the proteolytic system of the L. sakei strain depends on the type and nature of the substrate, which implies the need for an integrated approach when choosing a strain for starter cultures. The assessment of the proteolytic potential should be carried out not only by generally accepted methods, but also take into account the specificity of specific substrates and the realized production goals [39].

It seemed appropriate to establish the nature of hydrolysis of protein substrates by the L. sakei strain not only depending on the type of substrates, but also depending on the environmental conditions, in particular on the pH value. Proteolytic enzymes of lactobacilli are capable of exhibiting activity in a wide range of pH values, and according to this feature they are divided into slightly acidic, neutral and alkaline [10, 40]. The study of the dependence of the proteolytic activity of the studied strain on the pH value of the medium was carried out using 2% hemoglobin prepared in a universal buffer [28]. The studied L. sakei strain exhibits higher activity in the phase of intensive growth, which indicates active synthesis of proteases in the first hours of lactobacilli development. The obtained data are consistent with the literature data [20, 41]. The maximum proteolytic activity was recorded at pH = 8.0, while the optimal value for the growth and development of these strains lies in the range from 5.5 to 6.2. The results obtained in assessing the proteolytic activity of the strain under study show that the pH value has a significant effect on the activity of extracellular proteases produced by lactobacilli, which is due to a change in the hydrogen-ion balance, which results in modification of the enzyme structure and a decrease in substrate availability [39, 42]. Nevertheless, the strain under study exhibits proteolytic activity on various substrates and in a wide range of pH values, which indicates the universality of the strain's proteolytic complex and is confirmed by other researchers [38].

Thus, the proteolytic activity of lactic acid bacteria does not depend on their type and is an individual feature of a particular strain. That once again confirms the conclusions about the need for a thorough analysis of the functional and technological properties of each "candidate" for inclusion in starter cultures intended for fermented foods.

The production of fermented meat products is a complex technological process in which the key role is given to starter lactic acid microorganisms. To establish the prospects for using the L. sakei LSK-104 strain as a starter culture, an assessment of the quality indicators of dry fermented sausages produced using this strain was carried out. During the maturation and drying of sausage samples, an assessment of the main microbiological and physicochemical indicators was made. The results of the comprehensive assessment are presented in Table 2.

A microbiological analysis was carried out, in which the number of viable cells was counted during the maturation of sausage samples. Both experimental starter samples showed good survival. On the 9th day of the experiment, the population was 9.8-9.9 log cfu/g, decreasing by the end of drying to 7.9-8.0 log cfu/g. In the control samples, the amount of LAB at the beginning of fermentation was lower than in the experimental samples, especially on the first day of the experiment.

In terms of chemical composition assessment, the experimental samples of fermented sausages obtained

similar values for moisture, protein, fat and salt content by the end of the experiment on day 20, which allows us to speak about the high technological potential of the studied L. sakei strain, which showed efficiency comparable to the sample obtained using a commercial starter culture. A decrease in the pH level of the experimental samples, as well as an intensive accumulation of lactic acid, was also noted.

Table 2: Changes in the main parameters of fermented sausages during ripening and drying

Sample	Indicator	Days					
		1	3	6	9	15	20
Control	LAB, log cfu/g	2.2±0.1	7,8±0.1	8,8±0.1	8,9±0.1	9,0±0.1	7.1±0.1
	Lactic acid, mg/g	8.3±0.1	12.1±0.1	13.0 ±0.1	14.3±0.1	18.2±0.1	18.8±0.1
	Moisture content, %	58.3±0.5	55.0±0.5	52.8±0.5	51.4±0.5	46.4±0.5	42.4±0.5
	Salt content, %	3.10±0.12	3.34±0.12	3.54±0.12	4.08±0.12	4.49±0.12	4.69±0.12
	Protein content, %	17.85±0.3	18.01±0.3	18.17±0.3	18.83±0.3	19.40±0.3	19.81±0.3
	Fat content,%	18.4±0.7	23.7±0.7	28.5±0.7	32.3±0.7	35.9±0.7	41.3±0.7
	pH	5.9±0.2	5.4±0.2	5.2±0.2	5,0±0.2	4.9±0.2	4.8±0.2
Sample 1	LAB, log cfu/g	6.9±0.1	9.2±0.1	9.7±0.1	9.9±0.1	9.5±0.1	8.0±0.1
	Lactic acid, mg/g	8.7±0.1	15.4±0.1	16.3±0.1	18.9±0.1	19.4±0.1	21.7±0.1
	Moisture content, %	55.5±0.5	53.3±0.5	49.8±0.5	45.6±0.5	44.2±0.5	39.9±0.5
	Salt content, %	3.10±0.12	3.40±0.12	3.91±0.12	4.33±0.12	4.67±0.12	4.81±0.12
	Protein content, %	17.99±0.3	18.15±0.3	18.37±0.3	18.95±0.3	19.22±0.3	19.95±0.3
	Fat content,%	18.5±0.7	22.6±0.7	27.8±0.7	31.2±0.7	35.9±0.7	40.66±0.7
	pH	5.9±0.2	5.0±0.2	4.9±0.2	4.8±0.2	4.7±0.2	4.6±0.2
Sample 2	LAB, log cfu/g	5.7±0.1	9.3±0.1	9.5±0.1	9.9±0.1	9.7±0.1	7.9±0.1
	Lactic acid, mg/g	8.6±0.1	15,0±0.1	15.8±0.1	18.3±0.1	18.9±0.1	20.9±0.1
	Moisture content, %	55.5±0.5	52,9±0.5	51.2±0.5	49.6±0.5	45.3±0.5	40,1±0.5
	Salt content, %	3.11±0.12	3.36±0.12	3.81±0.12	4.22±0.12	4.61±0.12	4.77±0.12
	Protein content, %	17.94±0.3	18.24±0.3	18.67±0.3	18.95±0.3	19.45±0.3	20.12±0.3
	Fat content,%	18.5±0.7	23.0±0.7	28.2±0.7	30,9±0.7	36.4±0.7	40.90±0.7
	pH	5.9±0.2	5.4±0.2	5.1±0.2	4.8±0.2	4.8±0.2	4.7±0.2

The control sample containing microflora developed as a result of spontaneous fermentation by lactic acid bacteria due to natural microflora, received the expected worst results, but by the end of the experiment had indicators comparable to the experimental samples.

The theory of microbiological processes of "natural" maturation of fermented sausages has been well studied and is based on the fact that the combination of ingredients of meat products, as well as the parameters of maturation and drying, create conditions for the preferred development of lactic acid organisms [43]. Subsequently, the LAB initiates acidification of the medium, which makes it possible to suppress competing microflora by accumulating lactic acid and lowering the pH. This process is well-studied and is one of the factors that guarantees the stability of fermented meat products quality [44, 45].

However, with natural maturation, it is very difficult to predict which microorganisms will make up the main population, since even within one strain of microorganisms there are significant differences in the degree of resistance to environmental factors. Despite the fact that, as a rule, typical representatives of the lactic acid microflora of natural maturation are representatives of the genera L. sakei, L. curvatus, L. plantarum, other strains of lactic acid bacteria can get into the mince. The main disadvantage of the natural fermentation of sausages are defects in taste and smell. The growth of an unusual homofermentative microflora can lead to a pungent taste and odor, while the development of a heterotrophic microflora can cause defects such as gas formation, the appearance of pores, voids, tears, and color changes [46].

Assessment of the sensory characteristics of fermented sausages is a non-trivial task, since there are no clear evaluation criteria and a list of indicators reflecting the full sensory properties of fermented products [47]. During the study, such indicators as taste, appearance, color, aroma and cross-section appearance were evaluated. The evaluation of the obtained samples was carried out on the 20th day after the samples reached a humidity of 38-42%, which is a normalized factor indicating the completion of the drying process [1]. The results of the study are shown in Figure 1. The control samples received lower scores in terms of taste and aroma, and also had a less dense cross-section appearance. Despite the fact that this lowered the final score of the control samples, their sensory assessment was generally considered acceptable. The experimental samples of fermented sausages received a high organoleptic characteristic above 7 points. They had a pronounced taste and aroma, texture, consistency, and cross-section appearance typical of this type of food. When evaluating samples obtained using strain L. sakei LSK-104 sausages had a more pronounced pungent taste and aroma, which positively influenced the final assessment.

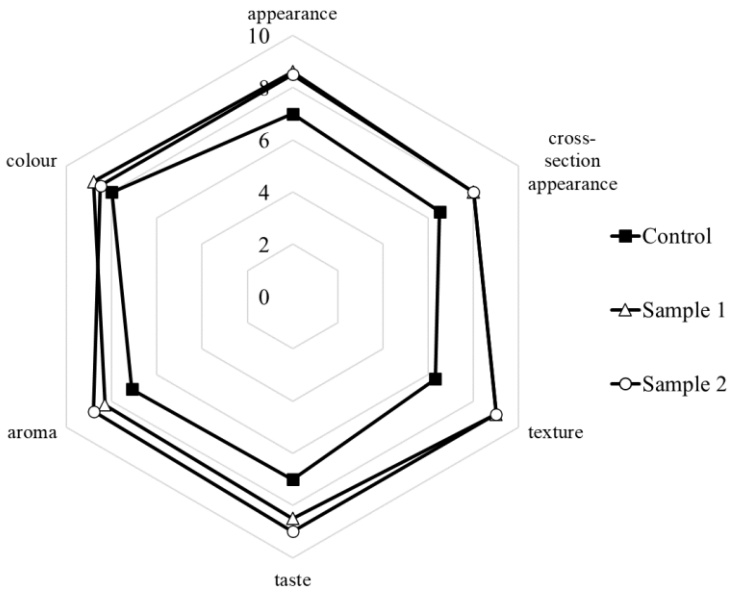

Figure 1: Sensory profile of fermented sausages

4. CONCLUSIONS

Analysis of the obtained data shows that the studied strain L. sakei LSK 104 (B-8936) is promising for use as a starter culture in the production of fermented meat products. A distinctive feature of the strain under study is its ability to form proteases that effectively hydrolyze protein fractions of muscle proteins and possess a universal proteolytic complex that is active in a wide range of pH values. At the same time, the L. sakei LSK 104 (B-8936) strain is adapted to meat raw materials and is able to reproduce in model minced meat systems intensively accumulating lactic acid. The technological characteristics of the fermented sausages obtained using the L. sakei strain were not inferior to the samples obtained using a commercial starter culture. At the same time, sausage samples with the L. sakei strain obtained a higher sensory characteristic. Based on the totality of the results, it can be concluded that the L. sakei LSK 104 (B-8936) strain has a high potential for use as a starter culture in the production of fermented sausages.

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