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Effect of lactic acid bacteria ferment cultures on pork freshness

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Abstract. During the primary pork processing at the stage of cooling half-carcasses, their mass is lost, leading to economic losses. One promising way to solve this problem is to wash half-carcasses with chilled water. This requires decontaminating meat with microflora, which causes

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its spoilage. The purpose of the study is to determine the effectiveness of the final treatment of pork half-carcasses with ferment cultures of SafePro® B-SF-443 (*Leuconostoc carnosum*) and SafePro® B-2 (*Lactobacillus sakei*) strains for their bioconservation and preservation of freshness. The paper uses organoleptic, chemical, and microbiological research methods. It was established that cooling half-carcasses of pork in the refrigerator using cold water washing contributes to the appearance of signs of meat spoilage in terms of microbial contamination and pH value already on the 4th day of storage. Surface treatment of half-carcasses with suspensions of lactic acid microorganisms of SafePro® B-SF-43 (*Leuconostoc carnosum*) and SafePro® B-2 (*Lactobacillus sakei*) strains at a dose of 10^7/cm^2 provides proper organoleptic parameters and the pH value of pork up to 7 days of storage, which correspond to high-quality meat. Both ferment cultures of lactic acid microorganisms reduce microbial contamination of meat due to the number of mesophilic aerobic and facultative anaerobic microorganisms in paired carcasses by 1.25 and 0.65 lg CFU/cm^2 and increase the number of lactic acid microorganisms by 3.47 and 3.43 lg CFU/cm^2 accordingly, this allows extending the shelf life of meat in the chilled form to at least 7 days. The most suitable culture for the final processing of half-carcasses of pork, which are cooled in the refrigerator in combination with washing with chilled water, is the SafePro® B-2 (*Lactobacillus sakei*) strain. The results obtained are promising for improving the technology of primary pork processing at the cooling stage using ferment cultures of lactic acid microorganisms as natural preservatives, which will increase the shelf life of meat, considering its quality and safety

Keywords: meat; cooling; washing; storage; microbial contamination

Introduction

Post-slaughter changes that occur during the maturation process of slaughtered animal meat play a crucial role in developing its qualitative characteristics and the overall perception of a fresh product. After animal slaughter, muscle glycogen, together with high-energy phosphate compounds, is anaerobically mobilised to maintain homeostasis by synthesising adenosine triphosphate (ATP). As a consequence of post-slaughter glycolysis, lactate and H^+ accumulate in the muscles and cause a decrease in pH. In the end, post-slaughter metabolism in the tissues of slaughtered animals stops due to substrate depletion or inactivation of phosphofructokinase-1, which leads to ATP deficiency and the beginning of meat freezing processes. Over time, animal muscles undergo proteolysis of structural proteins that change their properties. As noted by J.C. Wicks *et al.* (2022), during the breakdown of individual peptide bonds, the structure of proteins changes its configuration, which further improves the tenderness of muscle tissue.

Usually, muscle tissue is considered practically sterile before slaughter, but the environment during animal slaughter is not sterile, which causes a certain degree of microbial contamination, which leads to spoilage of meat. The sources of microbial contamination in this process can be generalised as endogenous and exogenous. K. Klaharn *et al.* (2021) report that the microbiological quality of fresh animal meat largely depends on its type, processing conditions, handling, and storage. In addition, according to this author, improper hygiene of slaughtering equipment, personnel and environmental factors (for example, water, air, and soil) lead to cross-contamination with microorganisms associated with spoilage of meat and processed products. During storage, the process of microbial contamination of meat is influenced by various internal and external factors, in particular, the presence of oxygen, pH level, temperature, and competing microorganisms in the environment. The diversity of these ecophysiological factors affects microbial growth dynamics, including the composition and ratio of the microbiota, which ultimately affects the type and rate of meat spoilage processes. K. Koutsoumanis *et al.* (2023) indicate that the main bacteria that cause meat spoilage include *Pseudomonas spp., Lactobacillus spp. Enterococcus spp., Weissella spp., Brochothrix spp., Leuconostoc spp., Lactobacillus spp., Shewanella spp., and Clostridium spp*. According to the traditional practice of primary processing of carcasses, microbiological risks that cause spoilage can affect the maturation of meat. Keeping meat under controlled conditions can reduce the burden of microbiological hazards to a certain extent.

In a study by A. Nakamura *et al.* (2023), it is noted that a fast decrease in the temperature of the meat before it reaches the proper level of acidification can lead to weight loss in the carcass. Shrinkage during rapid cooling can particularly affect carcasses obtained from high-performance meat breeds of pigs due to the lower subcutaneous fat content and faster heat release. A substantial number of technologies for their primary processing are used to reduce the weight loss of slaughtered animal carcasses, in particular, the use of washing, which allows quickly reducing the temperature of the half-carcass and the intensity of moisture evaporation. Therewith, this does not allow avoiding contamination of carcasses with microflora, which occurs during the time of slaughter of animals and subsequent operations of their primary processing. There is exogenous and endogenous contamination with microorganisms, the sources of which can be the skin, the contents of the digestive system, the air environment, technological equipment, vehicles, tools, hands, clothing, and shoes of workers who have contact with meat, and water used for processing carcasses. The air microflora in animal slaughtering and primary processing of carcasses is most often represented by various spore microflora, in particular, aerobic and anaerobic bacteria, and gram-negative non-spore rods, actinomycetes, mould fungi, and yeast. They get to the surface of meat from the external environment during skinning and subsequent stages of primary processing of slaughtered animals.

V. Vovkotrub *et al.* (2023) proved that one of the promising ways to reduce meat contamination are biotechnologies based on using lactic acid microorganisms of various strains that can show antagonism to putrefactive and pathogenic microflora. These cultures include SafePro® B-SF-43 (*Leuconostoc carnosum*) and SafePro® B-2 (*Lactobacillus sakei*) strains, the use of which in a dose of 10^7/cm^2 in half-carcasses of pork helped to reduce the intensity of autolysis of muscle tissue and, thus, extend the shelf life of meat to 7 days in a chilled form.

In most studies, lactic acid microorganisms are used in the technology of boiled or dried meat products, but for fresh meat, in particular, pork, they have not become widespread, which is due to the lack of scientifically based data on the effect of ferment cultures on the quality and safety of meat for storage in a chilled state. The purpose of the study is to determine the quality and safety indicators of pork in half-carcasses during cooling in the refrigerator compartment using washing and final treatment with suspensions *Lactobacillus sakei* and *Leuconostoc carnosum*.

Literature Review

Meat is a source of protein in the human diet, but simultaneously – a source of spread of pathogens of food origin around the world. Microbial contamination of meat can cause food infections, toxicoinfections, and toxicosis in humans. K. Klaharn *et al.* (2022) and Z. Rani *et al.* (2023) note that the risks associated with the threat to consumers of pathogenic microorganisms transmitted through meat require not only the examination of their distribution chains but also measures to prevent potential human diseases.

Under such conditions, there is a need to develop and implement scientifically based ways to reduce microbial contamination of animal carcasses using various means with an antimicrobial spectrum of action, in particular, organic acids, chlorine compounds, bacteriocins, essential oils, as described by F. Soyer (2020), Z.A. Ellatif *et al.* (2020), D.F. Benítez-Chao *et al.* (2021), and combining them with physical methods of meat decontamination. Because the above methods of reducing microbial contamination of meat contribute to the emergence of changes in the sensory properties of both fresh meat and products made from it, technologies that guarantee the safety of meat and improve its organoleptic characteristics come to the fore. S. Soltani *et al.* (2022) believe that one of the most promising ways to address this issue, along with good hygiene practices, is the treatment of the surface of half-carcasses of slaughtered animals with natural preservatives, in particular, suspensions of lactic acid bacteria, which can show antagonistic activity against undesirable and pathogenic microorganisms.

Bioconservation is a promising way to control the growth of unwanted bacteria on fresh meat. It is based on the ability to inhibit the growth of bacteria that spoil meat and thus increase the shelf life of meat. This aspect is much less studied compared to using an approach aimed at eliminating pathogenic bacteria in meat and processed products. Equally important is the influence of these microorganisms on the sensory characteristics of meat that attract the consumer. Studies have shown that microbial ferment cultures can delay the growth of bacteria that spoil meat, in particular, *Brochothrix thermosphacta*, *Pseudomonas* spp., and *Enterobacteriaceae*. However, despite the fact that the examination results give grounds for the introduction of such a technology in meat production, the concept of bioconservation using microorganisms for fresh meat is still limited. This is due to a lack of research in this area, especially in relation to the primary processing of pork. In addition, according to M.M. Xu *et al.* (2022), bioconservatives based on microbial ferment cultures require additional testing under conditions typical of specific meat processing plants.

The most promising microorganisms that can be used for primary processing and storage of pork include lactic acid bacteria of specialised strains that can form bacteriocins, have an antagonistic effect against pathogenic and conditionally pathogenic microorganisms, and improve the organoleptic properties of meat, especially during its storage in a chilled state. These microorganisms include bacteria of the *Lactobacillus* genus, in particular, *Lactobacillus sakei* (Costa *et al.*, 2019) and *Leuconostoc carnosum* (Raimondi *et al.,* 2019). As noted by A. Najjari *et al.* (2020), *Lactobacillus sakei* can withstand a substantial temperature range, pH level, and table salt content in the medium up to 8% and dominate among other microflora. In addition, this lactic acid microorganism forms a number of organic acids and aromatic compounds that give flavour to meat products, which makes it promising for use in meat production technology. *Leuconostoc carnosum* can grow at temperatures compatible with cooling and in the presence of up to 60 g/L of sodium chloride (NaCl) in many meat-based products.

Materials and Methods

The experiment used half-carcasses of pork, which were obtained after slaughter and primary processing in the conditions of LLC "Antonivsky meat processing plant", Kyiv region, during May 2021. 30 fattening pigs aged 6 months were used for slaughter. The slaughter of pigs was conducted in compliance with the current Order No. 28 "Rules of Pre-Slaughter Veterinary Inspection of Animals and Veterinary-Sanitary Examination of Meat and Meat Products" (2002), good hygiene practices (GMP). After slaughter, half-carcasses of pigs were transported to specialised cold storage rooms for cooling and further storage. The resulting pork half-carcasses were divided into 3 groups of 10 half-carcasses each, which were processed according to the scheme shown in Table 1. Suspensions of lactic acid microorganisms *Leuconostoc carnosum* and *Lactobacillus sakei* produced by *Chr. Hansen* (LLC "Chr. Hansen Ukraine") were used to treat the surface of pork half-carcasses.

Table 1. Scheme of the experiment to determine the effect of ferment cultures of lactic acid microorganisms on the freshness of pork during storage in a chilled state

Group	Experiment conditions
Control	Half-carcasses of pork are subjected to a wet toilet and washing
Experimental 1	Half-carcasses of pork are subjected to wet toilet and washing with the final treatment with ferment culture of the SafePro® B-SF-43 (*Leuconostoc carnosum*) strain at a dose of 10^7/cm^2
Experimental 2	Half-carcasses of pork are subjected to wet toilet and washing with the final treatment with ferment culture of the SafePro® B-2 (*Lactobacillus sakei*) at a dose of 10^7/cm^2

Source*: compiled by the authors*

Ferment cultures of lactic acid microorganisms were applied to the surface of the half-carcasses after the washing process ended. All half-carcasses of pork were stored in the industrial refrigerator of the meat processing plant at a temperature of 3±1°C. The results of the study were recorded 1 hour after cooling, on the 4th and 7th days of storage of half-carcasses. The mass of half-carcasses of pork was determined in a meat processing plant using industrial scales TV4-1500 with an accuracy of 0.1 kg. The temperature and pH of the meat were examined using EZODO MP-103M pH meter (Taiwan, GOnDO'Electronic Co., Ltd).

All microbiological studies were conducted in the Bila Tserkva city state laboratory of the State Food Safety and Consumer Protection Service of Ukraine. For determining the number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAM), bacteria of the *Salmonella spp., S. aureus*, *Lactobacillus*, *L. monocytogenes* genera, fungi, and mould were washed off from the surface of half-carcasses in the area of the collar. Consecutive ninefold dilutions using sterile saline solution were prepared for the study. The number of microorganisms in meat samples was determined in colony-forming units (CFU), and the results are presented in lg CFU/cm^2 of the surface. Generic and specific identification of microorganisms isolated from the meat of half-carcasses of the control and both experimental groups was conducted in accordance with the current methods.

The Plate count agar M091 medium (HiMedia, India) was used to determine the number of MAFAM. For isolation and quantitative counting of bacteria of the *Lactobacillus* genus – Lactobacillus MRS Agar M641 (HiMedia, India), for isolation of pathogenic and non-pathogenic staphylococci – Baird Parker, Agar M043

(HiMedia, India), for *Salmonella* – Bismuth Sulfite Agar M027 (HiMedia, India) and Xylose Lysine Deoxycholate Agar M031 (HiMedia, India), for *L. monocytogenes* – Agar Palcam, Agar Oxford (HiMedia, India). Organoleptic assessment of pork (colour, smell, consistency, cooking sample) was determined according to DSTU 7992:2015 (2017). Microscopic analysis of meat freshness was conducted in accordance with GOST 23392:2016 (2017). Moisture-retaining ability of meat was determined by pressing. Meat sampling of slaughtered animals and their preparation for microbiological tests were conducted in accordance with the requirements of DSTU 8381:2015 (2017). The selection of washes from the surface of half-carcasses of meat stored in the cold storage rooms of the meat processing plant was conducted in the collar area in accordance with the requirements of DSTU ISO 17604:2014 (2015). Statistical processing of the examination results was performed using the ANOVA programme, and the data in the tables are presented as x±SD (mean ± standard deviation). The difference between the groups was considered likely using the Tukey Test at $P<0.05$ (considering the Bonferroni correction).

Results and Discussion

Cooling pork in the refrigerator in combination with washing affected the appearance of the paired half-carcasses – the surface is wet, pale, and without a drying crust. When processing pork with microbial ferment cultures of SafePro® B-SF-443 (*Leuconostoc carnosum*) and SafePro® B-2 (*Lactobacillus sakei*) strains at a dose of 10^7/cm^2, it was determined that on the fourth day of storage, organoleptic indicators: the appearance, colour, consistency, and smell of meat of half-carcasses of the control and experimental groups did not differ. The meat had a pink colour, inherent in pork, on the cut – quite dense and elastic, and the smell was specific, characteristic of fresh meat. Organoleptic parameters of meat on the 4th day of storage of half-carcasses in these groups, compared with the initial data immediately after their washing (in paired half-carcasses) and ferment treatment, substantially improved. They have acquired an attractive pink colour, and their appearance is characteristic of pork.

As noted by S. Ali & A.F. Alsayeqh (2022), the degree of microbial contamination is one of the important indicators of meat safety, especially when storing it in a chilled state. Microscopic analysis of prints of pork samples from both the control and experimental groups on the 4th day of storage revealed that the number of microorganisms in the field of view did not depend on the method of processing half-carcasses (Table 2) and was within the limits characteristic of Information of N. Bogatko (2019) for chilled meat.

Table 2. Indicators of microscopic analysis of smears and moisture capacity of pork on the 4th day of storage, x±SD, n=5

Group	Number of microorganisms in the field of view	Moisture capacity, mg
Control	24.60 ± 4.15^a	32.44 ± 5.31^a
Experimental 1	9.20 ± 4.21^a	32.44 ± 3.87^a
Experimental 2	17.75 ± 3.69^a	52.28 ± 9.67^a

Note: *different superscript letters a, b indicate the values that considerably differed in the same row of the table (P<0.05) based on the results of comparison using the Tukey test*

Source*: compiled by the authors*

Half-carcass washing, in this case, did not affect the microbial contamination of pork, which is consistent with the results obtained by I.F. Jaja *et al.* (2018), before and after washing beef, lamb, and pork. In particular, as noted in this study, the most contaminated areas were the neck, sides, and chest, and washing carcasses did not change the amount of sanitary microflora in the neck and sternum of pork and beef carcasses. The largest number of *E. coli* colonies was identified after washing the samples in the neck area, and the highest number of *S. aureus* colonies was isolated from the washes on the sides. One of the main technological characteristics of meat is its water retention capacity since it substantially affects the yield of the finished product. Water retention capacity of meat, according to the data, obtained by B. Lebret & M. Čandek-Potokar (2022), is mainly determined by the changes that occur in it after slaughter, especially the rate and degree of pH decrease. The moisture capacity of pork in half-carcasses in the chilled form on the 4th day of storage did not substantially differ between the control and experimental groups. Therewith, the final treatment of half-carcasses of pork with ferment culture of the SafePro® B-2 (*Lactobacillus sakei*) strain at a dose of 10^7/cm^2 contributed to the tendency to increase the moisture capacity of meat, which indicates some improvement in its technological properties. The temperature in the thickness of half-carcasses of pork in both the control group and both experimental groups on the 7th day of storage did not substantially differ, which is associated with their storage at a stable temperature in one refrigerator at a temperature of 3±1°C (Table 3).

Table 3. Temperature and pH value of pork in half-carcasses on the 7th day of storage, x±SD, n=5

Group	Temperature in the thickness of the half-carcass, °C	pH of meat, units
Control	4.41 ± 0.17^a	6.71 ± 0.16^a
Experimental 1	4.39 ± 0.23^a	5.90 ± 0.12^b
Experimental 2	4.30 ± 0.10^a	5.86 ± 0.14^b

Note: *different superscript letters a, b indicate the values that considerably differed in the same row of the table (P<0.05) based on the results of comparison using the Tukey test*
Source*: compiled by the authors*

One of the critical indicators that indicate the freshness of meat is its pH value, which, in the case of cooling half-carcasses of pork in the refrigerator using washing and final treatment with ferment SafePro® B-SF-43 (*Leuconostoc carnosum*) and SafePro® B-2 (*Lactobacillus sakei*) strains at a dose of 10^7/cm^2 on the 7th day of storage was lower ($P<0.05$) by 0.81 and 0.85 units compared to the control. Therewith, on the 7th day of storage, the pH value of pork in both experimental groups was within the limits characteristic of fresh meat, and in the control group – higher and indicated the beginning of its spoilage. Notably, unlike live animals, which have a muscle tissue pH value in the range of 7.0-7.2, after slaughter, as a result of oxygen deficiency, anaerobic processes begin to prevail over aerobic ones, which are accompanied by the accumulation of lactic acid and rapid acidification of muscle tissue. Although carbohydrates make up a small percentage of muscle tissue (0.5-1.5% of muscle mass), they play an essential role in converting muscle into meat. In particular, glucose and glycogen are important for muscle metabolism and can be used in both aerobic and anaerobic environments. C. Álvarez *et al.* (2019) report that glycogen is converted to ATP with the accumulation of lactic acid until the pH value of meat reaches a 5.6-5.3.

The optimal pH value of pork is considered to be 5.6-5.9 units, which, according to A. Zmudzińska *et al.* (2020) and H. Jankowiak *et al.* (2021), indicates its freshness and proper quality. All properties of meat are directly or indirectly determined by the intensity of biochemical processes that occur in the muscle tissue of the animal during life, after slaughter, and during the maturation of meat, and the pH value of the medium largely regulates their course. The level of acidification (pH value) is associated with changes in muscle protein hydration and in the binding and retention of water in meat, which ultimately determines its tenderness. In addition, the changes in the pH values in pork during chilled storage reflect to a certain extent the ratio, total number, and metabolic activity of the dominant microorganisms that cause meat spoilage, in particular, species belonging to the *Pseudomonas* genus which are known for their proteolytic activity. This, in turn, can lead to the formation of alkaline bacterial metabolites, which lead to a shift in the pH value of meat to the alkaline side (Fengou *et al.,* 2019). Such changes were identified in the experiment in pork of the control group.

The obtained data on changes in the pH value of pork cooled in a refrigerator using washing and treatment of half-carcasses with ferment cultures of lactic acid microorganisms are consistent with the indicators of organoleptic research of meat of the control and both experimental groups (Table 4).

Table 4. Organoleptic parameters of pork meat on the 7th day of storage, n=10

Group	Appearance, colour	Consistency	Smell
Control	the surface is moistened, slightly sticky, darkened	the meat is not dense and elastic enough, the hole is made slowly during pressing (within 1 min.), the fat is soft.	slightly sour or with a hint of mustiness
Experimental 1 Experimental 2	the meat is pink or pale pink in colour, the surface is shiny, without pronounced stickiness	on the cut, the meat is dense, elastic, the hole is quickly filled when pressed.	specific, characteristic of pork that is ripe

Source*: compiled by the authors*

Thus, pork meat in half-carcasses of the first and second experimental groups showed signs of fresh. The meat of half-carcasses of pork in the control group was classified as questionable freshness according to the main organoleptic parameters since it showed signs of spoilage. Signs of meat spoilage that were observed during the storage of half-carcasses of pork in the control group include, according to information of Y. Zhu *et al*. (2022), substantial discolouration, unpleasant odours, and mucus formation, which define the main criteria for consumers' rejection of this food product. Considering the data of organoleptic studies, pork samples from half-carcasses of experimental groups were taken for further analysis of smear-preparations. The number of microorganisms in smears-preparation of pork that was cooled in a refrigerator using washing and a final treatment with ferment culture of the SafePro® B-SF-43 (*Leuconostoc carnosum*) strain, on the 7th day of storage was 2 times higher ($P<0.05$), than when treating the culture of SafePro® B-2 (*Lactobacillus sakei*) strain with ferment (Table 5).

Table 5. Indicators of microscopic analysis of smears-impressions and moisture capacity of pork for 7 days of storage of half-carcasses, x±SD, n=5

Group	Number of microorganisms in the field of view	Moisture capacity, mg
Experimental 1	505.67 ± 25.00^{b}	40.63 ± 9.94^{a}
Experimental 2	251.67 ± 16.42^{a}	46.53 ± 4.25^{a}

Note: *different superscript letters a, b indicate the values that considerably differed in the same row of the table (P<0.05) based on the results of comparison using the Tukey test*

Source*: compiled by the authors*

An increase in the number of microorganisms in smears-prints indicates the questionable freshness of meat, but the reason for such a large number of bacteria in the field of view of the microscope, in this case, was the processing of pork in half-carcasses with cultures of lactic acid bacteria that suppress putrefactive microflora and can penetrate the thickness for 7 days of its storage. Therewith, the absence of tissue breakdown, the presence of delineation of muscle fibres and organoleptic research data indicate the proper freshness of this meat. The moisture content of pork during this period did not substantially differ between the experimental groups (Table 5) and was in the optimal range characteristic of the meat of young pigs, which, according to O.M. Iakubchak *et al.* (2005), ranges from 38 to 48%. This indicator can be influenced by a number of factors: growing conditions, fattening, breed, primary processing, meat storage, and microflora contamination.

Although the surface of half-carcasses of pork of experimental groups was treated with suspensions of lactic acid bacteria of SafePro® B-2 (*Lactobacillus sakei*) and SafePro® B-SF-43 (*Leuconostoc carnosum*) strains at a dose of 10^7/cm^2, the number of MAFAM on the meat surface decreased ($P<0.05$) by 1.25 lg CFU/cm^2 and 0.65 lg CFU/cm^2, respectively, compared to the control. Therewith, the number of MAFAM on the surface of the meat of half carcasses of pork, which was subject to the final surface treatment with ferment culture of the SafePro® B-2 (*Lactobacillus sakei*) strain, increased ($P<0.05$) by 0.6 lg CFU/cm^2 compared to the same indicator of half-carcasses that were treated with ferment culture of the SafePro® B-SF-43 (*Leuconostoc carnosum*) strain (Table 6).

Table 6. Microbiological parameters of pork in paired half-carcasses, x±SD, n=5, lg CFU/cm^2

Group	MAFAM	Lactic acid bacteria
Control	5.29 ± 0.91^{a}	2.51 ± 0.94^{a}
Experimental 1	4.04 ± 0.67^{b}	5.98 ± 0.32^{b}
Experimental 2	4.64 ± 0.23^{c}	5.94 ± 0.51^{b}

Note: *different superscript letters a, b indicate the values that considerably differed in the same table row (P<0.05) based on the results of comparison using the Tukey test*

Source*: compiled by the authors*

The number of lactic acid bacteria on the surface of paired pork half-carcasses, which were treated with suspensions of lactic acid bacteria of SafePro® B-2 (*Lactobacillus sakei*) and SafePro® B-SF-43 (*Leuconostoc carnosum*) strains during cooling at a dose of 10^7/cm^2, increased ($P<0.05$) by 3.47 lg CFU/cm^2 and 3.43 lg CFU/cm^2, respectively, compared to the control group (Table 6). This is due to the ability of lactic acid microorganisms to penetrate the thickness of meat and show an antagonistic effect on other types of microbiota. The most common approach to producing quality meat is to use agents that have antimicrobial effects against bacteria responsible for food spoilage. The ideal bioconservation agent should exhibit only specific antimicrobial activity against targeted pathogens or microorganisms that cause spoilage and should not negatively affect the consumer's own gut microbiome.Lactic acid microorganisms *L. carnosum* and *L. sakei* correspond to these characteristics Therewith, an important property of *L. carnosum* and *L. sakei* is the ability to rapidly increase the number of colonies in food products, in particular, meat, acidify the environment in combination with antibacterial, bacteriostatic, and bactericidal properties due to the formation of organic acids, hydrogen peroxide, and bacteriocins (Barcenilla *et al.*, 2022). High adaptation of *Leuconostoc carnosum* and *Lactobacillus sakei*, as noted by M. Zagorec & M.C. Champomier-Vergès (2017) and F. Candeliere *et al.* (2021), also contributes to a nitrogen-rich environment, such as meat. Colonisation of meat with individual strains of these lactic acid microorganisms avoids changes in its sensory characteristics during storage in a chilled form.

In addition, meat, as noted by O.A. Odeyemi *et al.* (2020), is an ideal substrate for microbes due to its rich nutrient density, favourable water activity and pH value. On the 4^{th} day of storage of pork, which was treated with suspensions of lactic acid bacteria of SafePro® B-2 (*Lactobacillus sakei*) and SafePro® B-SF-43 (*Leuconostoc carnosum*) strains during cooling at a dose of 10^7/cm^2, a lower number of MAFAM was identified at 0.82 lg CFU/cm^2 and 0.53 lg CFU/cm^2 compared to control. Simultaneously, in pork of both experimental groups, which was] subjected to final treatment with suspensions of lactic acid microorganisms, an increase ($P<0.05$) in the number of lactic acid bacteria was observed by 1.65 lg Kuo/cm^2 and 1.17 lg Kuo/cm^2 compared to the control (Table 7).

Table 7. Microbiological parameters of pork in half-carcasses on the 4^{th} day of storage, x±SD, n=5, lg CFU/cm^2

Group	MAFAM	Lactic acid bacteria
Control	$5.34{\pm}0.87^a$	$4.08{\pm}0.16^a$
Experimental 1	$4.52{\pm}0.36^b$	$5.73{\pm}0.23^b$
Experimental 2	$4.51{\pm}0.07^b$	$5.25{\pm}0.18^b$

Note: *different superscript letters a, b indicate the values that considerably differed in the same row of the table (P<0.05) based on the results of comparison using the Tukey test*
Source*: compiled by the authors*

It was proved that in pork that was cooled in a refrigerator using washing without treatment with suspensions of lactic acid microorganisms (control group), lactic acid microorganisms dominated on the 4^{th} day of storage. The same pattern was found by C. Braley *et al.* (2023) for storing pork in vacuum packaging on days 15 and 29 at a temperature regime of -1.5°C, which was changed according to production risks at 2°C or 10°C for several hours. However, in this case, the researchers note that in contrast to SafePro® B-2 (*Lactobacillus sakei*) and SafePro® B-SF-43 (*Leuconostoc carnosum*) strain cultures, most types of lactic acid microorganisms belong to the microflora that cause spoilage of meat (EFSA Panel on Biological Hazards (BIOHAZ) (2023). Therewith, it is proved that after applying cultures of lactic acid microorganisms of the above-mentioned strains to half-carcasses for 4 days of storage in a chilled state, their number practically does not increase. This is regarded as a positive fact, provided that these lactic acid microorganisms, to some extent, restrain the development of other microflora, especially mould fungi and yeast, and do not form mucus and fermentation products with an unpleasant smell.

According to C.H. Jeong *et al.* (2022) and S.G. Kim & S.Y. Kim (2023), the property of individual strains of lactic acid microorganisms has been used for quite a long time for the production of various meat products, in particular, sausages and dried ham. However, in this case, it is desirable to achieve a substantial increase in the number of lactic acid bacteria that are involved in the fermentation of the food product. Thus, it not only ensures its preservation but also the maturation and acquisition of specific sensory properties.In the case of processing half-carcasses of pork with suspensions of lactic acid microorganisms SafePro® B-2 (*Lactobacillus sakei*) and SafePro® B-SF-43 (*Leuconostoc carnosum*) as bioconservants, the goal is to achieve a stable number of them in the product, which guarantees the suppression of the development and reproduction of unwanted microflora, including other types of lactic acid microorganisms that cause meat spoilage, but does not provide for their further rapid growth. This method of bioconservation of pork half-carcasses during primary processing and cooling has not yet become widespread in meat processing enterprises.

Considering the fact that the pork of the control group, which was subjected to cooling in a refrigerator using washing, according to organoleptic parameters, is not suitable for further storage, microbiological studies on the 7th day of storage were conducted on only half of the experimental groups. The data obtained in the course of the study are consistent with similar results of M. Kukhtyn *et al.* (2020), noted for storing beef chilled. Therewith, the authors established that storage of beef meat with an initial amount of mesophilic bacteria of about 4.88 log CFU/cm^2 and surface and psychrotrophic bacteria – 3.79 log CFU/cm^2 at a temperature of 0°C is possible only for 8 days. Further, microbiological indicators exceed the permissible norms and such meat is considered unsuitable. The number of MAFAM per 7 days of storage in pork during the final ferment treatment of the SafePro® B-SF-43 (*Leuconostoc carnosum*) strain culture increased (P<0.05) compared to similar indicators of pork during the final treatment of half-carcasses with a suspension of lactic acid microorganisms of the SafePro® B-2 (*Lactobacillus sakei*) strain (Table 8).

Table 8. Microbiological indicators of pork in half-carcasses by 7 storage day, x±SD, n=5, lg CFU/cm^2

Group	MAFAM	Lactic acid bacteria
Experimental 1	5.62±0.35^b	4.80±0.88^b
Experimental 2	4.82±0.51^a	5.47±0.08^a

Note: *different superscript letters a, b indicate the values that considerably differed in the same row of the table (P<0.05) based on the results of comparison using the Tukey test*

Source*: compiled by the authors*

Therewith, the number of lactic acid microorganisms in pork treated with ferment culture of the SafePro® B-SF-43 (*Leuconostoc carnosum*) strain decreased by 0.67 lg CFU/cm^2 (P<0.05) compared to pork treated with a suspension of lactic acid microorganisms of the SafePro® B-2

(*Lactobacillus sakei*) strain. It is difficult to compare the research results obtained with similar data from other researchers since they were performed on meat processing products and using different packaging options. In this case, S. Raimondi *et al.* (2019) established that the number of *Leuconostoc carnosum* in packaged meat products, in particular, in boiled ham, increases from the first days of the shelf life to several weeks, reaching its peak by 10^8 cells/g and dominates the microflora until the end of the product's shelf life. In an experiment on pork in half-carcasses treated with a spray suspension of the SafePro® B-SF-43 (*Leuconostoc carnosum*), this effect was not observed during the meat storage period.

This fact indicates the specificity of various strains of this microorganism and selective suitability for processing raw meat, in particular, half-carcasses of pork. Although the microorganisms of the SafePro® B-2 (*Lactobacillus sakei*) strain were not characterised by rapid reproduction in meat stored in a refrigerator, their numbers were fairly stable throughout the entire study period (Table 8). Furthermore, A. Najjari *et al.* (2020) indicate that microorganisms of this type, can substantially improve the organoleptic characteristics of meat products by creating an attractive aroma, in particular, during the fermentation of dry sausages. Therewith, the resistance of these microorganisms to bile acids, which, against the background of their entry into the small intestine and the ability to form bacteriocins, ensures the performance of a probiotic function and prevents colonisation by pathogenic microflora of the consumer's body. Therefore, it is necessary to focus further research on using ferment cultures of lactic acid microorganisms for preserving pork in half-carcasses after eliminating the risks of meat contamination at this meat processing enterprise and improving good hygiene practices.

Colonies of *S. aureus, Salmonella spp*. and *L. monocytogenes* were not identified on the surface of paired half-carcasses of pork of the control group and both experimental groups during the entire storage period, which indicates the safety of meat according to these pathogens. The data obtained do not contradict the results of the paper by T. Jacobsen *et al.* (2003), which indicate the advantages of using live lactic acid microorganisms in meat product technology over white blood cells or bacteriocins to suppress pathogenic microorganisms, especially *L. monocytogenes*. This is due to ensuring the stability of the number of colonies of lactic acid microorganisms in the food product, in contrast to the use of biotechnological synthesis products that can be destroyed during storage. Notably, the latest studies of a number of researchers, in particular, D.-M. Meng *et al.* (2020; 2021), are devoted to the use of impure cultures of living microorganisms and preservatives of microbial synthesis for storing pork in chilled, packaged form, in particular, Hispidalin and Mitihitin-A. These results indicate a reduction in meat weight loss during storage and suppression of pathogenic microflora, preventing an increase in pH and meat spoilage. However, the effectiveness of these preservatives for processing whole carcasses or half-carcasses of slaughtered animals during chilled storage is not fully understood.

Notably, each meat processing enterprise has its own microbial association, which must be considered when developing measures to improve hygiene practices. A comprehensive approach is needed to reduce the risks of contamination of meat with microorganisms, especially pork in half-carcasses that are stored in chilled form, which will allow combining measures to reduce air contamination of meat processing shops, equipment, and the pork itself during primary processing and, thus, increase the duration of its storage in fresh form.

Conclusions

Cooling half-carcasses using washing and final treatment with suspensions of lactic acid microorganisms of SafePro® B-SF-43 (*Leuconostoc carnosum*) and SafePro® B-2 (*Lactobacillus sakei*) strains at a dose of 10^7/cm^2 to extend the shelf life of pork in half-carcasses in chilled form up to 7 days and preserve its freshness is promising.

Cooling half-carcasses of pork in the refrigerator using washing without additional treatment with bioconservants contributes to meat spoilage already on the 4th day of storage. The use of washing to cool half-carcasses of pork in the refrigerator in combination with final treatment with suspensions of bacterial ferment cultures of SafePro® B-SF-43 (*Leuconostoc carnosum*) and SafePro® B-2 (*Lactobacillus sakei*) strains at a dose of 10^7/cm^2 contributed to a decrease in the number of MAFAM in fresh pork meat by 1.25 and 0.65 lg CFU/cm^2, respectively, with a simultaneous increase in the number of lactic acid bacteria by 3.47 and 3.43 lg CFU/cm^2, respectively, compared to the control. Storage of pork that was cooled using washing caused an increase in the number of MAFAM on day 4, which led to signs of meat spoilage. Pork in half-carcasses, which was subjected to final treatment with suspensions of bacterial ferment cultures of SafePro® B-SF-43 *(Leuconostoc carnosum*) and SafePro® B-2 (*Lactobacillus sakei*) strains, was characterised by a lower number of MAFAM by 0.82 and 0.53 lg CFU/cm^2 with the increasing number of lactic acid bacteria by 1.65 and 1.17 lg CFU/cm^2, respectively, compared to the control. Surface treatment of pork half carcasses with suspensions of bacterial ferment cultures of SafePro® B-SF-43 (*Leuconostoc carnosum*) and SafePro® B-2 (*Lactobacillus sakei*) strains at a dose of 10^7/cm^2 provided a stable number and dominance of lactic acid microorganisms in meat during 7 days of storage in a chilled state. Cooling of half-carcasses of pork in the refrigerator using washing and final treatment of the surface of half-carcasses with suspensions of lactic acid microorganisms of SafePro® B-SF-43 (*Leuconostoc carnosum*) and SafePro® B-2 (*Lactobacillus sakei*) strains at a dose of 10^7/cm^2 created conditions for ensuring the pH value of meat on the 7th day of storage at a level characteristic of high-quality meat and did not affect its moisture-retaining ability. The use of processing half-carcasses of pork with suspensions of lactic acid microorganisms will allow increasing the shelf life of chilled pork while maintaining its quality and ensuring safety. A promising area of research is to determine the effectiveness of cold water washing of pork in half-carcasses in combination with treatment with suspensions of lactic acid microorganisms and physical methods of meat bioconservation aimed at improving organoleptic parameters and preserving its freshness and biological value.

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None.

Conflict of Interest

None.

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Вплив заквасок молочнокислих бактерій на свіжість свинини

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Анотація. Під час первинної переробки свинини на етапі охолодження півтуш відбувається втрата їх маси, що призводить до економічних збитків. Одним із перспективних шляхів вирішення цієї проблеми є використання душування півтуш охолодженою водою, що потребує застосування засобів деконтамінації м'яса від мікрофлори, яка спричиняє його псування. Мета дослідження – визначити ефективність заключної обробки півтуш свинини заквасками культур штамів SafePro® B-SF-43 (*Leuconostoc carnosum*) і SafePro® B-2 (*Lactobacillus sakei*) для їх біоконсервації та збереження свіжості. В роботі використано органолептичні, хімічні та мікробіологічні методи дослідження. Встановлено, що охолодження півтуш свинини в холодильній камері з використанням душування холодною водою сприяє виникненню ознак псування м'яса за рівнем мікробної контамінації та величиною pH вже на 4 добу зберігання. Обробка поверхні півтуш суспензіями молочнокислих мікроорганізмів штамів SafePro® B-SF-43 (*Leuconostoc carnosum*) і SafePro® B-2 (*Lactobacillus sakei*) у дозі 10^7/см^2 забезпечує належні органолептичні показники і величину pH свинини до 7 доби зберігання, які відповідають якісному м’ясу. Обидві закваски молочнокислих мікроорганізмів знижують мікробну контамінацію м’яса за рахунок чисельності мезофільних аеробних і факультативно-анаеробних мікроорганізмів у парних тушах на 1,25 та 0,65 lg КУО/см^2 та збільшують чисельність молочнокислих мікроорганізмів на 3.47 і 3.43 lg КУО/см^2 відповідно, що дозволяє продовжити термін зберігання м’яса в охолодженому вигляді не менше 7 діб. Для заключної обробки півтуш свинини за охолодження в холодильній камері у поєднанні з душуванням охолодженою водою найпридатнішою є культура штаму SafePro® B-2 (*Lactobacillus sakei*). Отримані результати досліджень є перспективними для удосконалення технології первинної переробки свинини на етапі охолодження з використанням заквасок молочнокислих мікроорганізмів в якості натуральних консервантів, що дозволить збільшити термін зберігання м’яса з урахуванням його якості та безпечності

Ключові слова: м’ясо; охолодження; душування; зберігання; мікробна контамінація