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Influence of starter cultures of lactic acid bacteria on microbiological parameters and shelf life of sausages

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ABSTRACT

The main spoilage microorganisms of the vacuum-packaged sausages on the first day of chilled storage are the bacteria of the following families: Enterobacteriaceae (*Raoultella planticola, Raoultella ornithinolytica*, and *Citrobacter freundii*), Morganellaceae (*Morganella morganii*) and Staphylococcaceae (*Macrococcus caseolyticus*), and at the end of the shelf life (on the twenty-first day) - Enterobacteriaceae (*Proteus mirabilis, Moellerella wisconsensis* and *Serratia liquefaciens*). An appearance of cloudy juice, surface slime and delamination of the vacuum packaging characterises the sausage spoilage. QMAFAnM in the sausages was increased by 1.09 lg CFU/g and 1.53 lg CFU/g on the first day of storage, by 1.18 lg CFU/g and 1.54 lg CFU/g on the twelfth day, by 0.92 lg CFU/g and 1.96 lg CFU/g on the eighteenth day, respectively, compared to the control sample, because "Vienna sausages with chicken fillet" were treated with starter culture SafePro BLC-48 (*Lactobacillus curvatus*) or the mixture of starter cultures SafePro BLC-48 (*Lactobacillus curvatus*) + Bactoferm Rubis (*Lactococcus lactis subsp. Lactis*) before vacuum packaging. Because the sausages were treated with the mixture of starter cultures SafePro BLC-48 + Bactoferm Rubis, the lactic-acid microorganisms were increased by 0.63 lg CFU/g and 0.53 lg CFU/g on the twenty-fifth and thirtieth days, respectively, compared to the sausages that were treated with SafePro BLC-48. During the entire shelf life, no pathogenic and opportunistic pathogenic bacteria, in particular *S. aureus, L. monocytogenes, Salmonella spp., E. coli*, coliform bacteria, as well as yeast and mold, were detected in the sausages under all treatment options. The use of starter culture SafePro BLC-48 (*Lactobacillus curvatus*) or the mixture of starter cultures SafePro BLC-48 (*Lactobacillus curvatus*) + Bactoferm Rubis (*Lactococcus lactis subsp. Lactis*) increases the shelf life of the vacuum-packaged sausages if they are kept in a refrigerator for up to 30 days, which is 12 days longer than their shelf life without treatment. The sausage treatment with the mixed starter cultures of the lactic-acid microorganisms may be promising if the development of the aerobic spoilage bacteria is confirmed.

Keywords: safety, spoilage, vacuum packaging, minced meat, cooked meat products

INTRODUCTION

Animal meat is a valuable source of protein, lipids and biologically active substances **[1]**. It is processed into cold smoked meats, meatballs, sausages, nuggets and meat loaves, but the sausages remain one of the most popular products for consumers. Cooked meat products like sausages have a short shelf life and can spoil quickly. This not only leads to an increased risk to the consumer's health but also causes economic losses for the meat industry. The shelf life of the sausages depends on the quality and microbial contamination of raw materials, the casing type, and the packaging and storage conditions **[2]**.

The spoilage microflora of the meat products, in particular the sausages, consists of *Staphylococcus aureus, Streptococcus sp., Escherichia coli, Campylobacter jejuni, Shigella spp., Salmonella spp., Listeria monocytogenes, Clostridium botulinum/perfringens, Bacillus cereus*, which can be dangerous to the consumer health **[3]**, **[4]**. The microflora sources of the sausage products are the raw materials and technological operations of preparation and processing: carcass cutting, deboning, trimming, salt pickling, making of minced meat, filling of sausage casing with minced meat, as well as the air environment, staff hands, and technological equipment.

Reduced microbial contamination of sausages is the main condition for maintaining their quality, consumer demand, and safety **[5]**, **[6]**, **[7]**.

The most common preservation method of cooked meat products is to add chemicals to the raw materials. Synthetic chemical preservatives are widely used in the food industry to prevent spoilage caused by increased microorganisms, enzyme activity, and oxidative processes in cooked sausages. However, their consumer appeal is declining due to potential health risks and changes in organoleptic properties caused by synthetic preservatives in food products, particularly sausages **[8]**.

One of the most promising methods of reducing microbial contamination of meat [9] and meat products is using microbial cultures of lactic acid microorganisms that can produce bacteriocins that inhibit the growth and reproduction of spoilage bacteria **[10]**. The bacteriocins can interact with the microbial cell surface, increasing the permeability of its membrane, inhibiting the formation of the cell wall components, and synthesising the nucleic acid and protein **[11]**, **[12]**.

The most common bacteria used in the meat industry are the genus *Lactobacillus* **[13]**. They include *Lactobacillus curvatus*, a facultative anaerobe capable of synthesizing the lactic acid from sugars **[14]**, **[15]**. In addition, *L. curvatus* has a variety of genes that determine the bacteriocin synthesis **[16]** and is often used as a biological protective agent in fermented meat products that can inhibit the growth of spoilage bacteria. In addition, an equally important factor is the possibility of using the bacteriocin produced by this microorganism by spraying it onto a plastic film for active food packaging **[17]**. In addition to the bacteriocins, organic acids and hydrogen peroxide, produced by *L. curvatus* metabolism, can reduce the pH-value of the medium when the meat products are fermented and thus reduce their nitrite content **[18]**, **[19]**.

Due to the synthesis of the bacteriocins or antibacterial proteins the lactic-acid bacteria are highly effective against food-borne pathogens such as *Staphylococcus aureus, Pseudomonas fluorescens, P. aeruginosa, Salmonella typhi, Shigella flexneri, Listeria monocytogenes, Escherichia coli* O157:H7 and *Clostridium botulinum* **[20]**. Furthermore, the results show that the bacteriocins can effectively inhibit the formation of the biofilms of the pathogenic microorganisms **[21]**. It has been established that *L. curvatus* has a specific antimicrobial activity against *Campylobacter jejuni* ATCC 33560, *Camp. jejuni* NCTC 11168, *Listeria monocytogenes* ATCC 7644 and *Bacillus subtilis* ATCC 8633 **[22]**.

It is known that one of the prerequisites for the shelf life of cooked meat products, in particular the sausages, to be extended is the creation of anaerobic conditions, which are provided by the vacuum packaging, a modified gas environment **[23]**, as well as various oxygen absorbers based on iron nanocompounds, which are aimed at reducing the intensity of oxidative processes in the food products **[24]**, **[25]**. However, in that case this raises the question of controlling the residual content of any chemical components in the food product, particularly the sausages **[26]**. In this regard, *Lactobacillus curvatus* has advantages over any chemical preservatives, as it is also a promising microorganism as a probiotic **[27]**, with a pronounced resistance to the acidic pH-value of gastric juice, lysozyme and bile components. In 2012, it was included in the "Catalogue of Microorganisms of Technical Importance in Fermented Foods" of the Bulletin of the International Dairy Federation **[28]**, and in 2013, it was entered as a recommended biological agent in the qualification certification list of the European Food Safety Authority's **[29]**.

Scientific Hypothesis

Determination of the species composition of the spoilage microflora of the vacuum-packaged sausages while storing in a chilled state will make it possible to select the effective strains of lactic acid microorganisms, which are capable of inhibiting the growth of the spoilage bacteria. The sausage treatment with the starter culture of lactic-acid microorganisms SafePro BLC-48 (*Lactobacillus curvatus*) or the mixture of starter cultures SafePro BLC-48 (*Lactobacillus curvatus*) + Bactoferm Rubis (*Lactococcus lactis subsp. Lactis*) before vacuum packaging will make it possible to determine the most effective treatment option for extending their shelf life in a chilled state.

MATERIAL AND METHODOLOGY

Samples

The first-grade "Vienna sausages with chicken fillet", which are produced at a local meat-processing plant in Zakarpattia region, were used as the study material. The lactic-acid microorganisms SafePro BLC-48 (*Lactobacillus curvatus*) + Bactoferm Rubis (*Lactococcus lactis subsp. Lactis*), which Chr produces. Hansen (Chr. Hansen Ukraine, LLC), were used for these studies. The polyamide casing Mini Ralen (Devro, Czech Republic) and the vacuum packaging Amilen PA/PE (Kozak+, Ukraine) were used to produce these sausages.

Chemicals

The media and diagnostic tests HiMedia (India) manufactured were used for microbiological studies. Plate count agar M091 was used for QMAFAnM to be determined. For the isolation and quantification of bacteria of the genus *Lactobacillus* - Lactobacillus MRS Agar M641, for the isolation of pathogenic and non-pathogenic staphylococci - Baird Parker, Agar M043, for Salmonella - Bismuth Sulphite Agar M027 and Xylose Lysine Deoxycholate Agar M031, for *L. monocytogenes* - Agar Palcam, Agar Oxford, for *Bacillus cereus* - Bacillus cereus (Selective agar) M833, for enterobacteria - Endo Agar, for *E. coli* and the bacteria of the Escherichia coli group (*E. coli*) - Gissa's, Kessler, Endo Agar, Simmons Agar, XLD, for the isolation of yeast and mould - Sabouraud Agar.

Animals, Plants and Biological Materials

Sausages, minced meat, SafePro BLC-48 (*Lactobacillus curvatus*) Chr. Hansen (Chr. Hansen Ukraine, LLC), Bactoferm Rubis (*Lactococcus lactis subsp. Lactis*) Chr. Hansen (Chr. Hansen Ukraine, LLC)

Instruments

Petri dishes

Disposable microbiological tubes

Pipettes and dispensers of Eppendorf (Eppendorf, Germany)

The thermostat of Binder (Binder, Germany)

Mass-spectrometer of Bruker Daltonics, Maldi Tof microflex (Bruker Daltonics, Germany)

Laboratory Methods

The sausages were produced, treated and packed at a local meat-processing plant in Zakarpattia region according to standard TU U 15.1-00419880-049-2003 **[30]**.

The microbiological studies were conducted at the Transcarpathian Regional State Laboratory of the State Service of Ukraine for Food Safety and Consumer Protection, Uzhhorod, and "Expert Centre "Biolights" LLC, Ternopil. To determine the number of microorganisms, fungi and mould, the average samples of the sausages were taken, and the washes from the sausage casings were made of 5 vacuum packages of each variant. For this purpose, serial decimal dilutions were prepared in sterile normal saline. The number of microorganisms was determined in colony-forming units (CFU), the results were expressed in lg CFU/g for minced meat and lg CFU/cm2 for the surface of the sausage casings. The genus and species-level identifications of the isolated microorganisms were performed following the current methods. Organoleptic studies of the sausages were performed following standard DSTU 4823.2:2007 **[31]**.

Description of the Experiment

"Vienna sausages with chicken fillet" were produced as per the traditional technology according to the following recipe: main raw materials, kg/100 kg: greasy pork - 20, semi-greasy pork - 32, broiler chick fillet - 45, potato starch - 3, water (ice) - 25; auxiliary raw materials, kg/100 kg: table salt - 2.30, sodium nitrate - 0.0075, Emulin food additive (guar gum thickener (E412), milk protein mixture, sodium tripolyphosphate stabiliser (E451), table salt) - 2, Ham flavour food additive (extracts of black pepper, garlic, allspice and lovage, maltodextrin, soya protein hydrolysate, yeast hydrolysate, E621) - 0.2, enhanced milk food additive (spices and spice extracts: cardamom, nutmeg, garlic, meat flavour and aroma, blood haemoglobin (colour fixer), E120, milk proteins E451, E621, E316, E301, carriers (destractose, rice flour, salt) - 1.

Sample preparation: The casing, vacuum packaging of the sausages, minced meat and sausages were sampled and their preparation for the microbiological studies was performed in accordance with the requirements of standard DSTU 4823.2:2007 **[31]**.

A total of 70 packages weighing 200 g each were used. The indicators of the control sausage variant were compared with experimental sausage variant No. 1, which was treated with starter culture SafePro BLC-48 (*Lactobacillus curvatus*) before vacuum packaging, and experimental sausage variant No. 2, which was treated with the mixture of starter cultures SafePro BLC-48 (*Lactobacillus curvatus*) + Bactoferm Rubis (*Lactococcus lactis subsp. Lactis*) before vacuum packaging, as well as experimental sausage variant No. 1 with experimental sausage variant No. 2.

Number of repeated analyses: 5 to 10 samples were used in each study.

Number of experiment replication: 1

Design of the experiment: The studies were performed in two stages: the dominant species of the spoilage microorganisms of "Vienna sausages with chicken fillet", while storing the vacuum-packaged sausages in a refrigerator at a temperature of 4±1°C, were determined at the first stage. 10 vacuum packs of "Vienna sausages with chicken fillet" weighing 200 g each, which were studied for the species composition of the dominant microflora on the first day and for spoilage signs (appearance of cloudy juice and its haziness) on the 21st day of storage, were used for this purpose.

The treatment effectiveness of "Vienna sausages with chicken fillet" with the starter lactic-acid cultures intended to inhibit the spoilage microorganisms while storing the vacuum-packaged sausages in a refrigerator at a temperature of 4±1°C was studied at the second stage. The produced batch of the sausages was divided into 3 variants, which were treated according to the scheme given in Table 1.

Table 1 Experiment scheme for influence determination of starter cultures of lactic acid microorganisms on shelf life of vacuum-packaged "Vienna sausages with chicken fillet" in a chilled state, n = 20.

Variant	Study conditions
Control	Vacuum-packaged "Vienna sausages with chicken fillet" of first grade
Experimental No. 1	"Vienna sausages with chicken fillet" of first grade treated with starter culture SafePro BLC-48 (*Lactobacillus curvatus*) at the rate of 5x10^6 CFU/cm^2 of surface before vacuum packaging
Experimental No. 2	"Vienna sausages with chicken fillet" of first grade treated with the mixture of starter cultures SafePro BLC-48 (*Lactobacillus curvatus*) + Bactoferm Rubis (*Lactococcus lactis subsp. Lactis*) at the rate of 5x10^6 CFU/cm^2 of surface before vacuum packaging

Chr provided the starter cultures of the microorganisms. Hansen Ukraine and comply with the general food safety requirements under Regulation No. 178/2002/EC **[32]**.

All sausage variants were stored in a refrigerator until the spoilage signs appeared. The study results were recorded on days 1, 12, 18, 25 and 30 of the sausage storage in a refrigerator.

Statistical Analysis

The obtained results were statistically processed using Microsoft Excel 2016 in combination with XLSTAT, the tabulated data are presented as x ± SD (mean ± standard deviation). One-way analysis of variance was used to compare the data. The difference between the groups was considered significant using the Tukey test at $p \leq 0.05$.

RESULTS AND DISCUSSION

One of the most important factors determining the shelf life of cooked sausage products, including "Vienna sausages with chicken fillet", is the level of microbial contamination of the raw materials from which they are produced. Greasy and semi-greasy pork, broiler chickfillets, and a mixture of spices and food additives determine the microbial contamination of the minced meat for the sausages. As can be seen from the obtained data, QMAFAnM in the fresh minced meat for the sausages was at the level of 5.64 lg CFU/g. At the same time, after cooking, this figure decreased to 1.89 lg CFU/g (Fig. 1), which indicates that a significant part of the microorganisms was neutralised after being subjected to the heat treatment and the requirements of current standard TU U 15.1-00419880-049-2003 **[30]** are complied.

The casings used to produce the cooked sausages must be resistant to high temperatures and microorganisms **[34]**. An analysis of the microbial contamination of the surface of the polyamide casing Mini Ralen revealed that the number of QMAFAnM, lactic-acid microorganisms, yeasts, and moulds on its surface did not exceed the specified requirements. Similar results were obtained during the microbiological analysis of the vacuum multiwall bags Amilen PA/PE (Table 2).

Table 2 Microbiological indicators of casing and packaging for sausages, x ± SD, n = 5, lg CFU/cm^2.

Casing	Microbiological indicators		
	QMAFAnM	Lactic-acid microorganisms	Yeasts and moulds
Polyamide casing Mini Ralen	2.1 ±0.12	<1	<1
Vacuum multiwall bags Amilen PA/PE	<1	<1	<1

The shelf life of the sausages in a chilled state is also determined by using the casings and packaging materials that can ensure their complete isolation from environmental factors and guarantee the absence of microbial contamination **[33]**.

In “Vienna sausages with chicken fillet” on the first day of vacuum-packaging storage, QMAFAnM did not exceed 3 lg CFU/g, which complied with the requirements of standard TU U 15.1-00419880-049-2003 **[30]** (Table 3); *Macrococcus caseolyticus*, which are gram-positive cocci and belong to the family Staphylococcaceae, were detected among the dominant microorganisms. These microorganisms are often isolated from dairy products **[35]**, beef, pork and sausage products, which is consistent with the obtained study results. *Macrococcus caseolyticus* is conditionally pathogenic microorganisms, which can cause animal diseases **[36]**. Besides of that, as the most studied species of the genus *Macrococcus*, it is known for its ability to ferment with flavouring and tasting to food products **[37]**, as well as its ability to synthesise the lactic acid **[38]**. It is believed that no microorganism species of the genus *Macrococcus* is considered pathogenic to humans **[39]**, so it does not pose a danger to consumers as an infection.

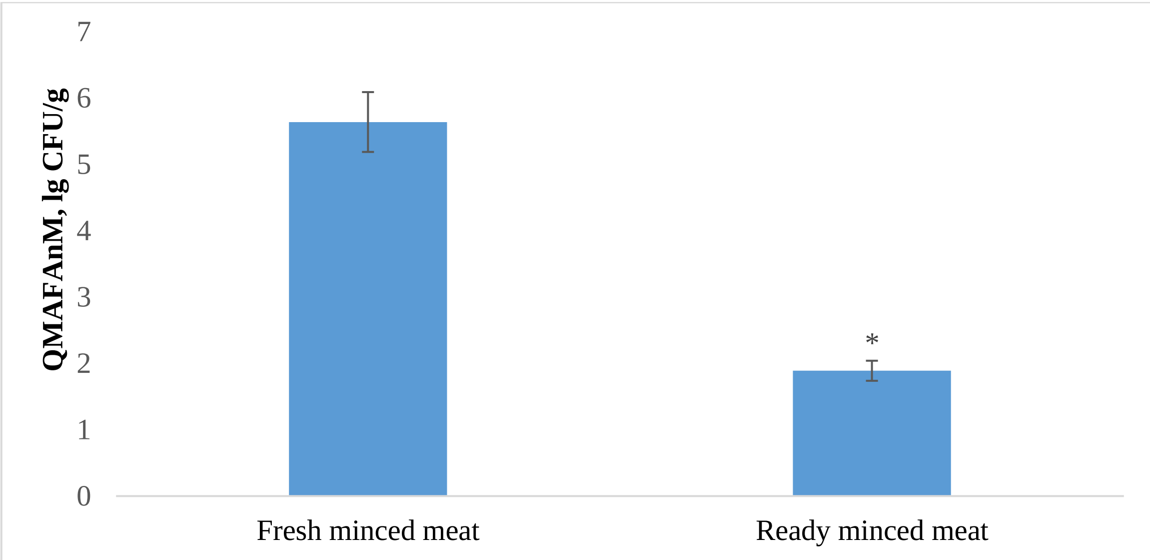

Figure 1 Microbiological indicators of minced meat for “Vienna sausages with chicken fillet”.
Note: x ±SD, n=5, * p≤0.05 compared to fresh minced meat.

The shelf life of the sausages in a chilled state is also determined by using the casings and packaging materials that can ensure their complete isolation from environmental factors and guarantee the absence of microbial contamination **[33]**.

The casings used to produce the cooked sausages must be resistant not only to high temperatures but also to microorganisms **[34]**. An analysis of the microbial contamination of the surface of the polyamide casing Mini Ralen revealed that the number of QMAFAnM, lactic-acid microorganisms, yeasts, and moulds on its surface did not exceed the specified requirements. Similar results were obtained during the microbiological analysis of the vacuum multiwall bags Amilen PA/PE (Table 2).

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Table 3 Microbiological indicators of vacuum-packaged "Vienna sausages with chicken fillet" while storing in a chilled state, x ± SD, n = 5, lg CFU/g.

Study period	QMAFAnM	Microbiological screening with the use of MALDI-TOF
Storage within the shelf life		
1-st day	2.78 ±0.55	*Macrococcus caseolyticus, Raoultella planticola, Raoultella ornithinolytica, Morganella morganii, Citrobacter freundii*
Storage until spoilage signs appeared (cloudy juice and its haziness)		
21-st day	8.12 ±1.05	*Proteus mirabilis, Moellerella wisconsensis, Serratia liquefaciens*

Raoultella planticola and *R. ornithinolytica*, which belong to the family Enterobacteriaceae - gram-negative, non-motile, anaerobic bacteria of the genus *Raoultella*, which are most commonly found in water, soil and aquatic environments, were also detected in the sausages with the shelf life of one day. They are capable of causing septic processes of urinoexcretory ways in immunocompromised people, although little evidence of their pathogenicity has been reported **[40]**, **[41]**. Their origin in the sausages is associated with the raw materials of animal origin, particularly with poultry meat **[42]**, which, according to the recipe, was used for "Vienna sausages with chicken fillet" to be produced.

Citrobacter freundii, which was detected in the sausages on the first day of storage, belongs to the genus *Citrobacter*, family Enterobacteriaceae. These gram-negative, motile, facultatively anaerobic bacteria are isolated from chicken meat, cold-stored minced meat, and cooked-stewed sausages **[43]**, consistent with our studies.

Morganella morganii, a gram-negative, facultatively anaerobic bacterium of the family Morganellaceae, was among the microorganisms that dominated the samples of "Vienna sausages with chicken fillet" on the first day of storage. This microorganism was detected in cheese and horse meat **[44]** and in fish products, where it forms biogenic amines, the content of which indicates the product spoilage **[45]**. Since the sausages consisted of food additives containing milk proteins, they could have been the source of this bacterium, but this needs to be confirmed.

Under standard TU U 15.1-00419880-049-2003 **[30]**, the shelf life of first-grade "Vienna sausages with chicken fillet" in a polyamide casing while storing in a refrigerator at a temperature of 0 to +6°C is 12 days. Still, the use of the vacuum packaging, as expected, extended this period and the organoleptic signs of the product spoilage, such as cloudy juice and packaging delamination, were detected on the 21st day of storage.

During this period, QMAFAnM in the sausages exceeded the permissible value of 3 lg CFU/g following standard TU U 15.1-00419880-049-2003 **[30]**, (Table 3). Among the dominant microorganisms which caused the sausage spoilage, no bacteria were detected that were the main ones in the sausages on the first day of storage, which indicates that the vacuum packaging or the temperature mode created the conditions that were unsuitable for the growth and reproduction of the microflora as mentioned earlier while storing the sausages in a refrigerator. It was also found that among the main bacteria which caused the sausage spoilage were representatives of the family Enterobacteriaceae, particularly *Proteus mirabilis*, *Moellerella wisconsensis* and *Serratia liquefaciens*.

Proteus mirabilis is a facultatively anaerobic, conditionally pathogenic microorganism, which is transmitted through food. It is one of the most common histamine-producing bacteria in fermented meat products, particularly in the sausages **[46]**. *Moellerella wisconsensis*, detected in the sausages with the spoilage signs, is a gram-negative, facultatively anaerobic, nitrate-reducing and oxidase-negative rod. They are also often detected in cooked ham with spoilage signs **[47]**. *Serratia liquefaciens* is a gram-negative, facultatively anaerobic microorganism often detected in chilled pork meat **[48]**. Likely, the storage of vacuum-packaged "Vienna sausages with chicken fillet" in a refrigerator has provided the conditions for the growth and reproduction of these microorganisms.

Thus, the main microorganisms which are capable of causing sausage spoilage at the early stages are the bacteria of the families Enterobacteriaceae, Morganellaceae and Staphylococcaceae, but at the end of the shelf life - only the bacteria of the family Enterobacteriaceae, which are widely distributed in the raw materials for the sausages to be produced and in the environment. Therefore, it is possible to inhibit the growth of the bacteria, which can cause their spoilage, using the starter cultures of the lactic acid microorganisms.

When "Vienna sausages with chicken fillet" were treated with the starter culture of lactic-acid microorganisms SafePro BLC-48 (*Lactobacillus curvatus*) or the mixture of SafePro BLC-48 (*Lactobacillus curvatus*) + Bactoferm Rubis (*Lactococcus lactis subsp. Lactis*) before vacuum packaging, QMAFAnM in the sausages on the first day of storage was increased by 1.09 lg CFU/g and 1.53 lg CFU/g, respectively, compared to the control ones.

When "Vienna sausages with chicken fillet" were treated with starter culture SafePro BLC-48, QMAFAnM was increased by 1.18 lg on the 12th day of storage in a chilled state; when "Vienna sausages with chicken fillet" were treated with the mixture of starter cultures SafePro BLC-48 + Bactoferm Rubis, QMAFAnM was increased by 1.54 lg CFU/g compared to the control ones. At the same time, in the experimental variant, where the mixture of starter cultures was used, QMAFAnM exceeded by 0.36 lg CFU/g compared to the same indicator in the variant using one starter culture. A similar pattern concerning QMAFAnM in the sausages of both experimental variants was observed on the 18th day of storage.

The sausages of the control sample showed signs of spoilage on the 21st day of storage, manifested by the appearance and haziness of the juice and the delamination of the vacuum packaging, which prevented further storage and study.

The storage of "Vienna sausages with chicken fillet" of both experimental variants for up to 25 days showed that they complied with the valid standard requirements for organoleptic indicators. However, QMAFAnM in them continued to increase. At the same time, when the sausages were treated with the mixture of starter cultures SafePro BLC-48 + Bactoferm Rubis, QMAFAnM in the sausages was 1.37 lg CFU/g higher than when the sausages were treated with starter culture SafePro BLC-48 (Table 4).

Table 4 QMAFAnM in "Vienna sausages with chicken fillet" if treated with starter cultures of lactic-acid microorganisms and stored in vacuum packaging in a chilled state.

Study period	**Variant**		
	Control	**Experimental No. 1 SafePro BLC-48**	**Experimental No. 2 SafePro BLC-48 + Bactoferm Rubis**
1-st day	2.36 ±0.32	3.45 ±0.35*	3.89 ±0.49*
12-th day	2.51 ±0.35	3.69 ±0.15*	4.05 ±0.28*,**
18-th day	3.01 ±0.27	3.93 ±0.22*	4.97 ±0.65*,**
25-th day	-	4.56 ±0.34	5.93 ±0.24**
30-th day	-	7.78 ±0.41	8.29 ±0.60**

Note: x ± SD, n = 5, lg CFU/g, * p≤0.05 compared to the control sample, ** p≤0.05 compared to experimental variant No. 1

On the 30th day of storage, under both variants of the treatment with the starter cultures, the organoleptic indicators of "Vienna sausages with chicken fillet" complied with the valid standard requirements. Still, QMAFAnM in them continued to increase, and if the mixture of the starter cultures was used, it exceeded by 0.51 lg CFU/g, the same variant where the single culture was used. Such an increase in QMAFAnM indicates that the starter lactic acid cultures of microorganisms continue their vital activity and growth in the sausages.

The number of the lactic-acid microorganisms in "Vienna sausages with chicken fillet" on the 1st and 12th days of storage if treated with starter culture SafePro BLC-48 did not differ from the control sample, while treating with the mixture of starter cultures SafePro BLC-48 + Bactoferm Rubis increased the number of the lactic-acid microorganisms by 0.3 lg CFU/g and 0.35 lg CFU/g, respectively, compared to the control sample (Table 5).

Table 5 Number of lactic-acid microorganisms in "Vienna sausages with chicken fillet" if treated with starter cultures of lactic-acid microorganisms and stored in vacuum packaging in a chilled state, x ± SD, n = 5, lg CFU/g.

Study period	**Variant**		
	Control	**Experimental No. 1 SafePro BLC-48**	**Experimental No. 2 SafePro BLC-48 + Bactoferm Rubis**
1-st day	1.75 ±0.13	1.88 ±0.33	2.05 ±0.29*
12-th day	1.80 ±0.16	2.01 ±0.25	2.15 ±0.22*
18-th day	2.03 ±0.27	2.13 ±0.41	2.37 ±0.68
25-th day	-	2.56 ±0.33	3.19 ±0.33**
30-th day	-	2.73 ±0.45	3.26 ±0.31**

Note: * – p≤0.05 compared to the control sample, ** – p≤0.05 compared to experimental variant No. 1

On the 18th day of sausage storage, the total number of lactic acid microorganisms did not differ significantly between the control sample and both variants of their treatment with the starter cultures.

The further storage of "Vienna sausages with chicken fillet" in a refrigerator for 25th and 30th days contributed to an increase in the number of the lactic-acid microorganisms if treated with the mixture of starter cultures SafePro BLC-48 + Bactoferm Rubis by 0.63 lg CFU/g and 0.53 lg CFU/g, respectively, compared to the treatment with starter culture SafePro BLC-48. At the same time, the number of lactic acid microorganisms was increased in the sausages under both treatment variants during the entire shelf life (Table 5).

The sausages' spoilage signs, such as the appearance of cloudy juice, surface slime, and delamination of vacuum packaging, were observed on the 36th day of storage both when treated with starter culture SafePro BLC-48 and the mixture of starter cultures SafePro BLC-48 + Bactoferm Rubis (Fig. 3 a, b) compared to their organoleptic indicators on the 1st day of storage (Figure 2 a, b, c).

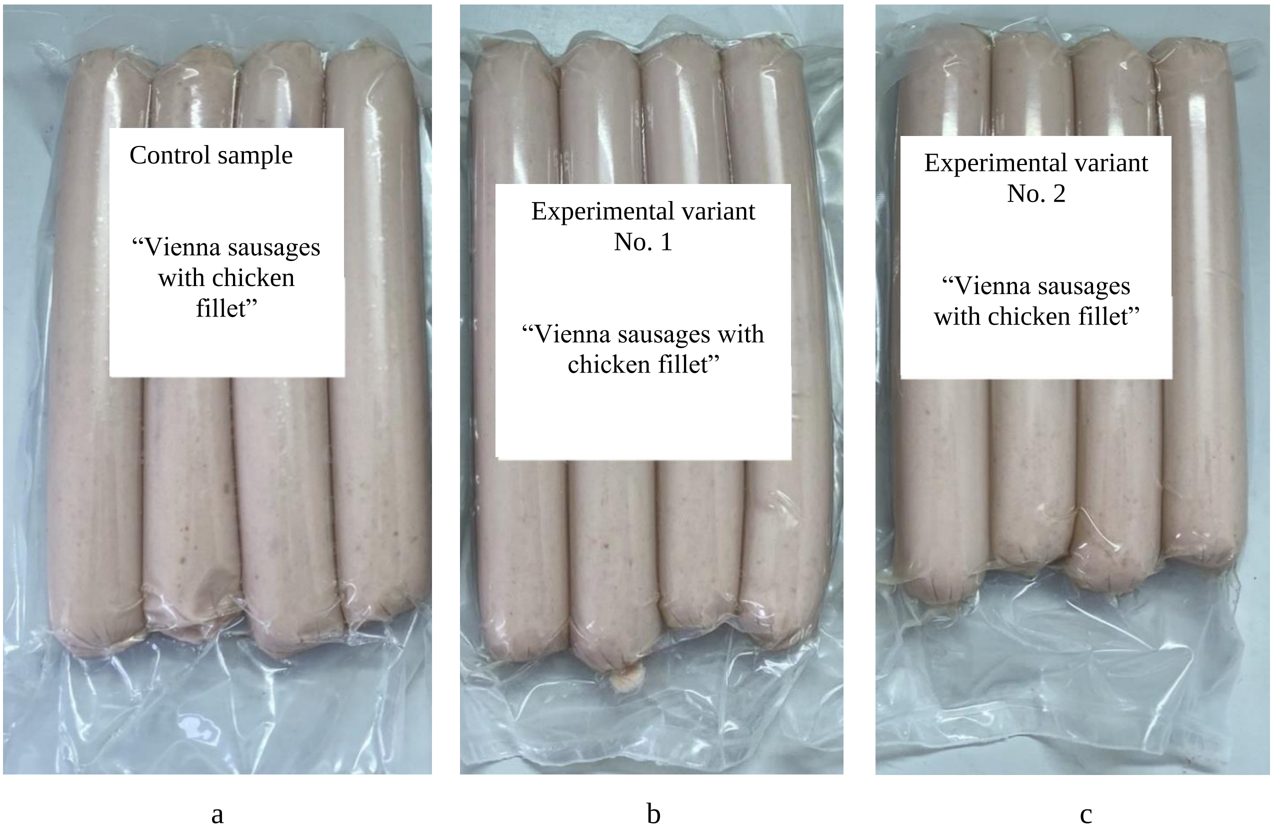

Figure 2. Sausages with a shelf life of one day: a - control sample, b - experimental variant No. 1 (treatment with starter culture SafePro BLC-48), c - experimental variant No. 2 (treatment with a mixture of starter cultures SafePro BLC-48 + Bactoferm Rubis)

a b

Figure 3 Sausages with shelf life of 36 days.
Note: a - experimental variant No. 1 if treated with starter culture SafePro BLC-48, b - experimental variant No. 2 if treated with mixture of starter cultures SafePro BLC-48 + Bactoferm Rubis

The lactic acid bacteria are the main microbial population found in various cooked sausages when stored in vacuum packaging. Various strains of *Lactobacillus sake* and *Lactobacillus curvatus* are also known to be common spoilage causes of cooked sausages. The growth of the lactic acid bacteria on the surface of the cooked sausages creates undesirable sensory properties such as a sour aroma and taste. Generally, during the cooking of the sausage products, high temperatures destroy the lactic acid bacteria on their surface. However, secondary contamination of the sausages with the lactic acid bacteria occurs during their cooling, prepackaging, and packing due to contact with contaminated air and technological equipment and workers **[49]**. In our experiment, the increase in the total number of the lactic-acid microorganisms in the sausages of experimental variant No. 1, which we observed throughout the entire shelf life, was probably due to the starter culture of strain SafePro BLC-48 (*Lactobacillus curvatus*), and in the sausages of experimental variant No. **-** due to the mixture of starter cultures SafePro BLC-48 (*Lactobacillus curvatus*) and Bactoferm Rubis (*Lactococcus lactis subsp. Lactis*), which contributed to an increase in their shelf life, unlike the sausages of the control sample.

According to the obtained data **[50]**, the mechanism of biopreservation, prevention of pathogen growth and reduction of microbial spoilage of the cooked meat products is based on the ability of the lactic-acid microorganisms and/or their metabolic products, in particular bacteriocins, to neutralise pathogenic and opportunistic pathogenic bacteria. This study shows that the use of sacacin G, isolated from *Lactobacillus curvatus* ACU-1, for Listeria and spoilage flora to be neutralised was effective when applied to the casing both before and after stuffing of “Vienna sausages with chicken fillet”. At the same time, applying the antimicrobial agent to the finished sausages inhibited the lactic acid bacteria that can spoil the meat products and the mesophilic microorganisms from the zero sampling time.

During the entire shelf life, no pathogenic and opportunistic pathogenic bacteria, including *S. aureus, L. monocytogenes*, *Salmonella spp.*, *E. coli*, bacteria of the *E. coli* group, as well as yeast and mould, were detected in the sausages of both the control sample and if treated with starter culture SafePro BLC-48 or the mixture of starter cultures SafePro BLC-48 + Bactoferm Rubis (Table 6). The results of the studies are consistent with the data **[51]**, which proves the complete elimination of *L. monocytogenes* in the sausage due to the use of the bacteria's lactic acid starter cultures. The increase in the shelf life of the sausages under both treatments in our experiment is due to the ability of *L. curvatus* to inhibit the growth of the bacteria, in particular, *Enterobacteriaceae, Pseudomonas fragi* and *Brochothrix thermosphacta*, which cause the spoilage of the meat products during their storage **[52]**, **[53]**.

Table 6 Number of pathogenic and opportunistic pathogenic microorganisms in "Vienna sausages with chicken fillet" if treated with starter cultures of lactic-acid microorganisms and stored in vacuum packaging in a chilled state, x ±SD, n =5, lg CFU/g.

Indicator	**Variant**		
	Control	**Experimental No. 1 SafePro BLC-48**	**Experimental No. 2 SafePro BLC-48 + Bactoferm Rubis**
	1^{st} , 18^{th} day	1^{st}, 18^{th}, 25^{th} and 30^{th} day	
S. aureus	not detected	not detected	not detected
L. monocytogenes	not detected	not detected	not detected
Salmonella spp.	not detected	not detected	not detected
bacteria of the E. coli group	not detected	not detected	not detected
E. coli	not detected	not detected	not detected
Yeast, mould	not detected	not detected	not detected

The obtained data show that the combination of starter culture Bactoferm Rubis with SafePro BLC-48 is ineffective for storing vacuum-packaged sausages, as it does not increase their shelf life. The use of starter culture Bactoferm Rubis as an aerobic microorganism, which was based on the absorption of residual oxygen, was probably not effective in this case, as the vacuum packaging of the sausages could provide a sufficient degree of oxygen removal, and the main pathogens that caused the sausage spoilage were enterobacteria, which belong to facultative anaerobes. The main effect on increasing the shelf life of the sausages was provided by starter culture SafePro BLC-48 (*L. curvatus*), which is also a facultative anaerobe, which in vacuum packaging could ensure its growth and reproduction in a refrigerator. This is confirmed by the data provided by several scientists **[27]**, which show the ability of different strains of L. curvatus to synthesize the bacteriocins, in particular curvacin A, saccacin G, saccacin P and saccacin X, curvacin 13, lactocin AL705 and curvacin 422, which tolerate a wide range of pH medium and temperature conditions, have antibacterial effects against a wide range of pathogenic bacteria and bacteria that cause the spoilage of the meat products such as *Bacillus cereus, L. monocytogenes, S. aureus* and *Enterococcus faecium*.

The idea of using oxygen absorbers for the extension of the shelf life of food products, particularly meat products, is currently realised mainly through the use of films, sachets, powders or components of packaging material in combination with chemicals such as metals and metal oxides, organic acids, antimicrobial peptides and bacteriocins, antimicrobial agents of plant origin, enzymes, lactoferrin, chitosan and bacteriophages, reduction of water activity, pH, use of multilayer composites and/or vacuum or modified atmosphere. The current demand increases their addition directly to the packaging material **[54]** rather than to the food product composition **[55]**, **[56]**. Among these preservatives, bacteriocins of lactic acid bacteria or their cultures are most often used **[57]**, **[58]**. It was proved that spraying the solution of bacteriocin *L. curvatus* on the polyethene films provided a stable antibacterial activity, and heat treatment at 70 °C did not affect the antibacterial activity of such films. Compared to the nisin-treated film, the lactocin-treated active polyethylene film inhibited Listeria more effectively, and this did not affect the functional properties of the film. At the same time, it was found that temperature and exposure time have a certain effect on the adsorption of the bacteriocin by the polyethylene film, provided that 60 minutes and 30°C are considered optimal conditions for the adsorption **[27]**.

In our experiment, the suspension spray of the starter lactic-acid cultures was applied to the polyamide casing of the sausages, which is in contact with the minced sausage on one side and the vacuum packaging on the other side, creating an additional barrier to the penetration of the microorganisms that cause the sausage spoilage. QMAFAnM on the sausage casing surface increased during the entire shelf life if treated with the suspension of starter culture SafePro BLC-48 or the mixture of starter cultures SafePro BLC-48 + Bactoferm Rubis. Under the given conditions, the treatment of the sausages with the mixture of starter cultures contributed to an increase in QMAFAnM on their surface on the 18th and 30th days of storage by 0.8 lg CFU/cm2 and 1.24 lg CFU/cm^2, respectively, compared to the treatment with the suspension of starter culture SafePro BLC-48 (Table 7).

Table 7 QMAFAnM in casing Mini Ralen of "Vienna sausages with chicken fillet" if treated with starter cultures of lactic-acid microorganisms and stored in vacuum packaging in a chilled state.

Study period	Variant	
	Experimental No. 1 SafePro BLC-48	**Experimental No.2 SafePro BLC-48 + Bactoferm Rubis**
1st day	6.78 ±1.32	7.35 ±1.21
18th day	7.12 ±0.59	7.92 ±0.84**
30th day	7.73 ±0.47	8.97 ±1.03**

Note: x ±SD, n =5, lg CFU/cm^2, ** – p≤0.05 compared to experimental variant No. 1

This makes it possible to suggest that the main species of the bacteria that colonised the polyamide casing of the sausages if treated with the spray of the starter cultures were the lactic-acid microorganisms, in experimental variant No. 1 - *Lactobacillus curvatus*, and in experimental variant No. 2 - a mixture of *Lactobacillus curvatus* and *Lactococcus lactis subsp. Lactis*.

Thus, the obtained study results show that the use of starter culture SafePro BLC-48 (*Lactobacillus curvatus*) or the mixture of starter cultures SafePro BLC-48 (*Lactobacillus curvatus*) + Bactoferm Rubis (*Lactococcus lactis subsp. Lactis*) for the sausage treatment makes it possible to increase the shelf life of the vacuum-packaged sausages in a chilled state by 12 days due to a complex and multicomponent inhibition mechanism of the spoilage microorganisms. The use of the mixture of starter cultures SafePro BLC-48 (*Lactobacillus curvatus*) + Bactoferm Rubis (*Lactococcus lactis subsp. Lactis*) for the sausage treatment may be promising if the development of the aerobic spoilage bacteria is confirmed. Along with this, the researchers emphasise the need to introduce effective protocols for producing meat products, which makes it possible to ensure the traceability of the production chain **[59]**.

In the future, it is necessary to determine the use effectiveness of the starter cultures of the lactic-acid bacteria in the association of different families and species of anaerobic or facultative anaerobic spoilage microorganisms of the sausages, which are produced following different recipes.

CONCLUSION

The main spoilage microorganisms of the vacuum-packed sausages on the 1st day of storage in a chilled state are the bacteria of the family Enterobacteriaceae (*Raoultella planticola, R. ornithinolytica, Citrobacter freundii*), Morganellaceae (*Morganella morganii*), and Staphylococcaceae (*Macrococcus caseolyticus*), and at the end of the shelf life for 21th day - the bacteria of the family Enterobacteriaceae (*Proteus mirabilis*, *Moellerella wisconsensis, Serratia liquefaciens*). The sausage spoilage is characterised by the appearance of cloudy juice, surface slime and delamination of vacuum packaging.

The treatment of "Vienna sausages with chicken fillet" with the starter culture of lactic-acid microorganisms SafePro BLC-48 (*Lactobacillus curvatus*) or the mixture of SafePro BLC-48 (*Lactobacillus curvatus*) + Bactoferm Rubis (*Lactococcus lactis subsp. Lactis*) before vacuum packaging contributed to an increase in QMAFAnM in the first-grade sausages on the 1st day of storage by 1.09 lg CFU/g and 1.53 lg CFU/g, on the 12th day - by 1.18 lg CFU/g and 1.54 lg CFU/g, on the 18th day - by 0.92 lg CFU/g and 1.96 lg CFU/g, respectively, compared to the control sample. In "Vienna sausages with chicken fillet", QMAFAnM was higher by 1.37 lg CFU/g on the 25th day of storage if treated with the mixture of starter cultures SafePro BLC-48 + Bactoferm Rubis, and by 0.51 lg CFU/g on the 30th day compared to the treatment with starter culture SafePro BLC-48.

The number of the lactic-acid microorganisms in "Vienna sausages with chicken fillet" on the 1st and 12th days of storage if treated with starter culture SafePro BLC-48 did not differ from the control sample, while treated with the mixture of starter cultures SafePro BLC-48 + Bactoferm Rubis increased the number of the lactic-acid microorganisms by 0.3 lg CFU/g and 0.35 lg CFU/g, respectively, compared to the control sample. Further storage of "Vienna sausages with chicken fillet" in a refrigerator for the 18th day did not affect, and on the 25th and 30th days it contributed to an increase in the number of the lactic-acid microorganisms if treated with the mixture of starter cultures SafePro BLC-48 + Bactoferm Rubis by 0.63 lg CFU/g and 0.53 lg CFU/g, respectively, compared to the treatment with starter culture SafePro BLC-48.

No pathogenic and opportunistic pathogenic bacteria, particularly *S. aureus, L. monocytogenes, Salmonella spp., E. coli*, bacteria of the *E. coli* group, yeast, and mould, were detected during the sausages' entire shelf life in both the control sample and if treated with starter culture SafePro BLC-48 or the mixture of starter culture SafePro BLC-48 + Bactoferm Rubis.

Thus, the obtained study results show that the use of starter culture SafePro BLC-48 (*Lactobacillus curvatus*) provides the extension of the shelf life of the vacuum-packaged sausages in a chilled state by 12 days due to the inhibition of their spoilage microorganisms. The use of the mixture of starter cultures SafePro BLC-48 (*Lactobacillus curvatus*) + Bactoferm Rubis (*Lactococcus lactis subsp. Lactis*) for the sausage treatment does not make it possible to improve the result obtained when using SafePro BLC-48 (*Lactobacillus curvatus*). The use of the starter culture mixture of the lactic-acid microorganisms for the sausage treatment, while stored in vacuum packaging, may be promising if the development of the aerobic spoilage bacteria is confirmed.

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