



Article

Application of *Lactiplantibacillus plantarum* SCH1 for the Bioconservation of Cooked Sausage Made from Mechanically Separated Poultry Meat

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Abstract: The aim of the research was an assessment of the effect of the *Lactiplantibacillus plantarum* SCH1 strain isolated from ecological raw fermented pork roast on pH, redox potential, nitrites, and nitrates content, L a* b* color parameters, total heme pigments content, nitrosyl myoglobin concentration, as well as the microbiological quality and sensory traits of cooked sausages produced from mechanically separated poultry meat (MSPM), cured with a lower sodium nitrite level (NaNO₂ 50 mg/kg) after production as well as after storage (1 and 3 weeks of storage). The biochemical identification of the *Lactobacillus* bacteria after storage was also performed. Tests were performed in two sausage treatments: C—control sausage made from MSPM and L—sausage made from MSPM inoculated with *L. plantarum* at approx. 10⁷ cfu/g. No negative effect of using the *L. plantarum* SCH1 strain on the physical and chemical MSPM sausage features was found. The treatment with *L. plantarum* SCH1 was of better microbiological quality after 3 weeks of storage. The sausages with *L. plantarum* SCH1 kept good sensory quality while the control treatment was spoiled after storage.

Keywords: *Lactiplantibacillus plantarum*; sausage with MSPM; biopreservation; quality



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1. Introduction

Mechanically separated meat (MSM) is widely used in the meat industry and is one of the cheapest animal origin materials [1]. In recent years, Poland has become one of the biggest poultry meat producers in Europe [2]. Mechanically separated poultry meat (MSPM) production can fully exploit soft tissue residues from the bones and poultry carcasses remaining after hand deboning [1].

The most commonly used separators in MSM production are high-pressure ones, low-pressure ones are used less commonly [3]. The risk of higher microorganism levels in the product increases together with the increased grinding level and increased muscle fiber degradation. Thus, MSPM obtained with the high-pressure method is a good substrate for microflora growth [3]. Excessive fragmentation of MSPM, decay of tissue structure and aeration during the separation process fosters microbiological pollution. What is more, the raw material for MSPM production might contain a relatively high number of microorganisms. In the MSM microflora, the following pathogenic may be present in the bacteria: *Salmonella* spp., *Campylobacter* spp., *Escherichia coli*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Staphylococcus aureus*, as well as saprophytic *Pseudomonas*, that speed up meat spoilage [4–8]. A substantial factor determining MSPM microbiological quality is the hygiene of carcass processing as well as the level and type of waste at the early production stage and during animal rearing [9]. MSPM is usually affected with microorganisms originating from chicken carcasses and bones as well as from the environment in which it is

processed (service, equipment, and environment). Additionally, if the temperature during the deboning process is not low enough, the risk of bacteria multiplying increases [10]. The presence of many bacteria genus, including the pathogenic ones like Enterobacteriaceae and *Salmonella* in the raw material of poultry is well documented [7,11–13].

Due to low microbiological MSPM stability it should be sent for processing immediately after production or should be chilled to a temperature of 2 °C. MSPM after chilling may be stored but not longer than 24 h, which significantly hinders the logistics of processing the MSPM in the plant.

One of the methods which enables the lengthening of MSPM shelf-life is to apply the freezing process to −18 °C [14]. Nevertheless, this process causes many negative changes within MSPM i.e., a worse water holding capacity, drip loss, color change, lower protein solubility and other changes [15]. In the industry the practice of curing fresh or defrosted MSPM with sodium nitrite is also applied. Considering the proven negative effect of nitrites and especially their derivatives N-nitrosamines (NAs) on human health it is recommended to reduce their level in the diet [16]. Research has indicated that NAs formation depends on the level of nitrites added to meat [17,18]. In some countries e.g., in Denmark internal regulations were introduced to lower the maximum level of nitrites from 150 mg/kg allowed in the European Union to 60 mg/kg [19]. Reducing the amount of nitrites in cured MSPM batters may have an influence on their microbiological quality and thus have a negative effect on shelf-life and the hygienic security of products with MSPM content. Thus, it is important to look for new technological solutions that will constitute an additional barrier in the ‘hurdle technology’ in producing meat products with MSPM and lower sodium nitrite levels.

Lactic acid bacteria (LAB) plays an important role in the production of fermented meat products, they affect the taste and texture as well as extend the shelf-life of these products. They also may assure meat safety without any negative effects on product quality [20–22]. Lactic acid bacteria—i.e., *Lactobacillus sakei*, *Pediococcus acidilactici*, *Lactobacillus curvatus*—are used in order to increase raw meat products shelf-life and health safety [22]. The antimicrobial LAB activity is attributed to the metabolites produced by the bacteria cell. Apart from organic acids, hydrogen peroxide, diacetyl, and CO₂, they also produce an abundant group of protein substances of significant antimicrobial and/or bacteriostatic activity called bacteriocins [23]. In the research of Łaszkiwicz et al. [24,25], it was proved that the *Lactiplantibacillus plantarum* SCH1 strain isolated from ecological raw fermented pork roast it is possible for it to be used to inhibit *E. coli* growth in raw uncured MSPM batter after 4 days of cold storage and also in raw MSPM batters cured with a lower amount of sodium nitrite (50 mg/kg) during storage. The results reported by other the authors indicate that some LAB strains are thermotolerant and thus could be used to elongate the shelf-life of cooked meat products [26,27]. In this research, the *L. plantarum* SCH1 strain was used in MSPM batter cured with a lower amount of sodium nitrite and next the model cooked meat product was formed for assessment.

The aim of the research was to assess the possibility of using *Lactiplantibacillus plantarum* SCH1 for the bioconservation of cooked sausage made from mechanically separated poultry meat cured with a reduced amount of sodium nitrite.

2. Materials and Methods

2.1. Bacteria Culture and Growth Condition

The *Lactiplantibacillus plantarum* SCH1 strain isolated from ecological raw fermented pork roast was used within the research. The strain originated from a microorganisms collection owned by the Facility of Food Hygiene and Quality Management, Warsaw University of Life Sciences in Poland and was chosen based on the research of Rzepkowska et al. [28] and Łaszkiwicz et al. [24,25]. Lactic bacteria cultures stored in 20% glycerol at −80 °C were defrosted to prepare the strain for the research. The 20 µL of the strain after mixing was put into 5 mL of MRS broth (Merck KGaA, Darmstadt, Germany) in sterile test tubes and incubated at 37 °C for 24 h. After the next decimal dilution and incubation in

the same conditions the cells were centrifuged in a J2-21 centrifuge (Beckman, Birkerød, Denmark) at 4500 RPM ($2313 \times g$). Then, after removing supernatant bacteria cells, it was suspended in a NaCl solution (0.9%) and applied into the MSPM batter to reach the bacteria concentration of approx. 10^7 cfu/g. All of the procedures were performed aseptically using sterile equipment.

2.2. Preparing Batters and Producing Sausages with MSPM

High pressure MSPM was obtained in industrial conditions (diameter of the holes was 1 mm). Non-frozen chicken carcasses were deboned using the separator (AM2C, Quimper, France) and after separation the obtained MSPM was divided into blocks of 10 kg each and frozen to -18 °C. The blocks of MSPM were stored frozen for 2–4 days. For producing the sausage batters, the blocks of MSPM were defrosted in refrigerated conditions (4–6 °C) for approx. 24 h. Two MSPM batter treatments were prepared for sausage production: C—control treatment produced with MSPM cured with 50 mg/kg of sodium nitrite, L—treatment produced with MSPM cured with 50 mg/kg of sodium nitrite with the addition of *Lactiplantibacillus plantarum* SCH1 at about 10^7 cfu/g.

In the L treatment, the bacterial biomass suspended in the physiological saline (1.25 kg) was added to 10 kg of defrosted MSPM. In the C treatment, physiological saline (1.25 kg) without bacteria was added to the 10 kg of MSPM. Next, the MSPM (C and L) were mixed separately in the mixer (N-50G, Hobart Corporation, Troy, OH, USA) for 1 min and left in polypropylene containers (48 L capacity) for 4 days in cold storage at a temperature of 4 °C. Time and storing conditions were established based on the preliminary research [24,25]. Next, water/ice, sodium nitrite (50 mg/kg), spices, and additional substances (Table 1) were added to the MSPM (C and L) and after that it was minced in a cutter K40 (Seydelmann, Stuttgart, Germany), reaching up to 10 °C in the batter. The MSPM batters were stuffed into the semi-permeable casings (DAT SCHAUB, Suchy Las, Poland). After stuffing, the sausages were allowed to settle for 45 min at a temperature of 20–25 °C.

Table 1. Model sausage with the MSPM formula.

Formula Ingredient [kg]/Experimental Treatment	C	L
MSPM	100.00	100.00
Bacterial biomass <i>Lactiplantibacillus plantarum</i> SCH1 suspended in NaCl 0.9% solution	-	1.25 *
NaCl 0.9% solution	1.25 *	-
Water/ice	30.00	30.00
Sodium nitrite	0.0070	0.0070
Sodium triphosphate (Tari P-31: 57% P ₂ O ₅)	0.33	0.33
Sodium ascorbate	0.05	0.05
Sodium chloride	2.30	2.30
SUPRO 595 protein	2.00	2.00
Potato starch	2.50	2.50
Carrageenan	0.40	0.40
Black pepper	0.25	0.25
Marjoram	0.07	0.07
Fresh garlic	0.05	0.05
Total	137.96	137.96

* Added before cold storage; C—control treatment with MSPM without a bacteria strain, L—treatment with MSPM and the bacteria *L. plantarum* SCH1 (10^7 cfu/g).

After that the sausages were smoked with hot smoke 56–65 °C for 50 min and then steamed to 70–72 °C up to reaching a final internal sausage temperature of 70 °C. The final products were chilled with water to 15–20 °C, and next additionally chilled with cold air in the cold store to 4–6 °C.

The tests of the model sausages with MSPM were performed after thermal treatment and chilling (after production) as well as at 1 and 3 weeks of storage. The experimental production was done in the technical hall of the Department of Meat and Fat Technology,

Institute of Agricultural and Food Biotechnology in Warsaw (Poland). The experiment described above was repeated three times ($n = 3$) in independent trials (replicates).

2.3. pH Measurement

The 10 g meat product samples were mixed with 50 mL distilled water and homogenized (14,000 RPM) for 1 min with an 800 W blender (MSM 66120, BSH Hausgeräte GmbH, Munich, Germany). To determine the pH value a digital pH-meter (Mettler Delta 350, Mettler Toledo, Schwerzenbach, Switzerland) with an automatic compensation of temperature and a glass-calomel In a Lab Cool electrode (Mettler Toledo, Greifensee, Switzerland) was used.

2.4. Oxidative-Reduction Potential (ORP) Measurement

To determine the ORP value the 10 g meat product samples were mixed with 50 mL distilled water and homogenized (14,000 RPM) for 1 min with an 800 W blender (MSM 66120, BSH Hausgeräte GmbH, Munich, Germany). The redox potential was performed using a digital pH-meter (Mettler Delta 350, Mettler Toledo, Schwerzenbach, Switzerland) equipped with an In Lab Redox Pro electrode (Mettler Toledo, Greifensee, Switzerland). The obtained results were calculated into the value of ORP in relation to the standard hydrogen electrode E_H (mv). The ORP value of the reference electrode at a temperature of $20\text{ }^\circ\text{C} - E_{\text{ref}} = 207\text{ mV}$ was summed up with the value obtained with the equipment.

2.5. Determining the Total Heme Pigments Content and Nitrosylmyoglobin Concentration

The total heme pigments content and nitrosylmyoglobin concentration was performed using the Hornsey method [29]. To determine the absorbance of the filtrates value, a U-2900 spectrophotometer (Hitachi, Tokyo, Japan) was used. The total heme pigment content was measured in hematin ppm and was calculated by multiplying the absorbance at 640 nm by 680. The nitrosylmyoglobin concentration (NO-Mb) content was calculated by multiplying the absorbance at 540 nm by 290 and was finally expressed in g/100 g according to the formula

$$\text{NOMb (g/100 g)} = \frac{\text{NO - Mb amount (ppm)}}{\text{total heme pigment amount (ppm)}} \times 100$$

2.6. Sodium Nitrite and Nitrate Content

The content of nitrites and nitrates was performed according to PN-EN 120414:2006 with an amendment of Siu and Henshall [30]. The 10 g homogenized sausage sample was mixed with deionized water added to the volume of 50 mL into a volumetric flask and heated for 20 min. at a temperature between $70\text{--}80\text{ }^\circ\text{C}$. After cooling, deionized water was added to the sample to a volume of 100 mL and mixed. The supernatant was filtered using cellulose acetate (CA) syringe filters (Alfatec Technology, Zagreb, Croatia) with a pore size of $0.45\text{ }\mu\text{m}$ and after that the filtrate was used for the HPLC analysis.

For the extracts analysis an Agilent 1200 liquid chromatograph (Agilent Technologies, Waldbronn, Germany) with a UV detector, analytical column IonPac[®] AS11-HC $4 \times 250\text{ mm}$ (Thermo Fisher Scientific, Sunnyvale, CA, USA) and pre-column AG11-HC $4 \times 50\text{ mm}$ (Thermo Fisher Scientific, Sunnyvale, CA, USA) was used. Nitrate and nitrite were separated in isocratic conditions: 10 mmol/L sodium hydroxide (Chempur, Piekary Śląskie, Poland) for 20 min, then the column was washed with 50 mmol/L sodium hydroxide for 10 min, and equilibrated with 10 mmol/L sodium hydroxide for 5 min. The volume of the injection was $25\text{ }\mu\text{L}$, the eluent flow-rate 1.5 mL/min and analytes were detected at 225 nm with UV detection.

The content of nitrite and nitrate anions was expressed in terms of the salts: NaNO_2 and NaNO_3 in mg/kg.

2.7. Determination of Color

To determine the color a spherical CR-300 spectrophotometer (Konica Minolta, Tokyo, Japan) with a measuring hole of 25.4 mm in diameter was used. The measurement was performed in a laboratory at a temperature of $24\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$. Computed trio-chromatic coordinates were expressed in the CIE $L^* a^* b^*$ system, where L^* means lightness, a^* chromaticity from green to red color and b^* chromaticity from blue to yellow color. For the measurement of the color, the standard CIE observer was used: 2° , illuminant D65, 8 mm measuring area and the white tile standard ($L^* = 95.87$, $a^* = -0.49$, $b^* = 2.39$) as a reference source was used. For each of the three replicate treatments (C, L) four measurements were carried out. The a^*/b^* ratio was also calculated [31].

2.8. Microbiological Tests

The scope of the MSPM sausages microbiological test in the first research stage (after production) was as follows: total viable counts (TVC), lactic acid bacteria counts, *Escherichia coli* counts, Enterobacteriaceae counts, coagulase positive *Staphylococcus* count and *Salmonella* spp., and *Campylobacter* spp. presence in 25 g. The range and methodology of the microbiological tests are described below.

Samples of 10 g or 25 g were taken aseptically for quantity or quality lab measurements and after mixing the samples was suspended in 100 mL or in 250 mL of peptone water, respectively. Then, the decimal dilutions were used and bacteria growth on different culture media was performed.

To determine the presence of *Salmonella* spp. in 25 g the Müller–Kauffman's medium with tetrathionate and novobiocin (Oxoid Ltd., Basingstoke, Hampshire, UK), medium according to Rappaport-Vassiliadis with soya (RVS, Oxoid Ltd., Basingstoke, Hampshire, UK), xylose lysine deoxycholate medium (Oxoid Ltd., Basingstoke, Hampshire, UK), and chromogenic medium Rambach (RA, Merck KGaA, Darmstadt, Germany) were used (37 °C for 24 h).

To determine the presence of *Campylobacter* spp. in 25 g a rapid serological test Singlepath *Campylobacter* (Merck KGaA, Darmstadt, Germany) was used. For incubation the Bolton broth medium (Oxoid Ltd., Basingstoke, Hampshire, UK) in a microaerophilic atmosphere (5% O₂, 10% CO₂ and 85% N₂) initially at 32 °C for 4 h was used and then at 41.5 °C for 44 h.

PCA (Plate Count Agar) medium (Oxoid Ltd., Basingstoke, Hampshire, UK) at 30 °C for 48 h was used to evaluate the number of the total viable counts (TVC).

For the mesophilic lactic acid bacteria counts and the determination of the MRS (Man Rogosa Sharpe) agar medium (Merck KGaA, Darmstadt, Germany) at 30 °C for 48 h was used.

Escherichia coli counts was determined using agar TBX (Tryptone Bile X-Glucuronide) medium (Oxoid Ltd., Basingstoke, Hampshire, UK) with incubation at 44 °C for 18–24 h.

To determine the Enterobacteriaceae counts VRBD (Violet Red Bile Glucose) agar medium (Merck KGaA, Darmstadt, Germany) at 37 °C for 24 h was used.

The positive coagulase *Staphylococcus* enumeration was performed on RPF (Rabbit Plasma Fibrinogen) medium (Oxoid Ltd., Basingstoke, Hampshire, UK) at 37 °C for 18–24 h.

The number of bacteria was expressed as log₁₀ of colony forming units per gram of MSPM (log cfu/g). In the case of *E. coli* and coagulase positive *Staphylococcus* if their presence was not confirmed in the sausage after production and 1 week of storage they were not determined after 3 weeks of refrigerated storage. In the case of *Salmonella* spp. and *Campylobacter* spp., if their presence was not confirmed in the sausage after production they were not determined in the further research stages during refrigerated storage. The remaining microbiological tests were performed on the products after production and also after 1 and 3 weeks of storage at 4 °C.

2.9. Biochemical Identification of LAB

The sausage samples were inoculated on plates with MRS agar as described above. After incubation the cultures were taken randomly, 10 colonies per each sample and placed in the MRS broth (LabM, Heywood, UK). Identification of isolated bacteria strains whether or not they belonged to the *Lactobacillus* genus was confirmed by Gram staining and by observing cells under a microscope as well as by a catalase test. Species identification was performed using an API CHL50 test (bioMérieux Polska Sp. z o.o, Warsaw, Poland) according to the producers' manual. Biochemical profiles obtained as carbohydrates fermentation results interpreted with an apiweb™ base allowed to qualify the tested isolates to the species. Next, they were compared to the *L. plantarum* SCH1 strain profile used within the experiment.

2.10. Sensory Assessment

The sensory assessment was performed using a Quantitative Descriptive Profile (QDP) method according to the ISO 13299:2016 norm. The assessing teams' task was to determine the intensity of each of the mentioned quality traits and to put their own assessment on the adequate non-structured graphic scale (0–10). In order to compare the sensory quality of the model meat products in the sensory assessment after production, the following parameters were used: four taste discriminants (of cured meat, acidic, fatty, salty, where 0—the least intense, 10—the most intense), three flavor determinants (of cured meat, acidic/spicy, fatty, where 0—the least intense, 10—the most intense) also the following traits were estimated: flavor desirability, color tone, tenderness, juiciness, taste desirability, general quality, where 0—the least desirable, 10—the most desirable. In order to compare the sensory quality of the model meat products after 3 weeks of cold storage the following was applied: two flavor discriminants (smoked, fatty, where 0—the least intense, 10—the most intense) and the following traits were assessed: flavor desirability, color tone, tenderness, taste desirability, general quality where 0—the least desirable, 10—the most desirable. The sensory assessment of the sausages after 3 weeks of cold storage was limited to seven discriminants due to the unacceptable taste and flavor of the control treatment of the product. Samples before assessment were stored in a room with 24 ± 1 °C for 30 min. Each time the assessing team obtained samples of the MSPM sausages, coded with a three-digit code, in the plastic 250 mL container with a lid, a dish with water for mouth rinsing and the assessment card. There were two slices of sausage; each slice was from a different sausage bar, in each container. The assessment was performed by a trained team of eight people and conducted in the sensory analysis studio with individual stands for assessors with artificial lighting. The sensory quality assessment of the model products was performed after production and after 3 weeks of storage.

2.11. Statistical Analysis

All the experiments were performed in triplicates in the independent trials (replicates) $n = 3$ and a completely randomized design were used. A one-way analysis of variance (ANOVA) was applied, first between treatments and then between the storage time of the samples for all parameters. Means and standard deviations were calculated and a probability level of $p < 0.05$ was used in testing the statistical significance of all of the experimental data. The Fisher test was used to determine the significance of the mean values for a multiple comparison ($p < 0.05$). Data analyses were conducted using the STATGRAPHICS v. 4.1 statistical program (Manugistics Inc., Rockville, MD, USA).

3. Results and Discussion

3.1. Determination of pH

In the control treatment the pH increase was observed during the whole storage period ($p < 0.05$). In the L treatment the pH value increase was observed only after 3 weeks of storage ($p < 0.05$). The slight pH value increase after 3 weeks of cold storage could be

the result of hydrolysis protein transformations that run in the heat treated product and microorganisms enzymes activity [32].

During the whole storage period, no differences ($p > 0.05$) in the pH values between the treatments were observed (Table 2). No differences in the samples acidity after production indicated similar pH changes in the dynamics of the raw MSPM during 4 days of cold storage (4 °C). In previous research this fact was also confirmed [24]. Relatively low sugar content in the MSPM could have an influence on the limited production of various metabolites including lactic acid by using LAB [33]. In the research of Vermeiren et al. [34] either the differences in acidity were not observed between cured cooked ham inoculated with *L. plantarum* (after cooking) and the sample without bacteria after 13 days of cold storage. The correlation between the amount of sugars in the medium and the level of the products acidification was also confirmed.

Table 2. pH and ORP changes (mean \pm SD) in sausages with MSPM during storage.

Parameter	Treatment	Storage Time (Weeks)		
		0	1	3
pH	C	6.72 \pm 0.01 ^{aA}	6.76 \pm 0.02 ^{aB}	6.80 \pm 0.01 ^{aC}
	L	6.74 \pm 0.02 ^{aAB}	6.72 \pm 0.02 ^{aA}	6.78 \pm 0.02 ^{aB}
ORP (mV)	C	284.3 \pm 3.0 ^{bA}	332.2 \pm 4.0 ^{bB}	331.7 \pm 2.5 ^{bB}
	L	271.5 \pm 2.9 ^{aA}	322.4 \pm 2.3 ^{aC}	311.6 \pm 2.8 ^{aB}

C—control treatment with MSPM without a bacteria strain, L—treatment with MSPM and the bacteria *L. plantarum* SCH1 (10^7 cfu/g). Various small letters (a–b) in the same column indicate significant differences between treatments ($p < 0.05$). Various capital letters (A–C) in the same line indicate significant differences while being stored ($p < 0.05$).

3.2. Oxidative-Reduction Potential

In both sausage treatments the increase ($p < 0.05$) of redox potential was observed during storage (Table 2). During the whole storage period a significantly higher ($p < 0.05$) redox value was observed in the C treatment, which means that adding *L. plantarum* SCH1 could inhibit the oxidation processes in the final product. Fine grinding, tissue structure decay, and aeration during the separation process caused the MSPM to be more prone to oxidation [6]. LAB belongs to organisms relatively anaerobic but in unfavorable conditions they might use oxygen as a final electrons acceptor. Nevertheless, oxygen might have a negative effect on the lactic acid bacteria cells. During oxygen reduction in the respiratory chain they appear damaging for the cells hydroxyl radicals, hydrogen peroxide and peroxide radicals. Reactive oxygen species (ROS) attack proteins, lipids and nucleic acids contribute directly to cells' death [35,36] Some LAB respond to oxygen stress with increased anti-oxidative enzymes synthesis [37]. In the case of *L. plantarum* the pseudocatalase containing manganese is produced (manganese-containing catalase) [38]. The other enzyme catalyzing hydrogen peroxide decomposition is glutathione peroxidase [39]. Moreover, the activity of the *L. plantarum* SCH1 anti-oxidative enzymes in raw MSPM batter during cold storage before cooking could have an influence on the redox potential value in the product after thermal treatment.

The highest redox potential in the C and L treatments was observed 1 week after storage. After 3 weeks of storage, the redox potential in the C treatment was similar (331.7 mV), but in sausage with the addition of *L. plantarum* SCH1 the redox potential was significantly lower ($p < 0.05$) and lowered from 322.4 mV to 311.6 mV. It should be pointed out that the significant anti-oxidative activity of *L. plantarum* SCH1 in the L treatment after 3 weeks of storage could have an effect on lower redox potential in this period [37].

3.3. Total Heme Pigments Content and Nitrosyl Myoglobin Concentration

The total heme pigments content did not differ significantly ($p > 0.05$) between the sausages with MSPM during storage (Table 3). After production the total heme pigments content reached 113.56 ppm in the C treatment and 116.51 ppm in the L treatment and

after 3 weeks of storage was 114.01 ppm and 112.20 ppm, respectively. In the treatment with *L. plantarum* SCH1, the heme pigments contents lowered significantly ($p < 0.05$) after 3 weeks of cold storage compared to their level after production (Table 3). Taking into consideration the fact that, after 3 weeks of cold storage, lactic acid bacteria were present in the sausages it may be assumed that they used heme from the meat environment for their growth. Abriouel et al. [40] found that *L. plantarum* CNRZ 1228 showed heme depended on catalysis activity on MRS broth with 30 mM hematin, whereas only on the MRS base it did not show any activity of this enzyme. heme delivered from the environment influences the faster growth of *L. plantarum* and enables longer bacteria life [41].

Table 3. Nitrite and nitrate (mean \pm SD), total heme pigments (mean \pm SD) and nitrosyl myoglobin content (mean \pm SD) changes in the model sausages with MSPM during storage.

Parameter	Treatment	Storage Time (Weeks)		
		0	1	3
Nitrite (mg/kg)	C	16.5 \pm 0.2 ^{aC}	15.4 \pm 0.1 ^{aB}	14.4 \pm 0.1 ^{aA}
	L	16.8 \pm 0.2 ^{aC}	15.7 \pm 0.1 ^{aB}	14.4 \pm 0.3 ^{aA}
Nitrate (mg/kg)	C	62.2 \pm 4.9 ^{aB}	49.1 \pm 0.8 ^{aA}	65.4 \pm 2.4 ^{aB}
	L	66.4 \pm 3.0 ^{aB}	49.3 \pm 1.1 ^{aA}	67.1 \pm 0.6 ^{aB}
Total heme pigments content (ppm)	C	113.56 \pm 0.68 ^{aB}	110.84 \pm 1.18 ^{aA}	114.01 \pm 0.39 ^{aB}
	L	116.51 \pm 1.96 ^{aB}	109.48 \pm 0.68 ^{aA}	112.20 \pm 1.80 ^{aA}
NOMb content (g/100 g)	C	75.93 \pm 1.52 ^{bB}	78.84 \pm 1.04 ^{aB}	58.93 \pm 2.73 ^{aA}
	L	70.86 \pm 0.76 ^{aA}	76.29 \pm 3.30 ^{aA}	64.96 \pm 1.07 ^{bA}

C—control treatment with MSPM without a bacteria strain, L—treatment with MSPM and the bacteria *L. plantarum* SCH1 (10^7 cfu/g). Various small letters (a–b) in the same column indicate significant differences between treatments ($p < 0.05$). Various capital letters (A–C) in the same line indicate significant differences while being stored ($p < 0.05$).

The C treatment after production was of significantly higher nitrosyl myoglobin (NOMb) concentration ($p < 0.05$). Lower NOMb content in the L treatment after production could be due to the negative effect of H_2O_2 produced by LAB on nitrosyl heme pigments in MSM batter [40]. After 3 weeks of storage, the tendency was the reverse. A significantly higher ($p < 0.05$) NOMb concentration level was found in the L treatment where *L. plantarum* SCH1 was applied (Table 3).

Significantly lower redox potential in the L treatment and in consequence higher environmental reductivity probably caused a higher nitrosyl pigment level in the L treatment after 3 weeks of storage compared to the control treatment (C) [42]. In both experimental sausages, the concentration of nitrosyl pigments after 3 weeks of storage was lower compared to the concentration determined after production. Reduced nitrosyl pigment levels in the meat products during storage is a commonly known fact and is caused by nitrosyl myochromogen oxidation [43].

3.4. Nitrates and Nitrites Content

The dynamics of nitrates and nitrites transformation in meat products during storage depends on many parameters including environment acidity, redox potential, and the amount of reducing substances [44]. In both experimental sausages a statistically significant ($p < 0.05$) reduction of nitrites level during storage was observed. No differences ($p > 0.05$) in NO_2 content was observed between the treatments during cold storage (Table 3). In the research of Terns et al. [45], the decrease of nitrites level was observed in cured pork sausages during 84 days of cold storage. Similar results were reported by Shin et al. [46] in the case of cured pork pates after 28 days of cold storage. Nitrites reduction in batters and meat products is connected with the reaction of these compounds with muscle and fat tissue components as well as with additional substances introduced into the meat batters [47].

The amount of nitrates in the sausages after production was similar and reached 62.2 mg/kg in the C treatment and 66.4 mg/kg in the L treatment. The amount of nitrates

decreased after 1 week of storage to approx. 49 mg/kg in both of the experimental sausages. Next, the significant increase of nitrates content was observed to the level of 65.4 mg/kg and 67.1 mg/kg respectively in treatment C and 7 after 3 weeks of storage ($p < 0.05$). Nitrates reduction in the tested meat products could be performed with the participation of bacterial enzymes. Whereas the observed nitrates increase in the products was probably caused by the transformation of residual nitrites during cold storage [42]. The introduction of lactic acid bacteria did not have a significant effect ($p > 0.05$) on nitrites and nitrates level in the L treatment (Table 3).

3.5. Color

The values of a^* and b^* parameters in the model products after production and after storage were similar and did not differ significantly ($p > 0.05$). Statistically significant differences ($p < 0.05$) were observed in the color lightness parameter (L^*) in both treatments after storage. The lowest L^* parameter values were observed 1 week after storage reaching 57.74 in the C treatment and 58.15 in the L treatment. A similar result was reported by Slima et al. [48], who observed color lightness decrease after 10 days in the storage of cured (50 mg/kg) raw beef sausages without significant differences between the control sample (without bacteria) and the sample with *L. plantarum*. The highest share of the red color (a^*) was reported in the C and L treatments directly after production reaching 17.33 and 17.22, respectively (Table 4).

Table 4. Color parameters (mean \pm SD) in the model sausages with MSPM during storage.

Parameter	Treatment	Storage Time (Weeks)		
		0	1	3
L^*	C	58.43 \pm 0.75 ^{aB}	57.74 \pm 0.63 ^{aA}	58.30 \pm 0.31 ^{aAB}
	L	58.82 \pm 0.48 ^{aB}	58.15 \pm 0.35 ^{aA}	58.41 \pm 0.39 ^{aAB}
a^*	C	17.33 \pm 0.52 ^{aA}	16.93 \pm 0.38 ^{aA}	17.15 \pm 0.36 ^{aA}
	L	17.22 \pm 0.83 ^{aA}	17.09 \pm 0.29 ^{aA}	17.12 \pm 0.33 ^{aA}
b^*	C	5.26 \pm 0.22 ^{aA}	5.49 \pm 0.22 ^{aB}	5.43 \pm 0.18 ^{aAB}
	L	5.36 \pm 0.25 ^{aA}	5.39 \pm 0.15 ^{aA}	5.48 \pm 0.09 ^{aA}
a^*/b^*	C	3.30 \pm 0.17 ^{aB}	3.09 \pm 0.13 ^{aA}	3.16 \pm 0.11 ^{aA}
	L	3.22 \pm 0.23 ^{aA}	3.17 \pm 0.09 ^{aA}	3.13 \pm 0.08 ^{aA}

C—control treatment with MSPM without a bacteria strain; L—treatment with MSPM and the bacteria *L. plantarum* SCH1 (10^7 cfu/g). Various small letters (a–b) in the same column indicate significant differences between treatments ($p < 0.05$). Various capital letters (A–B) in the same line indicate significant differences while storing ($p < 0.05$).

The b^* parameter value was similar in both experimental treatments in the whole storage period and ranged between 5.26 and 5.49. A significant decrease of the a^*/b^* factor was observed in the C treatment after storage which indicated a lower share of red color in the products [31] (Table 4). A lower share of red color in the C treatment might have been caused by the significant increase of redox potential and by NOMb concentration decrease in this sample after storage [42,49] (Table 3).

3.6. Microbiological Quality

Total viable counts (TVC) after production in the C treatment reached 2.16 log cfu/g and was significantly lower ($p < 0.05$) than in the L treatment (2.51 log cfu/g). After 1 week of storage TVC was at a similar level and reached 2.19 log cfu/g in the C treatment and 2.54 log cfu/g in the L treatment (Table 5). In the research of Zeleňáková, Kunová, and Lopašovský [50] TVC was determined in cooked pork sausages at the level of approx. 4 log cfu/g. Similar TVC values after production (4.05 log cfu/g) and after 3 weeks of storage (4.64 log cfu/g) was observed in the research of Eskandari et al. [51] in frankfurter-type sausages with low additive NaNO_2 (40 ppm) during refrigerated storage at 4 °C.

Table 5. Microbiological quality (mean \pm SD) of the model sausages with MSPM during storage.

Parameter	Treatment	Storage Time (Weeks)		
		0	1	3
Total viable counts (log cfu/g)	C	2.16 \pm 1.06 ^{aA}	2.19 \pm 0.76 ^{aA}	8.24 \pm 7.06 ^{bB}
	L	2.51 \pm 1.48 ^{bA}	2.54 \pm 1.06 ^{bA}	7.31 \pm 5.76 ^{aB}
Lactic acid bacteria counts (log cfu/g)	C	<1.00	<1.00	5.23 \pm 4.00 ^a
	L	<1.00	<1.00	5.92 \pm 4.64 ^b
<i>Escherichia coli</i> counts (log cfu/g)	C	<1.00	<1.00	na
	L	<1.00	<1.00	na
Enterobacteriaceae counts (log cfu/g)	C	<1.00	<1.00	<1.00
	L	<1.00	<1.00	<1.00
Coagulase-positive <i>Staphylococcus</i> count (log cfu/g)	C	<1.00	<1.00	na
	L	<1.00	<1.00	na
<i>Salmonella</i> sp. content in 25 g	C	nd	na	na
	L	nd	na	na
<i>Campylobacter</i> spp. content in 25 g	C	nd	na	na
	L	nd	na	na

C—control treatment with MSPM without a bacteria strain, L—treatment with MSPM and the bacteria *L. plantarum* SCH1 (10^7 cfu/g). Various small letters (a–b) in the same column indicate significant differences between treatments ($p < 0.05$). Various capital letters (A–B) in the same line indicate significant differences while storing ($p < 0.05$); na—not applicable, nd—not detected.

In sausages after production no *Salmonella* sp. nor *Campylobacter* spp. was confirmed. Counts of Enterobacteriaceae, *E. coli*, coagulase positive *Staphylococcus*, and LAB after production were <1 log cfu/g, which means that the heat dose used in the products thermal treatment effectively reduced their number. After 1 week of cold storage the microbiological quality of the sausages did not change (Table 5).

After 3 weeks of storage, the microbiological quality of the sausages changed. The total viable counts increased significantly in both treatments and the increase of mesophilic lactic acid bacteria was observed (Table 5). The TVC in the control C treatment (8.24 log cfu/g) was significantly higher than in the L treatment (7.31 log cfu/g); it might prove that the bacteria strain applied inhibited the growth of undesirable microflora. Reverse dependence was observed in the case of lactic acid bacteria. In the control treatment (C), a significantly lower ($p < 0.05$) LAB level was reported (5.23 log cfu/g) than in the L treatment (5.92 log cfu/g).

The TVC in the control C treatment (8.24 log cfu/g) was significantly higher than in the L treatment (7.31 log cfu/g); it might prove that the bacteria strain applied inhibited the growth of undesirable microflora. Reverse dependence was observed in the case of lactic acid bacteria. In the control C treatment a significantly lower ($p < 0.05$) LAB level was reported (5.23 log cfu/g) than in the L treatment (5.92 log cfu/g). The high level of TVC in both samples could be due to the increase in LAB also in the TVC cultivation, which is confirmed by the studies of other authors [52,53]. Lactic acid bacteria (LAB) are facultative anaerobic and are regarded as a specific spoilage organism in vacuum-packaged cooked meat products during long-term storage, probably due to its dominance in the total viable microflora as well as heat-resistance [54].

It may be assumed that the LAB level increase in the model products with MSPM at the early stage of storage was impeded due to bacterial cell damage caused by heat during thermal treatment. In the standard bacteria cultivation technique, only replicating cells are determined. Roszak et al. [55] showed that cells might enter a dormant state when the environmental conditions become unfavorable (lack of nutrients, temperature, and pH changes). VBNC (viable but nonculturable) cells exhibit active metabolism in the form of respiration or fermentation, incorporate radioactive substrates, and have active protein synthesis but cannot be cultured or grown on conventional laboratory media. The dormancy state is temporary and bacteria cells might reactivate the ability to replicate by corrective mechanisms [56–58], which was most probably observed after 3 weeks of storage. This hypothesis could be confirmed by flow cytometry, but in our study isolation

and identification by preliminary phenotypic and biochemical research were conducted (Table 6). It was found that bacteria isolated from the sausages was in the majority of bacilli, Gram-positive, catalase-negative. In the case of experimental treatment C—4 out of 10 isolates were excluded from further research whereas in the L treatment 1 isolate was excluded due to being atypical for the *Lactobacillus* cells shape.

Table 6. Biochemical estimation results of isolates.

Treatment	Isolate	Bacteria Type	Number of True Tests of Isolate Biochemical Activity with SCH1 Strain	Bacteria Species
C	1	<i>Lactobacillus</i>	40/49	<i>paracasei</i>
	2	nb	na	na
	3	nb	na	na
	4	<i>Lactobacillus</i>	41/49	<i>pentosus</i>
	5	<i>Lactobacillus</i>	48/49	<i>plantarum</i>
	6	nb	na	na
	7	<i>Lactobacillus</i>	45/49	<i>brevis</i>
	8	nb	na	na
	9	<i>Lactobacillus</i>	45/49	<i>brevis</i>
	10	<i>Lactobacillus</i>	41/49	<i>pentosus</i>
L	1	<i>Lactobacillus</i>	45/49	<i>plantarum</i>
	2	<i>Lactobacillus</i>	42/49	<i>rhamnosus</i>
	3	<i>Lactobacillus</i>	49/49	<i>plantarum</i>
	4	nb	na	na
	5	<i>Lactobacillus</i>	29/49	<i>buchneri</i>
	6	<i>Lactobacillus</i>	48/49	<i>plantarum</i>
	7	<i>Lactobacillus</i>	49/49	<i>plantarum</i>
	8	<i>Lactobacillus</i>	49/49	<i>plantarum</i>
	9	<i>Lactobacillus</i>	48/49	<i>plantarum</i>
	10	<i>Lactobacillus</i>	42/49	<i>rhamnosus</i>

C—control treatment with MSPM without a bacteria strain, L—treatment with MSPM and the bacteria *L. plantarum* SCH1 (10^7 cfu/g); na—not applicable.

Bacteria isolates were preliminarily attributed to the genus *Lactobacillus* according the appearance under the microscope, Gram staining and test for catalase, and next the species was identified using a set of 49 biochemical tests (Table 6). It was proved that, in the case of control treatment (C), only one isolate was in compliance with the biochemical activity profile of the SCH1 strain but in the case of the L treatment six isolates complied with the *plantarum* species (Table 6).

Slight differences in the biochemical profiles of isolates might be a sign that bacteria is in a lag phase accommodating to external environmental conditions, expressing or repressing genes responsible for certain traits [57,58]. However, it is clear that molecular biology techniques for identification, considered as the most precise ones, are recommended due to the confirmation of the results. In this study, our goal was to initially confirm the presence of *Lactobacillus* bacteria in the samples and to exclude the presence of other, e.g., spore-forming bacteria that could grow on MRS agar. The present results of the conducted research indicate that the *L. plantarum* bacteria specie has been confirmed in the L treatment. In addition, our previous studies have also shown that the meat environment is suitable for *L. plantarum* SCH1 bacterial growth [25,28], which is in agreement with the present results of research.

The use of nitrites in preserving meat products plays a key role in inhibiting *Clostridium botulinum* bacteria growth and preventing the production of botulinum toxins [44,59,60]. In this study, we did not analyze the antimicrobial efficacies of using of *L. plantarum* SCH1 and sodium nitrite in a reduced amount (50 mg/kg) against *C. botulinum*. However, the properties of curing with nitrite that also make it an effective antibotulinal compound are dependent on the interactions of nitrite with several other factors (salt, pH, heat

treatment, spore level, ingoing nitrite level during manufacture, and residual nitrite levels in the meat) [60,61]. Lövenklev et al. [62] indicated that use of NaNO₂ at 45.0 mg/kg effectively suppresses *C. botulinium* gene expression. Also other research proved applying NaNO₂ at 37.5 mg/kg or 75.0 mg/kg together with NaCl to the amount of 2.5% completely inhibits the growth of *C. botulinium* in an anaerobic environment [63].

3.7. Sensory Quality

After the production process the sensory quality of the MSPM sausages was at a similar level and no significant differences between the treatments were observed ($p > 0.05$). Using lactic acid bacteria did not have a significant ($p > 0.05$) effect on product taste and flavor desirability nor on the remaining sausage sensory traits assessed directly after production (Figure 1).

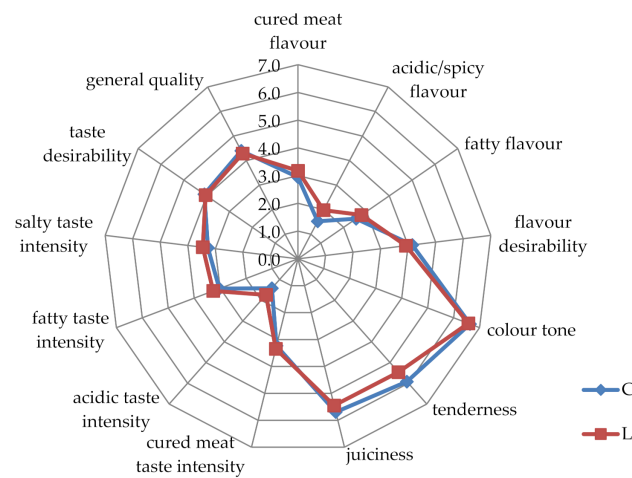


Figure 1. Sensory evaluation of MSPM sausages after production. C—control treatment with MSPM without a bacteria strain, L—treatment with MSPM and the bacteria *L. plantarum* SCH1 (10^7 cfu/g); No significant statistical differences were found ($p > 0.05$).

Interesting research results were obtained in terms of sausage sensory traits 3 weeks after storage. In the control treatment sensory assessment showed features proving that the product was spoiled. That is why, after 3 weeks of cold storage, only chosen indicators of sausage flavor and consistency as well as general desirability were assessed (Figure 2).

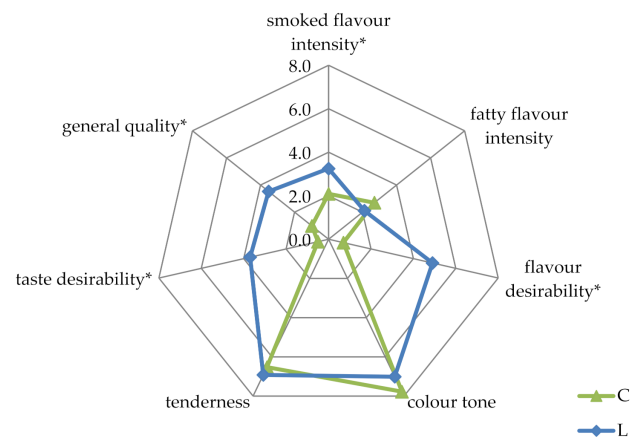


Figure 2. Sensory evaluation of MSPM sausages after 3 weeks of refrigerated storage. C—control treatment with MSPM without a bacteria strain, L—treatment with MSPM and the bacteria *L. plantarum* SCH1 (10^7 cfu/g); * Mean values of this attribute are significantly different ($p < 0.05$).

In the case of the sausage enriched with the *L. plantarum* SCH1 strain, no sensory disqualifying traits were found in the product. L sausage taste and flavor was of high desirability (Figure 2). In the research of Vermeiren et al. [34] assessed cured cooked ham products with *L. plantarum* addition (spread on the surface of slices) were rejected by the sensory panel after 13 days of storage due to the unacceptable taste. LAB addition, like in the tested MSPM sausages in this research, did not have an effect on the color and general appearance of the cured cooked ham products. Pérez-Chabela et al. [26] reported higher scores in the sensory tests of sausages with *L. plantarum* compared to the control treatment ($p < 0.05$). Whereas Victoria-León et al. [25] observed taste improvement and overall acceptance improvement in sausages with *L. lactis* and *L. piscicola* after thermal treatment (inoculated before scalding) after 2 days of cold storage. After 12 days of cold storage, sausages with the LAB addition obtained lower scores compared to the control sample.

4. Conclusions

The performed studies indicated the possibility of using *L. plantarum* SCH1 for the biopreservation of cooked sausages with MSPM cured with a lower sodium nitrite dose. The microbiological analysis and sensory assessment of MSPM sausages showed the protective activity of *L. plantarum* SCH1 against undesirable microorganisms causing product spoilage. No negative effect of *L. plantarum* SCH1 on the physical and chemical model sausages parameters after production and after storage was observed. Nevertheless, the study must be continued, especially in terms of analyzing the antimicrobial efficacies of using *L. plantarum* SCH1 and sodium nitrite in a reduced amount against microorganisms causing product spoilage and pathogens, including *Clostridium botulinum*. Further research will also embrace more detailed molecular identification tests of *L. plantarum* SCH1 bacteria applied to heat-treated meat products.

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