The effect of selected lactic acid bacterial strains on the technological and microbiological quality of mechanically separated poultry meat cured with a reduced amount of sodium nitrite

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ABSTRACT The aim of the research was to estimate the effect of selected lactic acid bacterial strains on the technological quality and microbiological stability of mechanically separated poultry meat (MSPM) cured with a reduced amount of sodium nitrite. The 5 different treatments of MSPM batters were prepared: C150 – control cured with sodium nitrite at 150 mg/kg, C50 – control cured with sodium nitrite at 50 mg/kg, PL1 – cured with sodium nitrite at 50 mg/kg and inoculated Lactobacillus plantarum SCH1 at about 10⁷ cfu/g, PL2 – cured with sodium nitrite at 50 mg/kg and inoculated Lactobacillus brevis KL5 at about 10⁷ cfu/g, and PL3 – cured sodium nitrite at 50 mg/kg and inoculated L. plantarum S21 at about 10⁷ cfu/g. The MSPM batters were tested at 1, 4 and 7 d of being in refrigerated storage. The scope of the research was as follows – physicochemical determinations: pH and redox, nitrates and nitrites as well as nitrosyl pigments levels, color estimation with a Commission Internationale de l’Eclairage Lab system and microbiological determinations: the total viable counts, the mesophilic lactic acid bacteria counts, Escherichia coli and Enterobacteriaceae counts. The inhibitory effect of L. plantarum SCH1 isolated from the ecological raw fermented meat product on E. coli in cured MSPM batters during refrigerated storage was proved (P < 0.05). The use of lactic acid bacterial strains in cured batters that were prepared and based on mechanically separated poultry meat did not have a negative effect on their technological quality. The positive effect of L. brevis KL5 on the level of nitrosyl pigments in the cured MSPM batters was observed (P < 0.05). The conducted research suggested the possibility of using the selected bacterial strains of the Lactobacillus genus to improve the microbiological quality of MSPM cured with a reduced amount of sodium nitrite.

Key words: mechanically separated poultry meat, curing, Lactobacillus, bioprotection

INTRODUCTION

In recent years, higher poultry meat consumption can be observed all over the world. It is forecasted that in 2020, global poultry meat production will reach a record level of 103.5 million tonnes and thus exceeding pork production (USDA, 2019). In Poland, the poultry slaughter market is currently the biggest of the remaining markets (e.g., pork, beef) (GUS, 2019). This increase in poultry meat production is accompanied by increased mechanically deboned poultry meat production and its use thereof in the meat industry (Froning and McKee, 2000). To obtain mechanically separated poultry meat (MSPM), high-pressure separators are used to destroy bone structure. As a result, such a raw material is of worse technological quality and lower stability compared with hand-deboned poultry meat (Stiebing, 2002; Viuda-Martos et al., 2012).

Mechanically separated meat is a cheaper product than hand-deboned meat and is commonly used in meat processing in Poland and worldwide. MSPM is mainly used in cooked or roasted meat products, canned meat, homogenized sausages and ready-to-eat products (Botka-Petrak et al., 2011). The MSPM chemical content is different from muscle tissue content. The raw
material obtained in the process of deboning has a high fat content (including phospholipids), high calcium, phosphorus, iron and heme pigments content (Daros et al., 2005; Botka-Petrak et al., 2011). Moreover, the bones used for MSPM production may contain a high number of microorganisms. The mechanically separated meat microflora may contain pathogenic bacteria, for example Salmonella spp., Campylobacter spp., Escherichia coli, Listeria monocytogenes, Yersinia enterocolitica, Staphylococcus aureus, as well as saprophytic bacteria, mainly the Pseudomonas, that accelerate meat spoiling (Gill, 2004; Daros et al., 2005; Ozkececi et al., 2008).

A lot of research has indicated some problems with microbiological MSPM quality. A total of 46 MSPM batches were tested within the studies performed in Poland, and Salmonella spp. was found in each batch of 25 g. In 59% of the MSPM samples, anaerobic spore bacteria were found in 0.01 g, whereas in 87% of the samples, E. coli bacteria were confirmed in 0.001 g. The coagulase-positive Staphylococci found in 0.1 g were confirmed in 76% of the meat samples (Pomykała and Michalski, 2008). In New Zealand, 145 MSPM samples from 3 meat plants were tested. The average amounts of E. coli in MSPM were 3.64 log cfu/g, average amounts of Campylobacter were 2.87 log cfu/g, and the coagulase-positive Staphylococci average were 2.72 log cfu/g (On et al., 2011).

To stabilize MSPM in the curing process, sodium nitrite is used in industrial practice. This method is used to stabilize fresh or defrosted MSPM. Curing plays an important role in forming the color and also contributes to the taste and flavor of products containing MSPM (Cammak et al., 1999; Majou and Christianas, 2018). Mechanically separated poultry meat is stabilized because of the bacteriostatic effect of nitrites added to MSPM at a certain level together with other substances, mainly with sodium chloride (Lücke, 2008; Sindelar and Milkowski, 2011). Most of all, sodium nitrite is an inhibiting factor to Clostridium botulinum growth and its toxin production (Sebranek and Bacus, 2007; Lücke, 2008; Sindelar and Milkowski, 2011). Nitrite has also a negative physiological effect on other bacteria such as Salmonella enterica serovar Typhimurium and Listeria spp. (Hospital et al., 2012; Majou and Christianas, 2018). An important nitrite function is also its antioxidative effect (Pegg and Shahidi, 2000).

The aforementioned facts legitimate the use of sodium nitrite as a preservative in the meat industry, but at the same time, its application has been raising concern for years. Sodium nitrite may be the precursor of nitrosamines, and many nitrosamines are considered to be carcinogenic (Pegg and Shahidi, 2000). European Union (EU) legislative solutions are aimed at limiting this ingredient in meat products (Commission Regulation (EU) No 257/2010). Some countries for example Denmark already have their own individual stricter regulations concerning the maximum level of sodium nitrite that is 60 mg/kg in some meat products, whereas in the EU, it is 150 mg/kg (Commission Decision (EU) 2018/702). Moreover, a significantly lower addition of sodium nitrite to MSPM batters may limit its stability. Thus, it is recommended and legitimate to look for new technological ways of enabling MSPM stabilization.

The lactic acid bacteria (LAB) are commonly used in meat processing to produce fermented sausages (Rubio et al., 2013). Lactic acid bacteria may be widely used in bioprotection because they may naturally dominate the microflora of many food products during their storage (Baleciunas et al., 2013). Furthermore, the antimicroorganism effect of LAB is possible owing to its extracellular protein substances. To provide proper conditions for its growth, the bacteria produce many antimicroorganism substances released outside the cell. Apart from organic acids, hydrogen peroxide, diacetly, and carbon dioxide, bacteria produce a wide variety of protein substances called bacteriocins that have a significant bactericidal and/or bacteriostatic effect (Rzepkowska et al., 2017a).

The LAB such as Lactobacillus sakei, Pediococcus acidilactici, and Lactobacillus curvatus are used to improve the stability and health security of raw meat products (Ammor and Mayo, 2007). In addition, there has been research performed on the use of various lactic acid bacterial strains for the bioprotection of steamed cured meat and poultry cutup carcass parts during storage (Bredholt et al., 2001; Enan, 2006; Perez-Chabela et al., 2008; Martinez-Romero et al., 2016). In addition, there were trials to use selected Pediococcus and Lactobacillus strains to improve the microbiological quality of uncurd MSPM (Raccach and Baker, 1979; Łaszkiewicz et al., 2019). The authors did not find research on LAB use in cured MSPM produced with reduced amounts of sodium nitrite in the available bibliography.

The use of LAB in cured MSPM production with reduced amount of sodium nitrite may be an additional hurdle in the “hurdle technology” improving MSPM microbiological quality. However, an important aspect in the practical use of LAB as cured MSPM bioprotection may be also the bacteria effect on the technological quality that is color, pH, redox, and nitrosyl pigments concentration in MSPM. The aim of this research was to estimate the effect of selected lactic acid bacterial strains on the technological and microbiological quality of MSPM cured with a reduced amount of sodium nitrite.

**MATERIAL AND METHODS**

**Lactic Acid Bacteria Strains and Culture Conditions**

Three lactic acid bacterial strains were used within this research that is: Lactobacillus plantarum SCH1 isolated from the ecological raw fermented pork roast, Lactobacillus brevis KL5 isolated from ecological raw fermented sausage, and L. plantarum S21 isolated from organic whey. The ecological raw fermented meat products were produced by the Meat Processing Plant “Jasiółka” in Dukla in Poland, in which meat from local
organic farms is being processed. Whey was derived from milk from an organic farm in Podkarpackie region in Poland. Bacterial strains originated from microorganism collection owned by the Chair of the Food Hygiene and Quality Management at the Warsaw University of Life Sciences were selected and based on the research of Rzepkowska et al. (2017a,b). To properly revive and prepare bacterial strains for the study each time, the LAB culture was defrosted from stocks containing 20% of glycerol with storage at −80°C. Each bacteria culture was mixed, and 20 μL of each strain was added to the sterile test tubes with 5 mL of sterile MRS broth (Merck, Darmstadt, Germany). Test tubes were incubated for 24 h at a temperature of 37°C. Then, 1 mL of bacterial culture was taken after 24 h and moved to 9 mL of fresh sterile MRS broth (Merck, Darmstadt, Germany) and was subjected to reincubation in the same conditions. After that, the bacterial cultures were centrifuged (J2-21; Beckman, Birkerød, Denmark) (4,500 rpm, 2,313 \times g), supernatant was removed, and bacteria were suspended in 0.9% NaCl solution. The bacterial biomass in this form was applied to MSPM batters. Suspended in 0.9% NaCl solution. The bacterial supernatant was removed, and bacteria were suspended in 0.9% NaCl solution. The bacterial biomass in this form was applied to MSPM batters. Finally, all of the procedures were performed in sterile conditions with the use of sterile equipment.

Preparing MSPM Batters

High-pressure MSPM was obtained by the deboning of unfrozen chicken carcasses in a separator (AM2C, Quimper, France) (whole diameter was 1 mm) in the conditions of the meat plant. The MSPM was divided into 10-kg blocks, frozen to −18°C, and stored at this temperature for 2 to 4 d. To produce batters, the MSPM blocks were defrosted in refrigeration conditions (4°C–6°C) for approximately 24 h. The 5 different treatments of MSPM batters were prepared (Table 1): C150 – control cured with sodium nitrite at 150 mg/kg, C50 – control cured with sodium nitrite at 50 mg/kg, PL1 – cured with sodium nitrite at 50 mg/kg and inoculated L. plantarum SCH1 at about 10^7 cfu/g, PL2 - cured with sodium nitrite at 50 mg/kg and inoculated L. brevis KL5 at about 10^7 cfu/g, and PL3 – sodium nitrite at 50 mg/kg and inoculated L. plantarum S21 at about 10^7 cfu/g. The batter ingredients were mixed for 3 min until they reached uniform consistency in the Hobart mixer (N-50G; Hobart Corporation, Troy, OH). The MSPM batters were canned in 190-g cans and kept for 7 d at a temperature of 4°C to 6°C to reduce microbiological pollution. All of the samples were tested at 24 h, 4 d, and 7 d of being stored. The experimental production of MSPM batters was performed in the half-technical hall of the Meat and Fat Technology Department in the Prof. Wacław Dąbrowski Institute of Agriculture and Food Biotechnology in Warsaw. The entire experiment was repeated 3 times.

**Physical and Chemical Analyses**

**pH and Oxidative Reduction Potential Measurement** Ten grams of MSPM batters was homogenized with 50 mL distilled water to determine the pH and oxidative reduction potential (ORP) using an 600-W blender (MSM 66120; BSH Hausgeräte GmbH, Munich, Germany) for 1 min at a speed equal to 14,000 rpm. Then, the digital pH-meter (Mettler Delta 350; Mettler Toledo, Schwerzenbach, Switzerland) was used to measure the pH value with an automatic compensation temperature, a glass-calomel electrode In Lab Cool (Mettler Toledo, Greifensee, Switzerland), and ORP value with an electrode In Lab Redox Pro (Mettler Toledo, Greifensee, Switzerland). The obtained result ORP value (mV) was calculated into the redox potential value in relation to the standard hydrogen electrode EH (mv). To this end, the potential value of the reference electrode at a temperature of 20°C (207 mV) was added to the readout value obtained with the equipment.

**Sodium Nitrate and Nitrite Content** The nitrate and nitrite level was determined as per PN-EN 120414:2006 changed by Siu and Henshall (1998). To perform the test, a liquid chromatograph (Agilent Technologies, Waldbronn, Germany) with a UV detector with an IonPac AS11-HC 4 × 250 mm analytical column (Thermo Fisher Scientific, Sunnyvale, CA) and precolumn AG11-HC 4 × 50 mm (Thermo Fisher Scientific, Sunnyvale, CA) was used. The nitrate and nitrite content in the tested samples was expressed as NaNO3 and NaNO2 salts content in mg/kg.

**Color Determination** The spherical spectrophotometer Minolta (CR-300; Konica Minolta, Tokyo, Japan) with a measuring hole diameter of 25.4 mm was used to determine the color parameters. The trio-chromatic

| Ingredients/experimental treatment | C150 | C50 | PL1 | PL2 | PL3 |
|-----------------------------------|------|-----|-----|-----|-----|
| Mechanically separated poultry meat (kg) | 10 10 | 10 10 | 10 | 10 | 10 |
| Salt (kg) | 0 2 | 0 2 | 0 2 | 0 2 | 0 2 |
| Sodium nitrite (kg) | 0.0016 0.00053 | 0.00053 0.00053 | 0.00053 | 0.00053 | 0.00053 |
| Bacterial biomass in NaCl solution 0.0% (kg) | - | - | 0.476 | 0.476 | 0.476 |
| NaCl solution 0.0% (kg) | 0.476 | 0.476 | - | - | - |

Abbreviations: C150, control cured with sodium nitrite at 150 mg/kg; C50, control cured with sodium nitrite at 50 mg/kg; MSPM, mechanically separated poultry meat; PL1, cured with sodium nitrite at 50 mg/kg and inoculated Lactobacillus plantarum SCH1 at about 10^7 cfu/g; PL2, cured with sodium nitrite at 50 mg/kg and inoculated L. brevis KL5 at about 10^7 cfu/g; PL3, cured sodium nitrite at 50 mg/kg and inoculated L. plantarum S21 at about 10^7 cfu/g.
coordinates were settled in a Comission Internationale de l’Eclairage system L* a* b*, where L* is for color brightness, a* for chromaticity in the range of red and green, and b* chromaticity in the range of yellow and blue. The standard Comission Internationale de l’Eclairage observatory was used at measurements: 2°, illuminant D65, 8 mm measuring area. The reference source was the white standard (L* = 95.87, a* = -0.49, b* = 2.39). For each of the 3 repeats of the treatment (C150, C50, PL1, PL2, PL3), 5 measurements were carried out.

**Nitrosyl Pigments Content Analysis**
The nitrosyl pigment content was determined with the Hornsey Method (1956). The absorbance of filtrate values was measured using a spectrophotometer (U-2900; Hitachi, Tokyo, Japan), and the nitrosyl pigment content was measured in hematin ppm.

**Microbiological Analysis**
The scope of the microbiological studies of cured MSPM batters was determined based on the preliminary tests. The preliminary tests included a microbiological analysis of an averaged sample of 3 batches of MSPM used in experiment. The range and methodology of the microbiological tests are described in the following text.

Ten grams of samples were taken for quantity laboratory measurements, and 25 g samples were taken for quality laboratory measurements. The sample after mixing was suspended in 100 mL of peptone water, then diluted many times and placed for growing on different culture media.

The presence of *Salmonella* spp. in 25 g of the product was determined using Müller-Kauffmann’s medium with tetrathionate and novobiocin (Oxoid Ltd., Basingstoke, Hampshire, England), RVS (medium in accordance with Rappaport-Vassiliadis with soya) (Oxoid Ltd., Basingstoke, Hampshire, England), xylose lysine deoxycholate medium (Oxoid Ltd., Basingstoke, Hampshire, England), and chromogenic medium Rambach (37°C for 24 h) (Merck KGaA, Darmstadt, Germany).

The presence of *Campylobacter* spp. in 25 g of the product was determined using a rapid serological test Singlepath Campylobacter (Merck KGaA, Darmstadt, Germany). Incubation was carried out on Bolton broth medium (Oxoid Ltd., Basingstoke, Hampshire, England) in a microaerophilic atmosphere (5% O₂, 10% CO₂, and 85% N₂) initially at 32°C for 4 h and then at 41.5°C for 44 h.

The number of the total viable counts (TVC) (30°C for 48 h) was determined on plate count agar medium (Oxoid Ltd., Basingstoke, Hampshire, England).

The mesophilic LAB counts (30°C for 48 h) was determined using MRS medium (Merck KGaA, Darmstadt, Germany).

The coagulase-positive *Staphylococci* counts (37°C for 18–24 h) were determined on RPF medium (Oxoid Ltd., Basingstoke, Hampshire, England).

The number of bacteria was expressed as log10 of cfu per gram of MSPM (log cfu/g). If the presence of the given microorganisms was not detected in the MSPM, no further tests were taken during storage. Microbiological tests were performed on MSPM batters after the first, fourth, and seventh day of storage at the temperature of 4°C.

**Statistical Analysis**
The experiment was carried out in 3 replications (n = 3) at different times, and a completely randomized design was used. All observations composing the experiment (5 treatments × 3 batches × 3 storage periods) were included in the statistical analysis. The results obtained in the research were statistically analyzed using the two-way ANOVA. The model included the treatment effect (LAB addition) of the time of storage (1, 4, 7 d) and their interaction (LAB addition x time of storage). The Bonferroni post hoc test was used to determine the significance of the mean values for a multiple comparison at P < 0.05. The Statistica package, version 13 (StatSoft Polska Sp. z o.o., Cracov, Poland), was used for the calculations.

**RESULTS AND DISCUSSION**

**pH and ORP**
The LAB treatment and storage time affected (P < 0.001) the pH value. Interaction between LAB addition and storage time for pH also was found (Table 2). The addition of LAB to the MSPM batters resulted in little but significant (P < 0.05) differences in the batter acidity and were observed between experimental treatment after the first day of storage (Table 2). The highest pH value was reported after the first day of storage in the PL2 treatment with the addition of *L. brevis* KL5 (pH1 = 6.99). In the remaining experimental treatments, the pH value was at a similar level (6.91–6.94). The acidity of the MSPM batters was higher with a longer refrigerated storage time (P < 0.05). The pH changes dynamics, which was similar in all of the experimental treatment (Table 2). The increase of the acidity was probably related to the metabolic activity of the added LAB or wild bacterial cultures present in MSPM (Ha et al., 2003). The LAB may ferment sugars into lactic acid or other organic acids (Balciunas et al., 2013). After 7 d of storing, the pH value of the MSPM batters did not differ significantly (P > 0.05) (Table 2). Taking into consideration the small differences between the pH values of the different treatment in all of the 3 storing periods (0.02–0.08), it can be assumed they had no effect on the water-holding capacity of the meat.

The chemical content of MSPM is different from hand-deboned meat. Mechanically separated poultry meat has a higher fat and collagen content and also lower water activity than hand-boned meat (Mayer et al., 2007). It can be assumed that the LAB environment
was unfavorable for its growth. In addition, a relatively low sugar content in MSPM could affect the limited production of some metabolites including lactic acid by applied LAB (Ha et al., 2003).

The significant ($P < 0.001$) influence of LAB treatment and storage time on the redox potential value was observed. In addition, interaction between LAB addition and storage time for redox potential was found (Table 2). There were no significant differences ($P > 0.05$) in ORP values between treatment at the first and fourth day of storage time. The significantly ($P < 0.05$) lowest ORP value was observed in C150 treatment after 7 d of being stored.

In the control treatment C50 (NaNO2 50 mg/kg), the redox potential value oscillated within 319.7–328.1 mV. In the control treatment C150 (NaNO2 150 mg/kg), the redox potential after 4 d of storage increased from 311.8 mV to 326.6 mV and then decreased by 21.0 mV after 7 d of being stored, reaching the lowest value of all of the treatments. Sodium nitrite is highly antioxidative (Sebranek, 2009), so it could be assumed that adding more NaNO2 caused a decrease of the redox potential of the MSPM batters after 7 d of storage.

In the treatments with the addition of LAB (PL1, PL2, PL3), an increase in ORP value during storage was observed. However, significant ($P < 0.05$) differences in ORP values during storage were found only for the PL2 treatment (Table 2).

In the research by Libera et al. (2015), the addition of Bifidobacterium BB12 did not affect the redox potential of dry-cured pork necks. It could be assumed that metabolites produced by the probiotic strains protect lipids from oxidation during refrigerated storage (Libera et al., 2015). In the case of MSPM in which the muscle tissue was crushed during deboning and the lipids were partially oxidized, damaged tissues were much more prone to oxidation (Stiebing, 2002). Moreover, MSPM is of higher heme pigment content than hand-deboned meat (Daros et al., 2005). Thus, heme pigments may initiate lipid oxidation processes, and lipid oxidation products contribute to pigment oxidation (Skibsted, 1996; Faustman et al., 1999).

### Sodium Nitrate and Nitrite Content

The LAB treatment and storage time affected ($P < 0.001$) NaNO2 content. Interaction between LAB addition and storage time for NaNO2 content was found (Table 3). It was confirmed that NaNO2 content in the experimental treatments decreased during storage (Table 3), and this fact indicated the reaction of added nitrites with heme pigments and other MSPM ingredients (Honikel, 2008). The sodium nitrite content in MSPM treatments cured at 50 mg/kg (C50, PL1, PL2, PL3) was similar after the first and fourth day of storage (Table 3). After 1 d of storage, sodium nitrite in the experimental treatments varied from 35.6 to 40.4 mg/kg, whereas after 4 d of storage, it ranged from 28.2 to 30.1 mg/kg. After 7 d of storage, the significantly ($P < 0.05$) lowest NaNO2 content (18.8 mg/kg) was reported in MSPM with L. brevis KL5 (PL2). The nitrites may be reduced by some bacterial strains of the Lactobacillus genus in the process called fermentation nitrate reduction (Hammes, 2012). In the control treatment C150, it was reported that after 4 d of being stored, the sodium nitrite content was significantly lower and reached 112.6 mg/kg, whereas after 7 d, it increased up to 122.0 mg/kg ($P < 0.05$). The higher nitrites content after 7 d may be caused by the reduction of nitrates by bacteria in the dismutation reaction (Honikel, 2008; Hammes, 2012). The effectiveness of the meat curing process depends on many factors including meat

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**Table 2.** The effect of lactic acid bacteria and refrigerated storage time on the pH and oxidative reduction potential of MSPM cured batters (average value ± SE).

| Treatment  | Storage time (day) | LAB treatment | Time | LAB treatment x time |
|------------|--------------------|---------------|------|----------------------|
|            | 1                  | 4             | 7    |                      |
| pH         |                    |               |      |                      |
| C150       | 6.94 ± 0.01c,d     | 6.77 ± 0.01b  | 6.66 ± 0.02* | **       |
| C50        | 6.92 ± 0.01*       | 6.76 ± 0.01b  | 6.64 ± 0.01* | **       |
| PL1        | 6.92 ± 0.01*       | 6.76 ± 0.01b  | 6.65 ± 0.01* | **       |
| PL2        | 6.99 ± 0.01d       | 6.78 ± 0.02b  | 6.68 ± 0.01* | **       |
| PL3        | 6.91 ± 0.01c       | 6.76 ± 0.01b  | 6.70 ± 0.01* | **       |
| ORP [mV]   |                    |               |      |                      |
| C150       | 311.8 ± 2.3b,c,e   | 326.6 ± 0.8c  | 305.6 ± 1.0c* | **       |
| C50        | 319.7 ± 1.9b,c,e    | 328.1 ± 3.0b  | 321.3 ± 5.6c,b,e | **       |
| PL1        | 330.2 ± 2.2b,c,e,d  | 332.6 ± 1.6b,c,d | 323.9 ± 3.2b,c,d | **       |
| PL2        | 308.9 ± 0.9b,c,d    | 315.2 ± 1.6b,c,d | 327.5 ± 1.8b,c,d | **       |
| PL3        | 318.3 ± 2.9b,c,e,d  | 318.4 ± 1.0b,c,d | 327.2 ± 1.7b,c,d | **       |

* Means with different superscript small letters differ significantly ($P < 0.05$).

n = 3.
P: significance of effects; LAB treatment; time; LAB treatment-time interaction.

*P < 0.01; **P < 0.001.

Abbreviations: C150, control cured with sodium nitrite at 150 mg/kg; C50, control cured with sodium nitrite at 50 mg/kg; MSPM, mechanically separated poultry meat; PL1, cured with sodium nitrite at 50 mg/kg and inoculated Lactobacillus plantarum SCH1 at about 10^7 cfu/g; PL2, cured with sodium nitrite at 50 mg/kg and inoculated Lactobacillus brevis KL5 at about 10^7 cfu/g; PL3, cured sodium nitrite at 50 mg/kg and inoculated L. plantarum S21 at about 10^7 cfu/g.
cured batters (average value $\pm$ SE).

| Treatment | 1 | 4 | 7 | LAB treatment | Time | LAB treatment x time |
|-----------|---|---|---|---------------|------|---------------------|
| NaNO₂ [mg/kg] | | | | | | |
| C150      | 131.2 ± 1.7<sup>c</sup> | 112.6 ± 2.1<sup>d</sup> | 122.0 ± 0.4<sup>b</sup> | ** | ** | ** |
| C50       | 35.6 ± 1.4<sup>f</sup> | 28.2 ± 0.9<sup>e</sup> | 22.7 ± 1.2<sup>d</sup> | ** | ** | ** |
| PL1       | 40.4 ± 1.3<sup>e</sup> | 28.4 ± 0.2<sup>d</sup> | 22.7 ± 0.9<sup>c</sup> | ** | ** | ** |
| PL2       | 38.1 ± 0.4<sup>e</sup> | 30.1 ± 1.6<sup>d</sup> | 18.8 ± 0.6<sup>c</sup> | ** | ** | ** |
| PL3       | 36.2 ± 1.2<sup>e</sup> | 29.1 ± 1.5<sup>d</sup> | 22.0 ± 0.2<sup>c</sup> | ** | ** | ** |
| NaNO₃ [mg/kg] | | | | | | |
| C150      | 15.5 ± 0.7<sup>b,c,d</sup> | 27.8 ± 0.7<sup>e</sup> | 15.4 ± 0.3<sup>b,c,d</sup> | ** | ** | ** |
| C50       | 14.7 ± 0.4<sup>b,c</sup> | 18.9 ± 0.4<sup>d</sup> | 7.3 ± 0.7<sup>e</sup> | ** | ** | ** |
| PL1       | 14.6 ± 1.0<sup>b,c</sup> | 17.5 ± 0.5<sup>d</sup> | 9.0 ± 1.4<sup>e</sup> | ** | ** | ** |
| PL2       | 14.9 ± 0.5<sup>b,c</sup> | 19.1 ± 0.4<sup>d</sup> | 7.7 ± 1.0<sup>e</sup> | ** | ** | ** |
| PL3       | 13.4 ± 0.5<sup>b</sup> | 15.1 ± 0.9<sup>b,c,d</sup> | 7.7 ± 0.6<sup>e</sup> | ** | ** | ** |
| Nitrosyl pigments [ppm hematin] | | | | | | |
| C150      | 59.45 ± 3.70<sup>b</sup> | n.a. | 82.94 ± 3.46<sup>b,c,d</sup> | ** | ** | ** |
| C50       | 62.21 ± 0.12<sup>b</sup> | n.a. | 85.98 ± 2.72<sup>e</sup> | ** | ** | ** |
| PL1       | 78.49 ± 5.7<sup>b</sup> | n.a. | 103.82 ± 3.31<sup>e</sup> | ** | ** | ** |
| PL2       | 48.29 ± 1.54<sup>a</sup> | n.a. | 127.31 ± 4.74<sup>e</sup> | ** | ** | ** |
| PL3       | 108.46 ± 3.79<sup>b</sup> | n.a. | 126.44 ± 8.52<sup>e</sup> | ** | ** | ** |

<sup>a</sup> Means with different superscript small letters differ significantly ($P < 0.05$).

<sup>n</sup> = 3.  
<sup>P</sup> = significance of effects; LAB treatment; time; LAB treatment-time interaction.

<sup>**</sup> $P < 0.001$.  
Abbreviations: C150, control cured with sodium nitrite at 150 mg/kg; C50, control cured with sodium nitrite at 50 mg/kg; MSPM, mechanically separated poultry meat; n.a., not applicable; PL1, cured with sodium nitrite at 50 mg/kg and inoculated *Lactobacillus plantarum* SCH1 at about $10^7$ cfu/g; PL2, cured with sodium nitrite at 50 mg/kg and inoculated *Lactobacillus brevis* KL5 at about $10^7$ cfu/g; PL3, cured sodium nitrite at 50 mg/kg and inoculated *L. plantarum* S21 at about $10^7$ cfu/g.

The significant ($P < 0.001$) influence of LAB treatment and storage time on NaNO₃ content in MSPM batters was observed. Interaction between LAB addition and storage time for NaNO₃ content was found (Table 3). After 1 d of storage, the nitrate content in composition (Honikel, 2008). High-fat and collagen protein content in MSPM (Botka-Petrak et al., 2011) may obstruct the nitrogen curing access to heme pigments and affect the final curing result (Mancini and Hunt, 2005).

### Table 4. The effect of lactic acid bacteria and refrigerated storage time on the colour parameters L* a* b* of MSPM cured batters (average value $\pm$ SE).

| Treatment | Storage time (day) | LAB treatment | Time | LAB treatment x time |
|-----------|-------------------|---------------|------|---------------------|
|           | 1 | 4 | 7 | P | P | P |
| L*        | | | | | | |
| C150      | 52.56 ± 0.16<sup>c</sup> | 51.45 ± 0.34<sup>d</sup> | 49.84 ± 0.29<sup>b</sup> | ** | ** | ** |
| C50       | 49.65 ± 0.28<sup>a</sup> | 51.88 ± 0.26<sup>b</sup> | 50.40 ± 0.33<sup>b,c</sup> | ** | ** | ** |
| PL1       | 53.95 ± 0.33<sup>c</sup> | 50.86 ± 0.39<sup>b,c</sup> | 51.25 ± 0.31<sup>b,c,d</sup> | ** | ** | ** |
| PL2       | 50.59 ± 0.25<sup>c</sup> | 49.54 ± 0.46<sup>a</sup> | 50.39 ± 0.29<sup>b,c</sup> | ** | ** | ** |
| PL3       | 53.29 ± 0.23<sup>f</sup> | 50.74 ± 0.34<sup>c</sup> | 49.68 ± 0.17<sup>e</sup> | ** | ** | ** |
| a*        | | | | | | |
| C150      | 13.78 ± 0.44<sup>b,c</sup> | 13.86 ± 0.63<sup>c</sup> | 22.00 ± 1.17<sup>f</sup> | * | ** | ** |
| C50       | 12.90 ± 1.23<sup>b</sup> | 14.47 ± 0.69<sup>e</sup> | 21.18 ± 1.15<sup>d</sup> | ** | ** | ** |
| PL1       | 12.61 ± 0.37<sup>b</sup> | 14.51 ± 0.75<sup>e</sup> | 20.43 ± 2.34<sup>d</sup> | ** | ** | ** |
| PL2       | 12.79 ± 0.67<sup>b</sup> | 14.33 ± 1.32<sup>e</sup> | 22.51 ± 1.30<sup>d</sup> | ** | ** | ** |
| PL3       | 12.24 ± 0.34<sup>b</sup> | 14.65 ± 0.48<sup>e</sup> | 20.93 ± 1.07<sup>e</sup> | ** | ** | ** |
| b*        | | | | | | |
| C150      | 8.88 ± 0.81<sup>b,c,d</sup> | 8.53 ± 0.58<sup>b,c</sup> | 8.40 ± 0.46<sup>b,c</sup> | N.S. | ** | ** |
| C50       | 8.85 ± 0.82<sup>b,c</sup> | 8.34 ± 0.60<sup>b,c</sup> | 8.29 ± 0.40<sup>b</sup> | ** | ** | ** |
| PL1       | 9.48 ± 0.56<sup>b</sup> | 8.20 ± 0.67<sup>b</sup> | 8.04 ± 0.47<sup>b</sup> | ** | ** | ** |
| PL2       | 9.18 ± 0.85<sup>b</sup> | 8.22 ± 0.82 | 8.63 ± 0.49<sup>b,c</sup> | ** | ** | ** |
| PL3       | 9.52 ± 0.47<sup>c</sup> | 8.26 ± 0.53<sup>b</sup> | 7.96 ± 0.71<sup>c</sup> | N.S. | ** | ** |

<sup>a</sup> Means with different superscript small letters differ significantly ($P < 0.05$).

<sup>n</sup> = 3.  
<sup>P</sup> = significance of effects; LAB treatment; time; LAB treatment-time interaction.

<sup>**</sup> $P < 0.001$.  
<sup>*</sup> $P < 0.001$.

Abbreviations: C150, control cured with sodium nitrite at 150 mg/kg; C50, control cured with sodium nitrite at 50 mg/kg; MSPM, mechanically separated poultry meat; N.S., not significant; PL1, cured with sodium nitrite at 50 mg/kg and inoculated *Lactobacillus plantarum* SCH1 at about $10^7$ cfu/g; PL2, cured with sodium nitrite at 50 mg/kg and inoculated *Lactobacillus brevis* KL5 at about $10^7$ cfu/g; PL3, cured sodium nitrite at 50 mg/kg and inoculated *L. plantarum* S21 at about $10^7$ cfu/g.
all of the experimental treatments was similar (13.4–
15.5 mg/kg). After 4 d, an increase in NaNO3 content in
all treatments was found; however, significant
($P < 0.05$) differences were observed in C150, C50,
and PL2 treatments (Table 3). The significantly
($P < 0.05$) highest nitrate content confirmed in the
C150 (27.8 mg/kg) and the lowest content in the PL3
(15.1 mg/kg). The nitrate increase in the treatments
was caused by the dismutation of nitric acid produced
from nitrites added to the MSPM and the secondary
responses of nitric oxide produced in the reaction
(Honikel, 2008). The NaNO3 content in each treatment signi-
ficantly ($P < 0.05$) diminished after 7 d of being stored.
The highest nitrate level was confirmed in the C150
treatment (15.4 mg/kg). In other treatments, nitrate
content was at a similar level (7.3–9.0 mg/kg). Nitrate
reduction in MSPM could run by the involvement of bac-
terial enzymes (Honikel, 2008). Furthermore, some bac-
terial strains of the Lactobacillus genus are able to
biochemically transform nitrogen into nitric oxide with
the use of nitrite reductase in both anaerobic and aerobic
conditions (Xu and Verstraete, 2001).

**Determination of Color**

The LAB addition and storage time affected
($P < 0.001$) lightness ($L^*$) in the tested MSPM batters.
Interaction between LAB treatment and storage time was
found for $L^*$ parameter (Table 4). The obtained re-
results point out that the addition of LAB has a significant
($P < 0.05$) effect on the lightness of the MSPM batters at
the beginning of the experiment (1 d). After 7 d of refrig-
erated storage, the increase of the $L^*$ parameter
compared with day 4 was reported in the PL1 and PL2
treatments. In the research of Zhang et al. (2018) also,
the influence of L. sakei and L. curvatus on the color
brightness ($L^*$) increases in vacuum-packed raw beef af-
ter cold storage was observed.

The LAB addition did not significantly affect the $b^*$
parameter value. The storage time affected
($P < 0.001$) the $b^*$ parameter. Interaction between
LAB treatment and storage time for the $b^*$ parameter
was found (Table 4). After 4 d, the significant
($P < 0.05$) decrease of the $b^*$ parameter was obser-
vied in all treatments with the LAB addition. After 7 d of
storage, decrease of the $b^*$ parameter was observed in
all treatments, except for the PL2 treatment with L. bre-
vis KL5, which presented an increase of the $b^*$ param-
eter (8.63). In the research of Kim et al. (2014), the
increase of $L^*$ and $b^*$ values was observed in chicken
breast with LAB added.

The significant influence of LAB treatment ($P < 0.01$)
and storage time ($P < 0.001$) on redness ($a^*$) was
observed. Interaction between LAB treatment and stor-
age time was found for the $a^*$ parameter (Table 4). At
the beginning of storage (1 d), the highest $a^*$ value
was observed in the C150 treatment. The highest red co-
lor share in the color tone after 7 d of storage was
observed in the PL2 treatment. The LAB effect on the
$a^*$ parameter increase was also reported by Zhang
et al. (2018) in raw beef after 38 d of being stored
($P < 0.05$). The $a^*$ value increased significantly
($P < 0.05$) in all of the experimental treatments during
storing. The higher share of red color was caused by the
overcuring of meat and the growing amount of nitro-
syl derivatives of heme pigments in the MSPM batters (Tables 3 and 4).

**Nitrosyl Pigments Content**

The LAB treatment and storage time affected
($P < 0.001$) nitrosyl pigment content. Interaction be-
tween LAB addition and storage time for nitrosyl pig-
ments content was also found (Table 3). One of the
factors determining the speed and efficiency of the curing
process is the concentration of hydrogen ions. Lower pH
influenced the reaction process between the heme pig-
ments (Mancini and Hunt, 2005; Faustman et al.,
2010). After 7 d of storage, the nitrosyl pigment levels
increased in all of the experimental treatments
($P < 0.05$). It was confirmed that nitrosyl pigment con-
tent in the MSPM batters after storage was the highest
($P < 0.05$) in treatments PL2 and PL3 with the addition
of LAB (Table 3). In the case of the PL2 treatment, the
high nitrosyl pigment level could be caused by a low
NaNO2 level after 7 d of storage. This fact indicated the
intensive reaction of heme pigments with sodium ni-
trite (Sebranek and Bacus, 2007; Sindelar and
Milkowski, 2011. The treatment with L. brevis KL5
showed the lowest ORP value after 1 and 4 d of storage,
among all of the experimental treatments (Table 2). The
higher reduction potential of the MSPM environment
could affect sodium nitrite reduction and thus produces
more nitrogen oxide. Nitrogen oxide may react with
MSPM heme proteins resulting in producing a complex
NO myoglobin (Honikel, 2008).

The PL3 treatment was a similar ORP value to the
control treatment (C50). This fact indicated the similar
reduction potential of the MSPM batters environment
(Table 2). It suggested that the mechanism behind the
higher amount of nitrosyl pigments in the PL3 treat-
ment was different (Table 3). Some lactic acid bacterial
strains may produce nitrogen oxide in the L-arginine
synthesis reaction (Zhang et al., 2007; Li et al., 2016). Ni-
trgen oxide produced in this way was observed in both
environments with and without nitrites (Morita et al.,
1997; Christensen et al., 1999).

**Microbiological Analysis**

**Microbiological Analysis of MSPM** In the prelimi-
inary tests, the TVC in MSPM was determined reaching
6.30 log cfu/g. A similar TVC level of 5.86 log cfu/g in
MSPM was reported by Hać-Szymańczuk et al. (2014).
In the research of On et al. (2011), the TVC in MSPM
was observed at a higher level reaching 7.26 log cfu/g.
Relatively high MSPM microbiological pollution is
caused by fine meat grinding, decomposition of tissue
structure, and aeration during the separation process,
which contributes to microorganism increase (Pomykała and Michalski, 2008).

In the tested MSPM, E. coli was at the 2.04 log cfu/g level, whereas Enterobacteriaceae reached 3.83 log cfu/g. E. coli counts observed in the MSPM crossed the limit of 1.6 log cfu/g settled as a criterium for a hygienic MSPM production process in EU Regulation 2073/2005. However, this parameter was settled for MSPM produced with a "low pressure" technique. In the research of other authors, E. coli in MSPM varied from 3.54 to 3.72 log cfu/g (On et al., 2011). Enterobacteriaceae counts in the MSPM ranged between 3.3 and 5.6 log cfu/g (Bijker et al., 1987; Hać-Szymańczuk et al., 2014).

The number of coagulase-positive Staphyloccoci in the MSPM was <10 log cfu/g. No Salmonella sp. or Campylobacter spp. in 25 g samples was detected (data are not presented in the table). The research of Hać-Szymańczuk et al. (2014) also did not confirm the presence of Salmonella sp. in 25 g MSPM samples. Other authors relatively commonly reported the presence of Salmonella sp. and Campylobacter sp. in MSPM (Pomykała and Michalski, 2008; On et al., 2011).

**Microbiological Analysis of Cured MSPM Batters During Refrigerated Storage** The LAB treatment and storage time affected (P < 0.001) the number of TVC, LAB, E. coli, and Enterobacteriaceae in the tested MSPM batters. Interaction between LAB addition and storage time for all microbiological analyses was found (Table 5). The significant (P < 0.05) increase of the TVC in MSPM batters was observed with the storage time. At the beginning of storage, the TVC of the treatments with LAB was higher because of bacteria addition, but after 7 d of storage, the TVC in treatments with LAB (PL2 and PL3) was lower than that in the control treatments C150 and C50 (Table 5). It may prove the inhibitory effect of LAB on other bacteria present in the MSPM batters.

The number of mesophilic LAB during the whole storage time was at a similar level in all of the experimental treatments with LAB addition (7.19–7.71 log cfu/g). The significant (P < 0.05) higher number of LAB in whole storage period was observed in treatments with LAB addition (Table 5). After 7 d of storage, it was significant (P < 0.05) that the highest number of LAB in the PL1 treatment was observed.

The significant (P < 0.05) inhibitory effect of L. plantarum SCH1 (PL1) on E. coli in the MSPM batters during the whole time of being stored was confirmed (Table 5). In the model research of Rzepkowska et al. (2017a), the inhibitory effect of L. plantarum SCH1 and L. brevis KL5 on E. coli, Salmonella Enteritidis, L. monocytogenes, and Pseudomonas fluorescens was proved. It can be assumed that LAB produced metabolites that hindered E. coli growth. Gong et al. (2010)

### Table 5. The effect of lactic acid bacteria and refrigerated storage time on the microbiological quality of MSPM cured batters (average value ± SE).

| Treatment | Storage time (day) | LAB treatment | Time | LAB treatment x time |
|-----------|--------------------|---------------|------|----------------------|
|           | 1                  | 4             | 7    |                      |
| Total viable counts, [log cfu/g] |                   |               |      |                      |
| C150      | 6.91 ± 5.57bc      | 7.19 ± 6.16abc | 8.42 ± 7.34d | ** | ** | ** |
| C50       | 6.61 ± 5.45bc      | 7.40 ± 6.32abc | 8.12 ± 7.22b | ** | ** | ** |
| PL1       | 7.80 ± 6.08ad      | 7.91 ± 6.16abc | 8.20 ± 7.00b | ** | ** | ** |
| PL2       | 7.71 ± 7.20ab      | 7.68 ± 6.16abc | 8.07 ± 6.52b | ** | ** | ** |
| PL3       | 7.36 ± 5.76abc     | 7.62 ± 5.95abc | 7.97 ± 6.70bc | ** | ** | ** |
| Escherichia coli counts, [log cfu/g] |                   |               |      |                      |
| C150      | 4.26 ± 2.52a       | 4.26 ± 2.52a  | 4.10 ± 2.52a | ** | ** | ** |
| C50       | 4.20 ± 2.76a       | 3.82 ± 2.53a  | 4.10 ± 2.95a | ** | ** | ** |
| PL1       | 7.71 ± 6.37d       | 7.65 ± 6.31d  | 7.87 ± 6.70d | ** | ** | ** |
| PL2       | 7.48 ± 5.52bc      | 7.33 ± 5.82bc | 7.30 ± 5.76b | ** | ** | ** |
| PL3       | 7.19 ± 5.52bc      | 7.17 ± 5.52bc | 7.16 ± 5.95b | ** | ** | ** |
| Enterobacteriaceae counts, [log cfu/g] |                   |               |      |                      |
| C150      | 2.07 ± 0.52a       | <1.00<sup>a</sup> | <1.00<sup>a</sup> | ** | ** | ** |
| C50       | 2.14 ± 0.82a       | 1.67 ± 0.52bc | <1.00<sup>a</sup> | ** | ** | ** |
| PL1       | <1.00<sup>a</sup>  | <1.00<sup>a</sup> | <1.00<sup>a</sup> | ** | ** | ** |
| PL2       | 1.82 ± 0.95<sup>a</sup> | 1.80 ± 0.52<sup>a</sup> | <1.00<sup>a</sup> | ** | ** | ** |
| PL3       | <1.00<sup>a</sup>  | 1.65 ± 1.18<sup>b</sup> | <1.00<sup>a</sup> | ** | ** | ** |

<sup>*Means with different superscript small letters differ significantly (P < 0.05). n = 3. P: significance of effects; LAB treatment; time; LAB treatment-time interaction. **P < 0.001. Abbreviations: C150, control cured with sodium nitrite at 150 mg/kg; C50, control cured with sodium nitrite at 50 mg/kg; MSPM, mechanically separated poultry meat; PL1, cured with sodium nitrite at 50 mg/kg and inoculated Lactobacillus plantarum SCH1 at about 10<sup>5</sup> cfu/g; PL2, cured with sodium nitrite at 50 mg/kg and inoculated Lactobacillus brevis KL5 at about 10<sup>7</sup> cfu/g; PL3, cured sodium nitrite at 50 mg/kg and inoculated L. plantarum S21 at about 10<sup>5</sup> cfu/g.</sup>
reported the inhibiting effect of bacteriocin (plantaricin MG) produced by *L. plantarum* on *E. coli*, *S. aureus*, *L. monocytogenes*, and *Salmonella* Typhimurium growth. The antagonistic LAB influence on gram-negative pathogens may be also caused by organic acids and hydrogen peroxide production (Laszlikiewicz et al., 2019). In the C50 treatment, the *E. coli* counts after 1 and 4 d of storage were 2.14 and 1.67 log cfu/g, respectively. It proved that the NaNO₂ dose (50 mg/kg) was not sufficient to hinder the growth of this bacteria. The inhibiting effect of *E. coli* growth after 4 and 7 d of storage was observed in the C150 treatment with the maximum NaNO₂ content (150 mg/kg). In the previous research performed on uncured MSPM batters, the inhibiting effect of *L. plantarum* SCH1 on *E. coli* was also observed. Nevertheless, the *E. coli* inhibiting effect was not as strong. A gradual reduction of *E. coli* counts from 2.28 log cfu/g (after 1 d of storage) to 1.94 log cfu/g (7 d of storage) was observed (Laszlikiewicz et al., 2019). The reported results indicate the synergy of a reduced amount of sodium nitrite (50 mg/kg) with *L. plantarum* SCH1 on *E. coli* in MSPM.

After 7 d of cold storage, the TVC in all of the experimental variants varied from 7.97 to 8.42 log cfu/g. The MSPM batters were already spoiled. After this duration, the *E. coli* counts in all of the treatments was lower than 1 log cfu/g. In all of the experimental treatments, the *Enterobacteriaceae* count increase was observed. It could be assumed that the reason for a lower *E. coli* count was the domination of the meat environment by other bacteria (including other bacteria from the *Enterobacteriaceae* family). Bacteria growth strictly depended on environmental conditions and nutrient availability that is essential for their growth (Tensink and Molenaar, 2017). The significant growth of *Enterobacteriaceae* after 7 d of storage was also observed in uncured MSPM with LAB (Laszlikiewicz et al., 2019). Hać-Szymańczuk et al. (2014) observed the increase of *Enterobacteriaceae* counts regardless of the MSPM stabilization method.

**CONCLUSIONS**

The application of the selected lactic acid bacterial strains did not have a negative effect on MSPM technological quality. The addition of LAB could be an effective alternative to the use of high amounts of sodium nitrite (150 mg/kg) for MSPM batters production. The inclusion of LAB improved the microbiological quality of MSPM batters. A significant inhibitory effect in terms of *E. coli* counts reduced by *L. plantarum* SCH1 was observed throughout the storage period. The conducted research suggests the possibility of using the selected bacterial strains of the *Lactobacillus* genus to improve the microbiological quality of MSPM cured with a reduced amount of sodium nitrite. However, research on the sensory quality and physicochemical parameters of products being produced with MSPM inoculated *L. plantarum* SCH1 should be carried out.

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**DISCLOSURES**

The authors declare no conflicts of interest.

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