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Research Article

Effects of the Use of *Staphylococcus carnosus* in the Curing Process of Meat with a Reduced Amount of Sodium Nitrite on Colour, Residue Nitrite and Nitrate, Content of Nitrosyl Pigments, and Microbiological and the Sensory Quality of Cooked Meat Product

Piotr Szymański,[1] **Beata Łaszkiewicz**,[1] **Urszula Siekierko**,[1] **and Danuta Kołożyn-Krajewska**[2]

[1]*Department of Meat and Fat Technology, Professor Waclaw Dabrowski Institute of Agricultural and Food Biotechnology, 36 Rakowiecka St., 02-532 Warsaw, Poland*
[2]*Institute of Human Nutrition Sciences, Warsaw University of Life Sciences-SGGW, 166 Nowoursynowska St, 02-787 Warsaw, Poland*

Correspondence should be addressed to Piotr Szymański; piotr.szymanski@ibprs.pl

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The aim of the work was to apply the bacteria *Staphylococcus carnosus* ATCC 51365 in the meat curing process with the use of a reduced amount of sodium nitrite and to evaluate the effects of bacteria on residual nitrites and nitrates, the content of nitrosyl pigments, colour, pH, oxidation-reduction potential, microbiological, and the sensory quality of a cooked meat product. Three meat batters in cans were prepared: (C) a control batter cured with $NaNO_2$—100 mg/kg, (L) a batter cured with $NaNO_2$—15 mg/kg, and (LS) a batter cured with $NaNO_2$—15 mg/kg and *S. carnosus* (10^7 CFU/g). The cans were stored at a temperature of 4°C for 24 h (curing time) and cooked. The analysis was carried out after production and after 4 and 8 weeks of storage. The use of denitrifying bacteria in the curing process with a reduced amount of sodium nitrite increased the availability of nitrite in the meat, by reducing nitrates formed as a result of a dismutation reaction. The reaction contributed to the formation of nitrosyl pigments in a larger quantity than in the treatment in which the denitrifying bacteria were not used. The LS treatment was characterized by the greatest redness. The colour of the LS treatment was stable during storage. No negative effect of *S. carnosus* on the sensory quality of the meat product was found. The use of *S. carnosus* had no influence on the microbiological quality of meat product during storage.

1. Introduction

Due to its specific properties, sodium nitrite is a substance commonly used in meat processing. The main functions of sodium nitrite in the meat curing process is the creation of the typical pink colour of meat products, generation of a flavour typical of cured meat, inhibition of the growth of certain undesirable microorganisms, including the *Clostridium botulinum* bacteria, and a slowing down of the oxidation processes [1]. Irrespective of the advantages resulting from the application of sodium nitrite, there is a potential health risk, coming from its use, specifically because of the participation of nitrites in the formation of carcinogenic N-nitrosamines (NAs) [2, 3] and due to its reaction in certain circumstances (lower pH, high temperature) with secondary amines in meat products [4]. The positive correlation between the amount of ingoing nitrite and the levels of NAs formation has been confirmed in meat

products [3, 5]. However, using an antioxidant such as ascorbate can minimize NAs formation [6].

Many studies have been concerned with attempts to eliminate the application of nitrites in meat products [7–9]. Until now, any alternative solution which would allow obtaining many positive effects of nitrites' use has not been found [10, 11].

The European Commission Regulation No. 1129/2011 [12] established a maximum amount of nitrite of 150 mg/kg that may be added during the processing of meat products. This legislation mentioned the possibility of reducing in the future the current maximum level of nitrite added to meat products in the European Union (EU). The European Commission has started consultations in this regard with member states [13]. Furthermore, certain countries, e.g., Denmark, have already introduced internal regulation, reducing the maximum level of the use of nitrites to 60 mg/kg in selected groups of meat products [14].

The nitrites, as added to meat, participate in many competitive chemical reactions during which they are subjected to alternations. In studies, related to nitrite balance, added to meat during the meat curing process, it was found that 5–15% of nitrite were bound with myoglobin and haemoglobin, 1–10% were transformed into nitrates, 5–20% remained in a free form, 1–5% was secreted as gas, 1–15% were bound with–SH groups, 1–15%—with other proteins and 1–15% with fats [15, 16]. Honikel [4] indicates that the quantity of nitrite which becomes converted into nitrates during meat curing may be greater and amount to 10–40%. A reduction of the ingoing amount of sodium nitrite to meat, with the simultaneous limitation of accessible nitrite, caused by its oxidation to nitrate during the curing process, may affect the effectiveness of the process and stability of the colour of meat products.

In case of nitrite level reduction, being added to meat, aimed at preserving an acceptable curing effect, it is important to keep the residual nitrite and nitrate at the lowest possible level. The low amount of residual nitrite and nitrate in the final meat product indicates a more complete use of the available nitrite [10].

In raw meat products, nitrates may be reduced to nitrites by microorganisms [4]. Many coagulase-negative nonpathogenic species of denitrifying bacteria from the *Staphylococcus* genus, interalia, *S. carnosus, S. xylosus S. simulans, S. equorum,* have been isolated from raw meat products [17]. The conversion of nitrates into nitrites in nitrite-cured meat batters, intended for the production of cooked meat products may be limited. The manufacturing process of such products is relatively short and may be insufficient for any effective action of desired meat microflora.

Staphylococcus genus as starter cultures is used in the production of fermented meat products, where they participate in the creation of the colour of the products by the reduction of nitrates to nitrites [18]. The utilization of nitrites and nitrates as final electron acceptors in the process of anaerobic respiration by the mentioned bacteria may also lead to NO formation [19].

The application of bacteria from *Staphylococcus* genus in the meat curing process may be suitable in reducing the level of the added sodium nitrite in a cooked meat product by increasing the efficiency of nitrite being added by reducing the nitrated formed back into nitrite. The aim of the work was to apply the bacteria *Staphylococcus carnosus* ATCC 51365 in the meat curing process with the use of a reduced amount of sodium nitrite and to evaluate the effects of bacteria on the residual nitrites and nitrates, the content of nitrosyl pigments, colour, pH, redox potential, and the microbiological and sensory quality of the cooked meat product.

2. Materials and Methods

2.1. Materials

2.1.1. Strain of Denitrifying Bacteria and Preparation Method. The denitrifying strain *Staphylococcus carnosus* (ATCC 51365) was used in this study. It was isolated from dried sausage. The bacterial culture was multiplied on a tryptic soy broth (TSB) (Becton, Dickinson and Company, Le Pont de Claix Cedex, France) for 20 h at 30°C. Then, bacteria from the third inoculation were separated in a separator type J2-21 (Beckman, Birkerød, Denmark) at 4500 RPM for 10 min. The obtained biomass was suspended in a physiological salt solution and added to meat batter.

2.1.2. Model Meat Product. Pork meat which was used in the experiment was obtained from cooled semicarcasses, 48 h after being slaughtered in a meat factory in Poland. The raw material was free from quality defects.

12 pieces of semimembranous (*M. Semimembranosus*) pork muscle from ham (derived from 12 pigs) were minced in a mincer, on a plate with a mesh diameter equal to 3 mm and mix. 3 treatments of meat batter from mixed meat were performed (Table 1): (C) the control one was cured with sodium nitrite, using $NaNO_2$—100 mg/kg (typical level of $NaNO_2$, applied in the production of cooked sausages), (L) batter cured with a reduced level of sodium nitrite $NaNO_2$—15 mg/kg, and (LS) batter cured with $NaNO_2$—15 mg/kg and the addition of *S. carnosus* ATCC 51365 bacteria at the number of 10^7 CFU/g. The meat with additional components and water were mixed in the non-vacuum mixer (Keripar, Troy (Ohio), USA) for 5 minutes and sealed in cans of 190 g in weight. The cans were stored at a temperature of 4°C for 24 h (curing time). Then, 24 cans of each treatment (C, L, LS) were cooked. The cooking was performed in three stages. In the first stage, a temperature of 40°C was maintained inside the product's block for 1 h. Then, heat treatment was conducted until a temperature of 70°C was obtained inside the block of the preserve. The cooking of the preserved products was carried out in cooker type B. 200l/E (Brokelmann, Ense-Höingen, Germany). The meat products were cooled down in water with ice to the temperature of 10–15°C and then additionally chilled in a refrigeration room to a temperature of 4–6°C. The products were stored in refrigeration conditions (4°C) for a period of 8 weeks. The analysis of the products was carried out after production (after cooling down the product to 4°C), and after 4 and 8 weeks of storage. The experiment described

Table 1: Formula of model meat product.

Component	Treatment		
	C	L	LS
Pork meat (*m. semimembranosus)* (kg)	100	100	100
Water/ice (kg)	10	10	10
Glucose (Cargill, Incorporated, Minneapolis, MN, United States) (kg)	1.2	1.2	1.2
Sodium triphosphate (57% P_2O_5, BK Giulini Chemie GmbH & OHG, Ludwigshafen/Rhein, Germany) (kg)	0.2	0.2	0.2
Sodium ascorbate (Hebei Welcome Pharmaceutical Co., Ltd, Hebei, Shijiazhuang, China)	0.06	0.06	0.06
NaCl (Salt Mine Kłodawa, Kłodawa, Poland) (kg)	2.0	2.0	2.0
$NaNO_2$ (Chempur, Piekary Śląskie, Poland) (kg)	0.0115	0.0017	0.0017
Bacterial biomass *S. carnosus* ATCC 51365 with physiological salt solution (NaCl 0.9%) (kg)	—	—	0.5
Physiological salt solution (NaCl 0.9%) (kg)	0.5	0.5	—

C: control treatment, quantity added $NaNO_2$ 100 mg/kg; L: quantity added $NaNO_2$—15 mg/kg; LS: quantity added $NaNO_2$—15 mg/kg + *S. carnosus* ATCC 51365 in number 10^7 cfu/g.

above was repeated four times ($n = 4$) in independent trials (replicates) using four lots of meat (4×12 pieces of semimembranous pork muscle).

2.2. Methods

2.2.1. Residual Nitrite and Nitrate Analysis. The content of nitrites and nitrates was determined according to PN-EN 120414:2006 with an amendment of Siu and Henshall [20]. The homogenized meat product (10 g) was weighed and put into a volumetric flask. Deionized water was added to the meat sample to the volume of 50 mL. The homogenized sample was heated and the temperature was maintained between 70°C and 80°C for 20 min. After cooling to room temperature, deionized water was added to the meat sample to a final volume of 100 mL and mixed. The supernatant was filtered through Cellulose Acetate (CA) syringe filters (Alfatec Technology, Zagreb, Croatia) pore size 0.45 μm. The filtrate was then collected for IC analysis.

Extracts of the samples were analyzed on an Agilent 1200 liquid chromatograph (Agilent Technologies, Waldbronn, Germany), equipped with a detector UV, analytical column IonPac® AS11-HC 4×250 mm (Thermo Fisher Scientific, Sunnyvale, USA) and precolumn AG11-HC 4×50 mm (Thermo Fisher Scientific, Sunnyvale, USA) was used. Nitrate and nitrite were separated using isocratic conditions - 10 mmol/L sodium hydroxide (Chempur, Piekary Śląskie, Poland) for 20 min., followed by a column wash with 50 mmol/L sodium hydroxide for 10 min. and equilibration with 10 mmol/L sodium hydroxide for 5 min. The injection volume was 25 μL and eluent flow rate was 1.5 mL/min. Analytes were detected using UV detection at 225 nm.

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

2.2.2. Nitrosyl Pigments Content Analysis. The content of the nitrosyl pigments was determined by the Hornsey method [21]. The obtained homogenized meat samples (5 g) were added 21.5 mL aqueous acetone solution acetone (Chempur, Piekary Śląskie, Poland) : distilled water = 40 : 3 and intensively mixed in a dark glass bottles for 3 minutes. Then the bottles were left in a dark room at a temperature of 20± 1°C for 30 minutes and were shaken several times. The resulting mixture was filtered using two quantitative filter papers (GF/A, Whatman, Lab-Sysytem-Service, Szczecin, Poland). Filtrate absorbance values were measured in a semi-micro quartz cuvette (Q104, Alchem, Toruń, Polnad) at 540 nm using a U-2900 spectrophotometer (Hitachi, Tokyo, Japan). The content of the nitrosyl pigments was expressed in ppm of haematin according to the formula:nitrosyl pigments (ppm of haematin) = $\text{Abs}_{540\,\text{nm}} * 290$.

2.2.3. Determination of pH Value. The pH value of the meat products was determined according to PN-ISO 2917:2001. A test sample of 10 g weight was mixed with 50 cm^3 of distilled water and homogenized using an 800 W blender (Bosch, Munich, Germany) for 1 minute at a speed equal to 14,000 RPM. Measurement of the pH of the homogenates was performed using a Delta 350 pH meter (Mettler Toledo, Schwerzenbach, Switzerland) with an automatic compensation temperature, using a glass-calomel electrode In Lab Cool (Mettler Toledo, Greifensee, Switzerland).

2.2.4. Redox Potential Determination. A sample of the meat product of 10 g in weight was mixed with 50 cm^3 of distilled water and homogenized using an 800 W blender (Bosch, Munich, Germany) for 1 minute at a speed equal to 14,000 RPM. In such a solution, the measurements were carried out, using a Delta 350 pH meter (Mettler Toledo, Schwerzenbach, Switzerland), with the application of an electrode In Lab Redox Pro (Mettler Toledo, Greifensee, Switzerland). The result was read after its stabilization. The measurement was conducted at a temperature of 20°C ± 2°C. The obtained result of the measurement (mV) was calculated into the value of redox potential in relation to the standard hydrogen electrode E_H (mv). To this end, the value of the potential of the reference electrode at a temperature of 20°C—E_{ref} = 210 mV was added to the readout value which was obtained with the equipment.

2.2.5. Instrumental Measurement of Colour. The measurement of the colour in the system CIELab, where L^* is brightness, a^* is chromaticity in the red and green range, b^*

is chromaticity in the yellow and blue range, was taken using a reflection colorimeter CR-300 (Konica Minolta, Tokyo, Japan). In the measurements, a standard observer CIE: 2°, illuminate D65 was employed; an area of 8 mm, calibration was conducted using a white colour pattern (L^* 99.18, a^* −0.07, b^* −0.05) (CIE, 1976). Components of the colour $L^*a^*b^*$ were determined in the meat products after production and 8 weeks of being stored at a temperature of 8°C. The measurement was conducted in a laboratory at a temperature of 24°C ± 2°C. Before the evaluation, the cans were opened and the meat products exposed to air in a room at 24 ± 1°C. For each of the four replicate of treatment (C, L, LS) four measurements were carried out.

The hue angle ($h°$) and chroma (saturation index) (C^*) was calculated according to the following equations:

$$h° = \tan^{-1}\frac{b^*}{a^*}, \qquad C^* = \sqrt{a^{*2} + b^{*2}}, \tag{1}$$

where a^* and b^* were data from an instrumental measurement of colour.

The total change in colour (ΔE^*) was calculated after storage according to the following equation:

$$\Delta E^* = \sqrt{(L1 - L2)^2 + (a1 - a2)^2 + (b1 - b2)^2}, \tag{2}$$

where 1—value before storage; 2—value after storage [22].

2.2.6. Microbiological Analysis. The samples of 20 g in weight were placed in plastic bags, 180 ml of peptone water for dilutions was added, mixed in a Stomacher blender (A.J. Seward, London, UK) for 2 minutes and the decimal dilutions were prepared. The surface inoculation was performed on the prepared media on Petri dishes. To determine the total number of aerobic mesophilic microorganisms-aerobic colony count (ACC), Tryptone Soya Agar (Oxoid, Basingstoke, UK) was used. Incubation was conducted at a temperature of 30°C, for 72 h. To determine the number of lactic acid bacteria (LAB), MRS agar (Merck KGaA, Darmstadt, Germany) was used; to determine bacteria from the *Staphylococcus* genus, *Staphylococcus* Medium 110 (Becton, Dickinson and Company, Le Pont de Claix Cedex, France) was employed. Both of the media were incubated at a temperature of 30°C for 48 h. After completion of the incubation period, the plates on which the number of colonies from two successive dilutions, at least on one plate, was equal to 15–300 were considered in the calculations. The bacteria count in the meat products was calculated according to PN-ISO 7218:2008. The results are expressed as the number of colonies-forming units per one gram (CFU/g).

2.2.7. Sensory Evaluation. Sensory evaluation was conducted by the Quantitative Descriptive Analysis method (QDA) (ISO 13299:2003). The task of the evaluators was to determine the intensity of the selected quality discriminants and place their evaluation on the appropriate nonstructured graphical scale (0–10 c.u.). To compare the sensory quality of the meat products, the following discriminants were used: salty taste (0 = not very strong; 10 = very strong), acid/sharp flavour (0 = not intensive; 10 = very intensive), sterilization flavour (0 = not intensive; 10 = very intensive), cure meat flavour (0 = not intensive; 10 = very intensive), fatty flavour (0 = not intensive; 10 = very intensive), acid/sharp odour (0 = not intensive; 10 = very intensive), sterilization odour (0 = not intensive; 10 = very intensive), cure meat odour (0 = not intensive; 10 = very intensive), fatty odour (0 = not intensive; 10 = very intensive), overall quality (0 = low; 10 = very high), colour tone (0 = light pink; 10 = dark pink), tenderness (0 = not very tender; 10 = very tender), and juiciness (0 = not very juicy; 10 = very juicy). Before the evaluation, the meat products were stored in a room at a temperature of 24 ± 1°C for 40 min. The test samples were prepared by cutting out the meat block with dimensions of 50 mm × 30 mm × 20 mm and placing them in closed plastic boxes (volume 250 ml). Water was provided to cleanse the palate between the samples. The evaluation was conducted with the participation of a trained team of 10 persons. The sensory evaluation of the products was conducted after 8 weeks of being stored. The evaluation was performed for two replications of variants (C, L, LS) in one repetition. The evaluation was performed in two sessions by the same panel in the same way.

2.3. Statistical Analysis. The experiment was carried out in four replications ($n = 4$) at different times using four lots of pork meat (batches), and a completely randomized design was used. All observations making up the experiment (3 treatments × 4 batches × 3 storage periods) were included in the statistical analysis. The normality and homoscedasticity were investigated using the Shapiro–Wilk test and the Bartlett test, respectively. The variables presented a normal distribution and homogeneous variances. The data from the physicochemical and microbiological analysis were evaluated by the analysis of variance (ANOVA) using a general linear model considering the treatments or storage time as a fixed effect and the replicates as a random effect. The significance of the differences between the means of treatment was analyzed by using the Fisher test ($P \leq 0.05$) using the STATGRAPHICS v. 4.1 statistical program (Manugistics Inc., Rockville, MD, USA). For analyzing sensory data, the model used treatments as a fixed factor (3 levels) and panellists (10 persons) as a random factor.

3. Results and Discussion

3.1. Analysis of the pH and Redox Potential. The effect of the *S. carnosus* ATCC 51365 on the increase of the pH value of a product was demonstrated ($P \leq 0.05$). The pH value of the LS treatment (6.36) was significantly higher than in the L (6.22) after production. The mentioned tendency was observed also after 4 and 8 weeks of storage (Table 2). Strains of the coagulase-negative *Staphylococcus* (CNS) perform nitrate respiration under anaerobic conditions and release ammonia [19, 23]. In this process nitrate is taken up by the cells of bacteria and reduced to nitrite, and nitrite is

Table 2: The content of nitrosyl pigments and values of redox potential and pH of model meat products (means ± standard deviation error).

	Sample	Storage time (weeks)		
		0	4	8
pH	C	6.25 ± 0.04^{aA}	6.23 ± 0.05^{bA}	6.25 ± 0.05^{bA}
	L	6.22 ± 0.05^{aB}	6.14 ± 0.06^{aA}	6.17 ± 0.03^{aA}
	LS	6.36 ± 0.07^{bB}	6.39 ± 0.05^{cB}	6.26 ± 0.05^{bA}
Redox (mV)	C	256.0 ± 2.1^{aA}	257.3 ± 2.4^{aA}	261.3 ± 2.9^{bB}
	L	263.1 ± 3.3^{bA}	265.3 ± 3.5^{bA}	263.0 ± 3.8^{bA}
	LS	263.3 ± 1.8^{bB}	263.8 ± 2.7^{bB}	257.5 ± 1.9^{aA}
Nitrosyl pigments (ppm)	C	40.5 ± 4.6^{bA}	41.7 ± 3.9^{bA}	37.9 ± 5.1^{bA}
	L	28.6 ± 1.9^{aB}	27.8 ± 2.7^{aAB}	24.9 ± 0.9^{aA}
	LS	36.5 ± 2.6^{bAB}	38.9 ± 4.2^{bB}	33.7 ± 2.4^{bA}

$^{a-c}$Means in the same columns with different superscript small letters differ significantly ($P \leq 0.05$). ^{A,B}Means in the same row with different superscript capital letters differ significantly ($P \leq 0.05$). C: control treatment, quantity added $NaNO_2$ 100 mg/kg; L: quantity added $NaNO_2$—15 mg/kg; LS: quantity added $NaNO_2$—15 mg/kg + *S. carnosus* ATCC 51365 in number 10^7 cfu/g.

subsequently excreted. After nitrate depletion, the externally accumulated nitrite is taken up by the cells and reduced to ammonia, which again is excreted into the medium [19, 24]. The accumulation of certain amounts of ammonia in the meat and other basic substances caused by proteolysis resulting from the growth of bacteria during curing time could have an effect on the observed increase in the pH of the final product in the LS treatment [25].

It is known that coagulase-negative staphylococci use carbohydrates and convert them into lactic acid and other organic acids, albeit slower and in lower quantities than, e.g., lactic acid bacteria [19]. Probably the amount of acid produced by *S. carnosus* ATCC 51365 during 24 hours of curing the meat was small and had no effect on the pH of the final product.

The pH value of the control treatment (C) (6.25) was similar to the L (6.22) after production. After 4 and 8 weeks of storage, the C treatment was characterized by a significantly higher pH value as compared to variant L ($P \leq 0.05$) (Table 2). Probably it was connected with the larger amount of sodium nitrite used in the control treatment. A similar relationship was demonstrated by Hayes et al. [26] who applied different amounts of sodium nitrite for curing meat (0, 50, 100 mg/kg). Along with an increase in the addition of sodium nitrite to meat they observed an increase in pH value in the meat product after heat treatment and storage.

In the control treatment, during the whole period of its storage any significant changes in the pH of the product were not found ($P > 0.05$). In the remaining treatments, the changes in the pH during storage were small but statistically significant ($P \leq 0.05$). In the treatment of L, a significant decrease of 0.08 in pH value was found after 4 weeks of storage. In the LS treatment, the increase of 0.03 in the pH value was observed after 4 weeks of storage, and a significant increase of 0.13 was found after 8 weeks of storage (Table 2).

Similar changes in the pH value of cured scalded pork-beef sausages were observed by Terns et al. [27] after 56 and 84 days of refrigeration. In turn, a significant increase in the acidity of the cured scalded pork sausages after 30 days of storage was observed by Wójciak et al. [28]. The changes of the pH value in the product during storage result from its own hydrolytic protein transformations and the activity of microbial enzymes [29].

After production and after 4 weeks of storage, the lowest, statistically significant value of oxidation-reduction potential was found in the control treatment (C) (256.0 mV and 257.3 mV, respectively) ($P \leq 0.05$). The lower value of redox potential in variant C could have been affected by the antioxidative effect of the residual nitrite [11]. No significant differences between treatments L and LS were found ($P > 0.05$). While, after 8 weeks of storage the LS treatment was characterized by the significantly lowest value of oxidation-reduction potential (Table 2).

3.2. The Content of Nitrosyl Pigments. It was found that in treatments LS and C, the content of nitrosyl pigments (36.5 ppm and 40.5 ppm, respectively) after production, was significantly higher ($P \leq 0.05$) than in treatment L (28.6 ppm). The same tendency was observed after storing the tested products (Table 2). Any statistically significant differences in the quantity of the nitrosyl pigment between treatments C and LS ($P > 0.05$) were not found.

Shin et al. [9] determined the content of nitrosyl pigments to be 49.3 ppm in cooked pork patties, cured with sodium nitrite in a quantity of 120 mg/kg. Heaton et al. [30] determined nitrosyl pigments as 26.2 ppm in pork shoulder rolls cured with sodium nitrite in an amount of 7 mg/kg.

In the control treatment (C), no statistically significant differences in the content of nitrosyl pigments during storage were found ($P > 0.05$). In treatment L, a significant decrease in the level of nitrosyl pigments after 8 weeks of being stored was observed. In the case of treatment LS, the statistically significant ($P \leq 0.05$) differences in the content of nitrosyl pigments were stated between 4 and 8 weeks of storage (Table 2).

Reduction in the quantity of nitrosyl pigments in the meat products during storage is a phenomenon connected with the oxidation of nitrosyl miochromogen [31]. Nitrites slow down the oxidation processes [1, 32], so it may be supposed that the higher content of nitrites and nitrates in the control treatment had an influence on slowing down the oxidation of nitrosyl miochromogen.

A mechanism which would explain the formation of a greater quantity of nitrosyl pigments in treatment LS as

compared to L may be multidirectional. One of the effects of a bacterial culture application included a small but statistically significant increase in the pH value of the finished product ($P \leq 0.05$). It is well-recognized that increased pH alone typically slows a reaction of the haeme pigments and nitrites [4, 31, 32]. Thus, the generation of a greater quantity of nitrosyl pigments in the products where *S. carnosus* ATCC 51365 was affected by other factors. In the environment of meat batter, lactic acid produced by *S. carnosus* can dissociate quickly, releasing hydrogen ions. Acid radicals can react with sodium cations present in meat batter to form sodium lactate. In the case of the addition of sodium lactate to meat, an increase in the pH of the environment can also be observed [33, 34]. The role of sodium lactate in creating the colour of cured meat is different from that of lactic acid [34]. Sodium lactate affects the increase in the level of coenzyme NADH derived from NAD by the conversion of lactate into pyruvate by the enzyme LDH (lactate dehydrogenase). In effect, the greater quantity of coenzyme NADH reduces metmyoglobin to deoxymyoglobin more effectively. The increasing content of deoxymyoglobin contributes to the generation of nitrogen oxide from nitrites due to the oxidation-reduction reaction with deoxymyoglobin. In effect, we may observe lower levels of nitrites in the system. NADH generated with the participation of lactates may also supply nitrogen oxide (NO) in the reaction process of haem pigments and may participate in the reduction of nitrosyl methmyoglobin to the nitrosyl myoglobin [34].

There is also another mechanism which can explain the high content of nitrosyl pigments in treatment LS. The study shows that *S. carnosus* ATCC 51365 reduced the nitrate formed (as a result of the dismutation reaction) back to nitrite and allowed it to react again. This could be done multiple times during 24 h of meat curing. The reduction of nitrates to nitrites by bacteria increased the accessibility of nitrites in meat, participating in the curing process. Furthermore, the utilization of nitrites and nitrates by bacteria and the generation of NO in meat batter cannot be excluded [19, 35]. NO may react with deoxymyoglobin ($MbFe^{2+}$) and, in effect, nitrosyl myoglobin is formed. There is also the possible reaction of NO with methmyoglobin ($MbFe^{3+}$), as a result of which the nitrosyl methmyoglobin complex is created [4]. The resulting nitrosyl methmyoglobin is reduced to nitrosyl myoglobin by NADH [34] or reducing substances chemically introduced to meat [4].

3.3. Residual Nitrites and Nitrates. The highest residual content of nitrites and nitrates in the meat products was determined in the control treatment (C), which was connected with the high amount of sodium nitrite introduced to the meat. The lower levels of nitrites and nitrates were found in treatments L and LS. The statistically significant ($P \leq 0.05$) lowest content of these compounds was demonstrated in treatment LS where the *S. carnosus* ATCC 51365 was employed (Figures 1(a) and 1(b)).

The lower level of nitrates in treatment LS as compared to treatment L was connected with the utilization of the mentioned compounds in the process of the anaerobic respiration of the *S. carnosus* ATCC 51365 bacteria. During the denitrification run, the electrons detached from the respiration substrate are attached to nitrates via the enzyme, nitrate reductase, and as a result, nitrites are generated [36]. The reduction of nitrites in the sample with the *S. carnosus* ATCC 51365 could occur with the participation of the enzyme-nitrite reductase [19]. The studies conducted by Gøtterup, Olsen, Knochel, Tjener, Stahnke and Møller [36] indicate that certain strains of denitrifying bacteria *S. carnosus, S. simulans, S. succinus* are characterized by the ability to produce this enzyme.

The content of nitrites and nitrates was reduced in all experimental treatments during the storage ($P \leq 0.05$). After 8 weeks of being stored, no presence of these compounds in the tested products was found. Terns et al. [27] stated a similar lowering of nitrites' content in cured pork sausages, during 84 days of refrigerated storage. The same relationship was demonstrated by Shin et al. [9] in the case of cured pork patties, stored in refrigerated condition for 28 days.

The reduction of nitrites and nitrates in meat products during storage is a known phenomenon and is related to the reaction of the mentioned compounds with the components of muscular and fat tissue and additional substances, which are added to meat batters [10].

3.4. Analysis of Colour. The level of the content of nitrosyl pigments in the meat product affects the values of the redness parameter (a^*) [30]. The LS treatment was characterized by the highest statistically significant value of redness (a^*) (Table 3). In this sample the significantly lowest b^* value was also detected ($P \leq 0.05$). This relationship was observed after production and after similar storage periods. After production, LS treatment also significantly had the highest value L^* ($P \leq 0.05$) (Table 3). A possible explanation for the observed high redness values in the treatment of LS after 8 weeks storage may be due to the relatively low ORP value that could preserve haem pigments at a reduced form. Ahn and Nam [37] showed that ascorbic acid had an effect on oxidation-reduction potential thus lowering and improving the colour stability compared to control.

The lowering of sodium nitrite concentration to the level of 15 mg/kg without the application of *S. carnosus* ATCC 51365 had a negative effect on the colour of the meat products. After 4 and 8 weeks of storage, L treatment was characterized by the lowest redness value (a^*) (Table 3). The negative effect of the low amount of sodium nitrite added for curing the meat on the colour of the finished product was demonstrated in Heaton et al. [30] studies. Nitrites present in the curing process participate in many competitive reactions [32] and the reduction of the rate of sodium nitrite, added to meat, has an influence on the efficiency of the reaction of nitrosylating haem pigments [1]. The authors indicate different levels of sodium nitrite addition, which are indispensable for obtaining an acceptable colour of a meat product; most frequently it is 25–50 mg/kg [1, 38].

A more complete description of the colour of meat products is possible due to an analysis of the hue angle (h°)

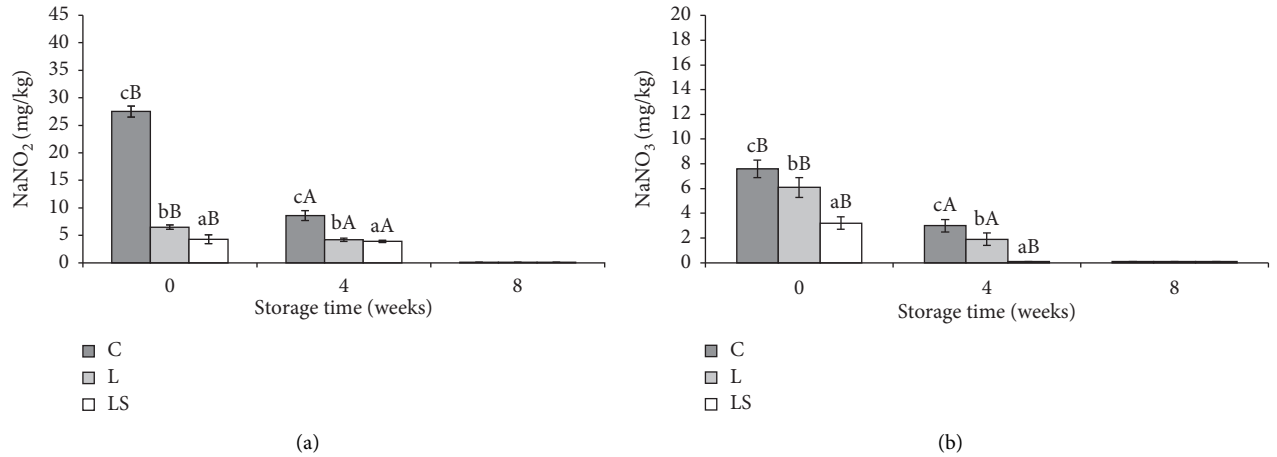

Figure 1: The content of nitrites (a) and nitrates (b) in model products. The figure shows mean values (bars) and standard deviation error (line segments). Mean values denoted by different capital letters (A, B) within the same sample and by small letters (a–c) among different samples differ statistically significantly ($P \leq 0.05$). C: control treatment, quantity added $NaNO_2$ 100 mg/kg; L: quantity added $NaNO_2$—15 mg/kg; LS: quantity added $NaNO_2$—15 mg/kg + *S. carnosus* ATCC 51365 in number 10^7 cfu/g.

Table 3: The influence of storage times on the colour parameters ($L^*, a^*, b^*, h°, C^*, \Delta E^*$) of the model meat products (means ± standard deviation error).

	Treatment	Storage time (weeks)		
		0	4	8
L^*	C	61.60 ± 0.37^{aB}	62.55 ± 0.40^{aC}	61.28 ± 0.32^{aA}
	L	61.70 ± 0.41^{aB}	62.79 ± 0.31^{bC}	61.43 ± 0.30^{abA}
	LS	62.26 ± 0.33^{bB}	62.86 ± 0.43^{bC}	61.61 ± 0.37^{bA}
a^*	C	10.73 ± 0.26^{aA}	11.30 ± 0.22^{bB}	10.74 ± 0.26^{bA}
	L	10.68 ± 0.25^{aA}	11.13 ± 0.22^{aB}	10.55 ± 0.19^{aA}
	LS	10.94 ± 0.17^{bA}	11.45 ± 0.18^{cC}	11.18 ± 0.23^{cB}
b^*	C	4.85 ± 0.21^{cA}	5.01 ± 0.20^{cAB}	5.16 ± 0.37^{cB}
	L	4.24 ± 0.18^{bA}	4.35 ± 0.22^{bA}	4.34 ± 0.24^{bA}
	LS	4.01 ± 0.24^{aA}	4.02 ± 0.14^{aA}	4.03 ± 0.14^{aA}
$h°$	C	24.26 ± 0.78^{cA}	23.84 ± 0.74^{cA}	25.57 ± 1.90^{cB}
	L	21.60 ± 0.75^{bAB}	21.32 ± 0.71^{bA}	21.96 ± 0.90^{bB}
	LS	20.09 ± 0.98^{aB}	19.33 ± 0.62^{aA}	19.81 ± 0.54^{aB}
C^*	C	11.78 ± 0.30^{bA}	12.36 ± 0.25^{cB}	11.92 ± 0.21^{bA}
	L	11.49 ± 0.27^{aA}	11.95 ± 0.27^{aB}	11.60 ± 0.24^{aA}
	LS	11.69 ± 0.22^{bA}	12.13 ± 0.19^{bB}	11.88 ± 0.24^{bA}
ΔE^*	C	—	—	0.78 ± 0.17
	L	—	—	0.69 ± 0.14
	LS	—	—	0.86 ± 0.12

[a–c]Means in the same columns with different superscript small letters differ significantly ($P \leq 0.05$). [A–C]Means in the same row with different superscript capital letters differ significantly ($P \leq 0.05$). C: control treatment, quantity added $NaNO_2$ 100 mg/kg; L: quantity added $NaNO_2$—15 mg/kg; LS: quantity added $NaNO_2$—15 mg/kg + *S. carnosus* ATCC 51365 in number 10^7 cfu/g.

and the chroma (C^*). It was revealed that treatments LS and C were characterized by a similar saturation of colour. It was also found that in the treatment of LS the values of the hue angle ($h°$) were significantly lower ($P \leq 0.05$) than in treatment C.

The observed relationships were found after the production and storage of the tested products (Table 3). Lower $h°$ values in cooked meat products indicate a more distinctive red colour [22]. The treatment produced with the reduced amount of sodium nitrite without the addition of bacteria was characterized by the significantly lowest value of chroma (C^*) ($P \leq 0.05$) indicating less saturation of the colour. In treatment L, the relatively low value of $h°$ was connected with the low value of the colour parameter b^* of the product.

No statistically significant differences in the total change of colour (ΔE^*) of the products before and after 8 weeks of storage were found ($P > 0.05$). It means that the stability of the colour of the products was at a similar level and the application of the bacterial strain *S. carnosus* ATCC 51365 during the curing process did not affect the dynamics of the changes in the colour of the products during the storage period.

3.5. Microbiological Quality of the Products. Nitrite is recognized for its bacteriostatic and bactericidal effect [4, 32]. It is known that the effectiveness of nitrite as an antimicrobial is dependent on several factors including residual nitrite level, pH, salt concentration, reductants present, iron content, heat treatment, and others [1, 32]. The conducted studies did not reveal differences in the microbiological quality of the model meat products. The total aerobic bacteria count (ACC) of treatments was found at a similar level from 2.66 to 2.84 log cfu/g (Table 4).

The statistical analysis did not show any significant differences in ACC between the treatments ($P > 0.05$). The number of lactic acid bacteria and *Staphylococcus* in the products was analyzed at a level below 1.2 log cfu/g during the whole storage period (Table 4). Similar results of total bacterial count (<2 log cfu/g) in cooked sausages with reduced fat content was observed by Jeong [8]. Shin et al. [9] determined ACC < 1 log cfu/g in cooked pork patties after production and after 28 days of refrigeration. Wójciak et al. [28] determined total bacteria count at 7.80 log cfu/g and the number of lactic acid bacteria at 7.17 cfu/g in cooked cured sausage with the addition of pork backfat after 28 days of refrigeration.

Table 4: Microbiological quality of model meat products (mean values).

(log CFU/g)	Treatment	Storage time (weeks)		
		0	4	8
Total aerobic bacteria count*	C	2.72	2.78	2.70
	L	2.66	2.79	2.80
	LS	2.68	2.69	2.84
Lactic acid bacteria*	C	<1.20	<1.20	<1.20
	L	<1.20	<1.20	<1.20
	LS	<1.20	<1.20	<1.20
*Staphylococcus**	C	<120	<1.20	<1.20
	L	<1.20	<1.20	<1.20
	LS	<1.20	<1.20	<1.20

*No statistically significant differences were found ($P > 0.05$). C: control treatment, quantity added $NaNO_2$ 100 mg/kg; L: quantity added $NaNO_2$—15 mg/kg; LS: quantity added $NaNO_2$—15 mg/kg + *S. carnosus* ATCC 51365 in number 10^7 cfu/g.

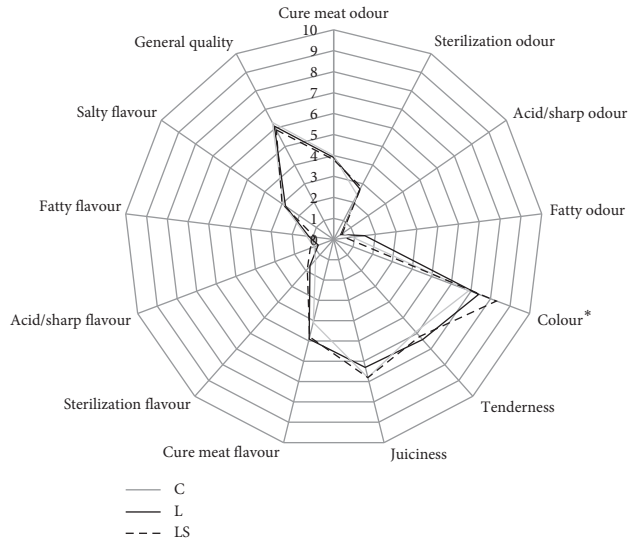

Figure 2: Sensory quality of model meat products after 8 weeks of refrigerated storage. C: control treatment, quantity added $NaNO_2$ 100 mg/kg; L: quantity added $NaNO_2$—15 mg/kg; LS: quantity added $NaNO_2$—15 mg/kg + *S. carnosus* ATCC 51365 in number 10^7 cfu/g. *Mean values of this attribute significantly different ($P \leq 0.05$).

The important function of nitrite is to suppress the outgrowth of *Clostridium botulinum* spores in cured meat products [1, 4, 32, 39]. In this study we did not analyze the antimicrobial efficacies of sodium nitrite in a reduced amount (15 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) [1, 32]. The characteristics of the competing flora accessibility of iron in the products and food additives added to meat including ascorbate, erythorbate, and phosphate are other additional factors [40]. Lövenklev et al. [39] proved that the combination consisting of 2.5% NaCl and $NaNO_2$ at 37.5 mg/kg or 75 mg/kg totally inhibited the growth of *C. botulinum* in the investigated model system. Other studies showed that sodium nitrite in the amount of 10 mg/kg can inactivate *C. botulinum* when combined with spice extracts (0.05% clove extract or 0.02% sage extract or 0.05% nutmeg extract) [25].

3.6. Sensory Quality. Sensory evaluation, as conducted after 8 weeks of being stored, showed statistically significant differences in the tone of the colour of the products. Treatment LS ($NaNO_2$—15 mg/kg) where the *S. carnosus* ATCC 51365 was employed was characterized by the most intensive pink colour; significantly lower values were detected for treatment C and L ($P \leq 0.05$). In other discriminants of sensory quality, no significant differences were stated ($P > 0.05$) (Figure 2).

Ahn and Maurer [41] recorded pinking effects in oven-roasted Turkey breasts with the addition of sodium nitrite with as little as 1 mg/kg. Other studies showed the same effect using sodium nitrite at the level of 4 mg/kg in pork shoulder [30]. However, significantly higher levels of sodium nitrite are required to prevent rapid fading and also maintain an acceptable cured colour, throughout extended shelf life. Also, the higher addition of nitrite to meat is important to be sufficient to induce an acceptable cured meat flavour [1]. In creating the flavour of cured meat products participation of the chemical products of a reaction of nitrites and proteins and the substances, generated as a result of the reaction between nitrogen oxide and free amino acids and their thiol (sulfhydryl) groups was observed [1, 26]. Deda et al. [42] showed that the amount of sodium nitrite added to frankfurters can be reduced from 150 to 100 mg/kg when using 12% tomato paste without any negative effect on the quality parameter of the sausage. Hayes et al. [26] proved that the amount of sodium nitrite could be reduced to 50 mg/kg when combined with 1.5% of tomato pomace powder with similar sensory qualities compared to a meat product with sodium nitrite used in the amount of 100 mg/kg. Other studies showed that the amount of nitrite added to Turkey meat sausages can be reduced from 72 mg/kg to 36 mg/kg when combined with 0.5% of carotenoproteins extract from blue crabs shells and getting greater colour stability in the final product [43].

4. Conclusion

This study shows that the enrichment of natural meat microflora with the *S. carnosus* ATCC 51365 has an influence on the transformations of nitrites during the curing process. The use of denitrifying bacteria in the curing process with a reduced amount of sodium nitrite increased the availability of nitrite in meat, by reducing nitrates formed as a result of a dismutation reaction. In addition, it was shown that *S. carnosus* ATCC 51365 has an influence on the rate of nitrosylation reactions of haem pigments. It has contributed to the formation of nitrosyl pigments in the quantity which is significantly higher than in the treatment where denitrifying bacteria were not used in the curing process of meat. The products produced with a reduced amount of sodium nitrite and *S. carnosus* ATCC 51365 were characterized by

the highest redness. Furthermore, the colour of the products was stable throughout the entire period of 8 weeks of storage. No negative effect of *S. carnosus* ATCC 51365 on the sensory quality of the meat product was found. The use of denitrifying bacteria had no influence on the microbiological quality of meat product during storage.

This study shows that the application of *S. carnosus* ATCC 51365 in the process of curing meat is heading in a promising direction and studies that are aimed at reducing the ingoing amount of sodium nitrite to meat and obtaining cooked meat products with lower residual nitrite have an important value. However, research on the colour stability of products being cured with a low amount of sodium nitrite and *S. carnosus* ATCC 51365 after exposure to light should be carried out. Studies on the microbiological stability of products made with a low amount of sodium nitrite inoculated with *S. carnosus* ATCC 51365 also need to be continued.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors. No ethical approval was required.

Conflicts of Interest

On behalf of all authors, the corresponding author states that there are no conflicts of interest.

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References

[1] J. J. Sindelar and A. L. Milkowski, "Sodium nitrite in processed meat and poultry meats: a review of curing and examining the risk/benefit of its use," *AMSA White Paper Series*, vol. 3, pp. 1–14, American Meat Science Association, Savoy, IL, USA, 2011.

[2] International Agency for Research on Cancer, *Ingested Nitrate and Nitrite, and Cyanobacterial Peptide Toxins*, vol. 94, International Agency for Research on Cancer, Lyon, France, 2010.

[3] S. S. Herrmann, K. Granby, and L. Duedahl-Olesen, "Formation and mitigation of N-nitrosamines in nitrite preserved cooked sausages," *Food Chemistry*, vol. 174, pp. 516–526, 2015.

[4] K.-O. Honikel, "The use and control of nitrate and nitrite for the processing of meat products," *Meat Science*, vol. 78, no. 1-2, pp. 68–76, 2008.

[5] G. Drabik-Markiewicz, B. Dejaegher, E. De Mey, T. Kowalska, H. Paelinck, and Y. Vander Heyden, "Influence of putrescine, cadaverine, spermidine or spermine on the formation of N-nitrosamine in heated cured pork meat," *Food Chemistry*, vol. 126, no. 4, pp. 1539–1545, 2011.

[6] L. Li, J. Shao, X. Zhu, G. Zhou, and X. Xu, "Effect of plant polyphenols and ascorbic acid on lipid oxidation, residual nitrite and N-nitrosamines formation in dry-cured sausage," *International Journal of Food Science & Technology*, vol. 48, no. 6, pp. 1157–1164, 2013.

[7] E. Vossen, M. Utrera, S. De Smet, D. Morcuende, and M. Estévez, "Dog rose (Rosa canina L.) as a functional ingredient in porcine frankfurters without added sodium ascorbate and sodium nitrite," *Meat Science*, vol. 92, no. 4, pp. 451–457, 2012.

[8] J. Y. Jeong, "Alternative curing technology in meat products," *Animal Food Science and Industry*, vol. 5, pp. 77–84, 2016.

[9] D.-M. Shin, K.-E. Hwang, C.-W. Lee, T.-K. Kim, Y.-S. Park, and S. G. Han, "Effect of Swiss chard (beta vulgaris var. cicla) as nitrite replacement on color stability and shelf-life of cooked pork patties during refrigerated storage," *Korean Journal for Food Science of Animal Resources*, vol. 37, no. 3, pp. 418–428, 2017.

[10] G. Barbieri, M. Bergamaschi, G. Barbieri, and M. Franceschini, "Kinetics of nitrite evaluated in a meat product," *Meat Science*, vol. 93, no. 2, pp. 282–286, 2013.

[11] X. Feng, C. Li, X. Jia et al., "Influence of sodium nitrite on protein oxidation and nitrosation of sausages subjected to processing and storage," *Meat Science*, vol. 116, pp. 260–267, 2016.

[12] Commission Regulation (EU) No 1129/2011 of 2011 Amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives.

[13] European Commission, "Ad-hoc study on the monitoring of the implementation of directive 2006/52/EC as regards the use of nitrites by industry in different categories of meat products," Revised interim report, European Commission, Brussels, Belgium, 2015.

[14] European Commission Decision (EU) 2018/702 of 2018 concerning national provisions notified by Denmark on the addition of nitrite to certain meat products (notified under document C(2018) 2721).

[15] R. G. Cassens, T. Ito, M. Lee, and D. Buege, "The use of nitrite in meat," *Bioscience*, vol. 28, no. 10, pp. 633–637, 1978.

[16] R. G. Cassens, "Use of sodium nitrite in cured meats today," *Food Technology*, vol. 49, no. 7, pp. 72–80, 1995.

[17] G. Landeta, J. A. Curiel, A. V. Carrascosa, R. Muñoz, and B. de las Rivas, "Characterization of coagulase-negative staphylococci isolated from Spanish dry cured meat products," *Meat Science*, vol. 93, no. 3, pp. 387–396, 2013.

[18] P. S. Cocconcelli and C. Fontana, "Characteristics and applications of microbial starters in meat fermentation," in *Meat Biotechnology*, F. Toldrá, Ed., Springer, New York, NY, USA, 2008.

[19] M. M. Mainar, D. A. Stavropoulou, and F. Leroy, "Exploring the metabolic heterogeneity of coagulase-negative staphylococci to improve the quality and safety of fermented meats: a review," *International Journal of Food Microbiology*, vol. 247, pp. 24–37, 2017.

[20] D. Siu and A. Henshall, "Ion chromatographic determination of nitrate and nitrite in meat products," *Journal of Chromatography A*, vol. 804, no. 1-2, pp. 156–160, 1998.

[21] M. Hornsey, "The colour of cooked cured pork," *The Journal of Agricultural Science*, vol. 9, no. 7, p. 534, 1956.

[22] M. Hunt and A. King, *AMSA Meat Color Measurement Guidelines*, American Meat Science Association, Savoi, IL, USA, 2012.

[23] S. Hartmann, G. Wolf, and W. P. Hammes, "Reduction of Nitrite by Staphylococcus carnosus and Staphylococcus piscifermen tans," *Systematic and Applied Microbiology*, vol. 18, no. 3, pp. 323–328, 1995.

[24] J. Löfblom, R. Rosenstein, M.-T. Nguyen, S. Ståhl, and F. Götz, "Staphylococcus carnosus: from starter culture to protein engineering platform," *Applied Microbiology and Biotechnology*, vol. 101, no. 23-24, pp. 8293–8307, 2017.

[25] H. Cui, A. A. Gabriel, and H. Nakano, "Antimicrobial efficacies of plant extracts and sodium nitrite against Clostridium botulinum," *Food Control*, vol. 21, no. 7, pp. 1030–1036, 2010.

[26] J. E. Hayes, I. Canonico, and P. Allen, "Effects of organic tomato pulp powder and nitrite level on the physicochemical, textural and sensory properties of pork luncheon roll," *Meat Science*, vol. 95, no. 3, pp. 755–762, 2013.

[27] M. J. Terns, A. L. Milkowski, J. R. Claus, and J. J. Sindelar, "Investigating the effect of incubation time and starter culture addition level on quality attributes of indirectly cured, emulsified cooked sausages," *Meat Science*, vol. 88, no. 3, pp. 454–461, 2011.

[28] K. M. Wójciak, M. Karwowska, and Z. J. Dolatowski, "Use of acid whey and mustard seed to replace nitrites during cooked sausage production," *Meat Science*, vol. 96, pp. 750–756, 2016.

[29] D. Kumar and V. K. Tanwar, "Effects of incorporation of ground mustard on quality attributes of chicken nuggets," *Journal of Food Science and Technology*, vol. 48, no. 6, pp. 759–762, 2011.

[30] K. M. Heaton, D. P. Cornforth, I. V. Moiseev, W. R. Egbert, and C. E. Carpenter, "Minimum sodium nitrite levels for pinking of various cooked meats as related to use of direct or indirect-dried soy isolates in poultry rolls," *Meat Science*, vol. 55, no. 3, pp. 321–329, 2000.

[31] N. Benjamin and J. Collins, "Nitrite," in *Food Preservatives*, N. J. Russell and G. W. Gould, Eds., Kluwer Academic/Plenum Publishers, New York, NY, USA, 2003.

[32] J. G. Sebranek and J. N. Bacus, "Cured meat products without direct addition of nitrate or nitrite: what are the issues?" *Meat Science*, vol. 77, no. 1, pp. 136–147, 2007.

[33] Y. H. Kim, J. T. Keeton, S. B. Smith, L. R. Berghman, and J. W. Savell, "Role of lactate dehydrogenase in metmyoglobin reduction and color stability of different bovine muscles," *Meat Science*, vol. 83, no. 3, pp. 376–382, 2009.

[34] B. N. McClure, J. G. Sebranek, Y. H. Kim, and G. A. Sullivan, "The effects of lactate on nitrosylmyoglobin formation from nitrite and metmyoglobin in a cured meat system," *Food Chemistry*, vol. 129, no. 3, pp. 1072–1079, 2011.

[35] J. Gøtterup, K. Olsen, S. Knochel et al., "Relationship between nitrate/nitrite reductase activities in meat associated staphylococci and nitrosylmyoglobin formation in a cured meat model system," *International Journal of Food Microbiology*, vol. 120, pp. 303–310, 2007.

[36] W. P. Hammes, "Metabolism of nitrate in fermented meats: the characteristic feature of a specific group of fermented foods," *Food Microbiology*, vol. 29, no. 2, pp. 151–156, 2012.

[37] D. U. Ahn and K. C. Nam, "Effects of ascorbic acid and antioxidants on color, lipid oxidation and volatiles of irradiated ground beef," *Radiation Physics and Chemistry*, vol. 71, no. 1-2, pp. 149–154, 2004.

[38] J. J. Sindelar and T. A. Houser, "Alternative curing systems," in *Ingredients in Meat Products: Properties, Functionality, and Applications*, R. Tarte, Ed., Springer, New York, NY, USA, 2009.

[39] M. Lövenklev, I. Artin, O. Hagberg, E. Borch, E. Holst, and P. Rädström, "Quantitative interaction effects of carbon dioxide, sodium chloride, and sodium nitrite on neurotoxin gene expression in nonproteolytic Clostridium botulinum type B," *Applied And Environmental Microbiology*, vol. 70, no. 5, pp. 2928–2934, 2004.

[40] T. A. Roberts and A. M. Gibson, "Chemical methods for controlling Clostridium botulinum in processed meats," *Food Technology*, vol. 40, pp. 163–171, 1986.

[41] D. U. Ahn and A. J. Maurer, "Concentration of nitrate and nitrite in raw turkey breast meat and the microbial conversion of added nitrate to nitrite in tumbled turkey breast meat," *Poultry Science*, vol. 66, no. 12, pp. 1957–1960, 1987.

[42] M. S. Deda, J. G. Bloukas, and G. A. Fista, "Effect of tomato paste and nitrite level on processing and quality characteristics of frankfurters," *Meat Science*, vol. 76, no. 3, pp. 501–508, 2007.

[43] M. Hamdi, R. Nasri, N. Dridi, H. Moussa, L. Ashour, and M. Nasri, "Improvement of the quality and the shelf life of reduced-nitrites turkey meat sausages incorporated with carotenoproteins from blue crabs shells," *Food Control*, vol. 91, pp. 148–159, 2018.