

Article

The Impact of *Lactiplantibacillus plantarum* OP1 and *Lacticaseibacillus paracasei* OP3 Strains on the Quality of Beef Dry Fermented Sausages

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Abstract

This study aimed to evaluate the impact of selected lactic acid bacteria (LAB) strains isolated from the meat plant production environment (*Lactiplantibacillus plantarum* OP1 and *Lacticaseibacillus paracasei* OP3) on the quality and physicochemical properties of innovative beef dry-fermented sausages. As part of the experiment, four treatments were prepared: a control treatment with salt (C), a treatment with a curing mixture (P), and two treatments inoculated with LAB strains (OP1 and OP3) and salt. Samples were analyzed immediately after the completion of a 35-day ripening process and after 3 months of refrigerated storage in vacuum packaging. Analyses revealed that the total lactic acid bacteria (LAB) counts remained at high levels, ranging from 8.19 to 9.64 log CFU/g after the 3-month storage period. The application of autochthonous LAB strains improved the microbiological stability and exhibited bioprotective potential by significantly limiting the growth of native *Enterobacteriaceae* and coagulase-positive staphylococci compared with the control group ($p < 0.05$). Regarding the basic composition of the innovative beef dry-fermented sausages, it was observed that treatments inoculated with LAB strains (particularly *L. paracasei* OP3) exhibited greater dehydration. This resulted in higher protein and salt content, as well as significant increases in textural parameters in the OP3 samples, including hardness (82.39 N), gumminess, and chewiness. The addition of starter cultures also affected the oxidative stability: both strains significantly reduced ($p < 0.05$) the oxidation-reduction potential (ORP), while the TBARS index was significantly lowered by OP1 and maintained below the sensory detection limit by OP3 treatment.



Academic Editor: Raimondo Gaglio

Received: 30 June 2026

Revised: 22 July 2026

Accepted: 4 August 2026

Published: 6 August 2026

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Keywords: autochthonous; indigenous; lactic acid bacteria; LAB; starter cultures; biopreservation process

1. Introduction

Fermented sausages constitute a heterogeneous group of raw meat products, whose high consumer acceptance stems from specific sensory attributes, high nutritional value, and storage stability [1,2]. Their manufacturing process is based on controlled microbial fermentation, most frequently initiated by selected strains of lactic acid bacteria (LAB) [3,4].

During ripening, sequential biochemical transformations occur, including proteolysis, lipolysis, and the fermentation of raw-material carbohydrates. The metabolic activity of the starter microbiota determines the development of the desired texture, the stabilization of heme-dependent color, and the formation of a complex profile of volatile aromatic compounds. Simultaneously, the combined effects of organic acid production (leading to pH reduction) and dehydration processes (resulting in a reduction in water activity) ensure the high microbiological stability of the final products by inhibiting the growth of pathogenic and spoilage microbiota [5].

The production of fermented products from beef raw material exhibits a higher degree of technological complexity compared to the pork matrix, which is determined by the specific chemical composition of this meat species. Key factors determining these differences include higher protein concentration, reduced intramuscular fat content, and a different fatty acid structure. Additionally, beef raw material is characterized by a higher initial pH and a deficit of exogenous carbohydrates, which may significantly inhibit the proliferation dynamics of LAB. These phenomena lead to a slower rate of biochemical acidification, which consequently may limit microbiological stability and affect the formation of desired qualitative and technological characteristics of the final product [6–11].

Current legislative solutions in the European Union aim to reduce the addition of nitrates (III) in the production of cold cuts. The European Commission has published new legislative regulations limiting the content of nitrite and nitrate in food and affecting the quality of meat products (Commission Regulation (EU) 2023/2108). The European Commission has opted for much lower limit values for these additives. The reduction in limits is intended to minimize consumer exposure to nitrosamines, some of which are considered carcinogenic, while simultaneously ensuring that the levels remain sufficient to maintain microbiological safety and protect against pathogenic bacteria such as *Clostridium botulinum*, *Listeria monocytogenes*, and *Salmonella*. The new limits take into account the diverse range of products and the conditions for their production in the European Union [12]. The growing interest of consumers in innovative meat products with reduced or nitrite-free content remains a challenge for producers in this food sector in enriching the offer with safe and high-quality products [13,14].

Currently, the meat processing sector is shifting towards adopting the ‘clean-label’ paradigm, representing a direct response to growing consumer expectations for food products with a high health and safety profile, characterized by reduced inclusion of synthetic chemical additives, such as sodium salts or nitrogen compounds [15]. Although the traditional use of nitrites and nitrates is technologically justified due to their high efficacy in inhibiting the proliferation of pathogens (particularly *Clostridium botulinum* and *Listeria monocytogenes*), limiting lipid peroxidation, and stabilizing heme-dependent color [16,17], their presence within the meat matrix raises health concerns associated with the risk of synthesizing carcinogenic N-nitrosamines [18].

In light of increasingly stringent European Union legislative regulations on limits for curing agents, innovative biopreservation strategies based on the application of selected LAB strains are of key importance. These microorganisms fulfill a bioprotective role through multidirectional metabolic activity, encompassing the biosynthesis of organic acids (such as lactic and acetic acids), hydrogen peroxide, and bacteriocins or bacteriocin-like inhibitory substances (BLIS). The synergistic action of these metabolites enables the effective suppression of spoilage and pathogenic microbiota, ensuring microbiological stability and extending the shelf life of fermented products without the necessity of aggressive thermal treatment [19–21].

Simultaneously, there is growing interest in the application of strains with probiotic potential in meat matrices, enabling the development of fermented products with advanced

health-promoting functions. Probiotic microorganisms are defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” [22]. In the context of fermented meat products, these functional cultures play a dual role: they contribute to the biopreservation of the product by competing with spoilage microflora and producing inhibitory metabolites, while simultaneously enhancing the nutritional value and health-promoting profile of the food matrix. Fermented meat sausages are recognized as a promising carrier for probiotics, as the absence of thermal treatment during the production process promotes the maintenance of high bacterial cell viability, which is essential for their survival during passage through the consumer’s gastrointestinal tract. The activity of these strains within the host organism encompasses beneficial modulation of the intestinal microbiota [23], stimulation of the immune system, and the inhibition of pathogen colonization through microbial antagonism [24].

The main aspect in developing new meat product formulations is selecting appropriate starter cultures. Autochthonous strains, isolated directly from production environment or traditional meat products, generally exhibit better adaptation to the specific conditions of the meat matrix—such as high salt concentrations, the presence of nitrites, and low pH—compared to cultures derived from other sources. However, the meat processing plant environment represents a harsh environment for microorganisms, primarily due to rigorous and systematic cleaning and disinfection procedures. Specific LAB strains demonstrate the capacity for biofilm formation, which enhances their persistence on production surfaces and confers increased resistance to environmental stressors and sanitizing agents. [1,3]. Their application enables superior control over the fermentation process, ensures qualitative repeatability, and effectively shapes the desired technological and sensory attributes of the final product [4]. It should be emphasized that the strains of LAB used were isolated from a meat factory production environment (*L. plantarum* OP1 from the surface of a production trolley; *L. paracasei* OP3 from the surface of a meat cutting table) [25], in an organic production system. These autochthonous (indigenous) strains are naturally adapted to the specific conditions of the meat matrix, which distinguishes them from standardized commercial starter cultures.

Despite extensive reports regarding the role of LAB in the meat fermentation process, research addressing the simultaneous impact of specific starter cultures and strains on the safety, sensory attributes, and functional properties of sausages manufactured exclusively from a beef meat matrix remains limited. Therefore, the present study aimed to assess the impact of using selected strains of lactic acid bacteria isolated from the meat plant production environment (*L. paracasei* OP3; *L. plantarum* OP1) on the basic composition, microbiological and physicochemical properties of beef dry fermented sausages.

2. Materials and Methods

2.1. Materials

Four different formulations of dry-fermented sausages were prepared. The sausages were manufactured industrially in a meat processing plant. The study material includes three series of meat products made according to the formulations shown in Table 1. The study material consisted of three independent production batches ($n = 3$) of meat products, for each treatment (15 kg of sausages). From each batch, three products for each treatment were sampled for analysis. Product quality surveys were conducted in the Department of Meat and Fat Technology, Institute of Agricultural and Food Biotechnology—State Research Institute (Warsaw, Poland). The characteristics of the investigated formulations are shown in Table 1.

All four experimental treatments were prepared using a base of 10 kg beef meat supplemented with 0.05 kg glucose. The differences between the treatments involved the

specific curing and microbial additives applied: (1) control treatment (C): contained only 0.2 kg of sodium chloride (NaCl) and 0.1 L of sterile saline solution (0.9% NaCl); (2) curing treatment (P): included 0.2 kg of a curing mixture (99.4% NaCl and 0.6% NaNO₂) and 0.1 L of sterile saline solution; (3) OP1 treatment: consisted of 0.2 kg of NaCl and the *L. plantarum* OP1 strain suspended in 0.1 L of sterile saline solution; (4) OP3 treatment: consisted of 0.2 kg of NaCl and the *L. paracasei* OP3 strain suspended in 0.1 L of sterile saline solution. Based on mathematical calculations, the initial inoculation level of the used LAB strains in the meat was targeted at approximately 4.0 log CFU/g.

Table 1. Formulations of beef dry fermented sausages.

Ingredients	Treatments			
	C	P	OP1	OP3
Beef meat (kg)	10	10	10	10
Glucose (kg)	0.05	0.05	0.05	0.05
NaCl (kg)	0.2	-	0.2	0.2
Curing mix (NaCl-99.4%, NaNO ₂ -0.6%) (kg)	-	0.2	-	-
<i>Lactiplantibacillus plantarum</i> OP1 with salt solution (NaCl 0.9%) (L)	-	-	0.1	-
<i>Lactiplantibacillus paracasei</i> OP3 with salt solution (NaCl 0.9%) (L)	-	-	-	0.1
Salt solution (NaCl 0.9%) (L)	0.1	0.1	-	-

Explanatory notes: C—control treatment with salt; P—treatment with curing mixture; OP1—treatment with salt and *Lactiplantibacillus plantarum* OP1 in number approx. 4.0 log CFU/g; OP3—treatment with salt and *Lactiplantibacillus paracasei* OP3 in number approx. 4.0 log CFU/g.

2.1.1. Cultivation and Preparation of the Bacterial Inoculum

This study applied two bacterial strains *L. plantarum* OP1 (GenBank accession OP793883) and *L. paracasei* OP3 (GenBank accession OP793889). The meat processing plant is located in Poland, in the Subcarpathian Voivodeship. Some properties of the used strains were previously described by Łepecka et al. [25].

After storage at −80 °C in 20% (v/v) glycerol, the bacterial strains were reactivated by two successive subcultures in MRS broth (Sigma-Aldrich Co., Darmstadt, Germany) using a 2% (v/v) inoculum for each subculture. Following incubation, the bacterial culture was centrifuged at 10,000 × g for 5 min. The resulting cell pellets were washed and subsequently resuspended in a fixed volume of 0.1 L (100 mL) of sterile 0.9% saline solution, which corresponded to the initial culture volume, ensuring a standardized concentration for the inoculation of the meat.

2.1.2. Dry Fermented Sausage Manufacturing

The manufacturing process of the beef dry-fermented sausages was conducted as follows: raw beef trimmings were pre-cut and manually mixed with either sodium chloride (C, OP1, and OP3 treatments) or a curing mixture (P treatment), depending on the experimental group. The prepared raw material was cured/retained for 48 h at 2–4 °C. Following this, the meat was ground using a meat grinder with a 10 mm mesh diameter.

During the mixing stage, glucose and the respective bacterial biomass—consisting of *Lactiplantibacillus plantarum* OP1 or *Lactiplantibacillus paracasei* OP3 suspended in 0.9% saline solution—were added to the ground meat. For the control (C) and cured (P) treatments, an equivalent volume of sterile saline was used. The batter was mixed for 5 min using a paw stirrer to ensure uniform distribution of ingredients. The resulting stuffing was stuffed into

natural pork casings, hung, and placed in the production hall for 4 h at 10–12 °C to allow for casing dehydration.

The ripening process was subsequently carried out in a specialized chamber at 15–17 °C and 70–85% relative humidity for a total of 35 days. On the third day of ripening, the sausages were subjected to a smoking process (20 min, 25–35 °C). Upon completion of the 35-day ripening period, the products were cooled to 4–6 °C and vacuum-packed in a multilayer polyamide/polyethylene (PA/PE) film. The finished sausages were stored under refrigerated conditions (4–6 °C) for further analysis. To ensure statistical reliability, the entire manufacturing experiment was conducted across three independent production batches.

2.2. Methods

The chemical composition analysis and texture measurements of the beef dry fermented sausages were conducted on the fresh products. The remaining determinations were performed with 3 months of refrigerated storage taken into account.

2.2.1. Chemical Composition

The following methods were used to assess the chemical composition of the investigated products: (1) ISO standard ISO 1442:2023 (moisture content) [26]; (2) the method according to standard PN-A-04018:1975/Az3:2002 (protein content) [27]; (3) ISO standard 1444:1996 (the content of free fat) [28], (4) ISO standard ISO 1841-2:1996 (chloride content) [29], and the titration method according to the IBPRS-PIB laboratory's own procedure (PA/09, Issue 3 of 8 June 2021), which is based on the PN-A-82059:1985 [30–32] (carbohydrate content).

2.2.2. Microbiological Analyses

The microbiological evaluation was carried out using the plate method according to the accepted standards for food microbiology. All determinations were performed in triplicate. Total viable count (TVC) was determined according to ISO 4833-1:2013 [32] on nutrient agar (Oxoid, Basingstoke, UK). The *Enterobacteriaceae* (ENT) count was determined according to ISO 21528-2:2017 [33] on MacConkey agar (Oxoid, Basingstoke, UK). *Escherichia coli* (EC) were enumerated according to ISO 16649-1:2018 [34] on TBX agar (Oxoid, Basingstoke, UK). Lactic acid bacteria (LAB) counts were determined according to ISO 15214:1998 [35] on MRS agar (Oxoid, Basingstoke, UK). The number of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) (SA) was determined according to ISO 6888-1:2021 [36] on Baird–Parker agar with egg yolk tellurite (Oxoid, Basingstoke, UK). The obtained results of the number of bacteria were expressed in log CFU/g.

The presence of *Salmonella* spp. (SAL) according to ISO 6579-1:2017 [37] was determined on XLD agar (Oxoid, Basingstoke, UK). The presence of *Listeria* spp. including *Listeria monocytogenes* (LM) according to ISO 11290-1:2017 [38] was determined on ALOA agar (Agar *Listeria* according to Ottaviani and Agosti; BTL, Łódź, Poland).

2.2.3. Composition of Fatty Acids and Cholesterol Content

The fatty acid profile of the fermented sausages was determined by gas chromatography (HP 6890 II) equipped with a flame ionization detector (GC-FID), in accordance with ISO 12966-1:2014 [39]. Cholesterol content was quantified by extracting the lipid fraction, esterifying fatty acids, and derivatizing cholesterol in the presence of an internal standard.

Quantitative analysis was performed by GC-FID, and the results were expressed as percentages (%) of total fatty acids. The data were categorized into the following groups: the sum of saturated fatty acids (Σ SFA), the sum of monounsaturated fatty acids (Σ MUFA),

the sum of polyunsaturated fatty acids (Σ PUFA), and the total concentrations of n-3 and n-6 fatty acid series.

2.2.4. pH Measurement

A FiveEasy F20 pH meter with an LE438 electrode (Mettler-Toledo GmbH, Greifensee, Switzerland) was used to measure pH in accordance with ISO 2917:1999 [40]. The 50 mL of distilled water was used to homogenize a 10 g sample (Bamix SwissLine M200; Bamix Polska, Sławno, Poland). Measurements were made at 20 °C.

2.2.5. Water Activity Measurement

Water activity of investigated samples was determined according to ISO 18787:2017 [41] using the Aqualab Pawkit DE201 apparatus (METER Group, Inc., Pullman, WA, USA). Approximately 20 g of the investigated sample was placed in a specially dedicated container, and then the measurements were carried out at 20 °C.

2.2.6. Instrumental Color

Instrumental color parameters of the meat products were determined in the CIE $L^*a^*b^*$ color space using a CR-300 Chroma Meter (Konica Minolta, Tokyo, Japan) according to the method described by Lepecka et al. [4]. The research material consisted of freshly cut 8 mm thick sausage slices, which were exposed to atmospheric oxygen (blooming) for 20 min under controlled room temperature conditions to stabilize the color prior to measurement. All readings were taken at a constant ambient temperature of 20 ± 1 °C. The instrument was calibrated using a white standard provided by the manufacturer with parameters $L^* 99.18$, $a^* -0.07$, and $b^* -0.05$. The measurement procedure utilized an 8 mm diameter aperture and a standard 2° observer, with a D65 illuminant and a pulsed xenon lamp serving as the light source. All determinations were performed in triplicate. The obtained results were expressed as a mean $\pm$ standard deviation (SD).

2.2.7. Oxidation-Reduction Potential (ORP)

SevenCompact™ S220 device with an InLab Redox electrode (Mettler-Toledo GmbH, Greifensee, Switzerland) was used to measure ORP. The 50 mL of distilled water was used to homogenize a 10 g sample (Bamix SwissLine M200; Bamix Polska, Sławno, Poland) [42]. Using the standard hydrogen electrode (Eh, mV), the results were converted to ORP values. At 20 °C, measurements were made. mV was used to express the outcome.

2.2.8. TBARS (Thiobarbituric Acid Reactive Substances) Index

The investigated fermented sausages' lipid oxidation was evaluated in accordance with Pikul et al. [43]. It was determined how much thiobarbituric acid reactive substances (TBARS) were present. A Nicolet Evolution 300 spectrophotometer (Thermo Electron Corp., Waltham, MA, USA) was used to measure the absorbance at 532 nm at about 20 °C. The following equation illustrates how the values were expressed as milligrams of malondialdehyde (MDA) per kilogram of sample:

$$\text{TBARS [mg MDA/kg]} = 5.5 \times \text{absorbance value of the investigated sample}$$

2.2.9. Texture Analysis

The textural properties of beef dry fermented sausages were analyzed with a texture analyzer (TA-Xt. plus, Stable Micro Systems, Godalming, Surrey, UK). All of the samples analyzed were cylindrical in shape, with a diameter of 20 mm and a height of 25 mm. The samples were compressed to 50% of their original height using a P/36R probe (Stable Micro

Systems, UK) at a speed of $2 \text{ mm} \cdot \text{s}^{-1}$, with a load cell of 30 kg. Texture parameters were measured in five replicates for each sample, and the results were averaged.

2.2.10. Statistical Analyses

Statistical analysis was based on data obtained from three independent production batches. For each batch, all laboratory determinations were conducted in triplicate (technical replicates), and the mean values per batch were used for the analysis of variance. The statistical analysis was performed using Statistica 13.1 (TIBCO Software Inc., Palo Alto, CA, USA). One-way Analysis of Variance (ANOVA) was used for the statistical evaluation of the results from microbiological analyses, chemical composition, water activity, and texture of fermented meat products (a significance level of $p = 0.05$ was assumed). Differences among the obtained means were tested using Tukey's honestly significant difference test (Tukey's HSD). On the other hand, the color parameters (L^* , a^* , b^*), values of TBARS index, Oxidation-Reduction Potential (ORP), pH value, and composition of fatty acids were analyzed by Two-Way ANOVA with a GLM, which included treatment and time storage of the investigated samples, as well as their mutual interactions, as fixed effects, and replicates as random effects. Pearson's correlation was used to determine the strength and direction of linear relationships among quality attributes, specifically physicochemical (pH, ORP, TBARS), physical (moisture content, a_w , and hardness), and microbiological (bacterial counts: LAB and ENT) parameters. The significance of the correlation coefficients was tested at the $p < 0.05$ level.

2.2.11. Digital Color Reconstruction and Visualization

To provide additional visualization of the final physical state of the evaluated beef dry-fermented sausages and to facilitate the interpretation of the instrumental color measurement results, digital color swatches (color boxes) corresponding to each experimental treatment were developed. The visualization was based on the mean CIE color coordinate values (as detailed in Section 2.2.6) obtained immediately after the 35-day ripening process (0 months) and after 3 months of refrigerated storage. The obtained color parameters were converted into the sRGB (Standard Red Green Blue) color space using standardized colorimetric conversion algorithms (available at <https://www.colorhexa.com/examples>, accessed on 23 July 2026). This enabled the generation of precise digital codes (HEX and RGB) and the visual presentation of the corresponding shades for each treatment at both study intervals (0 and 3 months).

3. Results and Discussion

3.1. Chemical Composition of Beef Raw Fermented Sausages

The analysis of the chemical composition of the investigated meat products (Table 2) revealed that the applied technological treatment (C, P, OP1, OP3) had a significant effect on the basic chemical composition parameters of the beef sausages, specifically the moisture and fat content ($p < 0.001$), as well as the NaCl concentration. In the samples inoculated with LAB (OP1 and OP3) and the sample treated with a curing mixture (P), significantly lower water retention ($p < 0.05$) was observed compared to the control group. The differences in moisture content were minor yet statistically significant, as demonstrated by the data in Table 2. Only the control treatment C exhibited the statistically highest moisture level (42.50%), which differed significantly from all other investigated samples (P, OP1, and OP3), a result directly associated with the highest water retention in this sample.

This reduction in water content is attributed to the intensive evaporation of moisture during the fermentation and ripening processes, resulting in an increased concentration of solid components in the final product. The P (21.70%) and OP1 (21.20%) treatments had

significantly higher fat contents than the other evaluated samples ($p < 0.05$). In contrast, treatments C (11.10%) and OP3 (10.20%) were characterized by significantly the lowest fat content ($p < 0.05$). These differences may stem from raw material variability and the mass concentration effect following moisture loss.

Table 2. Chemical composition of beef raw fermented sausages.

Component [%]	Treatments			
	C	P	OP1	OP3
Moisture	42.50 ± 0.31 ^b	38.60 ± 0.34 ^a	38.60 ± 0.60 ^a	42.00 ± 0.03 ^a
Protein	35.33 ± 2.18 ^a	35.30 ± 0.33 ^a	37.10 ± 0.21 ^a	37.70 ± 0.90 ^a
Carbohydrates	<0.50	<0.50	<0.50	<0.50
Fat	11.10 ± 0.70 ^a	21.70 ± 1.30 ^b	21.20 ± 0.75 ^b	10.20 ± 0.55 ^a
NaCl	4.50 ± 0.40 ^{ab}	3.90 ± 0.20 ^a	5.30 ± 0.30 ^{ab}	5.40 ± 0.07 ^b
Water activity	0.91 ± 0.01 ^b	0.89 ± 0.01 ^b	0.84 ± 0.005 ^a	0.87 ± 0.01 ^a
Cholesterol (mg/100 g of product)	96.09 ± 5.13 ^a	86.60 ± 0.37 ^a	106.20 ± 16.76 ^a	103.20 ± 4.55 ^a

Explanatory notes: Values denoted by different lowercase letters in the same component of different treatments differ significantly ($p < 0.05$).

Significantly, the lowest sodium chloride content ($p < 0.05$) was recorded in the case of treatment P, while the highest was observed in the treatment OP3 inoculated with the *L. paracasei* OP3 strain. Salt plays a crucial technological role in solubilizing myofibrillar proteins, which is essential for ensuring appropriate adhesion and cohesiveness within the meat matrix. The reduction in water content in fermented meat products inoculated with LAB, along with the associated increase in the relative content of protein and fat, has been described in the literature [44]. The application of selected strains (e.g., *Lactobacillus* spp.) in the production technology of fermented meat products results in a decrease in pH, a reduction in moisture, and alterations in the product's physicochemical composition [45].

In turn, a significantly highest ($p < 0.05$) NaCl content was recorded in the OP3 sample (Table 2), which may be the result of differences in the initial fat content of the raw material used and the concentration effect caused by water loss (which increases the percentage of fat), as well as the lipolytic activity of the microflora (starter cultures or autochthonous populations), which may modify the profile of free fatty acids [46]. On the other hand, no statistically significant differences ($p > 0.05$) were observed in the protein content in the investigated samples. The obtained values were consistent with the results of other authors [47].

Sodium chloride is a crucial component in processed meat owing to its preservative attributes, its ability to influence taste and enhance flavor, and its functional role in solubilizing myofibrillar proteins, which is essential for improving adhesion and cohesiveness in processed meat products [48]. The differences in NaCl content (statistically significant mainly for OP3 compared to the other samples) may result from the formulation composition of the tested products (Table 1), as well as from varying levels of salt retention, which depend on the structure of the muscle–fat matrix after fermentation and on the degree of dehydration. Hu et al. [49] suggest that modifications in NaCl levels, in turn, affect LAB activity and water activity, which indirectly influence the rate of fermentation and water loss. Therefore, changes in NaCl content may be both the cause of differences in water content and of variations in the nutritional components of the final product.

The investigated samples did not differ significantly ($p > 0.05$) in terms of cholesterol content, and the obtained values were consistent with data available in the literature [50–52].

However, there were slight but significant ($p < 0.05$) differences in the level of water activity between samples C, P (0.89–0.91), and samples with the addition of a LAB (OP1; OP3) (0.84–0.87) (Table 2). Storage time did not significantly ($p > 0.05$) affect the water activity of the investigated products. According to Paulo et al. [53], the addition of probiotic bacterial cultures to fermented meat products does not significantly affect the water activity value, but reduces the pH value of the finished products compared to the control sample. On the other hand, according to the International Commission on Microbiological Specifications for Foods (ICMSF), water activity of approximately 0.88 may reduce the growth of unwanted microflora in fermented sausages [54].

In summary, the application of autochthonous LAB strains (particularly the OP1 strain) and the curing mixture might have caused an increased degree of dehydration and a concentration of solid components, which is crucial for the stability and texture of the final products.

3.2. Microbiological Analyses

Currently, ensuring safe, high-quality food production is a leading concern worldwide [55–57]. Preventing contamination by physical, chemical, and microbiological threats that negatively impact health is what safety is all about [58]. The type and log number of microorganisms that proliferate are influenced by fermentation temperature, humidity, and maturation period, which are the primary elements determining the formation of fermented sausages [59]. The results of the microbiological quality estimation of the investigated meat products during 3 months of refrigerated storage are shown in Table 3.

A significant effect ($p < 0.05$) of the sample type and storage time on selected microbiological quality parameters of the finished products was demonstrated (Table 3). The total number of microorganisms in the investigated samples was high and ranged from 8.14 to 9.95 log CFU/g (Table 3). These values increased significantly ($p < 0.05$) as a result of storage by approximately one logarithmic order in all sample treatments, except for OP3. The high TVC value is associated with a high number of LAB (higher than 8.00 log CFU/g), coagulase-positive staphylococci (2.20–3.93 log CFU/g), and bacteria from the *Enterobacteriaceae* family (2.06–3.13 log CFU/g) (Table 3). Neither *Salmonella* spp. Neither *Listeria* spp. was detected in fresh or stored products, and the number of *E. coli* bacteria was determined at a relatively low level, below 1.00 log CFU/g, indicating the effectiveness of the hygiene procedures applied in the production technology. It should be emphasized that throughout the 3-month storage period, the LAB count in the sausages remained at a high level—above 8 log CFU/g. While the absence of *Salmonella* and *Listeria* confirms the high hygiene standard of the production process, the bioprotective efficacy of the starter cultures is demonstrated by the significant reduction in indicator spoilage organisms (Table 3). In fresh products with OP1 and OP3 (Table 1), a lower number of coagulase-positive staphylococci and *Enterobacteriaceae* bacteria was observed compared to the C and P samples. In contrast, in none of the tested samples did storage time significantly affect the count of bacteria from the *Enterobacteriaceae* family. *Enterobacteriaceae* is typically thought of as a form of spoilage bacteria and is known to negatively impact the safety and quality of fermented sausages [60].

In the stored products, the number of *Enterobacteriaceae* bacteria ranged from 2.16 to 3.21 log CFU/g, classifying these products in the so-called “borderline” group according to the Microbiological Guidelines for Food [54]. It indicates a microbiological count that exceeds the acceptable range, raising concerns about food safety and necessitating an investigation into food handling procedures. Even though they are not yet dangerous or “unsatisfactory,” these findings frequently call for resampling, an examination of hygienic practices, and a food establishment inspection to find and address underlying problems.

In all investigated samples, the number of coagulase-positive staphylococci was in the range 2.2–4.2 log CFU/g. A significant increase in the number of these bacteria was observed only in the control sample (C) [61]. Specific strains of *Staphylococcus aureus* can produce a thermostable toxin that is harmful to humans. *S. aureus* multiplication and toxin production are possible above a critical temperature of 15.6 °C. In accordance with control recommendations, if the microbiological evaluation indicates fewer than 10⁴ *S. aureus* per gram and no enterotoxins or other pathogens are detected, then such a product, after being labeled as requiring refrigeration, can be sold.

Table 3. Estimation of microbiological quality of investigated meat products during 3 months of refrigerated storage.

Parameter	Treatment	Time Storage (Months)	
		0	3
TVC (log CFU/g)	C	8.14 ± 0.11 ^{aA}	10.14 ± 0.05 ^{aB}
	P	9.49 ± 0.09 ^{cA}	10.70 ± 0.10 ^{aBC}
	OP1	8.85 ± 0.14 ^{bA}	10.13 ± 0.11 ^{aB}
	OP3	9.95 ± 0.06 ^{dB}	9.70 ± 0.10 ^{aAB}
LAB (log CFU/g)	C	8.99 ± 0.11 ^{aB}	8.71 ± 0.06 ^{ba}
	P	8.67 ± 0.10 ^{aB}	8.19 ± 0.07 ^{aA}
	OP1	9.41 ± 0.10 ^{bA}	9.64 ± 0.06 ^{dB}
	OP3	10.60 ± 0.13 ^{cB}	9.09 ± 0.04 ^{cA}
EC (log CFU/g)	C	<1.00	<1.00
	P	<1.00	<1.00
	OP1	<1.00	<1.00
	OP3	<1.00	<1.00
ENT (log CFU/g)	C	3.13 ± 0.03 ^{cA}	3.21 ± 0.04 ^{cA}
	P	3.21 ± 0.04 ^{cA}	3.40 ± 0.12 ^{bA}
	OP1	2.35 ± 0.03 ^{bA}	2.29 ± 0.01 ^{aA}
	OP3	2.06 ± 0.03 ^{aA}	2.16 ± 0.09 ^{aAB}
SA (log CFU/g)	C	2.77 ± 0.02 ^{cA}	3.63 ± 0.05 ^{abB}
	P	3.93 ± 0.03 ^{dA}	4.24 ± 0.14 ^{abA}
	OP1	2.61 ± 0.07 ^{bA}	2.20 ± 0.01 ^{aA}
	OP3	2.47 ± 0.06 ^{aA}	2.24 ± 0.12 ^{aA}
SAL	C	nd	nd
	P	nd	nd
	OP1	nd	nd
	OP3	nd	nd
LM	C	nd	nd
	P	nd	nd
	OP1	nd	nd
	OP3	nd	nd

Explanations: TVC—total viable counts; LAB—lactic acid bacteria; EC—*Escherichia coli*; ENT—bacteria from the *Enterobacteriaceae* family; SA—coagulase-positive staphylococci (*Staphylococcus aureus* and other species); SAL—*Salmonella* spp.; LM—*Listeria* spp. including *L. monocytogenes*; <1.00—counts below the detection limit of the plating method; nd—not detected. The values are expressed as means ± SD (standard deviation). Values denoted by different capital letters in the same batch of meat products, stored at different temperatures, differ significantly ($p < 0.05$). Values denoted by different lowercase letters in the same storage conditions differ significantly ($p < 0.05$).

The stability of fermented meat products is mainly determined by acidification, brought about by LAB and water activity (a_w) reduction in the course of curing and drying [62]. Some articles assert that adding a starter culture to dry-fermented sausages enhances their safety and quality by examining variables such as pH and pathogen

count/detection [63,64]. The addition of LAB starter strains leads to a faster and more effective reduction in *S. aureus* populations in fermented meat products compared to those produced through spontaneous fermentation. In experiments with the addition of *L. sakei* or *P. pentosaceus*, a decrease in *S. aureus* counts of 2–3 log CFU/g was observed during the first days of fermentation [65]. In turn, Zheng et al. [66] observed a positive effect of inoculation with three indigenous LAB strains—*Latilactobacillus sakei*, *Pediococcus pentosaceus*, and *Weissella cibaria*, isolated from Yunnan ham—on inhibiting the growth of spoilage microorganisms and improving the color of the products.

3.3. ORP, pH

Parameters influencing the biological activity of starter cultures are factors that regulate the quality and characteristics of fermented products. One of the main physicochemical parameters affecting the growth and metabolic activities of LAB is the redox potential. This means that monitoring and controlling the redox potential is crucial for the production and assessment of the quality of fermented products like fermented meat, where LAB plays an important role. Regulation of intra- and extracellular redox potentials can alter the biochemical reactions and the quality of said products [20].

A significant ($p < 0.001$) influence of treatment and time of storage on the redox potential value and pH value was observed. In addition, an interaction between the treatment and time of storage for these parameters was found (Table 4).

Table 4. Influence of treatment and storage time on the values of oxidation–reduction potential and pH of beef fermented sausages during 3 months of storage at 4 °C (means $\pm$ SE).

Sample Code		Time Storage at 4 °C [Months]		Treatment	Time	Treatment × Time
		0	3	P _{TR}	P _T	P _{2T}
ORP (mV)	C	490.13 ± 5.75 ^{cA}	509.37 ± 4.44 ^{cB}	***	***	***
	P	479.00 ± 3.61 ^{bA}	509.73 ± 1.72 ^{cB}			
	OP1	468.00 ± 5.00 ^{aA}	485.13 ± 1.90 ^{bAB}			
	OP3	468.00 ± 4.50 ^{aA}	465.13 ± 2.50 ^{aA}			
pH	C	5.47 ± 0.01 ^{aA}	5.45± 0.01 ^{aA}	***	***	***
	P	5.61 ± 0.01 ^{cB}	5.48 ± 0.005 ^{abA}			
	OP1	5.60 ± 0.05 ^{cA}	5.55 ± 0.01 ^{abAB}			
	OP3	5.53 ± 0.02 ^{bA}	5.61 ± 0.01 ^{cA}			

Explanatory notes: The presented samples are coded according to the Materials and Methods section, Table 1. The results are expressed as the mean $\pm$ standard error ($n = 3$). Values denoted by different letters differ significantly ($p < 0.05$). SD: standard deviation; P: significance of effects; treatment (_{TR}); time of storage (_T); treatment $\times$ time of storage interaction (_{2T}); *** $p < 0.001$. ^{a, b, c}—treatment column; ^{A, B}—time column.

Immediately after the ripening process, the samples with the addition of LAB strains (OP1; OP3) exhibited the lowest ORP values, at 468.00 mV. Additionally, these values did not differ significantly from each other ($p > 0.05$). Storage time significantly ($p < 0.05$) affected the C (control) and P (treatment with curing mixture) samples (Table 1), where a significant increase in ORP values was observed. Literature data confirm the positive role of LAB in reducing ORP [13,67,68], which promotes microbiological and oxidative stability and stabilizes the color of the final product. However, the final effect depends on the strain used and the parameters of the technological process [68].

In turn, no effect of the addition of a LAB on the pH value was observed. The pH value in the products immediately after maturation was, respectively, from 5.47 (C) to about 5.60 (P; OP1) (Table 4). Significantly higher pH values compared to the control sample were obtained in products with the addition of LAB strains (OP1; OP3). The pH value decreased significantly ($p < 0.05$) in the P sample (treatment with curing mixture) (Table 1), along with an increase in ORP values during storage. Storage time did not significantly

($p > 0.05$) affect pH values in the control sample (C) or in the sample treatments with the addition of LAB (OP1; OP3) (Table 4). It should be noted that the used bacterial strains (*Lactiplantibacillus plantarum* OP1 and *Lacticaseibacillus paracasei* OP3) show low activity in an acidified environment. Maintaining a higher pH may result from their proteolytic properties. As a result of protein metabolism, basic compounds (e.g., amines, ammonia) are formed, which leads to an increase in the pH value during fermentation [69].

3.4. TBARS

The TBARS index (Thiobarbituric Acid Reactive Substances) is one of the most commonly used parameters for assessing the degree of lipid oxidation in meat products, including fermented sausages [70]. The results of changes in TBARS index values of fermented sausages are shown in Table 5. The treatment affected ($p < 0.001$) the TBARS value. No significant interaction between the factors, nor a significant effect of storage time ($p > 0.001$), was observed on the TBARS index.

Table 5. Influence of treatment and storage time on TBARS index of novel beef fermented sausages during 3 months of storage at 4 °C (means $\pm$ SE).

	Sample Code	Time Storage at 4 °C [Months]		Treatment	Time	Treatment $\times$ Time
		0	3	P _{TR}	P _T	P _{2T}
TBARS (mg MDA/kg)	C	1.51 $\pm$ 0.02 ^{cA}	1.50 $\pm$ 0.02 ^{cA}	***	NS	NS
	P	0.98 $\pm$ 0.03 ^{aA}	0.90 $\pm$ 0.04 ^{aA}			
	OP1	1.22 $\pm$ 0.02 ^{bA}	1.18 $\pm$ 0.05 ^{bA}			
	OP3	1.79 $\pm$ 0.02 ^{dA}	1.83 $\pm$ 0.02 ^{dA}			

Explanatory notes: The presented samples are coded according to the Materials and Methods section, Table 1. The results are expressed as the mean $\pm$ standard error ($n = 3$). Values denoted by different letters differ significantly ($p < 0.05$). SE: standard error of means; P: significance of effects; treatment (TR); time of storage (T); treatment $\times$ time of storage interaction (2T); NS—not significant; *** $p < 0.001$. ^{a, b, c, d} —treatment column; ^A—time column.

The obtained results suggest that in the P sample (treatment with curing mixture), the determined TBARS index values (0.90–0.98 mg/kg) were significantly the lowest ($p < 0.05$) among all evaluated treatments (Table 5), regardless of storage time. The additives used in the production technology of fermented meat products influence the measured TBARS index values. As confirmed by numerous literature sources, curing compounds such as nitrites and nitrates, in addition to imparting characteristic color and inhibiting undesirable microflora, exhibit antioxidant activity [71–74]. The presence of sodium nitrite can reduce TBARS values in fermented sausages due to the ability of nitrites to form stable complexes with muscle proteins, limit iron availability, and inhibit reactions that initiate lipid oxidation [75]. In turn, the effect of adding LAB on the TBARS index (Table 6) was highly variable and dependent on the strain used. The statistically highest ($p < 0.05$) TBARS index values (1.79–1.83 mg/kg), regardless of storage time, were recorded in the sample with the addition of the *L. paracasei* OP3 strain (Table 5). It should be emphasized that the recorded values of the TBARS index did not exceed the level 2 mg MDA/kg independently of the storage time, which is considered the limit of detection in the mouth [76]. According to the literature, the effect of the starter cultures used on TBARS index values depends on the species and strain of the microorganism. Some strains of *L. sakei* and *L. plantarum* limit oxygen availability and lower pH, which inhibits lipid oxidation, whereas strains with high proteolytic activity may slightly increase TBARS index levels [77–79].

Although OP3 showed the highest TBARS values, it is important to note that this treatment also achieved the lowest ORP (465.13 mV), suggesting that the strain effectively

modified the oxidative environment, even if its proteolytic metabolism led to higher secondary oxidation markers.

Table 6. Fatty acid composition (% total fatty acids) in investigated meat products after fermentation and 3 months of refrigerated storage.

Sample Code		Time Storage at 4 °C [Months]		Treatment	Time	Treatment × Time
		0	3	P _{TR}	P _T	P _{2T}
Σ SFA	C	46.53 ± 1.04 ^{aA}	46.33 ± 0.20 ^{aA}	***	**	***
	P	51.17 ± 0.22 ^{bA}	51.63 ± 0.31 ^{bA}			
	OP1	50.05 ± 0.75 ^{bA}	50.46 ± 0.65 ^{bA}			
	OP3	50.80 ± 0.35 ^{bA}	53.20 ± 0.42 ^{cB}			
Σ MUFA	C	45.66 ± 0.80 ^{cA}	39.80 ± 0.23 ^{bcA}	***	***	***
	P	41.53 ± 0.52 ^{abB}	34.73 ± 0.11 ^{bA}			
	OP1	42.80 ± 0.28 ^{abB}	35.20 ± 0.26 ^{bA}			
	OP3	39.63 ± 0.95 ^{aB}	31.33 ± 0.15 ^{aA}			
Σ PUFA	C	3.63 ± 0.15 ^{cA}	3.92 ± 0.36 ^{bA}	***	NS	NS
	P	3.16 ± 0.11 ^{aA}	3.53 ± 0.15 ^{aB}			
	OP1	3.00 ± 0.64 ^{aA}	3.74 ± 0.06 ^{bA}			
	OP3	4.16 ± 0.37 ^{cA}	3.60 ± 0.15 ^{bAB}			
n-3	C	1.30 ± 0.05 ^{aB}	0.76 ± 0.05 ^{aA}	***	NS	***
	P	1.03 ± 0.21 ^{aA}	1.00 ± 0.10 ^{aA}			
	OP1	1.20 ± 0.28 ^{aB}	1.03 ± 0.09 ^{aA}			
	OP3	1.23 ± 0.27 ^{aB}	1.13 ± 0.08 ^{aAB}			
n-6	C	4.58 ± 0.02 ^{bC}	4.33 ± 0.05 ^b	***	NS	***
	P	3.46 ± 0.05 ^{aA}	3.70 ± 0.17 ^b			
	OP1	4.23 ± 0.11 ^{aB}	4.03 ± 0.06 ^b			
	OP3	4.36 ± 0.06 ^{bAB}	4.30 ± 0.25 ^{bA}			

Explanatory notes: The presented samples are coded according to the Materials and Methods section, Table 1. The results are expressed as the mean ± standard deviation ($n = 3$). Values denoted by different letters differ significantly ($p < 0.05$). SE: standard error of means; P: significance of effects; treatment (TR); time of storage (T); treatment × time of storage interaction (2T); NS—not significant; ** $p < 0.01$; *** $p < 0.001$. ^{a, b, c}—treatment column; ^{A, B}—time column.

3.5. Fatty Acid Profile

The fatty acid composition (% total fatty acids) of the investigated meat products after fermentation and 3 months of refrigerated storage is shown in Table 6.

The factor “treatment” significantly affected ($p < 0.001$) all assessed elements of the fatty acid profile. The obtained results of the two-way analysis of variance indicate a significant treatment effect and a strong „time × Treatment” interaction ($p < 0.001$), while the „time” factor alone did not show any clear significance when considered independently of treatment. The addition of sodium nitrite (P) and LAB bacterial strains in the recipes influenced Σ SFAs, Σ MUFAs, and Σ PUFAs in the investigated products. These samples varied in terms of the content of specific groups of fatty acids compared to the control sample.

The direction of observed changes in the fatty acid profile in fermented beef sausages depended on the strain of LAB used (Table 6), which is consistent with the observations of other authors. Wang et al. [69] reported that the application of probiotic/starter cultures (mainly *Lactobacillus*/*Lactiplantibacillus*, but sometimes in combination with strains of *Staphylococcus* or *Bacillus*) alters the lipolytic and oxidative activities during fermentation and maturation, which in turn influences the free fatty acid profile.

Fermented beef sausages show an advantage in SFAs, and MUFAs saturated fatty acid and monounsaturated fatty acids content, with a decrease in Σ PUFA relevance. The type of raw material and the recipe used determine these values. On the other hand, Skwarek & Karwowska [80] observed the contribution of Σ SFAs in raw fermented sausages

with the addition of tomato pomace. The fatty acids such as linoleic, oleic, palmitic, and stearic dominate in the fatty acid profile of this product. This is also confirmed by research by Arief et al. [81] regarding fermented sausages with the addition of probiotic strains.

Based on the results obtained in the case of the OP3 sample (treatment with salt and *Lactiplantibacillus paracasei* OP3; Table 1), a significant increase in the content of Σ SFAs was found with a simultaneous reduction in Σ MUFAs (Table 6). It has also been observed by Kuhl et al. [82]. Authors found that some strains of LAB (e.g., *Lactobacillus plantarum*, *L. sakei*, *Pediococcus pentosaceus*) can biohydrogenate, i.e., partially hydrogenate unsaturated fatty acids (MUFAs, PUFAs) to more saturated forms (SFAs). In turn, the use of probiotic/starter cultures (*Lactobacillus*/*Lactiplantibacillus* or mixtures with *Staphylococcus* or *Bacillus*) changes the lipolytic and oxidative activity during fermentation and maturation, which also affects the profile of free fatty acids. Lipolytic enzymes (esterases, lipases) of LAB can release fatty acids from triacylglycerols, with unsaturated acids often being released and oxidized faster than saturated ones. As a result of the maturation of fermented sausages, an increase in the relative share of Σ SFAs and a decrease in Σ PUFAs are often observed, even if the total amount of fat does not change [69].

In terms of the content of individual fatty acids during a given test period (after fermentation and 3 months of storage), it was similar to the tests with the addition of LAB (OP1; OP3, Table 1). In this case, a slight, although non-significant, increase in Σ SFAs was observed. However, the value of Σ MUFAs decreased significantly over time. Data available in the literature on the subject [83] also confirms that sodium nitrite can inhibit the autooxidation of unsaturated fatty acids, limiting the formation of undesirable oxidation products such as aldehydes, ketones, and free radicals. This helps maintain the proportions of n-3 and n-6 acids. Consequently, lipid stability and the characteristic taste and aroma of fermented sausages are maintained [71,83,84]. On the contrary, Skibstead et al. [85] reported that the effect of adding sodium nitrite may have a neutral character or a tendency to lower SFA levels.

3.6. Color Parameters

Table 7 presents the influence of treatment and storage time on color parameters (L^* a^* b^*) of beef fermented sausages during 3 months of storage at 4 °C. One of the most notable factors influencing consumer decisions to purchase is the color of meat [84]. The color parameter is used in product selection; mainly, it is influenced by myoglobin, the primary red pigment in animal flesh. Assessment of meat color is crucial in identifying issues related to processing. The color parameter is highly dependent on the level of meat oxygenation and its storage conditions [13,86].

The treatment and storage time affected ($p < 0.05$) lightness (L^*) coordinates. However, the color parameters L^* , a^* , and b^* differed significantly depending on the sample treatment used ($p < 0.001$). For the component value (L^*), a significant impact of both the type of sample ($p < 0.001$) and storage time ($p < 0.05$), as well as their mutual interaction ($p < 0.05$), was noted. The highest component value L^* among all assessed products was recorded in the case of the OP3 sample (42.33), and the highest in the case of the P sample (47.18). After 3 months of storage, the value of the color parameter L —brightness was reduced in all test treatments to the level of 37.39–44.58 (Table 7). The strongest effect of changes was observed in the OP3 sample (42.22–37.39). Lipid peroxidation is a factor influencing the brightness of color in meat, causing a decrease in its value in the product [87,88].

In fermented meat products, the change in L^* value is an important indicator of the processes occurring during fermentation, ripening, and drying. Changes in color parameters during storage indicate that the color stability of the samples was significantly dependent on the type of treatment used. The reduction in the L^* value after 3 months of storage suggests darkening of the samples, which is a typical phenomenon in products

stored under refrigerated conditions and may result from lipid oxidation reactions and degradation of natural dyes.

Table 7. Influence of treatment and storage time on color parameters (L^* a^* b^*) of novel beef fermented sausages during 3 months of storage at 4 °C (means $\pm$ SE).

Sample Code		Time Storage at 4 °C [Months]		Treatment	Time	Treatment $\times$ Time
		0	3	P _{TR}	P _T	P _{2T}
L^*	C	42.89 $\pm$ 1.25 ^{aB}	39.01 $\pm$ 0.97 ^{bA}	***	*	*
	P	47.18 $\pm$ 1.35 ^{dB}	44.58 $\pm$ 1.69 ^{dA}			
	OP1	45.27 $\pm$ 1.26 ^{cB}	42.00 $\pm$ 1.13 ^{cA}			
	OP3	42.22 $\pm$ 1.12 ^{abB}	37.39 $\pm$ 0.99 ^{aA}			
a^*	C	7.33 $\pm$ 0.57 ^{abB}	5.70 $\pm$ 0.37 ^{aA}	***	*	NS
	P	7.23 $\pm$ 0.40 ^{aA}	7.41 $\pm$ 0.52 ^{bAB}			
	OP1	7.58 $\pm$ 0.31 ^{cA}	9.82 $\pm$ 0.59 ^{cB}			
	OP3	8.86 $\pm$ 0.49 ^{dB}	8.34 $\pm$ 0.33 ^{cbA}			
b^*	C	7.61 $\pm$ 1.48 ^{bB}	5.14 $\pm$ 0.34 ^{aA}	***	NS	NS
	P	7.29 $\pm$ 0.29 ^{abB}	7.06 $\pm$ 0.35 ^{dAB}			
	OP1	7.10 $\pm$ 0.27 ^{aAB}	6.90 $\pm$ 0.27 ^{abB}			
	OP3	7.65 $\pm$ 0.34 ^{cB}	6.34 $\pm$ 0.38 ^{bA}			

Explanatory notes: The presented samples are coded according to the Materials and Methods section, Table 1. The results are expressed as the mean $\pm$ standard error ($n = 3$). Values denoted by different letters differ significantly ($p < 0.05$). L^* —Lightness, a^* —Redness, b^* —Yellowness; SE: standard error of means; P: significance of effects; treatment (TR); time of storage (T); treatment $\times$ time of storage interaction (TR $\times$ T); NS—not significant; * $p < 0.05$; *** $p < 0.001$. a, b, c, d—treatment column; A, B—time column.

In turn, the value of the color assessment parameter a^* , determining the contribution of red color, was significantly influenced by both the type of sample ($p < 0.001$) and storage time ($p < 0.05$). There was no significant interaction between factors (Table 7). The lowest share of red color (the value of the a^* parameter was recorded in the cured sample P (7.23), immediately after ripening, and the highest (8.86) in the OP3 sample. As a result of storage, a significant increase in the parameter value (a^*) was observed in the case of the OP1 sample from 7.58 to 9.82 (Table 7). a^* values showed a different direction of change depending on the treatment. The reduction in the share of red color in control samples may be related to the degradation of oxymyoglobin to metmyoglobin, while the increase in the OP1 treatment suggests a post-potential antioxidant effect of the additive used, which could stabilize the meat color [89,90].

For the value of the b^* parameter determining the share of yellow, a significant effect was demonstrated only for the type of sample ($p < 0.001$), without a significant effect of time and mutual interactions of these factors. Significantly, the highest ($p < 0.05$) values of the b^* parameter immediately after maturation were recorded in the OP3 sample (7.65), and the lowest in the OP1 sample (7.10). The influence of storage time on the value of the yellow component parameter was recorded only in the case of the OP3 sample (one of two samples with the addition of LAB), which may be caused by a reduction in the amount of oxymyoglobin as a result of the growth of oxygen-consuming bacteria during the maturation of the products [91,92].

Wei et al. [93] reported that the concentration of the *Lactobacillus plantarum* YR07 strain, which can reduce nitrites, leads to maintaining a stable, pink-red color in meat products. In turn, Cao Y. et al. [94] suggest that using microencapsulated strain *Bifidobacterium bifidum* with quercetin has a positive effect on the stability of color parameters in Harbin red sausage. In the research of Stadnik et al. [95], the role of bacterial enzymes in stabilizing the color parameters of fermented meat products by reducing peroxides was emphasized. In turn, Gu et al. [96] investigated the impact of using the *Limosilactobacillus fermentum* 332 strain on selected quality parameters of fermented sausage, including the improvement

of color parameters (L^* , a^* , b^*). The obtained results confirm a reduction in the rate of fat oxidation processes and maintain a stable color of the finished product, thanks to the concentration of LAB.

To visualize the investigated meat products' color, additional information has been provided in Table 8.

Table 8. CIELAB color parameters of investigated meat products and corresponding RGB/HEX values.

Sample Code	Time Storage at 4 °C [Months]	L^*	a^*	b^*	Color	RGB	HEX
C	0	42.89	7.33	7.61		(118,97,89)	#766159
C	3	39.01	5.70	5.14		(104,88,84)	#685854
P	0	47.18	7.23	7.29		(129,107,100)	#816B64
P	3	44.58	7.41	7.06		(122,101,94)	#7A655E
OP1	0	45.27	7.58	7.10		(124,102,96)	#7C6660
OP1	3	42.00	9.82	6.90		(119,93,88)	#775D58
OP3	0	42.22	8.86	7.65		(119,94,87)	#775E57
OP3	3	37.39	8.34	6.34		(105,83,78)	#69534E

3.7. Texture Analysis

The physicochemical properties of dry fermented beef sausages can be assessed during the maturing or storage process. One of these properties is texture profile, which determines the quality and consumer acceptance of food products, including meat products. It is a complex characteristic determined by the product's structure, shape, chemical composition, viscosity and other physical properties. The quality characteristics of texture include parameters such as hardness, adhesiveness, cohesiveness, gumminess, chewiness and resilience, although these vary in degree and intensity depending on the type of product [97]. Changes in these factors in fermented sausages may occur as a result of changes in pH (which promotes the aggregation and gelation of myofibrillar proteins) and the cooking of the product.

The textural properties also depend to a large extent on the product's moisture content and moisture loss [98]. The protein structure of fermented sausage is responsible for the proper development of its texture following salting, mincing and fermentation. The development of texture is strongly related to the drop in pH in fermented sausages. During the maturation process, the protein structure of fermented sausage is denatured by organic acids such as lactic acid, and the moisture content gradually decreases. Furthermore, the unstable coagulation bonds in the protein structure are replaced by condensed bonds, causing a transition from the sol state to the gel state. As the pH and moisture content of the sausages decrease, muscle fiber proteins coagulate, forming a gel-like structure. This gel-like structure increases the hardness and elasticity of the sausages [99].

The results of texture analysis of the investigated meat products after production are shown in Table 9.

Table 9. The texture parameters of the investigated beef dry fermented sausages.

Parameter	Treatment			
	C	P	OP1	OP3
Hardness (N)	57.95 ± 16.97 ^b	68.53 ± 15.76 ^{ab}	51.26 ± 6.89 ^b	82.39 ± 25.30 ^a
Adhesiveness (g·s)	−5.26 ± 7.62 ^a	−16.31 ± 15.41 ^a	−35.28 ± 20.05 ^b	−5.94 ± 8.52 ^a
Springiness	0.85 ± 0.07 ^a	0.81 ± 0.07 ^a	0.86 ± 0.06 ^a	0.84 ± 0.11 ^a
Cohesiveness	0.82 ± 0.02 ^a	0.79 ± 0.03 ^a	0.82 ± 0.02 ^a	0.82 ± 0.01 ^a
Gumminess	47.38 ± 14.36 ^b	54.33 ± 12.29 ^{ab}	41.84 ± 5.69 ^b	67.37 ± 20.85 ^a
Chewiness	40.48 ± 13.69 ^b	44.14 ± 10.24 ^{ab}	35.81 ± 4.87 ^b	56.97 ± 20.59 ^a

Explanatory notes: **C**—control treatment with salt; **P**—treatment with curing mixture; **OP1**—treatment with salt and *Lactiplantibacillus plantarum* OP1 in number 4.0 log CFU/g; **OP3**—treatment with salt and *Lactiplantibacillus paracasei* OP3 in number 4.0 log CFU/g. Different lowercase letters indicate statistically significant differences between groups according to Tukey's test ($p < 0.05$).

The hardness of the samples tested ranged from 51.26 to 82.39 N (Table 9), with significant differences, an increase in the hardness value compared to the control sample being observed in the case of research treatment OP3. Springiness and cohesiveness did not undergo any significant changes. Comparison to the control sample, however, there were significant changes in gumminess and chewiness, the values of which increased in the OP3 sample. Similar patterns were observed in previous studies [97,100], where dry-fermented sausages were inoculated with *Enterococcus faecium* CECT 410 using mixed starter cultures. Chewiness of the samples tested ranged from 35 to 56.97 (Table 9), but significant differences were observed in the case of the treatment OP3, stable gel networks during fermentation [101].

The analysis of the results for the investigated beef sausages (Table 2; Table 9) indicates a correlation between their texture profile and basic composition, particularly the dynamics of dehydration as well as fat and salt content. No significant differentiation in moisture content was noted between the samples OP1 and OP3 and sample P. However, the highest value for the texture attribute hardness was recorded only in the OP3 treatment (82.39 N), which is likely associated with the specific properties of the strain employed. This is related to moisture loss and the pH value reduction induced by the metabolic activity of the *L. paracasei* OP3 strain. The reduction in pH induces the coagulation of myofibrillar proteins, resulting in a structural transition from the sol state to the gel state, which significantly increases the hardness of the final product. Conversely, sample OP1, characterized by a substantially higher fat content (21.20%) compared to the OP3 treatment (10.20%), exhibited one of the lowest values for the texture parameter hardness (51.26 N). This suggests that a higher proportion of the lipid fraction in the meat matrix limits the compactness of the protein network, acting as a texture-relaxing factor. The OP3 treatment featured the highest NaCl concentration (5.40%). Salt plays a crucial technological role by solubilizing myofibrillar proteins, which are essential for ensuring appropriate adhesion and cohesiveness in processed meat products. The high salt content, in combination with low fat content and intensive dehydration in this sample, contributed to achieving the highest parameters for gumminess (67.37) and chewiness (56.97). The application of autochthonous LAB strains (*L. plantarum* OP1; *L. paracasei* OP3) influenced the rate of physicochemical processes through the production of organic acids. These bacteria determine the formation of a stable gel network, which results in the differentiation of textural characteristics relative to the control sample.

To provide a comprehensive understanding of the complex interactions between the physicochemical, microbiological, and textural parameters of the investigated beef dry-fermented sausages, a Pearson correlation analysis was conducted. This statistical approach allowed the identification of the strength and direction of relationships among the primary drivers of product quality and safety, including the degree of dehydration, acidification, oxidative stability, and starter microbiota viability. The calculated Pearson correlation coefficients for these key quality attributes are shown in Table 10.

A statistically significant negative correlation ($p < 0.05$) was observed between dehydration and texture parameters, including moisture and hardness, confirming that water evaporation is the primary factor determining the product's texture. The reduction in moisture during the 35-day ripening period leads to the concentration of solid components, including proteins and fats. This mechanism is closely linked to the transition of the matrix from a sol state to a gel state, resulting from the denaturation of myofibrillar proteins under the influence of organic acids produced by LAB. This explains the high hardness value (82.39 N, Table 9) recorded particularly in the OP3 treatment, which exhibited the most intensive dehydration.

Furthermore, the strong negative correlation ($p < 0.05$) between the lactic acid bacteria and Enterobacteriaceae counts confirms microbial antagonism. Autochthonous strains

L. plantarum OP1 and *L. paracasei* OP3 effectively dominate the beef matrix environment, maintaining viability at levels of 7–8 log CFU/g. Their bioprotective activity stems from the production of organic acids, hydrogen peroxide, and bacteriocin-like inhibitory substances (BLIS), resulting in selective inhibition of spoilage and pathogenic microflora.

Table 10. Pearson correlation matrix between the key physicochemical, microbiological, and textural parameters of beef dry-fermented sausages.

Parameters	Moisture	a_w	pH	ORP	TBARS	Hardness	LAB	ENT
Moisture	1.00							
a_w	0.85 *	1.00						
pH	0.32	0.41	1.00					
ORP	0.45	0.52	−0.38	1.00				
TBARS	−0.41	−0.39	0.55 *	0.62 *	1.00			
Hardness	−0.89 *	−0.78 *	0.28	−0.31	−0.097	1.00		
LAB	−0.48	−0.55 *	0.15	−0.74 *	0.22	0.58 *	1.00	
ENT	0.51	0.63 *	−0.24	0.68 *	−0.18	−0.52	−0.81 *	1.00

Explanation notes: a_w : water activity; ORP: oxidation–reduction potential; TBARS: thiobarbituric acid reactive substances; LAB: lactic acid bacteria count (log CFU/g); ENT: *Enterobacteriaceae* count (log CFU/g). The correlation is marked as significant at $p < 0.05$ *.

Correlation analysis shows that the metabolic activity of LAB is directly linked to a reduction in the oxidation-reduction potential (ORP) to approximately 468 mV (Table 4). The creation of a reducing environment by the starter cultures limits oxygen availability for oxidation processes, resulting in the stabilization of the TBARS index below the sensory detection limit (2 mg MDA/kg; Table 5). The positive correlation between ORP and TBARS confirms that redox potential control is a key element in protecting the lipid fraction from rancidity in ‘clean label’ products.

A positive correlation between water activity (a_w) and the count of spoilage bacteria indicates that reducing a_w to the 0.84–0.87 range in the inoculated treatments (OP1, OP3) constitutes a significant technological barrier. The synergy between reduced pH and low water activity (the so-called hurdle technology) ensures the microbiological stability of the final beef product, which is particularly important in reducing the need for nitrite additives.

In summary, selecting autochthonous strains with high adaptive potential for the meat matrix enables precise control of product quality characteristics, combining improved texture with high microbiological safety and stability.

4. Conclusions

The study demonstrates that the application of autochthonous *Lactiplantibacillus plantarum* OP1 and *Lactocaseibacillus paracasei* OP3 strains is an effective technological solution for shaping the quality of beef dry-fermented sausages. The investigated LAB strains maintain high viability (7–8 log CFU/g) throughout the 35-day ripening and 3-month storage period, ensuring their dominance in the meat matrix. Inoculation with the OP1 and OP3 strains provides a significant biopreservative effect, limiting the growth of *Enterobacteriaceae* and coagulase-positive staphylococci compared to control treatments ($p < 0.005$). The metabolic activity of the used strains, particularly OP3, accelerates dehydration and induces the transition of the protein matrix from a sol to a stable gel state, resulting in significant increases in hardness (82.39 N), gumminess, and chewiness. The metabolic activity of the LAB strains modulated oxidative stability; both strains established a strong reducing environment (low ORP), whereas enhanced protection against lipid peroxidation (lower TBARS) was specifically observed for the *L. plantarum* OP1 strain. The use of indigenous LAB strains allows for the production of microbiologically stable

beef sausages with appropriate color characteristics, constituting a viable “clean label” alternative to synthetic nitrite additives.

Author Contributions: Conceptualization: A.S. and A.Ł.; methodology: A.S., A.Ł. and A.O.; software: A.S.; validation: A.S.; formal analysis: A.S., A.Ł. and A.O.; investigation: A.S., A.Ł., K.M.-L. and U.S.; data curation: A.S.; writing—original draft preparation: A.S.; writing—review and editing: A.Ł., A.O. and P.S.; visualization: A.S.; supervision: P.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

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