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# Sucuk, Turkish-Style Fermented Sausage: Evaluation of the Effect of Bioprotective Starter Cultures on Its Microbiological, Physicochemical, and Chemical Properties

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**Abstract:** Bio-protection is one of the most popular natural protection methods to control food safety and shelf life. Lactic acid bacteria, especially *Lactobacilli* strains, are used in the food industry for this purpose due to their probiotic properties and, accordingly, bioprotective properties. We aimed to investigate the role of the bacteriocin-producing lactic acid bacteria *Lactobacillus sakei* and *Pediococcus acidilactici* in inducing microbiological, physicochemical, and chemical changes in the Turkish-style fermented sausage sucuk. The effects of protective cultures were compared with those of commercial starter cultures consisting of *Pediococcus pentosaceus* + *Staphylococcus carnosus*; a non-cultured group was used as a control. *L. sakei* inoculation and, to a lower extent, *P. acidilactici* inoculation resulted in the rapid domination of lactic acid bacteria (LAB) in the environment, whereas commercially used starter cultures and the non-cultured group showed lower counts of LAB. Moreover, *L. sakei* and *P. acidilactici* succeeded in inhibiting pathogens including *S. aureus*, *E. coli*, and *Enterobacteriaceae*. The number of enterococci decreased notably in the *L. sakei*-inoculated sucuk samples; however, an increase was determined in the samples inoculated with *P. acidilactici*. On the other side, the effect of commercial starter cultures was not sufficient for the inhibition of food-borne pathogens in the sucuk samples. Consequently, the inoculation of protective cultures, particularly of *L. sakei*, can provide a considerable contribution to improving microbial quality and food safety, retarding lipid oxidation, and increasing proteolytic activities in sucuk without important changes in its sensory properties.

**Keywords:** protective culture; *Lactobacillus sakei*; *Pediococcus acidilactici*; sucuk; fermented sausage

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## 1. Introduction

The Turkish-style fermented sausage sucuk is a highly demanded and popular meat product in Türkiye. The word "sucuk" was first used in 1072 by Mahmud Kashgari in his work "Dīwān Lughāt al-Turk (a book that refers to a compendium or collection of the languages spoken by the Turks)" [1]. Sucuk is a regional fermented product and is widely consumed in Türkiye as well as in the Middle East, Central Asia, the Balkans, and Northern European countries, where it is known by different names. For example, it is called "sujuk" in Bulgaria, "suxhuk" in Albania, "sudzuka" or "sujuka" in Bosnia, Macedonia, and Serbia, "sujuq" in Arabia, "soutzouki" in Greece, "suqiuc" in Romania, "sudjuk" in Russia, "sujouk" in Turkmenistan, "shujıq" in Kazakhstan, and "chuchuk" in Kyrgyzstan [2]. Sucuk has typical characteristics of both North and South European-style fermented sausages but differs from North European-style sausages by its short fermentation period. However, the smoking method that allows for achieving food safety with a rapid pH reduction in sausages is not applied in sucuk production [2].

According to the definition of Turkish Food Legislation, fermented sucuk is a meat product that consists in a mix of primarily bovine carcass meat and fat with other flavoring

ingredients including salt, sugar, nitrite, black pepper, red pepper, garlic, cumin, pimento. After the obtained meat batter is filled into natural or artificial sheaths, sucuk is exposed to fermentation and drying processes under specified conditions [3]. Fermentation is the most critical part of sausage production. Internal (salt and sugar amount, fat amount, meat size, casing type, calibration, and microflora in the sausage) and external (temperature, relative humidity (RH), and airflow) factors affect the fermentation process and final product directly [4].

Either natural microflora or starter cultures can be used to ferment the sausages. However, the characteristic properties of the sausages produced with a starter culture are quite different from those of traditionally produced sausages fermented with natural flora [4]. *Lactobacillus plantarum*, *Lactobacillus sakei*, *Lactobacillus curvatus*, *Lactobacillus pentosus*, *Pediococcus pentosaceus*, *Pediococcus acidilactici*, and *Micrococcus* (*Korucia varians*) are commonly used for fermented sucuk [5,6] and other traditional sausages such as Italian chorizo, Portugal alheira, and Greek loukaniko [7]. Achieving the optimal bacterial environment with the addition of a starter culture results in a better aroma, extended shelf life, accelerated color formation, inhibition of pathogenic bacteria, better product safety, and a uniform product [8,9] with a reduced nitrate residual [10].

Over the past few years, there has been a shift in consumer perceptions regarding food quality and safety, leading to a growing inclination towards natural methods requiring minimal processing and avoiding chemical preservatives or additives [11]. Therefore, research in this direction is changing towards using natural preservatives and additives. Bio-protection is the process of controlling the safety and shelf life of foods using natural preservation methods. Lactic acid bacteria (LAB), which comprise the natural microflora of meats, have an antagonist effect on pathogenic and unwanted microorganisms. Especially Lactobacilli strains have gained importance in the food industry thanks to their probiotic properties, their ability to be used as starter cultures, and their bioprotective properties [12,13]. LAB are considered the most important bio protectors because they are classified as GRAS (generally regarded as safe) and are dominant microorganisms in many foods during storage [8,14–16]. In this regard, in meat and meat products, these desired microorganisms (starter culture and protective cultures in the natural microflora of the meat or externally participating starter culture and protective cultures) dominate the environment and control pathogenic microorganisms [17] such as *Salmonella* and *Listeria monocytogenes* [6,18]. While a starter culture can provide safe products by quickly becoming dominant in the environment, protective cultures can decrease the health risk for consumers without causing any changes in the sensory properties of food [11]. This suggests that protective cultures offer enhanced food safety compared to commercial starter cultures. Bioprotective cultures not only improve food safety but also diversify product offerings by producing items with new sensory properties from raw materials. They inhibit pathogens, extend product shelf life, ensure product stability, and contribute to both gut microbiome and consumer health [6,19]. These beneficial properties distinguish bioprotective cultures from commercial starter cultures [7].

Bio-protection can be undertaken in two different ways in foods: (i) by the addition of microorganisms defined as protective; (ii) by the direct addition of bacteriocins (such as nisin) produced by those microorganisms. Bacteriocin addition may have some negative effects compared to bioprotective culture inoculation. For example, it is possible that the effect of bacteriocin decreases following its attachment to compounds such as fat and protein [20]. Protective cultures can be not only an antimicrobial peptide source but also a wide-spectrum source of other molecules including organic acids, ethanol, hydrogen peroxide, diacetyl, and bacteriocins [21].

Among LAB, *Lactobacillus sakei* has widespread use in the preservation and fermentation of pork meat products, mainly due to its production of organic acids and hydrogen peroxide in addition to bacteriocin [18,22]. Also, *P. acidilactici* has been reported to have a good bioprotective potential with an antilisterial effect and non-antibiotic resistance [23]. Because of its superior adaptive ability to the environment and capability to produce

bacteriocin, inhibition effects on pathogens and other unwanted microorganisms such as *S. aureus* and *Enterobacteriace,* and proteolytic activity, *L. sakei* either by itself or with a starter culture is used to obtain a protective culture [24]. Furthermore, it has been recently reported that *L. sakei* improved the volatile profile of heat-treated sucuk (which has a short fermentation time) [25], while *P. acidilactici* showed a limited effect [26].

Although the bioprotective effect of *L. sakei* and *P. acidilactici* in some fermented meat products prepared by using pork carcass has been reported [18,22,23,27], their effect on beef sausages was investigated only in relation to sausage volatile profiles [25,26]. This study aims to investigate the effects of *L. sakei* and *P. acidilactici* protective cultures, capable of producing bacteriocin, on fermented sucuk's microbiological quality and physicochemical, chemical, and sensory properties. The effects of *L. sakei* and *P. acidilactici* protective cultures were also compared to those of commercial starter cultures (*P. pentosaceus* and *S. carnosus* mix) for the first time, indicating that bioprotective cultures provide better protection than commercial starter cultures.

## 2. Materials and Methods

### *2.1. Sucuk Preparation*

In the production of the fermented sausage sucuk (Figure 1), chuck meat and fat obtained from 3-year-old Simmental-race carcasses were minced by using a grinder with 3 mm orifices (AlveoRed 32, İzmir, Turkey). Minced meat and fat materials stored frozen for a week were thawed at 4 °C for 12 h before the production process. Then, 2% salt (106404 Merck, Rahway, NJ, USA), 1.8% garlic (mashed with some water), 1% hot red pepper, 0.7 sweet red pepper, 0.8% cumin, 0.6% black pepper, 0.2% allspice, 0.5% dextrose (108337 Millipore-Merck, New Jersey, USA), and 0.015% sodium nitrite (106549 Merck, NJ, USA) were added to 100 kg of minced meat and tail fat (18%) mix.

In this study, *Lactobacillus sakei* (B2 SafePro®, CHR-Hansen, Hørsholm, Denmark) and *Pediococcus acidilactici* (B-LC-20 SafePro®, CHR-Hansen, Hørsholm, Denmark) were used to produce bioprotective cultures and a mix of *Pediococcus pentosaceus* and *Staphylococcus carnosus* (BFL-T03, CHR-Hansen, Hørsholm, Denmark) was used for the commercial starter culture.

The prepared sucuk batter was divided into four separate groups. One of the groups, which did not contain any starter culture, was the control group (C), whereas a 0.025% ($10^6$ units/g approximately) *P. pentosaceus* and *S. carnosus* starter culture mix, the *L. sakei* protective culture, and the *P. acidilactici* protective culture were used for the other groups (S1, S2, and S3, respectively). Each group was filled into a 38 mm diameter collagen casing using the filling apparatus of a kitchen-type meat grinder (Bosch, Gerlingen, Germany). The weight of each sucuk was 100 ± 2 g. The sucuks were produced in 12 days: 3 days of fermentation at 20–22 °C, 90–92% relative humidity and 9 days of ripening at 18–20 °C, 80–90% relative humidity.

### *2.2. Sampling and Experimental Design*

Samples were taken at the beginning of production (day 0) and on days 3, 6, 9, and 12 for microbiological, physicochemical, and chemical analyses. The sensory evaluation was carried out on day 12 after the ripening period was accomplished. The treatments were replicated three times, and all parameters were measured in duplicate.

### *2.3. Microbiological Analyses*

The collagen-cased sucuk samples were aseptically peeled using a sterile knife and a lancet. The peeled samples (10 g) were put in sterile bags with 90 mL of maximum recovery diluent (MRD, Merck, Darmstadt, Germany) and homogenized for 120 s at 230 rpm in a stomacher (Seward 400, London, UK). Then, 10-fold serial dilutions were prepared.

The count of microbial species was carried out according to AOAC (2006) [28]. The suitable media that were used following the pour plate techniques were Plate Count Agar (PCA, Merck code 1.05463) for total aerobic mesophilic bacteria (TAMB), de Man

Rogosa Sharpe (MRS, Merck code 1.10660) for lactic acid bacteria (LAB) (double-layer pouring), Mannitol Salt Phenol Red Agar (MSPR, Merck 1.05404) for coagulase-negative cocci (CNC), Chromocult TBX Agar (Merck 1.16122) for *Enterobacteria* and *E. coli*, Kanamisin Aesculin Agar (Merck, Millipore 105222) for *Enterococcus* and Baird Parker Agar (BPA, Merck 1.05406), with egg yolk for *Staphylococcus aureus*. The spread plate technique on Rose Bengal Chloramphenicol Agar (RBC, Merck code 1.00467) was used for mold and yeast counts. The colonies were counted at the end of the incubation period of 72 h at 28 °C for TMAB and LAB; of 48 h at 37 °C for CNC and *Staphylococcus aureus*; of 48 h at 42 °C for *Enterobacteria* and *E. coli* and *Enterococcus*, and of 5 days at 25 °C for mold and yeast. The results are expressed as log CFU (Colony-Forming Unit) per gram of sucuk.

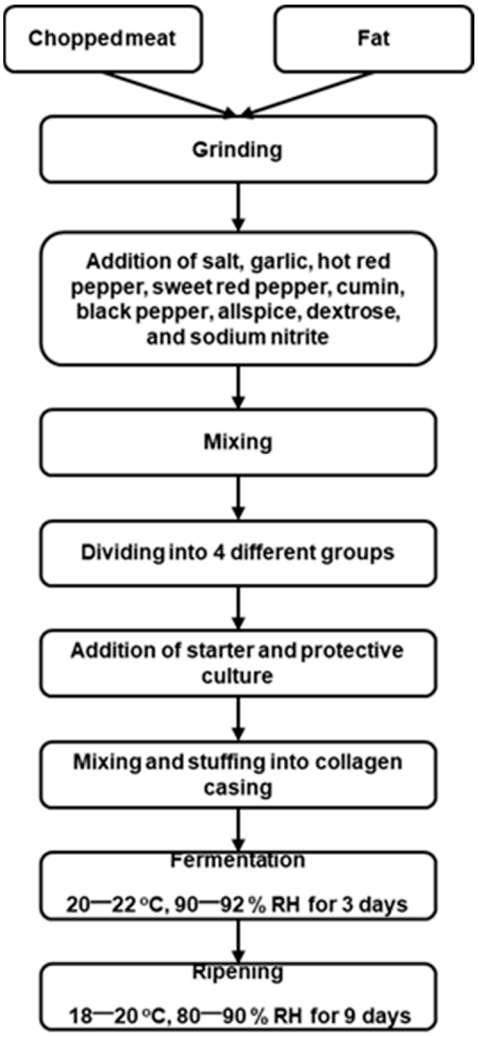



**Figure 1.** Flow chart of Turkish fermented sausage production (RH: relative humidity).

### *2.4. Physicochemical and Chemical Analysis*

Moisture content (950.46), ash content (920.153), crude fat (991.36), and crude protein (955.04) in the sucuk samples were determined in accordance with AOAC 2006 [28]. The pH value of 10 g of the sucuk samples homogenized (IKA T25, Breisgau, Germany) in 100 mL distilled water was measured by using a digital pH meter (Orion, Model 420A, Boston, MA, USA) 1993 [6].

The color measurements of the sucuk samples were taken immediately after cutting the samples in order to avoid color degradation due to oxygen and light. The lightness ($L^*$), redness ($a^*$, red ± green), and yellowness ($b^*$, yellow ± blue) of the samples were determined using a colorimeter (Minolta CR300 Reflectance Colorimeter, Osaka, Japan).

The color measurements were carried out six times for each sliced sucuk sample, and the average of these measurements was calculated.

To determine lipid oxidation, the TBARS (thiobarbituric acid-reactive substance) method was followed following Ahn et al. (1998) [29]. A 5 g minced sucuk sample was homogenized in 15 mL of distilled water in 50 mL Falcon tubes using a Polytron PT 10–35 GT probe (Kinematica, Lucerne, Switzerland) for 15 sec at high speed. The homogenized samples were centrifuged for 10 min at 2000× *g* (Hermle Z326K, Wehingen, Germany). Then, 1 mL of supernatant was transferred into a glass test tube, and 2 mL of TCA-TBA-HCl (%15 *w*/*v* TCA, %0.375 *w*/*v* TCA, 0.25 M HCl)-reactive solution was added. After vortexing for 15 sec, the glass tubes were put into a boiling water bath (WNB 7–45, Memmert, Germany) for 15 min to observe color changes. After the color change, the samples were cooled fast and centrifuged for 15 min at 2000× *g*. The absorbance of the supernatants was measured at a wavelength of 531 nm (Evolution 201, Thermo Scientific, Waltham, MA, USA). A blind sample was prepared as 1 mL of distilled water with 2 mL of the TCA-TBA-HCl reagent. The amount of TBARSs was calculated using a standard calibration curve prepared using 1,1,3,3-tetra-etoksipropan (TEP). The results are expressed as "mg malondialdehyde (MDA) per kg of sample".

The proteolysis index was determined following the method of Careri et al. (1993) [30] with some modifications. For this purpose, firstly, the nitrogen content of the sucuk samples was determined according to the Kjeldahl method. To determine the non-protein nitrogen content, 10 g of sucuk sample was mixed with 90 mL of distilled water and centrifuged at 1000 rpm and 5 °C for 15 min (Sigma 3K15 model, Osterode an Harz, Germany). The supernatant was filtered using Whatman No. 4 filter paper. Then, 25 mL of filtrate was mixed with 25 mL of a 10% TCA solution, and the mixture was left at room temperature for 30 min. After 30 min, the mixture was centrifuged at 1000 rpm 5 °C for 15 min. The supernatant was filtered using Whatman No. 4 filter paper. The amount of nitrogen in 20 mL of filtrate was determined with the Kjeldahl method following AOAC 2006 [28]. The results gave the amount of non-protein nitrogen in 100 g of sucuk. The proteolysis index was calculated by dividing the non-protein nitrogen by the total amount of nitrogen and multiplying the result by 100.

$$\text{Proteolysis index} = (\text{Non-protein nitrogen\%} \times 100)/\text{Nitrogen\%} \quad (1)$$

### *2.5. Sensory Analysis*

The aim of the sensory evaluation was to determine the effects of the bioprotective cultures on the sensory properties of sucuk. For this purpose, raw and grilled sucuk samples, sliced at a thickness of 4 mm, were tested organoleptically in terms of color, taste, odor, texture, and general acceptance using a non-structured hedonic scale whose levels varied from 1 (extremely undesirable) to 10 (extremely desirable). The sensory evaluation was carried out in individually well-designed sensory booths under white fluorescent lights. A total of 8 samples, 4 raw and 4 cooked, of the groups C, S1, S2, and S3 were evaluated by the panelists. The samples were served to the panelists in small uniform containers coded with 3-digit random codes and in random order. The raw sucuk samples were served directly in plastic containers, whereas the cooked samples were grilled (SM606 Model, Kenwood, Shanghai, China) for 1 min and 30 s.

### *2.6. Statistical Analysis*

One-way ANOVA using a randomized complete block design was conducted with a generalized linear model to assess the significance of the differences between the mean values of the microbiological and physicochemical data. The effects observed in starter culture-added sausages were considered fixed effects, while those observed in the other treated sucuk groups were considered random effects. When significant differences between the treatments were detected, the least-squares means (LSMs) were compared using Tukey's test at a 5% significance level ($p \leq 0.05$). The same statistical evaluation was also

used for the sensory analysis. The treatments' effects were considered as fixed effects, and the panelists' evaluations as random effects. All statistical analyses were conducted by using SPSS 20 (SPSS Inc., Chicago, IL, USA), Minitab 17, and MSTAT-C statistical analysis software. The results, reported in the tables, are expressed as "mean ± standard deviation" of 3 replicates.

## 3. Results

### *3.1. Microbiological Properties of Sucuk*

The TAMB, LAB, and CNC counts during the 12-day manufacturing process are shown in Table 1. The initial microbial count in the raw material with other added ingredients was determined; the addition of the starter culture and the fermentation temperature are the main factors affecting the number of TAMB in sucuk [4]. The lowest number of TAMB was counted in the control group, with 5.48 log CFU/g ($p < 0.05$), while the S1, S2, and S3 samples reported 7.05, 6.95, and 6.77 log CFU/g, respectively, at the beginning of production. A considerable increase was observed on the 6th day of production by approximately 2.5 logs in all groups ($p < 0.05$), whereas the numbers decreased on the following days. In each analysis period, the lowest TAMB counts were obtained in group C, which was significantly different from the other groups ($p < 0.05$).

**Table 1.** Total aerobic mesophilic bacteria, lactic acid bacteria, coagulase-negative cocci counts in sausage samples inoculated with commercial and protective starter cultures during ripening.

| | | TAMB (log CFU/g) | | | | | LAB (log CFU/g) | | | | | CNC (log CFU/g) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | DAYS | | | | | | | | | | | | | | |
| | | 0 | 3 | 6 | 9 | 12 | 0 | 3 | 6 | 9 | 12 | 0 | 3 | 6 | 9 | 12 |
| Groups | C | 5.48 ± 0.20 $^{Aa}$ | 8.78 ± 0.24 $^{Ac}$ | 8.83 ± 0.06 $^{Ac}$ | 8.63 ± 0.12 $^{Ac}$ | 8.22 ± 0.07 $^{Ab}$ | 5.22 ± 0.13 $^{Aa}$ | 7.48 ± 0.16 $^{Ab}$ | 8.53 ± 0.11 $^{Cd}$ | 8.47 ± 0.03 $^{Ad}$ | 8.34 ± 0.04 $^{Ac}$ | 4.17 ± 0.02 $^{Aa}$ | 5.35 ± 0.99 $^{Ab}$ | 5.71 ± 0.97 $^{Cd}$ | 5.77 ± 1.14 $^{Bd}$ | 5.57 ± 1.03 $^{Bbc}$ |
| | S1 | 7.05 ± 0.10 $^{Ba}$ | 9.28 ± 0.16 $^{Cb}$ | 9.40 ± 0.13 $^{Cc}$ | 9.29 ± 0.03 $^{Cb}$ | 9.16 ± 0.20 $^{Cb}$ | 6.64 ± 0.08 $^{Ba}$ | 9.31 ± 0.15 $^{Bc}$ | 9.48 ± 0.04 $^{Bd}$ | 9.30 ± 0.14 $^{Cc}$ | 9.11 ± 0.27 $^{Cb}$ | 6.86 ± 0.13 $^{Bb}$ | 7.00 ± 0.12 $^{Bb}$ | 6.90 ± 0.06 $^{Db}$ | 6.85 ± 0.14 $^{Cb}$ | 6.37 ± 0.38 $^{Ca}$ |
| | S2 | 6.95 ± 0.32 $^{Ba}$ | 9.38 ± 0.17 $^{Cc}$ | 9.75 ± 0.35 $^{Dd}$ | 9.08 ± 0.11 $^{Bb}$ | 9.05 ± 0.16 $^{Cb}$ | 6.68 ± 0.07 $^{Ba}$ | 10.03 ± 0.22 $^{Cd}$ | 10.14 ± 0.16 $^{Ad}$ | 9.62 ± 0.27 $^{Dc}$ | 9.25 ± 0.19 $^{Cb}$ | 4.30 ± 0.15 $^{Ab}$ | 4.88 ± 0.17 $^{Ad}$ | 4.68 ± 0.15 $^{Ac}$ | 4.31 ± 0.14 $^{Ab}$ | 3.97 ± 1.12 $^{Aa}$ |
| | S3 | 6.77 ± 0.33 $^{Ba}$ | 9.09 ± 0.39 $^{Bc}$ | 9.32 ± 0.20 $^{Bd}$ | 8.74 ± 0.24 $^{Ab}$ | 8.68 ± 0.17 $^{Bb}$ | 6.80 ± 0.23 $^{Ba}$ | 9.46 ± 0.18 $^{Bd}$ | 9.53 ± 0.10 $^{Bd}$ | 9.02 ± 0.03 $^{Bc}$ | 8.86 ± 0.08 $^{Bb}$ | 4.21 ± 0.09 $^{Aa}$ | 5.14 ± 0.16 $^{Ad}$ | 4.93 ± 0.26 $^{Bc}$ | 4.43 ± 0.16 $^{Ab}$ | 4.22 ± 0.07 $^{Aa}$ |

$^{A–D}$: Means with different uppercase letters in the same column are significantly different ($p < 0.05$). $^{a–d}$: Means with different lowercase letters on the same line are significantly different ($p < 0.05$). Results are given as 3 replicates' and 2 parallels' means ± standard deviation. TAMB: total aerobic mesophilic bacteria, LAB: lactic acid bacteria, CNC: coagulase-negative cocci, CFU: colony-forming unit, K: control group, S1: *P. pentosaceus*- and *S. carnosus*-inoculated sucuk samples, S2: *L. sakei* protective culture-inoculated sucuk samples, S3: *P. acidilactici* protective culture-inoculated sucuk samples.

LAB dominated the environment, with the addition of the protective and starter cultures. The non-cultured group had the lowest LAB count ($p < 0.05$), while the groups S1, S2, and S3 had 6.64, 6.68, and 6.80 log CFU/g, respectively. However, the highest counts of LAB were found for the group S2 during the ripening period.

The number of *Enterococci* (Figure 2A) was between 2.40 and 4.18 log CFU/g on the first day. The *Enterococci* count showed an increasing trend in sample C and a decreasing trend in samples S1, S2, and S3 during ripening. The counting pattern was similar to those of TAMB and CNC. At the end of the manufacturing process, the highest count of *Enterococci* was determined in the non-cultured samples with 6.37 log CFU/g, followed by S3 with 4.28 log CFU/g. On the other side, *L. sakei* inoculation caused a significant decrease in *Enterococci* ($p < 0.05$) at the end of production.

*Enterobacteriaceae* decreased in number steadily during the fermentation and ripening periods (Figure 2B), particularly in samples containing the starter and protective cultures. The interaction between inoculation of the cultures and treatment duration had a statistically significant effect on the number of *Enterobacteriaceae* ($p < 0.05$). The effect of the ripening time in the sucuk samples was observed after the 3rd day, when group C showed a significant increase in the number of *Enterobacteriaceae* ($p < 0.05$).

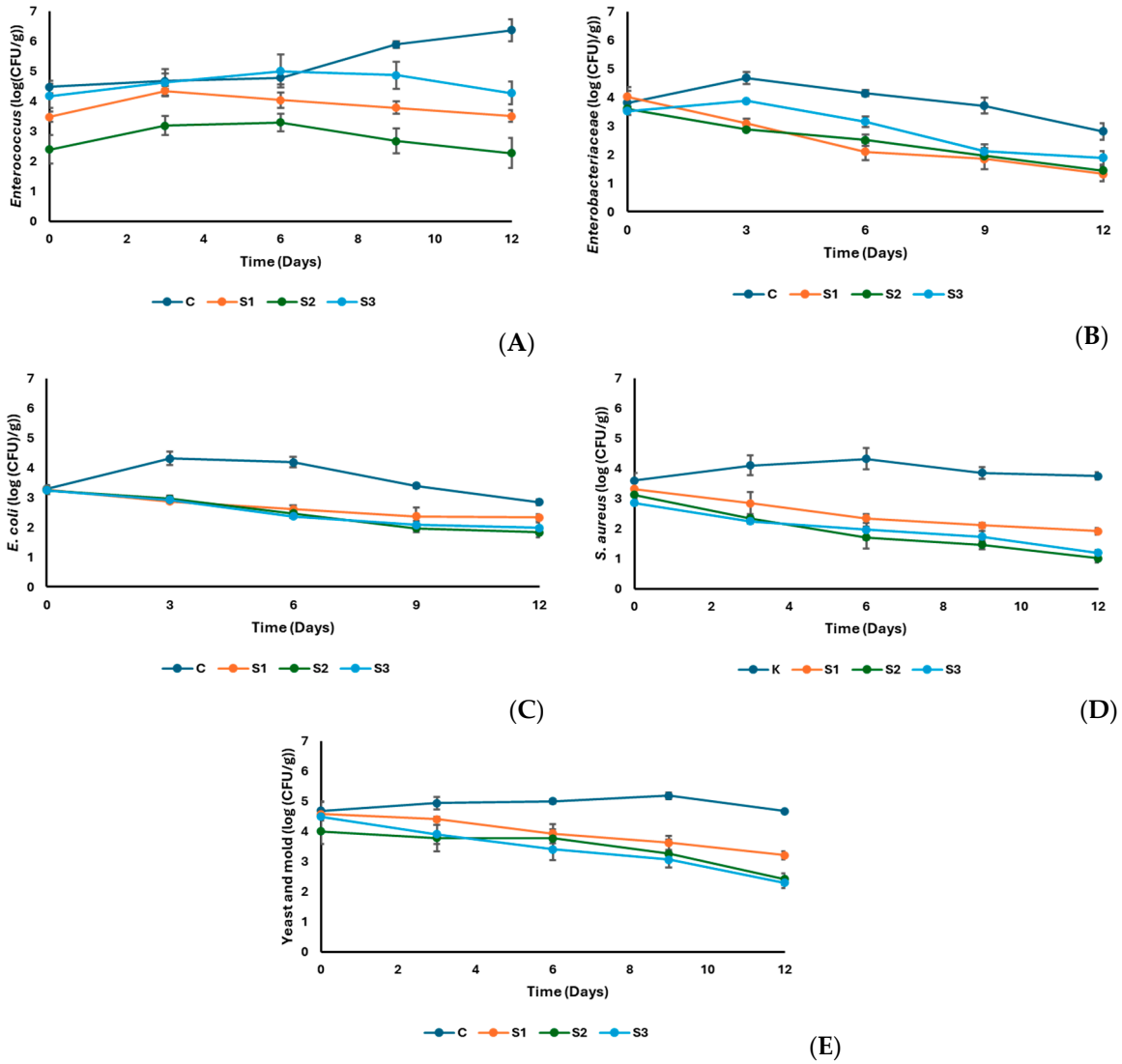



**Figure 2.** Graphs showing the growth rate of *Enterococcus* (**A**), *Enterobacteriaceae* (**B**), *E. coli* (**C**), *S. aureus* (**D**), yeast and mold (**E**) during the 12 days of ripening in non-cultured and culture-inoculated sucuk samples. C (blue lines): control, S1 (orange lines): *P. pentosaceus*- and *S. carnosus*-inoculated sucuk samples, S2 (grey lines): *L. sakei* protective culture-inoculated sucuk samples, S3 (yellow lines): *P. acidilactici* protective culture-inoculated sucuk samples. The lines represent the mean counts obtained from 3 replicates, and the error bars represent the standard deviations.

In the sausage batter, the number of *E. coli* varied between 2.24 and 2.75 log CFU/g (Figure 2C), and the effect of culture inoculation was observed on the first day. The non-cultured group C had a significantly higher *E. coli* count ($p < 0.05$) than the other groups initially and also in the following periods. Nonetheless, low counts were detected in the samples including *L. sakei* and *P. acidilactici* after the 6th day ($p < 0.05$). The addition of protective cultures redounded to the inhibition of *E. coli* compared with the addition of the commercial starter culture.

At the beginning of sucuk production, the number of *S. aureus* varied between 2.86 (*P. acidilactici*-inoculated) and 3.60 (non-cultured group C) log CFU/g; these values were statistically different ($p < 0.05$) (Figure 2D). Noteworthy decreases were observed in the number of *S. aureus* during fermentation in the samples inoculated with starter and protective starter cultures. The highest rate of *S. aureus* inhibition was detected in samples inoculated with the *L. sakei* preservative culture. Not surprisingly, the counts of *S. aureus* in

the C group were found to be two times higher ($p < 0.05$) than in the sucuk groups treated with a bioprotective starter culture in the last day of the microbiological analysis.

The mold and yeast count ranged between 4.68 log CFU/g (C) and 4.01 log CFU/g (S2) ($p < 0.05$) (Figure 2E). Although group C showed a slight increase till the 9th day ($p < 0.05$), a gradual decrease ($p < 0.05$) was observed in sucuks produced using *L. sakei* and *P. acidilactici*. At the end of the process, the difference between the protective culture-inoculated samples was not significant, whereas the other samples differed importantly ($p < 0.05$). The comparison of industrial sucuks and starter culture-inoculated sucuks showed that the count in the former ranged from 3.15 to 5.53 log CFU/g, whereas in the latter, it was between 3.48 and 5.80 log CFU/g (Figure 2).

### *3.2. Sucuk Composition and Physicochemical and Chemical Analysis Results*

A decrease in moisture content and an increase in fat, ash, and protein content was detected in the sucuks due to water loss depending on drying. The use of different types of starter cultures did not affect the composition of sucuk ($p < 0.05$). The moisture content of the sausage batter was at least 60.95%, reaching 62.28% at most ($p < 0.05$). Those contents changed in the range of 30.07–33.06% at the end of the process because of drying. The minimum loss of moisture content was recorded for the control samples ($p < 0.05$). Additionally, the initial fat, protein, and ash contents of the sucuk samples were determined as 17.83–18.80%, 16.70–17.88%, and 2.55–2.64% and, respectively, reached 30.72–33.79%, 29.23–30.54%, and 4.24–5.00%, increasing remarkably ($p < 0.05$).

The effect of culture application and time together on pH was found to be statistically important ($p < 0.05$). The inoculation of the starter culture showed its effect from the first day. The groups S2 (5.79) and S3 (5.76) had significantly lower pH values than C (5.95) and S1 (5.86) ($p < 0.05$). The starter culture addition caused a 0.09–0.19 unit decrease in pH at the beginning, which is an indicator of the adaptation of the starter cultures to the environment in a short time. The highest pH decrease was detected on the 3rd day in samples containing the starter and the protective cultures. Finally, the *L. sakei*-inoculated samples had the lowest pH value at 4.82. The difference between C and the other groups was significant ($p < 0.05$), while the difference between the three inoculated groups was not important statistically ($p < 0.05$) (Figure 3).

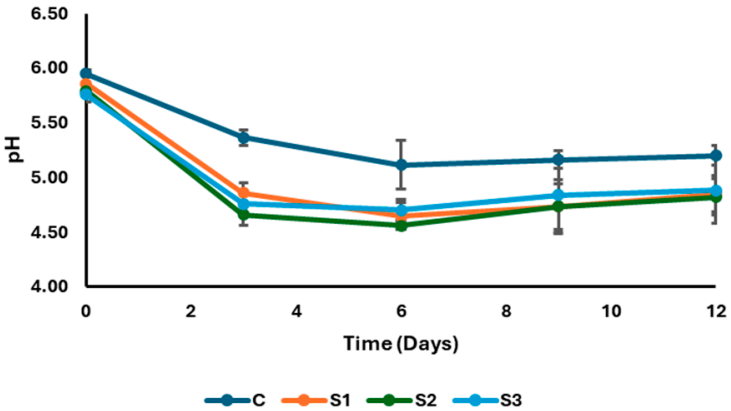



**Figure 3.** pH changes in the sucuk samples in the 12-day ripening process. C (blue line): control, S1 (orange line): *P. pentosaceus*- and *S. carnosus*-inoculated sucuk samples, S2 (grey line): *L. sakei* protective culture-inoculated sucuk samples, S3 (yellow line): *P. acidilactici* protective culture-inoculated sucuk samples. Lines represent average measurements of 3 biological and 2 technical replicates, and error bars represent standard deviations.

The $L^*$ (lightness–darkness) (A), $a^*$ (redness) (B), and $b^*$ (yellowness) (C) values of the sucuks are presented in Figure 4. The effect of the interaction between culture inoculation and ripening time on the $L^*$ and $a^*$ values of sucuk was found to be significant ($p < 0.05$); however, the addition of a culture was insignificant for the $b^*$ value ($p < 0.05$). The higher $a^*$

values of culture-added sucuks with respect to that of C sucuk indicated that the starter or protective cultures accelerated the curing color formation in sucuk.

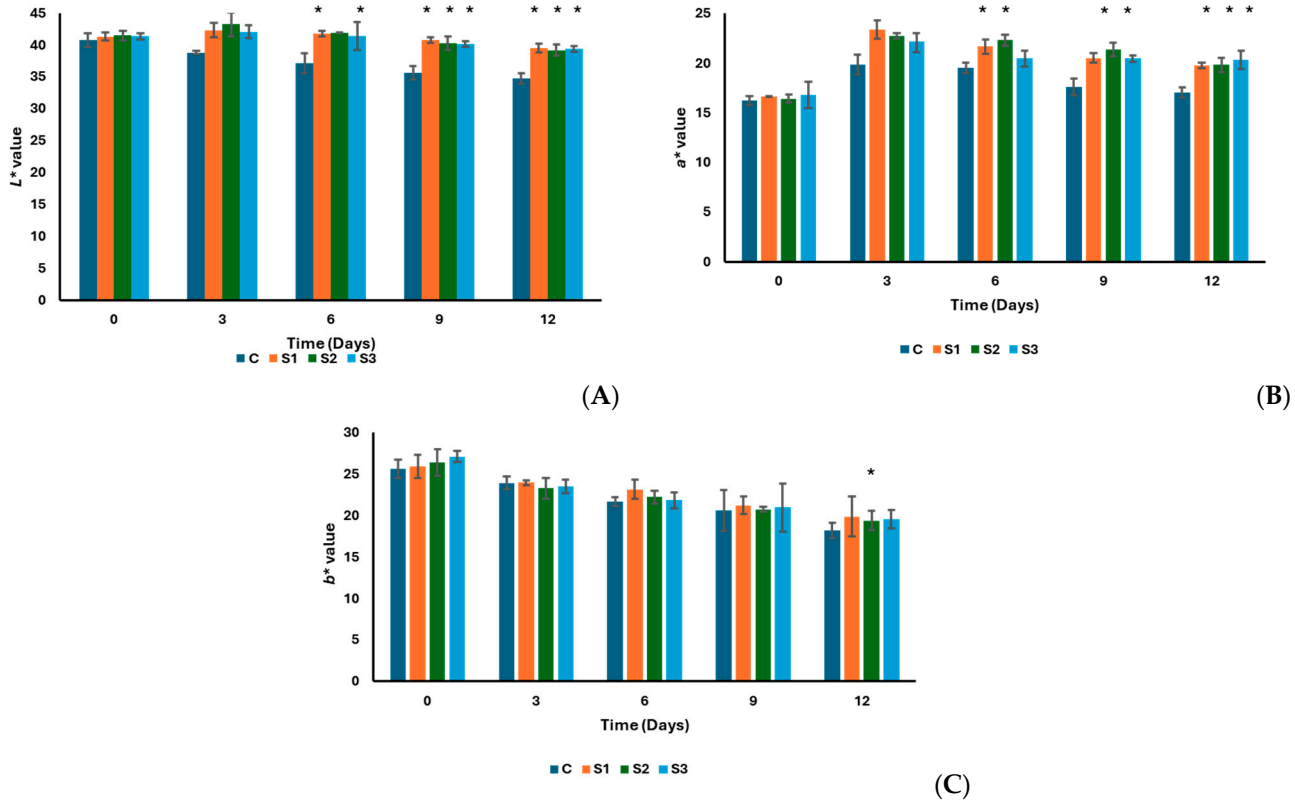



**Figure 4.** $L^*$ (**A**), $a^*$ (**B**), and $b^*$ (**C**) values obtained for different starter culture-inoculated and non-cultured sucuk samples in the 12-day production process. C (dark blue bars): control, S1 (orange bars): *P. pentosaceus*- and *S. carnosus*-inoculated sucuk samples, S2 (green bars): *L. sakei* protective culture-inoculated sucuk samples, S3 (light blue bars): *P. acidilactici* protective culture-inoculated sucuk samples, $L^*$: lightness, $a^*$: redness, $b^*$: yellowness. The mean of three biological replicates and two technical replicates are presented in bar graphs. Error bars present the standard deviations. Asterisks (*) show significant differences ($p < 0.05$) between the control group and the starter culture-including groups.

According to the variance analysis results, time and the addition of a culture affected the proteolysis index (PI) significantly ($p < 0.05$) (Table 2). The PI of the sucuk batter was found to be 10.14%, 13.51%, 12.84%, and 13.44% for the samples C, S1, S2, and S3, respectively ($p < 0.05$). The control group had a significantly lower PI ($p < 0.05$) than the other groups in all analysis periods. The highest proteolytic activity after ripening was determined in the sucuk ($p < 0.05$) samples in which the starter culture mixture was inoculated.

The TBARS activity in sample C increased significantly ($p < 0.05$) and differed from those in the other groups during fermentation. These results show that the inoculation of starter and/or protective cultures in sucuk retarded lipid oxidation dramatically. The TBARS activities in the starter and protective cultures were close to each other at the end of production, and no significant difference was found between them ($p < 0.05$) (Table 2).

**Table 2.** Proteolysis index and TBARS results obtained from sucuk samples during ripening.

| | PI (%) | | | | | TBARSs | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Groups** | DAYS | | | | | | | | | |
| | 0 | 3 | 6 | 9 | 12 | 0 | 3 | 6 | 9 | 12 |
| **C** | 10.14 ± 0.35 $^{Aa}$ | 9.83 ± 0.63 $^{Aa}$ | 10.37 ± 0.77 $^{A}$ | 10.86 ± 0.67 $^{Aa}$ | 12.30 ± 0.30 $^{Ab}$ | 0.51 ± 0.04 $^{Aa}$ | 0.66 ± 0.02 $^{Bb}$ | 0.75 ± 0.02 $^{Bc}$ | 0.84 ± 0.04 $^{Bd}$ | 0.98 ± 0.10 $^{Be}$ |
| **S1** | 13.51 ± 2.08 $^{Ba}$ | 13.26 ± 0.94 $^{Ba}$ | 14.81 ± 1.57 $^{Bb}$ | 17.14 ± 1.29 $^{Cc}$ | 19.31 ± 1.15 $^{Cd}$ | 0.50 ± 0.04 $^{Aa}$ | 0.54 ± 0.04 $^{Aa}$ | 0.59 ± 0.04 $^{Ab}$ | 0.67 ± 0.03 $^{Ac}$ | 0.62 ± 0.04 $^{Ab}$ |
| **S2** | 12.84 ± 0.93 $^{Ba}$ | 13.05 ± 0.90 $^{Ba}$ | 14.93 ± 0.46 $^{Bb}$ | 16.12 ± 0.73 $^{Cc}$ | 17.35 ± 1.24 $^{Bc}$ | 0.51 ± 0.04 $^{Aa}$ | 0.56 ± 0.02 $^{Ab}$ | 0.61 ± 0.03 $^{Abc}$ | 0.59 ± 0.04 $^{Ab}$ | 0.56 ± 0.02 $^{Ab}$ |
| **S3** | 13.44 ± 2.06 $^{Ba}$ | 13.49 ± 0.71 $^{Ba}$ | 13.11 ± 0.71 $^{Ba}$ | 14.63 ± 0.26 $^{Ba}$ | 16.24 ± 1.32 $^{Bb}$ | 0.52 ± 0.02 $^{Aa}$ | 0.56 ± 0.02 $^{Ab}$ | 0.58 ± 0.03 $^{Ab}$ | 0.64 ± 0.02 $^{Ac}$ | 0.58 ± 0.03 $^{Ab}$ |

$^{A–C}$: Means with different uppercase letters in the same column are significantly different ($p < 0.05$). $^{a–d}$: Means with different lowercase letters on the same line are significantly different ($p < 0.05$). Results are given as 3 replicates' and 2 parallels' means ± standard deviation PI: proteolysis index, TBARSs: thiobarbituric acid-reactive substances, C: control, S1: *P. pentosaceus*- and *S. carnosus*-inoculated sucuk samples, S2: *L. sakei* protective culture-inoculated sucuk samples, S3: *P. acidilactici* protective culture-inoculated sucuk samples.

### *3.3. Sensory Properties of the Sucuks*

The sensory evaluation results of the raw and grilled sucuk samples in terms of odor, taste, color, and overall liking are presented in Table 3. The average odor, color, taste, and general liking were evaluated as 7.38, 7.77, 7.56, and 7.48 out of 10 for the raw sucuks. S3 was the group with the highest scores for odor and taste, whereas S1 had the highest score for color. The lowest scores were found for C and were significantly different from those of the inoculated groups ($p < 0.05$)

**Table 3.** Sensory panel scores of sausages inoculated with different cultures.

| | Raw Samples | | | | Grilled Samples | | | |
|---|---|---|---|---|---|---|---|---|
| **Groups** | **Odor** | **Color** | **Taste** | **Overall Liking** | **Odor** | **Color** | **Taste** | **Overall Liking** |
| C | 6.89 ± 0.78 $^{a}$ | 6.98 ± 0.47 $^{a}$ | 6.91 ± 0.42 $^{a}$ | 6.83 ± 0.63 $^{a}$ | 7.74 ± 0.39 $^{a}$ | 7.64 ± 0.16 $^{a}$ | 6.96 ± 0.61 $^{a}$ | 6.95 ± 0.51 $^{a}$ |
| S1 | 7.52 ± 0.90 $^{b}$ | 8.44 ± 0.85 $^{c}$ | 7.76 ± 0.45 $^{b}$ | 7.76 ± 0.50 $^{b}$ | 8.47 ± 0.36 $^{b}$ | 8.44 ± 0.59 $^{b}$ | 8.30 ± 0.34 $^{b}$ | 7.84 ± 0.51 $^{b}$ |
| S2 | 7.48 ± 0.74 $^{b}$ | 7.85 ± 0.68 $^{b}$ | 7.52 ± 0.06 $^{b}$ | 7.44 ± 0.40 $^{b}$ | 7.56 ± 0.33 $^{a}$ | 8.37 ± 0.34 $^{b}$ | 7.67 ± 0.11 $^{b}$ | 7.87 ± 0.14 $^{b}$ |
| S3 | 7.63 ± 0.51 $^{b}$ | 7.82 ± 0.34 $^{b}$ | 7.93 ± 0.36 $^{b}$ | 7.89 ± 0.28 $^{b}$ | 7.85 ± 0.13 $^{a}$ | 8.04 ± 0.39 $^{b}$ | 8.00 ± 0.40 $^{b}$ | 8.11 ± 0.39 $^{b}$ |

$^{a–c}$: Means with different uppercase letters in the same column re significantly different ($p < 0.05$). C: control, S1: *P. pentosaceus*- and *S. carnosus*-inoculated sucuk samples, S2: *L. sakei* protective culture-inoculated sucuk samples, S3: *P. acidilactici* protective culture-inoculated sucuk samples.

The scores given to the grilled sucuks were slightly higher than those given to the raw sucuks. This was due to the preference of the consumer for cooked sucuk. On the other hand, 7.90, 8.12, 7.73, and 7.69 were the scores found for the grilled samples for odor, color, taste, and general liking, respectively. The highest scores for odor, taste, and color were observed for group S1, containing the *P. pentosaceus* + *S. carnosus* mixture; however, S3, including *P. acidilactici,* had the highest general liking score. Predictably, the lowest scores were obtained for the control group ($p < 0.05$).

## 4. Discussion

This study aimed to investigate the role of bioprotective starter cultures of *Lactobacillus sakei* and *Pediococcus acidilactici* in inducing microbiological, physicochemical, and chemical changes in the Turkish-style fermented sausage sucuk. The effect of these protective cultures was also compared with that of a *Pediococcus pentosaceus* + *Staphylococcus carnosus* commercial starter culture; a non-cultured sucuk group was used as a control.

During fermentation, a decrease in the number of TAMBs was observed (Table 1). This reductions was due to the inhibitory effect of LAB on competitive microorganisms [31]. The increase in acidity and the decrease in the $a_w$ value due to drying were the main cause

of the decrease in TAMB. It is known that the growth of LAB in sucuk produced by using starter cultures is quick in the first three days of ripening [32]. As expected, the effect of the starter cultures on LAB growth was considerable from the first day. The highest LAB counts were observed for the samples containing *L. sakei*. This shows that *L. sakei* has a better ability to adapt to the sausage environment than *P. acidilactici.* The reason for this is that the temperature of the sausage environment (between 18 to 22 °C) is very low for *P. acidilactici*, whose optimum growth temperature is 40 °C [33].

It was previously found that starter culture and duration of the ripening affected the number of LAB in sucuks produced using a mix of *Pediococcus acidilactici*, *Lactobacillus curvatus*, and *Staphylococcus xylosus* and mix of *Lactobacillus sakei* and *Staphylococcus carnosus* [34]. When fermented sausages produced with mixtures of different starter cultures (*L. sakei*, *S. xylosus*, *S. carnosus*, *L. sakei* + *S. carnosus,* and *P. pentosaceus* + *S. xylosus*) were investigated, the count of LAB almost doubled during 21 days. The changes in the number of LAB corresponded to changes in the pH value of the sausages [35]. In our study, the change in the number of TAMB and LAB (Table 1) throughout the production of the sausages is compatible with those reported in other studies [4,5,29]. Similar increasing numbers of LAB were also obtained in studies using *L. sakei* [36] and *P. acidilactici* S147 [26] cultures.

It was found that at the end of the manufacturing process, the highest counts of *Enterococci* were determined in the non-cultured samples. As reported in the literature, the presence of *Enterococci* in meat fermentation is constant [37], and they can be found in fermented meat products in significant numbers depending on the contamination [38,39]. However, their evaluation as GRAS is still controversial [38]. *Enterococci* can survive and replicate in fermented meat products that do not contain starter cultures [27,40]. The notable number of enterococci in *P. acidilactici*-inoculated samples showed that *P. acidilactici* promotes the development of enterococci. On the other side, with the inoculation of *L. sakei*, a significant decrease in *Enterococci* was observed at the end of production. This was due to the fact that *Enterococci* cannot survive and decrease in number in strong-acidity sausages [37,41]. The results obtained from our research correspond to those in the literature.

In dry-fermented sausages such as sucuk, the number of *Enterobacteriaceae* demonstrates the microbial quality and safety of the product [42]. *Enterobacteriaceae* cannot resist acidic environments and low water activity; therefore, they are eliminated in the ripening process in fermented meat products [42,43]. It was revealed that the inoculation of starter cultures, including *L. sakei*, *S. xylosus*, *S. carnosus*, and *L. plantarum,* was effective in decreasing the *Enterobacteriaceae* number during the ripening period [31,44,45]. The members of the *Enterobacteriaceae* family play a role in the formation of undesirable biogenic amines such as histamine, cadaverine, and putrescine in fermented meat products, and therefore, a reduction in their number is important [46,47].

*E. coli* can maintain its vitality by adapting to low-pH environments thanks to its acid resistance. *E. coli* strains, known to be resistant towards acids, were able to survive in sausages prepared using *S. carnosus*, *S. xylosus*, and *L. curvatus* starter cultures [48]. Moon et al. (2002) [49] reported that *P. acidilactici* K10 was not sufficient for the inhibition of *E. coli* O157: H7 in vitro by itself; however, a significant inhibition effect was observed in the presence of organic acids such as lactic, succinic, and acetic acid. Similarly, in studies conducted in in vivo environments, *E. coli* O157: H7 in minced meat decreased by 2.8 log units with the addition of *P. acidilactici* and lactic acid [49]. Correlatively, in our study, *E. coli* inhibition was observed in the lactic acid medium formed by the *P. acidilactici* protective culture.

*Enterobacteriaceae* and *E. coli* are organisms associated with hygienic quality. These organisms cannot adjust to decreasing pH levels and drying in sausages; therefore, their count decreases [50]. However, in productions with spontaneous fermentation like that of the control group used in this study, the decrease in their number is slow [51]. Some previous studies reported that a maximal decrease in the *Enterobacteriaceae* population,

including coliforms and *E. coli*, was observed during the ripening process due to the diminishing pH value, especially in the fermentation process [51].

It was shown that the counts of *S. aureus* in the control group without bioprotective starter cultures were doubled compared to those in the bioprotective starter culture-inoculated groups. This indicates that insufficient numbers of LAB and high-pH environments promote the growth of *S. aureus*. Kaban and Kaya (2006) [34] reported that the slow decrease in the pH value in samples without a starter culture created a suitable environment for *S. aureus* proliferation. The obtained results showed that the inhibition of *S. aureus* was better accomplished with *L. sakei* and *P. acidilactici* inoculation, while the commercial starter culture (S1) did not provide sufficient inhibition by itself. Similar to our findings, Kaban and Kaya (2006) [34] found that a *P. acidilactici*, *L. curvatus*, and *S. xylosus* mix and an *L. sakei* and *S. carnosus* mix in sucuk had a inhibition effect on *S. aureus* when compared to the control group. *S. aureus* is a food-borne pathogen that can also be present in fermented meat products. Retail product research has shown that *S. aureus* is frequently isolated from sucuk. Dry or semi-dry fermented meat products with a fermentation temperature of 30–40 °C are quite suitable for *S. aureus* development. The first days of the fermentation process are significant for *S. aureus* development due to the pH, water activity, and the changing microbial flora [34]. Lactic acid bacteria are competitive flora for coagulase-positive staphylococci. Therefore, the presence of a high number of LAB in the environment is important for the inhibition of these microorganisms [52]. The high number of *S. aureus* in the raw material, the low numbers of LAB, the high-pH environment, and the high $a_w$ are the main elements favoring the excretion of staphylococcal enterotoxins; therefore environment, equipment, raw material, and staff can cause *S. aureus* contamination in meat products [53]. The control group in our study could cause food safety concerns due to the lack of a starter culture. A low-pH environment, bacteriocin formation by the *L. sakei* and *P. acidilactici* protective cultures, and a fast drying promoted a decrease in the *S. aureus* number and provided more reliable products.

Even if LAB are the dominant bacteria in the sausage environment, there is a certain level of mold and yeast [19]. The mold and yeast numbers generally range between 2.0 and 4.5 log CFU/g in the sausage batter. However, their number does not exceed 5 logs CFU/g in general, even if an increase is observed during fermentation [30]. The inoculation of *L. sakei* can result in an increase in the mold and yeast count in 30 days in a long production period [54]; however, supplementation of a bacteriocin such as mesenterosin Y in addition to *L. sakei* can decrease the mold and yeast number for 28 days [27]. The similarity between the two different groups of samples derives from the lack of sanitation and hygiene rule application, the use of poor-quality raw materials, and/or differences between production methods [55].

The moisture content in the control group was the minimum among all groups. The reason for this is that drying [causing a rapid pH drop] was achieved quickly in the samples inoculated with the starter culture. The different moisture content when using bioprotective cultures corresponds with the results of other studies [5,43]. Sucuk composition is mainly affected by the amount of fat, the amount of meat, and the production conditions such as temperature, air speed, and RH [1]. It is obvious that the addition of a starter or a protective culture does not play a considerable role in sucuk composition.

The environment created in sucuk brings about LAB domination in the environment and the production of lactic acid as a result of fermentation. Therefore, the pH value of sucuk sharply declines, in particular, in the first three days of fermentation, as shown in Figure 3. The pH pattern we found complies with the study results obtained by Lorenzo et al. (2014) [43] and Kamiloglu et al. (2019) [56]. Moreover, Oz et al. (2018) [26] reported that the pH of a heat-treated sausage prepared using *P. acidilactici* was lower than the that of the control group.

The color, especially the redness of fermented meat products, is an important quality indicator [12]. In this study, the $a^*$ value (redness) increased in culture-added sucuks compared to the control sucuk, suggesting that the use of starter or protective cultures

accelerates the development of the cure color in sucuk. The studies conducted by Pérez-Alvarez et al. (1999) [57], Bozkurt and Bayram (2006) [58], and Wang et al. (2013) [54] showed consistency with our study.

Proteolysis is one of the fundamental biochemical reactions that is catalyzed by microorganisms in the environment or endogenous enzymes in meat tissues [59]. Proteolysis is effective in influencing the texture and flavor of meat products. The proteolysis index is an indicator of the nitrogen fraction, which contains non-protein peptides (NPN) and amino acids that remain after trichloroacetic acid precipitation. It is determined in % of the total protein nitrogen, considering the non-protein nitrogen ratio [30]. The better proteolytic activity of the *P. pentosaceus* + *S. carnosus* culture mixture (Table 2) can be explained by the fact that both single cultures have proteolytic activity [35,45,60,61]. It is known that *L. sakei* [45,62] has higher proteolytic activity than *P. acidilactici* [63,64]. However, according to the last-day data, the proteolytic activities of *P. acidilactici* and *L. sakei* were not significantly different from each other ($p > 0.05$) (Table 2). It was proven by other studies that, thanks to the proteolytic activities of starter cultures, proteins break down faster and more in fermented sausages [5,35,63]. However, in contrast to our findings, some studies could not find any effect of additional starter cultures on proteolytic activity [59,65].

The TBARS amount is used to evaluate secondary oxidation products occurring in meat and meat products, hence, lipid oxidation [66]. Oral and Kaban (2021) [25] reported that the lipolytic activity of *S. xylosus* resulted in an increased TBARS activity at the end of 14 days of storage. This may be due to the fact that the by-products of the *S. xylosus* strain GM92′s lipolytic activity are advantageous starting points for lipid oxidation [25]. Contrarily, our results showed that the addition of the starter and protective cultures reduced lipid oxidation (Table 2). It was stated in other studies that starter culture addition induces lipid oxidation in fermented meat products [67,68]. It was also indicated that this effect is caused by the antioxidant effect of coagulase-negative staphylococci (CNS). The catalase and superoxide dismutase activities of CNS bacteria cause peroxides to break down, thereby preventing lipid oxidation [69,70]. The lowest TBARS activity was measured in samples inoculated with *L. sakei* and *P. acidilactici* and can be associated with the antioxidative enzyme activity of LAB [71,72]. Correlatively to our findings, in another study by Bozkurt and Erkmen (2002) [73], the TBARS value of the sausage group without a starter culture was found to be higher than that of the sausage groups containing a starter culture.

It is known that "protective cultures", the purpose of which is to inhibit pathogenic and degrading microorganisms, should change the sensory properties of products as little as possible [6]. The control group had the lowest related scores among all groups (Table 3). This is related to the fact that protective and starter cultures dominate the environment fast and form more compounds that affect the taste as a result of acid production and proteolytic events [6,60]. The *P. acidilactici*-inoculated group had the highest general liking score when the samples were grilled (Table 3). Regarding the shown scores, it was seen that the panelists could not distinguish the S1 group, which, as demonstrated by chemical analysis, reported higher proteolytic changes, from the other groups in terms of taste, color, and general taste, with respect to both raw and cooked samples. Similar results were also previously obtained by Scannell (2004) [74]. The addition of the starter culture and the protective culture into the product improved the development of specific sensory properties of sausages in a shorter time. A rapid pH decrease, color formation and stabilization, the formation of an acidic taste, the activation of proteolytic enzymes in the low-pH environment, the breakdown of proteins into small-molecule compounds, and the change in the taste and aroma of the sausage affected the raw and the cooked sausages [5].

The exopeptidases of lactobacilli in meat affect the taste of products by helping to combine muscle aminopeptidases and free amino acids that provide flavor formation. However, lactobacilli and pediococci do not play an important role in the formation of typical aroma components such as 3-methyl butanol in fermented meat products due to their low catabolic activity [75]. It was stated that *L. sakei* [76,77] and *P. acidilactici* [78] protective

cultures have peptidase activity. For example, leucine and valine aminopeptidases generate free amino acids by hydrolyzing proteins. Those free amino acids are responsible for flavor formation [54,75–77]. In our study, sucuks inoculated with *L. sakei* and *P. acidilactici* reached a higher rank than the control group in terms of taste and general liking (Table 3).

## 5. Conclusions

Although fermented meat products contain microbiologically safe microorganisms, they are environments where pathogenic microorganisms can grow. The products produced without using a starter culture carry more risks; however, sausages produced by adding a starter culture also have the risk of allowing the growth of pathogens. It is recommended to use protective cultures as the best way to eliminate this risk. Our study revealed that the inoculation of starter and protective cultures provided better microbiological, chemical, and sensory qualities to sucuk than those of non-cultured sucuks. Considering that sucuk producers prefer short production times (e.g., a 12-day period, as tested in this study), it will be possible for them to use cultures that are functional and capable of producing bacteriocin rather than industrial starter cultures in order to ensure safe products and the development of sufficient sensory properties. The fact that protective cultures do not cause undesirable changes in sucuk composition and sensory properties or even improve the sensory properties and, on the other hand, prevent the development of unwanted microorganisms, shows that they can be used with starter cultures in sucuk production. Additionally, this capability enhances the commercial viability of sucuk by ensuring consistent quality and safety, which can be appealing to both producers and consumers. However, the use of bioprotective cultures for long-term fermentation periods requires more investigations in terms of microbial stability and safety, chemical stability, and sensory attributes.

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