



Characterization of Turkish dry fermented sausage produced with spontaneous microbiota and some lactic acid bacteria mixed culture

Características del embutido seco turco producido con microbiota espontánea y algunas culturas mixtas de bacterias lácticas

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Abstract

This study aimed to investigate the usability of lactic acid bacteria (LAB) strains (*Lactiplantibacillus plantarum*, *Enterococcus faecium*, *Lactococcus lactis* subsp. *lactis*) isolated from cheese whose properties and reliability being functional starter cultures were determined in previous studies in Traditional Turkish dry fermented sausage (sucuk) production. In addition to the control group (spontaneous microbiota), fermented sucuk samples were produced from six different mixed culture combinations. Quality characteristics of sucuk samples were determined during production (day 0 and day 7) and storage (days 7, 14, and 21). At the end of storage, the value ranges for moisture, water activity, pH, and ash content were found to be as 21.12-24.83, 0.862-0.872, 4.40-4.65, and 4.23-4.80%, respectively. Moreover, lactobacilli was 8.46 to 8.79 log cfu/g, total aerobic mesophilic bacteria ranged from 8.46 to 9.56 log cfu/g, and mold-yeast from 4.40 to 6.91 log cfu/g in groups with mixed culture. *E. faecium* and *L. lactis* has adapted well to sucuk ripening conditions. The group containing *Lpb. plantarum* + *E. faecium*; (1:2 ratio) showed the highest mean lactic acid bacteria count. The lowest thiobarbituric acid reactive substances (TBARS) value was determined in *Lpb. plantarum* + *Lc. lactis* (1:1 ratio) mixed culture. The addition of mixed culture caused an increase in the textural values. It can be concluded that the functional mixed cultures used in this study which exhibited good growth in sucuk microbiological quality parameters, contribute to sensory evaluation scores and physicochemical properties could be potential starter candidates or co-starter cultures.

Keywords: Lactic acid bacteria, fermented sausage, microbiological properties, sucuk, starter culture.

Resumen

Este estudio tuvo como objetivo evaluar la usabilidad de cepas de bacterias lácticas (LAB) (*Lactiplantibacillus plantarum*, *Enterococcus faecium*, *Lactococcus lactis* subsp. *lactis*) aisladas de queso, cuyas propiedades y confiabilidad como cultivos iniciadores funcionales se determinaron en estudios previos, en la producción tradicional de salchichas fermentadas secas turcas (sucuk). Además del grupo de control (microbiota espontánea), se produjeron muestras de sucuk fermentadas que contenían seis combinaciones diferentes de cultivos mixtos. Las características de calidad de las muestras de sucuk se determinaron durante la producción (día 0 y día 7) y el almacenamiento (días 7, 14 y 21). Al final del almacenamiento, se encontraron rangos de valores para la humedad, la actividad del agua, el pH y el contenido de cenizas de 21.12-24.83, 0.862-0.872, 4.40-4.65 y 4.23-4.80%, respectivamente. Además, los lactobacilos estuvieron en un rango de 8.46 a 8.79 log UFC/g, las bacterias mesófilas aeróbicas totales oscilaron entre 8.46 y 9.56 log UFC/g, y el moho-levadura de 4.40 a 6.91 log UFC/g en los grupos con cultivo mixto. *E. faecium* y *Lc. lactis* se adaptaron bien a las condiciones de maduración de sucuk. El grupo que contenía *Lpb. plantarum* + *E. faecium*; (proporción 1:2) mostró el recuento medio más alto de bacterias lácticas. El valor más bajo de sustancias reactivas al ácido tiobarbitúrico (TBARS) se determinó en el cultivo mixto *Lpb. plantarum* + *Lc. lactis* (proporción 1:1). La adición de cultivos mixtos provocó un aumento en los valores texturales. Se piensa que los cultivos mixtos funcionales utilizados en este estudio, que mostraron un buen crecimiento en los parámetros microbiológicos de calidad de sucuk, contribuyen a las puntuaciones de evaluación sensorial y a las propiedades fisicoquímicas, y podrían ser posibles candidatos como cultivos iniciadores o co-cultivos.

Palabras clave: bacterias lácticas, salchicha fermentada, propiedades microbiológicas, sucuk, cultivo iniciador.

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1 Introduction

Traditional Turkish dry-fermented sausages (sucuk) are usually spontaneously fermented in autumn, when the air temperature, airflow and humidity are optimal, using a method based on ancient and traditional experiences in Türkiye. Therefore, it is crucial to employ starter cultures when producing sucuk to standardize the production process and eliminate the impact of the raw material and spontaneous microbiota variability (Kaban *et al.*, 2022). Lactic acid bacteria (LAB) preventing the growth of undesirable microorganisms (Alp, 2022; Borrás-Enríquez *et al.*, 2023) the most prevalent species in fermented meat products, are often utilized as starter cultures to increase quality and safety (Dias *et al.*, 2020; Hu *et al.*, 2022). Lactobacillaceae species are the most frequently used strains within the LAB group (Zhang *et al.*, 2018). Some of those are *Lactiplantibacillus plantarum* (Blaiotta *et al.*, 2018; Pavli *et al.*, 2020), *Lactilactobacillus sakei* (Najjari *et al.*, 2020) and *Lacticaseibacillus paracasei* (Oliveira *et al.*, 2021). Enterococci play an important role in the formation of textural and organoleptic properties of various fermented foods (sausage, salami, cheese, etc.) thanks to their lipolytic, esterolytic and metabolic activities such as citrate-using (Cavalheiro *et al.*, 2021; Franz *et al.*, 2011). *Enterococcus faecium* used as a starter culture along with the genus *Lactiplantibacillus* in fermented meat products contribute to the flavor of the sausage. It has been used by researchers to shorten the fermentation period of fermented foods such as sausages and cheese thanks to its advantageous technical and physiological characteristics (Cavalheiro *et al.*, 2019; Schittler *et al.*, 2019). Antimicrobial peptides or proteins have inhibitory effects against bacteria that cause food spoilage. Some species of enterococci can increase the shelf life and stability of fermented foods by producing bacteriocins (enterocin) effective against various food-borne pathogenic bacteria (Kasimin *et al.*, 2022).

Standardization is ensured in the control end product in the fermentation process with starter cultures selected from microorganisms isolated from natural habitats with verified physiological and metabolic properties or from successful fermented products. All of these keep the screening and identification of starter strains up to date. In this study, the usability of some LAB strains (*Lpb. plantarum*, *E. faecium*, *Lactococcus lactis* subsp. *lactis*) whose functional starter culture properties and reliability have been confirmed in previous studies isolated from traditional cheeses was investigated as starter or co-

cultures. The effects of the strains were determined on various microbiological, physicochemical and sensory properties of fermented sucuk.

2 Materials and methods

2.1 Safety of LAB cultures

LAB strains (*Lpb. plantarum* PeLB75, *E. faecium* PeLC2 and *Lc. lactis* PeLC6) used in this study have previously been characterized in terms of their phenotypic definitions and identified by 16S rRNA gene sequencing with the universal primers 27F (5' AGAGTTTGATCMTGGCTCAG 3') and 1492R (5' TACGGYTACCTTGTTACGACTT 3'). Some technological safety properties like virulence factors (VanA, VanB, gelE genes) biogenic amine production, proteolytic activity, enterocin producing and safety features such as sensitivity to most antibiotics have been determined in our previous studies (Ertürkmen & Öner 2015; Ertürkmen *et al.*, 2015; Ertürkmen, 2024). Sterile glycerol was added to LAB and stored at -18 °C.

2.2 Preparation of sucuk dough

The flow chart of sucuk is presented in Figure 1. Meat from the thighs and shoulders of beef carcasses was separated from the coarse fat and connective tissues chopped. The spices that were added to the dough were provided from Mayafix (Istanbul, Türkiye) and natural sheath from Münsüroğlu (Afyon, Türkiye). The weight of the meat was then determined. The prescription stated by Gökalp *et al.* (2004) was based on the use of spices and additives in the formulation. First, a group of control sucuk dough including cubed meat, spices, and NaNO₂ was made. Then, six separate sucuk doughs containing the same quantity of nitrite and a combination of cultures were made at different ratios. Each sucuk group received an inoculation from the active strains at a concentration of 2×10^7 log cfu/g. Table 1 presents the starter cultures and usage ratios that were adopted in the study. The sucuk dough was allowed to rest at +4 °C for roughly 18 hours. Then fat was added and the mixture was run through a mill with a 3 mm diameter. It was kneaded until a homogenous structure was obtained. The collagen intestines (diameter: 40 mm) were filled with the meat in the grinder after the intestines were held in water at 15-20 °C for 5-10 minutes. This ensured that there were no air gaps. The sucuk samples were rested for 2-3 hour at room temperature in the balancing phase following the filling process.

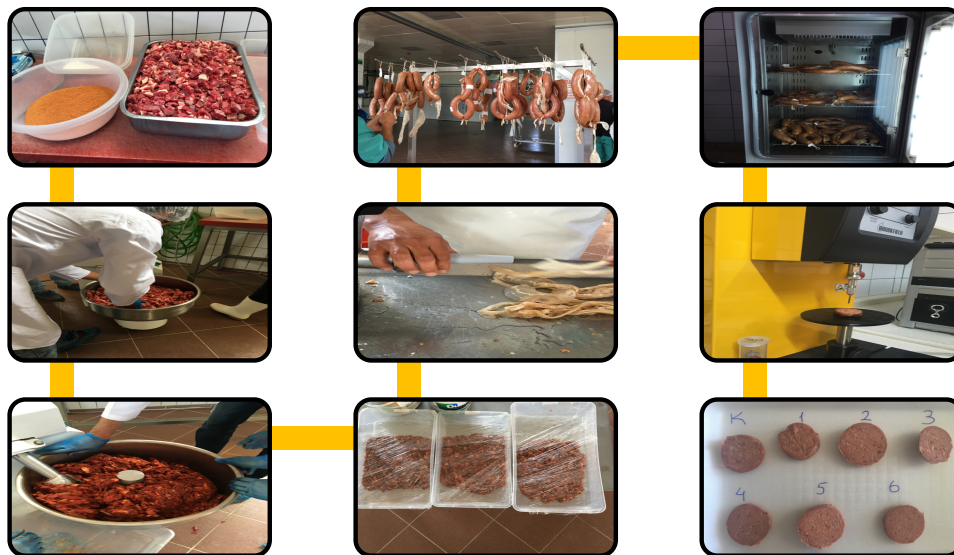


Figure 1. Flow chart of Turkish fermented sausage production (sucuk) *Chopping meat; Adding spices; Mixing; Dividing into 6 different groups; Adding mixed starter cultures; Resting at +4 °C, 18 hours; The fat adding; Grinding, kneading and collagen intestines filling; Fermentation in cabinet; Ripening.

Table 1. The names of the sucuk groups and starter culture combinations.

Sucuk groups	Starter culture combinations	Rate
Control	Any strain was not added	
S1	<i>Lpb. plantarum</i> (PeLB75)+ <i>Lc. lactis</i> (PeLC6)	1:1
S2	<i>Lpb. plantarum</i> (PeLB75)+ <i>E. faecium</i> (PeLC2)	1:2
S3	<i>Lpb. plantarum</i> (PeLB75)+ <i>E. faecium</i> (PeLC2)	1:1
S4	<i>Lpb. plantarum</i> (PeLB75)+ <i>E. faecium</i> (PeLC2)+ <i>Lc. lactis</i> (PeLC6)	1:1:1
S5	<i>Lpb. plantarum</i> (PeLB75) + <i>E. faecium</i> (PeLC2)+ <i>Lc. lactis</i> (PLC6)	1:2:1
S6	<i>E. faecium</i> (PeLC2)+ <i>Lc. lactis</i> (PLC6)	1:1



Figure 2. After the balancing phase, the sausage samples are kept in the fermentation cabin *24 hours (95% humidity at 24 °C), 24 hours (90% humidity at 22 °C), 12 hours (85% humidity at 20 °C), 12 hours (80% humidity at 20 °C) and 7 days (75% humidity at 18 °C).

2.3 Fermentation stage

Following the balancing phase, the sucuk samples were kept in a fermentation cabinet for 24 hours (95% humidity at 24 °C), 24 hours (90% humidity at 22 °C), 12 hours (85% humidity at 20 °C), 12 hours (80% humidity at 20 °C) and 7 days (75% humidity at 18 °C) (Figure 2). Following the fermentation, the samples were stored at +4 °C for 21 days. The microbiological analyses were performed during production (days 0 and 7) and storage (days 7, 14, and 21), followed by physicochemical and sensory analyses.

2.4 Microbiological analysis

Under aseptic conditions, 10 g samples of sucuk were taken from each group, and serial dilutions were made. Lactic acid bacteria were enumerated on de Man Rogosa Sharpe Agar (MRS, Merck, Darmstadt, Germany) after 48 h at 30 °C under anaerobic conditions; total aerobic mesophilic bacteria (TAMB, Merck, Darmstadt, Germany) were enumerated on Plate Count Agar (PCA, Merck, Darmstadt, Germany) after 48 h at 30 °C; coliform group microorganisms

on Violet Red Bile Agar (VRBA, Merck, Darmstadt, Germany); mold-yeast on Potato Dextrose Agar (PDA, Merck, Darmstadt, Germany) (pH 3.5) after 3-5 days at 25 °C. The results are presented as log cfu/g (Yilmaz-Oral & Kaban, 2021; Akköse *et al.*, 2023).

2.5 Physico-chemical analysis

The moisture values were determined according to Anonymous (2001) and ash value according to Gökalp *et al.* (1993). The measurements were recorded using a digital pH meter (Akcan *et al.*, 2023). The water activity values were determined according to Hughes *et al.* (2002). The Precise Color Reader TCR 200 was used to determine the L*, a* and b* color values of the samples (Wiegand & Waloszek, 2003). Thiobarbituric acid reactive substances (TBARS) analysis was carried out using the method modified by Kiliç & Richards (2003) to monitor lipid oxidation in sucuk samples. The results for TBARS were indicated in $\mu\text{mol/kg}$ of meat.

2.6 Textural profile analysis

Three different region analysis of the textural profile (TPA) was carried out using the TA.XT2 Plus Texture Analyzer (Stable Micro Systems, Godalming, UK) at room temperature. Before and after the analysis the probe speed was set to 2 mm/sec, and during the test, it was set to 5 mm/sec. In this analysis, the sucuks were compressed by 70% using a 50-kg load cell at room temperature. The analysis parameter included metrics for hardness (N), cohesiveness, springiness index, gumminess (N), chewiness (N), adhesiveness (mJ), and resilience.

2.7 Sensory analysis

Both surfaces were cooked for 2 minutes using a grill and pieces from the samples were then presented for the opinions of 20 semi-trained panelists who are academic staff at the Department of Food Processing and Faculty of Veterinary Medicine (Burdur Mehmet Akif Ersoy University, Burdur, Türkiye). In cooked samples nine-point hedonic-type scale was used for analysis, and attributes including color, smell, chewability, taste, scent and overall acceptability were assessed. In raw samples, attributes such as cross-sectional surface color, cross-sectional surface appearance and odor characteristics were assessed. According to the hedonic indicator chart, sensory results were rated as follows: Bad: 1-2-3 Medium: 4-5-6 Good: 7-8, and Very Good: 9 (Lawless & Heymann, 1999).

2.8 Statistical analysis

One-way analysis of variance (one-way ANOVA) and Tukey's multiple comparison tests ($p < 0.05$) were performed for the statistical evaluations utilizing the Minitab 17 statistical package program. The Kruskal-Wallis test was used to determine the differences in the sensory analyses. The results are presented as mean standard \pm deviation.

3 Results and discussion

3.1 Microbiological properties of sucuks

The lactobacilli count increased in all sucuk groups during the production days. The lactobacilli counts increased over 10^8 cfu/g in all sucuk groups produced using starter culture from the beginning of the storage period and maintained this value until the end of fermentation. Similar results were also determined by Pavli *et al.* (2020) has reported that in the ripening of dry-fermented sausages. There were significant differences between the average LAB counts of groups with starter culture and without starter culture ($p < 0.05$). Mix culture and storage day factors had a significant effect on the lactic acid bacteria count at the $p < 0.05$ level. The lowest mean value in mixed culture groups in terms of the storage days was determined in beginning of storage days. Higher lactic acid bacteria count was determined in S1 and S2 groups on storage day 21. This result is probably due to *E. faecium* and *Lc. lactis* has adapted well to ripening conditions. The groups with spontaneous microbiota (control) and without *Lpb. plantarum* strain S6 (*E. faecium*+*Lc. lactis*; 1:1 ratio) group yielded the lowest values in terms of lactic acid bacteria count. Further, the S2 (*Lpb. plantarum* + *E. faecium*; 1:2 ratio) group had a higher mean value than the control group (Table 2). These results were demonstrate that *Lpb. plantarum* exhibited good growth in the fermentation stage and continued its survival in the following days of sucuk ripening. Moreover, *Lpb. plantarum* together with *E. faecium* exhibits good growth in sucuk. Similar results have been determined by other researchers (Akköse *et al.*, 2023).

Throughout the whole production and storage period, there were statistically significant differences in terms of the TMAB counts between the sucuk groups ($p < 0.05$). All the sucuk groups showed an increase in terms of TMAB during production. The S6 group had the lowest TMAB count on the last day of storage (8.46 log cfu/g), whereas the S1 group had the highest TMAB count (9.56 log cfu/g). Similarly, Soyer *et al.* (2005) have reported that the TMAB counts increased to over 8.0 log cfu/g in the advanced stages of ripening.

Table 2. Microbial composition of sucuks (log cfu/g).

	Sucuk groups	Production day		Storage day		
		0. day	7. day	7. day	14. day	21. day
<i>Lactobacillus</i> sp.	Control	4.95±0.02 ^{Bd}	8.67±0.01 ^{Abc}	8.45±0.05 ^{Xc}	8.44±0.04 ^{Xb}	8.42±0.08 ^{Xb}
	S1	6.91±0.13 ^{Bbc}	8.74±0.03 ^{Aab}	8.54±0.06 ^{Ybc}	8.62±0.06 ^{Ya}	8.75±0.03 ^{Xa}
	S2	6.83±0.11 ^{Bc}	8.96±0.02 ^{Aa}	8.57±0.03 ^{Ybc}	8.73±0.03 ^{Xa}	8.79±0.04 ^{Xa}
	S3	6.97±0.13 ^{Babc}	8.59±0.07 ^{Abc}	8.56±0.05 ^{Xbc}	8.65±0.08 ^{Xa}	8.64±0.04 ^{Xab}
	S4	7.20±0.15 ^{Bab}	7.83±0.04 ^{Ad}	8.57±0.07 ^{Xbc}	8.68±0.07 ^{Xa}	8.67±0.04 ^{Xab}
	S5	7.23±0.14 ^{Ba}	8.46±0.08 ^{Ac}	8.67±0.07 ^{Xab}	8.66±0.07 ^{Xa}	8.56±0.10 ^{Xab}
	S6	7.05±0.11 ^{Babc}	8.51±0.20 ^{Abc}	8.76±0.07 ^{Xa}	8.67±0.05 ^{Xa}	8.46±0.21 ^{Yb}
Total aerobic mesophilic bacteria	Control	6.25±0.07 ^{Bd}	8.60±0.03 ^{Ab}	8.61±0.03 ^{Yb}	8.62±0.01 ^{Ybcd}	8.98±0.01 ^{Xbc}
	S1	7.38±0.06 ^{Bb}	8.59±0.01 ^{Aa}	8.62±0.01 ^{Yb}	8.68±0.04 ^{Ybc}	9.56±0.03 ^{Xa}
	S2	7.39±0.04 ^{Bb}	8.93±0.02 ^{Aa}	9.06±0.12 ^{Xa}	9.08±0.14 ^{Xa}	9.07±0.07 ^{Xb}
	S3	7.27±0.12 ^{Bb}	8.81±0.12 ^{Ab}	7.85±0.10 ^{Yd}	8.79±0.13 ^{Xb}	8.97±0.15 ^{Xbc}
	S4	8.07±0.02 ^{Ba}	8.60±0.04 ^{Aa}	7.94±0.11 ^{Zd}	8.28±0.09 ^{Ye}	8.74±0.03 ^{Xd}
	S5	7.25±0.22 ^{Bb}	8.91±0.13 ^{Aa}	8.34±0.33 ^{Ybc}	8.53±0.05 ^{XYcd}	8.81±0.09 ^{Xcd}
	S6	6.85±0.08 ^{Bc}	8.62±0.39 ^{Ab}	8.07±0.11 ^{Ycd}	8.41±0.04 ^{Xde}	8.46±0.05 ^{Xc}
Yeast	Control	7.41±0.04 ^{Aa}	5.45±0.05 ^{Ba}	5.69±0.16 ^{Za}	5.92±0.06 ^{Yb}	8.20±0.01 ^{Xa}
	S1	5.82±0.02 ^{Ad}	4.56±0.03 ^{Bbc}	4.22±0.05 ^{Zb}	4.76±0.16 ^{Yc}	5.14±0.17 ^{Xc}
	S2	5.88±0.01 ^{Accd}	3.44±0.27 ^{Bc}	3.69±0.16 ^{Ycd}	4.35±0.03 ^{Xf}	4.44±0.04 ^{Xf}
	S3	5.76±0.03 ^{Ac}	4.07±0.18 ^{Bcd}	3.81±0.09 ^{Zc}	5.40±0.06 ^{Yd}	5.59±0.02 ^{Xd}
	S4	5.94±0.02 ^{Abc}	3.76±0.12 ^{Bde}	3.69±0.16 ^{Ycd}	5.70±0.04 ^{Xc}	5.81±0.02 ^{Xc}
	S5	5.86±0.02 ^{Ad}	3.63±0.37 ^{Bde}	3.37±0.25 ^{Yd}	6.86±0.02 ^{Xa}	6.91±0.01 ^{Xb}
	S6	5.96±0.01 ^{Ab}	5.01±0.12 ^{Bab}	3.83±0.11 ^{Ybc}	5.53±0.04 ^{Xcd}	5.58±0.03 ^{Xd}
Coliform	Control	3.56±0.04 ^b	< 1.00	< 1.00	< 1.00	< 1.00
	S1	2.67±0.05 ^d	< 1.00	< 1.00	< 1.00	< 1.00
	S2	2.38±0.02 ^e	< 1.00	< 1.00	< 1.00	< 1.00
	S3	3.47±0.01 ^b	< 1.00	< 1.00	< 1.00	< 1.00
	S4	3.71±0.01 ^a	< 1.00	< 1.00	< 1.00	< 1.00
	S5	3.35±0.05 ^c	< 1.00	< 1.00	< 1.00	< 1.00
	S6	3.34±0.05 ^c	< 1.00	< 1.00	< 1.00	< 1.00

a, b, c, d (↓) The difference between the means with the same letters is not statistically significant ($p > 0.05$)
X,Y,Z,T (→) The difference between the means with the same letters on the storage days is not statistically significant ($p > 0.05$)

A,B,C,D (→) The difference between the averages with the same letters on the manufacturing days is not statistically significant ($p > 0.05$)

The highest mold-yeast count was determined in the control group. It was found that mold-yeast counts in all sucuk samples decreased during production days ($p < 0.05$). These results also show that the addition of starter culture, which reduces pH and a_w values, also significantly affects mold-yeast growth ($p < 0.05$). At the end of the storage, the mold-yeast counts in all experimental groups were 5-6 log cfu/g. Similarly, Teixeira *et al.* (2009), found the mold-yeast counts in traditional fermented sucuks produced using starter culture to be 5.1 log cfu/g.

Enterobacteriaceae count was determined below 10^2 cfu/g on production (day 0) and the other storage days. The fact that *Enterobacteriaceae* microorganisms were encountered in the groups at the end of fermentation we thought that may be due to the

fact that pH (< 5.3) and a_w (< 0.960) value. Similar results have been determined by other researchers (Wang *et al.*, 2021; Akköse *et al.*, 2023).

3.2 Physicochemical properties of sucuks

The effect of the addition of mixed starter culture to the sucuk formulation at different ratios on the moisture (%), pH, water activity, ash and TBARS values of sucuk groups is given in Table 3. In the early stages of production, the sucuk dough's moisture content ranged from 52.36 to 53.89%. There were no statistically significant differences between the sucuk groups ($p > 0.05$). Ercoşkun, (2010) found that the average moisture content of sucuk dough was among 57.97% and 59.41%. These results were consistent with those obtained in the present study.

Table 3. Physicochemical composition of sucuks.

	Sucuk groups	Production day		Storage day		
		0. day	7. day	7. day	14. day	21. day
Moisture (%)	Control	53.46±1.19 ^{Aa}	34.31±1.16 ^{Ba}	32.86±0.86 ^{Xa}	32.81±0.82 ^{Xa}	28.78±1.20 ^{Ya}
	S1	52.48±1.17 ^{Aa}	27.62±0.80 ^{Bd}	23.88±0.87 ^{Xc}	22.97±0.44 ^{XYc}	22.66±0.36 ^{Yc}
	S2	52.53±0.57 ^{Aa}	28.02±0.78 ^{Bd}	24.42±0.96 ^{Xc}	22.98±0.17 ^{Yc}	22.52±0.08 ^{Yc}
	S3	52.36±0.87 ^{Aa}	29.02±1.06 ^{Bcd}	28.61±0.78 ^{Xb}	22.91±1.09 ^{Yc}	22.25±1.32 ^{Yc}
	S4	53.89±0.25 ^{Aa}	30.57±0.77 ^{Bbc}	28.28±0.84 ^{Xb}	22.62±1.46 ^{Yc}	22.75±0.99 ^{Yc}
	S5	52.61±0.59 ^{Aa}	31.09±0.99 ^{Bbc}	28.69±1.09 ^{Xb}	22.46±0.80 ^{Yc}	21.12±0.77 ^{Yc}
	S6	52.60±0.37 ^{Aa}	32.22±1.30 ^{Bab}	31.78±0.78 ^{Xa}	29.82±0.45 ^{Yb}	24.83±0.71 ^{Zb}
pH	Control	6.21±0.15 ^{Aa}	4.87±0.11 ^{Ba}	4.81±0.13 ^{Xa}	4.78±0.10 ^{Xa}	4.75±0.08 ^{Xa}
	S1	6.17±0.19 ^{Aa}	4.58±0.04 ^{Babc}	4.42±0.02 ^{Xc}	4.41±0.02 ^{Xc}	4.40±0.02 ^{Xc}
	S2	6.16±0.13 ^{Aa}	4.50±0.05 ^{Bbc}	4.44±0.05 ^{Xc}	4.44±0.03 ^{Xc}	4.43±0.02 ^{Xc}
	S3	6.19±0.18 ^{Aa}	4.47±0.04 ^{Bc}	4.45±0.01 ^{Xc}	4.43±0.03 ^{Xc}	4.43±0.04 ^{Xc}
	S4	6.15±0.21 ^{Aa}	4.52±0.04 ^{Bbc}	4.47±0.03 ^{Xbc}	4.47±0.05 ^{Xc}	4.42±0.02 ^{Xc}
	S5	6.16±0.13 ^{Aa}	4.55±0.11 ^{Bbc}	4.53±0.08 ^{Xbc}	4.53±0.04 ^{Xbc}	4.52±0.02 ^{Xbc}
	S6	6.21±0.16 ^{Aa}	4.81±0.31 ^{Bab}	4.71±0.23 ^{Xab}	4.65±0.14 ^{Xab}	4.65±0.12 ^{Xab}
Water activity (a _w)	Control	0.935±0.00 ^{Aa}	0.908±0.00 ^{Ba}	0.890±0.00 ^{Xa}	0.881±0.002 ^{Ya}	0.873±0.00 ^{Za}
	S1	0.931±0.00 ^{Aa}	0.879±0.00 ^{Bc}	0.873±0.00 ^{Xb}	0.869±0.00 ^{Xab}	0.862±0.01 ^{Xa}
	S2	0.933±0.00 ^{Aa}	0.894±0.00 ^{Bb}	0.879±0.00 ^{Xab}	0.876±0.00 ^{Xab}	0.870±0.00 ^{Xa}
	S3	0.931±0.00 ^{Aa}	0.888±0.00 ^{Bbc}	0.884±0.00 ^{Xab}	0.868±0.00 ^{Yb}	0.869±0.00 ^{Ya}
	S4	0.932±0.00 ^{Aa}	0.885±0.00 ^{Bbc}	0.881±0.00 ^{Xab}	0.876±0.00 ^{Xab}	0.862±0.00 ^{Ya}
	S5	0.934±0.00 ^{Aa}	0.894±0.01 ^{Bb}	0.890±0.01 ^{Xa}	0.875±0.01 ^{Xab}	0.869±0.01 ^{Xa}
	S6	0.934±0.00 ^{Aa}	0.892±0.00 ^{Bb}	0.884±0.00 ^{Xab}	0.877±0.00 ^{Yab}	0.872±0.00 ^{Za}
Ash (%)	Control	2.94±0.12 ^{Ba}	4.12±0.62 ^{Aa}	4.31±0.86 ^{Xa}	4.51±1.06 ^{Xa}	4.57±1.13 ^{Xa}
	S1	3.01±0.54 ^{Ba}	4.25±0.50 ^{Aa}	4.34±0.61 ^{Ya}	4.56±0.91 ^{Xa}	4.63±1.00 ^{Xa}
	S2	3.18±0.67 ^{Aa}	3.90±0.67 ^{Aa}	4.04±0.71 ^{Xa}	4.15±0.78 ^{Xa}	4.29±0.95 ^{Xa}
	S3	3.18±0.31 ^{Aa}	3.76±0.92 ^{Aa}	3.98±1.10 ^{Xa}	4.10±1.22 ^{Xa}	4.23±1.19 ^{Xa}
	S4	3.11±0.21 ^{Aa}	3.96±0.85 ^{Aa}	4.05±0.92 ^{Xa}	4.25±1.04 ^{Xa}	4.42±0.75 ^{Xa}
	S5	3.72±0.93 ^{Aa}	3.87±0.80 ^{Aa}	4.05±0.92 ^{Xa}	4.10±0.97 ^{Xa}	4.37±0.74 ^{Xa}
	S6	2.85±0.37 ^{Aa}	3.03±0.43 ^{Aa}	3.65±0.76 ^{Ya}	3.80±0.64 ^{Ya}	4.80±0.15 ^{Xa}
TBARS (µmol/kg)	Control	3.46±0.00 ^{Ab}	3.60±0.04 ^{Ab}	4.06±0.06 ^{Xa}	4.13±0.01 ^{Xa}	4.29±0.08 ^{Xa}
	S1	2.88±0.04 ^{Be}	3.36±0.02 ^{Ad}	3.41±0.04 ^{Xd}	3.40±0.03 ^{Xd}	3.41±0.01 ^{Xc}
	S2	3.16±0.05 ^{Bd}	3.64±0.05 ^{Ab}	3.74±0.02 ^{Yb}	3.66±0.03 ^{Ybc}	3.95±0.05 ^{Xbc}
	S3	3.34±0.02 ^{Abc}	3.52±0.03 ^{Abc}	3.58±0.04 ^{Xbc}	3.54±0.01 ^{Xcd}	3.77±0.14 ^{Xcd}
	S4	3.40±0.03 ^{Abc}	3.52±0.03 ^{Abc}	3.56±0.01 ^{Yc}	3.79±0.06 ^{Xb}	3.83±0.04 ^{Xc}
	S5	3.87±0.04 ^{Aa}	3.86±0.01 ^{Aa}	3.95±0.06 ^{Ya}	4.15±0.04 ^{Xa}	4.18±0.02 ^{Xab}
	S6	3.31±0.01 ^{Ac}	3.43±0.04 ^{Ac}	3.37±0.04 ^{Yd}	3.44±0.05 ^{XYd}	3.53±0.01 ^{Xde}

a, b, c, d (↓) The difference between the means with the same letters is not statistically significant (p>0.05)
 X, Y, Z, T (→) The difference between the means with the same letters on the storage days is not statistically significant (p>0.05)
 A, B, C, D (→) The difference between the averages with the same letters on the manufacturing days is not statistically significant (p>0.05)

The water ratio in sucuk groups produced using starter cultures decreased during the production and storage period and this decrease was determined to be statistically significant (p<0.05). It was reported that using starter culture reduced the moisture level in sucuk samples, and it is easier to remove the water from the product during the ripening period (Kaban *et al.*, 2022). In the present study, the addition of mixed starter culture led to a further pH decrease during the fermentation process (p<0.05). At the end of the

storage period, the S1 (*Lpb. plantarum*+ *Lc. lactis*; 1:1 ratio) group had the lowest pH value (4.40) among the sucuk groups (p<0.05). This value was similar to the pH levels found in the previous study where pH of sucuk was found by Kizilkaya *et al.* (2023) to be below 5.4. The pH level of semi-dry fermented sausages is generally below 5.0 (Sallan & Kaya, 2021). Sucuk pH value affects the structural and sensory qualities, also ensuring the product's microbiological safety (Yilmaz-Oral & Sallan, 2023).

All of the sucuk samples water activity (a_w) values decreased during the production days ($p < 0.05$). The a_w value in sucuk groups containing mixed starter culture decreased faster in the initial days of drying and the water activity in the final product was lower. It was reported that a_w of sucuk values (min:0.880, max:0.950) for fermented sucuk. pH and a_w were considered as two significant hurdles in sausages (Yilmaz-Oral & Sallan, 2023). In this study, the decrease was parallel in a_w of S1 group (0.862) and pH value in during the production days.

In terms of the ash value, there were no statistically significant differences between the experimental groups ($p > 0.05$). Groups S1 and S6 showed an increase in ash value during the storage period. Soyer, (2005), has reported that the ash value increased in all groups related to the drying during production and there were no differences between the starter culture and the control group ash values increased.

Lipid oxidation is one of the main reasons for quality losses in meat products. There were significant differences between the sucuk groups that used starter culture in terms of the TBARS values during the production and storage stages ($p < 0.05$). The rapid pH decrease caused by the activity of starter cultures during fermentation and the strain's lipolytic activity

may be the cause of the increase in TBARS value in the experimental sucuk samples during the storage period. Moreover, the use of starter culture in sucuk decreased the TBARS value compared to the control group. The S1 (*Lpb. plantarum* + *Lc. lactis*; 1:1 ratio) group yielded a lower TBARS value than the other mixed culture groups. It can be considered that the development of the mixed starter cultures used in sausages increased the level of enzymes, which lowers the level of TBARS. It has been reported that factors such as iron content, the distribution of unsaturated fatty acids, pH and antioxidant value affect the level and/or ratio of lipid oxidation (Akköse *et al.*, 2023).

3.3 Colour results

The differences between the L* values in all sucuk groups were significant on days 0 and 14 of the storage ($p < 0.05$). The L* value was higher in the mixed starter groups than in the control groups. Starter cultures exhibited different effects during ripening. It was reported that LAB also increase the L* value in dry-cured meat products (Standnik *et al.*, 2022). L* value decreased in the S1 and S4 groups at the end of ripening compared to the beginning of the storage (Table 4).

Table 4. Color value of sucuks (L*, a*, b*).

	Sucuk	Production day			Storage day		
		7. day			7. day	14. day	21. day
L* value	Control	56.81±0.63 ^c			56.32±2.18 ^{Xa}	56.24±0.22 ^{Xb}	56.08±0.83 ^{Xa}
	S1	58.87±0.52 ^{ab}			58.81±0.20 ^{Xa}	58.76±1.00 ^{Xa}	57.25±0.57 ^{Ya}
	S2	57.84±0.42 ^{bc}			57.77±0.75 ^{Xa}	57.79±0.36 ^{Xab}	57.22±1.19 ^{Xa}
	S3	59.10±0.52 ^a			58.00±0.50 ^{Xa}	57.47±2.33 ^{Xab}	57.38±0.31 ^{Xa}
	S4	58.89±0.05 ^{ab}			58.89±0.18 ^{Xa}	58.82±0.45 ^{Xa}	57.33±0.67 ^{Ya}
	S5	57.35±0.44 ^c			57.16±0.56 ^{Xa}	56.76±1.13 ^{Xab}	56.73±0.48 ^{Xa}
	S6	58.81±0.54 ^{ab}			58.75±5.09 ^{Xa}	56.37±0.52 ^{Xab}	56.18±0.67 ^{Xa}
a* value	Control	23.96±0.57 ^a			23.83±0.70 ^{Xa}	23.76±0.48 ^{Xab}	23.15±0.57 ^{Xa}
	S1	25.12±0.38 ^a			24.68±0.33 ^{Xa}	24.60±0.27 ^{Xab}	24.38±0.65 ^{Xa}
	S2	24.39±0.61 ^a			24.35±0.71 ^{Xa}	24.17±0.63 ^{Xab}	24.25±0.75 ^{Xa}
	S3	24.79±1.21 ^a			24.52±0.97 ^{Xa}	24.19±0.29 ^{Xab}	23.75±0.34 ^{Xa}
	S4	24.60±0.29 ^a			23.31±0.96 ^{Xa}	23.27±0.24 ^{Xb}	23.27±0.20 ^{Xa}
	S5	25.09±0.89 ^a			24.88±0.83 ^{Xa}	24.79±0.21 ^{Xa}	23.61±2.38 ^{Xa}
	S6	24.88±1.53 ^a			23.45±0.42 ^{Xa}	24.72±1.45 ^{Xab}	23.82±1.02 ^{Xa}
b* value	Control	16.18±0.34 ^c			15.90±0.56 ^{Xb}	15.78±0.52 ^{Xa}	15.70±0.38 ^{Xa}
	S1	17.59±0.27 ^a			17.52±0.49 ^{Xa}	17.17±0.40 ^{Xa}	16.90±0.63 ^{Xa}
	S2	17.38±0.62 ^{ab}			16.71±0.42 ^{Xab}	16.61±0.37 ^{Xa}	16.58±0.21 ^{Xa}
	S3	16.55±0.45 ^{bc}			16.29±1.24 ^{Xab}	16.29±0.88 ^{Xa}	16.22±0.14 ^{Xa}
	S4	16.69±0.30 ^{abc}			16.44±0.42 ^{Xab}	16.32±0.35 ^{Xa}	16.16±0.79 ^{Xa}
	S5	16.88±0.47 ^{abc}			16.76±0.56 ^{Xab}	16.57±1.08 ^{Xa}	16.35±0.86 ^{Xa}
	S6	16.76±0.28 ^{abc}			16.11±0.78 ^{Xab}	16.09±0.51 ^{Xa}	16.07±0.94 ^{Xa}

a, b, c, d (↓) The difference between the means with the same letters is not statistically significant ($p > 0.05$)
 X, Y, Z, T (→) The difference between the means with the same letters on the storage days is not statistically significant ($p > 0.05$)

Table 5. Texture value of sucuks.

	Sucuk	Production day			
		Storage day			
		7. day	7. day	14. day	21. day
Hardness (N)	Control	2.54±0.12 ^c	5.89±0.04 ^{Ya}	6.20±0.17 ^{Xa}	6.36±0.17 ^{Xb}
	S1	3.67±0.41 ^a	5.42±1.29 ^{Yab}	6.32±0.35 ^{XYa}	7.43±0.07 ^{Xa}
	S2	3.17±0.16 ^{ab}	4.59±0.12 ^{Zabc}	6.23±0.19 ^{Ya}	7.23±0.16 ^{Xa}
	S3	2.35±0.34 ^c	5.01±0.39 ^{Yabc}	5.36±0.21 ^{Yb}	6.50±0.05 ^{Xb}
	S4	2.40±0.24 ^c	4.27±0.24 ^{Zbc}	5.54±0.31 ^{Yb}	6.44±0.42 ^{Xb}
	S5	2.62±0.17 ^{bc}	3.90±0.80 ^{Yc}	4.58±0.08 ^{Yc}	6.36±0.22 ^{Xb}
	S6	2.53±0.04 ^c	4.04±0.50 ^{Ybc}	4.21±0.48 ^{Yc}	6.45±0.45 ^{Xb}
Adhesiveness (N sec)	Control	1.87±0.35 ^a	2.61±0.19 ^{Xa}	2.62±0.17 ^{Xab}	2.64±0.11 ^{Xbc}
	S1	2.16±0.13 ^a	2.65±0.44 ^{Xa}	2.83±0.05 ^{Xa}	3.13±0.06 ^{Xa}
	S2	2.00±0.18 ^a	2.78±0.28 ^{Xa}	2.96±0.34 ^{Xa}	3.08±0.09 ^{Xa}
	S3	1.86±0.34 ^a	1.96±0.11 ^{Zb}	2.14±0.07 ^{Yc}	2.33±0.05 ^{Xd}
	S4	1.98±0.23 ^a	2.31±0.31 ^{Xab}	2.39±0.05 ^{Xbc}	2.60±0.04 ^{Xbcd}
	S5	1.86±0.34 ^a	2.22±0.17 ^{Yab}	2.46±0.11 ^{XYbc}	2.67±0.30 ^{Xb}
	S6	1.86±0.25 ^a	2.20±0.18 ^{Xab}	2.27±0.05 ^{Xbc}	2.36±0.05 ^{Xcd}
Cohesiveness	Control	0.31±0.01 ^c	0.27±0.01 ^{Xa}	0.28±0.01 ^{Xc}	0.25±0.01 ^{Yb}
	S1	0.32±0.01 ^{bc}	0.33±0.02 ^{Xa}	0.29±0.01 ^{Ybc}	0.27±0.02 ^{Yab}
	S2	0.39±0.01 ^a	0.35±0.07 ^{Xa}	0.32±0.01 ^{Xa}	0.29±0.01 ^{Xa}
	S3	0.29±0.04 ^c	0.31±0.04 ^{Xa}	0.30±0.01 ^{Xab}	0.29±0.01 ^{Xa}
	S4	0.33±0.01 ^{bc}	0.33±0.03 ^{Xa}	0.29±0.01 ^{XYabc}	0.27±0.01 ^{Yab}
	S5	0.33±0.03 ^{bc}	0.30±0.02 ^{Xa}	0.32±0.00 ^{Xa}	0.29±0.01 ^{Xa}
	S6	0.37±0.02 ^{ab}	0.33±0.01 ^{Xa}	0.29±0.02 ^{Ybc}	0.28±0.01 ^{Yab}
Springiness	Control	0.88±0.02 ^a	0.87±0.01 ^{Yb}	0.89±0.02 ^{XYbc}	0.92±0.02 ^{Xbc}
	S1	0.88±0.03 ^a	0.90±0.03 ^{Yab}	0.94±0.01 ^{XYab}	0.97±0.01 ^{Xa}
	S2	0.93±0.01 ^a	0.95±0.04 ^{Xa}	0.95±0.02 ^{Xa}	0.96±0.01 ^{Xabc}
	S3	0.85±0.04 ^a	0.87±0.04 ^{Xb}	0.89±0.02 ^{Xc}	0.92±0.02 ^{Xc}
	S4	0.87±0.03 ^a	0.93±0.02 ^{Xab}	0.93±0.03 ^{Xabc}	0.96±0.01 ^{Xab}
	S5	0.85±0.06 ^a	0.88±0.02 ^{Yb}	0.92±0.03 ^{Xabc}	0.95±0.01 ^{Xabc}
	S6	0.87±0.06 ^a	0.89±0.03 ^{Xab}	0.91±0.01 ^{Xabc}	0.92±0.02 ^{Xc}
Gumminess (N)	Control	1.12±0.26 ^{ab}	1.75±0.05 ^{Ya}	1.81±0.03 ^{Yabc}	1.93±0.03 ^{Xbc}
	S1	1.19±0.09 ^a	1.51±0.03 ^{Zabc}	1.64±0.03 ^{Ycd}	1.87±0.05 ^{Xc}
	S2	1.07±0.17 ^{ab}	1.64±0.35 ^{Yab}	1.95±0.14 ^{Ya}	2.42±0.02 ^{Xa}
	S3	0.72±0.15 ^c	1.27±0.06 ^{Yc}	1.72±0.03 ^{Ybc}	2.52±0.49 ^{Xa}
	S4	0.82±0.08 ^{bc}	1.23±0.02 ^{Zc}	1.48±0.06 ^{Yd}	2.34±0.04 ^{Xab}
	S5	0.86±0.07 ^{abc}	1.22±0.17 ^{Zc}	1.83±0.03 ^{Yab}	2.16±0.08 ^{Xabc}
	S6	0.84±0.12 ^{bc}	1.31±0.03 ^{Zbc}	1.73±0.11 ^{Ybc}	2.09±0.04 ^{Xabc}
Chewiness	Control	0.98±0.21 ^a	1.60±0.04 ^{Xa}	1.19±0.05 ^{XYb}	1.61±0.11 ^{Xc}
	S1	1.05±0.06 ^a	1.55±0.17 ^{Xab}	1.61±0.06 ^{Xa}	1.61±0.06 ^{Xc}
	S2	0.97±0.18 ^a	1.60±0.38 ^{Xa}	1.77±0.16 ^{Xa}	1.98±0.09 ^{Xbc}
	S3	0.63±0.28 ^b	1.61±0.31 ^{Ya}	1.67±0.06 ^{Ya}	2.47±0.43 ^{Xa}
	S4	0.74±0.06 ^{ab}	1.15±0.04 ^{Zab}	1.35±0.04 ^{Yb}	2.28±0.04 ^{Xab}
	S5	0.74±0.09 ^{ab}	1.08±0.16 ^{Zb}	1.77±0.05 ^{Ya}	2.06±0.07 ^{Xb}
	S6	0.73±0.15 ^{ab}	1.14±0.07 ^{Zab}	1.73±0.19 ^{Ya}	2.06±0.04 ^{Xb}

a, b, c, d (↓) The difference between the averages deciphering the same letters is not statistically significant (p>0.05)

X,Y,Z,T (→) The difference between the averages deciphering the same letters on the storage days is not statistically significant (p>0.05)

Reduced L* values in meat products may be related to oxidation-induced browning reactions during storage and ripening periods. In the present study, the highest mean a* value was determined in

S1 (*Lpb. plantarum* + *Lc. lactis*; 1:1 ratio) group. The lowest mean value was observed in the control group. On day 14 of the storage period, the addition of starter culture significantly affected the a* values among the

sucuk groups ($p < 0.05$). The a^* value of sucuk groups during the storage period was found to be unaffected by the starter culture addition ($p > 0.05$). This result demonstrates that color stability is better in the later stages of ripening. The addition of starter culture significantly affected the b^* values between the sucuk groups from the beginning of the production and day 7 of the storage period ($p < 0.05$). It has been reported that the formation and stability of color in cured meat products may vary based on several variables, including pH, reduction potential and the presence and concentration of reducing chemicals (Gökalp *et al.*, 2004; Akköse *et al.*, 2023).

3.4 Texture results

It was determined that the texture of the final sucuk product was significantly affected by the mixed starter cultures use that was added to the sucuk dough (Table 5). During the storage period, the hardness value increased in all sucuk groups ($p < 0.05$). The S1 and S2 sucuk groups had higher hardness values on the last day of storage ($p < 0.05$). It has been reported that the decrease in pH and water activity due to microbial activity during ripening process give final sucuk product its unique texture and consistency that makes it easy to slice (Hwang *et al.*, 2023). In terms of adhesiveness values, a significant difference was determined between the sucuk groups on all days of the storage ($p < 0.05$). On day 21 of the storage, the highest adhesiveness value was determined in the S1 and S2 groups. In terms of cohesiveness values, a significant difference was determined between the sucuk groups on days 14 and 21 of the storage ($p < 0.05$). At the end of the storage, the cohesiveness values of the samples varied between 0.25 and 0.29. It has been reported in a study on various quality parameters of fermented sausages produced using a starter culture (isolated from Siahmazgi cheese) that the cohesiveness values of the samples increased to 0.70 at the onset of the ripening process and dropped to approximately 0.45 at the last day of storage (Kargozari *et al.*, 2014). In the present study, during the whole storage period, there was a significant difference in the springiness values between the samples ($p < 0.05$). At the beginning of production, the springiness value in the experimental groups was determined between 0.85 and 0.93. The springiness value of fermented sausages was determined by Bozkurt & Bayram (2006) to be 0.82 at the beginning of the production process. Also, it was found that the gumminess value differed statistically among the groups on each storage day ($p < 0.05$). On the final day of the storage, the gumminess values of the control group were determined to be lower than those of the experimental sucuk. The S2 and S3 groups had the highest gumminess values at the end of ripening. When starter culture was added to groups S4,

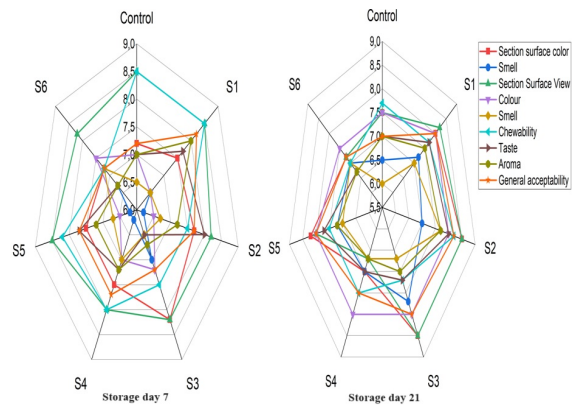


Figure 3. Sensory analysis of sucuks.

S5 and S6, the chewiness values of the sucuk groups increased during storage. This was associated with the increase in pH leading to an increase in drying rate. It has been reported that textural parameters such as hardness, gumminess and chewiness are related to pH and moisture content in parallel with the ripening conditions of sausage (Bozkurt & Bayram, 2006; Hu *et al.*, 2022).

3.5 Sensory assessment

The scores of the sensorial assessment of fermented sucuks at storage period are presented in Figure 3. Panelists in the sensory analysis panel evaluated sucuk groups both raw and cooked sucuks. The sensory analysis results revealed that the section surface color of the raw sucuk groups was affected ($p < 0.05$) by the starter addition at various rates on day 21 of the storage period. In general, small differences were observed between mixed cultures. The total assessment scores between mixed cultures were similar. Other studies, also reported high similarity regarding the flavor and taste profiles of traditional fermented sausages (Pavli *et al.*, 2020; Yilmaz-Oral & Sallan, 2023). Using starter culture during storage in the S1 group had a favorable effect on the color value, according to the sensory analysis of the cooked sucuk groups. The S2 group containing *Lpb. plantarum* + *E. faecium* (1:2 ratio) received a high general acceptability score (8.2) and was the most favored sucuk group by the panelists. The enzyme activities of the used starter cultures may have positively affected the sensory elements of fermented sucuks.

Conclusions

The use of *E. faecium* and *Lactococcus* combined as mix starter cultures containing the *Lactiplantibacillus* genus in sucuk production shortened the fermentation period. Hence, the production process has significantly affected the microbiological, physicochemical and

sensory properties of fermented sucuk. Functional LAB cultures decreased the pH by converting glucose into lactic acid, inhibiting coliform bacteria and can suppress lipid oxidation causing the typical organoleptic properties of the fermented meat product. The hardness and chewiness of the sucuk samples improved during the storage period with the addition of starter culture to the sucuk formulation. The results of the sensory evaluation of the final product revealed that groups created utilizing starter culture had higher sensory evaluation scores than those of the control group in terms of general acceptability. It was concluded that using separately or combined *E. faecium* and *Lc. lactis* with *Lpb. plantarum* as a mix starter culture in the production of Turkish dry-fermented sausage (sucuk) will have a significant effect on the quality parameters of sucuk.

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