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Effect of modified atmosphere and vacuum packaging on some quality characteristics of sliced 'sucuk' produced using probiotics culture

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Abstract: The effects of probiotics (*Lactobacillus acidophilus* and *Bifidobacterium lactis*), packaging (vacuum, 50% N_2 + 50% CO_2 or 100% CO_2), storage temperature (4 and 10 °C) and storage time (90 d) on lipid oxidation, colour and microbial properties of sliced dry fermented sausage 'sucuk', which has a long storage time at low temperatures, was investigated. Probiotic-added samples showed a significant reduction in the thiobarbituric acid-reactive substances (TBARS) values compared with a control and starter culture groups. The analysis showed that control groups had the highest pH and that the pH was not affected by packaging or storage temperature. Probiotic use had a significant effect on total aerobic bacteria, lactic acid bacteria and *Micrococcus*/*Staphylococcus* counts, and bacterial counts decreased with storage time. The Enterobacteriaceae count was below detectable levels (<2.00 CFU g^{-1}) throughout the storage period. The storage temperatures had no effect on the microbial counts. Packaging had a significant effect on lactic acid bacteria, and the highest value was determined with 50% N_2 + 50% CO_2. The addition of probiotics and the storage time resulted in a significant decrease of colour values (L*, a* and b*).
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Keywords: probiotic; *Lactobacillus acidophilus*; *Bifidobacterium lactis*; sucuk; dry fermented sausage; lipid oxidation; colour; microbial properties

INTRODUCTION

Sucuk, which is a dry-fermented meat product, is produced from beef and water buffalo meat, beef fat, sheep tail fat, salt, sugar, various spices, and nitrite, nitrate or nitrite/nitrate. It is the most popular dry-fermented meat product consumed in Turkey.[1,2]

Probiotics are living microbial food ingredients which benefit the health of humans by maintaining or improving their intestinal microbial balance.[3–5] Beneficial effects of specific probiotic microorganisms on human health, such as prevention of gastrointestinal tract infections, immune stimulation, and balancing of the intestinal microflora, have been established in numerous clinical trials. The successful probiotic strains, which are mainly members of the *Lactobacillus* genus and, more recently, the *Bifidobacterium* genus naturally found in the human intestinal tract, have been traditionally incorporated into fermented milk products but also have excellent potential for further inclusion in functional foods and health-related products.[6] Furthermore, probiotics are thought to have a range of potential health benefits, including cholesterol-lowering effects.[7]

Lactic acid bacteria may be able to protect the host by binding microbial toxins in the gastrointestinal tract.[8] Thus, probiotic lactic acid bacteria and bifidobacteria can act as promoters of a strong gut mucosal barrier and improve protection against disease and growth of pathogens.[9] In addition, *Bifidobacterium lactis* has important clinical effects in humans, such as prevention of traveller's diarrhoea, treatment of viral diarrhoea including rotavirus diarrhoea, modulation of intestinal flora, improvement of constipation and modulation of immune response.[5,10,11] *Lactobacillus acidophilus* from the lactic acid bacteria group has been widely utilized as a probiotic in various dairy products industrially.[12–14] *Lactobacillus gasseri* can also be utilized as a starter culture to develop probiotic meat products.[15] In addition *L rhamnosus* GC, E-97800 and LS-25 in fermented sausage[16] and probiotics (*L acidophilus*, *B lactis*) in dry-fermented sausage[17] can be utilized as starter and probiotic cultures.

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Probiotics are currently viewed as the world's biggest functional food products, and the probiotic concept is widely spread in the scientific and industrial fields today. We think that the use of probiotics in sucuk manufacturing as a new meat ingredient can improve human health and nutrition. So, the present study was conducted to determine the effect of commercial probiotics with starter culture used in sucuk manufacturing (control; *Staphylococcus xylosus* + *Pediococcus pentosaceus* (starter culture); starter culture + *B lactis*; starter culture + *L acidophilus*), packaging (vacuum; 50% N_2 + 50% CO_2; 100% CO_2), storage temperature (4 and 10 °C) and storage time (90 d) on the chemical and microbiological properties of sliced sucuk.

MATERIALS AND METHODS

Sucuk batters were manufactured according to the formulation reported by Kaya[1] and Aksu and Kaya.[18] The composition of batters used in sucuk production is shown in Table 1.[1,18] The batters were divided into four groups and starter culture and probiotics were added to each (12 kg) except for the control. The control, starter culture and probiotics groups were as follows: Group 1 (C): control group without starter culture or probiotics; Group 2 (SC): *S xylosus* + *P pentosaceus* (starter culture) (Bactoferm TM T-SPX, CHR HANSEN, Rudolf Müller, Polheim, Germany) without probiotics; Group 3 (SC+La 5): starter culture and *L acidophilus* (La-5, CHR HANSEN A/S, 10–12 Boge Alle, Horsholm, Denmark); Group 4 (SC+Bb 12): starter culture and *B lactis* (Bb-12, CHR HANSEN A/S, Denmark). All sucuk batters were kept at 3–4 °C for 12 h to increase the penetration of the ingredients into the meat. The mixture was then minced (3 mm diameter) and batters were stuffed into natural casings (38 mm diameter, collagen, Naturin Darm, Germany). The ripening programs of the sucuk were as follows: 3 d at 22 ± 1 °C and relative humidity (RH) 90% ± 2%, 4 d at 20 ± 1 °C and RH 85% ± 2%, 5 d at 18 ± 1 °C and RH 80% ± 2%.

The sucuks produced (C, SC, SC+La 5 and SC+Bb 12) were sliced (3–4 mm thick) using a slicing machine at the end of the ripening period.

Table 1. Composition of sucuk batters

Component	Content ($g\,kg^{-1}$)	Compound	Content ($g\,kg^{-1}$ meat and fat)
Beef meat	800	Saccharose	4
Beef fat	100	Red pepper	7
Tallow fat (sheep tail fat)	100	Black pepper	5
		Cumin	9
		Jamaican pepper (pimento)	2.5
		NaCl	25
		Garlic	10
		$NaNO_2$	150 ppm

The sliced sucuks for each group were further divided into three groups, and then the sliced-divided samples were packaged automatically and thermally sealed by a packaging unit (Multivac A 300/16, Sepp Haggenmüller, 87 787, Wolfertschwenden, Germany) in 100–120 g portions at vacuum, 50% N_2 + 50% CO_2 and 100% CO_2 conditions. The bag used for packaging was OPAEVOH/PE, with water vapour transmission rate of $15\,g\,m^{-2}\,(24\,h)^{-1}$ at 38 °C, 90% RH and 1 atm, O_2 transmission rate of $5\,cm^3\,m^{-2}\,(24\,h)^{-1}$ at 23 °C, 50% RH and 1 atm, N_2 transmission rate of $1\,cm^3\,m^{-2}\,(24\,h)^{-1}$ at 23 °C, 50% RH and 1 atm, and CO_2 transmission rate of $23\,cm^3\,m^{-2}\,(24\,h)^{-1}$ at 23 °C, 50% RH and 1 atm. The volume ratio (gas:product) was approximately 1:2.

The sliced-packaged sucuk samples were divided into two according to groups and types of packaging, and samples were stored at 4 and 10 °C for 90 d. The pH, thiobarbituric acid-reactive substances (TBARS), total aerobic bacteria, lactic acid bacteria, *Micrococcus*/*Staphylococcus*, and Enterobacteriaceae counts were observed at 0, 30, 60 and 90 d of storage.

The pH values of the samples were determined with a pH meter (SCHOTT L 6880, Lab Star pH) according to the methods of Gökalp *et al*.[19] The TBARS values were determined according to the methods of Lemon[20] and Kılıç and Richards.[21] In this analysis, 7.5% trichloroacetic acid, 0.01% propyl gallate and 0.01% Na-EDTA made up to 1000 mL with distilled water was used as the extraction solution. A standard curve was prepared using tetraethoxypropane, and the TBARS values were expressed as μmol malonaldehyde kg^{-1} meat.

The surface colour of the slice sucuks was determined by using a chromameter (model CR-200, Minolta, Japan), and expressed in terms of the Hunter colour indexes L^*, a^* and b^*, where L^* indicates the lightness, a^* is the colour axis from green to red and b^* represents the blue to yellow colour axis.

For microbiological analysis, sample solutions were prepared by homogenizing a 25 g sample with 225 mL physiological saline water (0.85% NaCl) in a stomacher (Laboratory Blender Stomacher 400, Seward Medical, London, UK) for 1 min. Total aerobic bacteria was enumerated on Plate Count Agar (Merck) at 30 °C for 3 d. Lactic acid bacteria and Enterobacteriaceae were incubated anaerobically on De Man Rogosa Sharpe Agar (Merck) for 3 d and Violet Red Bile Dextrose Agar (Merck) for 2 d at 30 °C, respectively. *Micrococcus*/*Staphylococcus* was incubated aerobically on Mannitol Salt Phenol-Red Agar (Merck) at 30 °C for 2 d. Bacteria counts were expressed as colony-forming units (CFU) per gram sample.

The experimental data were statistically processed using SPSS version 10.01 software (SPSS, 1996). Data were analyzed with the completely randomized design procedure. Differences between data were tested using the Duncan's multiple range test (significance $P < 0.05$). The model included probiotics (C, SC,

SC+La 5 or SC+Bb 12), packaging (vacuum, 50% N_2 + 50% CO_2 or 100% CO_2), storage temperature (4 or 10 °C), and storage time (0, 30, 60 or 90 d) as the main variables. The results of statistical analyses are shown as mean values ± standard error in the tables.

RESULTS AND DISCUSSION

Probiotics had a significant effect on TBARS values ($P < 0.01$), and the highest TBARS value was found in the control sucuks ($P < 0.05$). Packaging also affected the TBARS values ($P < 0.01$). Vacuum samples had the lowest TBARS value ($P < 0.05$). The Duncan's test showed that the TBARS value was higher in packaged sucuk with 100% CO_2 than in packaged sucuk with 50% CO_2 (Table 2). Also, there was a significant effect on TBARS values for each packaging type in among the probiotics groups and each storage temperature. As with the other groups, the TBARS values were decreased in both *B lactis* vacuum packaged and *L acidophilus* packaged samples with 50% N_2 + 50% CO_2 (Table 3). CO_2, the essential component of any effective modified atmosphere for meat and meat products, is highly soluble in both muscle and fat tissue.[22] However, Lawrie[23] reported that packaging under high concentration of CO_2 suppressed oxidative rancidity in meat products.

It was found that, as the storage temperature increased, the TBARS value also increased ($P < 0.01$). Significantly higher TBARS values were observed in sliced sucuk samples stored at 10 °C compared with samples stored at 4 °C (Table 2). The lowest TBARS value was observed in groups containing *B lactis* and *L acidophilus* at 4 °C (Table 3). The effect of storage time on TBARS values was found to be significant ($P < 0.01$, Table 2). The highest TBARS values of the starter culture were found at 60 days, while those of the probiotic and control groups were found at 90 days of storage time (Table 4).

The probiotic used and the storage period had a significant effect ($P < 0.01$) on the pH, but the packaging and storage temperature did not influence the pH ($P > 0.05$) (Table 2). While the highest pH value was determined in the control group, the lowest value was determined in sucuk with starter culture (*S xylosus* + *P pentosaceus*). During storage, the maximum pH value was determined in the sliced sucuk at 60 days of storage, and then decreased except for the SC+Bb 12 group (Table 2). The highest pH values were found in control groups for both packaging types and storage temperatures (Table 3). Öztürk Er[17] demonstrated that the use of probiotics in sucuk production had no effect on pH values at the end of ripening period. However, the pH of fermented salami produced with *L acidophilus* (4.38) was found to be lower than with *B lactis* (4.87) and *S xylosus* + *P pentosaceus* (4.71).[24]

The probiotic used ($P < 0.01$) and the storage period ($P < 0.01$) had significant effects on total aerobic bacteria (Table 2). The highest total aerobic bacteria count was found in the control group and the lowest value was found in the group with starter culture + *B lactis*. The total aerobic bacteria count decreased during the storage period. The smallest decrease in total aerobic bacteria count was found in the control group, in comparison with the other groups (Table 4). Bozkurt and Erkmen[25] reported that the count of aerobic bacteria in sucuk stored for 45 d decreased from 8.08–8.30 log CFU g^{-1} to 5.70–6.78 log CFU g^{-1}. Although the packaging and storage temperature had an insignificant effect ($P > 0.05$) on the total aerobic bacteria count (Table 2), the control group of packaged samples with 50% N_2 + 50% CO_2 had the highest the total aerobic bacteria count (Table 3).

The probiotic used ($P < 0.01$) and the storage period ($P < 0.01$) affected the *Micrococcus*/*Staphylococcus* count (Table 2). The highest *Micrococcus*/*Staphylococcus* count was found in the probiotic groups ($P < 0.05$). Although the *Micrococcus*/*Staphylococcus* counts decreased in all samples up to 30 d, the maximum decrease was found in the group with starter culture (Table 4). When compared with the control group, the *Micrococcus*/*Staphylococcus* counts of groups with *B lactis* and *L acidophilus* were higher in sucuk stored at 10 °C than in sucuk stored at 4 °C (Table 3).

The probiotic used had a significant effect on lactic acid bacteria count ($P < 0.01$). The highest lactic acid bacteria count was found in control sucuks ($P < 0.05$), and the lowest count was found in the group with *S xylosus* + *P pentosaceus* + *B lactis*. Packaging ($P < 0.01$) and storage time ($P < 0.01$) also had a significant influence on lactic acid bacteria count. The lowest lactic acid bacteria count was found at the end of the storage period (Table 2). The vacuum, 50% N_2 + 50% CO_2 and 100% CO_2 packaging affected the lactic acid bacteria count ($P < 0.01$) (Table 3). The lowest count was determined in the group with 100% CO_2 at the end of the storage period. Lawrie[23] indicated that packaging meat products under high concentrations of CO_2 suppressed microbiological growth. Öztürk Er[17] found that the counts of lactic acid bacteria at the end of the ripening period (12 d) in control sucuks, those with *S xylosus* + *P Pentosaceus*, those with *S xylosus* + *P pentosaceus* + *B lactis*, and those with *S xylosus* + *P pentosaceus* + *L acidophilus* were 7.78, 8.15, 8.36 and 8.33 CFU g^{-1}, respectively.

The Enterobacteriaceae counts of sliced sucuks were found to be under the detectable level (<2.00 CFU g^{-1}) throughout the storage period. An important property of probiotic strains is their antagonistic activity against pathogenic bacteria, by competitive exclusion, interbacterial aggregation or production of antimicrobial substances such as organic acids, bacteriocins and hydrogen peroxide.[1,6,26] However, Bifidobacteria are known to inhibit the growth of various pathogens *in vitro* and Bifidobacteria produce antimicrobial agents active against Gram-negative and Gram-positive

Table 2. Overall effect of probiotics, packaging, storage temperature and storage time on the pH, TBARS, microbiological counts and colour of sucuk (values are means ± SE)

	TBARS (μmol malonaldehyde kg^{-1})	pH	Total aerobic bacteria count (log CFU g^{-1})	*Micrococcus/ Staphylococcus* count (log CFU g^{-1})	Lactic acid bacteria count (log CFU g^{-1})	L*	a*	b*
Probiotics[A]								
Control	30.41 ± 0.02 a	4.83 ± 0.01 a	8.65 ± 0.03 a	4.57 ± 0.02 c	8.66 ± 0.03 a	38.6 ± 0.2 a	15.0 ± 0.1 a	9.25 ± 0.12 a
SC	29.10 ± 0.02 b	4.70 ± 0.01 c	8.52 ± 0.03 bc	4.58 ± 0.02 c	8.42 ± 0.03 b	37.0 ± 0.2 c	15.1 ± 0.1 a	8.45 ± 0.12 b
SC+La 5	27.45 ± 0.02 c	4.74 ± 0.01 b	8.56 ± 0.03 ab	4.83 ± 0.02 ab	8.42 ± 0.03 b	38.0 ± 0.2 b	14.4 ± 0.1 b	8.20 ± 0.12 b
SC+Bb 12	26.36 ± 0.02 c	4.74 ± 0.01 b	8.45 ± 0.03 c	4.79 ± 0.02 b	8.18 ± 0.03 c	36.6 ± 0.2 c	13.7 ± 0.1 c	7.50 ± 0.12 c
Significance	**	**	**	**	**	**	**	**
Packaging type[B]								
Vacuum	27.40 ± 0.02 c	4.75 ± 0.01	8.54 ± 0.03	4.74 ± 0.02	8.40 ± 0.02 b	37.3 ± 0.2 b	14.5 ± 0.1	7.77 ± 0.10 b
50% N2 + 50% CO_2	28.20 ± 0.02 b	4.76 ± 0.01	8.57 ± 0.03	4.79 ± 0.02	8.79 ± 0.02 a	37.9 ± 0.2 a	14.5 ± 0.1	8.72 ± 0.10 a
100% CO2	29.39 ± 0.02 a	4.76 ± 0.01	8.53 ± 0.03	4.76 ± 0.02	8.38 ± 0.02 b	37.4 ± 0.2 b	14.6 ± 0.1	8.56 ± 0.10 a
Significance	**	NS	NS	NS	**	**	NS	**
Storage temperature[C]								
4 °C	26.85 ± 0.02 b	4.75 ± 0.01	8.56 ± 0.02	4.75 ± 0.02	8.42 ± 0.02	37.2 ± 0.1 b	14.8 ± 0.1 a	8.17 ± 0.08 b
10 °C	29.81 ± 0.02 a	4.76 ± 0.01	8.53 ± 0.02	4.78 ± 0.02	8.42 ± 0.02	37.9 ± 0.1 a	14.3 ± 0.1 b	8.52 ± 0.08 a
Significance	**	NS	NS	NS	NS	*	*	*
Storage time[D]								
0 d	13.61 ± 0.02 d	4.72 ± 0.01 c	8.97 ± 0.03 a	5.71 ± 0.02 a	8.93 ± 0.03 a	39.4 ± 0.2 a	19.1 ± 0.1 a	10.50 ± 0.12 a
30 d	25.46 ± 0.02 c	4.69 ± 0.01 d	8.62 ± 0.03 b	4.59 ± 0.02 b	8.53 ± 0.03 b	37.1 ± 0.2 b	13.7 ± 0.1 b	7.28 ± 0.12 c
60 d	37.44 ± 0.02 a	4.83 ± 0.01 a	8.50 ± 0.03 c	4.56 ± 0.02 b	8.19 ± 0.03 c	37.4 ± 0.2 b	12.8 ± 0.1 c	7.90 ± 0.12 b
90 d	36.81 ± 0.02 b	4.78 ± 0.01 b	8.09 ± 0.03 d	4.20 ± 0.02 c	8.04 ± 0.03 d	36.2 ± 0.2 c	12.6 ± 0.1 c	7.70 ± 0.12 b
Significance	**	**	**	**	**	**	**	**

[A] Results are the mean of packaging type, storage temperature and storage time.
[B] Results are the mean of probiotics, storage temperature and storage time.
[C] Results are the mean of probiotics, packaging type and storage time.
[D] Results are the mean of probiotics, packaging type and storage temperature.
SC (starter culture) = *Staphylococcus xylosus* + *Pediococcus pentosaceus*; SC+La 5 = *S xylosus* + *P pentosaceus* + *Lactobacillus acidophilus*; SC+Bb 12 = *S xylosus* + *P pentosaceus* + *Bifidobacterium lactis*.
a–d: Any two means in the same column having the same letters in the same section are not significantly different at $P > 0.05$.
** $P < 0.01$; * $P < 0.05$; NS = non-significant; SE = standard error.

Table 3. TBARS values, pH, microbial counts and color values of sucuk treated with probiotics in different packaging type and storage temperatures (values are means ± SE)[A]

	Packaging type			Storage temperature	
Probiotics	Vacuum	50% N_2 + 50% CO_2	100% CO_2	4 °C	10 °C
TBARS (μmol malonaldehyde kg^{-1})					
Control	30.23 ± 0.05 a	28.81 ± 0.04 b	32.18 ± 0.04 a	30.24 ± 0.03 a	30.59 ± 0.04 b
SC	28.63 ± 0.05 b	30.34 ± 0.04 a	28.34 ± 0.04 b	28.65 ± 0.03 b	29.55 ± 0.04 c
SC+La 5	24.82 ± 0.05 d	28.80 ± 0.04 b	28.72 ± 0.04 b	23.71 ± 0.03 d	31.19 ± 0.04 a
SC+Bb 12	25.91 ± 0.05 c	24.84 ± 0.04 c	28.34 ± 0.04 b	24.80 ± 0.03 c	27.92 ± 0.04 d
Significance	**	**	**	**	**
pH					
Control	4.82 ± 0.02 a	4.83 ± 0.02 a	4.84 ± 0.02 a	4.84 ± 0.01 a	4.82 ± 0.01 a
SC	4.70 ± 0.02 b	4.72 ± 0.02 b	4.71 ± 0.02 b	4.70 ± 0.01 b	4.70 ± 0.01 b
SC+La 5	4.74 ± 0.02 b	4.73 ± 0.02 b	4.74 ± 0.02 b	4.74 ± 0.01 b	4.73 ± 0.01 b
SC+Bb 12	4.74 ± 0.02 b	4.75 ± 0.02 b	4.75 ± 0.02 b	4.74 ± 0.01 b	4.75 ± 0.01 b
Significance	**	**	**	**	**
Total aerobic bacteria count (log CFU g^{-1})					
Control	8.54 ± 0.05 b	8.82 ± 0.05 a	8.58 ± 0.06	8.76 ± 0.04 a	8.53 ± 0.04
SC	8.45 ± 0.05 b	8.51 ± 0.05 b	8.61 ± 0.06	8.48 ± 0.04 b	8.55 ± 0.04
SC+La 5	8.70 ± 0.05 a	8.45 ± 0.05 b	8.54 ± 0.06	8.56 ± 0.04 b	8.56 ± 0.04
SC+Bb 12	8.47 ± 0.05 b	8.49 ± 0.05 b	8.39 ± 0.06	8.44 ± 0.04 b	8.46 ± 0.04
Significance	**	*	NS	*	NS
Micrococcus/Staphylococcus Count (log CFU g^{-1})					
Control	4.78 ± 0.04	4.94 ± 0.04 a	4.88 ± 0.04 a	4.90 ± 0.03 a	4.83 ± 0.03 a
SC	4.63 ± 0.04	4.58 ± 0.04 c	4.54 ± 0.04 b	4.59 ± 0.03 c	4.58 ± 0.03 b
SC+La 5	4.72 ± 0.04	4.91 ± 0.04 a	4.86 ± 0.04 a	4.78 ± 0.03 b	4.88 ± 0.03 a
SC+Bb 12	4.85 ± 0.04	4.75 ± 0.04 b	4.76 ± 0.04 a	4.73 ± 0.03 b	4.84 ± 0.03 a
Significance	NS	**	*	**	*
Lactic acid bacteria count (log CFU g^{-1})					
Control	8.50 ± 0.04 a	8.78 ± 0.04 a	8.70 ± 0.06 a	8.83 ± 0.04 a	8.50 ± 0.04 a
SC	8.31 ± 0.04 b	8.47 ± 0.04 b	8.49 ± 0.06 a	8.30 ± 0.04 b	8.55 ± 0.04 a
SC+La 5	8.47 ± 0.04 a	9.36 ± 0.04 ba	8.44 ± 0.06 a	8.35 ± 0.04 b	8.50 ± 0.04 a
SC+Bb 12	8.30 ± 0.04 b	8.34 ± 0.04 b	7.90 ± 0.06 b	8.22 ± 0.04 b	8.14 ± 0.04 b
Significance	**	**	**	**	*
L* values					
Control	38.0 ± 0.4 a	38.6 ± 0.2 a	39.2 ± 0.3 a	38.2 ± 0.2 a	39.0 ± 0.2 a
SC	36.5 ± 0.4 a	37.9 ± 0.2 ab	36.6 ± 0.3 b	37.1 ± 0.2 b	36.9 ± 0.2 b
SC+La 5	38.5 ± 0.4 a	38.3 ± 0.2 a	37.2 ± 0.3 b	37.2 ± 0.2 b	38.8 ± 0.2 a
SC+Bb 12	36.3 ± 0.4 b	36.8 ± 0.2 b	36.6 ± 0.3 b	36.2 ± 0.2 c	36.9 ± 0.2 b
Significance	*	**	**	**	**
a* values					
Control	15.1 ± 0.1 a	15.0 ± 0.1 a	15.0 ± 0.2 a	15.3 ± 0.1 a	14.7 ± 0.1 a
SC	15.3 ± 0.1 a	14.7 ± 0.1 a	15.2 ± 0.2 a	15.3 ± 0.1 a	14.8 ± 0.1 a
SC+La 5	14.3 ± 0.1 b	14.4 ± 0.1 a	14.5 ± 0.2 b	14.7 ± 0.1 b	14.1 ± 0.1 b
SC+Bb 12	13.5 ± 0.1 c	13.8 ± 0.1 b	13.7 ± 0.2 c	13.7 ± 0.1 c	13.6 ± 0.1 c
Significance	**	**	**	**	**
b* values					
Control	8.78 ± 0.19 a	9.43 ± 0.25 a	9.53 ± 0.17 a	9.05 ± 0.15 a	9.44 ± 0.18 a
SC	7.96 ± 0.19 b	8.76 ± 0.25 a	8.63 ± 0.17 ab	8.52 ± 0.15 b	8.38 ± 0.18 b
SC+La 5	7.44 ± 0.19 b	8.77 ± 0.25 a	8.37 ± 0.17 b	7.85 ± 0.15 c	8.54 ± 0.18 b
SC+Bb 12	6.90 ± 0.19 c	7.89 ± 0.25 b	7.71 ± 0.17 c	7.27 ± 0.15 d	7.73 ± 0.18 c
Significance	*	*	**	**	**

[A] Results are the mean of storage time.

SC (starter culture) = *Staphylococcus xylosus* + *Pediococcus pentosaceus*; SC+La 5 = *S xylosus* + *P pentosaceus* + *Lactobacillus acidophilus*; SC+Bb 12 = *S xylosus* + *P pentosaceus* + *Bifidobacterium lactis*.

a–c: Any two means in the same column having the same letters in the same section are not significantly different at $P > 0.05$.

** $P < 0.01$; * $P < 0.05$; NS = $P > 0.05$; SE = standard error.

Table 4. Changes over the storage period in TBARS values, pH, microbial counts and color values of sucuk with probiotics (mean ± SE)[A]

Probiotics	Day 0	Day 30	Day 60	Day 90	Significance
TBARS (μmol malonaldehyde kg^{-1})					
Control	13.32 ± 0.06 d	29.73 ± 0.06 c	37.09 ± 0.06 b	41.52 ± 0.06 a	**
SC	13.60 ± 0.04 d	24.87 ± 0.04 c	40.30 ± 0.04 a	37.64 ± 0.04 b	**
SC+La 5	13.92 ± 0.05 d	24.39 ± 0.05 c	32.10 ± 0.05 b	39.38 ± 0.05 a	**
SC+Bb 12	13.60 ± 0.05 d	22.86 ± 0.05 c	32.98 ± 0.05 b	36.00 ± 0.05 a	**
pH					
Control	4.79 ± 0.02 c	4.75 ± 0.02 c	4.92 ± 0.02 a	4.86 ± 0.02 b	**
SC	4.64 ± 0.02 c	4.67 ± 0.02 c	4.79 ± 0.02 a	4.74 ± 0.02 b	**
SC+La 5	4.68 ± 0.02 c	4.68 ± 0.02 c	4.82 ± 0.02 a	4.77 ± 0.02 b	**
SC+Bb 12	4.78 ± 0.02 a	4.65 ± 0.02 b	4.79 ± 0.02 a	4.75 ± 0.02 a	**
Total aerobic bacteria count (log CFU g^{-1})					
Control	8.85 ± 0.06 b	9.03 ± 0.06 a	8.69 ± 0.06 b	8.02 ± 0.06 c	**
SC	8.85 ± 0.06 a	8.58 ± 0.06 b	8.54 ± 0.06 b	8.10 ± 0.06 c	**
SC+La 5	9.20 ± 0.07 a	8.38 ± 0.07 b	8.39 ± 0.07 b	8.27 ± 0.07 b	**
SC+Bb 12	8.98 ± 0.06 a	8.50 ± 0.06 b	8.38 ± 0.06 b	7.95 ± 0.06 c	**
Micrococcus/Staphylococcus count (log CFU g^{-1})					
Control	5.92 ± 0.05 a	4.92 ± 0.05 b	4.59 ± 0.05 c	4.03 ± 0.05 d	**
SC	5.63 ± 0.05 a	4.32 ± 0.05 b	4.38 ± 0.05 b	4.00 ± 0.05 c	**
SC+La 5	5.68 ± 0.03 a	4.51 ± 0.03 c	4.70 ± 0.03 b	4.43 ± 0.03 c	**
SC+Bb 12	5.64 ± 0.04 a	4.62 ± 0.04 b	4.57 ± 0.04 b	4.32 ± 0.04 c	**
Lactic acid bacteria count (log CFU g^{-1})					
Control	8.86 ± 0.03 a	8.86 ± 0.03 a	8.53 ± 0.03 b	8.39 ± 0.03 b	**
SC	8.91 ± 0.05 a	8.55 ± 0.05 b	8.18 ± 0.05 c	8.05 ± 0.05 c	**
SC+La 5	9.10 ± 0.06 a	8.32 ± 0.06 b	8.20 ± 0.06 ab	8.08 ± 0.06 c	**
SC+Bb 12	8.85 ± 0.07 a	8.40 ± 0.07 b	7.83 ± 0.07 c	7.64 ± 0.07 c	**
L* values					
Control	40.8 ± 0.4 a	38.9 ± 0.4 b	37.9 ± 0. ab	36.8 ± 0.4 c	**
SC	38.8 ± 0.3 a	36.9 ± 0.3 b	37.1 ± 0.3 b	35.2 ± 0.3 c	**
SC+La 5	39.4 ± 0.4 a	36.7 ± 0.4 c	38.3 ± 0.4 ab	37.5 ± 0.4 bc	*
SC+Bb 12	38.6 ± 0.3 a	35.8 ± 0.3 c	36.4 ± 0.3 b	35.3 ± 0.3 c	**
a* values					
Control	19.8 ± 0.2 a	14.0 ± 0.2 b	13.1 ± 0.2 c	13.1 ± 0.2 c	**
SC	18.9 ± 0.2 a	14.58 ± 0.2 b	13.6 ± 0.2 c	13.4 ± 0.2 c	**
SC+La 5	19.3 ± 0.1 a	13.5 ± 0.1 b	12.5 ± 0.1 c	12.3 ± 0.1 c	**
SC+Bb 12	18.3 ± 0.1 a	12.8 ± 0.1 b	11.7 ± 0.1 c	11.8 ± 0.1 c	**
b* values					
Control	11.74 ± 0.25 a	8.46 ± 0.25 b	8.44 ± 0.25 b	8.36 ± 0.25 b	**
SC	10.53 ± 0.21 a	7.57 ± 0.21 c	8.32 ± 0.21 b	7.39 ± 0.21 c	**
SC+La 5	10.22 ± 0.25 a	6.97 ± 0.25 c	7.75 ± 0.25 b	7.85 ± 0.25 b	**
SC+Bb 12	9.50 ± 0.23 a	6.12 ± 0.23 c	7.24 ± 0.23 b	7.24 ± 0.23 b	**

[A] Results are the mean of storage temperature and packaging type.
SC (starter culture) = *Staphylococcus xylosus* + *Pediococcus pentosaceus*; SC+La 5 = *S xylosus* + *P pentosaceus* + *Lactobacillus acidophilus*; SC+Bb 12 = *S xylosus* + *P pentosaceus* + *Bifidobacterium lactis*.
a–d: Any two means in the same line having the same letters in the same section are not significantly different at $P > 0.05$.
** $P < 0.01$; * $P < 0.05$; SE = standard error.

pathogens.[7,27] The growth of pathogens in fermented salami with *L acidophilus*, *B lactis* and *S xylosus* + *P pentosaceus* was found to decrease during fermentation.[24] Furthermore, Enterobacteriaceae counts in sucuks with probiotics (*L acidophilus*, *B lactis*) were found to decrease more rapidly than in control and starter culture groups.[17] However, modified atmospheres containing CO_2 have been shown to inhibit the normal aerobic spoilage bacteria and the possible growth of psychrotrophic food pathogens.[28] Gram-negative bacteria (aerobic-psychrotrophic) were found to be markedly inhibited in modified atmosphere package, and pathogen growth was less in modified atmosphere package than in vacuum packaging.[29]

Some researchers who have carried out studies on the microbiological counts of sucuk without probiotics produced in Turkey have reported similar findings in their control sucuks.[1,18,30,31] For instance, Aksu and Kaya[18] indicated that the count of total aerobic, lactic acid, *Micrococcus*/*Staphylococcus* and Enterobacteriaceae in Turkish dry-fermented sausage were 8.02, 7.92, 5.71 and <2.00 log CFU g^{-1}, respectively.

The L* values were significantly affected by probiotic treatment ($P < 0.01$), packaging ($P < 0.01$), storage temperature ($P < 0.05$) and storage time ($P < 0.01$) (Table 2). The use of *L acidophilus, B lactis* and starter culture in sucuk production decreased the L* values ($P < 0.05$), and the highest L* value was found in the control group. Differences in L* values were observed among packagings, and L* values in vacuum and 100% CO_2 were lower ($P < 0.05$) than in 50% N_2 + 50% CO_2 packaging. L* values in sucuks stored at 4 °C were lower ($P < 0.05$) than in the 10 °C groups. L* values decreased during the storage period and the lowest value was determined at the end of storage (Table 2). L* values rapidly decreased in the control, the *S xylosus + P pentosaceus + L acidophilus* and the *S xylosus + P pentosaceus + B lactis* groups stored at 4 °C, and there was no difference in the *S xylosus + P pentosaceus* groups between 4 and 10 °C (Table 3). The a* values were affected by probiotic treatment ($P < 0.01$), storage temperature ($P < 0.05$) and storage time ($P < 0.01$) (Table 2). The use of probiotics in sucuk production reduced the a* values ($P < 0.05$), and the highest a* value was found in the control and starter culture groups. The a* values in sucuks stored at 10 °C were lower ($P < 0.05$) than in the 4 °C groups. The a* values decreased during the storage period in all probiotic groups (Table 4). The yellowness (b*) was also affected by probiotic treatment ($P < 0.01$), packaging ($P < 0.01$), storage temperature ($P < 0.05$) and storage time ($P < 0.01$). The highest yellowness value was in the control group, and the use of starter culture and probiotic in sucuk production reduced the b* values ($P < 0.05$) (Table 3). The b* and TBARS values of the control group sucuks were higher than those in the other groups (Table 2).

The present study thus indicated that there was high correlation between a* and b* values and lipid oxidation. In this study, while lipid oxidation in the control group increased, the b* values also increased. Lipid oxidation may initiate the oxidation of myoglobin to metmyoglobin, and the change of meat colour from red to unattractive brown, so the rate of meat discoloration is closely related to the rate of myoglobin oxidation induced by lipid oxidation.[32] On the other hand, carotenoid pigments of meat and tail fats in sucuk batters may have an effect on b* value. The b* values in vacuum were lower ($P < 0.05$) than in 50% N_2 + 50% CO_2 and in 100% CO_2 (Table 2), and this was probably because of (1) the greater quantity of oxygen in the modified atmosphere packaging compared with vacuum samples, and (2) lipid oxidation being prevented by vacuum packaging. The b* values in sucuks stored at 4 °C were lower ($P < 0.05$) than in the 10 °C groups. The b* values decreased until 30 d of storage, and then increased (Table 2). The lowest b* values at the end of the storage period were found in the probiotic sucuks (Table 4). Öztürk Er[17] reported that *b values were low in sucuks with or without probiotics during the ripening period, while L* and a* values showed no change. Furthermore, Öztürk Er[17] established that L*, a* and b* values of ripened sucuks with *S xylosus + P pentosaceus + B lactis* and with *S xylosus + P pentosaceus + L acidophilus* were 39.6–42.7, 16.3–19.3 and 9.0–10.2, and 38.6–45.1, 17.3–18.3 and 9.5–12.2, respectively. Üren and Babayiğit[33] found that L*, a* and b* values of sausages obtained from various meat plants in Turkey were 35.9–45.9, 6.9–14.1 and 10.0–17.6, respectively. Kayaardı and Gök[34] found that the L* values of sucuk samples were in the range 41.5–45.3, the a* values between 7.8 and 12.3, and the b* values between 7.6 and 13.8. Aksu[35] reported that the mean colour values at the end of the ripening period of control sucuk were L* = 44.2, a* = 22.3 and b* = 12.1.

CONCLUSIONS

The results from this study showed that starter cultures with probiotics had significant effects on sliced sausages in vacuum or modified atmosphere packaging during storage. The use of probiotics in sausage manufacture prevented lipid oxidation, especially in samples vacuum-stored at 4 °C and/or packaged with 50% N_2 + 50% CO_2. The preventative effect of probiotics on lipid oxidation resulted in both the blocking of myoglobin oxidation and low a* and b* values in the probiotic-treated products. In addition, as lipid oxidation increased, the b* value increased. On the other hand, it was a positive result because the Enterobacteriaceae counts during storage were found to be below the limits of detection.

In conclusion, *Bifidobacterium lactis* and *Lactobacillus acidophilus* can be used together with starter cultures in sausage manufacture as probiotic sources. This procedure is suitable for a large-scale public consumption of probiotic bacteria together with fermented meat products. The probiotic-treated sausages may be submitted for consumption as 'functional meat products'.

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