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Production of Functional Fermented Sausage from Goat Meat with the Addition of Lactulose, *Lactobacillus acidophilus*, and *Bifidobacterium animalis*

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Abstract

The main goal of this study is to create safe and functional fermented sausages (Turkish dry-fermented sausage) from goat meat by combining probiotics and lactulose (1%) with traditional starter cultures. Group A was created with sausage dough to which only starter culture and spice mixture were added. *Bifidobacterium animalis* and *Lactobacillus acidophilus* cultures were added to group B, and 1% lactulose was added to group C, in addition to the control group's combination. By adding *Bifidobacterium animalis* and *Lactobacillus acidophilus* cultures, as well as lactulose, to group D, four distinct sausage samples were formed. Lactic acid bacteria levels increased throughout ripening, ranging from 5.27 to 6.98, and remained steady during storage, from 4.96 to 5.84. During ripening, the quantity of *B. animalis* increased, especially in groups B and D, which included lactulose. Water activity decreased during ripening and further decreased during storage. The latest water activity values fell below 0.79. pH values also decreased during ripening and storage. The final pH values were measured between 5.31 and 5.42. When the physico-chemical properties such as pH, dry matter, and water activity of the sausage samples were evaluated throughout the shelf life, although significant differences were observed in these parameters between the groups, they could not be associated with probiotic and prebiotic contributions. On the last day of storage, the samples with the addition of probiotic cultures and lactulose showed the lowest hardness values. In sensory analyses, it was observed that the groups containing lactulose received the highest scores and were generally accepted. The data obtained showed that adding probiotics and prebiotics to sausage caused positive changes in the quality of the product.

Anahtar Kelimeler: *Bifidobacterium animalis*, goat sausage, lactulose, *Lactobacillus acidophilus*

Laktuloz, *Lactobacillus acidophilus* ve *Bifidobacterium animalis* İlavesiyle Keçi Etinden Fonksiyonel Fermente Sucuk Üretimi

Öz

Bu çalışmanın temel amacı, geleneksel starter kültürlerin yanında probiyotik ve laktuloz (%1) eklenmesiyle keçi etinden güvenli ve fonksiyonel niteliklere sahip fermente sucuk elde etmektir. Yalnızca starter kültür ve baharat karışımı eklenerek A grubu sucuk örnekleri oluşturuldu. B grubuna *Bifidobacterium animalis* ve *Lactobacillus acidophilus* kültürleri eklenirken C grubuna kontrol grubunun kombinasyonuna ek olarak %1 laktuloz ilave edildi. Grup D, probiyotik kültürlerinin yanı sıra laktuloz da eklenerek dört farklı sucuk örneği oluşturuldu. Laktik asit bakterilerinin miktarı, olgunlaşma süresince artış göstermiştir. Laktik asit bakterilerinin son seviyeleri 5.27 ile 6.98 arasında değişmiştir. Depolama süresince bu seviyeler 4.96 ile 5.84 arasında sabit kalmıştır. *B. animalis* miktarı, olgunlaşma süresince artmış ve özellikle laktuloz içeren B ve D gruplarında yüksek seviyelere ulaşmıştır. Su aktivitesi olgunlaşma süresince düşmüş ve depolama süresince daha da azalmıştır. Son su aktivitesi değerleri 0.79'un altına inmiştir. pH değerleri, olgunlaşma ve depolama süresince de düşmüştür. Son pH değerleri 5.31 ile 5.42 arasında ölçülmüştür. Raf ömrü boyunca sucuk örneklerinin pH, kurumadde ve su aktivitesi gibi fiziko-kimyasal özellikleri değerlendirildiğinde gruplar arası bu parametrelerde anlamlı farklılıklar gözlemlense de probiyotik ve prebiyotik katkıları ile ilişkilendirilememiştir. Muhafazanın son gününde, probiyotik kültürler ve laktuloz ilave örneklerin en düşük sertlik değerine sahip olduğu tespit edilmiştir. Duyusal analizlerde, özellikle laktuloz içeren grupların en yüksek puanları aldığı ve genel olarak kabul gördüğü gözlemlenmiştir. Elde edilen veriler, sucuğa probiyotik ve prebiyotik eklenmesi ile ürünün niteliklerinde olumlu değişimler meydana getirdiğini göstermiştir.

Key Words: *Bifidobacterium animalis,* keçi sucuğu, laktuloz, *Lactobacillus acidophilus*

INTRODUCTION

Fermented sausage which has been produced for a long time, is characterized as minced meat combined with salt and curing agents, placed into casings, and exposed to a fermentation process in which microorganisms play a crucial role (1-3). While small-scale manufacturers frequently employ traditional processes and natural air drying to produce these butcher sausages locally, large-scale manufacturers utilize modern technology to manage the fermentation process (4).

In the recent decade, the increased use of starter cultures has ensured product safety by restricting the proliferation of uncultivated microflora, lowering the danger of

pathogenic and spoilage bacteria, preserving stability and shelf life, and improving sensory qualities. Starter cultures are mostly composed of lactic acid bacteria (LAB), including *Lactobacillus sakei* and *Lactobacillus curvatus*, and also coagulase-negative staphylococci such as *Staphylococcus xylosus, Staphylococcus carnosus,* and Micrococcaceae members (5). With lactic acid fermentation, the pH value of the product decreases; It helps create the color, taste, and texture of the product (6).

In recent years, numerous innovative methods have been developed to improve the nutritional value, flavors, and shelf life of traditional meat and meat products (7). One of these methods is the use of probiotics. Some researchers asserted that meat provides an ideal habitat for the proliferation of probiotic bacteria (8). Because of these considerations, the addition of probiotics to meat products was seen positively, resulting in the commercial production of many LAB-containing products (*L. acidophilus, L. casei,* and *Bifidobacterium* spp.) (9). Prebiotics are food components necessary for the growth of probiotic microorganism species (such as *Bifidobacterium*/*Lactobacillus*), which cannot be digested in the upper gastrointestinal tract but can be fermented in the colon (10). Prebiotic saccharides such as fructooligosaccharides and galactooligosaccharides are highly suggested for use in fermented meat products because they increase the growth of bifidobacterial (11). Numerous in *in vivo* studies have demonstrated that the administration of lactulose, an important prebiotic, leads *Bifidobacterium* species to become dominant in the environment and have a positive effect on the gut flora (12,13).

Various research suggests that different meats can be used in the preparation of fermented sausages (1, 14). Although it is an important source of red meat in underdeveloped countries, goat meat consumption is higher globally than the consumption of beef (15). In addition to being an essential source of readily available protein, goat meat has recently drawn consumers from all over the world looking for low-fat, low-calorie, and nutritious meat (16). While there is a common belief that goat meat may be met with prejudice due to its unique aroma and taste, research involving both expert and non-expert taste panels has shown that such biases do not hold (17).

This study aims to obtain functional fermented sausage by using probiotic bacteria (*Lactobacillus acidophilus* and *Bifidobacterium animalis*) and prebiotic (lactulose) along with traditional starter cultures in goat sausage production and to examine the quality parameters of the product during ripening.

MATERIAL AND METHODS

Probiotic Strains and Culture Conditions

Bifidobacterium animalis and *Lactobacillus acidophilus* cultures were purchased from Chr. Hansen (Hørsholm, Denmark). Both probiotics were separately incubated in MRS broth (Merck, Darmstadt, Germany) at 37 °C for 24 h, then centrifuged at 5000× g for 15 min and washed with physiological saline. After this procedure, which was performed twice, they were diluted with physiological saline to obtain a suspension containing 10 10 cfu/mL. At least 7 log cfu/g of each strain was added to the sausage dough from the solutions.

Sausage Manufacture

Burdur Mehmet Akif Ersoy University Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Meat and Meat Products Research and Development Unit performed experimental sausage manufacture. In experimental sausage samples, a mixture of 50% goat meat and 50% beef from Burdur province was used. The commercially supplied Selay Sucuk Kombi K (Istanbul) spice mix was applied at a rate of 5% by weight to all groups. Added 2% of curing salt containing 6% $NaNO_2$. Sausage starter culture (MITFER® 75MFV1-200) was applied to this meat combination for making control group (A) sausage samples. *Bifidobacterium animalis* and *Lactobacillus acidophilus* cultures were added to group B, and 1% lactulose was added to group C, in addition to the control group's combination. By adding *Bifidobacterium animalis* and *Lactobacillus acidophilus* cultures, as well as lactulose, to group D, four distinct sausage samples were formed.

The sausages produced underwent a 6-day fermentation process in the conditioning cabinet where the temperature was gradually reduced from 22°C to 18°C and the humidity from 95% to 75% before being kept at 4°C for 60 days. The experimental sausages were produced and analyzed in triplicate.

Microbiological Analyses

The following microbiological analyses were performed on sausages prepared and ripened under appropriate conditions by TS 1070 Sucuk Standard on the 0^{th}, 3^{rd}, and 6^{th} days of ripening as well as the 30^{th}, 45^{th}, and 60^{th} days of storage. Plates with between 30 and 300 colonies were evaluated after incubation. Samples were studied using 25 g of sample with 225 mL of peptone water that was 0.1% (v/v). In the stomacher (IUL instruments, Barcelona, Spain), the samples were homogenized. Homogenates were then serially diluted 10 times with 0.1% peptone water. These dilutions were collected, and 1 mL was used to inoculate appropriate agar plates.

Dilutions were plated on Plate Count Agar (PCA) (Oxoid CM 325) and incubated at 30±1°C for 48 hours to determine the total amount of mesophilic aerobic bacteria (18). Coliform bacteria, using the pour plate technique, dilutions were plated on Violet Red Bile Agar (Merck 1.01406). Typical colonies were enumerated after 24 hours of incubation at 30±1°C (19). The dilutions were plated on Potato Dextrose Agar (PDA) medium for yeast and mold. Colonies were counted after five days of incubation at 22±1°C (20). Dilutions of *Staphylococcus aureus* were plated on Baird-Parker Agar containing egg yolk tellurite and incubated at 37°C for 30 hours (21). MRS agar (Merck 1.10660) was used for lactic acid bacteria and the plates were incubated under anaerobic conditions at 42°C for 72 hours (22) The pour plate method was used to count *B. animalis* using a selective culture medium MRS-NNLP. It contained MRS Agar (Biolife LOT HC5002) supplemented with filter-sterilized solutions of nalidixic acid (15 mg/L), neomycin (100 mg/L), lithium (3 g/L), paromomycin (200 mg/L), and L-cysteine hydrochloride (0.5 g/L). The incubation period was 72 hours under anaerobic

conditions at 37°C (23). MRS agar (Merck 1.10661) was used to detect *Lactobacillus acidophilus* and anaerobic incubation was performed at 37±1°C for 72 hours (24).

Physicochemical Analyses

Physicochemical analyses were carried out on samples of sausage on the 0th, 3rd, and 6th days of ripening, as well as on the 30th, 45th, and 60th days of storage. The pH of sausage samples was tested using ISO 2917:1999 (25). The water activity (a_w) of sausage samples was measured using the technique provided in ISO 21527-2:2008 (26).

Organic Acid Analyses

Organic acid determinations were performed on the first and last days of ripening. For the detection of acetic acid and butyric acid, the instruments used are the Agilent 7697A Headspace and Agilent 7890A GC 5975C MS. The method includes a column temperature program where the temperature is held at 35°C for 5 minutes, then increased by 5°C per minute to reach 150°C and held for another 5 minutes. The detector and injector temperatures are set to 200°C and 180°C, respectively, with a flow rate of 25 psi (He). Other parameters include a needle temperature of 90°C, a transfer line temperature of 120°C, a vial oven temperature of 85°C, a thermostat time of 5 minutes, a pressurize time of 0.5 minutes, an inject time of 0.08 minutes, and a withdraw time of 0.5 minutes (27). Lactic acid detection was conducted using an HPLC method with the following setup: the system was a Shimadzu Prominence HPLC equipped with a CBM-20ACBM control unit, a DAD (SPD-M20A) detector, a CTO-10ASVp column oven, an LC20 AT pump, and a SIL-20ACHT autosampler. The analysis was controlled using the LC Solution software. The column used was an ODS 4 (250 mm x 4.6 mm, 5 µm) from GP Sciences, Inertsil ODS-4, Japan. The mobile phase was ultrapure water adjusted to pH 3 with orthophosphoric acid (28). The results were expressed as a percentage relative to the dry matter ratio.

Texture Analyses

Using a TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable MicroSystems, Godalming, UK), texture profile analysis (TPA) of sausage samples was performed successfully. The samples' values for hardness, adhesion, springiness, cohesiveness, gumminess, chewiness, and resilience were evaluated in the texture profiles. 5 cm x 4 cm aluminum rectangle probe, 1 mm/s test speed, 2 mm/s pretest speed, 1 mm/s post-test speed, 25% compression (tensile), and 25 kg load cell were the test parameters. Texture Expert Exceed Version 2V3 (Stable Micro Systems, 1998) was used for data collecting and calculation, while force deformation shape curves were used for calculations (3).

Sensory Analyses

The sensory evaluations were carried out by ISO 13299:2016, an international standard (29). Panelists were asked not to eat or drink anything at least 1 hour before the analyses. After each sensory test, panelists were allowed to drink water and eat breadcrumbs to cleanse the palate. To eliminate the risk of interference, panelists participating in the sensory assessment were placed in a white-light environment in an odor-free room. Using a hedonic scale from 1 to 9, ten experienced panelists rated the cooked sausage samples' color, odor, taste, texture, and general acceptability.

Statistical Analyses

Sausage production and analyses were carried out in triplicate. Results were subjected to one-way ANOVA using Statistical Package for the Social Sciences (SPSS) software (Version 25.0; SPSS, Chicago, IL, USA). Tukey's test was utilized as a statistical procedure to determine significant differences between mean values ($p<0.05$). Results are expressed as Mean ± Standard Deviation (SD) (SPSS, 2010).

RESULTS

Microbiological Changes

Microbiological analysis data for groups of sausages prepared by adding probiotic and prebiotic combinations on days 0, 3, and 6 of ripening and days 30, 45, and 60 of storage are presented in Table 1. Based on the microbiological analyses, no *Staphylococcus aureus* was detected in any of the sample groups. As compared to the levels obtained in the analysis on the first day of ripening, TAMB showed an overall decrease on the sixth day of ripening. Each group's values on the last day of storage are similar to those found in the initial analysis. Yeast and mold counts, like TAMB, declined on the sixth day of the study and developed on the following days, reaching statistically similar levels to those of the first day of analysis. Overall, yeast and mold results were less than 5.00 $\log_{10}$ cfu/g. Coliform the amount decreased throughout ripening, and there was no coliform showed on days 30, 45, and 60 of storage. The number of lactic acid bacteria at the end of ripening ranged from 5.27 to 6.98, with the groups supplemented with *B. animalis* and *L. acidophilus* having the highest levels ($p<0.05$). Their levels decreased to 4.96-5.84 ($p<0.05$) at the end of storage. Groups B and D, which were supplemented with *B. animalis* and *L. acidophilus*, had statistically higher levels of lactic acid bacteria at the end of storage ($p<0.05$). The study showed that the amount of *B. animalis* increased during ripening. At the end of the ripening period, *B. animalis* levels were higher in groups B and D, which were supplemented with *B. animalis* and *L. acidophilus* ($p<0.05$). When groups C and A were compared, it was found that the *B. animalis* level in group C, which had lactulose added, was higher than in group A at 45 and 60 days of storage ($p<0.05$). *L. acidophilus* levels were greater than 7 logs in the data obtained from analyses during ripening and storage. While group A had the most *L. acidophilus* on the first day of ripening, group B had the most on the last day ($p>0.05$). There were no significant changes in *L. acidophilus* levels within groups A, B, and C during storage; however, on the last day of analysis, group B had the highest value ($p>0.05$).

Table 1. Microbiological analysis results of sausage samples (log_{10} cfu/g) (Mean ± SD)

	DAYS						*p
	0	3	6	30	45	60	
Total mesophilic aerobe bacteria							
A	7.85±0.20^{a}	7.64±0.04^{Ba}	7.33±0.03^{Ab}	7.70±0.04^{Aa}	7.82±0.02^{Ca}	7.59±0.02^{Ca}	0.01
B	8.09±0.18^{a}	7.62±0.02^{Bbc}	6.97±0.29^{Bd}	7.26±0.01^{Bcd}	8.03±0.01^{Aa}	7.78±0.03^{Aab}	0.01
C	7.80±0.01^{b}	7.64±0.04^{Bc}	6.85±0.07^{Be}	7.06±0.03^{Cd}	7.91±0.01^{Ba}	7.69±0.05^{Bc}	0.01
D	7.83±0.02^{a}	7.73±0.01^{Ab}	7.65±0.01^{Acd}	7.65±0.02^{Acd}	7.61±0.01^{Dd}	7.66±0.02^{BCc}	0.01
*p	0.13	0.01	0.01	0.01	0.01	0.01	
Coliform							
A	3.02±0.03^{Aa}	2.65±0.14^{b}	2.02±0.11^{Ac}	-	-	-	0.01
B	2.99±0.04^{Aa}	2.73±0.09^{b}	2.08±0.04^{Ac}	-	-	-	0.01
C	2.80±0.05^{Ba}	2.65±0.01^{a}	1.54±0.20^{Bb}	-	-	-	0.01
D	3.00±0.02^{Aa}	2.82±0.02^{a}	1.88±0.22^{ABb}	-	-	-	0.01
*p	0.01	0.01	0.01				
Yeast and mold							
A	4.11±0.01^{b}	4.78±0.15^{Aa}	2.75±0.46^{Bc}	4.28±0.05^{Bab}	4.37±0.05^{Cab}	4.51±0.02^{Bab}	0.01
B	4.57±0.07^{ab}	3.49±0.04^{Bcd}	2.69±0.06^{Bd}	3.53±0.06^{Dbc}	4.59±0.02^{Ba}	4.57±0.12^{Ba}	0.01
C	4.90±0.01^{a}	3.38±0.02^{Bd}	3.10±0.04^{Ae}	3.74±0.07^{Cc}	4.71±0.02^{Ab}	4.79±0.01^{Ab}	0.01
D	4.53±0.45^{a}	3.57±0.09^{Bb}	2.82±0.52^{Bc}	4.47±0.02^{Aa}	4.63±0.04^{ABa}	4.68±0.06^{ABa}	0.01
*p	0.20	0.01	0.01	0.01	0.01	0.01	
Lactic acid bacteria							
A	7.50±0.02^{Aa}	4.90±0.03^{Cc}	5.27±0.13^{Cb}	5.30±0.02^{Cb}	5.39±0.03^{Cb}	5.51±0.17^{Bb}	0.01
B	5.64±0.41^{Bc}	7.94±0.06^{Aa}	6.98±0.01^{Ab}	5.78±0.02^{Ac}	5.62±0.05^{Bc}	5.84±0.09^{Ac}	0.01
C	5.54±0.04^{Bb}	6.65±0.31^{Ba}	6.26±0.05^{Ba}	5.78±0.03^{Ab}	5.83±0.03^{Ab}	4.96±0.01^{Cc}	0.01
D	7.65±0.03^{Aa}	7.47±0.85^{ABa}	6.86±0.01^{Aa}	5.71±0.02^{Bb}	5.31±0.07^{Cb}	5.77±0.05^{Ab}	0.01
*p	0.01	0.01	0.01	0.01	0.01	0.01	
Bifidobacterium animalis							
A	6.93±0.03^{Cd}	7.33±0.25^{c}	7.89±0.05^{ABa}	7.76±0.05^{Bab}	7.52±0.04^{Cbc}	6.89±0.04^{Dd}	0.01
B	6.87±0.02^{Ce}	7.40±0.07^{d}	7.92±0.01^{ABc}	8.21±0.17^{Ab}	8.71±0.03^{Aa}	7.56±0.09^{Ad}	0.01
C	7.15±0.03^{Ab}	7.67±0.27^{a}	7.74±0.16^{Ba}	7.80±0.05^{Ba}	7.86±0.01^{Ba}	7.10±0.02^{Cb}	0.01
D	7.07±0.03^{Bd}	7.37±0.05^{bc}	8.07±0.01^{Aa}	7.98±0.01^{ABa}	7.40±0.02^{Db}	7.28±0.05^{Bc}	0.01
*p	0.0q	0.24	0.01	0.01	0.01	0.01	
Lactobacillus acidophilus							
A	7.68±0.07^{a}	7.30±0.07^{BCb}	7.86±0.07^{Aa}	7.84±0.05^{Aa}	7.71±0.14^{a}	7.69±0.05^{ABa}	0.01
B	7.30±0.57^{b}	7.67±0.03^{ABab}	7.88±0.01^{Aa}	7.80±0.04^{Aa}	7.71±0.02^{ab}	7.74±0.03^{Aab}	0.02
C	7.03±0.04^{b}	7.09±0.02^{Cb}	7.78±0.06^{Aa}	7.68±0.04^{Aa}	7.63±0.05^{a}	7.62±0.01^{Ba}	0.01
D	7.05±0.12^{c}	7.84±0.02^{Aa}	7.41±0.14^{Bb}	7.43±0.19^{Bb}	7.55±0.08^{ba}	7.69±0.05^{ABba}	0.01
*p	0.08	0.01	0.00	0.01	0.13	0.05	

A: Control; B: Probiotic added; C: 1% Lactulose added; D: Probiotic and 1% Lactulose added. * Significance levels according to ANOVA test results. capital letters (A,B,C..) show statistical differences between groups, lower case letters (a,b,c..) show statistical differences between days

Physicochemical Changes

Data from physicochemical analyses carried out on groups of sausages on days 0, 3, and 6 of curing and days 30, 45, and 60 of storage are presented in Table 2. While the water activity was above 0.90 on the first day of ripening, it decreased to between 0.83 and 0.87 on the last day of ripening ($p<0.05$). On the last day of storage, group B had the lowest value (0.79) ($p<0.05$). The pH ranged from 6.69-6.82 at the beginning of ripening and decreased to 6.16-6.36 towards the end of ripening ($p<0.05$). The pH continued to decrease during storage, reaching 5.31-5.42 on the last day of analysis ($p<0.05$).

Table 2. Physicochemical analysis results of sausage samples (Mean ± SD)

	DAYS						*p
	0	3	6	30	45	60	
aw							
A	0.98±0.02^{Aa}	0.92±0.01^{b}	0.85±0.02^{c}	0.86±0.01^{ABd}	0.85±0.02^{Ad}	0.80±0.02^{ABe}	0.01
B	0.92±0.03^{Ba}	0.91±0.01^{ab}	0.87±0.01^{bc}	0.88±0.02^{Acd}	0.83±0.01^{ABde}	0.79±0.03^{Be}	0.01
C	0.96±0.01^{Ba}	0.92±0.01^{a}	0.86±0.01^{b}	0.82±0.03^{Bbc}	0.81±0.02^{Bc}	0.83±0.01^{Ac}	0.01
D	0.94±0.02^{Ba}	0.93±0.02^{a}	0.83±0.03^{b}	0.85±0.01^{ABb}	0.82±0.02^{ABb}	0.83±0.02^{Ab}	0.01
*p	0.00	0.03	0.14	0.03	0.03	0.02	
pH							
A	6.82±0.02^{Aa}	6.77±0.03^{ABa}	6.36±0.02^{Ab}	5.87±0.01^{BCc}	5.69±0.02^{Ad}	5.31±0.02^{Be}	0.01
B	6.70±0.03^{BCa}	6.75±0.01^{Ba}	6.16±0.01^{Cb}	5.94±0.02^{Ac}	5.66±0.01^{Bd}	5.40±0.03^{Ae}	0.01
C	6.69±0.01^{BCa}	6.80±0.02^{Ab}	6.27±0.01^{Bc}	5.90±0.03^{ABd}	5.61±0.02^{Ce}	5.42±0.01^{Af}	0.01
D	6.74±0.02^{Ba}	6.79±0.02^{ABb}	6.16±0.03^{Cc}	5.85±0.01^{Cd}	5.66±0.02^{Be}	5.40±0.02^{Af}	0.01
*p	0.01	0.05	0.01	0.01	0.01	0.01	

A: Control; B: Probiotic added; C: 1% Lactulose added; D: Probiotic and 1% Lactulose added. * Significance levels according to ANOVA test results
capital letters (A,B,C..) show statistical differences between groups, lower case letters (a,b,c..) show statistical differences between days

Changes in Organic Acids

Fermented sausages are characterized by increased acidity and unique flavors resulting from fermentation. For example, the breakdown of naturally occurring fats in meat by bacterial activity produces butyric acid, which gives the product a creamy flavor and aroma. Acetic acid is produced by lipid oxidation and amino acid catabolism, while lactic acid is released as a result of carbohydrate breakdown, both

of which contribute to the flavor profile of the sausage. The percentage increase in acetic, butyric, and lactic acid levels during the ripening period of the sausage samples is shown in Table 3. At the end of the ripening period, only group B, to which probiotics had been added, showed acetic, butyric, and lactic acid levels of 32.31, 22.39 and 2.25% respectively, whereas in group D, to which prebiotics had been added, these levels were 35.79, 24.23 and 2.48% respectively.

Table 3. Results of the analysis of organic acids in sausage samples (%)

	Acetic acid		Butyric acid		Lactic acid	
	Day 1	Day 6	Day 1	Day 6	Day 1	Day 6
A	28.57±0.93	28.61±1.59	18.80±1.09	18.13±1.12	1.68±0.06	2.13±0.08
B	28.57±2.61	32.31±2.03	18.80±1.02	22.39±1.20	1.12±0.20	2.25±0.03
C	28.19±2.91	30.41±1.95	18.14±0.80	20.42±1.08	1.60±0.03	2.21±0.04
D	28.56±0.76	35.79±1.74	18.50±1.42	24.23±1.04	1.69±0.02	2.48±0.04

A: Control; B: Probiotic added; C: 1% Lactulose added; D: Probiotic and 1% Lactulose added

Textural Changes

The data obtained from textural analyses conducted on experimental sausage samples on the first day of ripening, as well as on days 30 and 60 of storage, are presented in Table 4. The hardness values of groups A and B did not show any statistically significant differences throughout the analysis ($p>0.05$). While the hardness of sausages from group C decreased at the end of ripening, it increased again to 341.73 on the last day of storage ($p<0.05$). In contrast to group C, group D showed its highest hardness value on the 30th day of storage (534.56) ($p<0.05$). When examining the springiness value, Group B had the highest value on the first day of analysis ($p>0.05$), and no differences were observed between the groups on subsequent days of analysis ($p>0.05$). On the first day of analysis, Group B had the highest adhesiveness, whereas on the last day of analysis, Group C had greater adhesiveness ($p<0.05$). The gumminess value ranged from 36.67 to 66.71 on the first day of analysis and showed a decrease in all groups at the end of the storage period. Group C showed the lowest gumminess value on the 30th day of analysis, while group D showed the lowest value on the 60th day ($p<0.05$). The resilience analysis showed values ranging from 0.02 to 0.06, with no statistically significant difference between the groups in the 30th and 60th-day analyses ($p>0.05$).

Sensory Analysis Results

The data obtained from the sensory analysis of the sausage samples are presented in Table 5. As a result of the analyses, group C containing lactulose received the highest scores for all sensory parameters, including color, texture, aroma, flavor, and general acceptability ($p>0.05$). For the color parameter, group C was preferred more than the control group until the last day of analysis ($p<0.05$). In terms of texture evaluation, only group C was rated as very good or better on the last day of analysis ($p<0.05$). There was no significant difference between the analyzed sausage groups for the aroma parameter ($p>0.05$). For the flavor parameter, all groups except the control group were rated as good or above, with the sausage samples from group C being rated as very good, except on the final day of analysis. The general acceptability of group C samples containing lactulose received scores of 8 and above, making it the most preferred group. The second most preferred group was group D, which contained both lactulose and probiotics.

Table 4. Textural analysis results of sausage samples (Mean ± SD)

	DAYS			*p
	0	30	60	
Hardness (N)				
A	422.39±88.49	370.42±3.26^{B}	341.35±0.61^{B}	0.23
B	431.97±34.09	479.13±57.51^{A}	472.46±7.44^{A}	0.34
C	367.54±34.78^{a}	258.90±5.21^{Cb}	341.73±48.89^{Bab}	0.02
D	382.64±0.97^{b}	534.56±48.46^{Aa}	212.90±53.44^{Cc}	0.01
*p	0.39	0.01	0.01	
Springiness (mm)				
A	0.13±0.05^{AB}	0.04±0.01	0.07±0.03	0.06
B	0.19±0.01^{Aa}	0.07±0.01^{b}	0.07±0.02^{b}	0.01
C	0.12±0.01^{Ba}	0.07±0.02	0.17±0.12	0.29
D	0.11±0.01^{Ba}	0.07±0.02^{b}	0.03±0.01^{c}	0.01
*p	0.02	0.21	0.12	
Cohesiveness (N)				
A	0.10±0.03^{Ba}	0.06±0.00^{ab}	0.05±0.00^{Ab}	0.03
B	0.16±0.01^{Aa}	0.07±0.01^{b}	0.06±0.01^{Ab}	0.01
C	0.11±0.01^{Ba}	0.05±0.00^{b}	0.06±0.01^{Ab}	0.00
D	0.10±0.01^{Ba}	0.06±0.01^{b}	0.04±0.01^{Bc}	0.00
*p	0.01	0.07	0.01	
Adhesiveness (mJ)				
A	-6.81±2.61	-3.05±1.56^{A}	-7.70±5.48	0.32
B	-3.74±0.44^{a}	-6.87±1.42^{Bb}	-3.32±1.40^{a}	0.02
C	-6.23±0.36^{b}	-6.55±0.84^{ABb}	-1.72±0.59^{a}	0.01
D	-7.04±0.04	-7.25±1.48^{B}	-5.13±3.37	0.45
*p	0.06	0.02	0.22	
Gumminess (N)				
A	45.06±22.44	94.53±72.63	17.07±1.15^{B}	0.17
B	66.71±1.58^{a}	32.03±0.67^{b}	26.40±2.62^{Ac}	0.01
C	40.58±8.27^{a}	12.50±0.62^{b}	19.84±4.87^{ABb}	0.01
D	36.67±3.64^{a}	32.33±6.72^{a}	8.01±3.23^{Cb}	0.01
***p**	0.06	0.10	0.01	
Chewiness(N)				
A	6.97±5.14^{AB}	3.17±1.91	1.17±0.59^{A}	0.16
B	12.46±0.44^{Aa}	2.25±0.17^{b}	1.77±0.72^{b}	0.01
C	5.00±1.32^{B}	0.90±1.21	3.87±3.16	0.10
D	4.02±0.42^{Ba}	2.35±1.07^{a}	0.27±0.15^{b}	0.01
*p	0.02	0.17	0.13	
Resilience (N)				
A	0.04±0.01^{ABa}	0.03±0.01	0.02±0.00^{a}	0.15
B	0.06±0.00^{Aa}	0.03±0.00^{b}	0.03±0.00^{b}	0.01
C	0.04±0.01^{ABa}	0.02±0.00^{c}	0.03±0.00^{b}	0.01
D	0.03±0.00^{B}	0.03±0.00	0.02±0.00	0.18
*p	0.02	0.35	0.36	

A: Control; B: Probiotic added; C: 1% Lactulose added; D: Probiotic and 1% Lactulose added . * Significance levels according to ANOVA test results.
capital letters (A,B,C..) show statistical differences between groups, lower case letters (a,b,c..) show statistical differences between days

Table 5. Sensory analysis results of sausage samples (Mean ± SD)

	DAYS						* p
	0	3	6	30	45	60	
Appearance							
A	7.00±1.15^{B}	6.80±1.03^{B}	7.09±0.84^{B}	7.10±1.10^{AB}	7.05±1.02^{B}	7.30±1.23^{B}	0.80
B	7.60±0.52^{ABab}	6.80±0.63^{Bb}	7.36±0.48^{ABab}	7.20±1.14^{ABab}	7.20±0.69^{Bab}	7.95±0.64^{Aa}	0.23
C	8.50±0.85^{A}	8.00±1.63^{A}	8.32±0.82^{A}	8.70±0.67^{A}	8.48±0.46^{A}	7.98±0.89^{A}	0.34
D	7.60±0.97^{AB}	7.50±0.97^{AB}	7.68±0.69^{AB}	8.10±0.88^{A}	7.83±0.60^{AB}	7.90±1.04^{A}	0.58
*p	0.01	0.01	0.01	0.01	0.01	0.54	
Texture							
A	6.90±1.29^{B}	6.40±0.97^{C}	6.68±0.91^{BC}	7.20±0.42^{BC}	6.93±0.49^{BC}	7.08±0.77	0.41
B	6.30±1.16^{B}	7.10±1.20^{B}	6.82±1.54^{C}	6.55±1.54^{C}	6.63±0.89^{C}	6.93±1.12	0.52
C	8.40±0.84^{A}	8.10±0.88^{A}	8.14±0.74^{A}	8.10±0.74^{A}	8.18±0.50^{A}	7.73±0.70	0.27
D	7.40±1.07^{AB}	7.00±0.94^{B}	7.27±0.79^{AB}	7.60±0.70^{AB}	7.40±0.43^{AB}	7.48±0.77	0.42
*p	0.01	0.01	0.01	0.01	0.01	0.12	
Aroma							
A	7.60±1.07^{Bb}	7.50±0.71^{Bb}	7.68±0.50^{Bb}	7.20±1.14^{B}	7.38±0.76^{Bc}	7.95±0.90^{ABa}	0.34
B	7.70±1.06^{Ba}	7.50±1.08^{Bb}	7.73±0.70^{Ba}	7.35±1.45^{Bc}	7.48±0.70^{Bb}	8.00±0.81^{Aa}	0.57
C	8.50±0.85^{Aa}	8.40±0.84^{Aa}	8.50±0.55^{Aa}	8.45±0.50^{Aa}	8.45±0.37^{Aa}	8.15±0.74^{Ab}	0.91
D	7.70±0.95^{B}	7.70±0.95^{B}	7.73±0.71^{B}	7.75±1.27^{AB}	7.73±0.81^{B}	7.80±0.88^{B}	0.97
*p	0.16	0.16	0.16	0.16	0.16	0.66	
Taste							
A	7.20±1.03^{AB}	6.50±0.97^{AB}	6.86±0.67^{AB}	6.50±0.71^{AB}	6.68±0.50^{AB}	7.03±0.94	0.17
B	6.90±0.88^{B}	7.10±0.99^{B}	7.09±0.82^{B}	7.00±1.05^{B}	7.00±0.55^{B}	7.40±1.03	0.73
C	8.30±0.95^{A}	8.30±0.82^{A}	8.36±0.59^{A}	8.20±0.42^{A}	8.25±0.39^{A}	7.98±0.63	0.85
D	7.60±1.07^{AB}	7.40±0.97^{AB}	7.64±0.75^{AB}	8.30±0.67^{AB}	7.90±0.44^{AB}	7.80±0.77	0.23
*p	0.02	0.02	0.01	0.01	0.01	0.06	
General acceptability							
A	7.10±0.99^{B}	6.60±0.70^{B}	6.95±0.71^{B}	7.40±1.07^{B}	7.13±0.77^{B}	7.50±0.83	0.17
B	6.90±0.74^{B}	7.10±0.88^{B}	7.09±0.58^{B}	7.05±0.69^{B}	7.03±0.38^{B}	7.45±0.69	0.60
C	8.30±0.95^{A}	8.30±0.82^{A}	8.36±0.59^{A}	8.20±0.42	8.25±0.39^{A}	7.98±0.63	0.85
D	7.70±0.95^{AB}	7.70±0.82^{AB}	7.64±0.75^{AB}	8.30±0.67^{AB}	7.90±0.44^{AB}	7.80±0.77	0.28
*p	0.01	0.01	0.01	0.01	0.01	0.24	

A: Control; B: Probiotic added; C: 1% Lactulose added; D: Probiotic and 1% Lactulose added

* Significance levels according to ANOVA test results

capital letters (A,B,C..) show statistical differences between groups, lower case letters (a,b,c..) show statistical differences between days

DISCUSSION AND CONCLUSION

In this study, goat meat was used to produce fermented sausage with lactulose, *L. acidophilus,* and *B. animalis* and traditional starter cultures. The aim was to obtain a functional product and to use the meat of the goat, which is widely available around Burdur. In this context, four different sausage samples were prepared, and their microbiological, physicochemical, and textural changes were examined during ripening and storage.

The presence of microorganisms forms the foundation of fermented sausage production, and the microflora in the product determines its microbiological quality. In fermented sausages, the development of aroma, color, flavor, and texture occurs as a result of various biochemical reactions carried out by different microorganisms during the ripening process. In our study, *S. aureus* was not found in any sausage samples. In a study, it was observed that the number of *S. aureus* in the control group sausage samples prepared without adding starter culture, which has bioprotective properties, was twice as high as in the groups in which starter culture was added. It has been stated that the addition of LAB to sausages and low pH may prevent the development of *S. aureus* (30). To determine the count of mesophilic aerobic bacteria, data from the samples taken from the prepared sausages and cultured on PCA agar generally showed a decrease in counts on the 6th day of ripening, followed by an increase during storage, reaching the initial values by the end of storage. According to the Turkish Standards Institute (TSE), the TAMB (Total Aerobic Mesophilic Bacteria) count for fermented sausages should not exceed 5 $\log_{10}$ cfu/g. However, the microbial quality of the ingredients used in sausage production and the addition of starter cultures can affect the TAMB count. In our results, especially on the 45th and 60th days of storage, the TAMB count was higher compared to the other groups. Similar findings have been reported in other studies (31,32). Wang et al., (33) reported that the highest amount of TAMB was observed on the 5th day of fermentation and decreased thereafter. In another study, it was stated that the amount of TAMB was approximately 9 logs on the initial day and decreased at the end of ripening (34).

The yeast and mold count in sausages can originate from the environment during production as well as from raw materials. Additionally, yeast and mold, which can become active under low temperature and high humidity conditions, can develop on the surface of sausages. Research has shown that yeast and mold counts increase under low temperature and high humidity conditions during sausage ripening. The growth of some yeast and mold species can have positive effects on the color, aroma, and odor properties of sausages, while some species can lead to spoilage. In a study by Banjara et al. (35), it was reported that yeast and mold species and their quantities not used as starter cultures can vary between different products, among products produced by different manufacturers, and even among different batches of the same manufacturer. Our data are consistent with other

studies (31,36). In fermented sausages intended for consumption, it is important that the number of coliform microorganisms, which may be due to hygiene and technological errors, is not high (36).

In all sausage groups, including the control group, decreases in coliform microorganism counts were observed. The results of this study are in line with other studies (31,36) but different from the findings of Tekinşen et al (32). This difference may be due to the longer fermentation period in the study by Tekinşen et al (32). In a study reporting that the amount of coliform was below 10^2 $\log_{10}$ cfu/g both at the beginning of ripening (day 0) and on other analysis days, it was stated that this situation may be due to low pH and aw value (37). In another study, it was stated that on the 12th day of ripening, the amount of coliform decreased significantly compared to the initial level, reaching approximately 2 log levels (34). In the study reporting that the lowest amount of coliform was observed in the groups to which mixed starter culture was added, it was also stated that the addition of starter culture could positively affect the quality of product safety (33). Kamiloğlu (38), who examined 10 LAB strains isolated from sausages, examined their antimicrobial activities against the foodborne pathogens *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* Typhimurium and *Yersinia enterocolitica* and reported that most LAB strains showed antimicrobial effects. It was stated in the study that these strains would contribute to the technological and functional properties of foods and that they also provide antioxidant properties in addition to their antimicrobial effects (38).

In the experimental sausages prepared in this study, LAB (Lactic Acid Bacteria) counts ranged from 4.90 to 7.94 $\log_{10}$ cfu/g during ripening and storage. The highest levels were observed in the groups with probiotic supplementation. When our results are compared to those from other research, LAB levels generally look similar due to the presence of starter culture (39,40). In a study stating that conditions such as ripening speed and duration have a significant effect on LAB, it was stated that the amount of LAB in slow ripening is higher, especially on the 3rd day, and the lower increase in LAB in fast ripening may be due to a rapid decrease in pH (37). In the microbiological analysis conducted in this study, the amount of *B. animalis* was found to be between 6.03 and 7.88 $\log_{10}$ cfu/g, and it increased during ripening. At the end of storage, it was observed to be higher in the groups with probiotic supplementation compared to the others ($p < 0.05$). When compared between groups A and C, the addition of prebiotics was found to contribute to the development of *B. animalis* on the 45th and 60th days of storage ($p < 0.05$). During ripening and storage, *L. acidophilus* counts remained above 7 $\log_{10}$ cfu/g. Although there was no statistically significant difference in *L. acidophilus* counts between groups A, B, and C during the storage, the highest value was observed in group B on the last day of analysis ($p > 0.05$). In a study in which various sample groups were formed by adding *L. acidophilus* and *B. animalis* in addition to standard starter cultures to sausages made by combining lamb meat and pork fat with various ingredients, it was discovered that *L. acidophilus* was greater than 6 $\log_{10}$ cfu/g and *B. animalis* was greater than 3 $\log_{10}$ cfu/g. It was reported in the study that *B. animalis* is not suitable for use as an initial culture, but it may be possible in combination with another probiotic bacterial species (41). According to a study, in addition to their probiotic qualities, *B. lactis* and *L. acidophilus* can be used as starter cultures to produce fermented sausage. These cultures have an impact on the number of staphylococci, micrococci, lactic acid bacteria, and total aerobic bacteria in the final product, as well as decreasing lipid oxidation (42). It is thought that the microbiological properties of sausage may be affected by the natural meat microbiota and starter addition (34). A study conducted to evaluate how the addition of *Lactobacillus paracasei* (10^8 log cfu/g) and lactulose (2%) to fermented sausages affected the quality parameters of the product found that the added ingredients did not affect lactic acid bacteria count during the ripening process (X3).

Low pH values in products that ferment is one of the most significant factors affecting probiotic bacteria growth and stability (44). *L. acidophilus* grows better at a pH of 5.5-6.0, whereas bifidobacteria need a pH of 6.0-7.0 (45). The pH value was around 6.69-6.82 on the first day of analysis and decreased to levels between 6.16-6.36 by the end of ripening ($p < 0.05$). During storage, the pH value decreased further, reaching levels of 5.31-5.42 on the final day of analysis ($p < 0.05$). Several studies have observed changes in pH in fermented sausages during storage. According to Rebucci et al. (46), during sausage ripening, the pH was 5.29 in 30 days and reached 5.86 in 60 days, which was related to lactobacilli proteolysis and endogenous enzymes in meat (8). LABs in sausage samples create lactic acid, which lowers the pH in the surroundings. This happens particularly fast during fermentation. However, meat and meat products may exhibit a decrease in pH due to the production of alkaline nitrogenous substances such as ammonia and amines as a result of microbial proteolytic activities (47). The decrease in pH in sausages is a result of the fermentation process. During the production of fermented sausages, the pH decreases due to the action of microorganisms that live in acidic conditions, such as lactic acid bacteria. In their study with sausage samples of different brands and serial numbers from the market, Kızılkaya et al. (48) stated that the pH value was <5.36, and the use of LAB as a starter culture was common in commercial sausages. In a study investigating the microbiological and physicochemical properties of sausage samples prepared with added sugar beet as a carbohydrate source, it was observed that the pH, which was initially around 6.15 on the first day of the study, decreased to an average of 5.15 by the 5th day of ripening. At the end of ripening, the highest pH value was observed in the groups without sugar beet added, and it has been reported that the reason for the LAB addition may be a further pH decrease by fermenting the carbohydrate and producing lactic acid (49). It is thought that the addition of prebiotics may have an important role in the ripening of sausage by contributing to the development of LAB. Additionally, this pH decrease causes the denaturation of muscle proteins, leading to a decrease in their water-holding capacity (50). The inhibition of unwanted microorganisms in food is achieved by decreasing water activity (aw).

A study examining the water activity (aw) values of various commercially available sausage samples reported that even within different batches of the same brands, there were variations in aw values. Generally, the aw values ranged between 0.94 and 0.96. Based on these values, it has been stated that the sausages offered for sale are not dried sufficiently and that an arrangement must be made for this. Another study stated that the aw value, which was >0.95 at the beginning, was <0.75 on the 8th day with the addition of starter culture, and that a low aw value could improve the shelf life and food safety of the product (33).

Continuous reduction in water activity causes a significant portion of microorganisms to die. Therefore, water activity is an important factor in food products that affects shelf life and microbiological quality. Water activity in the sausages was determined to be above 0.90 on the first day of ripening, but it decreased during the ripening process to levels between 0.83 and 0.87 on the 6th day. While the aw values of the sausage varied between 0.88 and 0.90 on average according to the results of Ünal and Karakaya (51), in our study, they decreased as expected.

The characteristic taste and texture of fermented meats originate from the decomposition products of animal components. Using properly selected species that produce significant flavor components can enhance the quality of the senses. To acquire changed sensory qualities and the optimum amount of acidity during the fermentation process, probiotics must be co-cultured with non-probiotic starters. Probiotics may not have a significant impact on the end product's sensory acceptability, or they may have a beneficial effect when used in conjunction with conventional starter cultures throughout the sausage fermentation process (44).

The texture of sausage is known to be affected by its physicochemical properties, such as fat, salt, and pH values. The hardness of the sausages, as determined experimentally, ranged from 212.90 to 534.56. The stickiness value decreased in all groups as the storage period progressed. On the 30th day of storage, the lowest hardness and stickiness values were observed only in group C, which had lactulose added. Sensory evaluation results also showed that group C, which contained lactulose, was the most preferred group. There were no statistically significant differences in the aroma parameter within or between groups ($p>0.05$). The hardness value in the sausage samples examined on the 12th day of the texture profile increased as a result of the addition of LAB. It was stated that the situation, which is also associated with a decrease in pH, may also be due to excessive moisture loss. It was reported that the gumminess value was higher in the group without LAB addition (34). The increase in microorganisms affects the hardness of the product by reducing the pH of the product and causing the meat proteins to denature. The decrease in the aw value of the product causes the adhesion values to decrease. Therefore, it increases the cutting ability of sausage (3). The addition of *Lactobacillus plantarum* TN8 to sausages made with beef was found to cause a decrease in textural properties in terms of hardness, elasticity, and chewiness, which can be related to the decrease in pH (52). Kaban et al (53) reported that adding LAB as a starter affected pH change, especially in the first 3 days of the fermentation process.

In a study examining the effect of starter culture addition on sensory properties, it was stated that there was no significant difference in color, taste, and general acceptability, but sausage samples without starter culture received the lowest score in the texture parameter (53). It has been stated that the use of a starter and the rate of maturing have a significant effect on color and general acceptability, and rapid maturing in particular has a good effect on texture (37).

The addition of lactulose to sausages made with goat meat, which is common around Burdur, improved the textural and sensory properties of the product. The results obtained show that the addition of probiotic bacteria and lactulose to the traditional sausage production process has a positive and significant effect on the microbiological quality, textural characteristics, and sensory attributes of the product. These results offer significant potential for product improvement and diversification within the sausage industry.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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