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PRODUCTION OF FERMENTED MILK USING HUMAN ORIGIN *BIFIDOBACTERIUM LONGUM* BH28: A FUNCTIONAL AND TECHNOLOGICAL ASSESSMENT

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ABSTRACT
In the present study, fermented milk was produced using the local *Bifidobacterium longum* BH28 strain, which was previously isolated from newborn feces and determined to be safe for use in foods. The *Streptococcus thermophilus* 212S strain was used both alone and in combination with *B. longum* BH28 or *Lacticaseibacillus paracasei* Shirota strains. The number of *B. longum* BH28 decreased to approximately 5 log cfu/mL on the 7th day and lost its suitability for the probiotic definition. Principal Component Analysis results showed that the samples were classified into three distinct groups based on culture type and storage time. However, it was determined that the different culture combinations applied did not create a statistically significant difference in total phenolic content, antioxidant capacity, water-holding capacity, and sensory characteristics. It was concluded that further research and process optimization are needed to maintain the live cell count of *B. longum* BH28 above 6 log cfu/mL.
Keywords: *Bifidobacterium longum,* fermented milk, probiotic

İNSAN ORİJİNLİ *BIFIDOBACTERIUM LONGUM* BH28 KULLANILARAK FERMENTE SÜT ÜRETİMİ: FONKSİYONEL VE TEKNOLOJİK DEĞERLENDİRME

ÖZ
Sunulan çalışmada, daha önce yenidoğan feçesinden izole edilen ve gıdalarda kullanımının güvenli olduğu belirlenen yerel *Bifidobacterium longum* BH28 suşu ile fermente süt üretimi gerçekleştirilmiştir. Bu amaçla, *Streptococcus thermophilus* 212S suşu hem tek başına hem de *B. longum* BH28 veya *Lacticaseibacillus paracasei* Shirota suşları ile birlikte kullanılmıştır. *B. longum* BH28 sayısı 7. günde yaklaşık 5 log kob/mL seviyesine düşerek probiyotik tanımına uygunluk kriterini kaybetmiştir. Temel Bileşen Analizi sonuçları, örneklerin kültür tipine ve depolama süresine göre üç belirgin grupta sınıflandığını göstermiştir. Bununla birlikte, uygulanan farklı kültür kombinasyonlarının toplam fenolik madde içeriği, antioksidan kapasite, su tutma kapasitesi ve duyusal özellikler üzerinde istatistiksel olarak anlamlı bir farklılık oluşturmadığı tespit edilmiştir. *B. longum* BH28'in canlı hücre sayısının 6 log kob/mL'nin üzerinde tutulabilmesi amacıyla, daha ileri düzeyde araştırmalara ve süreç optimizasyonuna ihtiyaç duyulduğu sonucuna varılmıştır.
Anahtar kelimeler: *Bifidobacterium longum,* fermente süt, probiyotik

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INTRODUCTION

Fermented milk attracts consumer's attention with their beneficial effects on health, unique pleasant aroma, and high nutritional content. The functional, nutritional, and sensory properties of the final product are determined by antimicrobial metabolites, aroma compounds, organic acids, peptides, and other metabolites produced during the fermentation stage, which mainly involves lactic acid bacteria (LAB) (Galli et al., 2022). Probiotics are defined as live microorganisms that have positive effects on human health when taken in sufficient amounts (FAO/WHO 2022). Fermented milk is an ideal medium for the proliferation and transport of probiotics. The development of novel probiotic bacteria that are suited to multiplying in fermented milk is essential for improving the combination of probiotic bacteria and fermented milk (Tian et al., 2022).

Bifidobacteria are anaerobic, Gram-positive bacteria that live in the intestines of human and animals (Leahy et al., 2005). Studies have revealed many beneficial effects of bifidobacteria, including modulation of the immune system (Gavzy et al., 2023), prevention of constipation (Wang et al., 2022), reduction of symptoms of depression and anxiety (Li et al., 2023), facilitation of lactose digestion (Rasinkangas et al., 2022), and prevention of cancer (Parisa et al., 2020). The nutritional requirements of *Bifidobacterium* spp., their sensitivity to cold storage, and their anaerobic nature can limit their growth in fermented foods (Donkor et al., 2006; Shori, 2021). The use of *Bifidobacterium* spp. in co-culture with oxygen-using *Streptococcus salivarius* subsp. *thermophilus* (*S. thermophilus*) can create a favorable environment for their growth in fermented milk (Ma et al., 2025).

Lacticaseibacillus paracasei Shirota (formerly *Lactobacillus casei* Shirota, *L. paracasei* Shirota) is a probiotic strain that has been used in fermented milk production for more than 80 years. This strain, which is also employed in the commercial production of the fermented beverage Yakult, has been reported to regulate the immune system, protect against infections and depression, and contribute to the treatment of various illnesses, including constipation (Yang et al., 2023a). *Bifidobacterium longum* BH28 (*B. longum* BH28) was previously isolated from the feces of newborn, and *in vitro* probiotic and safety characterization was determined (Güler et al., 2024). This strain has been found to tolerate bile salts and simulated gastric conditions. It can auto-aggregate and has antimicrobial effects due to its production of organic acids. It is sensitive to many antibiotics recommended by the European Food Safety Authority (EFSA) and does not contain the investigated virulence genes. Additionally, it does not exhibit hemolytic or DNase activity. The objective of this study was to investigate the possibility of using potential probiotic and human origin *B. longum* BH28 in fermented milk production, in comparison with *L. paracasei* Shirota. Additionally, it was aimed to determine the microbiological and physicochemical properties, organic acid and total phenolic content (TPC), antioxidant capacity, and sensory evaluation of fermented milk samples.

MATERIALS AND METHODS

Bacterial strains and culture conditions

B. longum BH28 strain was obtained from newborn feces previously and was preserved at -80°C in MRS (de Man Rogosa Sharpe, Merck, Darmstadt, Germany) broth containing glycerol (40%) and L-cysteine (0.05%). According to the *in vitro* probiotic characterization and safety assessments, Güler et al., (2024) determined that *B. longum* BH28 is a promising probiotic candidate and that its use in food is safe. *S. thermophilus* 212S strain was isolated from yoghurt and proposed as a starter culture by Aktaş and Çetin (2024a) and stored in M17 (Merck, Darmstadt, Germany) broth containing glycerol (40%). A well-known probiotic *L. paracasei* Shirota was isolated from Yakult beverage and stored at -80°C in MRS broth containing glycerol (40%). All strains were stored at Atatürk University Food Engineering Department Microbiology Laboratory Culture Collection and activated by subculturing in related media.

Fermented milk production

Commercially available ultrahigh temperature sterilized milk (UHT milk; 3.3% fat content,

Torku, Konya, Türkiye) was used for fermented milk production. The milk was heated to 37 °C and then divided into three batches. Each batch with an initial concentration of 10^6 CFU/mL was inoculated with the corresponding starting culture combinations as follows: A: *S. thermophilus* 212S and *B. longum* BH28 (1:1), B: *S. thermophilus* 212S and *L. paracasei* Shirota (1:1), C: *S. thermophilus* 212S. After being moved to sterile glass bottles, the inoculated milk was incubated at 37 °C until its pH reached 4.6. After incubation, the samples were kept for 28 days at 4 °C (Figure 1). Fermented milk production was conducted in duplicate and analyzed at 1, 7, 14, 21, and 28 days of storage.

Figure 1. Sample image of a fermented milk sample undergoing incubation (A), Sample image of fermented milk stored in a refrigerator (B).

Microbiological analysis

Fermented milk was (10 mL) homogenized in Ringer's solution (90 mL) and serially diluted. For the enumeration of *S. thermophilus* 212S M17 agar was used according to Aktaş and Çetin (2024a) followed by incubation at 42 °C for 24 h aerobically. For *L. paracasei* Shirota enumeration, the method proposed by Irigoyen et al. (2005) was used with slight modification. Accordingly, MRS agar was used by adding 0.3% bile salt and after cultivation, the plates were incubated anaerobically at 37 °C for 48 hours. For the enumeration of *B. longum* BH28 MRS agar containing 0.05% L-cysteine and 50 mg/L

mupirocin (mMRS) was used according to Güler et al., (2024) followed by incubation at 37 °C for 48 h anaerobically. For the enumeration of coliforms, Violet Bile Red agar (VRB, Merck) was used according to Anonymous (2010) method and then the plates were incubated at 37 °C for 24 h. For the enumeration of yeast and mold Dichloran Rose Bengal Chloramphenicol agar (DRBC, Merck) was used according to Anonymous (2008) and then the plates were incubated at 25 °C for 5 days.

Physicochemical analysis

The pH of fermented milk samples was measured with a calibrated pH meter (Hanna Instruments, Italy). The titratable acidity of fermented milk samples was determined by titration method using a 0.1 N NaOH solution. The results were expressed as a lactic acid % (AOAC, 2005). To determine the water holding capacity (WHC), 10 g of the samples were weighed and centrifuged at 2750 ×g for 30 min (Allegra X–30R, Germany). The results were calculated using the following formula:

$$WHC\ (\%) = (W_2/W_1)\times 100$$

where W_1 is the amount of serum (g) separated from the sample after centrifugation, and W_2 is the initial sample weight (g) (Bensmira and Jiang 2012).

The viscosity of the samples was determined using a viscometer (Brookfield Viscometer Model DV-II, Stoughton, MA, USA) with spindle number 5. Viscosity measurements were conducted at 20 and 50 rpm for 30 s at 5 °C and the results were given in centipoise (cP) (El-Fattah et al., 2018).

Organic acid content

The quantification of organic acids (lactic, acetic, citric, malic and propionic acids) in fermented milk samples were conducted using high-performance liquid chromatography (HPLC) based on the method described by Atalar (2019), with slight modifications. Briefly, 5 g of each sample was extracted with 25 mL of 0.01 mol/L sulfuric acid (H_2SO_4). The mixture was homogenized and then centrifuged at 7000 ×g for 7 min at 4 °C. The resulting supernatant was filtered using a 0.45 µm nylon membrane filter (Supelco Iso-Disc™ N-25-4, 25 mm) and transferred into HPLC vials for analysis. Chromatographic separation was carried out on an HPLC system (Agilent Technologies, USA) equipped with a Spherisorb ODS2 column (4.6 × 250 mm, 5 µm particle size). The mobile phase consisted of 10 mM perchloric acid, delivered at a flow rate of 0.5 mL/min. Detection was performed at 210 nm, with the column temperature maintained at 35 °C (Figure 2).

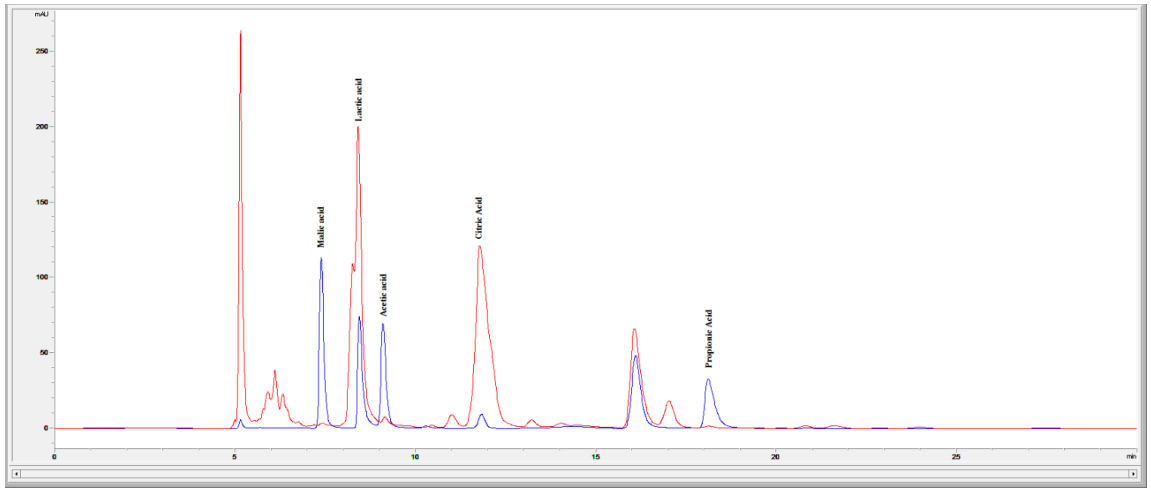

Figure 2. HPLC chromatograms of the organic acids. The blue chromatogram illustrates the standard mixture, while the red chromatogram displays the fermented milk sample.

Total phenolic content and antioxidant capacity

The extraction of fermented milk samples was conducted using the method described by Özcan et al., (2019), with slight modifications. Briefly, 10 g of each sample was extracted with 25 mL of an aqueous methanolic solution (80:20, v/v) and agitated with a shaker (SSL1, UK) for 6 h. The mixture was centrifuged at 1420 ×g for 10 min at 25 °C. The resulting supernatant was filtered with Whatman No. 1 filter paper and used to analyze TPC and antioxidant capacity.

TPC was determined using the Folin-Ciocalteu method. For this purpose, 100 µL extract, 500 µL Folin-Ciocalteu reagent, 400 µL of 1 M Na_2CO_3 solution and 4 mL deionized water were mixed. The absorbance of the mixture was determined using a spectrophotometer (Epoch, BioTek, Winooski, Vermont, USA) at 760 nm after standing for 1 hour. The total phenolic content of fermented milk samples was determined according to the standard curve to be obtained using the gallic acid standard and the results were expressed as mg of gallic acid equivalent per kg (mg GAE/kg) (Maleki et al., 2015).

For the determination of DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity of fermented milk samples, 3.0 mL DPPH methanol solution (25 mg/L), and 1 mL extract were mixed. After incubation for 30 min at room temperature and in the dark, the mixture was centrifuged at 3000 × g for 10 min. The absorbance values of the samples were then measured against the blind sample (methanol) at 517 nm using a spectrophotometer (Epoch, BioTek). The DPPH radical scavenging activity was determined using the following formula:

DPPH scavenging activity (%) = [1 -($A_{extract}$/ A_{DPPH})]×100

where $A_{extract}$ denotes the absorbance measured for the sample, and A_{DPPH} refers to the absorbance of the control solution containing DPPH without any sample (Maleki et al., 2015).

Sensory analysis

The sensory attributes of the fermented milk samples including appearance, color, texture, odor, taste, and overall acceptability were determined using a 9-point hedonic scale. The scale ranged from 1 to 9, with intermediate values representing varying degrees of preference (1-3: undesirable, 4-5: moderate, 6-7: desirable, 8-9: highly desirable). The samples were randomly coded and evaluated by a panel of 9 trained panelists who had completed formal sensory analysis training or relevant coursework (Meilgaard et al., 1999). All panelists were familiar with the sensory characteristics of fermented dairy products. The Atatürk University Faculty of Agriculture's Ethics Committee gave its ethical approval for the sensory evaluation (Protocol No. 2025/1).

Statistical analysis

The study involved the production of fermented milk samples in two distinct batches, with each sample undergoing analysis in at least two technical replicates. All the data are expressed as the mean±standard deviations. The results were analyzed via the SPSS 22.0 (SPSS Inc., Chicago, IL, USA), and the significant differences between groups were determined via ANOVA. Duncan's multiple comparison analysis was used to compare mean values at a significance level of $P<0.05$. Principal component analysis (PCA) was performed via the SIMCA 14.1 software (MKS UMETRICS, Umea, Sweden).

RESULTS AND DISCUSSION

Microbiological analysis

The microbiological changes in the fermented milk during storage are shown in Table 1. In the production of fermented milk, sample A was inoculated with approximately 6 log CFU/mL *B. longum* BH28. The viable cell count increased to 7.32 log CFU/mL during fermentation, but decreased to 5.03 log CFU/mL by the 7th day of storage ($P<0.001$). The count of *B. longum* BH28 did not change significantly at subsequent storage days and was found to be 4.95 log CFU/mL on day 28. According to the FAO/WHO (2002) definition, a probiotic food must contain a minimum of 6 log CFU/mL live cells to exert health benefits. As a result of this evaluation, the probiotic potential of sample A, produced with probiotic *B. longum* BH28 strain, was not

maintained after day 7. Similar decline trends in *Bifidobacterium* counts during refrigerated storage of dairy products have been reported by others. For instance, in the study, combinations of different yogurt starters and a human origin *B. longum* 5^{1A}, the researchers found that the count of *B. longum* 5^{1A} decreased from approximately 7 log CFU/mL to approximately 5 log CFU/mL on the 7th day of storage. The researchers also stated that the *B. longum* 5^{1A} count continued to decrease throughout the storage period, decreasing below 3 log CFU/mL on the 28th day (Souza et al., 2012). Similarly, in a study where *Bifidobacterium longum* subsp. *infantis* ATCC15697 (*B. longum* subsp. *infantis*) strain was used as an adjunct culture in yogurt production, the prebiotic effect of 2'-fucosyllactose was investigated. Accordingly, in both yogurts containing 2'-fucosyllactose at concentrations of 0% and 0.2%, the *B. longum* subsp. *infantis* ATCC15697 (initial concentration ~9 log CFU/mL) strain was found to be below the 6 log CFU/mL threshold after the 7th day (Xie et al., 2024). Damin et al. (2008) also found that *Bifidobacterium lactis* counts decreased from 9.15 log CFU/mL to around 7 log CFU/mL in fermented milk during the 21 days of storage. These findings are often attributed to the low acid and oxygen tolerance of bifidobacteria, as well as their sensitivity to cold storage conditions (Donkor et al., 2006; Shori, 2021). To enhance the viability of *Bifidobacterium* spp. in fermented products, strategies such as microencapsulation, the addition of protective carriers, like prebiotics, or oxygen-reducing packaging systems may be considered (Shori, 2021; Jena and Choudhury, 2025). The inoculation level of *B. longum* BH28 was determined based on preliminary trials, which showed that the strain exhibited weak fermentation activity alone but reached probiotic levels (>8 log CFU/mL) when co-cultured with *S. thermophilus*. The rapid loss of viability in this study highlights the need for more live cells to be added initially, and the need for optimization of the formulation to maintain the probiotic potential of *B. longum* BH28 throughout the shelf life of the product.

Table 1. Microbiological properties of fermented milk samples during storage[a]

Microbiological parameters (Log CFU/mL)	Sample	Storage period (days)					Sig.
		1	7	14	21	28	
B. longum BH28	A	7.32 ± 0.45^{a}	5.03 ± 0.05^{b}	5.02 ± 0.09^{b}	5.07 ± 0.10^{b}	4.95 ± 0.07^{b}	***
	B	nd	nd	nd	nd	nd	
	C	nd	nd	nd	nd	nd	
L. paracasei Shirota	A	nd	nd	nd	nd	nd	ns
	B	7.47 ± 0.67^{a}	8.05 ± 0.18^{a}	7.67 ± 0.10^{a}	7.94 ± 0.01^{a}	8.06 ± 0.16^{a}	
	C	nd	nd	nd	nd	nd	
S. thermophilus	A	8.95 ± 0.01^{aA}	8.91 ± 0.03^{aA}	8.70 ± 0.02^{cA}	8.84 ± 0.01^{bA}	8.85 ± 0.02^{bA}	***
	B	8.82 ± 0.04^{aB}	8.67 ± 0.05^{bB}	8.83 ± 0.06^{aA}	8.86 ± 0.17^{aA}	8.78 ± 0.03^{aA}	*
	C	8.89 ± 0.02^{bAB}	8.81 ± 0.03^{bA}	8.84 ± 0.02^{bA}	9.10 ± 0.15^{aA}	8.85 ± 0.02^{bA}	*
Sig.		*	*	ns	ns	ns	

[a] A: Fermented milk produced by using *S. thermophilus* 212S and *B. longum* BH28, B: Fermented milk produced by using *S. thermophilus* 212S and *L. paracasei* Shirota, C: Fermented milk produced by using *S. thermophilus* 212S. Values are expressed as mean ± standard deviation. nd: not detected. Sig.: Degree of statistical significance, ns: Not statistically significant, *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$. Lowercase and uppercase letter represent statistical differences in the same row and column, respectively.

The viable cell count of *L. paracasei* Shirota in sample B varied between 7.6 log CFU/mL and 8.06 log CFU/mL ($P>0.05$), and the probiotic properties of the product were preserved throughout storage. Similar to our study, the viable count of *L. paracasei* Shirota in commercial fermented milk varied between 8.57 log CFU/mL and 8.43 log CFU/mL during the 31 day storage period (Sumalapao et al., 2017). *S. thermophilus* 212S counts in fermented milk coded A, B, and C varied between 8.67 log CFU/mL and 9.10 log CFU/mL during storage and generally did not differ between the samples. Similarly, in the study conducted by Işık et al., (2023) *S. thermophilus* was

used as the starter culture, and the number of LAB growing on M17 agar was found to be around 8 log CFU/mL during storage. The presence of yeast, mold, and coliform group bacteria were not detected in any of the samples during the storage period.

Physicochemical analysis

Physicochemical properties of fermented milk during storage are presented in Table 2. The pH values of the samples ranged from 4.61 to 4.30. A general decrease in the pH values of the samples during storage were determined. Sample A, fermented with *B. longum* BH28 and *S. thermophilus* 212S, showed a pH decrease from 4.61 to 4.34 by day 28. Similarly, samples B and C followed a similar trend. A similar decreasing trend was observed by Yang et al. (2023b), who reported a significant decrease in the pH of goat milk fermented with yoghurt starter and *Bifidobacterium animalis* ssp. *lactis* (*B. animalis* ssp. *lactis*) as an adjunct probiotic during storage. This difference can be attributed to the metabolic activity of the adjunct probiotic strains, which continued to ferment residual lactose and other carbohydrates, producing additional organic acids such as lactic and acetic acid during cold storage. The titratable acidity (lactic acid %) of the samples ranged from 0.75% to 0.88%, and a significant increase in acidity was observed in all samples over the storage period (P <0.001). Sample B (fermented with *S. thermophilus* 212S and *L. paracasei* Shirota) exhibited the highest lactic acid content on day 28 (0.88%), while sample C, fermented with only *S. thermophilus* 212S, showed slightly lower acidity levels on all storage days. Physicochemical properties of fermented milk were investigated in a study by Tian et al. (2022) in which *B. longum* CCFM5871 strain was used as an adjunct in addition to the yogurt starter culture. Similar to our study, pH values of these samples decreased during storage, while %lactic acid values increased and this change was found to be higher in fermented milk produced by adding *B. longum* CCFM5871 strain compared to the control. According to the Codex Alimentarius Standard for Fermented Milks (2003), the titratable acidity in fermented milk should not be less than 0.3%. All fermented milks produced in this study comply with the codex.

Table 2. Physicochemical properties of fermented milk samples during storage[a]

Parameters	Sample	Storage period (days)					Sig.
		1	7	14	21	28	
pH	A	4.61±0.00^{aA}	4.43±0.01^{bAB}	4.41±0.00^{cA}	4.34±0.00^{dB}	4.34±0.00^{dB}	***
	B	4.51±0.01^{aB}	4.40±0.01^{bB}	4.30±0.06^{cA}	4.31±0.00^{cB}	4.31±0.01^{cB}	**
	C	4.60±0.00^{aA}	4.46±0.00^{bA}	4.40±0.00^{cA}	4.41±0.01^{cA}	4.41±0.00^{cA}	***
Sig.		**	*	ns	**	**	
lactic acid %	A	0.76±0.00^{dA}	0.82±0.00^{cB}	0.84±0.00^{bA}	0.86±0.00^{aB}	0.86±0.00^{aB}	***
	B	0.77±0.00^{dA}	0.83±0.00^{cA}	0.84±0.00^{bA}	0.88±0.00^{aA}	0.88±0.00^{aA}	***
	C	0.75±0.00^{dB}	0.80±0.00^{cC}	0.81±0.00^{bB}	0.82±0.05^{aC}	0.82±0.00^{aC}	***
Sig.		*	**	**	**	***	
WHC (%)	A	48.26±0.66^{dA}	59.48±0.89^{aA}	55.60±0.31^{cA}	57.86±0.35^{bA}	56.81±0.46^{bcA}	***
	B	48.42±0.14^{cA}	58.92±0.05^{aA}	54.98±0.26^{bA}	58.15±0.03^{aA}	58.08±0.70^{aA}	***
	C	48.86±0.67^{dA}	60.45±0.37^{aA}	55.43±0.29^{cA}	57.87±0.0^{bA}	58.20±0.26^{bA}	***
Sig.		ns	ns	ns	ns	ns	
ŋ20 (cP)	A	16151.5±818.2^{aA}	15013.9±554.4^{bB}	12568.5±703.5^{cA}	11977.6±634.8^{dA}	11320.0±597.6^{eB}	***
	B	16265.5±697.4^{aA}	15287.9±507.9^{bA}	12571.5±836.4^{cA}	12092.1±508.1^{dA}	11769.1±716.1^{dA}	***
	C	15905.5±961.5^{aA}	14475.8±340.5^{bC}	11022.4±707.2^{cB}	10577.6±828.4^{dB}	10053.3±800.1^{eC}	***
Sig.		ns	***	***	***	***	
ŋ50 (cP)	A	6960.7±210.1^{aA}	5272.7±387.9^{eB}	5482.3±180.4^{bA}	5546.2±372.9^{bA}	4946.8±212.6^{dB}	***
	B	6887.2±203.4^{aA}	5766.8±249.6^{bA}	5402.6±337.1^{cA}	5468.1±272.9^{cA}	5394.9±286.2^{cA}	***
	C	6808.5±474.7^{aA}	4581.1±251.4^{bC}	4414.1±317.8^{cB}	4678.1±166.5^{bB}	4672.9±137.2^{bC}	***
Sig.		ns	***	***	***	***	

[a] A: Fermented milk produced by using *S. thermophilus* 212S and *B. longum* BH28, B: Fermented milk produced by using *S. thermophilus* 212S and *L. paracasei* Shirota, C: Fermented milk produced by using *S. thermophilus* 212S. Values are expressed as mean ± standard deviation. Sig.: Degree of statistical significance, ns: Not statistically significant, *: P<0.05, **: P<0.01, ***: P<0.001. WHC: Water-holding capacity, ŋ20: Apparent viscosity at 20 rpm, ŋ50: Apparent viscosity at 50 rpm. Lowercase and uppercase letter represent statistical differences in the same row and column, respectively.

The water holding capacity (WHC) of fermented milk samples varied between 48.26 and 60.45% during storage. WHC increased significantly (P<0.001) on the 7th day of storage in all samples but showed no significant differences between samples (P>0.05). The highest WHC value (60.45%) was determined on day 7 only in sample C fermented with *S. thermophilus* 212S, but the fluctuations in WHC were small in all samples. Similar to our study, in a study by Yang et al. (2023b), the WHC of goat milk fermented using yogurt starter culture and *B. animalis* ssp. *lactis* strain ranged from about 50 to 62.5%, but decreased during storage. On the other hand, similar to our results, Aktaş and Çetin (2024b) reported that the WHC percentages of the yogurts produced using different starter culture combinations increased during storage and ranged between 46.52 and 55.17%. The significant increase in WHC determined at day 7 is likely related to changes in the milk gel microstructure during early storage. Acidification by starter cultures can generally increase WHC by promoting casein aggregation and network tightening, trapping more serum. Longer storage or increased proteolytic activity may subsequently weaken the protein network and increase syneresis (Arab et al., 2023).

The viscosity values of the fermented milk samples at 20 and 50 rpm ranged between 16265.5-10053.3 and 6960.7-4414.1 cP, respectively. On the first day of storage, at both 20 and 50 rpm, there was no significant difference between the viscosities of the samples (P>0.05) and the highest values were found on this day. On the other hand, viscosity values at both shear rates showed a consistent decline over time in all samples (P<0.001), with sample C exhibiting the steepest reduction. In addition, Sample C was found to have the lowest (P<0.001) viscosity values throughout storage except for day 1. Aktaş and Çetin (2024b) also reported that different starter cultures cause differences in the viscosity of fermented milk. Partly similar to our study, Son et al. (2023) reported that with higher *B. longum* inoculation levels, the viscosity of milk increased significantly during the fermentation stage, probably due to increased exopolysaccharide (EPS) production and its interaction with milk proteins. The use of *S. thermophilus* as a co-culture in fermented milk may have developed a symbiotic relationship with other bacteria and stimulate them to produce EPS.

Organic acid content

Organic acid contents of fermented milk during storage are presented in Figure 3 and Table 3. The flavor of fermented foods is significantly impacted by the presence of organic acids. These acids also play a crucial role in preserving the shelf life of these products (Ndhlala et al., 2022). The amounts of lactic acid in the fermented milks varied from 9149.5 to 11041.3 μg/mL for sample A, from 9465.2 to 11432.8 μg/mL for sample B, and from 9533.9 to 10998.4 μg/mL for sample C. In addition, the lactic acid content increased from day 1 to day 28 in all samples (P<0.001). The significantly higher level of lactic acid in sample B indicates the high metabolic activity of *L. paracasei* Shirota. In a study conducted by Xie et al. (2024), *Bifidobacterium longum* subsp. *longum* BB536 and *B. longum* subsp. *infantis* ATCC15697 strains were used as adjunct cultures in yogurt production, and it was also reported that lactic acid concentrations increased during 35 days of storage. Similar to our results, Nguyen et al. (2014) and Aktaş and Çetin (2024b) and stated that the lactic acid content in yogurts was around 10,000 μg/mL (ppm) and increased during storage. The amounts of acetic acid in the fermented milks varied from 211.19 to 337.16 μg/mL for sample A, from 125.63 to 178.61 μg/mL for sample B, and from not detected to 144.14 μg/mL for sample C. Acetic acid levels in the samples generally increased with storage duration, with the highest values found in sample A and the lowest values found in sample C. *B. longum* utilizes the fructose-6-phosphate phosphoketolase pathway (bifid shunt) to ferment carbohydrates, resulting in the concurrent production of lactate and acetate in varying proportions (Suzuki et al., 2010). Similar activity in mixed cultures of bifidobacteria and lactic acid bacteria have also been determined by others (Adhikari et al., 2002; Sun et al., 2023; Xie et al., 2024). The malic acid amounts of fermented milks varied from not detected to 118.37 μg/mL for sample A, from 122.87 to 148.29 μg/mL for

sample B, and from not detected to 138.57 μg/mL for sample C. The malic acid concentration in sample B remained relatively stable until day 28, while it was not detectable in samples A and C on day 28. This suggests strain dependent metabolism of malic acid. Malic acid is a naturally occurring intermediate in the tricarboxylic acid (TCA) cycle, formed during the sequence of oxidative reactions that begins with the condensation of oxaloacetate and acetyl-CoA to produce citrate (Wei et al., 2021). The increase in malic acid during storage may suggest that the bacteria needed more energy, which activated the citric acid cycle (Li et al., 2021). The amounts of citric acid in the fermented milks varied from 4885.93 to 5444.76 μg/mL for sample A, from 4899.37 to 5514.65 μg/mL for sample B, and from 5067.50 to 5569.61 μg/mL for sample C. All samples exhibited significant increases during storage (P<0.001). Similar to our study, Akgün et al. (2018) reported that lactic and citric acids were the predominant organic acids in buffalo yogurt produced with yogurt starters (*S. thermophilus*, and *Lactobacillus delbrueckii* subsp. *bulgaricus* (*L. bulgaricus*) and a combination of *Bifidobacterium animalis* subsp. *lactis* BB-12® (*B. animalis* subsp. *lactis*), *Lactobacillus acidophilus* LA-5® (*L. acidophilus*), *S. thermophilus*, and *L. bulgaricus*. They also found that lactic, acetic, and citric acids increased during storage. On the other hand, Aktaş and Çetin (2024b) reported that the citric acid content in the yogurts they produced using different starter cultures varied between 1912.91 and 2635.02 μg/mL. The fact that the results in our study are higher than this value may be due to the differences in the strains used. Propionic acid was not detected in any of the samples, suggesting that the culture and storage conditions were not conducive to propionic acid production.

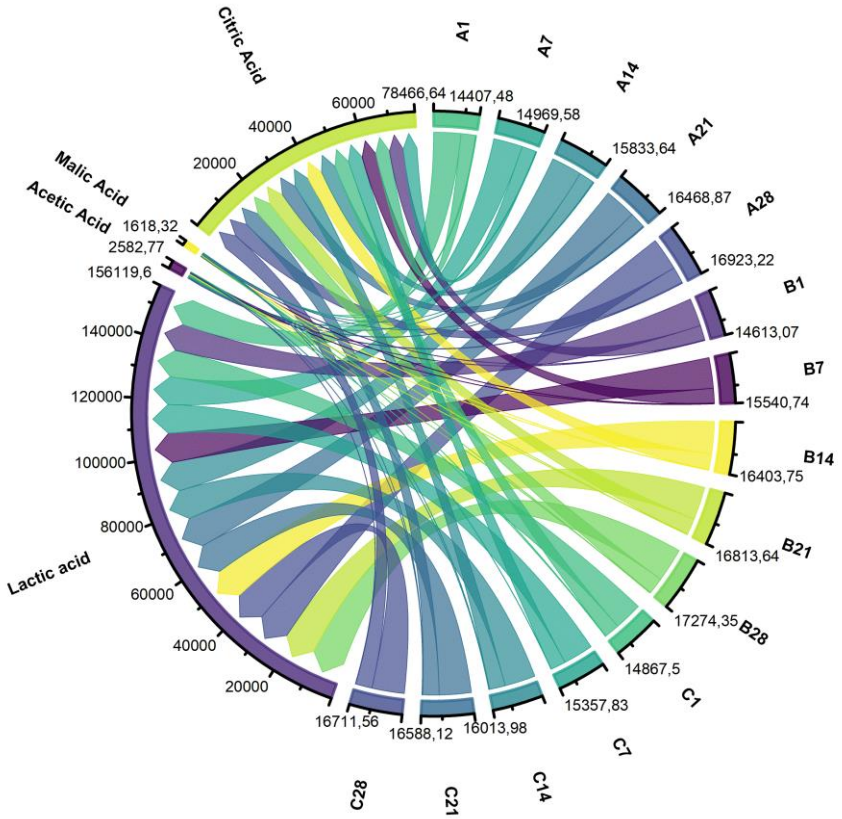

Figure 3. Chord diagram illustrating the results of the organic acid analysis of the fermented milk samples.

Table 3. Organic acid content of fermented milks during storage[a]

Organic acids (µg/mL)	Sample	Storage period (days)					Sig.
		1	7	14	21	28	
Lactic acid	A	9149.5±50.8^{eB}	9771.5±25.6^{dC}	10344.0±76.0^{eB}	10705.2±22.6^{bB}	11041.3±83,8^{aB}	***
	B	9465.2±66.5 ^{eA}	10316.1±60.3 ^{dA}	10902.2±29.8^{cA}	11085.2±43.5^{bA}	11432.8±51.4^{aA}	***
	C	9533.9±50.9^{eA}	10156.9±26.0^{dB}	10405.0±18.4^{cB}	10812.6±95.9^{bB}	10998.4±101.1^{aB}	***
Sig.		*	**	**	*	*	
Acetic acid	A	220.52±4.83^{dA}	211.19±7.08^{dA}	231.33±0.09^{cA}	286.80±0.64^{bA}	337.16±3.18^{aA}	***
	B	125.63±2.78^{cB}	140.00±4.59^{bcB}	161.51±20.78^{abB}	171.74±1.23^{aB}	178.61±1.53^{aB}	*
	C	103.56±2.44^{cC}	ND^{dC}	127.03±0.42^{bB}	144.14±1.24^{aC}	143.55±1.86^{aC}	***
Sig.		***	***	**	***	***	
Malic acid	A	118.37±20.47^{aA}	100.96±3.70^{aB}	109.06±3.36^{aA}	95.45±1.82^{aB}	ND^{bB}	***
	B	122.87±3.24^{aA}	142.36±3.58^{aA}	134.52±14.50^{aA}	141.72±8.04^{aA}	148.29±10.81^{aA}	ns
	C	138.57±1.99^{aA}	133.43±5.28^{aA}	128.98±7.69^{aA}	103.74±0.37^{bB}	ND^{cB}	
Sig.		ns	**	ns	**	***	
Citric acid	A	4919.09±27.06^{dB}	4885.93±26.22^{dB}	5149.25±34.69^{cA}	5381.62±25.46^{bB}	5544.76±27.03^{aA}	***
	B	4899.37±6.89^{cB}	4942.28±33.16^{cB}	5205.52±98.49^{bA}	5414.98±32.19^{aB}	5514.65±68.14^{aA}	***
	C	5091.47±34.58^{cA}	5067.50±25.10^{cA}	5352.97±13.76^{bA}	5527.64±26.67^{aA}	5569.61±0.48^{aA}	***
Sig.		**	*	ns	*	ns	
Propionic acid	A	ND	ND	ND	ND	ND	-
	B	ND	ND	ND	ND	ND	-
	C	ND	ND	ND	ND	ND	-
Sig.		-	-	-	-	-	

[a] A: Fermented milk produced by using *S. thermophilus* 212S and *B. longum* BH28, B: Fermented milk produced by using *S. thermophilus* 212S and *L. paracasei* Shirota, C: Fermented milk produced by using *S. thermophilus* 212S. Values are expressed as mean ± standard deviation. ND: Not determined, Sig.: Degree of statistical significance, ns: Not statistically significant, *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$. Lowercase and uppercase letter represent statistical differences in the same row and column, respectively.

Total phenolic content and antioxidant capacity

TPC and antioxidant capacity of fermented milk during storage are presented in Figure 4A, 4B, respectively, and Table 4. The TPC content of fermented milk varied between 23.40 and 26.92 mg GAE/L and there were no significant differences between the samples and storage time ($P>0.05$). Similar to TPC, the DPPH free radical scavenging activities (expressed as IC_{50} values) of the fermented milks did not differ statistically between the samples and throughout storage ($P>0.05$). IC_{50} values of fermented milk varied between 98.92 and 112.01 mg/mL during storage. Antioxidant activity can be exhibited by amino acids and small molecule peptides released during milk fermentation (Farvin et al., 2010). The literature contains different results regarding the TPC and DPPH scavenging activities of milk fermented using probiotic cultures. For example, in a study conducted by Hanum et al. (2022) IC_{50} values of fermented goat milk produced by adding different concentrations of *B. longum* varied between 99.12 and 123.46 ppm and antioxidant activity increased as the concentration of the culture increased. The TPC of cow's milk kefir produced by adding *Lactiplantibacillus plantarum* (*L. plantarum*), *B. longum*, and kefir starter cultures ranged from 63.82 to 82.62 mg GAE/L, with IC_{50} values ranging from 105.92 to 139.56 mg/mL, over 28 days of storage (Meral-Aktaş et al., 2025). On the other hand, Barat and Özcan (2018) determined that the TPC content of milk fermented using *S. thermophilus, L. bulgaricus, L. acidophilus,* and *Bifidobacterium animalis* spp. *lactis* (*B. animalis* spp. *lactis*) strains was 3.87 mg GAE/100 g dry weight. These differences in results may be due to variations in the cultures used, milk type, fermentation stage, and methodology.

Sensory analysis

Sensory analysis results of fermented milk during storage are presented in Figure 5, and Table 5. No differences were found between the samples in terms of all sensory properties during storage ($P>0.05$). On the other hand, there was a general decrease in the color scores of all samples during storage ($P<0.05$). Similarly, there was a non-linear decrease in the appearance score of samples A and B. Despite this decrease, all color and appearance scores of the samples were above 7.45 (6-7: desirable) throughout storage. In a study by

Casarotti et al. (2014), fermented milk was produced using *S. thermophilus*, *L. acidophilus,* and *B. animalis* spp. *lactis* strains as single and mixed cultures with *S. thermophilus*. The authors stated that the samples in which *B. animalis* subsp. *lactis* was used as a single culture and mixed culture with *S. thermophilus* received the highest overall acceptability scores. In the study conducted by Li et al. (2020), it was found that there was no significant difference in the sensory properties of fermented milks produced using different combinations of *S. thermophilus*, *L. plantarum,* and *B. animalis* strains on the 1st day of storage, but there were significant differences between the samples on the 21st day. Accordingly, on day 21, fermented milk produced with *S. thermophilus* with 2:1 of *L. plantarum* and *B. animalis* had the highest overall acceptability score, while fermented milk produced with *S. thermophilus* with 1:2 of *L. plantarum* and *B. animalis* had the lowest overall acceptability score. The lack of significant differences in sensory characteristics between samples and during storage suggest that the *B. longum* BH28 strain can be used in the production of fermented milk without adversely affecting its sensory characteristics.

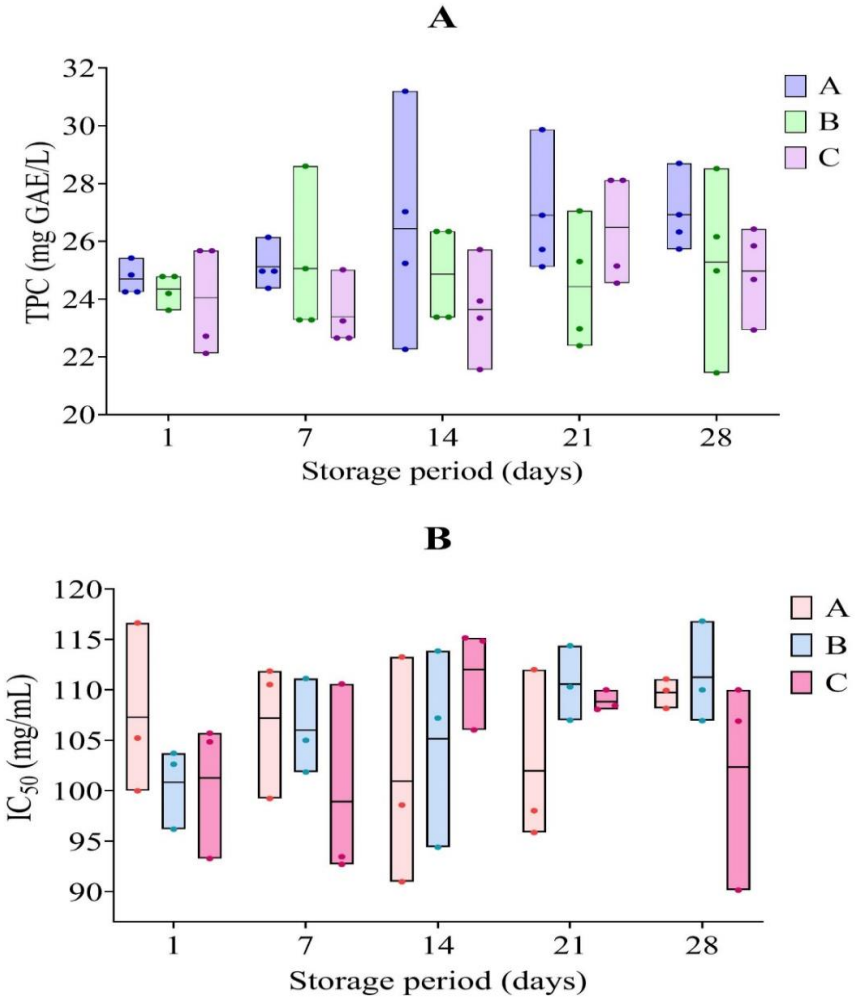

Figure 4. Total phenolic content (A) and antioxidant capability (B) of the fermented milk samples. A: Fermented milk produced by using *S. thermophilus* 212S and *B. longum* BH28, B: Fermented milk produced by using *S. thermophilus* 212S and *L. paracasei* Shirota, C: Fermented milk produced by using *S. thermophilus* 212S. Values are expressed as mean ± standard deviation.

Table 4. Total phenolic content and antioxidant activity of fermented milks during storage[a]

Parameters	Sample	Storage period (days)					Sig.
		1	7	14	21	28	
TPC (mg GAE/L)	A	24.69±0.56^{aA}	25.11±0.74^{aA}	26.43±3.73^{aA}	26.90±2.11^{aA}	26.92±1.28^{aA}	ns
	B	24.35±0.60^{aA}	25.06±2.51^{aA}	24.86±1.71^{aA}	24.43±2.16^{aA}	25.28±2.95^{aA}	ns
	C	24.05±1.89^{aA}	23.40±1.12^{aA}	23.64±1.71^{aA}	26.48±1.90^{aA}	24.97±1.54^{aA}	ns
Sig.		ns	ns	ns	ns	ns	
Antioxidant activity (IC_{50}, mg/mL)	A	107.28±8.51^{aA}	107.20±6.93^{aA}	100.94±11.32^{aA}	101.96±8.76^{aA}	109.72±1.47^{aA}	ns
	B	100.84±4.07^{aA}	106.00±4.71^{aA}	105.15±9.89^{aA}	110.56±3.70^{aA}	111.25±5.06^{aA}	ns
	C	101.28±6.94^{aA}	98.92±10.11^{aA}	112.01±5.18^{aA}	108.83±1.03^{aA}	102.35±10.68^{aA}	ns
Sig.		ns	ns	ns	ns	ns	

[a] A: Fermented milk produced by using *S. thermophilus* 212S and *B. longum* BH28, B: Fermented milk produced by using *S. thermophilus* 212S and *L. paracasei* Shirota, C: Fermented milk produced by using *S. thermophilus* 212S. Values are expressed as mean ± standard deviation. Sig.: Degree of statistical significance, ns: Not statistically significant, *: p<0.05, **: p<0.01, ***: p<0.001. Lowercase and uppercase letter represent statistical differences in the same row and column, respectively.

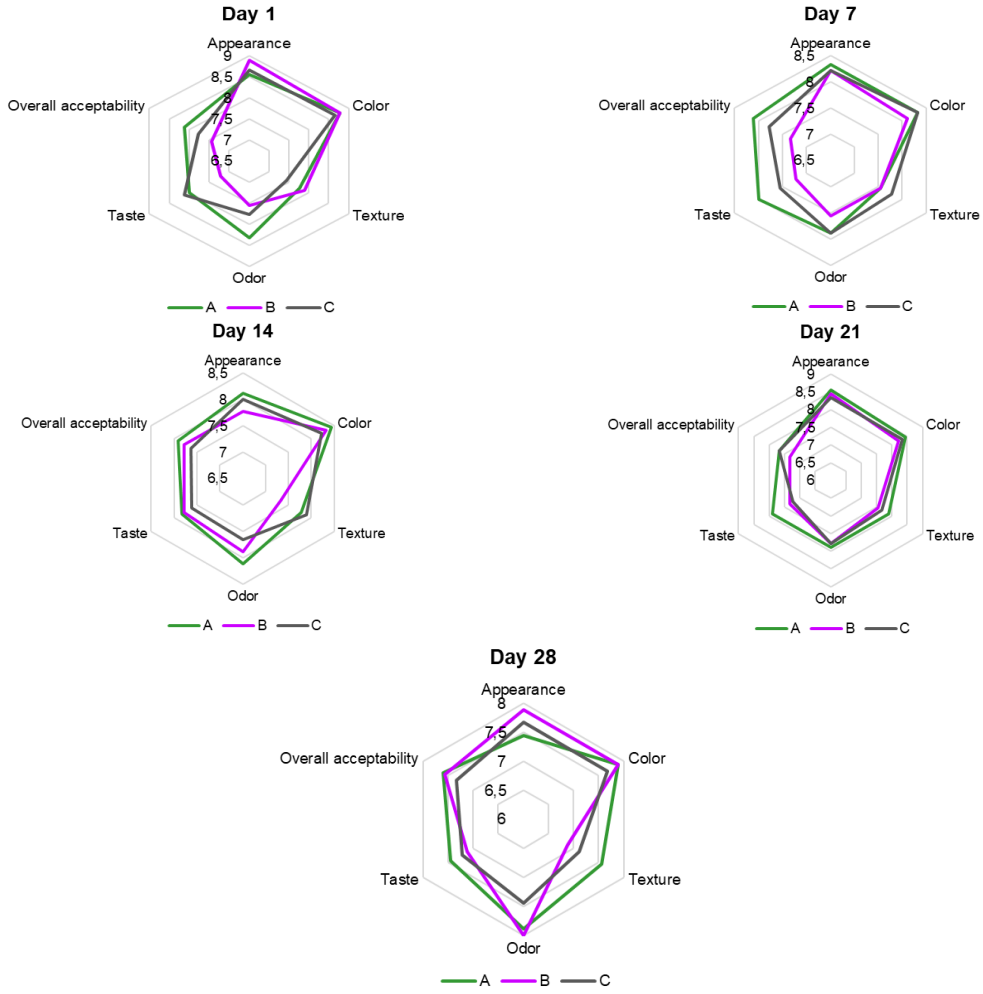

Figure 5. Sensory analysis results of fermented milk samples. A: Fermented milk produced by using *S. thermophilus* 212S and *B. longum* BH28, B: Fermented milk produced by using *S. thermophilus* 212S and *L. paracasei* Shirota, C: Fermented milk produced by using *S. thermophilus* 212S. Values are expressed as mean ± standard deviation.

Table 5. Sensory analysis results of fermented milks during storage[a]

Sensory parameters	Sample	Storage period (days)					Sig.
		1	7	14	21	28	
Appearance	A	8.56±0.53^{a}	8.34±0.71^{a}	8.12±0.71^{ab}	8.56±0.53^{a}	7.45±1.34^{b}	*
	B	8.89±0.34^{a}	8.22±0.44^{bc}	7.78±0.44^{c}	8.45±0.53^{ab}	7.89±0.78^{c}	***
	C	8.67±0.50^{a}	8.23±0.67^{a}	8.00±0.71^{a}	8.34±0.71^{a}	7.67±1.00^{a}	ns
Sig.		ns	ns	ns	ns	ns	
Color	A	8.77±0.44^{a}	8.34±0.71^{ab}	8.45±0.53^{ab}	8.45±0.52^{ab}	7.89±0.60^{b}	*
	B	8.78±0.44^{a}	8.11±0.60^{b}	8.34±0.50^{ab}	8.23±0.67^{ab}	7.89±0.60^{b}	*
	C	8.66±0.50^{a}	8.34±0.50^{a}	8.23±0.67^{a}	8.34±0.50^{a}	7.67±0.71^{b}	*
Sig.		ns	ns	ns	ns	ns	
Texture	A	7.77±0.67^{a}	7.56±1.24^{a}	7.78±0.44^{a}	7.89±.060^{a}	7.56±1.13^{a}	ns
	B	7.89±0.60^{a}	7.56±1.24^{a}	7.34±1.00^{a}	7.56±0.53^{a}	6.89±0.78^{a}	ns
	C	7.45±1.01^{a}	7.78±0.67^{a}	7.89±0.78^{a}	7.67±0.87^{a}	7.12±1.36^{a}	ns
Sig.		ns	ns	ns	ns	ns	
Odor	A	8.34±0.71^{a}	7.89±1.17^{a}	8.12±0.78^{a}	7.89±0.60^{a}	7.89±0.60^{a}	ns
	B	7.56±1.01^{a}	7.55±1.00^{a}	7.89±0.60^{a}	7.78±0.44^{a}	8.00±0.71^{a}	ns
	C	7.78±1.09^{a}	7.89±0.93^{a}	7.67±1.00^{a}	7.78±0.67^{a}	7.45±0.88^{a}	ns
Sig.		ns	ns	ns	ns	ns	
Taste	A	8.00±1.00^{a}	8.00±0.87^{a}	7.84±1.00^{a}	7.89±0.78^{a}	7.45±1.01^{a}	ns
	B	7.23±0.97^{a}	7.23±1.39^{a}	7.78±0.44^{a}	7.34±0.87^{a}	7.12±0.78^{a}	ns
	C	8.12±0.78^{a}	7.56±1.13^{a}	7.61±1.17^{a}	7.23±0.67^{a}	7.23±0.84^{a}	ns
Sig.		ns	ns	ns	ns	ns	
Overall acceptability	A	8.12±0.78^{a}	8.12±0.78^{a}	7.92±1.06^{a}	7.67±0.71^{a}	7.60±0.99^{a}	ns
	B	7.45±1.13^{a}	7.34±1.12^{a}	7.78±0.44^{a}	7.34±0.71^{a}	7.56±1.01^{a}	ns
	C	7.78±0.67^{a}	7.78±0.84^{a}	7.64±0.86^{a}	7.67±0.50^{a}	7.34±0.87^{a}	ns
Sig.		ns	ns	ns	ns	ns	

[a] A: Fermented milk produced by using *S. thermophilus* 212S and *B. longum* BH28, B: Fermented milk produced by using *S. thermophilus* 212S and *L. paracasei* Shirota, C: Fermented milk produced by using *S. thermophilus* 212S. Values are expressed as mean ± standard deviation. Sig.: Degree of statistical significance, ns: Not statistically significant, *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$. Lowercase and uppercase letter represent statistical differences in the same row and column, respectively.

Principal component analysis

Microbiological and physicochemical properties, organic acid, total phenolic content, antioxidant capacity, and overall acceptability scores of fermented milk samples were taken into consideration and analyzed by PCA, and the results are shown in Figure 6A-D. Three clusters of samples were created on hierarchical cluster analysis (HCA, Figure 6A). Group 1 included A1, B1, and C1, early storage points, indicating that these samples shared similar characteristics. Regarding sample C, there was no homogeneous distribution among groups 2 and 3. Sample A was generally clustered in group 2, and sample B was generally clustered in group 3. These patterns suggest that both the type of inoculum and the storage time significantly influenced the overall product profile. The PCA score plot (Figure 4B) revealed that PC1 and PC2 explained 41.3% and 22.1% of the total variance (62.4%), respectively. In alignment with the HCA results, samples clustered into three distinct groups. According to the biplot analysis (Figure 4D), samples A (A7, A14, A21, and A28) were separated as group 2, which probably indicated a distinct profile due to the presence of *B. longum* BH28 and its production of acetic acid. Conversely, the B samples (B1, B7, B14, and B21) formed a distinct group (group 3), likely due to the presence of *L. paracasei* Shirota and high malic acid content. In addition, group 1, which includes A1, B1, and C1, is characterized by high pH and viscosity.

CONCLUSION

In the present study, human origin *B. longum* BH28 and *L. paracasei* Shirota were cultured together with *S. thermophilus* 212S to produce fermented milk. These fermented milks differ from each other in terms of certain microbiological, physicochemical, and functional properties. The number of *B. longum* BH28 in sample A, which was produced using *B. longum*

BH28 and *S. thermophilus* 212S, was around 7 log CFU/mL on the first day of storage. On the seventh day, the number had fallen to 5 log CFU/mL, and it remained stable until the end of storage. The probable probiotic count, which fell below 6 log CFU/mL, led to a loss of probiotic properties in the product. An examination of the physicochemical properties of the products revealed that the viscosities of samples A and B, which were produced with the addition of probiotic cultures, were generally found to be higher than that of sample C (only *S. thermophilus* 212S). This phenomenon can be attributed to the enhanced EPS production potential of probiotics. During the storage period, lactic acid was identified as the predominant organic acid in all the fermented milk samples examined. Additionally, acetic acid levels were found to be elevated in sample B relative to the other samples. On the other hand, no significant differences were found between the samples in terms of TPC, antioxidant capacity, and sensory properties. Specifically, elevated sensory analysis scores indicated the potential for *B. longum* BH28 strain to be utilized in fermented dairy products and to gain approval among consumers. Further studies may undertake a more thorough examination of techniques such as high-rate culture addition, microencapsulation, and prebiotic utilization to ensure the maintenance of a minimum population of *B. longum* BH28 at 6 log CFU/mL during storage.

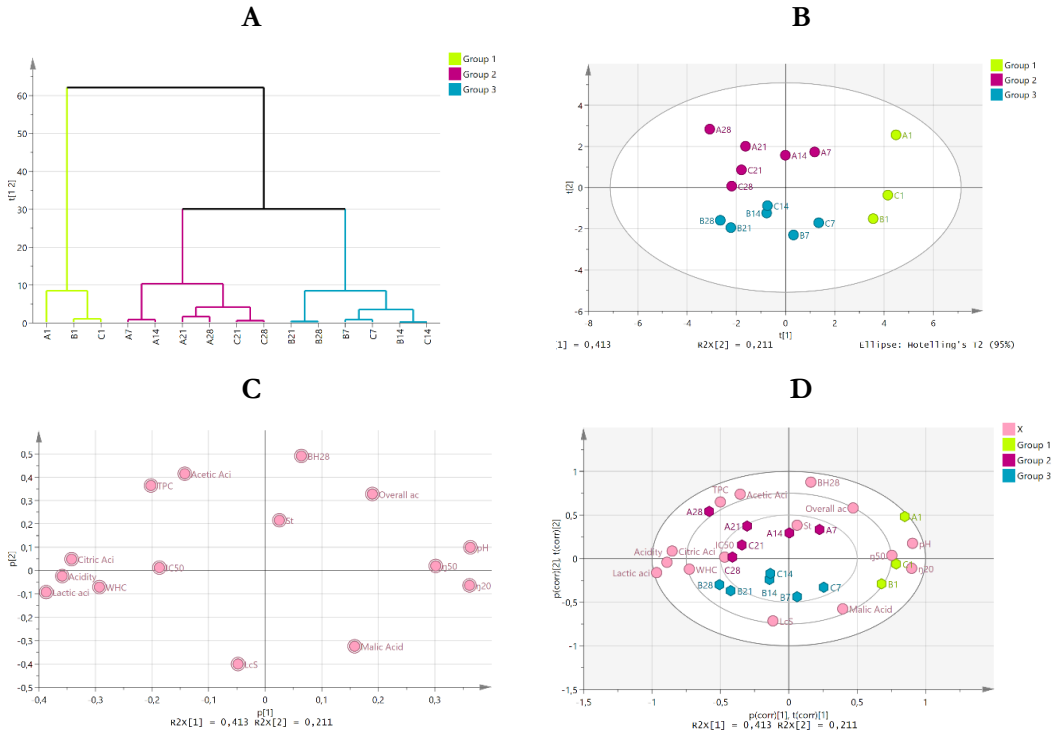

Figure 6. Hierarchical cluster analysis (A), score scatter plot (B), loading scatter plot (C), and biplot (D) of the principal component analysis. A: Fermented milk produced by using *S. thermophilus* 212S and *B. longum* BH28, B: Fermented milk produced by using *S. thermophilus* 212S and *L. paracasei* Shirota, C: Fermented milk produced by using *S. thermophilus* 212S, TPC: Total phenolic content, WHC: Water holding capacity, BH28: *B. longum* BH28, LcS: *L. paracasei* Shirota, St: *S. thermophilus*, Overall ac: Overall acceptability.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest with any individuals or institutions regarding this article.

AUTHOR CONTRIBUTIONS

Hacer MERAL-AKTAŞ: Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing-original draft preparation, writing-review and editing, visualization, supervision, project administration, and funding acquisition.

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REFERENCES

Adhikari, K., Grün, I.U., Mustapha, A., Fernando, L.N. (2002). Changes in the profile of organic acids in plain set and stirred yogurts during manufacture and refrigerated storage. *Journal of Food Quality*, 25(5): 435-451, doi: 10.1111/j.1745-4557.2002.tb01038.x.

Akgün, A., Yazıcı, F., Güleç, H.A. (2018). The combined effect of probiotic cultures and incubation final pH on the quality of buffalo milk yogurt during cold storage. *Food Science & Nutrition*, 6(2): 492-502, doi: 10.1002/fsn3.580.

Aktaş, H., Çetin, B. (2024a). Multidimensional evaluation of techno-functional properties of yoghurt bacteria. *International Dairy Journal*, 148: 105795. doi: 10.1016/j.idairyj.2023.105795.

Aktaş, H., Çetin, B. (2024b). Effects of novel autochthonous starter cultures on quality characteristics of yoghurt. *International Journal of Food Engineering*, 20(11-12): 799-808. doi: 10.1515/ijfe-2024-0103.

Anonymous (2008). Microbiology of food and animal feeding stuffs-horizontal method for the enumeration of yeasts and moulds-Part 1: Colony count technique in products with water activity greater than 0.95. International Standardization Organization. ISO 21527-1.

Anonymous (2010). Microbiology of food and animal feeding stuffs –horizontal method for the detection and enumeration of coliforms –most probable number technique. International Standardization Organization ISO 4831.

Arab, M., Yousefi, M., Khanniri, E., Azari, M., Ghasemzadeh-Mohammadi, V., Mollakhalili-Meybodi, N. (2023). A comprehensive review on yogurt syneresis: Effect of processing conditions and added additives. *Journal of Food Science and Technology*, 60(6): 1656-1665. doi: 10.1007/s13197-022-05403-6.

Atalar, İ. (2019). Functional kefir production from high pressure homogenized hazelnut milk. *LWT - Food Science and Technology*, 107: 256-263, doi: 10.1016/j.lwt.2019.03.013.

AOAC (2005). Official Methods of Analysis of AOAC INTERNATIONAL. 18th Edition, Gaithersburg, MD, the USA.

Barat, A., Özcan, T. (2018). Growth of probiotic bacteria and characteristics of fermented milk containing fruit matrices. *International Journal of Dairy Technology*, 71: 120-129. doi: 10.1111/1471-0307.12391.

Bensmira, M., Jiang, B. (2012). Effect of some operating variables on the microstructure and physical properties of a novel kefir formulation. *Journal of Food Engineering*, 108: 579-584, doi: 10.1016/j.jfoodeng.2011.07.025.

Casarotti, S.N., Monteiro, D.A., Moretti, M.M., Penna, A.L.B. (2014). Influence of the combination of probiotic cultures during fermentation and storage of fermented milk. *Food Research International*, 59: 67-75, doi: 10.1016/j.foodres.2014.01.068.

Codex Alimentarius (2003). Standard for fermented milks. https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%3A%2F%2Fworkspace.fao.org%2Fsites%2Fcodex%2FStandards%2FCXS%2B243-2003%2FCXS_243e.pdf (Erişim tarihi: 01.06.2025).

Damin, M.R., Minowa, E., Alcantara, M.R., Oliveira, M.N. (2008). Effect of cold storage on culture viability and some rheological properties of fermented milk prepared with yogurt and probiotic bacteria. *Journal of Texture Studies*, 39(1): 40-55, doi: 10.1111/j.1745-4603.2007.00129.x.

Donkor, O.N., Henriksson, A., Vasiljevic, T., Shah, N.P. (2006). Effect of acidification on the activity of probiotics in yoghurt during cold storage. *International Dairy Journal*, 16(10): 1181-1189.

El-Fattah, A.A., Sakr, S., El-Dieb, S., Eikashef, H. (2018). Developing functional yogurt rich in bioactive peptides and gamma-aminobutyric acid related to cardiovascular health. *LWT - Food Science and Technology*, 98: 390-397, doi: 10.1016/j.lwt.2018.09.022.

FAO/WHO (2002). Guidelines for the Evaluation of Probiotics in Food. FAO/WHO Working Group. https://www.fao.org/3/a0512e/a0512e.pdf (Erişim tarihi: 30.06.2025).

Farvin, K.S., Baron, C.P., Nielsen, N.S., Jacobsen, C. (2010). Antioxidant activity of yoghurt peptides: Part 1 - *in vitro* assays and evaluation in ω-3 enriched milk. *Food Chemistry*, 123(4): 1081-1089. doi: 10.1016/j.foodchem.2010.05.067.

Galli, V., Venturi, M., Mari, E., Guerrini, S., Granchi, L. (2022). Gamma-aminobutyric acid (GABA) production in fermented milk by lactic acid bacteria isolated from spontaneous raw milk fermentation. *International Dairy Journal*, 127: 105284, doi: 10.1016/j.idairyj.2021.105284.

Gavzy, S.J., Kensiski, A., Lee, Z.L., Mongodin, E.F., Ma, B., Bromberg, J.S. (2023). *Bifidobacterium* mechanisms of immune modulation and tolerance. *Gut Microbes*, 15(2): 2291164. doi: 10.1080/19490976.2023.2291164.

Güler, M.A., Çetin, B., Albayrak, B., Meral-Aktaş, H., Tekgündüz, K.Ş., Kara, M., Işlek, A. (2024). Isolation, identification, and *in vitro* probiotic characterization of forty novel *Bifidobacterium* strains from neonatal feces in Erzurum province, Türkiye. *Journal of the Science of Food and Agriculture*, 104(7): 4165-4175, doi: 10.1002/jsfa.13298.

Hanum, Z., Yurliasni, Y., Seutia, F.H. (2022, March). Antioxidant Activity of Fermented Goat's Milk with the Use of *Bifidobacterium longum*. *International Conference on Improving Tropical Animal Production for Food Security (ITAPS 2021)*, s. 374-380. Atlantis Press.

Irigoyen, A., Arana, I., Castiella, M., Torre, P., Ibanez, F.C. (2005). Microbiological, physicochemical, and sensory characteristics of kefir during storage. *Food Chemistry*, 90(4): 613-620. doi: 10.1016/j.foodchem.2004.04.021.

Işık, S., Dağdemir, E., Tekin, A., Hayaloğlu, A.A. (2023). Metabolite profiling of fermented milks as affected by adjunct cultures during long-term storage. *Food Bioscience*, 56: 103344. doi: 10.1016/j.fbio.2023.103344.

Jena, R., Choudhury, P.K. (2025). Bifidobacteria in fermented dairy foods: a health beneficial outlook. *Probiotics and Antimicrobial Proteins*, 17(3): 1-22. doi: 10.1007/s12602-023-10189-w.

Leahy, S.C., Higgins, D.G., Fitzgerald, G.F., Van Sinderen, D. (2005). Getting better with bifidobacteria. *Journal of Applied Microbiology*, 98(6): 1303-1315. doi: 10.1111/j.1365-2672.2005.02600.x.

Li, J., Wang, J., Wang, M., Zheng, L., Cen, Q., Wang, F., ... Zhang, A. (2023). *Bifidobacterium*: a probiotic for the prevention and treatment of depression. *Frontiers in Microbiology*, 14: 1174800. doi: 10.3389/fmicb.2023.1174800.

Li, S., Tang, S., He, Q., Gong, J., Hu, J. (2020). Physicochemical, textural and volatile characteristics of fermented milk co-cultured with *Streptococcus thermophilus*, *Bifidobacterium animalis* or *Lactobacillus plantarum*. *International Journal of Food Science and Technology*, 55(2): 461-474, doi: 10.1111/ijfs.14279.

Li, S.N., Tang, S.H., Ren, R., Gong, J.X., Chen, Y.M. (2021). Metabolomic profile of milk fermented with *Streptococcus thermophilus* cocultured with *Bifidobacterium animalis* ssp. *lactis*, *Lactiplantibacillus plantarum*, or both during storage. *Journal of Dairy Science*, 104(8): 8493-8505, doi: 10.3168/jds.2021-20270.

Ma, P., Li, Y., Hao, J., Lu, H., He, Y., Wei, L., ... Wang, S. (2025). Co-culture of *Lactobacillus bulgaricus* with *Streptococcus thermophilus* and *Bifidobacterium* impact the metabolism and flavor of fermented milk. *Food Science & Nutrition*, 13(5): e70182. doi: 10.1002/fsn3.70182.

Maleki, N., Khodaiyan, F., Mousavi, S.M. (2015). Antioxidant activity of fermented hazelnut milk. *Food Science and Biotechnology*, 24(1): 107–115, doi: 10.1007/s10068-015-0016-0.

Meral-Aktaş, H., Bazu-Çırpıcı, B., Aktaş, H., Kadiroğlu, H., Çetin, B. (2025). Comparative quality assessment of plant-based kefir as a vegan alternative to traditional kefir. *Food Bioscience*, 107062. doi: 10.1016/j.fbio.2025.107062.

Meilgaard, M.C., Carr, B.T., Civille, G.V. (1999). *Sensory Evaluation Techniques*. 3rd edn. CRC Press, Boca Raton, FL, USA.

Ndhlala, A.R., Kavaz Yüksel, A., Yüksel, M. (2022). Nutritional supplementation of yogurt with Jerusalem artichoke tubers: Organic acid profiles and quality parameters. *Plants*, 11(22): 3086. doi: 10.3390/plants11223086.

Nguyen, H.T., Ong, L., Kentish, S.E., Gras, S.L. (2014). The effect of fermentation temperature on the microstructure, physicochemical and rheological properties of probiotic buffalo yoghurt. *Food and Bioprocess Technology*, 7(9): 2538-2548. doi: 10.1007/s11947-014-1278-x.

Özcan, T., Şahin, S., Akpınar-Bayizit, A., Yılmaz-Ersan, L. (2019). Assessment of antioxidant capacity by method comparison and amino acid characterisation in buffalo milk kefir. *International Journal of Dairy Technology*, 72(1): 65–73. doi: 10.1111/1471-0307.12560.

Parisa, A., Roya, G., Mahdi, R., Shabnam, R., Maryam, E., Malihe, T. (2020). Anti-cancer effects of *Bifidobacterium* species in colon cancer cells and a mouse model of carcinogenesis. *PloS One*, 15(5): e0232930. doi: 10.1371/journal.pone.0232930.

Rasinkangas, P., Forssten, S.D., Marttinen, M., Ibarra, A., Bothe, G., Junnila, J., ... Ouwehand, A.C. (2022). *Bifidobacterium animalis* subsp. *lactis* Bi-07 supports lactose digestion in vitro and in randomized, placebo- and lactase-controlled clinical trials. *The American Journal of Clinical Nutrition*, 116(6): 1580-1594. doi: 10.1093/ajcn/nqac264.

Shori, A.B. (2021). Application of *Bifidobacterium* spp in beverages and dairy food products: An overview of survival during refrigerated storage. *Food Science and Technology*, 42: e41520. doi: 10.1590/fst.41520.

Son, J.K., Jo, Y.J., Jung, Y.J., Lee, Y.R., Lee, J., Jeong, H.S. (2023). Fermentation and quality characteristics of yogurt treated with *Bifidobacterium longum*. *Nutrients*, 15(15): 3490. doi: 10.3390/nu15153490.

Souza, T.C., Zacarías, M.F., Silva, A.M., Binetti, A., Reinheimer, J., Nicoli, J.R., Vinderola, G. (2012). Cell viability and immunostimulating and protective capacities of *Bifidobacterium longum* 51A are differentially affected by technological variables in fermented milks. *Journal of Applied Microbiology*, 112(6): 1184-1192. doi: 10.1111/j.1365-2672.2012.05280.x.

Sumalapao, D.E.P., Mesina, J.A.R.T., Cabrera, E.C., Gloriani, N.G. (2017). Viability kinetics of *Lactobacillus casei* Shirota strain in a commercial fermented milk drink during refrigerated storage. *National Journal of Physiology, Pharmacy and Pharmacology*, 7(11): 1242-1246. doi: 10.5455/njppp.2017.7.0621521072017.

Sun, Y., Guo, S., Wu, T., Yang, Y., Shen, T., Ma, X., Zhang, H. (2023). *Bifidobacterium adolescentis* B8589- and *Lacticaseibacillus paracasei* PC-01-co-fermented milk has more γ-aminobutyric acid and short-chain fatty acids than *L. paracasei* PC-01-fermented milk. *LWT*, 179: 114645. doi: 10.1016/j.lwt.2023.114645

Suzuki, R., Katayama, T., Kim, B.J., Wakagi, T., Shoun, H., Ashida, H., Fushinobu, S. (2010). Crystal structures of phosphoketolase: thiamine diphosphate-dependent dehydration mechanism. *Journal of Biological Chemistry*, 285(44): 34279-34287. doi: 10.1074/jbc.M110.156281.

Tian, R., Yu, Z., Yu, L., Tian, F., Zhao, J., Zhang, H., Zhai, Q. (2022). Effects of *Bifidobacterium longum* CCFM5871 as an adjunct starter culture on the production of fermented milk. *Food Bioscience*, 50: 102167. doi: 10.1016/j.fbio.2022.102167.

Xie, Z., Kim, C., Miller, M.J., Jin, Y.S. (2024). Effects of 2'-fucosyllactose on the viability of starter cultures and *Bifidobacterium* strains of human origin in yogurt during refrigerated storage. *Journal of Food Science*, 89(5): 2546-2556. doi: 10.1111/1750-3841.16996.

Wei, Z., Xu, Y., Xu, Q., Cao, W., Huang, H., Liu, H. (2021). Microbial biosynthesis of L-malic acid and related metabolic engineering strategies: advances and prospects. *Frontiers in Bioengineering and Biotechnology*, 9: 765685. doi: 10.3389/fbioe.2021.765685

Wang, L., Chai, M., Wang, J., Yu, Q., Wang, G., Zhang, H., Chen, W. (2022). *Bifidobacterium longum* relieves constipation by regulating the intestinal barrier of mice. *Food & Function*, 13(9): 5037-5049. doi: 10.1039/D1FO04151G.

Yang, X., He, X., Xu, S., Zhang, Y., Mo, C., Lai, Y., Xiao, Q. (2023a). Effect of *Lacticaseibacillus paracasei* strain Shirota supplementation on clinical responses and gut microbiome in Parkinson's disease. *Food & Function*, 14(15): 6828-6839. doi:10.1039/D3FO00728F.

Yang, Y., Zhang, R., Zhang, F., Wang, B., Liu, Y. (2023b). Storage stability of texture, organoleptic, and biological properties of goat milk yogurt fermented with probiotic bacteria. *Frontiers in Nutrition*, 9: 1093654. doi: 10.3389/fnut.2022.1093654.