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ORIGINAL PAPER

Innovations in dairy technology: probiotics in Turkish white cheese production

Halit Mazlum[1] · **Mustafa Atasever**[2]

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Abstract

Cheese offers significant advantages as a probiotic carrier food compared to fermented milk and yoğurt due to its fat content, solid matrix, higher pH, low oxygen levels, and longer shelf life. This study examined Turkish white cheeses ripened with both classical starter culture (*Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*) and various probiotic cultures (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus paracasei*, and *Bifidobacterium bifidum*). The quality and functional properties of these cheeses were investigated to evaluate the effect of adding probiotics to traditional starter culture and their potential for use as carrier food for probiotics. During the 90-days ripening period, the numbers of *L. acidophilus, L. casei, L. paracasei*, and *B. bifidum* were determined to be in the range of 8.09 ± 0.34–8.65 ± 0.30, 7.19 ± 0.28–8.12 ± 0.90, 7.01 ± 1.45–8.73 ± 0.98, and 7.16 ± 1.10–8.21 ± 1.19 log cfu/g, respectively. The study found that probiotic levels in the cheese remained above the effective threshold ($\geq 10^7$ cfu/g) throughout the ripening process. This was accompanied by an increase in water-soluble nitrogen, an indicator of proteolysis, leading to higher ripening index values in all cheese samples. In terms of sensory evaluation, cheeses with *L. acidophilus* and *L. paracasei* were particularly well-received, scoring higher (7.90 ± 0.30–8.47 ± 0.12, 7.80 ± 0.34–8.75 ± 0.12, respectively) in taste and aroma than the others. Overall, probiotics positively influenced the quality and functional properties of the white cheese. Notably, the *L. casei* ATCC 393 strain, used for the first time in Turkish white cheese production, proved highly compatible with existing production technologies. It was concluded from the study that Turkish white cheese is a suitable food for transporting probiotics to the intestinal environment.

Keywords Innovation · Dairy technology · Probiotic · Turkish white cheese · Probiotic cheese

Introduction

Recent scientific and technological advancements, coupled with the rise in pandemic diseases, have fueled consumer demand for health-beneficial foods [1, 2]. As a result, functional foods, which offer health benefits beyond traditional nutritional value, have gained prominence [3]. This category includes a variety of products like dairy items, cakes, beverages, and baby foods. Among these, foods enriched with live probiotic microorganisms stand out as the most significant and intriguing group in functional foods [2, 4].

Probiotics, when consumed in adequate amounts, exert beneficial health effects on the host by altering the intestinal microflora [5, 6]. These effects include oral health protection, control of gastrointestinal and urinary tract infections, reduction of lactose intolerance, cholesterol lowering, immune system enhancement, and prevention of colon cancer and allergies [6–8]. Probiotics can be incorporated into foods or administered orally as cachets, tablets, and capsules [8, 9]. For maximum health benefits, probiotic foods should contain at least 10^6–10^7 cfu/g or cfu/ml, and a daily intake of more than 100 g or ml is recommended [10, 11].

Dairy products are the largest category of probiotic foods. A major challenge in producing these products is ensuring the viability of the probiotics [12]. Most research on probiotic carrier foods within the dairy sector has focused on fermented milk and yoğurt [13–15]. However,

✉ Halit Mazlum
hmazlum@gumushane.edu.tr

[1] Department of Veterinary Medicine, Kelkit Aydın Doğan Vocational School, Gümüşhane University, Gümüşhane, Turkey

[2] Department of Food and Hygiene Technology, Faculty of Veterinary Medicine, Atatürk University, Erzurum, Turkey

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the short shelf life, low pH, and soft consistency of these products can impair probiotic survival under gastrointestinal conditions, such as enzymes, acidic environments, and bile salts [2, 16]. Consequently, in recent years, different food matrices (e.g., cheese, grains, fermented meats) have been evaluated for the passage of probiotics into the intestine [15].

Cheese offers significant advantages as a probiotic food compared to fermented milk and yogurt due to its fat content, solid matrix, higher pH, low oxygen levels, and longer shelf life [4, 17–21]. Additionally, cheese acts as a buffer against stomach acidity, thereby protecting probiotics in the gastrointestinal tract [20]. An in vitro study by Sharp et al. [22] found that cheese more effectively protected the *L. casei* strain in the acidic stomach environment (pH: 2.0) compared to yogurt.

When selecting probiotics for cheese, factors such as the type of cheese, its production technology, and the probiotics' survival in gastrointestinal conditions should be considered [21, 23, 24]. Commonly used species in probiotic cheese production include those from the *Lactobacillus* spp. and *Bifidobacterium* spp. genera, which are natural members of the intestinal microflora [5, 18, 25]. Their widespread use is attributed to their tolerance to acidity, salt, oxygen, temperature, gastrointestinal enzymes, and their ability to adhere well to the intestinal epithelium [24, 26].

Numerous studies in the literature have explored various types of cheese as carriers for probiotics. These include cheddar [1, 5, 9, 22, 27, 28], gouda [20, 29], edam [6, 19], minas frescal [30, 31], crescenza [14], domiati [32], mozzarella [8], feta [3, 7, 33], and ras [34] cheeses. In probiotic cheese production studies, probiotic addition to milk is applied by free, microencapsulation or immobilization methods [3, 7, 28, 35].

In Türkiye, white cheese is widely consumed for its unique taste, medium-hard texture, homogeneous appearance without holes, bright color, and white brine [36]. However, there are relatively few studies on probiotic white cheese production [37–40]. Further research is needed to understand the impact of different culture combinations on the quality and functional properties of white cheese under consistent production conditions. The objective of this study is to investigate the effect of probiotic addition on the quality of Turkish white cheese and the potential of using this cheese as a probiotic carrier food. In this study, white cheeses were produced with the addition of various probiotic cultures (*L. acidophilus* LA5, *L. casei* ATCC 393, *L. paracasei*, and *B. bifidum* BB12) alongside classical starter cultures (*Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris*). These cheeses were stored at 4 °C and their physicochemical, microbiological, and sensory properties were analyzed during a 90-day ripening period.

Materials and methods

Materials

Raw milk and cultures

For cheese production, cow's milk of appropriate quality was used, with the following characteristics: pH of 6.75 ± 0.05, titratable acidity of 0.171 ± 0.02, non-fat dry matter content of $10 \pm 0.5\%$, fat content of $3.97 \pm 0.12\%$, protein content of $3.35 \pm 0.25\%$, ash content of $0.82 \pm 0.02\%$, and specific gravity of 1.033 ± 0.001. A lyophilized DVS culture (R-708, Chr. Hansen, Denmark), consisting of an equal mixture of mesophilic *Lc. lactis* and *Lc. cremoris*, was used as the starter culture. The probiotic cultures used included *L. acidophilus* LA5, *L. paracasei* (*L. casei* 431), and *B. bifidum* BB12, provided by Christian Hansen, and *L. casei* ATCC 393, provided by the American Type Culture Collection (ATCC), United States.

Method

Preparation of cultures

L. acidophilus LA5, *L. paracasei*, and *B. bifidum* BB12 cultures were weighed (10 g each) and kept at 25 °C for approximately 1 h. These cultures were then activated in 1 L of pasteurized milk at 37 °C, and 1% of the culture (at least 10^7 cfu/ml) was added to the milk. The *L. casei* ATCC 393 culture was adjusted to a McFarland standard and added at a rate of 1% to 1 L of pasteurized milk at 37 °C. Mixture of *Lc. lactis* and *Lc. cremoris* were activated in pasteurized milk at 32 °C for use in 100 L of milk (50U bag per 1.5 tons of milk) and were added at a rate of 1% to the control group and 0.5% to the probiotic groups.

Turkish white cheese production

Turkish White cheese production was produced at the Atatürk University Food and Livestock Application and Research Center. The process was repeated 3 times, using 100 L of cow's milk for each batch. In this study, five different groups of cheese were produced:

Control Cheese: Made with 1% classic culture (CC), which includes mixture of *Lc. lactis* and *Lc. cremoris*.

LA Cheese: Consists of 0.5% CC and 1% *L. acidophilus* LA5.

LC Cheese: Comprises 0.5% CC and 1% *L. casei* ATCC 393.

LP Cheese: Contains 0.5% CC and 1% *L. paracasei*.

BB Cheese: Includes 0.5% CC and 1% *B. bifidum* BB12.

For cheese production, raw milk was pasteurized at 65 °C for 30 min and then cooled to 37 °C. At this temperature, 1% probiotic culture was added to the LA, LC, LP, and BB groups, followed by pre-ripening for 30 min. When the temperature reached 35 °C, 0.02% $CaCl_2$ was added. The classical starter culture was then added at 1% to the control group and 0.5% to the probiotic groups at 32 °C. Afterwards, rennet of 1/20,000 strength (Mayasan A.Ş.) was added at a rate of 15 ml per 100 L of milk at 30 ± 1 °C, allowing the milk to coagulate. 90 min post rennet addition, the curd was cut into about 1 cm^3 cubes with special knives and transferred to press cloths. The curd was then pressed at a weight ratio of 1:10 to the milk used for approximately 3 h. After pressing, the curd was cut into 7 × 7 × 7 cm cubes, placed into molds, and subjected to 2% dry salting overnight (about 18 h). The cheese molds were then placed in 500 g plastic containers, filled with 12% pasteurized brine, and ripened at 4 ± 1 °C for 90 days [41]. Microbiological and physicochemical analyses were performed on the 1st, 15th, 30th, 60th, and 90th days of the ripening period, while sensory analyses were conducted on the 15th, 30th, 60th, and 90th days.

Microbiological analyses

For the microbiological analyses, 10 g of cheese sample was transferred to stomach bags and diluted with 90 ml of Ringer's solution. This mixture was then homogenized in a stomacher device (Interscience-BagMixer 400, France) for 2 min. Serial dilutions were prepared by taking 1 ml of this homogenate and plantings were made using the pour plate method. Total aerobic mesophilic bacteria was measured on Plate Count Agar (PCA, Th. Geyer, Germany) at 37 °C for 3 days, as per Tabla and Roa [42]. For total aerobic psychrophilic bacteria, PCA was used at 4 °C for 7 days. Coliform group bacteria counts were done on Violet Red Bile Agar (VRBA, Th. Geyer, Germany) for 2 days at 37 °C, while yeast and mold counts were determined on Rose Bengal Chloramphenicol Agar (RBC, Merck) at 25 °C for 5 days. *Lactococcus* spp. counts were obtained after incubation on M17 agar (Liofilchem, Italy) at 37 °C in an aerobic environment for 2 days.

Lactobacillus spp. numbers were assessed in Man Rogosa Sharpe medium (MRS, Th. Geyer, Germany) following 3 days of anaerobic incubation at 37 °C [43]. Counts of *L. acidophilus* LA5 in MRS + Sorbitol (10% w/v) agar, *L. casei* ATCC 393, and *L. paracasei* in MRS + Vancomycin (1 mg/l) agar, along with *B. bifidum* BB12 in MRS + NNLP (neomycin sulfate, nalidixic acid, lithium chloride, paramomycin sulfate) + L cysteine hydrochloride (0.05%) on agar were determined after 3 days of incubation in an anaerobic environment at 37 °C [28, 44].

Physicochemical analyses

In the cheeses, pH was measured in lactic acid using a digital pH meter (inolab WTW), acidity was measured in lactic acid by the titration method, dry matter and ash content were determined by the gravimetric method, salt by the Mohr method, and fat content by the Gerber method as per Tekinşen et al. [41]. Protein content was determined by wet digestion using the micro-Kjeldahl method [45].

The water-soluble nitrogen ratio was determined using the method of Kuchroo and Fox [46]. For this, 40 ml of distilled water at 40 °C was added to 20 g of cheese sample and homogenized it in an ultra turrax mixer (Mtops sr30) for 2 min. The mixture was then incubated in a water bath at 40 °C for 1 h and centrifuged at 3000 rpm for 30 min at 4 °C. After removing the oily layer from the surface of the tubes, the liquid portion was filtered through Whatman filter paper. WSN values were ascertained by analyzing 10 ml of this filtrate using the micro-Kjeldahl method [45]. Maturation index values were calculated using the formula (Water-soluble nitrogen × 100)/ Total nitrogen [47]. Color (L, a*, b*) values were determined with a Chroma Meter color analyzer (Konica Minolta, Japan), and water activity (aw) values with the Aqua LAB 4TE water activity meter.

Sensory analysis

For sensory analysis, the scale with measurement criteria specified by Layne [48] was used. Expert panelists rated the Turkish white heese samples, coded with different numbers, on a scale from 1 (not good) to 9 (very good) before each analysis.

Statistical analysis

The SPSS statistics program (IBM SPSS Statistics 20) was used for analytical and descriptive analyses of the research. Prior to analysis, logarithmic transformation was applied to the microbiological data. Variance analysis were performed on the data using "one-way ANOVA" in the SPSS program. Duncan's multiple comparison test was applied to significant differences. Through statistical analysis, differences between ripening days and cheese groups were identified [49].

Results

Microbiological findings

Table 1 illustrates the variations in the counts of total aerobic mesophilic bacteria, total aerobic psychrophilic bacteria,

Table 1 Changes in the microbiological values of cheeses during maturation (Mean ± SD, log cfu/g)

Parameters		Groups	Ripening time (days)					
			1	15	30	60	90	F value
Total aerobic mesophilic bacteria	F value	Control	8.50 ± 0.30^{a}	7.64 ± 0.31^{a}	7.66 ± 0.57^{a}	5.78 ± 0.73^{bC}	5.29 ± 0.95^{bC}	14.38^{**}
		LA	8.38 ± 0.56^{a}	8.16 ± 0.25^{ab}	7.41 ± 0.24^{bc}	6.83 ± 0.49^{cBC}	5.41 ± 0.46^{dBC}	24.31^{**}
		LC	8.58 ± 0.30	7.92 ± 0.67	7.89 ± 0.50	7.88 ± 0.55^{AB}	7.17 ± 0.41^{A}	2.96
		LP	8.48 ± 0.28	8.29 ± 0.35	8.01 ± 0.78	8.06 ± 0.76^{A}	6.66 ± 1.03^{AB}	3.14
		BB	8.34 ± 0.39^{a}	8.14 ± 0.44^{ab}	7.28 ± 1.08^{ab}	7.17 ± 0.43^{bAB}	6.06 ± 0.18^{cABC}	7.20^{**}
			1.89	1.07	0.61	6.81^{**}	4.11^{**}	
Total aerobic psychrophilic bacteria	F value	Control	2.96 ± 0.56	3.25 ± 0.44	3.62 ± 0.58	2.94 ± 0.68	3.69 ± 0.82	0.94
		LA	3.29 ± 0.72	3.56 ± 0.16	3.94 ± 0.28	3.26 ± 1.04	3.63 ± 0.02	0.66
		LC	3.59 ± 0.77	3.63 ± 0.32	3.98 ± 0.36	3.29 ± 0.25	3.50 ± 0.34	0.95
		LP	2.96 ± 0.85	4.00 ± 0.31	4.00 ± 0.09	3.79 ± 0.36	4.05 ± 0.80	1.99
		BB	3.35 ± 0.84	3.45 ± 0.20	4.01 ± 0.36	3.52 ± 0.49	3.80 ± 0.88	0.59
			0.39	2.51	0.61	0.77	0.29	
Coliform group	F value	Control	3.43 ± 1.16	2.79 ± 0.99	2.52 ± 1.60	3.34 ± 0.65	1.71 ± 0.88	1.21
		LA	3.69 ± 1.04	3.11 ± 0.25	3.08 ± 0.68	3.22 ± 0.28	2.84 ± 0.58	0.72
		LC	3.73 ± 1.11	3.19 ± 1.10	3.26 ± 1.02	3.47 ± 0.33	2.04 ± 0.93	1.42
		LP	3.45 ± 0.97	2.96 ± 1.18	2.94 ± 1.42	3.12 ± 0.68	2.08 ± 1.21	0.62
		BB	3.55 ± 0.70	3.35 ± 1.02	3.37 ± 1.06	3.09 ± 0.82	2.30 ± 1.42	0.69
			0.06	0.15	0.23	0.21	0.49	
Yeast-mold	F value	Control	3.05 ± 1.10	3.54 ± 0.41	4.43 ± 0.27	3.94 ± 0.03	3.41 ± 0.81	1.10
		LA	3.10 ± 0.91	3.60 ± 0.44	3.90 ± 0.46	3.87 ± 0.21	3.51 ± 1.19	0.58
		LC	3.74 ± 0.37	3.79 ± 0.13	4.33 ± 0.46	4.01 ± 0.17	3.43 ± 1.10	1.06
		LP	3.41 ± 1.18	3.87 ± 0.16	3.26 ± 1.14	3.85 ± 0.21	3.64 ± 1.08	0.28
		BB	3.86 ± 0.83	3.74 ± 0.48	4.32 ± 0.44	4.16 ± 0.35	3.72 ± 0.82	0.55
			0.48	0.45	1.78	0.96	0.05	
Lactobacillus spp.	F value	Control	8.12 ± 0.69^{a}	8.26 ± 0.40^{a}	6.81 ± 0.37^{bC}	6.07 ± 0.23^{bB}	5.09 ± 0.53^{cB}	24.96^{**}
		LA	8.19 ± 0.83	8.60 ± 0.87	8.21 ± 0.56^{AB}	8.63 ± 0.07^{A}	7.70 ± 1.06^{A}	0.76
		LC	8.79 ± 0.32	8.38 ± 0.71	7.48 ± 0.49^{BC}	8.09 ± 0.92^{A}	7.62 ± 0.18^{A}	2.55
		LP	8.91 ± 0.43	8.66 ± 0.17	8.44 ± 0.56^{A}	8.41 ± 0.47^{A}	7.96 ± 0.18^{A}	2.39
		BB	8.78 ± 0.40	8.66 ± 1.12	8.49 ± 0.43^{A}	8.71 ± 0.18^{A}	8.08 ± 0.43^{A}	0.66
			1.31	0.19	6.58^{**}	15.52^{**}	14.10^{**}	
Lactococcus spp.	F value	Control	8.36 ± 0.57	8.09 ± 0.45	8.26 ± 0.66	6.91 ± 0.91	5.98 ± 1.78	3.25
		LA	8.60 ± 0.45^{a}	8.76 ± 1.03^{a}	7.92 ± 0.66^{a}	7.72 ± 0.82^{a}	6.36 ± 0.16^{b}	5.74*
		LC	8.54 ± 0.33	8.39 ± 0.91	7.46 ± 0.43	7.90 ± 0.94	7.17 ± 0.57	2.20
		LP	8.31 ± 0.83	8.06 ± 0.44	8.17 ± 0.57	8.32 ± 1.23	6.41 ± 0.43	3.39
		BB	8.52 ± 0.44^{a}	8.29 ± 0.35^{a}	8.28 ± 0.35^{a}	7.36 ± 0.62^{a}	5.58 ± 1.16^{b}	6.95^{**}
			0.16	0.35	1.17	0.99	1.03	
Probiotic	F value	LA	8.49 ± 1.0	8.53 ± 1.18	8.35 ± 0.41	8.65 ± 0.30	8.09 ± 0.34	0.25
		LC	7.19 ± 0.28	7.28 ± 0.92	7.53 ± 0.60	8.12 ± 0.90	7.61 ± 0.30	0.9
		LP	8.19 ± 1.20	8.22 ± 0.39	8.63 ± 0.55	8.73 ± 0.98	7.01 ± 1.45	1.41
		BB	7.95 ± 0.08	8.21 ± 1.19	8.07 ± 0.48	7.75 ± 0.75	7.16 ± 1.10	0.74
			1.47	0.93	2.45	1.07	0.81	

Control: Made with 1% classic culture (CC), which includes mixture of *Lc. lactis* and *Lc. cremoris*. LA: Consists of 0.5% CC and 1% *L. acidophilus* LA5. LC: Comprises 0.5% CC and 1% *L. casei* ATCC 393. LP: Contains 0.5% CC and 1% *L. paracasei*. BB: Includes 0.5% CC and 1% *B. bifidum* BB12

*$p < 0.05$, **$p < 0.01$. Differences between values marked with different letters in the same row (a–d, →) and in the same column (A–C, ↓) are statistically significant. 'Log cfu' stands for logarithmic colony forming unit, '$\bar{x}$' represents the mean value, and 'SD' denotes the standard deviation

coliform group bacteria, yeast-mold, *Lactobacillus* spp., *Lactococcus* spp., and probiotics during the ripening of the cheeses at + 4 °C.

During ripening, the total aerobic mesophilic bacteria numbers in control, LA, and BB cheeses decreased significantly ($p < 0.01$). On the 60th and 90th days, the total aerobic mesophilic bacteria numbers in LC and LP cheeses were found to be higher than in control cheese ($p < 0.01$). In control cheese, the numbers of *Lactobacillus* spp. decreased on the 30th and 90th days ($p < 0.01$) The numbers of *Lactobacillus* spp. in probiotic cheeses were higher than in control cheese on the 30th, 60th, and 90th days ($p < 0.01$). In LA and BB cheeses, *Lactococcus* spp. numbers decreased, with significance levels of $p < 0.05$ and $p < 0.01$, respectively, on the 90th day. It was determined that the probiotic numbers remained above the effective level ($\geq 10^7$ cfu/g) throughout the ripening process.

Physicochemical findings

During the ripening of cheeses at + 4 °C, changes were observed in pH, titratable acidity, dry matter, fat content, fat in dry matter, salt in dry matter, ash, protein, water-soluble nitrogen, ripening index, water activity (aw), and color values (L*, a*, b*), as shown in Table 2.

In control cheese, pH values increased on the 15th day ($p < 0.05$) and decreased in LP cheese on the 30th day ($p < 0.01$), with LP cheese having the lowest pH among all groups. Titratable acidity values increased on the 30th day in control and LP cheeses ($p < 0.01$) and in BB cheese ($p < 0.05$). Probiotic cheeses showed higher titratable acidity values than the control on the 15th day, but lower on the 90th day. Dry matter values were higher in control cheese on the 15th day ($p < 0.01$) and in LP cheese on the 60th day ($p < 0.05$) compared to other cheeses. Fat content decreased in control cheese on the 15th day ($p < 0.01$) and increased in LP and LC cheeses on the 15th and 90th days, respectively ($p < 0.05$). Fat in dry matter values in control cheese decreased on the 15th day and increased on the 90th day ($p < 0.01$). Salt in dry matter values rose in control and LA cheeses on the 15th day, and in LP cheese on the 30th day. An increase in ash content was observed in LA cheese on the 60th day ($p < 0.05$). Protein values decreased in LA and LC cheeses on the 15th and 30th days, respectively, and increased in LP cheese on the 60th day. The protein content of LC cheese was lower than that of other cheeses on the 60th day ($p < 0.05$). Water-soluble nitrogen values increased during ripening in control, LA, LP, and BB cheeses. These values were highest in control cheese on the 15th day, and in control and BB cheeses on the 60th day, but lowest in LP cheese on these days ($p < 0.01$). The ripening index values rose in all cheeses during ripening. They were highest in control and BB cheeses on the 60th day and in LA cheese on the 90th day, but lowest in LP cheese on these days ($p < 0.01$). A decrease in water activity (aw) values was noted in LA and LC cheeses on the 15th day ($p < 0.01$). The highest aw values were in LA cheese and the lowest in LP and BB cheeses on day 1 ($p < 0.05$). The a* values in LC, LP, and BB cheeses decreased on the 30th day and increased on the 60th day ($p < 0.01$). Probiotic cheeses had higher a* values than the control cheese on the 60th day ($p < 0.01$). During ripening, b* values increased in LA, LC, and BB cheeses. Control cheese had a higher b* value than the probiotic groups on the 15th and 60th days.

Sensory analysis findings

The sensory analysis values of cheese samples are presented in Table 3.

There was an observed increase in color scores for LC and LP cheeses on the 30th day ($p < 0.05$). An enhancement in the taste and aroma score of LP cheese was noted on the 30th day ($p < 0.05$). On the 90th day, the taste and aroma scores of LA and LP cheeses were found to be higher than those of control cheese ($p < 0.05$). The salinity scores of LA and LP cheeses were higher compared to other cheeses on the 90th day ($p < 0.05$). Overall acceptability scores showed a significant increase in LP cheese ($p < 0.01$).

Discussion

The total aerobic mesophilic bacteria number is a crucial parameter indicating food quality deterioration. During ripening, total aerobic mesophilic bacteria numbers decreased in control, LA, and BB cheeses ($p < 0.01$) (Table 1). Yangılar and Özdemir [39] linked this decrease in white cheeses produced with *L. acidophilus* LA5 and *Bifidobacterium* spp. cultures to increased acidity and salt levels. In contrast, the total aerobic mesophilic bacteria numbers in LC and LP cheeses were higher than in control cheese on the 60th and 90th days ($p < 0.01$) (Table 1). Yılmaztekin et al. [37] observed similar trends in cheeses with *L. acidophilus* LA5 and *B. bifidum* BB02 additions, suggesting that probiotics enhance the survival of these bacteria.

Lactobacillus spp. numbers in control cheese decreased on the 30th and 90th days ($p < 0.01$) (Table 1). This reduction could be attributed to bacterial lysis, increased salt and acidity, reduced water activity (aw), and nutrient competition with microorganisms, as noted by Yangılar and Özdemir [39], Kasımoğlu et al. [50], and Şahingil [51]. However, the numbers of *Lactobacillus* spp. in probiotic cheeses were higher than in control cheese on the 30th, 60th, and 90th days ($p < 0.01$) (Table 1). This increase may be due to the addition of probiotics, which are also countable in MRS culture mediums. This finding aligns

Table 2 The changes in the physicochemical properties of the cheeses during ripening (mean ± standard deviation, log cfu/g)

Parameters		Groups	Ripening time (days)					
			1	15	30	60	90	F value
pH	F value	Control	5.15 ± 0.10^{b}	5.33 ± 0.12^{aA}	5.23 ± 0.08^{abA}	5.28 ± 0.04^{aA}	5.26 ± 0.08^{abA}	3.39*
		LA	5.10 ± 0.05	5.08 ± 0.04^{AB}	5.02 ± 0.05^{B}	5.08 ± 0.17^{B}	5.05 ± 0.20^{A}	0.47
		LC	5.19 ± 0.13	5.23 ± 0.27^{A}	5.16 ± 0.10^{AB}	5.20 ± 0.09^{AB}	5.15 ± 0.05^{A}	0.30
		LP	5.09 ± 0.03^{a}	4.90 ± 0.20^{abB}	4.70 ± 0.13^{bcC}	4.56 ± 0.11^{cC}	4.74 ± 0.40^{bcB}	5.44**
		BB	5.20 ± 0.21	5.23 ± 0.29^{A}	5.15 ± 0.18^{AB}	5.15 ± 0.13^{AB}	5.09 ± 0.18^{A}	0.39
			1.01	3.87*	19.00**	36.14**	4.73**	
Titratable acidity (% l.a.)	F value	Control	0.82 ± 0.20^{ab}	0.68 ± 0.05^{bB}	0.91 ± 0.15^{a}	0.95 ± 0.10^{aA}	0.97 ± 0.07^{aA}	5.53**
		LA	0.79 ± 0.12	0.77 ± 0.04^{A}	0.88 ± 0.03	0.77 ± 0.05^{B}	0.78 ± 0.08^{C}	2.44
		LC	0.80 ± 0.28	0.74 ± 0.05^{A}	0.83 ± 0.06	0.88 ± 0.07^{A}	0.77 ± 0.10^{C}	0.79
		LP	0.72 ± 0.07^{c}	0.79 ± 0.05^{bcA}	0.86 ± 0.11^{ab}	0.89 ± 0.06^{aA}	0.91 ± 0.04^{aAB}	7.54**
		BB	0.73 ± 0.1^{b}	0.73 ± 0.06^{bAB}	0.88 ± 0.04^{a}	0.91 ± 0.12^{aA}	0.85 ± 0.09^{abBC}	4.21*
			0.40	4.20*	0.67	3.81*	7.4**	
Dry matter (%)	F value	Control	43.29 ± 1.50	43.79 ± 3.96^{A}	41.02 ± 2.81	39.57 ± 1.74^{AB}	40.36 ± 3.43	2.52
		LA	39.73 ± 3.63	38.41 ± 1.90^{B}	39.32 ± 1.03	38.41 ± 2.42^{B}	39.67 ± 1.95	0.47
		LC	39.60 ± 3.50	39.36 ± 1.66^{B}	38.39 ± 1.84	38.71 ± 1.16^{B}	39.70 ± 0.36	0.50
		LP	39.57 ± 3.35	38.87 ± 1.35^{B}	39.18 ± 1.08	40.74 ± 0.88^{A}	40.63 ± 0.66	1.40
		BB	38.82 ± 2.07	38.05 ± 1.62^{B}	39.05 ± 2.86	37.72 ± 0.24^{B}	38.89 ± 0.50	0.67
			2.16	6.21**	1.33	3.67*	0.85	
Fat (%)	F value	Control	20.25 ± 0.99^{ab}	18.25 ± 1.84^{c}	20.92 ± 1.36^{ab}	19.67 ± 1.30^{bc}	21.83 ± 1.60^{a}	4.81**
		LA	19.83 ± 2.29	19.83 ± 1.83	20.58 ± 1.50	19.83 ± 3.71	22.17 ± 1.44	1.15
		LC	19.42 ± 1.69^{b}	18.58 ± 2.15^{b}	20.42 ± 2.15^{ab}	20.25 ± 1.78^{ab}	22.25 ± 2.17^{a}	3.05*
		LP	18.58 ± 0.49^{c}	19.50 ± 2.35^{ab}	19.50 ± 1.67^{ab}	21.58 ± 2.06^{a}	21.75 ± 1.78^{a}	3.72*
		BB	18.67 ± 1.33	19.00 ± 1.90	19.83 ± 1.91	20.00 ± 2.88	20.67 ± 1.75	0.95
			1.42	0.61	0.65	0.55	0.86	
Fat in dry matter (%)	F value	Control	46.86 ± 3.47^{b}	41.85 ± 4.60^{c}	51.28 ± 5.87^{ab}	49.66 ± 2.61^{ab}	54.15 ± 1.56^{a}	8.54**
		LA	49.86 ± 2.30	51.62 ± 3.64	52.33 ± 3.22	51.55 ± 8.20	55.88 ± 2.26	1.46
		LC	49.11 ± 2.96	47.35 ± 6.59	53.19 ± 5.22	52.42 ± 5.64	56.04 ± 4.24	2.73
		LP	47.17 ± 2.96	50.27 ± 6.78	49.74 ± 3.60	53.09 ± 6.18	53.59 ± 5.12	1.57
		BB	48.14 ± 3.34	50.17 ± 6.95	50.84 ± 4.24	53.00 ± 740	53.16 ± 4.71	0.85
			1.06	2.66	0.52	0.30	0.71	
Salt in dry matter (%)	F value	Control	10.17 ± 3.42^{b}	13.61 ± 0.73^{a}	14.59 ± 3.49^{a}	14.31 ± 1.69^{a}	14.79 ± 1.97^{a}	3.51*
		LA	8.72 ± 3.41b	12.53 ± 2.20^{a}	13.27 ± 1.83^{a}	14.79 ± 1.74^{a}	14.42 ± 2.20^{a}	6.38**
		LC	11.80 ± 4.19	13.74 ± 2.18	15.30 ± 2.21	15.31 ± 1.30	14.82 ± 1.32	2.16
		LP	13.03 ± 0.63^{b}	13.11 ± 1.11^{b}	15.20 ± 1.88^{a}	12.92 ± 0.99^{b}	13.32 ± 1.85^{b}	2.84*
		BB	12.51 ± 2.95	13.20 ± 1.83	13.42 ± 1.85	15.41 ± 2.43	14.53 ± 2.22	1.51
			1.90	0.47	1.01	2.12	0.61	
Ash (%)	F value	Control	4.72 ± 0.80	5.47 ± 0.82	5.70 ± 0.80	5.80 ± 0.68	5.93 ± 0.50	2.61
		LA	3.82 ± 1.49^{b}	4.63 ± 0.52^{ab}	4.92 ± 0.60^{ab}	5.28 ± 0.90^{a}	5.63 ± 0.59^{a}	3.57*
		LC	5.27 ± 1.91	5.97 ± 0.58	5.81 ± 0.27	5.44 ± 0.82	6.16 ± 0.83	0.75
		LP	5.34 ± 0.23	5.62 ± 1.62	5.37 ± 0.91	4.64 ± 0.59	5.57 ± 0.33	1.17
		BB	5.71 ± 1.30	5.19 ± 1.05	5.76 ± 0.97	5.18 ± 0.82	5.49 ± 0.79	0.45
			1.97	1.50	1.48	1.82	1.16	
Protein (%)	F value	Control	19.97 ± 2.78	18.58 ± 3.52	16.91 ± 2.15	17.11 ± 0.89^{AB}	18.36 ± 2.95	1.35
		LA	18.66 ± 1.07^{a}	15.69 ± 1.38^{b}	15.73 ± 2.67^{b}	$16.02 \pm 2.08b^{BC}$	16.76 ± 1.08^{ab}	2.97*
		LC	18.53 ± 2.23^{a}	18.09 ± 2.30^{a}	14.87 ± 0.57^{bc}	14.71 ± 1.19^{cC}	16.79 ± 1.13^{ab}	7.08**
		LP	17.74 ± 1.19^{ab}	16.13 ± 1.33^{b}	16.20 ± 0.72^{b}	18.06 ± 1.34^{aA}	18.22 ± 1.73^{a}	3.69*
		BB	18.38 ± 3.01	16.06 ± 0.86	15.05 ± 1.69	16.49 ± 1.77^{ABC}	16.58 ± 0.52	2.72
			0.82	2.38	1.37	4.08*	1.59	

Table 2 (continued)

Parameters		Groups	Ripening time (days)					
			1	15	30	60	90	F value
Water-soluble nitrogen (%)	F value	Control	0.24 ± 0.07^{c}	0.31 ± 0.04^{abA}	0.27 ± 0.02^{bc}	0.35 ± 0.04^{aA}	0.32 ± 0.06^{ab}	4.45*
		LA	0.24 ± 0.05^{b}	0.26 ± 0.03^{bBC}	0.25 ± 0.02^{b}	0.26 ± 0.04^{bB}	0.36 ± 0.04^{a}	9.63**
		LC	0.25 ± 0.07	0.29 ± 0.02^{AB}	0.28 ± 0.02	0.27 ± 0.04^{B}	0.30 ± 0.06	1.13
		LP	0.21 ± 0.03^{b}	0.23 ± 0.04^{bC}	0.24 ± 0.08^{ab}	0.21 ± 0.01^{bC}	0.29 ± 0.04^{a}	3.72*
		BB	0.24 ± 0.04^{b}	0.26 ± 0.02^{bBC}	0.28 ± 0.06^{b}	0.33 ± 0.06^{aA}	0.35 ± 0.03^{a}	6.22**
			0.54	6.08^{**}	0.71	10.87^{**}	2.13	
Ripening index (%)	F value	Control	8.09 ± 2.97^{c}	11.11 ± 3.11^{a}	10.48 ± 1.48^{ab}	12.94 ± 1.71^{aA}	11.33 ± 1.92^{aBC}	3.41*
		LA	8.42 ± 2.08^{c}	10.78 ± 1.09^{b}	10.46 ± 2.16^{bc}	10.44 ± 1.48^{bcB}	13.90 ± 2.00^{aA}	7.13^{**}
		LC	8.90 ± 3.25^{b}	10.19 ± 0.89^{ab}	11.99 ± 1.30^{a}	11.91 ± 1.29^{aAB}	11.44 ± 1.51^{aBC}	3.10*
		LP	7.59 ± 1.22^{b}	9.26 ± 1.22^{ab}	9.53 ± 2.30^{ab}	7.40 ± 0.80^{bC}	10.37 ± 1.55^{aC}	3.30*
		BB	8.78 ± 2.82^{c}	10.16 ± 0.73^{bc}	11.79 ± 2.31^{ab}	12.90 ± 1.25^{aA}	13.35 ± 1.48^{aAB}	6.23**
			0.26	1.11	1.38	17.87^{**}	4.55^{**}	
aw	F value	Control	0.97 ± 0.01^{AB}	0.95 ± 0.01	0.95 ± 0.01	0.95 ± 0.01	0.95 ± 0.01	2.71
		LA	0.98 ± 0.00^{aA}	0.95 ± 0.00^{bc}	0.96 ± 0.01^{b}	0.95 ± 0.00^{c}	0.95 ± 0.00^{bc}	23.46^{**}
		LC	0.97 ± 0.01^{aAB}	0.94 ± 0.01^{c}	0.95 ± 0.00^{bc}	0.96 ± 0.02^{ab}	0.95 ± 0.01^{bc}	4.42^{**}
		LP	0.96 ± 0.01^{B}	0.95 ± 0.01	0.95 ± 0.01	0.95 ± 0.01	0.95 ± 0.01	1.63
		BB	0.96 ± 0.01^{B}	0.95 ± 0.02	0.95 ± 0.11	0.95 ± 0.15	0.96 ± 0.01	0.54
			3.29*	0.68	1.06	0.47	0.48	
L*	F value	Control	93.36 ± 2.21	92.91 ± 1.98	94.09 ± 1.69	93.73 ± 1.54	92.85 ± 2.85	0.38
		LA	95.00 ± 0.98	95.16 ± 0.96	94.92 ± 1.04	95.36 ± 1.15	93.08 ± 2.88	2.04
		LC	88.19 ± 12.52	92.77 ± 5.48	96.12 ± 0.48	94.87 ± 0.78	92.83 ± 3.86	1.35
		LP	93.23 ± 2.39	93.40 ± 2.17	95.20 ± 2.08	95.65 ± 1.10	92.84 ± 2.10	2.36
		BB	92.11 ± 3.29	91.23 ± 2.44	94.52 ± 1.67	94.41 ± 1.63	90.19 ± 4.68	2.52
			1.1	1.31	1.55	2.15	0.77	
a*	F value	Control	-2.63 ± 0.37	-2.70 ± 0.41	-3.01 ± 0.22^{B}	-2.68 ± 0.10^{C}	-2.89 ± 0.16	1.97
		LA	-2.51 ± 0.33	-2.35 ± 0.31	-2.55 ± 0.41^{A}	-2.36 ± 0.33^{B}	-2.77 ± 0.28	1.55
		LC	-2.45 ± 0.47^{ab}	-2.32 ± 0.45^{a}	-3.01 ± 0.12^{cB}	-2.56 ± 0.08^{abAB}	-2.77 ± 0.12^{bc}	4.81**
		LP	-2.36 ± 0.29^{bc}	-2.16 ± 0.46^{ab}	-2.48 ± 0.13^{bcA}	-1.88 ± 0.22^{aA}	-2.67 ± 0.18^{c}	7.06**
		BB	-2.50 ± 0.29^{abc}	-2.25 ± 0.46^{a}	-2.87 ± 0.14^{cB}	-2.29 ± 0.30^{abB}	-2.66 ± 0.22^{bc}	4.43**
			0.46	1.44	7.19^{**}	10.72^{**}	1.25	
b*	F value	Control	16.99 ± 1.64	17.36 ± 1.25^{A}	17.92 ± 1.02^{A}	18.43 ± 1.09^{A}	17.28 ± 1.69	1.07
		LA	15.37 ± 0.79^{b}	14.94 ± 0.64^{bB}	15.64 ± 1.21^{abB}	15.98 ± 1.07^{abCD}	16.69 ± 0.88^{a}	2.94*
		LC	15.26 ± 2.04^{b}	14.94 ± 1.81^{bB}	17.14 ± 0.73^{aA}	17.47 ± 0.84^{aAB}	16.53 ± 1.02^{ab}	3.90*
		LP	15.43 ± 1.85	14.85 ± 1.83^{B}	15.62 ± 0.90^{B}	14.92 ± 0.76^{D}	16.01 ± 1.41	0.70
		BB	15.71 ± 0.95^{bc}	15.21 ± 1.09^{cB}	17.03 ± 0.40^{aA}	16.43 ± 0.65^{abBC}	17.07 ± 0.75^{a}	6.25**
			1.29	3.50*	7.64^{**}	13.66**	1.02	

Control: Made with 1% classic culture (CC), which includes mixture of *Lc. lactis* and *Lc. cremoris*. LA: Consists of 0.5% CC and 1% *L. acidophilus* LA5. LC: Comprises 0.5% CC and 1% *L. casei* ATCC 393. LP: Contains 0.5% CC and 1% *L. paracasei*. BB: Includes 0.5% CC and 1% *B. bifidum* BB12

*$p < 0.05$, **$p < 0.01$. Differences between values marked with different letters in the same row (a–d, →) and in the same column (A–C, ↓) are statistically significant. 'Log cfu' stands for logarithmic colony forming unit, '$\bar{x}$' represents the mean value, and 'SD' denotes the standard deviation

with research by Terpou et al. [3] on feta, Leeuwendaal et al. [15] on cheddar, and Schoina et al. [11] on myzithra cheese. The number of *Lactococcus* spp. in cheese primarily reflects the starter cultures added during production. A decrease in *Lactococcus* spp. was observed in LA and BB cheeses on the 90th day (Table 1), potentially due to lactococci's reduced growth in acidic conditions, increased salt concentration, and nutrient competition. In this study, the

Table 3 Changes in sensory values of cheeses during their ripening (mean ± standard deviation, log cfu/g)

Parameters		Groups	Ripening time (days)				
			15	30	60	90	F value
Color	F value	Control	8.30 ± 0.02	8.34 ± 0.32	8.39 ± 0.27	8.39 ± 0.27	0.1
		LA	8.20 ± 0.28	8.62 ± 0.05	8.44 ± 0.27	8.57 ± 0.32	1.6
		LC	8.17 ± 0.29^{b}	8.64 ± 0.02^{a}	8.42 ± 0.03^{ab}	8.59 ± 0.18^{a}	4.63*
		LP	8.11 ± 0.29^{b}	8.80 ± 0.12^{a}	8.49 ± 0.16^{ab}	8.69 ± 0.28^{a}	5.61*
		BB	8.34 ± 0.30	8.58 ± 0.26	8.34 ± 0.11	8.57 ± 0.32	0.83
			0.4	2.24	0.25	0.42	
Texture	F value	Control	8.17 ± 0.66	7.38 ± 1.05	7.42 ± 1.62	7.13 ± 1.50	0.38
		LA	7.24 ± 0.68	7.64 ± 1.21	7.86 ± 0.28	8.36 ± 0.36	1.24
		LC	7.86 ± 0.50	7.46 ± 0.59	7.76 ± 0.40	8.09 ± 0.43	0.86
		LP	7.97 ± 0.27	8.51 ± 0.37	8.45 ± 0.43	8.84 ± 0.17	3.59
		BB	7.59 ± 0.48	7.82 ± 0.19	7.57 ± 0.19	7.53 ± 0.65	0.29
			1.38	0.98	0.77	2.29	
Taste and aroma	F value	Control	8.03 ± 0.55	7.63 ± 0.65	7.60 ± 0.30	7.72 ± 0.41^{C}	0.47
		LA	7.90 ± 0.30	8.24 ± 0.21	8.03 ± 0.34	8.47 ± 0.12^{AB}	2.82
		LC	7.76 ± 0.22	7.71 ± 0.26	7.92 ± 0.26	8.31 ± 0.64^{ABC}	1.53
		LP	7.80 ± 0.34^{b}	8.46 ± 0.44^{a}	8.51 ± 0.37^{a}	8.75 ± 0.12^{aA}	4.36*
		BB	7.63 ± 0.34	8.03 ± 0.36	7.88 ± 0.43	7.86 ± 0.37^{BC}	0.57
			0.50	2.16	2.82	3.77*	
Foreign taste and smell	F value	Control	8.68 ± 0.23	8.76 ± 0.20	8.78 ± 0.09	8.61 ± 0.38	0.33
		LA	8.72 ± 0.16	8.89 ± 0.19	8.52 ± 0.32	8.83 ± 0.10	1.78
		LC	8.63 ± 0.42	8.92 ± 0.14	8.83 ± 0.10	8.87 ± 0.02	0.92
		LP	8.58 ± 0.39	8.82 ± 0.22	8.74 ± 0.33	8.88 ± 0.11	0.65
		BB	8.75 ± 0.10	8.88 ± 0.21	8.83 ± 0.07	8.53 ± 0.32	1.78
			0.16	0.29	1.06	1.56	
Salinity	F value	Control	7.70 ± 0.73	6.58 ± 0.09	6.82 ± 0.59	6.63 ± 0.11^{B}	3.66
		LA	6.94 ± 0.97	7.05 ± 0.51	7.29 ± 0.47	7.71 ± 0.51^{A}	0.83
		LC	6.86 ± 0.37	6.54 ± 0.51	6.83 ± 0.72	7.25 ± 0.66^{AB}	0.75
		LP	7.10 ± 0.30	7.12 ± 0.28	7.21 ± 0.54	7.74 ± 0.36^{A}	1.88
		BB	7.06 ± 0.77	6.82 ± 0.53	6.43 ± 0.93	6.86 ± 0.28^{B}	0.46
			0.73	1.17	0.79	4.01*	
Overall acceptability	F value	Control	8.08 ± 0.52	7.91 ± 0.82	7.46 ± 0.65	7.72 ± 0.94	0.39
		LA	7.60 ± 0.72	8.07 ± 0.74	7.87 ± 0.02	8.40 ± 0.36	1.15
		LC	7.72 ± 0.16	7.88 ± 0.33	7.85 ± 0.60	8.24 ± 0.54	0.76
		LP	7.84 ± 0.17^{c}	8.51 ± 0.37^{ab}	8.35 ± 0.31^{b}	8.96 ± 0.06^{a}	9.63**
		BB	7.83 ± 0.64	7.99 ± 0.30	7.88 ± 0.56	7.72 ± 0.48	0.14
			0.40	0.63	1.28	2.65	

Control: Made with 1% classic culture (CC), which includes mixture of *Lc. lactis* and *Lc. cremoris*. LA: Consists of 0.5% CC and 1% *L. acidophilus* LA5. LC: Comprises 0.5% CC and 1% *L. casei* ATCC 393. LP: Contains 0.5% CC and 1% *L. paracasei*. BB: Includes 0.5% CC and 1% *B. bifidum* BB12

*$p < 0.05$, **$p < 0.01$. Statistically significant differences exist between values with different letters in the same row (a-c, →) and in the same column (A–C, ↓)

Lactococcus spp. numbers in probiotic groups with a 0.5% addition were not significantly different from the control cheese with a 1% addition, indicating a possible synergistic effect between starters and probiotics. Supporting this, Ortigosa et al. [52] reported a synergistic effect between probiotics and lactococci in Roncal cheese produced with the addition of *L. paracasei* and *L. plantarum*.

The concentrations of *L. acidophilus* LA5, *L. casei* ATCC 393, *L. paracasei*, and *B. bifidum* BB12 were measured to be in the range of 8.09 ± 0.34–8.65 ± 0.30,

7.19 ± 0.28–8.12 ± 0.90, 7.01 ± 1.45–8.73 ± 0.98, and 7.16 ± 1.10–8.21 ± 1.19 log cfu/g, respectively, as shown in Table 1. These values exceed the minimum level for probiotic effectiveness ($\geq 10^6$ cfu/g) as defined by the Turkish Food Codex [53]. The observed resilience to salt and acidity in these selected probiotics, along with the production methods and their integration with traditional starters, can explain these results. The probiotic counts in this study are comparable to those found in probiotic-enhanced cheddar [13, 54], feta [7, 55], mozzarella [8], and edam [19] cheeses. However, the numbers of *L. paracasei* reported by Gursoy and Kinik [38] and *B. bifidum* BB12 reported by Zomorodi et al. [56] were found to be lower than those determined in our study. This variation could be attributed to differences in the milk's microflora and the cheese production technologies used.

The pH of cheese plays a significant role in biochemical reactions and the growth of microorganisms, as noted by Tekinşen and Tekinşen [36]. The pH values of the cheeses ranged from 4.56 ± 0.11 to 5.33 ± 0.12, as shown in Table 2. Notably, the pH increased on the 15th day ($p < 0.05$) in the control cheese, while it decreased significantly ($p < 0.01$) on the 30th day in the LP cheese. Yangılar and Ozdemir [57] and Terpou et al. [43] reported similar findings in their studies. The increase in pH may be attributed to ammonia production by microorganisms and the alkalinization resulting from the breakdown of lactic acids [58]. Conversely, the decrease in pH is likely due to the conversion of lactose into organic acids. It was observed that the LP cheese had the lowest pH among all the tested groups, as detailed in Table 2. This trend aligns with the findings of Zomorodi et al. [56] and Burns et al. [59]. Titratable acidity reflects the total acidity derived from glycolysis, proteolysis, and lipolysis processes activated by microorganisms [60]. On the 30th day, titratable acidity values increased in the control, LP, and BB cheeses, as indicated in Table 2. This increase is primarily due to the enzymatic impact on lactose, particularly by the cultures used [61]. The titratable acidity values of probiotic cheeses were higher than those of the control cheese on the 15th day but were lower on the 90th day. It was found that the control cheese exhibited both the highest pH and the highest acidity on the 90th day, as reported in Table 2. Yılmaztekin [60] suggested that this condition might be related to the formation of basic buffer substances.

Dry matter in cheese, excluding moisture content, contains key nutritional elements of the cheese [36]. Dry matter values were higher in control cheese on the 15th day ($p < 0.01$) and in LP cheese on the 60th day ($p < 0.05$) compared to other cheeses, possibly due to the acid-forming properties of the cultures. Mantzourani et al. [62] linked higher dry matter ratios in feta cheese with *L. paracasei* addition to its lower pH value. The fat content in control cheese decreased on the 15th day ($p < 0.01$) and increased in LP and LC cheeses on the 15th and 90th days, respectively ($p < 0.05$) (Table 2). Uzun [63] noted a correlation between increased dry matter and fat content, while Mazou et al. [64] associated a decrease in fat content with the lipolytic activity of microbial enzymes. Fat in dry matter, a key parameter in cheese classification, showed a decrease in control cheese on the 15th day and an increase on the 90th day ($p < 0.01$), aligning with findings by Yılmaztekin [60]. Salt affects cheese's chemical, microbiological, and sensory properties [36]. Salt in dry matter values increased in control and LA cheeses on the 15th day, and in LP cheese on the 30th day (Table 2), likely due to osmotic pressure-driven salt migration. Abd-Elmonem et al. [34] observed a similar increase in salt in dry matter in probiotic ras cheese due to salt osmosis. An increase in ash content, possibly due to salt migration and moisture loss, was observed in LA cheese on the 60th day ($p < 0.05$).

Protein values decreased in LA and LC cheeses on the 15th and 30th days, respectively, but increased in LP cheese on the 60th day (Table 2). Mahmoudi et al. [18] reported that protein ratio changes were due to peptide and amino acid migration to the brine and moisture loss. The protein content of LC cheese was lower than other cheeses on the 60th day ($p < 0.05$) (Table 2), but Ong et al. [65] found no difference in protein ratios in cheddar cheeses with added *L. acidophilus*, *L. paracasei*, *L. casei*, and *Bifidobacterium* spp., suggesting the influence of production technology and culture combination rates. Water-soluble nitrogen in cheese, indicative of nitrogen fractions from casein breakdown, plays a vital role in texture and flavor development [32]. An increase in water-soluble nitrogen was noted in control, LA, LP, and BB cheeses (Table 2). Kamaly et al. [32] attributed this increase in domiati cheese to protein hydrolysis.

Water-soluble nitrogen levels were highest in control cheese on the 15th day, and in control and BB cheeses on the 60th day, but lowest in LP cheese on these days ($p < 0.01$) (Table 2), corroborating findings by Şahingil [51]. The ripening index in cheese is crucial for monitoring proteolysis and, by extension, ripening. All cheeses showed an increase in ripening index values during ripening (Table 2), likely due to enzymatic protein hydrolysis. Dabevska-Kostoska et al. [47] and Çetinkaya and Atasever [66] reported similar findings in their studies. The ripening index was notably higher in control and BB cheeses on the 60th day and in LA cheese on the 90th day compared to other cheeses, while LP cheese had the lowest values on these days ($p < 0.01$) (Table 2). Ong et al. [65] attributed differences in ripening indexes of cheddar cheeses to varying proteolytic enzyme activities among cultures.

The water activity (aw) value in foods is a measure of free water, impacting their biochemical, microbiological, and sensory properties. There was a significant decrease in aw values in LA and LC cheeses on the 15th day ($p < 0.01$)

(Table 2). The highest aw values were observed in LA cheese and the lowest in LP and BB cheeses on day 1 ($p < 0.05$). Hickey et al. [67] found a negative correlation between aw value and salt content in cheddar cheese, with nitrogen fractions from proteolysis being a major factor in reducing aw. Gomez-Torres et al. [68] linked decreased aw in experimental-model cheeses with *L. reuteri* supplementation to proteolysis and syneresis.

Cheese color is a key quality determinant. The a* values, indicating red-green color balance, in LC, LP, and BB cheeses decreased on the 30th day and increased on the 60th day ($p < 0.01$). Negative a* values suggest a greenish hue, which could be due to riboflavin, partial oxidation, and microbial growth. Akarca et al. [69] and De Almeida et al. [70] reported similar color changes in mascarpone and mozzarella cheeses, respectively. Probiotic cheeses exhibited higher a* values than control cheese on the 60th day ($p < 0.01$) (Table 2), contrasting with Dantas et al. [31], who observed different a* values in minas frescal cheeses with *L. casei* Zhang addition. The variations might stem from differences in culture types, riboflavin usage, and lipolytic properties. During ripening, b* values, indicating yellow-blue color balance, increased in LA, LC, and BB cheeses (Table 2). Fritzen-Freire et al. [71] linked the rise in b* value in ricotta cheese with *B. bifidum* to increased dry matter. The positive b* value in cheese often results from milk-derived beta-carotene, vitamin A, and vitamin E [70]. Control cheese had a higher b* value than probiotic groups on the 15th and 60th days (Table 2), aligning with findings by Akarca and Yildirim [72]. However, Amiri et al. [6] reported no difference in b* values of Edam cheeses with *L. casei* L26 addition.

Sensory properties significantly influence consumer preference. Cheese color received high scores ranging from 8.11 ± 0.29 to 8.80 ± 0.12 (Table 3), with LC and LP cheeses showing an increase in color scores on the 30th day ($p < 0.05$). This finding contrasts with Ahmed et al. [73], who reported decreased color scores in probiotic feta cheeses correlating with reduced moisture. Rehman et al. [9] emphasized the importance of proteolysis and acidity in cheese texture development. Texture scores varied between 7.13 ± 1.50 and 8.84 ± 0.17 (Table 3), indicative of a balanced casein breakdown. The cheeses received high scores for taste and aroma, ranging from 7.60 ± 0.30 to 8.75 ± 0.12 (Table 3). LP cheese exhibited an increase in taste and aroma score on the 30th day ($p < 0.05$), possibly due to proteolysis and lipolysis that form aroma compounds. On the 90th day, the taste and aroma scores of LA and LP cheeses were found to be higher than those of control cheese ($p < 0.05$) (Table 3). Mc Brearty et al. [27] and Garcia et al. [74] reported similar findings. Terpou et al. [43] suggested that feta cheese with *L. paracasei* K5 addition was more palatable than control cheese due to higher lactone content.

Salinity scores of the cheeses fell in the slightly salty to normal range (6.43 ± 0.93–7.74 ± 0.36) (Table 3). On the 90th day, LA and LP cheeses had higher salinity scores compared to other cheeses ($p < 0.05$). However, Demers-Mathieu et al. [5] and Mantzourani et al. [62] found no significant differences in salinity scores among groups in their studies. Despite similar dry matter salt values on the 90th day, the enhanced salinity and taste and aroma scores in LA and LP cheeses could be attributed to aroma compounds masking the salt perception. The overall acceptability of the cheeses was high, with scores ranging from 7.46 ± 0.65 to 8.96 ± 0.06 (Table 3). The overall acceptability significantly increased with LP cheese ($p < 0.01$), a finding echoed by Rehman et al. [9] and Özer et al. [35].

Conclusion

Probiotics generally had a positive impact on the quality of white cheese and enhanced its functional properties. Probiotic counts in cheeses with added probiotics were maintained above the effective level ($\geq 10^7$ cfu/g) during ripening. This indicates that cheeses preserved their probiotic qualities throughout ripening, making white cheese a suitable carrier for probiotics to the intestinal environment. In the sensory evaluation, the taste and aroma of the cheeses containing *L. acidophilus* and *L. paracasei* were more appreciated than the others. Furthermore, the *L. casei* ATCC 393 strain, used for the first time in Turkish white cheese, proved compatible with production technology. Future studies on probiotic white cheese should select cultures that withstand production processes and gastrointestinal conditions. It is advisable to add probiotics to pasteurized milk before traditional cultures and at higher temperatures (e.g., 37 °C). Additionally, reducing classical culture amounts to half their standard usage might prevent suppression of probiotics.

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Author contributions Halit Mazlum: Data curation (equal); investigation (equal); methodology (equal); resources (equal); writing—original draft (lead). Mustafa Atasever: Conceptualization (lead); formal analysis (equal); supervision (lead); writing—review and editing (equal).

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Data availability Data are available on request from the authors.

Declarations

Competing interest The authors declare that they have no competing interest.

Ethical approval Ethics committee approval was received for this study from the ethics committee of Atatürk University Veterinary Faculty (Date: 26/05/2021, Number: 2021/15).

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