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Effect of kefir addition on the microbiological, chemical and sensory properties of buffalo milk mozzarella cheese*

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Summary

The aim of this study was to determine the effects of kefir prepared using a commercial kefir starter culture on the microbiological, chemical, and sensory properties of mozzarella cheese produced from buffalo milk. In this study, kefir prepared using a lyophilized commercial kefir starter culture with a defined microbial composition was used. The culture contained *Lactococcus lactis* subsp. *lactis*, *L. cremoris*, *L. lactis* var. *diacetylactis*, *Leuconostoc mesenteroides* subsp. *cremoris*, *Streptococcus thermophilus*, *Kluyveromyces marxianus*, and *Debaryomyces hansenii*. A control group (without kefir addition) and four experimental groups supplemented with 0.5%, 1.0%, 1.5%, and 2.0% kefir were prepared. The cheeses were produced under laboratory conditions, vacuum-packed in PA/PE material, and stored at 4°C for 56 days. Microbiological, chemical, and sensory analyses were performed on days 0, 1, 7, 14, 21, 28, 42, and 56 of storage. Microbiological analyses included total aerobic, anaerobic, and psychrophilic bacterial counts, lactic acid bacteria (LAB), *Lactococcus* spp., yeasts, proteolytic, and lipolytic microorganisms. Chemical analyses involved pH, titratable acidity, fat, and dry matter determinations. The addition of kefir did not cause statistically significant differences in microbiological or chemical parameters ($p > 0.05$). However, kefir-supplemented cheeses exhibited a numerical increase in LAB and *Lactococcus* populations, lower pH values, and higher titratable acidity compared with the control. No significant differences were observed in fat and dry matter contents. Sensory evaluation showed numerically higher scores for appearance, texture, flavor, and sliceability in kefir-treated groups compared with the control. Statistical analysis revealed that only texture and sliceability scores were significantly higher in kefir-treated groups than in the control ($p < 0.05$). Kefir addition was associated with higher texture-related sensory scores without adversely affecting the microbiological quality or chemical composition of mozzarella cheese. These findings suggest that kefir prepared using a commercial kefir starter culture can be evaluated as a natural and functional starter culture alternative for buffalo milk mozzarella cheese production.

Keywords: Mozzarella cheese, buffalo milk, kefir, microbiological properties, sensory quality

Buffaloes in Türkiye belong to the Anatolian buffalo breed, which is part of the Mediterranean buffalo group. The Anatolian buffalo was registered and defined by the General Directorate of Agricultural Research and Policies (TAGEM) of the Ministry of Agriculture and Forestry in 2004 (43). According to 2021 data from the Turkish Statistical Institute, Diyarbakır ranks second in Türkiye in terms of native buffalo breeding, with 16,676 head of livestock (12).

Buffalo milk has a higher fat, dry matter, protein (especially casein), and mineral content compared to the milk of other farm animals (3). Due to these characteristics, it is widely used in the production of cream, yogurt, and various cheeses. Mozzarella cheese is a fresh, semi-soft cheese belonging to the pasta-filata group, originating in the Battipaglia region of Italy. Typically produced from buffalo milk, this cheese is characterized by its white color, elastic texture, and

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slightly salty taste. Due to its melting and stretching properties, it is particularly preferred in products such as pizza and lasagna (16).

Kefir is a fermented milk product with a slightly acidic taste, a unique aroma, and probiotic properties. The fermentation process carried out by the bacteria and yeast strains in kefir grains results in a microbially rich, symbiotic culture (38). According to the Turkish Food Codex Fermented Milk Products Regulation (2022/44), kefir is defined as a product fermented by *Lactobacillus kefiri*, *Leuconostoc*, *Lactococcus*, and *Acetobacter* species, along with yeasts that ferment lactose (*Kluyveromyces marxianus*) or do not ferment lactose (*Saccharomyces unisporus*, *S. cerevisiae*, *S. exiguus*) (42). It has been reported that the microorganisms present in kefir show resistance to low pH and bile acids, can adhere to the intestinal mucosa, and may exhibit antagonistic activity against pathogenic bacteria (13). In addition, *Lactobacillus brevis*, *L. delbrueckii ssp. bulgaricus*, *L. helveticus*, *Streptococcus thermophilus*, *L. casei ssp. pseudoplantarum*, *Kluyveromyces marxianus var. lactis*, *Saccharomyces cerevisiae*, *Candida inconspicua*, *C. maris*, and *L. lactis ssp. lactis* have been isolated (37).

In the literature, studies on mozzarella cheese produced from buffalo milk with the addition of kefir or kefir-based applications are limited. Most existing studies focus on cheese varieties produced from cow's milk. Ferawati et al. (11) reported that different kefir concentrations had no adverse effect on the microbiological and chemical properties of mozzarella cheese produced from cow's milk, and that cheeses produced with kefir addition showed suitable quality characteristics in terms of total mesophilic count, lactic acid bacteria content, pH, protein, fat, and moisture values (11).

Mozzarella cheese is generally produced from cow's milk and is widely consumed worldwide (6). Mesophilic (*Lactococcus lactis ssp. lactis*, *L. lactis ssp. cremoris*) and thermophilic (*Streptococcus salivarius ssp. thermophilus*, *Lactobacillus delbrueckii ssp. bulgaricus*, *L. helveticus*) cultures are used in production (26). These cultures increase the elasticity of the curd structure through lactic acid production (29). In pizza-type mozzarella production, thermophilic cultures are generally preferred because they provide the appropriate moisture content (26). Alternatively, direct acidification can be applied by adding organic acid to the milk. This method reduces insoluble calcium, maintains the pH in the range of 5.5-5.7, and increases the meltability of the cheese (15, 28, 31). Mozzarella cheeses produced by direct acidification can be used immediately after production, while those produced with starter cultures have a longer maturation period (26). It has also been noted that in cheeses using starter cultures, peptides and amino acids reacting with sugars during thermal processing may contribute to browning (33, 39).

The use of starter cultures with high microbial diversity, such as kefir, in mozzarella cheese production has been reported to be associated with changes of microbiological and sensory characteristics. Despite the growing interest in functional starter cultures, studies investigating the use of kefir in mozzarella cheese produced from buffalo milk remain limited. Most available research has focused on cheeses produced from cow's milk, while data on buffalo milk mozzarella are scarce. Moreover, the use of kefir prepared using a commercial kefir starter culture as a technological and functional additive in pasta-filata cheeses has not been sufficiently explored. Therefore, the aim of this study was to evaluate the effects of kefir prepared using a commercial kefir starter culture on the microbiological, chemical, and sensory properties of mozzarella cheese produced from buffalo milk during refrigerated storage.

Material and methods

Buffalo milk procurement and pasteurization. Raw buffalo milk samples were collected as three independent samples from milk sold in the Diyarbakır region. Each sample was analyzed in duplicate (parallel determinations). Milk samples were delivered to the Food Hygiene and Technology Department Laboratory of the Faculty of Veterinary Medicine at Dicle University within 30-45 minutes after morning milking, under cold chain conditions.

Raw milk samples were analyzed for pH, dry matter, fat, titratable acidity, total aerobic mesophilic bacteria, coliforms, *Escherichia coli*, *Staphylococcus–Micrococcus*, lactic acid bacteria (LAB), *Lactococcus* spp., proteolytic bacteria, lipolytic bacteria, yeast, and mold counts. Additionally, milk samples were analyzed for the presence of *Salmonella* spp., *Listeria monocytogenes*, and *E. coli* O157:H7.

The milk was pasteurized at 72°C for 15 seconds, and the same analyses were repeated on the pasteurized milk. The pasteurized milk was standardized to have a fat content of 3%.

After pasteurization, milk samples were analyzed for coliforms, *Escherichia coli*, *Staphylococcus–Micrococcus*, lactic acid bacteria (LAB), *Lactococcus* spp., proteolytic bacteria, lipolytic bacteria, yeast and mold counts, *Salmonella* spp., *Listeria monocytogenes*, and *E. coli* O157:H7. However, since no growth was detected in any of the microorganisms examined, the pasteurized milk analysis results are not presented in tabular form.

Kefir preparation and experimental groups. In this study, a commercial kefir culture (Vivo Gıda Sanayi ve Ticaret Ltd. Şti., Türkiye) in lyophilized form with a defined microorganism composition was used. The culture contains *Lactococcus lactis subsp. lactis*, *L. cremoris*, *L. lactis var. diacetylactis*, *Leuconostoc mesenteroides subsp. cremoris*, *Streptococcus thermophilus*, *Kluyveromyces marxianus*, and *Debaryomyces hansenii* species. The kefir culture was prepared according to the manufacturer's recommendations. For this purpose, 1 g/L lyophilized culture was added to UHT-sterilized cow's milk, incubated at 30°C for 16 hours, and then cooled in a refrigerator for 8 hours. It was stored at 4°C until used in the experiments.

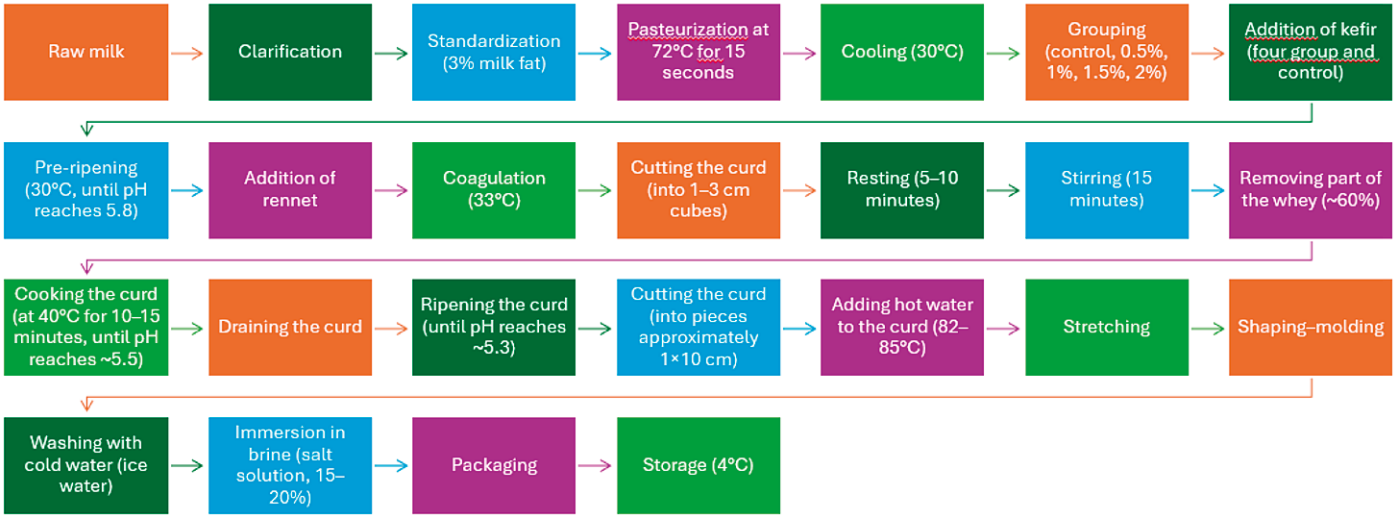

Fig. 1. Production flow chart for mozzarella cheese with kefir addition

In this study, the commercial kefir starter culture was used exclusively for kefir preparation and was not directly added to the milk during cheese production. The fermented milk obtained by this process (kefir) was used in cheese production.

Five experimental groups were established in cheese production, each containing 10 L of pasteurized milk: control (without kefir addition), and groups supplemented with 0.5%, 1.0%, 1.5%, and 2.0% kefir.

Mozzarella cheese production was carried out under laboratory conditions according to the method reported by Barbano et al. (4) and Bhattarai and Acharya (5). Kefir was added at different rates to pasteurized milk cooled to 30°C. After pre-maturation until the pH reached 5.8, rennet was added to the curd at 33°C, the curd was drained, and the curd was ripened until the pH reached 5.3. It was then boiled in water at 80-82°C and kneaded. The resulting cheeses were left in a brine containing 16% salt for 4 hours, then vacuum-packed in Polyamide/Polyethylene (PA/PE) packaging material and stored at 4°C for 56 days (Fig. 1).

Microbiological analyses. Microbiological analyses were performed on days 0, 1, 7, 14, 21, 28, 42, and 56 of storage. On each analysis day, a 25 g cheese sample taken under aseptic conditions was homogenized for 1 minute in a Stomacher device with 225 mL of 0.1% peptone water. Decimal dilutions of the homogenate were prepared and inoculated using the double plating method.

For each analysis day, samples were taken from individual cheese units, and each cheese sample was used only once.

The culture media, incubation conditions, and reference standards used in the analyses are provided in Table 1.

Chemical analyses. The dry matter content in cheese samples was determined using a dry matter determination device (Shimadzu MOC63u, Japan). Titratable acidity values were determined in terms of% lactic acid, salt content was determined according to the Mohr method, and fat content was determined using the Gerber method based on Turkish Standard TSE 591 (44), pH measurements were performed using a calibrated digital pH meter (Hanna HI 2211, Italy). All chemical analyses were performed in triplicate.

Sensory evaluation. Sensory analyses were conducted by 10 panelists from Dicle University Faculty of Veterinary Medicine who were trained on mozzarella cheese evaluation

Tab. 1. Microbiological analysis methods and reference standards used in mozzarella cheese samples

Microorganism	Culture medium	Temperature (°C)	Time (hours)	Reference method
LAB (*Lactobacillus*, *Leuconostoc*, *Pediococcus*)	MRS Agar	30	72	(20)
Lactococcus spp.	M17 Agar	22	72	(43)
Total aerobic mesophilic bacteria	Plate Count Agar (PCA)	30	72	(24)
Total anaerobic mesophilic bacteria	PCA (under anaerobic conditions)	30	72	(17)
Psychrophilic aerobic bacteria	PCA	7	168-240	(17)
Staphylococcus–Micrococcus spp.	Mannitol Salt Agar	37	36-48	(19)
Coliform bacteria	Violet Red Bile Agar	37	24	(23)
Escherichia coli	TBX Agar	44	24	(21)
Sulfite-reducing anaerobic bacteria	Sulfite Polymyxin Sulfadiazine Agar	37	24	(18)
Yeasts and molds	Potato Dextrose Agar (with 10% tartaric acid)	22	120	(22)
Proteolytic bacteria	Calcium Caseinate Agar	28-30	48	(17)
Lipolytic bacteria	Tributyrin Agar	28-30	48	(17)

criteria. Cheese samples were presented at 21 ± 1°C in a white-light environment with randomly coded three-digit numbers. The panelists evaluated the samples based on appearance, texture, flavor, and sliceability using a 5-point hedonic scale (1 = very poor, 5 = very good) (25). Sensory evaluation was conducted by trained adult panelists in accordance with institutional guidelines for sensory studies and therefore did not require formal ethical committee approval.

Statistical analysis. Microbiological data were converted to $\log_{10}$ cfu/g for evaluation. Data were analyzed using repeated measures ANOVA to determine changes during storage. The Tukey multiple comparison test was used to identify significant differences. Statistical analyses were performed using data obtained from three independent samples (n = 3), and each analysis was conducted in duplicate (parallel determinations). Statistical analyses were performed using the SPSS 16.0 software package, and the significance level was set at $p < 0.05$.

Results and discussion

Raw milk analysis results. The total mesophilic aerobic bacteria (TMAB) count in raw buffalo milk was 7.56 $\log_{10}$ cfu/mL (≈ 3.6×10^7 cfu/mL). The microorganisms detected in raw milk included *Escherichia coli* (1.57 $\log_{10}$ cfu/mL), *Listeria* spp. (2.33 $\log_{10}$ cfu/mL), *Staphylococcus–Micrococcus* (4.22 $\log_{10}$ cfu/mL), coliform bacteria (5.94 $\log_{10}$ cfu/mL), lactic acid bacteria (LAB) (5.75 $\log_{10}$ cfu/mL), and lactic streptococci (7.56 $\log_{10}$ cfu/mL). Yeast,

Tab. 2. Microbiological analysis results for raw buffalo milk used in the study

Microorganism type	Microorganism count ($\log_{10}$ CFU/mL)
Total mesophilic aerobic bacteria (TMAB)	7.56
Escherichia coli	1.57
Listeria spp.	2.33
Staphylococcus–Micrococcus	4.22
Coliform bacteria	5.94
Lactic acid bacteria (LAB)	5.75
Lactic streptococci	7.56
Yeast	2.73
Proteolytic microorganisms	5.30
Lipolytic microorganisms	6.46

Tab. 3. Microbiological analysis results for mozzarella cheeses ($\log_{10}$ cfu/g)

Groups	Initial (0 day)	Day 1	Day 7	Day 14	Day 21	Day 28	Day 42	Day 56
Total aerobic mesophilic bacteria								
Control	2.5	3.2	2.6	5.2	3.9	3.5	3.1	2.9
+ 0.5% kefir	3.3	3.8	3.3	5.4	5.8	4.8	4.1	2.8
+ 1% kefir	3.1	4.0	3.7	4.8	6.3	5.0	6.0	3.6
+ 1.5% kefir	2.8	3.2	4.1	6.4	5.8	6.4	3.8	3.4
+ 2% kefir	2.8	4.1	4.7	5.8	6.9	6.4	5.6	2.8
Total anaerobic bacteria								
Control	2.0	3.0	2.6	3.5	3.6	2.9	2.6	2.6
+ 0.5% kefir	3.0	3.5	2.4	3.1	3.6	4.5	2.9	3.8
+ 1% kefir	4.1	4.2	2.7	3.1	4.0	4.4	3.1	3.4
+ 1.5% kefir	3.6	3.8	3.5	3.1	3.7	4.3	3.1	3.2
+ 2% kefir	3.5	3.6	3.8	3.4	3.8	5.5	4.3	3.9
Psychrophilic aerobic bacteria								
Control	1.7	2.8	2.7	4.1	4.2	6.2	6.4	2.5
+ 0.5% kefir	3.2	3.8	2.8	3.1	3.5	4.6	4.5	4.8
+ 1% kefir	3.1	3.8	3.5	3.3	3.4	5.7	5.6	6.4
+ 1.5% kefir	2.9	3.4	3.9	3.2	3.8	3.5	4.1	4.2
+ 2% kefir	3.1	3.3	2.8	4.5	4.4	6.4	6.3	6.3
Lactic acid bacteria (LAB)								
Control	1.7	2.3	2.0	2.9	2.9	2.7	3.0	2.8
+ 0.5% kefir	2.5	3.3	3.5	3.3	3.7	3.2	3.2	3.4
+ 1% kefir	2.5	3.4	3.2	3.2	7.2	5.5	5.8	4.5
+ 1.5% kefir	2.3	3.0	3.5	3.9	7.5	6.0	5.2	3.6
+ 2% kefir	3.1	6.2	6.0	5.3	7.8	6.5	5.8	6.3
Lactococci								
Control	6.8	3.1	3.3	3.2	6.0	4.8	6.1	3.5
+ 0.5% kefir	4.4	4.1	7.5	3.8	6.1	6.3	7.1	2.8
+ 1% kefir	6.6	6.0	7.5	5.0	6.3	6.5	7.4	5.8
+ 1.5% kefir	6.5	5.5	7.4	6.4	7.2	5.5	5.8	5.2
+ 2% kefir	6.5	6.4	6.5	7.3	7.5	7.4	6.4	6.3
Yeast								
Control	3.1	ND	ND	ND	ND	3.2	ND	ND
+ 0.5% kefir	ND	ND	ND	ND	ND	1.5	ND	ND
+ 1% kefir	ND	ND	ND	0.8	ND	3.3	3.2	3.8
+ 1.5% kefir	ND	ND	ND	ND	ND	2.4	ND	0.8
+ 2% kefir	ND	ND	ND	ND	0.8	2.1	ND	ND
Proteolytic bacteria								
Control	1.7	1.5	1.8	2.1	2.2	1.9	2.3	2.0
+ 0.5% kefir	1.2	1.2	2.0	1.8	2.0	1.7	2.2	2.2
+ 1% kefir	1.1	1.8	2.6	1.2	1.8	2.0	2.0	2.0
+ 1.5% kefir	2.4	1.9	2.6	2.0	2.0	1.5	2.4	1.3
+ 2% kefir	2.3	2.4	2.4	1.8	1.7	2.0	2.4	1.6
Lipolytic bacteria								
Control	2.6	2.4	3.1	2.4	3.0	3.1	3.9	3.8
+ 0.5% kefir	3.2	2.0	3.3	2.8	3.2	3.7	2.6	2.3
+ 1% kefir	3.1	2.2	2.7	2.4	3.3	2.9	4.4	3.6
+ 1.5% kefir	2.0	2.4	3.2	1.8	2.8	2.6	3.4	2.2
+ 2% kefir	2.2	2.6	3.2	2.6	3.4	2.8	2.9	2.7

Explanations: No statistically significant differences were observed among groups at the same storage time ($p > 0.05$). ND – Not detected (below the detection limit of 1.0 $\log_{10}$ cfu/g).

proteolytic, and lipolytic microorganism counts were 2.73, 5.30, and 6.46 $\log_{10}$ cfu/mL, respectively.

These values exceeded the maximum limit of 1.5×10^6 cfu/mL permitted for non-cow milk species according to the Turkish Food Codex Regulation on the Supply of Raw Milk (41) and EU Regulation No. 853/2004 (10). This finding indicates a high microbial load in raw buffalo milk and suggests a potential risk of contamination originating from the raw material. *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7 were not detected in any of the samples.

The microbiological quality of raw buffalo milk reflected typical hygienic conditions associated with traditional production systems in the region and was not the primary focus of the present study, which evaluated cheese properties after pasteurization and pasta-filata processing.

Microbiological findings. Changes in microorganism counts in mozzarella cheeses during storage are presented in Table 3. In the control group, total mesophilic aerobic bacteria (TMAB) counts increased until day 14, reaching 5.2 $\log_{10}$ cfu/g, and then decreased to 2.9 $\log_{10}$ cfu/g by day 56. In contrast, in the kefir-supplemented groups, particularly at 1.5% and 2% levels, TMAB counts reached 6.9 $\log_{10}$ cfu/g on day 21 of storage and subsequently decreased.

LAB counts increased numerically with increasing kefir ratio. While the control group reached 2.9 $\log_{10}$ cfu/g on day 21, the group supplemented with 2% kefir reached 7.8 $\log_{10}$ cfu/g on the same day. Similarly, the *Lactococcus spp.* population remained in the range of 6-7.5 $\log_{10}$ cfu/g throughout storage in the kefir-supplemented groups, whereas lower values (3-6 $\log_{10}$ cfu/g) were observed in the control group.

The addition of kefir was associated with lower numerical levels of yeast, proteolytic, and lipolytic microorganisms; these groups generally remained at 1-3 $\log_{10}$ cfu/g throughout storage. Psychrophilic aerobic bacteria counts increased during the middle period of storage (days 21-28); however, kefir-added samples showed a more balanced numerical trend compared to the control group. Total anaerobic bacteria counts varied between 2-5 $\log_{10}$ cfu/g throughout storage, and no sulfite-reducing bacteria were detected in any of the samples.

Overall, although the addition of kefir did not result in statistically significant changes in the total microbial load ($p > 0.05$), numerically higher LAB and *Lactococcus* spp. counts were consistently observed in kefir-added groups. This increase can be primarily attributed to the microorganisms introduced with kefir. These findings suggest that the presence of kefir-derived microbiota influenced microbial profiles during storage, rather than indicating a statistically confirmed inhibitory effect on undesirable microflora.

Tab. 4. Chemical properties of mozzarella cheeses

Groups	pH	Titratable acidity (%LA)	Fat (%)	Dry matter (%)
Control	6.25	0.528	22.7	50.2
+ 0.5% kefir	6.10	0.612	20.3	45.3
+ 1% kefir	5.68	0.684	21.5	43.5
+ 1.5% kefir	5.63	0.732	22.3	40.4
+ 2% kefir	5.72	0.708	23.2	40.1

Explanations: The values represent measurements on day 56. No statistically significant differences were found between the groups ($p > 0.05$).

Chemical findings. The chemical analysis results for mozzarella cheeses are presented in Table 4. The addition of kefir did not have a statistically significant effect on pH, titratable acidity, fat, and dry matter content ($p > 0.05$). However, a gradual decrease in pH values and a corresponding increase in titratable acidity were observed with increasing kefir ratio. While the pH was determined as 6.25 in the control group, it was measured as 5.63 in the +1.5% kefir group and 5.72 in the +2% group. Titratable acidity reached 0.732% and 0.708% lactic acid levels in these groups, respectively. Fat and dry matter contents showed minor fluctuations in the kefir-supplemented groups but were generally lower than those of the control samples. These findings indicate a tendency toward increased acidity associated with kefir addition, without resulting in statistically significant changes in the overall chemical composition of the cheese.

Sensory findings. The sensory evaluation results for mozzarella cheeses are presented in Table 5. In the panel evaluation, higher mean scores were numerically observed for appearance, texture, flavor, and sliceability in the kefir-added groups compared with the control group.

According to statistical analysis, only the texture and sliceability parameters of the kefir-added groups, particularly at the 1.5% and 2% levels, were significantly higher than those of the control group ($p < 0.05$). In contrast, no statistically significant differences were detected among the groups in terms of appearance and flavor ($p > 0.05$).

Cheeses produced with kefir addition were described by panelists as having a more elastic and homogeneous

Tab. 5. Sensory evaluation results of mozzarella cheeses (hedonic scale, 1-5 points)

Attribute	Control	+ 0.5% kefir	+ 1% kefir	+ 1.5% kefir	+ 2% kefir
Appearance	2	3	4	4	5
Texture	2	3	4	4	5
Flavor	3	3	4	5	5
Sliceability	2	3	4	4	5

Explanations: A statistically significant difference was found between the control group and the kefir-added groups in the texture and sliceability parameters ($p < 0.05$)

structure. During storage, the control group exhibited an increase in texture hardness and a decrease in sliceability, whereas these changes were less pronounced in the kefir-added groups based on panelist evaluations.

Overall evaluation. The results obtained show that the addition of kefir did not cause statistically significant changes in the microbiological and chemical properties of mozzarella cheese produced from buffalo milk ($p > 0.05$). However, numerical differences were observed in several parameters, particularly higher LAB and *Lactococcus* populations, lower pH values, and higher titratable acidity levels in kefir-added groups.

Sensory evaluation revealed that the addition of kefir was associated with significantly higher texture and sliceability scores compared to the control group ($p < 0.05$). Kefir-added samples were described by panelists as more elastic and homogeneous in structure, based on sensory assessment results.

This study examined the effects of kefir addition on the microbiological, chemical, and sensory properties of mozzarella cheese produced from buffalo milk during storage. The findings show that kefir addition numerically increased LAB and *Lactococcus* populations, while pH values decreased and titratable acidity increased with increasing kefir levels. Sensory scores for appearance, texture, and flavor also improved as the kefir ratio increased.

The high TMAB and coliform levels detected in raw buffalo milk indicate a high microbial load prior to pasteurization, which does not comply with TGK (2017/16) (41) and EU Regulation 853/2004 (10). Although pasteurization (72°C/15 s) and the high temperatures applied during the pasta-filata stage reduce microbial risks, heat-resistant enzymes may still affect texture and fat–protein stability. The rapid acidification and LAB dominance associated with kefir addition may have partially compensated for these potential drawbacks, supporting the role of kefir as a bioprotective and functional starter culture.

Total mesophilic aerobic and psychrophilic bacteria counts showed an upward trend in kefir-supplemented groups during the middle storage period (days 21-28). For example, in the 2% kefir group, TMAB reached 6.9 $\log_{10}$ cfu/g on day 21, while psychrophilic bacteria reached 6.4 $\log_{10}$ cfu/g on day 28. This increase reflects the contribution of kefir-derived beneficial microbiota, rather than uncontrolled microbial growth.

Reale et al. (35) reported that probiotic counts decreased during the draining stage but recovered during ripening in mozzarella and scamorza cheeses. Similarly, the mid-storage increase observed in this study suggests the establishment and persistence of a kefir-derived probiotic ecosystem, highlighting the importance of shelf-life management.

Microbial changes observed in cheeses supplemented with kefir during storage reflect typical controlled fermentation dynamics. In kefir-supplemented groups, LAB and *Lactococcus* populations increased rapidly during the early stages, reached maximum levels between days 21 and 28, and remained relatively stable throughout storage. These microorganisms may contribute to limiting the development of psychrotrophic and proteolytic flora through organic acid production and antimicrobial metabolites (diacetyl, acetic acid, H_2O_2) under low pH conditions. In contrast, the absence of a starter culture in the control group resulted in more irregular microbial development.

The increase in LAB observed with kefir addition suggests the presence and activity of *Lactococcus lactis*, *Leuconostoc mesenteroides*, and *Streptococcus thermophilus* strains derived from the kefir within the cheese matrix. These bacteria are commonly associated with curd elasticity and textural properties in mozzarella production. Although no statistically significant differences were detected in microbial parameters ($p > 0.05$), the observed biological trends are considered technologically relevant. Similar observations were reported by Ferawati et al. (11) and Rehman (36), who noted maintenance of LAB viability in kefir-supplemented mozzarella-type cheeses. In this context, kefir may support the microbial ecosystem during storage, indicating a potential bio-protective role.

As reported for other kefir-fermented dairy products, increases in total viable counts accompanied by pH reduction are expected outcomes favoring LAB dominance (27). Consistent with this, LAB viability has been reported to be maintained throughout mozzarella production when kefir-derived *Lactobacillus kefiranofaciens* was used (36).

The natural microbiota of buffalo milk mozzarella and traditional yeast-starter communities play a key role in shaping product ecology, which may facilitate the integration of kefir-derived LAB into the cheese matrix. Findings by Ercolini et al. (9) supporting early pH reduction are consistent with the present results. Kefir addition levels reported in the literature vary widely (0-12%) (11), with higher levels generally leading to more pronounced pH decreases and LAB increases. The 0.5-2% range applied in this study produced moderate but consistent effects, which may be attributed to kefir concentration, kefir form, milk type, salting method, and storage conditions.

In kefir-supplemented groups, yeast counts generally remained at low levels throughout storage. Although limited increases were observed on day 28 in some groups (e.g., ≈3.3 $\log_{10}$ cfu/g in the 1% kefir group), elevated yeast levels were not reached. Despite the presence of yeasts in kefir microbiota, their contribution remained limited in the cheese matrix. Proteolytic and lipolytic microorganism counts also remained low (≈1.1-4.4 $\log_{10}$ cfu/g) and did not show a consistent increase with kefir addition, indicating no accelerating effect under the applied storage conditions.

Chemical data indicate that kefir addition promoted earlier acidification in mozzarella cheese during the early stages and maintained this effect throughout storage. The decrease in pH with increasing kefir level, together with the parallel increase in titratable acidity, can be attributed to the metabolic activity of kefir-derived lactic acid bacteria (LAB) and *Lactococcus* spp. within the cheese matrix. These results are consistent with the findings of Akarca et al. (1), who reported that the pH of mozzarella produced with different starters ranged from 5.89 to 6.05 and that the culture used had a significant effect on acidity and texture. In our study, the decrease in pH to approximately 5.6 with the addition of kefir indicates rapid acid production associated with the kefir-derived microbiota. The lower dry matter content in the kefir-containing groups may be attributed to the increased water-holding capacity due to the exopolysaccharide-producing bacteria in kefir. This contributes to maintaining the moist and elastic structure of the cheese. Similarly, Perry et al. (34) reported that exopolysaccharide-producing cultures improved texture by increasing moisture retention in low-fat mozzarella; Minervini et al. (30) reported that the addition of probiotic lactobacilli to *Fior di Latte* cheese improved aroma and texture development during the ripening process. Minor numerical variations in fat content are considered to be within the expected analytical variability of the Gerber method and cheese-making process, rather than reflecting true compositional differences, since the milk used in all groups was standardized to 3% fat. Overall, kefir addition was associated with a lower pH and higher titratable acidity profile without causing statistically significant changes in the overall chemical composition of mozzarella cheese.

As the kefir level increased, an increase in LAB and *Lactococcus* counts was observed, and this increase was found to be positively correlated with sensory quality. These findings are parallel to the results reported by Akarca and Yıldırım (2) in mozzarella cheeses supplemented with probiotic bacteria. In that study, the addition of *Lactobacillus acidophilus* and *Bifidobacterium* lactis increased acidity values, and the samples supplemented with probiotics received the highest sensory scores. Similarly, Mukhtar et al. (32) reported that probiotic viability was maintained in cheeses produced with *L. acidophilus* S2, and that pH reduction and sensory improvement were achieved. In this context, it can be said that kefir microbiota performs a similar function.

The positive effect of kefir addition on sensory properties was consistently observed. Appearance, texture, and flavor scores increased with increasing kefir levels, with the highest averages generally recorded in the 1.5% and 2% groups. This trend is consistent with microbial acidification dynamics and suggests that kefir addition positively influences sensory quality. Similar sensory improvements have been reported by Akarca and Yıldırım (2) and Mukhtar et al. (32). In addition, Minervini et al. (30) demonstrated that probiotic lactobacilli contributed to aroma development and consumer acceptance in Fior di Latte cheese. In the present study, kefir addition was associated with improvements in both the acidity profile and sensory attributes. Statistical analysis further demonstrated that texture and sliceability scores were significantly higher in kefir-supplemented samples ($p < 0.05$). These effects may be related to the activity of *Lactococcus* and *Leuconostoc* species through controlled acidification and potential exopolysaccharide production, as also reflected in panelist descriptions of more elastic, homogeneous, and glossy cheese surfaces.

Previous studies have shown that the use of kefir as a starter culture in white brined cheeses does not negatively affect sensory properties and may even enhance consumer acceptance when applied at appropriate levels (14). This observation is consistent with the present findings. During mozzarella production, increasing kefir levels were associated with greater curd pliability and ease of shaping during the cooking step, whereas control samples exhibited a relative decrease in elasticity.

Overall, kefir addition, particularly at 1-2% levels, contributed to regulating the microbiological ecology of buffalo milk mozzarella in favor of LAB and *Lactococcus*, while promoting a more pronounced acidity profile and improved sensory quality. The observed increases in total aerobic and psychrophilic populations during mid-storage highlight the importance of shelf-life monitoring. For future studies, combined evaluation of moisture–fat balance, instrumental texture measurements, and comprehensive pathogen screening may provide additional insight into product stability.

In conclusion, the addition of kefir at levels of 0.5-2% did not result in statistically significant changes ($p > 0.05$) in the microbiological or chemical properties of buffalo milk mozzarella cheese, but was associated with favorable numerical trends, including lower pH values and higher titratable acidity, in parallel with increased LAB and *Lactococcus* populations.

Sensory evaluation demonstrated that kefir addition resulted in significantly higher texture and sliceability scores compared with the control ($p < 0.05$). The most favorable scores were observed in the 1.5-2% kefir groups, where cheeses were described by panelists as more elastic, homogeneous, and easier to slice.

Overall, kefir may be considered a natural, functional starter alternative that supports the structural and sensory properties of buffalo milk mozzarella cheese without adversely affecting chemical composition, while maintaining microbial balance. Further studies are recommended to validate the 1.5-2% kefir addition range under industrial-scale conditions, using different kefir sources and processing parameters.

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