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Original Research Article

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Effects of fat level, prebiotics, and production methods on the quality and shelf life of synbiotic kefir from Buffalo milk

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Abstract

Kefir is a traditional and functional dairy product that is widely consumed around the world. The aim of this research is to produce synbiotic kefir to enhance the functional properties and storage stability. In this study, the effect of fat level (0.5%, 3.5%), prebiotics (galacto-oligosaccharides (GOS), inulin), production method (traditional, commercial), and storage period (21 days at 3 ± 1 °C) on the quality characteristics of synbiotic kefir produced from buffalo milk was investigated. Significant effects ($P < .01$) of production method, fat level, prebiotics, and storage period on the protein, pH, and total solids of kefir samples were observed, with the total solids content being higher in kefirs produced with 3.5% fat, the traditional method, and the addition of GOS compared to other samples ($P < .05$). The pH value increased in parallel with the increase in fat level ($P < .05$), and higher values ($P < .05$) were recorded in kefirs produced using GOS and commercial cultures. During storage, pH, protein, and total solids decreased ($P < .05$), while total titratable acidity and water activity values increased ($P < .05$). Higher lactic cocci and lactic bacilli counts were obtained in kefirs produced with commercial cultures compared to traditional methods ($P < .05$). Yeast counts were affected by all treatments ($P < .01$), with the lowest values found in samples containing 3.5% fat, commercial cultures, and inulin ($P < .05$). The *Enterobacteriaceae* count was below the detectable limit (<1 log cfu/ml) in all samples. The sensory properties of the kefir samples were influenced by the treatments, with the kefir samples produced using 3.5% fat, GOS addition, and traditional methods being more acceptable in terms of overall acceptability ($P < .05$).

Keywords

Synbiotic kefir, buffalo milk, prebiotic, galacto-oligosaccharides, inulin, quality and functional properties

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Introduction

Kefir is a fermented milk product and contains useful bacteria and yeasts, thus, is an important member of the functional foods rich in probiotic microorganisms. Kefir is a healthy and enjoyable fermented beverage obtained from the fermentation of kefir grains with water or milk (Kesenkaş et al., 2017). The kefir grains contain a rich and unique microflora, primarily consisting of lactic acid bacteria and yeasts, but this microflora can vary depending on the origin of the grains and the fermentation method used (Satir and Guzel-Seydim, 2015; Rosa et al., 2017). This variability, along with the type of milk used, the production method, and fermentation conditions, significantly affects the quality of kefir and consequently its functional properties. The functional characteristics of kefir result from probiotic microorganisms and bioactive compounds

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formed through microbial metabolism (Schwan et al., 2022).

The most important factors affecting the quality and stability of kefir are the type, quality, and characteristics of the milk used in production, the properties of the kefir grains, and the fermentation conditions. The use of buffalo milk in kefir production is more advantageous compared to cow's milk regarding functionality due to its high content of fat, protein, total solids, lactose, ash, calcium, and phosphorus, as well as its richness in antimicrobial substances, and better processing capabilities due to its high water-holding capacity and product yield (Khedkar et al., 2016). Also, the use of buffalo milk in kefir production increases total solids, protein and viscosity values compared to cow's milk, resulting in a higher yeast count and a more favorable sensory evaluation (Gul et al., 2018; Aktaş et al., 2024). However, in these studies, there are no findings regarding the fat level and prebiotic use on the quality characteristics of synbiotic kefirs produced using buffalo milk.

Kefir is produced using two methods: traditional and commercial. In the traditional method, kefir grains are used for fermentation, while in the commercial method, lyophilized starter culture is employed. Kefir obtained through the commercial method is reported to be more viscous and contain less yeast compared to the traditional method; however, kefir produced traditionally is noted to have superior sensory properties (Yildiz-Akgul et al., 2018). Although the production methods show some similarities, the resulting kefirs differ in sensory, microbiological, chemical, and even physical characteristics. It is often stated that the type of kefir culture used in production significantly influences these differences. Both kefir grains and starter cultures can possess unique microflora. The diversity of this microflora is one of the primary topics considered in optimizing production (Coşkun and Erol, 2023).

Products that utilize both probiotics and prebiotic substances, where probiotics are selectively targeted towards the prebiotics and exhibit more effects together than when alone, are referred to as synbiotics (González-Herrera et al., 2021). The addition of prebiotics such as GOS and inulin, which interact with the probiotics naturally present in kefir, results in the production of synbiotic kefir. As mentioned above, the numerous factors affecting kefir quality make it challenging to ensure a standard quality in the produced kefirs. This particularly negatively impacts the sensory and structural properties of kefir during storage, posing a significant problem. Therefore, producing structurally more stable products and enhancing the functional properties of kefir by imparting synbiotic characteristics is important. The aim of this study is to determine the effects of the fat level of the milk used in production (0.5% and 3.5%), the type of prebiotic (galacto-oligosaccharides and inulin), and the production methods (traditional and commercial) on the chemical, physicochemical, microbiological, and sensory quality characteristics of synbiotic kefir from buffalo milk, as well as to identify the changes that occur during a 21-day storage period at 3 ± 1 °C.

Materials and methods

Buffalo milk used in the production of kefir was sourced from a local business operating in Eskişehir/TURKEY. The kefir grains used for fermentation were obtained from the Food Engineering Department of Suleyman Demirel University; the FD Direct Vat Set (DVS) eXact Kefir mesophilic-thermophilic mixed culture used as a starter was supplied by Chr Hansen (Denmark); the galacto- oligosaccharides used as a prebiotic were obtained from Clasado Biosciences Ltd (Reading, UK), and inulin was sourced from Smart Kimya. The bottles made from high-density polyethylene (HDPE) used for storing the produced kefirs were procured from Petek Plastik (Konya, Turkey).

Kefir production and storage

Kefir production was carried out by modifying the method provided by Satir and Guzel-Seydim (2015), and the production flowchart is shown in Figure 1. The buffalo milk used as raw material in the study was subjected to preheating, after which the fat levels were adjusted to 0.5% and 3.5%. After pasteurization, each fat level was divided into three, with one group serving as the control and the other two groups receiving GOS and inulin to form two prebiotic groups. The control and prebiotic milk groups were then divided into two groups, and kefir grains and commercial starter culture were prepared (Figure 1). As shown in Figure 1, 12 different kefirs were produced as part of the study. The raw buffalo milk with a fat content of 6.5% was standardized to fat levels of 0.5% and 3.5% using a centrifugal separator. In kefir production, the usage rate of kefir grains was 3%, the usage rate of lyophilized kefir culture was 0.025 g/L, and the amount of prebiotic was used at 2% based on preliminary trials. The pasteurization process was performed at 95 °C for 5 min, and the pasteurized milk was cooled to 25 °C after the addition of prebiotics, after which the culture was added, and fermentation was carried out at 20 °C. The fermentation was terminated once the pH reached 4.6. A total of 48 liters of kefir were produced with each sample being 2 liters, using two replicates. The prepared samples were stored at 3 ± 1 °C in bottles made from high-density polyethylene (HDPE), and analyses were conducted on the 1st, 7th, 14th, and 21st days of storage.

Physicochemical analyses

The fat content of the kefir samples was determined using the Gerber method, and the results were expressed as a percentage. The determination of the protein content in kefir samples was based on the titration method provided by

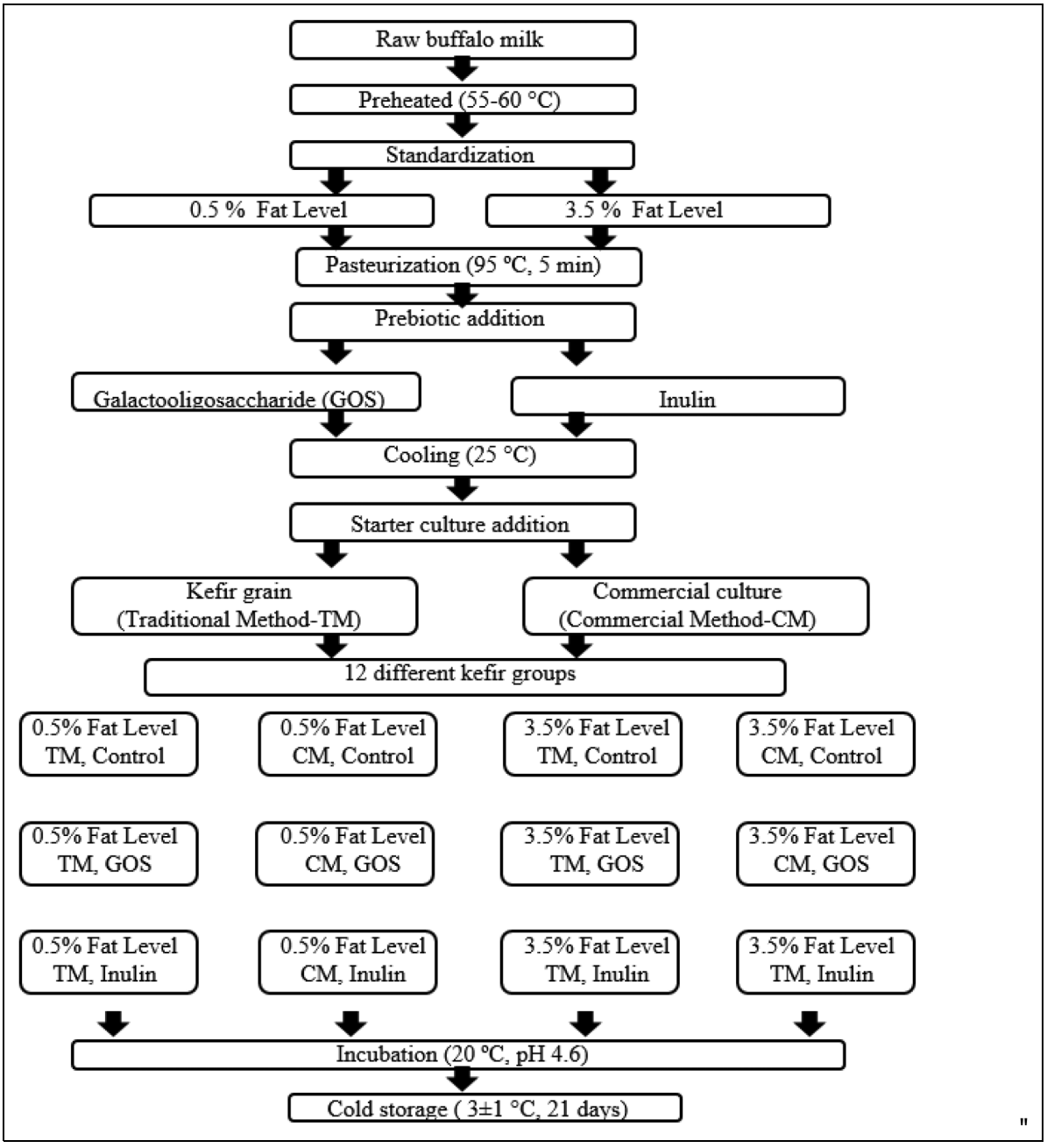

Figure 1. Flowchart of synbiotic kefir production.

Triwibowo et al. (2020). To determine titratable acidity (TA) of the samples, 10 mL of the homogenized sample were taken, and phenolphthalein was added. It was then titrated with 0.1N NaOH solution until a faint pink color was obtained. The titratable acidity was calculated as a percentage of lactic acid, taking into account the amount of NaOH used. The pH values of the samples were determined using a digital pH meter (Hanna HI 2020 Edge) calibrated with pH 4 and 7 buffer solutions. The dry matter content in the samples was determined using the drying method in an oven at 105 ± 2 °C. The fat-free dry matter content was determined using the Milkana Superior Milk Analyzer device with a sample size of 10 mL. The determination of water activity (a_w) of kefir was performed at a constant temperature of 20 ± 0.5 °C using a a_w determination system (Streoglass, Sweden).

Microbiological analyses

Microbiological analyses were performed using dilutions prepared with physiological saline solution (0.85% NaCl) and the pour plate method. For determining the total

aerobic mesophilic bacteria (TAMB) count, Plate Count Agar (Merck, 105463) was used, and incubated at 30 °C for 48 h. M17 Agar (Merck, 115108) was used for the count of lactic cocci, and the incubated at 30 °C for 24–48 h. The count of lactic bacilli was determined using MRS Agar (De Man, Rogosa and Sharpe, Merck, 110660), and anaerobically incubated at 37 °C for 48 h. For determining the yeast (aerobically at 25 ± 2 °C for 96 h) and *Enterobacteriaceae* spp. counts (anaerobically at 30 °C for 48 h), Potato Dextrose Agar (Merck, 110130) and Violet Red Bile Dextrose (VRBD) Agar (Merck, 107620) were used, respectively. Microbiological results were calculated as log cfu/mL.

Sensory analysis

In the sensory analyses, the appearance, aroma, taste, consistency, and overall acceptability criteria of the kefirs were assessed, with analyses conducted during the storage period. The trained panelist group consisted of 10–12 members from the Faculty of Agriculture at Eskişehir Osmangazi University. For this purpose, the kefir samples were numbered and presented to the panelists in transparent cups, ensuring they were of the same color and shape. During the sensory analysis, salted crackers and water were served alongside the kefirs. The evaluation was conducted based on the tests from Kök-Taş (2013) and Coşkun and Erol (2023), each sample was rated for overall acceptability on a scale of 1–5 points, with 1 point indicating very poor quality and 5 points indicating very good quality. A score of 5 (good kefir sample) was characterized by having a milk color, a frothy structure, being homogeneous and smooth, possessing a fermented taste and aroma, a flowing consistency, and being the most liked kefir in terms of taste, independent of the typical flavor of kefir.

Statistical analysis

The study was conducted with two replicates following a completely randomized block design. Data were analyzed using the SPSS software package (SPSS 23.0), and significant differences between the means of the main sources of variation found to be significant were determined using Duncan's Multiple Comparison Test at a 5% significance level ($P < .05$). The model included the fat levels in buffalo milk (0.5%,3.5%), the production method (traditional (TM) and commercial (CM)), prebiotics (galacto-oligosaccharides and inulin), and the storage period (21 days), as well as all interactions among these factors. All experimental data of the kefir samples [fat, protein, titratable acidity, pH, total solids, water activity, microbiological properties (TAMB, lactic cocci, lactic bacilli, *Enterobacteriaceae*, and yeast count), sensory properties] were analyzed using two-way ANOVA. The statistical analysis results are presented in tables as mean values and standard deviation, and in figures as mean ± standard error.

Results and discussion

Properties of buffalo milk

The values of dry matter, fat, protein, fat-free dry matter, pH, and titratable acidity of the raw buffalo milk used in kefir production were determined to be 16.81 ± 0.02%, 6.88 ± 0.01%, 4.79 ± 0.05%, 9.92 ± 0.03%, 6.81 ± 0.01, and 0.14 ± 0.01%, respectively. Various studies have reported that the dry matter content of buffalo milk ranges from 11.90% to 19.18%, the protein content ranges from 2.70% to 4.74%, and the fat content varies from 5.30% to 9.00% (Kapadiya et al., 2016; Fangmeier et al., 2019).

Physicochemical properties

The fat level, production method, addition of prebiotics, and storage duration have been found to have highly significant effects ($P < .01$) on the dry matter (DM) content of the synbiotic kefir (Table 1). The DM in the 0.5% fat kefirs was found to average 12.24 ± 0.63%, ranging from 11.19% to 12.92%, while in the 3.5% fat samples, it averaged 15.44 ± 0.80%, ranging from 14.14% to 16.53%. These results indicate an approximate 3% difference in DM content between kefirs produced using low-fat and high-fat buffalo milk. This difference is due to the higher DM content in fatty milk. Similarly, the study conducted by Tomar et al. (2020) noted that an increase in milk fat content used for kefir production resulted in an increase in the DM. In this study, the total DM of kefirs produced using CM was lower than that produced by TM ($P < .05$). The DM in kefirs produced with 100% buffalo milk using starter culture was determined to be 16.64% (Akdan et al., 2020), which is approximately 3% higher than our findings. This difference may be attributed to the high-fat content of the buffalo milk used by the researchers (Akdan et al., 2020). The DM of samples with prebiotics added was higher compared to the control group ($P < .05$). Among the prebiotic groups, the DM was higher with the addition of GOS compared to inulin, meaning that adding GOS significantly increased the DM of kefir more than adding inulin. This is probably due to the high water affinity of inulin, which has hydrophilic groups that bind strongly to water, affecting the hygroscopicity of the products to which it is added (Fernandes et al., 2014; Šeregelj et al., 2021). The addition of prebiotics increases the DM in kefir by holding more water. Similarly, Buran et al. (2021) found that the addition of prebiotics increased the DM ratios of kefirs. In their study, where they used FOS and inulin as prebiotics, the researchers found the DM to be approximately 2 units higher compared to the control. The DM of the produced kefirs began to decreases after the 7th day of storage, with a significant decrease ($P < .05$) observed on the 14th and

Table 1. Effect of fat levels, production methods, prebiotic and storage period on the dry matter, water activity, fat, protein, total titratable acidity, and pH values of synbiotic kefir samples during chilled storage.

	Dry matter (%)	Water activity (a_w)	Fat (%)	Protein (%)	Total titratable acidity (%)	pH
Fat levels (FL)						
0.5%	12.24 ± 0.63^{b}	0.986 ± 0.003^{a}	0.50 ± 0.04^{b}	4.06 ± 0.26^{b}	1.10 ± 0.09^{a}	4.55 ± 0.08^{a}
3.5%	15.44 ± 0.80^{a}	0.985 ± 0.002^{a}	3.40 ± 0.03^{a}	4.29 ± 0.29^{a}	1.07 ± 0.11^{a}	4.51 ± 0.14^{b}
P value	**	NS	**	**	NS	**
Production method (PM)						
Traditional	13.86 ± 1.77^{a}	0.986 ± 0.002^{a}	1.08 ± 0.09^{a}	4.52 ± 0.11^{b}	1.99 ± 1.52^{a}	4.52 ± 0.34^{b}
Commercial	13.82 ± 1.77^{b}	0.986 ± 0.003^{a}	1.09 ± 0.11^{a}	4.54 ± 0.12^{a}	2.00 ± 1.50^{a}	4.54 ± 0.19^{a}
P value	**	NS	NS	**	NS	*
Prebiotic (P)						
Control (C)	12.96 ± 1.56^{c}	0.986 ± 0.002^{a}	1.98 ± 1.52^{b}	4.27 ± 0.14^{a}	1.09 ± 0.11^{a}	4.53 ± 0.10^{b}
GOS (G)	14.49 ± 1.69^{a}	0.985 ± 0.003^{b}	2.00 ± 1.53^{a}	4.27 ± 0.28^{a}	1.06 ± 0.10^{a}	4.56 ± 0.12^{a}
Inulin (I)	14.08 ± 1.72^{b}	0.986 ± 0.002^{a}	1.99 ± 1.52^{ab}	3.97 ± 0.32^{b}	1.10 ± 0.09^{a}	4.50 ± 0.12^{b}
P value	**	**	**	**	NS	**
Storage period (SP, days)						
1	$14.01 \pm 1,98^{a}$	0.984 ± 0.002^{c}	2.01 ± 1.53^{a}	4.37 ± 0.21^{a}	1.01 ± 0.08^{d}	4.60 ± 0.09^{a}
7	14.03 ± 1.85^{a}	0.985 ± 0.001^{b}	2.00 ± 1.53^{a}	4.22 ± 0.24^{b}	1.05 ± 0.08^{c}	4.57 ± 0.10^{a}
14	13.69 ± 1.63^{b}	0.984 ± 0.001^{c}	1.96 ± 1.52^{b}	4.12 ± 0.27^{c}	1.11 ± 0.09^{b}	4.52 ± 0.09^{b}
21	13.64 ± 1.64^{c}	0.989 ± 0.002^{a}	2.00 ± 1.53^{a}	3.98 ± 0.31^{d}	1.17 ± 0.08^{a}	4.43 ± 0.11^{c}
P value	**	**	**	**	**	**
Interactions						
FL × PM	NS	NS	**	NS	NS	**
FL × P	**	NS	NS	**	**	NS
FL × SP	**	**	NS	**	NS	NS
PM × P	*	**	**	**	NS	NS
PM × SP	**	NS	NS	**	NS	NS
P × SP	**	**	NS	**	NS	NS
FL × PM × P	**	*	NS	**	NS	**
FL × PM × SP	NS	**	NS	*	NS	NS
FL × P × SP	**	NS	NS	**	NS	NS
PM × P × SP	**	NS	**	**	NS	NS

*$P < .05$; **$P < .01$; NS: $P > .05$.

[a–b:] Means in the same column and in the same section with the same letters are not significantly different at $P > .05$ (Duncan's test) between the means of the fat levels and production methods.

[a–c:] Means in the same column and in the same section with the same letters are not significantly different at $P > .05$ (Duncan's test) between the means of the prebiotic.

[a–f:] Means in the same column and in the same section with the same letters are not significantly different at $P > .05$ (Duncan's test) between the means of the storage periods.

21st days of storage. Similarly, the research conducted by Buran et al. (2021) noted a decrease in the DM ratio of all samples by the end of the 28-day storage. The DM contents of prebiotic-added kefirs with 3.5% fat are higher than those of kefirs with 0.5% fat. Among the prebiotics, the average DM is greater in the samples with GOS added (Figure 2(a)). It has been determined that the DM varies depending on the fat level. As shown in Figure 2(b), illustrating the interaction of fat level × storage, there is no significant change in the DM of kefirs produced from low-fat (0.5%) milk over the storage period, while there is a significant decrease in the 3.5% fat kefirs during the storage. In both production methods, the lowest DM were found in the control group. In the GOS group, the determined values were higher than those in the inulin group (Figure 2(c)). These findings indicate that the addition of prebiotics leads to greater water retention in kefir. During the 21-day storage period, a decrease in the average DM was observed in both kefir samples produced with culture and grains. In dry matter contents, the greatest decreases occurred on the 14th and 21st

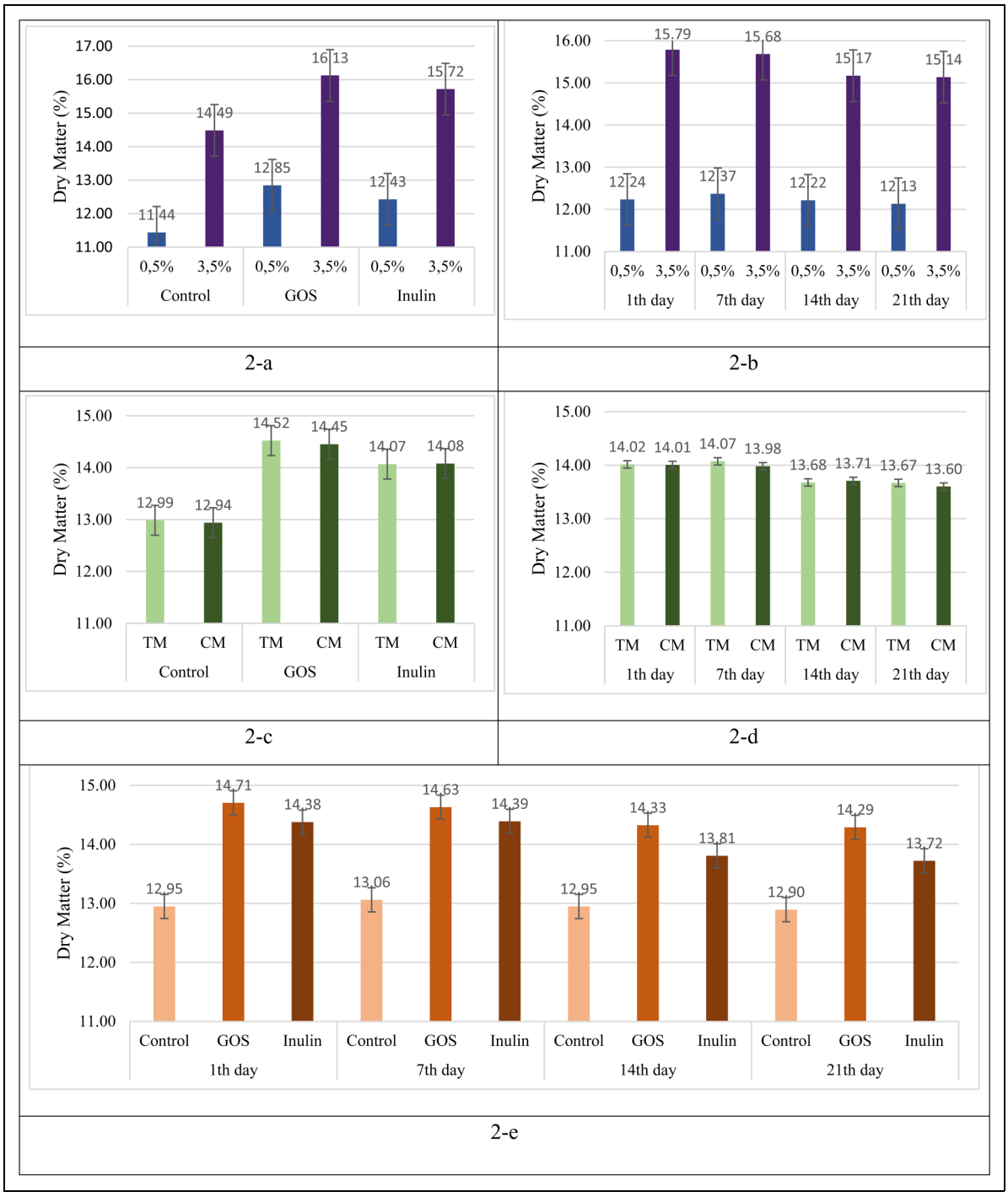

Figure 2. Effects of interactions on the dry matter content of kefirs: fat level × prebiotic (a), fat level × storage duration (b), production method × prebiotic (c), production method × storage duration (d), prebiotic × storage duration (e) (TM: Traditional Method, CM: Commercial Method, GOS: Galacto-oligosaccharides).

days of storage (Figure 2(d)). It was determined that the DM of kefirs with 3.5% fat and inulin added decreased by 1% after the 7th day of storage (Figure 2(e)). Corona et al. (2016) stated that while the soluble solid content in kefir decreases after fermentation, the volatile organic compounds increase. The decrease in the average DM of the kefir samples during storage may be due to the conversion of lactose into lactic acid, acetic acid, alcohol, and CO_2 during fermentation (Laureys and Vuyst, 2014).

The addition of prebiotics and storage had effect on the water activity (a_w) of the kefir samples ($P < .01$). On the 21st day of storage, the a_w of the kefirs reached its highest value

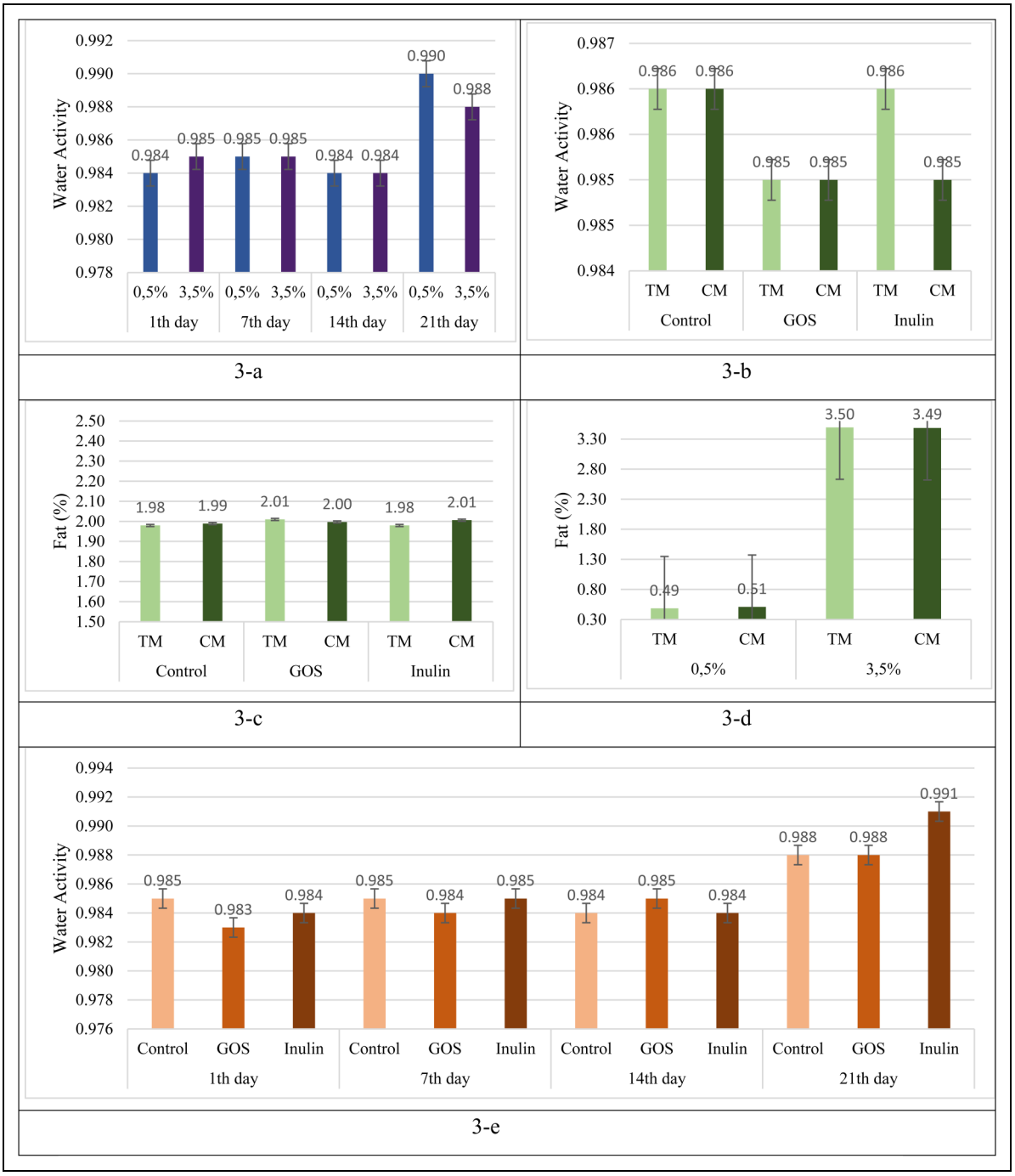

Figure 3. Effects on the water activity of kefirs: fat level × storage duration (a), production method × prebiotic (b), production method × prebiotic (c), effects on fat content: fat level × production method (d), prebiotic × storage duration (e) interactions (TM: Traditional Method, CM: Commercial Method, GOS: Galacto-oligosaccharides).

(Table 1). The a_w values of the control and inulin-added samples were higher than those of the GOS-added samples ($P < .05$). At the beginning of storage, the a_w values in high-fat kefirs were higher than those in low-fat samples; however, on the 21st day of storage, the activity of the low-fat samples was found to be higher. Additionally, the highest values during the storage period were determined on the 21st day (Figure 3(a)). It was found that the control group had the highest average a_w of 0.986 in both TM and CM. In samples with added prebiotics, GOS addition reduced a_w in samples produced with grains, while both GOS and inulin additions reduced a_w in samples produced with cultures (Figure 3(b)). In kefirs with GOS, a_w increased throughout the storage. In

samples with inulin, the a_w that decreased on the 14th day increased again on the 21st day, reaching its highest value. Although the control kefirs showed a decrease in a_w on the 14th day, they reached the same level as the GOS-added kefirs on the 21st day (Figure 3(c)). a_w is influenced by the type and amount of prebiotics as well as their combinations. The molecular structure of stabilizers or emulsifiers, their water absorption capacities, and their interactions with each other and other components in the beverage can affect a_w in different ways (Peredo et al., 2016). Every product has a limited shelf-life, and its quality tends to decline over time. When the water retention capacities and solubilities of prebiotics are considered together, it is expected that the highest value will be observed on the 21st day of storage due to the increasing solubility over time. The water retention capacities of prebiotics have positive effects on the structure. Inulin and GOS are water-soluble prebiotics. As their solubility increases during the storage period, their water retention capacities decrease. However, the structure of the kefir begins to deteriorate, and a_w increases. Kefir samples can remain stable for a long time without spoilage, but after a certain period, lipid oxidation and other harmful reactions increase, accelerating spoilage (Buran et al., 2021).

The fat content and quality of the milk used in kefir production are important for the formation of the final product's quality, as they influence the sensory attributes of the product such as texture, flavor, and color (Mei et al., 2017). The fat level ($P < .01$), prebiotics ($P < .01$), and storage duration ($P < .01$) had very significant effects on the fat of the produced kefir (Table 1). The high fat in kefir samples containing 3.5% fat is due to the standardization process applied to raw milk prior to kefir production. No significant difference in fat levels was observed between the two production methods (Figure 3(d)). The addition of prebiotics to the kefir composition increased the fat, and the highest fat was found in the GOS-added kefir group ($P < .05$). In a study conducted by Ender (2009) testing the addition of prebiotic (fructoolisaccharide) to kefir, it was noted that 30 days of storage did not affect the fat, and kefirs produced with fructoolisaccharide had a higher fat compared to those produced with cultures. Although the fat was high in the control and inulin-containing groups in the commercial production method, it was higher in the GOS-containing group produced by the GM method (Figure 3(e)). In a study conducted by Arda (2020) on kefirs produced from cow and sheep milk using grains and cultures, it was found that the fat ratios increased by 0.3–0.1 until the 14th day, except for the kefir sample obtained from the fermentation of cow's milk with grains, while on the 28th day it either decreased or remained the same. Khaze Hagh (2018) stated that the fat ratios of kefirs produced from cow, goat, camel, and buffalo milk decreased during storage.

The protein content of the kefir samples varied between 3.48% and 4.69% (Table 1). According to the Turkish Food Codex (TGK, 2022) criteria, the protein content in kefir must be at least 2.7%. The protein amounts determined in all the kefirs produced in our study comply with this criterion. Researchers producing kefir from buffalo milk or from a combination of buffalo milk with other milks have reported protein contents ranging from 4.00% to 4.80% (Akdan et al., 2020; Khaze Hagh, 2018; Tomar et al., 2020). Proteins play a crucial role not only as fundamental components of foods but also in enhancing sensory and functional properties (Paraskevopoulou et al., 2003) and are composed of amino acids that are involved in cell repair and growth (Hoffman and Falvo, 2004). In the current study, the fat level ($P < .01$), addition of prebiotics ($P < .01$), production method ($P < .01$), and storage ($P < .01$) had an effect on the protein content of the kefirs. Interactions such as fat level × prebiotic (Figure 4(a)), fat level × storage (Figure 4(b)), production method × prebiotic (Figure 4(c)), production method × storage (Figure 4(d)), and prebiotic × storage (Figure 4(e)) also had significant effects on these values (Table 1). According to the results, the average protein value of the 3.5% fat group was determined to be 4.287%, which was found to be higher compared to the average value of the 0.5% fat group ($P < .05$). The average protein amount (4.273 ± 0.19%) of kefir samples produced using CM was higher than that produced using TM ($P < 0.05$). Among the prebiotic-added kefirs, the lowest protein was found in samples with inulin added ($P < .05$), while no statistically significant difference was observed between the control and GOS-added samples ($P > .05$). The highest protein was determined at the beginning of storage ($P < .05$) and decreased over the storage. In the separation process applied to milk for fat level standardization, the membrane proteins on the surface of fat globules decrease along with the fat content. Additionally, the protein may decrease as a result of proteolytic activity carried out by microorganisms (Tomar et al., 2020). In the study, the highest average protein was found in samples with 3.5% fat and GOS, while the lowest value was observed in samples with 0.5% fat and inulin added (Figure 4(a)). The highest protein amounts were determined in the 3.5% fat groups, both at the beginning of storage and throughout the storage period. The protein contents of the two different fat groups of kefir gradually decreased over the storage period (Figure 4(b)). This decrease was influenced by the increase in the amount of lactic acid produced by the lactic acid bacteria present in the kefir, leading to a drop in pH and the denaturation of proteins. The lowest protein content was found in kefirs produced using the CM with inulin added (Figure 4(c)), and it was determined that the protein amounts decreased over the storage regardless of the production method. The use of cultures led to a higher average protein during storage compared to the use of grains. In the samples using grains, the average protein, which was 4.32% on the first day, dropped to an average of 3.84% on the 21st day (Figure 4(d)). The rate of this decrease was found

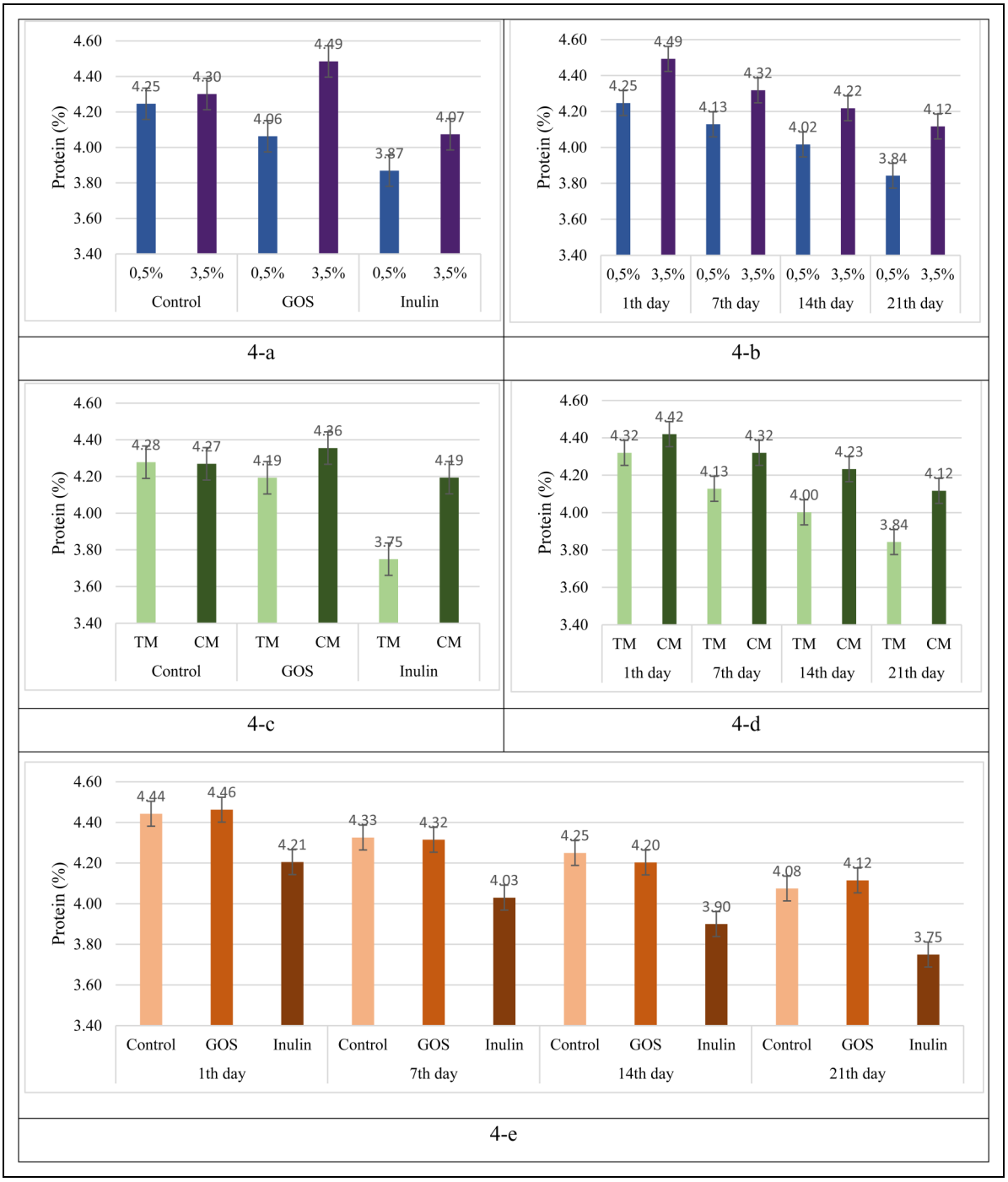

Figure 4. Effects on the protein content of kefirs: fat level × prebiotic (a), fat level × storage duration (b), production method × prebiotic (c), production method × storage duration (d), prebiotic × storage duration (e) interactions (TM: Traditional Method, CM: Commercial Method, GOS: Galacto-oligosaccharides).

to be lower in samples using cultures (Figure 4(e)). It is thought that this decrease is caused by the proteolytic microorganisms that develop in kefir during storage (Tomar et al., 2020).

Storage had a very significant effect on titratable acidity (TA) ($P < .01$), with TA increasing over the storage ($P > .05$; Table 1). It is thought that the changes in titration acidity are primarily due to the dense microbial flora in kefir. The interaction of fat level × prebiotic also had very significant effects on TA (Figure 5(a)), with the highest TA value found in the 3.5% fat group with inulin added, in contrast to the inulin group, the TA in 0.5% fat kefirs was higher in the control and GOS-added groups.

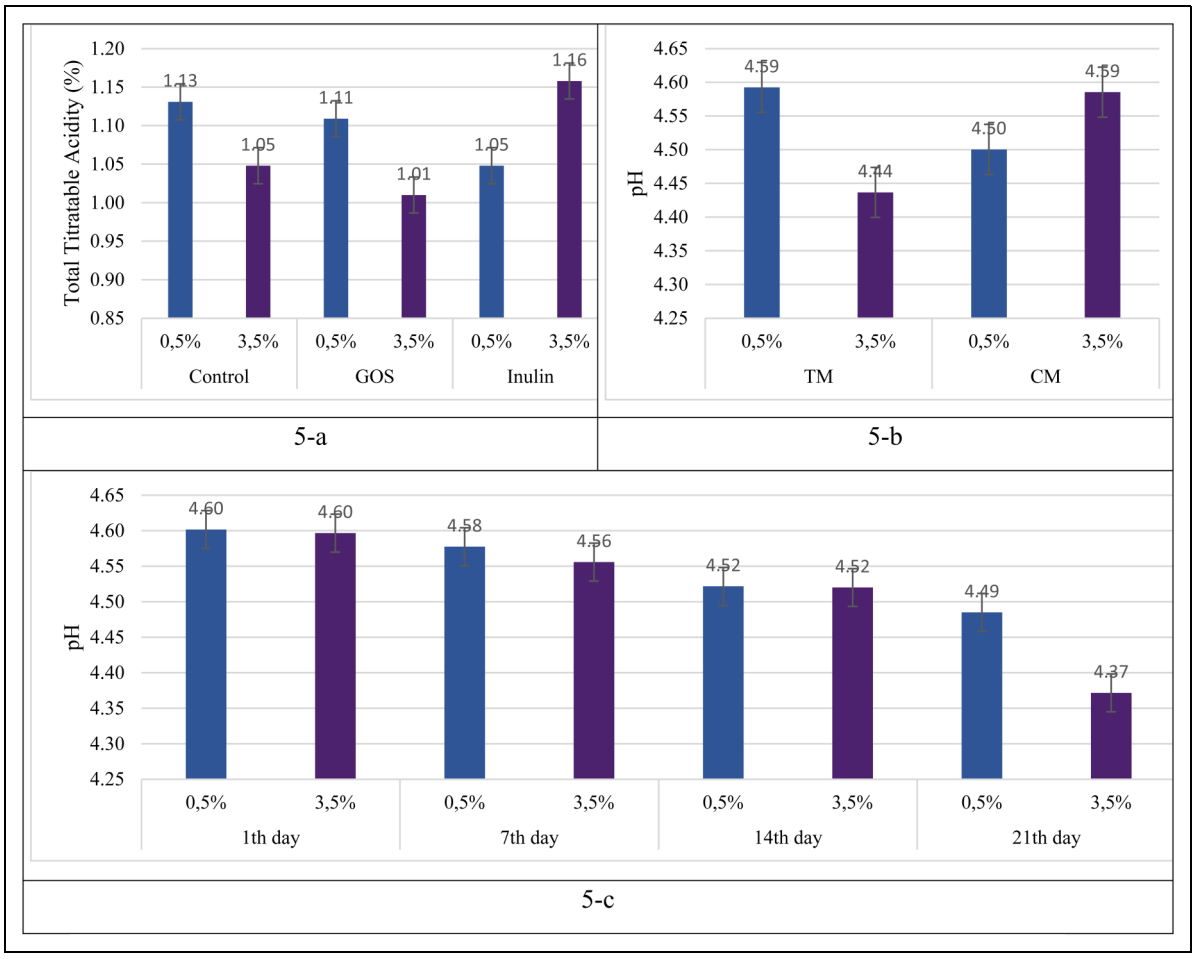

Figure 5. Effects on the total titratable acidity of kefirs: fat level × prebiotic (a); effects on pH value: fat level × production method (b), fat level × storage duration (c) interactions (TM: Traditional Method, CM: Commercial Method, GOS: Galacto-oligosaccharides).

This observation was also made by Tomar et al. (2020), who found that the acidity levels in kefirs produced from buffalo milk ranged from a minimum of 0.72% to a maximum of 0.98%, emphasizing that lower fat content and the use of grains increased acidity, and that buffalo milk kefirs had higher acidity compared to those produced from cow milk. Ender (2009) noted that the kefirs produced with grains added with FOS had the highest acidity level at 0.9%, stating that at the end of the storage period, acidity reached its highest level across all samples. In a study by Akdan et al. (2020) where kefir was produced using buffalo and other milks, acidity was found to range from 0.91% to 1.04%, with the highest result occurring in kefir produced from a mixture of buffalo and sheep milk. Buran et al. (2021) produced kefir with probiotic and prebiotic additions from cow and goat milk, stating that the acidity of the probiotic-added samples reached its highest level due to microbial activity.

The pH value of the kefirs produced in the study was affected by fat level ($P < .01$), addition of prebiotics ($P < .01$), storage ($P < .01$), and production method ($P < .05$; Table 1). The pH value of kefir samples with 0.5% fat level was higher than that of samples with 3.5% fat. Therefore, increasing the fat content of the buffalo milk used in kefir production reduced the pH value of the final product. It is believed that the decrease in pH is due to the free fatty acids produced as a result of the lipolytic activity of the microorganisms, particularly yeasts, present in the kefir. The use of cultures in kefir production increased the pH value compared to grain usage ($P < .05$). In a study by Tomar et al. (2020) analyzing cow and buffalo milk kefirs, the pH values were determined to range from 4.21 to 4.70. They concluded that kefirs fermented with grains had lower pH levels compared to those produced with starter cultures, particularly in low-fat samples. No statistically significant difference was found between the control and inulin-added kefirs ($P > .05$); however, it was observed that the addition of GOS significantly increased the pH value ($P < .05$). Buran et al. (2021) recorded pH values between 4.18 and 4.47 in their kefirs made from cow and

goat milk. Kef and Arslan (2021) determined the pH range of kefirs to be 4.46–4.28 when they added inulin and quinoa flour, and they found no significant difference between samples using inulin and FOS ($P > .05$). In the current study, the highest average pH of 4.6 was determined on the first day of storage, and it was found to decrease after the 7th day of storage. The increase in acidity contributed to this change. Yildiz-Akgul et al. (2018) reported that the pH values of kefirs stored for 23 days ranged from 4.66 to 4.14 and decreased regularly. The researchers indicated that the decrease in pH was the result of lactose being broken down into lactic acid by lactic acid bacteria, leading to an increase in the acidity level in the environment. The interaction of fat level × production method had a very significant effect on the pH value ($P < .01$), and the average pH of kefirs produced with grains in the 0.5% fat group was 4.59, while the closest result to this average pH value in the 3.5% fat group was observed in samples produced with cultures. This means that the pH value of kefirs produced by the CM in the higher fat group decreased more than in the other groups (Figure 5(b)). This may be due to the differences in both the number and type of microorganisms capable of fermenting lactose between the grain and commercial culture samples (Tomar et al., 2020). As shown in the fat level × storage interaction in Figure 5(c), the pH value decreased during the storage in both fat level kefirs, with the most significant decrease occurring in the 3.5% fat group. This reduction is consistent with the changes in titration acidity values within the same group, and the use of inulin played a role in this change (Figure 5(a)). Inulin affects microorganisms, resulting in greater carbohydrate breakdown and increased acidity formation during fermentation. Because inulin is more easily or extensively broken down by microorganisms, fermentation is enhanced in products containing it, leading to increased acidity formation (Chawla and Patil, 2010; Kef and Arslan, 2021).

Microbiological properties

The fat level and production method had very significant effects on the total aerobic mesophilic bacteria (TAMB) count ($P < .01$), with higher counts observed in kefirs with 0.5% fat and those produced using commercial starter cultures. The TAMB counts identified in these studies are generally at levels of 8–9 log cfu/mL (Table 2). In various studies (Yildiz-Akgul et al., 2018; Khaze Hagh, 2018) have indicated significant differences in TAMB counts depending on the production method, storage, and the source and characteristics of the milk used in kefir production.

The production method ($P < .01$) and storage ($P < .05$) had effects on the lactic cocci count of the kefir (Table 2). The use of CM in kefir production resulted in a count of 8.959 log cfu/mL, which is higher than that obtained using grains ($P < .05$). The lactic cocci increased until the 7th day of storage and then decreased by 0.1 log. The lactic cocci were influenced by the interaction of fat level × production method ($P < .05$), with the highest values determined in samples with commercial starter cultures. Among the starter culture samples, the count in kefirs with 3.5% fat (9.01 log cfu/mL) was higher than that in kefirs with 0.5% fat (8.91 log cfu/mL). In contrast to kefirs with added cultures, the development of lactic cocci decreased as the fat content increased in those produced with grains, with the lowest count of 8.35 log cfu/mL found in kefirs with 3.5% fat (Figure 6(a)). The interaction of production method × storage also had effects on the lactic cocci count ($P < .01$; Figure 6(b)). On the 7th day of storage, lactic cocci development in the group with commercial starter cultures was greater than in the grain group. However, in the later days of storage, a decrease was observed in both groups, with a more significant decrease in the grain group (Figure 6(b)). These results indicate that due to the effectiveness of commercial cultures in kefir production, kefirs produced using commercial methods contain a higher number of lactic cocci. Ender (2009) reported that there was an increase in lactococcal counts during the first 10 days of the storage process, and this increase was much greater in kefirs that were fermented with grains and had FOS added. Aktaş et al. (2024) found that the counts of lactic cocci in concentrated kefir made from buffalo milk were 8.46 ± 0.20 log cfu/g at the 1st day of storage, stayed the same for the first 14 days, and then decreased between the 14th and 28th days. The development of lactic bacilli in kefir samples was found to be significantly affected by production method, addition of prebiotics, and storage duration ($P < .01$), while the effect of fat level was not significant ($P > .05$; Table 2). The use of cultures had a positive effect on the development of lactic bacilli compared to grains ($P < .05$), with an average count of 7.391 log cfu/mL in CM kefirs, while an average count of 7.101 log cfu/mL was observed in TM kefirs. The count of lactic bacilli was lower in kefirs with inulin added compared to control and GOS-added kefirs ($P < .05$). The count of lactic bacilli increased until the 7th day of storage but decreased after that, with the most significant decrease occurring between the 7th and 14th days (Table 2). The use of CM in kefir production increased the count of lactic bacilli. The combined use of starter cultures and GOS in production led to a greater increase in this count (Figure 6(c)). It is thought that the different effects of GOS and inulin on the development of lactic bacilli are due to the higher polymerization degree of inulin compared to GOS. Short-chain GOS dissolves in water more quickly than long-chain inulin, and considering that the development of probiotic bacteria is selectively stimulated, this situation leads to differences in the mechanisms of action (Buran et al., 2021). The lactic bacilli in kefir are important for the functional properties of kefir. Lactic acid bacteria

Table 2. Effect of fat levels, production methods, prebiotic and storage period on the total number of aerobic mesophilic bacteria, lactic cocci, lactic bacilli, and yeast counts of synbiotic kefir samples during chilled storage.

	TAMB (log kob/mL)	Lactic cocci (log kob/mL)	Lactic bacilli (log kob/mL)	Yeast (log kob/mL)
Fat levels (FL)				
0.5%	8.668 ± 0.24^{a}	8.689 ± 0.35^{a}	7.336 ± 0.60^{a}	3.764 ± 1.19^{b}
3.5%	8.544 ± 0.23^{b}	8.679 ± 0.48^{a}	7.156 ± 0.66^{a}	4.109 ± 1.02^{a}
P value	**	NS	NS	**
Production method (PM)				
Traditional	8.496 ± 0.17^{b}	8.408 ± 0.27^{b}	7.101 ± 0.64^{b}	4.773 ± 0.72^{a}
Commercial	8.716 ± 0.25^{a}	8.959 ± 0.36^{a}	7.391 ± 0.59^{a}	3.100 ± 0.75^{b}
P value	**	**	**	**
Prebiotic (P)				
Control (C)	8.636 ± 0.25^{a}	8.658 ± 0.47^{a}	7.284 ± 0.60^{a}	4.090 ± 1.04^{a}
GOS (G)	8.609 ± 0.23^{a}	8.737 ± 0.41^{a}	7.416 ± 0.61^{a}	4.145 ± 1.16^{a}
Inulin (I)	8.573 ± 0.24^{a}	8.657 ± 0.38^{a}	7.038 ± 0.64^{b}	3.574 ± 1.08^{b}
P value	NS	NS	**	**
Storage period (SP, days)				
1	8.631 ± 0.16^{a}	8.490 ± 0.23^{c}	7.494 ± 0.57^{a}	4.465 ± 1.21^{a}
7	8.657 ± 0.23^{a}	8.864 ± 0.38^{a}	7.629 ± 0.53^{a}	4.046 ± 1.06^{b}
14	8.616 ± 0.26^{a}	8.762 ± 0.44^{ab}	7.066 ± 0.49^{b}	3.771 ± 0.99^{c}
21	8.521 ± 0.27^{a}	8.620 ± 0.51^{bc}	6.795 ± 0.57^{b}	3.464 ± 0.99^{d}
P value	NS	**	**	**
Interactions				
FL × PM	NS	*	NS	**
FL × P	NS	NS	NS	**
FL × SP	NS	NS	NS	NS
PM × P	NS	NS	*	*
PM × SP	NS	**	NS	NS
P × SP	NS	NS	NS	NS
FL × PM × P	NS	NS	**	**
FL × PM × SP	NS	NS	NS	NS
FL × P × SP	NS	NS	NS	NS
PM × P × SP	NS	NS	NS	NS

*$P < .05$; **$P < .01$; NS: $P > .05$.

[a–b:] Means in the same column and in the same section with the same letters are not significantly different at $P > .05$ (Duncan's test) between the means of the fat levels and production methods.

[a–c:] Means in the same column and in the same section with the same letters are not significantly different at $P > .05$ (Duncan's test) between the means of the prebiotic.

[a–f:] Means in the same column and in the same section with the same letters are not significantly different at $P > .05$ (Duncan's test) between the means of the storage periods.

have antagonistic effects, the ability to produce antimicrobial metabolites, and the capacity to produce exopolysaccharides. Exopolysaccharides contribute to texture and organoleptic properties, while also possessing functional properties such as prebiotic, antioxidant, antitumor, and antidiabetic effects (Özoğlu et al., 2022; Şentürk et al., 2022). In the current study, the highest count of lactic bacilli among the treatment groups was found in kefirs that were fermented with cultures and had GOS added, along with a fat content of 0.5%. In a study conducted by Ender (2009), it was observed that the storage caused changes in the count of lactobacilli in kefirs, while the same effect was not seen with the use of FOS, grains, or cultures ($P > .05$). Khaze Hagh (2018), Yildiz-Akgul et al. (2018) and Aktaş et al. (2024) determined the lactic bacilli count in kefirs decreased during storage. Kef and Arslan (2021) indicated that the addition of inulin promotes bacterial development, stating that a kefir sample containing 2% inulin had the highest lactic bacilli count (9.40 log cfu/mL).

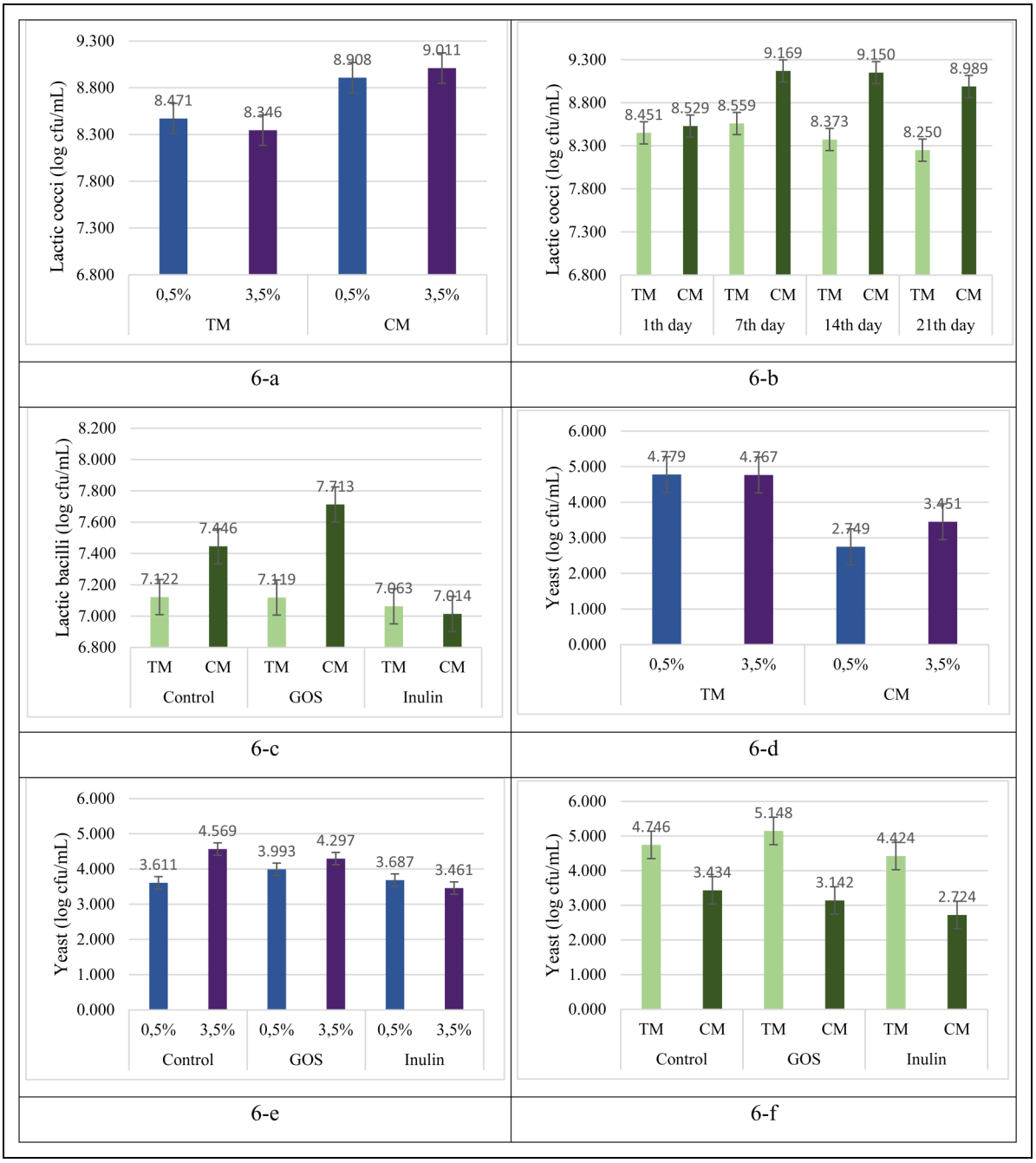

Figure 6. Effects on the quantity of lactic cocci in kefirs: fat level × production method (a), production method × storage duration (b); effects on the quantity of lactic bacilli: production method × prebiotic (c); effects on yeast count: fat level × production method (d), fat level × prebiotic (e), production method × prebiotic (f) interactions (TM: Traditional Method, CM: Commercial Method, GOS: Galacto-oligosaccharides).

The yeast count was influenced by fat level ($P < .01$), production method ($P < .01$), addition of prebiotics ($P < .01$), and storage ($P < .01$), as well as the interactions of fat level × production method ($P < .01$), fat level × prebiotic ($P < .01$), and production method × prebiotic ($P < .05$) (Table 2). The increase in the fat level of the milk used in kefir production increased the yeast count in kefir ($P < .05$). The predominant growth of lactic acid bacteria due to the use of commercial starter cultures, along with changes in pH and acidity in these products, inhibited yeast development ($P < .05$), resulting in a higher yeast count in kefirs produced by CM (Table 2). The addition of inulin decreased the yeast count ($P < .05$), leading to an approximate difference of 0.5–0.6 logarithmic units compared to the control and GOS groups. As the storage duration increased,

Table 3. Effect of fat levels, production methods, prebiotic and storage period on the sensory properties of synbiotic kefir samples during chilled storage.

	Appearance	Smell	Taste	Consistency	General acceptability
Fat levels (FL)					
0.5%	3.802 ± 1.03^{b}	3.721 ± 0.88^{b}	3.406 ± 1.11^{b}	3.560 ± 1.09^{b}	3.573 ± 0.96^{b}
3.5%	4.077 ± 0.90^{a}	3.842 ± 0.91^{a}	3.640 ± 1.11^{a}	3.819 ± 0.97^{a}	3.769 ± 0.95^{a}
P value	**	*	**	**	**
Production method (PM)					
Traditional	4.092 ± 0.90^{a}	3.869 ± 0.88^{a}	3.537 ± 1.17^{a}	3.890 ± 0.94^{a}	3.800 ± 0.97^{a}
Commercial	3.788 ± 1.03^{b}	3.694 ± 0.90^{b}	3.508 ± 1.06^{a}	3.490 ± 1.09^{b}	3.542 ± 0.92^{b}
P value	**	**	NS	**	**
Prebiotic (P)					
Control (C)	3.925 ± 0.97^{ab}	3.716 ± 0.88^{b}	3.350 ± 1.09^{b}	3.616 ± 1.04^{b}	3.572 ± 0.92^{b}
GOS (G)	4.038 ± 0.90^{a}	3.881 ± 0.88^{a}	3.722 ± 1.11^{a}	3.847 ± 0.99^{a}	3.816 ± 0.92^{a}
Inulin (I)	3.856 ± 1.06^{b}	3.747 ± 0.92^{ab}	3.497 ± 1.12^{b}	3.606 ± 1.07^{b}	3.625 ± 1.01^{b}
P value	*	*	**	**	**
Storage period (SP, days)					
1	3.883 ± 0.97^{bc}	3.750 ± 0.92^{b}	3.508 ± 1.10^{b}	3.654 ± 1.04^{b}	3.658 ± 0.95^{a}
7	3.925 ± 0.97^{b}	3.704 ± 0.85^{b}	3.425 ± 1.08^{b}	3.638 ± 1.10^{b}	3.683 ± 0.95^{a}
14	3.738 ± 1.10^{c}	3.704 ± 0.93^{b}	3.442 ± 1.21^{b}	3.617 ± 1.13^{b}	3.588 ± 0.99^{a}
21	4.213 ± 0.81^{a}	3.967 ± 0.85^{a}	3.717 ± 1.05^{a}	3.850 ± 0.86^{a}	3.754 ± 0.95^{a}
P value	**	**	*	*	NS
Interactions					
FL × PM	*	NS	NS	NS	NS
FL × P	*	NS	NS	NS	NS
FL × SP	NS	NS	NS	NS	NS
PM × P	**	NS	NS	*	*
PM × SP	NS	NS	NS	NS	NS
P × SP	NS	NS	NS	NS	NS
FL × PM × P	*	NS	**	**	**
FL × PM × SP	NS	NS	NS	NS	NS
FL × P × SP	NS	NS	NS	NS	NS
PM × P × SP	NS	NS	NS	NS	NS

*$P < .05$; **$P < .01$; NS: $P > .05$.

[a–b:] Means in the same column and in the same section with the same letters are not significantly different at $P > .05$ (Duncan's test) between the means of the fat levels and production methods.

[a–c:] Means in the same column and in the same section with the same letters are not significantly different at $P > .05$ (Duncan's test) between the means of the prebiotic.

[a–f:] Means in the same column and in the same section with the same letters are not significantly different at $P > .05$ (Duncan's test) between the means of the storage periods.

the yeast count decreased ($P < .05$). This change was influenced by the high yeast development in the control and GOS-added kefirs with 3.5% fat (Figure 6(e)). The highest average yeast counts were determined to be 3.99 log cfu/mL in the GOS-added 0.5% fat group and 4.57 log cfu/mL in the control 3.5% fat group (Figure 6-(e)). The use of grains, compared to cultures, resulted in high levels of yeast development in both fat levels, with higher counts detected in the 3.5% fat group of cultured kefirs (3.45 log cfu/mL) than in the 0.5% fat group (2.75 log cfu/mL) ($P < .05$) (Figure 6(d)). As seen in Figure 6(f), which presents the interaction of production method × prebiotic addition, the use of grains in kefir production promoted yeast development, with the lowest yeast counts observed in the inulin-added groups (Figure 6(f)). The highest average yeast count in the current study was found in kefirs that were 3.5% fat, GOS-added, and fermented with grains. Yeast count of kefir is affected by various factors (amount of fat, production method, storage, etc.) (Tomar et al., 2020; Buran et al. (2021). *Enterobacteriaceae* were not detected in kefir samples and the this bacteria

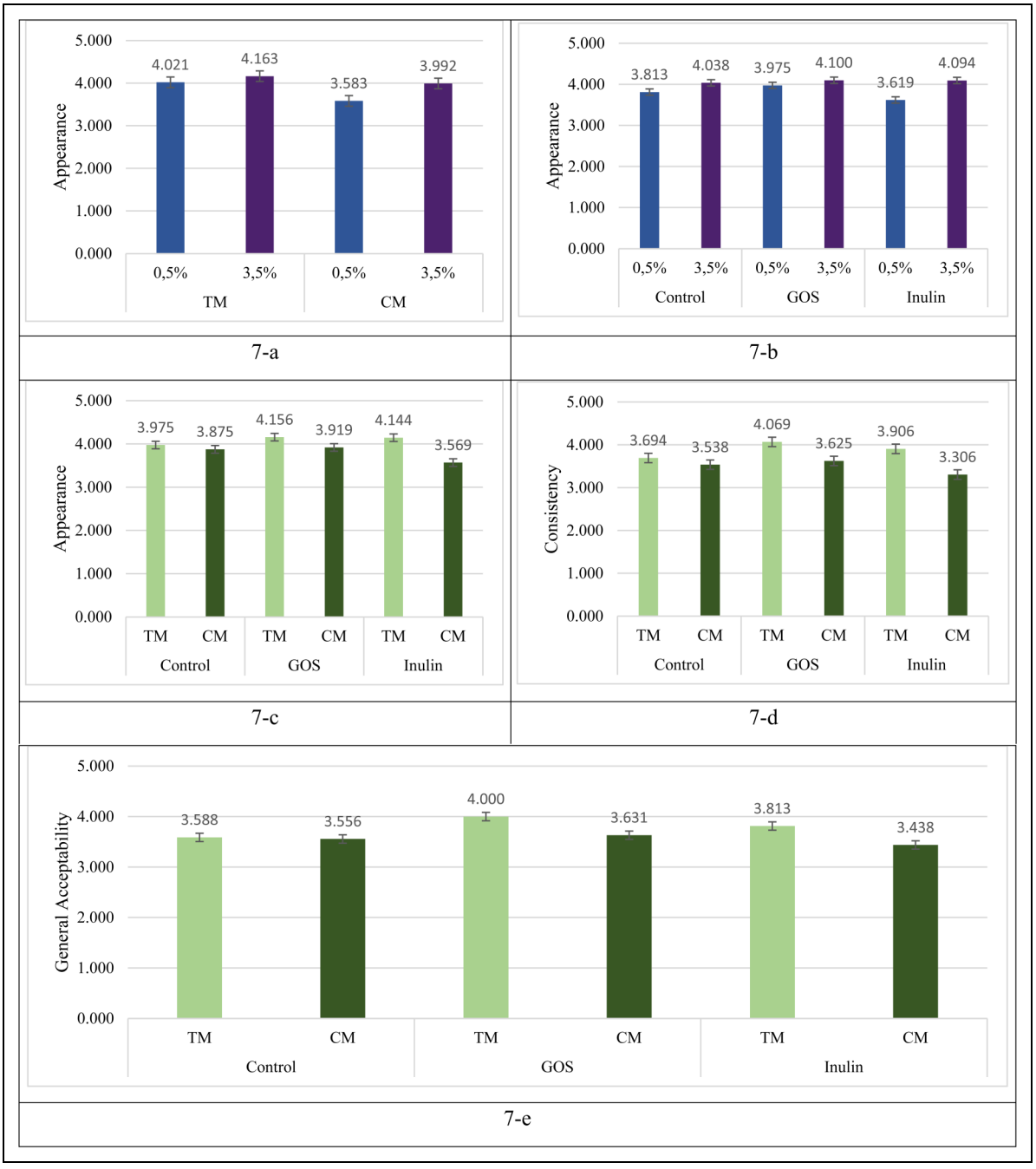

Figure 7. Effects on the appearance scores of kefirs: fat level × production method (a), fat level × prebiotic (b), production method × prebiotic (c); effects on consistency scores: production method × prebiotic (d); effects on overall acceptability scores: production method × prebiotic (e) interactions (TM: Traditional Method, CM: Commercial Method, GOS: Galacto-oligosaccharides).

count remained below the detectable limit (<1 log cfu/mL) in all samples during storage.

Sensory analysis results

The average scores for appearance, aroma, taste, consistency, and overall acceptability of the kefir samples determined in the sensory analyses are presented in Table 3. The sensory parameters identified in the study were significantly affected by fat level ($P < .01$), production method (excluding taste) ($P < .01$), addition of prebiotics ($P < .01$), and storage (excluding overall acceptability) ($P < .01$) (Table 3). Increasing the fat level of the milk used in kefir production, producing using the traditional method, and the addition of prebiotics (especially

GOS) improved the sensory properties of the kefir. The interaction effect of fat level × production method on the appearance of the kefir samples was observed (Figure 7(a)), with the highest appearance score obtained for kefirs with 3.5% fat and the lowest score for kefirs fermented with cultures at 0.5% fat. The interactions of fat level × prebiotic ($P < .01$; Figure 7(b)) and production method × prebiotic addition ($P < .01$; Figure 7(c)) were also effective, with GOS-containing kefirs receiving the highest average scores in both fat groups. The least liked group in terms of appearance criteria was the 0.5% fat and inulin group (average: 3.62). The average appearance scores of kefirs produced using commercial method were lower than those produced using TM, and within this group, the inulin plus commercial culture group was the least liked (average: 3.57). The use of grains as cultures and GOS as a prebiotic improved the consistency of the kefir (Figure 7(d)). In terms of overall acceptability, kefirs produced using both TM and CM containing GOS were rated more favorably (Figure 7(e)).

In this study, it was generally found that in sensory analyses, kefirs with 3.5% fat received higher scores compared to those with 0.5% fat, kefirs produced with grains were rated higher than those produced with cultures, and kefirs with GOS added received higher scores compared to those without prebiotics and those with inulin added, particularly on the 21st day of storage. The flavor-enhancing properties of fat in food are thought to have contributed to the preference for the 3.5% fat kefirs. Aroma, due to fermentation and the source of the milk, is likely influenced by the support of microorganisms through the addition of prebiotics, the use of grains with rich microflora, and the more pronounced aroma in kefirs with 3.5% fat due to the buffalo milk fat. Moreover, the partial increase in pH on the 21st day of storage and the better development of the characteristic sour taste of kefir are thought to contribute to its higher preference. Gul et al. (2018), in their study using cow and buffalo milk, indicated that the buffalo milk kefirs obtained received higher scores in terms of appearance, consistency, taste, and overall acceptability compared to kefirs produced from cow milk. In research conducted by Tomar et al. (2020), kefirs produced using 3% fat buffalo milk were the most liked group, while the least liked group was the 0.5% fat buffalo milk kefir. In a study where kefirs were produced using cow and buffalo milk with grains and starter cultures, the use of buffalo milk increased the viscosity of the kefir but decreased its sensory properties (Elgarhy et al., 2018). In another study producing kefir from mixtures of buffalo milk and other milks, it was found that the sensory properties deteriorated towards the end of the shelf life, and buffalo milk kefirs were not well received by those who dislike the intense fatty taste, while the kefir made from a mixture of buffalo, sheep, goat, and cow milk was the most preferred (Kırmaz et al., 2023). In another study (Aktaş et al. 2024), among the concentrated kefirs produced with cow, sheep, goat, and buffalo milk, the most appreciated kefir in sensory properties (color and appearance, general acceptability, mouthfeel, flavor, and syneresis) after production (1st day) and the end of storage (28th) was the kefir produced from buffalo milk.

Conclusions

In conclusion, it has been determined that buffalo milk can be successfully used as an alternative to cow milk in synbiotic kefir production and that the use of GOS yields more efficient results in terms of microbiological, physicochemical, and sensory evaluations compared to the use of inulin. Hence, its production should be encouraged as an easily accessible prebiotic. It was also found that fatty products generally appeal more to people's taste preferences compared to low-fat products and that the use of grains provides organoleptic advantages while the use of cultures offers microbiological benefits.

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Data availability statement

Research data are not shared.

Declaration of conflicting interests

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