

Enriching kefir produced from cow and buffalo milks with different doses of partially hydrolyzed guar gum

İnek ve manda sütleriyle üretilen kefirin farklı dozlarda kısmen hidrolize guar zamkı ile zenginleştirilmesi

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ABSTRACT

Kefir is an acidic dairy product that is obtained through the fermentation of different types of milk. It has several health-promoting properties due to its probiotic content. Kefir fortification with dietary fibers is a potential method to further increase the quality of kefir. While several studies have been conducted on this topic, choosing the appropriate dietary fiber that provides both conditions of increasing the number of probiotics along with improving the physical and chemical properties of kefir is challenging. Partially hydrolyzed guar gum (PHGG) is a non-viscous water-soluble dietary fiber with high fermentability by microorganisms. The aim is to produce cow and buffalo kefir enriched with PHGG and to investigate the effects of the PHGG on proximate, microbial, and sensory analyses. Kefir was produced using commercial kefir grains and 1% and 2% PHGG at 25 °C for 36 hours. Then, the products were kept at 4 °C for 21 days. As a result, there was no effect of PHGG on pH value. At the end of fermentation, the viscosity of kefir samples containing 1% and 2% PHGG decreased, except for buffalo kefir containing 2% PHGG. Fortifying cow kefir with 2% of PHGG gained the highest acceptability score.

Key Words: Buffalo kefir, cow kefir, partially hydrolyzed guar gum, fortification, sensory analysis

Öz

Kefir, farklı süt türlerinin fermantasyonu ile elde edilen asidik bir süt ürünüdür. Probiyotik içeriği nedeniyle sağlığı geliştirici özelliklere sahiptir. Kefirin diyet lifleriyle zenginleştirilmesi, kefirin kalitesini daha da artırmak için yapılabilecek potansiyel bir yöntemdir. Bu konu üzerinde birçok çalışma yürütülmüş olsa da, hem probiyotik sayısını artırma hem de kefirin fiziksel ve kimyasal özelliklerini iyileştirme koşullarını sağlayan uygun diyet lifini seçmek zordur. Kısmen hidrolize guar zamkı (PHGG), mikroorganizmalar tarafından yüksek fermente edilebilirliğe sahip, viskoz olmayan, suda çözünür bir diyet lifidir. Bu çalışmanın amacı, PHGG ile zenginleştirilmiş inek ve manda kefirini üretmek ve PHGG'nin fiziksel, kimyasal, mikrobiyal ve duyu analizler üzerindeki etkilerini araştırmaktır. Kefir, ticari kefir taneleri ve %1 ve %2 PHGG kullanılarak 25 °C'de 36 saat boyunca üretildi. Daha sonra üretilen kefirler 4 °C'de 21 gün tutuldu. Sonuç olarak, PHGG'nin pH değeri üzerinde bir etkisi olmadığı görülmüştür. Fermantasyonun sonunda, %1 ve %2 PHGG içeren kefir örneklerinin viskozitesi, %2 PHGG içeren manda kefirini hariç, azalmıştır. %2 PHGG ile zenginleştirilmiş inek kefirinin duyu analizler sonucunda en yüksek kabul edilebilirlik puanını kazandığı görülmüştür.

Anahtar Kelimeler: Manda kefir, inek kefir, Kısmen hidrolize guar zamkı, Zenginleştirme, Duyusal analiz

Introduction

Western eating habits, which is characterized by inadequate intake of essential nutrients such as vitamins, minerals, and proteins, have been widely adopted around the world due to it is hyper palatability (Adolph & Tilg, 2024; Fazzino et al., 2021). In addition, people have a tendency to consume ready-to-eat food products aiming to reduce time required for food preparation as a way to adapt with the required modern lifestyle. Subsequently, the prevalence of chronic diseases such as obesity, diabetes, and cardiovascular disease has significantly increased (Jardim et al., 2021; Lane et al., 2024). However, the awareness of people regarding the impact of eating patterns on our health has contributed to an increase the demand of alternative healthy choices (e.g. fermented food products) in the markets (Embling et al., 2024; Firoozzare et al., 2024).

Fermentation is a natural process that is applied to food to improve its properties. Kefir, which is widely consumed beverage around the world, is the result of fermented milk by certain beneficial microorganisms. In this context, lactic acid and acetic acid producing bacteria along with yeasts are the major contributors in milk fermentation to kefir (Azizi et al., 2021; Hikmetoglu et al., 2020). Clinical trials have demonstrated diverse health benefits of kefir consumption including decrease in serum inflammatory markers, blood glucose, and blood lipids (Bellikci-Koyu et al., 2022; Ghizi et al., 2021; Ostadrahimi et al., 2015).

While the exact mechanism remains unclear, probiotics, which defined as living microorganisms when consumed adequately provide additional health benefits to host, content is suggested to play a vital role in these outcomes (Culpepper, 2022; Peluzio et al., 2021). Despite this, kefir is not highly preferred by people, especially young adults due to its acidic taste (Kabakcı et al., 2020).

Moreover, the growing trend in kefir

consumption has led to a rise in research on fortifying kefir with different bioactive substances to improve its sensory properties, boost its health benefits, and improve its quality (Yiğit Ziolkowski et al., 2024). One of the well-studied options to meet these requirements is the addition of prebiotic, probiotic food substances, to kefir such as water-soluble dietary fibers (WSDF) (Farag et al., 2020; Kef & Arslan, 2021; Özbey et al., 2018).

Guar gum is widely used in food industries as stabilizer and thickener in various food products such as sauces, ketchup, and ice creams. Although gums are classified as WSDF, guar gum has high molecular weight, high viscosity in aqueous solution and is poorly utilized by probiotics making it difficult to be added to liquid fermented food products such as kefir and ayran (Mudgil et al., 2017b; Phillips et al., 2008). Therefore, researchers have produced partially hydrolyzed guar gum (PHGG) by the application of various techniques on guar gum to reduce its molecular weight and viscosity. PHGG is a colorless, odorless, and non-viscous WSDF that has shown promising results in different food products such as yogurt, cookies, and noodles (Mudgil, Barak, & Khatkar, 2018; Mudgil et al., 2016, 2017a, 2017b).

The sensory acceptance and health-promoting properties of kefir make it a valuable addition to the daily diet. In this study, it was aimed to investigate the effects of adding certain amounts of PHGG to kefir produced by traditional methods using pasteurized cow and buffalo milk on the probiotic microorganism content, quality, and sensory properties of kefir.

Materials and Methods

Kefir production

Pasteurized cow and buffalo milk were used in this experiment and were obtained from (Sütiş Çiftliği and Saray Muhallebicişi, Türkiye). PHGG was obtained from (TAIYO GmbH, Germany). Kefir grains were obtained from (Doğadan Bizim, Türkiye). Two grams of kefir grains were added to

each bottle (1 liter) of milk at room temperature (25 °C). Then, each type of milk was separated into 3 groups, control, 1% and 2%. Nothing was added to control group in terms of dietary fiber, while 1% and 2% of PHGG was added to the experimental groups. PHGG was added to milks in a sterile glass jar covered with a lid. Fermentation process was achieved by keeping the milks at room temperature for 36 hours as recommended by the company. Fermented kefir samples were stored at 4 °C for 21 days. Sensory, and chemical analyses (except for viscosity analysis) were performed after the end of the fermentation on the 1st, 7th, 14th, and 21st days of storage, whereas viscosity analysis was conducted on 7th, 14th, and 21st days. Microbial analysis was performed in all days except the 1st day.

pH Analysis

To determine the pH value of the sample, 1 mL of kefir sample was added into a beaker and diluted with 9 mL of distilled water. The pH values of the samples were determined using a Hanna pH 211 (Hanna Instruments, USA) bench type pH meter. Before each analysis, the pH meter was calibrated as pH 4 and 7 at 20 °C using standard solutions, and then the pH values of the homogenized samples were read directly at 20 °C. Two parallel analyses were carried out (Güler-Akın & Akın, 2007).

Titratable acidity

To determine titratable acidity, 10 mL of kefir sample was added into a beaker and diluted with 100 mL of distilled water. Then, 3 drops of phenolphthalein solution were added and titrated with 0.1 M NaOH. Two parallel analyses were carried out, and the titratable acidity of kefir samples was calculated as % of lactic acid (Abdo et al., 2022; AOAC, 2000).

$$\% \text{ Acidity} = V \times N \times 0.09 \times 100 / W$$

V = mL of NaOH consumed during the titration

N = normality of NaOH

W = weight of the sample.

Dry matter analysis

After weighing 10 g of kefir samples in tared containers, the samples were dried at 105 °C until their weight remained constant. After the samples were cooled in a desiccator at room temperature (20 °C), they were weighed and the dry matter was calculated. Two parallel analyses were carried out (AOAC, 2000). Dry matter was calculated using the following equation:

$$\% \text{ dry matter} = (M1 - (M2 - P)) / M1 \times 100$$

M1 = Sample weight (g)

M2 = Crucible after incineration + ash weight (g),

P = Weight of the crucible brought to constant weighing (g).

Ash analysis

A 10 g of kefir sample was placed in porcelain crucibles that were brought to a constant weight and tared. After pre-burning, the samples were burned in a muffle furnace at 550°C until white ash was formed. Then, the samples were taken into a desiccator and allowed to cool, and after cooling, they were weighed, and the % ash amount was calculated according to the formula below (AOAC, 2000).

$$\% \text{ Ash} = (M1 - (M2 - P)) / M1 \times 100$$

M1 = Sample weight (g)

M2 = Crucible after incineration + ash weight (g)

P = Weight of the crucible brought to constant weighing (g).

Viscosity measurement

Viscosity was determined by a viscometer with LV-4 (64) spindle (Brookfield DV-II viscometer, USA). The apparent viscosity measurements were conducted at 40 rpm and 10 s for the kefir samples at room temperature (Kef & Arslan, 2021).

Microbiological analysis

A dilution series was prepared from the samples. To prepare the first dilution, 25 mL of kefir sample was mixed with 225 mL of sterile physiological salt solution (PSS) containing 0.85%

(w/v) sodium chloride. To continue the dilution series, 1 mL from the first dilution was taken and added into the 9 mL of PSS. The other dilutions followed this procedure. From the dilution series, 0.1 mL was taken and put in Petri dishes containing Man Rogosa Sharpe (MRS) agar (Merck) to count the rod-shaped bacteria (*Lactobacillus*), Sabouraud Dextrose Agar (SDA) (Merck) to count the yeasts and M17 agar to count round-shaped bacteria (*Lactococcus*). The bacteria and yeast counting procedures were carried out at the 48th hour after the incubation at 37 °C and after 5 days of incubation at 25 °C, respectively (Halkman, 2005).

$$N = C / (V \times d \times (n1 + (0.1 \times n2)))$$

N = Number of microorganisms in 1 g or 1 mL of food sample

C = Total number of colonies in all petri dishes counted

V = Volume (mL) transferred to the petri dishes being counted

n1 = Number of petri dishes counted in the counts made from the first dilution

n2 = Number of petri dishes counted in the counts made from the second dilution

d = dilution ratio of the more concentrated of the 2 consecutive dilutions in which the count is made.

Sensory analysis

Sensory analysis of kefir samples was carried out to evaluate their acceptability by the consumer. Hedonic test was used in the analysis. In the sensory analysis laboratory, 50 mL of sample was given to 4 people who had not received sensory analysis training but were explained how to perform the analysis. They were provided with water, as well as a napkin and a spitting cup. Panelists inspected and analyzed the kefir samples, asking them to score the samples using full-score levels for flavor, odor, color, and texture quality factors (0-1: it is not taken as a human food, 2: unpleasant, and 3: mildly 4: decent, 5: excellent).

Statistical analysis

All the statistical analysis were performed in the SPSS software (Version 29.0 IBM SPSS Software, USA) including Duncan^a and one-way ANOVA tests on the analytical analysis data at a significance level of 5%.

Results and Discussion

There was no significant difference in moisture content between the samples during the study. Also, moisture content of cow kefir samples didn't reveal any changes during the study. However, buffalo kefir control, 1%, and 2% significantly differed between the weeks ($p=0.005$; $\eta^2=0.04$), ($p=0.004$; $\eta^2=0.06$), and ($p=0.005$; $\eta^2=0.94$) respectively (Table 1). At the beginning of the study, no difference in ash content was found. There was significant difference between the sample in day 7, 14, and 21 ($p=0.000$; $\eta^2=0.96$; $\eta^2=0.99$; $\eta^2=0.99$, respectively). In addition, the ash % was significantly increased during the study in all the samples except cow milk (2%). Titratable acidity of cow kefir (control), and buffalo kefir (1% and 2%) was not changed during the weeks ($p=0.902$, $p=0.104$, and $p=0.252$ respectively). The organic compounds of the kefir samples, which reflect macronutrients regarding the extraction of ash and moisture, remained stable. However, there was an increasing trend in titratable acidity from day 1 to 21 in cow kefir (1% and 2%) ($p=0.004$; $\eta^2=0.95$). Similarly, buffalo kefir (control) showed significant changes in titratable acidity ($p=0.009$; $\eta^2=0.92$). This change reflects the increase of lactic acid in kefir (Ntsame Affane et al., 2011). In addition, there was no significant difference in titratable acidity on day 1 and 21 between the samples. However, a significant difference was found on day 7 ($p=0.007$) and 14 ($p=0.000$).

Furthermore, pH values ranged from 4.85 to 5.30. Cow and buffalo kefir samples (1% and 2% PHGG) reveal significant reductions in pH values at day 21 compared to day 1 (Table 1). The initial decline in pH values is mainly due to the fermentation process occurring on milk. The pH

value was further declined due to the utilization of milk nutrients and PHGG by the microorganisms which led to acids production. Notably, the pH of cow kefir (control), (2%), and buffalo kefir (2%) reached the lowest values on day 14 and then showed a mild increase on day 21. However, kefir samples fortified with 1% of PHGG showed a gradual decline in pH values reaching the lowest value on day 21 which could indicate an increase in shelf life. Kefir fortification with 1% and 2% inulin resulted in decreased pH

values which ranged from 4.49 to 4.61 (İrkin & Berkkaçan, 2022). Another study fortified goat kefir with inulin found pH values between 4.57 and 4.63 (Agata & Jan, 2012). Unlike kefir, a study on yogurt found that addition of PHGG increased the pH level (Mudgil et al., 2016). To our knowledge, this is the first experiment examining kefir fortification with PHGG which make it difficult to compare with the results of other studies.

Table 1. Physicochemical properties of the kefir samples

Analysis	Kefir type	Day 1	Day 7	Day 14	Day 21
Titrateable acidity	Cow milk (control)	1.37±0.55 ^{Ba}	1.17±0.1 ^{Ca}	1.32±0 ^{Aa}	1.32±0 ^{ABa}
	Cow milk (1%)	0.75±0.13 ^{ABa}	1.04±0.11 ^{Cb}	1.3±0.02 ^{Ac}	1.53±0.03 ^{Bc}
	Cow milk (2%)	0.37±0.2 ^{Aa}	1.12±0.07 ^{Cb}	1.2±0.02 ^{Bb}	1.19±0.04 ^{ABb}
	Buffalo milk (control)	0.81±0.04 ^{ABa}	0.99±0.03 ^{BCc}	0.85±0 ^{Aab}	0.91±0.03 ^{Ab}
	Buffalo milk (1%)	0.59±0 ^{Aa}	0.75±0.01 ^{Aab}	0.87±0.08 ^{Aab}	1.24±0.37 ^{ABb}
	Buffalo milk (2%)	0.85±0.13 ^{ABa}	0.86±0.05 ^{ABa}	0.9±0.01 ^{Aa}	1.01±0.04 ^{Aa}
Ash (%)	Cow milk (control)	0.73±0.04 ^{Aa}	0.95±0.04 ^{Cb}	1.11±0.01 ^{Dc}	1.12±0 ^{Ed}
	Cow milk (1%)	0.51±0.02 ^{Aa}	0.75±0.06 ^{Bb}	0.79±0.01 ^{Eb}	0.9±0 ^{Dc}
	Cow milk (2%)	0.55±0.62 ^{Aa}	0.58±0.02 ^{Aa}	0.51±0.01 ^{Aa}	0.61±0.01 ^{Ba}
	Buffalo milk (control)	0.62±0.01 ^{Aa}	0.77±0.01 ^{Bb}	0.73±0.01 ^{Bb}	0.84±0.03 ^{Cc}
	Buffalo milk (1%)	0.97±0.02 ^{Ad}	0.61±0.01 ^{Ab}	0.78±0.01 ^{Cc}	0.52±0.02 ^{Aa}
	Buffalo milk (2%)	0.63±0.02 ^{Ab}	0.73±0.02 ^{Bc}	0.5±0.03 ^{Aa}	0.86±0.02 ^{Cd}
pH	Cow milk (control)	5.25±0.01 ^{BCd}	4.97±0 ^{Ab}	4.88±0.03 ^{Aa}	5.03±0.01 ^{Cc}
	Cow milk (1%)	5.11±0.01 ^{ABc}	5.17±0.04 ^{Dc}	5.01±0.01 ^{Bb}	4.85±0 ^{Aa}
	Cow milk (2%)	5.3±0.01 ^{Cd}	5±0 ^{ABb}	4.94±0.01 ^{Aa}	5.08±0.01 ^{Dc}
	Buffalo milk (control)	4.95±0.16 ^{Aa}	5.07±0 ^{Ca}	5.07±0.05 ^{Ba}	4.92±0.03 ^{Ba}
	Buffalo milk (1%)	5.02±0.01 ^{Ac}	4.98±0.01 ^{Ab}	5.01±0.01 ^{Bbc}	4.9±0.01 ^{Ba}
	Buffalo milk (2%)	5.03±0.01 ^{Ab}	5.03±0 ^{Bb}	4.94±0.01 ^{Aa}	5.01±0.02 ^{Cb}
Moisture (%)	Cow milk (control)	85.06±2.05 ^{ABa}	84.35±0.21 ^{BCa}	86.4±0.04 ^{Ca}	86.4±0.08 ^{BCa}
	Cow milk (1%)	88.87±3.54 ^{Bb}	73.39±7.54 ^{Aa}	85.1±0.11 ^{Bab}	85.15±1.48 ^{Bab}
	Cow milk (2%)	82.79±3.09 ^{Aa}	78.94±2.82 ^{ABa}	84.48±0.4 ^{Aa}	82.79±1.2 ^{Aa}
	Buffalo milk (control)	89.87±0.05 ^{Bbc}	88.64±0.18 ^{Ca}	90.04±0.21 ^{Fc}	89.53±0.21 ^{Cb}
	Buffalo milk (1)	89.95±0.42 ^{Bc}	88.09±0.13 ^{Ca}	89.05±0.11 ^{Eb}	88.4±0.02 ^{Ca}
	Buffalo milk (2%)	89.62±0.45 ^{Bc}	87.54±0.06 ^{Ca}	88.31±0.15 ^{Db}	88.27±0.13 ^{BCb}
Organic Compounds (%)	Cow milk (control)	14.20	14.70	12.49	12.47
	Cow milk (1%)	10.63	25.86	14.11	13.95
	Cow milk (2%)	16.66	20.49	15.02	16.60
	Buffalo milk (control)	9.51	10.59	9.23	9.63
	Buffalo milk (1)	9.08	11.30	10.18	11.09
	Buffalo milk (2%)	9.75	11.73	11.19	10.88

^{a-d}: Different lowercase letters show the statistical differences in the same sample in different weeks – in the horizontal line (ANOVA. Duncan^a test; p<0.05)

^{A-F}: Different uppercase letters show the statistical differences in the same week in different samples – in the vertical line (ANOVA. Duncan^a test; p<0.05)

±: Standard Deviation

Viscosity is one of the most important parameters contributing to product texture and acceptability (Gomes et al., 2013). Viscosity analysis was conducted on days 14 and 21 (Table 2). The results were heterogeneous among the samples which ranged from 67.5 to 2312 cP. At the end of the study, cow kefir (control) had the

highest viscosity of 2312 cP. Interestingly, cow kefir fortified with 2% of PHGG had the lowest viscosity (112.5 cP) among cow kefir samples. Cow and buffalo kefir fortified with 1 and 2% PHGG had approximately equivalent viscosity on day 14 ranging from 240 to 335 cP. Several studies have found some increase in viscosity

when fortified with PHGG. The addition of 1% and 2% inulin to kefir produced from cow and goat milk resulted in slightly higher viscosity compared to our results of 488 and 365 cP, respectively (İrkin & Berkkaçan, 2022). Yogurt viscosity was increased with an increase in incubation time of PHGG at higher levels, but this effect was not observed when the incubation time increased with low amounts of PHGG (Mudgil et al., 2016, 2017b). Fortifying tomato soup with 15% of PHGG led to significantly lower viscosity compared to guar gum (3600 vs 15 cP) (KS et al., 2021). Moreover, Mudgil et al. (2016) found positive relation of PHGG dose with viscosity in which utilizing 1-5% PHGG led to 46-131 cP viscosity results in butter milk (Mudgil & Barak, 2016). Another study fortified kefir with apple and

lemon fiber with different percentages ranging from 0.25 to 1%. The viscosity gradually increased with the increase of the dose of dietary fiber (Goncu, 2017).

Furthermore, our results can be compared to the findings of Ünal et al. (2020), who studied the quality of six commercial kefir samples (Ünal et al., 2020). They reported viscosity values between 286 and 462 cP, depending on the product. In contrast, our cow kefir (control) showed much higher viscosity (2312 cP), while the sample fortified with 2% PHGG had the lowest viscosity (112.5 cP). This shows how PHGG can significantly affect the texture of kefir. The mechanism by which PHGG impacts kefir's viscosity remains unclear, future investigations are needed.

Table 2. Viscosity analysis of the kefir samples

Analysis	Kefir type	Day 14	Day 21
		40 rpm	40 rpm
Viscosity (cP)	Cow milk (control)	1200±233.35	2312.5±491.44
	Cow milk (%1)	315±275.77	172.5±10.61
	Cow milk (%2)	335±289.91	112.5±10.61
	Buffalo milk (control)	195±21.21	67.5±10.61
	Buffalo milk (%1)	247.5±180.31	185±49.5
	Buffalo milk (%2)	240±169.71	870±21.21

±: Standard Deviation

In addition, the type of kefir exerted different microorganisms changing patterns due to milk composition variations (Table 3). For instance, our microbial analysis shows a higher increase in lactobacillus genera in cow kefir, particularly control and 1%, compared to buffalo kefir (Figure 1). On the other hand, yeast proliferation in buffalo kefir was high relative to cow kefir, although kefir had similar values at the end of the study. Another study found higher concentrations of ethanol in buffalo kefir compared to cow kefir. Similar to our findings, yeast growth was more rapid in buffalo kefir samples compared to cow kefir samples (Gul et al., 2015). Kefir generally contains both types of yeasts, lactose fermenting and non-fermenting species. However, several studies have found non-fermenting species (e.g. *Saccharomyces cerevisiae*, *Saccharomyces*

pastoriansporianus, *Saccharomyces pastoriansatanis*, *Istria orientalis*, and *Debaryomyces occidentalis*) are the most abundant (Ding Fan et al., 2022; Saygili et al., 2023; Taş et al., 2012). These yeasts contribute to the production of ethanol and CO₂ which are responsible for the yeasty taste in kefir. A good kefir quality exerts a slightly yeasty taste (Gul et al., 2015). In addition, our experiment reveals that buffalo kefir had a higher amount of streptococcus bacteria than cow kefir regardless of PHGG addition. PHGG was previously examined as a potential prebiotic source of 6 probiotic strains belonging to lactobacillus and streptococcus genera. All the 6 species had the ability to grow and proliferate in a similar manner with those using glucose, but not guar gum, as a substrate (Mudgil et al., 2018).

Table 3. Microbial analysis of the kefir samples

Kefir Samples	Count on round-shape bacteria (log CFU/mL)			Count on yeast (log CFU/mL)			Count on rod-shape bacteria (log CFU/mL)		
	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
Cow milk (control)	8.4728	9.2279	9.3222	8.4728	9.2279	9.3222	8.4728	9.2279	9.3222
Cow milk %1	8.5527	9.3522	9.4669	8.5527	9.3522	9.4669	8.5527	9.3522	9.4669
Cow milk %2	8.7177	8.7782	8.9590	8.7177	8.7782	8.9590	8.7177	8.7782	8.9590
Buffalo milk (control)	8.0253	8.7324	8.8633	8.0253	8.7324	8.8633	8.0253	8.7324	8.8633
Buffalo milk %1	8.3304	7.0000	8.2788	8.3304	7.0000	8.2788	8.3304	7.0000	8.2788
Buffalo milk %2	8.1614	7.4771	8.4472	8.1614	7.4771	8.4472	8.1614	7.4771	8.4472

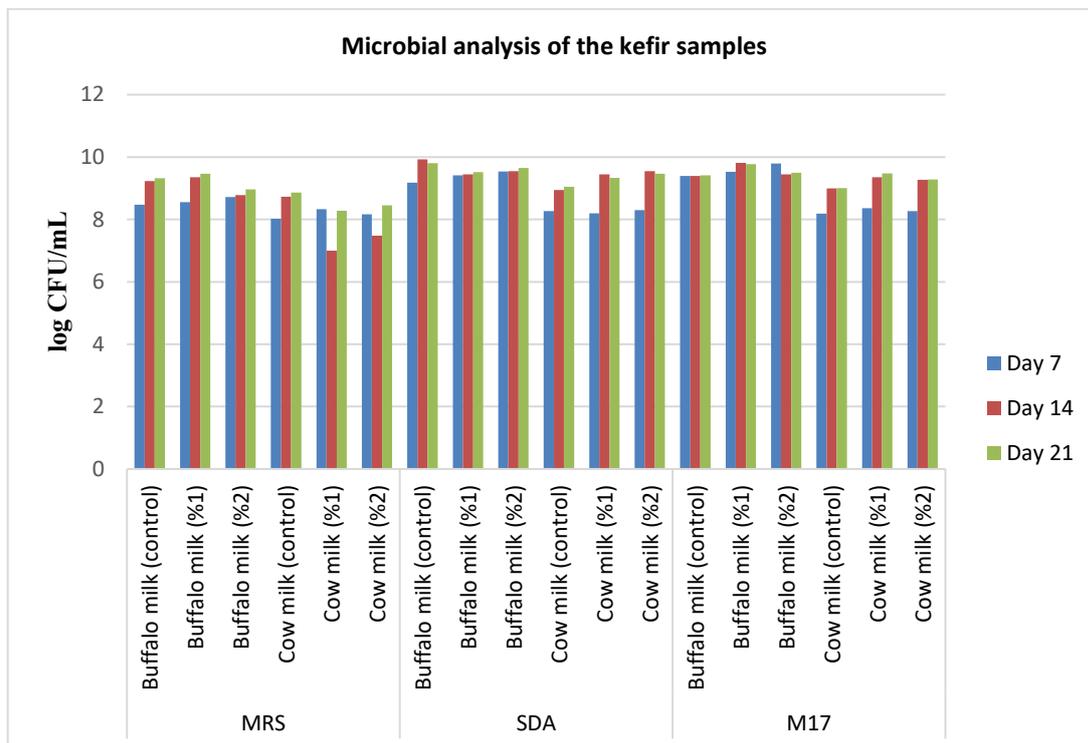


Figure 1. Changes in microbial counts during the study

Furthermore, sensory analysis of the kefir samples was evaluated for color, odor, taste, texture, and overall acceptability (Table 4). Odor, taste, texture, and overall acceptability were significantly different between the samples on days 7, 14, and 21. Buffalo samples had higher acceptability on the 1st day of the experiment. However, the evaluation was declined until the end of the study in which the least acceptable sample was buffalo kefir 2%. While the overall acceptability of cow kefir samples control and 1% started to decline on day 14, cow kefir 2% reached the highest level on day 21 with 3.8 points which could be explained by gaining the highest points of all other sensory analysis

domains including texture, taste, odor, and color. Similarly, yogurt fortified with PHGG had increased viscosity, and the highest acceptability value was at the dose of 2% of PHGG (Mudgil et al., 2016). Noodles fortified with 2.23% of PHGG showed an increase in overall acceptability. Beyond this dose there was a decrease in overall acceptability which could be explained by water holding capacity of PHGG (Mudgil et al., 2018). Adding 2-4% of PHGG increased the overall acceptability of butter milk compared to control, 1, and 5% (Mudgil & Barak, 2016). Therefore, our experiment reveals that 2% of PHGG is the optimum amount to be added to enhance the acceptability of cow kefir for a longer duration.

İrkin and Berkkaçan (2021) found that 1% of inulin is the optimum value to enhance kefir acceptability.

A recent comparative study by Meral-Aktaş et al. (2025) evaluated kefir produced from cow, buffalo, ewe, and goat milks, analyzing their microbiological, physicochemical, sensory, and volatile compound profiles (Meral-Aktaş et al., 2025). The authors found that cow milk kefir received the highest consumer preference, whereas goat milk kefir was the least favored. Interestingly, ewe milk kefir exhibited the highest levels of benzoic acid, a volatile compound contributing to its distinct flavor. Microbial dynamics varied as well—lactic cocci and bacilli counts declined during storage, while yeast populations tended to increase. These findings underscore how milk origin affects not only fermentation and microbiota but also sensory acceptability and aroma profiles (Meral-Aktaş et al., 2025). Our work likewise revealed differences in texture, microbial behavior, and sensory ratings between cow and buffalo kefir, particularly in response to PHGG fortification. The fact that cow milk kefir tends to be more accepted in sensory evaluations aligns with our observation that 2% PHGG-fortified cow kefir achieved the highest overall acceptability. Similarly, the microbial shifts over storage seen in Meral-Aktaş et al.'s study—decreasing lactic bacteria and rising yeast counts—mirror our findings of microbial

population dynamics in both milk types during cold storage. While our study did not profile volatile compounds, the observation of such differences in benzoic acid levels for ewe milk kefir suggests that similar volatile variations might exist between cow and buffalo kefir and would be a fruitful avenue for future research.

Moreover, one of the important aspects of kefir beverage contributing to human health is antioxidant properties (Güven, 2025). Although antioxidant capacity was not directly measured in the present study, previous literature has demonstrated notable antioxidant properties in kefir, particularly when derived from buffalo and cow milk. Ozcan et al. (2018) assessed buffalo milk kefir and reported high antioxidant activity, which increased during storage, likely due to the continued release of bioactive peptides and amino acids generated through microbial fermentation (Ozcan et al., 2018). Similarly, Bengi et al. (2023) demonstrated that fortifying kefir with propolis extract significantly enhanced its antioxidant capacity. This enrichment also improved the total phenolic content (TPC), further confirming the role of functional ingredients in boosting the antioxidant profile of kefir (Bengi et al., 2023). These findings highlight kefir's potential as a functional food with health-promoting benefits, even though antioxidant parameters were not analyzed in the current work.

Table 4. Sensory analysis

Sensory	Kefir types	Day 1	Day 7	Day 14	Day 21
Color	Cow milk (control)	4±1.22 ^a	4.8±0.45 ^a	4.4±0.55 ^{ab}	4.2±0.84 ^a
	Cow milk (%1)	4.2±0.84 ^a	4.2±0.84 ^a	4.4±0.55 ^{ab}	4.2±0.84 ^a
	Cow milk (%2)	4.2±0.84 ^a	4.6±0.55 ^a	4.8±0.45 ^b	4.8±0.45 ^a
	Buffalo milk (control)	3.8±1.3 ^a	4.6±0.55 ^a	4.8±0.45 ^b	4.4±0.89 ^a
	Buffalo milk (%1)	4.4±0.89 ^a	4.2±0.45 ^a	3.8±0.84 ^a	4.2±0.84 ^a
	Buffalo milk (%2)	3.8±1.3 ^a	4.4±0.55 ^a	4.2±0.84 ^{ab}	4.2±0.84 ^a
Odor	Cow milk (control)	2.6±1.14 ^a	3.6±0.89 ^{bc}	4.2±0.45 ^b	3.6±0.89 ^{ab}
	Cow milk (%1)	2.6±0.89 ^a	3.2±1.3 ^{abc}	3.6±0.55 ^b	3.6±0.89 ^{ab}
	Cow milk (%2)	2.6±1.52 ^a	3.6±0.89 ^{bc}	4±0 ^b	4.2±0.84 ^b
	Buffalo milk (control)	4.2±0.84 ^b	4.6±0.89 ^c	3.8±0.84 ^b	3.6±1.14 ^{ab}
	Buffalo milk (%1)	4±0.71 ^{ab}	1.8±0.84 ^a	1.8±0.84 ^a	3.2±0.84 ^{ab}
	Buffalo milk (%2)	3±1.22 ^{ab}	3±1.22 ^{ab}	3.4±1.14 ^b	2.8±0.84 ^a
Taste	Cow milk (control)	2.4±1.14 ^a	3.6±0.55 ^b	4.2±0.84 ^d	3±1.22 ^{bc}
	Cow milk (%1)	2±1.22 ^a	2.6±0.89 ^{ab}	3.2±0.84 ^c	3.2±1.1 ^{bc}
	Cow milk (%2)	2.2±1.1 ^a	3.4±1.52 ^b	3.8±0.45 ^{cd}	3.8±1.3 ^c
	Buffalo milk (control)	3.4±0.55 ^a	4±1.22 ^b	3±0.71 ^c	2.8±1.1 ^{abc}
	Buffalo milk (%1)	3.2±0.84 ^a	1.4±0.55 ^a	1±0 ^a	2.2±1.1 ^{ab}
	Buffalo milk (%2)	2±1.73 ^a	1.4±0.89 ^a	2±0.71 ^b	1.4±0.55 ^a
Texture	Cow milk (control)	2±1 ^a	4.2±0.45 ^b	4.2±0.45 ^c	2.6±1.14 ^{ab}
	Cow milk (%1)	2±1.22 ^a	2.4±1.14 ^a	2.8±1.3 ^b	2.6±0.89 ^{ab}
	Cow milk (%2)	1.8±1.1 ^a	3.6±0.89 ^{ab}	4.2±0.45 ^c	4.2±1.1 ^c
	Buffalo milk (control)	2.4±1.14 ^a	2.8±0.84 ^a	2.6±0.55 ^{ab}	3.8±1.3 ^{bc}
	Buffalo milk (%1)	2.4±1.14 ^a	2.6±1.14 ^a	2.6±0.55 ^{ab}	2.4±0.55 ^a
	Buffalo milk (%2)	2.4±1.14 ^a	2.4±0.89 ^a	1.6±0.89 ^a	1.4±0.55 ^a
Overall	Cow milk (control)	2.4±1.14 ^a	3.8±0.45 ^c	3.6±0.55 ^b	2.6±0.89 ^{abc}
	Cow milk (%1)	2±1 ^a	2.6±1.14 ^{abc}	3.2±0.84 ^b	2.8±0.84 ^{bc}
	Cow milk (%2)	2.2±1.3 ^a	3.2±1.48 ^{bc}	3.6±0.55 ^b	3.8±1.3 ^c
	Buffalo milk (control)	3±0.71 ^a	3.4±0.55 ^c	2.8±0.45 ^b	3±1 ^{bc}
	Buffalo milk (%1)	3.2±0.84 ^a	1.6±0.55 ^a	1.2±0.45 ^a	2.2±1.1 ^{ab}
	Buffalo milk (%2)	2.2±1.3 ^a	2±1.22 ^{ab}	1.4±0.55 ^a	1.4±0.55 ^a

^{a-d}: Different uppercase letters show the statistical differences in the same week in different samples – in the vertical line (ANOVA). Duncan^a test; $p < 0.05$

±: Standard Deviation

Conclusion

This study aimed to produce a novel dairy product by enriching kefir with partially hydrolyzed guar gum. PHGG didn't have a significant impact on kefir pH. However, there was a gradual increase in titratable acidity in samples fortified with PHGG which might be due to lactic acid production. 1 and 2% of PHGG have caused reductions in kefir viscosity at the end of the study, but this effect was not observed in 2% buffalo kefir. Microbial count was slightly increased due to PHGG addition to kefir. Fortifying cow kefir with 2% of PHGG gained the highest acceptability score compared to other kefir samples. To note, this is the first experiment that examines the impact of kefir fortification

with PHGG. Future studies will help us to better understand significant aspects of kefir fortified with PHGG. Some aspects include, but not limited to, physicochemical properties, mechanisms of interaction between different doses of PHGG and kefir derived from different milk types (e.g. goat) on viscosity and microbial dynamics, and antioxidant capacity. Such investigations will be determined to consider kefir fortification with PHGG as functional food in industrial field.

Conflict of Interest

The authors declare that there is no conflict of interest between them.

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Author's Contributions

All authors contributed equally to the manuscript. The final version of the manuscript was reviewed and approved by all authors.

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