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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Production of Functional Kombucha Enhanced with Mormix, Mulberry Powder and Different Concentrations of Whey*

Farklı Konsantrasyonlarda Peynir Altı Suyu, Mormix ve Dut Tozu ile Zenginleştirilen Fonksiyonel Kombucha Üretimi

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Abstract

In this study, the fortified fermented beverages with Kombucha culture (SCOBY) and different concentrations of whey (25%, 50%, 75%) by using mulberry powder to give natural sweetness with mormix rich in bioactive components during fermentation (1st, 5th, 7th, 9th, 11th and 13th days) microbiological, physicochemical and sensory properties were planned. This effort was to develop an innovative, functional, and immune-boosting product by combining Mormix, which is rich in bioactive compounds, and nutritionally valuable whey in the production of Kombucha tea. The lowest acetic acid bacteria count (6.87 $\log_{10}$ cfu mL^{-1}) was found in the control sample, and the highest (8.07 $\log_{10}$ cfu mL^{-1}) was found in the sample produced with green tea+75% whey+mormix+mulberry powder (KMP_3). *Lactobacillus* spp. and *Lactococcus* spp. counts were determined to be the highest in the KMP_3 sample. The effect of the samples on the mold counts developed in Kombucha beverages was statistically significant (p<0.01). Using green tea and different raw materials for Kombucha fermentation caused changes in dry matter, titration acidity, and pH values. The viscosity results were very significant during fermentation and adding different concentrations of whey and mormix+mulberry powder (p<0.01). It was determined that the $L*$, $a*$, $b*$, C, $\alpha°$, and $\Delta E*$ color parameters of Kombucha enriched with whey and mormix+mulberry powder changed during fermentation. In fermented beverage samples made with Kombucha culture, an increase was observed in total phenolic content (981.33-1515.30 mg GAE g^{-1}) and antioxidant capacity (DPPH 59.03-94.29%) during fermentation depending on the raw materials added. The sensory analysis showed that the most preferred sample was green tea+25% whey+mormix+mulberry powder (KMP_1). Increasing the fermentation time caused an increase in acidity, and on the 13th day, KMP_2 and KMP_3 were the samples with the lowest taste scores. It has been obtained from the thesis findings that Kombucha can be evaluated as a new fermented beverage because raw materials such as whey and mormix used in its production provide strong antioxidant properties.

Keywords: Kombucha, Functional beverage, Mormix, Mulberry powder, Whey

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*This study was summarized from the Esra Bebek's MSc thesis.

Öz

Bu çalışmada Kombu çayı kültürü (SCOBY) ile peyniraltı suyunun (PAS) farklı konsantrasyonları (%25, %50, %75) ve biyoaktif bileşenlerce zengin olan mormiks ile doğal tatlılık vermesi amacıyla dut tozu kullanılarak zenginleştirilen fermente içeceklerin fermantasyon süresince (1., 5., 7., 9., 11. ve 13. günler) mikrobiyolojik, fiziko-kimyasal ve duyusal özelliklerinin incelenmesi planlanmıştır. Buradaki amaç biyoaktif bileşik içeriği çok fazla olan mormiks ile besinsel yönden çok değerli olan peynir altı suyunun Kombu çayı üretiminde kullanılarak yenilikçi, fonksiyonel ve bağışıklık sistemini güçlendirici bir ürün geliştirilmesine imkân sunmaktır. Mikrobiyolojik analizlerin sonucunda, en düşük asetik asit bakteri sayısı (6,87 $\log_{10}$ kob mL^{-1}) kontrol örneğinde, en yüksek (8,07 $\log_{10}$ kob mL^{-1}) ise yeşil çay+%75 PAS+mormiks+dut tozu (KMP_3) ile üretilen örnekte bulunmuştur. *Lactobacillus* spp. ve *Lactococcus* spp. sayıları en yüksek bulunan örnek KMP_3 örneği olmuştur. Kombu çayı içeceklerinde gelişen maya sayıları üzerinde örneklerin etkisi çok önemli tespit edilmiştir (p<0,01). Kombu çayı fermentasyonu için yeşil çay ile farklı hammaddelerin kullanımı kurumadde, titrasyon asitliği ve pH değerlerinde değişikliklere neden olmuştur. Farklı konsantrasyonlarda PAS ve mormiks+dut tozu ile katkılandırmanın ve fermantasyonun viskozite sonuçları üzerindeki etkisi çok önemli (p<0,01) bulunmuştur. PAS ve mormiks+dut tozu ile zenginleştirilen Kombu çaylarının L^*, a^*, b^*, C, $\alpha°$ ve ΔE^* renk parametrelerinin fermentasyon süresince değiştiği belirlenmiştir. Kombu çayı kültürü ile yapılan fermente içecek örneklerinde ilave edilen hammaddelere bağlı olarak toplam fenolik madde (9813,33-1515,3 mg GAE g^{-1}) ve antioksidan kapasitelerinde (DPPH %59,03-94,29) fermantasyon boyunca artış saptanmıştır. Duyusal analiz sonucuna göre en beğenilen ürün yeşil çay+%25 PAS+mormiks+dut tozu (KMP_1) ile hazırlanan fermente içecek olmuştur. Fermastasyon gününün artması asitlik miktarında artışa neden olmuş ve 13. gününde KMP_2 ve KMP_3 tat puanını en düşük alan örnekler olmuştur. Üretiminde kullanılan PAS ve mormiks gibi hammaddeler kuvvetli antioksidan özellik kazandırdığı için yeni bir fermente içecek olarak Kombu çayının değerlendirilebileceği sonucu çalışma bulgularından elde edilmiştir.

Anahtar Kelimeler: Kombucha, Fonksiyonel içecek, Mormiks, Dut tozu, Peynir altı suyu

1. Introduction

A healthy diet requires an adequate intake of nutrients. Diversity is equally crucial to nutritional intake (Yılmaz and Çolakoğlu, 2024). Fermented foods are reliable products, with taste and aroma serving as additional factors influencing their consumption (Dağlıoğlu et al., 2002; Şensoy and Tarakçı, 2023). Kombucha beverages are functional foods, and their popularity is due to their multiple functional properties, such as anti-inflammatory potential and antioxidant activity (Villarreal-Soto et al., 2018). Among the beneficial components of Kombucha are polyphenols, amino acids, vitamins, antibiotics, and various micronutrients produced during fermentation (Jayabalan et al., 2007). The various effects of Kombucha on human health, such as accelerating digestion, preventing microbial infections, protecting against stress and cancer, and providing prophylactic and therapeutic benefits for hemorrhoids, have been determined. It has also been reported that it is effective at lowering cholesterol levels, removing toxins from the body, and cleansing blood (Dufresne and Farnworth, 2000; Malbaša et al., 2011; Jayabalan et al., 2014; Amarasinghe et al., 2018). The incidence of metabolic disorders such as diabetes, cardiovascular diseases, and obesity is associated with increased sugar consumption (Özdemir et al., 2015; Arslaner and Salık, 2017). As consumer demands for low-calorie foods have increased, products produced with alternative sweeteners have become more popular in recent years. One of the fruits that is appetizing and low in calories is mulberry (Wang et al., 2013). It has been reported that this fruit has pharmacological effects through its anti-inflammatory, anticholesterol, antidiabetic, antioxidant, and antiobesity properties (İnanç et al., 2020; Kang et al., 2006; Kim and Park, 2006; Ye et al., 2002; Yu et al., 2021; Zhang and Shi, 2010; Özbalcı et al., 2023). However, whey proteins have high biological value and contain many essential amino acids (lysine, tryptophan, isoleucine, threonine, etc.). Whey proteins have a higher protein efficiency ratio (PER) than casein (Walzem et al., 2002). Moreover, it contains higher concentrations of essential and sulphur-containing amino acids than egg, milk, casein, red meat, soy, corn, and wheat proteins. (Smithers, 2008; Kumar et al., 2018). In addition, whey proteins are also known to be effective as antioxidants, antibacterial agents, antihypertensive agents, antitumor agents, antiviral agents, and hypolipidemics (Rezende et al., 2014; Bilal and Altıner, 2017; Yüksel et al., 2019).

The presence of mormix, a product extremely rich in bioactive compounds, is extremely important for improving the immune system. It is the concentrated color substance of the color components of purple-colored fruits and vegetables and provides the purple color resulting from the purple pigment content in these fruits (Baylan and Badem, 2023). The mormix makes important contributions, especially to blood vessels, by preventing blockage in the heart, brain, and leg vessels. Regulating the body's glycemic index protects against many chronic diseases, especially diabetes. Mormix, which is required to be produced and consumed in Japan, has become increasingly popular due to its effects on human health, and their production has begun in many countries (Cömert and Gün, 2020).

When preparing innovative foods, they must be produced by considering the acceptance conditions of consumers. In this context, it has been shown that the nutritional content of Kombucha, an innovative food, can be further improved by the use of whey, which is used as waste in the dairy by product industry, and mormix a good source of antioxidants. Upon reviewing current literature, no nutritionally rich Kombucha made with whey, mormix, and mulberry powder was found. Given the prevalence of chronic diseases in today's world, creating a health-promoting nutraceutical product has made this study particularly significant compared to existing research.

2. Materials and Methods

2.1. Materials

Green tea leaves (*Camellia sinensis*), starter culture (SCOBY) [(CENTRAGEN Laboratory Products Informatics and Consultancy Company)], dried mulberry, sucrose, and mormix (Sankara Food) were obtained from the market.

2.2. Kombucha production

The ratios determined after preliminary trials, taking into account previous studies, were used to formulate the Kombucha samples (*Table 1*).

Table 1. Kombucha sample formulations

Groups	Pure water (mL)	Whey (mL)	Mulberry powder (g/3L)	Mormix (g/3L)
K	3000	0	0	0
KMP$_1$	2250	750	180	90
KMP$_2$	1500	1500	180	90
KMP$_3$	750	2250	180	90

*K: Control (only green tea); KMP$_1$: Green tea+25% Whey+mormix+mulberry powder; KMP$_2$: Green tea+50% whey+mormix+mulberry powder and KMP$_3$: Green tea+75% whey+mormix+mulberry powder.

Fermented beverages were produced by brewing at 90°C for 10 min according to the methods of Jayabalan et al. (2007). The filtered mixture was cooled to 28–30°C, after which mulberry powder and mormix were added. Then, 10% SCOBY Kombucha culture was added. The brewed teas were left to ferment at 25±2°C. During fermentation, analyses were performed on the 1st, 3rd, 5th, 7th, 9th, 11th, and 13th days. A flow chart of the production of Kombucha fruits produced with different concentrations of whey or mormix+mulberry powder is shown in *Figure 1*.

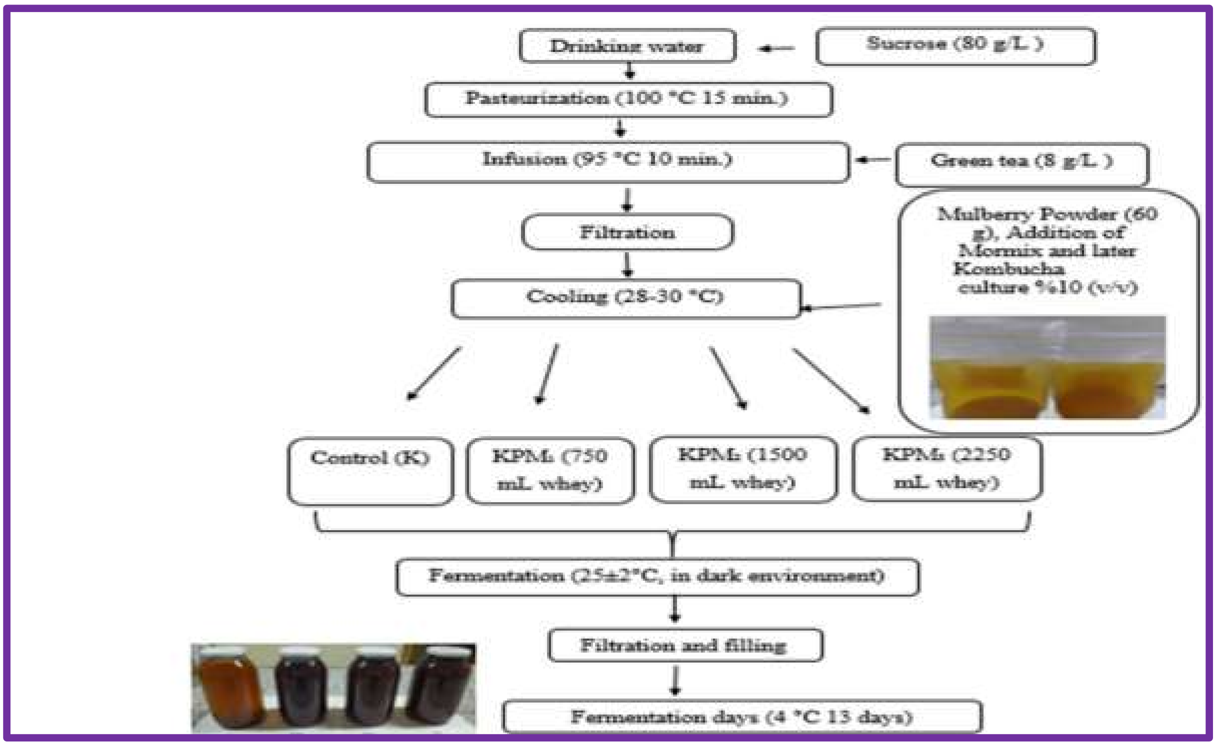

Figure 1. Production flow chart of Kombucha produced with different concentrations of whey, mormix+mulberry powder

2.3. Microbiological analyses

The enumeration of acetic acid bacteria was conducted using the pour plate method on Glucose Yeast Extract Calcium Carbonate Agar (GYC) at 30°C for 3-5 days under aerobic conditions, as described by Irigoyen et al. (2004). Following incubation, colonies with a cream color and a clear precipitation zone were identified as typical AAB colonies. *Lactococcus* spp. enumeration was carried out on M17 agar (pH 7.2+/-0.2) using the pour plate method, with petri dishes incubated at 30°C for 2 days in an aerobic environment, following the method by Irigoyen et al. (2004). For *Lactobacillus* spp. count, MRS agar (pH 6.5+/-0.2) and the pour plate method were employed, with inoculated petri dishes incubated at 30°C for 3 days in an anaerobic environment using double-layered inoculation according to Irigoyen et al., (2004). Mold counting was performed using Yeast Extract Glucose Chloramphenicol (YGC) agar under aerobic conditions at 25°C for 4 days based on the method by Witthuhn et al. (2005).

2.4. Physicochemical and chemical analyses

The dry matter content of Kombucha samples was assessed according to the method in AOAC (1990). Measurement of pH values was conducted using a pH meter (Eutech PH 150 Model), while total acidity was determined as method by Cemeroğlu (2007). Viscosity values were measured using a viscometer (Model DV-1; Brookfield Engineering Laboratories, Inc., MA, USA) in line with the procedure detailed by Aktan and Yıldırım (2011). Color values ($L*$, $a*$, and $b*$) were determined employing a HunterLab device (Colorflex-EZ; HunterLab, Virginia, USA) following the methodology established by Cemeroğlu (2007). Furthermore, the Hue angle value ($\alpha°$), Chroma value (*C*), and total color difference values ($\Delta E*$) were calculated based on Equation 1.

$$C = [(a*^2 + b*^2)]^1/^2 \qquad \alpha° = \tan -^1(b*/a*) \qquad \Delta E = [(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2]^1/^2 \qquad \text{(Eq.1).}$$

2.5. Total phenolic contents and antioxidant capacities

The total phenolic content (TPC) of Kombucha samples was determined using a UV spectrophotometer (UV-180, Shimadzu, Kyoto, Japan) based on a colorimetric oxidation/reduction reaction as described by Škerget et al. (2005). The oxidizing reagent used was Folin–Ciocalteu reagent by AOAC guidelines (2019). A 5 mL aliquot of diluted extract (10 mg in 10 mL solvent), 2.5 mL of Folin–Ciocalteu reagent (diluted tenfold with distilled water), and 2.5 mL of Na_2CO_3 solution (75 g/L) were combined. The mixture was incubated for five minutes at 50°C and then allowed to cool. A control sample used 0.5 mL of water instead of the extract. The absorbance was measured at 760 nm, and the TPC is expressed as gallic acid equivalents mg GAE g^{-1} of dried extract. To determine the antioxidant activities of the Kombucha beverage samples (0.025 mL) were diluted with 4 mL of methanol. After adding a 1 mM 2,2 diphenyl-1-picrylhydrazyl (DPPH) solution prepared with 0.6 mL of methanol, the samples were homogenized by vortexing for 30 min. Samples were measured at 517 nm, and the antioxidant capacity was calculated as % inhibition by Equation (2) below (Chu and Chen, 2006):

$$Inhibition\ rate\ (\%) = [(Ao - A1)/Ao] \times 100 \qquad \text{(Eq.2)}$$

A_o: Absorbance of the blank (control group); A_1: absorbance of the sample

2.6. Sensory analysis

In the sensory evaluation of the Kombucha samples, each sample was subjected to sensory analysis with a panelist group of eight people; the samples were evaluated in terms of color and appearance, odor, sweetness, acidic taste, and general acceptability.

2.7. Statistical analyses

The study was set up and conducted according to a randomized complete block experimental plan with 2 replications, 3 different whey concentrations (25%, 50%, and 75%) x 6 different fermentation days (1^{st}, $5^{th,}$ 7^{th}, 9^{th}, 11^{th}, and 13^{th} days). Statistical analyses of the obtained results were performed in the SPSS 22 package program (SPSS Inc., Chicago, IL, USA) and Duncan's multiple comparison test was applied to those whose averages were found significant. The LSD (Least Significant Difference) test was used to determine statistical differences between the obtained average values at a probability level of $p<0.05$.

3. Results and Discussion

3.1. Acetic acid bacteria (AAB) count

The microbiological analysis results of the Kombucha beverages are shown in *Figure 2a–d*. The lowest number of acetic acid bacteria in the drinks (6.87 $\log_{10}$ cfu mL^{-1}) was detected on the 1^{st} day of fermentation in the K sample, and the highest number (8.07 $\log_{10}$ cfu mL^{-1}) was detected on the 7^{th} day of fermentation in the KMP_3 sample. Upon examination of the averages based on concentrations, it was observed that the AAB counts of the KMP_3 sample were higher compared to other samples. This difference in the samples is believed to be attributed to the quantity of whey added. Furthermore, fermentation times impacted the AAB counts of the samples, with the highest viability (7.65 $\log_{10}$ cfu mL^{-1}) being recorded on the 9^{th} day of fermentation according to the averages. In his study using Kombucha culture to produce fermented milk beverages, Şarkaya (2019) reported that the AAB numbers were close to each other on the 1^{st} and 10^{th} days of fermentation and that there was an increase in the AAB numbers on the 20^{th} and 30^{th} days.

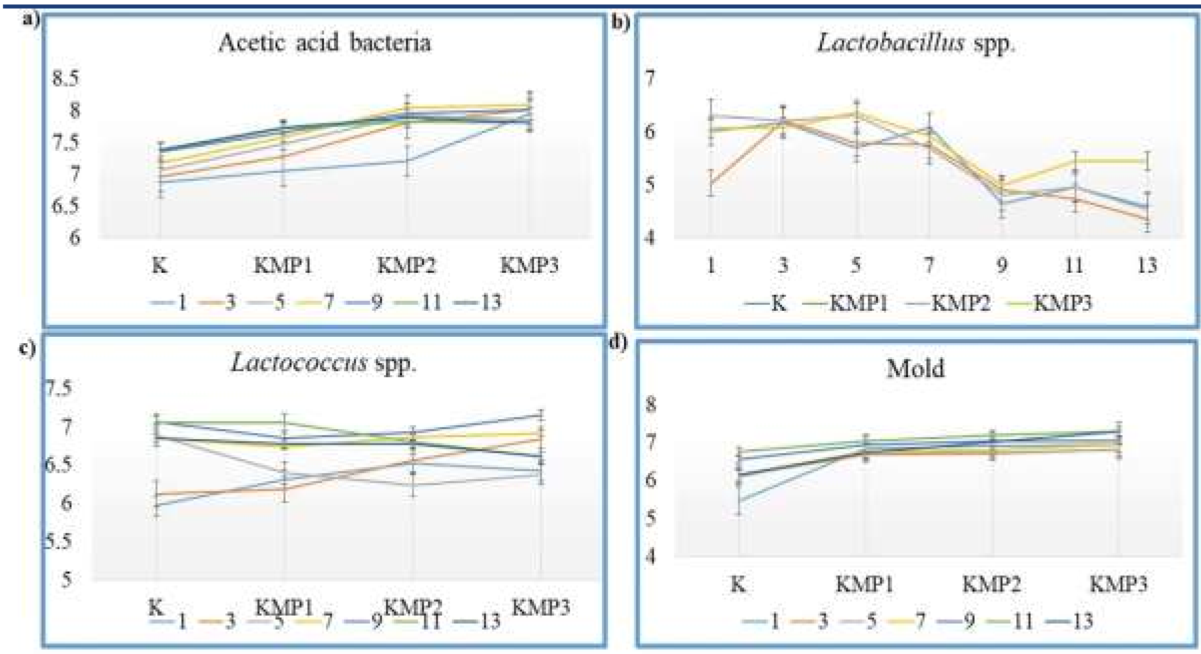

Figure 2a-d. Microbiological changes in Kombucha samples during fermentation (log_{10} cfu mL^{-1})

The count of *Lactobacillus* spp. increased in Kombucha beverages with increasing whey concentration. It was determined that there were fluctuations in counts, but the number of living organisms was above 4.00 log_{10} cfu mL^{-1} on all fermentation days. The effect of using different concentrations of whey or mormix+mulberry powder and fermentation time on *Lactobacillus* counts was found to be significant (p<0.05). The lowest *Lactobacillus* count was detected on the 13th day of fermentation in the KMP_1 sample (4.36 log_{10} cfu mL^{-1}), and the highest number (6.35 log_{10} cfu mL^{-1}) was detected on the 5th day of fermentation in the KMP_3 sample.

The effect of Kombucha beverages on *Lactococcus* spp. was found to be statistically significant (p<0.05). Şarkaya (2019) assessed the lactococci counts of Kombucha samples to be between log 5.64 and 7.15 cfu mL^{-1} and found that their numbers were greater on the 1st and 30th days of fermentation and lower on the 10th and 20th days. *Lactococcus* spp. counts in fermented beverages with sage and Kombucha were determined to be between log 6.55 and 6.56 cfu mL^{-1}. It was reported that the sample with the lowest *Lactococcus* count (log_{10} cfu mL^{-1}) was the green tea sample.

The effect of whey and mormix+mulberry powder addition and fermentation time on mold numbers was found to be significant (p<0.05). The highest number of mold in the beverage samples (7.30 log_{10} cfu mL^{-1}) was detected on the 11th day of fermentation in the KMP_3 sample, and the lowest number (5.46 log_{10} cfu mL^{-1}) was detected on the 1st day of fermentation in the K sample. Sreeramulu et al. (200) found 4.48 log cfu mL^{-1} mold cells and 5.3 log_{10} cfu mL^{-1} bacterial cells in the fermented liquids after six days of fermentation. Teoh et al. (2004) reported mold counts between 5 and 7 log_{10} cfu mL^{-1} on the sixth day of the process. In addition, Akarca and Tomar (2020) used red carrots, red beets, and purple cabbage in the production of Kombucha tea and reported that the interaction between sample type, fermentation time, and sample type×fermentation time was highly significant for determining the number of osmophilic molds (p<0.0001). These findings are similar to the findings of the present study. The results are believed to be influenced by the raw materials utilized in the production process. This assumption is supported by the finding of the highest mold count in sample KMP_3, which had the highest amount of whey. It is speculated that the nutrient-rich composition of whey, combined with mulberry powder, may promote mold growth.

3.2. Physicochemical analysis results

The statistical differences in dry matter content, pH, total acidity, and viscosity values of the Kombucha beverages during fermentation are presented in *Table 2*. It was determined that as the concentration of whey increased, along with the use of mulberry powder and mormix, the dry matter values of the samples also increased. The lowest dry matter content (8.40%) was observed on the 11th day of fermentation in the control group (K), while the highest value (18.67%) was recorded on the 13th day of fermentation in the KMP_3 group. The findings of this study indicate that the dry matter values of each sample were statistically influenced by the fermentation days ($p<0.05$). Naji et al. (2021) reported the dry matter content of iced teas produced with varying concentrations of Hibiscus extract powder to be between 7.50% and 7.84%. Giritlioğlu et al. (2020) found that Kombucha tea samples produced through the fermentation of caper buds and green tea exhibited higher dry matter content compared to the control group containing only capers. The results of these researchers are consistent with the findings of the current study.

Table 2. Changes in physicochemical parameters determined during fermentation in Kombucha samples

Analyses	**Days**	**Samples**				
		K	**KPM$_1$**	**KPM$_2$**	**KPM$_3$**	**Average**
Dry matter (%)	1	8.48±0.23	14.46±0.01	16.02±0.60	17.91±0.72	14.22±3.79^{ab}
	3	8.98±0.94	14.20±0.28	16.07±0.02	16.33±0.05	13.89±3.18^{ab}
	5	8.66±0.47	12.59±4.86	16.94±0.73	18.15±1.11	14.08±4.44^{ab}
	7	8.62±0.63	14.31±0.43	16.19±0.61	17.14±0.24	14.06±3.55^{ab}
	9	8.49±0.14	10.96±1.24	16.29±0.41	17.25±0.35	13.24±3.92b
	11	8.40±0.14	12.02±0.11	16.43±0.60	17.45±0.63	13.57±3.88^{ab}
	13	8.56±0.20	14.32±0.60	17.54±0.08	18.67±0.10	14.77±4.20^{a}
	Average	8.60±0.39^{d}	13.26±1.95^{c}	16.49±0.64^{b}	17.55±0.85^{a}	
pH	1	6.47±0.02	4.43±0,01	4.41±0.00	4.38±0.01	4.92±0.95^{a}
	3	6.04±0.04	4.46±0.02	4.42±0.02	4.39±0.00	4.83±0.74^{abc}
	5	6.04±0.04	4.46±0.02	4.43±0.02	4.40±0.00	4.83±0.74^{abc}
	7	6.05±0.03	4.37±0.07	4.40±0.03	4.62±0.03	4.86±0.73^{ab}
	9	5.81±0.15	4.40±0.00	4.49±0.00	4.53±0.31	4.81±0.63^{bc}
	11	5.68±0.25	4.25±0.02	4.36±0.02	4.41±0.01	4.67±0.62^{d}
	13	5.99±0.01	4.27±0.01	4.33±0.07	4.40±0.07	4.75±0.77^{cd}
	Average	6.01±0.25^{a}	4.38±0.08^{b}	4.40±0.05^{b}	4.44±0.12^{b}	
Total acidity (%)	1	0.45±0.07	3.15±0.21	3.05±0.07	3.40±0.14	2.51±1.28^{ab}
	3	0.25±0.07	2.95±0.07	3.05±0.35	3.55±0.07	2.45±1.38^{abc}
	5	0.25±0.07	2.95±0.07	3.05±0.35	3.55±0.07	2.45±1.38^{abc}
	7	0.25±0.07	2.65±0.07	3.00±0.14	3.35±0.07	2.31±1.30^{c}
	9	0.50±0.14	2.95±0.07	3.25±0.07	3.60±0.28	2.57±1.31^{a}
	11	0.35±0.07	2.95±0.07	3.45±0.01	3.65±0.07	2.60±1.41^{a}
	13	0.35±0.07	2.75±0.07	3.05±0.01	3.35±0.07	2.37±1.27^{bc}
	Average	0.34±0.11^{d}	2.90±0.17^{c}	3.12±0.21^{b}	3.49±0.15^{a}	
Viscosity (cP)	1	225±35.35	150±42.43	290±127.2	230±155.5	223±95.00^{d}
	3	270±42.42	375±106.6	450±70.7	587±77.7	420±136.65^{c}
	5	225±77.78	586±93.33	435±49.4	955±35.3	550±289.5^{b}
	7	261±69.29	555±134.3	469±195.1	694±149.9	494±200.2^{bc}
	9	325±7.07	502±110.3	743±34.6	538±36.7	527±165.4^{b}
	11	347±52.32	498±4.94	673±45.2	790.5±12.0	577±182.3^{ab}
	13	348±16.26	586±3.90	690±98.9	949±26.8	643±236.3^{a}
	Average	285±63.1^{d}	464±165.2^{c}	535±177.8^{b}	677±254.1^{a}	

K: Control (only green tea); KMP$_1$: Green tea+25% Whey+mormix+mulberry powder; KMP$_2$: Green tea+50% whey+mormix+mulberry powder and KMP$_3$: Green tea+75% whey+mormix+mulberry powder. *The difference between values with different letters is statistically significant ($p<0.05$).

The pH of the whey-containing samples was lower than that of the control. It is also thought that the presence of mormix, which is acidic and supplemented with whey, affects this result. The lowest pH was measured as 4.25 in the KMP$_1$ sample, while the highest was determined as 6.47 in the K sample. Among the tea samples, the Kombucha tea type with the lowest pH values at the beginning and end of fermentation compared to other samples is the KPM$_1$ sample ($p<0.05$). Primiani et al. (2018) reported that the lowest pH value of the Kombucha produced from different plants (green tea, rosella, mangosteen peel, cinnamon, and seduhan leaf) on mango peel was 2.57, and the highest value was 3.05 for green tea. This result may be due to increased concentration of other organic acids, especially acetic and lactic acid, during fermentation (Chen and Liu, 2000).

The total acidity varied alongside pH values across different fermentation days. These variations are believed to be attributed to the raw materials used in Kombucha production and the fermentation process. The average total acidity values for the control group (K), and the groups with 25% whey + mormix + mulberry powder (KMP1), 50% whey + mormix + mulberry powder (KMP_2), and 75% whey + mormix + mulberry powder (KMP_3) were determined to be 0.34, 2.90, 3.12, and 3.49, respectively. The increase in acidity was notably higher in Kombucha samples containing whey (KMP_3 and KMP_2) and relatively lower in the Kombucha tea made solely with green tea (K). Significant differences were observed in the samples on the 9th day of fermentation ($p<0.05$). It is thought that the differences in acidity values occurred as a result of the microbial activity from molds and acetic acid bacteria (AAB), combined with varying sugar concentrations and compositions in the samples over different storage times. Cardoso et al. (2020) reported that the predominant organic acid found in Kombucha samples is acetic acid, at 3 g L^{-1}, and that this is produced through the oxidation of ethanol during fermentation by acetic acid bacteria. Kallel et al. (2012) noted that the total acidity (in terms of acetic acid) of Kombucha produced from green and black teas was 0.35 and 0.45 g L^{-1}, respectively, on the first day, and 5.4 and 8 g L^{-1} on the 15th day.

The effect of Kombucha samples, fermentation, and the interaction between sample and fermentation on viscosity results was statistically significant ($p<0.01$). The use of varying amounts of whey in Kombucha production increased the viscosity values of the samples throughout fermentation. The KMP_3 sample exhibited the highest average viscosity value (677 cP). On the first day of fermentation, the lowest viscosity value (150 cP) was recorded for the KMP_1 sample, while on the 5th day of fermentation, the highest value (955 cP) was observed for the KMP_3 sample. Akarca and Tomar (2020) measured the viscosity values of red and purple vegetables prepared from Kombucha teas at 50 rpm (cP) for red carrot, red beet, and purple cabbage samples, reporting values of 57.19, 72.50, and 42.38, respectively. Balcıoğlu (2013) reported viscosity values ranging from 967 to 1515 cP in a study of strawberry fermented milk drinks, emphasizing that both strawberry concentration and fermentation time increased the viscosity of the samples. Additionally, Watawana et al. (2016) noted that the viscosity of Kombucha tea from coconut water increased due to fermentation. It is believed that the differences observed between these studies are due to variations in production methods, raw materials, and fermentation conditions.

3.3. Color results

The color values of foods are among the evaluation criteria that affect consumers' preference points. The differences between the Kombucha beverages and the L^*, a^*, b^*, C, $\alpha°$, and ΔE^* values at different fermentation times are shown in *Figure 3a-f.* As the whey concentration increased, the L^* values of the beverage samples fluctuated. It is generally thought that the mormix is effective at decreasing the L^* value as the concentration increases. The lowest L^* value of the beverage samples (2.69) was detected on the 1st day of fermentation in the KMP_3 sample, and the highest value (34.55) was detected on the 13th day of fermentation in the K sample.

Watawana et al. (2016) emphasized that the decrease in the L^* value in Kombucha samples may be due to the decrease in pH affected by fermentation and the breakdown of color pigments and polyphenolic components resulting from the development of microorganisms. Whey and mormix additions caused a decrease in a^* parameter values, and the lowest a^* was found in the KMP_2 and KMP_3 groups. It is thought that the presence of whey, especially the mormix, affects these findings. The average a^* values of the Kombucha samples during the fermentation periods were determined to be between 9.36 and 13.63. Savan (2022) stated that during the first fermentation of Kombucha samples produced with persimmon, the a^* value decreased in black and oolong tea samples and increased in green tea. Depending on the persimmon concentration, the a^* value of the samples decreased with the addition of 5% persimmon to green tea and was reported to decrease further with the addition of 10% persimmon. The addition of mormix decreased the b^* values of the beverage samples. This color difference may be due to the use of the mormix as a raw material. The lowest b^* values of the beverages were found in the KMP_2 and KMP_3 samples, and the highest value was found in the K sample. Giritlioğlu et al. (2020) reported b^* values of 4.29, 2.74, and 3.45 for samples produced from Kombucha green tea, a caper bud, and a green tea + caper bud, respectively.

The lowest C value of the beverage samples (4.60) was detected on the 3rd day of fermentation in KMP_2, and the highest value (52.64) was detected on the 13th day of fermentation in the K sample (*Figure 3d*). The lowest $\alpha°$ value of Kombucha beverages (-0.03) was determined on the 1st day of fermentation in the KMP_1 and KMP_2 samples, and the highest value (86.19) was determined on the 7th day of fermentation in the K sample. With increasing mormix+mulberry powder and whey concentration, the C values in the beverage samples generally increased, and the $\alpha°$ values were lower than the control. The lowest ΔE^* value (5.88) was found in the KMP_2 group on the 3rd day of fermentation, and the highest value (62.97) was found in the K group on the 13th day of fermentation. An increase in whey concentration caused Kombucha beverages to obtain positive *ΔE** values.

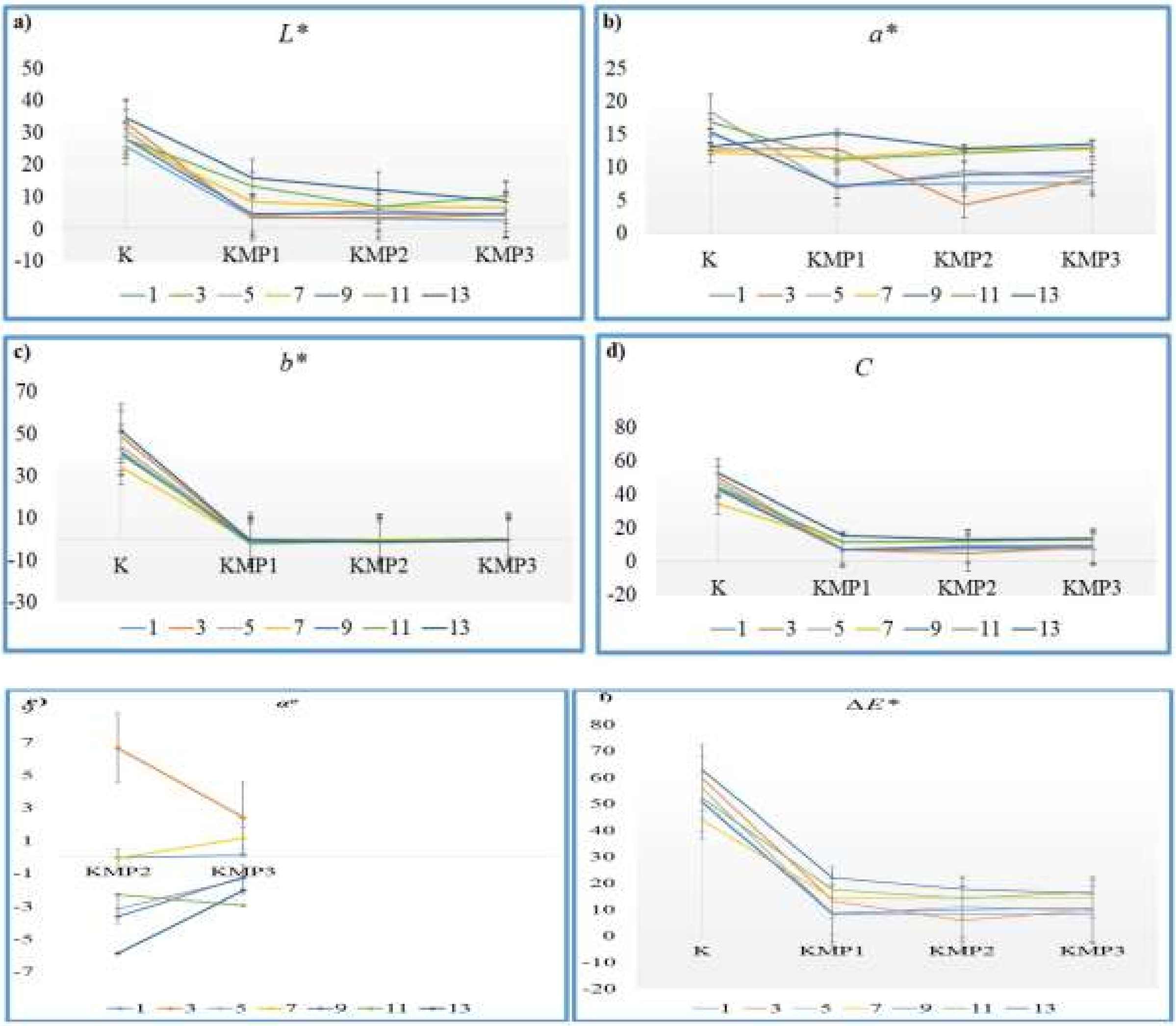

Figure 3a-f. Changes in color parameters (L*, a*, b*, C, α°, and ΔE*) during fermentation in Kombucha samples

3.4. Total phenolic content and antioxidant capacity

The effect of enrichment with different concentrations of whey or of mormix+mulberry powder and fermentation on the TPC was determined to be significant (p<0.05). The lowest TPC was found in the K group (981.33 mg GAE g^{-1}) on the 5th day of fermentation, and the highest value was found in the 1% KMP_1 group (1515.3 mg GAE g^{-1}) on the 3rd day (*Figure 4a*). During the fermentation process, the formation of small molecules such as phenolic substances, tannins, and flavonoids with higher antioxidant activity, depending on the activities of microorganisms in the SCOBY culture, can increase the TPC (Chu and Chen, 2006; Jayabalan et al., 2007; Bhattacharya et al., 2016; Gamboa-Gómez et al., 2016; Liamkaew et al., 2016; Ulusoy, 2019). However, long-term fermentation causes a decrease in polyphenol concentration due to microorganisms using phenolic compounds to provide themselves with energy (Watawana et al., 2016).

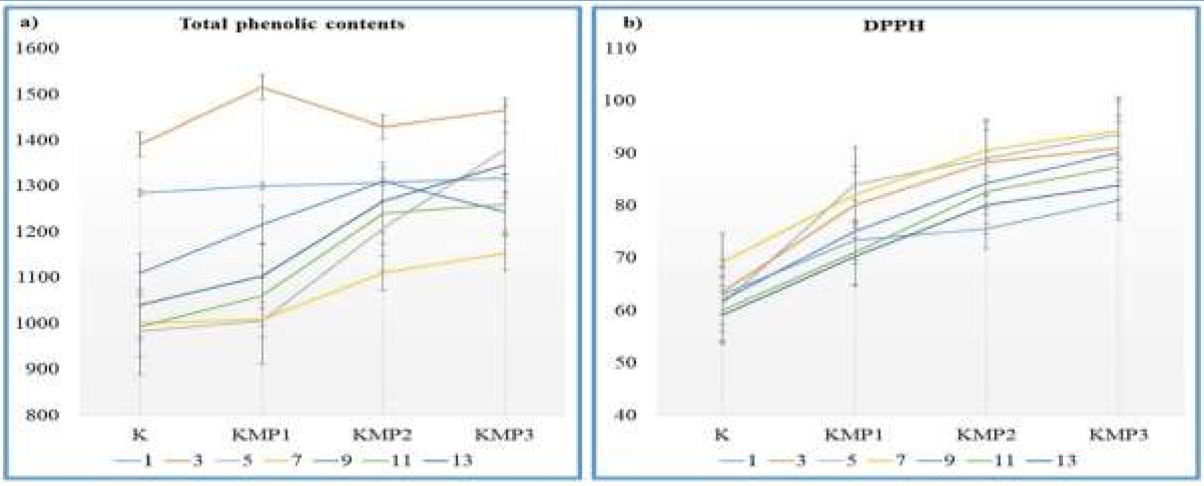

Figure 4. a) Total phenolic contents (mg GAE g^{-1}) and b) Antioxidant capacities (DPPH %) determined in Kombucha samples during fermentation

Pereira et al. (2014) reported the TPC in Kombucha samples produced with green and black teas as 1080 mg GAE L^{-1} and 1120 mg GAE L^{-1}, respectively. Bhattacharya et al. (2013) reported that Kombucha species produce Kombucha fruits using black and green tea and sucrose (for a fermentation period of 14th days) and that the phenolic compound content in Kombucha beverage samples was greater than that in black and green tea samples. According to the data in *Figure 4b*, while the concentrations of the components in the control sample decreased, the DDPH values of the Kombucha tea samples increased as the whey concentration increased and with the influence of the mormix. The lowest total antioxidant value (59.03%) of the Kombucha beverage was found on the 13th day of fermentation in the K sample, and the highest value (94.29%) was found on the 7th day of fermentation in the KMP_3 sample. In different studies, it was determined that the antioxidant capacity and TPC decreased in Kombucha tea samples during fermentation (Chu et al., 2006; Yıkmış and Tuğgüm, 2019). Differences were observed in the antioxidant rates in the beverages compared to those in the control sample ($p<0.05$). The reason for this, the presence of lactic acid bacteria in SCOBY may release many bioactive substances in mormix and whey, such as exopolysaccharides, which contribute to the free radical scavenging ability of Kombucha (Amjadi et al. 2023).

Jakubczyk et al. (2020) reported that fermentation time and tea type (green, black, white, or red tea) are effective at enhancing the antiradical properties of Kombucha and in terms of tea type, Kombucha prepared from green tea has the highest antioxidant potential. The results of the present study, which are similar to the findings of previous researchers, suggest that whey and mormix can be used in Kombucha samples together with green tea as good sources of antioxidants.

3.5. Sensory results

The results of the sensorial evaluation of the Kombucha samples are given in *Table 3*. An increase in the mormix+mulberry powder and whey concentrations caused differences in the scores fluctuations given by the panelists regarding color and appearance. In general, color and appearance values were found to be higher than the control group on the 1st day of fermentation. The lowest color and appearance score of the samples (5.65) was determined on the 7th day of fermentation in the K sample, and the highest score (8.45) was determined on the 1st day of fermentation in the KMP_3 sample. With increasing whey concentration, the odor values of the beverage samples differed during fermentation. The panelists gave the lowest odor score (2.95) to the KMP_3 sample, which had the highest whey concentration on the 13th day of fermentation, and the highest score (8.35) to the KMP_2 sample on the 9th day of fermentation. Giritlioğlu et al. (2020) reported that the odor scores of Kombucha fruits produced with caper buds ranged from 4.85 to 5.65. The researcher stated that since the acid content of Kombucha is a product with a sharp odor, the panelists used scores that were not high and very close to each other when evaluating the odor scores. Differences in taste scores were found throughout the fermentation period for all the samples. Additionally, it was determined that the acidic taste scores of the beverage samples decreased with increasing whey concentration. The lowest acidic taste score of the beverages (2.82 points) was found in the KMP_3 group on the 13th day of fermentation, and the highest

Table 3. Changes in sensory parameters of Kombucha samples during fermentation

Parameters	Kombucha	Fermentation Time (days)							
		1	3	5	7	9	11	13	Average
Color and appearance	K	6.90±1.97	6.65±2.19	7.04±2.62	5.65±0.91	7.55±0.77	7.53±0.66	6.03±0.04	6.76±1.34^{a}
	KPM$_1$	7.30±0.42	7.32±0.45	7.09±0.12	6.60±0.56	7.82±1.15	8.20±0.28	6.15±0.21	7.21±0.78^{a}
	KPM$_2$	8.23±0.32	7.96±0.05	5.83±0.24	7.47±0.66	7.15±1.62	7.82±0.25	5.88±0.16	7.19±1.06^{a}
	KPM$_3$	8.45±0.63	6.20±0.28	5.90±0.14	7.20±1.13	6.28±1.81	7.25±1.76	5.73±0.38	6.71±1.22^{a}
	Average	7.72±1.06^{a}	7.03±1.11^{abc}	6.46±1.18^{bc}	6.73±0.98^{abc}	7.20±1.22^{ab}	7.70±0.82^{a}	5.94±0.24^{c}	
Odor	K	6.86±1.22	5.24±1.75	5.11±0.16	5.28±2.43	6.30±0.98	6.11±0.16	5.67±0.94	5.79±1.16^{a}
	KPM$_1$	5.79±0.29	6.30±1.83	7.65±0.49	7.79±1.12	8.10±1.55	6.75±0.35	5.89±1.25	6.89±1.23^{a}
	KPM$_2$	7.63±0.89	7.85±1.20	4.85±1.20	6.30±1.83	8.35±0.49	7.17±1.17	5.75±1.06	6.84±1.48^{a}
	KPM$_3$	6.25±1.76	3.45±0.63	3.25±1.76	3.40±3.39	4.45±0.63	3.85±2.61	2.95±2.75	3.94±1.92^{b}
	Average	6.63±1.15^{a}	5.71±2.03^{a}	5.21±1.87^{a}	5.69±2.46^{a}	6.80±1.84^{a}	5.97±1.75^{a}	5.06±1.82^{a}	
Taste	K	6.06±4.32	6.83±3.06	4.80±1.69	4.90±0.14	6.25±1.06	6.32±0.96	4.68±0.45	5.69±1.80^{ab}
	KPM$_1$	6.87±0.17	6.64±1.91	5.17±4.00	6.82±1.66	6.81±0.26	7.36±0.50	5.90±1.27	6.51±1.54^{a}
	KPM$_2$	7.30±0.42	6.98±0.02	5.11±2.99	6.57±0.81	7.32±0.45	5.29±0.41	3.83±0.23	6.06±1.55^{ab}
	KPM$_3$	8.42±0.59	4.73±1.03	5.80±2.54	5.85±1.20	4.32±0.96	3.14±1.61	2.83±0.23	5.01±2.08^{b}
	Average	7.16±1.89^{a}	6.29±1.72^{a}	5.22±2.24^{ab}	6.03±1.15^{ab}	6.17±1.34^{ab}	5.52±1.82^{ab}	4.31±1.31^{b}	
Acidic Taste	K	8.91±0.12	7.82±1.66	4.67±3.29	7.37±2.30	7.86±0.19	4.80±0.28	5.47±2.16	6.70±2.11^{a}
	KPM$_1$	6.12±0.17	6.25±1.06	7.82±0.25	6.58±0.82	7.32±1.86	6.89±0.14	4.17±0.24	6.45±1.29^{a}
	KPM$_2$	8.74±1.04	4.82±3.08	5.39±0.55	6.63±0.51	5.67±0.95	5.17±1.16	3.82±1.15	5.75±1.84^{a}
	KPM$_3$	5.83±1.64	3.82±0.25	3.43±0.60	5.73±3.20	3.73±0.38	4.79±1.11	2.82±1.15	4.30±1.58^{b}
	Average	7.40±1.69^{a}	5.67±2.12^{bc}	5.32±2.14^{bc}	6.58±1.65^{ab}	6.14±1.90^{ab}	5.41±1.11^{bc}	4.07±1.44^{c}	
General acceptability	K	6.82±1.15	7.10±1.55	6.68±0.44	6.80±1.13	6.07±0.09	5.18±3.08	3.95±1.47	6.08±1.58^{a}
	KPM$_1$	7.17±0.24	5.78±0.31	7.81±1.14	7.57±0.60	6.75±0.35	8.41±0.57	3.82±0.25	6.76±1.54^{a}
	KPM$_2$	8.09±0.00	7.42±0.59	5.74±1.05	7.41±0.83	7.67±0.94	4.97±2.87	2.76±1.07	6.29±2.09^{a}
	KPM$_3$	5.82±2.57	3.75±1.06	3.32±0.96	6.41±2.24	5.09±1.54	4.68±2.38	1.73±1.03	4.40±2.02^{b}
	Average	6.97±1.38^{a}	6.01±1.71^{a}	5.89±1.90^{a}	7.05±1.14^{a}	6.39±1.22^{a}	5.81±2.45^{a}	3.06±1.25^{b}	

*K: Control (only green tea); KMP$_1$: Green tea+25% Whey+mormix+mulberry powder; KMP$_2$: Green tea+50% whey+mormix+mulberry powder and KMP$_3$: Green tea+75% whey+mormix+mulberry powder.
*The difference between values with different letters is statistically significant (p<0.05).

score (8.91) was found in the K group on the 1st day of fermentation. Panelists showed a negative approach toward an increase in whey concentration when general acceptability scores were given. In the first days of fermentation, the KMP_2 and KMP_1 samples were especially appreciated. Odor and taste changes, which are thought to be caused by whey during the last days of fermentation, may have negatively affected panelist preferences. Giritlioğlu et al. (2020) reported the highest overall liking scores for Kombucha samples containing a caper bud and green tea in the (5.61 point) sample, which they obtained via fermentation with a caper bud and green tea.

4. Conclusions

This study aimed to prepare a new product with improved nutritional and physiological properties by adding whey and mormix+mulberry powder to Kombucha samples, which are functional products. The effects of whey and mormix+mulberry powder addition and fermentation variables on the number of acetic acid bacteria were found to be statistically significant ($p<0.01$). *Lactobacillus* spp. were 4.36–6.35 $\log_{10}$ cfu mL^{-1}, *Lactococcus* spp. were 5.64–7.15 $\log_{10}$ cfu mL^{-1}, and mold counts were 5.46–7.30 $\log_{10}$ cfu mL^{-1}. Content and fermentation time had a statistically significant effect on the viscosity of the samples ($p<0.01$). The total phenolic content of the Kombucha samples was the highest in the green tea+75% whey+mormix+mulberry powder (KMP_3) sample. In addition, the antioxidant capacities of the functional drinks were greater than control sample. The KMP_1 sample was more liked than the other samples were. Taken together, the results of the present study suggested that the use of green tea, whey, mulberry powder, or mormix fermented with Kombucha can significantly contribute to the health of these plants, considering the effect of consuming foods with high biological value. Moreover, these newly obtained functional drinks with high nutritional value can be considered a refreshing beverage type, especially in the summer months, because of their color, slight sour taste, and aroma originating from whey.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Authorship Contribution Statement

Concept: Bebek E., Yangılar, F.; Design: Yangılar, F.; Data Collection or Processing: Bebek E., Yangılar, F.; Statistical Analyses: Yangılar, F.; Literature Search: Bebek E., Yangılar, F.; Writing, Review and Editing: Yangılar, F.

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