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Prunus mahaleb'in Fermente Çay İçeceklerinde Biyokimyasal ve Mikrobiyolojik Etkilerinin Araştırılması

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Öne Çıkanlar:

- Kombucha dünya genelinde oldukça popüler fermente bir içecektir
- Mahlep, geleneksel kombu çayını tatlandırmak için kullanılabilecek tıbbi bir bitkidir
- Bu bitki ile fermente edilen kombu çayı, geleneksel kombu çayı ile benzer biyoaktivite göstermiştir

Anahtar Kelimeler:

- Antibakteriyel aktivite
- Biyoaktivite
- Fenolik içerik
- Flavonoid içerik
- Kombu çayı fermantasyonu,
- Duyusal analiz

ÖZET:

Günümüzde kombu çayı gibi fermente içecekler, sağlığa yararlı etkileri sebebiyle tüketiciler arasında oldukça popülerdir. Kombu çayı fermantasyonu için genellikle siyah çay kullanılsa da çeşitli bitkilerin eklenmesi ile hazırlanan kombu çayları da giderek daha popüler hale gelmektedir. Bu çalışmada, kombu çayı fermantasyonu için tıbbi öneme sahip olan mahlep kullanılmıştır. Fermente edilen aromalı kombu çaylarının antioksidan aktivitesini belirlemek için DPPH yöntemi, toplam fenolik ve flavonoid içeriğini belirlemek için sırasıyla Folin-Ciocalteu ve alüminyum klorür kolorimetrik yöntemi ve antibakteriyel etkinliğini belirlemek için disk difüzyon yöntemi kullanılmıştır. Ayrıca kombu çaylarının mikrobiyolojik bileşimi ve duyusal değerlendirmeleri de bu çalışmada yapılmıştır. Sonuç olarak, mahlep aromalı kombu çayının antioksidan aktivitesinin, geleneksel kombu çayından istatistiksel olarak farklı olmadığı gösterilmiştir ($p>0.05$). Diğer yandan içeceklerin fenolik madde miktarları karşılaştırıldığı zaman, geleneksel kombu çayının fermantasyonun 7, 14 ve 21. günlerinde en yüksek fenolik içeriğe sahip olduğu bulunmuştur ($p<0.05$). İçeceklerin toplam flavonoid içeriği karşılaştırıldığında mahlep aromalı kombu çayının 7. günde geleneksel kombu çayı ile benzer içeriğe sahip olduğu görülmüştür. Ancak 21. günde mahlep aromalı kombu çayının toplam flavonoid madde miktarının daha yüksek olduğu bulunmuştur ($p<0.05$). İçeceklerin mikrobiyolojik profilleri karşılaştırıldığı zaman fermentasyonun 7. ve 14. günlerinde maya miktarının geleneksel kombu çayında, toplam mezofilik bakteri ve asetik asit bakteri miktarlarının ise mahlep aromalı kombu çayında yüksek olduğu görülmüştür. Duyusal değerlendirmelerde mahlep aromalı kombu çayı tüm kriterler değerlendirildiğinde en yüksek puanları almış ve katılımcılar arasında en popüler fermente içecek olmuştur. Gerçekleştirilen bu çalışma kombu çayı fermantasyonunda mahlep bitkisinin kullanıldığı ilk çalışmadır.

Investigation of Biochemical and Microbiological Effects of *Prunus mahaleb* in Fermented Tea Beverage

Highlights:

- Kombucha is a very popular fermented beverage around the world
- Mahaleb is an medicinal herb can be used to flavour the traditional kombucha
- Kombucha fermented with this herb showed similar bioactivityt to traditional kombucha

Keywords:

- Antibacterial effect
- Bioactivity
- Phenolic content
- Flavonoid content
- Kombucha fermentation
- Sensory analysis

ABSTRACT:

Nowadays, fermented beverages such as kombucha are particularly popular among customers because of their health benefits. Although black tea is often used as a substrate for kombucha fermentation, kombucha drinks prepared with various herbal teas are becoming increasingly popular. In this study, the medicinal plant, mahaleb was used for kombucha fermentation. The DPPH method was used to determine the antioxidant activity of kombucha drinks, the Folin–Ciocalteu and aluminium chloride colorimetric method was used to determine the total phenolic and flavonoid content of the drinks, respectively, and the antibacterial activity was determined by the disc diffusion method of the drinks. In addition, the microbiological composition and sensory analysis of the kombucha drinks were investigated. The antioxidant activity of the mahaleb-flavoured kombucha was not statistically different from those of traditional kombucha ($p>0.05$). As for phenolic content, traditional kombucha had higher content on all fermentation days ($p<0.05$). The total flavonoid content of mahaleb-flavoured kombucha was similar to traditional kombucha on day 7 of fermentation ($p>0.05$), but higher than traditional kombucha on day 21 of fermentation ($p<0.0.5$). In the analysis of the microbiological profile, the highest values of total mesophilic bacteria and acetic acid bacteria content were observed in mahaleb-flavoured kombucha and yeast in traditional kombucha on the 7th and 14th day of fermentation. Mahaleb-flavoured kombucha scored highest on all criteria and was the most popular beverage among participants. This is the first study in which mahaleb was used in kombucha fermentation.

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INTRODUCTION

Mahaleb (*Prunus mahaleb* L.) is a medicinal plant with different effects and biochemical properties and is widely used among the public as traditional medicine. Although mahaleb is generally widespread in Mediterranean countries, it is also popular in North Africa, West and Central Asia and European countries (Farag et al., 2021). Mahaleb is an annual shrub or deciduous small tree belonging to the Rosaceae family (Özçelik et al., 2012). This plant has a high content of polyphenolic compounds and has dark purple fruits with a bitter cherry-like taste (Gerardi et al., 2016; Dadalı and Elmacı, 2021). While the seeds are also used as a spice with beneficial properties, ground seed kernels and fruits are used in some bakeries in Mediterranean countries as a pleasant spice source (Oskoueian et al., 2012; Özçelik et al., 2012). The seeds are rich in protein (30.98%) and fat (40.40%) as well as antioxidant and phenolic substances (Mariod et al., 2010; Güven, 2020). It has also been reported that the seeds of this plant are effective against diabetes, used in traditional medicine against gastrointestinal problems, and recognized as a natural bioactive compound for medicinal purposes (Al-Said and Hifnawy, 1986; Oskoueian et al., 2012; Özçelik et al., 2012). Seyyednejad et al. (2008) stated that mahaleb can be used in folk medicine and can be considered a disinfectant or antiseptic.

Nowadays, the protection of the microbiota, together with the awareness of a healthy diet is gaining importance worldwide (Selhub et al., 2014; Galimberti et al., 2021; Mohammed et al., 2021). In this context, consumers are rediscovering traditional fermented beverages and foods (Marco et al., 2017). One of the most popular traditional fermented beverages is kombucha. Its history dates back to antiquity times and is becoming increasingly popular in western countries (Pure and Pure, 2016; Coelho et al., 2020). This beverage is made by fermenting sweetened black tea with a special symbiotic mixture of different yeast (*Saccharomyces* sp., *Zygosaccharomyces kombuchensis*, *Candida* sp., *Pichia fluxum* etc.) and bacteria (*Acetobacter aceti*, *Gluconobacter oxydans*, *Acetobacter pasteurianus*, *Bacterium gluconicum*, *Acetobacter xylinum* etc.) living in a specific cellulosic matrix (Kurtzman et al., 2001; Marsh et al., 2014; Morales, 2020). The symbiotic relationship of microorganisms in kombucha can also be referred to as “tea fungus” or “SCOBY” (Santos et al., 2009; Villarreal-Soto et al., 2018). Fermentation is started by adding a sample of the tea fungus and a liquid portion of the previous fermentation, called “soup”, to freshly prepared sugary black tea (Ayed et al., 2017). During fermentation, the taste of the sweetened black tea transforms into a pleasant, sparkling beverage, and as the fermentation time increases, a slight vinegar taste becomes noticeable, which is due to the production of acetic acid (Blanc, 1996). The fermentation period of this symbiotic culture in kombucha is generally between 7 and 14 days under aerobic conditions in the traditional fermentation process. Kombucha has been reported to help prevent various metabolic diseases, psoriasis, hypertension, constipation, antihyperglycemic, and chronic diseases, but mostly in models (Sreeramulu et al., 2000; Mo et al., 2008; Chakravorty et al., 2016; Neffe-Skocińska et al., 2017). In addition, this drink has been reported to regulate metabolism and intestinal flora as it is also classified as a probiotic drink (Magalhães-Guedes et al., 2019; Mousavi et al., 2020). Kombucha is very popular among consumers and is combined with other medicinal plants because of its positive effects on human health. For this reason, it is important to know the biochemical properties of the beverage and track the changes that occur when this beverage is combined with other medicinal plants. There are many studies covering the use and biochemical activities of different medicinal plants for kombucha fermentation (Velićanski et al., 2014; Shahbazi et al., 2018; Vitas et al., 2020; Eroğlu et al., 2021; Zou et al., 2021; Tefon Öztürk et al., 2023).

Medicinally important plants are known to be natural sources of antioxidants (Mohammed et al., 2019; Mohammed et al., 2020), and the variety of kombucha products on the market can be increased by using these natural antioxidants as substrates in kombucha fermentation (Vitas et al., 2020).

In this study, changes in bioactivities such as total phenolic and flavonoid content, antioxidant capacity, antibacterial properties, pH changes, microbiological profiles, and sensory analyses of traditional kombucha beverages flavoured with a traditional medicinal plant (*P. mahaleb*) were investigated. Mahaleb was selected for fermentation because of its medicinal importance. To investigate the effects of this plant on kombucha tea, flavoured and fermented kombucha beverages were prepared with ground seeds of mahaleb, as these plant parts are commonly used by the public as a spice. Moreover, this is the first study to show the results of its use in kombucha fermentation.

MATERIALS AND METHODS

Plant Materials

The powder of the mahaleb seeds was purchased from a local market in Kepez, Antalya.

Preparation of Culture Media

In this study, five different infusions were used as culture media. The protocol described by Marsh et al. (2014) was slightly modified for the preparation of the infusions. In general, 5% dry leaves (w v^{-1}) were added to 1 litre of boiled water and then soaked for 15 min. The sugar solution, boiled elsewhere for 1 min to sterilise, was added to this solution so that the final volume was 9% (w v^{-1}). The solution was cooled to room temperature (RT) and the dry leaves were removed by filtration. For the samples of traditionally fermented kombucha and non-fermented black tea, 5% (w v^{-1}) dry black tea leaves (Lipton, Turkiye) were used to prepare a black tea infusion. For the samples containing fermented mahaleb and non-fermented mahaleb, the infusion of mahaleb was used instead of black tea, and the same procedure was followed. To prepare infusions for mahaleb-flavoured kombucha (KM), 5% (w v^{-1}) dry herbs were combined with 5% (w v^{-1}) dry black tea leaves, and the same procedure was followed.

In this study, regional kombucha tea (Antalya, TURKIYE) was used. 10% soup and 2% (w v^{-1}) tea fungi of the culture previously grown for 14 days were inoculated into 100 mL of the corresponding broth for each fermentation. The non-fermented samples were not inoculated. All these samples were then incubated for 21 days at RT. On days 0, 7, 14, and 21 of incubation, samples were collected from the liquid part of the culture for bioactivity analysis and microbiological profiling.

Microbiological Composition Dynamics

The changes in microbiological profiles of mahaleb-flavoured kombucha and traditional kombucha due to fermentation were studied on days 0, 7, 14, and 21. Serial dilutions of each sample were prepared in 0.85% saline (NaCl solution) (Sigma, USA) with 10 mL final volume. Then the samples were inoculated into selective media; Glucose Yeast Extract Agar (for acetic acid bacteria), Yeast Extract Glucose Chloramphenicol Agar (for yeast) (Merck, Germany), De Man Rogosa and Sharpe Agar (for *Lactobacillus*) (Merck, Germany)) and Plate Count Agar (for total mesophilic bacteria) (Merck, Germany). The samples were incubated for 5 days and after the incubation colony forming units per mL (cfu mL^{-1}) were calculated according to Equation 1:

$$N = \frac{C}{[V \times (n_1 + (0.1 \times n_2))]} \times d \qquad (1)$$

In this equation, N stands for the total number of microorganisms mL^{-1}, C for the total number of colonies counted in the samples, V for the inoculation volume in the samples, n_1 for the replicates number of prepared samples with first serial dilution, n_2 represents the replicate number of prepared samples with the second serial dilution and for the ratio of the most concentrated serial dilution (Halkman, 2000).

pH Measurement

On days 0, 7, 14, and 21 of incubation, samples were taken from the liquid part of the culture for pH measurement with pH meter (Isolab, Germany). Then, the samples were prepared for further analysis by centrifugation at 3200 rpm for 10 minutes, and the supernatant was sterilised with a 0.45 µm pore diameter filter (GVS North America Sanford, USA).

Antibacterial Activity Tests

Six different well-defined bacteria were tested with disc diffusion method for antibacterial activity (Bauer, 1966). *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus cereus* (DSM 22648), *Staphylococcus aureus* (ATCC 29213), *Klebsiella pneumoniae* (ATCC 13883) and *Staphylococcus epidermidis* (ATCC 12228) were used as test bacteria for antimicrobial activity of both fermented and non-fermented samples. For this assay, fresh overnight cultures of each bacterium were inoculated into a sterile 0.85% NaCl solution (Merck, Germany) until the mixture was adjusted to 0.5 McFarland. Once this concentration was reached, Nutrient Agar Medium (NA) (Merck, Germany) was inoculated with 200 µL of the culture via a drigalski. After inoculation, impregnated discs were placed on the inoculated samples. As a test group, 50 µL of the sterile samples were impregnated into blank antibiogram discs (Bioanalyses, Turkiye). For the control group, 20 µL of kanamycin (30 µg mL^{-1}) (Cayman, ABD) and 20 µL of ampicillin (30 µg mL^{-1}) (Sigma, USA) were impregnated into antibiogram discs for each test organism. The inoculated Petri dishes containing the discs were incubated for 24 h at 37°C. The diameter of the inhibition zones formed after the incubation period was measured. All experiments were repeated in 3 replicates.

DPPH Antioxidant Capacity

The protocol described by Von Gadow et al. (1997) was used to measure antioxidant capacity. For this purpose, 100 µL of each sterilised sample was mixed with 4 mL of $6x10^{-5}$ M DPPH (2,2-diphenyl-1-picrylhydrazyl) (Sigma, USA) in methanol. The mixtures were incubated 30 min in the dark. After incubation, absorbance was measured at OD516. The antioxidant capacities of each sample were calculated, and the percentage of inhibition was calculated according to Equation 2 (Hrnjez et al., 2014):

$$\%Inhibiton = \frac{(Absorbance_{Control} - Absorbance_{Sample})}{Absorbance_{Control}} \times 100 \qquad (2)$$

In addition, the antioxidant capacity of each sample was calculated in ascorbic acid equivalents (AAE µM mL^{-1}) using the ascorbic acid calibration curve.

Determination of Total Phenolic Content (TPC)

The Škerget et al. (2005) method was used to investigate the TPC of beverages. The protocol described by Singleton and Rossi (1965) was used to prepare the Folin-Ciocalteu reagent. According to the protocol, 500 µL of the sterilised samples were mixed with 2.5 mL of the FC reagent (1:10 diluted) and incubated for 2 min at room temperature (RT). Then 2 mL of sodium carbonate (Na_2CO_3) (7.5%) (Merck, Germany) was added to the mixture and vortexed for 30 s. To complete the reaction, the samples were incubated at 50 °C for 5 min and then cooled at RT for 5 min. The absorbance of the samples was measured at OD760 using a spectrophotometer. Then, the gallic acid equivalent (GAE) of the samples was calculated in µg mL^{-1} using the previously generated calibration curve.

Determination of Total Flavonoid Content (TFC)

A colorimetric method based on aluminium chloride described by Dwiputri and Feroniasanti (2019) was used to determine TFC. According to this protocol, 500 µL of sterile samples were mixed in the order 2.8 mL distilled water, 100 µL $AlCl_3$ (10%) (Merck, Germany), 100 µL 1 M CH_3CO_2K (Merck, Germany) and 1.5 mL methanol (Isolab, Germany). Then, the mixtures were incubated for 30 min at RT. The absorbance at 415 nm was measured with a spectrophotometer, and the quercetin equivalent (QE) in µg mL^{-1} of the samples was calculated from the calibration curve.

Evaluation of Consumer Preferences- Sensory Analysis

Specific properties such as acidity, taste, appearance, odor, and overall evaluation that affect consumer preferences for fermented products were investigated in sensory analysis. This test used the protocol described by Irigoyen et al. (2005) with slight modifications. The participants were selected by Akdeniz University, their ages ranged from 18 to 45 years, and they were not informed about beverages. The drinks were rated by the participants on a 5 hedonic scale, with 1 representing the worst and 5 representing the best. Informed consent was obtained from each participant.

Statistical Analysis

Data from experiments in this study were expressed as mean ± standard deviation of at least 3 independent experiments. One-way ANOVA was used for statistical analysis, which was performed using IBM SPSS 22 software (SPSS, Inc., Chicago, IL, USA). A 95% confidence interval was set for all statistical analyses ($p<0.05$).

RESULTS AND DISCUSSION

Microbiological Composition Dynamics

Table 1 shows the microbial composition profiles and their change with time. Before fermentation, the content of total mesophilic bacteria, *Lactobacillus*, acetic acid bacteria, and yeast in the cultures varied around 10^3 CFU mL^{-1}. Traditional kombucha reached the highest concentration of acetic acid bacteria, *Lactobacillus* and yeast on day 14, after which the number of these microorganisms decreased. However, the highest yeast concentration was reached on day 21 in mahaleb-flavoured kombucha, and the highest concentration of mesophilic bacteria was reached on day 14. Teoh et al. (2004) showed in their experiments that there were 6 dominant yeast species in kombucha cultures: *Brettanomyces bruxellensis, Rhodotorula mucilaginosa, Candida stellata, Zygosaccharomyces bailii, Schizosaccharomyces pombe* and *Torulospora delbreuckii*.

Table 1. Microbiological profiles (CFU mL^{-1}) of kombucha beverages on day 0, 7, 14 and 21

	Lactobacillus	Yeast	TMB	AAC
DAY 0				
K	3±0.05×10^3	1.8±0.1×10^3	3±0.05×10^3	2±0.001×10^3
DAY 7				
K	1.45±0.05×10^7	8±0×10^6	1.15±0.049×10^7	1.05±0.05×10^7
KM	1.15±0.049×10^7	7.05±0.049×10^6	1.25±0.05×10^7	1.4±0.001×10^7
DAY 14				
K	1.75±0.05×10^7	1.4±0.05×10^7	1±0.15×10^7	1.6±0×10^7
KM	1.88±0.001×10^7	1.2±0.05×10^7	1.80±0.05×10^7	1.80±0.005×10^7
DAY 21				
K	1.02±0.05×10^7	7.6±0.5×10^6	1.8±0.05×10^6	8±0.5×10^6
KM	1.5±0.1×10^7	1.5±0×10^7	1.4±0.05×10^7	1.03±0.5×10^7

K: Traditional kombucha, KM: Mahaleb-flavoured kombucha, AAC: Acetic acid bacteria, TMB: Total mesophilic bacteria

They also found that the yeast species reached the highest levels on the 6th and 8th day of fermentation and then remained stable in the range of 10^4 -10^7. However, in this study, yeast levels peaked at the highest concentration on day 14 (10^7) and remained stable between 10^6 and 10^7. In another study, Sreeramulu et al. (2000) showed that acetic acid bacteria and yeast increased over time and pH decreased as a result of fermentation. Interestingly, they found that the number of acetic acid bacteria in their study increased rapidly up to day 4 and decreased rapidly from day 6 onwards. Cardoso et al. (2020) argued that high amounts of sugar and SCOBY could cause an increase in the number of microorganisms in the microbial composition, as is the case in this study.

pH Measurements

After 21 days of incubation, the pH values in the herbal infusions decreased slightly; however, the changes in the pH values of the kombucha samples were remarkable (Table 2). Similarly, many studies have been published stating that pH decreases as a function of fermentation time (Ayed & Hamdi 2015; Neffe-Skocińska et al., 2017; Vitas et al., 2020; La Torre et al., 2021; Zou et al., 2021). The initial pH of the fermented products is more acidic than that of the infusions, and the lowest pH is that of the fermented mahaleb tea. After 21 days of incubation, the pH values decreased further, and at the end of fermentation, the fermented mahaleb tea was the sample with the highest acidity. The studies by Chen and Liu (2000) and Lopes et al. (2021) reported that the decrease in pH in the beverages during fermentation could be because of the concentration of organic acids produced by the yeasts and bacteria during fermentation. Ayed et al. (2017) found that the pH values of the fermented products reduce in parallel with the rise in organic acid during the fermentation period.

Table 2. pH values of kombucha samples and tea infusions from 0, 7, 14 and 21 days

Days	K	KM	FM	B	M
0	3.1±0	3.1±0	2.9±0	4.9±0	5.7±0
7	3.0±0	3.0±0	2.9±0	4.9±0	4.5±0
14	2.7±0	2.7±0	2.6±0	4.8±0	4.9±0
21	2.5±0	2.5±0	2.4±0.1	4.5±0	4.4±0

K: Traditional kombucha, KM: Mahaleb-flavoured kombucha, FM: Fermented Mahaleb tea, B: Black tea, M: Mahaleb tea

Antibacterial Activity

On days 0 and 7, none of the beverages showed any antibacterial activity. Inhibition zones began to appear on days 14 and 21, but no inhibition zone was observed in any of the test organisms on these days in the non-fermented plant infusions (Table 3). There are similar studies that found the absence of antibacterial activity in unfermented teas (Battikh et al., 2012; Ayed et al., 2017). However, Sreeramulu et al. (2000) observed in their study that unfermented black tea was effective against *Campylobacter jejuni* strain.

Traditional kombucha was ineffective against *S. aureus* and *P. aeruginosa* strains on day 14, while it showed antibacterial activity against all bacterial strains on 21st day of fermentation. The only strain on which mahaleb-flavoured kombucha had no effect was *S. aureus*. On the 21st day of fermentation, the fermented mahaleb showed more effective antibacterial activity than the mahaleb-flavoured kombucha. Although Pure and Pure (2016) used *E. coli* and *S. aureus* in their 21-day fermentation studies, they did not observe any antibacterial activity in any of the traditional kombucha and other fermented samples they prepared. In their study, Al-Mohammadi et al. (2021) observed that 14-day-old kombucha samples had antibacterial effects against *B. cereus*, *S. aureus*, and *E. coli* strains. Generally, no antibacterial activity was observed in the non-fermented beverages, whereas mahaleb-flavoured kombucha had higher antibacterial activity than the non-fermented samples ($p<0.05$).

Table 3. The average diameter (mm) of inhibition zones for day 14 and day 21

		INHIBITION ZONES					
DAYS	SAMPLES	EC	SA	PA	BC	KP	SE
14	KAN	16±0.3	14±0.05	7.3±0.02	14±0.06	14.3±0.03	16±0.04
	AMP	6±0	17.3±0.2	9±0.1	7.3±0.04	10.3±0.2	11±0.03
	K	7±0.07	6±0	6±0	7.3±0.06	8.3±0.06	7±0
	KM	7±0	6±0	8±0.06	7±0	7.5±0.07	7±0
	FM	6±0	6±0	6±0	8±0.06	7.5±0.07	7±0
	B	6±0	6±0	6±0	6±0	6±0	6±0
	M	6±0	6±0	6±0	6±0	6±0	6±0
21	KAN	17±0	15.3±0.06	8±0.1	18±0.1	20±0	18.3±0.1
	AMP	6±0	18±0.2	8±0.1	11.3±0.06	12±0.06	11±0.06
	K	8±0	10±0.3	10±0.07	8±0	8.3±0.1	8.3±0.06
	KM	7±0	6±0	8±0.1	7±0	7±0	7.3±0.06
	FM	9±0.06	8±0.1	7.5±0.07	8±0	7±0	8.3±0.06
	B	6±0	6±0	6±0	6±0	6±0	6±0
	M	6±0	6±0	6±0	6±0	6±0	6±0

The antibiotic discs used in this study have a diameter of 6 mm. The value 6 given in the table indicates that no zone of inhibition has not developed. KP: *K. pneumonia,* SA: *S. aureus,* EC: *E. coli,* SE: *S. epidermidis,* BC: *B. cereus*, PA: *P. aeruginosa*, K: Traditional kombucha, KM: Mahaleb-flavoured kombucha, FM: Fermented Mahaleb tea, B: Black tea, M: Mahaleb tea, KAN: Kanamycin, AMP: Ampicillin

DPPH Antioxidant Capacity

The results of DPPH antioxidant capacity are shown in Figure 1. All samples except the fermented mahaleb beverage and mahaleb tea had higher antioxidant activity on the 7th day of fermentation. Among the non-fermented beverages, the antioxidant activity of the mahaleb sample was significantly lower than the black tea. The antioxidant activities of the samples in terms of AAE showed a similar trend.

According to the antioxidant capacity results, the mahaleb-flavoured kombucha had a higher antioxidant capacity. The antioxidant activity of this beverage was also different from the others ($p<0.05$). On day 14, the traditional kombucha and the mahaleb-flavoured kombucha were the beverages with the highest antioxidant activity ($p<0.05$) and there was no statistically significant difference between these two beverages (591 µM mL^{-1} AAE and 615 µM mL^{-1} AAE, respectively, $p>0.05$). Additionally, after 21 days, mahaleb-flavoured kombucha and traditional kombucha had similar antioxidant activity (560 µM mL^{-1} AAE and 554 µM mL^{-1} AAE, respectively, $p>0.05$) (Figure 1). Like this study, Vitas et al. (2020) reported a rise in the antioxidant activity on 7th day of fermentation and a reduction on 10th day in their study with 6 different herbs.

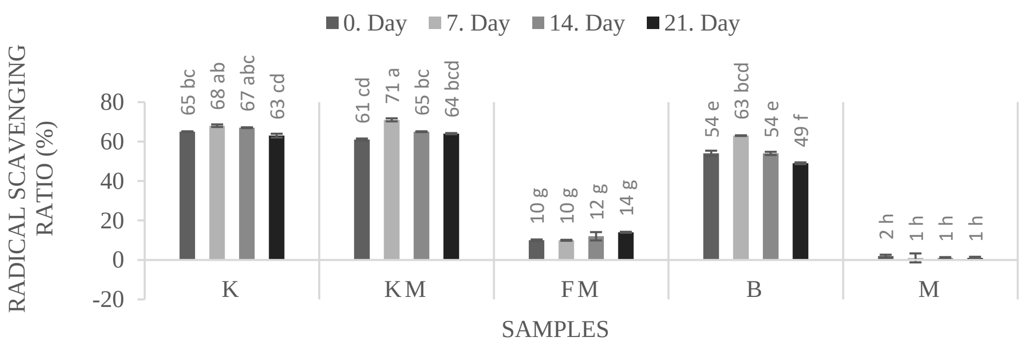

Figure 1. Antioxidant activity (%) of samples on days 0, 7, 14, and 21 (KM: Mahaleb-flavoured kombucha, K: Traditional kombucha, FM: Fermented Mahaleb tea, B: Black tea, M: Mahaleb tea, different letters in superscripts indicate a statistically significant difference ($p<0.05$))

However, Tanticharakunsiri et al. (2021) reported that in 21-day kombucha fermentation with mint and oolong tea leaves, the antioxidant capacity of the samples increased during fermentation and were at the highest level on the 14th day. According to Tosun and Karadeniz (2005), black tea has strong antioxidant activity. Therefore, it can be said that fermented beverages containing black tea may have higher antioxidant activity than others.

Determination of Total Phenolic Content (TPC)

Phenolic compounds are natural phytochemicals that play a protective role against cancer, strengthen the neurological system, and have antioxidative and antibacterial effects (Karaman et al., 2022; Şahin et al., 2022). According to the results, traditional kombucha and mahaleb-flavoured kombucha had the highest TPC on the 7th day. Fermented mahaleb and mahaleb tea had the lowest phenolic content among all samples. Among the fermented kombucha beverages, the mahaleb-flavoured kombucha had the highest TPC on day 0 and was significantly higher from the other samples ($p<0.05$). After 7, 14, and 21 days of fermentation, traditional kombucha had the highest TPC ($p<0.05$) (Figure 2). Chu and Chen (2006) reported that in a 15-day kombucha fermentation, the TPC increased as a function of fermentation time and peaked at the end of fermentation. However, in this study, it was observed that the flavoured kombucha drinks had the highest phenolic content on the 7th day. In an 8-week kombucha fermentation study, the TPC of some fermented samples peaked on day 21 in some fermented samples (Amarasinghe et al., 2018). According to the researchers, an increase in TPC may be related to an increase in microbiological activity, while a decrease in phenolic content may be caused by the utilisation of phenolic compounds by tea fungus (Amarasinghe et al., 2018).

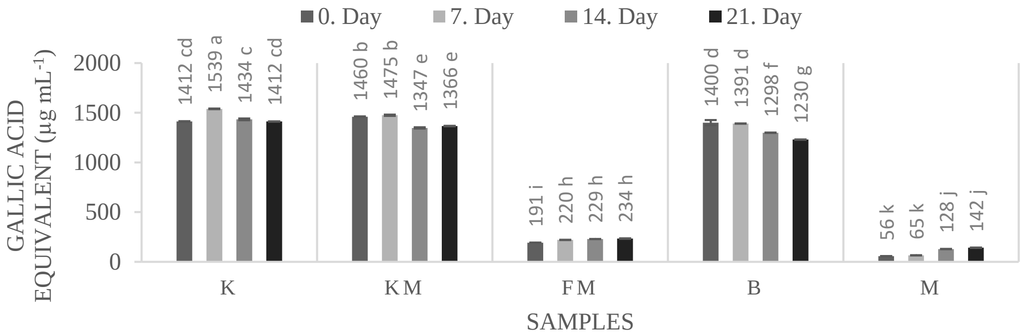

Figure 2. TPC of samples (µg/mL GAE) on days 0, 7, 14, and 21 (KM: Mahaleb-flavoured kombucha, K: Traditional kombucha, FM: Fermented Mahaleb tea, B: Black tea, M: Mahaleb tea, different letters in superscripts indicate a statistically significant difference ($p<0.05$))

Determination of Total Flavonoid Content (TFC)

Flavonoids are phenolic compounds with antioxidant, anti-allergic, anti-inflammatory, and antiviral activity that can be isolated from many plants (Pietta, 2000). Our result shows that the flavoured kombucha beverages had the highest content of total flavonoids on day 14 (Figure 3). Shahbazi et al. (2018) reported that the total quantity of flavonoids in the cinnamon, cardamom, and shirazi thyme flavoured kombucha samples increased during fermentation. They explained that this increase in flavonoid amount was caused by catechins released by acid-sensitive microorganisms on the 12th day of fermentation, forming total flavonoids (epicatechin isomers). In their study, Chakravorty et al. (2016) tried to observe the changes in the biochemical properties of kombucha tea at various time points of fermentation and showed that the total amount of flavonoids increased on day 21. Interestingly, Jakubczyk et al. (2020) stated that in a 14-day kombucha fermentation of green, black, red and white tea, the total flavonoid content was highest on the first day of fermentation and declined during the fermentation period.

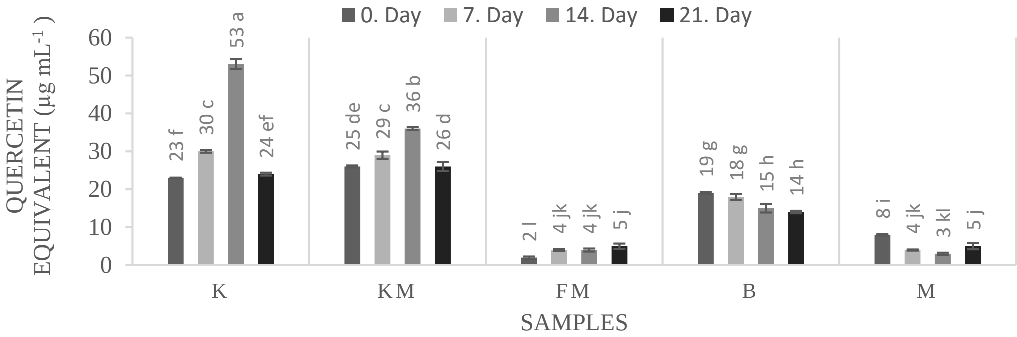

Figure 3. TFC of samples (µg/mL QE) on days 0, 7, 14, and 21 (KM: Mahaleb-flavoured kombucha, K: Traditional kombucha, FM: Fermented Mahaleb tea, B: Black tea, M: Mahaleb tea, different letters in superscripts indicate a statistically significant difference (p<0.05))

Evaluation of Consumer Preferences- Sensory Analysis

Figure 4 shows the sensory test results of the fermented kombucha beverages on days 7, 14, and 21, respectively. When the participants rated the 7-day fermented drinks, mahaleb-flavoured kombucha received the highest average score. Traditional kombucha, on the other hand, was the beverage that received the highest average score from the point of taste only (Figure 4). Among the samples that were fermented for 14 days, traditional kombucha was considered the preferred drink, and overall, the highest mean score overall was for this sample (Figure 4). When consumer preferences were assessed on day 21, the mahaleb-flavoured kombucha received the highest mean score. Traditional kombucha has been rated as the beverage with the best taste. Ayed and Hamdi (2015) reported that a 6-day fermentation might be sufficient in terms of sensory properties as the production of organic acids is responsible for forming the vinegar flavour. Similarly, in this study, drinks produced with a 7-day fermentation received the highest average scores.

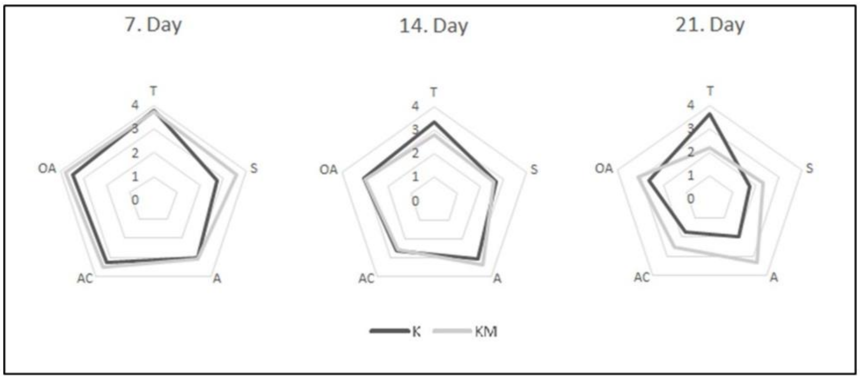

Figure 4. The sensory test results of the fermented kombucha beverages on days 7, 14, and 21 (KM: Mahaleb-flavoured kombucha, K: Traditional kombucha, T: Taste, S: Smell, A: Appearance, AC: Acidity, OA: Overall Assessment)

CONCLUSION

In this study, the effect of mahaleb in kombucha beverages was investigated in terms of antimicrobial activity, antioxidant activity, microbial profile, and sensory properties. Kombucha cultures were successfully fermented using these alternative substrates. Mahaleb is also a promising kombucha substrate that is at least as successful as black tea in terms of other beneficial bioactive properties. It is also particularly preferred for its sensory properties. It can be said that mahaleb could be one of the alternative substrates for developing functional kombucha beverages. However, its sensory properties still need to be improved. Determining the optimal fermentation time, sugar, herb, and black tea concentrations, and studying the formation of compounds beneficial to human health in these alternative kombucha beverages require further research. We believe that improving the sensory properties of this newly developed product with its beneficial effects will lead people to prefer these products more, which will have a positive impact on human health.

Conflict of Interest

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

Author's Contributions

Burcu Emine TEFON ÖZTÜRK, Berfin EROĞLU and Eda DELİK have designed the study and collected the data. No support was received from any institution to carry out the study. Burcu Emine TEFON ÖZTÜRK, AYSUN TÜRKANOĞLU ÖZÇELİK, Volkan YILDIRIM, Berfin EROĞLU and Eda DELİK executed the experiment. Eda DELİK and Berfin EROĞLU wrote the article, and critically reviewed by Burcu Emine TEFON ÖZTÜRK.

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