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Determination of some quality properties and antimicrobial activities of kombucha tea prepared with different berries

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Abstract: Kombucha is a slightly sweet, acidic, carbonated, fermented beverage obtained by fermenting sugared tea (Camellia sinensis) with symbiotic bacteria. In the present study, kombucha was produced from black tea (Camellia sinensis), black grape (Vitis labrusca), black mulberry (Morus nigra), and rosehip (Rosa canina) fruits. Its physicochemical, microbiological, sensory properties and antimicrobial effects were investigated. During the fermentation, pH, Brix (%), viscosity, total antioxidant, and phenolic substance values decreased (p < 0.05). Total aerobic mesophilic bacteria (TAMB) and yeast/mold, Lactococcus/Streptococcus counts decreased, while the osmophilic yeast, acetic acid, and lactic acid bacterial counts increased (p < 0.05). The samples produced using black mulberry had the highest antibacterial activity (26.58 mm zone diameter), lowest minimum inhibitory concentration (MIC) (0.012 mg·L⁻¹), and minimum bactericidal concentration (MBC) (0.008 mg·L⁻¹) on Staphylococcus aureus. The highest antifungal activity was in the rosehip on Mucor racemosus (p < 0.05). The a* values increased during fermentation, while L* and b* values decreased. Based on the sensory analysis, black mulberry was the most preferred sample concerning all assessment criteria.

Key words: Kombucha, black mulberry, antimicrobial, antifungal, Staphylococcus aureus, Mucor racemosus

1. Introduction
Kombucha is a slightly sweet, acidic, carbonated, fermented beverage obtained by fermenting sugared tea (Camellia sinensis) with symbiotic bacteria with the dominant genera Komagataeibacter, Acetobacter, and Gluconobacter, and yeasts, mainly from genera Brettanomyces and Zygosaccharomyces (Villarreal-Soto et al., 2020). The word "Kombucha" originates from the Japanese words "seaweed" (Kombu) and "tea" (cha). Kombucha has been consumed in China for 2000 years. Also, it is enjoyed in Middle Eastern countries, Germany, and Russia (Amarasinghe et al., 2018). Conventionally, the fermentation of Kombucha lasts between 7 and 10 days under domestic conditions (at 20–28 °C). As the fermentation period progresses, fermentation increases consumers' acceptance, affecting the tea's sourness, aroma, radiant color, and other sensory properties (Marsh et al., 2014). In addition to tea leaves, Kombucha influences the final product and its sensory properties via the phenolic and antioxidant traits in other herbs (mint, sage, black mulberry, black grape, rosehip, thyme, strawberry, etc.). They were added to soften the sour taste, providing nutritional enrichment (Martínez Leal et al., 2018).

Berries have an important place in the food industry with their unique taste, aroma, and colors besides the components they contain (Celik and Islam, 2019). These fruits are exceptionally rich in polyphenols, including flavonoids, anthocyanins, procyanidins, hydroxylases, and ellagitannins. They are food groups with high-calorie values due to their high sugar content. Moreover, they are abundantly rich in any metabolites, such as organic acids (glucuronic, acetic), vitamins (C, B₁, B₂, B₁₂), and minerals (Ca, K, Na, and Fe) (Coton et al., 2017).

Some benefits proven in animal studies include the prevention of diabetes, reduction in cholesterol, triglyceride levels (Hosseini et al., 2016), hepatoprotein (Hyun et al., 2016), and oxidative stress control (Lobo et al., 2017). Kombucha contains phenolic compounds such as catechins and flavonoids having bioactive properties. These compounds are the essential antioxidant group naturally occurring in kombucha and are responsible for the beverage's health benefits (Jayabalan et al., 2011).

Kombucha has antimicrobial (Ivanišová et al., 2019), antioxidant, antiproliferative, and antidiabetic (Bhattacharya et al. 2013), anticarcinogenic (Jayabalan et al., 2011) effects in treating various diseases such as gastric ulcers (Banerjee et al., 2010) and high cholesterol (Yang et al., 2009). Moreover, tea has impacted the immune response (Ram et al., 2000) and liver detoxification
In this research, the used microorganisms were *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 6538), *Salmonella Typhi* (ATCC 6539), *Enterobacter aerogenes* (ATCC 13048), *Pseudomonas aeruginosa* (ATCC 10145), *Bacillus subtilis* (ATCC 6633), *Listeria monocytogenes* (ATCC 51774), *Aspergillus flavus* (ATCC 204304), *Penicillium citrinum* (ATCC 38065), *Botrytis cinerea* (ATCC 26943), *Mucor racemosus* (ATCC 42647), *Rhizopus nigricans* (ATCC 6227), *Cladosporium cladosporioides* (ATCC 26688), and *Byssoschlamys fulva* (ATCC 10099) determining antimicrobial activity of kombuchas. The American Type Culture Collection (ATCC, Rockville, MD, US) provided the microorganism strains in the present study. The faculty members of Afyon Kocatepe University, Faculty of Science and Literature, Department of Molecular Biology and Genetics (Afyonkarahisar, Turkey) identified microorganisms’ species, subspecies, and variety levels.

2.3. Physical and chemical analysis

During the fermentation period, the samples’ pH was measured weekly using a Hanna (HI 2215, Germany) pH-meter, while Brix (% soluble dry matter content) was measured using a hand refractometer (Atago Refractometer N-1E, Japan) specified by Budak (2015). Viscosity was measured using spindle No. 2 (Brookfield, Middleboro, MA, USA) at 100 RPM, room temperature (Ryan et al., 2019), while the color values were measured using a colorimeter (Konika Minolta Chroma Meter CR-400) according to Gök et al., (2015). Total antioxidant capacity (% inhibition) and the total amount of phenolic substance (mg GAE/L) were determined based on Yikmis and Tuggum (2019).

2.4. Determination of antibacterial and antifungal activity by the disk diffusion method

1.0 mL sample was taken from the tea dilutions using a sterile automatic pipette (Research Plus, Eppendorf, Germany) and analyzed by the spread plate method, and incubated for the periods and conditions specified in Table 1. Samples from single colonies of the bacteria and mold species used in the study were taken using a sterile loop and set to 0.5 McFarland turbidity standard in physiological saline (Merck, 115525, Germany) using a densitometer (Bios 1B, Turkey). Then 0.1 mL (10−1–10 CFU/mL) sample was taken from this inoculum using a sterile pipette. Then, Mueller Hinton Agar (Merck 1.05437) (MHA) for antibacterial activity and Mueller Hinton Agar (Merck 1.05437) (MHA) modified with 2% glucose and 0.5 mg-L−1 methylene blue for antifungal activity was added and spread uniformly on the surface of the medium using a sterile transport swap (Fıratpen, Turkey). The standard blank antibiogram discs (Bio-Disk 316010001, 6 mm in diameter) impregnated with 100 µL of tea dilutions were placed in the medium where the zones would not touch each other (Alästrüey-Izquierdo et al., 2015). The bacteria (*Listeria monocytogenes* in 5% CO2 medium) were incubated for 16 to 20 h at 35 °C, while molds were incubated for 4–7 days at 25 °C (EUCAST, 2018) in an incubator (Incucell, MMM, Germany). The diameters of the zones formed after incubation were measured in mm using a digital caliper (Mitutoyo, 500-181-30, Japan) under daylight.
2.5. Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC)

MIC of fermented teas was determined based on the macro dilution method (Aamer et al., 2014; CLSI, 2015). Following the incubation, tubes with the membrane, turbidity, and sediment formation at the bottom were evaluated as positive (+). The MIC value was determined by taking the average of the sum of the concentration of the tube with microbial growth in the tubes and the concentration of the previous tube without growth (Castro et al., 2015).

1 µL sample was taken from each of the first tubes in the MIC analysis and subsequent tubes and inoculated in Mueller-Hinton agar for MBC analysis and modified-Mueller Hinton agar (2% glucose and 0.5 mg·L⁻¹ methylene blue) for MFC analysis, determining MBC and MFC values. The Petri dishes were incubated at 35 °C for MBC (Listeria monocytogenes in 5% CO₂ medium) for 16 to 20 h and at 25 °C for MFC for 4–7 days. After the incubation, the lowest concentration without growth was evaluated as the MBC value against the bacterial species tested and MFC value against the mold species (Aamer et al., 2014; Castro et al., 2015).

2.6. Sensory analysis

Sensory evaluation of kombuchas was conducted after the fermentation. Scorecards formed by modifying the sensory test parameters specified by Malbaša et al., (2014) were used in these analyses. The panel included twenty panelists (ten men and ten women, average age 29), Faculty, and Ph.D. students of Afyon Kocatepe University Food Engineering Department. In a randomized order, the samples were served under artificial light (incandescent) at room temperature (22 °C) in a randomized order. Panelists evaluated samples with a 1–10–hedonic (lowest to highest) scale for taste, acidity structure, color, odor, and general appreciation.

2.7. Statistical analysis

All the production and analyses were duplicated with double parallels, and data were analyzed with the SPSS V 23.0.0.0 statistical program software.

3. Results and discussion

The pH, Brix, viscosity, total phenolic contents, and total antioxidant values depending on the fermentation period of the kombucha samples are in Table 2. The concentration of organic acids was not measured. During the three-week-fermentation, the pH of all samples decreased (p < 0.05; Table 2). According to variance analysis results, the effect of the sample type on pH was highly significant (p < 0.0001), while the effect of the fermentation period on pH was significant (p < 0.05, Table 2). At the end of the period, the lowest pH values were in the BG (2.53) and RS samples (2.58), respectively (p < 0.05; Table 2). Likewise, Ayed et al., (2017) determined that the pH value of the kombuchas decreased (p < 0.05) during the fermentation period. This decrease in pH was caused by increased organic acids concentration formed during fermentation.

The effect of sample type on the Brix (%) value was highly significant (p < 0.0001), while the effect of the fermentation period was significant (p < 0.01). In all samples, a decrease (p < 0.05) in the Brix values was observed during the fermentation. The highest decline during the fermentation period was in the BT sample (1.49%) (p < 0.05; Table 2).

Abuduaibifu and Tamer (2019) reported that the Brix values of three different kombucha samples produced by using black tea, black goji berry, and red goji berry decreased during the 11-day fermentation. These results are in line with our findings. The Brix value decreased due
<table>
<thead>
<tr>
<th>Samples (S)</th>
<th>pH</th>
<th>Brix (%)</th>
<th>Viscosity (cP)</th>
<th>Total phenolic (mg GAE/L)</th>
<th>Total antioxidant (% Inhibition)</th>
<th>TAMB</th>
<th>Mold/yeast</th>
<th>Osmophilic yeast</th>
<th>Lactic acid bacteria</th>
<th>Acetic acid bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BT</strong></td>
<td>2.98 ± 0.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.81 ± 0.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.25 ± 9.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>231.59 ± 80.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>60.88 ± 25.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.24 ± 0.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.00 ± 0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.74 ± 0.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.66 ± 0.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.28 ± 0.94&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>BG</strong></td>
<td>2.82 ± 0.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.28 ± 0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.00 ± 10.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>151.37 ± 77.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91.94 ± 23.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.75 ± 0.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.99 ± 0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.82 ± 0.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.55 ± 0.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.06 ± 0.85&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>RS</strong></td>
<td>2.87 ± 0.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.48 ± 0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.00 ± 11.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70.05 ± 76.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>43.64 ± 11.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.26 ± 0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.99 ± 0.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.44 ± 0.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.76 ± 0.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.28 ± 0.86&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>BM</strong></td>
<td>3.21 ± 0.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.60 ± 0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.00 ± 9.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>252.28 ± 73.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>64.44 ± 20.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.40 ± 0.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.00 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.01 ± 0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.63 ± 0.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.33 ± 0.63&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Table 2.** Physical, chemical, and microbiological (log CFU/mL) analysis results of samples*.

<table>
<thead>
<tr>
<th>pH</th>
<th>Brix (%)</th>
<th>Viscosity (cP)</th>
<th>Total phenolic (mg GAE/L)</th>
<th>Total antioxidant (% Inhibition)</th>
<th>TAMB</th>
<th>Mold/yeast</th>
<th>Osmophilic yeast</th>
<th>Lactic acid bacteria</th>
<th>Acetic acid bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S × FT</strong></td>
<td><strong>0</strong></td>
<td>3.52 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.97 ± 0.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.00 ± 6.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>184.08 ± 6.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.64 ± 18.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.81 ± 0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.23 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.05 ± 0.52&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>7</strong></td>
<td>2.96 ± 0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.75 ± 0.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.00 ± 6.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>178.84 ± 6.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>66.03 ± 19.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.53 ± 0.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.03 ± 0.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.75 ± 0.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.35 ± 1.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>14</strong></td>
<td>2.75 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.35 ± 0.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.50 ± 6.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>174.08 ± 2.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.14 ± 18.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.81 ± 0.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.85 ± 0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.00 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.72 ± 0.97&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>21</strong></td>
<td>2.65 ± 0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.09 ± 0.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.75 ± 7.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>168.29 ± 10.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.14 ± 18.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.51 ± 0.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.64 ± 0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.21 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.90 ± 1.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* (Mean ± standard deviation), Black Tea: BT, Black Grape: BG, Rosehip: RS, Black Mulberry: BM, α-l (↓): Values with the same capital letters in the same column for each analysis differ significantly (p < 0.05).
to the yeast hydrolysis of sucrose, glucose, and fructose to ethanol during fermentation. Acetic acid bacteria utilize ethanol to produce acetic acid (Ayed et al., 2017; Abduaibu and Tamer, 2019).

The viscosity values decreased during the fermentation period in all samples (p < 0.05). The effect of the fermentation period on viscosity value was highly significant (p < 0.0001). At the end of the fermentation period (21st day), the highest viscosity value was in the BM sample (42 cP), while the lowest one was in the BG sample (34 cP) (p < 0.05; Table 2). The decrease in total soluble solids affected the decrease in the viscosity values of the samples during fermentation. Watawana et al. (2016) reported that the kombucha produced from coconut juice increased viscosity due to fermentation. The difference detected between the studies might be due to differences in production, raw material, and fermentation conditions.

Based on variance analysis results, the effects of the differences in fermentation period and kombucha samples were highly significant (p < 0.0001) on total phenolic substance and antioxidant values. The total amount of phenolic substances and antioxidants decreased during fermentation (p < 0.05; Table 2). The lowest total phenolic and antioxidant values during fermentation were 168.29 mg GAE/L and 63.14%, respectively, on day 21 (p < 0.05; Table 2). The decrease in the total amount of TPC at the end of fermentation may be due to the polymerization of phenolic compounds (Wang et al., 2000). Similarly, Yikmis and Tuggum (2019) found that the total phenolic contents and antioxidants decreased in kombuchas during fermentation. During the fermentation, factors such as hydrolysis, isomerization, and polymerization were influential under acidic conditions lowering the total phenolic contents and antioxidants.

The change in the TAMB, yeast/mold, osmophilic yeast, lactic acid bacteria, Lactococcus/Streptococcus, and acetic acid bacteria counts during Kombucha samples' fermentation is in Table 2. On the TAMB counts, the effect fermentation period was significant at p < 0.0001, while the sample type was significant at p < 0.01. Moreover, the effect of the fermentation period on the yeast/mold counts was significant (p < 0.01). The TAMB and yeast/mold counts decreased (p < 0.05) in all samples depending on the fermentation period. At the end of the period, the lowest TAMB and yeast/mold counts were 2.51 CFU/mL and 4.64 CFU/mL, respectively, on the 21st day (Table 2). Yikmis and Tuggum (2019), agreeing with the present study results, reported that all kombucha samples’ TAMB counts increased up to day 7 of the fermentation period, then decreased (p < 0.05) later (Table 2). The suppression of the Lactococcus/Streptococcus growth due to the increased environmental acidity and the number of other fermentative microorganisms may have been the reason.

The antibacterial and antifungal effects of Kombucha samples on seven different food-borne bacteria and mold species are depicted in Table 3. In the present study, sample, bacteria species, and sample x bacteria species interactions had a significant effect (p < 0.0001) on the antibacterial effect, MIC, and MBC values. Moreover, sample, mold species, and sample x mold interactions were significant (p < 0.0001) for the antifungal effect, MIC, and MFC values. The highest antibacterial effect was on Staphylococcus aureus in the BM (26.58 mm zone diameter) and BT (24.99 mm zone diameter) samples. In comparison, the highest antifungal effect was on Mucor racemosus in the RS (18.77 mm zone diameter) samples and BM (18.30 mm zone diameter) (p < 0.05; Table 3).

Akarca and Tomar (2018) posed that the antibacterial effect was the highest on Staphylococcus aureus in black kombucha (24 mm zone diameter) and on Enterobacter aerogenes and Pseudomonas aeruginosa (9 mm zone diameter) in green kombucha. Their findings were similar to those of the present study. Yuniarto et al., (2016), on the 18th day of the fermentation of kombucha, reported the highest antifungal effect on Microsporum gypseum with a zone diameter of 21.16 mm. In contrast, the lowest effect was on Candida albicans with a diameter of 11.00 mm. The differences between the studies were likely due to the differences in the raw materials and mold used in the production of teas.

The sample type had a highly significant effect (p < 0.0001) on the lactic and acetic acid bacteria counts and a significant effect (p < 0.01) on the osmophilic yeast counts. Furthermore, the fermentation period had a highly significant effect (p < 0.0001) on lactic acid bacteria, acetic acid bacteria, Lactococcus/Streptococcus, and osmophilic yeast count.
Antibacterial and antifungal effects of Kombucha were due to organic acids formed during fermentation and other bioactive compounds and metabolites such as polyphenols (flavonoids), bacteriocins, and enzymes biosynthesized during fermentation (Bhattacharya et al., 2016).

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the Kombucha samples on seven different food-borne pathogens are in Table 4. The lowest MIC value on seven different bacterial species was on *Staphylococcus aureus* with 0.012 mg·L⁻¹ in the BM sample (p < 0.05). In contrast, the highest MIC values were RS in *Listeria monocytogenes*, BG in *Pseudomonas aeruginosa*, and BT in *Enterobacter aerogenes* with 0.375 mg·L⁻¹ (p < 0.05; Table 4).

The lowest MBC value on different bacterial species was in *Staphylococcus aureus* with 0.008 mg·L⁻¹ in the BM sample (p < 0.05). The highest MBC value, 0.250 mg·L⁻¹, was on three different samples and bacterial species (RS, *Listeria monocytogenes*; BG, *Pseudomonas aeruginosa*; BT, *Enterobacter aerogenes*) (p < 0.05, Table 4). In their research, Bhattacharya et al. (2016) informed the lowest MIC values of kombucha samples to be 3.125 mg·mL⁻¹ on *Vibrio cholerae, Escherichia coli*, and *Salmonella Typhimurium*. The results were similar to those obtained in the present study.

MIC and minimum fungicidal concentration (MFC) values of four different kombucha samples on seven different mold types are in Table 4. The lowest MIC value was 0.070 g·L⁻¹ on *Mucor racemosus* in the RS and BM samples, while the highest was 0.750 mg·L⁻¹ on *Cladosporium cladosporioides* in the BT sample (p < 0.05; Table 4).

The lowest MFC values were 0.047 mg·L⁻¹ in RS and BM samples on *Mucor racemosus*. However, the highest MFC value was on *Cladosporium cladosporioides* in the CT sample with 0.500 mg·L⁻¹ (p < 0.05; Table 4). Nazemi et al. (2019) declared that the MIC values of kombucha samples on *Aspergillus fumigatus* ranged from 6170 to 24,700 μg·mL⁻¹ and MFC values from 12,300 to 98,800 μg·mL⁻¹. The results were similar to those of the present study.

### Table 3. Antibacterial and antifungal effects of tea samples on some food-borne pathogenic microorganisms used in the research (mm zone diameter)*.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Samples</th>
<th>BT</th>
<th>BG</th>
<th>RS</th>
<th>BM</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td></td>
<td>20.53 ± 0.11b</td>
<td>17.56 ± 0.14b</td>
<td>18.29 ± 0.17c</td>
<td>22.29 ± 0.17ab</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td></td>
<td>24.99 ± 0.14a</td>
<td>17.40 ± 0.16a</td>
<td>17.64 ± 0.15c</td>
<td>26.58 ± 0.16a</td>
</tr>
<tr>
<td><em>Salmonella Typhi</em></td>
<td></td>
<td>17.37 ± 0.10bc</td>
<td>15.61 ± 0.12bc</td>
<td>16.29 ± 0.16bc</td>
<td>18.76 ± 0.13bc</td>
</tr>
<tr>
<td><em>Enterobacter aerogenes</em></td>
<td></td>
<td>10.27 ± 0.14c</td>
<td>12.19 ± 0.16bc</td>
<td>14.57 ± 0.11bc</td>
<td>12.40 ± 0.15c</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td></td>
<td>13.39 ± 0.15bc</td>
<td>11.80 ± 0.13bc</td>
<td>13.47 ± 0.15bc</td>
<td>15.26 ± 0.12bc</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td></td>
<td>15.54 ± 0.13cd</td>
<td>16.70 ± 0.15cd</td>
<td>15.43 ± 0.16cd</td>
<td>17.52 ± 0.16cd</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td></td>
<td>14.68 ± 0.12bc</td>
<td>13.59 ± 0.17cd</td>
<td>12.53 ± 0.20bc</td>
<td>15.59 ± 0.14bc</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td></td>
<td>11.31 ± 0.13bc</td>
<td>10.56 ± 0.13cb</td>
<td>11.38 ± 0.15bc</td>
<td>13.61 ± 0.13bc</td>
</tr>
<tr>
<td><em>Penicillium citrinum</em></td>
<td></td>
<td>10.57 ± 0.15cb</td>
<td>11.46 ± 0.11bc</td>
<td>11.64 ± 0.11bc</td>
<td>14.43 ± 0.15cd</td>
</tr>
<tr>
<td><em>Botrytis cinerea</em></td>
<td></td>
<td>10.01 ± 0.14bc</td>
<td>8.96 ± 0.17bc</td>
<td>11.25 ± 0.12bc</td>
<td>17.22 ± 0.20ab</td>
</tr>
<tr>
<td><em>Mucor racemosus</em></td>
<td></td>
<td>10.54 ± 0.12cb</td>
<td>11.70 ± 0.16bc</td>
<td>18.77 ± 0.14bc</td>
<td>18.30 ± 0.13bc</td>
</tr>
<tr>
<td><em>Rhizopus nigricans</em></td>
<td></td>
<td>9.91 ± 0.17bc</td>
<td>10.55 ± 0.14bc</td>
<td>10.39 ± 0.18bd</td>
<td>15.29 ± 0.15bc</td>
</tr>
<tr>
<td><em>Cladosporium cladosporioides</em></td>
<td></td>
<td>8.99 ± 0.13cd</td>
<td>9.33 ± 0.24bc</td>
<td>10.97 ± 0.16bc</td>
<td>13.36 ± 0.12bc</td>
</tr>
<tr>
<td><em>Byssochlamys fulva</em></td>
<td></td>
<td>9.23 ± 0.14bd</td>
<td>9.19 ± 0.23bc</td>
<td>10.19 ± 0.21bd</td>
<td>14.66 ± 0.14bd</td>
</tr>
</tbody>
</table>

* (Mean ± standard deviation, Black Tea: BT, Black Grape: BG, Rosehip: RS, Black Mulberry: BM, a-g (↓): Values with the same capital letters in the same column for each analysis differ significantly (p < 0.05), A-D (→): Values with the same capital letters in the same rows for each analysis differ significantly (p < 0.05).
Table 4. MIC, MBC, and MFC values of the samples used in the research on some food-borne pathogen bacteria and mold species (mg·L⁻¹).

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Samples</th>
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<tbody>
<tr>
<td></td>
<td>BT</td>
<td>BG</td>
<td>RS</td>
<td>BM</td>
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<tr>
<td></td>
<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
<td>MBC</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0.035 ± 0.00 b</td>
<td>0.023 ± 0.00 d</td>
<td>0.094 ± 0.00 c</td>
<td>0.063 ± 0.01 b</td>
<td>0.070 ± 0.00 d</td>
<td>0.047 ± 0.01 b</td>
<td>0.023 ± 0.00 e</td>
<td>0.016 ± 0.00 e</td>
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<tr>
<td>Staphylococcus aureus</td>
<td>0.024 ± 0.00 b</td>
<td>0.016 ± 0.00 d</td>
<td>0.070 ± 0.00 c</td>
<td>0.047 ± 0.00 b</td>
<td>0.094 ± 0.01 a</td>
<td>0.063 ± 0.02 a</td>
<td>0.012 ± 0.00 f</td>
<td>0.008 ± 0.00 f</td>
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<tr>
<td>Salmonella Typhi</td>
<td>0.094 ± 0.00 b</td>
<td>0.063 ± 0.00 c</td>
<td>0.094 ± 0.00 c</td>
<td>0.141 ± 0.03 b</td>
<td>0.094 ± 0.00 c</td>
<td>0.069 ± 0.00 b</td>
<td>0.047 ± 0.00 c</td>
<td>0.188 ± 0.04 a</td>
<td></td>
<td></td>
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<tr>
<td>Enterobacter aerogenes</td>
<td>0.375 ± 0.03 a</td>
<td>0.250 ± 0.03 a</td>
<td>0.281 ± 0.04 b</td>
<td>0.188 ± 0.04 b</td>
<td>0.188 ± 0.02 c</td>
<td>0.125 ± 0.02 b</td>
<td>0.281 ± 0.05 b</td>
<td>0.188 ± 0.04 a</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>0.188 ± 0.04 c</td>
<td>0.125 ± 0.03 b</td>
<td>0.375 ± 0.03 a</td>
<td>0.250 ± 0.03 a</td>
<td>0.281 ± 0.05 a</td>
<td>0.188 ± 0.04 a</td>
<td>0.094 ± 0.00 b</td>
<td>0.063 ± 0.00 b</td>
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<tr>
<td>Bacillus subtilis</td>
<td>0.141 ± 0.03 a</td>
<td>0.094 ± 0.01 b</td>
<td>0.094 ± 0.00 c</td>
<td>0.063 ± 0.00 b</td>
<td>0.141 ± 0.03 a</td>
<td>0.094 ± 0.01 a</td>
<td>0.070 ± 0.00 b</td>
<td>0.047 ± 0.01 b</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>0.141 ± 0.02 b</td>
<td>0.094 ± 0.00 b</td>
<td>0.141 ± 0.03 c</td>
<td>0.094 ± 0.02 b</td>
<td>0.375 ± 0.04 a</td>
<td>0.250 ± 0.04 a</td>
<td>0.141 ± 0.03 b</td>
<td>0.094 ± 0.00 b</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Aspergillus flavus</td>
<td>0.281 ± 0.04 c</td>
<td>0.188 ± 0.03 c</td>
<td>0.281 ± 0.04 b</td>
<td>0.188 ± 0.04 b</td>
<td>0.281 ± 0.05 b</td>
<td>0.188 ± 0.05 b</td>
<td>0.188 ± 0.03 b</td>
<td>0.125 ± 0.02 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillium citrinum</td>
<td>0.375 ± 0.05 a</td>
<td>0.250 ± 0.03 a</td>
<td>0.188 ± 0.03 c</td>
<td>0.125 ± 0.02 b</td>
<td>0.188 ± 0.04 b</td>
<td>0.125 ± 0.01 b</td>
<td>0.141 ± 0.02 b</td>
<td>0.094 ± 0.00 b</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Botrytis cinerea</td>
<td>0.563 ± 0.03 b</td>
<td>0.375 ± 0.02 b</td>
<td>0.563 ± 0.04 a</td>
<td>0.375 ± 0.03 a</td>
<td>0.375 ± 0.06 b</td>
<td>0.250 ± 0.03 b</td>
<td>0.095 ± 0.00 c</td>
<td>0.063 ± 0.00 b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucor racemosus</td>
<td>0.375 ± 0.04 c</td>
<td>0.250 ± 0.01 c</td>
<td>0.281 ± 0.05 b</td>
<td>0.188 ± 0.03 b</td>
<td>0.070 ± 0.01 b</td>
<td>0.047 ± 0.01 b</td>
<td>0.070 ± 0.00 b</td>
<td>0.047 ± 0.01 b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhizopus nigricans</td>
<td>0.563 ± 0.03 b</td>
<td>0.375 ± 0.03 b</td>
<td>0.375 ± 0.03 b</td>
<td>0.250 ± 0.02 b</td>
<td>0.375 ± 0.05 b</td>
<td>0.250 ± 0.02 b</td>
<td>0.094 ± 0.01 c</td>
<td>0.063 ± 0.00 c</td>
<td></td>
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</tr>
<tr>
<td>Cladosporium cladosporoides</td>
<td>0.750 ± 0.02 a</td>
<td>0.500 ± 0.02 a</td>
<td>0.563 ± 0.02 a</td>
<td>0.375 ± 0.05 a</td>
<td>0.281 ± 0.04 c</td>
<td>0.188 ± 0.04 b</td>
<td>0.281 ± 0.03 c</td>
<td>0.188 ± 0.05 b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bysschlamys fulva</td>
<td>0.563 ± 0.04 b</td>
<td>0.375 ± 0.03 b</td>
<td>0.563 ± 0.04 a</td>
<td>0.375 ± 0.06 a</td>
<td>0.563 ± 0.03 a</td>
<td>0.375 ± 0.03 a</td>
<td>0.188 ± 0.04 b</td>
<td>0.125 ± 0.01 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Black Tea: BT, Black Grape: BG, Rosehip: RS, Black Mulberry: BM, a-e (↓): Values with the same capital letters in the same column for each analysis differ significantly (p < 0.05), A-C (→): Values with the same capital letters in the same rows for each analysis differ significantly (p < 0.05).
On the last day of the fermentation, the highest $a^*$ value was in the RS sample (25.19), while the lowest $a^*$ value was in the BM sample (12.21) ($p < 0.05$; Figure 1). The $b^*$ values of all samples decreased during fermentation ($p < 0.05$). On day 21 of the fermentation, the lowest $b^*$ values were in the BM (4.41), RS (12.25), and BT (15.06) samples, respectively ($p < 0.05$; Figure 1).

Abuduaibifu and Tamer (2019) presented that the color values ($L^*$, $a^*$, $b^*$) increased during the 11-day fermentation in kombuchas. There were differences between these results in that study and the results in the present study. These differences could be due to differences in raw material, fermentation period, and fermentation conditions.

In kombucha samples, the reasons for the increase in $a^*$ value, decrease in $L^*$ and $b^*$ values depending on fermentation might be the deterioration of color pigments and polyphenolic components (Watawana et al., 2018) due to the decrease in pH and microorganism growth.

The effect of the sample type, fermentation period, and sample type × fermentation period interaction was highly significant ($p < 0.0001$) for the sensory properties. The sensory scores were based on the 10-point hedonic scale ratings assigned by the panelists at the end of the last day ($21^{st}$) of fermentation (Figure 2). Based on the sensory evaluation results of the kombuchas produced with different grape berries, the samples produced with black mulberry had the highest scores in all assessed parameters. Samples produced with black tea received the second-highest scores in all parameters. However, the panelists rated the samples produced with rosehip the lowest in all parameters except structure.
To sum up, Kombucha is a fermented beverage traditionally produced with black tea. As an alternative to black tea in the production of Kombucha, panelists rated only black mulberry as an acceptable replacement.

4. Discussion
In the present study, four different kombucha samples were studied for their physical, chemical, microbiological, and sensory characteristics and antibacterial and antifungal activities. The antimicrobial effects of kombuchas produced from grape berries might have originated from organic acids formed during fermentation, and other bioactive compounds and metabolites such as biosynthesized polyphenols and bacteriocins and enzymes.

Using different grape berries as a substrate in kombucha production can be preferable concerning taste, aroma, and functional properties. The popularity of kombucha has increased thanks to its beneficial effects on health recently. The therapeutic effects are associated with its chemical composition, essentially polyphenols and secondary metabolites produced during fermentation. Kombucha may replace carbonated beverages due to its health benefits and therapeutic properties. Moreover, kombucha can be an alternative to artificial additives in the food industry due to its superior antimicrobial effects.

Conflict of interest
The authors declare that there is no conflict of interest.

References


