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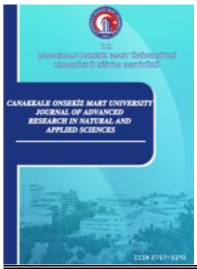

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Kombucha Beverage: Comparative Study Based on Bioactive Properties and Antimicrobial Potentials of Different Plant Infusion

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Abstract –Kombucha, a fermented beverage, is popular for its prophylactic and therapeutic properties. Kombucha is a traditionally black tea infusion fermented with a symbiotic bacteria and yeast consortium (SCOBY) under aerobic conditions for 7-21 days. However, the beneficial properties of kombucha vary according to the substrate kind, fermentation conditions, and SCOBY consortium. The present study has screened the physicochemical, bioactive, antimicrobial, and sensory properties of beverages produced by fermenting black, green, rosehip, and licorice tea infusions with kombucha starter culture for 21 days. Tea infusions before and after fermentation; pH value, titratable acidity (TA), pellicle weight, color values (L^*, a^*, b^*, ΔE), total phenolic content (TPC), antioxidant capacity against DPPH (2,2-diphenyl-1- picrylhydrazil) radicals, and antimicrobial activity was measured. Antimicrobial activity is applied to various foodborne pathogens such as *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), and *C. albicans* with based disc diffusion method and spectrophotometric technique. In this study, tea type statistically affected all parameters except pH in kombucha beverages ($p<0.05$). The highest TPC and antioxidant activity were determined in the green tea kombucha sample. All kombucha beverages, especially those prepared by fermentation of licorice and green tea infusions, showed the highest antimicrobial potential against *E. coli* and *S. aureus*, respectively. Consequently, it is vital to prefer kombucha fermented with SCOBY instead of consuming beverages prepared with various plants' infusions to increase many beneficial properties and provide additional benefits.

Keywords *− Antimicrobial activity, bioactive potential, fermented beverage, Kombucha, SCOBY*

1. Introduction

The popularity of foods that provide additional health benefits beyond basic nutrition has accelerated over the past two decades (Sun-Waterhouse, 2011). Especially beverage industry is among the most dynamic food categories open to applications in the field of functional foods (Corbo et al., 2014). In addition to its thirst-quenching and refreshing/cooling, consumers expect functionality from beverages such as increasing energy, anti-aging, decreasing stress, and so on. even more so, they demand a positive effect in treating some diseases (Sethi et al., 2016). While new products are being developed to meet this demand in the beverage industry (Poveda-Castillo et al., 2018; Rojo-Poveda et al., 2019), some traditional drinks that are not valued enough are extremely striking.[1]

Kombucha is a refreshing beverage known for its added health benefits that have been traditionally consumed for thousands of years in many countries (Sun et al., 2015). The fermentation process includes sweetening the infused black tea and inoculating it with a Symbiotic Bacteria and Yeast Colony (SCOBY) and then incubating under aerobic conditions for 7-10 days (Villarreal-Soto et al., 2018). The new beverage formed after fermentation is rich in organic acids, vitamins, minerals, dietary fiber, essential amino acids, various enzymes, and secondary metabolites (Chu & Chen, 2006; Miranda et al., 2016). Its metabolites have beneficial effects such as antimicrobial (Cardoso et al., 2020), antitumor (Rasu Jayabalan et al., 2011), antihypertensive (Vitas et al., 2020), immune-modulatory (Villarreal-Soto et al., 2020), antioxidant, anticancer, antiinflammatory

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(Villarreal-Soto et al., 2019), and so on. The microflora of SCOBY, which is a cellulosic biofilm used as the starter culture of kombucha beverage, contains acetic acid bacteria (De Roos & De Vuyst, 2018), lactic acid bacteria (Marsh et al., 2014), and osmophilic yeasts (Coton et al., 2017). Probiotic microorganisms in the microbiota of SCOBY and the pharmacological activities of its biologically active ingredients in its liquid attracted the attention of researchers, and studies fermented using alternative substrates (raw material) other than black tea gained momentum (Oh et al., 2013; Emiljanowicz & Malinowska-Pańczyk, 2020).

Ultimately the substrate, fermentation time, sweeteners, and starter culture microflora used in kombucha production lead to the emergence of beverages with different chemical compositions and biological activities (Rasu Jayabalan et al., 2014). Especially in certain regions and cultures, plants that are traditionally consumed as cold or hot beverages and also have therapeutic potential can be preferred (Silva et al., 2021).

Rosa canina L. (Rosehip) is a wild rose in the *Rosaceae* family, known as the false fruit of its kind. Rosehip is a remarkable fruit with its high phenolic and antioxidant content (Medveckiene et al., 2020). It is used to treat infections, inflammatory diseases, chronic pain, ulcers etc., (Chrubasik et al., 2008). Therefore, rosehip is widely used in traditional medicine and is preferred in various foods (tea, jelly, jam, probiotic beverage, yoghurt, etc.) (Demir et al., 2014).

Glycyrrhiza glabra L. (Licorice) is a plant widely used, especially in the field of food. (Yang et al., 2020). Since licorice is characterized by its sweet taste and high nutritional value, it is preferred in desserts, beverages, snacks, and specialty foods (Fujii et al., 2014). In addition, the reason why licorice root is preferred in foods is associated with its antioxidant, anti-inflammatory, antimicrobial and antiviral activity originating from the triterpenoids and approximately 300 flavonoids it contains (Wang et al., 2019).

Although it varies according to society, tea is one of the most preferred beverages in the world after water. When it comes to tea, the leaves of the *Camellia sinensis* plant come to mind, and many types of tea are categorized as white, green, black and oolong tea according to the harvest time and fermentation degree of this plant (Khan & Mukhtar, 2019). Although the composition of tea changes depending on many factors, the most important active compound is polyphenols, which decrease due to fermentation. The antioxidant, anticancer, anti-hyperlipidemia, and antidiabetic effects of these compounds, which are high in green tea, are shown by studies (Kamal et al., 2021).

In this study, black tea was traditionally used in kombucha production and rosehip, licorice, and green tea were fermented separately with the kombucha liquid culture. Fermented kombucha beverages were analyzed for their physicochemical, bioactive, antimicrobial, and sensory properties.

2. Materials and Methods

Black tea (Doğuş, Turkey)/green tea leaves (Doğadan, Turkey), rosehip (Mest, Turkey)/licorice plants (Ramco, Turkey), and sugar were purchased from suppliers in Yozgat province. Kombucha liquid culture was provided from Yozgat Bozok University, Boğazlıyan Vocational School (Yozgat, Turkey). The chemicals were purchased from Merck (Darmstadt, Germany) unless otherwise stated.

2.1. Preparation and fermentation of Kombucha Beverages

Kombucha beverages were produced according to Tapias et al. (2022) with minor modifications. For this purpose, it was dissolved by adding sucrose (90 g/L) to the boiling drinking water, and then ten grams of the plant (4 g/L) were added in filtering bags for each tea sample and infused for 20 minutes, cooled to 25°C. After that, it was inoculated with 10% starter kombucha liquid culture and incubated in filtered glass jars for 21 days under dark room conditions (25±2 °C) (Figure 1). Tea samples were analyzed before fermentation (BF) and after fermentation (AF).

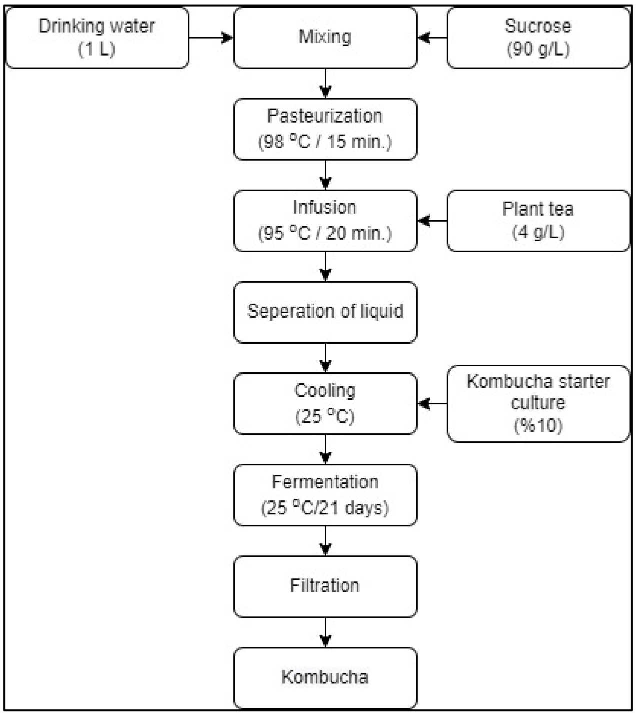

Figure 1. Kombucha production flowchart

2.2. Determination of Some Physicochemical Properties of Kombucha Beverages

Standard methods of AOAC (2002) were used pH, titratable acidity (%), and pellicle weight of the kombucha samples was determined BF and AF.

The pH value, color changes and pellicle weight of the kombucha samples were recorded using a digital pH meter (Hanna, HI 99131, U.S.A.), colorimeter (Lovibond, England), precision scale (Radwag AS 220/C/2 Poland), respectively. The titration acidity was performed by titration in the presence of 0.1 N NaOH and expressed as g/L of acetic acid. The color values (L^*, a^*, b^*) of the kombucha samples were determined according to Ramaswamy ve Richards (1980). ΔE was calculated as;

$$\Delta E = \left[\left(L_{bf} - L_{af}\right)^2 + \left(a_{bf} - a_{af}\right)^2 + \left(b_{bf} - b_{af}\right)^2\right]^{\frac{1}{2}} \tag{2.1}$$

Where *bf* and *af* express the condition of the samples before and after fermentation, respectively.

2.3. Bioactive Properties of Kombucha Beverages

2.3.1. Total Phenolic Content (TPC) Analysis

TPC was performed Folin-Ciocalteu reagent assay (AOAC, 1975). For this purpose, 0.4 mL of kombucha was vortexed in a test tube with 2 mL of Folin-Ciocalteu (10-fold diluted) and 1.6 mL of 7.5% Na_2CO_3, respectively. After 1 hour of incubation in the dark, absorbance was recorded at 765 nm (Optizen POP, Mecasys Co., Ltd, Korea). The TPC value of the samples was calculated according to a standard gallic acid curve and expressed in mg gallic acid equivalent (GAE)/mL kombucha.

2.3.2. Antioxidant Activities Analysis

The antioxidant capacity of kombucha samples was performed to the DPPH method. In context, 0.1 mL kombucha and 3.9 mL (25 mg/L) ethanolic DPPH solution were mixed in a test tube and incubated in a dark place for 30 minutes at room temperature. After incubation, the absorbance of the samples was recorded at 515 nm using a spectrophotometer (Brand-Williams et al., 1995; Sharma & Bhat, 2009).

The antioxidant activity (DPPH inhibition (%)) was calculated according to the formula:

$$DPPH\ inhibition\ (\%) = \frac{A_B - A_S}{A_B} \times 100 \quad (2.2)$$

Where A_B is the absorbance of the blank and A_S is the absorbance of the kombucha.

2.4. *In Vitro* Antimicrobial Activity

The antimicrobial activity of kombucha was determined on several food-borne pathogens, namely gram-negative bacteria *Escherichia coli* (*E. coli*), gram-positive bacteria *Staphylococcus aureus* (*S. aureus*), and *Candida albicans* (*C. albicans*).

Antimicrobial activity was performed based on the agar-based disc diffusion method and spectrophotometric technique (Chiu et al., 2021). Stock cultures were transferred to 5 mL Mueller Hinton Broth for each microorganism and incubated at 35 °C until they reached 10^8 CFU/mL based on OD 600 absorbance. 100 μL of the diluted bacterial load was inoculated onto Mueller Hinton Agar and spread over the entire petri dish with a sterile swab. After waiting for 5 minutes for surface absorption, 6 mm diameter sterile discs were placed on the agar and 25 μL of filtered tea samples were adsorbed on the discs. After the petri dishes were incubated for 24 hours at 35 °C, the zone diameters formed around the discs were measured with a caliper and recorded.

2.5. Sensory Evaluation

Kombucha samples were evaluated at the end of 21 days of aerobic fermentation in terms of color, odor, appearance/brilliant, taste/aroma, and general acceptability. Fermented and unfermented tea samples were presented to each panelist in random order with three-digit codes, respectively.

Samples were rated on a typical 9-point hedonic scale by a consumer panel of 25 panelists. In scoring, (1) dislike extremely, (2) dislike so much, (3) dislike moderately, (4) dislike slightly, (5) neither like nor dislike, (6) like slightly, (7) like moderately, (8) like so much (9) like extremely (Maughan et al., 2012).

2.6. Statistical Analysis

IBM- SPSS 22.0 statistical package (SPSS Inc., Chicago, IL, USA) was used to determine the significance of the differences ($p<0.05$) between the data, and group means were compared with the Duncan multiple comparison test and independent samples T-test. Results were analyzed using one-way analysis of variance (ANOVA) and expressed as mean ± standard deviation. The intensity of the parameters of all samples was visualized using a color-coded heatmap. The corresponding two-dimensional plot was prepared using the software OriginPro 2021 (OriginLab Corporation, Northampton, UK).

3. Results and Discussion

The physicochemical bioactive, antimicrobial, and sensory properties of kombucha beverages are shown in Table 1.

Table 1
Physicochemical, bioactive, antimicrobial, and sensory properties of kombucha beverages

	Tea samples							
	Black tea		Green tea		Rosehip tea		Licorice tea	
Parameters	**BF**	**AF**	**BF**	**AF**	**BF**	**AF**	**BF**	**AF**
pH	6.58±0.02	3.20±0.01*[a]	6.84±0.01	3.12±0,01*[a]	4.13±0.05	3.22±0.03*[a]	5.19±0.01	3.12±0.02*[a]
Titratable acidity (%)	0.44±0.01	9.60±0.00*[c]	0.51±0.01	18.60±0.02*[a]	0.87±0.01	9.00±0.06*[d]	0.33±0.01	18.00±0.08*[b]
Pellicle weight (g/L)	-	19.15±0.07[c]	-	26.88±0.11[a]	-	23.33±0.22[b]	-	15.1±0.05[d]
L	50.87±2.2	49.6±1.34*[b]	58.33±1.06	59.23±0.60*[a]	29.60±1.10	31.30±0.80*[c]	30.50±0.80	17.73±0.66*[d]
a	7.17±1.01	5.83±0.85*[c]	-3.03±0.40	-1.87±0.15*[d]	39.03±1.53	48.87±0.35*[a]	20.43±1.10	21.37±0.66*[b]
b	46.3±0.90	47.97±0.55*[a]	47.1±0.10	39.33±0.66*[c]	27.97±1.17	37.77±0.66*[d]	38.13±1.38	47.77±0.06*[b]
ΔE	-	2.48±0.04[d]	-	7.90±0.01[c]	-	13.99±0.05[b]	-	16.02±0.04[a]
TPC (mg GAE/mL)	61.08±3.02	123.41±1.85*[c]	169.41±2.63	717.08±2.89*[a]	61.26±1.55	77.71±2.33*[d]	50.04±1.47	225.05±3.00*[b]
DPPH inhibition (%)	24.21±0.10	57.76±0.77*[b]	76.29±0.11	93.24±0.63*[a]	24.47±0.09	33.89±1.1*[c]	15.53±0.05	27.38±1.22*[d]
***E.coli* (mm)**	N.D	8.00±0.58[d]	N.D	12.00±0.89[b]	N.D	9.00±1.10[c]	N.D	13.00±0.75[a]
***S.aureus* (mm)**	N.D	15.00±0.22[c]	N.D	20.00±0.30[a]	N.D	11.00±0.35[d]	N.D	16.00±1.00[b]
Color	5.52±0.06	4.92±0.01*[b]	4.72±0.07	4.04±0.3*[c]	6.64±0.01	7.64±0.45*[a]	5.00±0.69	3.72±0.03*[d]
Odor	4.84±0.02	2.8±0.24*[b]	5.72±0.34	2.8±0.22*[b]	6.2±0.74	5.04±0.12*[a]	4.88±0.31	2.76±0.02*[b]
Appearance/Brightness	5.8±0.01	5.04±0.11*[b]	5.24±0.21	4.28±0.13*[c]	7.08±0.36	7.28±0.14*[a]	5.76±0.47	3.64±0.01*[d]
Taste/Aroma	6.04±0.02	3.64±0.26*[b]	5.52±0.14	2.76±0.04*[d]	6.76±0.57	5.92±0.03*[a]	5.32±0.29	3.28±0.07*[c]
Overall acceptability	6.68±0.23	4.00±0.06*[b]	5.48±0.36	3.04±0.47*[d]	7.12±0.06	5.88±0.05*[a]	6.12±0.15	3.56±0.43*[c]

BF: Before fermentation, AF: After fermentation, significant differences ($p<0.05$) between parameters after and before fermentation are expressed with *, the AF tea samples' significant differences ($p<0.05$) were expressed in lowercase letters., N.D: Non-determined.

The pH values of the tea samples before fermentation varied between 4.13 and 6.84. The pH values of the tea samples decreased dramatically after fermentation with the starter kombucha liquid culture, and this decrease was found to be statistically significant ($p<0.05$). This decrease is due to formation of organic acids associated with biological activity because of fermentation. The pH value of tea samples decreases due to the production of organic acids (acetic acid, glucuronic acid, citric acid, etc.) during fermentation. The resulting acidity has a positive effect on the activities of the yeasts, increasing the ethanol concentration and then allowing the acetic acid bacteria to dominate the environment (Dufresne & Farnworth, 2000). The rapid decrease in pH value in the first days of fermentation is important to prevent the development of microorganisms that can spoil (Sharma & Bhat, 2009). The decrease in pH values due to fermentation is similar to other studies (Battikh et al., 2013; Chakravorty et al., 2016). However, the pH change between tea samples at the end of the 21st day was statistically insignificant ($p>0.05$). Different substrate types show distinct changes in pH depending on the starting culture (yeast and acetic acid bacteria). Still, they show similar trends at the end of fermentation (final pH) (Sun et al., 2015). Differences between total acidity values for fermented and unfermented tea samples were statistically significant ($p<0.05$). Although no significant differences were observed between the tea samples at the end of the fermentation period in pH values ($p>0.05$), the total acidity values were found to be statistically significant ($p<0.05$).

There is a negative correlation between pH and total acidity in all tea samples ($r= -0.78$). It is known that the acidity value increases and the pH level decrease after fermentation in tea samples (Velićanski et al., 2014). In addition, towards the end of the fermentation, there was no significant change in the pH values of the samples, while an increase was observed in the total acidity values. On the other hand, acetic acid, one of the dominant acids of kombucha, can be broken down into water and carbon dioxide with over fermentation (R. Jayabalan et al., 2007). Therefore, pH and total acidity should be evaluated together in determining the fermentation time of kombucha samples.

SCOBY forms a film of bacterial cellulose (pellicle) on the surface during fermentation. The pellicle to be formed as a result of fermentation depends on the microorganism load/type of the kombucha starter culture, the substrate, the metabolites produced during fermentation, the fermentation time and incubation conditions, etc. (Mukadam et al., 2016). As fermentation progresses, the pellicle forms thicker layers and protects the kombucha against contaminants (May et al., 2019). Moreover, pellicle can be evaluated as an indicator at the completion of fermentation. The suspended pellicle on the surface tends to sink to the bottom when the fermentation ends. In addition, in recent years, due to its cellulosic composition, it has been used in bio-textile and biomedical fields and as a source of food packaging materials, etc. (Azeredo et al., 2019; Cottet et al., 2020). Moreover, pellicle could be edible and used for gastronomy/culinary (Torán-Pereg et al., 2021). Pellicle; exhibits antioxidant and antimicrobial activity due to its phytochemical characterization (Ramírez Tapias et al., 2020).

Pellicle weight in kombucha teas was determined as 19.15±0.07, 26.88±0.11, 23.33±0.22, and 15.1±0.05 for black, green, rosehip, and licorice teas, respectively. The highest pellicle weight was detected in green tea kombucha (Table 1). The highest bioactivity and antimicrobial activity in fermented green tea are due to the bioactive and antimicrobial compounds provided by both the substrate source and the pellicle thickness.

Table 1 gives the color values (L^*, a^*, b^*, and ΔE). Fermented and unfermented samples of the same teas showed significance ($p<0.05$). While the L^* value increased in green tea and rosehip teas due to fermentation, it decreased in black and licorice teas. Depending on the fermentation, an increase was observed in a* values excluding black and green fermented tea, an addition was found in b* values excluding green tea. The high color change due to fermentation was observed in all tea samples. The differences in brightness in kombucha tea are due to the transformation of polyphenols as a result of the enzymatic activities of microorganisms in its natural flora (R. Jayabalan et al., 2007). Color values may vary due to phenolics, carotenoids, microbial transformation, substrate source, etc., in fermentation and storage periods (Watawana et al., 2018).

TPC and antioxidant potential of fermented tea was in the range between 77.71- 717.08 mg GAE/mL and 27.38-93.24% DPPH inhibition, respectively (Table 1). Before fermentation, black tea, green tea, rosehip, and licorice tea had high TPC and antioxidant capacity and showed statistical differences ($p<0.05$). TPC and DPPH inhibition (%) increased significantly with fermentation in four different tea samples ($p<0.05$).

The intensity of parameters such as physicochemical, bioactive, antimicrobial, and sensory properties of teas obtained from different plant infusions before and after the fermentations were visualized using a color-coded heat map in Figure 2.

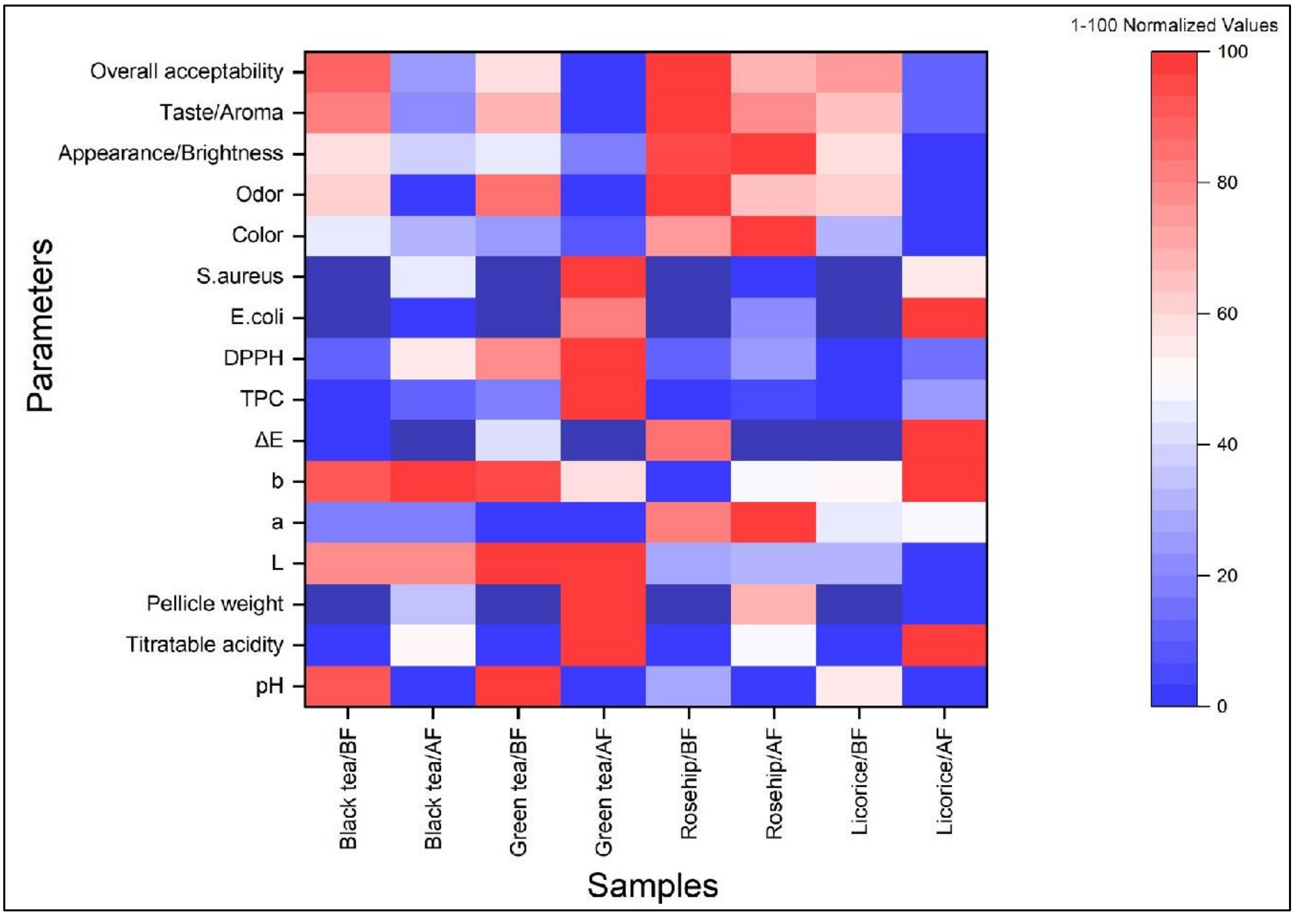

Figure 2. Heat map plot of each sample according to parameters.

In the heatmap, red tones indicate a high parameter value, while blue tones indicate visa versa. In Figure 2, when plant infusions are fermented with SCOBY, it can be observed that their bioactive properties and antimicrobial activities increase. The highest bioactive properties and antimicrobial activities were observed in kombucha samples prepared with green tea (Figure 2).

Higher TPC levels in kombucha are associated with the breakdown of complex phenolic compounds by enzymes in yeast and bacteria (Bhattacharya et al., 2013). Green tea showed the highest TPC (169.41±2.63) and antioxidant activity (76.29±0.11) before fermentation. The highest increase in TPC value (328.28%) was seen in fermented green tea. There is a positive correlation (r=0.77) between TPC and DPPH inhibition %. Kombucha exhibits high free radical scavenging activities. The potency of antioxidant activity also depends on the fermentation time, substrate, fermentation conditions, and culture microbiota. In different kombucha samples, polyphenol compounds and antioxidants activity increased in proportion to the increase in fermentation time. There is a statistical correlation between the polyphenol content of kombucha and its antioxidant capacity. In addition, the substrate used in kombucha production and the fermentation time also affects the bioactive character (Jakubczyk et al., 2020). R. Jayabalan et al. (2007) investigated kombucha phenolics such as epicatechin isomers EGCG ([-]-epigallocatechin-3-gallate), EGC ([-]-epigallocatechin), ECG (-]-epicatechin-3-gallate), and EC ([-]-epicatechin). The results showed modifiable stability of these components throughout the fermentation process. Before fermentation in green tea, EC, ECG, EGC, and EGCG polyphenols predominate, but when fermented, ECG, CG, GA, and EGCG polyphenols dominate the environment. The concentration of polyphenols (catechins and flavonols) responsible for antioxidant activity was reduced in brewed green and black teas (Record & Lane, 2001). Catechins lost in the final product, are further polymerized into high molecular weight molecules leading to the detection of higher phenolic contents in kombucha. Thus, the high phenolic component and antioxidant capacity of kombucha teas in the study can be explained by this phenomenon (Chu & Chen, 2006).

The fact that the antioxidant molecules of tea extracts have a high scavenging effect against free radicals and that this feature increases further and probably provides protection against oxidative damage gives this beverage a functional character. In previous studies, similar results were obtained with biological activities in kombucha made with different plant extracts (Gaggìa et al., 2018; Massoud et al., 2022). Many benefits of kombucha's antioxidant activity are known, such as preventing cancer, boosting immunity, and anti-inflammatory. Beneficial effects of kombucha, it is generally associated with phenolic compounds, acids (gluconic acid, glucuronic acid, acetic acid, lactic acid), amino acids, lipids, proteins, some hydrolytic enzymes, carbon dioxide, vitamins, minerals, etc. (Rasu Jayabalan et al., 2014).

None of the unfermented tea samples showed antimicrobial activity. High antimicrobial activity was detected in fermented teas against *E. coli* and *S. aureus* (Table 1). The highest antimicrobial activity against *E. coli* was determined in licorice fermented tea with inhibition zone diameters of 13 mm. The highest activity against *S. aureus* was detected in green tea (20 mm). In addition, four different fermented tea varieties showed inhibition zone diameters against *E. coli* and *S. aureus*, and the difference between them was statistically significant ($p<0.05$). Antimicrobial activity was not detected in tea samples against *C. albicans*. It is in agreement with the literature that kombuchas did not exhibit antimicrobial activity against *C. albicans* (Greenwalt et al., 1998). In another study reported by Battikh et al. (2013), although it does not show as much inhibition zone diameter as bacteria, *C. albicans* have been shown to be effective. Based on this difference, factors such as kombucha consortium, substrate type, acidity level and type, fermentation conditions, etc., can be effective.

The antimicrobial activity of kombucha against pathogenic microorganisms has been emphasized by many researchers, and this activity has been associated with acetic acid, proteins and catechins (Greenwalt et al., 1998; Sreeramulu et al., 2001). It has been reported that the antimicrobial effect of kombucha is positively related to some organic acids (Greenwalt et al., 1998). Currently, organic acids are a common practice in food preservation against some foodborne pathogenic microorganisms. In addition, Liu et al. (1996) reported that kombucha has antimicrobial activity against pathogenic bacteria, and this is due to acetic acid. High positive correlations coefficient of 0.95 and 0.82 were determined between the antibacterial activities of fermented tea samples against *E. coli* and *S. aureus* and their titratable acidity, respectively.

The sensory parameters such as over color, odor, appearance/brightness, taste/aroma, and overall acceptability of fermented and unfermented tea samples was evaluated (Table1). Sensory score of fermented teas lower than unfermented teas, and the difference is significant ($p<0.05$). There is a statistically difference between kombucha samples prepared with different herbal infusions in terms of overall acceptability ($p<0.05$). A decrease was observed in color and appearance parameter values due to fermentation in other plant infusions except for rosehip. However, these values increased in rosehip. Color is an important criterion for consumers and is associated with appearance. It is thought that rosehip kombuchas are more appreciated in terms of color and appearance, due to the positive effect of the reddish color on the panelists. As clearly seen in Figure 2, rosehip was the most popular (in terms of general acceptability) among the kombucha samples. It was followed by samples of kombucha, in which black, licorice, and green tea infusions were prepared, respectively. However, a decrease in odor, taste, and overall acceptability was observed in all plant infusions due to fermentation. The reason for the low sensory parameters in kombucha samples is the increase in acidity and the dominant taste of acetic acid, which is one of the dominant acids, and the negative effect on consumers due to the smell of vinegar. The negative correlation ($r= -0.80$) between the titratable acidity obtained in the fermented tea samples and the overall acceptability confirms our hypothesis. Although the fermentation process negatively affects the sensory parameters, this problem can be overcome with the conscious consumers' search for healthy beverages and familiarity with long-term consumption.

Determining an optimum fermentation time to produce kombucha can significantly impact consumption acceptability. Otherwise, high acidity limits the amount of use and poses a potential risk, especially for people with stomach-related ailments. However, there are limited studies that reveal the sensory parameters of kombucha. The inclusion of gastronomic considerations in kombucha studies over the past two decades is promising (Kim & Adhikari, 2020).

4. Conclusion

The increasing consumer interest in functional foods has recently affected the soft drink industry. This interest is more in traditional fermented beverages because of their positive effects on health. Kombucha, known and consumed as fermented tea for centuries, is still consumed with or without knowing its positive effects on health, thanks to its primary and secondary components. In order to explain the health benefits of kombucha, its antioxidant and antimicrobial capacity is emphasized. Since the beneficial properties and sensory parameters of kombucha usually depend on the fermented tea type when some other parameters remain constant (the content of SCOBY, fermentation conditions, etc.); in this study, physicochemical, bioactive, antimicrobial, and sensory properties were tested by using four different plant infusions. Kombucha samples were tested on day 21. The results showed that each fermented beverage had higher antioxidant activity than unfermented ones. DPPH inhibition activity by fermentation of teas increased by 138%, 22.22%, 38.50%, and 76.30% for black, green, rosehip, and licorice samples, respectively. Tea samples that did not show antimicrobial activity showed effective inhibition zone diameter against *E. coli* and *S. aureus* after fermentation. The most beneficial fermented beverage in terms of bioactive properties was observed in kombucha prepared from green tea. It also showed the highest antimicrobial activity against *S. aureus*. The highest antimicrobial activity against *E. coli* was detected in kombucha produced from licorice. However, kombucha prepared with rosehip infusions was most appreciated by the panelists. While fermented rosehip sample is lower than fermented green tea in terms of bioactive and antimicrobial properties, they are higher in antioxidant capacity than fermented licorice tea. In kombucha production, the kombucha starter culture, the substrate, the fermentation time, and incubation conditions affect the physicochemical, bioactive, antimicrobial, and sensory properties of the final product. Here, the final decision should be left to the consumers, depending on personal needs and demands.

Although kombucha consumption has increased rapidly in recent years, it has not come to the place it deserves. Low sensory acceptability scores can be resolved by optimizing the fermentation time and/or flavoring. Moreover, natural or artificial carbonation can positively affect consumers' purchasing.

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Author Contributions

Cemhan Doğan: Provided the concept of the study, drafting of the article and final approval of the submitted version

Nurcan Doğan: Provided the analysis and interpretation of data, drafting and revision of the article.

Conflicts of Interest

The authors declare no conflict of interest.

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