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Determination of Potential Antimicrobial Activities of some Local Berries Fruits in Kombucha Tea Production

Gökhan Akarca[1*]
https://orcid.org/0000-0002-5055-2722

[1]Afyon Kocatepe University, Engineering Faculty, Food Engineering Department, Afyonkarahisar, Turkey.

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*Correspondence: gakarca@aku.edu.tr; Tel.: +90-272-2182300 (G.A.).

HIGHLIGHTS

- Kombucha is a tea formed by symbiotic combination of bacteria and yeasts.
- Kombucha teas produced with blackberry, raspberry, and red goji berry.
- Tea samples produced with blackberry were the most appreciated ones in all criteria.
- Potassium, magnesium, catechin and gallic acid were detected in all samples.

Abstract: In this study, physicochemical, microbiological, and sensory properties, antibacterial and antifungal effects of kombucha teas produced with some small berry fruits (blackberry, raspberry, and red goji berry) were investigated. During fermentation, titratable acidity and pellicle biomass weights increased whereas water activity, brix, viscosity, L* and b* values decreased. At the end of fermentation, the highest minerals determined in the samples were potassium and magnesium. Also, catechin and gallic acid were detected in all samples. Samples produced with blackberry were the most appreciated ones in all criteria. The highest antibacterial and antifungal effects were determined in samples containing blackberries on *Staphylococcus aureus* and *Rhizopus nigricans* (24.36 and 20.53 mm zone diameters). The antibacterial effect, MIC, and MBC values (0.023 and 0.016 mg/L) on *Staphylococcus aureus*. Regarding the antifungal effect, the MIC and MFC values were determined in tea produced with blackberry on *Rhizopus nigricans* with 0.035 mg/L, and 0.023 mg/L.

Keywords: Anti-Microbial Effect; Blackberry; Gallic Acid; Kombucha Tea; Potassium.

INTRODUCTION

Medicinal plants are vital to human life. These plants contain many biologically valuable natural components such as alkaloids, flavonoids, iridoids, coumarins [1-4]. In addition, studies have shown that these components of plants are antimicrobial, antioxidant, anti-inflammatory, anticarcinogenic, etc. has also demonstrated the characteristics. Today, most of these herbs are considered to be the main parts of ethnobotanical and traditional folk medicine of different countries around the world [5-7].

Kombucha or pellicle layer is defined as a system formed by the symbiotic combination of acetic and lactic acid bacteria and osmophilic yeasts as the starting culture [8,9]. Kombucha tea, with this pellicle layer, is a non-alcoholic, gas-free, slightly acidic beverage with a taste similar to sparkling apple juice, which is obtained as a result of a fermentation lasting approximately 14 days [10,11]. Tea was first consumed in east Asia and then has become a beverage consumed worldwide [12,13]. Kombucha tea, which is mostly produced under domestic conditions, albeit limited, is also commercially produced. Kombucha tea is a very medicinal drink that contains numerous good bacteria and beneficial yeasts. It has been used for a long time in treating of various diseases in alternative medicine in many Far East countries, especially in China. [14,15].

The composition of tea includes organic acids such as acetic, gluconic, gluconic, citric, L-lactic, malic, tartaric, malonic, oxalic, succinic, pyruvic and usnic acid, and sugars such as sucrose, glucose, and fructose. It also contains vitamins B1, B2, B6, B12 and vitamin C, 14 amino acids, pigments, lipids, proteins, some hydrolytic enzymes, antimicrobial substances including carbon dioxide, phenols, polyphenols and minerals [15-18]. Such rich content makes tea more valuable than its counterparts. Also, its bioactive phenolic components (flavonoids and catechin) increase the functional value of tea [19,20].

Many fruits contain ingredients called "nutraceuticals" such as phenolics, anthocyanins and other flavonoid compounds that may play an important antioxidant role by preventing the spread of new free radical species [21,22]. Small berries contain these compounds, which have extremely positive effects on human health, at a higher level compared to other fruits [23,24]. Kombucha tea can also be produced by using various berries, e.g., blackberry, black mulberry, rosehip, currant, black grape, raspberry, etc. In this way, the acidic and sour taste softens. In addition, a product with increased attractiveness for consumers and added value in nutritional and functional aspects is produced [25,26].

The present study aimed to determine the physical, chemical, microbiological and sensory properties, and antibacterial and antifungal effects of Kombucha teas produced with Black tea and some small berries (blackberry, raspberry, and red goji berry).

MATERIAL AND METHODS

Materials

Black tea and dried berries used in the study were obtained from a local company in Afyonkarahisar, Turkey. The identification of fruits at the sub-species and variety level was made by the faculty members of Afyon Kocatepe University, Faculty of Sciences and Literature Department of Biology.

Microorganisms used in the study

In this study; *Escherichia coli* O157:H7 (ATCC 35150), *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica* (ATCC 35664), *Enterococcus faecalis* (ATCC 29212), *Vibrio vulnificus* (ATCC 27562), *Bacillus cereus* (ATCC 14579) and *Listeria monocytogenes* (ATCC 51774), *Aspergillus fumigatus* (ATCC 1022), *Penicillium chrysogenum* (ATCC 10106), *Byssochlamys fulva* (ATCC 10099), *Mucor ramosissimus* (ATCC 90286), *Rhizopus nigricans* (ATCC 6227), *Cladosporium sphaerosumum* (ATCC 11289) and *Geotrichum candidum* (ATCC 16635) microorganisms belonging to the strains were used.

Preparation of Kombucha teas

The production of Kombucha teas was made by modifying the conditions specified by Akarca and Tomar [27]. Accordingly, 60 g of Blackberry (*Rubus fruticosus* L.) (BB), raspberry (*Rubus idaeus* L.) (RB) and red goji berry (*Lycium barbarum* L.) (GB) and 25 g black tea (*Camellia sinensis*) (BT) were mixed. Then 1000 mL drinking water and sucrose (100 g/L) were added to the mixture. The mixture was dissolved for 20 min. at 95°C. After waiting for the mixture to infuse for 20 min. The samples were filtered through a sterile filter paper (Whatman, Grade 54, Diameter 55 mm) and then subsequently autoclaved at 121°C for 20 min. in an autoclave (Nuve, OT 90L, Turkey).

After being sterilized in sealed glass bottles, they were cooled at 25˚C and, from previously produced tea samples, 45 g/L pellicle from biomass phase and 150 mL liquid phase was added. The samples were left to ferment for 21 days at 24±3°C in a dark environment.

Physicochemical analyses

Brix (% soluble dry matter content) values of Kombucha tea samples were determined by a refractometer (Atago, N-1E, Japan) at 25°C [11] while titratable acidity was determined according to Yıkmıs and Tuggum [28]. Color measurements (L*, a* and b*) were carried out using the Hunter Lab colorimeter (Konika Minolta Chromium Meter, CR-400, Japan) [7]. Viscosity values (Brookfield, Middleboro, MA, USA) were measured at 25°C with spindle (No.2) at 50 rpm [29] while pellicle biomass weight was measured using a precision balance

(Radwag PS-1000 R2, Poland) and water activity (aw) values were determined according to AOAC 978.18 using a water activity device (Novasina, LabTouch, Switzerland) [30,31]. Phenolic components of the tea samples were determined by High-Performance Liquid Chromatography (HPLC) (Agilent 1200 series HPLC, Waldbronn, Germany) and as described by Vulić and coauthors [32]. Mineral concentrations in samples were measured using ICP-OES (Plasma Quant PQ 9000) [33].

Microbiological analysis

Microbiological analyses of the samples were made according to the smear plate method. Accordingly, 1 mL dilutions prepared from tea samples were taken using automatic pipettes (Research Plus, Eppendorf, Germany) and inoculated in suitable media. Then, using a sterile Drigalski spatula (Firatpen, Turkey), the samples were uniformly spread on the surface of the media [34]. Lactic acid bacteria count was determined using Man Rogasa and Sharpe (MRS) Agar (Merck 1.10661, Germany) at 30°C under anaerobic conditions for 24-48-h. Acetic acid bacteria count analyses were carried out using Yeast Extract Calcium Carbonate Glucose (YCG) Agar (Himedia M1182, India) at 30°C under aerobic conditions for 5-10 days [35]. Lactococcus/Streptococcus bacteria counts analysis was carried out using M-17 Agar (Merck, 1.15108, Germany) with 30°C in aerobic conditions for 24-48 h. [36]. Total aerobic mesophilic bacteria (TAMB) count analyses were carried out using Plate Count Agar (PCA) (Merck 1.05463, Germany) for 48-72 h. under aerobic conditions [37]. Yeast/mold count analyses were carried out using Rose Bengal Chloramphenicol Agar (Merck 1.00467) under aerobic conditions for 3-5 days at 22°C [38,39]. Osmophilic yeast analyses were carried out using Dichloran Glycerol (DG-18) Agar (Merck 1.04092, Germany) under aerobic conditions at 30°C for 5-7 days [38,39].

Antibacterial and antifungal activity analyses

Antibacterial and antifungal activity analyses were performed by the disc diffusion technique [40]. Samples from 24-hour-old colonies produced using specific media were taken in physiological saline solution (Merck, 115525, Germany) and adjusted to 0.5 McFarland using a densitometer (Biosan, 1B, Turkey). Then 0.1 mL (106-107 cfu/mL) from each inoculum was taken using a sterile pipette and Mueller Hinton Agar (MHA) (Merck 1.05437) was used to determine the antibacterial activity while MHA modified with the addition of glucose (2%) and methylene blue (0.5 mg/L) was used for the determination of antifungal activity and was transferred to the surface of the medium and spread homogeneously.

After absorbing 200 µL of Kombucha tea samples, blank antibiogram discs (6 mm, Bio-Disk 316010001, Oxoid, Basinstoke, UK) were dried in the laminar flow cabinet (Cryste, Puricube 1200, Thailand) and were added to the different regions of Mueller Hinton Agar media in such a way that they cannot contact each other [41]. For the determination of antifungal activity, the Petri dishes were incubated for 5-7 days at 25°C and for the determination of antibacterial activity the Petri dishes were incubated for 16-20 hours at 37°C (Listeria monocytogenes in 5% CO_2 medium) (Incucell, MMM, Germany) [42]. The diameters (mm) of the zones formed at the end of the incubation were measured using a digital caliper (Mitutoyo, 500-181-30, Japan) in sufficient daylight [27]. The inoculations were carried out according to the spread plate method.

Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

The minimum inhibitory concentrations (MIC) of Kombucha tea samples were determined according to the macro dilution method. The MIC value was determined by taking the arithmetic average of the concentrations of the first tubes which were determined to have sediment, turbidity, membrane, and micelle development on their surface after the incubation and the concentrations of the previous tube that did not exhibit a growth [43,44].

The minimum bactericidal (MBC) and minimum fungicidal (MFC) concentration values were determined by taking 1 µL from the first tube observed to have microbial development and from all other tubes following this tube and inoculating on the surface of MHA (for MFC modified with the addition of methylene blue and 2% glucose) and inoculating at the appropriate temperature (for MFC, 24±2°C 5/7 days, for MBC, 16/20 h. at 35±2°C, for Listeria monocytogenes % 5 CO_2 media). At the end of incubation, no microbial growth was observed, and the determined concentration value was taken as MBC for bacteria and as MFC for molds [45].

Sensory analysis

Sensory analysis of tea samples was carried out at the end of fermentation in the sensory analysis laboratory by a team of 20 panelists. Accordingly, 200 mL from each tea sample was taken in glasses and encoded with letters. The samples were evaluated by the panelists three times in terms of taste, odor, texture, appearance, and general taste. Panelists valued each parameter between 1-10, according to the hedonic scale [36,46].

Statistical analysis

The results of the study were analyzed using the SPSS V 23.0.0 statistical package software program. The study was conducted in duplicate and two parallels, and the data obtained as a result of the analyses were evaluated by the variance analysis. The level of significance was determined by the Duncan test (P<0.05).

RESULTS

Brix (%) of samples

The Brix (%) values of all Kombucha tea samples are shown on Table 1.

Titratable acidity (%)

Fermentation time, sample type and fermentation time x sample type interactions had a significant effect (P<0.0001) on titratable acidity. During the three-week fermentation, titratable acidity values of samples are shown on Table 1.

Table 1. Physicochemical analysis results of samples.

	%Brix	Titration Acidity (%)	Viscosity(cP)
Samples (S)			
BT	5.55±2.30^{a}	12.27±0.24^{d}	47.00±11.46^{c}
RB	7.25±3.40^{a}	14.88±1.05^{b}	55.50±10.30^{ab}
BB	9.63±3.09^{a}	16.16±1.82^{a}	61.50±7.39^{a}
GB	6.75±2.99^{a}	14.18±0.38^{c}	52.00±11.56^{bc}
P Value	0.238	<0.0001	0.005
Fermentation Time (FT)			
0	7.90±4.37^{a}	13.29±0.80^{d}	64.00±6.05a
7	7.55±3.45^{a}	14.09±1.26^{c}	60.00±7.17a
14	7.03±2.80^{a}	14.71±1.76^{b}	47.50±9.61b
21	6.70±2.35^{a}	15.40±2.36^{a}	44.50±8.12b
P Value	0.927	<0.0001	<0.0001
S x FT			
BT x 0	6.10±3.00^{a}	12.05±0.15^{h}	58.00±4.00^{abcd}
RB x 0	8.00±5.00^{a}	13.53±0.21^{g}	66.00±4.00^{a}
BB x 0	10.00±4.00^{a}	13.86±0.13^{fg}	68.00±2.00^{a}
GB x 0	7.50±3.00^{a}	13.73±0.20^{g}	64.00±6.00^{ab}
BT x 7	5.70±2.00^{a}	12.26±0.21^{h}	54.00±8.00^{abcde}
RB x 7	7.60±3.00^{a}	14.56±0.15^{e}	62.00±3.00^{abc}
BB x 7	9.80±2.00^{a}	15.46±0.19^{d}	66.00±3.00^{a}
GB x 7	7.10±4.00^{a}	14.06±0.11^{efg}	58.00±4.00^{abcd}
BT x 14	5.30±1.00^{a}	12.36±0.19^{h}	40.00±6.00^{ef}
RB x 14	6.90±2.00^{a}	15.23±0.18^{d}	48.00±5.00^{bcdef}
BB x 14	9.50±3.00^{a}	16.89±0.16^{b}	58.00±6.00^{abcd}
GB x 14	6.40±2.00^{a}	14.37±0.16^{ef}	44.00±6.00^{def}
BT x 21	5.10±2.00^{a}	12.42±0.12^{h}	36.00±2.00^{f}
RB x 21	6.50±1.00^{a}	16.19±0.14^{c}	46.00±2.00^{cdef}
BB x 21	9.20±2.00^{a}	18.42±0.24^{a}	54.00±4.00^{abcde}
GB x 21	6.00±1.00^{a}	14.57±0.13^{e}	42.00±5.00^{def}
P Value	1.000	<0.0001	0.992

Black Tea: BT, Raspberry: RB, Blackberry: BB, Red Goji Berry: GB, a-h (↓): Values with the same capital letters in the same column for each analysis differ significantly (P<0.05).

Viscosity values

Fermentation time (P<0.0001) had a significant effect, and the sample type has an effect (P<0.01) on viscosity. Viscosity values of Kombucha tea samples were measured at 22ºC and 50 rpm and decreased during fermentation (P<0.05; Table 1).

Microbiological analysis results

Sample type and fermentation time had a significant effect (P<0.0001) on acetic acid bacteria counts. Also, fermentation time (P<0.0001) had a significant effect on TAMB, osmophilic yeast, *Lactococcus/Streptococcus* bacteria and lactic acid bacteria counts. On the other hand, the sample type has an effect (P<0.01) on osmophilic yeast, and *Lactococcus/Streptococcus* bacteria counts. Microbiological analysis results of Kombucha tea samples are shown in Table 2.

Table 2. Microbiological analysis results of samples.

	TAMB Count	Yeast/Mold Count	Osmophilic Yeast Count	L/S Species Bacteria	Lactic Acid Bacteria	Acetic Acid Bacteria
Samples (S)						
BT	3.47±0.59[a]	5.02±0.30[a]	4.40±0.43[c]	3.19±0.65[c]	3.26±0.66[c]	3.79±0.91[d]
RB	3.19±0.67[ab]	4.89±0.36[a]	4.79±0.48[ab]	3.67±0.68[ab]	3.60±0.64[ab]	4.90±1.32[b]
BB	3.03±0.66[b]	4.82±0.39[a]	5.00±0.55[a]	3.84±0.70[a]	3.82±0.66[a]	5.57±1.55[a]
GB	3.30±0.65[ab]	4.93±0.33[a]	4.62±0.40[bc]	3.46±0.65[bc]	3.44±0.65[bc]	4.42±1.19[c]
P Value	0.045	0.587	0.008	0.002	0.06	<0.0001
Fermentation Time (FT)						
0	3.97±0.26[a]	5.19±0.20[a]	4.18±0.27[c]	2.79±0.21[d]	2.59±0.30[c]	3.24±0.34[d]
7	3.55±0.28[b]	5.08±0.19[ab]	4.56±0.33[b]	4.39±0.36[a]	3.52±0.33[b]	4.02±0.75[c]
14	2.99±0.27[c]	4.83±0.25[bc]	4.89±0.39[a]	3.68±0.32[b]	3.88±0.27[a]	5.19±0.88[b]
21	2.47±0.30[d]	4.55±0.28[c]	5.18±0.35[a]	3.29±0.46[c]	4.12±0.31[a]	6.23±0.98[a]
P Value	<0.0001	0.002	<0.0001	<0.0001	<0.0001	<0.0001
S x FT						
BT x 0	4.13±0.17[a]	5.21±0.18[a]	3.96±0.28[f]	2.63±0.10[h]	2.32±0.22[g]	2.87±0.19[h]
RB x 0	3.96±0.23[ab]	5.19±0.21[ab]	4.25±0.18[def]	2.85±0.21[gh]	2.68±0.12[fg]	3.35±0.15[gh]
BB x 0	3.77±0.22[abc]	5.17±0.20[ab]	4.31±0.19[def]	2.97±0.11[fgh]	2.85±0.24[efg]	3.56±0.21[fg]
GB x 0	4.03±0.18[a]	5.20±0.16[a]	4.18±0.19[ef]	2.72±0.13[h]	2.51±0.19[g]	3.16±0.23[gh]
BT x 7	3.74±0.26[abc]	5.16±0.20[ab]	4.26±0.21[def]	4.02±0.23[bcd]	3.21±0.16[def]	3.16±0.23[gh]
RB x 7	3.49±0.17[abcd]	5.06±0.18[abc]	4.65±0.23[bcdef]	4.51±0.18[ab]	3.59±0.18[bcd]	4.25±0.18[e]
BB x 7	3.34±0.24[bcde]	4.98±0.18[abc]	4.85±0.22[abcde]	4.72±0.22[a]	3.86±0.20[abc]	4.98±0.19[d]
GB x 7	3.61±0.20[abcd]	5.11±0.13[ab]	4.46±0.21[cdef]	4.32±0.24[abc]	3.42±0.23[cde]	3.67±0.14[efg]
BT x 14	3.21±0.20[cde]	4.94±0.24[abc]	4.52±0.25[cdef]	3.41±0.18[defg]	3.64±0.18[bcd]	4.15±0.15[ef]
RB x 14	2.94±0.18[defg]	4.81±0.21[abc]	4.98±0.28[abcd]	3.76±0.27[cde]	3.96±0.22[abc]	5.42±0.23[cd]
BB x 14	2.79±0.16[efgh]	4.74±0.23[abc]	5.27±0.25[ab]	3.99±0.14[bcd]	4.12±0.17[ab]	6.33±0.22[b]
GB x 14	3.05±0.24[def]	4.86±0.19[abc]	4.79±0.16[bcde]	3.55±0.17[def]	3.81±0.14[abcd]	4.87±0.16[d]
BT x 21	2.78±0.18[efgh]	4.75±0.21[ab]c	4.85±0.18[abcde]	2.69±0.25[h]	3.86±0.19[abc]	4.96±0.26[d]
RB x 21	2.37±0.21[gh]	4.49±0.23[bc]	5.26±0.24[ab]	3.54±0.22[def]	4.16±0.26[ab]	6.59±0.28[b]
BB x 21	2.21±0.13[h]	4.38±0.22[c]	5.56±0.20[a]	3.67±0.25[de]	4.43±0.12[a]	7.39±0.13[a]
GB x 21	2.52±0.21[fgh]	4.56±0.25[abc]	5.06±0.11[abc]	3.25±0.12[efgh]	4.02±0.18[abc]	5.98±0.20[bc]
P Value	1.000	1.000	0.997	0.892	1.000	0.038

Black Tea: BT, Raspberry: RB, Blackberry: BB, Red Goji Berry: GB, L: Lactococcus, S*: Streptococcus*, a-h (↓): Values with the same capital letters in the same column for each analysis differ significantly (P<0.05).

Water activity (aw)

Water activity (a_w) values of kombucha samples decreased during fermentation (Figure 1.; P<0.05).

Pellicle biomass weight

Fermentation time, sample type and fermentation time x sample type interactions had a significant effect (P<0.0001) on pellicle biomass weight. Pellicle biomass weight increased in all samples during fermentation (Figure 2., P<0.05).

Color values

Sample type and fermentation time interactions had a significant (P<0.0001) effect on L*, a* and b* values. In addition, the sample type x fermentation time (P<0.05) is effective on the b* value. The changes of the L*, a* and b* values of the samples during storage are shown in Figures 3, 4 and 5.

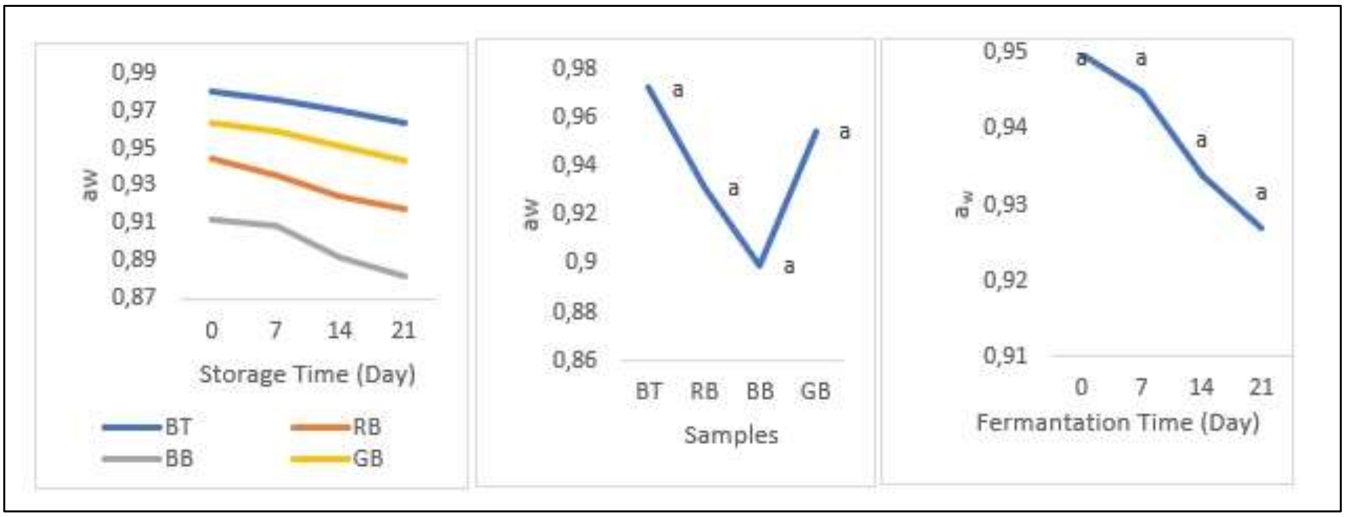

Figure 1. The variation of the water activity values of the samples depending on the storage time and the variation of the water activity depending on the samples and the storage time. Black Tea: **BT**, Raspberry: **RB**, Blackberry: **BB**, Red Goji Berry: **GB**.

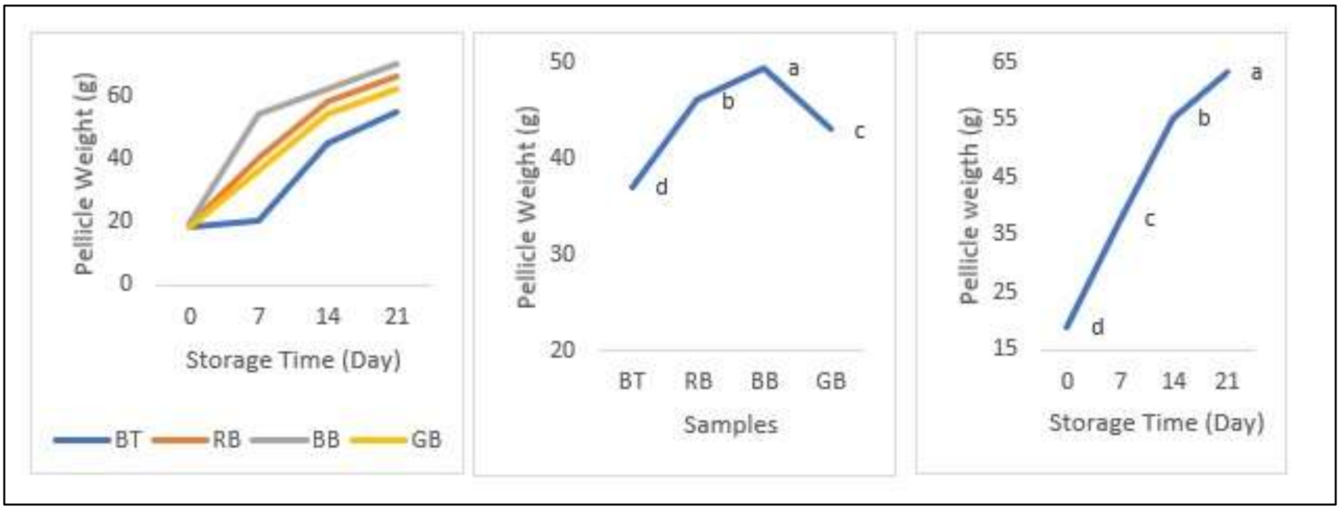

Figure 2. Variation of pellicle biomass weight based on storage time and variation of pellicle biomass weight based on samples and storage time. Black Tea: BT, Raspberry: RB, Blackberry: BB, Red Goji Berry: GB.

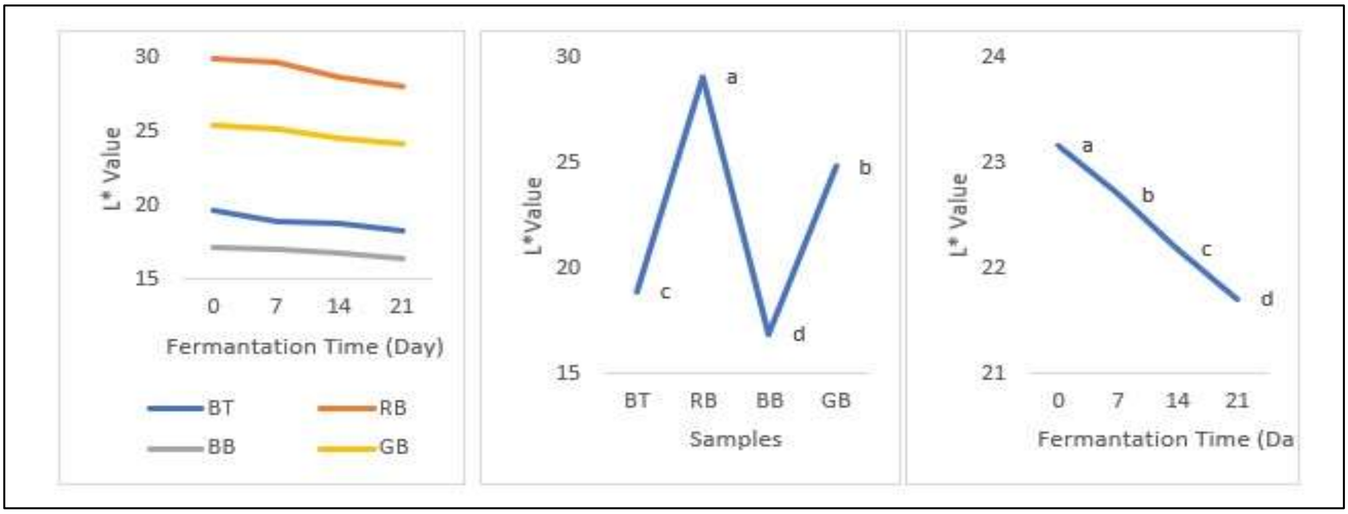

Figure 3. The variation of L* values of the samples depending on the storage time and the change of the L* value depending on the samples and fermentation time. Black Tea: **BT**, Raspberry: **RB**, Blackberry: **BB**, Red Goji Berry: **GB.**

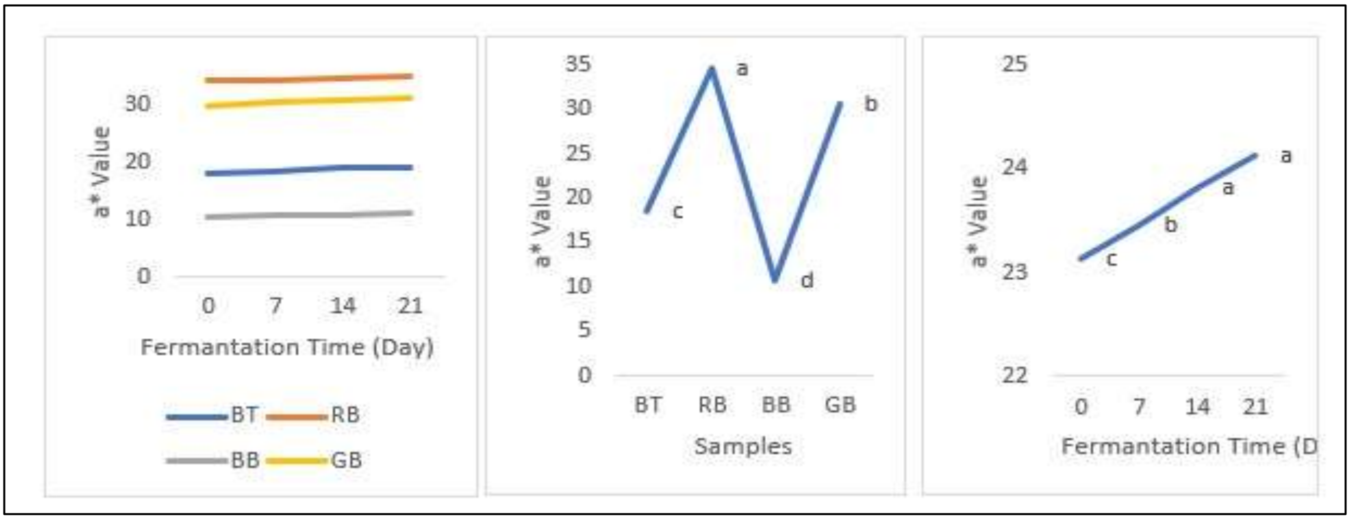

Figure 4. The variation of a* values of the samples depending on the storage time and the change of the a* value depending on the samples and fermentation time. Black Tea: **BT**, Raspberry: **RB**, Blackberry: **BB**, Red Goji Berry: **GB**.

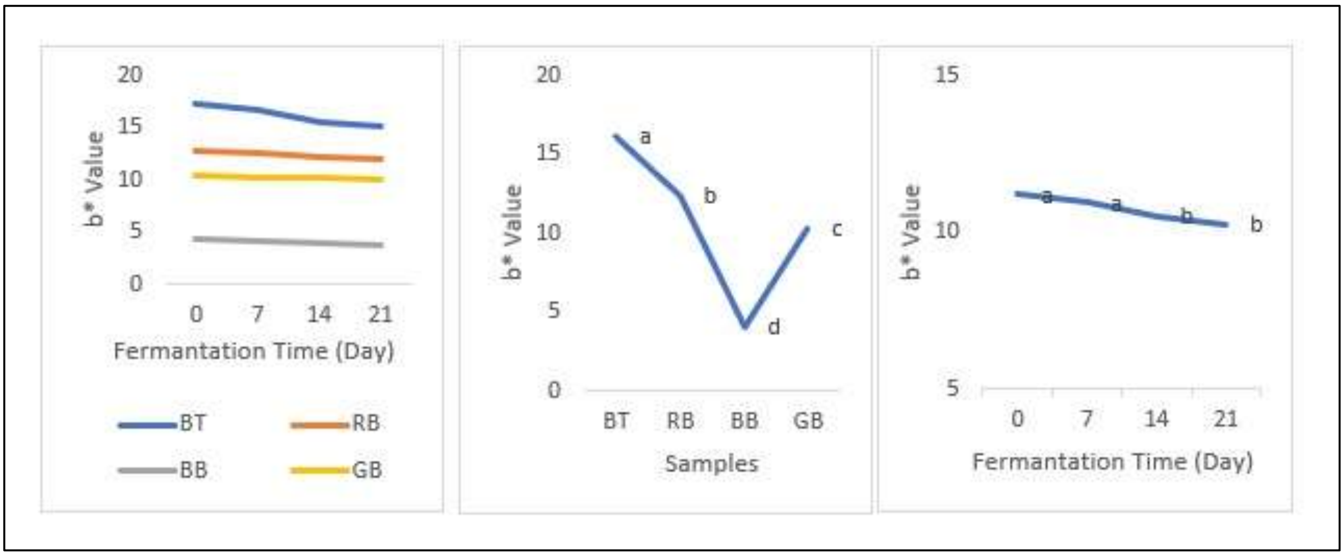

Figure 5. The variation of b* values of the samples depending on the storage time and the change of the b* value depending on the samples and fermentation time. Black Tea: **BT**, Raspberry: **RB**, Blackberry: **BB**, Red Goji Berry: **GB**.

Amounts of mineral substances

Amounts of mineral substances of Kombucha tea samples are shown in Table 3.

Table 3. Mineral substance values (mg/kg) of the samples at the end of fermentation.

	Minerals (mg/kg)				
Sample	**Calcium (Ca)**	**Potassium (K)**	**Magnesium (Mg)**	**Phosphorus (P)**	**Copper (Cu)**
BT	0.00±0.00[c]	8498.12±123.41[b]	763.36±0.65[a]	5461.19±116.12[a]	10.21±0.12[a]
RB	236.47±23.14[b]	1486.32±112.25[c]	149.23±33.41[b]	197.52±25.78[c]	0.16±0.08[c]
BB	271.45±33.14[b]	1487.52±123.41[c]	236.41±28.14[b]	169.45±25.42[c]	0.87±0.12[c]
GB	998.19±54.23[a]	12421.52±153.42[a]	765.12±42.13[a]	976.36±52.31[b]	9.12±0.41[b]
Sample	**Manganese (Mn)**	**Iron (Fe)**	**Zinc (Zn)**	**Sodium (Na)**	
BT	156.32±33.12[a]	99.24±22.13[a]	15.49±0.16[a]	296.36±0.12[a]	
RB	3.97±0.13[b]	6.49±0.21[b]	0.97±0.15[d]	0.00±0.00[b]	
BB	26.12±0.54[b]	9.85±0.33[b]	2.12±0.12[c]	0.00±0.00[b]	
GB	4.23±0.25[b]	43.12±19.63[ab]	6.58±0.26[b]	0.00±0.00[b]	

Black Tea: **BT**, Raspberry: **RB**, Blackberry: **BB**, Red Goji Berry: **GB.** a-d (↓): Values with the same capital letters in the same column for each analysis differ significantly (P<0.05).

The phenolic compounds

The phenolic compounds determined in the kombucha tea samples at the end of the three-week fermentation period and their values are given in Table 4.

Table 4. Phenolic content of the samples at the end of the fermentation period (mg/100g).

Phenolics (mg/100g)	**Samples**			
	Black Tea	**Raspberry Tea**	**Blackberry Tea**	**Red Goji Berry Tea**
Gallic Acid	6.50±0.29[b]	1.85±0.12[c]	0.64±0.19[d]	40.44±0.48[a]
Coumaric Acid	0.00±0.00[b]	3.76±0.34[a]	0.00±0.00[b]	0.63±0.18[b]
Chlorogenic Acid	0.95±0.15[b]	0.22±0.11[c]	0.00±0.00[c]	3.19±0.24[a]
Catechin	21.13±0.33[c]	58.34±0.69[b]	92.38±0.38[a]	21.07±0.34[c]
Kaempferol	0.00±0.00[b]	0.27±0.12[a]	0.00±0.00[b]	0.00±0.00[b]
Quercetin	0.00±0.00[b]	0.65±0.11[a]	0.00±0.00[b]	0.00±0.00[b]
Epicatechin	1.47±0.17[b]	6.99±0.21[a]	0.00±0.00[c]	0.00±0.00[c]
Epigallocatechin	2.58±0.23[b]	5.92±0.23[a]	0.00±0.00[c]	0.00±0.00[c]
Ellagic acid	0.00±0.00[b]	0.00±0.00[b]	1.29±0.24[a]	0.00±0.00[b]
Gallocatechin	19.15±0.56[a]	0.00±0.00[b]	0.96±0.15[b]	0.00±0.00[b]
Syringic acid	0.00±0.00[b]	0.00±0.00[b]	1.91±0.22[a]	0.00±0.00[b]
Salicylic acid	0.00±0.00[b]	0.00±0.00[b]	17.64±0.42[a]	0.00±0.00[b]
Protocatechuic acid	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	14.75±0.39[a]
Vanillic acid	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	4.19±0.17[a]
Caffeic acid	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	7.18±0.26[a]
Ferulic acid	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	0.70±0.12a
Rutin	2.18±0.19[a]	0.00±0.00[b]	0.00±0.00[b]	0.43±0.14[b]
Caffeine	20.18±0.86[a]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]

a-d (→): Values with the same capital letters in the same rows for each analysis differ significantly (P<0.05).

Sensory analysis

Sensory analysis results at the end of the fermentation period in Kombucha tea samples are shown in Figure 6.

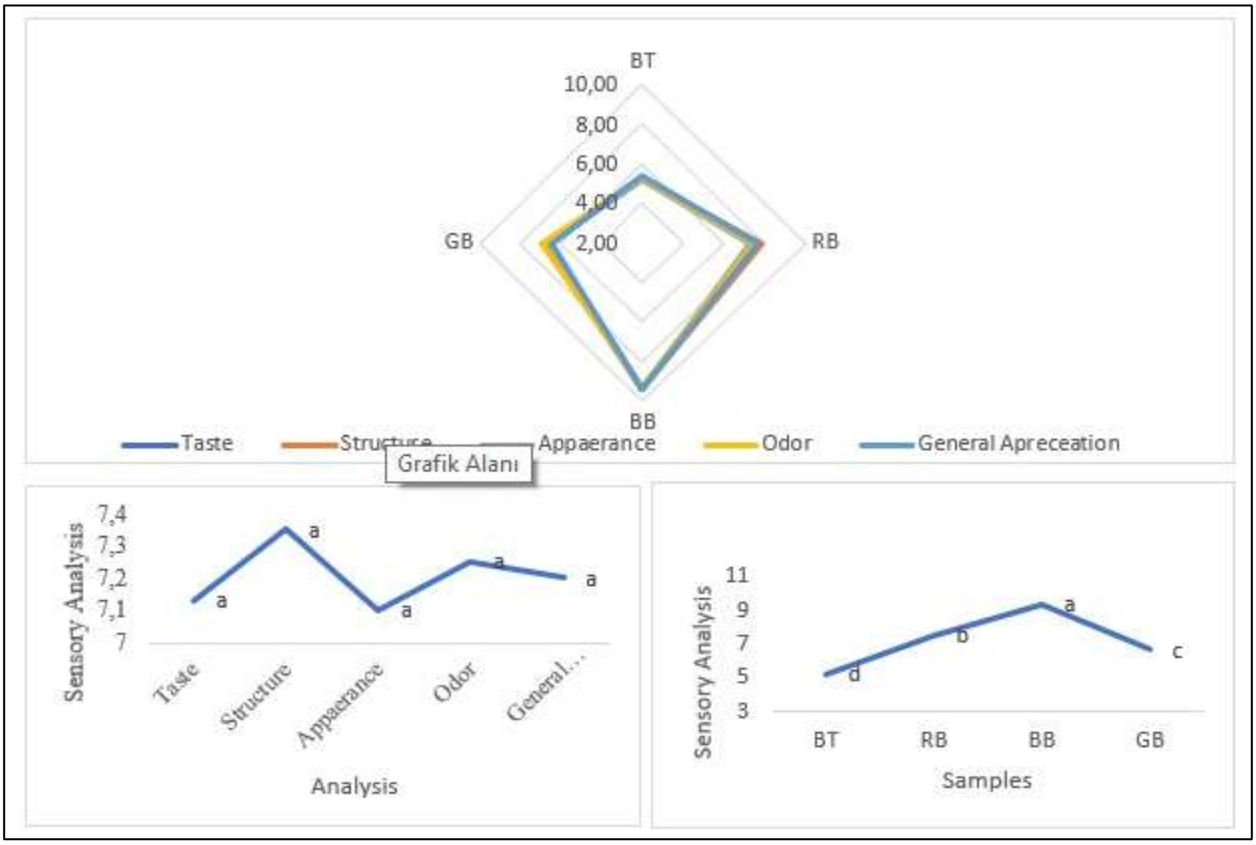

Figure 6. Sensory analysis results of the samples as a result of fermentation and analysis and variation of sensory analysis results depending on the sample type Black Tea: BT, Raspberry: RB, Blackberry: BB, Red Goji Berry: GB, <3: Unacceptable, 3-4: Weakly, 5-6: Acceptable 7-8: Very Good 9-10: Perfect.

The antibacterial and antifungal effects

Bacteria type, sample type and bacteria type x sample type interactions had significant effects (P<0.0001) on antibacterial effect. Likewise, mold type, sample type and mold type x sample type interactions had significant effects (P<0.0001) on antifungal effect, The antibacterial and antifungal effects of Kombucha samples on seven different food-borne bacteria and saprophytic mold species are shown in Table 5.

Table 5. Antimicrobial effects of the kombucha tea samples (mm zone diameter).

Microorganism	Samples			
	Black Tea	Raspberry	Blackberry	Red Goji Berry
Escherichia coli O157:H7	13.32±0.15^{Ca}	16.38±0.14^{Bd}	18.44±0.15^{Ae}	16.25±0.19^{Bc}
Staphylococcus aureus	12.64±0.17^{Db}	18.45±0.23^{Bb}	24.36±0.21^{Aa}	17.49±0.23^{Cb}
Salmonella enterica	13.37±0.16^{Da}	19.36±0.12^{Ba}	21.33±0.18^{Ab}	18.25±0.15^{Ca}
Enterococcus faecalis	11.26±0.19^{Dc}	17.52±0.17^{Bc}	19.28±0.16^{Ad}	16.24±0.26^{Cc}
Vibrio vulnificus	13.39±0.21^{Da}	16.59±0.15^{Bd}	19.61±0.22^{Ad}	15.32±0.17^{Cd}
Bacillus cereus	12.54±0.23^{Db}	16.04±0.19^{Bd}	20.48±0.1^{Ac}	14.25±0.14^{Ce}
Listeria monocytogenes	10.13±0.12^{Dd}	14.26±0.15^{Be}	15.36±0.21^{Af}	13.23±0.21^{Cf}
Aspergillus fumigatus	8.16±0.13^{Ce}	11.22±0.12^{Bd}	12.25±0.13^{Ae}	11.51±0.15^{Bc}
Penicillium chrysogenum	9.57±0.15^{Ccd}	10.12±0.13^{Ce}	15.96±0.17^{Ad}	11.26±0.23^{Bc}
Byssochlamys fulva	10.16±0.14^{Db}	13.02±0.16^{Cb}	18.28±0.15^{Ac}	14.23±0.12^{Ba}
Mucor ramosissimus	10.54±0.19^{Cab}	13.27±0.14^{Bb}	18.92±0.19^{Ab}	12.91±0.19^{Bb}
Rhizopus nigricans	10.02±0.21^{Dbc}	14.23±0.19^{Ba}	20.53±0.21^{Aa}	11.42±0.16^{Cc}
Cladosporium sphaerospermum	11.03±0.18^{Da}	12.41±0.21^{Bc}	17.86±0.12^{Ac}	10.19±0.14^{Cd}
Geotrichum candidum	9.23±0.12^{Cd}	10.89±0.16^{Bd}	16.37±0.22^{Ad}	11.56±0.12^{Bc}

Black Tea: **BT,** Raspberry: **RB,** Blackberry: **BB,** Red Goji Berry: **GB**
a-f (↓): 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).

MIC, MBC and MFC values

Mold type, sample type and mold type x sample type interactions had significant effects (P<0.0001) on MIC (Molds) and MFC. Also, bacteria species x sample type (P<0.05) influenced MIC (bacteria) and MBC. The MIC and MBC values of Kombucha samples on food-borne bacteria are shown in Table 6 and the MIC and MFC values of Kombucha samples on foodborne saprophytic mold species are shown in the Table 7.

Table 6. MIC and MBC values (mg/L) of Kombucha tea samples on microorganisms used in the research (mg/L).

Bacteria	Samples			
	Black Tea		**Raspberry**	
	MIC	**MBC**	**MIC**	**MBC**
Escherichia coli O157:H7	0.188±0.02^{Ac}	0.125±0.03^{Ac}	0.188±0.03^{Aab}	0.125±0.02^{Aa}
Staphylococcus aureus	0.281±0.03^{Abc}	0.188±0.05^{Abc}	0.070±0.02^{Bcd}	0.047±0.01^{Ba}
Salmonella enterica	0.281±0.04^{Abc}	0.188±0.04^{Abc}	0.047±0.02^{Bd}	0.031±0.01^{Ba}
Enterococcus faecalis	0.375±0.05^{Ab}	0.250±0.02^{Ab}	0.094±0.04^{Bbcd}	0.047±0.00^{Ba}
Vibrio vulnificus	0.281±0.02^{Abc}	0.188±0.02^{Abc}	0.141±0.03^{BCbc}	0.094±0.03^{BCa}
Bacillus cereus	0.281±0.05^{Abc}	0.188±0.01^{Abc}	0.188±0.05^{ABab}	0.125±0.03^{ABa}
Listeria monocytogenes	0.563±0.04^{Aa}	0.375±0.03^{Aa}	0.281±0.02^{Ba}	0.125±0.05^{Ba}
	BlackBerry		**Red Goji Berry**	
Bacteria	**MIC**	**MBC**	**MIC**	**MBC**
Escherichia coli O157:H7	0.070±0.02^{Bb}	0.047±0.03^{Aa}	0.188±0.02^{Aab}	0.125±0.02^{Aab}
Staphylococcus aureus	0.023±0.01^{Bb}	0.016±0.00^{Ba}	0.094±0.03^{Bb}	0.063±0.03^{Bb}
Salmonella enterica	0.035±0.02^{Bb}	0.023±0.00^{Ba}	0.070±0.01^{Bb}	0.047±0.04^{Bb}
Enterococcus faecalis	0.035±0.03^{Bb}	0.031±0.02^{Ba}	0.141±0.04^{Bab}	0.094±0.02^{Bb}
Vibrio vulnificus	0.047±0.02^{Cb}	0.023±0.01^{Ca}	0.188±0.05^{ABab}	0.125±0.01^{ABab}
Bacillus cereus	0.070±0.01^{Bb}	0.031±0.03^{Ca}	0.188±0.05^{ABab}	0.125±0.02^{ABab}
Listeria monocytogenes	0.188±0.00^{Ba}	0.063±0.04^{Ba}	0.281±0.06^{Ba}	0.188±0.03^{Ba}

Black Tea: **BT**, Raspberry: **RB**, Blackberry: **BB**, Red Goji Berry: **GB**.
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).

Table 7. MIC and MFC values (mg/L) of Kombucha tea samples on microorganisms used in the research (mg/L).

Mold	Samples			
	Black Tea		**Raspberry**	
	MIC	**MFC**	**MIC**	**MFC**
Aspergillus fumigatus	0,750±0.04^{Aa}	0,500±0.05^{Aa}	0,375±0.03^{Bb}	0,250±0.04^{Bb}
Penicillium chrysogenum	0,563±0.05^{Ab}	0,375±0.03^{Ab}	0,563±0.03^{Aa}	0,375±0.03^{Aa}
Byssochlamys fulva	0,563±0.03^{Ab}	0,375±0.02^{Ab}	0,281±0.04^{Bbc}	0,188±0.03^{Bbc}
Mucor ramosissimus	0,375±0.04^{Ac}	0,250±0.02^{Ac}	0,188±0.03^{BCcd}	0,125±0.03^{BCc}
Rhizopus nigricans	0,563±0.03^{Ab}	0,375±0.03^{Ab}	0,141±0.03^{Cd}	0,094±0.04^{BCc}
Cladosporium sphaerospermum	0,375±0.02^{Ac}	0,250±0.02^{Ac}	0,281±0.02^{Abc}	0,188±0.04^{Abc}
Geotrichum candidum	0,563±0.03^{Ab}	0,375±0.03^{Ab}	0,375±0.02^{Bb}	0,250±0.02^{Bb}
Mold	**Blackberrry**		**Red Goji Berry**	
	MIC	**MFC**	**MIC**	**MFC**
Aspergillus fumigatus	0,281±0.03^{Ba}	0,188±0.03^{Ba}	0,281±0.02^{Bab}	0,188±0.03^{Bab}
Penicillium chrysogenum	0,094±0.01^{Bb}	0,063±0.01^{Cb}	0,375±0.03^{Ca}	0,250±0.02^{Ba}
Byssochlamys fulva	0,070±0.00^{Cbc}	0,047±0.02^{Cb}	0,188±0.01^{Bb}	0,125±0.04^{BCb}
Mucor ramosissimus	0,070±0.00^{Cbc}	0,047±0.02^{Cb}	0,281±0.04^{ABab}	0,188±0.02^{ABab}
Rhizopus nigricans	0,035±0.00^{Cc}	0,023±0.03^{Cb}	0,281±0.05^{Bab}	0,188±0.01^{aBb}
Cladosporium sphaerospermum	0,070±0.00^{Bbc}	0,047±0.02^{Bb}	0,375±0.05^{Aa}	0,250±0.04^{Aa}
Geotrichum candidum	0,094±0.02^{Cb}	0,063±0.01^{Cb}	0,281±0.06^{Bab}	0,188±0.03^{Bab}

Black Tea: **BT**, Raspberry: **RB**, Blackberry: **BB**, Red Goji Berry: **GB**.
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).

DISCUSSION

The Brix (%) values of all samples decreased during fermentation (P<0.05). The highest reduction rate among the samples was found in raspberry (RB) and red goji berry GB samples (1.50%) (Table 1). Abuduaibifu and Tamer [47] reported that, in parallel with the findings of the present study, there was a decrease in the Brix (%) values of tea samples with fermentation. The decrease in Brix value during fermentation was associated with the hydrolysis of sucrose to glucose and fructose by yeasts and lactic acid bacteria while the glucose formed and found in the environment to be metabolized by microorganisms to different products (ethanol, lactic and acetic acid).

During the three-week fermentation, titratable acidity increased in all samples (P<0.05). On the last day of fermentation, the highest titratable acidity values were found in blackberry (BB) (18.42% w/v) and raspberry (RB) (16.19% w/v) samples, respectively (P<0·05; Table 1). Similarly, Chakravorty and coauthors [48] and Tu and coauthors [49] reported that titratable acidity increased depending on the fermentation time. The increase in titratable acidity was due to the fermentation of glucose to organic acids by microorganisms, especially lactic acid bacteria.

Viscosity values were found to be between 58.00 cP and 68.00 cP at the beginning of fermentation and 36.000 and 54.00 cP at the end of fermentation (Table 1). Watawana and coauthors [50] have reported that the viscosity values of Kombucha teas increased due to fermentation in their study. This difference between the study and the findings of the present study research was associated with the differences in production method, raw material and fermentation conditions. The decrease in viscosity value was associated with a decrease in the water-soluble dry matter values by breaking down the sugars, bacteria, and yeasts in the environment.

TAMB and yeast/mold counts decreased in all samples depending on the fermentation time (P<0.05; Table 2). At the end of the 21-day fermentation period, the lowest TAMB and yeast-molds counts were determined in tea samples produced with blackberries with 2.21 and 4.38 log cfu/mL, respectively (P<0.05). Like our research results, Yıkmış and Tuggum [28] have reported that TAMB and yeast/mold counts decreased depending on the fermentation time. During fermentation, acetic and lactic acid bacteria count and increases in the concentration of organic acids resulting from the metabolic activities of these bacteria affected the decrease in TAMB and yeast/mold counts.

An increase was observed in the osmophilic yeast, acetic acid bacteria and lactic acid bacteria counts during the fermentation period (P<0.05; Table 2). On the last day of fermentation, the highest osmophilic yeast (5.56 log cfu/mL), lactic (4.43 log cfu/mL) and acetic acid (7.39 log cfu/mL) bacteria counts were determined in the samples produced with blackberry.

Similarly, Marsh and coauthors [51] and Ayed and coauthors [52] have reported that during the fermentation period at different temperatures, the osmophilic yeast, acetic acid bacteria, and lactic acid bacteria counts increased in all samples. Suitable environmental conditions, sufficient fermentable carbohydrate, and ethanol levels influenced the increase in osmophilic yeast, lactic and acetic acid bacteria count during fermentation.

The counts of *Lactococcus/Streptococcus* strains decreased after an increase until the second week of fermentation (P<0.05; Table 2). The increase in lactic and acetic acid bacteria counts and organic acid concentrations in the medium influenced *Lactococcus/Streptococcus* counts.

The highest water activity (a_w) decrease in tea samples was determined in samples produced with blackberry (BB) with a ratio of 0.030, while the lowest decrease was in the samples produced with black tea (BT) with a ratio of 0.017 (Figure 1). The decrease in water activity values during fermentation was associated with the use of water in microorganism metabolism and the binding of metabolites such as extra polysaccharides to free water.

At the end of the fermentation period, the highest pellicle biomass weights were determined in BB (70.18 g) and SB (66.19 g) samples, respectively (Figure. 2.; P<0.05). Watawana and coauthors [50] and Hassan and Al-Kalifawi [53] have reported that pellicle biomass increased during the fermentation period. The increase in pellicle biomass was due to extracellular polysaccharides, especially synthesized by acetic acid bacteria. Also, the pellicle biomass weight was higher in the blackberry sample compared to other samples because the amount of fermented sugar in this fruit was higher.

L* values decreased during fermentation in all samples (P<0.05; Figure 3.). L* values at the end of fermentation were determined to be BT (18.25), SB (28.04), BB (16.36) and GB (24.12), respectively (P<0.05). a* values increased during the three-week fermentation period (P<0.05; Figure 4.). At the end of fermentation, the highest a* values were determined in SB (35.08) and GB (31.15) samples, respectively (P<0.05). The b* values of all samples decreased during fermentation (P<0.05). 21. On the final day of the fermentation, the highest b* values were determined in the BT sample (15.06) whereas the lowest a* value was in the BB sample (3.76) (P<0.05; Figure 5.). Abuduaibifu and Tamer [47] have reported that, in kombucha teas, the color values (L*, a*and b*) increased during the 11-day fermentation. The raw materials used in the studies, the fermentation conditions, and the duration, formed the existing difference between the studies.

During the fermentation of Kombucha tea samples, an increase of a* value, decrease of L* and b* values; In particular, due to the increase in the number of lactic and acetic acid bacteria, the breakdown of polyphenolic components and color pigments was effective as a result of the increase in the organic acid concentration in the environment (decrease in pH value) [50].

At the end of the fermentation period, the highest amounts of mineral substances in Kombucha tea samples were potassium (12.421 mg/kg), calcium (998.19 mg/kg) and phosphorus (976.36 mg/kg) in red goji berry sample, potassium (1487.52 mg/kg), calcium (271.45 mg/kg), magnesium (236.41 mg/kg) in blackberry sample, potassium (1486.323 mg/kg), calcium (236.47 mg/kg), phosphorus (197.52 mg/kg) in raspberry sample, potassium (8498.12 mg/kg), and phosphorus (5461.19 mg/kg) and magnesium (763.36 mg/kg) in black tea sample (Table 3).

It was determined that the phenolic components varied depending on the raw material used in the production of teas. Catechin and gallic acid were detected in all samples. The highest catechin value was detected in the blackberry sample (92.38 mg/100g) whereas the lowest amount of catechin was detected in the red goji berry sample (21.07 mg/100g). The highest gallic acid was determined in the samples produced with the black tea (6.50 mg/100g), whereas the lowest was in the samples produced with blackberry (0.64 mg/100g) (Table 4).

Eight different phenolic substances were determined in the samples produced with black tea, including catechin (21.13 mg/100g), caffeine (20.18 mg/100g) and gallo catechin (19.15 mg/100g) while eight different phenolic substances were determined in the samples produced with raspberry including catechin (58.34 mg/100g), epicatechin (6.99 mg/100g) and epigallocatechin (5.92 mg/100g), six different phenolic substances were determined including catechin (92.38 mg / 100g), salicylic acid (17.64 mg/100g) and syringic acid (1.91 mg/100g) and nine different phenolic substances were determined in kombucha teas produced with red goji berry, including gallic acid (40.44 mg/100g), catechin (21.07 mg/100g) and protocatechuic acid (14.75 mg/100g) (Table 4). Sun and coauthors [54] have reported that the highest phenolic components were caffeic acid (49.98 mg/100 g) and gallic acid (27.49 mg/100g) on the 12th day of fermentation in traditional Kombucha teas.

The most appreciated sample in terms of all sensory criteria (taste, structure, appearance, smell, and general taste) was blackberry (BB) sample whereas the least appreciated example was the tea samples produced using black tea (BT) (P<0.05; Figure 6.). Taste, structure, appearance, smell, and general appreciation scores were given to the BB sample by panelists were as follows 9.37, 9.44, 9.39, 9.26 and 9.39 while the scores given to the CT sample were 5.27, 5.21, 5.15, 5.30 and 5.35, respectively (P<0·05).

The highest antibacterial effect was determined on *Staphylococcus aureus* (24.36 mm zone diameter) and *Salmonella enterica* (21.33 mm zone diameter) while the highest antifungal effect was detected on Rhizopus nigricans (20.53 mm zone diameter) and *Mucor ramosissimus* (18.92 mm zone diameter) in tea samples produced blackberry. In contrast, the lowest antibacterial effect was detected on *Listeria monocytogenes* (10.13 mm zone diameter) and *Enterococcus feacalis* (11.26 mm zone diameter) while the lowest antifungal effect was detected on *Aspergillus fumigatus* (8.16 mm zone diameter) and *Geotrichum candidum* (9.23 mm zone diameter) in samples produced with black tea (P<0.05, Table 5). Similarly, Kalkan and coauthors [55] have reported that goji berry fruit extracts prepared with various solvents had a strong antimicrobial effect against *Staphylococcus aureus* (zone diameter 15.00 -15.67 mm) and *Listeria monocytogenes* (14.33-16.33 mm zone diameter). Yuniarto and coauthors [56] have stated that the highest antifungal effect in Kombucha tea samples was on *Microsporum gypseum* (21.16 mm zone diameter).

The lowest MIC and MBC values in seven different foodborne pathogenic bacterial species were found in samples produced with blackberry against *Staphylococcus aureus* (BB) with 0.023 mg/L and 0.016 mg/L, respectively (P<0.05; Table 6). On the other hand, the highest MIC and MBC values were in the samples produced with black tea (BT) against *Listeria monocytogenes* with 0.563 mg/L and 0.375 mg/L, respectively (P<0.05; Table 6). Like the results obtained in the present study, Bhattacharya and coauthors [57] have reported the lowest MIC values of Kombucha tea samples were 3.125 mg/mL on *Vibrio cholerae, Escherichia coli*, and *Salmonella Typhimurium*.

The lowest MIC and MFC values in seven different saprophyte mold species were 0.035 mg/L, 0.023 mg/L in black tea samples (BB) and *Rhizopus nigricans* (P<0.05; Table 7). On the other hand, the highest MIC and MFC values were determined in the samples produced with black tea on *Aspergillus fumigatus* (BT) with 0.750 mg/L, 500 mg/L. (P<0.05; Table 7). Nazemi and coauthors [58], in line with the results of the present study, have reported that the lowest MIC and MFC values in the Kombucha tea samples were on *Aspergillus fumigatus* with 6.170 µg/mL, 12.300 µg/mL. The antibacterial and antifungal effects shown by Kombucha teas were associated with organic acids, bacteriocins, and enzymes produced during the fermentation period by other microorganisms, and polyphenolic compounds originating from raw materials [57].

CONCLUSION

In the present study, physical, chemical, microbiological and sensory properties, and antimicrobial activities of kombucha tea samples produced with small berry fruits were determined. Teas produced with berry fruits were much richer in mineral and phenolic contents compared to the samples produced with black tea. Also, the antibacterial and antifungal activities of these teas were higher compared to the samples produced with black tea. Sensory analysis results showed that small berry fruits, especially blackberry, can be used in Kombucha tea production.

Due to the beneficial effects of Kombucha tea on human health, its popularity is increasing day by day. This beverage is known to be very rich in bioactive components that can be digested, metabolized, and absorbed by the body. Also, it was thought that the use of different berry fruits as a raw material in the production of Kombucha tea will be more preferred by consumers in terms of flavor, taste and functional properties. It is evident that Kombucha teas will be a good alternative to carbonated beverages due to their therapeutic and health benefits. As a result of the study on the effects of different berry fruits in Kombucha production with both in vitro and in vivo studies, this product can easily replace many expensive and artificial antimicrobial additives used in the food industry.

This research revealed that kombucha teas produced with berries are rich in phenolic and mineral content. In addition, it has been determined that teas have important effects on food-borne pathogens and saprophytic microorganisms. Therefore, in future studies, the chemical composition of tea, the properties of the substances in this composition, and their effects on human health should be determined by in vivo and in vitro studies, and the usability of tea in medical and pharmacological fields should be revealed. It is also thought that this product will create a serious alternative to the use of unnatural additives in food production, which has become a major problem in the food industry in recent years and causes serious concerns on the consumer.

Conflicts of Interest: I have no conflicts of interest with anyone.

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