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Evaluation of bioaccessibility and functional properties of kombucha beverages fortified with different medicinal plant extracts

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Abstract: In this study, sweetened black and green tea were utilized as substrate for kombucha fermentation. Linden, lemon balm, sage, echinacea, mint, and cinnamon infusions were added to kombucha to design a novel beverage with improved functional and organoleptic characteristics. After fermentation, the antioxidant capacity (AC) of the kombucha increased by 13.96% 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), 48.90% ferric reducing antioxidant power (FRAP), and 55.54% cupric reducing AC (CUPRAC). On days 0 and 9 of storage, the bioaccessibility of the total phenolics and AC (FRAP and CUPRAC) in all of the samples showed a significant increase after gastric and intestinal digestion when compared to pregastric digestion ($P < 0.05$). The AC (DPPH) after in vitro digestion at the beginning and end of storage in all of the beverages also increased after gastric digestion when compared to pregastric digestion ($P < 0.05$); however, it decreased after intestinal digestion ($P < 0.05$). By conducting in vitro and in vivo studies, the effects of kombucha on health and nutrition need to be further investigated.

Key words: Antioxidant capacity, bioaccessibility, fermentation, kombucha, medicinal plants

1. Introduction

Fermented foods and beverages have begun to be consumed more during recent years because of their improved nutritional value, antioxidant capacity (AC), and several health benefits (Dufresne and Farnworth, 2000). In the world functional drink market, kombucha is the fastest growing fermented beverage[1] (Baschali et al., 2017). Kombucha is obtained by fermenting sweetened black and green tea with yeast (*Saccharomyces*, *Zygosaccharomyces*, *Saccharomycodes*, *Schizosaccharomyces*, *Torulopsis*, *Pichia*, *Brettanomyces*, *Dekkera*, *Candida*, *Koleckera*, *Mycoderma*, *Mycotorula*, *Lachancea*, *Hanseniaspora*, *Torulospora*) and predominantly acetic acid bacteria (*Acetobacter aceti*, *A. pasteurianus*, *A. peroxydans*, *Glucobacter oxydans*, *G. europaeus*, *G. saccharivorans*, and *Komagataeibacter xylinus*) consortium (Dutta and Gachhui, 2006, 2007; Jayabalan et al., 2014; Marsh et al., 2014; Chakravortyet al., 2015, 2016, 2019; Coton et al., 2017). Several biochemical reactions take place during aerobic fermentation (Neffe-Skocińska et al., 2017). First, the yeast uses the existing carbon source to produce ethyl alcohol, which is afterward transformed into organic acids by the bacteria (Jayabalan et al., 2014). Due to microbial activity, a cellulose-like pellicle (biofilm) forms on a liquid-air interface (Chakravorty et al., 2015). Although the exact composition of kombucha tea is still under investigation, the reported principle components are organic acids (acetic, lactic, malic, tartaric, citric, malonic, oxalic, succinic, pyruvic, 2-keto-D-gluconic, glucuronic, and usnic acids), amino acids (lysine, methionine, arginine), sucrose, glucose, fructose, ethanol, vitamins (B_1, B_2, B_6, B_{12}, C, E), minerals (K, Mn, Zn, Cu, Fe, Ni, Co, and F), phenolics, probiotics, biogenic amines, purines, pigments, CO_2, glycerol, enzymes, etc. (Greenwalt et al., 1998; Dufresne and Farnworth, 2000; Teoh et al., 2004; Kumar et al., 2008; Nguyen et al., 2015; Neffe-Skocińska et al., 2017; Leal et al., 2018). Kombucha has gained popularity for its prophylactic and therapeutic features that have been generally reported based on observations, survey-based reports, and testimonials (Battikh et al., 2012a; Vīnaet al., 2014). Although some have

[1] Troitino C (2017). Kombucha 101: Demystifying the past, present and future of the fermented tea drink [online]. Website https://www.forbes.com/sites/christinatroitino/2017/02/01/kombucha-101-demystifyingthe-past-present-and-future-of-the-fermented-tea-drink/#261ea4684ae2

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been demonstrated by scientific studies, evidence based on human models is still absent (Chakravorty et al., 2019). It has a high antioxidant potential that has been related with prevention of cancer, immune system support, and improvement of joint rheumatism (Deghrigue et al., 2013; Srihari et al., 2013a; Jayabalan et al., 2014; Shenoy et al., 2019). Antihypertensive (Elkhtab et al., 2017), antidiabetic (Srihari et al., 2013b), hepatoprotective (Yarbrough, 2017; Bhattacharya et al., 2011), antiinflammatory (Vázquez-Cabral et al., 2017), antimicrobial (Sreeramulu et al., 2000; Battikh et al., 2012a, 2012b; Bhattacharya et al., 2016), antihyperlipidemic (Yang et al., 2009; Dias and Shenoy, 2016) cytoprotective, genoprotective (Cavusoglu and Guler, 2010; Yapar et al., 2010), and renoprotective (Bellassouedet al., 2015) effects of kombucha have also been reported. Various substrates other than black and green tea could be used in kombucha fermentation, such as fruits (i.e. raspberry, goji berry, red grape, cactus pear, blackthorn, snake fruit, date, coconut, melon, watermelon, and sour cherry) (Yavari et al., 2011; Kubilay, 2014; Ayed and Hamdi, 2015; Watawana et al., 2016b; Ayed et al., 2017; Zubaidah et al., 2018; Abuduaibifu and Tamer, 2019; Khosravi et al., 2019; Ulusoy and Tamer, 2019), herbs (i.e. lemon balm, rooibos, yarrow, wheatgrass, guava leaves, cinnamon, cardamom, Shirazi thyme, African mustard leaves, *Eucalyptus camaldulensis*, *Litsea glaucescens*, *Thymus vulgaris L.*, *Lippia citriodora*, *Rosmarinus officinalis*, *Foeniculum vulgare*, and *Mentha piperita*) (Battikh et al., 2012a; Velicanski et al., 2014; Vīna et al., 2014; Sun et al., 2015; Gamboa-Gómez et al., 2016; Moreno-Jiménez et al., 2018; Shahbaziet al., 2018; Vitas et al., 2018; Gaggìa et al., 2019; Rahmani et al., 2019), and others (i.e. milk, coffee, whey, wine, vinegar, Jerusalem artichoke, black carrot juice concentrate, and oak) (Malbasa, 2004; Malbasa et al., 2009; Watawana et al., 2015; Vázquez-Cabral et al., 2017; Tu et al., 2019; Ulusoy and Tamer, 2019). The health benefits of tea, which are closely related to the structure and composition of polyphenols, have been extensively investigated (Zhang et al., 2019). There are various studies related to the beneficial effects of green tea, including antioxidative, antiinflammatory, antibacterial, antiviral, and antiangiogenic effects and against cancer, obesity, diabetes, and cardiovascular diseases (Retoet al., 2007; Suzuki et al., 2016). Medicinal plants have long been stated as an expected source of natural antioxidants (Heim et al., 2002). They are increasingly accepted by the public and medical professionals (Adegbola et al., 2017). Natural phenolic compounds play a fundamental role in the prevention and treatment of cancer. Several bioactivities of phenolics are responsible for their chemopreventive features and they also contribute to the induction of apoptosis by arresting the cell cycle, regulating carcinogen metabolism and ontogenesis expression, inhibiting DNA binding and cell adhesion, migration, proliferation or differentiation, and blocking signaling pathways (Huang et al., 2010). In this study, it was aimed to investigate the possibility of the fortification of the traditional kombucha beverage with different medicinal plant infusions, such as linden, lemon balm, sage, echinacea, mint, and cinnamon, to produce a functional beverage with high AC and bioaccessibility. Concurrently, the sensory properties (color, appearance, odor, and taste) of the kombucha beverage were intended to be increased by the utilization of these ingredients to receive the appreciation of wider consumer groups.

2. Materials and methods

2.1. Materials

Black tea, green tea, and sucrose were purchased from a local market in Bursa. Medicinal plants were obtained from Sani&Çay Food Company (İzmir, Turkey). Kombucha culture was obtained by the Food Engineering Department Laboratory, Bursa Uludağ University. The liquid kombucha inoculum used [10% (v/v)] in this study was the fermentation liquid of kombucha obtained after a 14-day-long fermentation of a black tea (10 g/L) and green tea (3 g/L) infusion sweetened with sucrose (60 g/L) at 28 ± 2 °C.

2.2. Methods

2.1.1. Preparation and cultivation of the kombucha samples

Production of the kombucha beverage samples is shown in Figure 1. According to the preliminary results, fermentation was terminated when the acidity (in acetic acid) reached 0.28%. Briefly, 10 g of linden, lemon balm, sage, echinacea, mint, and cinnamon were infused in 1 L of water at 98 °C for 5 min, separately, and then the infusions were added [10% (v/v)] to the fermented kombucha tea. The kombucha samples were stored at 4 °C in glass jars with lids.

2.2.2. Analyses

The Brix° was measured using a RA-500 KEM refractometer (Shinjuku-ku, Tokyo, Japan). Samples were titrated with 0.1 N of NaOH to pH 8.1, and the total acidity (TA) value, in terms of acetic acid, was calculated as g/100 mL (AOAC 2000). The pH was measured using a Mettler Toledo SevenCompact pH/Ion pH meter (Columbus, OH, USA). A Shimadzu UV 1208 spectrophotometer (Kyoto, Japan) was used for the total phenolics and AC analyses. The color parameters (L^*, a^*, b^*) of the kombucha beverages were assessed using a Hunterlab D25A-PC2Δ colorimeter (Reston, VA, USA) (Bakker et al., 1986). Afterwards, the total color difference (ΔE^*), chroma (C_{ab}^*), and hue angle ($h°$) were calculated according to the following equations, respectively (method OIV-MA-AS2-112006). All results were given as the mean ± standard deviation.

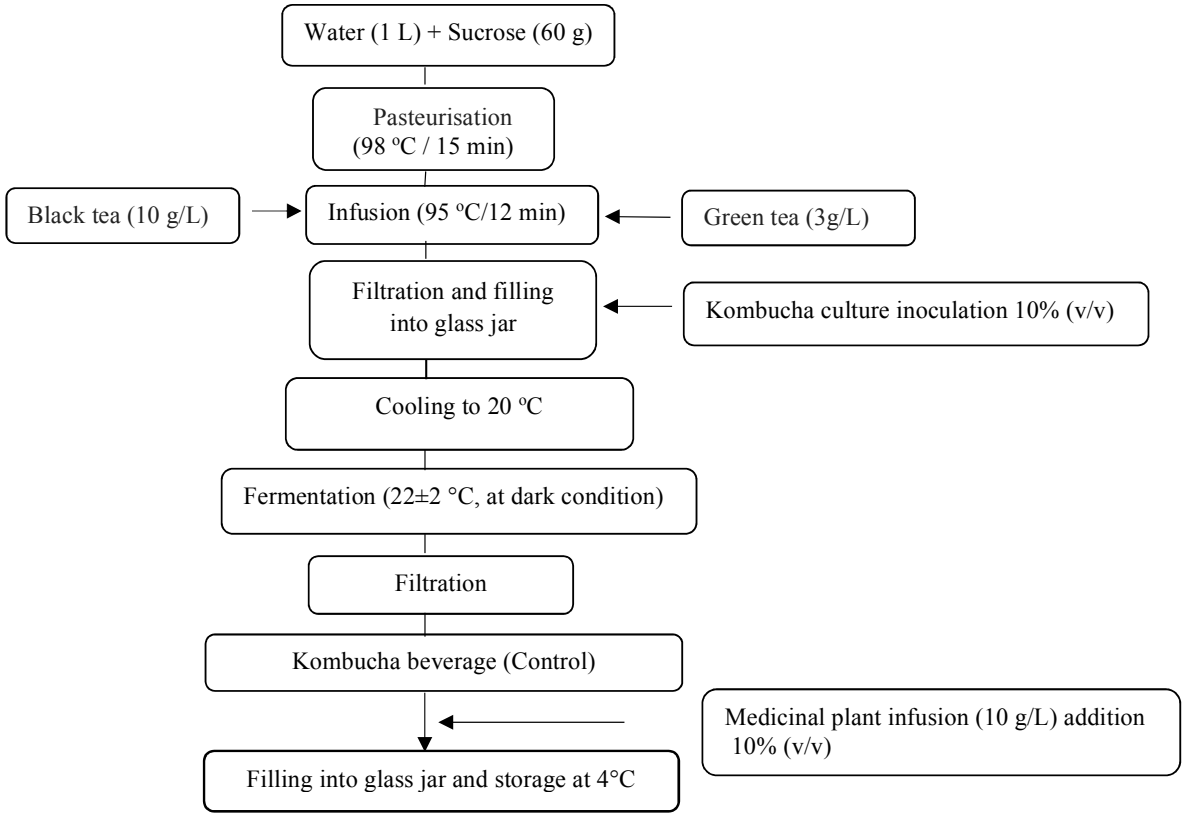

Figure 1. Kombucha beverage production flow chart

$$\Delta E = \sqrt{(L0*-L*)^2 + (a0*-a*)^2 + (b0*-\mathrm{b}*)2}$$

$$\text{Chroma} = \sqrt{(a)^2 + (b)^2}$$

$$\text{Hue Angle} = \arctan\left(\frac{b}{a}\right)$$

2.2.2.1. Preparation of the extracts for the total phenolic compound content and antioxidant capacity

The extracts of the kombucha beverages were prepared according to method defined by Vitali et al. (2009). The extraction of the samples was conducted in triplicate, and the extracts were stored at –20 °C until used.

2.2.2.2. Total phenolic matter content

Briefly, 0.2 mL of extract, 2.3 mL of distilled water, and 0.15 mL of Folin-Ciocalteu reagent were put into capped glass scoops, and vortexed (Vortex Mixer Classic, Velp Scientifica, Italy) for 15 s. After 5 min, 0.3 mL of saturated Na_2CO_3 (35%) solution was added, and the tube contents were agitated and allowed to stand in the dark for 2 h. The absorbance of the sample was measured at 725 nm and the result was expressed as mg gallic acid equivalent (GAE)/100 g determination (Zhang and Hamauzu, 2004).

2.2.2.3. Antioxidant capacity determination

The AC was analyzed by conducting the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) (Zhang and Hamauzu, 2004), ferric reducing antioxidant power (FRAP) (Benzie and Strain, 1996), and cupric reducing AC (CUPRAC) methods (Apak et al., 2008). In the DPPH method, 0.1 mL of sample was added to 3.9 mL of DPPH solution and vortexed for 30 s. The test tubes were kept in the dark at room temperature for 30 min. A Trolox calibration curve (R^2 = 0.9997) was obtained by measuring the reduction in the absorbance of the DPPH solution in the presence of different concentrations of Trolox (10–100 µmol/L). In the FRAP method, 3 mL of daily prepared FRAP reagent was mixed with 300 µL of distilled water and 100 µL of the sample or blank. The test samples, digested extracts, and blank were incubated at 37 °C for 30 min then the absorbance was measured immediately at 595 nm. The results were calculated from the calibration curve (R^2 = 0.9934). In the CUPRAC method, 100 µL of the sample was mixed with 300 µL of distilled water and CUPRAC reagent, which was prepared with the equal amounts of $CuCl_2$, neocuproine, and ammonium acetate solutions, and the final absorbance was measured at 450 nm after 30 min (R^2 = 0.9933). The results were expressed as µmol Trolox equivalent (TE) per g water soluble dry matter (µmol TE/g w.s.d.m).

2.2.2.4. In vitro gastrointestinal digestion assay

In vitro digestion of the samples was employed according to the method of Minekus et al. (2014), with minor

modifications. Samples were passed through a two-step gastrointestinal digestion stage representing the aliquots of the stomach phase and intestinal phase, which were collected during the procedure. Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared, as reported previously by Minekus et al. (2014). The samples were combined with the SGF, porcine pepsin solution (25,000 U/mL, Sigma-Aldrich P6887; St. Louis, MI, USA), and $CaCl_2$ during the gastric phase. The pH of the mixture was then adjusted to 3 with HCl and incubated at 37 °C for 2 h in a shaking water bath (Memmert SV 1422, Memmert GmbH & Co., Germany), and afterwards, 4 mL aliquots were collected for each sample. The remainder of the mixture was mixed with the SIF solution, pancreatin solution (800 U/mL, Sigma-Aldrich P3292), and bile solution (160 mM), one-by-one, to simulate intestinal digestion. The pH was adjusted to 7.0 with NaOH, and the samples were incubated at 37 °C for 2 h in a shaking water bath. Subsequently, the samples were centrifuged at 3500 rpm for 10 min, filtered, and the supernatant was collected. After that, the extracts were kept at –20 °C until analyzed.

2.2.2.5. Ultraperformance liquid chromatography analysis of the organic acids

D-glucuronic acid, D-gluconic acid, acetic acid, and lactic acid were determined using Dionex UltiMate-3000 ultra-performance liquid chromatography (UPLC) (Dreieich, Germany) according to the method of Jayabalan et al. (2007), with some modifications. Briefly, the samples were passed through a 0.45-µm membrane filter and injected into the UPLC equipped with a UV-Visible diode array detector. A Nucleogel Sugar 810H column (300 mm × 7.8 mm, Macherey-Nagel, Düren, Germany) was used. Next, 5 mM of sulfuric acid (95%–97%, ACS Reagent; Merck, Darmstadt, Germany), with a flow rate of 0.65 mL/min and injection volume of 5 µL, was used for the measurements at 210 nm. The concentrations of the organic acids were calculated by comparing relative peak area values from standard curves with good linearity ($R^2 > 0.990$). The analyses were conducted in triplicate and the total concentration was given as mg/L.

2.2.2.6. Mineral analysis

The mineral analysis of the samples was conducted using the TS EN 13805:2004 standard method, and a Perkin-Elmer 2100 inductively-coupled plasma optical emission spectrometer (Waltham, MA , USA) was used (Anonymous, 2004). According to this method, the sample digestion of 5 mL was performed in a Milestone microwave oven (Sorisole, Italy) with the addition of 6 mL of HNO_3 (65%) (Sigma-Aldrich) and 1 mL of H_2O_2 (35%) (Merck). Potassium, magnesium, calcium, and iron were detected at 766.49, 285.21, 317.93, and 238.20 nm, respectively. All analyses were conducted in triplicate and the results were expressed as mg/kg.

2.2.2.7. Microbiological analysis

Microbiological growth of the samples was monitored during the fermentation and storage periods. The total mesophilic aerobic bacteria were counted on plate count agar medium at 30 °C under aerobic conditions for 3 days. For total yeast counting, the samples were plated on oxytetracycline glucose-yeast extract agar medium (CM545, Oxoid) containing oxytetracycline (SR73, Oxoid), as defined by Sreeramulu et al. (2000), and the plates were incubated at 25 °C for 5 days. Acetic acid bacteria counts were conducted on a medium prepared from 5% glucose, 1% yeast extract, and 2% agar, with added cycloheximide to inhibit yeast growth, and incubated at 25 °C for 3–5 days (Irigoyen et al., 2005). The viability value of the microorganisms was calculated according to the method of Bruno et al., (2002), using the following equation:

Viability index = [log cfu/mL of the latest storage or fermentation time / log cfu/mL of the first storage or fermentation time] × 100.

2.2.2.8. Sensory evaluation

Sensory analysis was performed during the storage days (days 0, 3, 6, and 9) with 8 panelists from Bursa Uludağ University Food Engineering Department. The 8 panelists (6 females and 2 males) from the academicians and graduate students of the Food Engineering Department, who were between the ages of 25 and 40, were briefed before the sensory analysis. Samples were presented to each panelist in 200 mL glass cups at a recommended consumption temperature of 7–9 °C. The panelists evaluated the control sample and also the kombucha beverages fortified with different medicinal plant infusions. They were asked to score the samples, which were coded with three digits of random numbers, for appearance, color, odor, and taste, using a 9-point hedonic scale according to the descriptive analysis technique. The proposed categories were 1) dislike extremely, 2) dislike very much, 3) dislike moderately, 4) dislike slightly, 5) neither like nor dislike, 6) like slightly, 7) like moderately, 8) like very much, and 9) like extremely (Altuğ, 1993).

2.2.2.9. Statistical analysis

The assessment was conducted using the SAS Statistical Program (SAS Institute Inc., Cary, NC, USA) and the data were analyzed using ANOVA. Further analysis was conducted with the least square difference test and $P < 0.05$ was considered statistically significant (Granato et al., 2014).

3. Results and discussion

3.1. pH, Brix°, and total acidity

In this study, prefermentation, fermentation, and postfermentation (storage) analyses of the kombucha culture, infusion of sweetened black and green tea, medicinal plant infusions, kombucha tea (control), and

kombucha beverages supplemented with medicinal plant infusion were performed. It is known that during fermentation, microorganisms convert sugars into ethanol and then into acetic acids, and also various organic acids. As a result of this mechanism, the amount of sugar in the medium was reduced. Therefore, the Brix° decreased. It was determined that the pH decreased and TA increased during fermentation (Table 1). The results of this study were similar to those reported by Yavari et al. (2011) and Ayed and Hamdi (2015). The decrement in the pH value was found to be significant due to the increase in organic acid content (Sievers et al., 2006; Shahbazi et al., 2018). As a result of the current sensory analysis, considering that the panelists did not prefer an extreme acidic taste, the fermentation time was kept short in this study and the kombucha tea was obtained when the TA reached 0.28 g/100 mL (Table 1). Amarasinghe et al. (2018) reported that consumer appreciation decreased with increasing acidity in kombucha. The results of the pH, Brix°, and TA analysis of the medicinal plant infusions and kombucha beverages to which the plant infusions were added are given in Tables 2 and 3, respectively. While the difference between the TA values of the infusions was insignificant ($P > 0.05$), the cinnamon infusion had the highest pH value. The lowest Brix° was determined in the sage and cinnamon infusions. However, the highest Brix° was determined in the mint infusion. Changes in the pH, TA, and Brix° values indicated that fermentation was ongoing, even though the samples were kept in closed glass jars at 4 °C. In this case, while the pH value was expected to decrease over time, slight increases and decreases in the pH were observed on days 0, 3, 6, and 9, and fluctuations in the results were determined. When previous studies were examined, it was thought that the buffer effect of the fermentation liquid could have caused these fluctuations (Chen and Liu, 2000; Sreeramulu et al., 2000). The Brix° values of all of the beverages reduced after 9 days of storage. When comparing days 0 to 9, the TA of the samples increased significantly, except in the control sample and cinnamon infusion + kombucha (CK) ($P < 0.05$).

3.2. Organic acids

The arrival order of the identified peaks was D-glucuronic acid, D-gluconic acid, lactic acid, and acetic acid, respectively. The inoculated kombucha culture contained 235.623 ± 2.49 mg/L of D-glucuronic acid, 1045.57 ± 0.76 mg/L of D-gluconic acid, 706.183 ± 63.82 mg/L of lactic acid and 6546.86 ± 81.34 mg/ L of acetic acid. While the D-gluconic acid, lactic acid, and acetic acid were not detected before fermentation, the amount of these acids reached 237.52 ± 2.35 mg/L, 257.08 ± 18.22 mg/L, and 1776.31 ± 3.70 mg/L, respectively, after fermentation. D-glucuronic acid increased from 34.22 ± 3.77 mg/L to 37.71 ± 1.30 mg/L during fermentation. Shahbazi et al. (2018) reported an organic acid change in green tea kombucha fermented at 28 °C for 16 days. Although the D-glucuronic acid was not detected at the beginning, it increased to 839.06 ± 94.13 mg/L after fermentation. Lactic acid increased from 48.35 ± 3.21 mg/L to 145.71 ± 17.76 mg/L and acetic acid increased from 944.54 ± 81.12 mg/L to 2395.64 ± 189.12 mg/L during fermentation. The difference between the values was thought to have been due to the fermentation time, temperature, and substrate composition. The highest levels of D-glucuronic acid (61.92 ± 0.19 mg/L) and lactic acid (915.07 ± 14.61 mg/L) were determined in the linden + kombucha (LK). The highest amounts of D-gluconic acid (298.74 ± 2.68) and acetic acid (1776.31 ± 3.70) were determined in the lemon balm infusion + kombucha (LBK) and control sample, respectively (Table 4). Jayabalan et al. (2007) monitored the change in organic acid levels during

Table 1. The pH, Brix°, and TA values determined during the kombucha fermentation.

	pH	Brix (°Bx)	TA*
Day 0	3.67 ± 0.01^{a}	6.27 ± 0.06^{a}	0.10 ± 0.00^{d}
Day 1	3.66 ± 0.01^{a}	6.30 ± 0.00^{a}	0.12 ± 0.00^{c}
Day 2	3.63 ± 0.02^{a}	6.00 ± 0.00^{b}	0.12 ± 0.01^{c}
Day 3	3.48 ± 0.05^{b}	5.70 ± 0.00^{c}	0.17 ± 0.00^{b}
Day 4	3.17 ± 0.05^{c}	5.50 ± 0.00^{c}	0.28 ± 0.01^{a}

*Acetic acid (g/100 mL)
Values with different lowercase letters for days (a–d) in the same column are significantly different ($P < 0.05$).
Data are expressed as the mean ± standard deviation (n = 3).

Table 2. The pH, Brix°, and TA values of the medicinal plant infusions.

	pH	Brix (°Bx)	TA*
Linden	6.49 ± 0.05^{e}	0.27 ± 0.06^{b}	0.01 ± 0.00
Lemon balm	6.54 ± 0.08^{de}	0.30 ± 0.00^{b}	0.01 ± 0.00
Mint	6.63 ± 0.05^{d}	0.40 ± 0.00^{a}	0.01 ± 0.00
Echinacea	7.17 ± 0.03^{b}	0.20 ± 0.00^{c}	0.01 ± 0.00
Sage	7.01 ± 0.04^{c}	0.10 ± 0.00^{d}	0.01 ± 0.00
Cinnamon	7.31 ± 0.04^{a}	0.10 ± 0.00^{d}	0.01 ± 0.00

*Acetic acid (g/100 mL), Values with different lowercase letters (a–e) in the same column are significantly different ($P < 0.05$). There were no statistically significant differences between the unlettered samples ($P > 0.05$). Data are expressed as the mean ± standard deviation (n = 3).

Table 3. The pH, Brix°, and TA values of the kombucha samples during storage.

	Day 0	Day 3	Day 6	Day 9	% Change*
pH					
C	3.17 ± 0.04^{bC}	3.21 ± 0.04^{abAB}	3.21 ± 0.05^{abAB}	3.28 ± 0.02^{aAB}	3.47 (+)
LK	3.28 ± 0.01^{aAB}	3.21 ± 0.04^{aB}	3.26 ± 0.06^{aA}	3.29 ± 0.02^{aAB}	0.30 (+)
LBK	3.21 ± 0.02^{aC}	3.27 ± 0.08^{aAB}	3.21 ± 0.03^{aAB}	3.22 ± 0.01^{aC}	0.31 (+)
MK	3.30 ± 0.05^{aA}	3.26 ± 0.03^{abAB}	3.21 ± 0.02^{bAB}	3.30 ± 0.01^{aA}	0.00
EK	3.22 ± 0.04^{bBC}	3.28 ± 0.03^{aA}	3.18 ± 0.02^{bB}	3.30 ± 0.00^{aA}	2.48 (+)
SK	3.19 ± 0.03^{bC}	3.21 ± 0.00^{bAB}	3.16 ± 0.04^{bB}	3.27 ± 0.02^{aAB}	2.51 (+)
CK	3.21 ± 0.00^{bcC}	3.22 ± 0.01^{bAB}	3.19 ± 0.02^{cB}	3.26 ± 0.01^{aB}	1.56 (+)
Brix (°Bx)					
C	5.50 ± 0.00^{aA}	5.40 ± 0.00^{bA}	5.20 ± 0.00^{cA}	5.00 ± 0.00^{dA}	9.09 (-)
LK	5.00 ± 0.00^{aB}	4.90 ± 0.00^{bB}	4.60 ± 0.00^{cC}	4.47 ± 0.05^{dC}	10.60 (-)
LBK	4.90 ± 0.00^{aC}	4.70 ± 0.00^{bD}	4.60 ± 0.00^{cC}	4.40 ± 0.00^{dD}	10.20 (-)
MK	5.00 ± 0.00^{aB}	4.70 ± 0.00^{bD}	4.50 ± 0.00^{cD}	4.40 ± 0.00^{dD}	12.00 (-)
EK	5.00 ± 0.00^{aB}	4.80 ± 0.00^{bC}	4.60 ± 0.00^{cC}	4.57 ± 0.05^{cB}	8.60 (-)
SK	5.00 ± 0.00^{aB}	4.80 ± 0.00^{bC}	4.60 ± 0.00^{cC}	4.60 ± 0.00^{dB}	8.00 (-)
CK	4.90 ± 0.00^{aC}	4.90 ± 0.00^{aB}	4.70 ± 0.00^{bB}	4.60 ± 0.00^{cB}	6.12 (-)
TA [as acetic acid (g/100 mL)]					
C	0.28 ± 0.01^{aA}	0.26 ± 0.01^{bA}	0.28 ± 0.02^{aA}	0.27 ± 0.01^{abB}	3.57 (-)
LK	0.24 ± 0.01^{bC}	0.26 ± 0.01^{bA}	0.28 ± 0.02^{aA}	0.28 ± 0.01^{aA}	16.67 (+)
LBK	0.26 ± 0.01^{bAB}	0.26 ± 0.00^{bA}	0.29 ± 0.01^{aA}	0.28 ± 0.01^{aA}	7.69 (+)
MK	0.25 ± 0.01^{cBC}	0.26 ± 0.01^{bA}	0.28 ± 0.01^{aA}	0.26 ± 0.00^{bBC}	4.00 (+)
EK	0.24 ± 0.00^{bC}	0.25 ± 0.01^{bA}	0.29 ± 0.02^{aA}	0.28 ± 0.01^{aA}	16.67 (+)
SK	0.25 ± 0.01^{bcBC}	0.24 ± 0.00^{cB}	0.27 ± 0.01^{aA}	0.26 ± 0.01^{bC}	4.00 (+)
CK	0.23 ± 0.01^{aD}	0.22 ± 0.00^{aC}	0.23 ± 0.01^{aB}	0.23 ± 0.00^{aD}	0.00

C: control, LK: Linden infusion + kombucha, LBK: Lemon balm infusion + kombucha, MK: Mint infusion + kombucha, EK: Echinacea infusion + kombucha, SK: Sage infusion + kombucha, CK: cinnamon infusion + kombucha, * Increase (+) % or decrease (–) % between days 0 and 9 day of storage. Different lowercase letters (a–d) within the row are significantly different ($P < 0.05$). Different uppercase letters (A–D) within the column are significantly different ($P < 0.05$). Data are expressed as the mean ± standard deviation (n = 3).

fermentation (24 ± 3 °C for 18 days) in green and black tea kombucha. Initially, only D-glucuronic acid was detected. Lactic and acetic acid were also determined at the end of fermentation. Glucuronic acid has been reported to be one of the important compounds of kombucha due to its detoxification effect (Vīna et al., 2014). Chakravorty et al. (2016) reported that the initial amount of acetic acid in kombucha was 0.65 ± 0.06 g/L and it reached 16.57 ± 0.09 g/L after 3 weeks of fermentation. Moreover, the gluconic acid value increased from 0.33 ± 0.03 g/L to 7.36 ± 0.87 g/L at the end of fermentation (Chakravorty et al., 2016). The source of inoculum (Nguyen et al., 2015), sugar and tea concentrations (Fu et al., 2014), duration of fermentation (Chen and Liu, 2000), and temperature (Jayabalan et al., 2014) were effective on the results.

3.3. Color parameters

The highest increment of the L^* value was caused by the addition of echinacea infusion ($P < 0.05$). The values of b^*, ΔE^*, and $C_{ab}{}^*$ were found to be the highest in the control sample ($P < 0.05$). However, the addition of the plant infusions decreased these parameters. The cinnamon and echinacea infused samples (CK and EK) had the lowest a^* value, but highest $h°$ value ($P < 0.05$) (Table 5). Chakravorty et al. (2016) stated that during fermentation,

Table 4. Organic acid content (mg/L) of the kombucha beverages (day 0 of storage)

	D-glucuronic acid	D-gluconic acid	Lactic acid	Acetic acid
C	37.71 ± 1.30^{c}	237.52 ± 2.35^{d}	257.08 ± 18.22^{b}	1776.31 ± 3.70^{a}
LK	61.92 ± 0.19^{a}	193.77 ± 1.97^{f}	380.19 ± 14.61^{a}	1469.56 ± 34.24^{e}
LBK	42.98 ± 0.74^{b}	298.74 ± 2.68^{a}	109.54 ± 2.13^{d}	1774.83 ± 9.67^{a}
MK	26.41 ± 1.46^{d}	271.86 ± 4.50^{b}	135.79 ± 3.22^{cd}	1637.08 ± 0.07^{c}
EK	42.07 ± 1.16^{bc}	268.85 ± 5.27^{b}	166.00 ± 9.65^{c}	1740.09 ± 34.05^{b}
SK	41.06 ± 1.16^{bc}	257.30 ± 2.46^{c}	116.01 ± 0.49^{d}	1766.80 ± 1.13^{ab}
CK	28.55 ± 7.10^{d}	227.88 ± 4.60^{e}	99.91 ± 2.30^{d}	1528.41 ± 0.50^{d}

Different lowercase letters (a–f) within the column are significantly different ($P < 0.05$).
Data are expressed as the mean ± standard deviation (n = 3).

Table 5. Color parameters of the kombucha samples (L^*, a^*, b^*, ΔE^*, C^*, and $h°$).

	L*	a*	b*	ΔE*	C_{ab}*	h°
C	21.87 ± 0.12^{bc}	4.50 ± 0.62^{a}	$9.6\ 3\pm 0.06^{a}$	24.32 ± 0.22^{a}	10.64 ± 0.30^{a}	65.02 ± 2.99^{bc}
LK	21.57 ± 0.06^{cd}	4.10 ± 0.75^{ab}	9.07 ± 0.40^{bc}	23.76 ± 0.33^{bc}	9.96 ± 0.68^{b}	65.80 ± 2.98^{bc}
LBK	21.93 ± 0.06^{bc}	3.30 ± 0.44^{bc}	8.78 ± 0.21^{cd}	23.85 ± 0.09^{abc}	9.37 ± 0.32^{bc}	69.42 ± 2.21^{ab}
MK	21.30 ± 0.20^{de}	4.33 ± 0.35^{a}	9.00 ± 0.26^{bc}	23.53 ± 0.25^{cd}	9.99 ± 0.30^{b}	64.30 ± 1.86^{c}
EK	22.50 ± 0.36^{a}	2.97 ± 0.31^{c}	8.50 ± 0.10^{d}	24.24 ± 0.40^{ab}	9.01 ± 0.18^{c}	70.78 ± 1.69^{a}
SK	20.97 ± 0.12^{e}	4.47 ± 0.38^{a}	8.60 ± 0.10^{d}	23.10 ± 0.13^{d}	9.69 ± 0.26^{b}	62.58 ± 1.71^{c}
CK	22.10 ± 0.36^{b}	2.97 ± 0.84^{c}	9.23 ± 0.06^{b}	24.14 ± 0.35^{ab}	9.72 ± 0.27^{b}	72.28 ± 4.73^{a}

Values with different lowercase letters (a–e) in the same column are significantly different ($P < 0.05$). Data are expressed as mean ± standard deviation (n = 3).

thearubigin may have been partially transformed into theaflavin, and with the progression of fermentation, it may have been effective in the transformation of the kombucha tea color from reddish-brown to light brown. In the study by Abuduaibifu and Tamer (2019), the color parameters (L^*, a^*, b^*, ΔE^*, $C_{ab}{}^*$, and $h°$) were measured after a 48-h fermentation (28 ± 2 °C) of black tea with kombucha culture as 4.72 ± 0.23, 5.91 ± 0.19, 4.52 ± 0.27, 8.81 ± 0.38, 7.44 ± 0.31, and 37.35 ± 0.76, respectively. When the results were compared, it was found that keeping the fermentation time longer and using black and green tea together as a substrate increased all of the color values, except a^*. Watawana et al. (2016) compared nonfermented coconut water and coconut kombucha fermented at 24 ± 3 °C for 7 days in terms of the color parameters. Although there was no significant change in the a^* value, the L^* and b^* values of the fermented product increased over time.

3.4. Minerals

The K, Mg, Ca, and Fe contents of kombucha culture fermented at 28 ± 2 °C for 14 days were 154.50 ± 6.82, 12.80 ± 1.58, 27.41 ± 5.11, and 0.41 ± 0.07 mg/L, respectively. In terms of the minerals in the infusion samples, the mint infusion had the highest K, Mg, and Ca values. In contrast, the cinnamon infusion had the lowest K, Mg, and Ca contents. While the lemon balm infusion had the highest Fe value, the sage infusion had the lowest (Table 6). The mineral analysis results of the sweetened black and green tea substrate with no kombucha culture added (before fermentation) and day 0 of storage (after fermentation) changed from 143.86 ± 4.18 mg/L to 173.04 ± 1.68 mg/L for K, 8.03 ± 0.18 mg/L to 9.76 ± 0.18 mg/L for Mg, 14.80 ± 1.00 mg/L to 14.94 ± 0.76 mg/L for Ca, and 0.34 ± 0.00 mg/L to 0.27 ± 0.04 mg/L for Fe. The increase in K and Mg levels and decrease in Fe level were significantly different ($P < 0.05$) with the addition of the kombucha culture, but the change in Ca level was not significantly different ($P > 0.05$). The control sample (173.04 ± 1.68 mg/L) and mint infusion + kombucha (MK) (170.96 ± 3.36 mg/L) had the highest K level. Moreover, MK had the highest Mg (12.34 ± 0.10 mg/L) and Ca (22.94 ± 0.76 mg/L) levels. However, LK and LBK had the highest Fe level (0.54 ± 0.06 mg/L). The results were consistent with the mineral values

Table 6. Mineral content of the medicinal plant infusions and kombucha samples (mg/L).

	K	Mg	Ca	Fe
Medicinal plant infusions				
Linden	61.50 ± 0.02^{d}	5.52 ± 0.09^{d}	16.80 ± 0.28^{d}	0.21 ± 0.02^{bc}
Lemon balm	140.44 ± 0.12^{b}	15.86 ± 0.24^{b}	34.29 ± 0.48^{b}	0.28 ± 0.00^{a}
Mint	202.14 ± 2.98^{a}	33.74 ± 0.48^{a}	72.28 ± 0.88^{a}	0.24 ± 0.04^{ab}
Echinacea	77.96 ± 0.92^{c}	12.44 ± 0.08^{c}	35.47 ± 0.69^{b}	0.19 ± 0.04^{c}
Sage	77.60 ± 4.72^{c}	5.57 ± 0.47^{d}	19.10 ± 1.48^{c}	0.11 ± 0.01^{d}
Cinnamon	14.34 ± 0.27^{e}	2.65 ± 0.06^{e}	13.43 ± 0.33^{e}	0.14 ± 0.02^{d}
Kombucha samples				
C	173.0 4 ± 1.68^{a}	9.76 ± 0.18^{b}	14.94 ± 0.76^{e}	0.27 ± 0.04^{b}
LK	159.44 ± 1.04^{b}	9.65 ± 0.04^{b}	17.27 ± 0.01^{c}	0.48 ± 0.04^{a}
LBK	151.44 ± 3.04^{c}	9.99 ± 0.11^{b}	19.40 ± 0.02^{b}	0.54 ± 0.06^{a}
MK	170.96 ± 3.36^{a}	12.34 ± 0.10^{a}	22.94 ± 0.76^{a}	0.32 ± 0.07^{b}
EK	153.32 ± 1.80^{c}	9.19 ± 0.03^{c}	16.52 ± 0.07^{cd}	0.20 ± 0.01^{c}
SK	151.42 ± 4.38^{c}	8.74 ± 0.47^{d}	15.96 ± 0.66^{d}	0.19 ± 0.01^{c}
CK	138.00 ± 0.68^{d}	8.24 ± 0.15^{e}	15.72 ± 0.50^{de}	0.19 ± 0.00^{c}

Values with different lowercase letters (a–e) in the same column are significantly different ($P < 0.05$). Data are expressed as the mean ± standard deviation (n = 3).

determined in the plant infusions (Table 6). In the study by Bauer-Petrovska and Petrushevska-Tozi (2000), the Fe values of black tea infusion and kombucha tea (fermented at 23–24 °C for 8 days) were 0.26 ± 0.013 µg/mL and 0.353 ± 0.018 µg/mL, respectively. Factors such as the difference in kombucha culture, tea substrate, preparation method, and mineral content of the water used for the kombucha preparation may have affected the amount of minerals in the kombucha beverage.

3.5. Total phenolic compound content and antioxidant capacity

Mint infusion was the most important source of total phenolic compounds (TPCs) and AC (FRAP and CUPRAC methods). The highest AC determined by DPPH assay was found in the sage infusion (Table 7). Before fermentation, the TPC content was determined as 1145.74 ± 18.89 mg GAE/100 mL. The DPPH, FRAP, and CUPRAC values were measured as 78.67 ± 0.66, 150.28 ± 5.32, and 87.04 ± 2.77 µmol TE/g w.s.d.m. respectively. After fermentation, the TPC content increased to 1865.28 ± 44.76 mg GAE/100 mL. The DPPH, FRAP, and CUPRAC values also increased to 89.65 ± 5.63, 223.85 ± 5.79, and 135.38 ± 1.50 µmol TE/g w.s.d.m., respectively. The increases determined in the TPC content and AC after fermentation were significant ($P < 0.05$). The increase rates of the TPC content, DPPH, FRAP, and CUPRAC were 62.80%, 13.96%, 48.96%, and 55.54%, respectively. Ayed and Hamdi (2015) also suggested that this increase might be related to the biotransformation and acid hydrolysis of phenolics. Additionally, Chu and Chen (2006) reported that after fermenting for 15 days, the AC of kombucha was increased to about 70% (DPPH), 40% (ABTS) and 49% (inhibition of linoleic acid peroxidation), respectively. On the contrary, the ferrous ion binding ability of kombucha was reduced by 81%. The TPC content increased up to 98%, which indicated that the thearubigin might have been subjected to depolymerization throughout the fermentation process, resulting in the release of smaller molecules exerting higher antioxidant potential. Abuduaibifu and Tamer (2019) determined TPC content and AC after the fermentation of black tea kombucha as 1079.67 ± 7.46 mg GAE/100 mL, 93.99 ± 0.24 µmol Trolox/g w.s.d.m (DPPH), 56.08 ± 0.71 µmol Trolox/g w.s.d.m (CUPRAC), and 81.77 ± 1.48 µmol Trolox/g w.s.d.m (FRAP). The difference between the values was thought to have been caused by changes in the substrate and production procedure, differences in the fermentation time, and temperature. Zubaidah et al. (2018) fermented sugared snake fruit juices for 2 weeks with kombucha consortium (a natural symbiotic consortium of acetic acid bacteria and yeast). They reported the increase in the TPC content and AC after fermentation similarly. Gaggia et al. (2019) used green, black, and rooibos tea as a substrate for kombucha fermentation at 27 ± 1 °C for 14 days. The highest AC (by DPPH and FRAP) was determined in the green

Table 7. TPC content and AC of the medicinal plant infusions.

	TPC (mg GAE*/100 mL)	DPPH (μmol TE/g w.s.d.m**)	FRAP (μmol TE/g w.s.d.m**)	CUPRAC (μmol TE/g w.s.d.m**)
Linden	4530.08 ± 221.03^{e}	2061.75 ± 6.03^{d}	47.21 ± 5.45^{d}	33.79 ± 5.09^{d}
Lemon balm	7440.15 ± 136.23^{c}	1845.04 ± 12.29^{e}	147.66 ± 1.83^{c}	110.98 ± 9.05^{b}
Mint	14534.42 ± 147.35^{a}	1364.05 ± 25.67^{f}	998.60 ± 2.38^{a}	1107.54 ± 15.55^{a}
Echinacea	6582.80 ± 255.22^{d}	2753.64 ± 6.97^{c}	48.67 ± 2.75^{d}	60.71 ± 6.78^{c}
Sage	12740.88 ± 141.57^{b}	5344.77 ± 8.04^{a}	160.77 ± 9.51^{b}	121.43 ± 13.57^{b}
Cinnamon	6747.75 ± 216.25d	5205.48 ± 62.81b	33.93 ± 5.49e	113.59 ± 13.57b

Values with different lowercase letters (a–f) in the same column are significantly different ($P < 0.05$).* Gallic acid equivalent. ** Water soluble dry matter. Data are expressed as the mean ± standard deviation (n = 3).

tea kombucha on day 7 of fermentation. The highest TPC content was found in MK (2055.27 ± 12.54 mg GAE/100 mL) on day 3 of storage, while the lowest TPC was found in CK (1564.14 ± 26.59 mg of GAE/100 mL) on day 6 of storage (Table 8). The highest AC (DPPH) was determined in LBK (114.40 ± 5.41 μmol TE/g w.s.d.m) on day 6 of storage, and in CK (114.07 ± 0.35 μmol TE/g w.s.d.m) on day 9 of storage. The control sample (89.66 ± 5.64 μmol TE/g w.s.d.m) had the lowest DPPH value on day 0 of storage (Table 8). The difference between the FRAP values of the medicinal plant infusion + kombucha samples and the control sample was found to be significantly different at the beginning of storage ($P < 0.05$). The highest FRAP value (256.30 ± 3.51 μmol TE/g g w.s.d.m) was determined in the control sample on day 3 of storage. However, on day 9 of storage, the lowest FRAP value (155.08 ± 7.02 μmol TE /g w.s.d.m) was found in the sage infusion + kombucha (SK) (Table 8). The highest CUPRAC value (177.34 ± 7.76 μmol TE/g w.s.d.m) was found in MK on day 9 of storage. However, the lowest CUPRAC value (108.23 ± 1.28 μmol TE/g w.s.d.m) was detected in SK on day 9 of storage. When the related tables were examined, it was observed that the addition of mint and cinnamon infusion may have been effective in increasing the AC of kombucha. It was found that the addition of the sage and cinnamon infusions caused a significant difference when compared with the control sample at the beginning of storage. In addition to this, the addition of the other plants, except the linden and echinacea infusions, caused a significant difference when compared to the control sample at the end of storage ($P < 0.05$).

Although the medicinal plant infusions generally resulted in significant increases in the AC on days 6 and/or 9 of storage, there were some exceptions (Table 8). During fermentation, the antioxidant properties of some of the samples could have been reduced. Similarly, Vohra et al. (2018) reported that fermentation could reduce the antioxidant properties of the samples after day 7 of fermentation. Researchers have determined the antioxidant activity (DPPH assay) of kombucha using different carbon sources (i.e. sugar, jaggery, and Kelulut honey in black tea and green tea media) over a period of 7, 14, 28, and 60 days. The antioxidant activity was measured postfermentation. All of the samples demonstrated antioxidant activity on day 7 of fermentation, but a subsequent decrease was observed with a longer fermentation time, except for the combination of green tea with jaggery. Dwiputri and Feroniasanti (2019) also indicated that there was a fluctuation in the antioxidant activity (DPPH) that occurred during the butterfly pea kombucha fermentation process. The antioxidant activity increased dramatically on day 4 of fermentation and then continued to drop over the next 4 days. After 8 days of fermentation, the antioxidant activity increased gradually, then remained almost stable until day 20 of fermentation. They explained that the reason for this fluctuation might have been the presence of some antioxidant compounds (flavonoids, tannins, and phenols) contained in the butterfly pea flowers, as well as the microbial activity degrading polyphenols during fermentation.

3.6. Post in vitro digestion of total phenolics and antioxidant capacity

The portion of a compound that is released from its matrix in the gastrointestinal tract, and becomes available for absorption is described as bioaccessibility. The term bioaccessibility involves digestive transformations of foods into material ready for assimilation, absorption/assimilation into intestinal epithelium cells, as well as presystemic, intestinal, and hepatic metabolism. In vitro digestion procedures, generally simulating gastric and intestinal digestion, sometimes followed by Caco-2 cell uptake, have been generally used for the evaluation of

Table 8. Results of the TPC content and AC of the kombucha beverages during storage.

	Day 0	Day 3	Day 6	Day 9	% Change*
TPC content (mg GAE**/100 mL)					
C	1865.28 ± 44.76^{aB}	1740.78 ± 54.74^{bcD}	1701.55 ± 66.07^{cC}	1825.30 ± 21.99^{abBC}	2.14 (–)
LK	1846.06 ± 42.48^{bB}	1840.40 ± 67.94^{bC}	1984.04 ± 59.14^{aA}	1740.03 ± 20.78^{cCD}	5.74 (–)
LBK	1686.31 ± 10.14^{cD}	1835.38 ± 21.79^{bC}	1887.59 ± 29.20^{aB}	1638.77 ± 39.71^{cE}	2.82 (–)
MK	1990.46 ± 69.41^{abA}	2055.27 ± 12.54^{aA}	1983.02 ± 9.61^{abA}	1972.24 ± 40.28^{bA}	0.92 (–)
EK	1719.59 ± 13.38^{bCD}	1861.05 ± 13.51^{aC}	1911.71 ± 39.25^{bAB}	$1757.73 \pm 111.77^{abBCD}$	2.22 (+)
SK	1733.75 ± 10.72^{bCD}	1957.39 ± 28.34^{aB}	1654.02 ± 43.46^{cC}	1671.13 ± 6.15^{cDE}	3.61 (–)
CK	1779.73 ± 15.02^{cC}	1947.30 ± 27.56^{aB}	1564.14 ± 26.59^{dD}	1842.45 ± 38.73^{bB}	3.52 (+)
DPPH (µmol TE/g w.s.d.m ***)					
C	89.66 ± 5.64^{bC}	92.61 ± 3.32^{abD}	93.95 ± 2.08^{abE}	99.10 ± 1.89^{aB}	10.53 (+)
LK	112.10 ± 1.21^{aA}	108.60 ± 0.59^{aA}	94.57 ± 2.33^{bDE}	92.22 ± 8.09^{bC}	17.73 (–)
LBK	113.06 ± 0.33^{aA}	109.47 ± 3.70^{aA}	114.40 ± 5.41^{aA}	97.62 ± 1.14^{bBC}	13.66 (–)
MK	110.33 ± 1.85^{aA}	103.84 ± 0.78^{bB}	99.07 ± 1.47^{cCD}	102.41 ± 3.32^{bcB}	7.18 (–)
EK	98.08 ± 3.23^{abB}	94.71 ± 1.16^{bD}	100.16 ± 2.64^{aC}	99.14 ± 0.35^{aB}	1.08 (+)
SK	94.55 ± 0.74^{dBC}	111.44 ± 1.43^{aA}	102.13 ± 1.14^{bBC}	98.22 ± 2.93^{cBC}	3.88 (+)
CK	92.02 ± 3.75^{dC}	99.13 ± 1.15^{cC}	105.16 ± 1.90^{bB}	114.07 ± 0.35^{aA}	23.96 (+)
FRAP (µmol TE/g w.s.d.m ***)					
C	223.85 ± 5.79^{bAB}	256.30 ± 3.51^{aA}	218.24 ± 7.37^{bBC}	225.56 ± 3.14^{bAB}	0.76 (+)
LK	212.51 ± 5.76^{bB}	223.76 ± 16.81^{abBC}	238.63 ± 5.00^{aA}	216.08 ± 11.20^{bB}	1.68 (+)
LBK	217.69 ± 9.26^{abB}	200.22 ± 13.63^{bcD}	227.64 ± 8.48^{aAB}	191.82 ± 7.04^{cC}	11.88 (–)
MK	218.66 ± 3.53^{bAB}	229.44 ± 4.29^{abB}	233.57 ± 11.45^{aA}	233.08 ± 4.28^{aA}	6.59 (+)
EK	232.35 ± 2.56^{aA}	206.50 ± 4.88^{bCD}	205.38 ± 4.07^{bCD}	214.26 ± 12.90^{bB}	7.79 (–)
SK	224.67 ± 7.93^{aAB}	222.47 ± 5.96^{aBC}	183.05 ± 5.84^{bE}	155.08 ± 7.02^{cD}	30.97 (–)
CK	214.12 ± 15.38^{aB}	211.20 ± 15.48^{aBCD}	193.41 ± 6.94^{aDE}	197.00 ± 4.50^{aC}	8.00 (–)
CUPRAC (µmol TE/g w.s.d.m ***)					
C	135.38 ± 1.50^{bAB}	140.94 ± 5.44^{abB}	145.91 ± 2.14^{aBC}	148.30 ± 7.10^{aB}	9.54 (+)
LK	134.98 ± 3.59^{bAB}	135.34 ± 2.42^{bBC}	151.91 ± 3.89^{aAB}	145.13 ± 6.58^{aB}	7.52 (+)
LBK	131.98 ± 4.33^{bBC}	131.09 ± 2.75^{bCD}	140.92 ± 0.62^{aC}	115.11 ± 4.24^{cD}	12.78 (–)
MK	143.60 ± 10.24^{bA}	155.43 ± 4.65^{bA}	156.07 ± 2.47^{bA}	177.34 ± 7.76^{aA}	23.50 (+)
EK	132.95 ± 2.59^{bBC}	126.57 ± 2.59^{cD}	147.27 ± 1.57^{aB}	144.53 ± 2.84^{aB}	8.71 (+)
SK	124.33 ± 2.49^{bCD}	133.42 ± 0.98^{aC}	130.44 ± 7.86^{abD}	108.23 ± 1.28^{cD}	12.95 (–)
CK	122.87 ± 5.28^{abD}	125.42 ± 1.44^{abD}	121.51 ± 1.02^{bE}	129.18 ± 4.29^{aC}	5.14 (+)

Values with different letters (a–d) in the same row are significantly different ($P < 0.05$). Values with different letters (A–E) in the same column are significantly different ($P < 0.05$). * Increase (+) % or decrease (–) % between days 0 and 9 of storage. ** Gallic acid equivalent. *** Water soluble dry matter. Data are expressed as the mean ± standard deviation (n = 3).

bioaccessibility[2]. As human studies are time-consuming, sumptuous, and restricted by ethical concerns, in vitro models for analyzing the effects of digestion on secondary plant metabolites have been developed and employed to anticipate their release from the food matrix, bioaccessibility, and evaluate changes in their profiles prior to absorption. However, the variation in conditions employed in in vitro gastrointestinal system models have made it difficult to compare the results across different studies (Alminger et al., 2014). Minekus et al.

[2] Galanakis C (2017). What is the difference between bioavailability bioaccessibility and bioactivity of food components? Online. Food Science & Nutrition [online]. Website http://scitechconnect.elsevier.com/bioavailability-bioaccessibility-bioactivity-food-components/

(2014) proposed a standard and practical static digestion model based on physiologically suitable conditions in order to overcome this problem. This model has been used previously to evaluate the bioaccessibility of some food wastes rich in polyphenols (Kamiloglu et al., 2017; Guven et al., 2018; Kamiloglu, 2019). In this study, in vitro bioaccessibility analyses of TPC and AC were performed by simulating the digestive system conditions in pre- and postdigestion at the beginning and at the end of storage. The % recoveries were given as the ratios of the values determined after intestinal digestion to the values determined for the initial (predigestion) values, and then multiplied by 100. The results are shown in Figures 2–5. The TPC contents of all of the samples increased postdigestion (gastric and intestinal) when compared to predigestion on days 0 and 9 of storage. The lowest recovery was determined in the control sample, both at the beginning (426.50%) and end (484.59%) of storage. On the other hand, LBK had the highest recovery on days 0 (569.51%) and 9 (593.98%) of storage. The enzymes that participate in digestion and changes in the pH of the digestion fluids cause the release of phenolics from food matrix (Bouayed et al., 2011). For this reason, recovery of the TPC content increased after gastric and intestinal digestion (Figure 2). Hollman et al. (1997) stated that several factors, such as the cell wall structure, position of the glycosides, binding on the food matrix, and releasing from this matrix during digestion, may affect the stability of phenolics and flavonoids. Gut flora hydrolyses, glycosides, glucuronides, sulphates, amides, esters, and lactons, and then microflora, alter polyphenols with antioxidant characteristics into different compounds (Espin et al., 2009). Horasan Sağbasan (2015) determined the bioaccessibility of the antioxidant content of dried red fruits using the in vitro model developed by Minekus et al. (2014). Villarreal-Soto et al. (2019) stated that fermentation can increase the bioavailability of tea polyphenols and there may be changes in the production of bioactive compounds, depending on the fermentation conditions. Jayabalan et al. (2007) found that the catechins in tea during kombucha fermentation were degraded. Their results demonstrated that theaflavin and thearubigin were relatively more stable than epicatechin isomers during fermentation. The AC (DPPH) of all of the kombucha samples decreased after intestinal digestion on days 0 and 9 of storage. While the lowest DPPH recovery (1.87%) was determined in EK, the highest value (10.53%) was determined in CK at the beginning of storage. Similarly, the lowest DPPH recovery (3.72%) was determined in EK, while the highest value (27.06%) was determined at the end of storage. Another result obtained in this study was that the DPPH values determined after gastric digestion of all of the samples were higher than the predigestion values at the beginning and end of storage (Figure 3). Bermudez-Soto et al. (2007) reported that polyphenols are very sensitive to the alkaline conditions of the intestines and they might turn into different compounds that have distinct bioaccessibility. The FRAP values of all of the samples increased postdigestion when compared to predigestion on days 0 and 9 of storage. While SK had the lowest recovery value (342.87%), CK had the highest recovery value 447.38% at the beginning of storage. However, MK had the lowest recovery value (369.84%) and CK, again, had the highest recovery value (487.88%) at the end of storage (Figure 4). The CUPRAC values of all of the samples increased postdigestion when compared to predigestion on days 0 and 9 of storage. At the beginning of storage, MK and CK had the lowest (655.31%) and highest (824.70%) recovery values, respectively. On the other hand, MK and LBK had the lowest (597.48 %) and highest (881.96%) recovery values, respectively, at the end of storage (Figure 5). When the current in vitro bioaccessibility results were compared to those of Abuduaibifu and Tamer (2019), the increment or reduction trend that was determined postdigestion was similar. According to their results, except for DPPH, the TPC, FRAP, and CUPRAC values all increased postdigestion. Abuduaibifu and Tamer (2019) used black tea kombucha fermented at 28 ± 2 °C for 2 days as the control sample. The recovery results of the in vitro bioaccessibility of the TPC content, DPPH, FRAP, and CUPRAC values of this sample at the beginning and end of storage (11 days) were 704.45%–699.41%, 24.24%–19.18%, 595.61%–577.66%, and 841.07%–889.74%, respectively. In this study, both green tea and black tea were chosen as the control sample and fermentation was performed at 22 ± 2 °C for 4 days. The recovery results of the post in vitro digestion of the total phenolics, DPPH, FRAP, and CUPRAC of this sample at the beginning and end of storage (9 days) were 426.50%–484.59%, 8.38%–24.05%, 394.92%–458.03%, and 745.53%–792.60%, respectively. The disparity of the results might have originated from the differences in the kombucha culture and substrate, temperature, and time.

3.7. Microbiological analysis

The total aerobic mesophilic bacteria, yeast, and acetic acid bacteria counts of the kombucha culture fermented at 28 ± 2 °C for 14 days were 6.32, 6.53, and 6.30 log cfu/mL. The change in the microbial counts during fermentation are given in Table 9. The total aerobic mesophilic bacteria, yeast, and acetic acid bacteria counts increased during fermentation. The viability value (124.05%) of the yeast count was higher than that of the total aerobic mesophilic bacteria and acetic acid bacteria. The change in the total aerobic mesophilic bacteria, yeast, and acetic acid bacteria counts of the kombucha during storage are given in Table 10. The total mesophilic aerobic bacteria count in the kombucha beverages, except in LBK, increased during

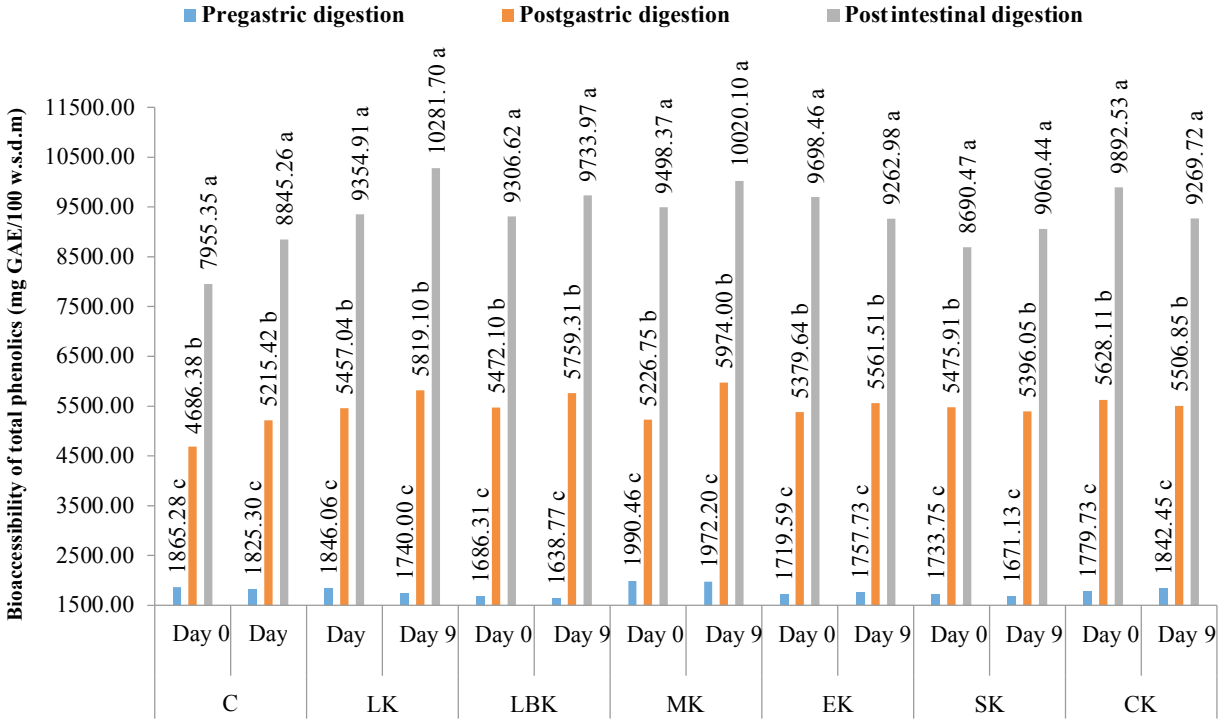

Figure 2. TPC content of the kombucha samples before digestion, after gastric digestion, and after intestinal digestion. a–b: Statistically significant differences between the pregastric digestion, postgastric digestion, and postintestinal digestion results ($P < 0.05$).

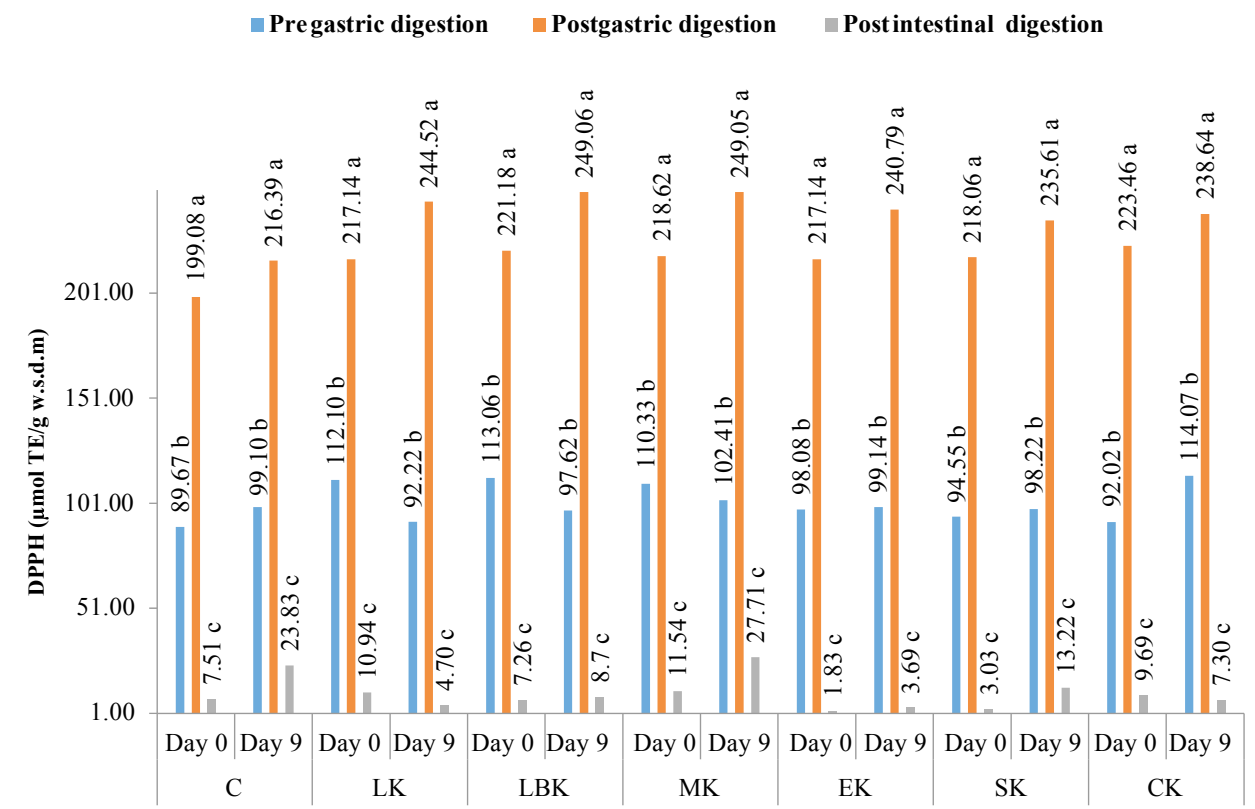

Figure 3. Total AC (DPPH) of the kombucha samples before digestion, after gastric digestion, and after intestinal digestion. a–c: Statistically significant differences between the pregastric digestion, postgastric digestion, and postintestinal digestion results ($P < 0.05$).

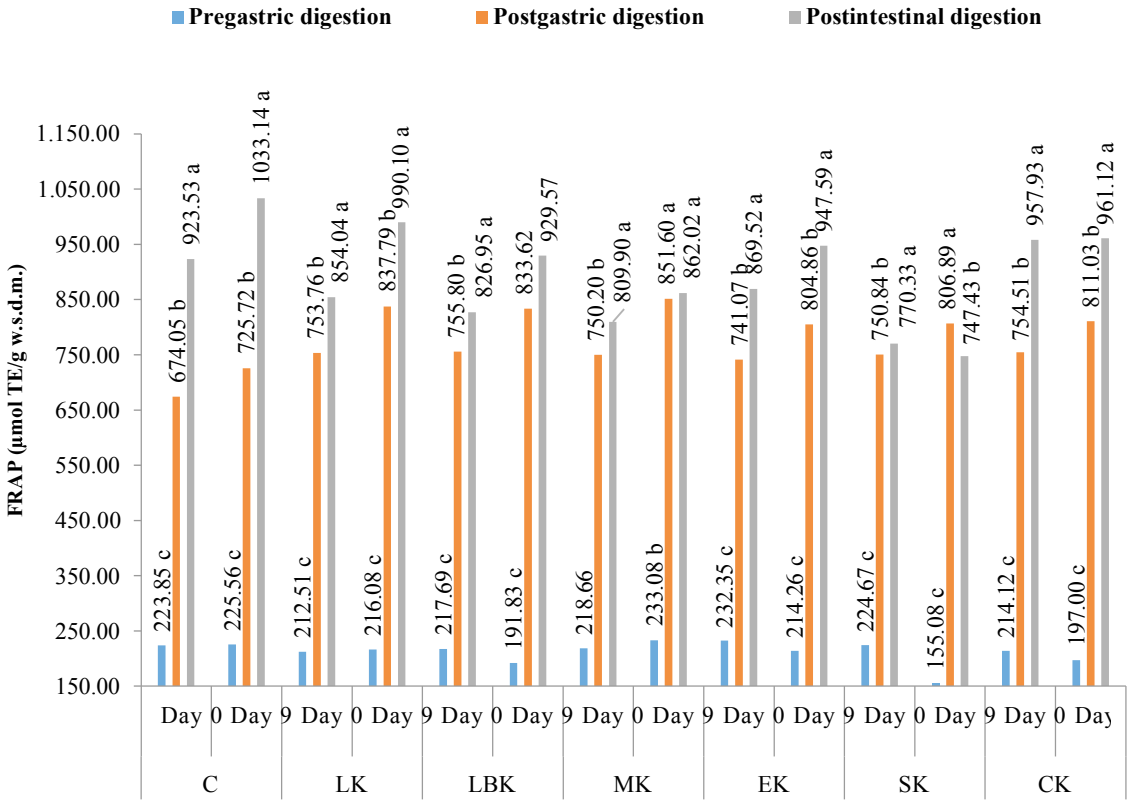

Figure 4. Total AC (FRAP) of the kombucha samples before digestion, after gastric digestion, and after intestinal digestion. a–c: Statistically significant differences between pregastric digestion, postgastric digestion, and postintestinal digestion results ($P < 0.05$)

storage. The highest viability value was determined in SK. The acetic acid bacteria count determined during storage increased in all of the beverages at the end of storage when compared to the initial counts, except for MK (Table 10). It can be understood that although the kombucha samples were stored in the refrigerator, fermentation still continued. When compared to the control sample, the acetic acid bacteria count of MK was lower. It was thought that the peppermint infusion may have had inhibitory properties on fermentation and the other plant infusions may have had fermentation-activating properties. Shazbazi et al. (2018) reported that kombucha samples containing cinnamon exhibited higher antimicrobial activity on *E. coli*, *S. typhimurium*, and *S. aureus*. The yeast count increased during storage in the control sample, LK, MK, and CK. However, LBK, EK and SK had lower viability values at the end of storage when compared to the beginning of storage (Table 10). Ayed and Hamdi (2015) investigated the microbiological properties of kombucha prepared with cactus pear juice. Similar to the current results, they stated that the yeast count was higher than the acetic acid bacteria count. Ayed et al. (2017) also reported that the yeast count was ten-fold higher than that of the acetic acid bacteria because the metabolic activity of acetic acid bacteria was lower than that of the yeast in red grape juice kombucha. Fermentation was conducted for 10 days at 20, 25, and 30 °C in the production of kombucha, where green tea, black tea, and sucrose were used as substrates in the study by Neffe-Skocinska et al. (2017). The highest acetic acid bacteria count (7.61 log cfu/mL) was reached after 10 days of fermentation at 25 °C. The yeast count was 4 log cfu/mL at all temperatures before fermentation. The fastest increase was achieved at 25 °C on day 3 of fermentation and reached 7 log cfu/mL after fermentation, and this count remained constant. Değirmencioğlu et al., (2019) produced kombucha samples using white, green, oolong, black, and pu-erh tea leaves. Lactic acid bacteria, yeast, acetic acid, and gluconobacter counts were determined during fermentation at 30 °C for 21 days. There was a rapid increase in all of the microorganisms on day 3 of fermentation, and this increment continued until day 12 in the lactic acid bacteria and yeast counts. At the end of fermentation, the acetic acid and gluconobacter counts were 7.77–10.66 and 7.68–9.68 log cfu/mL, respectively. The lactic acid bacteria count increased first, followed by a reduction of 60% due to high acidity. As a result of the higher fermentation temperature and longer fermentation period, the microbial counts were higher.

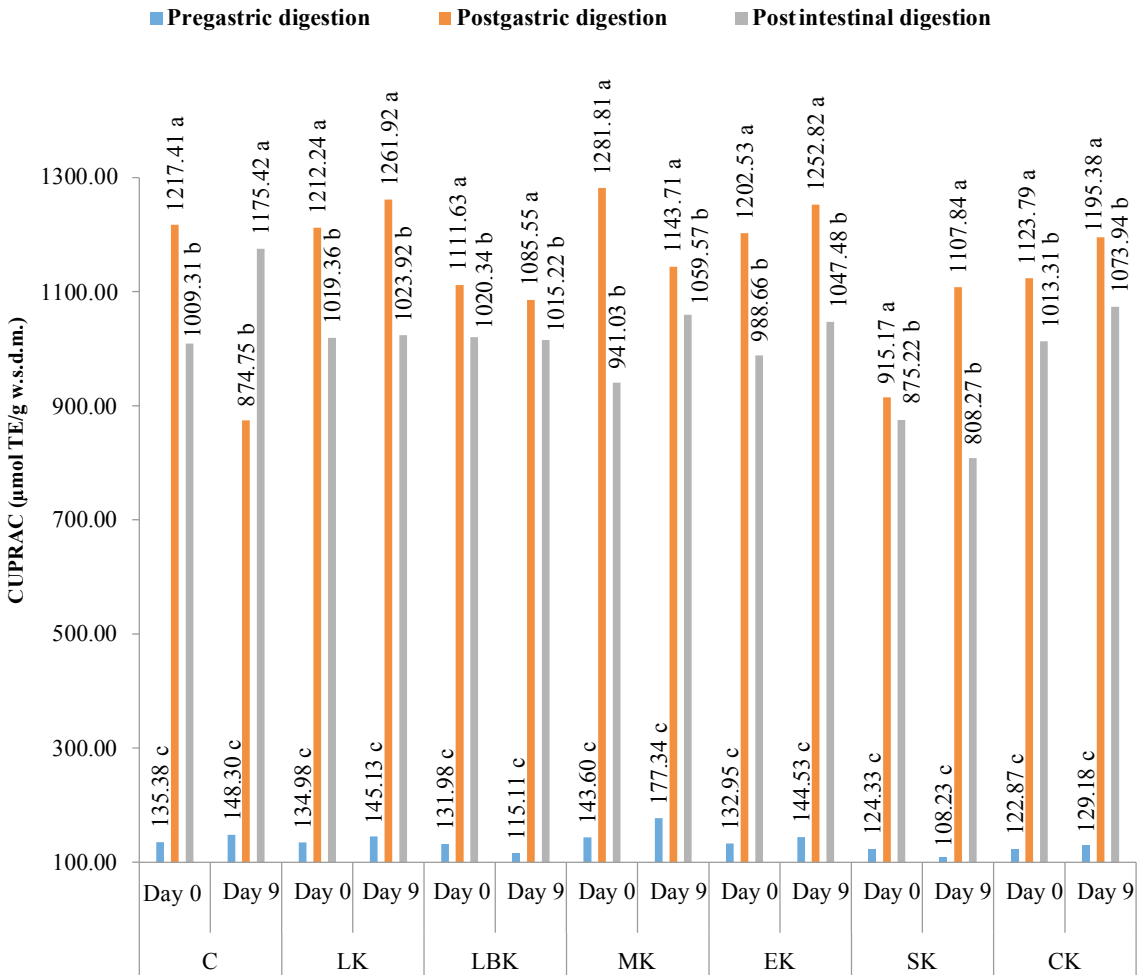

Figure 5. Total AC (CUPRAC) of the kombucha samples before digestion, after gastric digestion, and after intestinal digestion. a–c: Statistically significant differences between the pregastric digestion, postgastric digestion, and postintestinal digestion results ($P < 0.05$).

Table 9. Changes in the microbial counts during fermentation.

Fermentation time	Total aerobic mesophilic bacteria (log cfu/mL)	Yeast (log cfu/mL)	Acetic acid bacteria (log cfu/mL)
Day 0	5.65 ± 0.07^{cA}	5.28 ± 0.11^{cB}	5.86 ± 0.08^{bA}
Day 1	6.19 ± 0.13^{bA}	6.48 ± 0.14^{aA}	5.16 ± 0.23^{dB}
Day 2	5.30 ± 0.26^{dB}	5.63 ± 0.27^{bA}	5.53 ± 0.25^{cAB}
Day 3	6.66 ± 0.08^{aA}	6.59 ± 0.00^{aA}	5.75 ± 0.07^{bcB}
Day 4	6.10 ± 0.26^{bB}	6.55 ± 0.26^{aA}	6.41 ± 0.24^{aA}
Viability (%)	107.96%	124.05%	109.39%

a, b: Different lowercase letters denote significant differences ($P < 0.01$) between the different days of fermentation. A, B: Different capital letters denote significant differences ($P < 0.01$) between the different microorganisms. Data are expressed as the mean ± standard deviation (n = 3).

3.8. Sensory analysis

Sensory analysis of the kombucha samples was performed on days 0, 3, 6, and 9 of storage. Güldane et al. (2017) stated that sensory analysis should be used to determine the fermentation time in kombucha production, and TA and pH analysis are also necessary to terminate fermentation.

Table 10. Change in the microorganism counts during storage.

	Day 0	Day 3	Day 6	Day 9	Viability (%)
Total aerobic mesophilic bacteria (log cfu/mL)					
C	6.65 ± 0.07^{bC}	6.74 ± 0.06^{cdC}	6.99 ± 0.01^{abB}	7.69 ± 0.01^{bA}	115.64%
LK	6.75 ± 0.03^{abC}	6.93 ± 0.04^{bcBC}	7.15 ± 0.21^{aB}	7.80 ± 0.14^{abA}	115.56%
LBK	6.87 ± 0.10^{abC}	7.93 ± 0.00^{aA}	6.93 ± 0.03^{abC}	7.73 ± 0.00^{abB}	112.52%
MK	6.77 ± 0.10^{abA}	6.89 ± 0.00^{bcA}	7.26 ± 0.00^{aA}	7.08 ± 0.32^{cA}	104.58%
EK	6.98 ± 0.31^{aA}	6.41 ± 0.33^{dB}	7.13 ± 0.07^{aA}	7.11 ± 0.35^{cA}	101.86%
SK	6.70 ± 0.00^{bC}	7.17 ± 0.16^{bA}	6.98 ± 0.18^{abBC}	7.85 ± 0.16^{abA}	117.16%
CK	6.89 ± 0.06^{abB}	6.66 ± 0.23^{cdB}	6.60 ± 0.08^{bB}	7.93 ± 0.01^{aA}	115.09%
Acetic acid bacteria (log cfu/mL)					
C	5.76 ± 0.07^{aA}	5.87 ± 0.08^{cA}	5.88 ± 0.10^{bA}	5.77 ± 0.00^{dA}	100.17%
LK	5.49 ± 0.07^{abC}	5.62 ± 0.03^{cC}	6.43 ± 0.18^{aB}	6.94 ± 0.06^{aA}	126.41%
LBK	5.21 ± 0.08^{bB}	5.60 ± 0.30^{cAB}	5.92 ± 0.07^{bA}	5.95 ± 0.03^{cdA}	114.20%
MK	5.52 ± 0.06^{aB}	6.61 ± 0.251^{bA}	6.47 ± 0.08^{aA}	4.90 ± 0.03^{eC}	88.77%
EK	4.89 ± 0.06^{cC}	5.15 ± 0.00^{dC}	6.39 ± 0.21^{aA}	5.79 ± 0.13^{dB}	118.40%
SK	4.78 ± 0.045^{cC}	7.18 ± 0.00^{aA}	6.16 ± 0.04^{abB}	6.21 ± 0.29^{bcB}	129.91%
CK	5.56 ± 0.11^{aB}	5.76 ± 0.03^{cB}	6.18 ± 0.03^{abA}	6.36 ± 0.10^{bA}	114.39%
Yeast (log cfu/mL)					
C	6.35 ± 0.07^{dC}	6.71 ± 0.07^{aA}	6.51 ± 0.01^{cdB}	6.76 ± 0.08^{abA}	106.49%
LK	6.44 ± 0.07^{cdB}	6.40 ± 0.06^{abB}	6.91 ± 0.07^{abA}	6.92 ± 0.03^{aA}	107.45%
LBK	6.49 ± 0.71^{cdA}	5.49 ± 0.00^{abA}	6.18 ± 0.03^{dA}	6.11 ± 0.16^{cA}	94.14%
MK	6.74 ± 0.58^{bAB}	6.20 ± 0.30^{bcB}	7.21 ± 0.30^{aA}	6.93 ± 0.02^{aA}	102.82%
EK	6.98 ± 0.02^{aA}	6.17 ± 0.30^{bcC}	6.58 ± 0.05^{bcB}	6.87 ± 0.02^{aAB}	98.42%
SK	6.59 ± 0.13^{bcA}	5.91 ± 0.01^{cA}	6.63 ± 0.08^{bcA}	6.26 ± 0.37^{cA}	94.99%
CK	6.36 ± 0.08^{cdA}	6.21 ± 0.30^{bcA}	6.59 ± 0.01^{bcA}	6.40 ± 0.14^{bcA}	100.63%

a, b: Different lowercase letters denote significant differences ($P < 0.01$) between the different samples. A, B: Different capital letters denote significant differences ($P < 0.01$) between the different days of storage.

As can be seen in Table 3, the TA was determined as 0.28 g/100 mL in LK, LBK, and EK on day 9 of storage. At that time, these samples were evaluated as nondrinkable for taste characteristics. For this reason, the storage period was limited as 9 days. The control sample and plant-infusion-fortified kombucha samples were served to the panelists. They rated the samples according to their color, appearance, odor, and taste from 0 to 9 points, where 5 points was set as the acceptable limit and anything below that value was rejected. The sensory properties of the kombucha beverages are given in Figure 6. There were no significant differences determined in the color and odor criteria between samples during storage ($P > 0.05$). The opinions of the panelists showed that all of the beverages were appreciated at the beginning; however, the slight fruity taste and smell in the beverages turned into a vinegar-like taste and smell towards the end of storage. Moreover, slight turbidity in the drinks became visible at the end of storage. In terms of the taste criteria, the highest-rated samples at the beginning of storage were the control sample and MK. MK again was the most preferred sample at the end of the storage. It was thought that there might have been a negative relationship between the increasing acidity level of the beverages and the liking status. Tarhan (2017) stated that a hibiscus extract + kombucha beverage was the most admired sample, while kombucha prepared with sage extract was the least admired drink.

4. Conclusion

In this study, kombucha beverages with enhanced functional and sensory properties were obtained by adding linden, lemon balm, sage, echinacea, mint, and cinnamon infusions after the completion of fermentation. According to the results of the sensory analysis, the

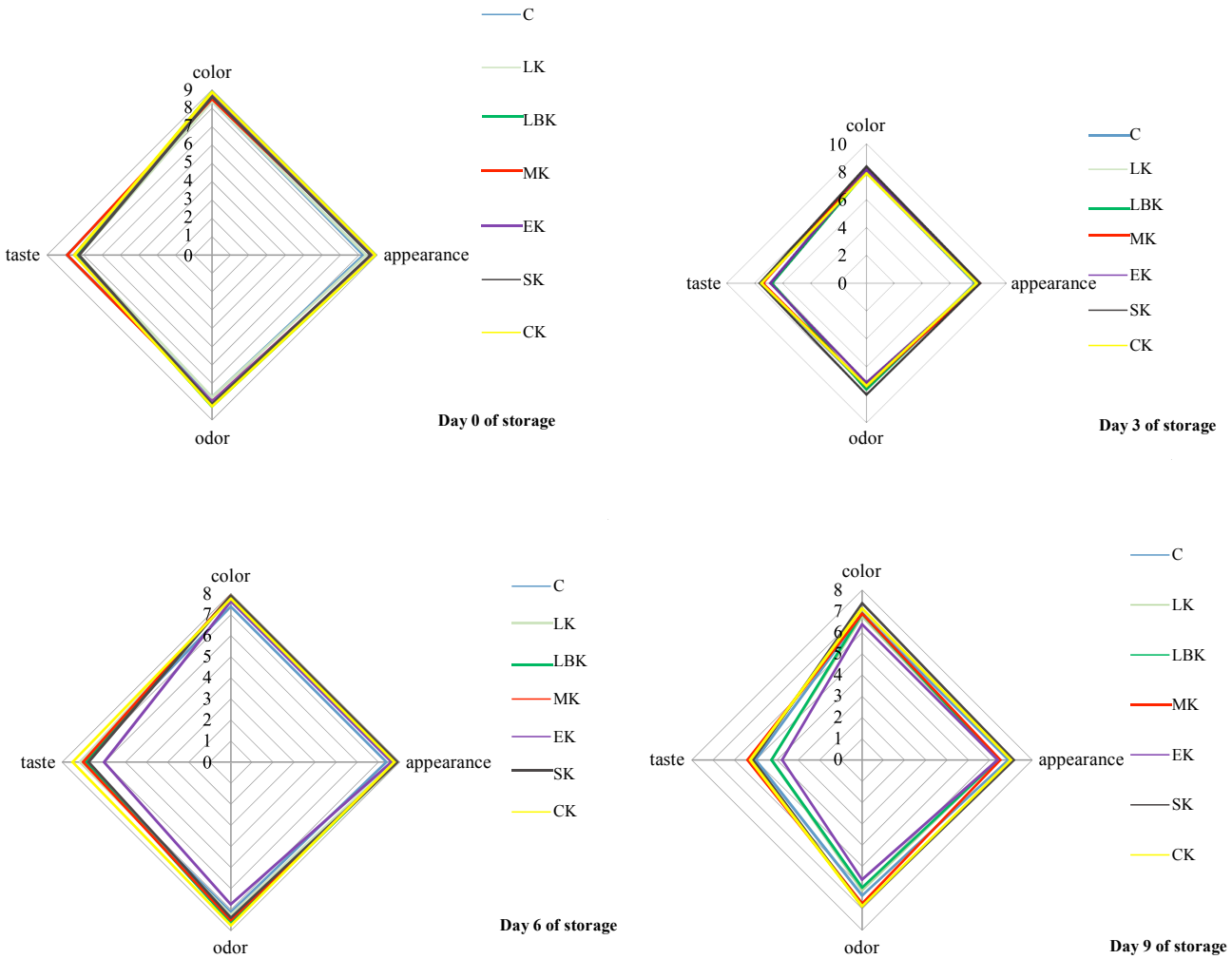

Figure 6. Sensory evaluation of kombucha samples during the storage.

most preferred samples for taste were the control sample and MK at the beginning of storage. MK, again, had the highest score among samples at the end of storage. The TPC content and AC values determined using the DPPH, FRAP, and CUPRAC methods increased as a result of fermentation. The TPC contents of all of the samples increased postdigestion (gastric and intestinal) when compared to predigestion on days 0 and 9 of storage. The lowest recovery was determined in the control sample at both the beginning (426.50%) and end (484.59%) of storage. On the other hand, LBK had the highest recovery on days 0 (569.51%) and 9 (593.98%) of storage. The AC (DPPH) after in vitro digestion at the beginning and end of storage in all of the beverages also increased after gastric digestion when compared to pregastric digestion ($P < 0.05$); however, it decreased after intestinal digestion ($P < 0.05$). Today, it is known that people have increased interest in functional foods, especially fermented products that are both beneficial to health and appeal to the palate. By conducting in vitro and in vivo studies on kombucha, the effects of this functional beverage on health and nutrition need to be further investigated.

Conflicts of interest

The authors have no conflicts of interest to declare.

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