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Korrespondenzadresse:
gulden-gk@hotmail.com

Ege University, Engineering Faculty, Food Engineering Department, Izmir, Turkey

Microbiological, physicochemical and sensory properties of kombucha beverages produced with Anatolian Hawthorn *(Crataegus orientalis)* and Nettle *(Urtica dioica)* leaves

Mikrobiologische, physikalisch-chemische und sensorische Eigenschaften von Kombucha-Getränken, die mit Blättern von Anatolischem Weißdorn (Crataegus orientalis) und Brennnessel (Urtica dioica) hergestellt wurden

Gulden Kilic, Ilkin Yucel Sengun

Summary

In the study, Kombucha beverages produced with Anatolian hawthorn (KH) or nettle leaves (KN) were examined in terms of some microbiological and chemical properties during fermentation (at 25 °C for 21 days) and storage (at 4 °C for 120 days) periods. The samples were also examined for color and sensory properties during storage. The numbers of acetic acid bacteria (AAB) and yeast for fermentation period were in the range of 3.08–6.15 and 3.30–6.21 log CFU/mL in KH, 3.74–6.02 and 3.85–6.85 log CFU/mL in KN, and 3.63–6.13 and 3.86–6.70 log CFU/mL in Kombucha produced from black tea (control samples-KC), respectively. At the end of the storage, AAB and yeast counts of the samples were mostly lower than the beginning. Besides, AAB counts of KH and KN were higher than KC (except KC1). The highest acidity was determined in KN (except KH4) at the end of the fermentation period. The results of the sensory evaluation showed that in general, KH had higher scores in terms of color, clarity, taste, odor, and general acceptance, depending on the storage time. As a result, this study indicated that Anatolian hawthorn and nettle leaves have significant potential to be used as a substrate for developing Kombucha having better sensory properties than Kombucha produced from black tea.

Keywords: Kombucha, Anatolian Hawthorn, Nettle, AAB, yeast, sensory

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Introduction

Kombucha is a traditional fermented beverage of Manchurian origin, but it is popular in many countries for its health benefits including antimicrobial, antioxidant, detoxification, hypoglycemic and hepatoprotective effects, neurodegenerative disease prevention, blood pressure reduction, and anticancer properties (Watawana et al., 2015; Kapp and Sumner, 2019). It is produced by fermenting sugared tea with a symbiotic culture, which mainly contains various acetic acid bacteria (AAB) (*Acetobacter* spp., *Gluconacetobacter* spp., *Gluconobacter* spp., *Komagataeibacter* spp.), yeast (*Saccharomyces* spp., *Schizosaccharomyces* spp., *Zygosaccharomyces* spp., *Brettanomyces* spp.), and rarely lactic acid bacteria (LAB) (*Lactobacillus* spp., *Lactococcus* spp., *Streptococcus* spp.) (Teoh et al., 2004; Jayabalan et al., 2010; Petrušic et al., 2011; Marsh et al., 2014; Coton et al., 2017; Villarreal-Soto et al., 2018).

Kombucha fermentation typically occurs for 3 to 60 days at room temperature (Chen and Liu, 2000; Xia et al., 2019; Leonarski et al., 2021). In the production, Kombucha cellulosic layer generally known as SCOBY (symbiotic culture of bacteria and yeast) or Kombucha liquid from a previous fermentation is utilized as starter culture, while sucrose is generally used at 5–20% of concentration as the main sugar source for the growth of microorganisms (Vina et al., 2013; Watawana et al., 2015). The taste of the beverage is slightly acidic and it is accepted as safe for consumption due to containing organic acids such as acetic acid, gluconic acid, etc., which are produced during fermentation (Watawana et al., 2015). The composition of the beverage produced depends on the variables such as fermentation time and temperature, microbial composition of SCOBY, raw material, and sugar source used. These variables affect the quality properties of the beverage such as microbiological, nutritional, and sensory properties (Vina et al., 2013; Martínez Leal et al., 2018; Emiljanowicz and Malinowska-Pańczyk, 2020).

Kombucha, which has come up with its positive effects on health in recent years, is traditionally produced by using black or green tea and sucrose (Jayabalan et al., 2016). In several studies, various raw materials such as white tea, oolong tea, extracts of thyme, lemon flower, rosemary, fennel and mint, yarrow herb, rooibos leaves, and also tofu and soy whey have also been used in the Kombucha production as alternative sources to black and green tea (Battikh et al., 2012; Watawana et al., 2016; Vitas et al., 2018; Gaggìa et al., 2019; Tu et al., 2019; Jakubczyk et al., 2020). However, there are no studies examining the possibility of using Anatolian hawthorn (*Crataegus orientalis* M Bieber.) and nettle (*Urtica dioica* L.) leaves in Kombucha production.

Crataegus orientalis belongs to the Rosaceae family, and commonly called as Anatolian hawthorn and also "geyikdikeni" in Turkey. It is native to the Mediterranean region, Turkey, Iran and Crimea (Donmez, 2004). The extract of *Crataegus orientalis* leaves contain flavonoids such as apigenin, apigenin-7-glucoside and vitexin. Hence, it has antioxidant activity, and also the potent antiinflammatory and antinociceptive effects (Melikoglu et al., 1999; Bor et al., 2012).

Urtica dioica L. belongs to the Urticaceae family, also known as nettle and commonly used by local people of Turkey (Gozum et al., 2003; Cakilcioglu and Turkoglu, 2010). The stinging nettle leaves contains pantothenic acid, chlorophyll, carotenoids, B1 and B2 vitamins, vitamin C and K, tannins, essential oil, proteins, and minerals, so the leaves are used for the treatment of anemia as well as general well-being, and natural food colorant (Kukrić et al., 2012). Recently, it is stated that it can be used as a prebiotic source in functional food production (Bonetti et al., 2016; Sengun et al., 2020).

In the present study, it is aimed:

1) to carry out Kombucha production using Anatolian hawthorn and nettle leaves,
2) to investigate and compare the microbiological, physical, chemical and sensory properties of these samples versus black tea Kombucha during fermentation and storage period.

Materials and methods

Plant samples

Anatolian hawthorn *(Crataegus orientalis)* and nettle *(Urtica dioica)* leaves were supplied from an indigenous producer from Nizip, Gaziantep and Serik, Antalya in Turkey, respectively. The plant leaves were dried in a tray dryer at 50 °C, rate of 1 m/s until the moisture content reaches to <7% (g/g) (TK Lab Model, Eksis Industrial Drying Systems, Turkey) and stored after vacuum packing (Henkelman Boxer 42, Holland). Black tea (Lipton, London, United Kingdom), sucrose (Balkupu, Istanbul, Turkey) and honey (Balparmak, Istanbul, Turkey) were purchased from a local market in Izmir, Turkey.

Production of plant tea

Plant tea samples were prepared with Anatolian hawthorn (TH), nettle (TN) and black tea (TC, as control) by infusion method. Firstly, samples were separately prepared with 1 L of drinking water by using two different sources of sugar (sucrose and honey) in different proportions (75 and 100 g) (Table 1). After the mixtures were heated to 85 °C, 10 g of plant leaves was added, and the mixture was allowed to infuse for 10 minutes. Brewed teas were filtered through a cheesecloth, placed in 1.5 L of sterile jars under aseptic conditions and cooled at room temperature. The final liquid was called as plant tea and stored at +4 °C until microbiological (AAB, LAB and yeast counts), chemical (pH, total acidity and brix), physical (color) and sensory (color, odor, clarity, taste and general acceptance) analysis were performed.

Production of Kombucha

Anatolian hawthorn tea (TH), nettle tea (TN) and black tea (TC) samples were separately inoculated with Kombucha culture to obtain Anatolian hawthorn Kombucha (KH), nettle Kombucha (KN) and black tea Kombucha (KC), respectively. Kombucha cellulosic layer including starter cultures were supplied from a local producer in Ankara, Turkey (Mayakoy Food Co. Ltd., Turkey) and transferred to the laboratory under cold chain, and stored at +4 °C until analysis. For the production of Kombucha samples, 50 g of Kombucha cellulosic layer and 50 mL of Kombucha liquid, were separately inoculated to 1 L of plant tea (TH, TN or TC), prepared as described in **Production of plant tea.** Then the jars were covered with sterile gauze to ensure aerobic conditions. The fermentation process was conducted at 25 °C for 21 days (Neffe-Skocińska et al., 2017).

Microbiological and chemical analyses were conducted on the 1st, 3rd, 5th, 7th, 10th, 14th, 18th, and 21st days of the fermentation process. After fermentation, cellulosic

TABLE 1: *Formulations of plant teas and Kombucha.*

Ingredients of Plant Tea	TH1	TH2	TH3	TH4	TN1	TN2	TN3	TN4	TC1	TC2	TC3	TC4
Water (L)	1	1	1	1	1	1	1	1	1	1	1	1
Sucrose (g)	75	100	-	-	75	100	-	-	75	100	-	-
Honey (g)	-	-	75	100	-	-	75	100		-	75	100
Crataegus orientalis (g)	10	10	10	10	-	-	-	-		-		-
Urtica dioica (g)	-	-	-	-	10	10	10	10		-		-
Camellia sinensis (g)	-	-	-	-	-	-	-	-	10	10	10	10
Ingredients of Kombucha	**KH1**	**KH2**	**KH3**	**KH4**	**KN1**	**KN2**	**KN3**	**KN4**	**KC1**	**KC2**	**KC3**	**KC4**
Water (L)	1	1	1	1	1	1	1	1	1	1	1	1
Sucrose (g)	75	100	-	-	75	100	-	-	75	100	-	-
Honey (g)	-	-	75	100	-	-	75	100		-	75	100
Biofilm (g)	50	50	50	50	50	50	50	50	50	50	50	50
Kombucha (mL)	50	50	50	50	50	50	50	50	50	50	50	50
Crataegus orientalis (g)	10	10	10	10	-	-	-	-		-		-
Urtica dioica (g)	-	-	-	-	10	10	10	10		-		-
Camellia sinensis (g)	-	-	-	-	-	-	-	-	10	10	10	10

layers were removed and the remaining liquids, Kombucha, were stored at +4 °C for 120 days. Kombucha were investigated in terms of microbiological, physical, chemical, and sensory properties on the 0th, 15th, 30th, 60th, 90th, and 120th days of storage.

Microbiological analysis

Twenty-five milliliter sample was aseptically transferred in a sterile stomacher bag containing 225 mL of 0.1% peptone water (PW, pH 6.8±0.2, Oxoid Ltd., Basingstoke, Hampshire, England, CM0009), homogenized in a Stomacher (Stomacher Lab-Blender 400, Seward Medical, London, UK) and then appropriate 10-fold dilutions were prepared in PW.

The enumeration of AAB was performed on Glucose Yeast Extract $CaCO_3$ Agar [GYC, 10% D-glucose (Merck KGaA, Darmstadt, Germany, 108337), 1% yeast extract (Merck, 103753), 2% calcium carbonate (Carlo Erba Reagents Co., Val-de-Reuil, France, 327059), 1.5% agar (Sigma-Aldrich Co., St. Louis, MO, USA, 05039), 3% ethanol (Sigma-Aldrich, 162973), 0.5% glacial acetic acid (Merck, 100063), pH 6.8±0.2] by spread plate method and the plates were incubated at 30 °C for 5–10 days. At the end of the incubation, colonies having creamy color and clean precipitation zone on GYC plates were considered typical AAB colonies (De Vero et al., 2006; De Vero and Giudici, 2013).

The enumeration of LAB was carried out on Man Rogosa and Sharp Agar (MRS, pH 6.2±0.2, Merck, 110660) containing 0.1% cycloheximide (Sigma-Aldrich, C7698) by using the double-layer technique and the plates were incubated at 30 °C for 3–5 days (Gaggìa et al., 2019).

The enumeration of yeast was determined on Potato Dextrose Agar (PDA, pH 5.6±0.2, Merck, 110130) acidified with tartaric acid (10%, Merck, 100804) by using pour plate method and the plates were incubated at 25 °C for 3–5 days (FDA-BAM, 2001).

Chemical and physical analysis

The pH values of the samples were determined using a pH meter (Hanna HI 2002-02, Woonsocket, Rhode Island, USA) (AOAC, 2007).

Titrimetric method was used for detecting total acidity of the samples. The results were expressed as g/L acetic acid (AOAC, 2007).

Brix values of the samples were measured by refractometer (Hanna HI 96801) calibrated with distilled water (AOAC, 2007).

Color properties of the samples were measured using a HunterLab Colorflex (Management Company, USA) and the results were expressed as L^* (lightness-darkness), a^* (redness-greenness), and b^* (yellowness-blueness) (Rommel et al., 1990). The assays were performed in triplicate and four readings were taken for each sample.

Sensory evaluation

Sensory evaluations were conducted in individual air-conditioned booths under white light at the Sensory Laboratory of Food Engineering Department, Ege University. Ten panelists (aged 20–35) semi-trained about the products consisting of students and staff members from Food Engineering Department, Ege University evaluated the samples. Before serving, the samples were coded with three-digit numbers. Bread and water were also served between the samples to avoid carry-over effects. Then the color, odor, clarity, taste and general acceptance properties of the samples were evaluated using nine-point hedonic scale (9-like extremely, 1-dislike extremely) (Altug and Elmaci, 2015).

Statistical analysis

All analyses were performed in triplicate. Data were examined by one-way analysis of variance (ANOVA) and the differences among the means were compared by Duncan's Multiple Range Test using the SPSS software for Windows Version 20.0. A statistically significant level of $P < 0.05$ was determined in all analyses. All results obtained in the study were expressed as the mean value ± standard deviation in tables and figures.

Results and discussions

Microbiological properties of the samples

Microbiological properties of plant tea samples were investigated in terms of AAB, LAB and yeast counts, and the counts were found under detection limit. Kombucha

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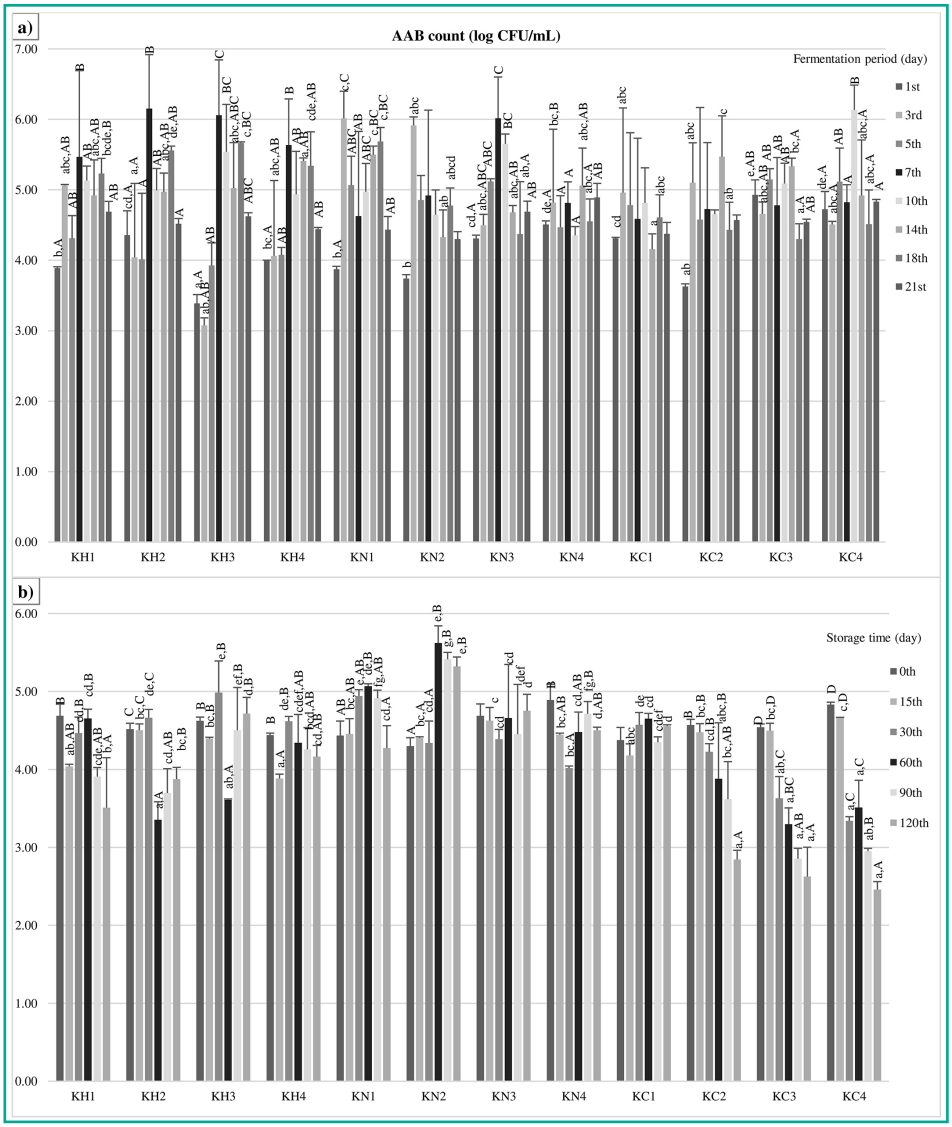

FIGURE 1: *AAB counts of Kombucha samples during fermentation (at 25 °C for 21 days) (a) and storage (at 4 °C for 120 days) (b). Different lower case (a, b, c, d, e, f, g) on the graph show statistical differences between the samples, the different upper case (A, B, C, D) show the statistical differences between fermentation/ storage times (P < 0.05).*

samples were also microbiologically tested during fermentation and storage period. The counts of AAB in Kombucha samples were detected as ranging from 3.08 to 6.15 log CFU/mL during fermentation at 25 ºC for 21 days ($P < 0.05$) (Fig. 1a). AAB counts of Kombucha samples were generally increased until the 5th and 7th days of the fermentation and then decreased till the end of the fermentation. There was no statistical difference between the samples during fermentation at 5th, 7th, and 10th days, and also at the end of the fermentation period (21st) ($P > 0.05$). Additionally, the counts were not changed during fermentation period for KN2, KC1 and KC2 ($P > 0.05$) (Fig. 1a).

During storage period at 4 °C for 120 days, AAB counts of Kombucha samples were ranged between 2.46 and 5.62 log CFU/mL (Fig. 1b) ($P < 0.05$). Only the counts of KN3 were not significantly changed during storage period ($P > 0.05$). However, AAB counts of all samples except KH3, KN2, and KC1 were decreased at the end of the storage, when compared to the beginning (0th day) (Fig. 1b). Besides, no significant difference was observed between AAB counts of the Kombucha samples produced with different plant samples in the scope of the study ($P > 0.05$). However, AAB counts of KH and KN samples was statistically higher than KC samples (except KC1) for 30th, 90th and 120th days of the storage ($P < 0.05$).

Limited studies have been reported the presence of LAB in Kombucha fermentation (Marsh et al., 2014; Coton et al., 2017). In this study, no LAB could be isolated from all of the samples, as reported similarly in the previous studies (Neffe-Skocińska et al., 2017; Gaggìa et al., 2019; Barbosa et al., 2021). Microbiological properties of Kombucha may change depending on the source of the Kombucha cellulosic layer, intrinsic and extrinsic factors such as the climatic and geographical conditions, and the available nutrients found in the environment (Malbaša et al., 2011a, b; Fu et al., 2014; Jayabalan et al., 2014).

The yeast counts of the samples during fermentation period were given in Figure 2a. At the beginning of the fermentation, the yeast counts of the samples were ranged from 4.49 to 5.66 log CFU/mL ($P < 0.05$), while these were ranged from 4.23 to 5.30 log CFU/mL at the end of the fermentation ($P > 0.05$). During fermentation period, the yeast counts were decreased till the end of fermentation (except KC2), while the lowest numbers were detected from the 18th day. There was no statistical difference between the samples during fermentation at 3rd, 5th and 10th days, and also at the end of the fermentation period (21st day) ($P > 0.05$).

Moreover, during the storage period, yeast counts of the samples were ranged between 1.51 and 5.30 log CFU/mL (Fig. 2b). No difference was observed between the yeast counts of the samples stored at 0th and 15th days ($P > 0.05$). When compared to the beginning (0th day), yeast counts of all samples were decreased at the end of the storage. Consequently, there were no significant differences between Kombucha samples produced with different plant samples (at 25 ºC for 21 days) in terms of yeast counts, however yeast counts of KN1 and KC1 samples were statistically higher than other samples at the end of the storage ($P < 0.05$).

It has been reported that the microbiological properties of Kombucha may change depending on the raw materials used. When compared with our results, the higher yeast counts for Kombucha produced from black, green and rooibos tea were reported by Gaggìa et al. (2019) as ranging from 6.83 to 7.97 log CFU/mL. Similarly, Kaewkood et al. (2019) determined that the counts of AAB and yeast increased from ~5–6 to ~7–8 log CFU/mL during fer-

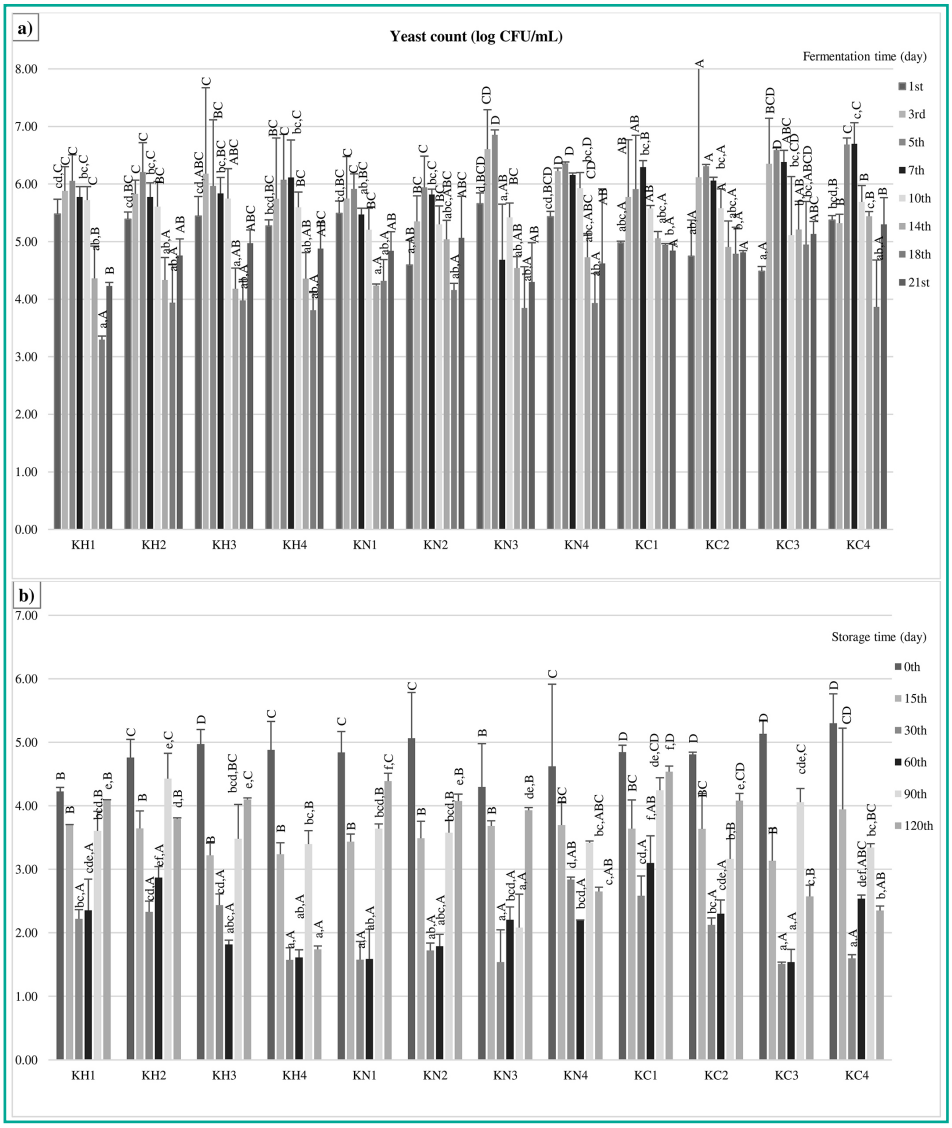

FIGURE 2: *Yeast counts of Kombucha samples during fermentation (at 25 °C for 21 days) (a) and storage (at 4 °C for 120 days) (b). Different lower case (a, b, c, d, e, f, g) on the graph show statistical differences between the samples, the different upper case (A, B, C, D) show the statistical differences between fermentation/storage times ($P < 0.05$).*

mentation (at room temperature for 15 days) of Kombucha samples prepared from green, oolong, and black teas. In contrary, in our study, AAB and yeast counts of the samples were generally decreased till the end of fermentation.

On the other hand, AAB and yeast counts found in Kombucha samples produced with Anatolian hawthorn, nettle, and black tea were at similar levels (Fig. 1 and 2). However, in the study performed by Goh et al. (2012), the yeast counts of Kombucha prepared with black tea is higher than the counts of AAB. Similarly, in another study, the yeast and AAB counts in Kombucha-like beverages containing 1%, 3%, and 5% acerola by-product were determined between 13.05×10^3–53.00×10^5 and 25.50×10^2–64.00×10^3 CFU/mL, respectively (Leonarski et al., 2021).

Different sugar sources may affect the growth of microorganisms during fermentation. Generally, yeasts belonging to *Zygosaccharomyces* genus dominantly found in Kombucha fermentation show the ability to ferment hexose sugars, such as glucose and fructose. These yeasts convert the available sugar into ethanol at the beginning of the fermentation, and AAB species metabolize the ethanol and produce organic acids (Wang et al., 2018; Barbosa et al., 2021). However, when sucrose is added as the sugar source, it is firstly hydrolyzed into glucose and fructose with the invertase enzyme biosynthesized by yeasts, then these monosaccharides are converted to ethanol and organic acids by yeasts and AAB during fermentation (Goh et al., 2012). On the other part, AAB species such as *Komagataeibacter,* commonly involved in Kombucha fermentation, can use sucrose, glucose and fructose, thereby competing with yeasts for sugar since the commencement of fermentation. Moreover, *Komagataeibacter* can produce acids from glucose and/or ethanol and cellulose from glucose (Komagata et al., 2014; Wang et al., 2018; Emiljanowicz and Malinowska-Pańczyk, 2020). Nevertheless, in this study, honey and sucrose were separately used as sugar sources in different proportions (75 and 100 g/L), but the results showed that the microbiological properties of Kombucha samples produced were generally not affected by the sugar sources used (Fig 1a, b; Fig 2a, b). All these results showed that changes of microbial communities can be due to raw materials distinction.

Chemical properties of the samples

The pH values of tea samples were ranged from 5.49 to 8.11 (Table 2), while the values ranged between 2.73 and 5.38 during fermentation period ($P < 0.05$). The pH values of the samples were decreased significantly till the end of fermentation ($P < 0.05$) while the lowest pH values were detected in KH and KC samples (Fig. 3a). During storage period, the values were ranged from 2.73 to 3.32 at the beginning and ranged from 2.92 to 3.73 at the end of the storage (120th day) (Fig. 3b). The highest pH values were generally determined in KN samples, which is in parallel with the pH values of the tea samples. However, the pH values of the samples were significantly increased till the end of storage except KH3 and KH4 ($P < 0.05$). At the end of the storage, the pH values of KN samples were still higher than KH and KC samples (except KC1) ($P < 0.05$).

Acetic acid is the main organic acid produced by AAB, especially by *Gluconobacter* and *Komagataeibacter* spp. found in the Kombucha beverage and biofilm (Chakravorty et al., 2016). In the present study, the total acidity of tea samples was ranged of 0.03–0.05 g acetic acid/L ($P > 0.05$) (Table 2). As it was expected, the total acidity of the samples was increased after fermentation process (except KC1 and KC2) to the range of 0.90–3.82 g acetic acid/L ($P < 0.05$) and the highest acidic values were detected in KN

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TABLE 2: *Some chemical and physical properties of plant tea samples.*

Samples	pH	Total acidity (g/L*)	Brix°	Color properties L*	a*	b*
TH1	7.43±0.37^{cde}	0.03±0.02^{a}	7.67±0.81^{bcde}	0.61±0.05^{a*}	0.48±0.05^{e}	0.10±0.20^{b}
TH2	7.58±0.24^{cde}	0.03±0.01^{a}	9.52±0.38^{f}	0.61±0.10^{a}	0.44±0.06^{e}	0.10±0.05^{b}
TH3	6.92±0.28^{bcde}	0.04±0.01^{a}	6.12±0.24^{ab}	4.62±0.03^{d}	-0.01±0.11^{d}	-1.67±0.29^{a}
TH4	6.05±0.85^{ab}	0.03±0.02^{a}	8.17±0.74^{def}	7.48±0.29^{f}	-0.37±0.22^{cd}	-1.35±0.05^{a}
TN1	8.11±0.44^{e}	0.03±0.02^{a}	7.60±0.28^{bcde}	0.80±0.06^{a}	-0.13±0.04^{d}	0.41±0.00^{bc}
TN2	7.90±0.09^{de}	0.05±0.00^{a}	9.67±0.31^{f}	0.72±0.03^{a}	-0.02±0.21^{d}	0.49±0.08^{bc}
TN3	7.92±0.56^{de}	0.03±0.02^{a}	6.35±0.07^{abc}	4.35±0.12^{c}	-1.50±0.25^{a}	1.99±0.14^{e}
TN4	7.59±0.70^{cde}	0.03±0.01^{a}	8.15±0.42^{def}	5.79±0.03^{e}	-1.10±0.47^{b}	1.79±0.45^{de}
TC1	6.67±0.26^{bc}	0.04±0.01^{a}	7.02±0.67^{abcd}	1.28±0.04^{b}	0.84±0.24^{f}	1.51±0.07^{d}
TC2	6.71±0.12^{bcd}	0.04±0.01^{a}	9.12±0.67^{ef}	0.73±0.01^{a}	0.76±0.05^{ef}	0.74±0.00^{c}
TC3	5.73±0.58^{ab}	0.04±0.01^{a}	5.97±0.88^{a}	10.66±0.16^{g}	-0.73±0.23^{c}	5.90±0.29^{f}
TC4	5.49±0.74^{a}	0.04±0.01^{a}	7.92±1.37^{cde}	10.73±0.10^{g}	-0.71±0.15^{c}	5.78±0.30^{f}

*: acetic acid. Values in the same column with different lower case (a, b, c, d, e, f, g) are significantly different ($P < 0.05$).

samples at the end of the fermentation (Fig. 4a). At the end of the storage period, the values were ranged between 0.71 and 4.33 g acetic acid/L ($P < 0.05$) (Fig. 4b). The highest acidic values were detected in KN3, KN4 and KC4 ($P < 0.05$), while no significant difference was observed among KH2, KH4, KN1, KN2, KN4, KC1 and KC2 samples during storage time ($P > 0.05$). Although high levels of acidity were correlated with the high numbers of AAB in Kombucha fermentation (Coton et al., 2017), in the present study, it was observed that the numbers of AAB were decreased at the final stage of the fermentation due to the high levels of acidity of the samples (Fig. 1a, Fig. 4a).

The pH and titratable acidity changes during fermentation process because of the formation of organic acids (Barbosa et al., 2021). As in parallel, in our study, the acidic values of Kombucha samples were significantly increased till the end of fermentation, while the pH values of the samples were decreased (Fig. 3a and 4a). In a study performed by Leonarski et al. (2021), the initial pH values of the beverages prepared with 1%, 3%, and 5% of acerola by-products were 3.24, 3.34, and 3.27, respectively, while these values were decreased to 2.49, 2.54, and 2.58 at the 15th day of fermentation. In the study, it was also reported that the production rate of acetic acid was higher from the 3rd day, and the final acidic values varied as 6.58, 11.98, 16.35 g/L according to the amount of acerola by-product added.

Barbosa et al. (2021) determined that the pH value of green and black tea Kombucha decreased from 3.8 to 3.1 during fermentation (0th to 15th days), while the total acidity values increased from 2.5 to 24 g/L for black tea Kombucha and from 4.5 to 27.3 g/L for green tea Kombucha. In the literature, the acidic values of Kombucha samples prepared with green, black and oolong tea by 15 days of fermentation were found between 9.51 and 11.15 g/L of acetic acid (Jayabalan et al., 2007; Kaewkod et al., 2019). These results are higher than our findings, which could be linked with the differences of Kombucha formulation (plant type, sugar source, SCOBY etc.) and fermentation conditions (time, temperature etc.).

The acidic values of the samples produced by honey were found higher than the samples produced by sucrose (*P*

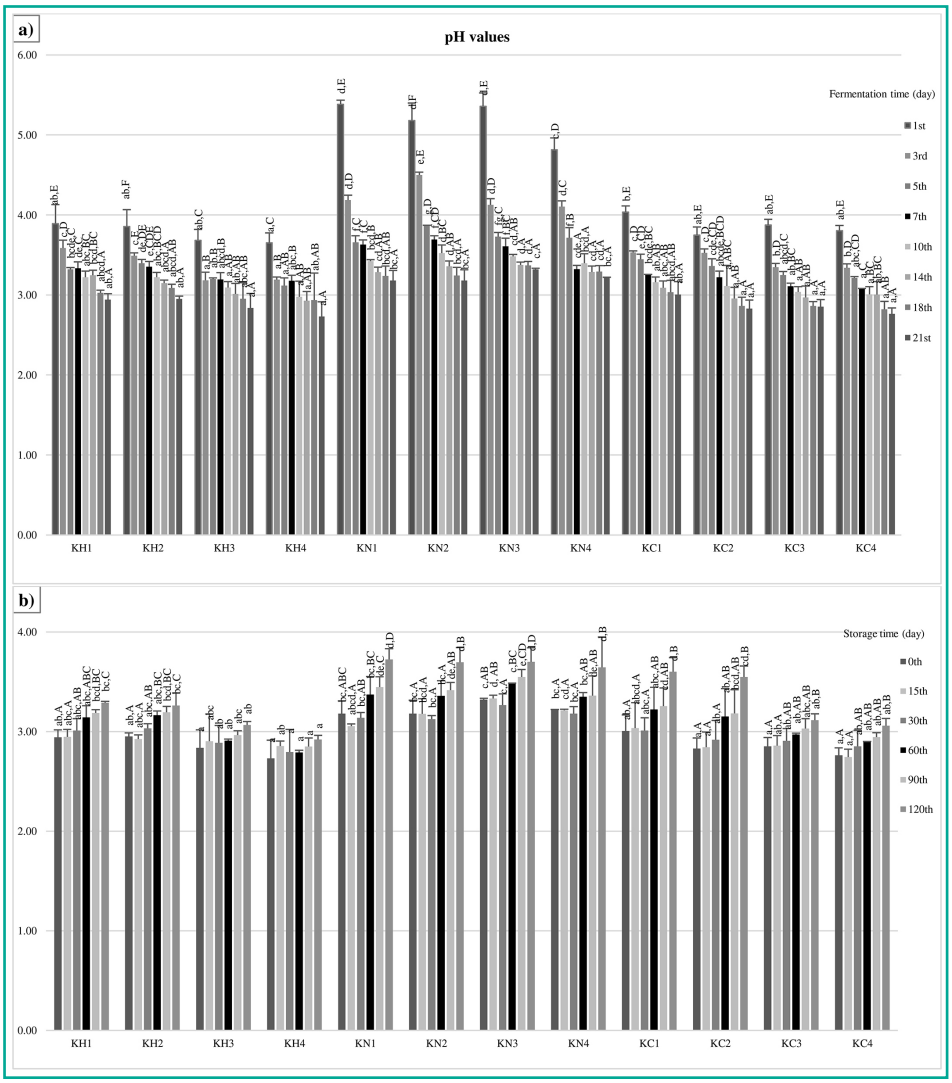

FIGURE 3: *pH values of Kombucha samples during fermentation (at 25 °C for 21 days) (a) and storage (at 4 °C for 120 days) (b). Different lower case (a, b, c, d, e, f, g) on the graph show statistical differences between the samples, the different upper case (A, B, C, D) show the statistical differences between fermentation/storage times ($P < 0.05$).*

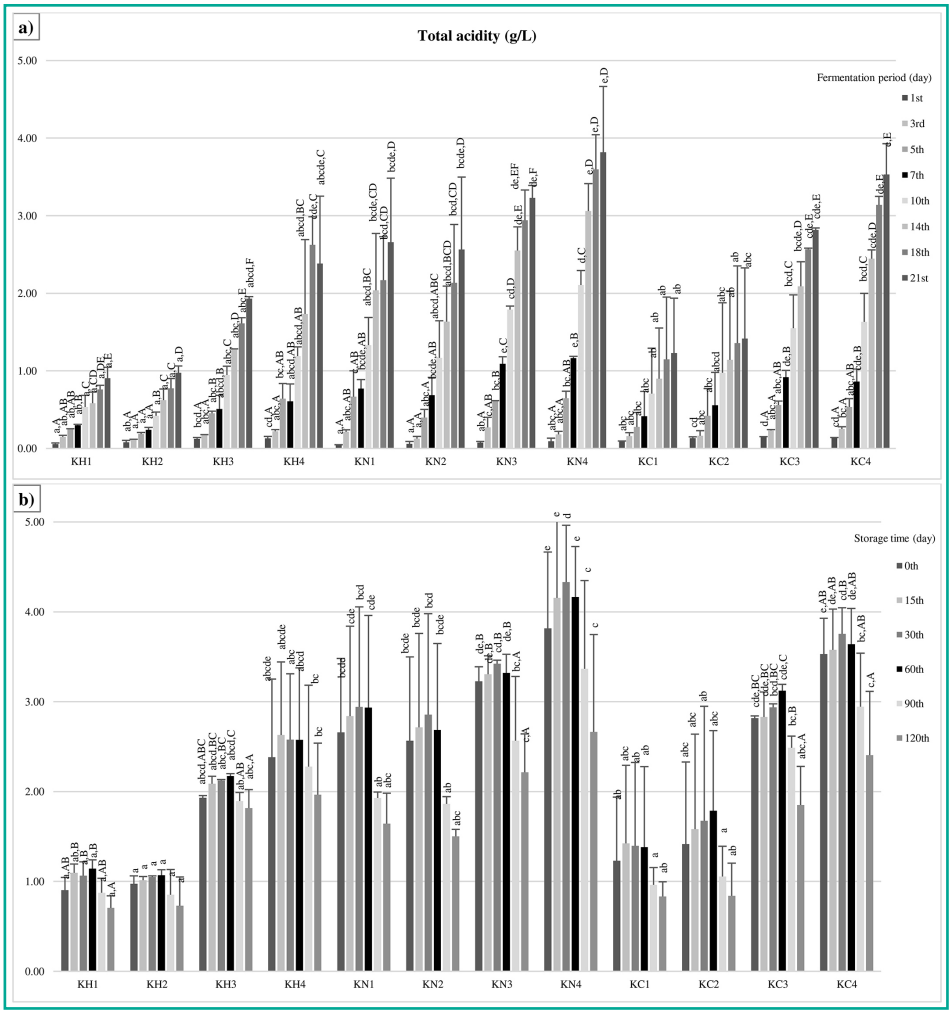

FIGURE 4: *Total acidic values of Kombucha samples during fermentation (at 25 °C for 21 days) (a) and storage (at 4 °C for 120 days) (b). Different lower case (a, b, c, d, e, f, g) on the graph show statistical differences between the samples, the different upper case (A, B, C, D) show the statistical differences between fermentation/storage times ($P < 0.05$).*

< 0.05). These results confirm that quantities of organic acids occurred during Kombucha fermentation vary according to the different substrates utilized (Malbasa et al., 2008; Chakravorty et al., 2016). In a study carried by Watawana et al. (2017), it was determined that the use of honey as a sugar source resulted higher organic acid content in the final product than using sucrose in the formulation. In another study, where lower acidic values were reported than our findings, molasses obtained from the sugar beet was used in Kombucha formulation instead of sucrose, and the results showed that acetic acid content of Kombucha produced by molasses (0.28 g/L) was lower than the samples prepared with sucrose (0.53 g/L) at the end of fermentation (14 days) (Malbasa et al., 2008). On the other hand, when sucrose is used as sugar source in Kombucha production, it is firstly hydrolyzed into glucose and fructose by yeasts and then these monosaccharides are used during fermentation (Kallel et al., 2012; Jayabalan et al., 2014). However, it was also reported that fructose compared to glucose was utilized faster during fermentation (Neffe-Skocinska et al., 2017). In this regard, in the present study, honey which containing glucose and fructose, were used faster compared to sucrose by microorganisms and total acidity of the samples including honey was higher than the samples including sucrose (Fig. 4a).

Sugar decreases linearly during Kombucha fermentation, which correlates with the reduction in brix value (total soluble solids, TSS) that indicates the amount of remaining sugar in the product (Randazzo et al., 2016; Wang, 2018). In this study, brix values of tea samples (TH, TN and TC) were ranged between 5.97 and 9.67 (Table 2), while these values were ranged between 2.83 and 9.23 in Kombucha samples ($P < 0.05$) (Table 3). When compared to control samples (TC), TH and TN have the higher brix values. Moreover, TH2, TN2 and TC2 samples prepared with 10% sucrose have the higher brix value, similarly, the highest brix values were also determined in Kombucha samples produced with TH2, TN2 and TC2 during fermentation period (Table 3). These results demonstrated that sugar concentration had significant effects on the sugar content found in the product at the end of the fermentation of Kombucha and so on the microbial activities of the Kombucha starter culture, as reported similarly by Wang, (2018). During storage period, the brix values of Kombucha samples were ranged between 2.60 and 8.85 ($P < 0.05$) (Table 3). The highest and lowest brix values were observed in KH2 and KN3 samples during all storage periods, respectively ($P <$ 0.05), while the values were not significantly changed for the remaining samples ($P > 0.05$). In a study performed by Aung and Eun (2021), the brix values of Kombucha produced by laver *(Porphyra dentata)* at 25 ºC for 14 days, decreased from the range of 5.37-6.30 to the range of 3.83–6.20 during fermentation period. Although it was stated that the brix and acidity of Kombucha conversely related with each other (Aung and Eun, 2021), this type of correlation was not observed in our study.

Color properties of the samples

Color is one of the most important quality parameters of beverages, and also it is the first organoleptic feature perceived by the consumers (Vázquez-Cabral et al., 2014; Lima et al., 2019). As it can be seen from Table 2, *L*, a*, b** values of tea samples (TH, TN and TC) were in the range of 0.61–10.73, –1.50–0.84 and –1.67–5.90, respectively ($P < 0.05$). The highest *L** values (whiteness (+)) and *b** values (yellowness (+)) were determined in TC3, while the highest *a** values (greenness (–)) were observed in TN3 (P < 0.05) (Table 2).

The color properties of Kombucha samples during storage period was given in Table 4. *L** values were significantly increased in most of the samples during storage period of Kombucha samples and ranged between 0.09 and 30.53 ($P < 0.05$). a* values were ranged from –2.49 to

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TABLE 3: *Brix values of Kombucha samples during fermentation and storage period.*

Samples	Fermentation time (Day)							
	1st	3rd	5th	7th	10th	14th	18th	21st
KH1	7.18±0.04^{b,A*}	7.15±0.07^{b,A}	7.05±0.21^{d,A}	6.80±0.28^{c,A}	6.83±0.18^{cd,A}	6.98±0.04^{def,A}	6.85±0.14^{de,A}	6.93±0.18^{def,A}
KH2	9.15±0.07^{c,B}	9.03±0.04^{c,B}	9.00±0.07^{e,B}	8.93±0.05^{d,AB}	8.18±0.60^{de,A}	8.80±0.07^{g,AB}	8.63±0.46^{f,AB}	8.60±0.42^{f,AB}
KH3	5.78±0.32^{a,B}	5.83±0.25^{a,B}	6.10±0.71^{bcd,B}	5.05±0.78^{ab,AB}	4.20±0.49^{ab,A}	3.78±0.25^{a,A}	3.80±0.71^{abc,A}	3.80±0.57^{abc,A}
KH4	7.55±0.21^{b,B}	7.25±0.35^{b,AB}	6.65±0.28^{cd,AB}	6.23±0.67^{bc,AB}	6.15±1.06^{c,AB}	5.90±1.27^{cd,AB}	5.50±1.34^{cd,AB}	5.33±0.88^{cd,A}
KN1	7.40±0.14^{b,A}	7.28±0.04^{b,A}	6.65±0.57^{cd,A}	6.50±0.57^{c,A}	6.03±0.95^{c,A}	5.43±1.24^{bcd,A}	5.13±1.31^{bcd,A}	5.18±1.59^{bcd,A}
KN2	8.98±0.74^{c,C}	9.05±0.42^{c,C}	8.55±0.07^{e,BC}	8.60±0.57^{d,BC}	8.15±0.78^{de,ABC}	7.78±0.95^{efg,ABC}	6.85±0.78^{de,AB}	6.65±0.92^{de,A}
KN3	6.20±0.07^{a,D}	5.50±0.14^{a,C}	5.52±0.14^{ab,C}	4.28±0.32^{a,B}	3.03±0.04^{a,A}	2.95±0.07^{a,A}	2.83±0.11^{a,A}	2.83±0.32^{a,A}
KN4	7.38±0.67^{b,E}	7.03±0.18^{b,D}	5.93±0.67^{abc,CD}	5.08±0.60^{ab,BC}	4.05±0.64^{ab,AB}	3.95±0.49^{ab,AB}	3.75±0.28^{ab,A}	3.75±0.21^{abc,A}
KC1	7.35±0.07^{b,A}	7.05±0.14^{b,A}	7.00±0.42^{d,A}	6.70±0.49^{c,A}	6.65±0.78^{c,A}	6.23±0.74^{de,A}	6.13±0.53^{d,A}	6.18±1.17^{d,A}
KC2	9.23±0.04^{c,A}	9.05±0.07^{c,A}	8.68±0.32^{e,A}	8.75±0.35^{d,A}	8.50±0.64^{e,A}	8.08±0.67^{fg,A}	8.03±1.03^{ef,A}	8.05±0.85^{ef,A}
KC3	6.10±0.07^{a,C}	5.60±0.35^{a,C}	4.95±0.35^{a,B}	4.53±0.32^{a,B}	3.63±0.39^{ab,A}	3.43±0.25^{a,A}	3.38±0.18^{a,A}	3.40±0.00^{ab,A}
KC4	7.75±0.07^{b,D}	6.75±0.21^{b,C}	5.00±0.57^{a,AB}	5.15±0.64^{ab,B}	4.63±0.25^{b,AB}	4.45±0.35^{abc,AB}	4.35±0.28^{abc,AB}	4.20±0.28^{abc,A}

Samples	Storage time (Day)					
	0th	15th	30th	60th	90th	120th
KH1	6.93±0.18^{def,A}	6.85±0.21^{def,A}	6.93±0.18^{def,A}	6.85±0.28^{def,A}	6.90±0.28^{ef,A}	6.78±0.39^{cd,A}
KH2	8.60±0.42^{f,A}	8.78±0.53^{f,A}	8.85±0.35^{f,A}	8.75±0.42^{f,A}	8.60±0.14^{f,A}	8.83±0.32^{e,A}
KH3	3.80±0.57^{abc,A}	3.75±0.64^{ab,A}	3.83±0.67^{ab,A}	3.75±0.57^{ab,A}	3.78±0.60^{abc,A}	3.80±0.49^{ab,A}
KH4	5.33±0.88^{cd,A}	5.40±1.13^{cd,A}	5.40±1.06^{bcd,A}	5.23±1.03^{bcd,A}	5.45±1.13^{cde,A}	5.40±0.99^{bc,A}
KN1	5.18±1.59^{bcd,A}	5.30±1.56^{bcd,A}	5.43±1.45^{bcd,A}	5.28±1.66^{bcd,A}	5.33±1.66^{bcde,A}	5.33±1.59^{bc,A}
KN2	6.65±0.92^{de,A}	7.18±1.52^{def,A}	7.18±1.66^{def,A}	7.25±1.70^{def,A}	7.25±1.77^{ef,A}	7.28±1.66^{cde,A}
KN3	2.83±0.32^{a,A}	2.60±0.21^{a,A}	2.95±0.14^{a,A}	2.90±0.28^{a,A}	2.68±0.11^{a,A}	2.93±0.18^{a,A}
KN4	3.75±0.21^{abc,A}	3.83±0.32^{ab,A}	3.85±0.35^{ab,A}	3.73±0.25^{ab,A}	3.83±0.25^{abc,A}	3.68±0.25^{ab,A}
KC1	6.18±1.17^{d,A}	6.10±0.78^{de,A}	6.25±0.92^{cde,A}	6.18±0.95^{cde,A}	6.23±1.10^{de,A}	6.20±0.99^{cd,A}
KC2	8.05±0.85^{ef,A}	7.90±0.71^{ef,A}	8.03±0.88^{ef,A}	7.95±1.06^{ef,A}	6.88±0.74^{ef,A}	7.43±0.39^{de,A}
KC3	3.40±0.00^{ab,A}	3.35±0.21^{ab,A}	3.40±0.14^{ab,A}	3.35±0.07^{ab,A}	3.28±0.11^{ab,A}	3.25±0.07^{a,A}
KC4	4.20±0.28^{abc,A}	4.15±0.49^{abc,A}	4.33±0.39^{abc,A}	4.15±0.14^{abc,A}	4.13±0.46^{abcd,A}	4.18±0.25^{ab,A}

*Different lower case (a, b, c, d, e, f, g) in the same column show statistical differences between the samples, the different upper case (A, B, C, D) in the same line show the statistical differences between fermentation/storage times ($P < 0.05$).

2.54, and the highest greenness (–) and redness (+) values were found in KC1 (in 60nd day) and KN4 (in 0th day), respectively. a^* (redness) values of the samples were significantly decreased in KN3, KN4 and KC4 at the end of the storage ($P < 0.05$). In addition, b^* values were ranged between 0.21 and 18.74 at the beginning of the storage period ($P < 0.05$) and these values were significantly increased in KN1, KN3 and KC3 during storage period ($P < 0.05$). At the end of the storage, the highest yellowness (+) values were found in KC3 (Table 4). In a study conducted by Aung and Eun (2021), the color properties of Kombucha produced from laver *(Porphyra dentata)* determined as ranging between 59.46 and 60.45 for L^* values, –0.03 and 0.30 for a^* values, 2.50 and 3.18 for b^* values. These results confirm that Kombucha had diverse color values because of the nature of color compounds found in different raw materials (Aung and Eun, 2021; Zou et al., 2021). Similarly, the color properties of Kombucha produced from Anatolian hawthorn (KH), nettle leaves and black tea also demonstrated significant differences according to the raw material used in the production (Table 4).

Sensory properties of the samples

The sensorial properties of Kombucha are influenced by some factors including raw materials, fermentation temperature and time. Its taste is slightly acidic, sparkling and sweet (Senanayake, 2013; Jayabalan et al., 2014; Gramza-Michałowska et al., 2016). Especially the sour taste of Kombucha is considered as a distinctive quality property in functional beverages (Lončar et al., 2006). In this study, sensorial evaluation of plant tea and Kombucha samples were performed to find out the effects of raw materials on sensory properties (color, odor, clarity, taste and general acceptance) of the products.

The sensory scores of the plant tea samples were given in Table 5. TC1 was found as the most admired sample in terms of color and clarity characteristics, while TH1 and TH2 was found as the best in terms of odor properties ($P < 0.05$). Besides, it was determined that TH3 were found the most admired one in terms of taste and general acceptance ($P < 0.05$). The results of the sensory evaluation showed that tea samples including sucrose as sugar source generally had higher scores than the sample produced with honey in terms of general acceptance properties ($P < 0.05$).

The sensory evaluation results showed that the usage of different plants in Kombucha production caused a significant difference between the sensory properties of the samples ($P < 0.05$) (Fig. 5). During storage period, the most admired samples were KC2 (at 0th, 60th and 90th day), KC1 (at 15th and 120th day), KN1 (at 30th day) and KN2 (at 30th day) in terms of color properties ($P < 0.05$). It was determined that the most admired samples in terms of odor properties were in KH1, KH2, KC1 and KC2 for 15th, 30th, 60th and 90th day of the storage, respectively. Storage period did not significantly affect the odor properties of KH2, KN2, KN3 and KC1 ($P > 0.05$). In addition, the highest clarity scores were given for KH1 and KH2 at the end of storage ($P < 0.05$). However, the clarity scores were changed significantly (except KC1) during storage period ($P < 0.05$) (Fig. 5).

Besides, KH2 (at 0th, 30th and 15th days), KH1 (at 15th, 30th and 90th days), KC2 (at 60th day) and KC1 (at 120th day) were the most admired samples in terms of taste ($P < 0.05$). Storage period did not significantly affect the taste of KH2, KH4, KN1, KC1 and KC2. In general, the highest general acceptance scores were given for KH1 and KH2. As similar with the taste scores of the samples, no significant difference was observed among KH2, KH4, KN1, KC1 and KC2 during storage time in terms of general acceptance ($P > 0.05$) (Fig. 5). These results demonstrated that the taste of the samples mainly influence the general acceptance of the samples.

In the study carried by Zubaidah et al. (2018) the sensory properties of Kombucha produced from four different snake fruit *(Salak doyong, Salak madu, Salak pondoh, Salak segaran, Salak suwaru)* were investigated. Although no significant differences were found between the color of the samples, taste and aroma scores showed

TABLE 4: *Color properties of Kombucha samples during storage period.*

	Samples	Storage time (Day) 0th	15th	30th	60th	90th	120th
*L**	KH1	3.20±0.46^{abc,B*}	0.10±0.02^{a,A}	0.13±0.03^{a,A}	0.43±0.02^{a,A}	0.14±0.04^{a,A}	0.10±0.01^{a,A}
	KH2	1.69±0.47^{a,B}	0.25±0.16^{a,A}	0.20±0.07^{ab,A}	0.19±0.02^{a,A}	0.09±0.00^{a,A}	0.11±0.03^{a,A}
	KH3	1.49±0.24^{a,B}	0.11±0.02^{a,A}	0.14±0.06^{a,A}	0.27±0.02^{a,A}	0.10±0.01^{a,A}	0.10±0.01^{a,A}
	KH4	3.04±0.21^{abc,C}	0.14±0.01^{a,A}	0.63±0.31^{bc,B}	0.24±0.02^{a,A}	0.12±0.05^{a,A}	0.13±0.06^{a,A}
	KN1	5.53±0.20^{e,E}	0.18±0.06^{a,A}	2.30±0.27^{e,D}	1.45±0.05^{b,C}	0.83±0.08^{c,B}	10.79±0.02^{e,F}
	KN2	11.80±0.01^{d,E}	0.39±0.02^{a,A}	4.55±0.05^{f,B}	4.51±0.01^{d,B}	4.97±0.04^{e,C}	5.69±0.05^{d,D}
	KN3	4.93±1.38^{bc,B}	0.13±0.01^{a,A}	0.51±0.02^{ab,A}	0.49±0.01^{a,A}	1.26±0.08^{d,A}	21.23±0.06^{g,C}
	KN4	22.80±3.18^{e,B}	1.86±1.03^{b,A}	0.23±0.14^{ab,A}	0.37±0.06^{a,A}	1.14±0.07^{d,A}	3.13±0.03^{c,A}
	KC1	2.42±0.29^{ab,D}	0.24±0.12^{a,A}	1.30±0.01^{d,C}	6.59±0.04^{e,E}	0.45±0.02^{b,AB}	0.70±0.05^{b,B}
	KC2	2.48±0.56^{ab,B}	0.33±0.12^{a,A}	0.51±0.37^{ab,A}	4.26±0.14^{d,C}	0.11±0.00^{a,A}	0.16±0.01^{a,A}
	KC3	4.93±0.07^{bc,B}	4.47±1.53^{c,B}	1.35±0.28^{d,A}	3.33±0.92^{c,B}	16.19±0.05^{g,C}	30.53±0.04^{h,D}
	KC4	11.23±1.24^{d,D}	1.43±0.23^{ab,A}	0.97±0.17^{cd,A}	4.73±0.43^{d,B}	8.73±0.11^{f,C}	13.05±0.05^{f,E}
*a**	KH1	-0.21±0.22^{d,A}	0.06±0.03^{b,B}	0.08±0.03^{bc,B}	0.34±0.02^{e,C}	0.04±0.01^{b,B}	1.05±0.06^{e,D}
	KH2	-0.17±0.25^{d,A}	-0.06±0.02^{b,AB}	0.10±0.01^{bc,BC}	0.30±0.00^{e,C}	0.00±0.00^{b,AB}	0.00±0.00^{d,AB}
	KH3	-0.14±0.01^{d,A}	0.09±0.04^{b,C}	0.02±0.01^{bc,BC}	0.28±0.06^{e,D}	0.01±0.01^{b,BC}	-0.01±0.02^{d,B}
	KH4	-0.32±0.17^{cd,A}	0.13±0.01^{b,B}	0.19±0.02^{c,B}	0.37±0.03^{e,C}	0.01±0.01^{b,B}	0.02±0.01^{d,B}
	KN1	-0.71±0.21^{bcd,A}	0.03±0.01^{b,A}	-0.22±0.03^{b,A}	0.11±0.06^{c,A}	-1.49±2.06^{a,A}	-1.60±0.07^{b,A}
	KN2	-1.40±0.66^{a,A}	0.06±0.01^{b,C}	-0.62±0.15^{a,BC}	-0.44±0.01^{d,BC}	-0.95±0.30^{ab,AB}	-1.12±0.04^{c,AB}
	KN3	-0.89±0.12^{abc,B}	0.12±0.03^{b,D}	0.03±0.02^{bc,D}	0.07±0.08^{e,D}	-0.24±0.10^{ab,C}	-1.94±0.03^{a,A}
	KN4	2.54±0.39^{e,C}	-0.83±0.45^{a,A}	0.05±0.04^{bc,B}	0.09±0.04^{e,B}	0.05±0.04^{b,B}	-1.21±0.14^{c,A}
	KC1	-0.55±0.21^{bcd,B}	0.05±0.04^{b,C}	-0.28±0.39^{ab,BC}	-2.49±0.20^{a,A}	-0.01±0.00^{b,C}	0.12±0.00^{d,C}
	KC2	-0.47±0.03^{bcd,B}	0.15±0.05^{b,D}	-0.20±0.25^{bc,BC}	-1.45±0.10^{b,A}	0.04±0.01^{b,CD}	0.04±0.04^{d,CD}
	KC3	-0.55±0.09^{bcd,AB}	-0.86±0.20^{a,A}	-0.18±0.26^{bc,B}	-0.86±0.22^{c,A}	-0.85±0.01^{ab,A}	2.42±0.02^{f,C}
	KC4	-1.00±0.36^{ab,AB}	-0.50±0.35^{a,B}	0.17±0.04^{c,C}	-0.56±0.25^{d,AB}	-0.76±0.06^{ab,AB}	-1.18±0.18^{c,A}
*b**	KH1	0.41±0.36^{ab,B}	0.05±0.02^{ab,AB}	-0.07±0.05^{bc,A}	-0.01±0.02^{de,A}	0.03±0.02^{c,AB}	0.01±0.01^{b,A}
	KH2	0.19±0.18^{a,B}	0.03±0.01^{ab,AB}	0.05±0.06^{c,AB}	-0.14±0.07^{cd,A}	-0.01±0.01^{c,AB}	-0.01±0.01^{b,AB}
	KH3	0.41±0.24^{ab,B}	0.02±0.03^{ab,A}	0.00±0.00^{bc,A}	-0.04±0.02^{cde,A}	0.01±0.01^{c,A}	0.01±0.00^{b,A}
	KH4	0.21±0.03^{a,B}	0.06±0.02^{ab,AB}	0.23±0.18^{cd,B}	0.05±0.01^{de,AB}	-0.01±0.01^{c,A}	0.01±0.01^{b,A}
	KN1	0.47±0.13^{abc,C}	0.03±0.03^{ab,B}	-0.35±0.07^{b,A}	-0.25±0.13^{c,AB}	-0.35±0.09^{b,A}	1.84±0.19^{e,D}
	KN2	0.50±0.16^{abc,D}	-0.19±0.02^{a,C}	-1.87±0.10^{a,B}	-2.64±0.01^{a,A}	-2.33±0.37^{a,A}	-0.59±0.09^{a,C}
	KN3	1.31±0.12^{bcd,C}	0.03±0.01^{ab,A}	0.16±0.02^{cd,AB}	0.30±0.02^{f,B}	0.02±0.03^{c,A}	4.68±0.08^{g,D}
	KN4	18.74±0.71^{f,B}	1.05±0.77^{c,A}	0.09±0.07^{c,A}	0.06±0.02^{de,A}	0.07±0.10^{c,A}	0.75±0.03^{c,A}
	KC1	1.40±0.06^{cd,B}	0.05±0.02^{ab,A}	0.48±0.46^{d,A}	0.09±0.04^{e,A}	0.08±0.09^{c,A}	1.04±0.08^{d,B}
	KC2	0.72±0.72^{abc,B}	-0.02±0.05^{ab,B}	0.00±0.10^{bc,B}	-1.10±0.09^{b,A}	0.02±0.02^{c,B}	0.14±0.02^{b,B}
	KC3	2.06±0.57^{d,C}	1.20±0.18^{c,B}	0.18±0.04^{cd,A}	2.05±0.10^{h,C}	4.82±0.10^{e,D}	19.06±0.09^{h,E}
	KC4	3.82±0.52^{e,C}	0.60±0.46^{bc,A}	0.53±0.14^{d,A}	1.13±0.25^{g,A}	2.26±0.21^{d,B}	4.52±0.04^{f,C}

*Different lower case (a, b, c, d, e, f, g) in the same column show statistical differences between the samples, the different upper case (A, B, C, D) in the same line show the statistical differences between storage times ($P < 0.05$).

differences, as inherent properties of the raw materials manifested, Kombucha prepared with the *Salak segaran* was the least preferred. In another study, it was reported that Kombucha produced from green and white teas were more preferred than black tea Kombucha, in terms of general acceptance (Gramza-Michałowska et al., 2016).

The sensory properties of Kombucha may also be affected by the source of sugar used. Neffe-Skocinska et al. (2017) reported that fructose was consumed faster than glucose during Kombucha fermentation, leaving glucose as the main residual carbohydrate in the Kombucha. Nevertheless, it was also stated that fructose (relative sweetness 115–180) is sweeter than sucrose (relative 100), which is sweeter than glucose (relative 50–70) (Clemens et al., 2016). However, in the current study, it was determined that the usage of different sugar sources in Kombucha production did not cause a significant difference between most of the samples ($P > 0.05$). In general, the results showed that sensory properties of the samples were greatly influenced by the raw material used in Kombucha formulation and the sensory characteristics of Kombucha samples produced from Anatolian hawthorn and sucrose (KH1 and KH2) had mostly higher scores than KN and KC samples.

TABLE 5: *Sensory evaluation scores of plant tea samples.*

Samples	Color	Odor	Clarity	Taste	General acceptance
TH1	5.87±0.53^{ab*}	6.63±0.18^{c}	7.06±0.09^{abc}	6.94±0.09^{e}	6.25±0.71^{cd}
TH2	6.43±0.09^{ab}	6.55±0.42^{c}	7.31±0.27^{bc}	3.88±0.18^{a}	4.63±0.18^{a}
TH3	5.88±0.53^{ab}	6.44±0.27^{bc}	6.69±0.27^{abc}	6.94±0.09^{e}	7.06±0.09^{e}
TH4	5.94±0.27^{ab}	6.38±0.18^{bc}	6.75±0.35^{abc}	6.44±0.27^{de}	6.38±0.18^{d}
TN1	6.75±0.71^{ab}	5.69±0.44^{abc}	7.06±0.62^{abc}	5.88±0.53^{bcd}	6.25±0.35^{cd}
TN2	6.88±0.53^{b}	6.38±0.18^{bc}	7.44±0.09^{bc}	5.69±0.27^{bcd}	6.06±0.09^{cd}
TN3	6.19±0.09^{ab}	5.44±0.27^{ab}	6.75±0.35^{abc}	5.38±0.18^{bc}	5.56±0.09^{bc}
TN4	5.75±0.71^{a}	5.94±0.27^{abc}	6.38±0.18^{ab}	5.25±0.71^{b}	5.85±0.14^{cd}
TC1	6.69±0.27^{ab}	6.38±0.53^{bc}	7.56±0.09^{c}	6.13±0.53^{cde}	6.06±0.44^{cd}
TC2	6.38±0.18^{ab}	5.81±0.44^{abc}	6.81±0.44^{abc}	5.69±0.27^{bcd}	6.31±0.09^{d}
TC3	5.81±0.09^{ab}	5.00±0.71^{a}	5.94±0.27^{a}	6.13±0.18^{cde}	6.31±0.27^{d}
TC4	6.44±0.44^{ab}	5.81±0.62^{abc}	6.38±1.24^{ab}	5.50±0.35^{bc}	5.19±0.09^{ab}

*Values in the same column with different lower case (a, b, c, d, e, f) are significantly different ($P < 0.05$).

Conclusion

In this study, Kombucha was produced from Anatolian hawthorn and nettle leaves by using honey or sucrose as sugar sources for the first time. The results demonstrated that the microbiological and chemical properties of the samples varies in a wide range. In general, AAB and yeast counts of Kombucha samples were increased until the 5th/7th days and 3rd/5th days, respectively, and then decreased till the end of fermentation. Depending on microbial activity, acidic values of the samples increased till the end of fermentation. In addition, using different plants in the production of Kombucha affected the color and also sensory properties of the samples. As a result, microbiological, chemical, physical and sensory properties of Kombucha samples showed differences depending on the plant material, the source and amount of sugar used. Further studies are needed to determine the bioactive properties of Kombucha produced from Anatolian hawthorn and nettle leaves. Especially consumer preferences of Kombucha and its health-promoting

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properties should be investigated in more detail and the microbial composition associated with the preferred product quality should also be defined.

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Conflict of interest

The authors have declared no conflicts of interest for this article.

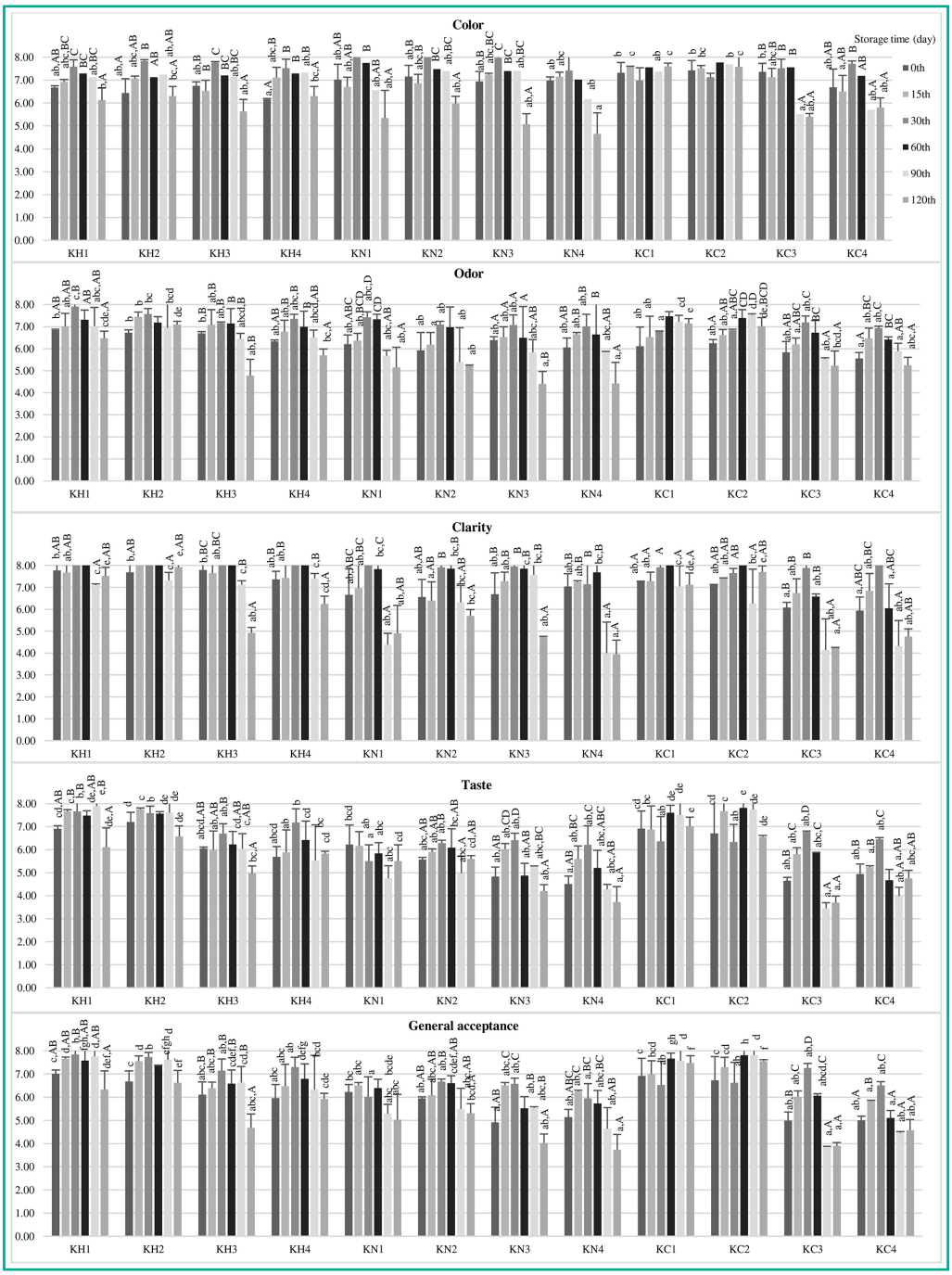

FIGURE 5: *Sensory evaluations of Kombucha samples during storage time (at 4 °C for 120 days). Different lower case (a, b, c, d, e, f, g) on the graph show statistical differences between the samples, the different upper case (A, B, C, D) show the statistical differences between storage times ($P < 0.05$).*

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Address of corresponding author:
Gulden Kilic
Ege University
Engineering Faculty
Food Engineering Department
35100, Bornova
Izmir
Turkey
gulden-gk@hotmail.com