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Research Article

Development of an alternative kombucha drink from gilaburu juice: Gilaburu-flavoured kombucha

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ABSTRACT

Kombucha is a Far East-originated fermented beverage that is valued by people for its health benefits worldwide. Fruit and fruit juice of the gilaburu plant is used in traditional medicine. In this study, fruit juice of gilaburu is used in a 21-day kombucha fermentation. Sensory properties, microbiological profile, total phenolic and flavonoid content, and antioxidant and antibacterial activity of the prepared beverages for days 0, 7, 14, and 21st were investigated. In addition, chemical compound profiles were determined using LC-MS/MS on the 7th and 14th days. When evaluated in general, gilaburu-flavoured kombucha yielded better results than traditional kombucha in terms of microbiological analysis, antibacterial activity, total phenolic content, and sensory properties. In addition, the LC-MS/MS analysis of the beverages revealed fourteen active compounds. Notably, on the 7th day, gilaburu-flavoured kombucha exhibited elevated levels of fumaric acid, quinic acid, chlorogenic acid, catechin, 4-OH-benzoic acid, epicatechin, vitexin and hesperidin compared to traditional kombucha.

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INTRODUCTION

In recent years, the consumption of fermented beverages has emerged as a prominent trend in nutrition. Although many consumers prefer kombucha due to its therapeutic effects, this fermented drink is also classified as a probiotic beverage. In general, the content of a kombucha consists of sugars, organic acids, tea polyphenols, water-soluble vitamins, ethanol, and various micronutrients produced during fermentation [1]. Additionally, probiotics such as acetic acid and lactic acid bacteria contribute to its overall content [2]. The symbiotic microbial combination is mainly formed by *Acetobacter* and *Gluconobacter* species [3,4]. The positive effects of Kombucha on gastric, intestinal, and glandular activities are emphasised, indicating its potential efficacy against digestive disorders, various types of cancer, hepatotoxicity, toxin excretion, diabetes, irritability, and ageing [5,6]. Also, there are many studies on the free radical scavenging ability and antioxidant capacity of various kombucha teas [7-10]. In fermented kombucha culture, it has been reported that metabolites, catechins, and vitamins have a positive effect on the antioxidant effect of the drink [11].

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In Ukraine and other countries in Eastern Europe, gilaburu has been widely used in traditional medicine [12,13]. It has been stated that the fruit juice of this plant is a good source of polyphenols [14] and the fruits are used as antidiabetic agents [15]. The bioactive substances in the fruit of the gilaburu plant also have antimicrobial effects against many pathogenic bacteria [16,17].

This study aims to investigate the antibacterial effect, antioxidant activity, total phenolic, and total flavonoid content, as well as microbiological profiling and sensorial properties of kombucha cultures fermented using gilaburu. Gilaburu was chosen due to its beneficial effects on human health, and in this study, fermented kombucha products were prepared with fruit juice, taking into account the consumption behaviour. This is the first study that investigates the bioactivity of kombucha fermented with gilaburu.

MATERIAL AND METHODS

Preparation of Fermented Beverages

In this study, 5 different culture media were prepared. Kombucha drink prepared by the fermentation of black tea (B) is named as traditional kombucha (K). K fermented with gilaburu fruit juice (G) is named gilaburu-flavoured kombucha (KG). G fermented with only SCOBY (symbiotic culture of bacteria and yeast), without B, is named as fermented gilaburu drink (FG). Non-fermented B infusion and G were used as control groups. KG cultures were established to investigate the influence of gilaburu fruit juice on the biological activities of traditional kombucha. FG cultures were set up to investigate the influence of black tea on gilaburu fermentation. K was prepared as a positive control to compare the alternative beverages. In addition, non-fermented B and G cultures without kombucha were prepared as negative controls.

In order to prepare infusions, the protocol described by Marsh et al. [18] was used with slight modifications. For K fermentation, 100 mL B infusion with 9% sucrose, inoculated with 2% (w/v) SCOBY and 10% (v/v) soup (starter culture) from the previous culture. The KG drink was prepared by adding 15% G to the K drink. The samples were incubated at RT for 21 days, collected every 7 days, and subjected to experiments. After 14 days of incubation, the liquid part of the kombucha beverages was collected and centrifuged at 1370 rcf for 10 min for further analysis.

Microbiological Profile Determination Experiments

KG and K were used for microbiological profiling. Serial dilutions of the samples were prepared using 0.85% NaCl (Sigma Aldrich, USA) and inoculated onto selective media. In this study, Glucose Yeast Extract Agar (GYC) media was used for acetic acid bacteria selection, Yeast Extract Glucose Chloramphenicol Agar (YGC) (Merck, Germany) was used for yeast count, De Man Rogosa Sharp Agar (MRS) (Merck, Germany) was used for *Lactobacillus* count and Plate Count Agar (PCA) (Merck, Germany) was used for total mesophilic bacterial counts. The inoculated samples were incubated for 5 days, after which the colonies were counted. The colony forming units (cfu/mL) were calculated according to Equation 1:

$$N = \frac{c}{[V \times (n_1 + (0.1 \times n_2))]} \times d \quad (1)$$

According to this equation, the most concentrated dilution ratio of two consecutive dilutions expressed as d; the number of petri dishes inoculated by the first dilution expressed as n_1 and number of petri dishes inoculated by the second dilution expressed as n_2; the total number of microorganisms per 1 ml as N; inoculation volume to petri dishes as V; total counted number of colonies in petri dishes (mL) C [19].

pH Changes of Samples

Samples of the kombucha drink were taken every 7 days and the pH values of these samples were measured with a pH metre (Isolab, Germany).

Antibacterial Activity and Bacterial Strains

The antibacterial effect of all samples was investigated via the disk diffusion method, as described by Bauer [20]. The antibacterial effect was tested against 6 well-defined bacteria which are *Klebsiella pneumonia* (ATCC 13883), *Staphylococcus aureus* (ATCC 29213), *Bacillus cereus* (DSM 22648), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 35218). The overnight culture of each bacteria was sampled into 0.05 M NaCl (Merck, Germany), and the concentration of the mixture was adjusted to 0.5 McFarland. Then 200 µl inoculums were spread on Nutrient Agar Medium (NA) (Merck, Germany), and inoculated petri dishes were incubated at room temperature for 30 min. After 30 min, as a test group, 60 µL of previously prepared samples, and as a control group 20 µL of 30 µg/mL Ampicillin (Sigma Aldrich, USA) and 20 µL of 30 µg/mL Kanamycin (Cayman Chemical, USA) was impregnated into antibiogram disks (Bioanalyse Turkey). After impregnation, disks were placed on top of these inoculated petri dishes and incubated at 37 °C for 24 hours. Inhibition zones were measured after 24-h incubation. 3 replicates were applied in this experiment.

Determination of Free Radical Scavenging Activity with DPPH Assay

2,2 -Diphenyl-1-picrylhydrazyl (DPPH) method described by Von Gadow et al. [21] was used to investigate the free radical scavenging ability (RSA) of samples. 100 µl centrifuged and filter-sterilized kombucha samples were mixed with 4 mL of methanolic DPPH solution (Sigma Aldrich, USA) and then incubated in dark for 30 min. After incubation, the absorbance of samples was measured in a

spectrophotometer (SOIF, China) at 516 nm and the ascorbic acid equivalent (AAE) was calculated in µM/ml.

Total Phenolic Content Measurement

The colorimetric method, based on the Folin-Ciocalteu (FC) reagent, described by Škerget et al. [22] was used to determine the total phenolic content of samples. The filter-sterilized kombucha samples (500 µl) were mixed with 2.5 ml of 1:10 diluted FC reagent [23], and the mixtures were incubated for 2 min at the RT. After incubation, 2 ml of 7.5% Na_2CO_3 (Merck, Germany) was added to the mixtures and vortexed for 30 s. Mixtures were stored at 50 °C for 5 min. The absorbance of samples was measured at 760 nm. The total phenolic content of samples was calculated as gallic acid equivalent (GAE) in µL/mL.

Total Flavonoid Content

The total flavonoid content of samples was determined by using aluminium chloride method [24]. 1.5 ml of methanol (Isolab, Germany) was mixed with 500 µl of samples. 100 µL of 10 % $AlCl_3$ (Merck, Germany), 100 µL of 1 M potassium acetate (CH_3CO_2K) (Merck Darmstadt, Germany), and 2.8 mL distilled water were added to the mixtures respectively, and then incubated at 25 °C for 30 min. After incubation, the absorbance of the samples was measured at 415 nm, and total flavonoid content was expressed as quercetin equivalent (QE) in µg/mL.

Determination of Phenolic Content of Kombucha Beverages By LC-MS/MS

LC-MS/MS analysis was carried out with the Agilent 6460 Triple Quad LC-MS/MS system (Waldbronn, Germany) equipped with a reversed-phase C18 HPLC column (particle size 5 µm, 150 mm×4.6 mm i.d.) using Multiple Reaction Monitoring (MRM) mode. 5 µL of all samples were injected into the device. Mobile phase a consisted of ultrapure water with 0.1% formic acid (CH_2O_2), and mobile phase b consisted of acetonitrile with formic acid. The gradient program and flow rate were optimised according to Gai et al. [25].

Sensory Analysis

Consumer preferences based on taste, odour, acidity, appearance, and overall assessment of fermented products were investigated via the protocol described by Altuğ [26] with slight modifications. Twenty participants between the ages of 18 and 45 from the Akdeniz University Campus were selected for this blind test analysis. Since the test was designed as a blind test, the content of the drinks was not shared by the attendants. On a 5-point hedonic scale, an acceptability degree from very bad to very good (1 to 5 respectively) was expressed by all participants for each drink. Besides the taste, visual and olfactory properties of drinks were also evaluated by participants on the same scale as well.

Statistical Analysis

All experiments were conducted with three independent replicates, and all data were expressed as mean ± standard deviation of these three replicates. For statistical comparisons of the samples, One-way analysis of variance (ANOVA) was performed for statistical comparisons of the samples using IBM SPSS software version 22 (SPSS, Inc., USA). Additionally, Tukey's post-hoc analysis was used as a supplementary test to determine which groups exhibited differences in multiple comparisons [8]. Statistical significance was considered for p-values < 0.05.

RESULTS AND DISCUSSION

Although black tea is commonly used in kombucha tea fermentation, nowadays, various herbal materials are being added with the aim of enhancing the health benefits of this fermented product. In this study, a kombucha fermentation was conducted using gilaburu juice. Before fermentation, the number of *Lactobacillus*, yeast, total mesophilic bacteria, and acetic acid bacteria in all cultures ranged from 1.8 to 3×10^3 CFU / mL (Table 1). The microbial count of all samples reached its peak on the 14th day of fermentation and gradually decreased throughout the remaining fermentation period. After fermentation, the amount of *Lactobacillus*, yeast, total mesophilic bacteria, and acetic acid bacteria in the gilaburu-flavoured kombucha was higher than in the traditional kombucha, except for day 14 of acetic acid bacteria.

According to Goh et al. [5], the amount of sugar used in kombucha culture affects the density of acetic acid bacteria

Table 1. Microbiological profile of kombucha cultures

	Day 0	Day 7		Day 14		Day 21	
	K	K	KG	K	KG	K	KG
Lactobacillus	$3.00\pm0.05\times10^3$	$1.45\pm0.05\times10^7$	$1.60\pm0.10\times10^7$	$1.75\pm0.05\times10^7$	$2.05\pm0.05\times10^7$	$1.02\pm0.05\times10^7$	$1.50\pm0.0\times10^7$
Yeast	$1.80\pm0.10\times10^3$	$8.00\pm0.00\times10^6$	$1.35\pm0.05\times10^7$	$1.40\pm0.05\times10^7$	$1.65\pm0.05\times10^7$	$7.60\pm0.50\times10^6$	$1.30\pm0.10\times10^7$
TMB	$3.00\pm0.05\times10^3$	$6.85\pm0.15\times10^6$	$1.70\pm0.00\times10^7$	$1.00\pm0.05\times10^7$	$6.85\pm0.15\times10^7$	$1.80\pm0.05\times10^6$	$1.10\pm0.00\times10^7$
AAC	$2.00\pm0.001\times10^3$	$1.05\pm0.05\times10^7$	$1.40\pm0.10\times10^7$	$1.60\pm0.00\times10^7$	$1.45\pm0.01\times10^7$	$8.00\pm0.50\times10^6$	$1.30\pm0.00\times10^7$

(AAC: Acetic acid bacteria, TMB: Total mesophilic bacteria, K: Traditional kombucha, KG: Gilaburu-flavoured kombucha)

and yeasts. According to Sreeramulu et al. [27], the proliferation of acetic acid bacteria in cultures, depending on the fermentation period, also leads to a decrease in pH value. In this study, all beverages reached their most acidic values after a 21-day fermentation period. However, in contrast to the results reported by Sreeramulu et al. [27], our study found a reduction in the number of acetic acid bacteria in both traditional kombucha and gilaburu-flavoured kombucha on the 21st day (Table 2). According to Chen and Liu [28], the decrease in pH due to fermentation may be caused by the amount of organic acid produced by the metabolic activities of bacteria and yeast in fermented beverages. Similarly, various studies, including those by Vitas et al. [7], Wang et al. [29], and Tefon-Öztürk et al. [8], have reported a decrease in pH values over time during the fermentation process. According to Chu and Chen [30], a low pH value reduces the acceptance rate in relation to the sensory quality of the beverage. In this study, the lowest pH value for traditional kombucha was recorded on the 14th and 21st day of fermentation. The prolonged fermentation time may lead to a drop in the pH value, possibly resulting in an antibacterial effect.

In this study, there was no antibacterial effect in any of the samples on the 0th and 7th days of fermentation. The formation of the inhibition zone was observed from the 14th day onward and was only observed in the fermented cultures (Table 3). On fermentation day 14, the traditional kombucha showed antibacterial activity against four strains, whereas the gilaburu-flavoured kombucha showed antibacterial activity against three strains. Both traditional kombucha and gilaburu-flavoured kombucha were effective against all six strains used in this study on day 21. Similarly, Sreeramulu et al. [27] could not find any antibacterial effect in their studies with unfermented teas, only fermented products could exert an antibacterial effect by forming an inhibition zone. They also claimed that the components exhibiting an antibacterial effect are produced by bacteria or yeasts in kombucha.

Foods with high antioxidant content are important because they can balance free radicals in the cell [31]. Therefore, high antioxidant values of the prepared samples gain importance. In this study, on days 7, 14, and 21 of the fermentation periods, traditional kombucha and gilaburu-flavoured kombucha had the highest antioxidant activity ($p < 0.05$) and there was no statistical difference between these two samples ($p > 0.05$), as indicated in Table 4. While the fermented kombucha samples showed increased antioxidant activity on the 7th and 14th days, a decrease in antioxidant activity was observed in all samples after the 21st day. Amarasinghe et al. [32] showed that the antioxidant activity

Table 2. pH values on day 0, day 7, day 14 and day 21 of kombucha beverages and tea infusion

	Length of fermentation (days)			
Samples	**0**	**7**	**14**	**21**
K	3.4±0.0	3.3±0.0	2.9±0.1	2.8±0.1
KG	3.3±0.0	3.2±0.0	3.1±0.1	2.9±0.0
FG	3.2±0.0	3.1±0.0	3.0±0.0	2.9±0.0
B	5.1±0.1	4.9±0.0	4.8±0.0	4.6±0.1
G	4.0±0.0	3.6±0.0	3.6±0.0	3.5±0.0

(K: Traditional kombucha, KG: Gilaburu-flavoured kombucha, FG: Fermented gilaburu, B: Black tea, G: Gilaburu fruit juice)

Table 3. Average diameter (in mm) of the inhibition zones of the kombucha samples

	Day 14							Day 21						
	KAN	**AMP**	**K**	**KG**	**FG**	**B**	**G**	**KAN**	**AMP**	**K**	**KG**	**FG**	**B**	**G**
EC	16±0.2	6±0	7±0.07	7±0	8±0.1	6±0	6±0	17±0	6±0	8±0	8±0.07	6±0	6±0	6±0
SA	14±0.06	17.3±0.3	6±0	6±0	6±0	6±0	6±0	15.3±0.06	18±0.2	10±0.3	8±0.06	7.3±0.06	6±0	6±0
PA	7.3±0.06	9±0.1	6±0	6±0	6±0	6±0	6±0	8±0.1	8±0.1	10±0.07	9±0.07	6±0	6±0	6±0
BC	14±0.06	7.3±0.06	7.3±0.06	8.3±0.06	7±0	6±0	6±0	18±0.1	11.3±0.06	8±0	7±0	6±0	6±0	6±0
KP	14.3±0.06	10.3±0.2	8.3±0.06	7.3±0.06	6±0	6±0	6±0	20±0	12±0.06	8.3±0.1	7±0	7±0	6±0	6±0
SE	16±0.06	11±0.06	7±0	6±0	6±0	6±0	6±0	18.3±0.1	11±0.06	8.3±0.06	7.3±0.06	6±0	6±0	6±0

(The discs used in the study have a diameter of 6 millimetres. A value of 6 in the table indicates that no inhibition zone is formed. SA: *S. aureus,* PA: *P. aeruginosa,* SE: *S. epidermidis,* KP: *K. pneumonia,* BC: *B. cereus*, EC: *E. coli.* K: Traditional kombucha, KG: Gilaburu-flavoured kombucha, FG: Fermented gilaburu, B: Black tea, G: Gilaburu fruit juice, KAN: Kanamycin, AMP: Ampicillin)

Table 4. Antioxidant activity (μM/mL AEE) of fermented beverages on day 0, 7, 14, and 21

	Ascorbic Acid Equivalent (μM/mL)			
	Day 0	**Day 7**	**Day 14**	**Day 21**
K	(529±0.7)^{b}	(585±3.7)^{*a}	(580±4.5)^{*a}	(528±3.8)$^{ns, a}$
KG	(579±1.8)^{a}	(573±5.2)$^{ns, a}$	(588±2.6)$^{ns, a}$	(531±4.5)^{*a}
FG	(278±5.0)^{e}	(325±4.6)^{*c}	(393±0.7)^{*c}	(241±4.5)^{*c}
B	(481±5.1)^{c}	(479±2.9)$^{ns, b}$	(430±5.2)^{*b}	(359±5.1)^{*b}
G	(343±4.9)^{d}	(206±1.4)^{*d}	(216±2.6)^{*d}	(191±0.8)^{*d}

(Different letters indicate statistical differences in the same column, statistically significant difference between day 0 indicated with * and no significant difference indicated with ns. K: Traditional kombucha, KG: Gilaburu-flavoured kombucha, FG: Fermented gilaburu, B: Black tea, G: Gilaburu fruit juice)

Table 5. Total phenolic content (μg/mL GAE) of fermented beverages on day 0, 7, 14, and 21

	Gallic Acid Equivalent (μg/mL)			
	Day 0	**Day 7**	**Day 14**	**Day 21**
K	(1330±1.6)^{c}	(1484±2.8)^{*b}	(1451±0.8)^{*b}	(1262±4.9)^{*b}
KG	(1469±3.2)^{a}	(1572±2.5)^{*a}	(1476±0.6)^{*a}	(1342±2.7)^{*a}
FG	(598±0.7)^{d}	(663±1.2)^{*d}	(616±0.3)^{*d}	(635±1.0)^{*c}
B	(1350±1.0)^{b}	(1357±0.3)^{*c}	(1298±1.3)^{*c}	(1257±1.8)^{*b}
G	(481±1.2)^{e}	(461±0.4)^{*e}	(424±0.4)^{*e}	(438±0.3)^{*d}

(Different letters indicate statistical differences in the same column, statistically significant difference between day 0 indicated with * and no significant difference indicated with ns. K: Traditional kombucha, KG: Gilaburu-flavoured kombucha, FG: Fermented gilaburu, B: Black tea, G: Gilaburu fruit juice)

decreased in all samples at the end of the 8-week fermentation, noting that this is an indicator of the declining functional properties of the beverages. Lee et al. [1] reported an increase in DPPH activity of kombucha cultures during a 14-day fermentation period, but a decrease on the 14th day. Chu and Chen [30] showed that the DPPH activity of various Taiwanese home kombucha samples increased with fermentation. Additionally, they have reported that the total phenolic content of the beverages increased by up to 98%, indicating that polyphenols can be biodegraded during fermentation, leading to the release of smaller molecules with higher antioxidant activity.

Phenolic compounds and flavonoids are considered as high-level antioxidants because of their ability to remove free radicals [33]. However, in contrast to Chu and Chen [30], all prepared beverages in this study had the highest total phenolic content on the 7th day (Table 5).

When the fermentation days with the highest total flavonoid content were analysed individually for each beverage, the total flavonoid content was highest in traditional kombucha on the 14th day, and highest in gilaburu-flavoured kombucha on the 7th day (Table 6). Gilaburu-flavoured kombucha had the highest total phenolic and flavonoid content overall during the fermentation period ($p < 0.05$). Amarasinghe et al. [32] reported that the use of phenolic compounds in fermented tea by tea fungus could lead to a decrease in total phenolic content. Similarly, Vitas et al. [7] observed a decrease in the phenolic substance content in beverages after the 7th day. In our study, the total amount of flavonoid content in the gilaburu-flavoured kombucha culture continued to increase on the 21st day, but the total flavonoid amount of the traditional kombucha culture increased until the 14th day and started to decrease on the 21st day. In their study, Chakravorty et al. [34] stated that during the 21-day fermentation, the total flavonoid content in the kombucha drink increased continuously until the end of the fermentation. They also found that black tea fermented with kombucha had a higher total flavonoid content than plain black tea. Bhattacharya et al. [33] stated that enzymes released by bacteria and yeasts during kombucha fermentation may break down the polyphenols, thereby increasing the flavonoid and phenolic compounds.

Kombucha beverages contain many phenolic compounds that may vary depending on the type of plant used and the steeping procedure [35]. In this study, traditional kombucha and gilaburu-flavoured kombucha generally exhibited higher activities on the 7th and 14th days, and therefore, the phenolic substance contents on these days were investigated and compared. Fourteen phenolic contents were identified from beverages: quinic acid, fumaric acid, gallic acid, chlorogenic acid, catechin, 4-OH-benzoic acid, epicatechin, epigallocatechin gallate, vitexin, naringin,

Table 6. Total flavonoids (μg/mL QE) of fermented beverages on day 0, 7, 14, and 21

	Quercetin Equivalent (μg/mL)			
	Day 0	**Day 7**	**Day 14**	**Day 21**
K	$(20.8\pm0.7)^{b}$	$(24.7\pm0.5)^{*a}$	$(26.7\pm0.3)^{*a}$	$(23.3\pm0.2)^{*b}$
KG	$(23.8\pm0.3)^{a}$	$(23.8\pm0.3)^{ns,\ a}$	$(26.6\pm0.1)^{*a}$	$(28.4\pm0.3)^{*a}$
FG	$(7.4\pm0.1)^{d}$	$(9.7\pm0.1)^{*c}$	$(6.1\pm0.1)^{*d}$	$(9.6\pm0.6)^{*d}$
B	$(17.6\pm0.2)^{c}$	$(18.2\pm0.6)^{*b}$	$(16.7\pm0.2)^{*b}$	$(15.1\pm0.2)^{*c}$
G	$(5.8\pm0.3)^{e}$	$(7.9\pm0.7)^{*d}$	$(7.9\pm0.7)^{*c}$	$(6.7\pm0.2)^{ns,\ e}$

(Different letters indicate statistical differences in the same column, statistically significant difference between day 0 indicated with * and no significant difference indicated with ns. K: Traditional kombucha, KG: Gilaburu-flavoured kombucha, FG: Fermented gilaburu, B: Black tea, G: Gilaburu fruit juice)

Table 7. The concentration of polyphenols (ng/mL) in kombucha samples

	Day 7		Day 14	
Contents	**K**	**KG**	**K**	**KG**
Quinic Acid	1812.1 ± 0.9^{b}	1980.6 ± 0.4^{a}	1702.4 ± 0.8^{c}	1523.8 ± 1.5^{d}
Fumaric Acid	370.9 ± 0.4^{d}	803.8 ± 0.5^{b}	521.0 ± 0.6^{c}	1948.3 ± 0.9^{a}
Gallic Acid	18970.9 ± 1.0^{c}	18048.5 ± 0.8^{d}	21875.6 ± 1.5^{a}	19679.3 ± 0.9^{b}
Chlorogenic Acid	258.9 ± 0.3^{c}	11890.1 ± 0.9^{a}	244.2 ± 0.3^{c}	11539.4 ± 0.4^{b}
Catechin	89.4 ± 0.1^{c}	196.0 ± 0.3^{b}	98.6 ± 0.1^{c}	277.2 ± 0.4^{a}
4-OH-Benzoic Acid	0 ± 0.0^{d}	21.9 ± 0.0^{c}	38.7 ± 0.1^{b}	82.0 ± 0.1^{a}
Epicatechin	361.8 ± 0.5^{c}	273.1 ± 0.2^{d}	384.7 ± 0.5^{b}	438.8 ± 0.6^{a}
Epigallocatechin Gallate	4752.6 ± 0.9^{a}	3116.2 ± 1.5^{d}	4553.2 ± 0.5^{b}	3939.3 ± 0.7^{c}
Vitexin	306.2 ± 0.4^{b}	322.5 ± 0.3^{a}	323.7 ± 0.1^{a}	299.7 ± 0.1^{b}
Naringin	41.3 ± 0.1^{a}	31.3 ± 0.0^{b}	39.9 ± 0.1^{a}	35.3 ± 0.2^{ab}
Ellagic Acid	526.8 ± 0.5^{a}	367.4 ± 0.6^{b}	516.9 ± 0.8^{a}	353.4 ± 0.4^{b}
Hesperidin	826.7 ± 0.1^{b}	907.5 ± 0.4^{a}	812.3 ± 0.4^{bc}	810.0 ± 0.9^{c}
p-Coumaric Acid	17.1 ± 0.0^{a}	6.2 ± 0.0^{b}	0 ± 0.0^{c}	0 ± 0.0^{c}
Rosmarinic Acid	39.5 ± 0.1^{a}	29.9 ± 0.0^{bc}	33.1 ± 0.1^{ab}	22.4 ± 0.2^{c}

(Different letters indicate statistical differences in the same line. K: Traditional kombucha, KG: Gilaburu-flavoured kombucha)

ellagic acid, hesperidin, p-coumaric acid, and rosmarinic acid (Table 7). Gallic acid, which has antioxidant, anti-inflammatory, antimicrobial, and anticancer activities [11], was the main phenolic compound in both kombucha beverages, traditional kombucha and gilaburu-flavoured kombucha. Gilaburu-flavoured kombucha had a higher content of fumaric acid, chlorogenic acid, catechin, and 4-OH-benzoic acid than traditional kombucha on days 7 and 14. It is known that chlorogenic acid, exhibits antioxidant, carcinogenic, and antimutagenic activities [36] and Karaçelik et al. [37] reported that chlorogenic acid is one of the main components of gilaburu. Catechins, which belong to the group of polyphenols that play an important role in human nutrition, are bioactive compounds involved in scavenging reactive oxygen species and free radicals and have antimicrobial activity [38]. Similarly, 4-OH benzoic acid is known to have antimicrobial and antioxidant activity and inhibits human carbonic anhydrase and phenoloxidase activity [39]. Additionally, fumaric acid is a beverage ingredient frequently used as a food acidulant and food additive due to its taste and non-toxicity [40].

Upon evaluating the prepared kombucha beverages in terms of taste, smell, appearance, acidity, and overall quality, participants generally found the gilaburu-flavoured kombucha to be more flavourful than the traditional kombucha (Figure 1). Participants gave higher scores for the gilaburu-flavoured kombucha and traditional kombucha sample for acidity on day 7 of fermentation, while scores for the gilaburu-flavoured kombucha and traditional kombucha samples decreased on day 21 of fermentation. At the same time, the participants said that the acidity and foul odour increased during fermentation. Both samples had the lowest scores on day 21 of fermentation. Similarly, Ayed et al. [41], in their sensory analysis, stated that the scores of the kombucha samples decreased during fermentation, especially after the 6th day, they turned into a vinegar-like

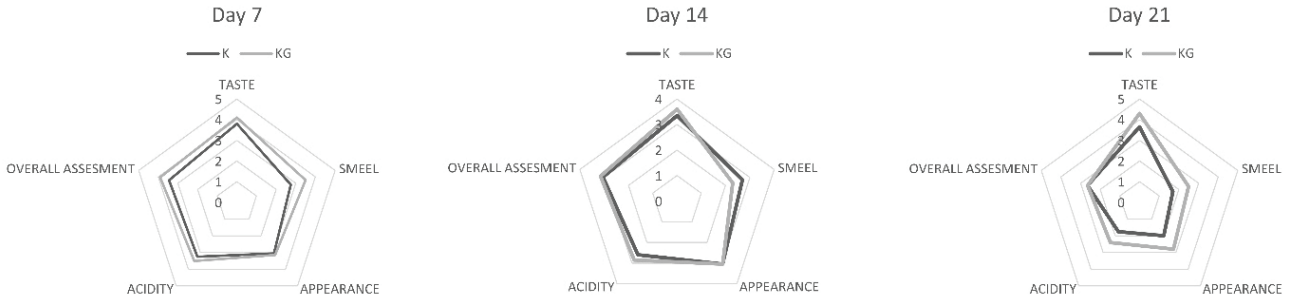

Figure 1. Sensory test results for samples after 7, 14, and 21 days of fermentation. (K: Traditional kombucha, KG: Gilaburu-flavoured kombucha).

drink with an extremely acidic taste. In addition, in this study, the participants stated that the kombucha samples looked blurry. Gramza-Michałowska et al. [42] claimed that *Acetobacter xylinum*, which is present in the structure of kombucha and has a fibrous structure, may cause the kombucha samples to appear cloudy.

CONCLUSION

In this study, we investigated the effect of using a medicinal herb gilaburu in kombucha fermentation, in terms of antimicrobial, total phenolic, and flavonoid content, microbial profile, and sensory properties. The kombucha culture was successfully fermented with gilaburu juice, and the best results in terms of studies conducted were generally obtained in the sample of gilaburu-flavoured kombucha. Moreover, it has been shown that the gilaburu-flavoured kombucha drink is rich in ingredients with high antioxidant activity, such as 4-OH-benzoic acid, fumaric acid, chlorogenic acid, and catechin, and has a high total phenolic content. At the same time, in the sensory analysis, the participants found the gilaburu-flavoured kombucha sample tastier than traditional kombucha. For the beverage to be preferred by consumers, the acidity and bad smell produced during the fermentation process need to be improved. Further studies on the *in vivo* effects of the drink are also needed.

AUTHORSHIP CONTRIBUTIONS

Authors equally contributed to this work.

DATA AVAILABILITY STATEMENT

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICS

There are no ethical issues with the publication of this manuscript.

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