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Preparation and Analysis of Physicochemical Properties, Antioxidant and Antibacterial Activities of Kombucha Tea Produced from Beijing Bamboo Leaf Tea (*Dendrocalamus* sp.) and Green Tea

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Abstract

Beijing bamboo (*Dendrocalamus* sp.), belonging to the Poaceae family, has been largely neglected and underutilized, particularly its leaves. This study aims to investigate the physicochemical properties, antioxidant, and antimicrobial activities of Beijing bamboo leaf and Beijing bamboo leaf kombucha. Hot air drying was conducted at temperatures of 60, 65, and 70ºC, with results indicating that 65ºC is the optimal drying temperature for achieving the highest levels of total phenolic compounds, flavonoids, and antioxidant activities. Beijing bamboo leaf tea underwent a 21-day fermentation process with a kombucha consortium comprising yeasts and acetic acid bacteria. The results showed that the changing trends of pH, total acidity, and total soluble solids were similar between kombucha produced from Beijing bamboo leaf tea and green tea. Fermentation notably enhanced the antioxidant activity of the kombucha, as evidenced by DPPH and FRAP assays, which correlated with increases in phenolics and flavonoids. The antimicrobial efficacy was assessed through the agar diffusion method and minimum inhibitory concentration (MIC), revealing that both Beijing bamboo leaf tea and green tea suppressed the growth of pathogenic bacteria, including *Salmonella* Typhimurium DMST 562, *E. coli* DMST 4242, *Bacillus cereus* DMST 5040, *Staphylococcus aureus* DMST 8840, and *Pseudomonas aeruginosa* DMST 4739. Notably, kombucha from green tea exhibited superior antimicrobial effects compared to Beijing bamboo leaf tea kombucha. Additionally, fermentation of Beijing bamboo leaf tea resulted in higher counts of yeast, acetic acid bacteria, and lactic acid bacteria throughout the fermentation process than those observed in green tea kombucha. These findings highlight the potential of Beijing bamboo leaves as a valuable resource for developing functional properties in underutilized plant resources, offering promising applications in the food and health industries.

Keywords: *Beijing bamboo leaves; antioxidant; antimicrobial activity; green tea; kombucha*

1. Introduction

Bamboo belongs to the Poacease family, specifically the subfamily Bambusoideae, and predominantly thrives in tropical regions. Nevertheless, bamboo species exhibit adaptability to diverse climatic conditions and ecosystems. Bamboo leaves, regarded as renewable natural resources, offer an eco- friendly avenue for healthcare within sustainable resource frameworks (Nirmala, & Bisht, 2017). Since ancient times, they have been processed and utilized as tea in China and South East Asia (Nirmala et al., 2018) due to their considerable therapeutic potential, including alleviating digestive issues, reducing blood pressure, and possessing antioxidant, anti-inflammatory, antidiabetic, anti-bacterial, anti-fungus, anti-carcinogenic properties (Ghasemzadeh, & Ghasemzadeh, 2011; Rathod et al., 2011; Singhal et al., 2013; Wasnik, & Tumane, 2014; Hossain et al., 2015). The composition of bamboo leaves includes fiber, protein, silica, choline, cyanogenic glycoside, albuminoids, oxalic acid, reducing sugar, resin, wax, benzoic acid, arginine, cysteine, arginine, histidine, niacin, riboflavin, thiamine, glutelin, lysine, methionine, nuclease, and urease (Rathod et al., 2011). Moreover, bamboo leaves contain flavonoids such as orientin, isoorientin, vitixin, and isovitixin, along with other phenolic compounds including *p*-coumaric, chlorogenic, caffeic and ferulic acids (Zhang et al., 2005; Gong et al., 2016). Considerable attention has been directed towards extracting beneficial compounds from various by-products, including bamboo leaves, for potential applications in developing functional foods or nutraceutical products (Sharma et al., 2019).

Kombucha, also known as tea fungus, is a globally consumed health-promoting beverage. Its fermentation process is facilitated by a symbiotic consortium of acetic acid bacteria and yeasts acting on sweetened black or green tea. As a result from this process, kombucha has a slightly acidic, carbonated, and somewhat sweet taste. The beverage is abundant in organic acids, vitamins, minerals, dietary fiber, essential amino acids, various enzymes, and secondary metabolites (Antolak et al., 2021). Studies have shown that kombucha exhibits functional properties, including antimicrobial, antioxidant, anticancer, and antidiabetic activities (Chakravorty et al., 2016; Villarreal-Soto et al., 2019). While black and green tea are the most common substrates for its preparation, there are also reports indicating that infusions made from substrates other than tea have been studied as well (Emiljanowicz, & Malinowska-Pańczyk, 2019; Xiong et al., 2023). A recent study has reported that kombucha fermented with bamboo leaf residues increased its antioxidant capacities and total phenolic contents (Xiong et al., 2023). Despite these findings, there remains a lack of comprehensive data regarding optimal drying methods for bamboo leaf tea and the antimicrobial activities of kombucha derived from Beijing bamboo leaf tea. To the best of our knowledge, this study represents the first investigation into kombucha produced specifically using Beijing bamboo leaves (*Dendrocalamus* sp.).

2. Objectives

The objectives of this study were to determine the optimal drying temperature for Beijing bamboo (*Dendrocalamus* sp.) leaves and to compare the physicochemical properties, antibacterial activity, and other biological activities of kombucha produced from Beijing bamboo leaf tea with those of green tea kombucha.

3. Materials and methods

3.1 Chemicals, reagents, and bacterial strains

Folin - Ciocalteu’s phenol reagent was purchased from Loba Chemie (Mumbai, India). 2,4,6-tripyridyl-S-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, and quercetin were obtained from Sigma-Aldrich (MO, USA). Brain Heart Infusion (BHI) broth was obtained from HiMedia (Mumbai, India). Antibiotics (penicillin/streptomycin) were brought from Gibco (New York, USA). Other chemicals used in the experiments were of analytical grade.

Escherichia coli DMST 4242, *Salmonella* Typhimurium DMST 562, *Staphylococcus aureus* DMST 8840, *Bacillus cereus* DMST 5040, and *Pseudomonas aeruginosa* DMST 4739 were obtained from the National Institute of Health of Thailand.

3.2 Preparation of bamboo leaves

Fresh leaves of Beijing bamboo (*Dendrocalamus* sp.) were harvested from Meesuk bamboo farm located in Rayong province, Thailand. The bamboo leaves were thoroughly cleaned and washed with water, then cut into small pieces approximately 1 cm in width. Subsequently, the samples were subjected to drying at temperatures of 60, 65, and 70ºC using a tray dryer (OFM, Thailand) until achieving a moisture content of less than 8%. Once dried, the leaves were ground into a fine powder and sieved through a 35-mesh sieve. The resulting bamboo leaf powder was stored in an airtight container until further use.

3.3 Measurement of physicochemical properties of bamboo leaves

Moisture content was determined according to AOAC (2000). Water activity (a_w) was measured using a water activity meter (Aqualab, Cx3TE, USA). Color parameters CIE L*, a* and b* were assessed using a colorimeter (Konica Minolta, Japan).

3.4 Preparation of aqueous infusion of bamboo leaf tea

Infusions were prepared by adding 2 g of bamboo leaf tea, contained within a tea bag, to 100 mL of deionized boiling water. The infusions were then brewed under cover for 5 min in hot water ranging from 95º to 100ºC. Determinations were carried out using samples from three different batches, each analyzed in triplicate**.** Fresh infusions were prepared for each experiment to ensure consistency and accuracy of the results.

3.5 Preparation and fermentation of kombucha

Bamboo leaf tea and green tea (Raming tea, Thailand) at a concentration of 2% (w/v) were steeped in boiling water for 10 min. Afterward, the tea was filtered, and 10% (w/v) sugar was added, allowing the temperature to decrease naturally until it reached 30ºC. The filtered tea was transferred to a clean jar, and a symbiotic culture of bacteria and yeast (SCOBY) was added at a concentration of 10% (w/v), along with 10% (v/v) of its liquid from a previous batch. The jar was covered with cheesecloth and incubated at 30ºC for a duration of 21 days. Samples of the kombucha, derived from both bamboo leaf tea and green tea, were collected on days 0, 1, 7, 14, and 21 for subsequent analysis.

3.6 Measurement of physicochemical properties of kombucha

pH was measured using a pH meter. Total acidity was determined using the titration technique (AOAC, 2002). Total soluble content was assessed using a hand refractometer (Atago, Japan). Alcohol content was analyzed employing an ebulliometer (Dujardin-Salleron, France).

3.7 Determination of total phenolic content (TPC)

The total phenolic content was determined using the Folin Ciocalteu reagent (Dudonne et al., 2009). Two hundred microliters of the sample were mixed with 250 µl of Folin-Ciocalteu reagent, followed by the addition of 5.8 mL of dH_2O. The mixture was then incubated at room temperature for 6 min, after which 2.5 mL of Na_2CO_3 was added. The mixture was then left at room temperature for 30 min. Subsequently, the absorbance was measured at 765 nm using a UV-Vis spectrophotometer. The total phenolic content was calculated in mg gallic acid equivalent (GAE)/L.

3.8 Determination of total flavonoid content (TFC)

The total flavonoid content was determined following the method described by Zhishen et al. (1999) with some modifications. Briefly, 0.5 mL of the sample was mixed with 2 mL of dH_2O and 150 µl of $NaNO_2$. After incubation for 6 min at room temperature, 150 µl of $AlCl_3$ and 2 mL of 1 M NaOH were added. The mixture was then incubated at room temperature for 15 min, and the absorbance was measured at 415 nm using a UV-Vis spectrophotometer (Shimadzu, Japan). The total flavonoid content was expressed in mg quercetin equivalent (QE)/ L.

3.9 Determination of antioxidant activities

3.9.1 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method

Antioxidant activity was assessed using the DPPH radical scavenging method described by Braca et al. (2001). A DPPH solution was prepared at a concentration of 0.004%. Then, 4.8 mL of this solution was mixed with 0.2 mL of the sample, and the mixture was incubated at 37ºC for 30 min. Subsequently, the solution was measured at 517 nm using a UV-Vis Spectrophotometer (Shimadzu, Japan). The antioxidant activity of the samples was quantified as mg gallic acid equivalent (GAE)/L.

3.9.2 Ferric reducing ability power (FRAP) assay

The FRAP assay was performed as previously described (Benzie, & Strain, 1996). A volume of 100 mL of the sample extract was combined with 3 mL of FRAP reagent, consisted of 300 mM Acetate buffer pH 3.6, 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$. The solution was left in the dark for 15 min, after which the absorbance was measured at 593 nm. The antioxidant activity of the samples was then quantified in mg gallic cid equivalent (GAE)/ L.

3.10 Determination of anti-microbial activity of kombucha

3.10.1 Agar well diffusion of kombucha

The antimicrobial activity was assessed using the agar well diffusion method against pathogenic bacteria including *Escherichia coli*, *Salmonella* Typhimurium, *Staphylococcus aureus*, *Bacillus cereus,* and *Pseudomonas aeruginosa*. These bacterial strains were cultured in brain heart infusion (BHI) broth and incubated at 37 °C for 18 h until reaching a density of 10^8 cells/mL. The bacterial suspensions were then swabbed onto BHI agar plates. Wells with an 8 mm diameter were punched into the agar using a Cork borer, and 100 µl of kombucha was added into each well. Penicillin and streptomycin at the concentration of 10 mg/mL were employed as positive controls. All samples were incubated at 37ºC for 24 h, following which the diameter of the inhibition zone was measured in mm. The experiments were conducted in triplicate to ensure reliability and reproducibility of results.

3.10.2 Minimum inhibitory concentration (MIC) of kombucha

This method was adapted from Silva et al. (2021). In brief, kombucha at an initial concentration of 1000 µl/mL was twofold diluted with BHI broth in a 96 well- microplate. Subsequently, bacterial suspensions with a density of 10^8 cells/mL were added to each well, and the microplate was incubated at 37ºC for 24 h. MIC was determined by observing the lowest concentration at which bacterial growth was visibly inhibited, indicated by the absence of turbidity as detected by naked eye.

3.11 Microbiological characterization

The study of microorganism's changes in kombucha was adapted from Cardoso et al. (2020). Yeast, acetic acid bacteria, and lactic acid bacteria counts were conducted on days 0, 7, 14, and 21 of the fermentation process. Initially, 10 mL of kombucha was added to 90 mL of 0.1% peptone solution and thoroughly mixed. Serial dilutions were prepared using an optimal 3-level dilution scheme, and the speared plate method was employed for plating. Acetic acid bacteria were cultured on glucose yeast calcium carbonate agar (GYCA) medium. Lactic acid bacteria were cultured on De Man Rogosa and Sharpe (MRS) agar plates and incubated at 35ºC for 48 h. Yeasts were cultured on Sabouraud Dextrose Agar (SDA) plates and incubated at 30ºC for 48 h.

3.12 Statistical analysis

All assays were conducted in triplicate. The resulting data were recorded as means ± standard deviation. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS version 16.0). The obtained data were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range tests to determine the significance of differences between the means at $p < 0.05$

4. Results and Discussion

4.1 Physicochemical properties of bamboo leaf tea at different temperatures

Using different temperatures of 60, 65 and 70ºC resulted in varying outcomes. The results indicated a decrease in moisture content as the drying temperature increased, which aligns with findings from prior research on herbal tea (Thaiwong, & Chaiwong, 2020). Notably, the moisture content of the bamboo leaves dried at 60, 65, and 70ºC met the standards set by the Thai Industrial Standards Institute (TISI) for tea, which mandates a maximum moisture content of 8%. Water activity (a_w) did not exhibit statistically significant differences ($p < 0.05$). The water activity value for dried bamboo leaves fell within the safe margin, ranging from 0.35 to 0.37. Generally, a water activity level below 0.60 ensure microbial stability.

The appearance of dried bamboo leaves is shown in Figure 1. In Table 1, there was a trend observed in L* value, which tended to decrease with increasing drying temperature. The use of high drying temperatures led to a loss of brightness. Additionally, the b* value decreased as the drying temperature increased. The color of bamboo leaf tea exhibited a green hue, attributed to the negative value of a*. Furthermore, the positive value of b* indicated a yellowish color (26.34 ± 0.38 - 27.69 ± 0.30) instead of blue.

4.2 Bioactivities of bamboo leaves at different temperature

The results of the total phenolic contents (TPC) and total flavonoid contents (TFC) are presented in Table 1. Significant differences were observed among the different drying temperatures. At 65ºC, the TPC and TFC exhibited the highest levels of activity ($p < 0.05$). A low drying temperature (60ºC) did not cause the rupture of the whole cellular structures to release of phenolic and flavonoid compounds (Patrón-Vázquez et al., 2019), whereas at 65ºC, higher total polyphenols were produced due to the use of higher temperatures and faster drying times. The higher temperature can improve the solubility of phenolic compounds. However, total polyphenol contents decreased at a drying temperature of 70ºC, likely due to disruptions caused by the high drying temperature (Ismanto et al., 2020).

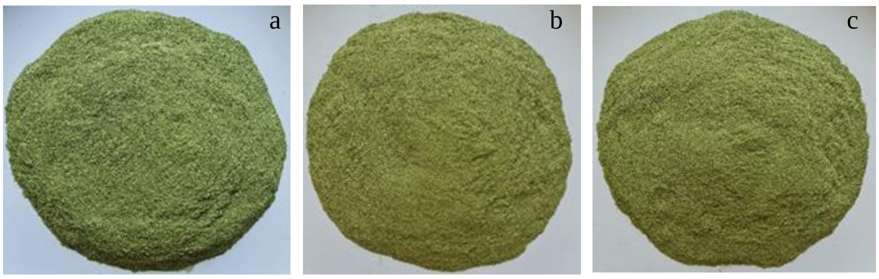

Figure 1 Bamboo leaf tea with drying temperature at a) 60ºC b) 65ºC, and c) 70ºC

Table 1 Physicochemical properties of bamboo leaves with different drying temperatures

Physicochemical properties	60ºC	65ºC	70ºC
Moisture (%)	7.07 ± 0.02[c]	6.38 ± 0.08[b]	5.09 ± 0.02[a]
Drying time (min)	90	60	60
Water activity	0.35 ± 0.002	0.36 ± 0.005	0.37 ± 0.001
L*	57.63 ± 0.32[c]	57.20 ± 55.0[b]	57.03 ± 0.59[a]
a*	-4.39±0.04[c]	-4.09±0.14[b]	-3.84±0.25[a]
b*	27.03±0.42[b]	27.69±0.30[b]	26.34±0.38[a]
TPC (mg GAE/L)	70.98 ± 14.32[a]	123.98 ± 10.53[b]	66.18 ± 8.21[a]
TFC (mg QE/L)	141.72 ± 4.19[a]	273.26 ± 9.95[c]	232.49 ± 7.74[b]
Antioxidant activity (DPPH) (mg GAE/L)	8.65 ± 0.93[b]	14.89 ± 0.81[c]	6.35 ± 0.66[a]
Antioxidant activity (FRAP) (mg GAE/L)	38.60 ± 9.55[a]	60.99 ± 5.60[b]	58.47 ± 11.77[b]

Values are expressed as mean ± standard deviation. Different letters in the same row indicate significantly different values ($p < 0.05$)

The DPPH and FRAP results of this study are presented in Table 1. Drying temperature at 65ºC recorded the highest level of antioxidant activities. In this study, it was shown that the TPC and TFC were positively correlated with their antioxidant properties. There was a previous study reported that rapid and short time of heating may cause inactivation of oxidative enzymes and help to preserve phenolic compounds (Roslan et al., 2020). This would explain the increasing in the antioxidant properties of the studied dried bamboo leaves

4.3 Physicochemical properties of kombucha

The development of kombucha beverage derived from bamboo leaf tea was performed and compared with green tea kombucha. The pH values, total acidity, and total soluble solids (TSS) are parameters that indicate the success of the fermentation process. Bamboo leaf tea kombucha and green tea kombucha exhibited lower pH values compared to the initial values, with a decrease observed from day 0 to day 21, indicating the metabolic activity of kombucha. Additionally, the total acidity of both bamboo leaf tea kombucha and green tea kombucha showed a statistically significant increase ($p < 0.05$). In the study by Cardoso et al., (2020), it was reported that kombucha from green and black tea, when fermented for 10 days, had pH values of 3.2 and 3.5, respectively. Additionally, Zou et al. (2021) found that kombucha fermented from green tea, black tea, and zijuan tea over a period of 14 days had pH values that decreased with increasing fermentation time. However, it is important to note that the safe pH range for consumer safety is 2.5-4.2 (Nummer, 2013). From this study, it is evident that kombucha from green tea and bamboo leaf tea has a safe pH level for consumers and helps reduce the risk of contamination from harmful bacteria. The decrease in pH is related to the increase in acid content throughout the fermentation process. The increase in acidity is attributed to the production of organic acids, particularly acetic acid, gluconic acid, and glucuronic acid, by acetic acid bacteria present in the SCOBY. However, the different acid levels depend on the type of tea, the diversity of strains of acetic acid bacteria, and lactic acid bacteria used in kombucha fermentation (Velićanski et al., 2013).

Table 2 Physicochemical properties of kombucha produced from bamboo leaf tea and green tea

Physicochemical properties	Kombucha	Days				
		0	1	7	14	21
pH	Bamboo leaf tea	3.55 ± 0.03^b	3.63 ± 0.03^b	3.07 ± 0.08^b	2.87 ± 0.01^a	2.73 ± 0.04^a
	Green tea	3.51 ± 0.01^d	3.53 ± 0.03^d	3.14 ± 0.02^c	3.10 ± 0.02^b	3.10 ± 0.01^a
% Acidity	Bamboo leaf tea	0.24 ± 0.06^a	0.21 ± 0.03^a	0.50 ± 0.04^b	1.00 ± 0.06^c	1.71 ± 0.23^d
	Green tea	0.18 ± 0.05^a	0.21 ± 0.03^a	0.42 ± 0.07^b	0.69 ± 0.05^c	1.60 ± 0.18^d
Total soluble solid (ºBrix)	Bamboo leaf tea	10.43 ± 0.06^a	10.43 ± 0.06^a	11.07 ± 0.21^b	11.93 ± 0.12^c	12.65 ± 0.07^d
	Green tea	10.60 ± 0.00^a	10.77 ± 0.21^a	11.63 ± 0.06^b	12.53 ± 0.12^c	12.77 ± 0.15^c

Values are expressed as mean ± standard deviation. Different letters in the same row indicate significantly different values ($p < 0.05$).

Table 3 Bioactivities of kombucha produced from bamboo leaf tea and green tea

Bioactivities	Bamboo leaf tea kombucha		Green tea kombucha	
	Fermentation Day 0	Fermentation Day 21	Fermentation Day 0	Fermentation Day 21
TPC (mg GAE/L)	122.02 ± 11.74^a	238.78 ± 3.73^b	955.60 ± 36.32^a	1548.80 ± 130.69^b
TFC (mg QE/L)	231.07 ± 8.43^a	543.46 ± 5.97^b	1544.40 ± 72.65^a	2039.57 ± 22.16^b
Antioxidant activity (DPPH) (mg GAE/L)	18.84 ± 0.49^a	21.06 ± 0.13^b	423.42 ± 2.51^a	753.76 ± 34.27^b
Antioxidant activity (FRAP) (mg GAE/L)	43.98 ± 0.79^a	58.77 ± 1.89^b	387.88 ± 0.74^a	826.85 ± 59.92^b

Values are expressed as mean ± standard deviation. Different letters in the same row within each sample indicate significantly different values ($p < 0.05$).

The increasing trends in total soluble solids (TSS) were observed in both bamboo leaf tea kombucha and green tea kombucha, as represented in Table 2. For bamboo leaf tea kombucha, the TSS significantly increased ($p < 0.05$) from 10.43 ± 0.06 on day 0 to 12.65 ± 0.07 by day 21 of fermentation. Similarly, for green tea kombucha, the TSS had also significantly increased ($p < 0.05$) from 10.60 ± 0.00 to 12.77 ± 0.15 by day 21. This result contradicted with previous studies that reported a decrease in TSS during fermentation (Kaewkod et al., 2019; Jakubczyk et al., 2020). TSS values represent the content of a combination of all substances (inorganic and organic) dissolved in food or beverages. During kombucha fermentation, microorganisms consume sugars as a source of energy and metabolize them into other compounds, including organics acid, glycerol and ethanol. These compounds contribute to the increasing TSS in kombucha samples. Future research should aim to confirm the identity of the putative acids and sugars found in bamboo leaf tea kombucha.

4.4 TPC, TFC, and antioxidant activities of kombucha

The results in Table 3 indicated a significant difference in TPC, TFC, and antioxidant activities when compared to the initial day of fermentation for both bamboo leaf tea kombucha and green tea kombucha. For bamboo leaf tea kombucha, the initial TPC value of day 0 was 122.02 ± 11.74 mg GAE/L, which significantly increased to a final value of 238.78 ± 3.73 mg GAE/L after 21 days of fermentation, representing 1.96 times compared with those of day 0. The tendency was similar to the previous study (Xiong et al., 2023) Meanwhile, green tea kombucha exhibited a significantly higher concentration of TPC compared to bamboo leaf tea kombucha. The TPC on day 0 was 955.60 ± 36.32 mg GAE/L and increased to a final value of 1548.80 mg GAE/L after 21-day fermentation (1.62 times). Indeed, green tea is known for its rich polyphenol contents, which includes catechins such as epigallocatechin gallate (EGCG), epicatechin (EC), epigallocatechin (EGC), and epicatechin gallate (ECG) (Zhao et al., 2019).

In bamboo leaf tea kombucha, the flavonoid contents increased from 231.07 ± 8.43 mg QE/L to 543.46 ± 5.97 mg QE/L after fermentation for 21 days, representing an increase of 2.35 times compared with those of day 0. Bamboo leaves are a rich source of flavone glycosides, including orientin, isoorientin, vitixin, and isovitixin, which possess antioxidant, anti-aging, anti-bacterial, and anti-inflammatory properties (Zhang et al., 2005, Lam et al., 2016). Similarly, in green tea kombucha, the flavonoid content increased from 1544.40 ± 72.65 mg QE/L to

2039.57 ± 22.16 mg QE/L after 21-day fermentation (1.32 times). Moreover, the flavonoid contents in green tea kombucha were higher than those in bamboo leaf tea kombucha. The increase in flavonoid contents after fermentation were observed in both beverages. Polyphenol contents, including flavonoids, are naturally present in tea, and during fermentation, microbes from the SCOBY produce various enzymes such as cellulose, glucanase, and glucosidase. These enzymes may break down large molecules of polyphenols in the tea into free phenolics (Chu, & Chen, 2006). Additionally, microbial metabolism during fermentation generates organic acids, alcohols, and esters, leading to the release of insoluble bound phenols (Zhang et al., 2020). Consequently, the total phenolic and flavononid contents increase in kombucha during fermentation.

The DPPH assay evaluates the ability of antioxidants to scavenge DPPH radicals. In bamboo leaf tea kombucha, the antioxidant activity assessed using DPPH method significantly increased during the 21-day fermentation period from 18.84 ± 0.49 mg GAE/L to 21.06 ± 0.13 mg GAE/L (1.12 times). However, the antioxidant activity of green tea kombucha was notably higher, with levels increasing from 423.42 ± 2.51 mg GAE/L to 753.76 ± 34.27 mg GAE/L after 21 days of fermentation (1.78 times).

The FRAP assay measures the ability of antioxidants to reduce ferric ions to ferrous ions. In bamboo leaf tea kombucha, the FRAP values increased progressively over the fermentation period and reached their peak on day 21, which was 1.34 times higher than those observed on day 0 (Table 3). Consequently, the antioxidant activities of bamboo leaf tea kombucha significantly increased after 21 days of fermentation. This might be due to the production of some bioactive compounds by microorganisms during fermentation, leading to increased antioxidant activity. In green tea kombucha, the FRAP values increased from 387.88 ± 0.74 mg GAE/L to 753.76 ± 34.27 mg GAE/L (1.94 times). Nonetheless, green tea kombucha exhibited stronger antioxidant activity compared to bamboo leaf tea kombucha, attributed to its higher content of free radical-scavenging catechins. Catechins are known to exhibit potent antioxidant properties (Yang, & Liu, 2012). Additionally, green tea catechins are stable in acidic pH, which is consistent with the acidic environment of kombucha fermentation (Li et al., 2012). This stability further supports the superior antioxidant activity of green tea kombucha compared to bamboo leaf tea kombucha.

4.5 Antimicrobial activity of kombucha

The study investigated the antimicrobial properties of kombucha derived from green tea and bamboo leaf tea, both fermented for 21 days. It was found that kombucha from both bamboo leaf tea and green tea fermented for 21 days, exhibited inhibitory effects against *S.* Typhimurium, *E. coli*, *B. cereus*, *S. aureus*, and *P. aeruginosa*. Notably, kombucha from green tea fermented for 21 days exhibited superior inhibitory effects against pathogenic bacteria compared to kombucha from bamboo leaf tea, particularly against *S.* Typhimurium, *S. Aureus*, and *P. aeruginosa* ($p < 0.05$).

The minimum inhibitory concentration (MIC) testing of kombucha against pathogenic growth revealed that bamboo leaf tea kombucha fermented for 21 days, exhibited MIC values of 12.88 mg/mL (for *S.* Typhimurium, *S. aureus*, and *P. aeruginosa*), 51.50 mg/mL (for *E. coli*), and 103.00 mg/mL (for *B. Cereus*). Meanwhile, kombucha from green tea fermented for 21 days, exhibited MIC values of 13.38 mg/mL (for *S.* Typhimurium, *S. aureus*, and *P. aeruginosa*), 52.50 mg/mL (for *E. coli*), and 105.00 mg/mL (for *B. cereus*). From this study, it can be observed that both types of kombucha effectively inhibited pathogenic bacteria in the digestive system, consistent with previous research of Deghrigue et al., (2013). Their findings showed that green tea kombucha inhibited both gram-negative (*E. coli*, *P. aeruginosa*, *S.* Typhimurium) and gram-positive bacteria (*Listeria monocytogenes*, *Enterococcus faecalis*, *Micrococcus aureus*, and *S. aureus*). Similarly, Cardoso et al., (2020) reported that green tea kombucha fermented for 12 days exhibited inhibitory effects against *E. coli*, *Salmonella*, *S. aureus*, and *L. monocytogenes*, with MIC values of 250 μL/mL. This antimicrobial activity of kombucha is primarily attributed to organic acids, particularly acetic acid, and bioactive compounds. Moreover, during the fermentation process involving various types of bacteria, synergistic effects of different components occur, resulting in metabolites such as volatile organic acids that contribute to antimicrobial properties (Coelho et al., 2020). Additionally, green tea contains high levels of catechins, which possess antibacterial properties against various bacteria (Bhattacharya et al., 2016), while bamboo leaves contain various phytochemicals with inhibitory properties against bacteria as well (Menchavez et al., 2018).

Table 4 Antimicrobial activity and minimum inhibitory concentration (MIC) of bamboo leaf tea kombucha and green tea kombucha on day 21 of fermentation

Physicochemical properties	Inhibition zone (mm)				MIC (mg/mL)	
	Bamboo leaf tea kombucha	Green tea kombucha	Pennicilin	Streptomycin	Bamboo leaf tea kombucha	Green tea kombucha
S. Typhimurium	14.7 ± 0.06[a]	17.3 ± 0.06[b]	ND	25.0 ± 1.00	12.88	13.38
E. coli	13.3 ± 0.21[a]	15.3 ± 0.21[a]	ND	24.7 ± 0.58	51.50	52.50
B. cereus	15.0 ± 0.10[a]	16.0 ± 0.00[a]	17.3 ± 2.89	ND	103.00	105.00
S. aureus	12.7 ± 0.12[a]	15.7 ± 0.11[b]	23.3 ± 1.15	ND	12.88	13.38
P. aeruginosa	12.0 ± 0.20[a]	16.0 ± 0.10[b]	ND	22.7 ± 1.15	12.88	13.38

Values are expressed as mean ± standard deviation. Different letters in the same row indicate significantly different values ($p < 0.05$).
ND = Not detected

4.6 Microbial changes in the kombucha during fermentation

Kombucha fermentation is a collaborative process involving various microorganisms, including yeast, acetic acid bacteria, and lactic acid bacteria. Yeast initiates the fermentation process, demonstrating resilience to osmotic stress. Subsequently, acid-tolerant bacteria contribute to the fermentation process (Villarreal-Soto et al., 2020).

In a study investigating changes in yeast, acetic acid bacteria, and lactic acid bacteria during kombucha fermentation for 21 days, the initial counts of yeast in bamboo leaf tea kombucha and green tea kombucha were 4.01 and 3.08 log CFU/mL, respectively. These counts significantly increased ($p < 0.05$) on days 1, 7, 14, and 21. Kombucha from bamboo leaf tea had the highest yeast count on day 7, reaching 6.29 log CFU/mL. Meanwhile, kombucha from green tea reached maximum yeast counts of approximately 4.36 to 4.65 log CFU/mL from days 1, 7, and 14, gradually decreasing to 3.68 log CFU/mL thereafter. Throughout the fermentation period, yeast counts in bamboo leaf tea kombucha remained higher than those in green tea kombucha ($p < 0.05$) (Figure 2a). Additionally, a study by de Noronha et al., (2022) reported yeast counts during kombucha fermentation to be approximately 5.92 log CFU/mL, showing an increasing trend in the early stages of fermentation. This increase may be due to yeast metabolizing sucrose into monosaccharides, particularly fructose and ethanol, during the initial fermentation stages (Chen, & Liu, 2000; Coton et al., 2017; Tran et al., 2020).

Regarding acetic acid bacteria, kombucha from bamboo leaf tea and green tea initially had acetic acid bacteria counts of 3.07 and 2.49 log CFU/mL, respectively, which increased on days 1, 7, and 14. Kombucha from bamboo leaf tea had counts of 3.42, 3.87, and 4.04 log CFU/mL, while kombucha from green tea had acetic acid bacteria counts of 3.33, 3.23, and 3.28 log CFU/mL, respectively. However, by day 21 of fermentation, the contents of acetic acid bacteria decreased to 3.59 and 2.82 log CFU/mL, respectively. A higher count of acetic acid bacteria was observed in bamboo leaf tea kombucha than those in green tea kombucha throughout the fermentation period ($p < 0.05$) (Figure 2b). Consistent with this, de Noronha et al., (2022) found that acetic acid bacteria counts decreased from approximately 5.91 to 3.90 log CFU/mL during the first ten days of black tea fermentation. This decrease may be due to a reduction in nutrients available in the medium for bacterial growth (Chen, & Liu, 2000; Coton et al., 2017).

For lactic acid bacteria counts, kombucha from bamboo leaf tea and green tea had counts of 3.73 and 2.32 log CFU/mL, respectively, on day 0. These counts increased rapidly on days 1 and 7 (reaching 5.45 and 5.74 log CFU/mL) and then gradually decreased by days 14 and 21 (to 4.16 and 4.39 log CFU/mL) of fermentation ($p < 0.05$). Kombucha from green tea had lactic acid bacteria counts of 4.61 log CFU/mL on day 1, then decreased gradually on days 7, 14, and 21 (to 4.11, 3.53, and 3.51 log CFU/mL) ($p < 0.05$). Meanwhile, lactic acid bacteria counts in bamboo leaf tea kombucha increased to 4.61 log CFU/mL on day 1, then decreased gradually on days 7, 14, and 21 (to 4.16, 3.73, and 3.47 log CFU/mL) of fermentation ($p < 0.05$). Nevertheless, lactic acid bacteria counts in bamboo leaf tea kombucha were significantly higher than those in green tea kombucha throughout the fermentation period ($p < 0.05$) (Figure 2c). The majority of lactic acid bacteria found in kombucha were heterofermentative lactic acid bacteria, particularly *Oenococcus oeni*, which accounted for 62.8% of identifiable lactic acid bacteria in green tea kombucha and 57.1% in black tea kombucha (Coton et al., 2017).

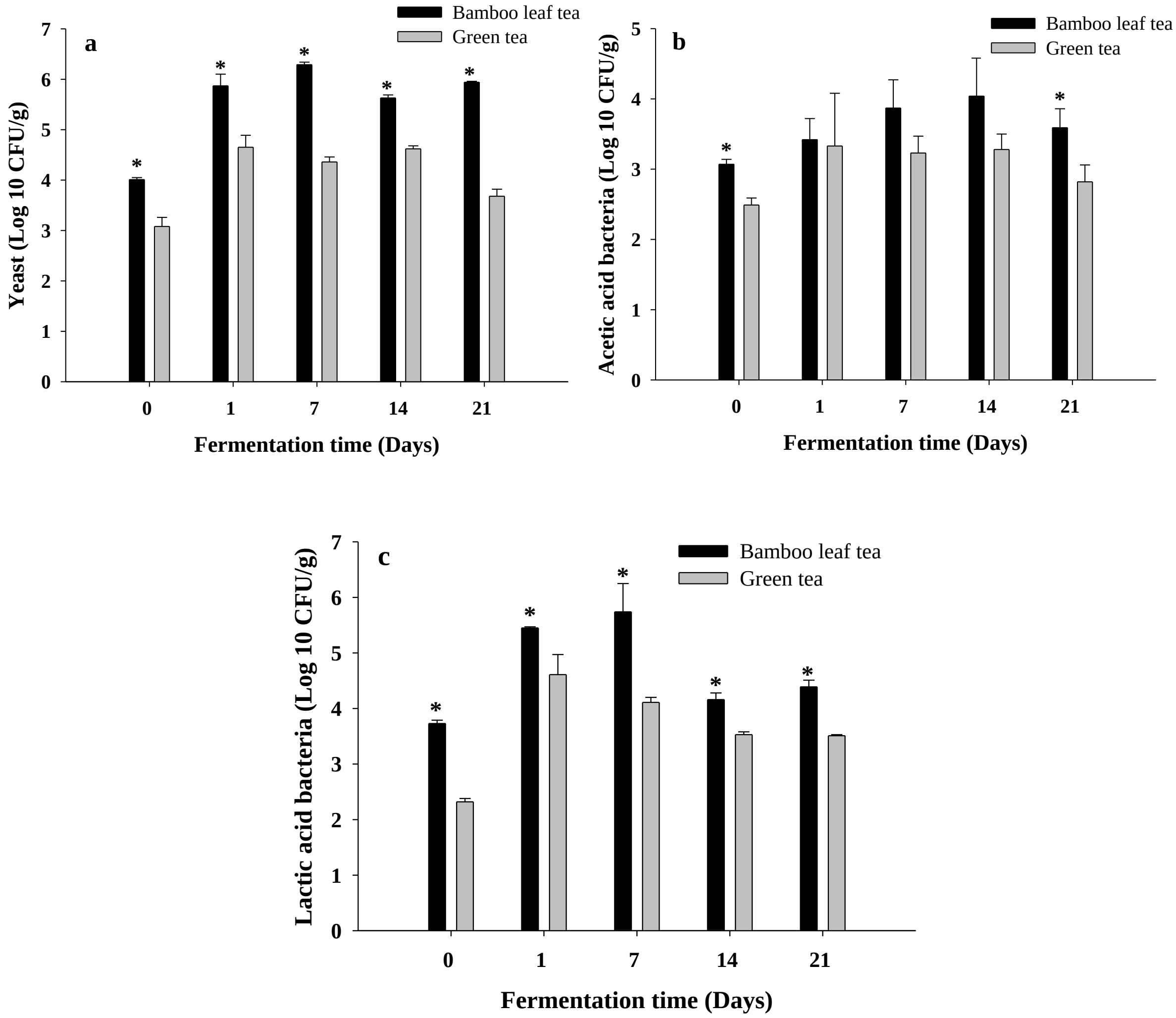

Figure 2 Microbial growth of (a) yeast, (b) acetic acid bacteria, and (c) lactic acid bacteria in bamboo leaf tea kombucha and green tea kombucha during fermentation for 21 days. The asterisk (*) represents significance difference between bamboo leaf tea kombucha and green tea kombucha at the same fermentation time ($p < 0.05$).

5. Conclusion

This study was undertaken to explore the optimal drying temperature concerning both bioactivities and physicochemical properties of Beijing bamboo leaves. The optimal drying temperature of bamboo leaves was 65 ºC based on TPC, TFC as well as antioxidant activities. Interestingly, the pH, total acidity, and total soluble solids of bamboo leaf tea and green tea kombucha were similar by the end of fermentation. Furthermore, fermentation of bamboo leaf tea could remarkably enhance TPC, TFC, and antioxidant activities compared to the initial day, although these values remained lower than those of green tea kombucha. This discrepancy was attributed to the green tea's naturally higher total phenolic, flavonoid, and antioxidant properties compared to bamboo leaf tea. Moreover, yeast, acetic acid bacteria, and lactic acid bacteria counts revealed that bamboo leaf tea kombucha promoted the growth of these microorganisms to a higher extent compared to green tea kombucha. Overall, this study sheds light on the potential of utilizing Beijing bamboo leaves as a tea base for brewing kombucha. It is suggested to explore the infusion of Beijing bamboo leaf tea kombucha with other herbs to further enhance phenolic compounds and antioxidant activities.

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