


Enhanced Biochemical and Health-promoting Properties of Novel Substrate-based Kombucha Drink

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Abstract: Kombucha is a distinctive fermented beverage recognized for its antioxidant activity and essential nutritional value, which contribute to its potential health benefits. Traditionally prepared from black tea, Kombucha production is expanding to include alternative plant substrates and herbs that may enhance its functional properties. This study explored the preparation and physicochemical characterization of Kombucha produced from fresh and dried basil (*Ocimum tenuiflorum*) and curry leaves (*Murraya koenigii*), collectively termed “Green Leaves Kombucha,” and compared it with control Kombucha prepared by fermenting dried black tea (*Camellia sinensis*). Fermentation was conducted using SCOBY at a controlled temperature for 14 days. This study investigates the impact of fresh and dried basil and curry leaves on the antioxidant activity (AA_{DPPH}), chemical composition, and sensory qualities of Kombucha. Results showed that green leaves Kombucha had significantly higher antioxidant activity and TPC compared to the control, although TFC was relatively lower. Despite the differences in flavonoid levels, green leaves Kombucha exhibited slightly lower sensory scores for aroma and taste compared to the control Kombucha, though the overall quality remained acceptable. These findings suggest that basil and curry leaves can be effective substrates for producing Kombucha with enhanced functional properties.

Keywords: Kombucha; fermentation; health benefits; probiotics; antioxidant; biochemical analysis.

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1. Introduction

Kombucha is a tangy, slightly sweet traditional fermented beverage with a long history. In recent years, it has surged in worldwide popularity [1-3]. Its distinct aroma, flavor, and perceived health benefits have made it a sought-after drink among health-conscious consumers [4]. It has multiple beneficial functional properties, including anti-inflammatory and antioxidant activity, maintaining blood pressure and cholesterol levels, reducing cancer cell proliferation, improving liver and gastrointestinal function, and lowering the risk of diabetes [3-6]. Kombucha has emerged as a popular functional beverage with potential health benefits and refreshing properties, developed using various natural substrates. Studies have extensively explored the preparation of kombucha tea through the fermentation of green and black tea and have investigated changes in functional antioxidant capacity and flavoring substances [7,8]. Recent studies have demonstrated that, beyond black and green tea, Kombucha has also been developed using many other natural herbs, such as mint (*Mentha spicata*), nettle (*Urtica*

dioica), and blackcurrant (*Ribes nigrum*) leaves [9]. This divergence aims to provide additional antioxidant activity, health benefits, and improved sensory qualities. Similarly, fermentation with traditional medicinal plants like hop (*Humulus lupulus*), madimak (*Polygonum cognatum*), and hawthorn (*Crataegus monogyna*) has revealed significant antioxidant, antibacterial, and cytotoxic activities, with madimak kombucha exhibiting the highest phenolic and flavonoid content [10]. Further research showed that Kombucha brewed with alternative herbal infusions such as peppermint, winter savory, stinging nettle, wild thyme, elderberry, and quince exhibits higher antioxidant activity, total phenolic content, and flavonoid content than traditional black and green tea substrates [11]. Beyond these, recent investigations suggest the production of novel substrate-based Kombucha using native green leaves as starting substrates, a method not reported earlier in the literature. Given the growing interest in Kombucha, it is essential to conduct scientific studies to understand the advantages and potential physicochemical properties of novel substrate-based green leaves Kombucha. Curry and basil leaves are excellent candidates for Kombucha due to their rich content of natural antioxidants, flavonoids, phenolics, tocopherols, and carotenoids, and their proven antibacterial and antifungal activities [12-14]. These bioactive compounds can enhance the functional, antioxidant, and antimicrobial properties of the fermented beverage.

This study explored the preparation and physicochemical characterization of the innovative formulation “Green Leaves Kombucha” as an alternative to traditional tea in kombucha production for potential health benefits. Green leaves kombucha was prepared with the combination of fresh and dried basil leaves (*Ocimum tenuiflorum*) and curry leaves (*Murraya koenigii*), while control kombucha was prepared using dried organic tea (*Camellia sinensis*) for comparison. The organic tea and green leaves were fermented with a SCOBY (Symbiotic Culture of Bacteria and Yeast) at controlled temperatures for approximately 14 days to prepare Control Kombucha (Ctrl.K) and Green Leaves Kombucha (GLK), respectively. To achieve favorable kombucha properties, it is essential to understand the factors affecting the fermentation rate as well as the characteristics of the final product.

This study investigated the influence of fermentation time and temperature on various physicochemical properties of Kombucha, including pH, total dissolved solids (TDS), total acidity (TA), volatile and non-volatile acidity, alcohol content, phenolic and flavonoid compounds, antioxidant activity (DPPH assay), and sensory attributes. The fermentation characteristics of Kombucha prepared with green leaves were evaluated and compared with those prepared with traditional tea substrates.

2. Materials and Methods

2.1. Materials.

Dried organic tea leaves (*Camellia sinensis*) were procured from Darjeeling, India. Fresh basil leaves (*Ocimum tenuiflorum*) and curry leaves (*Murraya koenigii*) were freshly plucked from a local garden in Alwar, Rajasthan, and thoroughly washed with distilled water. Portions of these fresh leaves were oven-dried at 60°C and combined in equal weight ratios to prepare a mixed dried leaf sample. Food-grade sucrose was purchased from the local market in Alwar. A live kombucha starter culture (SCOBY) was obtained from Cultures Market, Tamil Nadu, India. The received SCOBY was activated in starter tea (a pre-acidified liquid medium) according to the supplier’s protocol. All materials were used as received, without further purification.

2.2. Preparation of control tea liquid base for Kombucha using organic tea leaves.

The primary substrates required for kombucha preparation are organic tea leaves, a sweetener, and a SCOBY. To prepare the control tea base, 70 g of sucrose was dissolved in 200 mL of boiling deionized water (DI). Subsequently, 4 g of dried organic tea leaves were added to the boiling solution in a borosilicate brewing jar and steeped for 10–15 minutes. The mixture was then allowed to cool to room temperature (approximately 25°C). After steeping, the tea leaves were removed using a cotton filter cloth, and the filtrate was transferred to a clean borosilicate jar. The volume was adjusted to 1000 mL with additional deionized water. Finally, the SCOBY and starter tea were introduced to initiate fermentation, ensuring the desired pH and acidity levels were achieved.

2.3. Preparation of green leaves liquid base for Kombucha using basil and curry tree leaves.

For the preparation of green leaves Kombucha, a mixture of fresh and dried basil leaves and curry leaves, along with sucrose and a SCOBY, was used as the substrate. To prepare the infusion, 70 g of sucrose was dissolved in 200 mL of boiling deionized water in a borosilicate jar. Then, 3 g of dried mixed basil and curry leaves, 2 g of fresh basil leaves, and 2 g of fresh curry leaves were added and steeped for 10–15 minutes. All subsequent steps were performed as per the control protocol.

Fresh basil and curry tree leaves equivalent to 1 g of dried weight were used in this study. To maintain an equivalent weight ratio of dried and fresh leaves in both control and green leaves Kombucha, ensuring a well-controlled comparative analysis.

2.4. Fermentation process for the preparation of Kombucha.

The systematic fermentation process was carried out following the previously described method [15-16]. To initiate fermentation, the appropriate quantity of SCOBY culture was added to a borosilicate brewing jar containing the liquid base. The pH of the liquid base was ensured to be between 4.5 and 4.6; if outside this range, the pH was adjusted by adding 1–5 mL of starter tea. The brewing jar was covered with a cotton cloth. Fermentation was conducted in a dark environment at 25–30°C. If sugar levels decrease significantly during fermentation, additional sucrose may be added to sustain microbial activity and ensure the fermentation is completed.

To monitor the fermentation process, pipette out samples every other day during the fermentation period and analyze for pH, temperature, TDS, TA, volatile acidity, non-volatile acidity, alcohol content, phenolic compounds, flavonoids, and AA_{DPPH}. If no change in pH is observed during fermentation, it indicates that fermentation has not occurred and Kombucha has not been formed.

2.5. Physicochemical characterization.

2.5.1. pH and temperature.

pH and temperature were measured using an Eutech digital pH Meter to monitor changes in pH and temperature during the fermentation process.

2.5.2. Total dissolved solids (TDS).

TDS of fermented Kombucha was measured using an Eutech Digital TDS meter to monitor changes throughout the fermentation process. The unit of measurement is ppm or mg L⁻¹.

2.5.3. Titratable acidity (TA).

The TA of fermented Kombucha was measured using the acid-base titration method. In the standard procedure, a 5 mL fermented kombucha sample was pipetted and diluted to 10 mL with deionized water. This solution was then titrated against a N/40 standard sodium hydroxide (NaOH) solution using phenolphthalein as the indicator to determine the TA.

2.5.4. Volatile and non-volatile acidity.

Volatile and non-volatile acidity of fermented Kombucha were analyzed using the acid-base titration method. In the standard procedure, a 10 mL kombucha sample was pipetted and heated at 25–30°C for 5-6 minutes. The heated solution was then titrated against an N/40 standard sodium hydroxide (NaOH) solution to determine the non-volatile acidity. Volatile acidity was calculated as the difference between total titratable acidity (TA) and non-volatile acidity.

2.5.5. Alcohol content.

Alcohol content in the fermented Kombucha was determined using the Redox titration method. The ethanol is oxidized to ethanoic acid by reacting it with excess potassium dichromate in an acidic solution. The amount of unreacted dichromate is analyzed by Iodometric titration. In the typical procedure, pipette out 5 mL of the fermented kombucha sample and dilute to 10 mL with deionized water. This solution is then titrated against standard sodium thiosulfate (Na₂S₂O₃ x 5H₂O) solution to measure alcohol content.

2.5.6. Total phenolic content (TPC).

TPC was determined using a previously described UV–Visible spectrophotometric method [17]. Measurements were taken at a wavelength of 745 nm using a UV-Vis spectrophotometer (Thermo Scientific Orion AquaMate 8000). TPC in Kombucha was quantified by constructing a calibration curve with Gallic acid as the standard (ESI Figure S1). Results were expressed as milligrams of gallic acid equivalents per liter (mg GAE/L) of Kombucha.

2.5.7. Total flavonoid content (TFC).

TFC was determined using a UV–visible spectrophotometric technique reported earlier [18]. It was measured at a wavelength of 415 nm using a UV-Vis spectrophotometer. Quantification in Kombucha was performed using a calibration curve constructed with quercetin as the standard (ESI Figure S2). Results were expressed as milligrams of quercetin equivalents per liter (mg QE/L) of Kombucha.

2.5.8. DPPH radical antioxidant activity (AA_{DPPH}).

AA_{DPPH} was determined by the previously described radical scavenging activity method [19,20]. It was measured at a wavelength of 517 nm using a UV-Visible Spectrophotometer. In this method, 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used as a radical for adaptation, and methanol was used as a solvent and for blank reading. DPPH is light sensitive; the whole experiment is performed under dark conditions or covered with aluminum foil. Always prepare a fresh DPPH solution for AA_{DPPH} determination and use it the same day. AA_{DPPH} of Kombucha was calculated using the following formula:

$$AA_{DPPH} (\%) = \frac{A_{Blank} - A_{Sample}}{A_{Blank}} \times 100 \quad (1)$$

Where A_{Sample} is the absorbance of the Kombucha sample, and A_{Blank} is the absorbance of the blank (Methanol) at wavelength 517 nm.

The schematic overview of the fermentation process and sampling schedule for the control and green leaves kombucha formulations is summarized in the flow diagram (Figure 1).

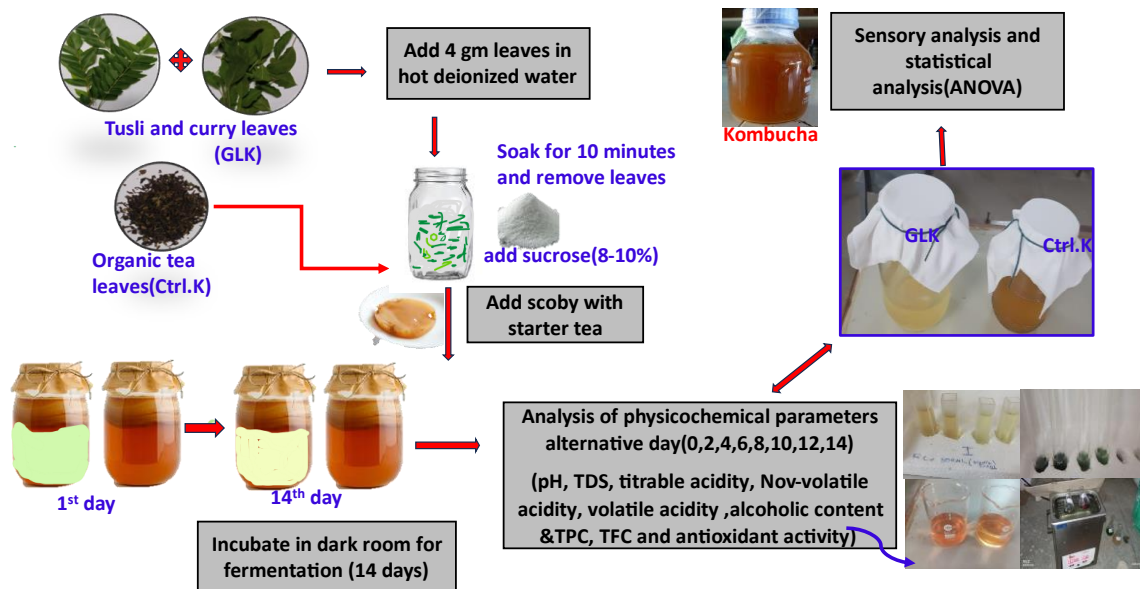


Figure 1. The schematic overview of the key stages and analytical measurements during the 14-day fermentation.

2.6. Sensory evaluation.

In the sensory evaluation, the standard and green leaves kombucha samples were first filtered, arranged in order, and served slightly chilled to volunteer participants. A 5-point hedonic scale was employed to assess the sensory attributes of the samples. This scale ranged from 1 to 5, where 5 indicated “very good”, 4 “good”, 3 “acceptable”, 2 “poor”, and 1 “very poor”. The participants evaluated each sample based on five parameters: taste, aroma, color, sourness, and overall acceptability [21].

2.7. Statistical analysis.

All parameters of control Kombucha and green leaves Kombucha were analyzed in triplicate, with results expressed as the mean \pm standard deviation. Statistical comparison

between control and green Kombucha was performed using two-way ANOVA (MS Excel 2021). In all analyses, the calculated F values exceeded the critical F values, with statistical significance determined at $p < 0.05$.

3. Results and Discussion

The fermentation process of both control and green leaf kombucha was systematically monitored over 14 days to evaluate changes in key physicochemical properties. These included pH, total dissolved solids (TDS), titratable acidity (TA), volatile and non-volatile acidity, alcohol content, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (AA_{DPPH}). The measured parameters provide insights into the biochemical and microbial transformations occurring during fermentation. A comparative summary of all measured properties for control and green leaf kombucha is presented in Table 1. The following sections discuss the observed changes and underlying mechanisms driving these variations throughout the fermentation period.

3.1. pH of Kombucha.

A marked decrease in pH was observed in both control and green leaves kombucha during the fermentation period (Figure 2a). The pH dropped sharply from day 1 to day 8 of fermentation. This acidification continued after day 8, with further substantial decreases observed up to day 14, as pH values declined from 2.12 to 1.5 in the control and from 1.28 to 1.01 in green leaves of Kombucha. These results indicate acidification continued throughout the entire fermentation period. The significant decrease in pH during fermentation results from the buffering effect and synthesis of weak organic acids, which lower the kombucha pH and contribute to its characteristic taste [18,22,23]. The pH of green leaves Kombucha was observed to be lower compared to control Kombucha, likely due to a higher concentration of organic acids. These changes make the Kombucha more biochemically enriched, potentially affecting its sensory qualities and functional properties. Further studies are needed to understand their potential health effects.

Table 1. Physicochemical properties of Control and Green Leaves Kombucha during fermentation.

Fermentation time (Days)	Ctrl. K pH	GLK pH	Ctrl. K TDS	GLK TDS	Ctrl. K TA	GLK TA	Ctrl. K NVA	GLK NVA	Ctrl. K VA	GLK VA	Ctrl. K alcohol (%)	GLK alcohol (%)
0	4.68±0.11	4.75±0.12	155±6.35	355±15.26	0.15±0.00	0.23±0.00	0.08±0.00	0.14±0.00	0.07±0.00	0.09±0.00	0	0
2	3.89±0.07	4.65±0.11	168±6.38	372±14.14	0.6±0.02	1.05±0.03	0.36±0.00	0.41±0.01	0.24±0.00	0.64±0.01	0.24±0.00	0.29±0.00
4	3.65±0.11	2.46±0.07	179±6.26	402±18.09	1.08±0.03	1.74±0.05	0.5±0.01	0.72±0.02	0.58±0.01	1.02±0.03	0.32±0.00	0.38±0.00
6	2.67±0.05	1.75±0.05	193±8.29	442±18.12	1.56±0.05	2.25±0.07	0.69±0.02	0.93±0.03	0.87±0.03	1.32±0.03	0.35±0.01	0.44±0.02
8	2.36±0.06	1.34±0.04	230±10.35	465±18.13	2.19±0.07	2.94±0.11	1.5±0.05	1.71±0.05	0.69±0.02	1.23±0.05	0.39±0.01	0.5±0.02
10	2.12±0.06	1.28±0.04	278±13.06	496±19.84	3.12±0.11	4.35±0.15	2.25±0.08	2.46±0.09	0.87±0.03	1.89±0.06	0.47±0.02	0.56±0.02
12	1.76±0.05	1.20±0.04	302±13.28	626±23.79	4.14±0.16	5.94±0.24	3±0.11	2.97±0.16	1.14±0.05	2.97±0.13	0.62±0.02	0.71±0.03
14	1.50±0.04	1.01±0.03	318±12.40	680±23.81	4.62±0.17	6.42±0.23	3.42±0.11	3.87±0.14	1.2±0.03	2.55±0.08	0.65±0.03	0.73±0.03

Ctrl. K: Control Kombucha; GLK: Green leaves Kombucha; pH=acidity level; TDS: Total Dissolved Solids ppm; TA: Total acidity (%); NVA: Non-volatile acidity (%); VA: Volatile acidity (%); Alcohol (%); Alcohol content.

The ideal pH range for drinking kombucha tea is typically between 2.5 and 3.5. Consuming Kombucha with a pH below 2.5 is not recommended due to potential health concerns related to acidity. Generally, Kombucha fermentation lasts 5–8 days, after which the pH is measured to assess progress and ensure the beverage has reached a safe, acceptable acidity level. Based on our observations, fermentation beyond 8 days results in increased

acidity and reduced palatability, potentially limiting the health benefits and consumer acceptability. Within the 5 to 8 day window, Kombucha develops a balanced profile of organic acids, phenolic compounds, and antioxidant activity, which contributes to its functional properties. Therefore, halting fermentation at this stage not only optimizes flavor and carbonation but also maximizes the beverage's potential health benefits. Prolonged fermentation up to 14 days, while informative for understanding microbial and biochemical changes, may result in over-acidification and diminished sensory qualities, which could be undesirable for regular consumption.

3.2. Total dissolved solids (TDS) of Kombucha.

During fermentation, both control and green leaves kombucha exhibited a significant increase in TDS (Figure 2b). This rise in TDS primarily resulted from the synthesis of weak organic acids, alcohols, and the increased concentration of soluble minerals. The upward trend in TDS indicates active microbial fermentation [24]. Green leaves kombucha consistently showed higher TDS levels than control kombucha, indicating a greater concentration of beneficial compounds. The TDS of green leaves kombucha varied from 355 ppm to 680 ppm, whereas the TDS of control kombucha ranged from 155 to 318 ppm. This higher TDS likely reflects enhanced microbial activity and a richer accumulation of fermentation metabolites, contributing to its distinct chemical profile.

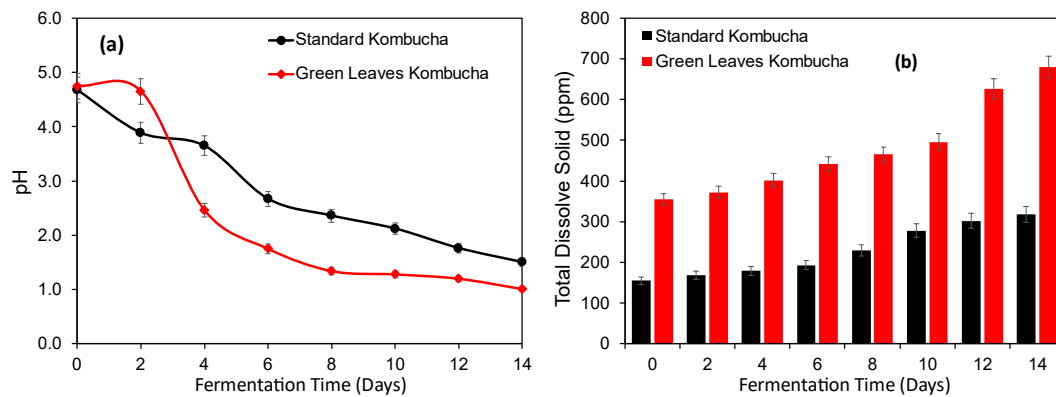


Figure 2. The characteristic changes in (a) pH; (b) total dissolved solids with respect to the fermentation time of Control Kombucha and Green Leaves Kombucha.

3.3. Acidity of Kombucha.

The fermentation of both control and green leaves kombucha was monitored over a 14-day period to track significant changes in TA, volatile, and non-volatile acidity. As shown in Figures 3a and 3b, both types of Kombucha exhibited a significant increase in TA and non-volatile acidity during fermentation. Volatile acidity showed an increasing trend from the 1st day to the 12th day, after which it stabilized in both control and green leaves kombucha (Figure 3c). This increased acidity is primarily due to the production of weak organic acids, such as acetic acid, lactic acid, citric acid, oxalic acid, gluconic acid, glucuronic acid, and amino acids, by the microbial community during fermentation [25,26]. Acidity and pH in Kombucha are strongly correlated; increasing acidity coincides with decreasing pH, as the concentration of these weak organic acids increases (Figures 2a and 3a). Additionally, green leaves kombucha consistently exhibited higher acidity levels compared to control Kombucha.

3.4. Alcohol content of Kombucha.

Alcohols produced during kombucha fermentation have a significant effect on aroma and flavor profile [27]. Alcohol content significantly increased throughout fermentation in both types of Kombucha (Table 1). The concentration steadily rose from the 1st day to the 14th day, reflecting active microbial fermentation and yeast metabolism. SCOBY fermentation primarily produces alcoholic compounds such as ethanol, making kombucha a popular low-alcohol fermented beverage. Kombucha contains a low alcohol content of about 0.5 %, which depends on fermentation duration and sugar concentration, as sugar is metabolized during fermentation [1,4,5]. The results showed that green leaves kombucha consistently produced higher alcohol levels than control kombucha, reaching 0.73 % after 14 days of fermentation compared to 0.65% in control kombucha (Figure 3d). Therefore, green leaves kombucha exhibited a higher alcohol content than control kombucha. Based on these findings, the ideal fermentation period for human consumption is 6–8 days, as green leaves kombucha attains desirable acidity and alcohol levels within this timeframe, offering enhanced health benefits.

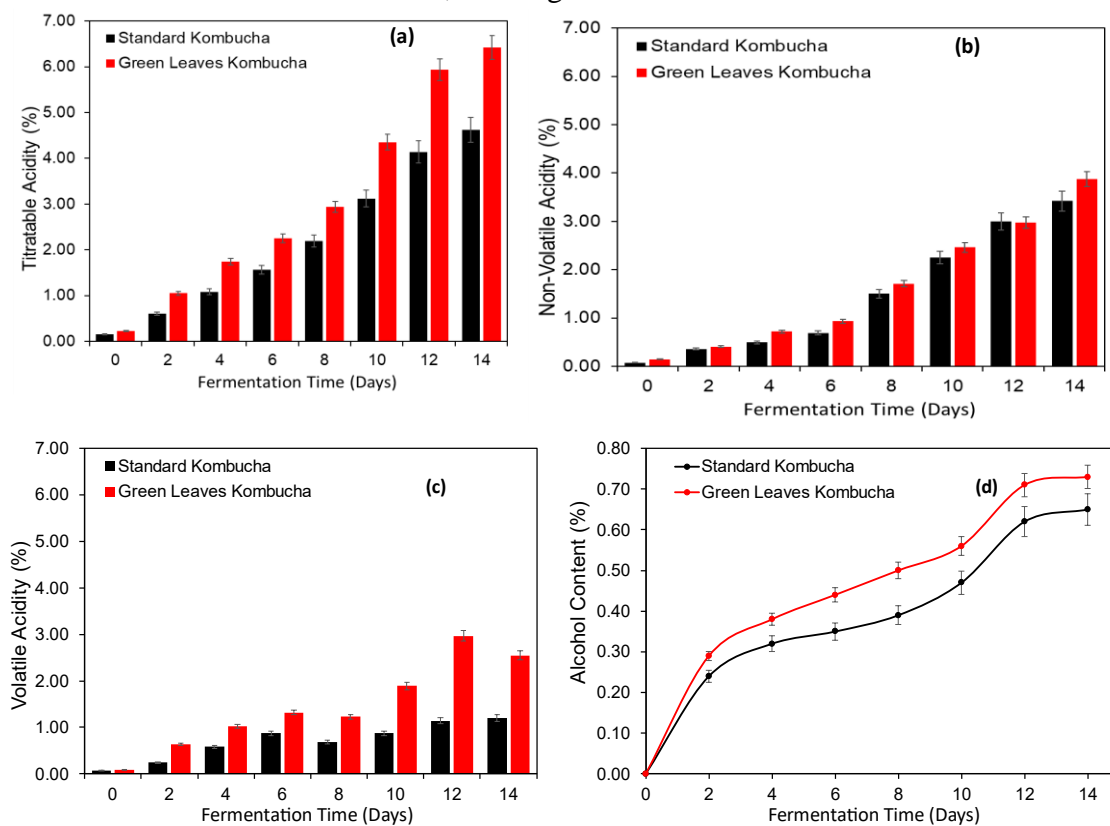


Figure 3. The characteristic changes in (a) titratable acidity; (b) non-volatile acidity; (c) volatile acidity; (d) alcohol content with respect to the fermentation time of Control Kombucha and Green Leaves Kombucha.

3.5. Total phenolic and total flavonoid content of Kombucha.

The fermentation of control and green leaves kombucha was monitored over 14 days by measuring total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity(AA_{DPPH}). The results of these analyses are summarized in Table 2. As shown in Figures 4a and 4b, both types of Kombucha showed significant increases in TPC and TFC during fermentation. TPC showed a steady increase from day 1 to day 12, followed by a slight decrease observed on day 14 in both control and green leaves kombucha. The TPC in green leaves kombucha increased from 177.98 mg GAE/L to 597.73 mg GAE/L, higher than that of control Kombucha, which ranged from 50.23 mg GAE/L to 333.48 mg GAE/L (Table 2). Green leaves

kombucha had significantly higher TPC than control kombucha, likely due to the inclusion of fresh and dried basil leaves and curry tree leaves in its preparation.

Table 2. Total phenolic content, total flavonoid content, and antioxidant activity during fermentation.

Fermentation Time (Days)	Ctrl. K TPC (mg GAE/L)	GLK TPC (mg GAE/L)	Ctrl. K TFC (mg QE/L)	GLK TFC (mg QE/L)	Ctrl. K AA (DPPH % Inhibition)	GLK AA (DPPH % Inhibition)
0	50.23±2.06	177.98±7.47	16.14±0.45	13.28±0.35	5.12±0.13	27.72±0.70
2	100.73±3.92	262.98±9.73	22.65±0.68	19.26±0.60	10.22±0.30	38.01±1.22
4	157.98±6.00	360.48±14.07	34.29±1.13	28.68±0.98	20.53±0.58	48.85±1.66
6	219.73±7.25	443.98±14.21	53.17±1.64	42.12±1.34	21.7±0/59	53.3±1.55
8	270.98±8.67	564.98±19.77	60.63±2.18	47.51±1.66	14.06±0.41	49.72±1.54
10	316.98±11.09	571.48±16.57	54.55±1.58	47.88±1.39	11.05±0.33	49.6±1.49
12	333.48±14.00	597.73±17.93	59.58±1.84	47.2±1.41	19.87±0.62	57.37±2.0
14	320.98±9.30	587.48±18.80	61.64±1.78	47.3±1.46	17.33±0.49	54.71±1.86

TPC: Total Phenolic content; TFC: Total flavonoid content; AA: Antioxidant activity.

TFC of green leaves kombucha increased from 13.28 mg QE/L to 47.88 mg QE/L, which was lower than the TFC of control kombucha, ranging from 16.14 mg QE/L to 61.64 (Figure 3 b and Table 2). Both control and green leaves kombucha showed an increasing trend in TFC from day 1 to day 8 of fermentation. After day 8, TFC levels in green leaves Kombucha remained relatively stable, while in the control kombucha, TFC continued to increase slightly until day 14. Green leaves kombucha exhibited significantly lower TFC compared to control Kombucha. This difference aligns with USDA data, which indicates that organic black tea extract has a higher flavonoid content than basil leaves [28]. Therefore, the lower TFC in green leaves kombucha is attributed to the composition of its ingredients relative to the control kombucha.

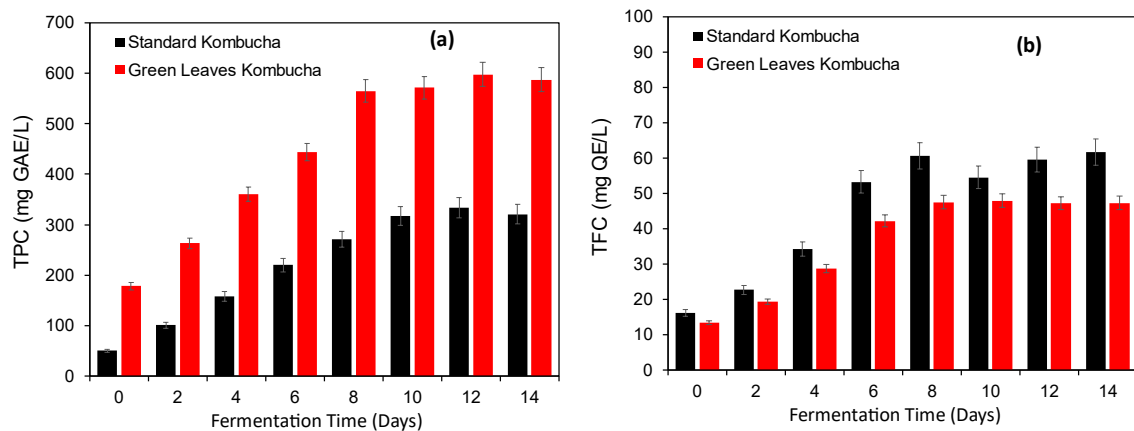


Figure 4. The characteristic changes in (a) total phenolic content; (b) total flavonoid content with respect to the fermentation time of Control Kombucha and Green Leaves Kombucha.

3.6. Antioxidant activity of Kombucha.

The antioxidant activity (AA_{DPPH}) of both samples exhibited significant changes in AA_{DPPH} throughout fermentation (Figure 5). Green leaves kombucha demonstrated significantly higher AA_{DPPH} levels compared to control Kombucha, likely due to the enhanced antioxidant properties of basil and curry tree leaves. The enhanced antioxidant capacity is a major contributor to Kombucha's widespread recognition as a health-promoting beverage [29,30]. A sharp increase in antioxidant activity (AA_{DPPH}) was observed in both control and green leaves Kombucha from day 1 to day 6 of fermentation (Table 2 and Figure 5). After this period, AA_{DPPH} displayed fluctuating patterns, with alternating decreases and increases during the remaining fermentation process. These variations likely reflect dynamic biochemical

processes occurring during fermentation, such as microbial metabolism and substrate utilization. Overall, green leaves kombucha exhibited comparatively higher antioxidant activity, suggesting its potential for greater health benefits.

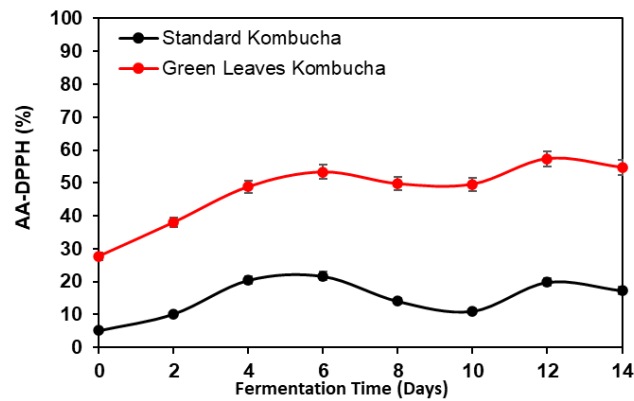


Figure 5. The characteristic changes in antioxidant activity (AA_{DPPH}) with respect to the fermentation time of control Kombucha and green leaves Kombucha.

3.7. Sensory evaluation of Kombucha.

The sensory evaluation of both control kombucha and green leaves Kombucha assessed four distinct characteristic properties: aroma, color, taste, and sourness (Figure 6). Initially, green leaves kombucha exhibited a greenish-yellow color, which transitioned to light yellow or yellow post-fermentation. Organic tea kombucha, conversely, started dark brown and lightened to a light brown hue over the fermentation period (Figure 7).

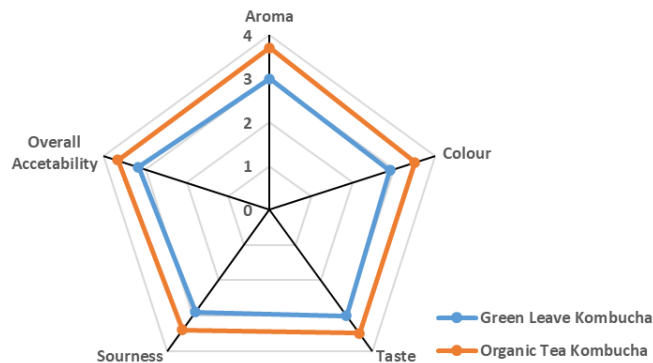


Figure 6. Sensory evaluation of control organic tea Kombucha, and green leaves Kombucha.



Figure 7. Green leaves Kombucha (Light yellow liquid) and control organic tea Kombucha (Amber brown liquid).

Further sensory analysis revealed distinct differences between the two Kombucha formulations. The Green leaves Kombucha exhibited a characteristic herbal and acetic aroma <https://nanobioletters.com/>

profile, whereas the control Kombucha was defined by a more pronounced vinegar-like and traditional tea aroma. In terms of visual appearance, the Green leaves Kombucha appeared slightly turbid, while the control sample showed a relatively hazy appearance. Moreover, the final color of the Green leaves Kombucha was observed as light greenish-yellow, in contrast to the light amber-brown color of the control kombucha. With respect to taste, the Green leaves Kombucha imparted a herbal, mildly sour flavor, whereas the control Kombucha demonstrated a more pronounced sourness and a characteristic tea-based taste.

4. Conclusions

This study successfully developed and characterized “Green Leaves Kombucha,” a novel formulation that provides an alternative to traditional tea-based Kombucha and demonstrates promising health benefits. This research meticulously analyzed the impact of fermentation time and temperature on various physicochemical properties, including pH, total dissolved solids (TDS), titratable acidity (TA), volatile and non-volatile acidity, alcohol content, phenolic compounds, flavonoid compounds, antioxidant activity, and sensory evaluation.

The findings definitively demonstrate several key advantages of the novel Green leaves Kombucha. The prepared Green leaves Kombucha exhibited consistently enhanced physicochemical properties compared to the control formulation. Furthermore, the use of basil and curry tree leaves as fermentation substrates resulted in significantly higher antioxidant activity than Kombucha prepared from organic tea leaves. In addition, both Kombucha formulations demonstrated acceptable sensory qualities, particularly in aroma and taste.

These findings highlight the critical influence of fermentation time on the biochemical composition and functional properties of Kombucha and suggest that incorporating antioxidant-rich botanicals can enhance the beverage's health-promoting potential. Optimizing fermentation parameters can thus be a valuable strategy to improve both the quality and bioactive properties of Kombucha.

Despite the comprehensive analysis conducted, this study was limited by the absence of microbiome characterization and *in vivo* testing to assess the biological effects of Kombucha fully. Future research should prioritize microbial community profiling and extended shelf-life testing to enhance understanding and ensure product stability. Furthermore, research could further explore the mechanistic basis of these changes and investigate fermentation at varying temperatures and durations to tailor kombucha characteristics for specific health benefits and meet diverse consumer preferences.

Author Contributions

Conceptualization, P.C. and K.G.; methodology, K.G.; validation, K.G.; formal analysis, K.G.; investigation, P.C.; data curation, P.C.; writing—original draft preparation, P.C.; writing—review and editing, K.G.; supervision, K.G.; project administration, K.G. All authors have read and agreed to the published version of the manuscript.

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Not Applicable.

Data Availability Statement

All the data presented in this study are shown in the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Supplementary Material

Gallic acid solution complies with Beer's Law with the regression coefficient (R^2) = 0.9931. The standard curve equation: $y = 0.0283x + 0.0468$ (Figure S1).

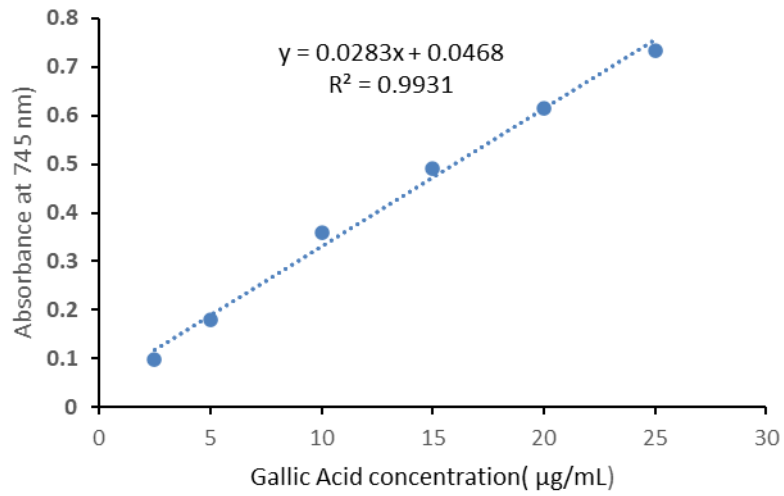


Figure S1. Total phenolic content for standard gallic acid.

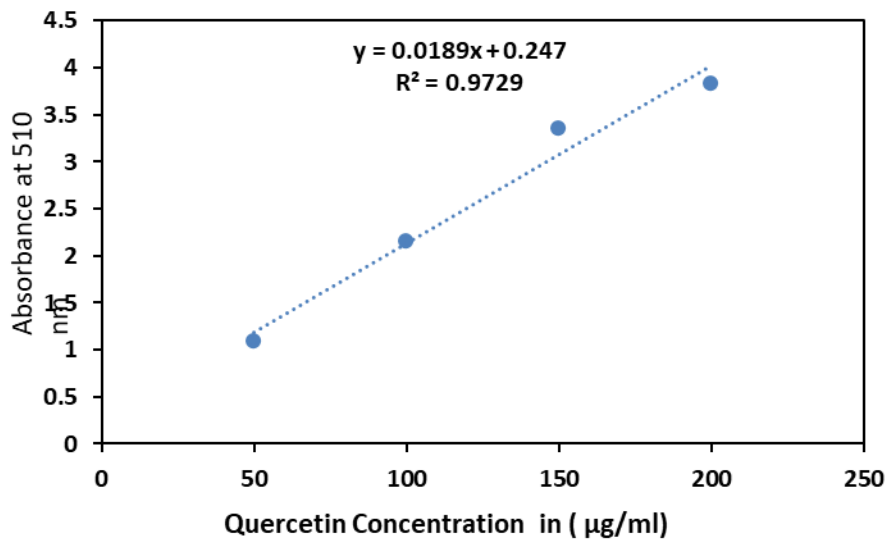


Figure S2. Total flavonoid content for standard quercetin.