Comparative Evaluation of Microbiological, Physicochemical, Nutritional, and Probiotic Properties of Kombucha Produced by Single and Mixed Fermentation of Green Tea Extract and Pineapple Juice

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Abstract

This study investigated the microbial dynamics, physicochemical changes, nutritional profiles, antinutritional content, and probiotic potential of kombucha produced via two fermentation approaches: single fermentation of green tea extract and mixed fermentation involving green tea extract and pineapple juice using symbiotic culture of bacteria and yeast (SCOBY). Microbial enumeration and identification revealed a greater diversity of beneficial microorganisms, particularly *Lactobacillus spp.*, *Acetobacter spp.*, and fermentative yeasts in the mixed fermentation. Notably, mixed fermentation enhanced the presence of probiotic candidates such as *Lactobacillus casei*, *L. plantarum*, *L. fermentum*, and *Saccharomyces spp.* Physicochemical analysis showed a progressive decrease in pH and a corresponding increase in total titratable acidity, indicating active organic acid production. The final pH of mixed fermentation kombucha dropped to 2.99 by day 7 compared to 3.1 in the single fermentation. Proximate analysis indicated increased protein content (23.1 g/100g) and lower moisture content (78.3 g/100g) in mixed fermentation, suggesting better nutrient concentration and shelf stability. Mineral analysis demonstrated appreciable levels of potassium (5.8 mg/100g), magnesium (5.3 mg/100g), and iron (2.6 mg/100g) in the mixed-fermented product, surpassing the levels in the single fermentation counterpart. Antinutritional factors such as tannins, lectins, and trypsin inhibitors were significantly reduced post-fermentation, especially in the mixed fermentation sample, enhancing safety and nutritional quality. Probiotic characterization of seven bacterial isolates under simulated gastrointestinal conditions showed that most isolates exhibited bile salt tolerance and acid resistance, with these strains *Lactobacillus casei*, *L. acidophilus*, *Pediococcus sp.*, and *Streptococcus sp.*) surviving all tested conditions, indicating strong probiotic potential. Sensory evaluation scores were higher for mixed-fermentation kombucha in all parameters, particularly taste (4.7), aroma (4.6), and appearance (4.8), confirming consumer acceptability. The findings suggest that the inclusion of pineapple juice in kombucha fermentation not only enhances microbial diversity and probiotic quality but also improves nutritional value, safety, and sensory appeal, offering a promising functional beverage in africa.

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1. Introduction

Kombucha, a fermented beverage derived from sugared tea using a symbiotic culture of bacteria and yeast (SCOBY), has gained significant attention for its functional health benefits, particularly due to its probiotic potential and associated bioactive compounds. Traditionally brewed from black or green tea, kombucha fermentation yields organic acids, vitamins, and microbial metabolites that contribute to gut health, antioxidant properties, and antimicrobial activity (Greenwalt *et al.*, 2000; Jayabalan *et al.*, 2014) [6, 8].

The integration of fruit substrates such as pineapple, rich in fermentable sugars, enzymes, and vitamins, offers an opportunity to enhance kombucha's nutritional value, microbial diversity, and sensory profile (Malbaša *et al.*, 2008). In sub-Saharan Africa, such bio-enriched functional beverages are of growing importance for addressing micronutrient deficiencies and promoting gut health naturally.

This study compares the microbial, nutritional, antinutritional, and probiotic characteristics of kombucha fermented using (1) green tea extract alone and (2) a combination of green tea extract and pineapple juice. It aims to establish the impact of substrate composition on fermentation dynamics, microbial succession, and product quality.

2. Materials and Methods

2.1. Preparation of Substrates and SCOBY Inoculation

The modified method of Leal *et al*. (2018) was adopted, 200ml of boiled water was added to 3 bags of green tea in a jar for 15 minutes for extraction to take place. Three (3) tablespoonful of sugar was then added, mixed and allowed to cool. The 200ml green tea (*Camellia sinensis*) extract was divided into two equal portions, the first portion containing the green tea extract serve as a control while 100mls of a freshly extracted pineapple juice, was added to the other portion at a ratio of 1:1(green tea extract to pineapple juice). Old *kombucha* drink and SCOBY were added to both portions to initiate the fermentation by providing the initial bacteria and yeast as starter cultures. The set-up was fermented at 35 ^{0}C for 7 days. Sample was taken for analysis at interval (day 1, 3 and 7). During and after the fermentation, physiochemical analysis (temperature, pH, titratable acidity) and microbiological, nutritional and antinutritional analyses were conducted on the fermenting mixtures.

2.2. Experimental Design

- **K**: Green tea extract before fermentation
- **AfGt**: After fermentation of green tea extract
- **B4Gt+Pj**: Before fermentation of green tea + pineapple juice
- **Kbh**: After fermentation of green tea + pineapple juice

2.3. Microbial Enumeration and Isolation

The growth media were measured in grams according to the manufacturers specification and dissolved in a given milliliters (mls) of distilled water in a conical flask, the conical flask was corked with cotton wool and covered with aluminium foil and sterilized in the autoclave at 121°C for a period of 15 minutes. Samples were serially diluted and plated on the growth media ((Potato Dextrose agar, Nutrient Agar, MRS media, Yeast Extract Peptone Dextrose (YPD)) to enumerate fungi, total viable bacteria, lactic acid bacteria (LAB), and yeasts. Isolates were characterized morphologically and biochemically and identified using standard taxonomic keys.

2.4. Probiotic Characterization

2.4.1. Probiotic testing with bile salt solution.

Bile salt solution (0.3%) was prepared by dissolving 0.3 gram of bile salts in 100 ml sterile distilled water and sterilized in an autoclave. Fresh culture of the test probiotic strains was inoculated into 10 ml of sterilized MRS broth and incubated overnight at 37 °C, serial dilution of the overnight culture was done to obtain a suitable concentration for testing. One ml of the diluted probiotic culture (10^3) was mixed with an equal volume (1ml) of the 0.3% bile salt solution (this simulated the conditions of the small intestine where bile salts are present). The mixture was incubated at 37 °C for 24 hrs to assess survival over time. After incubation period, turbidity was an indication of growth under bile salt conditions, it was then inoculated on selective agar (MRS) and the plates were incubated at 37 °C for 24-48 hrs and observed for growth.

2.4.2. Probiotic testing using simulated gastric juice

Simulated gastric juice was prepared by mixing 0.2% hydrochloric acid (HCl) and 0.3% pepsin and pH adjusted to 2.0, it was then sterilized. Single colony of test isolate was inoculated into 10 ml of MRS broth and incubated overnight at 37 °C. A serial dilution of the overnight culture was performed to obtain a suitable concentration for testing. One ml of the diluted probiotic culture (10^3) was mixed with an equal volume (1ml) of the of the simulated gastric juice, this step simulated the acidic environment of the stomach. This mixture was allowed to stay for 4 hrs to assess survival of the test organism. The mixture was incubated at 37 °C for 24 hrs to assess survival over time. After incubation period, turbidity was an indication of growth under bile salt conditions, it was then inoculated on selective agar (MRS) and the plates were incubated at 37 °C for 24-48 hrs and observed for growth.

2.4.3. Probiotic testing using pH 3

Single colony of test isolate was inoculated into 10 ml of MRS broth and incubate overnight at 37 °C, pH was adjusted to 3.0 by dissolving sodium hydroxide (NaOH) and citric acid in distilled water, adjusting with hydrochloric acid, a pH meter was used to confirm the pH. Serial dilution of the overnight culture was performed to obtain a suitable concentration for testing. 1ml of the diluted probiotic culture (10^3) was mixed with an equal volume (1ml) of the pH 3 buffer solution this step simulated the acidic environment of the stomach, incubated at 37 °C for 24 hrs. It was then inoculated on selective agar (MRS) and the plates incubated at 37 °C for 24-48 hrs and observed for growth.

2.5. Physicochemical Analysis

pH, temperature, and total titratable acidity (TTA) were measured at fermentation days 1, 3, and 7 using standard AOAC (2016) [4] methods.

2.6. Proximate and Mineral Analysis

Moisture, crude protein, fat, carbohydrate, and ash were analyzed using AOAC (2016) [4] methods. Mineral content was determined using atomic absorption spectrophotometry and flame photometry.

2.7. Antinutrient Determination

Tannins, lectins, and trypsin inhibitors were analyzed using standard spectrophotometric methods.

2.8. Sensory Evaluation

A 5-point hedonic scale was used to evaluate taste, aroma, texture, and appearance by 20 trained panelists. The cumulative average of the Scores were determined and analyzed for acceptability.

3. Results and discussion

The bar chart illustrates the changes in mean microbial counts (cfu/mL or cfu/g) for yeast, mold, total bacterial count (TBC), and lactic acid bacteria (LAB) at three different stages of fermentation: Before Fermentation, Day 3 of Fermentation, and Day 7 of Fermentation, alongside control samples.

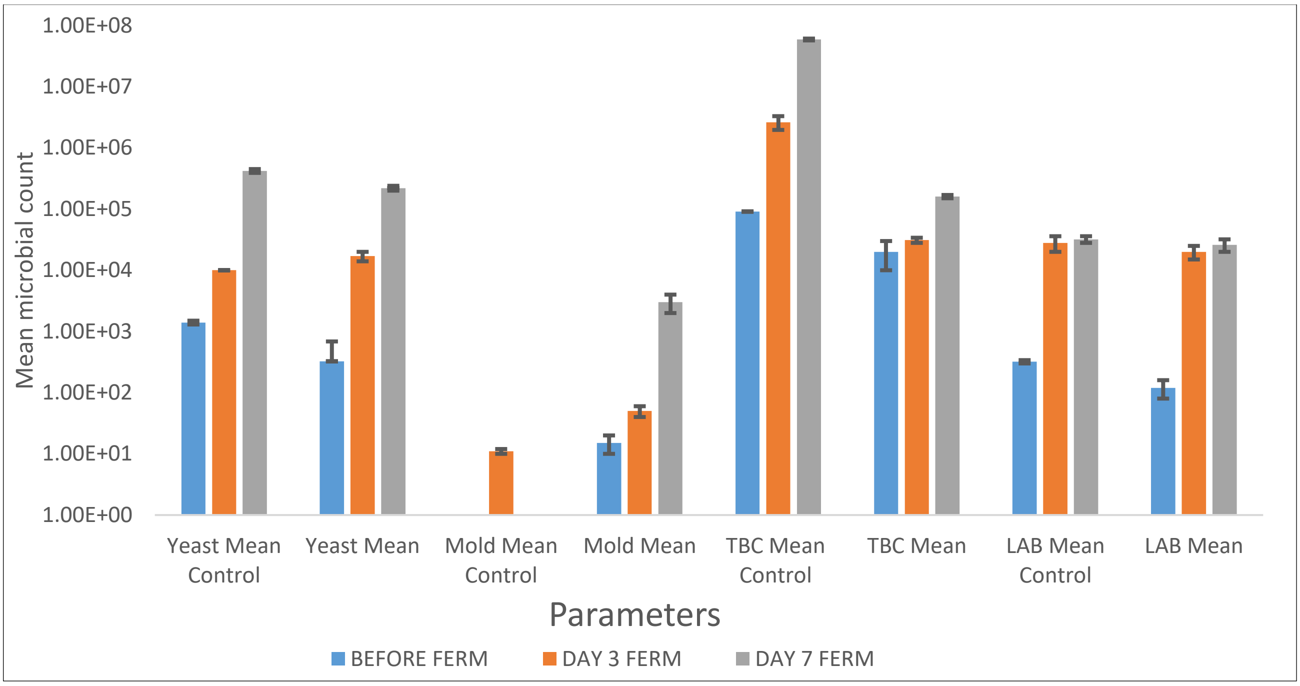

Fig 1: Mean microbial count (cfu/ml) of green tea (control) and *kombucha* (green tea + pineapple juice during fermentation.

The progressive increase in yeast and LAB counts aligns with typical kombucha fermentation dynamics, where yeasts hydrolyze sucrose and produce ethanol, while LAB and acetic acid bacteria ferment the ethanol into organic acids, enhancing probiotic value and acidity (Greenwalt *et al*., 2000; Villarreal-Soto *et al*., 2018) [6, 14]. The significant rise in LAB and TBC by Day 7 confirms active microbial proliferation and metabolic activity, particularly acid production, which contributes to flavor development and preservation. The increase in yeast counts suggests a stable fermentative environment suitable for synergistic microbial interactions. Mold growth, although present, was relatively controlled, likely due to increasing acidity and microbial antagonism, which inhibit mold proliferation—a crucial safety consideration (Marsh *et al*., 2014) [11]. These findings support the microbial robustness and potential probiotic enrichment of the fermented product, especially by Day 7. The combined increase in LAB and yeast counts also signifies potential functional food benefits, as both groups contribute to gut health (Ashaolu, 2020) [2].

Table 1 presents the occurrence of microbial isolates in kombucha made from green tea and a mixture of green tea and pineapple juice, observed at three stages: green tea (K), before fermentation (B4 ferm.), and after fermentation (Af ferm.) of the green tea–pineapple mixture. A total of 20 microbial genera were detected across the fermentation phases, comprising both bacteria and fungi. Before fermentation, a moderate presence of bacteria was observed, including *Streptococcus*, *Micrococcus*, *Bacillus*, and lactic acid bacteria such as *L. acidophilus* and *Pediococcus sp.*, alongside fungal isolates like *Saccharomyces*, *Candida*, and *Rhizopus*. After fermentation, the microbial profile shifted significantly. Notably, *Acetobacter sp.*, *Gluconobacter sp.*, and multiple *Lactobacillus* species (*L. casei*, *L. plantarum*, *L. fermentum*, *L. bulgaricus*) were exclusively detected in the fermented green tea–pineapple kombucha. Fungal species such as *Zygosaccharomyces*, *Brettanomyces*, and *Penicillium* emerged only post-fermentation, whereas *Rhizopus* and *Micrococcus* were absent.

Table 1: Occurrence of Microorganisms in the *Kombucha* (green tea and pineapple juice)

Isolate	Green tea (K)	Before fermentation	After fermentation (Green + pine)
Bacteria			
Bacillus sp.	–	+	–
Streptococcus sp.	+	+	+
Micrococcus sp.	+	+	–
Propionibacter	+	–	+
Gluconobacter sp.	+	–	+
Acetobacter sp.	–	–	+
Lactic acid bacteria			
Lactobacillus casei	–	–	+
Lactobacillus plantarum	–	–	+
L. acidophilus	+	–	+
Pediococcus sp.	+	–	–
Lactobacillus fermentum	–	–	+
Lactobacillus bulgaricus	+	–	+
Fungi			
Zygosaccharomyces	+	–	+
Brettanomyces	+	–	+
Candida tropicalis	+	–	+
Penicillium sp.	–	–	+
Mucor sp.	–	+	–
Saccharomyces sp.	+	+	+
Rhizopus sp.	+	+	–

Key: += Detected; - = None detected

The changes in microbial diversity before and after fermentation indicate active microbial succession driven by substrate composition and environmental conditions. The presence of *Acetobacter* and *Gluconobacter* after fermentation is characteristic of kombucha and essential for the oxidation of ethanol to acetic acid, contributing to acidity and flavor development (Jayabalan *et al.*, 2014) [8]. The detection of diverse *Lactobacillus* species (*L. fermentum, L. plantarum*, *L. casei*) post-fermentation suggests favorable conditions for probiotic growth, likely due to the sugar and micronutrient content of pineapple juice, which may have acted as a prebiotic (Malbaša *et al.*, 2008; Vitas *et al.*, 2013) [15].

Fungal dynamics also reflected typical fermentation trends. The persistence of *Saccharomyces* across all stages, and the emergence of *Zygosaccharomyces* and *Brettanomyces* post-fermentation, align with their roles in ethanol and CO_2 production, contributing to carbonation and flavor (Greenwalt *et al*., 2000) [6]. The decline in spoilage-associated genera such as *Mucor* and *Rhizopus* post-fermentation could be attributed to acidification and competitive exclusion by fermentative microbes.

Table 2 presents the results of the probiotic characterization of six bacterial isolates under simulated gastrointestinal conditions—specifically their tolerance to bile salts (0.3%), low pH (<3), and gastric juice. These conditions mimic the human gastrointestinal tract to evaluate the probiotic potential of the isolates. Lactobacillus casei, L. acidophilus, Pediococcus sp., and Streptococcus sp. demonstrated positive growth in all three conditions, indicating high resilience and potential for survival in the human gut. Lactobacillus bulgaricus and L. plantarum tolerated bile and gastric juice but failed to grow at pH <3, suggesting a limitation in acid tolerance.

Table 2: The probiotic characterization of isolates (simulated conditions)

Isolate	Bile (0.3%)	pH (<3)	Gastric juice
Lactobacillus casei	+	+	+
Lactobacillus bulgaricus	+	–	+
Lactobacillus plantarum	+	–	+
Lactobacillus acidophilus	+	+	+
Pediococcus sp.	+	+	+
Streptococcus sp.	+	+	+

Key: + = Presence of growth, - = Absence of growth

Probiotic efficacy is highly dependent on a microorganism's ability to survive the harsh conditions of the gastrointestinal tract, including acidic stomach pH, presence of bile salts, and gastric enzymes (Monteagudo-Mera *et al*., 2019). The positive responses of *Lactobacillus casei*, *L. acidophilus*, *Pediococcus sp.*, and *Streptococcus sp.* to all tested conditions suggest their suitability as effective probiotic candidates. These species have been widely reported in literature for their acid and bile tolerance, which is critical for colonization and functionality in the intestine (Ashaolu, 2020; FAO/WHO, 2002) [2].

The bile salt resistance exhibited by all isolates aligns with the ability of probiotic bacteria to deconjugate bile salts via bile salt hydrolase (BSH) activity, which aids their survival and helps reduce cholesterol in the host (Begley *et al*., 2005).

Meanwhile, the absence of growth of *L. bulgaricus* and *L. plantarum* at pH <3 may limit their ability to pass through the stomach intact when consumed orally. However, these strains may still confer benefits when used in combination with more robust strains or encapsulated to enhance acid resistance (Ranadheera *et al*., 2014).

Moreover, the overall strong tolerance pattern seen in the isolates confirms their probiotic viability, particularly for potential use in functional beverages like kombucha, which was the fermentation medium in this study. These findings support previous reports that *Lactobacillus* and *Pediococcus* strains isolated from fermented foods often possess desirable probiotic properties (Lawal *et al*., 2022).

Table 3 presents the physicochemical changes observed in the kombucha produced from the mixed fermentation of green tea extract and pineapple juice over a 7-day fermentation period. The data show a continuous decline in pH from 3.53 on day 1 to 2.99 on day 7, indicating increasing acidity. Correspondingly, total titratable acidity (TTA) increased from 0.54% on day 1 to 1.71% by day 7, suggesting progressive organic acid accumulation. Temperature remained relatively stable, ranging between 30°C and 32°C, typical for mesophilic fermentation.

Table 3: characteristics of Kombucha (green tea + pineapple sample during fermentation

Day	pH	Total acid (%)	Temperature (°C)
1	3.53	0.54	32
3	3.13	1.32	30
7	2.99	1.71	31

The progressive decrease in pH and increase in total acid content are indicative of active microbial metabolism during fermentation. The presence of acetic acid bacteria (*Acetobacter*, *Gluconobacter*) and lactic acid bacteria (*Lactobacillus* spp.) likely contributed to the accumulation of acetic, gluconic, and lactic acids, leading to the observed acidification (Jayabalan *et al*., 2014; Malbaša *et al*., 2008) [8]. This acid production not only lowers the pH but also improves product stability by inhibiting spoilage organisms (Vitas *et al*., 2013) [15]. The final pH of 2.99 aligns with reported values for well-fermented kombucha and is considered microbiologically safe for consumption due to the inhibition of pathogenic bacteria under acidic conditions (Greenwalt *et al*., 2000) [6]. The significant rise in TTA, particularly between days 1 and 3 (from 0.54% to 1.32%), reflects rapid acid synthesis during the early stages of fermentation when microbial activity is most intense. The relatively stable temperature range (30–32°C) supports optimal activity of both yeasts and bacteria involved in kombucha fermentation (Teoh *et al*., 2004) [13]. The addition of pineapple juice, rich in sugars, organic acids, and vitamins, likely enhanced microbial growth and fermentation efficiency compared to green tea alone (Malbaša *et al*., 2008; Vitas *et al*., 2013) [15]. In summary, the physicochemical profile during the 7-day fermentation confirms that the mixed substrate (green tea and pineapple juice) created a favorable environment for fermentative microbes, resulting in significant acid production and product acidification. These changes are essential for flavor development, microbial safety, and functional properties of the final kombucha product.

Table 4 shows the progression of pH, total titratable acidity (TTA), and temperature during the 7-day fermentation of green tea kombucha without pineapple juice. The pH of the substrate decreased from 6.53 on day 1 to 3.1 by day 7, reflecting increased acidity. Conversely, TTA increased from 0.3% to 1.5% over the same period. The fermentation temperature showed slight variation, ranging from 29°C to 38°C, with a notable spike on day 7.

Table 4: Characteristics of Kombucha (green tea only) sample during fermentation

Day	pH	Total acid (%)	Temperature (°C)
1	6.53	0.3	30
3	4.2	1.2	29
7	3.1	1.5	38

The gradual reduction in pH and increase in titratable acidity during fermentation suggest active metabolic conversion of sugars to organic acids by the kombucha microbiota, particularly *Acetobacter* and *Lactobacillus* species (Jayabalan *et al*., 2014) [8]. This acidification process is essential for preserving the kombucha, inhibiting spoilage organisms, and enhancing its functional qualities (Greenwalt *et al*., 2000) [7].

Compared to the mixed-substrate fermentation (green tea + pineapple juice), the green tea-only fermentation began at a significantly higher pH (6.53 vs. 3.53), reflecting a lower initial acidity. This difference is likely due to the absence of naturally occurring organic acids and sugars that pineapple juice would otherwise contribute (Malbaša *et al*., 2008). The slower acidification in the early phase (day 1 to day 3) may have allowed for more extended growth of early-colonizing bacteria and yeasts, which were later succeeded by acid-tolerant strains.

The increase in TTA from 0.3% to 1.5% indicates the efficient production of acetic, gluconic, and possibly lactic acids. However, the final pH (3.1) was slightly higher than in the mixed-substrate kombucha (2.99), suggesting that the inclusion of pineapple juice enhances acidification, possibly by stimulating microbial growth with additional fermentable substrates (Vitas *et al*., 2013) [15].

The temperature increase to 38°C by day 7 is noteworthy, as it may have influenced fermentation kinetics and microbial composition. While most kombucha fermentations are carried out between 25–32°C, temperatures above 35°C can favor the growth of thermotolerant acetic acid bacteria but may inhibit some probiotic LAB species (Teoh *et al*., 2004) [13]. In summary, fermentation of green tea alone supports typical kombucha acidification trends but demonstrates slower pH reduction and less pronounced acid accumulation than the green tea–pineapple mixture. These findings suggest that substrate enrichment with fruit juices like pineapple can accelerate fermentation dynamics and enhance microbial metabolism.

Table 5 presents the proximate composition (g/100g) of three kombucha variants: kombucha from green tea after fermentation (AfGt), unfermented mixture of green tea and pineapple juice (B4Gt+Pj), and the mixed fermentation product of green tea and pineapple juice (Kbh). The key parameters assessed include moisture, protein, fat/lipids, mineral content, and carbohydrates. The mixed fermentation sample (Kbh) had the lowest moisture content (78.3 g/100g) and highest protein content (23.1 g/100g) among all samples. The carbohydrate content decreased from 40.2 g/100g in AfGt and 33.3 g/100g in B4Gt+Pj to 30.2 g/100g in Kbh. Notably, mineral content was significantly higher in AfGt (9.8 g/100g) than in both B4Gt+Pj (1.4 g/100g) and Kbh (1.5 g/100g). Fat content remained low across all samples, with a slight decrease observed post-fermentation.

Table 5: The proximate contents of *Kombucha* (green tea and pineapple juice)

Component	AfGt	B4Gt+Pj	Kbh
Moisture	97.8	83.2	78.3
Protein	21.1	19.4	23.1
Fat/Lipids	0.12	0.76	0.52
Minerals	9.8	1.4	1.5
Dietary fibre	ND	ND	ND
Carbohydrate	40.2	33.3	30.2

Key:
B4Gt+Pj – before fermentation of green tea and pineapple juice
AfGt – after fermentation of green tea
Kbh – After mixed fermentation of green tea and pineapple

The results demonstrate that fermentation significantly altered the proximate composition of the kombucha samples, with notable improvements in nutritional quality following mixed fermentation with pineapple juice.

The decrease in moisture content in Kbh compared to B4Gt+Pj and AfGt suggests increased microbial metabolism and evaporation during fermentation, which is typical in kombucha production (Jayabalan *et al*., 2014) [8]. Lower moisture levels may enhance product shelf stability and microbial safety.

The higher protein content in Kbh (23.1 g/100g) can be attributed to microbial biomass production, particularly from *Lactobacillus*, *Saccharomyces*, and *Acetobacter* species, which are known to proliferate during fermentation and contribute to increased nitrogenous compounds (Greenwalt *et al*., 2000) [7]. This result aligns with studies where protein content increased following fermentation of plant-based beverages with probiotic cultures (Malbaša *et al*., 2008).

The carbohydrate reduction in Kbh (30.2 g/100g) compared to the unfermented blend (33.3 g/100g) and green tea kombucha (40.2 g/100g) reflects substrate utilization by fermenting microorganisms, which consume sugars and convert them into organic acids, alcohols, and gases (Vitas *et al*., 2013) [15]. This sugar reduction supports the use of kombucha as a lower-calorie functional drink alternative.

Fat content remained very low in all samples, which is expected as tea and fruit-based substrates contain minimal lipids. The slight reduction in fat post-fermentation may be due to enzymatic breakdown or microbial assimilation (Sreeramulu *et al*., 2000) [12].

Interestingly, mineral content was highest in AfGt (9.8 g/100g), followed by a sharp decline in the mixed fermented product (1.5 g/100g). This may be due to complex formation with organic acids or microbial uptake during mixed fermentation. The mineral leaching effect from tea leaves during extraction could also explain the higher values in AfGt compared to the mixed variants (Battikh *et al*., 2013) [5]. Nonetheless, Kbh retained an appreciable mineral level. Although dietary fiber was not detected in any of the samples (ND), kombucha may still contain non-digestible polysaccharides or microbial metabolites that contribute to gut health but are not captured in standard proximate fiber analysis.

Table 6 outlines the concentrations of selected minerals (mg/100g) in kombucha produced from fermented green tea (AfGt), unfermented green tea + pineapple juice (B4Gt+Pj), and the mixed fermentation of green tea and pineapple juice (Kbh). The minerals analyzed include phosphorus, potassium, magnesium, calcium, sodium, zinc, iron, manganese, and copper.

The fermented green tea (AfGt) exhibited the highest phosphorus content (59 mg/100g), while the unfermented mixture (B4Gt+Pj) and the mixed fermentation product (Kbh) recorded significantly lower values (3.4 and 4.2 mg/100g, respectively). Potassium, magnesium, and zinc were relatively more abundant in the mixed fermentation (Kbh) than in either of the other samples. Notably, calcium, sodium, and copper concentrations declined after fermentation, especially in the Kbh sample. Manganese and iron showed a moderate increase in Kbh, suggesting possible microbial release or concentration during fermentation.

Table 6: Minerals (mg/100g) for Fermented green tea extract and with pineapple juice (kombucha)

Mineral	B4Gt+Pj	AfGt	Kbh
Phosphorus	3.4	59	4.2
Potassium	2.1	4.8	5.8
Magnesium	8.4	4.2	5.3
Calcium	3.2	2.8	1.6
Sodium	1.00	0.52	0.22
Zinc	2.1	4.5	2.6
Iron	1.4	1.54	2.6
Manganese	0.5	0.7	0.9
Copper	0.09	0.33	0.29

Key:
B4Gt+Pj – before fermentation of green tea and pineapple juice
AfGt – after fermentation of green tea
Kbh - After mixed fermentation of green tea and pineapple

Fermentation significantly modulates mineral content in kombucha, and the observed differences among samples may be attributed to substrate composition, microbial metabolism, and elemental interactions during fermentation.

The exceptionally high phosphorus concentration in AfGt (59 mg/100g) compared to the mixed fermentation (4.2 mg/100g) and unfermented blend (3.4 mg/100g) is likely due to tea polyphenol–mineral interactions and extractability from tea leaves.

Tea leaves are known to be rich in phosphorus-containing compounds, and hot water extraction during brewing may liberate these elements (Jayabalan *et al.*, 2014; Malbaša *et al.*, 2008) [8]. However, the significant reduction in phosphorus in Kbh may be due to its utilization by microbes during mixed fermentation or binding with organic acids, making them less extractable (Battikh *et al*., 2013) [5].

The increase in potassium (5.8 mg/100g) and magnesium (5.3 mg/100g) in Kbh suggests that pineapple juice enrichment contributed additional electrolytes and micronutrients. Pineapple is naturally rich in potassium and magnesium, and their retention in the final kombucha product enhances its functional beverage profile (Sreeramulu *et al*., 2000) [12]. Similarly, the moderate increase in iron (2.6 mg/100g) and manganese (0.9 mg/100g) in Kbh compared to AfGt and B4Gt+Pj implies microbial biotransformation or release from bound forms during fermentation.

In contrast, calcium and sodium were significantly reduced post-fermentation, particularly in Kbh, possibly due to microbial assimilation or precipitation with organic acids such as gluconic and acetic acids (Greenwalt *et al*., 2000) [7]. This trend is consistent with earlier findings indicating that prolonged fermentation can reduce certain minerals due to complex reactions or uptake by growing microbial cells (Vitas *et al*., 2013) [15].

Zinc and copper, important co-factors for enzymatic activity, also showed slight variation across the samples. Zinc was highest in AfGt (4.5 mg/100g) and moderately retained in Kbh (2.6 mg/100g), while copper was slightly reduced during fermentation, reflecting its sensitivity to complexation and microbial uptake.

Table 7 presents the concentrations of selected antinutritional factors—tannins, lectins, and trypsin inhibitors—in kombucha formulations at different stages of fermentation. The samples evaluated include the unfermented mixture of green tea and pineapple juice (B4Gt+Pj), the fermented green tea only (AfGt), and the fermented green tea–pineapple juice blend (Kbh).

Tannins were highest in B4Gt+Pj (0.43 mg/100g) but were not detected (ND) in AfGt, and reduced to 0.12 mg/100g in Kbh. Lectin content decreased markedly from 1.96 mg/100g in B4Gt+Pj to 0.14 mg/100g in AfGt and 0.15 mg/100g in Kbh. Similarly, trypsin inhibitors dropped from 0.37 mg/100g in B4Gt+Pj to 0.23 mg/100g in AfGt and further to 0.13 mg/100g in Kbh.

Table 7: Antinutrients for kombucha

Parameter	B4Gt+pj	AfGt	Kbh
Tannins	0.43	ND	0.12
Lectin	1.96	0.14	0.15
Trypsin inhibitors	0.37	0.23	0.13

Key:

B4Gt+pj – Before fermentation of mixture of green tea extract and pineapple juice

AfGt – After fermentation of green tea extract

Kbh – After fermentation of mixture of green tea extract and pineapple juice

The results reveal that fermentation substantially reduces the levels of antinutritional compounds in kombucha beverages, in agreement with previous studies on the beneficial effects of microbial fermentation on food safety and nutrient bioavailability (Adeyemo *et al*., 2022; Jayabalan *et al*., 2014) [1, 8].

Tannins, which are known to interfere with iron absorption and protein digestibility (Awika & Rooney, 2004) [3], were completely eliminated in the green tea-only fermented sample (AfGt) and significantly reduced in the mixed fermentation (Kbh). This reduction is likely due to the tannase enzyme activity of microbial strains such as *Acetobacter* and *Lactobacillus*, which are known to degrade tannins during fermentation (Battikh *et al*., 2013; Sreeramulu *et al*., 2000) [5, 12].

Lectins, carbohydrate-binding proteins that can impair nutrient absorption and cause gastrointestinal discomfort, were reduced by over 90% post-fermentation in both AfGt and Kbh. The near-complete degradation of lectins is likely attributable to proteolytic enzymes produced by fermenting microbes, which hydrolyze complex protein structures (Malbaša *et al*., 2008). This detoxifying effect improves the safety and digestibility of the kombucha beverage.

Trypsin inhibitors, which block protein digestion by inhibiting trypsin enzyme activity, also declined during fermentation. The lowest level (0.13 mg/100g) was observed in Kbh, suggesting that the mixed substrate (green tea and pineapple juice) not only supports robust microbial growth but may also facilitate a more efficient breakdown of antinutrients due to synergistic enzymatic actions from diverse microbial populations (Vitas *et al*., 2013) [15].

The overall trend confirms that kombucha fermentation improves the nutritional profile and safety of the beverage by significantly reducing or eliminating harmful antinutritional factors. The slightly higher retention of tannins in Kbh (0.12 mg/100g) compared to ND in AfGt may be due to residual tannins from pineapple or less complete degradation under the mixed fermentation conditions.

Table 8 shows the cumulative average score of the sensory evaluation of the two kombucha products. Sample G: Green tea kombucha, Sample H: Green tea + pineapple juice kombucha. Sensory attributes assessed by a trained panel using a 5-point hedonic scale included taste, aroma, texture (mouthfeel), and appearance. Sample H consistently scored higher across all parameters: Taste: H (4.7) > G (4.1), Aroma: H (4.6) > G (4.0), Texture: H (4.7) > G (4.6), Appearance: H (4.8) > G (4.6). This indicates that the green tea + pineapple juice kombucha (Sample H) was more preferred in every sensory dimension evaluated.

Table 8: Sensory Evaluation of the Kombucha Products.

Samples	Taste	Aroma	Texture	Appearance
G	4.1	4	4.6	4.6
H	4.7	4.6	4.7	4.8

The sensory results confirm that substrate enrichment with pineapple juice significantly enhances the sensory appeal of kombucha. The higher taste score (4.7) for Sample H reflects the naturally sweet, fruity, and slightly acidic profile imparted by pineapple juice, which balances the astringency of fermented tea. This aligns with earlier findings that fruit-flavored kombucha is generally more palatable to consumers due to its lower perceived bitterness and smoother acidity

(Jayabalan *et al*., 2014; Malbaša *et al*., 2008) [8].

The aroma of Sample H (4.6) was also preferred, likely due to the presence of volatile esters, alcohols, and organic acids derived from both pineapple and microbial metabolism. The integration of pineapple juice during fermentation promotes the formation of fruity and pleasant aromatic compounds, which contribute to consumer acceptability (Vitas *et al*., 2013) [15].

Although texture differences between both samples were minimal (4.7 vs. 4.6), Sample H slightly outperformed G. This may be due to a smoother mouthfeel from pineapple-derived pectins and sugars, which can affect viscosity and sensory perception during fermentation (Greenwalt *et al*., 2000) [7].

In terms of appearance, the marginally higher score for Sample H (4.8) suggests better color appeal and clarity. Pineapple juice likely contributed to a brighter, golden hue, making the beverage more visually attractive, which is a crucial factor influencing consumer preferences (Lima *et al.*, 2018) [9].

Overall, the data indicate that the inclusion of pineapple juice in kombucha production enhances its sensory characteristics, supporting its suitability as a value-added functional beverage with broader market appeal.

4. Conclusion

This study successfully demonstrated the potential of green tea and pineapple juice as complementary substrates for the production of kombucha with improved nutritional, sensory, and functional properties. The use of a mixed fermentation approach, involving symbiotic culture of bacteria and yeast (SCOBY), led to marked changes in the microbial profile, physicochemical characteristics, and nutritional composition of the final beverage.

The microbial succession analysis revealed that while *Streptococcus sp.*, *Saccharomyces sp.*, and *Lactobacillus spp.* were dominant in the post-fermentation phase of the green tea–pineapple kombucha, spoilage organisms such as *Rhizopus* and *Micrococcus* were eliminated. The emergence of beneficial fermentative organisms such as *Acetobacter sp.*, *Gluconobacter sp.*, and *Lactobacillus plantarum* highlighted the selective advantage of the acidic and sugar-rich pineapple–green tea matrix in supporting probiotic development.

The fermentation profile showed a sharper pH decline and greater titratable acid production in the green tea–pineapple kombucha (pH 2.99, TTA 1.71%) compared to green tea alone (pH 3.10, TTA 1.5%), confirming enhanced microbial activity and acidification in the enriched substrate. Temperature remained stable in the mixed fermentation, favoring microbial stability.

Proximate analysis indicated higher protein content (23.1 g/100g) and lower carbohydrate content (30.2 g/100g) in the green tea–pineapple kombucha, suggesting improved nutritional density and possible caloric reduction. Although moisture content decreased, mineral retention was significant, particularly for potassium (5.8 mg/100g), magnesium (5.3 mg/100g), and iron (2.6 mg/100g), which are essential for human metabolic functions.

Fermentation also resulted in the significant reduction of antinutrients, with tannins completely eliminated in green tea-only kombucha and reduced by over 70% in the mixed product. Lectins and trypsin inhibitors followed similar trends, affirming the role of fermentation in detoxification and enhancement of nutrient bioavailability.

Furthermore, several of the microbial isolates demonstrated robust probiotic potential, tolerating low pH, bile salts, and simulated gastric conditions, particularly *L. casei*, *L. acidophilus*, *Pediococcus sp.*, and *Streptococcus sp*. These findings suggest potential application in functional food development.

Lastly, sensory evaluation confirmed that the green tea–pineapple kombucha was significantly more acceptable in terms of taste (4.7), aroma (4.6), texture (4.7), and appearance (4.8) than the green tea-only variant, making it more marketable and consumer-friendly.

In conclusion, the incorporation of pineapple juice into green tea kombucha production enhanced microbial diversity, improved fermentation dynamics, reduced antinutrients, enriched nutritional content, and improved sensory attributes, thereby validating its suitability as a health-promoting, functional beverage with probiotic potential.

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