Fortified Kombucha: A Functional Beverage Infused With Banana And Pineapple By-products For Nutritional Enrichment

Krithikashruthi Parthasarathy ,Sneha Naidu, David Elisha Henry*, Hanumantharaju K N, Dr Rajadurai M

Dept. of Food Technology, FLAHS, MS Ramaiah university of Applied Sciences, New BEL Road, Bengaluru- 560 054

*Corresponding author: Dr. David Elisha Henry.

Abstract

The current study focuses on developing a kombucha with value addition, combining convenience, portability, and its nutritional content. Kombucha, a fermented tea beverage rich in bioactive compounds such as antioxidants and phenolics, is recognized for its health-promoting properties . Our study evaluates green and black tea variations, sweetened with sugar, undergoing fermentation for 7–14 days using a culture called symbiotic culture of bacteria and yeast (SCOBY). Significant pH reductions were observed during fermentation, with green tea kombucha decreasing from pH 5 to 3. To enhance flavour and sustainability, we infused the fermented kombucha with pineapple pulp, pineapple peel, and banana peel, effectively reducing food waste (Phung et al., 2023a). This innovative approach ensures portability, offering a refreshing, convenient, and eco-friendly beverage option. By leveraging the nutritional richness of kombucha and infusing it with sustainable ingredients, this project addresses the growing demand for nutritious, flavourful, and sustainable beverages. It not only promotes health and wellness but also aligns with eco-conscious practices, making kombucha accessible and appealing to a broader audience. This effervescent format provides a versatile and easy-to-use product, encouraging sustainable consumption while delivering the health benefits of kombucha in an innovative and practical form.

Introduction

Kombucha is a fermented tea beverage that has garnered widespread attention for its potential health benefits, especially due to its probiotic, antimicrobial, and antioxidant properties. It is believed to have originated in Northeast Asia, with historical accounts tracing its use back to ancient China around 220 BCE. Early uses of kombucha included its role in promoting vitality, boosting energy, and assisting with detoxification (Costa et al., 2023; B. Wang et al., 2022a). In ancient China it was revered as the "tea of immortality" (Chou et al., 2024). The process of making kombucha involves fermenting sweetened tea with a symbiotic culture of bacteria and yeast (SCOBY). This fermentation process generates the drink's characteristic tangy, effervescent flavour and is the result of a complex interplay between various microorganisms (Antolak et al., 2021a). Over centuries, its use expanded globally, with increasing recognition of its potential benefits, including digestive health support, antimicrobial activity, and detoxification (Júnior et al., 2022a). The objectives of this research is to evaluate the impact of kombucha on gut health and its antioxidant properties, formulate a kombucha product using banana and pineapple pulp with their peels, develop a shelf-stable dehydrated formulation, and assess its microbiological, nutritional, and sensory characteristics. (Nyhan et al., 2022)

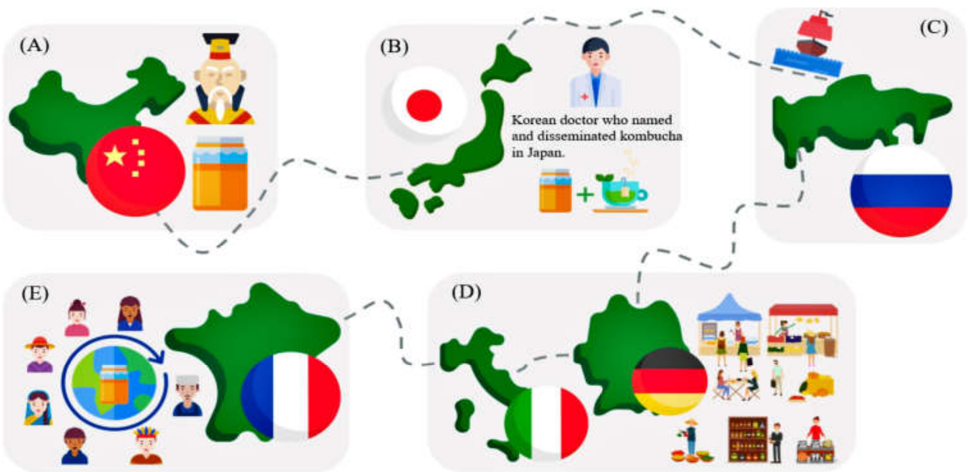


Figure 1: The journey of kombucha from its origins in China to global popularity (Leal et al., 2018)

The essential ingredients in kombucha as mentioned above include tea (typically black or green), sugar, and the SCOBY. Tea serves as a foundation rich in polyphenols and caffeine, which are essential for the metabolic activities of the microorganisms during fermentation. The sugar added to the tea acts as a fuel source for fermentation, where it is converted by the SCOBY into a variety of organic acids, alcohols, and other metabolites(Júnior et al., 2022b; Nyhan et al., 2022). The SCOBY itself is composed of a mix of bacteria and yeast, such as *Acetobacter*, *Gluconacetobacter*, and *Saccharomyces*. These microbes collaborate in the fermentation process to produce kombucha's unique properties (Antolak et al., 2021b; Júnior et al., 2022b)

Probiotics: Live microorganisms that, when consumed in adequate amounts, offer health benefits by helping to balance gut microbiota and modulate the immune system. In kombucha, these probiotics are thought to enhance digestive health by supporting the growth of beneficial bacteria and inhibiting the proliferation of harmful microorganisms (Kozyrovska et al., n.d.; Sengun & Kirmizigul, 2020)

Health benefits

Antioxidants

Bioactive compounds that protect cells from oxidative damage by neutralizing harmful free radicals. These antioxidants play a critical role in preventing cellular damage, reducing inflammation, and minimizing the risk of chronic diseases(Anantachoke et al., 2023; Kitwetcharoen et al., 2023a). Kombucha’s antioxidant properties are largely attributed to the tea polyphenols and the metabolites produced during fermentation (Jakubczyk et al., 2024). The gut microbiome plays a critical role in maintaining overall health by influencing digestion, immunity, and mental well-being. Kombucha, a fermented tea known for its probiotic content, has emerged as a versatile functional beverage that supports gut health, metabolic functions, and cognitive wellness (Selvaraj & Gurumurthy, 2023a)

Gut Health and Probiotic Benefits

Kombucha promotes a healthy gut microbiome by enhancing microbial diversity and functionality (Costa et al., 2022). Its live cultures improve nutrient absorption, helping the body derive maximum benefits from food. Additionally, the probiotics in kombucha reduce inflammation in the gut, which is essential for preventing digestive issues. Studies have highlighted its effectiveness in managing conditions such as irritable bowel syndrome, making it a valuable aid for digestive health (Ecklu-Mensah et al., 2024; Fraiz et al., 2024)

Antimicrobial Properties

One of kombucha's standout qualities is its ability to inhibit harmful pathogens. Acetic acid and polyphenols formed during fermentation exhibit strong antimicrobial properties, effectively targeting bacteria like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* (Greenwalt et al., 2020). These effects vary depending on the type of tea and fermentation process, emphasizing the importance of how kombucha is brewed to maximize its health benefits (Al-Mohammadi et al., 2021)

Support for Metabolic Health

Kombucha shows promise in improving metabolic health, particularly in managing type 2 diabetes. A recent study demonstrated that consuming kombucha daily for four weeks significantly reduced fasting blood sugar levels from 164 to 116 mg/dL (Rahimi et al., 2023). Its antioxidant properties help combat oxidative stress and inflammation, two key factors contributing to insulin resistance. Furthermore, kombucha enhances lipid metabolism, reducing triglyceride levels and supporting overall metabolic function (Kitwetcharoen et al., 2023b).

The Gut-Brain Connection and Cognitive Health

A healthy gut microbiome is closely linked to cognitive function, and kombucha plays a role in maintaining this balance. Its probiotics help stabilize gut bacteria, positively impacting mental health and cognitive performance(Ecklu-Mensah et al., 2024; H. X. Wang & Wang, 2016). Additionally, the antioxidants in kombucha protect brain cells from oxidative stress, which is often associated with neurodegenerative diseases (Birben et al., n.d.; Davies, 2000).This dual action highlights kombucha's potential in supporting both gut and brain health.

Anti-Inflammatory and Chronic Disease Management

The anti-inflammatory properties of kombucha, driven by organic acids and polyphenolic compounds, make it beneficial for managing chronic diseases. It has been shown to alleviate inflammation-related conditions such as arthritis and inflammatory bowel disease (Ivanišová et al., 2019;Al-Mohammadi et al., 2021).Moreover, kombucha's antioxidant and gut-enhancing effects support the management of chronic conditions like hypertension, obesity, and diabetes by regulating metabolic processes and reducing oxidative stress (Ecklu-Mensah et al., 2024; Selvaraj & Gurumurthy, 2023b).

Materials:

Chemicals:

All the chemicals and reagents used in the present investigation were of analytical grade and procured from NICE Chemicals (P) LTD., Kerala and S D fine-chem limited, Mumbai. The microbiological media used in the present investigation were procured from Himedia Pvt. Ltd, Mumbai.

Culture collection: SCOBY

The Symbiotic Culture Of Bacteria and Yeast (SCOBY) the probiotic culture that is used in this is maintained at -80°C and subculture prior to use. The SCOBY was procured from a high-end platform named "Happy Live Culture" which is located in Bangalore and they have in house production of SCOBY. SCOBY, a Symbiotic Culture of Bacteria and Yeast, is crucial for kombucha production. It contains lactic acid bacteria (LAB), acetic acid bacteria (AAB), and yeasts that ferment sweetened tea, producing its tangy taste, fizz, and probiotics. During fermentation, SCOBY consumes sugar, creating organic acids, ethanol, and carbon dioxide. LAB provides probiotics that boost digestion and immunity, while kombucha also offers B vitamins and bioactive compounds. The composition of SCOBY varies with tea type, fermentation time, and storage conditions, optimizing health benefits as the culture matures.

Food grade:

The ingredients used in the kombucha formulations included tea bags, sulphur-less sugar, *Poovan* variety of ripe bananas, and mature *Smooth Cayenne* variety of pineapple, all sourced from the local market. Ripe banana peels were specifically utilized for their antioxidant content (Júnior et al., 2022a; Phung et al., 2023a). Taj brand black tea leaves and Tetley brand green tea leaves were chosen for their quality and antioxidant properties. The *Poovan* banana variety is known for its antioxidant-rich profile, including high levels of vitamin C and polyphenols, while the mature *Smooth Cayenne* pineapple variety is recognized for its rich antioxidant content, particularly due to bromelain and phenolic compounds, enhancing its health benefits (Phung et al., 2023a).

Methodology:

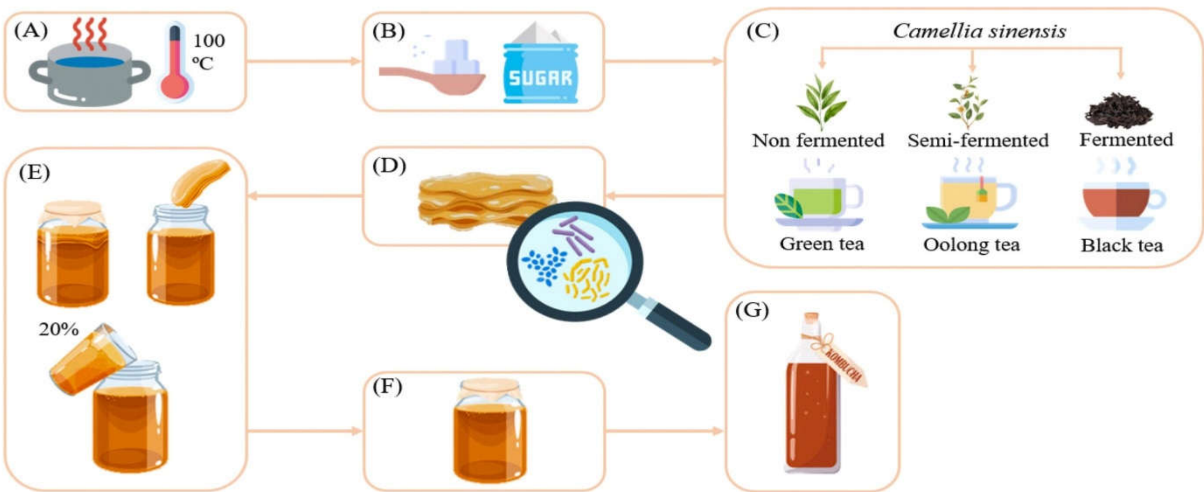


Figure 2: Process of kombucha preparation (Leal et al., 2018)

Preparation of Kombucha: To prepare kombucha, boil 500 mL of water and steeping two green tea bags. Once the tea cools to 75°C, boil it for 3 more minutes and add 2 tablespoons of sulfur-free sugar.

Allow it to cool further to 20°C, then pour 250 mL of the tea into glass jar.(Akbarirad et al., 2017; B. Wang et al., 2022b)

Addition of probiotic culture: Introduce SCOBY (Symbiotic Culture Of Bacteria and Yeast). Cover the jars with a cotton cloth and let them ferment at room temperature for 14 days, during which a baby SCOBY will develop. Once this process is completed the unflavoured kombucha is obtained as it is shown in the

Incorporation of fruit pulp and peels: After fermentation, wash the pineapple and the banana in a clean water and remove the peels and weigh it. Make the pineapple into pulp and add it into the fermented unflavoured green tea kombucha. Now add the pineapple peels and the banana peels to it and allow it to ferment for an additional 7 days. So, this process enhances the flavour and the nutritional properties of the kombucha.

Analysis:

Proximate Analysis of Kombucha- Green Tea and Black Tea Infused with Pineapple Peel, Pulp, and Banana Peel

Proximate analysis is a critical step in determining the nutritional composition of food or beverage samples. In this study, the proximate composition of kombucha prepared using green tea and black tea, infused with pineapple peel, pulp, and banana peel, was analysed following the standardized **AOAC 2016 (Association of Official Analytical Chemists)** methods. The parameters analysed include energy, carbohydrates, protein, fat, total fiber, soluble fiber, and ash content. The results provide insight into the nutritional value of the kombucha samples and their potential health benefits.

Below is the table format designed to include the values obtained for each parameter after analysis for both **green tea kombucha** and **black tea kombucha**:

Table 1: Comparative Analysis of Green Tea and Black Tea Kombucha Nutritional Values

Parameters	Green Tea Kombucha (/100ml)	Black Tea Kombucha (/100ml)
Energy (kcal)	17.6	18.8
Carbohydrates (g)	3.6	3.9
Protein (g)	0.8	0.8
Fat (g)	Nil	Nil
Total Fiber (g)	0.4	0.4
Soluble Fiber (g)	0.3	0.3
Ash (mg)	0.2	0.2

Description of Parameters

1. **Energy:** Represents the caloric content of the kombucha samples, calculated based on macronutrient composition.
2. **Carbohydrates:** Quantifies the sugar and other carbohydrate content available in the beverage.
3. **Protein:** Measures the nitrogen content to estimate the protein level, which contributes to the overall nutritional profile.
4. **Fat:** Indicates the total fat content, including lipids and fatty acids, providing insights into the beverage's lipid profile.
5. **Total Fiber:** Refers to the sum of soluble and insoluble fibers in the kombucha, essential for digestive health.
6. **Soluble Fiber:** Assesses the fraction of fiber that dissolves in water and supports gut health.
7. **Ash:** Represents the total mineral content in the sample, an indicator of its inorganic constituents.

Methodology: The analysis was performed following AOAC-recommended methods to ensure accuracy and reproducibility. Each parameter was measured in triplicate to account for variability, and results will be expressed as means ± standard deviations.

This proximate analysis aims to compare the nutritional profiles of kombucha brewed from green tea and black tea, identifying potential differences and the influence of pineapple peel, pulp, and banana peel infusion on the final composition.

Total Sucrose Content

The sucrose content in kombucha was determined using a spectrophotometric method. First, the kombucha sample was treated with a reagent that reacts specifically with sucrose. The resulting colour change was measured using a spectrophotometer, and the concentration of sucrose was determined based on the absorbance at a specific wavelength. This method allowed for the quantification of sucrose levels before and after fermentation, offering insights into the fermentation dynamics. **This test was performed to determine the amount of sucrose utilized during fermentation and to monitor the progress of microbial activity** (B. Wang et al., 2022a)**.**

Table 2: Total Sucrose content in Green Tea and Black Tea Kombucha

Test	**Green Tea Kombucha (**/100ml)	**Black Tea Kombucha (**/100ml**)**
Total Sucrose Content(g)	0.6	0.8

Vitamin C (Ascorbic Acid)

The Vitamin C content was quantified using the DCPIP titration method. In this method, the kombucha sample was titrated with a standard DCPIP (2,6-dichlorophenol-indophenol) solution, which acts as an indicator. The titration involves the reduction of DCPIP by ascorbic acid, causing the solution to change from blue to colourless. The volume of DCPIP solution required to reach the endpoint was recorded, and the results were used to calculate the concentration of Vitamin C in the sample. **This test was performed to assess the antioxidant potential and nutritional content of the kombucha** (Phung et al., 2023b)

Table 3: Vitamin C content in Green Tea and Black Tea Kombucha

Test	**Green Tea Kombucha(/100ml)**	**Black Tea Kombucha (/100ml)**
Vitamin C Content (mg)	22	20

Titratable Acidity

Titratable acidity (TA) is a key parameter in kombucha as it reflects the overall acid concentration, which directly impacts its tartness, flavor profile, and microbial stability. TA was measured by titrating a diluted kombucha sample with 0.1 N sodium hydroxide using phenolphthalein as an indicator. The endpoint, marked by a light pink colour, was used to calculate acidity as grams of acetic acid per 100 mL. Higher TA values indicate longer fermentation times, with increased organic acid production and reduced sugar content, shaping the sensory and functional qualities of kombucha.

Table 4: Total Titratable Acidity in Green Tea and Black Tea Kombucha

Test	**Green Tea Kombucha (/100ml)**	**Black Tea Kombucha (/100ml)**
Titratable Acidity (g)	0.41	0.48

Anti-oxidant Analysis

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay

The DPPH assay was used to evaluate the free radical scavenging ability of kombucha. A DPPH solution was mixed with kombucha, and the decrease in absorbance was measured at 517 nm after a period of incubation. The decrease in absorbance corresponds to the neutralization of free radicals, indicating the antioxidant activity of the kombucha(Lushchak, 2015). **This test was performed to evaluate the antioxidant capacity of kombucha, which may contribute to its potential health benefits** (Chou et al., 2024; Jakubczyk et al., 2020)**.**

Table 5: Total Antioxidant capacity in Green Tea Kombucha

Test	**Green Tea Kombucha (/100ml)**
DPPH Scavenging Activity (mg)	92.8

Total Flavonoids

Total flavonoid content was determined using the aluminum chloride colourimetric method. Kombucha was mixed with an aluminum chloride reagent, and the absorbance was measured at 415 nm. The concentration of flavonoids was calculated by comparing the absorbance to a standard curve of quercetin. **This test was performed to quantify the flavonoid content in kombucha, which are known for their antioxidant properties** (Jakubczyk et al., 2020)**.**

Table 6: Total flavonoid content in Green Tea Kombucha

Test	**Green Tea Kombucha (/L)**
Total Flavonoids (mg)	424

Microbiological Analysis

Microbiological analysis was performed using selective agar media to assess the presence of specific microorganisms in kombucha.

Potato Dextrose Agar (PDA)

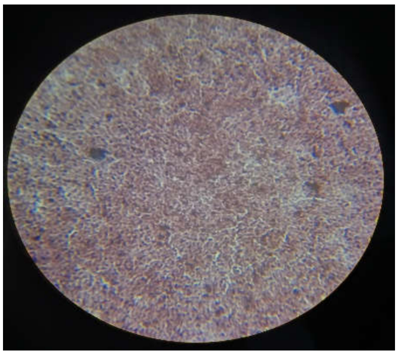


Figure 3: PDA agar plate (10^{-6} dilution) observed under the microscope at 100x magnification

To isolate and enumerate yeast, a sample of kombucha was plated onto Potato Dextrose Agar (PDA). The plates were incubated at 25°C, and the yeast colonies were counted after 48-72 hours. **This test was performed to evaluate the yeast content in kombucha, which is crucial for the fermentation process and the production of beneficial metabolites (Zhao et al., 2020).**

Observation: The PDA plates showed growth of single white colonies, but Gram staining revealed the presence of red, rod-shaped bacteria instead of yeast.

Interpretation: Although PDA typically supports yeast and fungal growth, the presence of rod-shaped bacteria suggests that bacteria are predominant in the kombucha sample. The absence of yeast growth indicates that either the yeast population was too low to thrive under the conditions or it was outcompeted by the bacteria.

Rose Bengal Agar: Total Fungi

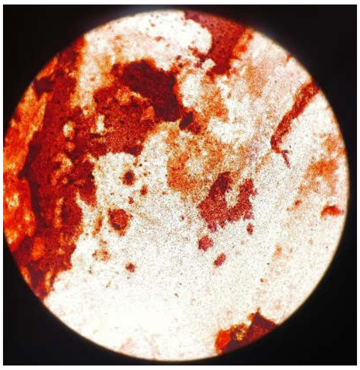


Figure 4.1

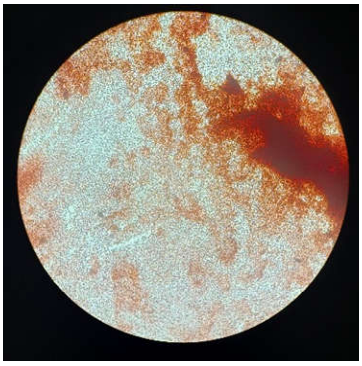


Figure 4.2

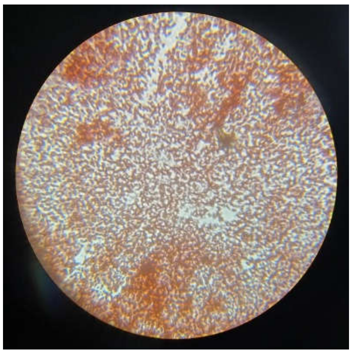


Figure 4.3

Figure 4.1:RBA agar plate (10^{-5} dilution) observed under the microscope at 10x magnification

Figure 4.2:RBA agar plate (10^{-5} dilution) observed under the microscope at 40x magnification

Figure 4.3:RBA agar plate (10^{-5} dilution) observed under the microscope at 100x magnification

For fungal enumeration, the kombucha sample was plated onto Rose Bengal Agar, which is selective for fungi. The plates were incubated at 25°C for 3-5 days, and fungal colonies were counted. **This test was performed to determine the presence of fungi in kombucha, ensuring the quality and safety of the product** (La Torre et al., 2021)**.**

Observation: White colonies appeared on RBA plates, but Gram staining revealed red, rod-shaped bacteria instead of fungal growth.

Interpretation: The observation of white colonies on Rose Bengal Agar (RBA), later identified as Gram-negative, red, rod-shaped bacteria, suggests the presence of Acetobacter and Gluconobacter, which are common and abundant in kombucha. These bacteria are integral to the fermentation process, contributing to the production of acetic acid and gluconic acid. Their growth on RBA, despite its selectivity for fungi, highlights their prevalence in kombucha. This finding does not necessarily indicate contamination but rather reflects the microbiota typical of kombucha fermentation. However, monitoring their levels is essential to ensure product quality and safety.

MRS Agar: Lactic Acid Bacteria

Lactic acid bacteria (LAB) were isolated by plating kombucha on MRS agar, a selective medium for LAB. The plates were incubated at 30°C for 48 hours, and LAB colonies were counted . **This test was performed to quantify the presence of beneficial lactic acid bacteria in kombucha, which are important for fermentation and contribute to its probiotic potential** (Bishop et al., 2022)**.**

Observation: White colonies were observed on MRS agar plates after incubating the kombucha sample at 30°C for 48 hours.

Interpretation: The white colonies observed on MRS agar are used to indicate the presence of lactic acid bacteria (LAB), as MRS agar is selective for their growth. LAB are essential contributors to the fermentation process in kombucha, producing lactic acid and other metabolites that enhance its probiotic properties. The growth of these colonies confirms the presence of beneficial LAB, supporting the functional and probiotic potential of the kombucha.

EMB Agar: Coliforms

For coliform detection, kombucha was plated on Eosin Methylene Blue (EMB) agar, a selective medium for coliforms. The plates were incubated at 37°C for 24-48 hours, and the presence of coliform colonies was observed . **This test was performed to assess the safety of kombucha by detecting potential contamination by coliform bacteria** (Bishop et al., 2022)**.**

Observation: White colonies were observed on EMB agar, plates EMB agar plate (10^{-6} dilution), EMB agar plate (10^{-5} dilution), EMB agar plate (10^{-5} dilution) after incubating the kombucha sample at 37°C for 48 hours.

Interpretation: EMB agar is designed to detect coliform bacteria, which usually show a characteristic metallic sheen. The white colonies indicate non-coliform bacteria, possibly LAB or other aerobic bacteria, confirming the absence of coliforms and ensuring the kombucha sample’s safety.

Kombucha Sample Stained with Methylene Blue

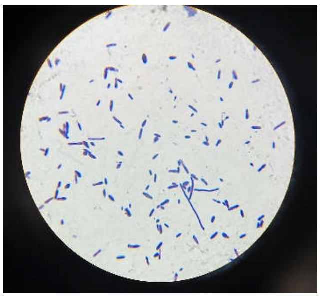


Figure 5: Kombucha stained with Methylene Blue and observed under microscope (100x)

Observation: Upon staining the kombucha sample with methylene blue, rod-shaped bacteria were observed alongside round structures with vacuoles.

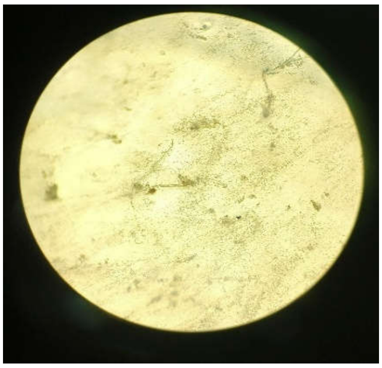
Microscopic Observation: Rod-Shaped Bacteria: At low magnification (10x), clusters of rod-shaped bacteria became visible. Upon increasing the magnification to 40x, the cells exhibited distinct, elongated, and thin shapes typical of Acetobacter or Gluconobacter . These bacteria stained blue, indicating their ability to retain the methylene blue stain, which highlights the cytoplasm.

Round Structures with Vacuoles: Under higher magnification (100x), round cells with vacuoles inside them were observed. These structures appeared less stained compared to the rod-shaped bacteria, indicating that they may be yeast cells or certain bacterial forms. The vacuoles within these cells were visible as clear, empty spaces, a characteristic of yeast cells undergoing fermentation.

Interpretation:

The presence of rod-shaped bacteria alongside round structures with vacuoles in the methylene blue-stained kombucha sample suggests the presence of both bacterial and possibly yeast cells. The rod-shaped bacteria are likely to be Acetobacter or Gluconobacter, which are typical in kombucha and contribute to its fermentation process. The round structures with vacuoles are likely yeast cells, which are also integral to kombucha fermentation. The vacuoles in the yeast cells could indicate active metabolism or storage of nutrients. This mixed microbial population is typical of kombucha, with both bacteria and yeast working together to produce the characteristic flavor and probiotic benefits of the beverage.

SCOBY observation under the Microscope



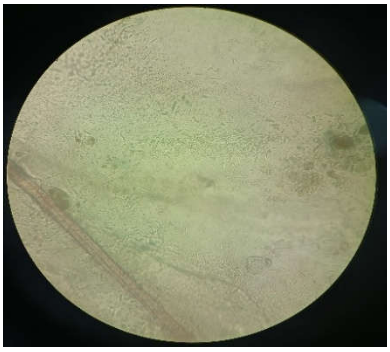


Figure 6.1: SCOBY observed under the microscope at 10x magnification

Figure 6.2: SCOBY observed under the microscope at 40x magnification

Observation: Upon examining the SCOBY under the microscope, rod-shaped bacteria and cellulose were clearly observed.

Microscopic Observation: Rod-Shaped Bacteria: When the sample was viewed under low magnification (10x), rod-shaped bacteria were clearly visible within the gelatinous matrix. Upon switching to a higher magnification (40x), the bacteria appeared as distinct, well-defined rods, consistent with Acetobacter species. These bacteria appeared to be arranged in long chains, indicating a high density within the SCOBY.

Cellulose Structure: The cellulose fibers were visible as long, thread-like structures throughout the sample, particularly at high magnification (100x). These cellulose strands, produced by the bacteria, formed the matrix of the SCOBY, providing a gelatinous structure.

Interpretation:

The rod-shaped bacteria observed in the SCOBY are likely Acetobacter or Gluconobacter or Lactic Acid Bacteria (LAB), important for the fermentation process. These bacteria play a important role in the production of lactic acid, contributing to its characteristic sourness of kombucha.

The cellulose fibers observed are primarily produced by Acetobacter species, which are responsible for the production of cellulose in the SCOBY. These bacteria form the gelatinous structure that forms a layer in the surface of kombucha fermentation, aiding in the separation of the liquid from the yeast and bacteria.

Sensory analysis:

A sensory evaluation was conducted to assess the characteristics of three kombucha formulations: a control sample (without any infusion), Sample 1 (green tea-based kombucha infused with pineapple peel, pulp, and banana peel), and Sample 2 (black tea-based kombucha infused with pineapple peel, pulp, and banana peel). A total of 29 participants were selected for the evaluation. The sensory attributes evaluated included appearance, colour, taste, flavour, aroma, and overall acceptability. Panellists rated each parameter on a 9-point hedonic scale.

Figure 7: Fermentation Profile: pH, Total solids, Alcohol Content, and Time for Green Tea vs. Black Tea Kombucha

Contents	**Black tea with sugar infused with banana peel , pineapple peel and pulp**	**Green tea with sugar infused with banana peel , pineapple peel and pulp**
Basic kombucha fermentation	14 days	14 days
Total Days	21 days	21 days
Start	24/10/24	24/10/24
End	06/11/24	13/11/24
Total solids	12 g	12 g
pH	Plain kombucha- 3.8 pH Infused kombucha-4.1 pH	Plain kombucha- 3.8 pH Infused kombucha-4.1 pH
Alcohol percentage	<1%	<1%

Appearance:

Table 7: Appearance Data: Comparison of Control and kombucha Samples

Groups	Count	Sum	Average ± SD	Variance
Control	28	209	7.46 ± 0.79	0.63
Sample 1 (Green Tea)	28	229	8.18 ± 0.72	0.52
Sample 2 (Black Tea)	28	186	6.64 ± 1.98	3.94

Interpretation:

The **p-value** of 0.00016 indicates a highly significant difference between the groups. The p-value is less than the significance level (α = 0.05), hence the null hypothesis is rejected and concluded that the infusion of pineapple peel, pulp, and banana peel significantly improved the appearance of the green tea-based kombucha (Sample 1). The **average** score for Sample 1 is significantly higher than the other samples, with a **standard deviation (SD)** of 0.72, indicating relatively consistent ratings.

Colour:

Table 8: Colour Data: Comparison of Control and Kombucha Samples

Groups	Count	Sum	Average ± SD	Variance
Control	28	204	7.29 ± 1.72	0.95
Sample 1 (Green Tea)	28	215	7.68 ± 2.16	1.86
Sample 2 (Black Tea)	28	189	6.75 ± 2.65	2.79

Interpretation:

The **p-value** of 0.0434 is less than the significance level (α = 0.05), which indicates that the infusion had a statistically significant effect on the colour of the kombucha. The **average** for Sample 1 (7.68) is higher than the other two samples, suggesting a more vibrant and appealing colour. The **standard deviation (SD)** of 2.16 for Sample 1 is the highest, indicating a greater variability in the panellists' ratings compared to the control and Sample 2.

Taste:

Table 9: Taste Data: Comparison of Control and Kombucha Samples

Groups	Count	Sum	Average ± SD	Variance
Control	28	193	6.89 ± 0.92	0.84
Sample 1 (Green Tea)	28	196	7.26 ± 1.35	1.81
Sample 2 (Black Tea)	27	196	7.26 ± 1.35	1.81

Interpretation:

The **p-value** of 0.146 is greater than the significance level ($\alpha = 0.05$), meaning that the difference in taste ratings is not statistically significant. Therefore, the infusion of pineapple and banana peel did not significantly affect the taste perception. The **average** taste ratings are quite similar for all samples, with a **standard deviation (SD)** of 1.35, indicating some variation in the panellists' assessments.

Flavour:

Table 10: Flavour Data: Comparison of Control and Kombucha Samples

Groups	Count	Sum	Average ± SD	Variance
Control	28	190	6.79 ± 0.88	0.77
Sample 1 (Green Tea)	28	198	7.07 ± 1.36	1.85
Sample 2 (Black Tea)	28	179	6.39 ± 1.85	3.43

Interpretation:

The results shows that there is no significant difference was found in the flavour ratings ($p = 0.2057$), as the **p-value** is greater than 0.05. The secondary infusion did not significantly affect the perceived flavour. The **average** ratings are relatively close for all samples, with a **standard deviation (SD)** of 1.85 for Sample 2, indicating higher variability compared to the others.

Aroma:

Table 11: Aroma Data: Comparison of Control and Kombucha Samples

Groups	Count	Sum	Average ± SD	Variance
Control	28	158	5.64 ± 1.95	3.79
Sample 1 (Green Tea)	28	191	6.82 ± 1.65	2.74
Sample 2 (Black Tea)	28	191	6.82 ± 1.65	2.74

Interpretation:

The **p-value** of 0.0186 indicates a significant difference in the aroma ratings, as it is less than 0.05. Both Sample 1 and Sample 2 received higher ratings for aroma than the control, showing that the infusion enhanced the aroma profile. The **average** aroma rating for both Sample 1 and Sample 2 is 6.82, with a **standard deviation (SD)** of 1.65, indicating moderate variability in panellists' perceptions.

Overall Acceptability:

Table 12: Overall Acceptability Data: Comparison of Control and Kombucha Samples

Groups	Count	Sum	Average ± SD	Variance
Control	28	194	6.93 ± 0.83	0.44
Sample 1 (Green Tea)	28	207	7.39 ± 1.34	1.80
Sample 2 (Black Tea)	28	182	6.50 ± 1.57	2.48

Interpretation:

The **p-value** of 0.0334 is less than 0.05, indicating a statistically significant difference in overall acceptability. Sample 1 (green tea-based) received the highest rating, suggesting that the infusion improved the kombucha's overall sensory profile. The **average** rating for Sample 1 is 7.39, with a **standard deviation (SD)** of 1.34, indicating moderate variability in the responses.

Statistical analysis:

Sensory analysis with ANOVA:

- A group of 29 people tasted three types of kombucha: a plain one (control), a green tea kombucha flavoured with pineapple and banana peels, and a black tea version with the same flavours.
- Participants rated these on looks, colour, taste, flavour, aroma, and overall liking. Statistical analysis (ANOVA) helped identify which version people preferred and if the differences were real or just by chance.
- Results showed the green tea kombucha was most liked for its appearance, colour, and aroma, while taste and flavour were similar for all versions.

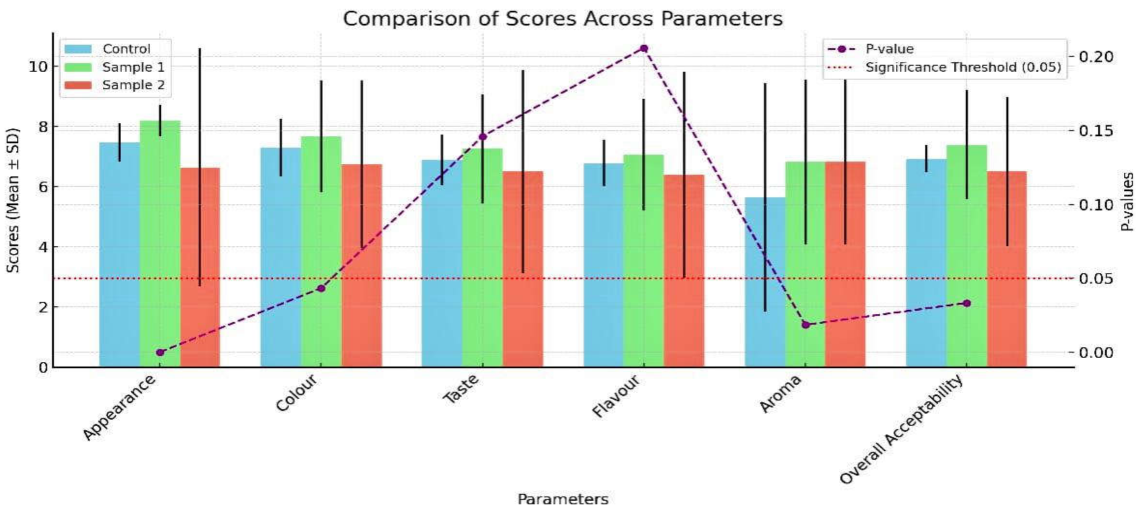


Figure 8: Sensory Evaluation of Kombucha Samples Across Different Parameters (Sample 1- Green Tea infused Kombucha ,Sample 2- Black Tea infused Kombucha)

Tools Used for Analysis:

- The team used Microsoft Excel year 2021 with 18.2412.1221.0 version is used to calculate scores and compare results.
- They emphasized repeating tests at least three times to ensure findings are trustworthy.

Results

Nutritional Composition:

We analysed the nutrient content of both green tea and black tea kombucha made with pineapple peel, pulp, and banana peel. The comparison of both the sample-1 which is green tea and the sample-2 is black tea is shown in the below table:

Table 13: Comparative Analysis of Green Tea and Black Tea Kombucha Nutritional Values

Parameters	**Green Tea Kombucha (/100ml)**	**Black Tea Kombucha (/100ml)**
Energy (kcal)	17.6	18.8
Carbohydrates (g)	3.6	3.9
Protein (g)	0.8	0.8
Fat (g)	Nil	Nil
Total Fiber (g)	0.4	0.4
Soluble Fiber (g)	0.3	0.3
Ash (mg)	0.2	0.2

Green tea kombucha was slightly lower in carbohydrates and energy than black tea kombucha. Both had negligible fat and similar amounts of protein, fiber, and ash.

Sucrose Breakdown:

The fermentation process significantly reduced sugar levels, with green tea kombucha ending up with 0.6 g/100 mL of sucrose and black tea kombucha with 0.8 g/100 mL.

Vitamin C:

Green tea kombucha with a slightly higher vitamin C content (22 mg/100 mL) compared to black tea kombucha (20 mg/100 mL), boosting its antioxidant value.

Antioxidant Power:

- **Free Radical Scavenging**: Green tea kombucha was a strong performer, showing 928 mg/L of scavenging activity.
- **Flavonoid Content**: This was measured at 424 mg for green tea kombucha, highlighting its antioxidant potential.

Sensory Experience:

We asked a panel of people to taste and rate the kombuchas. The green tea version infused with fruit peel (Sample 1) was preferred for appearance, colour, and aroma. Here's a summary of the key findings:

- **Appearance and Colour**: Sample 1 that is green tea stood out with a significant advantage ($p = 0.00016$ for appearance, $p = 0.0434$ for colour). Sample 1 (green tea-based kombucha) received the highest rating for appearance, showing a highly significant improvement, with a consistent response from panellists. The infusion impacted the colour significantly, with Sample 1 displaying the most vibrant hue, though panellist assessments showed some variation.

- **Taste and Flavour**: The differences in taste and flavour weren't significant (p = 0.146 and p = 0.2057). There was no significant difference in taste ratings across the samples, although moderate variability in the panellists' ratings was observed. Flavour ratings did not differ significantly, but Sample 2 showed the highest variability.
- **Aroma**: Both Sample 1 and the black tea kombucha (Sample 2-black tea) scored better than the plain control (p = 0.0186). Aroma ratings saw a significant enhancement, with both Sample 1 and Sample 2 outperforming the control. Panellists showed moderate variation in their assessments.
- **Overall**: Sample 1- green tea was rated the most enjoyable overall (p = 0.0334). There was a significant difference in overall acceptability, with Sample 1 receiving the highest score, though panellists' opinions varied moderately.

The microbial analysis:

The microbial analysis of kombucha samples provided valuable insights into the microbial community that influences both the fermentation process and the safety of the kombucha. On PDA, which supports yeast and fungal growth, rod-shaped bacteria dominated, indicating that Lactic Acid Bacteria (LAB), Acetobacter and Gluconobacter were more abundant than yeast in the kombucha culture. Similarly, selective growth observed on RBA further supported the idea that LAB had a competitive edge over fungal species in this environment. Growth on MRS agar confirmed the presence of LAB, which are crucial for both the fermentation and the probiotic qualities of kombucha. The white vacuolated structures observed in the samples may have been yeast or bacterial remnants, with LAB, Acetobacter and Gluconobacter clearly taking precedence due to their strong growth. EMB agar analysis showed white colonies, signalling the absence of coliform bacteria, which is an important indicator of kombucha's microbiological safety. Further observations indicated that the LAB, Acetobacter and Gluconobacter which plays a significant role in creating the acidic, tangy flavour of kombucha. The vacuolated structures likely represented remnants of yeast or bacteria, but the yeast population remained low, possibly due to nutrient competition that favoured Bacteria. Additionally, cellulose fibers present in the SCOBY were linked to Acetobacter species, responsible for forming the cellulose structure that is crucial to kombucha's fermentation process.

Discussion

In our analysis of green tea and black tea kombucha infused with pineapple peel, pulp, and banana peel, we found some interesting differences and similarities in their nutritional and sensory properties. Both types of kombucha were low in calories and carbohydrates, with green tea kombucha slightly lower in both (14.4 kcal and 3.6 g of carbohydrates) compared to black tea kombucha (14.5 kcal and 3.9 g of carbohydrates). The fermentation process didn't significantly change the overall macronutrient profile, keeping the kombucha a light, refreshing, and low-calorie drink.

When it comes to micronutrients, green tea kombucha had a bit more vitamin C (22 mg/100 mL) compared to black tea kombucha (20 mg/100 mL). This slight edge in vitamin C gives green tea kombucha a stronger antioxidant boost. Vitamin C is well-known for its immune-supporting and antioxidant properties, and its increase during fermentation suggests that the microorganisms responsible for the brewing process may be helping to boost its levels. Additionally, the green tea kombucha showed excellent antioxidant activity, with free radical scavenging at 928 mg/L and a flavonoid content of 424 mg, further supporting its health-promoting potential.

In terms of sensory qualities, the green tea kombucha with the fruit peel infusion (Sample 1) stood out in terms of appearance, colour, and aroma. Panellists rated it highly for its appearance (with a significant difference, $p = 0.00016$) and colour ($p = 0.0434$), suggesting the fruit infusion brightened the kombucha's visual appeal. The infusion also made a noticeable difference in the aroma, with both the green tea (Sample 1) and black tea kombucha (Sample 2) scoring better than the control. The differences in taste and flavour weren't as pronounced ($p = 0.146$ and $p = 0.2057$), but there was still some variability in how the panellists rated them, indicating that the fruit infusions added an interesting dimension to the flavour.

From a microbial perspective, we found that Lactic Acid Bacteria (LAB) were the dominant microorganisms in both types of kombucha. LAB are vital for producing the lactic acid that gives kombucha its signature tangy flavour. These bacteria seem to have a competitive advantage over yeast and fungi, likely due to their ability to thrive in the kombucha environment. This microbial balance ensures that the kombucha remains both safe and beneficial, offering its probiotic qualities without being overtaken by harmful microbes. EMB agar analysis showed no coliform bacteria, further confirming that the kombucha is microbiologically safe to drink.

The cellulose fibers found in the SCOBY, which are linked to Acetobacter species, play a major role in the fermentation process. The bacteria produce the cellulose matrix that supports the growth of beneficial microorganisms and contributes to the characteristic texture of kombucha. This shows how important the microbial community is in shaping kombucha's final taste, texture, and overall quality.

In conclusion, infusing kombucha with pineapple peel, pulp, and banana peel enhanced its appearance, colour, and aroma without compromising its nutritional value or safety. The green tea kombucha, in particular, offered a slight advantage in terms of vitamin C and antioxidant content, making it a healthy and appealing option. With LAB playing a major role in the fermentation process and the absence of harmful bacteria, both types of kombucha remain safe and beneficial for gut health. This study shows that fruit peel infusions can not only improve kombucha's sensory appeal but also help maintain its probiotic benefits, providing a flavourful, healthy beverage.

Summary

In conclusion, this study shows that both green tea and black tea kombucha infused with pineapple peel, pulp, and banana peel are tasty, low-calorie drinks that offer plenty of nutrients and probiotics.

Green tea kombucha had a slight advantage in terms of vitamin C and antioxidant levels, adding even more health benefits. When it comes to sensory qualities, the fruit peel infusion made the kombucha look better, brighter, and smell more appealing, although the flavour differences weren't as significant. The microbial analysis confirmed that Lactic Acid Bacteria (LAB), which are key to the fermentation and tangy taste, dominate the brew, ensuring it's both safe and full of probiotics. With no harmful bacteria present, the kombucha remains safe to drink. All in all, infusing kombucha with fruit peels not only improves its sensory qualities but also keeps its nutritional value intact, making it a flavourful and healthy option.

Conclusion

This study successfully crafted a nutritious, eco-friendly kombucha using fruit peels. Green tea kombucha shined with higher antioxidants, better flavour, and safety assurance. It's a great step toward making sustainable, health-boosting beverages that are both delicious and kind to the planet. This study successfully created a unique and sustainable kombucha by incorporating pineapple peel, pulp, and banana peel into traditional green and black tea recipes. The green tea kombucha has a higher antioxidant levels, more vitamin C, and better ratings for appearance, colour, and aroma. The microbial analysis confirmed the dominance of Lactic Acid Bacteria (LAB), which not only ensured the product was safe to drink but also boosted its probiotic benefits. By finding a way to repurpose fruit peels, this research shows how we can create eco-friendly drinks that are both healthy and delicious. It's an exciting step toward meeting the growing demand for sustainable and nutritious options while offering a refreshing new take on functional beverages.

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