The effects of green tea and black tea on the total acid, alcohol content, and antioxidant levels in kombucha

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Abstract. This research aimed to determine the effects of green tea and black tea on the total acid content, alcohol concentration, and total phenolic content of kombucha. The fermentation process was carried out over 14 days to monitor changes in key characteristics, including total titrated acid, pH, reducing sugar content, alcohol content, and Total Polyphenol Content (TPC). The results indicated that kombucha made from green tea contained higher alcohol levels and greater phenolic content, whereas kombucha from black tea had a higher acid concentration. By the end of the 14th day of fermentation, the acid content of green tea kombucha reached 0.240 M with a pH of 2.56, while the black tea variant reached 0.246 M with a pH of 2.61. Additionally, the alcohol content peaked on day 14 for both green tea and black tea, at 0.84% and 0.52% (v/v), respectively. The highest Total Polyphenol Content for green tea was recorded on day 12, measuring 344.4 mg/L, while black tea reached its peak of 333.89 mg/L on day 7. Based on the organoleptic tests and the analysis of kombucha contents, the optimal fermentation time for both green tea and black tea kombucha is concluded to be 7 days.

Keywords: Alcohol, Kombucha, Polyphenol, Total Acid

1 Introduction

Kombucha is a widely consumed beverage among various fermented foods, characterized by a sweet, sour, carbonated, and mildly alcoholic taste. The global kombucha market size was valued at USD 2.64 billion in 2021 and is expected to expand at a compound annual growth rate (CAGR) of 15.6% from 2022 to 2030 [1]. It consists of two distinct phases: a liquid phase and a floating pellicle (biofilm). Kombucha is produced through the fermentation of tea over a period of 7 to 10 days under aerobic conditions, utilizing a SCOBY (Symbiotic Culture of Bacteria and Yeast) [2].

The SCOBY used in the kombucha fermentation process consists of a combination of yeast and bacteria that metabolize aerobically. This metabolism converts the sugars in tea into acidic compounds and alcohol, which give kombucha its distinctive taste [3]. Yeast converts sucrose into glucose and fructose, which are then further converted into ethanol, glycerol, and CO_2. Meanwhile, bacteria convert ethanol into acetic acid and glucose into gluconic acid and glucuronic acid. The gluconic and glucuronic acids then contribute to the formation of cellulose, creating a new pellicle [2]. The bacteria and yeast work symbiotically, where the presence of acetic acid stimulates ethanol production by yeast, while low concentrations of ethanol stimulate cellulose formation by acetic acid bacteria (AAB) [4].

The unique flavor and potential health benefits have led to kombucha's rising popularity as a health beverage. The health benefits attributed to kombucha include aiding digestion, reducing inflammation, supporting liver, gut, and stomach function, boosting the immune system, and lowering cholesterol and blood pressure [5], [6].

Kombucha also contains a high level of antioxidants due to its production from tea, which is rich in polyphenols. Tea is the 7th largest plantation commodity in Indonesia, with black tea and green tea making up the majority of the country's total tea production [7], [8]. Tea is also the most consumed beverage in the world after water [9]. According to the World Tea Export Council, black tea is the most widely consumed tea globally, accounting for approximately 75% of total tea consumption, largely due to its strong flavor and high caffeine content [10]. The production of kombucha from tea can enhance the value of downstream tea products and increase community income.

In green tea production, the leaves are rolled and steamed to inactivate polyphenol oxidase, thereby minimizing oxidation before the drying process. In contrast, during black tea production, the leaves are rolled to rupture cellular constituents, which facilitates contact between phenolic compounds and polyphenol oxidase, promoting oxidation [11], [12].

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Green tea contains polyphenols as its main antioxidant component, consisting of various types of flavonoids, primarily catechins [13]. Catechins, such as epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin-3-gallate (EGCG), are among the most abundant flavonoid groups in green tea. EGCG, the primary polyphenol in green tea, is recognized as an active compound with significant anti-inflammatory and antioxidant properties [14], [15]. Many clinical diseases have been shown to be associated with oxidative damage. Tea polyphenols are an effective antioxidant that scavenge free radicals directly, thus protecting human body from oxidative stress-induced damage.

In black tea, the compounds acting as antioxidants are theaflavins, thearubigins, and their derivatives, as well as tannins. These compounds are formed as a result of polyphenol enzyme oxidation during the fermentation process. Although their structures are similar to catechins, these compounds still possess antioxidant capacity [11], [13], [16].

The type of tea used can lead to variations in the taste of kombucha as well as differences in its components, including acid content, alcohol content, and antioxidant levels. Therefore, this study aims to examine the effects of green tea and black tea on the acid, alcohol, and antioxidant content in kombucha. Additionally, the study explores the trends of these components throughout the fermentation period to determine the optimal fermentation time, balancing kombucha composition with preferred organoleptic characteristics.

2 Materials and Methods

2.1 Materials

The materials used in this study included green tea (Pekoe, Sukabumi, West Java, Indonesia), black tea sourced from a farmer in Central Java Province, Indonesia, sucrose ('Gulaku' brand), and SCOBY obtained from a commercial supplier in Indonesia.

2.2 Preparation of Kombucha Tea

Eight grams of black tea and green tea were placed in tea bags, which were then added to 750 mL of boiling water and allowed to infuse for approximately 3 minutes. Sucrose (140 g) was dissolved in the hot tea, and the preparation was allowed to cool.

The cooled tea solution was transferred into 1 L glass jars that had been sterilized by rinsing with hot water (90°C). The cooled tea solution was inoculated with 80 gram of freshly cultured tea fungus that had been grown in the same medium for 7 days, along with 10% (v/v) of previously fermented liquid tea broth, added aseptically. The kombucha starter cultures, commonly referred to as SCOBY (Symbiotic Culture of Bacteria and Yeast), consisting primarily of *Acetobacter xylinum*, *Gluconobacter*, and *Saccharomyces cerevisiae*, [17] were sourced from a commercial supplier in Indonesia. The jars were then covered with clean cloths and secured. Fermentation was carried out at room temperature for approximately 14 days.

2.3 Sampling

The experiment was conducted in two batches for each tea variant. Sampling was conducted periodically on days 0, 1, 4, 7, 9, 12, and 14 from all batches, followed by analysis. Each measurement was conducted once, and the data was presented as the mean of two replications. Prior to each sampling, all equipment was thoroughly rinsed to prevent potential contamination. Samples were frozen immediately after collection and analyzed on the 14th day, following the completion of the fermentation process.

Both black tea and green tea samples were analyzed for pH, total acid concentration, reducing sugar concentration, and total phenolic content. For alcohol content, samples were collected and measured in real-time on the corresponding fermentation days. Samples for the organoleptic test were prepared in separate jars and collected according to the fermentation timeline.

2.4 Analysis of pH and Total Acid Concentration

pH was measured using a pH meter (Sartorius, range pH 0-14). Total acid concentrations were determined by titration with a 0.1 M NaOH solution (ROFA, Indonesia).

2.5 Analysis of Alcohol Concentration

Alcohol concentrations were measured using a hydrometer (Brewer's Elite, SG 0.980-1.160). The difference in density was calculated to determine the volumetric content of alcohol.

2.6 Analysis of Reducing Sugar Concentration

The concentration of reducing sugar was determined by reacting it with Benedict's reagent, following a modified procedure based on [18]. One milliliter of the standard solution was reacted with 4 mL of 0.1 M Benedict's reagent (ROFA, Indonesia). The mixture was then placed in boiling water for 5 minutes and allowed to cool. Subsequently, 2 mL of the supernatant was diluted with 1 mL of demineralized water. The solution was centrifuged, and the supernatant was collected and measured using a UV-Vis spectrophotometer (Hanon i3, China, with a wavelength range of 320-1100 nm) at a wavelength of 745 nm.

A calibration curve was created using a glucose standard solution (ROFA, Indonesia) with concentrations ranging from 0 to 2000 ppm. The sample was diluted 100 times prior to measurement.

2.7 Analysis of Total Phenolic Content

The antioxidant content in the tea was assessed using the Total Phenolic Content (TPC) measurement method, which follows a similar trend to the DPPH assay but detects a broader range of phenolic compounds compared to the DPPH method [19], [20]. The procedure followed was based on [20] with modification. Standard gallic acid solutions ranging from 20 to 100 ppm were prepared by dissolving gallic acid (Merck, Germany) in 100 ml methanol (Emsure, Germany) and reacting it with Folin-Ciocalteu reagent.

For each variation, 0.3 ml of the standard solution was combined with 1.5 ml of 10% (v/v) Folin-Ciocalteu reagent 2N (Merck, Germany) and homogenized using a vortex mixer (Corning CLS 6776, US). After incubating for 5 minutes in a dark room, 1.2 ml of 7.5% (w/v) Na_2CO_3 solution (Pudak, Indonesia) was added, followed by further incubation for 30 minutes. The resulting solution was homogenized and diluted with distilled water at a 1:7 ratio. Absorbance was measured using a UV-Vis Spectrophotometer (Hanon i3, China) at a wavelength of 765 nm. Absorbance data were used to construct the calibration curve for the standard gallic acid solutions.

Distilled water was used as the blank following the same procedure as the standard solution, and its absorbance was zeroed during measurement. Sample concentration measurements were conducted similarly to the standard solution, with 0.3 ml of sample solution prepared for each variation. The final concentration values were then converted into total phenolic content expressed in milligrams of gallic acid equivalents (GAE) per gram.

2.8 Organoleptic Test

The organoleptic test was conducted on four samples from each tea variant, collected on days 7, 9, 12, and 14, as the kombucha flavor begins to develop from day 7 onward [5,6]. Six sensory characteristics of kombucha were evaluated: sweetness, sourness, flavour, colour, alcohol flavour intensity, and carbonation intensity. The organoleptic assessment was performed by a panel of 20 untrained panelists as a mean of preliminary consumer preference evaluation. All panelists were 18 years or older, had consumed kombucha previously, and were not allergic to fermented products.

Samples from days 7, 9, 12, and 14 were collected during fermentation and kept in a refrigerator. Each panellist was given a 15 ml sample to sip; then they scored six sensory characteristics of kombucha. The scoring system ranged from 1 to 5, where 1 indicated the most disliked and 5 the most liked. After finishing each sample, panellists were given still water to neutralize their palate before moving to the next sample.

2.9 Statistical Method

In all measurements, each sample was analyzed, and all assays were performed in duplicate. Statistical analysis was conducted using Microsoft Excel 2017, with results expressed as mean values and standard deviations (SD). For the organoleptic test, data are presented as mean values and standard deviations from 20 samples.

3 Results and Discussions

3.1 Total Acid Concentration

The acid concentration values in Figure 1 were determined through titration using a 0.1 M NaOH solution. The trend of acid concentration over the fermentation period is depicted in Figure 1.

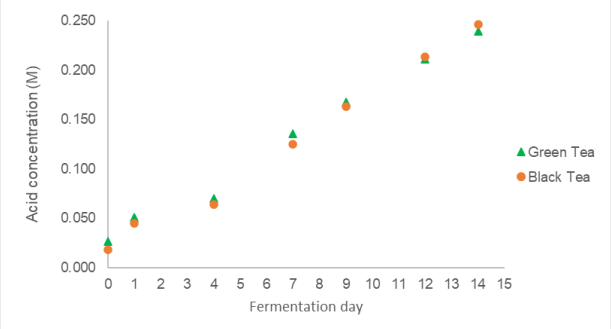

Figure 1. Acid Concentration during kombucha fermentation. Data are expressed as mean ± SD of n = 2 samples.

Based on Figure 1, it can be concluded that during fermentation up to the 14th day, the total titratable acid concentration in kombucha continued to increase. The increase in titratable acid levels in green tea kombucha was not as pronounced as in black tea kombucha. On the final day of fermentation, the total titratable acid concentration in green tea kombucha reached only 0.240 M, whereas black tea kombucha reached 0.246 M. The acid concentration starts to increase significantly from day 4 of fermentation, for both green tea & black tea. These figures represent the combined concentrations of acetic acid and glucuronic acid. Acetic acid is the result of ethanol conversion by acetic acid bacteria (AAB), while glucuronic acid is a product of glucose conversion by acetic acid bacteria (AAB) [4].

3.2 pH

The pH of kombucha decreases as fermentation progresses. The drastic drop in pH from day 0 to day 1 is caused by an increase in organic acid content due to acetic acid bacteria (AAB) and lactic acid bacteria (LAB). Furthermore, during the fermentation process from day 1 to day 14, the pH gradually decreases, as shown in Figure 2.

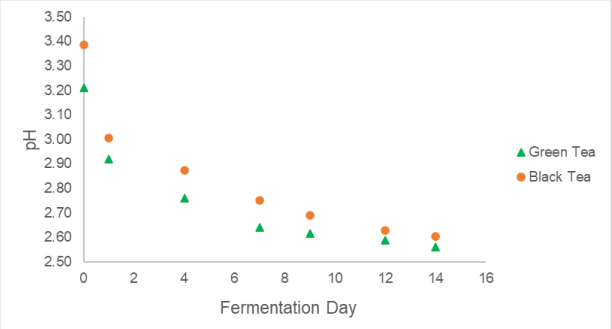

Figure 2. Trend of pH during kombucha fermentation. Data are expressed as mean ± SD of n = 2 samples.

Figure 2 illustrates the decrease in kombucha's pH over time during fermentation. A significant decline is observed starting on day 4 for both green tea and black tea. This trend corresponds to the formation of organic acids, as shown in Figure 1. When compared to day 0 of fermentation, black tea exhibited a greater reduction in pH by the end of the fermentation period (day 14) compared with green tea.

The final pH of fermented kombucha is usually around 2.5 [21]. Black tea has a lower pH compared with green tea because its leaves undergo fermentation, leading to an increase in amino acids and catechin gallates. As a result, black tea is more acidic than green tea [22]. In addition to tea type, the fermentation process in black tea generates more acids (such as acetic acid and glucuronic acid). This is consistent with data from [5] and [6], which demonstrate that the total acid content (acetic acid and glucuronic acid) produced by black tea is higher compared to green tea.

3.3 Alcohol Content

The trend of alcohol content in kombucha over the fermentation period is shown in Figure 3.

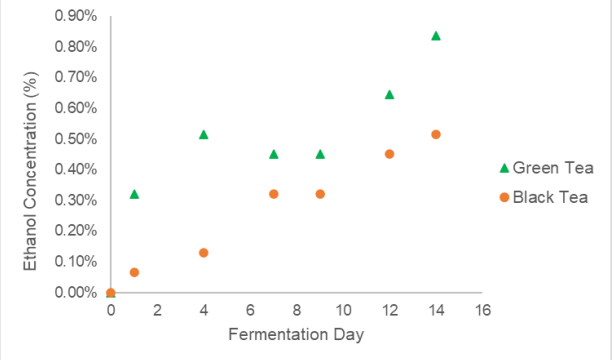

Figure 3. Alcohol Concentration During Kombucha Fermentation. Data are expressed as mean ± SD of n = 2 samples.

Figure 3 shows that the alcohol concentration in both types of tea increases throughout the fermentation period. This is related to the decrease in reducing sugar concentration, as illustrated in Figure 4. The declining sugar levels indicate that reducing sugars have been converted into ethanol.

During fermentation, reducing sugars (glucose and fructose) are metabolized by yeast to produce alcohol, glycerol, and carbon dioxide. Glucose is also metabolized by acetic acid bacteria (AAB) into gluconic and glucuronic acids, which subsequently lead to the formation of a cellulose pellicle [6].

In green tea kombucha, alcohol levels begin to rise significantly on day 4, which corresponds with the decline in reducing sugar concentration on the same day (figure 4). In contrast, alcohol levels in black tea kombucha begin to rise significantly after day 6, matching the sharp decline in reducing sugar concentration on day 6.

Figure 3 also shows that the highest ethanol content for green and black tea occurs on day 14, at 0.84% and 0.52% (v/v), respectively. The lower alcohol content in black tea, when associated with its higher total acid content, suggests that more ethanol is converted into acetic acid in black tea.

3.4 Concentration of Reducing Sugar

Figure 4 illustrates the trend of reducing sugar concentration throughout the fermentation period.

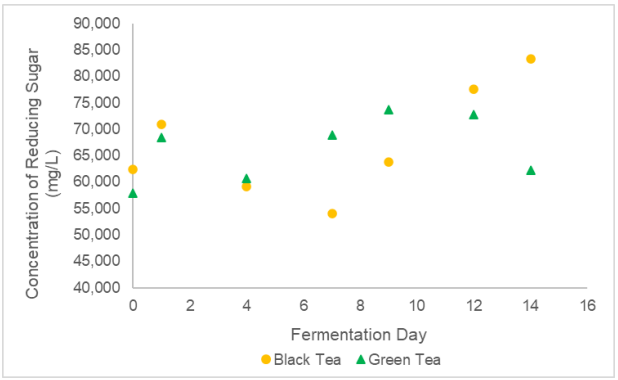

Figure 4. Reducing Sugar Concentration During Kombucha Fermentation. Data are expressed as mean ± SD of n = 2 samples.

During fermentation, sucrose is broken down by yeast into its monosaccharide components (glucose and fructose), after which glucose and fructose are converted into alcohol by yeast and into organic acids by acetic acid bacteria (AAB). Thus, reducing sugars serve as both a product and a reactant in the kombucha fermentation process, causing their concentration to fluctuate over time [23].

Throughout the fermentation period, reducing sugar concentration increases on day 2, then decreases on day 4 for green tea and on day 6 for black tea. This decline is due to the metabolism of reducing sugars into alcohol, as shown in Figure 3. In green tea, the concentration then rises until day 9, after which it decreases until day 14. This trend occurs because as fermentation progresses, more sucrose is converted into reducing sugars, which are subsequently metabolized into alcohol and organic acids.

In black tea kombucha, the concentration of reducing sugars continues to increase until day 14. A greater amount of reducing sugars is further metabolized into organic acids from alcohol, as reflected by the lower alcohol content and higher acid levels compared to green tea kombucha.

3.5 Total Phenolic Content

Figure 5 illustrates the trend of Total Phenolic Content throughout the fermentation period.

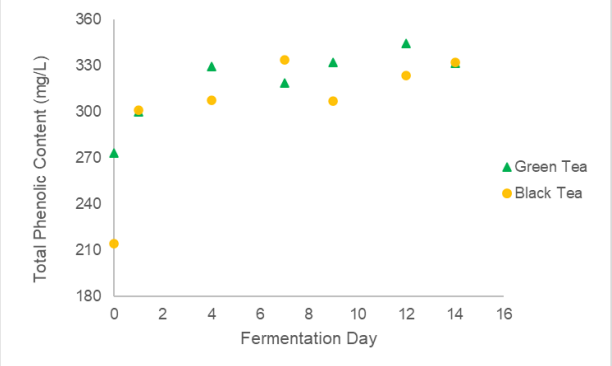

Figure 5. Total Phenolic Content during Kombucha Fermentation. Data are expressed as mean ± SD of n = 2 samples.

From Figure 5, it can be observed that total phenolic content increases over time for both types of tea during fermentation. A high correlation was demonstrated between the total polyphenol content and antioxidant capacities both for green tea and black tea. Antioxidant capacity is measured using the DPPH method, which evaluates antioxidants based on their ability to bind free radicals or act as hydrogen donors [20]. The antioxidant properties of tea are derived from its polyphenol components, which inhibit oxidation reactions and scavenge free radicals due to the presence of hydroxyl groups in their chemical structure [13].

The higher total phenolic content observed during fermentation suggests that the fermentation process enhances the antioxidant levels in tea, making kombucha richer in antioxidants compared to pure tea. Consequently, kombucha has greater potential to combat free radicals compared tob pure tea.

The antioxidant content continues to rise until day 14 for green tea, reaching a maximum of 344.4 ± 2.5 mg/L on day 12, while for black tea, the antioxidant content peaks on day 7 at 333.89 ± 15.1 mg/L and then decreases. This is consistent with polyphenol measurements conducted by [17], where the highest polyphenol concentration in green tea kombucha was 320.1 mg/L on day 14, compared to only 219.5 mg/L in black tea kombucha on day 7.

Originally infused green tea contains more polyphenol content than black tea. This could be due to deactivation of enzymes that can catalyze oxidative polimerisation of catechins after harvesting, while for black tea, the production involve leaf-breaking step that promotes enzymatic oxidation of catechins, therefore decrease polyphenol content [11].

Microbial metabolism in kombucha enhances phenolic compounds through biotransformation, a process that utilizes enzymes secreted by microorganisms in kombucha [6]. As fermentation progresses, acidic conditions cause phenolic compounds to become more stable, making it harder for them to release protons that can bind with DPPH, thereby reducing their antioxidant activity [24] [25].

3.6 Organoleptic Test Result

An organoleptic test involving 20 panelists was conducted to evaluate kombucha samples on days 7, 9, 12, and 14 for both green tea and black tea [26]. The results of this sensory evaluation are presented in Figures 6 and 7.

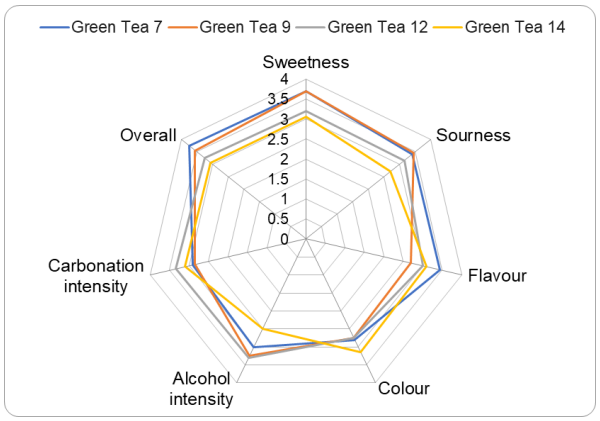

Figure 6. Organoleptic Evaluation Results of Green Tea Kombucha. Data are expressed as mean ± SD of n = 20 samples.

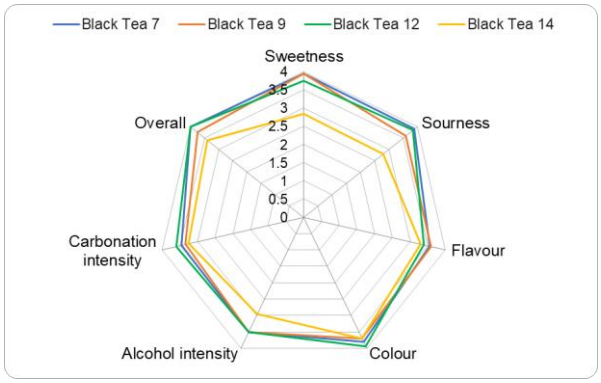

Figure 7. Organoleptic Evaluation Results of Black Tea Kombucha. Data are expressed as mean ± SD of n = 20 samples.

For green tea, Figure 6 shows that the most preferred samples were those fermented on days 7 and 9. The organoleptic test revealed the highest average scores for overall sweetness, acidity, carbonation intensity, alcohol flavour intensity, colour, and aroma, with a score of 3.75 on day 7 and 3.55 on day 9.

For black tea, Figure 7 indicates that the most favored samples were those fermented on days 7 and 12. The organoleptic test recorded the highest average scores for overall sweetness, acidity, carbonation intensity, alcohol flavour intensity, colour, and aroma, with a score of 4 on both day 7 and day 12.

This organoleptic test result aligned with other findings, where on day 7 of fermentation, the antioxidant content showed the highest levels, followed by a decrease thereafter [26]. Considering total acid content, alcohol content, and antioxidant content, along with the organoleptic test results, day 7 of fermentation was concluded to be the most optimal fermentation day, producing high antioxidant content while maintaining preferable sensory qualities.

4 Conclusion

Green Tea Kombucha fermentation results in kombucha with higher alcohol and antioxidant content, while Black Tea Kombucha fermentation produces kombucha with higher acid levels. The optimal fermentation time both for Green Tea Kombucha and for Black Tea Kombucha is 7 days, based on acid content, alcohol, antioxidants, and organoleptic properties.

As the fermentation period increases, kombucha becomes more acidic. The highest acid content for both green tea and black tea kombucha was recorded on day 14, with green tea kombucha reaching 14.4 mg/L and a pH of 2.56, while black tea kombucha reached 14.78 mg/L and a pH of 2.61. Over 14 days of fermentation, reducing sugars are initially formed and then converted into ethanol, acetic acid, and glucuronic acid.

The highest antioxidant content was achieved during kombucha fermentation, with Green Tea Kombucha reaching 344.44 mg/L and Black Tea Kombucha reaching 333.89 mg/L. The longer the fermentation, the higher the alcohol content produced. The highest alcohol content for both Green Tea Kombucha and Black Tea Kombucha was recorded on day 14, with values of 0.84% for green tea and 0.52% (v/v) for black tea.

Conflict of Interest

The authors would like to thank Calvin Institute of Technology for providing research funding. The authors declare no conflict of interest.

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