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MORALITY BEFORE KNOWLEDGE

Changes in the quality of kombucha during fermentation: A study of microbial, physicochemical and sensory attributes

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ABSTRACT

Background: Kombucha is a fermented tea beverage known for its health-promoting properties, largely due to its antioxidant-rich polyphenolic compounds. This study aims to examine how fermentation influences the microbial, physicochemical and sensory qualities of kombucha, focusing on microbial growth, polyphenols, total acids, total solids, and sensory attributes. **Methods:** A literature review approach was employed to synthesize findings from relevant studies on kombucha fermentation and its biochemical mechanisms. **Findings:** Results show that extended fermentation reduces microbial growth through the antimicrobial activity of phenolic compounds, increases polyphenol and organic acid content via biotransformation. Total solids decline over time due to the conversion of components into volatile metabolites, while acidity, sour aroma, and color brightness intensify with longer fermentation. **Conclusion:** These findings highlight fermentation duration as a critical factor shaping kombucha's functional properties and consumer acceptability. **Novelty/Originality of this article:** The novelty of this article lies in its integrated review of fermentation effects across multiple quality parameters, offering valuable insights for optimizing kombucha as a functional beverage.

KEYWORDS: fermentation; kombucha; quality.

1. Introduction

Kombucha tea is known for its diverse variations, both in terms of tea type, kombucha starter, and manufacturing process. Kombucha tea contains a beneficial composition that makes it a functional beverage with antioxidant properties, primarily due to its polyphenol content. Polyphenolic compounds in tea, particularly EGCG (epygalochaetekin gallate), play a role in cancer prevention due to their antioxidant activity, which suppresses the growth of cancer cells (Naland, 2008).

The kombucha tea processing process involves fermentation, involving yeast-like organisms and bacteria that live in the tea water. This distinguishes kombucha tea from other teas. The kombucha culture is a symbiotic collaboration of yeasts (*Saccharomyces cerevisiae, Saccharomyces ludwigii, Saccharomyces bisporus, Zygosaccharomyces sp.*) (Chakravorty et al., 2016), and bacteria (*Acetobacter xylinum*). Yeast hydrolyzes sucrose to form glucose and fructose for ethanol production, while bacteria convert glucose to gluconic acid and fructose to acetic acid. Acetobacter sp. in kombucha cultures oxidizes ethanol to acetaldehyde, which then forms acetic acid. The accumulation of each metabolite produces

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glucuronic acid, lactic acid, vitamins, amino acids, antibiotics, and other substances that are beneficial for health and create a distinctive aroma. These two fermentation agents live symbiotically, making it difficult for pathogens to contaminate the collaboration between these two types of microorganisms. Furthermore, the acidic conditions created by the fermentation process further complicate the survival of foreign organisms that cannot tolerate low pH environments. In this article, we will discuss the effect of processing and fermentation time of kombucha tea on the physicochemical characteristics.

Kombucha tea was first consumed by people in mainland China as an herbal medicine. Kombucha tea is also known as Manchurian tea. Kombucha tea was also introduced to several countries, including Indonesia in the 1930s because of its health benefits. Kombucha tea is a fermented beverage product made from a solution of tea and sugar with the addition of the kombucha microbial starter, Acetobacter xylinum, and several types of yeast. Kombucha is actually a symbiosis of bacteria and yeast. Kombucha yeast has a thin sheet form, 0.3-1.2 cm thick, resembling white gelatin (Naland, 2008). The symbiotic bacteria and kombucha yeast during the fermentation process of kombucha tea break down sugar into compounds such as acids, vitamins, and nutritious alcohol. The types of yeast found in kombucha tea are *Saccharomyces cerevisiae, Saccharomyces ludwigii, S. apiculatus varieties,* and *Schizosaccharomycespombe*. Together with yeast, bacteria play a significant role in the production of kombucha tea (Villarreal-Soto et al., 2019).

Kombucha is generally made from a black tea solution with granulated sugar , but now there is a lot of research on kombucha using leaves, which have high antioxidant and tannin content. In Suhardini'sresearch, kombucha is made from a variety of leaves, including bay leaves, guava leaves, betel leaves, soursop leaves, coffee leaves, and tea leaves (Suhardini & Zubaidah, 2015). One leaf that also has a high antioxidant content is moringa. A popular processed product from moringa leaves is tea. A brew of moringa leaves becomes more nutritious when fermented into kombucha, as kombucha has higher antioxidant activity than unfermented beverages. Recently, fruit-based kombuchas have begun developed (Zubaidah et al., 2023).

Sugar is the most important ingredient in making kombucha, as it serves as a food source for the kombucha culture's microbes . Granulated sugar is often used as a carbon source in kombucha production. Research by Marwati et al. on the effect of sugar concentration and kombucha starter on the quality of kombucha tea showed that the best tasting kombucha tea was obtained from a combination of 20% sugar concentration and 20% kombucha starter concentration (Marwati & Handria, 2013). Both sugar concentration and kombucha starter concentration significantly influenced the flavor characteristics of kombucha tea. The fermentation process is also crucial in making kombucha (Coton et al., 2017).

Fermentation in kombucha is carried out by the kombucha culture by converting glucose into alcohol and CO_2, which then react with water to form carbonic acid. The alcohol is oxidized to acetic acid. Gluconic acid is formed from the oxidation of glucose by bacteria of the genus *Acetobacter*. The culture simultaneously produces other organic acids. *A. xylinum* bacteria convert sugar into cellulose, called nata, which floats on the surface of the medium.

Fermentation is the conversion of sugars in wort (a clear cooking liquid) into alcohol, CO_2, energy, and other substances, particularly organoleptic substances that produce distinctive aromas and flavors. The fermentation stage is divided into two stages: aerobic and anaerobic fermentation. During aerobic fermentation, sugars are converted into CO_2, H_2O, and energy. The aerobic fermentation process is often referred to as the yeast growth period. After the yeast growth period ends, marked by the depletion of oxygen, the anaerobic fermentation process begins. At this stage, yeast is able to convert sugars into alcohol, CO_2, and energy (Antolak et al., 2021; Gaggìa et al., 2018; Jayabalan et al., 2014).

Kombucha is ready to drink after its pH ranges from 2.5-3.5 with a fermentation period of 8-12 days. Kombucha is a fermented beverage product that contains several vitamins, minerals, enzymes, and organic acids. Kombucha tea is a traditional beverage product resulting from the fermentation of tea and sugar solution using kombucha starter culture

(*Acetobacter xylinum* and several types of yeast). Kombucha has several health effects, including acting as an antioxidant, antibacterial, improving intestinal microflora, increasing body resistance, and lowering blood pressure.

Kombucha fermentation time ranges from 8-12 days at a temperature of 18-20°C, while at higher temperatures the fermentation process is shorter. Generally, areas with high climates (22-26°C) ferment kombucha for 4-6 days. The length of kombucha fermentation affects the physical, chemical, and organoleptic qualities of kombucha. The fermentation results in a suspension that can produce organic acids such as glucuronic acid, acetic acid, lactic acid, folic acid, as well as amino acids, vitamins, antibiotics, enzymes, and other products. Glucuronic acid is the most important product in kombucha because it functions to detoxify toxins.

The use of kombucha as a beverage to prevent and treat various diseases has long been practiced by households in several Asian countries. This beverage has been proven to increase stamina, improve small intestine function, promote weight loss, lower cholesterol, normalize organ function, treat gout, prevent cancer, and boost immunity. As a beverage, kombucha has never caused fatal side effects in consumers.

Kombucha can be made according to the procedure described by Naland (2008). First, bring 1 L of water to a boil, then add 4 tea bags or 4-8 teaspoons (12g) of chopped black tea. The tea is allowed to steep for 15 minutes, then strained. Next, add 70-100 g of granulated sugar and cool to 20-25°C. Once cooled, the tea solution is poured into a glass container along with the kombu starter. The container is then covered with a clean cloth and fermented for 7-10 days at 23-27°C. Once the tea solution has reached the correct acidity level (pH 2.5-3.5), the kombu starter is removed, and the tea solution is ready to be consumed.

Besides brewed black tea, there are two important components in the kombucha-making process: kombu starter and sucrose (granulated sugar). In Indonesia, kombu starter is more often called mushroom dipo, while in other countries it is known by other names such as cajnyj kvas, heldenpilz, mandarin tea mushroom, fungus japonicum, tea kwass, olinka, mogu, kargasok tea, zauberpilze, olga spring, and super mushroom. Kombu starter is a white gel-like organism, 0.3-1.2 cm thick, enclosed in a clay membrane. This starter is a colony of yeast and several bacteria. In foreign terms, kombu mushrooms are commonly known as SCOBYs (Symbiotic Colonies of Bacteria and Yeast). The yeast found in kombucha is *Candida albicans*, *Saccharomyces* and *Pichia* fermentants, while the bacteria are *Acetobacter xylinum*, *A. ketogenum*, and *Bacterium gluconicum*.

Considering the complexity of microbial interactions, the variability of raw materials, and the significant influence of fermentation conditions, kombucha presents a unique potential as a functional beverage with diverse health benefits. However, differences in fermentation duration and processing methods greatly determine its physicochemical and organoleptic qualities, which in turn affect its nutritional value and consumer acceptance. Therefore, it is essential to further examine how fermentation time influences the quality characteristics of kombucha, particularly in terms of bioactive compounds, acidity, and sensory attributes. This article aims to provide an in-depth discussion on the effect of processing and fermentation time on the physicochemical properties of kombucha tea, offering insights that may serve as a foundation for optimizing its production as a functional beverage.

2. Methods

A comprehensive literature search was conducted utilizing the keyword "Kombucha" across various databases, including Google Scholar, ScienceDirect, Scopus, and Web of Science. The author gathered information from scientific journals, books, book chapters, reports, and conference proceedings published from 2010 onwards. The data collection process involved several stages, including the identification of relevant articles through keyword searches, the application of inclusion criteria to categorize articles, and the analysis and comparison of findings with related data. The inclusion criteria for this study specified that articles must be published between 2010 and 2024, written in English or Indonesian, and accessible in full text. Subsequently, the articles were organized based on their thematic discussions, and data were systematically collected.

3. Results and Discussion

3.1 The effect of fermentation time on microbial growth in kombucha

Fermentation time has a critical impact, particularly on microbial growth and development in kombucha. If fermentation is extended too long, excessive acid production can inhibit microbial growth and result in an overly acidic product, making it important to determine the optimal fermentation duration for balanced quality (Sarmila & Romadhan, 2025). These findings further highlight that fermentation duration not only affects microbial dynamics in general, but also plays a key role in determining the specific growth patterns of lactic acid bacteria in kombucha.

Microbial growth and the formation of secondary metabolites are the result of substrate conversion, which serves as a nutrient source. Acetic acid bacteria such as *Acetobacter sp.* utilize glucose produced from sucrose hydrolysis by yeast to synthesize cellulose on the surface of the medium. The increase in bacterial colonies is attributed to the rise in reducing sugars generated from yeast-mediated sucrose hydrolysis in kombucha. In the study conducted by Anggraini et al. (2023) bacterial colonies increased on the day 7 of fermentation and subsequently decreased on the 15th day. On the day 15, the decrease in bacterial colony count was caused by the depletion of nutrients in the medium and the increasingly acidic conditions. Thus, optimizing fermentation duration is essential to ensure balanced activity of acetic acid bacteria and to achieve desirable quality characteristics in kombucha.

Acetic acid bacteria (AAB) are essential microorganisms in kombucha, responsible for oxidizing ethanol into organic acids and synthesizing cellulose that supports SCOBY formation (Chong et al., 2024). The types of acetic acid bacteria (AAB) present in kombucha depend on the initial microbial composition of the SCOBY, the available substrates (sugars and ethanol), fermentation conditions such as temperature and pH, and interactions with other microorganisms like yeast and lactic acid bacteria. These factors determine which AAB strains become dominant during the fermentation process. The types of AAB bacteria commonly found in kombucha include the species *Acetobacter pasteurianus*, *Acetobacter orientalis*, *Acetobacter cibinongensis*, *Acetobacter pasteurianus*, *Acetobacter pomorum*, and *Acetobacter ascendens* (Lee et al., 2024).

In addition to acetic acid bacteria, lactic acid bacteria (LAB) also show dynamic changes during fermentation. The longer the fermentation, the more inhibited and decreased microbial growth. The results show that the decrease in microbial counts varies for each kombucha. This is influenced by the phenolic compounds formed during fermentation, which have antimicrobial properties, inhibiting and decreasing microbial growth. A study conducted by Wistiana showed that total lactic acid bacteria (LAB) increased from day 0 to day 8, while decreasing from day 8 to day 14 (Wistiana & Zubaidah, 2015). This is similar to the total microbial count, where the increase in LAB is thought to be due to the presence of dissolved solids in various kombuchas, such as sugars, amino acids, and caffeine. These substances in the tea are utilized as a source of nutrients and energy, thus increasing LAB growth. The longer the fermentation, the more inhibited and decreased LAB growth (Firdaus et al., 2020). The results show that the decrease in LAB varies for each kombucha. This is influenced by the kombucha environment, which produces alcohol during fermentation and production of organic acids, which can inhibit microbial growth (Sari et al., 2022). The increase in LAB is also due to the use of sugar, and the decrease is also due to the fact that during the kombucha fermentation process, organic acids, alcohol and other substances are produced which inhibit the growth of LAB (Rihibiha et al., 2022; Shafira et al., 2022).

In certain kombucha products, LAB have been reported to constitute up to 30% of the bacterial community. The predominant LAB species typically detected during kombucha fermentation include *Lacticaseibacillus casei, Lactiplantibacillus plantarum, Lactobacillus nagelii, Lactobacillus rhamnosus, and Lactobacillus mali*. Moreover, the presence of LAB throughout the fermentation process has been associated with enhanced production of D-saccharic acid-1,4-lactone (DSL) and glucuronic acid. DSL is known for its detoxifying properties and is believed to play a key role in the potential hepatoprotective effects attributed to kombucha (Wang et al., 2022).

The fermentation duration significantly influences microbial growth in Curcuma mangga kombucha, as demonstrated by the increased antibacterial activity observed on the 10th day of fermentation, with the largest inhibition zones against Escherichia coli and Salmonella typhi measuring 8.2 mm and 9.2 mm, respectively. Although these values fall within the resistant category according to CLSI standards, the presence of inhibition zones indicates that the fermentation process produces antimicrobial compounds, including organic acids such as acetic acid and lactic acid, that are capable of suppressing bacterial growth. This finding aligns with previous studies on black tea kombucha, kecombrang flower kombucha, and Curcuma longa kombucha, which reported increased antimicrobial activity with longer fermentation periods, although the effectiveness varies depending on the substrate and target bacteria. The relatively lower inhibition observed in this study can be attributed to the complex cell wall structure of the Gram-negative bacteria tested, which provides stronger resistance to antimicrobial agents. Therefore, prolonged fermentation enhances the production of bioactive compounds contributing to microbial inhibition, although further optimization is needed to improve the antimicrobial effectiveness of Curcuma mangga kombucha (Ratna et al., 2025).

3.2 The effect of fermentation time on physicochemical properties of kombucha

In Susilowati's research, kombucha tea was made from green tea extracts (*permeates*) of *Camellia assamica* grade Pekoe & Dewata and *Camellia sinensis* grade Arraca Kiara & Arraca Yabukita with variations in fermentation time for 1 and 2 weeks with commercial black tea as a comparison (Susilowati, 2013). Kombucha fermentation produced total polyphenols and reducing sugars in kombucha tea. The addition of sucrose during fermentation activates microbial enzymes (invertase), thereby increasing the degradation of tea components (TF/Theaflavin and TR/Thearubigins) as epicatechin isomers (EGCG, EGC, ECG, and EC) and increasing total polyphenols. Tea polyphenols can transform into other compounds through assimilative and dissimilative chemical reactions during fermentation.

There are variations in the stability of four types of epicatechin isomers during Kombucha black tea fermentation: epigallocatechin gallate, epicatechin gallate, epigallocatechin, and epicatechin. All catechins undergo degradation at the beginning of fermentation, with degradation increasing by the 12th day of fermentation. This increase in polyphenols may be due to the biotransformation process of epigallocatechin gallate into epigallocatechin and epicatechin gallate into epicatechin by enzymes produced by microorganisms in Kombucha culture as well as the release of catechins from acid-sensitive microorganism cells. Overall, total polyphenol optimization was achieved by Arraca Kiara (8.006% db), higher than Pekoe (3.271% db), Dewata (2.1521% db) and Arraca Yabukita (2.165% db) and commercial black tea control (0.8610% db) at 2 weeks fermentation time. Under these conditions, kombucha tea from Arraca Kiara, Arraca Yabukita, Dewata and Pekoe green tea extracts produced total polyphenols that were respectively higher by 893% or 8.93x, 151% or 15.1x, 280% or 28x and 193% or 19.3x, compared to total polyphenols of kombucha tea from commercial black tea (0.861%).

Research conducted by Wistiana showed that the longer the fermentation period, the higher the total phenol content (Wistiana & Zubaidah, 2015). The results showed a difference in the total phenol content of each kombucha sample. This is likely due to the different phenolic compounds contained in the tea leaves. Phenolic compounds are influenced by the flavonoid content, which is influenced by the growing location and the availability of sufficient sunlight for photosynthesis. Therefore, the total phenol content of each kombucha product varies. Phenolic compounds can be increased through the fermentation process. The depolymerization of thearubrigine may occur during fermentation, which may explain the increase in total phenol content that occurs during fermentation.

Reducing sugars in kombucha tea are residual metabolites of glucose and fructose that are not hydrolyzed by *Acetobacter xylinum* bacteria and therefore remain detectable (Susilowati, 2013). Kombucha fermentation for 1 and 2 weeks reduced reducing sugars in the green tea extract biomass of A. Kiara, A. Yabukita, and Dewata, but increased them in Pekoe and the black tea control. This is likely related to the tea processing process, in addition to the influence of the initial polysaccharide concentration, invertase enzyme activity, and conditions. A. Kiara and A. Yabukita were steam-processed at a low temperature (80ºC), thus preserving their polysaccharides. Dewata and Pekoe were fan-firing at temperatures >175ºC (Susilowati, 2013), which resulted in the degradation of many tea components. Commercial black teas underwent natural fermentation, resulting in different compositions. In A. Kiara and A. Yabukita, all substrate was hydrolyzed by week 1, leaving only a small amount remaining by week 2. However, in Dewata and Pekoe, the cultures had a more difficult time adapting, and different reducing sugars only showed up after 2 weeks of fermentation. Compared with the tea extract before fermentation (without sucrose), fermentation increased reducing sugars both at 1 week and 2 weeks of fermentation. Optimization of reducing sugars was achieved in A. Kiara with reducing sugars of 42.25 mg/mL, lower (51 mg/mL) than the reducing sugars of commercial black tea during 2 weeks of fermentation.

Total acids are an important parameter in kombucha fermentation as metabolites produced by microbes during fermentation (Susilowati, 2013), and total solids represent the accumulation of all tea components, both soluble and insoluble. In kombucha fermentation, L-theanine and other amino acids play a role in the formation of the overall tea aroma through the biogenesis process of the kombucha culture, in addition to other components (chlorophyll, carotenoids, lipids, and volatile components, known to be present in more than 600 types of molecules in tea leaves). During the 2-week fermentation period, there was a decrease in L-theanine in all types of tea extracts, which was suspected to be due to the use of L-theanine as a source of kombucha culture protein for its growth or reactions related to microbial metabolism, in addition to the suspicion that L-theanine had reacted with other tea components to produce a specific kombucha tea aroma so that it was no longer detected as L-theanine. However, compared to green tea extracts before fermentation, there was an increase in L-theanine in A. Yabukita and Pekoe, but a decrease in L-theanine in A. Yabukita and Dewata at 1 week of fermentation. Optimization of L-theanine was achieved in A. Yabukita with L-theanine of 2.082% (dry weight), lower (2.514% dry weight) than L-theanine in commercial black tea during 1 week of fermentation.

The antioxidant activity observed in green tea kombucha in the study by Salsabilah & Handayani (2024) increased on day 3, which was attributed to the biotransformation carried out by microorganisms in the fermentation system. These microorganisms transformed compounds by utilizing enzymes present in plant cells, such as those in green tea. However, by day 5 of fermentation, antioxidant activity decreased. This decline was due to the acidic environment, which stabilizes phenolic compounds, making it more difficult for them to release protons that can interact with DPPH. The acids present in both substrates can inhibit further increases in antioxidant activity, resulting in an overall decrease. In contrast to previous studies, the duration of fermentation showed a significant effect on increasing antioxidant activity in turmeric kombucha from day 3 to day 15 of fermentation. Polyphenol and flavonoid compounds also influenced antioxidant properties, where the increase in total phenolic content was directly proportional to antioxidant activity. During fermentation, the concentration of free phenols increased, resulting in higher antioxidant activity. Turmeric infusion contains active phenolic compounds, namely diarylheptanoids and diarylpentanoids, one of which is curcumin, known for its antioxidant and antibacterial properties, with curcumin levels ranging from 3–15% (Nafisah et al., 2024).

Kombucha fermentation shows a significant effect on its physicochemical properties, particularly through the increase of essential micronutrients such as Mn, Cu, Fe, Cr, and Zn over time, with peak concentrations generally observed on day 14 of fermentation. The mineral concentration pattern follows the order Zn > Mn > Fe > Cu > Cr, and the variation is influenced by the type of tea used, where green tea yields the highest manganese levels, black tea produces the highest copper and zinc levels, and white tea contains the highest iron levels, while chromium is found in greatest amounts in kombucha made from green and red tea. The peak mineral concentration on day 14 is likely due to optimal microbial activity during the mid-fermentation phase. At this stage, mineral extraction from tea leaves into the liquid medium reaches its maximum as a result of increased acidity (declining pH) and microbial enzymatic activity that facilitates mineral solubilization. In addition, microbial metabolism is at its peak, resulting in biotransformation and release of mineral ions such as Mn, Cu, Zn, Fe, and Cr from the substrate by acetic acid bacteria and yeasts (Jakubczyk et al., 2022).

Titratable Acidity (TA) and pH are the main parameters indicating the progress of kombucha fermentation. In the study by Aung & Eun (2022) using laver kombucha, TA increased significantly with longer fermentation time. A gradual increase in TA was observed during the first 7 days of fermentation, followed by a sharp rise reaching the highest value at the end of the process. In contrast, pH showed a sharp decline throughout fermentation. This inverse relationship between TA and pH reflects successful kombucha fermentation, driven by the production of organic acids, particularly acetic acid. The formation of organic acids leads to an increase in TA, which is clearly observed in samples with longer fermentation times, with higher TA values recorded at lower pH levels. Overall, significant change points were observed during the initial period (days 2–4), the middle (days 6–7), and the end (days 12–14) of the fermentation process, while the following days showed considerably higher TA, which can affect the taste of the final product.

According to the findings of Vohra et al. (2019), ethanol concentration in kombucha is significantly influenced by both the carbon source and the duration of fermentation. Kombucha produced using tea combined with honey demonstrated a reduction in ethanol levels on day 14, followed by an increase up to day 60 of fermentation. Conversely, kombucha prepared with tea and white sugar exhibited the highest ethanol concentration on day 60, indicating that white sugar serves as an effective carbon source for sustained ethanol production during prolonged fermentation. Fermentation using carbon sources without tea resulted in peak ethanol levels on day 7, which subsequently declined, likely due to the microbial conversion of ethanol into acetic acid by SCOBY-associated bacteria or depletion of available carbon substrates. Meanwhile, kombucha fermented without additional carbon sources contained the lowest ethanol levels. Overall, these findings suggest that kombucha may be classified as an alcoholic beverage, as ethanol content increases with extended fermentation and varies according to the substrate utilized.

Regarding total acid, 2 weeks of kombucha fermentation produced higher total acid than 1 week in all green tea extracts and black tea controls as shown in Figure 3b. This is thought to be related to the occurrence of assimilative and dissimilative chemical reactions by the kombucha culture during fermentation. Biotransformation of glucose and fructose by acetic acid bacteria (Acetobacter), especially A. xylinum, will produce gluconic acid and other organic acids, namely lactic acid, malic acid, oxalic acid, butyric acid, glucuronic acid, which are counted as total acid. Dewata green tea extract produced the highest total acid (0.659%) compared to Arraca Kiara (0.395%), Arraca Yabukita (0.285%), Pekoe (0.263%) and commercial black tea (0.573%) in 2 weeks of fermentation by the activity of the Acetobacter invertase enzyme and the initial concentration and processing method. In 2 weeks of fermentation, the yield of reducing sugar as a residual metabolite that was not hydrolyzed as shown in Figure 3 shows that Dewata green tea extract was not higher (10 mg/mL) than Pekoe (27.13 mg/mL), A. Yabukita (6.13 mg/mL) and A.Kiara (7.6 mg/mL), in other words, almost all of the glucose and fructose yield in Dewata, A. Yabukita and A.Kiara was hydrolyzed to form organic acids as total acids. Compared with the total acid of microfiltration tea extract, kombucha fermentation increased the total acid in Arraca Kiara, Arraca Yabukita, Dewata and Pekoe by 55.45, 53.75, 86.64 and 16.86% respectively in 2 weeks of fermentation, or optimization of total acid was achieved in Dewata with a total acid of 0.659% (dry weight), higher than the total acid of commercial black tea (0.5733% dry weight) during 2 weeks of fermentation. Another study found that kombucha has a pH ranging from 3.0 to 5.5 (Marwati & Handria, 2013). The higher the concentration of kombucha starter, the lower the pH of the resulting kombucha tea. During the initial fermentation process, the pH decrease is caused by bacteria and yeast converting sucrose into organic acids.

The fermentation rate increased in the first week of total solids, but decreased in the second week of fermentation for all types of tea extracts in total solids (Susilowati, 2013). The increase in total solids in the first week is thought to be due to the contribution of sucrose (10% w/v) and kombucha culture (0.5% v/v), as well as the intermediate metabolites of kombucha culture, namely glucose and fructose. As the fermentation time increases, these components form volatile components (organic acids) and ethanol which are more volatile and polyphenols with low molecular weight and smaller particle size which overall reduce the total solids. Compared to the initial total solids of the material, 2 weeks of fermentation increased the total solids in Arraca Kiara and Arraca Yabukita by 30.82 and 30.75%, respectively, but decreased the total solids in Dewata and Pekoe by 42 and 34.64%, respectively. Optimization of total solids was achieved in Arraca Kiara with a total solid of 1.295%, lower than the total solids of commercial black tea (11.841%) during 2 weeks of fermentation.

Color is one of the most influential characteristics of food products, including kombucha. In study Evangelina & Nugerahani (2025), differences in the color of green tea kombucha were observed from the beginning of the fermentation process until day 12. It was found that the red color intensity in green tea kombucha was relatively low at the early stage, while the green and blue color intensities were also low. After 12 days of fermentation, the red color intensity increased, indicating that the tea infusion became brighter. The green and blue intensities did not show significant increases. As fermentation progressed, the brightness intensity of the green tea kombucha infusion continued to increase. In addition to increased brightness, the infusion also became more turbid due to microbial activity, such as the breakdown of substrates (granulated sugar or sucrose) into organic acids as one of the metabolic products, as well as the increase in microbial population in green tea kombucha. Overall, the color of green tea kombucha tended to shift toward a combination of yellow and orange.

Based on study Nurhayati et al. (2020), the addition of cascara concentration in kombucha production influences its physical characteristics, particularly viscosity. Kombucha with 2% cascara addition exhibited higher viscosity compared to kombucha with 1% cascara, as a higher amount of cascara increases the total dissolved solids and specific gravity, resulting in increased viscosity. This finding is consistent with Setianto et al. (2014), who stated that the amount of dissolved solids affects the viscosity of liquids. Based on observations on day 4, day 8, day 12, and day 16 of fermentation, the viscosity of cascara kombucha decreased as the fermentation progressed. This reduction in viscosity indicates microbial activity that degrades sucrose and other soluble components in the fermentation medium, leading to a decrease in total dissolved solids and consequently a decline in viscosity.

3.3 The effect of fermentation time on sensory properties of kombucha

Taste is one of the determinants of panelist acceptance. The longer the fermentation period, the lower the panelists' average preference for the kombucha flavor (Wistiana & Zubaidah, 2015). This is due to the higher levels of organic acids produced over time, resulting in a strong sour taste. In addition to its sour taste, kombucha is also known for having a complex flavor profile, including sweet, fruity, slightly spicy, and mildly bitter notes, depending on the raw materials and fermentation conditions. At the initial stage of fermentation, the tea exhibits a sweet taste; however, this sweetness progressively diminishes as the sugar is metabolized by microorganisms. Concurrently, bacterial activity leads to the production of organic acids, resulting in a gradual sensory transition from a sweet to a sour taste (Akbar et al., 2023). The longer the fermentation period, the more sour the taste and aroma of kombucha. This is because yeast and bacteria metabolize sucrose and produce a number of organic acids such as acetic acid, glunoronic acid, and gluconic acid. The organoleptic assessment reported by Josephine et al. (2023) indicated that a 15-day fermentation period resulted in a diverse flavor profile across different tea-based kombucha samples. Black tea kombucha exhibited fruity notes with hints of green apple and passion fruit, while green tea kombucha demonstrated floral and fruity sensations with peach and apple nuances. Oolong tea kombucha showed general flavor characteristics without specific descriptors, whereas white peony kombucha presented a delicate floral taste. In sencha tea kombucha, panelists perceived fruity and floral notes with a peach-like nuance. Another oolong-based kombucha displayed a combination of fruity and milky attributes with white pear characteristics. Jasmine tea kombucha exhibited distinctive floral notes dominated by jasmine aroma, and white tea kombucha presented an earthy profile with herbal and smoky undertones. Overall, Josephine et al. (2023) confirmed that a 15-day fermentation period produces complex and varied flavor characteristics depending on the type of tea used. Kombucha made from coffee leaves has a bitter and astringent taste. Caffeine and tannins contribute to the development of this bitterness and astringency. The longer the fermentation process, the more the tannin and caffeine levels decrease due to microbial activity that degrades these compounds. *Acetobacter sp., Lactobacillus sp., and Saccharomyces sp.* produce the enzyme tannase, which breaks down tannins during kombucha fermentation (Nurhayati et al., 2020).

Kombucha exhibits a unique and characteristic aroma that can be readily distinguished. The aroma of kombucha is due to the presence of organic acids and aromas produced by the leaves. The longer the fermentation lasts, the stronger the aroma becomes this is because yeast and bacteria metabolize sucrose and produce a number of organic acids, such as acetic acid, gluconic acid, and glucuronic acid. The aroma of kombucha is caused by the volatile compounds formed, producing a distinctive sour aroma (Shafira et al., 2022). The lactic acid and acetaldehyde produced lower the pH of the fermentation medium or increase the acidity, creating a distinctive aroma. However, according to the study by Gumanti et al. (2023), kombucha fermented for 10 days exhibited more pronounced acidic and alcoholic aromas compared to kombucha fermented for 14 days. This phenomenon may be attributed to a reduced availability of nutrients, particularly sugars, which are metabolized by the bacteria in the SCOBY for alcohol production, thereby diminishing the intensity of kombucha’s characteristic pungent aroma. Therefore, the duration of fermentation constitutes a critical factor in influencing the aroma of kombucha that is favored by consumers.

Color is an important sensory attribute that influences consumer perception and acceptance of kombucha. The color of kombucha not only reflects the type of raw materials and fermentation conditions but also provides an indication of product quality, including clarity, brightness, and pigment intensity developed during the fermentation process. Variations in color can be affected by the type of tea used, fermentation duration, and interactions between phenolic compounds and organic acids formed during fermentation . A common color change in kombucha is the transition from dark to bright and clear hues over the course of fermentation, which is caused by the ability of the microbial consortium to degrade pigments (Wahyuningtias et al., 2023). Kombucha tea from Arraca Kiara and Arraca Yabukita is brownish-yellow, brighter and clearer than Dewata and Pekoe. Compared to green tea extract before fermentation, the biomass appears cloudier overall because it is only filtered through a 0-mesh filter to separate the kombucha microbes/cultures.

The clarity of kombucha is influenced by the cellulose production of acetic acid bacteria within the biomass. An increase in turbidity may indicate a higher release of cellulose and other fibrous materials into the fermentation medium, as well as microbial growth during the fermentation process (Dartora et al., 2023). The clarity of each sample used in kombucha production increased from day 0 to day 14 during the fermentation process (Wistiana & Zubaidah 2015). This is suspected to be due to the pH of the tea brew, with pH <7 resulting in a lighter or brighter color. Furthermore, the results show that the clarity of each kombucha varies, influenced by the phenolic compounds they contain. Tannins are damaged by the acid, causing the kombucha's bright color to fade, and the reddish-brown color of the thearubrigin to fade.

The longer the fermentation time, the more nutrients in the tea solution are used up by the bacteria. The thickness of each kombucha leaf differed from leaf to leaf with varying phenol content (Wistiana & Zubaidah, 2015). The more nutrients available, the more cellulose strands are produced as secondary metabolites. These cellulose strands continue to bond, forming strong and compact bonds. Nata biomass is derived from the growth of Acetobacter xylinum during the fermentation process in a medium containing sugar and acid.

Fermentation time has a significant impact on the sensory properties of kombucha, including taste, aroma, and color. Longer fermentation generally increases acidity and aroma intensity, while also affecting the clarity and pigment profile of the beverage through microbial activity and chemical interactions. Therefore, controlling the fermentation duration is essential for achieving the desired sensory characteristics and ensuring overall consumer acceptance of kombucha.

Table 1. Summary of key findings on microbiological, physicochemical, and sensory changes during kombucha fermentation

No	Aspect	Parameter	Key Findings	Interpretation/Implication
1	Microbiology	Microbial growth during fermentation	The population of acetic acid bacteria and lactic acid bacteria increases at the beginning of fermentation, then decreases at the end due to acidity and nutrient depletion.	Optimal fermentation (7–10 days) maintains microbial balance, while over-fermentation suppresses growth due to low pH.
2		Activity of Acetic Acid Bacteria (AAB)	Acetobacter species oxidize ethanol into acetic acid and produce cellulose (SCOBY).	AAB play a vital role in producing the sour flavor, characteristic aroma, and formation of the kombucha biofilm.
3		Activity of Lactic Acid Bacteria (LAB)	LAB populations rise until day 8 and then decrease as the environment becomes more acidic. Dominant species include L. plantarum, L. casei, and L. mali.	LAB contribute to organic acid and detoxifying compound production (DSL and glucuronic acid).
4		Antimicrobial activity	Fermentation lasting 10–15 days enhances organic acid formation (acetic, lactic, gluconic acids), inhibiting E. coli and Salmonella typhi.	Longer fermentation strengthens antimicrobial properties, though efficacy varies with substrate and bacterial target.
5	Physicochemical	Total polyphenols	Polyphenol levels increase during fermentation due to microbial	Fermentation enhances antioxidant potential through increased

			biotransformation of catechins into simpler phenolics.	bioactive phenolic compounds.
6		Reducing sugars	Reducing sugars decline as microorganisms utilize sucrose and convert it into glucose, fructose, and volatile metabolites.	Prolonged fermentation reduces residual sugars, producing a less sweet beverage.
7		Total acids and pH	Total acidity rises while pH decreases (down to 2.5–3.5) as fermentation progresses.	Higher organic acid levels indicate successful fermentation and contribute to kombucha's sour taste and preservation.
8		Antioxidant activity	Antioxidant activity increases up to day 10, then slightly decreases as phenolic compounds stabilize in the acidic environment.	Moderate fermentation (5–10 days) achieves optimal antioxidant capacity.
9		Mineral content (Mn, Cu, Fe, Cr, Zn)	Mineral concentrations rise until day 14 due to enhanced solubilization from tea leaves by microbial enzymes and acidic pH.	Kombucha becomes a rich source of micronutrients during mid-fermentation.
10		Total solids	Total solids decrease after the first week as sugars and polyphenols convert to organic acids and ethanol.	Solid reduction signifies fermentation maturity and metabolic activity.
11		Color changes	The color shifts from dark to bright yellow-orange due to pigment degradation and increased brightness.	Lighter color indicates active fermentation and product stability.
12		Viscosity	Viscosity decreases as sugars and soluble solids are degraded over time.	Fermented kombucha becomes lighter and less viscous during progression.
13	Sensory	Taste	Sweetness decreases and sourness intensifies with fermentation time; best balance occurs at 7–10 days.	Extended fermentation yields an overly acidic flavor, reducing consumer preference.
14		Aroma	Acidic and fermentative aroma strengthens over time due to acetic acid and acetaldehyde production.	Fermentation duration determines the desirable aromatic intensity of kombucha.
15		Color and clarity	The color brightens and clarity increases as pigments and tannins degrade during fermentation.	Visual clarity serves as an indicator of proper fermentation.
16		SCOBY texture	SCOBY thickness and compactness increase as Acetobacter xylinum activity continues.	SCOBY formation reflects fermentation progress and microbial health.

4. Conclusions

Kombucha is a functional fermented beverage produced from tea and sugar solutions through the activity of a starter culture, resulting in a complex composition of vitamins, minerals, enzymes, and organic acids. The duration of fermentation has been shown to significantly influence its nutritional, functional, and sensory characteristics. Prolonged fermentation suppresses microbial growth due to the accumulation of antimicrobial phenolic compounds, while increasing phenolic and organic acid contents through depolymerization and microbial metabolism. Conversely, L-theanine levels decline as it is utilized in microbial growth or transformed into volatile compounds that contribute to the distinctive aroma of kombucha. Extended fermentation also decreases total solids as they are converted into smaller metabolites, and further enhances acidity, sour aroma, and color brightness, reflecting changes in pH and organic acid production. These findings underscore the critical role of fermentation time in shaping the overall quality of kombucha and provide valuable insights for optimizing its production as a functional beverage with improved health benefits and consumer acceptance.

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