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Physicochemical Characteristics of Watermelon (*Citrullus lanatus*) Peel Kombucha as Influenced by Fermentation Duration

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Abstract: This study aimed to determine the physicochemical characteristics of watermelon rind (*Citrullus lanatus*) kombucha, including antioxidant activity, vitamin C content, pH, and alcohol content, based on variations in fermentation duration. The findings were used to identify the most effective fermentation period for producing a beverage rich in natural antioxidants while adding value to agro-industrial waste utilization. This quantitative experimental study employed a single-factor completely randomized design (CRD) with two replications. The fermentation periods were 8, 10, and 12 days. Physicochemical analyses included antioxidant activity using the DPPH method, vitamin C content using iodometric titration, alcohol content using an alcohol meter, and pH measurement using a pH meter. Data were analyzed using the Kruskal–Wallis test, followed by Duncan's test to compare the effectiveness of fermentation treatments. The results showed that 10 days of fermentation produced the highest physicochemical characteristics, with antioxidant activity of 49.26 ± 0.11c, vitamin C content of 75.49 ± 1.50b, pH of 4.14 ± 0.35a, and alcohol content of 0.6 ± 0.00c. However, the alcohol content on day 10 exceeded the safe limit for beverage products; therefore, this treatment is not recommended for consumption. In contrast, the 8- and 12-day fermentation treatments produced physicochemical characteristics with alcohol levels within the safe limit. On day 12, the antioxidant activity was 45.66 ± 0.11b, vitamin C content was 64.42 ± 1.49b, pH was 3.99 ± 0.31a, and alcohol content was 0.4 ± 0.00a. On day 8, the antioxidant activity was 43.54 ± 0.11a, vitamin C content was 67.48 ± 3.07a, pH was 4.59 ± 0.02a, and alcohol content was 0.5 ± 0.00b. Overall, although the 10-day fermentation treatment yielded superior physicochemical properties, the 8- and 12-day treatments are more recommended because their alcohol contents remained within the safe limit.
Keywords: Watermelon rind kombucha; fermentation duration; physicochemical properties; antioxidant activity

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INTRODUCTION

Public awareness of healthy lifestyles continues to increase, leading to growing consumer interest in new functional food products with proven health benefits (Petrescu & Vermeir, 2019). Functional foods are associated with improved health and quality of life and are generally defined as food products that, when consumed regularly, can enhance health status and reduce disease risk. Probiotic consumption has also increased, accompanied by the emergence of various probiotic products, among which fermented foods are widely available (Batista et al., 2023). The selection of new and innovative food products, including fermented products, has become a global trend (Czarnowska-kujawska et al., 2024). This shift toward healthier lifestyles is reflected in consumer dietary behavior. Kim et al. (2022) reported several indicators of healthy lifestyle practices, including limiting sugar intake (68.6%), reducing junk food consumption (73.65%), and paying greater attention to the nutritional content of food products consumed (58.4%). These criteria can be addressed by kombucha, a fermented functional food containing antioxidants and probiotic properties that may support digestive health and promote a healthy lifestyle.

Kombucha is a functional fermented beverage with a sour, sweet, and slightly carbonated taste. It has been consumed traditionally since 220 BC and is known for its detoxifying properties and refreshing characteristics (Alves et al., 2024; Boying et al., 2022). Kombucha is fermented using a SCOBY (Symbiotic Culture of Bacteria and Yeast). During fermentation, sugar is converted into ethanol, which is subsequently oxidized by acetic acid bacteria into acetic acid (Kelly et al., 2025). Green tea and black tea are the most commonly used substrates in kombucha production. However, several studies have highlighted the use of alternative ingredients, such as herbal teas and fruits, to develop innovative kombucha flavor combinations with unique functional health benefits (Kitwetcharoen et al., 2025; Nurul & Swasono, 2025). The use of alternative substrates may also enhance the content of bioactive compounds and antioxidants (Barros et al., 2024).

Watermelon (*Citrullus lanatus*) is a member of the Cucurbitaceae family and is one of the most widely cultivated horticultural crops in temperate regions worldwide. Watermelon consists of three main parts: the thick green outer rind or exocarp, the white middle layer or mesocarp, and the edible flesh or endocarp (Bagas et al., 2020). The rind, which is often discarded as waste, contains high levels of fiber, vitamins, minerals, amino acids, and phenolic compounds with strong antioxidant activity (Rico et al., 2020; Rocchetti et al., 2021). The inner watermelon rind is also rich in citrulline (Rezagholizade Alieh et al., 2023). As an agro-industrial by-product, watermelon rind accounts for approximately 30–41% of total fruit waste and contains substantial dietary fiber (David et al., 2024).

The nutritional and fiber content of watermelon rind indicates its strong potential as an additional ingredient in kombucha production. Watermelon rind may support SCOBY metabolism during fermentation by serving as an additional nutrient substrate. Furthermore, its bioactive compounds may interact with acetic acid produced during fermentation, resulting in a healthier kombucha product with enhanced antioxidant properties. The utilization of fruit rind waste as a source of natural antioxidants may also contribute to waste reduction in the agro-industrial sector.

Several studies have reported that, among fruit-processing by-products, fruit peels often contain higher antioxidant levels and greater antioxidant capacity than the fruit flesh (Rico et al., 2020). Watermelon rind was therefore selected as a kombucha substrate rather than the flesh because of its higher antioxidant content. Neglo et al. (2021) compared the antioxidant activity of different watermelon parts and found that watermelon rind exhibited the highest antioxidant activity, reaching 55.75 ± 2.44, whereas the antioxidant activity of the flesh was 41.46 ± 3.45.

Previous research on watermelon rind kombucha was conducted by Gunawan et al. (2023), who demonstrated that watermelon rind kombucha functions as a probiotic beverage with high antioxidant activity. The product also showed potential antidiabetic and anti-aging properties, with the highest antiglycation activity and α-amylase inhibition reaching 55.65 ± 1.25% and 61.25 ± 0.90%, respectively. In addition, the incorporation of orange peel, apple peel, and golden kiwi peel has been shown to increase antioxidant activity compared with the control, as determined using the DPPH assay (Lee & Yi, 2023).

Based on previous studies on fruit peel kombucha, the compositional characteristics of fruit peels play an important role in determining the physicochemical properties of kombucha products. Therefore, this study aims to develop a functional food product while contributing to the reduction of agro-industrial waste through the production of watermelon rind kombucha. The physicochemical characteristics evaluated include antioxidant activity, vitamin C content, pH, and alcohol content under different fermentation durations, with the potential to provide health-related benefits.

METHOD

This study was conducted from October 2025 to March 2026 at the Industrial Microbiology Laboratory, Biology Education Program, Universitas Muhammadiyah Surakarta, located at Jl. Ahmad Yani, Pabelan, Sukoharjo. This research employed a quantitative experimental approach using a single-factor Completely Randomized Design (CRD). The factor examined was fermentation duration of watermelon rind (*Citrullus lanatus*) kombucha, with two replications, as follows:

Factor: Fermentation Duration

F1: Watermelon rind (*Citrullus lanatus*) kombucha fermented for 8 days
F2: Watermelon rind (*Citrullus lanatus*) kombucha fermented for 10 days
F3: Watermelon rind (*Citrullus lanatus*) kombucha fermented for 12 days

Each treatment produced one batch of kombucha, followed by two technical replications during the analytical stage. Each sample was tested twice according to the analytical method used for each parameter, namely antioxidant activity, vitamin C content, pH, and alcohol content. One sample represented each treatment, resulting in six analytical data points for each parameter.

Research Preparation

Preparation of materials and equipment

The preparation stage involved preparing the main materials, including fresh watermelon rind, green tea, palm sugar, water, and SCOBY starter, while ensuring their quality. Equipment such as pans, blenders, filters, measuring cups, and glass jars was cleaned and checked for proper function. The fermentation environment was maintained in a dark room at a stable temperature of 25–30°C.

Preparation of watermelon rind tea

The preparation of watermelon rind tea began with sterilizing all equipment used during tea and kombucha production, including stainless-steel pans, glass jars, wooden spoons, knives, and basins. The equipment was then dried before use. Drying is an important step in sample preparation because it reduces water content to below 15%, thereby preventing microbial growth (Ho et al., 2018). Watermelon rind contains approximately 95% water, making it highly susceptible to spoilage, particularly during fermentation. However, prolonged drying may alter the sensory, nutritional, and physical properties of the product (Rezagholizade Alieh et al., 2023).

Ho et al. (2018) compared watermelon rind drying temperatures of 40°C and 60°C using an oven and reported that oven drying was more effective than freeze-drying for red-fleshed watermelon varieties. In addition, samples dried at 60°C showed higher antioxidant values than those dried at 40°C. Because drying duration is a key factor affecting antioxidant activity, reducing drying time is important to maximize antioxidant retention.

Based on these findings, this study used oven drying at 50°C for 2 × 24 hours. Each watermelon was weighed and peeled into several pieces. The white rind was obtained by removing the outer green peel using a sharp knife and separating the rind from the flesh so that no red flesh or green peel remained. The wet weight of the watermelon rind was recorded before drying (±1200 g), and the dry weight was measured after drying (±25 g). The ratio of wet to dry weight was used as an indicator of drying success. Considering that watermelon rind contains approximately 95% water, controlling drying temperature and duration was essential to achieve a low moisture content (<15%) and ensure that the material was suitable for kombucha tea preparation.

Kombucha preparation began by boiling approximately 3.5 L of clean water in a pan. Green tea and dried watermelon rind were used at a 1:1 ratio (5 g/L:5 g/L). A total of 15 g of green tea and 15 g of dried watermelon rind were placed in a filter bag and immersed in boiling water. The tea was boiled for approximately 10 minutes, after which 300 g of palm sugar was added and stirred until dissolved. The infusion was then allowed to cool to a warm temperature. The cooled tea infusion was transferred into sterile 250 mL glass jars. Each jar was inoculated with 3 g of SCOBY and 1 mL of starter culture, then tightly closed. The watermelon rind tea was fermented according to the treatment duration, namely 8, 10, or 12 days, in a glass cabinet at room temperature of 25–30°C.

Research Implementation

1. Antioxidant activity test

Antioxidant activity was determined using the radical scavenging activity (RSA) method. The stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) was used to measure the antioxidant activity of the samples through spectrophotometric analysis (Ghaffar et al., 2021). The percentage of antioxidant activity was calculated using the following equation (Rodhiyah et al., 2024):

DPPH antiradical activity (%) = $\frac{A\ blanko - A\ sampel}{A\ blanko} \times 100\%$

Note: A represents the absorbance value.

2. Vitamin C analysis

Vitamin C content was analyzed using the iodimetric titration method. A 25 mL kombucha tea sample was measured using a pipette and recorded as the initial sample volume. The sample was diluted with distilled water to 100 mL. A 10 mL aliquot was then transferred into a 250 mL Erlenmeyer flask. Next, 2 mL of 1% starch indicator solution was added, and the sample was titrated with 0.01 N iodine solution until a blue color appeared. Vitamin C content was calculated using the following equation (Qutrunnadakhairunnisa et al., 2024):

Vitamin C content (mg/100 g) = $\frac{ml\ iod \times 0{,}88 \times dulation\ factor}{sample\ weight} \times 100$

3. Alcohol Content Analysis

Alcohol content (%) was measured using the immersion method with an Alla France alcohol meter. The instrument was first calibrated using distilled water until the scale indicated 0% to ensure measurement accuracy. The alcohol meter was then immersed in a tube containing the kombucha sample until it floated stably. The value indicated at the liquid surface was recorded as the final alcohol content.

4. pH Measurement

The acidity level (pH) was measured using a Mediatech pH meter. Before measurement, the instrument was calibrated using a pH 4.00 buffer solution. The pH meter was then rinsed with distilled water and dried. The electrode was immersed in the kombucha sample according to treatment until the reading stabilized for approximately 2 minutes. The pH meter was rinsed with distilled water each time it was transferred to another kombucha sample.

Data Collection Techniques and Instruments

1. Experimental method

This study used an experimental approach by producing watermelon rind kombucha with different fermentation durations. The experiment was conducted to compare the quantitative data obtained from each treatment.

2. Observation method

Observation was used as a data collection technique to record experimental results and document the research process, including the preparation of materials, equipment, and kombucha production.

Data Analysis Technique

Data analysis was conducted to test the proposed hypotheses. The null hypothesis (H0) stated that there was no difference in the quality and physicochemical characteristics of watermelon rind (*Citrullus lanatus*) kombucha based on fermentation duration. The alternative hypothesis (H1) stated that there was a difference in the quality and physicochemical characteristics of watermelon rind (*Citrullus lanatus*) kombucha based on fermentation duration.

Statistical analysis was performed quantitatively. The normality test was conducted using the Kolmogorov–Smirnov test, while homogeneity was assessed using Levene's test. Because the data were not normally distributed and were not homogeneous, the analysis was continued using the non-parametric Kruskal–Wallis test to detect significant differences among fermentation-duration treatments. Duncan's test was then used to determine the most effective fermentation duration for watermelon rind kombucha.

RESULTS AND DISCUSSION

The physicochemical characteristics of watermelon rind (*Citrullus lanatus*) kombucha were evaluated based on fermentation duration. The parameters analyzed included antioxidant activity, vitamin C content, alcohol content, and pH. The Kruskal–Wallis test was used to determine whether fermentation duration significantly affected these parameters. The results are presented in Table 1.

Table 1. Kruskal–Wallis test results

Parameter	Antioxidant activity (%)	Vitamin C (mg/100 g)	Alcohol content (%)	pH
Kruskal–Wallis	4.571	3.714	4.571	3.715
df	2	2	2	2
Asymp. Sig.	0.102	0.156	0.102	0.156

Based on Table 1, all parameters had p-values greater than 0.05. Antioxidant activity and alcohol content had the same p-value of 0.102, whereas vitamin C content and pH had p-values of 0.156. These results indicate that fermentation duration did not produce statistically significant differences in the physicochemical characteristics of watermelon rind kombucha based on the Kruskal–Wallis test. The interpretation of these results is summarized in Table 2.

Table 2. Hypothesis test results

Parameter	p-value Interpretation	Conclusion
Antioxidant activity	0.102 > 0.05	H0 accepted; no significant difference among fermentation durations
Vitamin C	0.156 > 0.05	H0 accepted; no significant difference among fermentation durations

Parameter	p-value Interpretation	Conclusion
Alcohol	0.102 > 0.05	H0 accepted; no significant difference among fermentation durations
pH	0.156 > 0.05	H0 accepted; no significant difference among fermentation durations

Although the Kruskal–Wallis test showed no significant differences among treatments, the mean values in each treatment showed different descriptive trends. Therefore, the mean values and Duncan notation were used to describe the pattern of changes in each physicochemical parameter during fermentation. The results are presented in Table 3.

Table 3. Duncan test results

Fermentation duration	Antioxidant activity (%)	Vitamin C (mg/100 g)	Alcohol (%)	pH
F1	43.5443 ± 0.117[a]	67.4847 ± 3.07[a]	0.5060 ± 0.0011[b]	4.59 ± 0.0282[a]
F2	49.2647 ± 0.117[c]	75.4954 ± 1.50[b]	0.6126 ± 0.0023[c]	4.14 ± 0.3535[a]
F3	45.6699 ± 0.115[b]	64.4028 ± 1.49[a]	0.4979 ± 0.0012[a]	3.99 ± 0.3111[a]

Values are presented as mean ± standard deviation. Different superscript letters indicate significant differences at P < 0.05. Values with the same superscript letter are not significantly different.

Antioxidant Activity

As shown in Table 3, antioxidant activity increased from F1 to F2 and then decreased in F3. The antioxidant activity was 43.5443 ± 0.117% in F1, increased to 49.2647 ± 0.117% in F2, and decreased to 45.6699 ± 0.115% in F3. The highest antioxidant activity was observed in F2, indicating that the 10-day fermentation treatment produced the most favorable antioxidant profile compared with the other fermentation durations.

The increase in antioxidant activity during fermentation may be associated with the activity of bacteria and yeasts, which produce enzymes capable of degrading complex compounds into simpler bioactive compounds. According to (Hunandar, 2016), biotransformation of phenolic compounds occurs during kombucha fermentation due to enzymatic activity, accompanied by the release of catechins from acid-sensitive microorganisms. Similarly, (Coelho et al., 2020) reported that sugars are hydrolyzed by yeasts and enzymes produced by lactic acid bacteria, resulting in increased phenolic compounds.

The higher antioxidant activity in F2 is consistent with (Kuzu et al., 2023), who reported that total phenolic compounds increased after 10 days of fermentation. (Sutthiphatkul et al., 2023) also found that black tea kombucha supplemented with roselle and fermented for 10 days showed higher antioxidant activity than black tea kombucha without roselle. However, the decrease in antioxidant activity in F3 suggests that prolonged fermentation may reduce some antioxidant compounds due to further degradation or changes in the stability of bioactive compounds.

The antioxidant activity of watermelon rind kombucha may also be influenced by the substrate and sugar source. In this study, watermelon rind and palm sugar/brown sugar were used as the main fermentation materials. (Suciati et al., 2024) reported that the addition of coconut sugar or brown sugar produced high antioxidant activity because brown sugar contains glucose, fructose, and natural bioactive compounds such as phenolics and minerals. In addition, (Gerek, 2025) found that fruit peel substrates may contribute substantially to phenolic content. In watermelon rind, microbial degradation of the pectin–polysaccharide matrix during fermentation may release bound phenolics and increase the bioaccessibility of L-citrulline, thereby increasing free phenolic content and total antioxidant activity (Kumar et al., 2025).

Vitamin C

Table 3 shows that vitamin C content varied among fermentation treatments. The vitamin C content was 67.4847 ± 3.07 mg/100 g in F1, increased to 75.4954 ± 1.50 mg/100 g in F2, and then decreased to 64.4028 ± 1.49 mg/100 g in F3. The highest vitamin C content was observed in F2, indicating that fermentation for 10 days was more effective in maintaining or increasing vitamin C content than fermentation for 8 or 12 days.

This increase may be related to the release of bioactive compounds from watermelon rind during fermentation. (Almugni et al., 2025) reported that watermelon rind from Redin and Amara varieties contains vitamin C and has potential as a source of this nutrient. In addition, (Soto et al., 2018) observed an increase in vitamin C content in kombucha tea on the 10th day of fermentation, which supports the finding that moderate fermentation duration may improve vitamin C availability.

However, vitamin C content decreased in F3. This decline may be caused by oxidative degradation during prolonged fermentation. (Kuzu et al., 2023) reported that vitamin C content in green tea kombucha supplemented with fruit decreased as fermentation duration increased. Oxidative degradation of vitamin C is influenced by temperature, oxygen exposure, water activity, pH, and the presence of metal ions that act as catalysts (Aji et al., 2023).

According to (Ebrahimi & Dabbagh, 2019), L-ascorbate can be oxidized into unstable dehydroascorbic acid, which subsequently decomposes into inactive compounds. Longer fermentation duration increases cumulative oxygen exposure and microbial metabolite accumulation, thereby accelerating vitamin C degradation. Therefore, the lower vitamin C content in F3 may reflect the instability of ascorbic acid under prolonged fermentation conditions. This interpretation is also supported by (Gerek, 2025), who stated that the reduction in vitamin C during fermentation can be caused by oxidation and enzymatic degradation by microorganisms.

Alcohol Content

As presented in Table 3, alcohol content increased from F1 to F2 and then decreased in F3. The alcohol content was 0.5060 ± 0.0011% in F1, increased to 0.6126 ± 0.0023% in F2, and decreased to 0.4979 ± 0.0012% in F3. These results indicate that alcohol production reached its highest level in F2, corresponding to the 10-day fermentation treatment, before declining during further fermentation.

Alcohol is naturally produced during kombucha fermentation through sugar metabolism by yeasts. The ethanol formed may subsequently be oxidized by acetic acid bacteria into organic acids, particularly acetic acid (Nasution & Pasaribu, 2023). Therefore, the increase in alcohol content in F2 indicates active ethanol production during the middle phase of fermentation, whereas the decrease in F3 suggests that ethanol was further converted into organic acids during extended fermentation.

The alcohol level in F2 exceeded 0.5%, which has important implications for product acceptability in Indonesia. Based on the Indonesian Ulema Council Fatwa No. 10 of 2018 concerning food and beverage products containing alcohol/ethanol, fermented beverages containing alcohol at 0.5% or higher are categorized as haram. Therefore, although F2 showed the highest antioxidant activity and vitamin C content, its alcohol content may limit its suitability for consumption and commercial distribution under Indonesian halal regulations.

The observed increase in alcohol content up to day 10 is consistent with (Chen & Liu, 2000), who reported that alcohol content in kombucha increased with fermentation duration and generally reached its maximum value around day 10. A similar finding was reported by (Tanushree & Katyal, 2024), who found that kombucha fermentation could produce ethanol levels of up to 0.69%.

In contrast, the decrease in alcohol content in F3 indicates the occurrence of advanced fermentation. At this stage, acetic acid bacteria may have converted accumulated ethanol into acetic acid. This finding is supported by (Kallel et al., 2012), who reported that ethanol content decreased to 0.3% after 12–15 days of fermentation. Similarly, (Cheepchirasuk et al., 2025) stated that the reduction in alcohol content during the later stage of fermentation is associated with the bioconversion of ethanol into acetic acid by acid-producing bacteria.

pH

Table 3 shows that the pH of watermelon rind kombucha decreased as fermentation duration increased. The pH was 4.59 ± 0.0282 in F1, decreased to 4.14 ± 0.3535 in F2, and further decreased to 3.99 ± 0.3111 in F3. Although the decrease was observed descriptively, the same superscript notation indicates that the pH values were not significantly different among treatments.

The decrease in pH reflects the accumulation of organic acids during fermentation. In kombucha, organic acids are mainly produced by acetic acid bacteria through the oxidation of ethanol into acetic acid. Therefore, the lower pH in F3 is consistent with the decrease in alcohol content observed in the same treatment, suggesting that ethanol conversion into organic acids occurred during prolonged fermentation.

pH is an important parameter for evaluating kombucha safety and stability. (Kitwetcharoen et al., 2025) stated that pH is used to determine the safety of kombucha products and the consistency of their chemical composition. Low pH conditions also help inhibit the growth of pathogenic and spoilage microorganisms (Suffys et al., 2023).

The pH values obtained in this study remained within the acceptable range for kombucha consumption. (Puspaningrum et al., 2022) stated that kombucha is considered safe for consumption when its pH is not below 3. This statement is supported by (Rezende et al., 2020), who reported that pH values above 3 are generally acceptable because lower values may indicate excessive acetic acid content. However, pH values above 4.2 may increase the risk of microbial growth and compromise the microbiological safety of fermented beverages. Based on this consideration, F2 and F3 showed more favorable acidity profiles than F1, although microbiological testing would still be required to confirm product safety.

CONCLUSION

This study showed that fermentation duration influenced the physicochemical profile of watermelon rind (*Citrullus lanatus*) kombucha. Although the Kruskal–Wallis test indicated no significant differences among treatments, the mean values showed clear descriptive changes in antioxidant activity, vitamin C content, alcohol content, and pH. The 10-day fermentation treatment produced the highest antioxidant activity and vitamin C content, indicating its potential to improve the functional quality of watermelon rind kombucha. However, this treatment also resulted in alcohol content exceeding 0.5%, which may limit its suitability for consumption and commercial distribution under Indonesian halal regulations. In contrast, the 12-day fermentation treatment produced alcohol content below 0.5%, acceptable pH, and relatively high antioxidant activity. Therefore, the 12-day fermentation duration is considered the most appropriate treatment when functional quality, acidity, alcohol content, and regulatory acceptability are considered together. This finding suggests that watermelon rind has potential as a substrate for functional kombucha production and as an alternative strategy for utilizing fruit waste.

RECOMMENDATION

The selection of raw materials (substrates) and fermentation duration are key factors influencing the quality of watermelon rind kombucha (*Citrullus lanatus*). Appropriate determination of sampling intervals is also essential, as excessively short intervals may not produce statistically meaningful differences among kombucha samples. Furthermore, kombucha products should be safe for consumption. In the present study, the kombucha fermented for 10 days contained alcohol levels exceeding the recommended safety threshold; therefore, it is not suitable for consumption. Future studies are recommended to employ longer fermentation intervals when evaluating variations in fermentation duration to obtain more distinct and significant differences among treatment groups.

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