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Effect of Fermentation Time on the Microbiological, Chemical and Sensory Characteristics of Kombucha Genitri Leaf

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***ABSTRACT**: Genitri leaves contain secondary metabolites, including alkaloids, flavonoids, and phenolic compounds, which exhibit potent antioxidant activities. The quality and profile of these secondary metabolites can be enhanced through microbial fermentation, such as in the production of kombucha. This study aimed to determine the optimum fermentation duration for genitri kombucha. The experiment was arranged in a Completely Randomized Design (CRD), and the gathered data were statistically analyzed to evaluate significant differences among the treatments. The results demonstrated a progressive decline in pH and Total Soluble Solids (TSS) as the fermentation period extended. Conversely, the concentration of flavonoids, phenolic compounds, and the total counts of Lactic Acid Bacteria (LAB) and yeast significantly increased with longer fermentation. Sensory evaluation revealed that panelists preferred the kombucha fermented for 14 days, citing a well-balanced acidity. In conclusion, a 14-day fermentation period is optimal for achieving the most desirable sensory, chemical, and microbiological characteristics of genitri kombucha.*

***Keywords:** fermentation duration, genitri, kombucha.*

1. INTRODUCTION

Functional foods have received increasing attention in line with the growing public awareness of the relationship between dietary patterns and health. The production of functional foods is often associated with fermentation processes. Fermentation is a method employed to modify the nutritional quality of food (Krogerus et al., 2018). Through the fermentation process, complex compounds are hydrolyzed into simpler forms, thereby enhancing their digestibility in the human body (Rokhmah et al., 2009; Śliżewska & Chlebicz-Wójcik, 2020). During fermentation, microorganisms produce various metabolites, such as organic acids, vitamins, and bioactive compounds, which contribute to both the sensory characteristics and the potential health benefits of the resulting products.

One of the fermented products that utilizes various types of microorganisms and is recognized as a wild fermentation product is kombucha. Kombucha is a beverage produced from brewed tea leaves inoculated with a SCOBY (Symbiotic Culture of Bacteria and Yeast) starter culture. Within a kombucha starter, groups of bacteria and yeasts interact synergistically to produce distinctive flavors, aromas, and metabolic compounds that are beneficial to the human body. A study conducted by Yaa'ri et al.,

(2024) demonstrated that kombucha fermentation produces compounds such as ethyl 3-methylbutanoate, phenethyl acetate, ethyl hexanoate, and 2-methyl-1-propyl acetate, which contribute to fruity, sweet, and floral aroma characteristics. In addition, these compounds are components of bioactive substances that provide health benefits. Differences in the raw materials used for kombucha production can influence the flavor, aroma, and bioactive compounds generated during fermentation.

A plant with potential for development as a raw material for kombucha production is genitri (*Elaeocarpus ganitrus*). To date, genitri has been more widely recognized for the utilization of its seeds in handicrafts, accessories, and spiritual practices. From a physiological perspective, genitri leaves are known to contain various secondary metabolites that play important roles in the plant's defense mechanisms against environmental stress. Studies conducted by Khushwaha et al., (2023) and Kiromah et al., (2021) reported FTIR analysis results indicating the presence of alkaloids, flavonoids, phenolic compounds, tannins, and saponins. Furthermore, research by Khushwaha et al., (2023) identified several compounds in genitri leaves through LC-MS analysis, including gallic acid, chlorogenic acid, pinocembrin, epicatechin, caffeic acid, and naringenin. The presence of these bioactive compounds makes genitri leaves an attractive candidate for development as a raw material for kombucha production.

The quality of kombucha is strongly influenced by fermentation duration, as this factor determines the metabolic activity of microorganisms throughout the fermentation process. Yeasts play a role in hydrolyzing sucrose into glucose and fructose, which are subsequently fermented into ethanol and carbon dioxide (Timmermans et al., 2022). The metabolites produced are then utilized by fermentative bacteria to generate various organic acids. The activity of these microorganisms leads to changes in the chemical characteristics of the product, including a decrease in total soluble solids (TSS) due to the utilization of sugars as fermentation substrates and a reduction in pH resulting from the accumulation of organic acids (Jiang et al., 2020; Yaa'ri et al., 2024). Therefore, monitoring the total lactic acid bacteria and total yeast populations is important to describe the dynamics of microbial communities involved in the development of kombucha characteristics during fermentation (Kaashyap et al., 2021).

Microbiological and chemical changes occurring during fermentation can influence the sensory characteristics of the product. Reductions in total soluble solids (TSS) and pH may affect sweetness, acidity, fermentative aroma, and overall consumer acceptance of kombucha. A fermentation period that is too short may result in a product with underdeveloped fermentation characteristics, whereas excessively prolonged fermentation may produce an overly acidic taste, thereby reducing consumer preference(Zubaidah et al., 2019). Therefore, organoleptic evaluation is an important aspect in determining the fermentation duration that can achieve an optimal balance among the microbiological, chemical, and sensory characteristics of genitri leaf kombucha.

Although numerous studies have investigated kombucha produced from various plant materials, information regarding the utilization of genitri leaves as a raw material for kombucha remains very limited. In addition, the effects of fermentation duration on microbial dynamics, changes in chemical characteristics, and the sensory quality of genitri leaf kombucha have not been extensively reported. Based on this research gap, the present study was conducted to evaluate the effect of fermentation duration on total lactic acid bacteria, total yeast population, total soluble solids, pH, and the organoleptic characteristics of genitri leaf kombucha.

2. MATERIALS AND METHODS

The primary materials used in this study consisted of genitri leaf powder, black tea,

granulated sugar, and a 14-day-old kombucha starter culture. All chemical reagents employed throughout the study were obtained from Merck (Darmstadt, Germany) and Oxoid (Basingstoke, Hampsphire, United Kingdom).

The equipment utilized in this research included cooking pots (Lionstar, Indonesia), glass jars (Iwaki, Japan), spatulas (Lionstar, Indonesia), a stove (Rinnai, Indonesia), burettes (IWAKI, Japan), erlenmeyer flasks (IWAKI, Japan), a pH meter (Hach, USA), a laminar airflow cabinet (Biobase, Jinan-Tiongkok), petri dishes (IWAKI, Japan), a spectrophotometer (Hach, USA), cuvettes (IWAKI, Japan), and other supporting laboratory instruments required for the experimental procedures.

2.1. Genitri Kombucha Production

The preparation of kombucha started with the sterilization of all equipment using boiling water. Tea, genitri leaf powder, and granulated sugar were brewed for 10 minutes until a concentrated infusion was obtained, followed by a filtration process. After brewing with hot water, room-temperature water was added to accelerate cooling. Once the solution reached a lukewarm temperature, kombucha starter culture and SCOBY obtained from a previous fermentation batch were added. The kombucha starter used consisted of SCOBY and kombucha from the previous fermentation batch, which had been fermented for 14 days. The SCOBY had a diameter of 20 cm and a thickness of 3 cm. The formulation used for kombucha production was as follows (Table 1).

Table 1. Kombucha Genitri Formulation

Ingredients	Amounts
Sucrose	25 g
Kombucha starter culture	25 ml
Scooby culture	20 cm (diameter) 3 cm (thickness) 50 g (weight)
Genitri leaf powder	1,25 g
Black tea	3,75 g
Water	250 ml

2.2. Determination of Yeast

A total of 1 mL of kombucha sample was diluted in 9 mL of 0.85% NaCl solution. Serial dilutions were then performed up to 10^{-7}. Three tubes from the final dilution were used for total yeast enumeration. Subsequently, the samples were inoculated onto SDA medium supplemented with chloramphenicol. The inoculated plates were incubated at 37°C for 72 hours(Rizqiati et al., 2023).

2.3. Determination of Lactic Acid Bacteria

A total of 1 mL of kombucha sample was diluted in 9 mL of 0.85% NaCl solution. Serial dilutions were subsequently performed up to 10^{-8}. Three tubes from the final dilution were used for microbiological analysis. The samples were then incubated at 37°C for 48 hours (Rizqiati et al., 2023).

2.4. Determination of pH

Measurement of kombucha pH was carried out using a pH meter. The instrument was calibrated beforehand using standard buffer solutions of pH 3, 5, and 7. Subsequently, the electrode was immersed in the prepared kombucha sample, and the pH value was recorded once a stable reading was obtained (Rizqiati et al., 2023).

2.5. Determination of Total Soluble Solid

Measurement of total soluble solids (TSS) was carried out using a refractometer. The prepared kombucha sample from each treatment was placed onto the prism surface of the refractometer, and the TSS value was determined directly from the reading displayed on the instrument. The results were expressed as °Brix (Rizqiati et al., 2023).

2.6. Determination of Total Phenolic Content

Total phenolic content was determined using the Folin–Ciocalteu colorimetric method with gallic acid as the standard compound. A series of gallic acid standard solutions was prepared to construct a calibration curve. Each standard solution was reacted with Folin–Ciocalteu reagent,

followed by the addition of 20% sodium carbonate solution. The reaction mixture was then diluted with demineralized water and incubated at room temperature for 30 min to allow color development. Absorbance was measured using a UV–Vis spectrophotometer at a wavelength of 740 nm. The total phenolic content of the samples was subsequently calculated based on the gallic acid calibration curve (Sintya Hunandar, 2016)

2.7. Determination of Total Flavonoid

Total flavonoid content was determined using the aluminum chloride colorimetric method with quercetin as the standard reference compound. The maximum absorption wavelength and optimum incubation time were established using a 100 ppm quercetin solution reacted with 10% $AlCl_3$ and 5% acetic acid. A quercetin standard curve was subsequently prepared at concentrations ranging from 40 to 120 ppm under the same reaction conditions. For sample analysis, the kombucha extract was prepared at a concentration of 1000 ppm and reacted with 10% $AlCl_3$ and 5% acetic acid. After incubation for the predetermined optimum time, the absorbance was measured at the maximum wavelength using a UV–Vis spectrophotometer. All measurements were performed in triplicate, and the total flavonoid content was calculated based on the quercetin calibration curve (Lee et al., 2024).

2.8. Sensory Analysis

Sensory evaluation was conducted using a hedonic test to determine the panelists' level of preference for genitri kombucha. Each panelist was provided with 50 mL of kombucha from each predetermined treatment. The panelists were asked to assess their degree of liking for the products using a five-point hedonic scale. A score of 1 indicated that the panelist disliked the product, whereas a score of 5 indicated that the panelist liked the product very much.

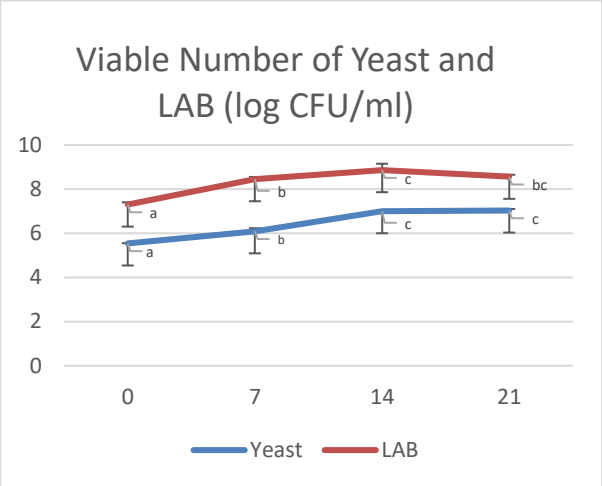
2.9. Data Analysis

All data obtained were statistically analyzed using Analysis of Variance (ANOVA) to determine the effect of the treatments. Microbiological and chemical data were obtained from three replicates for each treatment and were subsequently subjected to statistical analysis. When a significant treatment effect was observed, Duncan's Multiple Range Test (DMRT) was performed as a post hoc analysis to identify significant differences among treatments. Organoleptic data were analyzed using the Kruskal–Wallis test, followed by the Mann–Whitney test to determine significant differences between treatments.

3. RESULTS AND DISCUSSION

3.1. Microbiological Analysis

The microbiological characteristics of kombucha were evaluated based on the total viable yeast and lactic acid bacteria (LAB) counts during fermentation. The results are presented below (Table 2) and (Figure 1).



Note: Results presented as mean ± standard deviation of triplicates (n=3). Values marked with different superscript letters showed significant difference between group ($p<0.05$)

Figure 1. Viable Number of Yeast and Lactic Acid Bacteria (LAB)

The results of the study showed that the total yeast counts of genitri kombucha at fermentation periods of 0, 7, 14, and 21 days were 5.54, 6.09, 7.00, and 7.03 log CFU/mL, respectively. Statistical analysis indicated that fermentation duration had a significant

effect on total yeast counts. Significant differences were observed among the 0-, 7-, and 14-day fermentation periods, whereas no significant difference was found between the 14- and 21-day fermentation periods. As the fermentation period increased, the number of viable yeasts in the kombucha also increased. However, no significant yeast growth was observed between days 14 and 21 of fermentation.

The total lactic acid bacteria (LAB) counts of genitri kombucha at fermentation periods of 0, 7, 14, and 21 days were 7.30, 8.45, 8.86, and 8.56 log CFU/mL, respectively. Statistical analysis revealed that fermentation duration had a significant effect on total LAB counts. Significant differences were observed among the 0-, 7-, and 14-day fermentation periods, whereas no significant difference was found between the 14- and 21-day fermentation periods. The increase in fermentation duration was associated with an increase in the total LAB population; however, no significant difference in the viable LAB counts was observed between days 14 and 21 of fermentation.

Both parameters exhibited a similar trend. On day 0, yeasts and LAB were still adapting to the genitri kombucha medium. As the fermentation period increased, both yeasts and LAB demonstrated active growth, indicating an exponential growth phase (Krogerus et al., 2018; Śliżewska & Chlebicz-Wójcik, 2020). A study conducted by (Tran et al., 2020) also reported an increase in microbial populations with increasing fermentation duration.

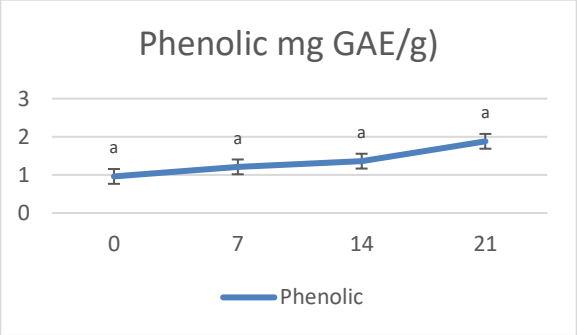
A study conducted by (Yang et al., 2022) reported that the LAB present in kombucha generally belong to the genus Lactobacillus. Not all LAB species found in kombucha exhibit probiotic properties; however, they may contribute to the improvement of digestive health. Furthermore, a study by (Yuliana et al., 2023) demonstrated an increase in microbial populations with increasing fermentation duration in kombucha.

The results of total yeast and total LAB analyses indicate the number of viable microorganisms present in kombucha. Yeasts convert sucrose into glucose, ethanol, and CO_2 (Da Silva Fernandes et al., 2022; Wilson et al., 2019). Lactic acid bacteria also metabolize substrates to produce lactic acid (Cvetković et al., 2019).

During the fermentation process, LAB and yeasts produce various metabolites as end products of fermentation, including ethanol, CO_2 and organic acids (Lee et al., 2024; R. Li et al., 2022; Zubaidah et al., 2018). The organic acids produced during kombucha fermentation may act as antibacterial agents, thereby inhibiting the growth of pathogenic microorganisms throughout the fermentation process. Meanwhile, the CO_2 and ethanol generated during kombucha production contribute to the sparkling sensation experienced when consuming kombucha (Nyhan et al., 2022; Tran et al., 2020).

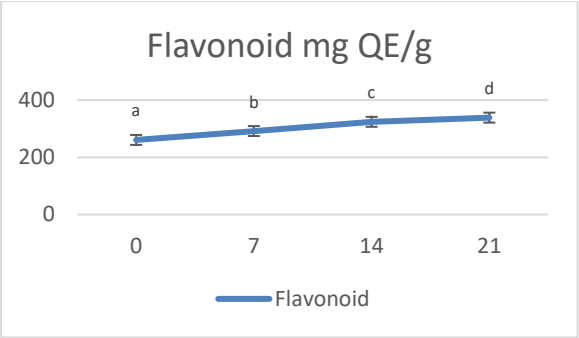
3.2. Phenolic and Flavonoid Analysis

The total phenolic and flavonoid contents of kombucha during the fermentation process were analyzed, and the results are presented below (Figure 2 and Figure 3).



Note: Results presented as mean ± standard deviation of triplicates (n=3). Values marked with different superscript letters showed significant difference between group ($p<0.05$)

Figure 2. Phenolic Compound Kombucha Genitri Leaves (mg GAE/g)



Note: Results presented as mean ± standard deviation of triplicates (n=3). Values marked with different superscript letters showed significant difference between group (p<0.05)

Figure 3. Flavonoid Compound Kombucha Genitri Leaves (mg QE/g)

The results of the study indicated that fermentation duration had no significant effect on the phenolic content of genitri kombucha. The phenolic contents of kombucha fermented for 0, 7, 14, and 21 days were 0.96, 1.21, 1.36, and 1.88 mg GAE/g), respectively.

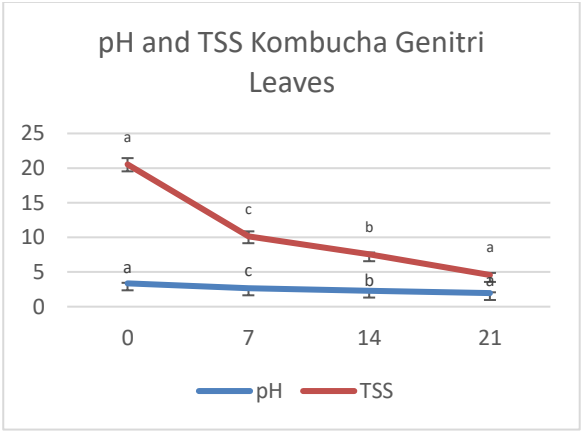
During the fermentation process, microorganisms are presumed to carry out biotransformation, converting complex phenolic compounds into simpler forms (Gaur & Gänzle, 2023; F. Yang et al., 2023). However, the analysis performed in this study measured total phenolic content rather than individual phenolic compounds. Therefore, no significant differences in phenolic content were observed across the different fermentation periods.

The flavonoid contents of genitri kombucha fermented for 0, 7, 14, and 21 days were 260.67, 291.67, 324.00, and 338.67 mg QE/g), respectively. These results indicate that flavonoid content increased with increasing fermentation duration. The increase in this phytochemical content is attributed to the metabolic activity of microorganisms during the fermentation process (Saharan et al., 2020; Tomassi et al., 2025).

A study conducted by (S. Li et al., 2022) demonstrated that increasing fermentation time resulted in higher phenolic and polyphenol contents. This increase was attributed to the activity of microorganisms in degrading the substrate cell walls during the fermentation process. The concentrations of flavonoids and phenolic compounds in kombucha are highly influenced by the microbial strains involved in fermentation Tomassi. This phenomenon represents a limitation of the present study, as the kombucha starter culture used was not derived from specific microbial strains.

3.3. Chemical Analysis

Chemical analysis of the kombucha samples was conducted by determining pH and total soluble solids (TSS) at different fermentation periods. The results of the chemical characterization are presented below (Figure 4).



Note: Results presented as mean ± standard deviation of triplicates (n=3). Values marked with different superscript letters showed significant difference between group (p<0.05)

Figure 4. pH and TSS Kombucha Genitri Leaves

The pH values of genitri kombucha fermented for 0, 7, 14, and 21 days were 3.36, 2.64, 2.31, and 1.96, respectively. Fermentation duration had a significant effect on the pH of genitri kombucha, with significant differences observed among the fermentation periods. As fermentation time increased, the pH of the kombucha decreased. This reduction in pH was attributed to the production of organic acids resulting from microbial metabolic activity during the fermentation process.

A study conducted by (Tran et al., 2020) reported that the decrease in pH during kombucha fermentation was caused by the production of lactic acid and acetic acid

through microbial activity. Similarly, (Tomassi et al., 2025) observed a reduction in pH with increasing fermentation duration. The organic acids produced during the fermentation process include acetic acid, lactic acid, glucuronic acid, and citric acid.

The total soluble solids (TSS) of genitri kombucha fermented for 0, 7, 14, and 21 days were 20.53, 10.16, 7.56, and 4.57, respectively. Fermentation duration had a significant effect on the TSS of genitri kombucha, with significant differences observed among the fermentation periods. As fermentation time increased, the TSS values decreased. This reduction in TSS was attributed to the degradation and utilization of substrates by microorganisms during the fermentation process. At the end of the fermentation period, sucrose was no longer detected, resulting in a substantial decline in TSS values.

A study conducted by (Zubaidah et al., 2018) reported a decrease in the total soluble solids (TSS) of kombucha with increasing fermentation duration. Similarly, (Tran et al., 2020) demonstrated that *Saccharomyces cerevisiae* hydrolyzes sucrose and subsequently utilizes the resulting monosaccharides without inducing glucose-mediated catabolite repression.

3.4. Sensory Analysis

The sensory characteristics of kombucha were evaluated through a hedonic test based on color, aroma, taste, and overall acceptability. The hedonic scale used was a 5-point scale, where 1 indicated that the panelist strongly disliked the product, while 5 indicated that the panelist strongly liked the product. Panelists preference scores for each sensory attribute are presented in the following Table 5.

Kombucha fermented for 21 days received the highest color preference score compared with kombucha fermented for 0, 7, and 14 days. On day 14 of fermentation, an increase in panelists' liking was observed, as indicated by the higher hedonic score compared with those of the previous fermentation periods. As fermentation time increased, the color changed from a dark brown-black appearance to a lighter shade. This color fading was attributed to the degradation of color pigments during fermentation. The production of organic acids throughout the fermentation process may reduce pigment stability, resulting in a decrease in color intensity and a lighter product appearance.

Table 2. Sensory Analysis

Day	Color	Flavor	Taste	Overall
0	3.62 ± 1.14[a]	3.38 ± 0.98[c]	2.42 ± 1.53[a]	2.3 ± 0.86[a]
7	3.76 ± 0.17[b]	3.82 ± 0.91[d]	3.06 ± 1.64[b]	2.96 ± 1.29[a]
14	3.88 ± 0.07[c]	3.66 ± 1.11[b]	3.94 ± 1.34[b]	3.46 ± 1.28[b]
21	3.98 ± 1.03[d]	2.82 ± 1.36[a]	1.98 ± 1.22[a]	2.54 ± 1.01[a]

Kombucha with a dark brown color (day 0, and 7) was less preferred by the panelists because it created the impression of a herbal tonic with a bitter taste. Dark-colored products tend to generate a negative perception among consumers, as they are often associated with being less palatable and having a bitter flavor. Therefore, the panelists showed a greater preference for kombucha with a lighter brown color than for kombucha with a dark brown-black appearance. A study conducted by (Yuliana et al., 2023) also reported that the color of kombucha became lighter brown as fermentation time increased.

The flavor evaluation revealed that kombucha fermented for 0 days received the highest aroma preference score from the panelists. As fermentation duration increased, acidic flavors derived from organic acids became more pronounced. Excessively strong acidic aromas were less preferred by the panelists because they created the impression of a pungent and overpowering product. A study conducted by (Luo et al., 2023) reported that the aroma of kombucha results from the formation of volatile compounds during the fermentation process. Furthermore, prolonged

fermentation tends to produce a more intense acidic flavors.

The kombucha samples most preferred by the panelists in terms of taste were those fermented for 7 and 14 days. From a flavor perspective, kombucha fermented for 7 and 14 days still retained a degree of sweetness and was not dominated by sourness. In addition, the carbonation characteristics produced during these fermentation periods were not as intense as those observed in the kombucha fermented for 21 days. In contrast, the kombucha fermented for 0 days was less preferred by the panelists because it had not yet developed the acidic taste typically associated with fermented products, and carbonation had not formed, making it less acceptable as a kombucha beverage (Luo et al., 2023).

Overall, the panelists showed the highest preference for kombucha fermented for 14 days. The overall acceptability assessment was based on the panelists evaluation of all sensory attributes contributing to product preference. These findings indicate that, in product development, a fermentation period of 14 days can be used to produce kombucha with the highest level of consumer acceptance.

Based on the overall results of the data analyses, the optimal fermentation duration was determined to be 14 days, as it resulted in optimal microbial growth, desirable chemical characteristics in terms of pH and total soluble solids (TSS), and the highest level of panelist acceptance. A study conducted by (Gumanti et al., 2023) similarly reported that dragon fruit kombucha fermented for 14 days exhibited the best organoleptic characteristics. Likewise, Siti Nuraliah et al., (2025) observed the same trend, reporting that a fermentation period of 14 days was required to produce the best-quality kombucha from *Turbinaria conoides*.

4. CONCLUSIONS AND RECOMMENDATIONS

Fermentation duration significantly affected the microbiological, chemical, and sensory properties of genitri kombucha. Increasing fermentation time reduced pH and total soluble solids while enhancing total flavonoid content, viable yeast populations, and lactic acid bacteria counts. No significant effect of fermentation duration on the phenolic content was observed. Based on the overall analytical and sensory evaluation results, a fermentation period of 14 days was determined to be the optimum condition for producing genitri kombucha with desirable microbiological, chemical, and sensory characteristics.

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