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Antioxidant activity (IC_{50}) and physicochemical characteristics of mango ginger (*Curcuma mangga*) kombucha

Dewandhaka Putra Andaru[1], Gemala Anjani [1,2]*, Anang M. Legowo[2,3], Fitriyono Ayustaningwarno[1,2], Ahmad Ni'matullah Al-Baarri[3]

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ABSTRACT

Background: Kombucha is a traditional fermented beverage produced by the symbiotic culture of bacteria and yeast (SCOBY) in sweetened tea. Fermentation is a metabolic process in which microorganisms convert carbohydrates, such as sugars, into other chemical compounds typically acids, gases, and alcohol, under controlled environmental conditions. In recent years, kombucha has gained considerable attention as a functional food, defined as a food that, beyond its basic nutritional value, provides health benefits and may reduce the risk of chronic diseases.

Objective: This study aimed to evaluate the physicochemical characteristics and antioxidant activity (IC_{50}) of mango ginger kombucha at various fermentation durations.

Method: An experimental study using a completely randomized design was conducted with four fermentation durations (5, 7, 10, and 14 days), each with three replicates (n=12). Parameters measured included pH, total sugar (°Brix), total dissolved solids (TDS), color (L*, a*, b*), and antioxidant activity (IC_{50} using DPPH assay). Data were analyzed using ANOVA and Bonferroni post-hoc tests at a 95% confidence level.

Result: Fermentation time reduced pH, total sugar, and TDS, while increasing brightness (L*) and decreasing redness (a*) and yellowness (b*). Antioxidant activity (IC_{50}) remained very strong in all samples, peaking at 2.60 ppm on day 10 and 2.41 ppm on day 14. A significant ($p < 0.05$) drop on day 7 followed by a sharp rise on day 10 reflected intensified microbial metabolism and bioactive compound transformation. The 14-day fermentation was optimal, as antioxidant activity stabilized after its peak, while pH and TDS continued to decline, indicating maximal release of bioactive compounds.

Conclusion: Fermentation significantly affects the physicochemical properties and enhances the antioxidant potential of mango ginger kombucha. The optimal antioxidant activity was achieved by day 14, suggesting a promising functional beverage formulation with high therapeutic potential.

Keywords: *Antioxidant; DPPH; IC_{50}; kombucha; mango ginger*

BACKGROUND

Kombucha is a traditional fermented beverage produced by the symbiotic culture of bacteria and yeast (SCOBY) in sweetened tea. Fermentation is a metabolic process in which microorganisms convert carbohydrates, such as sugars, into other chemical compounds typically acids, gases, and alcohol, under controlled environmental conditions.[1] In kombucha, this process leads to the formation of organic acids, ethanol, carbon dioxide, and a wide range of bioactive metabolites that contribute to its characteristic acidity, flavor, and potential health benefits.[2] In recent years, kombucha has gained considerable attention as a functional food, defined as a food that, beyond its basic nutritional value, provides health benefits and may reduce the risk of chronic diseases. This is largely attributed to the presence of bioactive compounds such as polyphenols, flavonoids, organic acids, vitamins, and microbial metabolites produced during fermentation. Several studies have linked these compounds to antioxidant, antimicrobial, and anti-inflammatory properties, making kombucha a promising candidate for functional beverage development.[3]

Kombucha fermentation can be made not only from tea but also from various herbs and rhizomes such as turmeric and ginger. Rhizomes have significant health benefits, making them commonly used in traditional medicine and enhancing the health effects of functional food products. Numerous studies have been conducted on various rhizomes, such as ginger and turmeric.[4, 5] In fact, turmeric (*Curcuma longa*) is often referred to as a superfood due to its health benefits and is included in the World Health Organization (WHO) priority list as one of the most widely used medicinal plants worldwide.[6] Fermentation is a method of processing plant-based

[1]Department of Nutrition Science, Faculty of Medicine, Universitas Diponegoro, Semarang, Central Java, Indonesia
[2]Center of Nutrition Research (CENURE), Universitas Diponegoro, Semarang, Central Java, Indonesia
[3]Department of Agricultural, Faculty of Agricultural and Animal Sciences, Universitas Diponegoro, Semarang, Central Java, Indonesia
***Correspondence** : gemanjani@gmail.com

food ingredients that not only improves their sensory quality but also enhances antioxidant activity and provides additional therapeutic properties.[7, 8]

Mango ginger belongs to the Curcuma genus and contains curcumin, which exhibits high antioxidant activity. It is a locally grown rhizome in Indonesia that contains various organic compounds, including curcumin, demethoxycurcumin, alanine, methionine, threonine, lysine, alpha-glucose, beta-glucose, sucrose, alpha-fructose, beta-fructose, fumaric acid, tyrosine, quercetin, terpenoids, and curcumanggoside.[9] These compounds contribute to its antioxidant, antibacterial, and other functional properties. Therefore, fermentation into kombucha is conducted to enhance its antioxidant activity and improve its sensory aspects. Microorganisms involved in fermentation have the ability to release active compounds from the plant matrix and other components, as well as modify the raw materials into bioactive compounds.[10] There has been limited research on the processing and utilization of mango ginger, despite its abundant availability in the market, particularly regarding the fermentation of mango ginger extract using kombucha culture.

Fermentation duration plays a key role in determining the physicochemical characteristics and bioactive compound composition of kombucha. Over time, microbial metabolism alters parameters such as pH, total acidity, total soluble solids (TSS), and these changes directly influence the antioxidant activity of kombucha. Physicochemical properties also serve as key indicators of the fermentation process and as important benchmarks for further analyses, such as sensory evaluation and consumer acceptance. Despite the growing interest in functional beverages, studies investigating the relationship between fermentation duration, physicochemical characteristics, and antioxidant activity in mango ginger kombucha remain limited. Therefore, this study aims to determine the effect of fermentation duration on the antioxidant activity and physicochemical characteristics of mango ginger kombucha.

MATERIALS AND METHODS

The design of this research is experimental with a completely randomized design. The research was conducted at the UPT Integrated Laboratory of Universitas Diponegoro, aiming to test the activity of antioxidant IC_{50} with DPPH methods and physicochemical characteristics of mango ginger kombucha consist of pH, total sugar, total dissolved solid, and color. This study involved 12 samples with four treatments, each repeated three times with a volume of 1 liter per jar. Samples were made through the kombucha fermentation process with the addition of mango ginger for 5, 7, 10, and 14 days.

The preparation of kombucha began with the production of mango ginger (*Curcuma mangga*) extract at a concentration of 0.7% as the substrate for kombucha fermentation. Mango ginger was selected according to the general quality requirements based on SNI 7953:2014 regarding turmeric quality standards. Sugar and mango ginger materials were purchased from a local supermarket, while the SCOBY starter culture was obtained from an online marketplace. The mango ginger extract was prepared using the infusion method, which is a hot extraction technique for extracting bioactive and aromatic compounds from mango ginger using water as the solvent at a temperature of 90–100°C for 15 minutes. The mango ginger extract was then supplemented with 10% sucrose and filtered using an 80-mesh sieve to obtain sweetened mango ginger extract. In the next step, the extract used for each experimental treatment was inoculated with kombucha vinegar and SCOBY, each at 10% (v/v) of the total extract volume, and gently stirred to ensure uniform distribution. With details per jar, mango ginger extract of 1 liter (0.7% w/v: 7g/1000ml water), with 100g of granulated sugar, 100g of SCOBY, and 100ml of kombucha vinegar (starter). The fermentation process was carried out in jars covered with cloth, at room temperature, and protected from direct light. The fermentation durations were set according to the treatments: Day-5 (T1), Day-7 (T2), Day-10 (T3), and Day-14 (T4). After the fermentation period ended, the SCOBY was removed by filtration.[11-13]

After the fermentation process, pH testing was conducted using a pH meter, while the total sugar measurement was performed with a Brix refractometer. TDS was measured using a Tekcplus Multi-Function TDS Meter, and color was analyzed using a colorimeter.[11-13] The color test results based on the parameters of brightness (L*), redness-greenness (a*), and yellowness-blueness (b*). The activity of antioxidant IC_{50} with DPPH was tested using the UV-Vis spectrophotometry method at a wavelength of 517 nm. The working standard solutions with concentrations of 1, 5, 10, 20, 50, and 100 ppm were measured for their absorbance at the obtained maximum wavelength, and then the linear regression equation was determined using the formula $y=bx+a$. The analysis of antioxidant activity IC_{50} was measured following formula, then, the IC_{50} value was calculated and interpreted in terms of antioxidant activity (in ppm units).[14, 15] Antioxidant activity is classified into several categories. If the IC_{50} value is below 50 ppm, the antioxidant activity is considered very strong;

between 50–100 ppm, it is classified as strong; between 100–150 ppm, it is moderate; between 150–200 ppm, it is weak; and if the IC_{50} value is above 200 ppm, the antioxidant activity is categorized as very weak.[16, 17]

$$\% \textit{ Inhibition} = \frac{(\textit{Blank Absorbance} - \textit{Sample Absorbance})}{\textit{Blank Absorbance}} \times 100$$

The data analysis was conducted using SPSS 25.0 for Windows with a 95% confidence level. The normality of the distribution was tested using the Shapiro-Wilk test. To ascertain whether the average antioxidant activity and bioactive substances among these groups differed significantly, an analysis of variance (ANOVA) was performed. If the test results indicate a significant difference ($p < 0.05$), a post-hoc Bonferroni test is performed to determine which groups differ.

RESULT

The results of the physicochemical characteristics, including pH, total sugar, total dissolved solids, color, and antioxidant activity (IC_{50}) of mango ginger kombucha, are shown in Table 1 based on the fermentation duration.

Total sugar was measured using a Brix refractometer, which indicates the percentage of sucrose and dissolved sugars present in mango ginger kombucha, while pH shows the acidity level of the solution. As the fermentation duration increases, both total sugar and pH values decrease. The total sugar shows a decline over time due to the degradation of sucrose into simpler sugars, glucose and fructose, by yeast. These glucose and fructose are then utilized by acetic acid bacteria to produce organic acids, which contribute to the decrease in pH over the fermentation period.[18, 19]

Table 1. Results of physicochemical and antioxidant activity of Mango Ginger Kombucha

Parameter	Duration of fermentation			
	Day-5 (T1)	Day-7 (T2)	Day-10 (T3)	Day-14 (T4)
pH	4.20±0.06	3.70±0.00	3.70±0.00	3.56±0.08
Total Sugar (°Brix)	11.00±0.00	11.00±0.00	10.50±0.00	8.50±0.00
TDS (ppm)	551.80±1.09^{a}	536.00±0.50^{b}	497.80±1.09^{c}	405.40±0.54^{d}
Color L*	58.64±1.33^{a}	68.58±1.29^{b}	71.00±2.67^{b}	74.92±1.43^{c}
Color a*	8.73±0.99^{a}	6.82±1.87^{a}	6.66±1.27^{a}	-0.35±0.14^{b}
Color b*	60.80±2.69^{a}	46.78±7.42^{b}	49.24±5.18^{b}	14.63±1.22^{c}
Antioxidant Activity IC_{50} (ppm)	6.74±0.09^{a}	8.85±0.07^{b}	2.60±0.08^{c}	2.41±0.94^{c}

Data is presented as the mean of three repetitions ± standard deviation (SD). n=12 (4 treatments, 3 repetitions). Superscript in parameter TDS, Color, and Antioxidant activity indicates a significant difference.

DISCUSSION

The Physicochemical Characteristics

Sucrose serves as the primary substrate and carbon source for the metabolic activities of various microorganisms, including yeast for alcohol production and acetic acid bacteria for the synthesis of organic acids such as acetic acid. During fermentation, sucrose undergoes hydrolysis into glucose and fructose, resulting in a progressive decline in Brix values as fermentation time increases.[18] The glucose and fructose produced are subsequently metabolized by bacteria to generate organic acids, contributing to the observed reduction in pH. In this study, the Brix values ranged from 8.5 to 11, which is consistent with the findings of the total sucrose content in kombucha prepared from green, black, white, and red teas ranged from 7.5 to 10.75, depending on the tea type and fermentation duration[18]. Similarly, the pH decreased from 4.2 to 3.6 throughout the fermentation process, aligning with the results of pH of kenitu leaf kombucha ranged from 2 to 4 and declined progressively with extended fermentation time.[19,20] The best treatment, based on physicochemical properties such as pH, total sugar, and TDS, was observed in the 14-day fermentation sample. This is because, on day 14, the lowest pH value was recorded, and a lower pH indicates higher production of organic acids, which are potential antioxidant compounds. According to FDA standards, the pH must be below 4.6; a more acidic condition also reflects optimal fermentation and the inhibition of spoilage and pathogenic bacteria. Although no specific standards exist for TDS and total sugar, a rapid decrease was observed on day 14, where the reduction in total solids and sugar was closely associated with the pH decline, resulting from the metabolic activity of microorganisms.[21]

The results of total dissolved solids in mango ginger kombucha based on fermentation duration on days 5, 7, 10, and 14 are presented consecutively in Table 1. This indicates that, as the fermentation time increases, the total dissolved solids show a decreasing trend.[22] Dissolved solids in kombucha consist of sucrose added at the beginning of fermentation, dissolved starch content in the substrate, and other dissolved components. The decrease in values is due to the degradation of sucrose into simple sugars during fermentation, which then transform into organic acids. Starch degradation into simpler compounds is also possible, providing an additional carbon source for microorganisms.[23] The process of decreasing total dissolved solids may also indicate an increase in microbial activity as fermentation time progresses. Total dissolved solids can indirectly reflect the availability of substrates that microorganisms can utilize, so as they decrease, it suggests that the availability of substrates is also diminishing. This decrease also correlates with the reduction in total sugar measured using a refractometer, which shows the activity of microorganisms in breaking down complex substrates into simpler molecules.[24] Kombucha fermentation is characterized by a symbiotic association between bacteria and yeast. The process begins with yeast hydrolyzing sucrose and other macronutrients, such as soluble starch present in the substrate, into simpler nutrients. The resulting simple sugars, along with the ethanol produced by yeast, are subsequently utilized by bacteria to synthesize a cellulose biofilm and organic acids. This sequential metabolic interaction forms the basis of the symbiotic relationship between the bacteria and yeast.[21]

Figure 1. Differences in brightness of mango ginger kombucha with various fermentation durations. T1: 5-day fermentation; T2: 7-day fermentation; T3: 10-day fermentation; T4: 14-day fermentation.

The decrease in dissolved solids also affects the color change in kombucha as fermentation time increases. The color parameters are shown through the values of L*, a*, and b*, which represent lightness/brightness, redness-greenness, and yellowness-blueness, respectively are shown in Table 2. Based on the results, it shows that as the fermentation time increases, it influences the brightness level of mango ginger kombucha shown in Fig 1.[25] The increase in the brightness of kombucha over fermentation time is related to the decrease in total dissolved solids. The reduction in the amount of solids in kombucha, as a result of microbial activity during fermentation, leads to a decrease in the cloudiness of the solution and enhances the brightness of the kombucha color. The cloudiness of kombucha is caused by the presence of various solids and macronutrients, which are then broken down during the fermentation process.[26]

The results of the redness-greenness color test for the four samples showed that the fermentation durations from day 5 to day 10 did not show significant differences. A difference was observed in the fermentation duration on day 14 with a value of -0.346. The result of -0.35 indicates that the solution color in the a* value test begins to approach a green/greenness hue. In other results, the low values indicate that there was no red or reddish color (redness) in the sample.[27] The relatively high b* values indicate that the solution color is in the yellowish/yellowness range, while the decrease in values shows that the color is fading. The yellow color in mango ginger kombucha is influenced by the curcuminoid compounds present in mango ginger.[28]

Antioxidant Activity

The antioxidant activity was tested using the IC_{50} method with a DPPH solution. A smaller IC_{50} value indicates stronger antioxidant activity.[29] Based on the antioxidant activity test of mango ginger kombucha, the results are shown in Table 1. Antioxidant activity with varying fermentation durations showed a significant difference ($P<0.05$) between the fermentation treatments on days 5, 7, and 10. However, no significant difference was observed between the fermentation durations from day 10 to day 14. Overall, the antioxidant activity of mango ginger kombucha falls into the very strong category.[16] Antioxidant activity significantly decreased from fermentation day 5 to day 7, then significantly increased on day 10, and showed no significant difference up to day 14 of fermentation. The change in antioxidant activity during fermentation is due to the activity of microorganisms in degrading various nutrients present in the mango ginger substrate.[30, 31]

The decrease in antioxidant activity on day-7 may be due to the degradation of bioactive compounds, including phenolic compounds, in the mango ginger substrate. Antioxidant activity on day-5 showed stronger activity because, at the beginning of fermentation, microorganisms are actively breaking down macronutrients such as sugars and starch, leading to the release of bioactive compounds.[32, 33] However, on day-7, the availability of sugars started to become limited, as indicated by the significant decrease in total dissolved solids. This leads to nutrient competition among microbial colonies, with some beginning to utilize phenolic compounds as a carbon source.[34, 35] This explains the decrease in antioxidant activity on day-7 in *Curcuma mangga* kombucha and is consistent with a study on butterfly pea flower kombucha, where a decline was observed on day 9 compared to day 6.[15] This is also consistent with the study on agarwood kombucha, where antioxidant activity showed a decrease on day 7 compared to day 10.[36]

Antioxidant activity increased on day 10 and remained relatively stable until day 14. This enhancement during fermentation may result from: 1) the release of bioactive compounds following macronutrient degradation;[37] 2) the conversion of phenolic, flavonoid, and terpenoid compounds into simpler analogs;[38] and 3) the formation of new compounds through the conjugation of simpler molecules.[39,40] While total phenolic content is a key determinant of antioxidant activity in kombucha, other constituents such as terpenoids, alkaloids, organic acids, volatile compounds, and various metabolites also play important roles in *Curcuma mangga* kombucha.[14,30] Previous studies have shown that microbial activities such as deglycosylation, oxidation, and ring-cleavage reactions can convert complex curcuminoids, flavonoids, and terpenoids into simpler analogs with higher antioxidant potential. This is consistent with the findings of a study on *Curcuma xanthorrhiza* kombucha, which showed that after 12 days of fermentation, curcumin degradation products were formed, resulting in conjugated curcumin compounds such as curcumin monoacetate, curcumin monoglucoside, and curcumin monoglucuronide.[30] Another study on *Curcuma zedoaria* kombucha showed that after fermentation, curcumin was broken down into tetrahydrocurcumin and conjugated compounds similar to those found in *Curcuma xanthorrhiza* kombucha.[14]

The results of the antioxidant activity in mango ginger kombucha based on fermentation duration showed significant changes in some treatments. Overall, the antioxidant activity of mango ginger kombucha falls into the very strong category, as it is below 50 ppm. This is consistent with other studies on kombucha, such as those made from butterfly pea flower[15], robusta coffee[34], coffee leaves and tea infusions[41], cherries[42], and cocoa kombucha.[16] The optimal treatment based on physicochemical characteristics and antioxidant activity was the 14-day fermentation period, as it exhibited the strongest antioxidant activity while the total dissolved solids had not yet experienced a significant decrease. The trend in changes in antioxidant activity in mango ginger kombucha also aligns with the research on kombucha from blacktea, greentea, and redtea which showed both a decrease and increase during the fermentation process.[18] This indicates that across different substrates, kombucha exhibits a similar pattern of chemical changes, driven by the activity of yeasts and bacteria in metabolizing sugars and other chemical compounds.[43, 44]

CONCLUSION

Overall, the 14-day fermentation period offers the best compromise between maintaining physicochemical quality and maximizing bioactive compound release, positioning mango ginger kombucha as a promising functional beverage with potential health benefits. The 14-day fermentation period of mango ginger kombucha demonstrated the optimal balance between antioxidant activity and physicochemical characteristics. While antioxidant activity (IC_{50}) reached its peak on day-10 and remained stable until day-14, the total dissolved solids and pH values decreased significantly in day-14, indicating that this period provided the highest bioactive compound release. The enhanced antioxidant activity during fermentation is closely associated with microbial metabolism, particularly the synergistic roles of yeasts and acetic acid bacteria.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

DECLARATION USE AI

The authors utilized QuillBot Paraphrasing Tool to paraphrase the manuscript and DeepL Translator to translate it during the development of this work. Following the use of these tools, the author or authors took full responsibility for the publications content and reviewed and amended it as necessary.

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