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Antioxidant Activity of Red Ginger Kombucha (*Zingiber officinale* var. *rubrum*) with Various Fermentation Time and Palm Sugar Concentrations

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Abstract

The use of red ginger (*Zingiber officinale* var. *rubrum*) as the primary ingredient of kombucha is a diversification to increase the antioxidant activity of kombucha. The presence of gingerol dan shogaol in red ginger acts as a potential antioxidant, while the use of palm sugar serves as an additional source of antioxidants and alternative source of sucrose from regular granulated sugar. The fermentation time and the amount of sugar used are two variables that affect the kombucha's fermentation process. This study aims to examine the effect of various fermentation times and palm sugar concentrations on the antioxidant activity, pH, alcohol content, and organoleptic properties of red ginger kombucha. The methods used were antioxidant test with DPPH, pH test, alcohol content, and organoleptic test with 20 panelist. The results indicate that variations in fermentation time and concentration of palm sugar have a significanr effect ($\alpha = 0.05$) on the antioxidant activity with IC_{50} values ranging from 27.89 ppm to 46.77 ppm, categorized as very strong antioxidant activity. The characteristics of red ginger kombucha includes pH 3.6 to 4.6, alcohol content 0.2% to 0.3%, and organoleptic properties varying from moderately liked to liked. Red ginger kombucha fermented for 12 days with 20% palm sugar showed an IC_{50} value of 40.28 ppm, which categorized into very strong antioxidant activity and is most preferred by the panelists with organoleptic scores of color attribute at 4, aroma at 3.65, and taste at 4.

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Introduction

Exposure to free radicals in the environment has also increased along with the advancement of time and mobility. Free radicals are highly reactive molecules or atoms with unpaired electrons that can damage healthy tissues (Sanjay and Shukla, 2021). The risk of disease increases with exposure to free radicals from UV rays, cigarette smoke, and vehicle emissions in the environment. Antioxidants are compounds that can protect cells and tissues from damage caused by free radicals (Agustiarini and Wijaya, 2022). Additional antioxidant intake is important for maintaining health. Additional antioxidant intake is important for maintaining health and reducing the risk of disease. Consuming antioxidant-rich foods and drinks can be a way to maintain health. Kombucha is a fermented drink made with SCOBY (Symbiotic Culture of Bacteria and Yeast). SCOBY consists of acetic acid bacteria that produce cellulose fibers in the form of nata, and yeast that convert the sugar in kombucha into alcohol and stimulate the growth of acetic acid bacteria (Kamelia et al., 2023). Kombucha contains organic acids, catechins, flavonoids, and phenolic compounds, which contribute to its therapeutic effects. Komucha also exhibits antioxidant, antimicrobial, antidiabetic, and anticancer properties (Soares et al., 2021). Typically, kombucha is made with black tea and sugar, but over time, variations using ingredients such as butterfly pea flowers, roselle flowers, coffee, and cocoa have also been used. The composition of the ingredients determines the metabolic characteristics of the kombucha (Jakubczyk et al., 2020).

Red ginger is a type of ginger with higher essential oil and oleoresin content compared to other types of ginger (Sandrasari et al., 2023). Red ginger contains 23-25% gingerol and 18% shogaol as parts of its oleoresin content, which shows antibacterial, anticancer, anti-inflammatory, and antimicrobial activities (Prasad and Tyagi, 2015; Dalsasso et al., 2022). Gingerol and shogaol possess antioxidant potential as they can break free radical chains and enhance antioxidant enzyme activity (Andriani and Murtisiwi, 2020). This makes red ginger a promising base for kombucha. In kombucha fermentation, sugar acts as a carbon source for SCOBY. Palm sugar, derived from palm sap, contains 83.31% sucrose (Setyoningsih et al., 2020). It can be used as an alternative to cane sugar due to its lower glycemic index of 35. Palm sugar also contains the natural antioxidant DDMP (Saputro et al., 2017). Kombucha fermentation typically lasts 14 days under facultative anaerobic conditions and is influenced by factors such as fermentation time, temperature, pH, and sucrose concentration (Nur et al., 2018).

This study uses red ginger as the base for kombucha to diversify the product, with palm sugar as an alternative sucrose source. This diversification aims to enhance the antioxidant activity and quality of kombucha both functionally and organoleptically. Previous researvh showed that red ginger kombucha with 20% honey had high antioxidant activity (Pebiningrum et al., 2017). However, there has been no research on the antioxidant activity of red ginger kombucha with varying fermentation times and the use of palm sugar. Therefore, this study will analyze the effects of different fermentation times and palm sugar concentrations on the antioxidant activity, pH, alcohol content, and organoleptic characteristics of red ginger kombucha.

Materials and Methods

Materials

Red ginger and palm sugar was obtained from local market, as well as commercialized SCOBY. The chemicals used in this researvh were analytical reagent grade.

Method

The study was conducted from September 2023-February 2024. The study includes the process of making kombucha and analyzing its organoleptic characteristics. A total of 200 mL of red ginger extract was poured into three different jars, then palm sugar was added at concentrations of 10%, 15%, and 20% respectively. Kombucha starter, amounting to 10% of the total solution, was added to the ginger extract and palm sugar mixture. The jars were then covered with cotton cloth and fermented for 8, 12, and 16 days (Astuti et al., 2017). The organoleptic analysis includes DPPH testing where each kombucha sample was diluted to 10, 20, 30, 40, and 50 ppm. Then, 600 µL of each sample was added to 2.4 mL of DPPH solution and homogenized. The samples were then incubated for 30 minutes. Subsequently, absorbance was measured using a spectrophotometer DW-721G VIS at λ 517 nm (Alyaqoubi et al., 2017). The free radical inhibition activity is then calculated using the following formula.

$$\%\ \text{inhibition} = \frac{(A0-A1)}{A0}\ x\ 100\%$$

Where :
% inhibition : Antioxidant inhibition
A0 : Absorbance of DPPH
A1 : Absorbance of sample

The percentage inhibition results are plotted on a graph to obtain the equation y = a + bx, where x is the sample concentration and percentage inhibition is y. The IC_{50} value is calculated by replacing the y value with 50 to find the x value. Then, the antilog of x is calculated to obtain the IC_{50} value. A lower IC_{50} value indicates better antioxidant activity (Badriyah et al., 2017).

pH testing was carried with pH meter where the electrode was rinsed with distilled water and then dried. After calibration, the pH meter was immersed in a 10 mL kombucha sample. The pH value will be displayed shortly after the pH meter shows a consistent reading (AOAC, 1995). Alcohol content testing is done with a total of 50 mL of kombucha was distilled to obtain alcohol distillate. Then, an empty 10 mL picnometer was weighed. Next, the alcohol distillate sample was poured into the picnometer until full, then reweighed. We also weighed the picnometer containing distilled water. The alcohol content was calculated using the following equation (Ningsih et al., 2018):

$$\%\ \text{Alcohol content} = \frac{W_3 - W_1}{W_2 - W_1}$$

Where :
W_3 : Weight of picnometer + alcohol
W_2 : Weight of picnometer + aquadest
W_1 : Weight of empty picnometer

Hedonic testing is done with 20 panelists, each one were asked to rate their preferences on color attribute, aroma, and taste using a scale of 1 (strongly dislike), 2 (dislike), 3 (moderately like), 4 (like), 5 (strongly like) (Simanungkalit et al., 2018). The data obtained were analyzed using the Two-Way ANOVA method and post-hoc DMRT test using SPSS 25.0 Statistical Software. The significance level set was α = 0.05.

Results and Discussion

Antioxidant Activity Test

Red ginger kombucha shows IC_{50} values ranging from 27.89 ppm to 46.77 ppm, categorized as strong to very strong antioxidant activity. The lowest IC_{50} value was found in kombucha fermented for 8 days with 10% palm sugar, while the highest IC_{50} value was found in kombucha fermented for 16 days with 20% (Figure 1). Sylvi et al. (2022) stated that a smaller IC_{50} value indicates stronger antioxidant activity. Based on the ANOVA test, a significance value of $p < 0.05$ was found, indicating a significant influence of fermentation time and palm sugar concentration treatments. The small IC_{50} value is based on the usage of palm sugar because palm sugar is one of the natural antioxidant sources. Srikaeo et al. (2019), stated that palm sugar has higher polyphenol and flavonoid content compared to regular refined sugar, hence showing higher antioxidant activity.

In the graph (Figure 1), it can be seen that antioxidant activity significantly increased from day 0 to day 8, indicated by the decreasing IC_{50} value. This indicates that the fermentation of ginger extract into kombucha can enhance its antioxidant activity because fermentation can release phenolic compounds found in ginger extract and also palm sugar. The phenolic compounds that play a role in the antioxidant activity of red ginger are gingerol and shogaol. According to Handito and Amaro (2021), during the fermentation process, microbes can produce enzymes that can break ester bonds, releasing bound phenolic compounds, thereby increasing the availability of phenolic compounds potentially as antioxidants. The IC_{50} value began to increase from day 12 to day 16, indicating a decrease in antioxidant activity. This can occur due to the increasingly acidic condition of the kombucha

Table 1. Average Organoleptic Scores of Red Ginger Kombucha

Indicators	Samples								
	F8 A10	F8 A15	F8 A20	F12A10	F12 A15	F12 A20	F16 A10	F16 A15	F16 A20
Color	3^{a}	3^{b}	$3,3^{cd}$	$3,25^{bc}$	$2,45^{cde}$	4^{h}	$3,85^{gh}$	$3,7^{f}$	$3,8^{fg}$
Aroma	$2,8^{c}$	$2,45^{a}$	$3,1^{bc}$	$3,3^{ef}$	$3,6^{gh}$	$3,65^{h}$	$3,2^{cd}$	$3,2^{de}$	$3,45^{f}$
Taste	$2,5^{cd}$	$2,1^{a}$	$3,35^{f}$	$3,15^{ef}$	$3,65^{g}$	4^{h}	$2,8^{de}$	2,75	$2,4^{b}$

Different superscript letters in the same column indicates the significant differences ($p < 0.05$).

solution with prolonged fermentation and increasing palm sugar concentration used. Villareal-Soto et al. (2018) stated that an increasing acidic environment could affect the phenolic compound state to become more stable, making it more difficult to donor its protons to DPPH molecules, thus decreasing antioxidant activity.

pH Assay

The pH testing of red ginger kombucha was conducted with ginger extract as control with starter on day 0 at a pH of 5.2. The pH values (Figure 2) indicate that the average pH of red ginger kombucha ranges from 3.4 to 4.6. Based on ANOVA test results, it was found that fermentation time and palm sugar concentration had a significant effect ($p < 0.05$) on the pH value of red ginger kombucha. Figure 2 shows that as the fermentation time increases and the palm sugar concentration used increases, the acidity level (pH) will decrease. According to Hapsari et al. (2021), the decrease in pH in kombucha is caused by the growth and metabolism of SCOBY as a starter that produces organic acids. In the kombucha fermentation process, SCOBY consisting of yeast, acetic acid bacteria, and a number of lactic acid bacteria (LAB) will use sugar as a fermentation substrate. This also indicates thath the higher the sugar concentration available to microorganisms, the more organic acids can be produced, thus affecting the acidity level. According to Zulaikhah et al. (2021), with higher sugar concentrations, the availability of substrates for microorganisms will increase, leading to increased microbial activity.

Alcohol Content Assay

The alcohol content in kombucha is a byproduct of fermentation. Verawati (2019), stated that the presence of alcohol in kombucha products is due to the role of yeast in SCOBY, which converts sucrose into alcohol during fermentation. In this study, alcohol content testing was conducted by weighing a pycnometer. The empty pycnometer weighed 18.8310 grams, and the pycnometer filled with aquadest was weighed 28.8369 grams. The results of the alcohol content test (Figure 3) indicate an increase in alcohol content with the time of fermentation and the increasing concentration of palm sugar used. Based on the ANOVA test, a significance value of $p < 0.05$ was found, indicating that fermentation time and palm sugar concentration treatments had a significant effect on alcohol content in kombucha.

The higher the concentration of sugar used, the higher the alcohol content will increase. According to Rosyada et al. (2023), this occurs because sugar concentration can affect yeast consumption, where the more sugar, the more substrate can be converted into ethanol. With longer fermentation, the alcohol content will also increase. Khotib (2018) explains that the longer the fermentation, the greater the microbial activity in producing alcohol, leading to an increase in alcohol content. The results of the alcohol content test of red ginger kombucha (Figure 3) indicate that its alcohol content is still below 0.5%, which is still classified as a halal beverage. This is in accordance with the Fatwa of the Indonesian Ulama Council (MUI) Number 10 of 2018 on Food and Beverage Products Containing Alcohol/Ethanol, where fermented beverages containing alcohol or ethanol with a concentration below 0.5% are classified as halal, as long as the manufacturing process does not involve forbidden items and the content is not medically harmful.

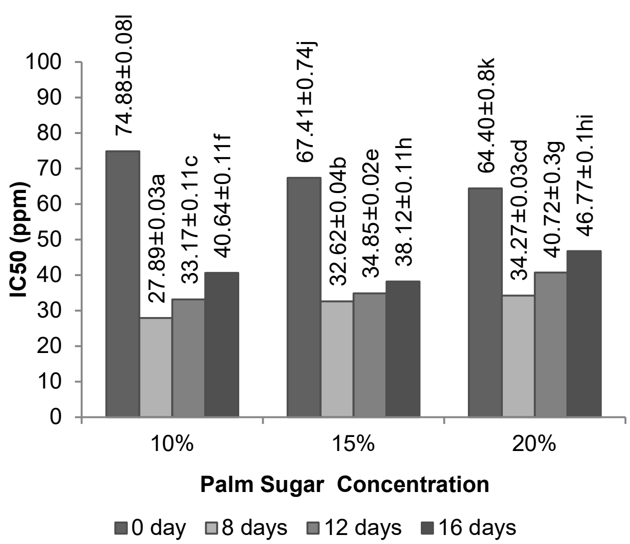

Figure 1. IC_{50} Value of Red Ginger Kombucha

Organoleptic Test

The organoleptic test of red ginger kombucha was conducted by 20 panelists with three parameters: color, aroma, and taste. The hedonic scale used ranged from 1 to 5, where 1 was categorized as very disliked, 2 disliked, 3 moderately liked, 4 liked, and 5 well liked. Based on the average hedonic test results for color (Table 1), scores 3 to 4 were obtained. These scores can be categorized into moderately liked and liked categories. The highest score by the panelists was a scale of 4 on kombucha with a fermentation period of 12 days and a palm sugar concentration of 20%, falling into the liked category. Based on ANOVA test, it was found that fermentation time and palm sugar concentration treatments had a significant effect ($p < 0.05$) on the color hedonic preference or liking by the panelist. The color changes that occur in red ginger kombucha can influence the panelists' preference for the product. The color of kombucha may turn brighter due to the SCOBY's ability to use total soluble solid as an energy source. The concentration of palm sugar in a certain amount can also affect the color of kombucha. Red ginger kombucha shows a dark brown color derived from the combination of red ginger extract and palm sugar. Crum and Alex (2016) state that the color of sugar can affect the color of kombucha liquid and also the color of SCOBY nata produced.

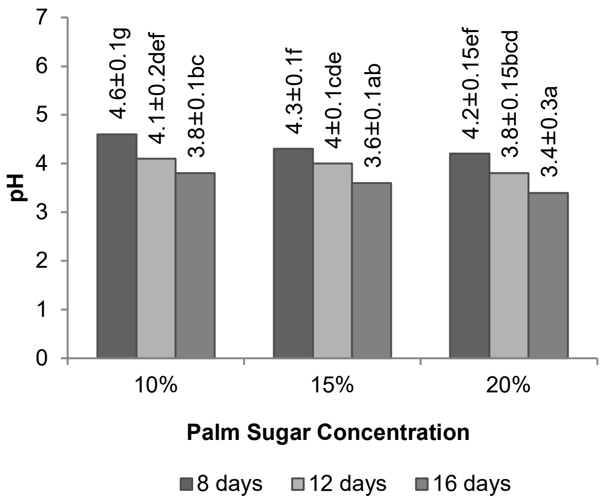

Figure 2. pH of Red Ginger Kombucha

The average results of the aroma hedonic test (Table 1) indicate the panelists' preference for the aroma of red ginger kombucha ranging from scale 2 to 3, which can be categorized as disliked to moderately liked. The product with the highest preference score is the result of 12 days of fermentation and 20% palm sugar concentration, scoring 3.65 in the moderately liked category. Based on the ANOVA test, it was found that the treatment of fermentation time and palm sugar concentration had a significant effect ($p < 0.05$) on the preference for the aroma attribute of red ginger kombucha by the panelists. According to the panelists' assessment, the kombucha aroma exhibits a distinctive sour smell, similar to apple cider vinegar. This makes the panelists less fond of the kombucha aroma as a beverage for daily consumption. The distinctive aroma of kombucha is influenced by the acids formed during the fermentation process. According to Bishop et al. (2022), the yeast in SCOBY will convert sugar sources into ethanol, carbon dioxyde, and glycerol, while acetic acid bacteria will convert ethanol into acetic acid. This acid contributes to the distinctive aroma of kombucha.

The average hedonic test results for taste attributes (Table 1) ranged from scale 2 to 4, categorized as disliked to liked. The highest preference score was found in kombucha produced with 12 days of fermentation and 20% palm sugar concentration. Based on the ANOVA test, both fermentation period and palm sugar concentration were found to have a significant effect ($p < 0.05$) on the preference for taste attribute by the panelists. According to the panelists' assessment, the taste of red ginger kombucha has a carbonated and sour taste with the distinctive hint of a slightly spicy ginger taste. The presence of carbonation is caused by the carbon dioxide produced during the fermentation. Tran et al. (2022) state that when yeast convert sugar into alcohol, the process will produce carbon dioxide bubbles, thus creating a carbonated sensation in kombucha. As fermentation prolonged more than 12 days and palm sugar concentration increases, kombucha will become more acidic, causing panelists' preference to decrease, while at the beginning of fermentation, kombucha taste is still too spicy, thus less preferred by the panelists. According to Puspaningrum et al. (2022), a longer fermentation process will cause kombucha to have a more acidic taste due to a decrease in total sugar content and an increase in total acid content caused by sucrose metabolism by bacteria and yeast. According to Sinamo et al. (2022), sugar will be used as a carbon source and converted into organic acids, so the higher the sugar concentration used at the beginning of fermentation, the higher the organic acid content that causes acidity in kombucha.

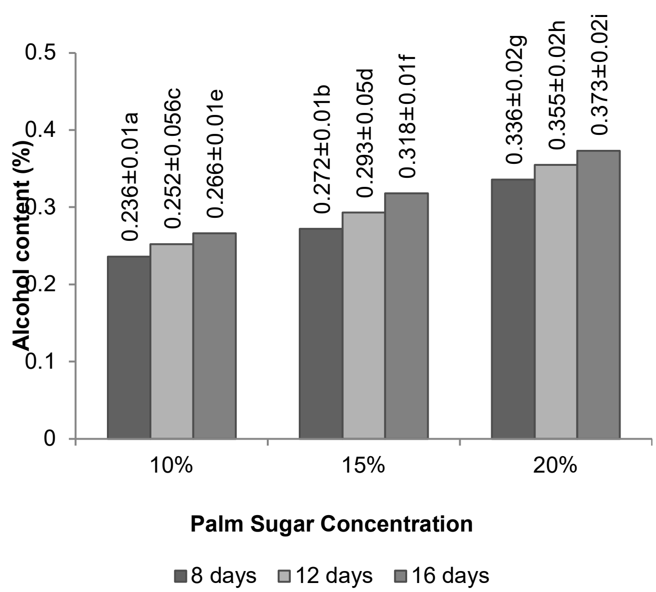

Figure 3. Alcohol Content of Red Ginger Kombucha

Conclusion

Fermentation time and variations in palm sugar concentration (10%, 15%, 20%) had a significant effect ($\alpha = 0.05$) on antioxidant activity with IC_{50} values ranging from 27.89 ppm to 46.77 ppm, categorized as very strong antioxidant activity, and the characteristics or red ginger kombucha including pH ranging from 3.4 to 4.6, alcohol content ranging from 0.2% to 0.3%, and organoleptic characteristics in the moderately liked to liked category. The kombucha produced with 12 days of fermentation and 20% palm sugar concentration was found to be most likely suitable to be produced and consumed daily, resulting in an IC_{50} value of 40.72 ppm, categorized in very strong antioxidant activity and is the most preferred by panelists, with organoleptic scores for color attribute at 4 (liked), aroma at 3.65 (moderately liked), and taste at 4 (liked).

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