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Antioxidant Activity in Kombucha (Scooby) Tea Based on Fermentation Duration with DPPH (1,1-Diphenyl-2-Picrylhydrazyl) Method

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ABSTRACT
Kombucha tea is the result of fermentation of sugar and tea carried out by symbiotic cultures. The ingredients that can be used in making kombucha tea are green tea leaves. The fermentation process is carried out for 7-12 days with the help of Acetobacter xylinum bacteria and several types of yeast or other yeast. This study aims to determine whether there are differences in antioxidant activity, determine the total acid content, and organoleptic in kombucha tea. The method used is the Completely Randomized Design (CRD) method including two factors. The first factor is the length of fermentation (1, 2, 3 and 5 days) and the second factor is vitamin C. Data analysis using ANOVA with a confidence level of 95%. Based on the results of this study, it can be concluded that the antioxidant activity value in kombucha tea (SCOOBY) on the 2nd day of fermentation has the best antioxidant activity inhibition capacity, namely 7.00 ppm. The total acid obtained on the 5th day was 0.67%.

Keywords: kombucha; antioxidant activity; long fermentation.

INTRODUCTION

Kombucha tea is a fascinating traditional beverage as it is the result of sugar and tea fermentation carried out by a symbiotic culture (1). Kombucha, also known as SCOBY (Symbiotic Cultures of Bacteria and Yeasts), has a sour and tart taste similar to apple cider. Kombucha tea is fermented by microorganisms from yeast and bacterial groups. The symbiotic culture includes acetic acid bacteria (*Acetobacter xylinum, Acetobacter aceti*), lactic acid bacteria (*Lactobacillus, Lactococcus), Saccharomyces ludwigii, Saccharomyces bisporus, Zygosaccharomyces sp*., and several types of yeast such as *Torulopsis sp*. These bacteria and yeasts can inhibit contamination by other microorganisms. Zygosaccharomyces is the dominant yeast with a relative abundance of 84.1%, followed by Dekkera and Pichia species with 6% and 5% respectively (2). Acetic acid bacteria work slowly without direct use of sucrose, while yeasts break down sucrose into glucose and fructose, which are then further fermented to produce ethanol (2).

The fermentation process is carried out over 7–14 days, and after 10–14 days of fermentation, the new SCOBY reaches a thickness of 8–12 mm, separating from the inoculated mother culture (2). Changes in Kombucha culture during fermentation lead to an increase in phenolic content due to different fermentation times, though the difference is not very significant. Most polyphenols, such as flavonoids and phenolics, have antioxidant properties due to hydroxyl groups on their aromatic rings that can neutralize free radicals (3). Thus, the levels of flavonoids and phenolics are directly proportional to antioxidant activity—the higher the levels, the greater the antioxidant activity produced (4). In addition, the fermentation duration and the type of tea used, such as green tea, can affect the taste, aroma, composition, and quantity of chemical content. Since Kombucha is a fermented drink, it has a long shelf life when stored in an airtight bottle, even at room temperature. As long as the bottle

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remains unopened, the kombucha remains preserved and unspoiled. This is because Kombucha contains natural substances that prevent the growth of spoilage-causing bacteria (4).

Antioxidants are substances that can prevent or inhibit damage caused by oxidation (5). Various natural substances contain antioxidants, including Kombucha tea. Antioxidants are needed to prevent oxidative stress. Oxidative stress is a condition of imbalance between the number of free radicals and the amount of antioxidants in the body, including reactive oxygen species (ROS) (6). Kombucha tea contains many antioxidants that function as antitumor, anticarcinogenic, and antimutagenic agents (7). Kombucha is composed of bioactive compounds, with a focus on phenolic compounds. Fermentation affects the increase in antioxidant properties, depending on the tea used, fermentation duration, and type. Fermentation can also reduce reductive properties, again depending on the type of tea. The polyphenols in tea are responsible for Kombucha's antioxidant activity. During fermentation, polyphenols and flavonoids increase, while thearubigin is converted into theaflavin, causing a color change in Kombucha from dark to light as fermentation time progresses. Thearubigin is a further oxidation product of theaflavin, while theaflavin contributes color and is slightly acidic (8). The antioxidant activity of Kombucha tea can increase due to the presence of free phenolics produced during fermentation; the longer the fermentation, the higher the antioxidant activity (9). The activity in Kombucha tea offers more benefits than unfermented tea, as microbial fermentation alters its contents (10).

DPPH is a free radical capable of accepting electron (hydrogen radical) donors from other compounds to form more stable molecules and is stable in aqueous solution (11). The DPPH method is based on the antioxidant's ability to inhibit free radicals by donating a hydrogen atom (12). The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical method is considered simple, fast, easy to perform, and low-cost. Antioxidant activity is measured by its ability to scavenge DPPH radicals. Antioxidants neutralize DPPH radicals by donating electrons to DPPH (13). Antioxidant activity is expressed in terms of IC_{50} value, which indicates the concentration required to inhibit 50% of oxidation. The lower the IC_{50} value, the higher the antioxidant activity. Fermentation time affects the IC_{50} value. The longer the fermentation, the higher the IC_{50} value, meaning the antioxidant activity decreases (14).

This study was conducted to determine whether Kombucha tea has antioxidant activity using the DPPH method and to assess the effect of fermentation time on antioxidant activity. This research also aims to determine the optimal fermentation time for Kombucha tea to achieve the highest antioxidant activity using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. Based on this, the study examine the antioxidant activity of Kombucha tea (SCOBY) based on fermentation durations of 1 day, 2 days, 3 days, and 5 days using the DPPH method.

METHODS

Tool

The equipment used in this study includes a stove, autoclave, beaker glass, 50 mL volumetric flask, glass jars, strainer, stirrer, digital scale, measuring glass, petri dishes, dropper pipette, Erlenmeyer flask, and UV-Vis spectrophotometer.

Materials

The materials used in this study are kombucha culture (obtained from www.wikikombucha.com), water, sugar, green tea, distilled water, 2,2-diphenyl-1- picrylhydarzyl (DPPH) ALDRICH brand, methanol (p.a), NaOH, aluminum foil, clean cloth, rubber band, label paper.

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Kombucha Tea Preparation

Kombucha tea was prepared using standard fermentation procedures. The equipment used included a stove, autoclave, beakers, 50 mL volumetric flasks, glass jars, strainers, stirrers, digital scales, measuring cylinders, petri dishes, dropper pipettes, Erlenmeyer flasks, and a UV-Vis spectrophotometer.

Organoleptic Analysis

Organoleptic properties such as appearance, odor, taste, and color of the kombucha tea were evaluated using sensory observations.

pH Measurement

The pH of the kombucha samples was measured using a pH meter calibrated with buffer solutions at pH 4 and pH 7 prior to use (15).

Total Acid Content Determination

The total acid content was analyzed by titration following the Indonesian National Standard (SNI 01-3546-2004). The procedure involved standardizing the NaOH solution and titrating the kombucha tea samples. The total acid content (%) was calculated using the following formula (15):

$$\text{TA}\ (\%) = \frac{V\ tritan\ \times n\ titran\ \times FP\ \times BM\ CH_3COOH}{V\ Sample\ \times 1000} \times 100\%$$

Description:
V titrant: amount of NaOH solution for titration (ml)
N titrant: normality of NaOH
FP: dilution factor
BM: molecular weight of acetic acid (60)
V sample: volume of kombucha sample (ml)

Antioxidant Activity Assay

The antioxidant activity was measured based on DPPH radical scavenging. After incubation, the absorbance of the reaction mixture was measured at 517 nm using a UV-Vis spectrophotometer. The percentage of DPPH inhibition was calculated using the formula:

$$\%\text{inhibition} = \frac{Abs.\,Control - Abs.\ \ Sample}{Abs.\,Control} \times 100\%$$

The %inhibition value is then processed using Microsoft Excel to obtain a linear regression value, namely y=ax+b.
Description:
y = Percent (%) inhibition
x = log concentration

One-Way ANOVA Analysis

All data were analyzed quantitatively to assess the effect of fermentation time on antioxidant activity. A one-way Analysis of Variance (ANOVA) was conducted at a 95% confidence level using the Statistical Package for the Social Sciences (SPSS) version 23 (16). Normality tests were conducted to determine if the data were normally distributed; data were considered normally distributed if the significance value was greater than α

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(0.05), and not normally distributed if less than α (0.05). Homogeneity tests were also conducted to check if variances across groups were equal. If the significance value was less than α (0.05), the data were considered not homogeneous; if greater, the data were considered homogeneous. Only data that were both normally distributed and homogeneous proceeded to ANOVA testing.

RESULT AND DISCUSSION

The results of making kombucha tea and fermentation for 1 day, 2 days, 3 days and 5 days can be seen in Table 1, which describes the characteristics of each kombucha tea fermentation result.

Tabel 1. Organoleptical Testing Results of Fermented Kombucha Tea

Treatment	Assessment		
	Color	Aroma	Taste
1 day	Dark brown	Sour-scented	Sour and slightly sweet
2 days	Golden brown	Sour-scented	Sour and slightly sweet
3 days	Golden brown	Sour-scented	Sour and slightly sweet
5 days	Dark brown	Sour-scented	Sour and slightly sweet

Based on Table 1, it can be observed that the resulting colors vary, ranging from golden brown to dark brown. The test samples that produced a dark brown color were the kombucha solutions fermented for 1 day and 5 days. Meanwhile, the samples that exhibited a golden brown color were the kombucha solutions fermented for 2 days and 3 days. This observation aligns with the findings of Nasution et al. (17), which state that the longer the fermentation period and the longer the SCOBY is submerged in green tea or black tea kombucha solution, the more it affects the SCOBY's color.

Table 1 also shows that all four samples had similar aromas and tastes, characterized by a sour aroma and a slightly sweet and sour taste.

Total Acid Measurement

The total acid content was analyzed using a titration method, which included two stages: the standardization of NaOH and the measurement of total acid content in kombucha tea (15). The following are the results of the total acid content measurement of the fermented kombucha tea samples.

Tabel 2. Measurement Result %Total Acid

Treatment	Fermentation			
	1	2	3	5
%Total Acid	0,09	0,28	0,43	0,67

Based on Table 2, it can be seen that the longer the fermentation period, the higher the percentage of total acid produced. This finding is consistent with the study by Simanjuntak et al. (18) on the chemical characteristics and antioxidant activity of kombucha from water lettuce (Pistia stratiotes), which showed that the total acid content increases over time during fermentation. This increase occurs because during the fermentation

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process, yeast and bacteria metabolize sucrose and produce several organic acids, such as acetic acid and gluconic acid. As a result, the concentration of these organic acids increases, leading to a higher total acid content.

The level of acidity or pH is a measurement used to determine the acidity or alkalinity of a solution. The pH is measured on a scale from 0 to 14, where a neutral pH is 7. A pH value below 7 indicates an acidic solution, while a value above 7 indicates a basic (alkaline) solution (19).

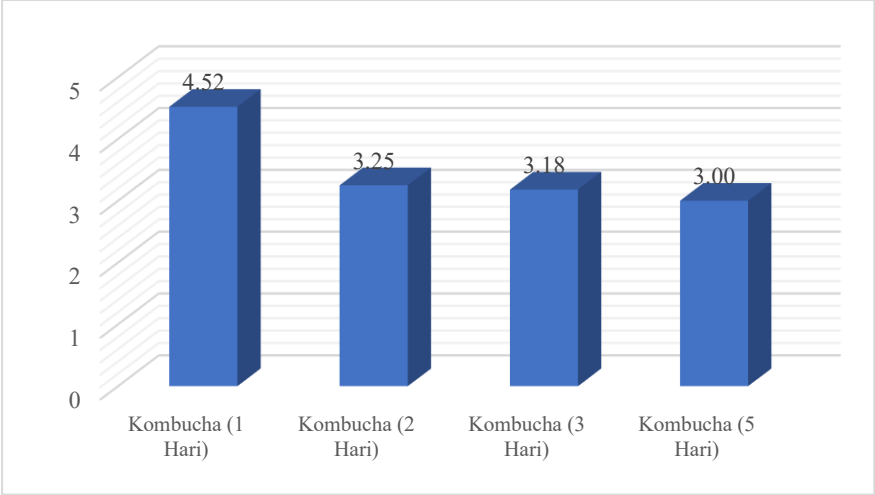

Figure 1. Diagram of pH Degree Measurement Results

Based on Figure 1, the highest pH value was observed in the K1F1 treatment (kombucha fermented for 1 day), while the lowest pH value was found in the K3F5 treatment (kombucha fermented for 5 days). Furthermore, Figure 1 also shows that the pH values of all four kombucha tea samples decreased during the fermentation process. The pH declined with increasing fermentation time, which is consistent with the findings of Indrawan et al. (19), who reported that longer fermentation leads to a decrease in kombucha pH.

Antioxidant Activity Test

The antioxidant activity of a compound is determined using the IC_{50} parameter. IC_{50} refers to the Inhibition Concentration at which 50% of DPPH radicals are neutralized. The antioxidant activity test in this study was performed on kombucha tea samples using five concentration variations: 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm. This study examined the effect of fermentation duration on the antioxidant activity of four different kombucha tea samples: kombucha fermented for 1 day, 2 days, 3 days, and 5 days. The IC_{50} values for the kombucha tea samples are presented in Table 3.

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Tabel 3. IC50 Value of Fermented Kombucha Tea

Treatment	Fermentation			
	1	2	3	5
IC_{50}	11.07	7.00	7.01	8.48

Antioxidant Activity

Antioxidant activity can be evaluated based on the IC_{50} value. The lower the IC_{50} value, the higher the antioxidant activity. Fermentation time affects the IC_{50} value—longer fermentation durations result in higher IC_{50} values, indicating a decrease in antioxidant activity (14). Based on the antioxidant activity test conducted on kombucha tea fermented for 1, 2, 3, and 5 days, it was observed that the antioxidant activity increased on the 2nd day of fermentation. However, after this peak on day 2, antioxidant activity decreased on days 3 and 5.

One-Way ANOVA Test

This analysis used the One-Way ANOVA method to determine the effect of fermentation duration on the antioxidant activity of kombucha tea. Prior to performing the ANOVA test, normality and homogeneity tests were conducted. The results showed a significance value greater than 0.05, indicating that the data were normally distributed and homogeneous. Therefore, the data were suitable for ANOVA testing. The result of the One-Way ANOVA test, conducted at a 95% confidence level ($\alpha = 0.05$), showed a significance value less than 0.05, indicating that fermentation duration significantly affects the antioxidant activity of kombucha tea.

CONCLUSION

Based on the tests conducted, it can be concluded that fermentation duration influences antioxidant activity. From the study on the antioxidant activity of kombucha tea (SCOBY) fermented for 1, 2, 3, and 5 days using the DPPH method, it was found that kombucha tea fermented for 2 days exhibited the highest antioxidant activity, with an IC_{50} value of 7.00 ppm.

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