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Analysis of the Antioxidant Activity of Strawberry Kombucha using the UV–Vis Spectrophotometric Method

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ABSTRACT

The development of modern lifestyles, which tend to favor instant foods and beverages, has increased the risk of degenerative diseases associated with oxidative stress. This condition has driven the demand for foods and beverages rich in antioxidants. Kombucha is a fermented beverage recognized for its probiotic and antioxidant properties, while strawberries are rich in natural antioxidant compounds. The combination of these two components has the potential to produce a functional beverage beneficial to health, particularly as a source of antioxidants. Therefore, this study aimed to determine the effect of fermentation duration on the antioxidant activity of strawberry kombucha and to identify the optimal fermentation duration for achieving the highest antioxidant activity. This study employed a quantitative research design using a laboratory experimental method. Strawberry kombucha was prepared from a mixture of fresh strawberries, water, granulated sugar, SCOBY starter solution, and SCOBY, followed by fermentation for 7, 10, and 13 days. Antioxidant activity was evaluated using the UV-Vis spectrophotometric method with DPPH reagent. The results demonstrated that fermentation duration significantly affected the antioxidant activity of strawberry kombucha. The highest antioxidant activity was achieved on day 10, with an IC_{50} value of 24.69 ppm, which was categorized as very strong antioxidant activity. Fermentation on day 13 resulted in an IC_{50} value of 30.69 ppm, which also fell within the very strong category, whereas fermentation on day 7 produced an IC_{50} value of 74.38 ppm, categorized as strong antioxidant activity. Therefore, a fermentation duration of 10 days was identified as the optimal condition for producing the highest antioxidant activity. The findings of this study are expected to contribute to the development of beverage formulations rich in antioxidant compounds.

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INTRODUCTION

The development of modern lifestyles has influenced people's consumption patterns. Increasing work demands and busy schedules have led individuals to prefer practical instant foods and beverages, which are often low in nutritional value and high in preservatives or artificial sweeteners. In addition, many people adopt unhealthy lifestyle habits and engage in insufficient physical activity. These habits contribute to the increasing prevalence of degenerative diseases such as diabetes, hypertension, and cardiovascular diseases, which are largely associated with oxidative stress caused by free radicals [1]. Therefore, the demand for nutritious food and beverage products rich in antioxidants has become increasingly important in supporting public health. In

recent years, kombucha has emerged as one of the fermented beverages gaining growing public interest due to its claimed health benefits [2].

Kombucha is a fermented beverage made from tea and sugar, widely recognized for its probiotic and antioxidant properties. The fermentation process involves a Symbiotic Culture of Bacteria and Yeast (SCOBY). SCOBY initiates the fermentation process when tea is mixed with approximately 10% sugar, producing new bioactive compounds at room temperature over a fermentation period of 7–14 days [1]. Kombucha contains polyphenolic compounds that function as antioxidants capable of neutralizing free radicals associated with degenerative diseases. Fruit-based kombucha may provide additional health benefits [3], particularly when strawberries are used as the primary ingredient. Strawberries are rich in natural antioxidant compounds, including vitamin C, anthocyanins, flavonoids, and polyphenols, all of which have been shown to play important roles in scavenging free radicals [4]. The combination of strawberry constituents and the kombucha fermentation process presents an opportunity to produce a functional beverage that is not only refreshing but also beneficial to health. Fermentation may modify the chemical structure of strawberries and generate new compounds with potentially enhanced antioxidant bioactivity. In accordance with the study conducted by Pramono et al. (2024), which investigated the bioactive components present in strawberry kombucha fermented for 12 days, the beverage demonstrated antioxidant activity, where a concentration of 17.28 ppm was capable of reducing 50% of free radical concentration [2]. However, the study did not investigate the effect of fermentation duration on the antioxidant activity of strawberry kombucha.

Fermentation duration is one of the primary factors influencing the chemical composition of kombucha. During fermentation, microorganisms such as acetic acid bacteria and yeast work synergistically to metabolize the main components, particularly sugars, while interacting with phenolic compounds. This activity results in changes in the concentration of antioxidant compounds in kombucha [5]. Excessively prolonged fermentation may increase the concentration of certain compounds; however, it may simultaneously lead to the degradation of some antioxidant compounds. The degradation of antioxidant compounds during fermentation is closely associated with changes in pH and enzymatic activity. A significant decrease in pH due to the production of organic acids may destabilize certain phenolic compounds, causing their degradation. Furthermore, excessive enzymatic activity by microorganisms during extended fermentation may break down bioactive compounds into components that are less effective as antioxidants [6].

Several previous studies have examined the relationship between fermentation duration and antioxidant activity in various types of kombucha. A study by Nizioł-Łukaszewska et al. (2020) [7], demonstrated that the antioxidant activity of green coffee-based kombucha increased with longer fermentation time but began to decline after exceeding the optimal fermentation point. Similarly, Tejedor-Calvo and Morales (2023) [8], reported that the fermentation process of Arbutus unedo fruit kombucha influenced changes in chemical compounds, thereby affecting antioxidant activity throughout fermentation. Pramono et al. (2024) [2], showed that the free radical scavenging activity of strawberry kombucha was strongly influenced by bioactive compounds produced through microbial metabolism at specific fermentation durations. Furthermore, Xiong et al. (2023) [9], found that kombucha made from bamboo leaves and mulberry exhibited the highest antioxidant activity at a certain fermentation duration, after which the activity declined due to the degradation of phenolic compounds. Overall, these studies indicate that fermentation duration significantly affects the antioxidant activity of kombucha. Therefore, it is important to determine the optimal fermentation duration to achieve the highest antioxidant activity.

This study employed a laboratory experimental approach aimed at measuring the antioxidant activity of strawberry kombucha at different fermentation durations, specifically on days 7, 10, and 13. The analysis was conducted using the UV-Vis spectrophotometric method with DPPH reagent at a concentration of 40 ppm. This method was selected due to its high level of accuracy and sensitivity in measuring the antioxidant activity of compounds. The obtained data were analyzed to determine the relationship between fermentation duration and antioxidant activity.

RESEARCH METHODS

This study employed a quantitative research design using a laboratory experimental method. The research was conducted to analyze the effect of fermentation duration on the antioxidant activity of strawberry kombucha by comparing the IC_{50} values of the three fermentation durations using a One-Way ANOVA statistical test. The study commenced in February 2025 and continued until June 2025. It was carried out at the Laboratory of Sekolah Tinggi Farmasi Mahaganesha, located on Jalan Tukad Barito Timur No. 57, Renon, South Denpasar, Denpasar City, Bali, Indonesia.

Instruments and Materials

This study utilized several instruments, including a blender, saucepan, analytical balance, filter paper, aluminum foil, flannel cloth, water bath, volumetric flask, vial, dropper pipette, volumetric pipette, pipette filler, glass funnel, stirring rod, beaker glass, graduated cylinder, thermometer, pH meter, and UV-Vis spectrophotometer. The materials used in this study consisted of fresh strawberries, granulated sugar, SCOBY starter solution, SCOBY, bottled drinking water, distilled water (aquadest), 95% methanol, and 2.2-diphenyl-1-picrylhydrazyl (DPPH).

Research Procedure

a. Preparation of Strawberry Kombucha

The formulation of strawberry kombucha is presented in Table 1. The preparation process began by boiling water (±80°C) in a saucepan, followed by the addition of 120 g of granulated sugar. The mixture was stirred until homogeneous, after which 9.6 g of strawberries (previously blended and filtered) were added. The solution was then stirred until its color changed to a dark reddish-brown, after which the heat was turned off, the saucepan was covered, and the mixture was allowed to cool. The solution was subsequently transferred into a sterile 1000 mL bottle together with the SCOBY starter solution and SCOBY (ensuring that the solution pH ranged between 4.0 and 4.5 prior to fermentation). A clean cheesecloth was placed over the bottle opening and securely tied, after which the bottle was stored at a temperature of 20–25°C for 7, 10, and 13 days. Upon completion of the fermentation process, all samples were subjected to further analysis.

Table 1. Formulation of Strawberry Kombucha

Material	Quantity
Strawberry fruit	9.6 g
Granulated sugar	120 g
SCOBY	7 g
SCOBY starter solution	200 mL
Water	Ad 1000 mL

b. **Antioxidant Activity Assay**

The assay began with the preparation of a 1000 ppm stock solution of strawberry kombucha fermented for 7, 10, and 13 days using methanol as the solvent. The stock solution was

subsequently diluted with methanol to obtain test sample solutions with concentrations of 50, 100, 150, 200, and 250 ppm. Furthermore, a DPPH working standard solution with a concentration of 40 ppm was prepared in methanol, and its absorbance was measured within a wavelength range of 400–800 nm using a UV-Vis spectrophotometer to determine the maximum wavelength. Subsequently, the antioxidant activity assay was conducted by measuring the absorbance of the 40 ppm DPPH solution mixed with methanol (negative control) and the absorbance of the test sample solution mixed with 40 ppm DPPH after incubation for 30 minutes using a UV-Vis spectrophotometer at the maximum wavelength. The antioxidant activity assay was performed in triplicate. The absorbance values obtained from each concentration were then used to calculate the percentage inhibition (% inhibition) using the following equation:

$$\% \text{ Inhibition} = \left(\frac{\text{DPPH Absorbance} - \text{Test Sample Absorbance}}{\text{DPPH Absorbance}}\right) \times 100\% \quad (1)$$

c. **Data Analysis**

The obtained data were analyzed to determine the antioxidant activity of strawberry kombucha using Microsoft Excel software. Based on the percentage inhibition values at each concentration, a calibration curve was constructed to obtain a linear regression equation in the form of $y = bx + a$, where the concentration of strawberry kombucha (ppm) was plotted on the x-axis (abscissa) and the percentage inhibition value was plotted on the y-axis (ordinate). Subsequently, the IC_{50} value was calculated as the concentration of the sample required to inhibit 50% of DPPH activity in order to determine its antioxidant activity, using the following equation:

$$IC_{50} = \frac{50 - a}{b} \quad (2)$$

Where:
a = intercept
b = slope

The IC_{50} data obtained from the three different fermentation durations (7, 10, and 13 days) were statistically compared. Prior to further analysis, several assumptions had to be fulfilled, namely that the data were normally distributed and homogeneous; therefore, normality and homogeneity tests were conducted.

If the data met these assumptions, the analysis was continued using a One-Way ANOVA test. A significance value of $p<0.05p < 0.05p<0.05$ indicated a statistically significant difference among the groups. The analysis was subsequently followed by a Post Hoc Tukey HSD test, in which a significance value of $p<0.05p < 0.05p<0.05$ indicated a significant difference in the mean antioxidant activity of kombucha fermented for different durations.

RESULTS AND DISCUSSION

Preparation of Strawberry Kombucha

The kombucha used in this study was strawberry kombucha formulated using strawberries, granulated sugar, SCOBY, SCOBY starter solution, and water. All ingredients produced a total volume of 1000 mL, which was subsequently fermented at a temperature of 20–25°C for 7, 10, and 13 days. After the fermentation process was completed, all samples were further analyzed for research purposes. The resulting strawberry kombucha can be seen in Figure 1.

Figure 1. Strawberry Kombucha

Determination of Maximum Wavelength

The 40 ppm DPPH solution used as the free radical reagent was measured using a UV-Vis spectrophotometer to determine its maximum wavelength. The results of the maximum wavelength determination are presented in Table 2.

Table 2. Maximum Wavelength of 40 ppm DPPH Solution

Observation Result	Literature [10]
516 nm	515-520 nm

Based on the observation results, the maximum wavelength of the 40 ppm DPPH solution was determined to be 516 nm. This indicates that at this wavelength, the solution exhibits maximum sensitivity, resulting in the greatest absorbance change for each unit concentration. The value of 516 nm is also consistent with the literature, which states that the maximum wavelength of DPPH ranges between 515–520 nm [10]. The maximum wavelength serves as an important reference in measuring the antioxidant capacity of a sample. If an inappropriate wavelength is used, the measurement results may become less accurate.

Antioxidant Activity Assay

The antioxidant activity of strawberry kombucha was evaluated at concentrations of 50, 100, 150, 200, and 250 ppm. The samples were incubated for 30 minutes with a 40 ppm DPPH solution dissolved in methanol, after which their absorbance values were measured using a UV-Vis spectrophotometer. The results of the antioxidant activity assay are presented in Table 3.

Table 3. Results of the Antioxidant Activity Assay of Strawberry Kombucha

Day	Concentration (ppm)	Abs of DPPH + Methanol	Abs of Sample + DPPH		
			R 1	R 2	R 3
7	50	0.803	0.469	0.468	0.468
	100		0.328	0.325	0.323
	150		0.266	0.265	0.263
	200		0.191	0.189	0.187
	250		0.102	0.100	0.098
10	50	1.134	0.538	0.537	0.536
	100		0.421	0.421	0.420
	150		0.381	0.381	0.380
	200		0.285	0.284	0.283
	250		0.213	0.199	0.194
13	50	1.119	0.535	0.534	0.533
	100		0.419	0.419	0.418
	150		0.380	0.379	0.379
	200		0.281	0.280	0.279
	250		0.185	0.185	0.180

Based on the observation results, both fermentation duration and test sample concentration influenced the antioxidant activity of strawberry kombucha. As the sample concentration increased, the resulting absorbance values decreased. This indicates that the antioxidant compounds present in the test samples were capable of inhibiting DPPH, which acts as a free radical reagent.

Calculation of Percentage Inhibition (% Inhibition)

The calculation of percentage inhibition (% inhibition) was performed to determine the ability of antioxidant compounds present in the test samples to inhibit DPPH as a free radical compound. This activity was indicated by a reduction in the purple color intensity of the DPPH solution after reacting with antioxidant compounds. The absorbance values obtained at each concentration were subsequently used to calculate the percentage inhibition values. The results of the percentage inhibition calculations are presented in Table 4.

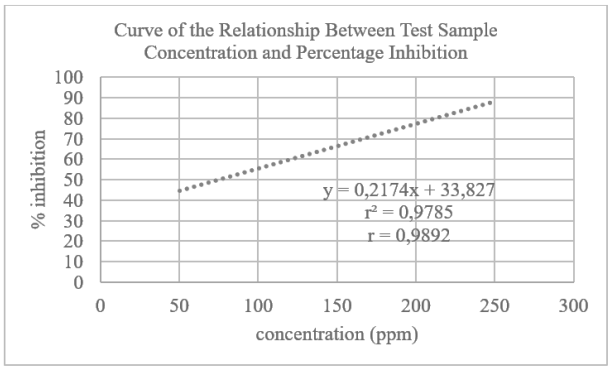
Table 4. Results of Percentage Inhibition (% Inhibition) Calculation

Day	Concentration (ppm)	Inhibition (%)			Average Inhibition (%)
		R 1	R 2	R 3	
7	50	41.59	41.72	41.72	41.68
	100	59.15	59.53	59.78	59.49
	150	66.87	67.00	67.25	67.04
	200	76.21	76.46	76.71	76.46
	250	87.30	87.55	87.80	87.55
10	50	52.56	52.65	52.73	52.65
	100	62.87	62.87	62.96	62.90
	150	66.40	66.40	66.49	66.43
	200	74.87	74.96	75.04	74.96
	250	81.22	82.45	82.89	82.19
13	50	52.19	52.28	52.37	52.28
	100	62.56	62.56	62.65	62.59
	150	66.04	66.13	66.13	66.10
	200	74.89	74.98	75.07	74.98
	250	83.47	83.47	83.91	83.62

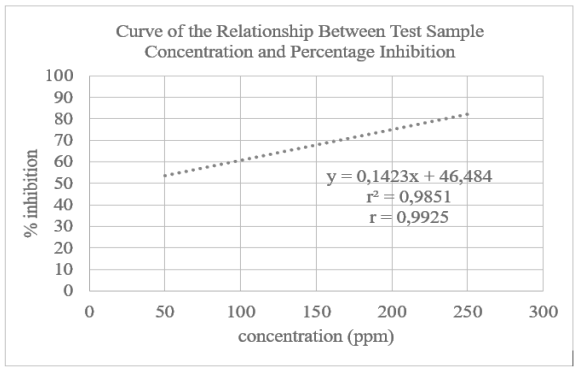
Based on the calculation results, the antioxidant activity of strawberry kombucha increased with increasing concentrations of the test samples. This was indicated by the increase in percentage inhibition values at each concentration for the 7-day, 10-day, and 13-day fermentation periods. Higher concentrations resulted in greater free radical scavenging activity, indicating that the antioxidant compounds present in the samples became increasingly potent.

Preparation of the Calibration Curve

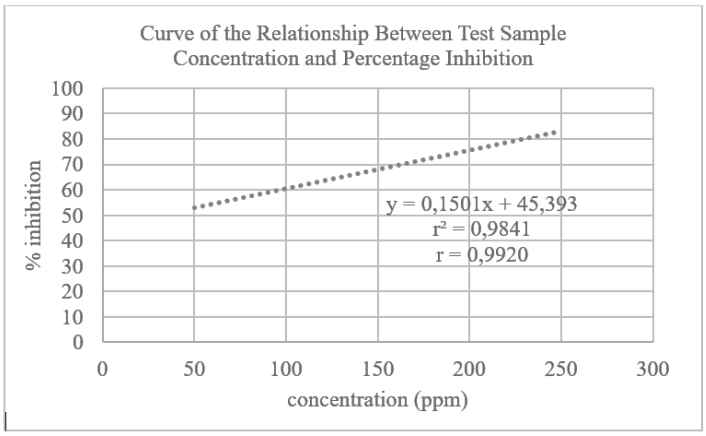
The preparation of the calibration curve aimed to determine the relationship between the concentration of the test samples and the percentage inhibition values. This curve facilitated the generation of a linear regression equation, which was subsequently used to calculate the IC_{50} value. The calibration curve enabled the IC_{50} calculation to be performed in a more accurate, objective, and measurable manner based on the obtained data. The results of the calibration curve construction are presented in Figure 2.



(A) Day 7



(B) Day 10



(C) Day 13

Figure 2. Calibration Curves Showing the Relationship between Test Sample Concentration and Percentage Inhibition (% Inhibition) for the 7-Day (A), 10-Day (B), and 13-Day (C) Fermentation Periods

Based on the curves illustrating the relationship between test sample concentration and percentage inhibition values of strawberry kombucha fermented for 7, 10, and 13 days, the correlation coefficient (r) values obtained from the three curves were 0.9892, 0.9925, and 0.9920, respectively, indicating a strong correlation between test sample concentration and percentage inhibition. These r values are close to the maximum value of +1, suggesting that the obtained data were highly reliable and linear [11].

Determination of IC_{50} Value

After obtaining the linear regression equation for each test sample, the IC_{50} value was determined to evaluate the antioxidant activity of strawberry kombucha. The results of the IC_{50} determination are presented in Table 5.

Table 5. Results of IC_{50} Determination

Day	IC_{50} Value (ppm)			Average IC_{50} Value	Standard Deviation (SD)	Persentage SD (%)	Significance.
	R1	R2	R3				
7	75.21	74.25	73.68	74.38	0.78	1.04	$p<0.001$
10	23.16	25.40	25.52	24.69	1.33	5.38	$p<0.001$
13	30.96	30.47	30.66	30.69	0.25	0.81	$p<0.001$

Based on the statistical analysis of the IC_{50} values, the data satisfied the assumptions of normality and homogeneity tests and were subsequently analyzed using a One-Way ANOVA test. The obtained p-value (p<0.001p < 0.001p<0.001) was considerably lower than the significance level of 0.05, indicating that the three IC_{50} values differed significantly. Further analysis using the Post Hoc Tukey HSD test demonstrated that the fermentation durations of 7, 10, and 13 days each showed significant differences (p<0.001p < 0.001p<0.001).

The results of this study indicate that fermentation duration significantly affected the antioxidant activity of strawberry kombucha. Lower IC_{50} values reflect a greater ability to scavenge free radicals. During the fermentation process, the IC_{50} values of kombucha changed from 74.38 ppm on day 7, decreased to 24.69 ppm on day 10, and slightly increased to 30.69 ppm on day 13. Strawberry kombucha harvested on days 10 and 13 was categorized as having very strong antioxidant activity, whereas kombucha harvested on day 7 was classified as having strong antioxidant activity. However, when compared with previous studies using positive controls such as vitamin C, quercetin, and glutathione, which are recognized as very strong antioxidants against DPPH radicals, strawberry kombucha still required higher concentrations (higher IC_{50} values) to inhibit 50% of free radicals. The IC_{50} values reported for vitamin C, quercetin, and glutathione were 3.701 ppm [12], 3.089 ppm [13], and 3.625 ppm [2], respectively.

Standard deviation (SD) was used to indicate the degree of variation or dispersion of the data relative to the mean value. Smaller SD values indicate that the replicate data are more homogeneous and consistent, whereas larger SD values indicate greater variability or inconsistency among replicates [11]. On day 7, the obtained SD value was 0.78, representing 1.04% of the mean IC_{50} value of 74.38 ppm. This result indicates that the variation among replicates was relatively small, suggesting that the measurements were sufficiently consistent and stable. On day 10, the SD value was 1.33, representing 5.38% of the mean IC_{50} value of 24.69 ppm. This was the highest SD value among all fermentation durations, indicating slightly greater variability among replicates, although the results were still considered reasonably consistent. Meanwhile, on day 13, the SD value was 0.25, representing 0.81% of the mean IC_{50} value of 30.69 ppm. This was the lowest SD value, indicating highly consistent results with minimal data variation. Based on the SD percentages, all obtained results remained within the acceptable range, namely less than 10% of the mean value. This range is generally considered acceptable, indicating that the data were homogeneous and suitable for further analysis [11].

The lowest IC_{50} value was obtained on day 10, namely 24.69 ppm, indicating that the antioxidant activity reached its optimum level at this fermentation duration. This condition may be attributed to the fermentation process reaching its peak stage, during which the microorganisms present in the SCOBY actively and efficiently converted substrates into various bioactive compounds that play important roles as antioxidants. In contrast, the IC_{50} value on day 7 was higher than that on day 10, reaching 74.38 ppm, indicating lower antioxidant activity compared with day 10, although it still fell within the strong antioxidant category. This may be explained by the fermentation process still being in its early stage, during which the microorganisms in the SCOBY had not yet functioned optimally in converting substrates into bioactive compounds.

The IC_{50} value on day 13 was lower than that on day 7 but higher than that on day 10, with a value of 30.69 ppm, indicating that its antioxidant activity was higher than that observed on day 7 but lower than that on day 10. Although the antioxidant activity on day 13 was lower than that on day 10, it still remained within the very strong antioxidant category. This phenomenon may be attributed to the degradation of antioxidant compounds during prolonged fermentation. The degradation of antioxidant compounds during fermentation is closely related to changes in pH and enzymatic activity. A significant decrease in pH due to the production of organic acids may

destabilize certain antioxidant compounds, leading to their degradation. In addition, excessive enzymatic activity of microorganisms during prolonged fermentation may break down bioactive compounds into components that are less effective as antioxidants. This finding is consistent with the study conducted by Morales et al. (2023), which reported that strawberry kombucha experienced a decrease in pH from 3.3 on day 0 to 2.6 on day 21. Furthermore, the total phenolic content decreased with increasing fermentation time, from 13.66 ± 0.43 mg/100 mL on day 7 to 7.06 ± 0.13 mg/100 mL on day 21 [14].

CONCLUSION

Based on the results of this study, it can be concluded that fermentation duration significantly affects the antioxidant activity of strawberry kombucha. This was demonstrated by the variation in IC_{50} values observed on fermentation days 7, 10, and 13, indicating that each fermentation duration produced different levels of antioxidant activity. The highest antioxidant activity was achieved on day 10 of fermentation, with an IC_{50} value of 24.69 ppm, which falls within the category of very strong antioxidant activity. This finding indicates that day 10 represents the optimal fermentation duration for producing the highest antioxidant activity. Although antioxidant activity increased during certain fermentation periods, further analysis of the bioactive compounds contributing to kombucha activity throughout the fermentation process is still necessary. Therefore, further studies should be conducted to evaluate the content of bioactive compounds such as flavonoids, total phenolics, and organic acids. These analyses should employ more specific analytical techniques, such as High-Performance Liquid Chromatography (HPLC) or Liquid Chromatography–Mass Spectrometry (LC-MS). Such investigations are important for gaining a deeper understanding of the chemical transformation mechanisms that occur during the fermentation process.

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