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COMPARISON OF ANTIOXIDANT ACTIVITY AND CHEMICAL PROFILE BETWEEN GREEN TEA KOMBUCHA WITH GREEN TEA AND ROSELA COMBINATION KOMBUCHA

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ABSTRACT: This study investigates the antioxidant potential and chemical profile of kombucha made from green tea leaves and roselle flowers, analyzing pH, alcohol content, and the impact of fermentation time. Kombucha was prepared with green tea and a 1:1 mixture of green tea and roselle, with samples analyzed on days 1, 4, 8, 12, 16, and 24. Antioxidant activity, pH, and alcohol content were measured, and statistical analyses including one-way ANOVA, Tukey HSD, and Spearman correlation tests were conducted. Results indicated that the combination kombucha exhibited higher antioxidant activity (96.39% DPPH inhibition) compared to green tea kombucha (79.62%). There was no significant difference in pH and alcohol content between the two types ($p > 0.05$). A strong negative correlation was found between pH and fermentation time (-0.589, $p < 0.001$) and pH and alcohol content (-0.571, $p < 0.001$), while a strong positive correlation was observed between % inhibition and fermentation time (0.637, $p < 0.001$). The findings suggest that combining green tea and roselle enhances antioxidant activity in kombucha, and fermentation time significantly affects antioxidant activity, pH, and alcohol content.

Keywords: Antioxidant Activity, Fermentation Dynamics, Green Tea, Kombucha, Rosella.

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INTRODUCTION

Degenerative diseases are medical conditions that last for a long period of time, such as heart disease, hypertension, diabetes, and the like (Hamka, 2020). These diseases have become one of the leading causes of death worldwide, including in Indonesia, where the number of chronic degenerative disease cases increases every year. The main causes of degenerative diseases are related to unhealthy lifestyles such as smoking, consumption of alcoholic beverages, unhealthy diet, lack of physical activity, and the impact of environmental pollution that can trigger the emergence of free radicals and oxidative stress that can potentially damage the body. The increase in per capita income and the development of lifestyle, especially in big cities, has also led to an increase in degenerative diseases (Amila et al., 2021). Based on the Riskesdas (2018), the Ministry of Health, patients with degenerative diseases in Indonesia have always increased from year to year, from 9.4% in 2007 to 13.3% in 2013.

The human body needs antioxidants to protect its cells from the negative effects of free radicals (Fadlilah & Lestari, 2023). Antioxidants are components that can prevent or inhibit the oxidation of fats, nucleic acids, or other molecules by preventing the initiation or progression of oxidizing chain reactions. Consumption of antioxidants in adequate amounts can reduce the risk of developing degenerative diseases. One drink that has a role as an antioxidant is kombucha.

Kombucha is a beverage resulting from a fermentation process involving SCOBY (Symbiotic Culture of Bacteria and Yeast), which is a colony of microorganisms that work symbiotically to turn a mixture of ingredients into a sour and fizzy drink. The basic ingredient commonly used in making kombucha is tea. (Khaerah & Akbar, 2019) distinctive flavor and aroma. Apart from tea, several other ingredients can also be used as substrates for kombucha fermentation, such as coffee leaves, betel leaves, fruits, or other materials that contain high phenolic compounds (Khaerah & Akbar, 2019).

As a functional beverage, the demand for kombucha is increasing due to its superior nutritional content. The global kombucha market increased rapidly with a compound annual growth rate of 23% in the 2014-2018 period and is expected to maintain its rapid growth in the coming years (Zou et al., 2021). Estimates of enthusiast growth are supported by data from Kapp & Sumner (2019), that by 2028, the kombucha market is expected to reach more than six billion US dollars. Kombucha's popularity as a functional food is driven by its health benefits, which include dual functional properties such as anti-inflammatory potential and antioxidant activity.

The utilization of kombucha as a functional drink that is high in antioxidants has been widely carried out, such as in the research of (Khaerah & Akbar, 2019) conducted antioxidant tests on green tea kombucha, black tea, white tea, and oolong tea, then it was found that the highest antioxidant activity was possessed by green tea kombucha. Kombucha tea is one of the drinks that has been known to have antioxidant activity. The increased antioxidant activity in kombucha tea is due to the presence of free phenolics produced during the fermentation process, so the higher the phenolic content produced, the higher the antioxidant activity (Bishop et al., 2022). Furthermore, the optimal antioxidant activity of green tea kombucha is on days 1 to 5 with a value of 90.835% - 91.853% (Khamidah & Antarlina, 2020). The antioxidant activity value of green tea kombucha decreased after day 15. Research on kombucha tea by Nasution & Nasution (2022) shows that the antioxidant activity of rosella flower tea kombucha is high compared to tea leaves (down as much as 85% after fermenting for ten days) while the lowest antioxidant activity is owned by black tea kombucha (Nasution & Nasution, 2022). Then the research of Winandari et al. (2022) showed that rosella kombucha tea increased antioxidant activity at a fermentation time of 9 days, namely 0.9818% (Winandari et al., 2022). In the other similar studies, state that, the treatment combination of moringa leaves and rosella flowers ratio 1:2 has high antioxidant activity (Wahyudi et al., 2018).

Based on previous research, no one has ever conducted research on kombucha beverages combined with green tea leaves and rosella. From previous studies, it is known that green tea leaves have high antioxidant activity compared to other teas and rosella flowers also have higher antioxidant activity than tea leaves. So, this study aims to determine the antioxidant potential of the combination of the two ingredients, which are known to have high antioxidant activity. However, it is not only limited to the determination of percent inhibition as an antioxidant, but chemical profile analysis is carried out, such as pH analysis, alcohol content and variation of testing on the length of fermentation time to determine whether there is an effect of time on pH, alcohol and antioxidant parameters.

METHOD

Materials and Tools

The materials used in this research are green tea and dried rosella flowers sold kilos in one of the markets in Yogyakarta, kombucha mushroom inoculants with a diameter of ± 10 cm, sugar, distilled water, ascorbic acid (MERCK), 2,2-diphenyl-1- picrylhydarzyl (DPPH) (Sigma-Aldrich), strips pH (MERCK) and 96% ethanol (MERCK). The tools used in this research are vacuum glass jars, Agilent Carry 60 Spectrophotometer, Thermo Nicolet IS 10 FTIR, and ATC Alcoholmeter.

Kombucha Tea Preparation

Kombucha tea was made in two variations of the formula: green tea kombucha and mixed kombucha of green tea and rosella flowers. Green tea kombucha was made using 30 grams of green tea, while green tea and rosella flower blend kombucha was made with a mixture of green tea and rosella flowers in a 1:1 ratio. Both variations were steeped in 1 liter of hot water along with 100 ml of starter and 120 grams of sugar. After a 15-minute soaking process, the tea solution was filtered and allowed to settle to room temperature. The next step involves the addition of kombucha mushroom inoculant with a diameter of about ±10 cm or weighing about 70-75 grams.

Collection of Samples

The kombucha tea was sampled on several different days, namely on days 1, 4, 8, 12, 16, and 24. Thus, a total of 12 samples were taken. The sampling process was carried out by paying attention to the sterility and cleanliness of the equipment so as not to affect the kombucha mushroom inoculant and cause contamination.

Determination of pH

Determination of pH was carried out using a pH meter on each sample on days 1, 4, 8, 12, 16, and 24.

Determination of Alcohol Level

Alcohol content testing refers to the method of (Jakubczyk et al., 2020), where alcohol content is measured using an alcoholometer. The alcoholometer is immersed in the liquid, and the results are read from the available scale. Then, the alcohol content is checked in units of %.

Determination of % Inhibition as an Indication of Antioxidant Ability

The antioxidant activity of the samples was measured by a spectrophotometric method using DPPH radical according to (Pekkarinen et al.,

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1999; Williams et al., 1995). The spectral absorbance was immediately measured at a wavelength of 518 nm (Agilent 8453UV). All assays were performed in triplicate. The results were expressed as % inhibition of DPPH radicals. The antioxidant potential (antioxidant activity, inhibition) of the tested solutions has been expressed as a percentage of DPPH inhibition using the following formula (1).

$$\% \textbf{ Inhibition} = \frac{A_0 - A_s}{A_0} \times 100 \text{ ....(1)}$$

Where on formula (1):
A_0 = Absorbance of DPPH solution at 518 nm without tested sample; and
A_s = Absorbance of DPPH solution at 518 nm with tested sample.
(**Source:** Jakubczyk et al., 2020).

Data Analysis

All quantitative data to determine the correlation between fermentation time, alcohol, and pH on antioxidant activity were statistically analyzed using One-Way ANOVA method at 95% confidence level, Tukey HSD post hoc test, and Spearman's Correlation test was used for further analysis. Statistical analysis was performed using the JASP (Jeffreys's Amazing Statistics Program) application program.

RESULTS AND DISCUSSION

Antioxidant Testing Results (as % Inhibition)

Analysis of the antioxidant potential of the studied samples revealed that the content of antioxidant compounds ranged from 69.22% to 96.39% DPPH inhibition (Table 1). Fermentation time and tea type affect the anti-radical properties of kombucha. The comparative analysis between the two types of Kombucha showed that the antioxidant activity of kombucha combined with green tea and rosella was higher than that of green tea kombucha, with significant differences in percentage inhibition. The results of statistical analysis using the Mann-Whitney U Test (Table 2) showed a significant difference in antioxidant activity between the two types of kombucha ($p < 0.05$). The Mann-Whitney U Test was chosen because the One-Way ANOVA test results found that the data were not normally distributed (reject H_0) and had homogeneous data

Table 1. Antioxidant Testing Results (as % Inhibition).

Type of Kombucha	Day	DPPH (%)
Green Tea Kombucha	0 Day	70.99 ± 0.22[b]
	1st Day	79.62 ± 0.56[f]
	4th Day	78.83 ± 0.43[e,f]
	8th Day	77.94 ± 0.35[e]
	12th Day	74.98 ± 0.21[d]
	16th Day	73.11 ± 0.29[c]
	24th Day	69.22 ± 0.29[a]
Green Tea and Rosella Kombucha	0 Day	87.48 ± 0.72[g,h]
	1st Day	96.39 ± 0.52[k]
	4th Day	95.30 ± 0.12[k]
	8th Day	93.26 ± 0.17[j]
	12th Day	90.85 ± 0.42[i]

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Type of Kombucha	Day	DPPH (%)
	16th Day	88.06 ± 0.24^{h}
	24th Day	86.38 ± 0.57^{g}

Letters next to numbers represent significant differences between treatments at specific fermentation time measurements based on Tukey HSD Post Hoc Test. The same letter indicates no significant difference between the treatments, while different letters indicate a statistically significant difference (Hidayah et al., 2022).

Table 2. Uji Mann-Whitney U Test.

Parameters	Test	Statistic	df	P
pH	Welch	1.459	37.412	0.153
	Mann-Whitney	257.000		0.365
% Alcohol	Welch	1.672	36.855	0.103
	Mann-Whitney	276.000		0.165
Days	Welch	0.000	40.000	1.000
	Mann-Whitney	220.500		1.000
Percent_Inhibisi	Welch	-13.718	39.994	< .001
	Mann-Whitney	0.000		< .001

In terms of tea type, kombucha prepared from green tea and rosella was characterized by the highest antioxidant potential, reaching the highest value on the first day of fermentation. Combined kombucha containing additional raw materials such as rosella, has high potential as a source of antioxidant compounds. Rosella is known to contain flavonoids, anthocyanins, and phenolic acids that may contribute to antioxidant activity (Hapsari et al., 2021; Safnowandi, 2022). Combination kombucha exploits the synergistic effect of the combination of raw materials. Combining green tea with rosella may result in a more complex and diverse variety of antioxidant compounds, which may contribute to higher antioxidant activity. (Etxebarria et al., 2020). The fermentation process may play a role in enhancing the bioavailability and activity of antioxidant compounds. Fermentation can increase the availability of bioactive compounds and trigger the formation of new compounds with antioxidant activity (Zubaidah et al., 2022). Increasing fermentation time can increase antioxidant activity due to the free phenolic compounds produced during the fermentation process, but at a certain point, antioxidant activity may decrease (Hassmy, 2017; Kuncoro, 2019). Based on the test results, the ability of both kombucha formulations to inactivate free radicals decreased as the fermentation time increased due to the influence on antioxidant activity caused by changes in compounds during the fermentation process. In addition, the decrease in antioxidant activity can also be caused by an increase in alcohol content and a reduction in pH during fermentation. Therefore, although fermentation time may increase the antioxidant activity initially, at some point, the antioxidant activity may decrease as the fermentation time increases (Rajma et al., 2016; Rahayu & Abram, 2023). A graph of the increase and decrease in % inhibition at each fermentation time can be seen in Figure 1.

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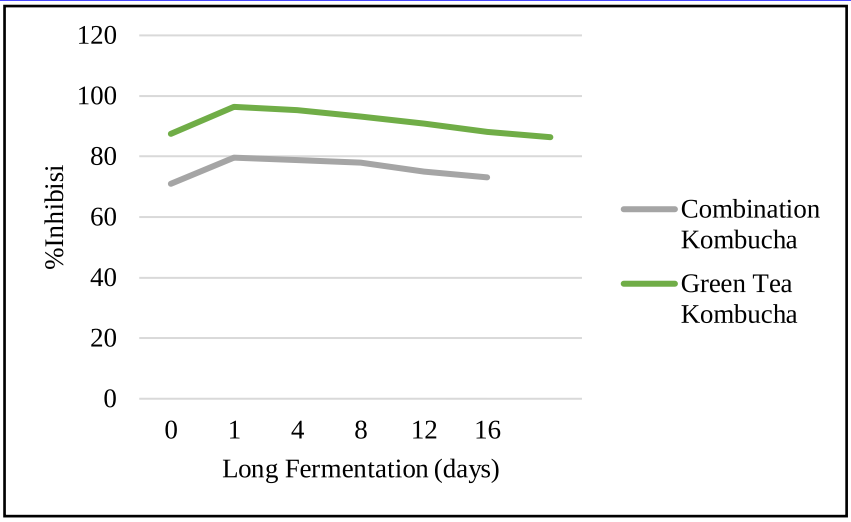

Figure 1. Graph of % Inhibition between Green Tea Kombucha and Combination Kombucha against Fermentation Time.

Chemical Profile Testing Results (pH and Alcohol)

During the analysis of pH values, it was observed that the pH of all the samples studied decreased as the fermentation duration increased (Table 3). No significant difference was observed in pH between beverages prepared from different tea types. This was supported by the results of the Mann-Whitney U Test (Table 2). The analysis of pH and alcohol content in the two types of kombucha, namely green tea kombucha and green tea with rosella kombucha, reveals no significant differences between them. This can be attributed to the similar fermentation processes they undergo. Both types of kombucha involve the same types of bacteria and yeast, which convert sugars into various organic acids, decreasing the pH of the beverage over time. The fundamental fermentation process is the same, resulting in similar pH changes regardless of whether the kombucha is made with green tea alone or with a combination of green tea and rosella.

The results of the Mann-Whitney U Test support this observation, showing a p-value of 0.365, which is greater than the significance level of 0.05. This indicates that the differences in pH values between the two types of kombucha are not statistically significant. Therefore, the hypothesis (H_0) that there is no significant difference in pH between green tea kombucha and green tea with rosella kombucha is accepted.

Regarding alcohol content, both types of kombucha exhibit consistent fermentation dynamics. During fermentation, yeast converts sugars into alcohol and carbon dioxide, and then bacterial cultures convert the alcohol into acetic acid and other organic acids (Luyt et al., 2021). Given that both kombucha types use the same microbial cultures and have similar initial sugar content, the resulting alcohol levels are comparable. The primary difference between the two kombucha types is the addition of rosella, which does not significantly alter the fermentation process to impact alcohol content dramatically. The slight variations in alcohol content seen in Table 3 are within the range of normal fermentation variability.

The pH values for both kombucha types decrease consistently over the fermentation period, indicating acid production from microbial activity. The end pH values for both types are very close, especially from the 8th day onward, showing a convergence in acidity levels. Similarly, the alcohol content increases initially as yeast converts sugars into alcohol, peaking around the 8th day, and then decreases slightly as bacteria convert the alcohol into acetic acid and other compounds. Both kombucha types show this trend, with similar alcohol content across the different fermentation days. The p value in the pH value data is 0.365 > 0.05 to accept H_0, meaning there is no significant difference in pH between green tea kombucha and kombucha combination of green tea with rosella.

Table 3. Chemical Profile Testing Results (pH and Alcohol).

Type of Kombucha	Day	pH	Alcohol (%)
Green Tea Kombucha	0 Day	6.13 ± 0.13^{g}	0.00 ± 0.00^{a}
	1^{st} Day	4.56 ± 0.05^{e}	0.43 ± 0.06^{b}
	4^{th} Day	4.25 ± 0.26^{e}	2.27 ± 0.12^{d}
	8^{th} Day	$2.99 \pm 0.20^{c,d}$	4.60 ± 0.00^{h}
	12^{th} Day	2.31 ± 0.06^{a}	3.43 ± 0.15^{g}
	16^{th} Day	2.03 ± 0.12^{a}	$2.80 \pm 0.00^{e,f}$
	24^{th} Day	2.16 ± 0.25^{a}	2.27 ± 0.15^{d}
Green Tea and Rosella Kombucha	0 Day	5.39 ± 0.13^{f}	0.00 ± 0.00^{a}
	1^{st} Day	3.25 ± 0.10^{d}	$0.20 \pm 0.00^{a,b}$
	4^{th} Day	$2.79 \pm 0.23^{b,c}$	1.30 ± 0.10^{c}
	8^{th} Day	$2.43 \pm 0.10^{a,b}$	2.90 ± 0.10^{f}
	12^{th} Day	$2.37 \pm 0.06^{a,b}$	$2.67 \pm 0.06^{e,f}$
	16^{th} Day	2.05 ± 0.07^{a}	2.60 ± 0.10^{e}
	24^{th} Day	2.03 ± 0.14^{a}	1.27 ± 0.06^{c}

The decrease in pH during fermentation is due to the production of organic acid compounds such as acetic and gluconic acid by SCOBYs involved in the fermentation process (Sintyadewi et al., 2021). The longer the fermentation time, the more organic acid compounds are produced so that the pH value of the fermentation product will decrease. This can be seen in the results of the study which showed that the pH of all samples studied decreased as the duration of fermentation increased, and further fermentation had no significant effect on changes in pH value (Sandi et al., 2023). Therefore, a decrease in pH during fermentation is a consistent result in various types of beverages and fermented ingredients. The graph of the pH decrease at each fermentation time can be seen in Figure 2. The graph of the pH value shows that the pH value of the green tea kombucha drink is higher than the pH value of the combined kombucha drink, and the pH value both decreases with the length of fermentation.

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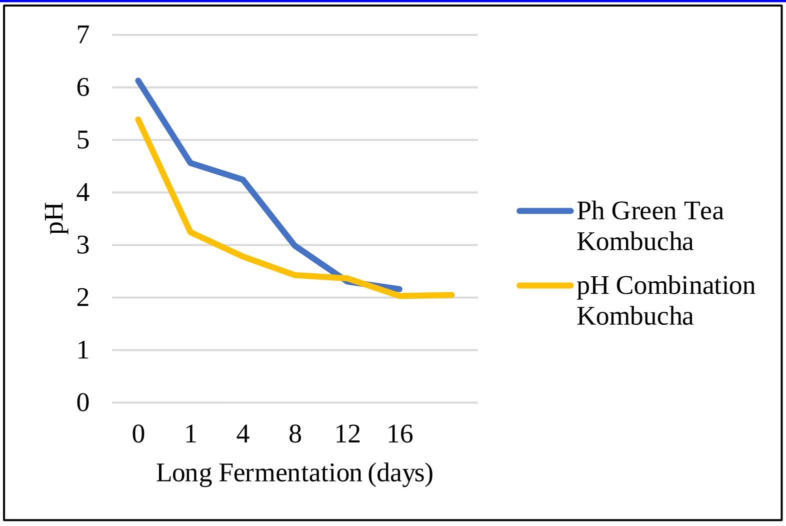

Figure 2. Graph of pH Decrease between Green Tea Kombucha and Combination Kombucha against Fermentation Time.

The p value (Table 2) in % alcohol content is 0.165 > 0.05, thus accepting H0, meaning that there is no significant difference in % alcohol between green tea kombucha and green tea with roselle combination kombucha.

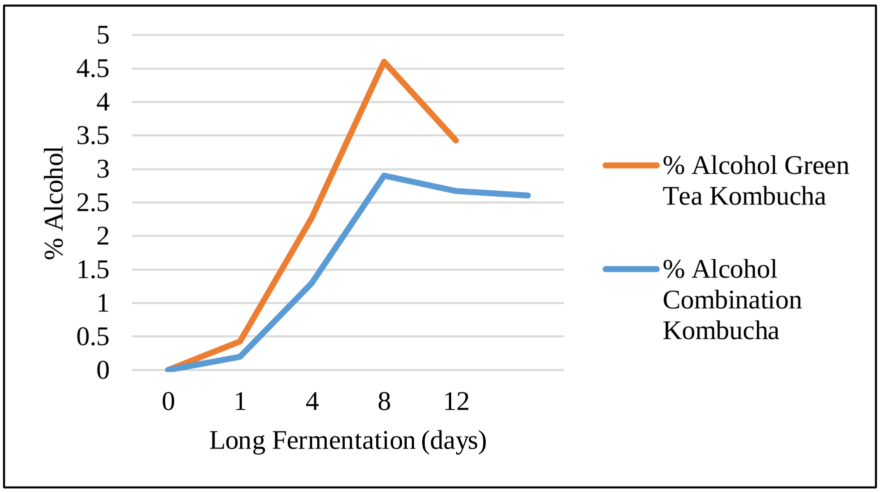

Figure 3. Graph of Alcohol Percentage Increase between Green Tea Kombucha and Combined Kombucha against Fermentation Time.

It can be seen from the graph (Figure 3) that the alcohol percentage of the green tea kombucha drink is higher than that of the combined kombucha. Over time, the alcohol content increased in the fermentation process, reaching a peak on day 8 between 2.90% to 4.60%, depending on the tea formulation used. However, at a later stage, there was a decrease in alcohol concentration in all types of kombucha beverages (on day 12 of the fermentation process). An increase in alcohol content in kombucha also occurred during fermentation. This is due to SCOBY activity that converts glucose in the tea liquid into alcohol. The length of fermentation time also affects the increase of alcohol content in kombucha. As the

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duration of fermentation increases, the alcohol content tends to increase, but this increase is not always linear (Sulistiawaty & Solihat, 2022).

The decrease in alcohol content in kombucha occurs because alcohol is used by SCOBY to form acetic acid, causing the alcohol content to decrease. During the fermentation process, the yeast in SCOBY will produce alcohol anaerobically. However, the bacteria in SCOBY use the alcohol to form acetic acid, one of the primary organic acids in kombucha. This process leads to a decrease in alcohol content along with an increase in acetic acid and other organic acids, which also contribute to the flavor and characteristics of kombucha. Therefore, the decrease in alcohol content in kombucha is mainly due to the conversion of alcohol to acetic acid by the bacteria in SCOBY during the fermentation process (Hafsari et al., 2021; Riswanto & Rezaldi, 2021).

Correlation Analysis of Antioxidant Activity and Chemical Profile with Fermentation Time

This analysis will evaluate the relationship between antioxidant inhibition % and pH, alcohol content, and fermentation duration. This analysis uses Spearman correlation analysis because the data is not normally distributed or homogeneous. The following Table 4 shows the results of Spearman correlation analysis on green tea kombucha drinks.

Table 4. Spearman's Correlations Test on Green Tea Kombucha Beverage.

Variable		Day	Alcohol	pH	Inhibisi
1. Days	Spearman's rho	—			
	p-value	—			
2. Alcohol	Spearman's rho	0.101	—		
	p-value	0.524	—		
3. pH	Spearman's rho	-0.589	-0.571	—	
	p-value	< .001	< .001	—	
4. Inhibisi	Spearman's rho	0.637	-0.159	0.064	—
	p-value	< .001	0.315	0.687	—

The variables tested in the Spearman correlation analysis were % inhibition, pH value, % alcohol value, and fermentation time in green tea kombucha drink and green tea with rosela combination kombucha drink. However, it should be noted that many test results have p values that are only close to general significance or even insignificant. This is due to the small number of samples tested. Small sample sizes can have a significant impact on the reliability and reproducibility of research findings. This is due to reduced statistical power, which reduces the likelihood of detecting true impacts and increases the risk of overestimating the magnitude of impacts. Sample size plays a crucial role in achieving statistical significance by influencing the power of a study, accuracy of estimates, and reliability of research findings. A well-designed study with an appropriate sample size enhances the ability to detect true differences, control errors, and ensure validity (Gumpili & Das, 2022; Mishra, 2020). However, there is a common misconception among researchers that larger sample sizes always yield more reliable results, which is not necessarily the case (Sapra, 2022).

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The results of Spearman's correlation analysis on the variable between alcohol content and fermentation duration (days) showed a very weak positive correlation (0.101) between alcohol percentage and number of fermentation days. However, the high p value (0.524) indicates this relationship is not statistically significant. A positive correlation indicates that as one variable's value increases, the other variable's value tends also to increase, and vice versa. Thus, the correlation between alcohol content in kombucha and fermentation time suggests that more extended fermentation periods can lead to a significant increase in ethanol content (Talebi et al., 2017). This is consistent with the finding that fermentation duration affects the pH, total phenolic, and antioxidant activity of kombucha (Hapsari et al., 2021).

The results of the analysis on the variable pH value with the length of fermentation (days) showed a strong negative correlation (-0.589) between pH and the number of days of fermentation. The deficient p value (<0.001) indicates this relationship is highly statistically significant. With an increase in fermentation days, pH tends to decrease. This is in accordance with the study Hapsari et al. (2021) and Sinamo et al. (2022) both observed a decrease in pH with fermentation time. However, Vohra et al. (2019) noted that although the antioxidant activity of kombucha peaked on fermentation day 7, the activity decreased with a longer fermentation time. This suggests a potential trade-off between pH and antioxidant activity in kombucha.

The results of the analysis on the variable pH value with alcohol content showed that there was a strong negative correlation (-0.571) between pH and alcohol percentage. The deficient p-value (<0.001) indicates this relationship is highly statistically significant. With an increase in alcohol percentage, pH tends to decrease. The pH value and alcohol content of kombucha are closely related. As the fermentation process progresses, the pH value decreases due to the formation of acetic acid and gluconic acid, while the alcohol content increases (Agustiani et al., 2020). This relationship is further supported by research that has developed methods to accurately measure alcohol levels in kombucha, indicating the need for such methods due to the potential health risks associated with high alcohol levels (Chan et al., 2021; Liu et al., 2019) In addition, the temperature at which the fermentation process occurs can also affect the ethanol content in kombucha, with higher temperatures leading to higher alcohol levels (Vitas et al., 2021).

The results of the analysis on the variable of the percentage value of inhibition with the length of fermentation showed a strong positive correlation (0.637) between the percentage of inhibition and the number of days of fermentation. The deficient p-value (<0.001) indicates this relationship is highly statistically significant. The percentage inhibition tended to increase with an increase in the number of days of fermentation. The percentage of antioxidant inhibition in kombucha beverages with different fermentation lengths depends on the type of tea and carbon source used (Vohra et al., 2019). Fermentation time has a significant effect on the chemical characteristics and antioxidant activity of kombucha, with longer fermentation times generally producing higher antioxidant activity (Hapsari et al., 2021; Simanjuntak et al., 2016; Widyasari, 2016).

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The results of the analysis on the variable of percentage inhibition value and alcohol content showed a weak negative correlation (-0.159) between percentage inhibition and alcohol percentage. However, the high p-value (0.315) indicates this relationship is not statistically significant. This weak negative correlation is likely because the percentage of alcohol in kombucha drinks is generally low, with one study finding that the alcohol content was 0.055% (Riswanto & Rezaldi, 2021). This low alcohol content may not significantly correlate with the percentage of antioxidant inhibition in the beverage. However, the antioxidant activity of kombucha was found to be significantly higher than that of black tea (Ivanišová et al., 2020).

The results on the variable percentage inhibition with pH value showed a very weak positive correlation (0.064) between percentage inhibition and pH. However, the high p value (0.687) indicated this relationship was not statistically significant. This may also be because the antioxidant activity in kombucha beverages can decrease as in the study of Nasution & Nasution (2022), the antioxidant activity value of green tea kombucha decreased after day 15, as well as the antioxidant activity of rosella flower tea kombucha was high compared to tea leaves (decreased by 85% after fermentation for 10 days). So, it makes a positive but very weak correlation result, as in the graph that at a high pH at the beginning of the fermentation time, there are also high antioxidant levels because it has not decreased.

CONCLUSION

Based on the results of the study, it can be concluded that: 1) the antioxidant activity of kombucha combined green tea with rosella is higher than that of green tea kombucha and based on the results of the Mann-Whitney U Test the two kombuchas have significant differences; 2) the results of the Mann-Whitney U Test no significant differences were observed in terms of pH and % alcohol in green tea kombucha drinks and kombucha combined green tea with rosella, The pH value in both kombuchas decreased as the fermentation progressed, while the % alcohol of both kombuchas increased with the length of fermentation until the peak point on the eighth day after which the % alcohol decreased; and 3) The results of Spearman's correlation test, the correlation between % alcohol and the length of fermentation (days) showed a very weak positive correlation (0. 101). The result of the correlation test between pH value and fermentation duration (days) showed a strong negative correlation (-0.589). The result of the correlation test between pH value and % alcohol showed a strong negative correlation (-0.571). The correlation test result of % inhibition with fermentation duration showed a strong positive correlation (0.637). The correlation test result of % inhibition with % alcohol showed a weak negative correlation (-0.159). The correlation test result of % inhibition with pH value showed a very strong positive correlation (0.064).

ADVICE

Optimize the concentration data so that the IC50 value can be calculated. Further research should be carried out with larger samples to improve statistical quality results.

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REFERENCES

Agustiani, E., Susilaningrum, D., Rahmawati, A., L., F. Z., & R., D. L. (2020). Study the Effect of pH on the Fermentation Anaerobic-Aerobic Siwalan (*Borassus flabellifer* L.) Sap to Produce Acetic Acid. *Eksakta : Berkala Ilmiah Bidang MIPA, 21*(1), 29-35. https://doi.org/10.24036/eksakta/vol21-iss1/220

Amila, A., Sembiring, E., & Aryani, N. (2021). Deteksi Dini dan Pencegahan Penyakit Degeneratif pada Masyarakat Wilayah Mutiara *Home Care*. *Jurnal Kreativitas Pengabdian Kepada Masyarakat, 4*(1), 102-112. https://doi.org/10.33024/jkpm.v4i1.3441

Bishop, P., Pitts, E. R., Budner, D., & Witrick, K. A. T. (2022). Chemical Composition of Kombucha. *Beverages, 8*(3), 1-17. https://doi.org/10.3390/beverages8030045

Chan, M., Sy, H., Finley, J., Robertson, J., & Brown, P. N. (2021). Determination of Ethanol Content in Kombucha Using Headspace Gas Chromatography with Mass Spectrometry Detection: Single-Laboratory Validation. *Journal of AOAC International, 104*(1), 122-128. https://doi.org/10.1093/jaoacint/qsaa094

Etxebarria, N., Clark, B., Ross, M. L., Hui, T., Goecke, R., Rattray, B., & Burke, L. M. (2020). Quinine Ingestion During the Latter Stages of a 3,000-m Time Trial Fails to Improve Cycling Performance. *International Journal of Sport Nutrition and Exercise Metabolism, 31*(1), 9-12. https://doi.org/10.1123/ijsnem.2020-0265

Fadlilah, A. R., & Lestari, K. (2023). Review : Peran Antioksidan dalam Imunitas Tubuh. *Farmaka, 21*(2), 171-178. https://doi.org/10.24198/farmaka.v21i2.45929

Gumpili, S. P., & Das, A. V. (2022). Sample Size and its Evolution in Research. *IHOPE Journal of Ophthalmology, 1*(1), 9-13. https://doi.org/10.25259/ihopejo_3_2021

Hafsari, A. R., A, G. A., Farida, W. N., & S, M. A. (2021). Karakteristik pH Kultur Kombucha Teh Hitam dengan Jenis Gula Berbeda pada Fermentasi *Batch-Culture*. In *Prosiding Seminar Nasional Biologi 6* (pp. 228-232). Bandung, Indonesia: UIN Sunan Gunung Djati.

Hamka, Z. (2020). *Skrining* Antioksidan Ekstrak Buah Buni (*Antidesma bunius* (L) spreng.) Asal Kabupaten Enrekang dengan Metode Peredaman Radikal DPPH. *Jurnal Kesehatan Yamasi Makassar*, *4*(2), 1-8.

Hapsari, B. W., Manikharda., & Setyaningsih, W. (2021). Methodologies in the Analysis of Phenolic Compounds in Roselle (*Hibiscus sabdariffa* L.): Composition, Biological Activity, and Beneficial Effects on Human Health. *Horticulturae*, *7*(2), 1-41. https://doi.org/10.3390/horticulturae7020035

Hapsari, M., Rizkiprilisa, W., & Sari, A. (2021). Pengaruh Lama Fermentasi terhadap aktivitas antioksidan minuman fermentasi kombucha lengkuas merah (*Alpinia purpurata*). *Agromix*, *12*(2), 146-149. https://doi.org/10.35891/agx.v12i2.2647

Hassmy, N. P. (2017). Analisis Aktivitas Antioksidan pada Teh Hijau Kombucha berdasarkan Waktu Fermentasi yang Optimal. *Pharmacon*, *6*(4), 67-74. https://doi.org/10.35799/pha.6.2017.17719

Hidayah, M. A., Tunjungsari, G. P., Fikroh, R. A., Mashadi, H., Handayani, T., & Nurohmah, B. A. (2022). Antihyperuricemia Potential in Ethanol Extract of Siamese Bamboo (*Thyrsostachys siamensis*) Leaves on Male White House Mice (*Mus musculus* L.). *Walisongo Journal of Chemistry*, *5*(2), 120-129. https://doi.org/10.21580/wjc.v5i2.10666

Ivanišová, E., Meňhartová, K., Terentjeva, M., Harangozo, Ľ., Kántor, A., & Kačániová, M. (2020). The Evaluation of Chemical, Antioxidant, Antimicrobial and Sensory Properties of Kombucha Tea Beverage. *Journal of Food Science and Technology*, *57*(5), 1840-1846. https://doi.org/10.1007/s13197-019-04217-3

Jakubczyk, K., Kalduńska, J., Dec, K., Kawczuga, D., & Janda, K. (2020). Antioxidant Properties of Small-Molecule Non-Enzymatic Compounds. *Polski Merkuriusz Lekarski: Organ Polskiego Towarzystwa Lekarskiego*, *48*(1), 128-132.

Kapp, J. M., & Sumner, W. (2019). Kombucha: A Systematic Review of the Empirical Evidence of Human Health Benefit. *Annals of Epidemiology*, *30*(1), 66-70. https://doi.org/10.1016/j.annepidem.2018.11.001

Khaerah, A., & Akbar, F. (2019). Aktivitas Antioksidan Teh Kombucha dari Beberapa Varian Teh yang Berbeda. In *Prosiding Seminar Nasional LP2M UNM* (pp. 472-476). Makassar, Indonesia: Universitas Negeri Makassar.

Khamidah, A., & Antarlina, S. (2020). Peluang Minuman Kombucha sebagai Pangan Fungsional. *Agrika : Jurnal Ilmu-ilmu Pertanian*, *14*(2), 184-200. https://doi.org/10.31328/ja.v14i2.1753

Kuncoro, G. C. A. (2019). Pengaruh Waktu Lama Fermentasi terhadap Aktivitas Antioksidan dan Karakteristik Fisik Teh Kombucha Daun Ginseng Jawa (*Talinum paniculatum*). *Skripsi*. Universitas Sanata Dharma Yogyakarta.

Liu, Y., Chan, M., Blake, E., Sy, H., & Brown, P. N. (2019). Determination of Ethanol Content in Kombucha Products by Gas Chromatography with Flame Ionization Detection: A Multilaboratory Study. *Journal of AOAC International*, *102*(3), 878-882. https://doi.org/10.5740/jaoacint.18-0190

Luyt, N. A., Beaufort, S., Divol, B., Setati, M. E., Taillandier, P., & Bauer, F. F. (2021). Phenotypic characterization of cell-to-cell interactions between two yeast species during alcoholic fermentation. *World Journal of Microbiology and Biotechnology*, *37*(1), 186-196. https://doi.org/10.1007/s11274-021-03154-8

Mishra, P. (2020). The Importance of Sample Size in Research Studies. *Journal of Health & Medical Economics*, *6*(2), 1-1.

Nasution, I. W., & Nasution, N. H. (2022). Peluang Minuman Teh Kombucha dan Potensinya sebagai Minuman Kesehatan Pencegah dan Penyembuh Aneka Penyakit. *Journal of Comprehensive Science*, *1*(1), 9-16. https://doi.org/10.59188/jcs.v1i1.2

Pekkarinen, S. S., Stöckmann, H., Schwarz, K., Heinonen, I. M., & Hopia, A. I. (1999). Antioxidant Activity and Partitioning of Phenolic Acids in Bulk and Emulsified Methyl Linoleate. *Journal of Agricultural and Food Chemistry*, *47*(8), 3036-3043. https://doi.org/10.1021/jf9813236

Rahayu, E. S., & Abram, P. H. (2023). Pengaruh Variasi Waktu Fermentasi terhadap Aktivitas Antioksidan pada Minuman Kombucha Pulpa Kakao (*Theobroma cacao* L). *Media Eksakta*, *19*(1), 1-8. https://doi.org/10.22487/me.v19i1.2405

Rajma, F., Nahariah, N., & Maruddin, F. (2016). Optimasi Antioksidan dengan Lama Fermentasi yang Berbeda pada Telur Infertil Sisa Hasil Penetasan. *Jurnal Ilmu dan Teknologi Peternakan*, *5*(1), 58-65. https://doi.org/10.20956/jitp.v5i1.1530

Riskesdas. (2018). *Laporan Nasional Riskesdas 2018*. Jakarta: Kementrian Kesehatan Republik Indonesia.

Riswanto, D., & Rezaldi, F. (2021). Kombucha Tea: A Study on the Halal of Fermented Drinks. *International Journal Mathla'ul Anwar of Halal Issues*, *1*(2), 71-77. https://doi.org/10.30653/ijma.202112.28

Safnowandi, S. (2022). Pemanfaatan Vitamin C Alami sebagai Antioksidan pada Tubuh Manusia. *Biocaster : Jurnal Kajian Biologi*, *2*(1), 6-13. https://doi.org/10.36312/bjkb.v2i1.43

Sandi, S., Pratama, A. N. T., Sahara, E., Yosi, F., Sari, M. L., & Nurdin, A. S. (2023). Pengaruh Lama Fermentasi terhadap pH, Total Asam, dan Amonia Ampas Jus Limbah Sayur sebagai Pakan. *Jurnal Ilmu Peternakan Terapan*, *6*(2), 51-57. https://doi.org/10.25047/jipt.v6i2.3640

Sapra, R. L. (2022). How to Calculate an Adequate Sample Size? In Nundy, S., Kakar, A., & Bhutta, Z.A. (2021). *How to Practice Academic Medicine and Publish from Developing Countries?* (pp. 81-93). The Gateway: Springer Nature Singapore.

Simanjuntak, D. H., Herpandi, H., & Lestari, S. D. (2016). Karakteristik Kimia dan Aktivitas Antioksidan Kombucha dari Tumbuhan Apu-apu (*Pistia stratiotes*) selama Fermentasi. *Jurnal Fishtech*, *5*(2), 123-133. https://doi.org/10.36706/fishtech.v5i2.3940

Sinamo, K. N., Ginting, S., & Pratama, S. (2022). Effect of Sugar Concentration and Fermentation Time on Secang Kombucha Drink. *IOP Conference*

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Sintyadewi, P. R., RS, I. G. A. Y. R., & Wulansari, N. T. (2021). Analysis of Chemical Characteristics and Antioxidant Activity Test of Kombucha Black Tea and Butterfly Pea Flower (*Clitoria ternatea* L.) Based on Fermentation Time. *International Journal of Chemical and Material Sciences*, *4*(1), 27-32. https://doi.org/10.31295/ijcms.v4n1.1768

Sulistiawaty, L., & Solihat, I. (2022). Kombucha: Fisikokimia dan Studi Kritis Tingkat Kehalalan. *Jurnal Warta Akab*, *46*(1), 21-27. https://doi.org/10.55075/wa.v46i1.80

Talebi, M., Frink, L. A., Patil, R. A., & Armstrong, D. W. (2017). Examination of the Varied and Changing Ethanol Content of Commercial Kombucha Products. *Food Analytical Methods*, *10*(12), 4062-4067. https://doi.org/10.1007/s12161-017-0980-5

Vitas, J., Banjac, M. K., Kovačević, S., Vukmanović, S., Jevrić, L., Malbaša, R., & Kuzmanović, S. P. (2021). Chemometric Approach to Quality Characterization of Milk-Based Kombucha Beverages. *Mljekarstvo : Časopis Za Unaprjedjenje Proizvodnje i Prerade Mlijeka, 71*(2), 83-94. https://doi.org/10.15567/mljekarstvo.2021.0201

Vohra, B. M., Fazry, S., Sairi, F., & Airianah, O. B. (2019). Effects of Medium Variation and Fermentation Time on The Antioxidant and Antimicrobial Properties of Kombucha. *Malaysian Journal of Fundamental and Applied Sciences*, *15*(2), 298-302. https://doi.org/10.11113/mjfas.v15n2-1.1536

Wahyudi, H., Mustofa, A., & Widanti, Y. A. (2018). Aktivitas Antioksidan Teh Daun Kelor (*Moringa oliefera*)-Rosela (*Hibiscus sabdariffa* L) dengan Variasi Lama Pengeringan. *JITIPARI : Jurnal Ilmiah Teknologi dan Industri Pangan UNISRI*, *3*(2), 106-112. https://doi.org/10.33061/jitipari.v3i2.2692

Widyasari, A. (2016). Aktivitas Antioksidan dan Organoleptik Kombucha Daun Kelor dengan Lama Fermentasi dan Konsentrasi Daun Kelor yang Berbeda. *Thesis*. Universitas Muhammadiyah Surakarta.

Williams, W. B., Cuvelier, M. E., & Berset, C. (1995). Use of A Free Radical Method to Evaluate Antioxidant Activity. *LWT - Food Science and Technology*, *28*(1), 25-30. https://doi.org/10.1016/S0023-6438(95)80008-5

Winandari, O. P., Widiani, N., Kamelia, M., & Rizki, E. P. (2022). Potential of Vitamin C and Total Acid as Antioxidants of Rosella Kombucha with Different Fermentation Times. *Jurnal Pembelajaran dan Biologi Nukleus, 8*(1), 141-148. https://doi.org/10.36987/jpbn.v8i1.2471

Zou, C., Li, R. Y., Chen, J. X., Wang, F., Gao, Y., Fu, Y. Q., Xu, Y. Q., & Yin, J. F. (2021). Zijuan Tea-Based Kombucha: Physicochemical, Sensorial, and Antioxidant Profile. *Food Chemistry*, *363*(1), 1-20. https://doi.org/10.1016/j.foodchem.2021.130322

Zubaidah, E., Effendi, F. D., & Afgani, C. A. (2022). *Kombucha: Mikrobiologi, Teknologi, dan Manfaat Kesehatan*. Malang: Universitas Brawijaya Press.