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Antioxidant Activity and Consumer Acceptance Level of Kombucha Tea With Noni Fruit Extract (*Morinda citrifolia*)

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Abstract. This study aimed to investigate the impact of fermentation time and the best formulation of black tea and noni fruit juice on the production kombucha with the highest hedonic value and antioxidant activity. The research method involved (1) preparing kombucha tea, (2) assessing antioxidant activity, Total Solids value, pH value of the sample, and (3) conducting statistical analysis using One-Way ANOVA followed by Tukey's Post Hoc Test at a confidence level $\alpha = 5\%$ and T test. The addition of noni juice was found to increase the antioxidant activity in kombucha; however, it reduced the overall hedonic value of kombucha. In conclusion, this study determined that sample P2 (200 mL of noni juice in 800 mL of black tea) was the best formulation. Statistical tests revealed that both fermentation time and formulation P2 significantly influenced the increase in antioxidant activity, reaching 41.20%. Additionally, the highest hedonic score was achieved by the control sample fermented for 7 days, which scored to 6.68%.

Keywords: antioxidant activity, kombucha tea, noni fruit

1. Introduction

Based on the latest assessment, the Global Burden of diseases, Injuries, and Risk Factors Study (2019), estimates that 8 million deaths were attributable to dietary risk factors. The adoption of healthy diets has the potential to significantly decrease premature fatalities, all while staying within the boundaries of a sustainable global ecosystem [1]. As time goes by, public awareness of the importance of maintaining a healthy body condition is increasing, especially in Indonesia [2]. Tea is a functional beverage that is beneficial for the body. According to 2018 data, tea consumption in Indonesia society by 4%, reaching 105 thousand tons or around 75% of the total national tea production [3]. The benefits of consuming tea include cancer prevention, richness in vitamins C and B, antioxidant properties, and its refreshing nature [4]. Various innovations can be applied to enhance the nutritional value of tea, and one such innovation is the fermentation process to create kombucha tea [5].

Kombucha tea is a fermented beverage that utilizes the symbiotic relationship between yeast and bacteria. To make kombucha tea, black tea can be used, which contains numerous compounds such as antioxidants, vitamin C, phenolic acid, kauramic acid, methylxanthines (caffeine), more. The fermentation process in making black tea kombucha can increase the antioxidant activity and vitamin C in the tea [6]. Functional fruit can be added to extract their juice to further optimize the antioxidant and nutritional activity of black tea kombucha [7]. Noni fruit a high-antioxidant functional fruit, offers various advantages, including antitumor, anthelmintic, analgesic,



hypotensive, anti-inflammatory, immune system enhancement, cholesterol level regulation, antihypertensive properties (scopoletin substances that help prevent plaque formation or atherosclerosis), and blood pressure reduction [8].

Therefore, this research aims to develop kombucha tea products with the addition of noni juice. We hope that the results of this research will not only enhance the nutritional content of kombucha tea but also have the potential to support the realization of the third Sustainable Development Goal, which focuses on ensuring healthy lives and promoting well-being across all age groups, starting from adopting a healthy life by consuming functional drinks.

2. Materials and Method

The equipments used in this research included spoon, stove, ladle, pot, large glass jar, plastic cup, sieve, watch glass, spatula, measuring flask, beaker, blender, measuring cup, measuring pipette, dropper pipette, pH meter, test tube, bulb, micropipette, micropipette tip, falcon tube, digital balance, oven, timer, vortex, spectrophotometer and centrifuge. Materials used in this research are 150 g black tea, 8 L mineral water, 1 kg sugar for kombucha fermentation, 1200 mL noni juice, 600 g sugar to be added to samples P1, P2, and P3, SCOBY (Symbiotic Culture of Bacteria and Yeast), distilled water, 80% methanol, and 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution. This research was done at Cibubur dan PT Indolakto, Jl. Raya Bogor Km. 26,6 Jakarta 13710 from January to April 2023.

2.1 Formula Determination

This research design is a Completely Randomized Design (CRD) with the addition of noni juice extract with 4 treatments as much as 0 mL, 100 mL, 200 mL, and 300 mL. For hedonic test, 100 grams of sugar was added to each sample.

2.2 Sample Production

Based on the results of the previous analysis, noni juice extract was produced by starting with 2 kg of noni fruit, which was washed and reduced in size to facilitate the crushing process. Then, 550 mL of water was added. The brewed black tea was subsequently mixed with the noni juice extract.

Table 1. Amount of noni extract added

	Control (mL)	P1 (mL)	P2 (mL)	P3 (mL)
Black tea	1000	900	800	700
Noni extract	0	100	200	300

The kombucha was divided into four containers: one for control and the others were mixed with noni extract. Each sample was fermented for seven days, with daily pH value checks using a pH meter. The sample production was conducted twice.

2.3 Antioxidant analysis

Antioxidant activity analysis was performed using the DPPH method. The analysis started with the preparation of a 0.1 mM DPPH solution. Subsequently, 2 mL of each sample solution was mixed with 2 mL of the 0.1 mM DPPH solution and left in a dark place for 30 minutes. The absorbance of both the sample and control solutions was then measured at a wavelength of 517 nm using a Thermo Scientific UV-Vis Spectrophotometer. This analysis was conducted in duplicate. The % antioxidant activity value can be calculated using the following equation:

$$\% \text{ antioxidant activity} = 1 - \frac{\text{sample absorbance}}{\text{control absorbance}} \times 100\%$$

2.4 Analysis of TS value

The total solids testing involved weighing the kombucha sample, drying the sample, and weighing the residue of the kombucha sample. Samples were predried for 25 minutes in a steam bath and

then further dried in a Shel Lab Forced Air Oven at $1000 \pm 10^\circ\text{C}$. Total solids analysis was conducted in duplicate. The total solids content represents the weight of the remaining kombucha sample as a percentage of the initial kombucha sample weight. % Total solids can be calculated using the following equation:

$$\text{Total Solid\%} = \frac{\text{remaining sample weight}}{\text{initial sample weight}} \times 100\%$$

2.5 Sensory evaluation

A total of 40 panelists who had an interest in kombucha tea products or health drinks and were in the age range of 18 to 50 years and residing in Jabodetabek participated in the study. These panelists conducted direct sample tests and were subsequently asked to provide preference scores for five test attributes: color, aroma, taste, texture, and overall impression. The assessment was made on a hedonic scale ranging from 1 to 9, with the following criteria: (1) dislike extremely (2) dislike very much, (3) dislike moderately, (4) dislike slightly, (5) neither like nor dislike, (6) like slightly, (7) like moderately, (8) like very much, and (9) like extremely. Panelists filled out the evaluation forms online using Google Forms.

2.6 Statistical analysis

The data obtained from the hedonic results, DPPH test, and total solids test will be analyzed using the SPSS method, employing One-Way ANOVA, followed by the Tukey follow-up test at a confidence level of $\alpha = 5\%$. A statistically significant difference will be indicated by the T-test results.

3. Result and Discussion

3.1 Antioxidant activities

Based on the results of the antioxidant analysis using DPPH on the samples (Figure 1), it is evident that the antioxidant activity in the kombucha tea samples increased with the increasing concentration of noni juice. The results indicate significant differences between control, P2, and P3 samples, whereas the Control sample did not significantly differ from the P1 sample. Therefore, based on the test results, it can be concluded that the addition of 200 mL and 300 mL of noni juice with a fermentation time of 7 days had a significant effect on increasing the antioxidant activity of kombucha. The DPPH test measures antioxidant activity by involving the oxidation and reduction process of antioxidant compounds, such as phenols produced in the fermentation process by SCOBY microorganisms, with DPPH compounds [9]. A fermentation time of 7 days is more optimal for increasing antioxidant activity because drinks fermented for more than 10 days are at risk of damage due to organic acid fertilization. The increase in antioxidant activity in kombucha tea is a result of the metabolic processes of microorganisms during the fermentation process. The addition of noni fruit extract, in fact, can enhance the antioxidant activity of kombucha. This is because noni fruit contains ascorbic acid compounds that have the potential to act as very strong antioxidants. These compounds are capable of donating hydrogen atoms to free radicals, and their activity can be measured using the DPPH method. Ascorbic acid in noni fruit can capture free radicals and prevent the occurrence of a chain reaction [10].

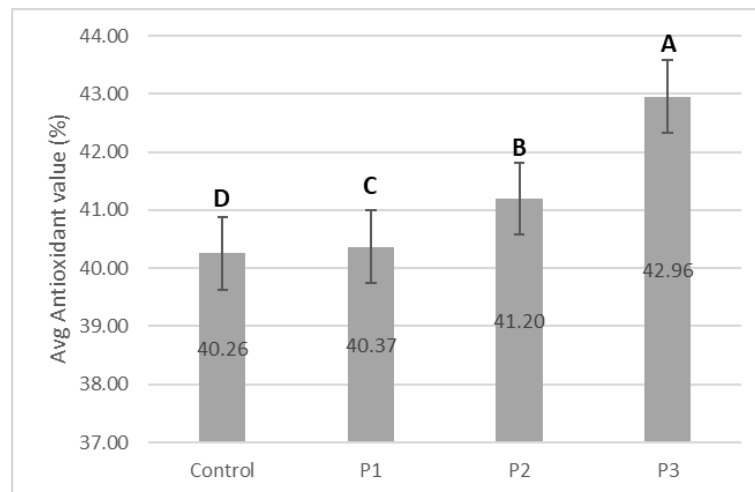


Figure 1. Effect of noni extract concentration on antioxidant activity

3.2 Total Solid

Based on the result of Total Solid (Figure 2), the highest % of total solids was found in the control kombucha sample with a fermentation time of 7 days. A statistical test was then performed to determine significant differences between samples. It can be concluded that, with a fermentation time of 7 days, the addition of 100 mL, 200 mL, and 300 mL of noni juice has a significant effect on changes in total solids in kombucha. Total dissolved solids measure the number of compounds or molecules dissolved in a solution. The decrease that occurred in the sample could be attributed to the activity of microorganisms in the SCOBY, which break down dissolved sugars in the tea solution and noni extract for metabolism and growth. *Saccharomyces cerevisiae* is believed to be a microbe that degrades sugar into ethanol, carbon dioxide, and simple sugar (glucose). Ethanol is then metabolized and oxidized to acetic acid, carbon dioxide evaporates, and glucose and fructose are used as energy sources in microbial metabolic activities to form a new layer or nata in kombucha [11]

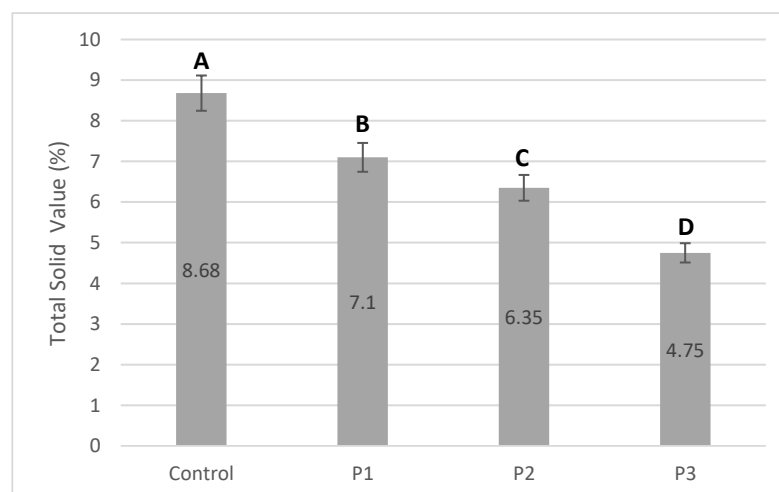


Figure 2. Effect of noni extract concentration on total solid value

3.3 pH

Based on the results of the pH value test (Figure 3), the highest pH value was found in the control sample with a fermentation time of 7 days. A statistical test was conducted to determine significant differences between samples. It can be concluded that, with a fermentation time of 7 days, the addition of noni juice did not have a significant effect on changes in pH value in kombucha. The decrease in the pH value is influenced by the maturation of *Acetobacter xylinum*, which synthesizes sugar into cellulose, resulting in the formation of acetic acid that reduces the pH value to 2. The decrease in the pH of the kombucha drink is thought to be caused by an increase in the concentration of acidic substances during the fermentation process. Acids dissolved in the solution release protons, causing a decrease in pH. Sucrose is synthesized into organic acids (e.g., acetic acid and gluconic acid) by yeast and bacteria during the fermentation process. An increase in the concentration of organic acids results in a decrease in the pH of the fermentation medium [12].

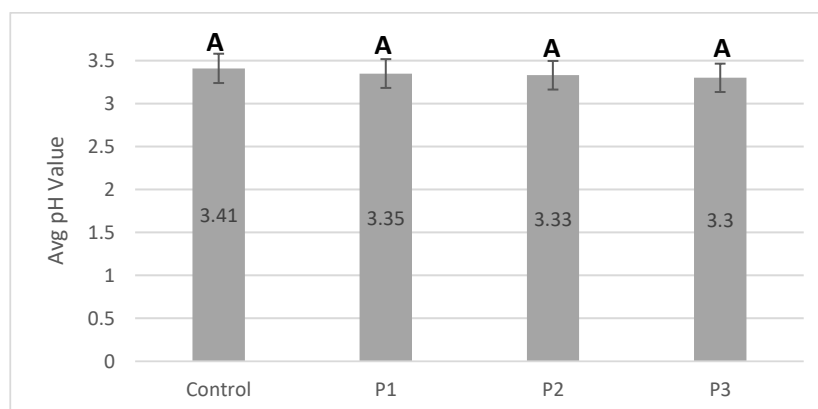


Figure 3. Effect of noni extract concentration on pH value

3.4 Sensory Evaluation

The parameters used in this organoleptic test involve the panelists' preference for the color, aroma, taste, aftertaste, and overall taste of kombucha tea with noni juice extract. Overall, in the hedonic test (see Figure 4), the addition of noni juice caused a decrease in the panelists' acceptance of kombucha tea. This is evidenced by the value of the P3 sample with a fermentation time of 7 days, which significantly differs from the other treatments. Organoleptic tests rely on human senses, including sight, smell, and taste [13]. Noni juice has a cloudy brown color, and as more noni juice is added, the intensity of the brown color becomes stronger. One factor influencing this is the vitamin C browning reaction followed by the Maillard reaction, causing the color of kombucha tea with noni fruit extract to darken with longer fermentation times [14]. Additionally, kombucha tea has a sharp aroma due to its acetic acid content [15].

Table 2. Effect of noni fruit extract on sensory attributes

	Color	Aroma	Taste	Aftertaste	Overall
C	6.78 ± 1.60 a	5.15 ± 1.68 a	6.63 ± 1.61 a	6.68 ± 1.44 a	6.68 ± 1.12 a
P1	6.68 ± 1.44 a	5.45 ± 1.81 a	5.83 ± 1.97 ab	5.98 ± 1.99 a	6.00 ± 1.92 ab
P2	5.98 ± 1.60 a	5.73 ± 1.90 a	6.45 ± 1.96 ab	6.58 ± 1.57 a	6.48 ± 1.52 ab
P3	4.90 ± 1.79 b	4.93 ± 1.69 a	5.475 ± 1.76 b	5.73 ± 1.60 a	5.70 ± 1.67 b

4. Conclusion

The test results indicate that the best combination of black tea and noni juice formulation was sample P2 (200 mL of noni juice in 800 mL of black tea), resulting in a significant increase in antioxidant activity (41.20%). Even though the antioxidant activity of P2 is not the highest, it was still chosen as the best formulation because it is supported by the panelists' preference, which is

not significantly different from the control, which is the sample with the highest preference. In contrast, sample P3 has the highest antioxidant activity, but its hedonic value is significantly different from the control. Sample P2 yielded a rating of 6.48 (like moderately) on a hedonic scale of 1-9.

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