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THE EFFECT OF FERMENTATION TIME ON ANTIOXIDANT AND ORGANOLEPTIC ACTIVITIES OF BIDARA (*Zizipus spina* CRISTI L.) KOMBUCHA DRINK

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ABSTRACT

Bidara leaves contain antioxidants that are good for the body because they play a role in warding off free radicals. Kombucha made with bidara leaves is an innovative tea variant product that comes from the symbiotic culture of fermentation between bacteria and fungi. The purpose of this study is to determine the effect of fermentation time on antioxidant and organoleptic activity of kombucha and to determine the value of antioxidant activity of kombucha. The parameters measured were the antioxidant activity using the DPPH (2,2-diphenyl-picrilhydrazine) radical method and the organoleptic test with the hedonic scale including taste, aroma and color. The analysis showed that the length of fermentation time affected both the antioxidant activity and organoleptic test which included the taste, aroma and color of kombucha leaves of bidara. Based on the mean value of DPPH radical scavenging percentage, the antioxidant activity at fermentation time of 1, 8 and 12 days are 24,72%; 35,98%; and 7,05% with the highest antioxidant activity of kombucha made with bidara leaves occurred at 8 days fermentation is 35.87%. The IC50 value of kombucha made with bidara leaves product at fermentation time of 1, 8 and 12 days are 524,63 ppm; 441,03 ppm; and 1442,3 ppm.

Keywords: Antioxidant Activity, Kombucha, Leaf of Bidara, Organoleptic.

PENDAHULUAN

Kombucha is a fermented beverage derived from a consortium of bacteria and yeast as the initial culture or better known as SCOBY. The chemical characteristics of the fermented drink are enough to make the body healthy because it contains organic acids, minerals, various vitamins derived from tea, various amino acids, and bioactive compounds

that act as sources of polyphenols. [1]. Kombucha is generally made with the basic ingredients of black tea or green tea accompanied by sugar. However, there have also been many research results using other basic ingredients such as leaves, flowers, fruit which have high sources of antioxidants and high sources of secondary metabolites such as tannins. Bidara leaves are one of the plants that have a high source of antioxidants and tannins.

Antioxidants are substances that function as inhibitors of oxidation reactions caused by the presence of free radicals, causing various damage to both cells and body tissues. Damage that occurs at the cellular level to molecules including cell wall membranes, and genetic material, especially DNA that will cause a mutation towards cancer or other degenerative diseases. A high source of antioxidants can potentially inhibit oxidation reactions caused by free radicals so that they become neutral when exposed to them [2].

The process of making kombucha derived from ingredients sourced with high antioxidants can affect the quality of kombucha both from the concentration of raw materials and the length of time for fermentation. The results of research conducted by Widyasari (2016) stated that the highest antioxidant activity made from Moringa leaves was in the 30 g treatment with 8 days of fermentation. The highest organoleptic quality is found in Moringa leaves 20 g with a fermentation time of 4 days and has a brown color, aroma and slightly sour taste [3].

The results of other studies that have been carried out by Falahudin et al., (2017) state that there is a very significant effect on the fermentation process and fermentation time and also the use of different levels of soursop tea leaves on vitamin C levels carried out by kombucha fermentation. The higher the duration of fermentation and the higher the levels of soursop tea leaves used, the levels of vitamin C will increase, so that the highest levels of vitamin C are found in samples with 12 days of treatment and a sample weight of 60 g [4].

A similar study conducted by Kuncoro (2019), stated that the length of time for kombucha fermentation of Javanese ginseng leaves had a

significant effect on antioxidant activity and did not significantly affect physical characteristics. The highest antioxidant activity was found at the 8th day of fermentation [5].

The availability of natural raw materials that have the potential to make kombucha because it is efficacious as a source of high antioxidants. Materials that have the potential to make kombucha include dates, dragon fruit, mango fruit, ginger, as well as those with high potential as a source of antioxidants such as rosella flowers, telang flowers, kecombrang flowers, and bidara leaves. In this study, we will use bidara leaves because bidara leaves have the potential as a source of antioxidants. This statement has been supported by the results of research conducted by Saminara et al (2017), which states that the ethanol extract of bidara leaves has antioxidant activity with an IC_{50} value of 59.52 + 1.2 g/mL compared to Vitamin C with an IC_{50} value of 2, 73+0.05 g/mL [6].

Bidara plants, especially on the leaves, have been empirically known to contain betulinic, seanotic acid, and various flavonoids and triterpenoids. The results of research conducted by Kusriani et al., (2015) stated that the ethanolic extract of bidara leaves contains alkaloids, flavonoids, saponins, tannins, quinones, and triterpenoids. The results of this study also found that bidara leaves have the best antioxidant activity with an IC_{50} value of 127.87 ppm [7].

Apart from being efficacious as a source of antioxidants, bidara leaves have a fairly low cytotoxic effect. The results of research conducted by Jafarian et al (2014) stated that bidara leaves have a fairly low cytotoxic effect based on the IC_{50} value of 0.02 mg/mL. The descriptions regarding the potential of kombucha and bidara leaves as a source of antioxidants made us interested researchers. to conduct research on the length of time for kombucha fermentation of bidara leaves on antioxidant and organoleptic activity.

METHOD

1. Research Time and Place

This research has been carried out at the Laboratory of the Chemical Analyst School (STAK) Cilegon for 3 months, from January to March 2021.

2. Research and Material Tools

The materials used in this study include bidara leaves from the Cilegon area. Sugar, water, methanol, kombucha starter, DPPH, and aquadest.

The tools used in this study were panic, hot plate, analytical balance, 500 mL glass bottle, spoon, rubber band, UV-Vis spectrophotometer, 50 mL and 100 mL volumetric flask, test tube, and 100 mL beaker glass.

3. Work Procedures

The stages of work in this study were drying bidara leaves and sterilizing tools, making bidara leaf solution, fermenting kombucha bidara leaves, and testing antioxidant activity which consisted of stages of making 0.1 mM DPPH solution, making standard solutions, making standard solutions, and measuring sample. Kombucha dau bidara organoleptic test.

a. Bidara Leaf Drying and Equipment Sterilization:

Wash the bidara leaves then drain in the shade until completely dry. Prepare 3 glass bottles, pans. Put in the glass bottle after the water boils for 15 minutes. Drain, and let stand before use [3].

b. Preparation of Bidara Leaf Solution:

Prepare 10 grams of dried bidara leaves and boil 300 mL until it boils and add bidara leaves for 5 minutes. Strain the boiled water from the bidara leaves and add sugar as much as 20% and 60 grams [3].

c. Kombucha Fermentation Process Of Bidara Leaves

Place the decoction of bidara leaves in each glass bottle, wait until it is lukewarm or cold then add 20% kombucha starter and 60 mL of the volume of the bottle's contents. Cover the top of the bottle with a cloth and tie the cloth with a rubber band. Store at room temperature to observe the fermentation results for 1 day, 8 days, and 12 days. Condition the bottles during fermentation to avoid being moved, bumped, and exposed to direct sunlight.

d. Antioxidant Activity Testing

Testing of antioxidant activity consists of 4 stages including:

Preparation of 0.1 mM . DPPH Solution

The antioxidant activity of bidara leaf kombucha was tested using the DPPH method. DPPH powder with a molecular weight of 394.32 as much as 10 mg was dissolved with 62.5 mL of methanol and then put into a 100 mL volumetric flask as a producer of DPPH solution with a concentration of 0.5 mM and diluted until the concentration of the DPPH solution became 0.1 mM.

Preparation of Standard Solution

Weigh 0.05 grams of kombucha, dissolve it with methanol, put it in a 50 mL volumetric flask and add methanol to the mark.

Preparation of Standard Solution

Prepare standard solutions with each concentration of 0; 50; 100; 150; 200; and 250 ppm. Standard solution pipette as much as 0; 0.5; 1; 1.5; 2; and 2.5 mL. Put it in a 10 mL volumetric flask, and add methanol to threshold.

Measurement of Absorption with UV-Vis Spectrophotometer

Pipette 1.2 mL of each standard solution, put it in a test tube. Add 1 mL of DPPH solution. Homogenize. Incubate at room temperature for 30 minutes, then measure the absorption at a wavelength of 515 nm.

e. Kombucha Bidara Leaf Organoleptic Test

The organoleptic test and the acceptability of bidara leaf kombucha were carried out by 20 panelists. Panelists will be asked to observe, smell, taste and give an assessment of the research results.

f. Data Analysis Technique

Data collection techniques and instruments were experimental methods, antioxidant activity tests, and organoleptic tests. The analysis technique uses qualitative descriptive and quantitative description [3]. The results of antioxidant activity were calculated by presentation using the formula:

$$\% \text{ Antioxidant Activity} = \frac{a\ balangko - a\ sampel}{a\ balangko} X\ 100\% \ldots\ldots\ldots$$

.[1].

HASIL DAN PEMBAHASAN

1. Sampling

The sample used in this study was bidara leaves obtained from bidara trees in the Tegal Cabe Environment, Citengkil District, Cilegon City, Banten Province.

2. Preparation of Bidara Leaf Samples and Equipment Sterilization

Kombucha is a traditional beverage product from fermented tea and sugar solution which has a distinctive taste and aroma, namely sweet-sour taste, contains various vitamins and minerals as well as organic acids. Kombucha is efficacious to aid digestion, lower cholesterol, lose weight, stabilize blood glucose levels, and help boost the immune system. Besides that, Kombucha is also efficacious as a probiotic drink that is rich as a source of antioxidants. The source of antioxidants found in kombucha comes from compounds found in the basic ingredients of kombucha.

In this study, the tea leaves used were replaced with bidara leaves. Bidara leaf tea was fermented until the 12th day with a fermentation time of 1 day, 8 days and 12 days. The parameters measured in this study were the antioxidant and organoleptic activity of kombucha bidara leaves.

The preparatory stage in making bidara leaf kombucha is washing the bidara leaves, draining and placing in a shady place until the bidara leaves to be used are completely dry. The tools that will be used in making kombucha bidara leaves must be sterilized first by putting a glass bottle into boiling water for 10 to 15 minutes. Then drained, and let stand for a while before use.

3. Preparation of Bidara Leaf Solution

As an ingredient in making kombucha, 10 g of dried bidara leaves are prepared. Then boil for +5 minutes, after that the decoction of the bidara leaves is filtered and added sugar. Prior to fermentation to obtain kombucha, the antioxidant activity was tested in the bidara leaf solution with the results presented in Table 1.

Tabel 1. Aktivitas Antioksidan Larutan Daun Bidara

Sample	Concentration	I	Abs II	Average	% Inhibition
Control (Bidara Solution)	0	0,958	0,954	0,956	0
	50	0,892	0,889	0,891	6,85
	100	0,829	0,825	0,827	13,49
	150	0,816	0,810	0,813	14,96
	200	0,792	0,790	0,791	17,26
	250	0,761	0,757	0,759	20,61

To see the statistical differences in the antioxidant activity values of several concentrations of bidara solution, the data in Table 1 is converted into a graphic form as shown in Figure1.

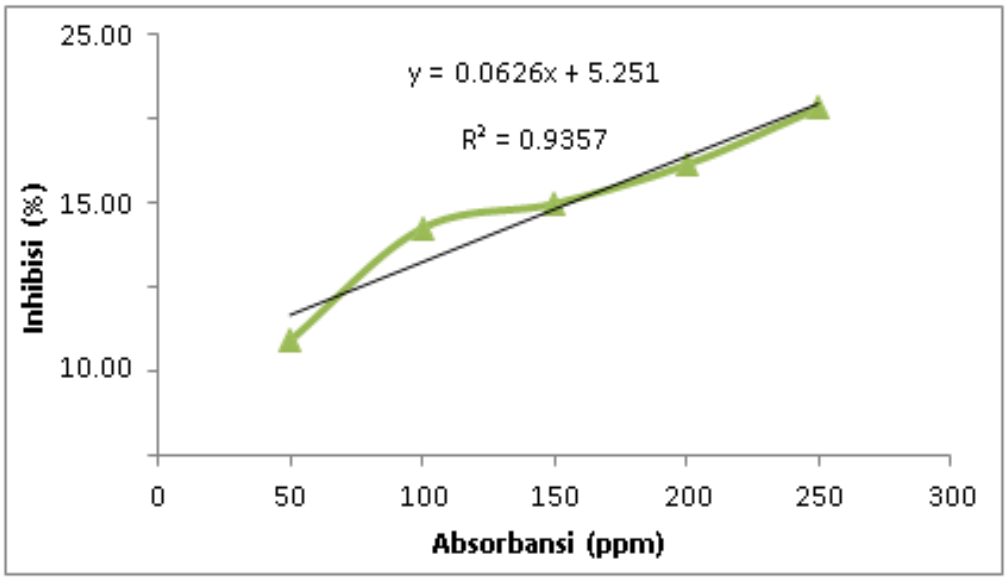


Figure 1. Inhibition Value of Bidara Solution

Figure 1 it can be seen that the bidara leaf solution contains phenol which can be used as an antioxidant. The correlation curve between the concentration of bidara solution and the percentage of inhibition was made to calculate the IC50 value based on the regression equation obtained by entering the number 50 in the equation (y = 50) and the linear regression line equation y = 0.0624x + 5.254 was obtained. The IC_{50} value obtained is 714.84 ppm and this value can still

increase after the bidara leaf solution is fermented.

3. Kombucha Fermentation Process of Bidara Leaves

In the fermentation process, there is an overhaul with the breakdown of sucrose into glucose and fructose. Then, there is the breakdown of glucose and fructose into organic acids and alcohol continuously until the sugar contained in the kombucha solution runs out. So that the acid produced will continue to increase during the longer fermentation time.

In the fermentation process occurs the breakdown of carbohydrates, amino acids and fats which can produce organic acids, carbon dioxide and other substances. The fermentation process can cause changes in the physical and chemical properties of foodstuffs which include alcohol content, total acid and pH. The longer kombucha fermentation will produce high acidity. Fermentation (the conversion of glucose to alcohol and carbon dioxide) occurs through the following reactions:

$$C6H12O6 \rightarrow 2C2H5OH + 2CO2$$

$(Glucose)$ $(Ethanol)$ $(Carbon\ Dioxide)$

In the fermentation process, the kombucha starter used produces alcohol anaerobically, then alcohol stimulates the growth of Acetobacter xylinum to produce acetic acid aerobically, while acetic acid stimulates the growth of the starter. This continues until the sugar in the kombucha solution turns into organic acids needed by the body such as acetic acid and others. Kombucha starter culture is called "kombu mushroom" or "dipo mushroom" or in foreign terms it is called SCOBY (Symbiotic Culture of Bacterial and Yeast) which contains bacteria and yeast. The kombucha starter used in this study was the yeast Saccharomyces cerevisiae. This yeast can produce 70% organic acids such as acetic acid, malic acid, succinic acid and pyruvic acid during the fermentation process.

During the kombucha fermentation process, there is activity from the yeast to break down the sugars contained in the medium as energy for growth. As a result of this

activity, a floating layer will be formed on the top of the medium called nata. The percentage of sugar affects the thickness of the resulting nata.

The microorganisms in kombucha use carbon and produce cellulose which appears as a thin film on the surface. During the fermentation process, the yeast used to metabolize sucrose and produce a number of organic acids. Therefore, there was an increase in organic acid levels and a decrease in the pH of the tea steeping water during the fermentation process and after that it became stable. Although no other nitrogen source was added to the tea prior to fermentation, the protein content increased slightly with increasing fermentation time.

4. Bidara Leaf Antioxidant Activity

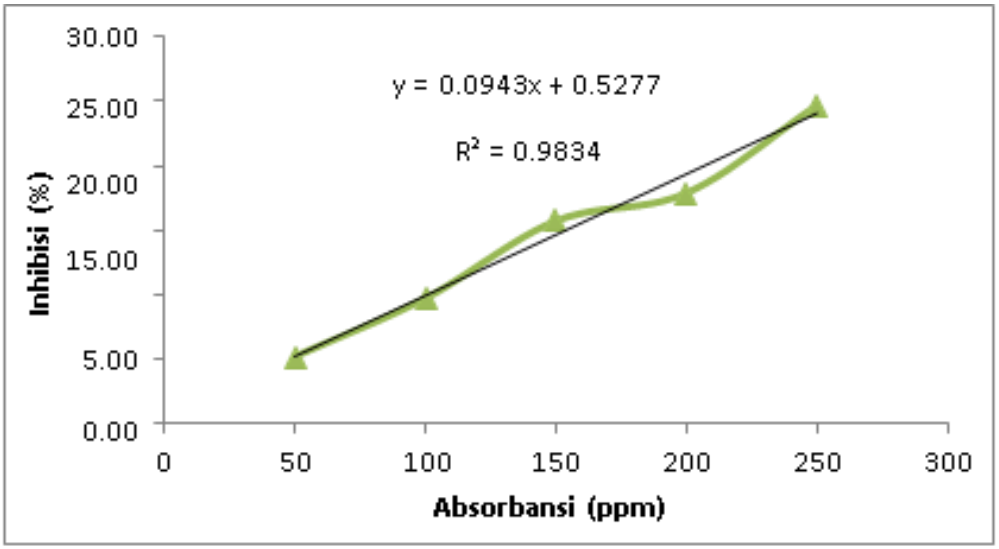
Antioxidants are compounds that play a role in counteracting free radicals and have a good impact on the body's physiological activities. Bidara leaves used as ingredients in this study have various types of secondary metabolites that act as natural antioxidants. Determination of free radical immersion power is carried out using the DPPH method because it is easier and simpler. In addition, DPPH is a free radical that is relatively stable when compared to other free radicals so that it is easily reacted with the test solution [8]. Quantitative antioxidant activity testing using the DPPH method was tested using a UV spectrophotometer at a wavelength of 515 nm. The obtained wavelength is then used to measure the sample with the results in Table 2.

To see the statistical differences in the antioxidant activity values of several variations of the fermentation time used, the data in Table 2 is converted into a graphic form as shown in Figures 2a, 2b, and 2c.

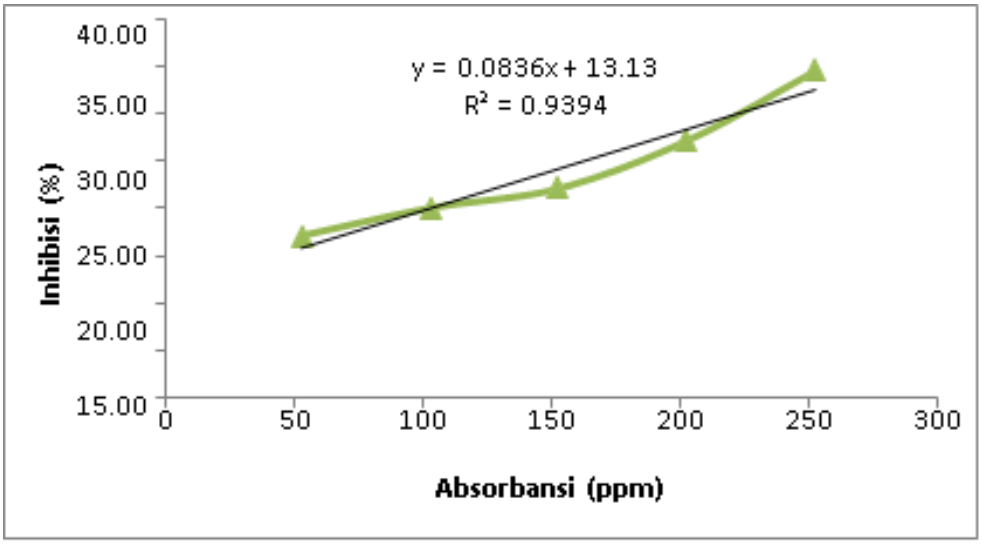
Table 2. Kombucha Leaf Antioxidant Activity

Fermentation Time (Days)	Code Sample	Antioxidant Activity (%)							
		0 ppm	50 ppm	100 ppm	150 Ppm	200 ppm	250 ppm	Linear Equation	IC_{50} value (ppm)
1	A1B	0	5,23	9,74	15,74	17,91	24,72	y = 0,0943x + 0,5277	524,63

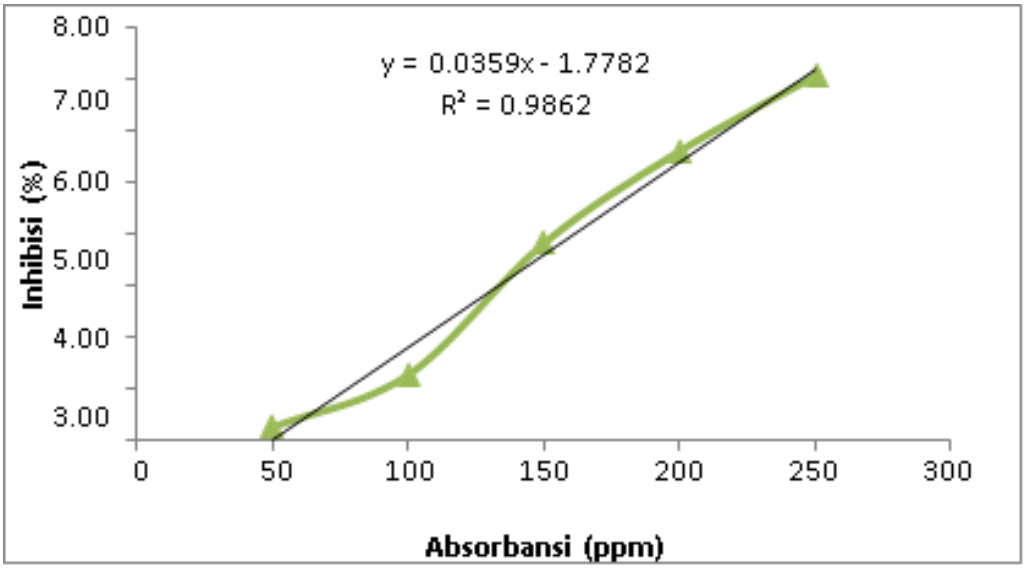
Fermentation Time (Days)	Code Sample	Antioxidant Activity (%)							
		0 ppm	50 ppm	100 ppm	150 Ppm	200 ppm	250 ppm	Linear Equation	IC_{50} value (ppm)
8	A2B	0	18,62	21,54	23,60	28,64	35,98	y = 0,0836x + 13,13	441,03
12	A3B	0	0,25	1,27	3,82	5,60	7,05	y = 0,0359x – 1,7782	1.442,3



(a)



(b)



(c)

Figure 2. Kombucha Product Inhibition Value
Description: (a) 1 Day; (b) 8 Days; and (c) 12 Days

Antioxidant activity (expressed in % inhibition) in kombucha bidara leaves is shown in Table 3. From the results of the study, it can be seen that there were fluctuations in the antioxidant activity produced during the fermentation process of kombucha bidara leaves. The lowest antioxidant activity was shown by sample A_3B, namely the sample with a long fermentation time of 12 days with a value of 7.05%, while the antioxidant activity The highest sample was shown by sample A_1B, namely the sample with a long fermentation time of 8 days with a value of 35.98%. The antioxidant activity of bidara leaf kombucha with 12 days of fermentation time tends to be lower than the other bidara leaf kombucha antioxidant activity. Kombucha leaves of bidara have increased antioxidant activity as seen from the percentage value of DPPH radical capture (%) due to the increase in fermentation time of 1-8 days. This could be due to the presence of phenolic compounds in bidara leaves which increased along with the fermentation process. The increase in antioxidant activity is due to the presence of free phenolic compounds produced during the fermentation process, so the higher the phenolic content produced, the higher the antioxidant activity. [9].

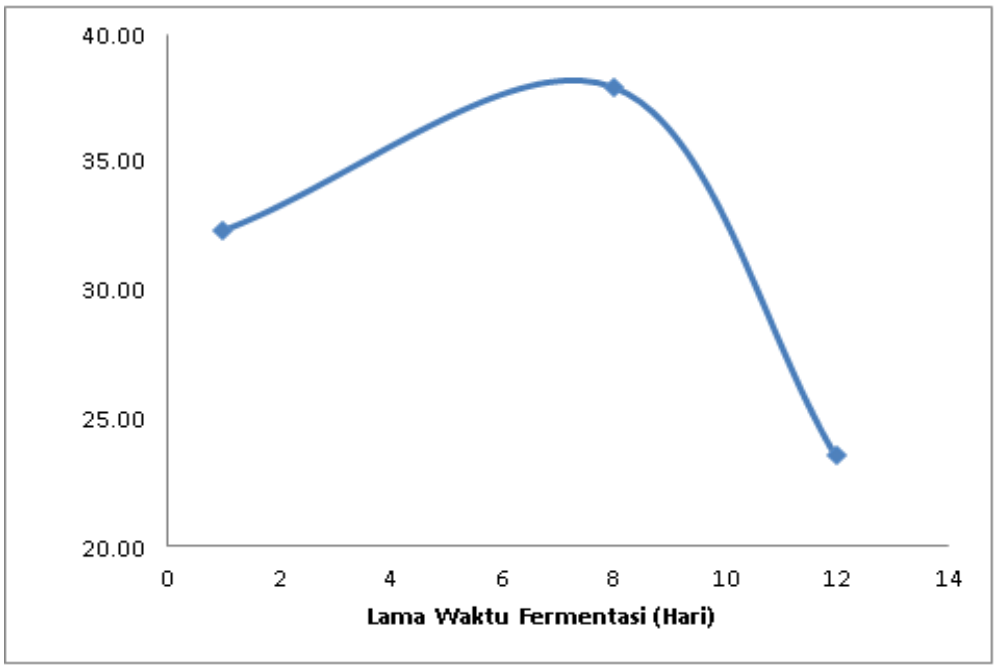


Figure 3. The Relationship of Fermentation Time with Antioxidant Activity of Kombucha Bidara Leaves

In addition, the increase in antioxidant activity is also caused by the metabolism of microorganisms during the fermentation process which produces tannase enzymes in degrading tannin compounds. During the fermentation process, microorganisms will carry out metabolic processes that increase phenolic compounds due to a biotransformation process by utilizing the enzymes of a plant cell to increase biological activity, resulting in modification of polyphenolic compounds to form phenolic compounds that have antioxidant activity such as flavonoids, tannins, and other compounds.

It can be seen when the fermentation took place on the 12th day there was a decrease in antioxidant activity. This can be caused because during the fermentation process, there was an increase in the amount of organic acids due to the activity of yeast and bacteria in kombucha. Acidic conditions cause phenolic compounds to become more stable and difficult to release protons that can bind to DPPH so that their antioxidant activity decreases [10].

Data describing antioxidant activity by looking at the percentage value of DPPH radical capture (%) of

kombucha bidara leaves were then tested statistically.

Based on Table 1, it can be seen that the smallest antioxidant activity is found in sample A3B with a fermentation time of 12 days with a percentage of 7.05% and the largest antioxidant activity is in sample A2B with a fermentation time of 8 days with a percentage of 35.98%. Based on the percentage of antioxidant activity in bidara leaf kombucha, it can be suggested that in making bidara leaf kombucha it takes 8 days of fermentation to get maximum antioxidant results.

The results of the analysis showed that the length of time of fermentation affected the IC50 value of kombucha bidara leaves. An ingredient can be said to be a strong antioxidant if it has an IC_{50} value of less than 200 ppm (Simanjuntak et al., 2016) [11]. Based on the IC_{50} values obtained, all samples had an IC_{50} value of more than 200 ppm (Figure 4), namely 524.84 ppm (A_1B sample with 1 day fermentation time), 441.03 ppm (A_2B sample with 8 days fermentation time) , and 1442.3 ppm (sample A_3B with a fermentation time of 12 days).

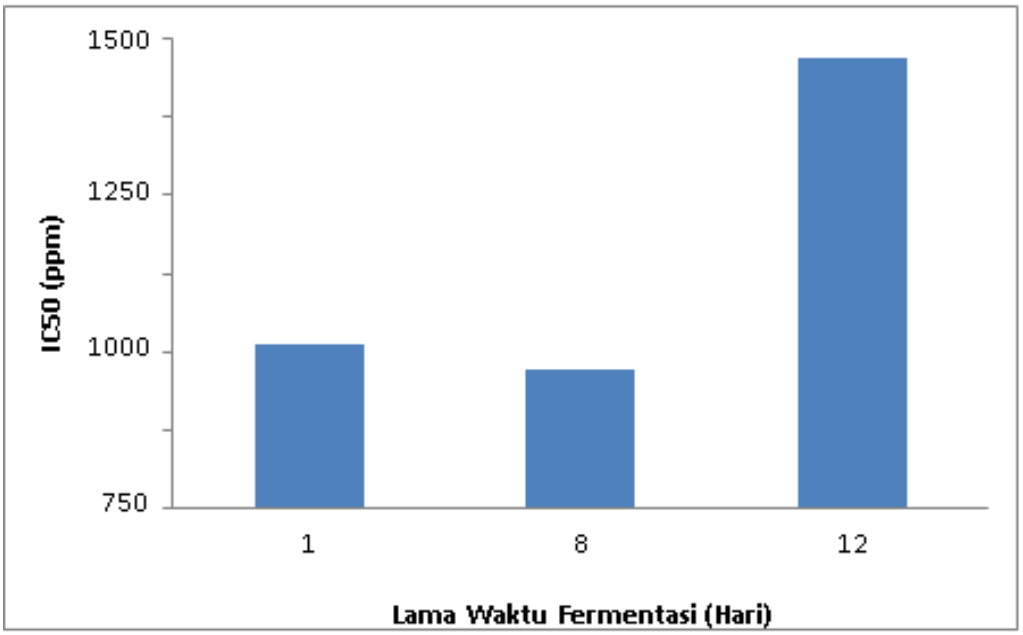


Figure 4. Kombucha Bidara IC_{50} Value

Thus, the sample that has the highest antioxidant activity is the sample that has the lowest IC_{50} value, namely the A2B sample with a fermentation time of 8 days.

4. Organoleptic Testing of Kombucha Bidara Leaves

Organoleptic test was carried out as a physical characteristic test of bidara leaf kombucha which was carried out by 20 untrained panelists based on the level of preference for bidara leaf kombucha products. Parameters assessed in this organoleptic test include the taste, aroma and color of each bidara leaf kombucha product which was carried out directly by the panelists.

5. Parameters of Kombucha Leaf Bidara Flavor:

Consumer acceptance of a food product, in this case is kombucha bidara leaves, tends to be influenced by the taste of the product itself. The results of the organoleptic tests that have been carried out show that the average value of the panelists' preference for the taste of kombucha from bidara leaves ranges from 3.25 (slightly dislike) to 4.55 (somewhat likes). The level of taste preference for each bidara leaf kombucha product can be seen in Figure 5.

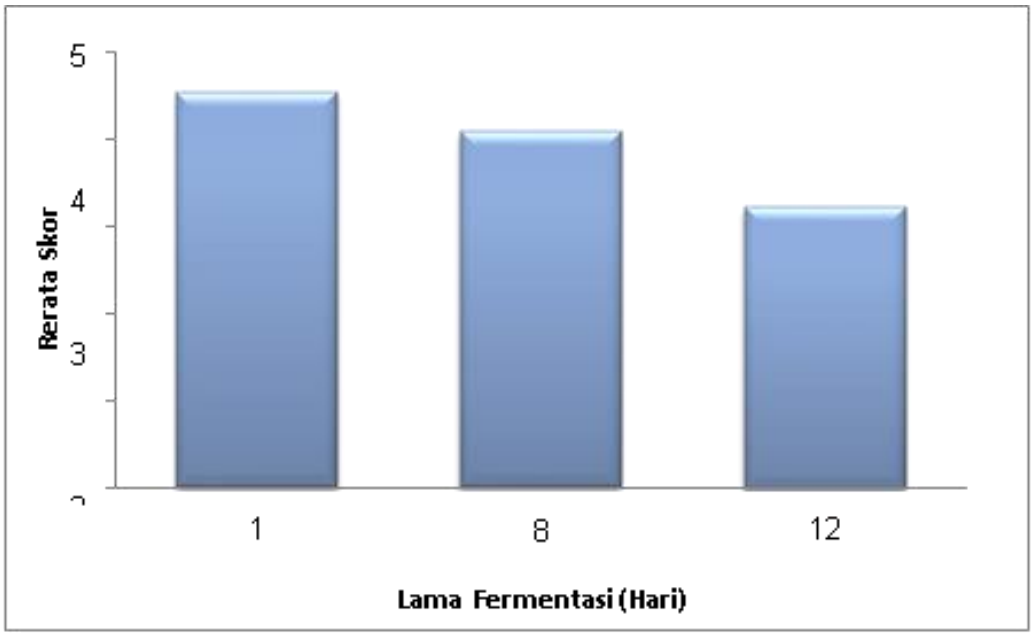


Figure 5. Average Panelist's Likeness Rating for Taste

Based on Figure 5, it can be seen that the highest appreciation of the panelists for the taste of kombucha bidara leaves was obtained from sample A_1B, namely with a 1-day fermentation time treatment with a value of 4.55 then followed by A2B sample, namely 8-day fermentation time treatment with a value of 4 ,10 and the last sample A_3B is the treatment with a long fermentation time of 12 days with a value of 3.25. The length of time of fermentation affects the panelists' perception of the product. The longer the fermentation time lasts, the panelists' preference for the product will decrease. Panelists tend to prefer sample A_1B because the product has a sweeter and slightly sour taste due to the addition of kombucha liquid starter compared to other samples. This is because sample A_1B undergoes the shortest fermentation process. The bacteria and yeasts contained in sample A_1B have not undergone a complete metabolic process so that only a small amount of organic acids is produced. The sweet taste contained in a product will provide pleasure compared to the sour taste for the panelists. Panelists will give a higher response to products that have a sweet taste. The kombucha product itself still feels foreign, so the panelists are not too familiar with and accustomed to the sour taste produced from kombucha. It can be seen that the longer the time used in the fermentation process, the average preference of the panelists for the taste of bidara leaf kombucha decreased. This is influenced by the higher levels of organic acids produced during the fermentation process. Bacteria and yeast will convert sucrose and other organic substances into organic acids, so the product has a strong sour taste. The longer the time required for the fermentation process, the more sour the taste produced by kombucha [12]. Yeast plays a role in breaking down sucrose into glucose to produce ethanol and carbon dioxide, then bacteria will oxidize ethanol to acetaldehyde which is converted into organic acids such as acetic acid, glunoronic acid and gluconic acid.

6. Parameters of Kombucha Leaf of Bidara

Aroma testing was carried out by panelists by smelling or smelling the product at a distance of about 3cm

from the nose. The results of the organoleptic test that have been carried out show that the panelists' preference for the aroma of kombucha from bidara leaves ranges from 3.40 (slightly dislike) to 4.65 (somewhat likes). The level of preference for the aroma of each bidara leaf kombucha product can be seen in Figure 6.

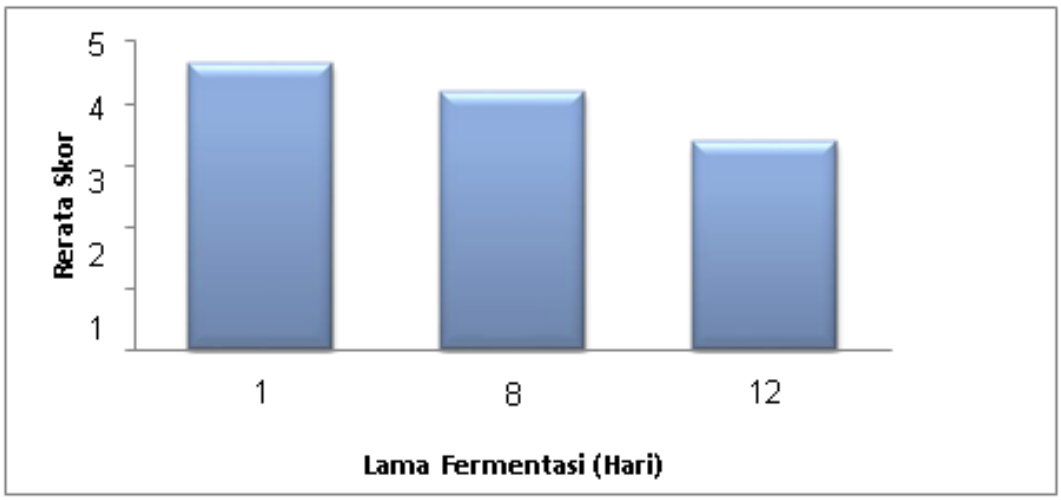


Figure 6. Average Panelist's Likeness Rating for Aroma

Based on Figure 6, it can be seen that the highest appreciation of the panelists for the aroma of kombucha bidara leaves was obtained from sample A_1B, namely with a 1-day fermentation time treatment with a value of 4.65 followed by A_2B sample, namely with 8-day fermentation time treatment with a value of 4.20 and the last sample A_3B is the treatment with a long fermentation time of 12 days with a value of 3.40.

Through the data obtained, it can be said that the perception of the panelists is determined by the length of time for kombucha fermentation of bidara leaves. The longer the fermentation time, the panelists' preference for the product tends to decrease. The aroma of sample A_1B was preferred over that of the other samples. Sample A_1B is preferred over the others because the sour aroma that comes from the addition of the kombucha starter is not overpowering. The aroma generated from a product determines the quality of the product. The pungent aroma of a product tends to reduce consumer interest [13].

Panelists were able to identify the presence of sour aroma, alcohol and the aroma of bidara leaf tea produced from the fermentation process. Kombucha bidara leaves have a strong and pungent aroma due to the fermentation process of alcohol and acetic acid. During the fermentation process, alcohol will be formed which gives a refreshing aroma effect. The metabolism of bacteria and yeasts that break down sucrose will increase the concentration of organic acids during the fermentation process, so that organic acid components will accumulate and lower the pH of the media. Fermentation is said to run well if it produces alcohol and there is a decrease in pH [14].

5. Kombucha Bidara Leaf Color Parameters

The test was carried out by panelists by observing the color of the kombucha of bidara leaves. Observation of this color is related to the level of clarity of the product. Clarity determines whether or not a solution is cloudy, while color determines the type of color produced from bidara leaf kombucha. Clear criteria can be determined by the absence of deposits or other particles that pass during the filtering process in the brew. The results of the organoleptic test that have been carried out show that the average value of panelists' preference for the color of bidara leaf kombucha ranges from 3.50 (slightly dislike) to 4.90 (close to like). The level of color preference for each bidara leaf kombucha product can be seen in Figure 7 below:

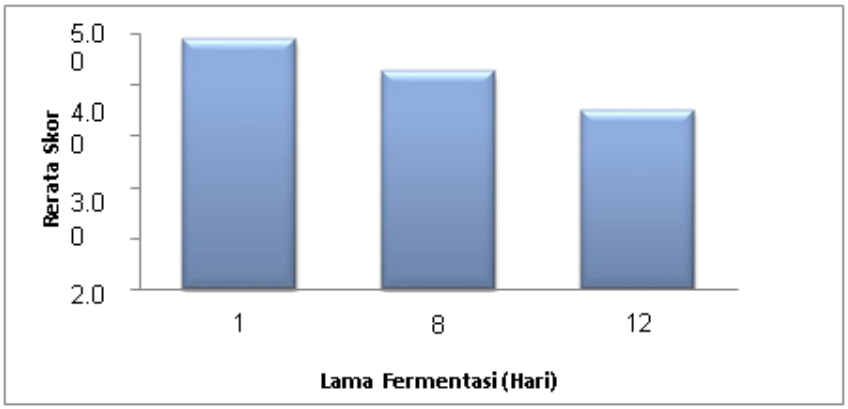


Figure 7. Average Panelist's Likeness Rating for Color

Based on Figure 7, it can be seen that the highest appreciation of the panelists for the color of kombucha bidara leaves was obtained from sample A_1B, namely with a 1-day fermentation time treatment with a value of 4.90, then followed by A_2B sample, namely with 8-day fermentation time treatment with a value of 4 .25 and the last sample A_3B is the treatment with a long fermentation time of 12 days with a value of 3.50. Through the data obtained, it can be said that the length of time of fermentation affects the panelists' perception of the product. Panelists' preference for the product tends to decrease along with the longer fermentation time.

When observed, the color produced from sample A1B (1 day fermentation time) tends to be brownish yellow like the color of bidara leaf tea steeping. In general, kombucha has a base color that matches the color of the material used. Dried bidara leaves when brewed will produce a brownish yellow color, while samples A2B and A3B have the final color of fermentation, which is pale yellow. Bidara leaves basically contain chemical compounds such as alkaloids, saponins, phenols, flavonoids, and tannins. The brownish yellow color is due to the phenol content in bidara leaves.

The color change occurs due to the oxidation process that occurs during the fermentation process depending on the length of time used for the fermentation process. The content of tannin compounds contained in bidara leaves will be damaged over time during the fermentation process due to the acidic atmosphere caused by a decrease in pH and the decomposition of the components in the solution, thereby reducing the concentration, so that the color of kombucha will fade [12].

Based on the results of the research conducted, the first color change occurred on the 3rd day of fermentation, namely from brownish yellow to pale yellow, then after fermentation for 7 days to 12 days, the yellow color looked more faded (pale). The color of the product will affect the perception of the panelists. This can be seen from the average preference level of panelists for the color of kombucha bidara leaves which decreases with the length of time the fermentation takes place.

CONCLUTION

The conclusions obtained from this research are as follows:

1. The length of time of fermentation affects the antioxidant activity of bidara leaf kombucha and the physical characteristics of the organoleptic test results which include the taste, aroma and color of each bidara leaf kombucha product.
2. The antioxidant activity value of bidara leaf kombucha product is 24.72% (sample A_1B with 1 day fermentation time); 35.98% (A_2B sample with 8 days of fermentation); and 7.05% (sample A_3B with 12 days of fermentation).
3. The IC_{50} value of bidara leaf kombucha products has a value of more than 50 ppm, namely 524.63 ppm (sample A_1B with a fermentation time of 1 day), 441.03 ppm (sample A2B with a fermentation time of 8 days), and 1,442.3 ppm (sample A_3B with a fermentation time of 12 days). The higher the IC_{50} value, the lower the antioxidant activity of kombucha.

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