# The Effect of Fermentation Time on Antioxidant Activity of Vanilla (Vanilla planifolia) Leaf Kombucha Tea

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**Abstract :** Free radicals can come from exposure to pollution such as motor vehicle fumes, industrial fumes, heavy metal contamination and consumption of unhealthy food and drinks. One of the innovations in beverage products that are rich in antioxidants and popular with the public is kombucha tea. Plant leaves that have the potential to be used as basic ingredients for making kombucha tea are vanilla leaves (Vanilla planifolia). The purpose of this study was to determine the effect of the length of fermentation time on the manufacture of vanilla leaf kombucha tea on antioxidant activity. The research method used was experimental research by the titrated total acid content test, pH test and levels of antioxidants of kombucha tea vanilla leaves test with variations of 0, 4, 8, and 12 days. The results of this study obtained IC50 value was 18% on day 0.

## INTRODUCTION

Health is one of the important things that must be maintained by humans to be able to carry out daily activities. Regular exercise, eating nutritious food, adequate rest, and managing stress properly can be done to maintain health so that our bodies are protected from disease. Recently, many diseases have arisen due to pollution caused by exposure to industrial fumes, motor vehicle fumes, etc., as well as from consuming unhealthy foods. Free radicals from outside can be in the form of cigarette smoke, radiation will be bad for health. Degenerative diseases such as cardiovascular disease, diabetes, stroke, cancer, Parkinson's disease are formed due to an imbalance between the number of free radicals and antioxidants that cause cellular oxidative stress (Simanjuntak, 2012).

Exposure to these free radicals can be avoided by consuming healthy foods and drinks. Healthy food or drink is food or drink that contains antioxidant compounds such as polyphenols, flavonoids and tannins which function to counteract free radicals. Antioxidants are compounds that are able to delay, slow down or inhibit oxidation reactions and can fight free radicals that are formed as a result of metabolism in the body (Agustina, 2017). Natural antioxidants can be obtained from natural ingredients. Thus, it is necessary to innovate food and beverage products that contain these antioxidant-rich ingredients. One of the innovations in beverage products that are rich in antioxidants and popular with the public is tea.

Kombucha tea is a type of tea made by fermenting sweet tea using a symbiotic culture of bacteria and yeast (Leal et al., 2018). Microbes that play a role in making kombucha tea from the acetic acid bacteria group are Acetobacter aceti, Acetobacter pausterianus Acetobacter xylinum, and Bacterium gluconicum and Schizosaccharomyces pombe, Candida sp., Kloeckera sp., S. ludwigii, S. cerevisiae, Torulospora sp., Zygosaccharomyces

bailii and Pichia species belonging to the yeast (Goh et al., 2012).

Kombucha tea can be made from plants that have high antioxidant content such as flavonoids and tannins. One of the plants that can be made for kombucha tea is vanilla leaves. Vanilla plant (Vanilla planifolia) is a plant that is widely used by the community. The plant has a stem with a smooth surface and knuckles. The diameter of the tree is 1-2 cm, the length of the trunk is up to 50 meters and the color is light or dark green (Ruhnayat, 2004). Generally, vanilla is used as a food and beverage flavoring ingredient, as well as a raw material for perfume (Nurcahyani, 2013). The results of research by Menon and Nayeem (2013) on vanilla plants show that vanilla plants are able to treat and prevent toothaches, menstrual pain, stomach ulcers, fever, coughs and sore throats.

Wet vanilla water content is 80% and will be 20% when dried. Vanilla beans weighing 100 grams contain 20 grams of water, 3-5 grams of protein, 11 grams of fat, 7-9 grams of sugar, 15-20 grams of fiber, 5-10 grams of ash, 1.5-3 grams of vanillin, 2 grams of resin. and vanillin acid. Compounds that play a very large role in the organoleptic properties are 4-hydroxy3-methoxy benzaldehyde (vanillin). Based on the isolation results, it can be seen that the components of fresh and dry vanilla glycosides include acids, fatty aldehydes, alcohols, fatty esters, alkanes, alkanones, amines, benzene aldehydes, benzene esters, benzene alcohol and phenols, as well as other substances (Melawati, 2006).

In the manufacture of kombucha tea, one of the factors that can affect the resulting product is the fermentation time. Fermentation time will affect the alcohol content and content of active compounds in kombucha tea. Fermentation time affects the antioxidant activity in kombucha tea. Antioxidants are compounds that have inhibitory power against oxidation reactions so that free radicals can be bound. New radical compounds are produced from a chain reaction between free radicals and the molecules around them. The structure of an antioxidant molecule can give electrons to free radical molecules without disrupting their function. Free radical chain reactions can also be broken in the presence of antioxidants (Azhar & Yuliawati, 2021).

Puspitasari et al., (2010) conducted a study on the antioxidant activity of kombucha tea. Kombucha tea antioxidant activity increases with increasing fermentation time. The increase in antioxidant activity on day 1 (88.88%) and day 3 (90.51%) was 1.63%, then on day 5 (91.88%) it increased by (1.37 %), and optimum antioxidant activity on the 7th day of fermentation (93.79%). The increase in antioxidant activity on the 7th day is the optimum condition based on the length of fermentation, this can be seen by the decrease in anti-oxidant activity on the 9th day (93.56%) and it decreases on the 11th day (93.21%) (Puspitasari et al., 2017).

Research by Suhardini & Zubaidah (2016), analyzed the antioxidant activity of kombucha tea using various leaf substrates containing phenols to obtain optimum antioxidant activity results of 88.24% to 92.97% on the 8th day of fermentation and decreased antioxidant activity on the 8th day. -14 (Suhardini & Zubaidah, 2016).

From the research conducted by Rosyada et al., (2023) regarding the antioxidant activity of kombucha tea from belimbing wuluh leaves (Avverhoa bilimbi Linn), it is known that the higher the sugar concentration, the higher the IC50 value which indicates a decrease in antioxidant activity. The kombucha leaf sample is a strong antioxidant because it has an IC50 value <50 (Rosyada et al., 2023).

The results of research conducted by Lalong et al., (2022) showed an increase in the antioxidant activity of DPPH in kombucha tea which was fermented for 14 days. Differences in the concentration of tea made from faloak stem bark had a significant effect ($p<0.05$) on changes in kombucha's antioxidant ability. There was an increase in antioxidant activity on the 14th day each of 1.8; 5.62; 11.42; 10.30; and 9.74% for concentrations 0.8;1.2; 1.6; and 2% and black tea kombucha. The purpose of this study was to determine the effect of fermentation time on the antioxidant levels of vanilla leaf kombucha tea.

## METHOD

### Research design

This study used an experimental research type with a completely randomized design (CRD) with variations in fermentation time of 0, 4, 8, 12 days. The treatment in this study are:
P1 = 0 days fermentation time treatment P2 = 4 days fermentation time treatment P3 = 8 days fermentation time treatment P4 = 12 days fermentation time treatment The research variables are:
Independent variable: length of fermentation time. Dependent variable: antioxidant activity. Control variables: concentration of kombucha culture, temperature, process of making kombucha tea.

### Tools and materials

**Tool**

Glass containers, spoons, analytical scales, stoves, stirrers, beakers, erlenmeyer, 10 ml volumetric flasks, 100 ml volumetric flasks, dropping pipettes, volume pipettes, test tubes, vortexes, aluminum foil, pH meters, UV-VIS spectrophotometer.

**Material**

Vanilla (Vanilla planifolia) leaves, water, tea, kombucha culture starter, distilled water, granulated sugar, PP indicator, NaOH, methanol pa, DPPH.

### Procedure

**1. Making Vanilla Leaf Tea**

Vanilla leaves are washed under running water until clean and cut into small pieces. Then dried in the sun until the leaves wilt for 30-60 minutes. The wilted vanilla leaves are dried in an oven at 55°C℃Until the leaves are completely dry. The dried leaves are used as vanilla leaf tea.

**2. Kombucha Starter Preparation**

2000 ml of water is boiled until boiling and 200 grams of sugar (10% w/v) is added to the amount of water used and 10 grams of 0.5% (w/v) tea is added. Then filtered and the filtrate is covered with aluminum foil and let stand until the tea has room temperature. After that, 200 ml (10% b/v) of kombucha culture starter was added to the brewed tea and the container was tightly closed. The propagation of the kombucha starter culture was left for 14 days.

**3. Making Vanilla Leaf Kombucha Tea**

36 grams (0.5% w/v) of vanilla leaf tea is brewed using 7200 ml of boiling water. Then added sugar with a concentration of 20 grams (10% w/v) stirred. The infusion of vanilla leaf tea is tightly closed with aluminum foil and let stand until room temperature. 20 ml of liquid kombucha starter was added and fermented for 0, 4, 8 and 12 days in a closed container and filtered after the fermentation time was complete.

**4. Test Levels of Total Titrated Acid**

The titrated total acid level test was carried out using the principle of acid-base titration. The test was carried out by placing 10 ml of the sample in a 100 ml volumetric flask and then adding distilled water up to the mark. After that, 10 ml of the filtrate was taken and put into the Erlenmeyer and 3 drops of PP indicator were added. The solution was titrated with 0.1 N NaOH solution until the color of the sample solution changed from clear to pink. Total acid is calculated using the formula:

$$\text{Total Acid (\%)} = \frac{V_{NaOH} \ X \ N_{NaOH} \times BM \times 100\%}{V_{sampel} \times 1000} \quad (1)$$

Information :
VNaOH : Volume of NaOH used for titration
NNaOH : Standard concentration of NaOH
Vsamples : The volume of the sample used for the titrate
BM : Molecular weight of acetic acid

**5. Test pH levels**

pH measurement was measured using a pH meter. Vanilla leaf kombucha tea is put in a beaker glass and immersed in a pH meter.

**6. Antioxidant Activity Test**

Vanilla leaf kombucha tea was tested for its antioxidant activity using the DPPH (-

diphenyl-αβ-picrylhydrazyl). The antioxidant activity test was carried out by mixing 2 ml of 50 ppm DPPH solution with 2 ml of kombucha sample solution. Kombucha tea concentration variations were 10%, 15%, 20%, 25% and 30% with distilled water up to 10 ml.Blank using 4 ml of distilled water.The solution was incubated for 30 minutes at room temperature and its absorbance was measured at a wavelength of 524 nm. The formula for the percentage inhibition of DPPH uptake is as follows:

$$\% \text{ inhibition} = \frac{(A_0 - A_S)}{A_0} \times 100$$

Description:
A0: Absorbance control (DPPH + Aquades)
As: Sample absorbance and DPPH

The control absorbance was prepared by adding 2 ml of 50 ppm DPPH and 2 ml of distilled water and then measuring it at a wavelength of 524 nm. After that, the IC50 value is determined from the linear regression equation.

## RESULTS AND DISCUSSION

In general, Indonesian people are considered to frequently consume tea. Tea is believed to be able to ward off toxins, eliminate alcohol, streamline urine and blood, relieve joint pain, and boost immunity (Sa'diyah & Lestari, 2020). As time goes by, the types of tea continue to develop, one of which is kombucha. Kombucha is a traditional or ancient drink that originated in China. Kombucha is produced from the fermentation of green tea or black tea by the SCOBY (Symbiotic Culture of Bacteria and Yeast) biofilm (Cardoso et al., 2020). SCOBYs are colonies of fermenting bacteria that act as starters in making kombucha. SCOBY is made from the bacteria Acetobacter xylinum, Gluconobacter, Ascomycetous, and Saccharomyces ludwigii (Mousavi et al., 2020).The role of these microorganisms is to change the texture, aroma, color and quality of the fermented product (Riadi et al., 2020).

The main ingredients for making kombucha tea are green tea and sugar which are then fermented for 7 to 10 days with the help of microorganisms. The added sugar stimulates the growth of microorganisms and becomes a natural biocontrol during fermentation (Negara & Meilani, 2023). During the fermentation process, sugar is converted by bacteria and yeast into main compounds such as acetic acid, ethanol and glucuronic acid, as well as additional compounds such as lactic acid, phenolics, B vitamins and enzymes. The fermentation process increases the concentration of the active ingredient in kombucha tea (Purnami et al, 2018). Kombucha contains epicatechin gallate, epigallocatechin, catechin, epicatechin, and epigallocatechin gallate which are useful as anti-carcinogenic and antioxidants (Mousavi et al., 2020).

Sugar is the most important basic ingredient in making kombucha tea. Sugar is a food source for microorganisms in the kombucha culture process. In general, the type of sugar used to make kombucha tea is granulated sugar. Kombucha bacteria convert glucose into acids, vitamins and alcohol during kombucha tea fermentation. Yeast produces glucose and fructose from the inversion of sucrose. Glucose will be converted into gluconic acid by the Acetobacter xylinum enzyme via the pentose phosphate pathway, while most of the fructose is converted into acetic acid and small amounts of gluconic acid. Therefore, glucose is very useful as a substrate for cell growth and formation of products such as acetic acid (Puspitasari et al., 2017).

The manufacture of kombucha tea, a factor that can affect the resulting product is the length of time it ferments. Kombucha tea fermentation time ranges from 8-12 days at 18-280C (Wistiana & Zubaidah, 2015). Kombucha tea with a long fermentation time is believed to have a more complete composition compared to tea before fermentation (Puspitasari et al., 2017). Fermentation time will affect the alcohol content and content of active compounds in kombucha

tea. According to research conducted by Goh et al., (2012) using black tea-based kombucha tea, it can be seen that the best product was fermented for 8 days because it produced the highest cellulose precipitate of 66.9%.

Kombucha tea has a sour taste caused by an increase in organic acid compounds during the fermentation process so that the pH in kombucha tea decreases. According to research Wistiana & Zubaidah (2015), the longer the fermentation time, the total acid increases. This happens because of the metabolic processes of yeast and bacteria towards sucrose which produce a number of organic acids such as acetic acid, gluconic acid, and glucuronic acid. Thus, the higher the organic acids in kombucha tea, the more sour it tastes.

Level acidity something material usually stated with mark pH. pH product fermentation relate tightly with amount sour Which generated. Mark pH own connection backwards with mark total sour Which titrated. The more low mark pH, the more tall amount total sour Which titrated (Prastujati et al., 2018). The pH value and total titrated acid of vanilla leaf kombucha tea can be seen in table 1. According toSulistiawaty & Solihat (2022), the pH value produced during the fermentation process is said to be still safe for consumption if the taste is between 4.28 to 3.36. A pH value below 2.5 poses a health risk because the acetic acid content is too high.

Table 1: pH value and total titrated acid

| Treatment | pH value | Titrated Total Acid Content (%) |
|---|---|---|
| P1 | 5.56 | 0.003 |
| P2 | 3.67 | 0.012 |
| P3 | 3.06 | 0.024 |
| P4 | 2.37 | 0.048 |

Description: P1 (0 day fermentation time), P2 (4 days fermentation time), P3 (8 days fermentation time), P4 (12 days fermentation time)

According to research Puspitasari et al., (2017), on the 1st day of fermentation (pH 5.93) gradually decreased on the 3rd day of fermentation (pH 5.31), the 5th day (pH 5.12), the 9th day (pH 4.06 ) and the 11th day (pH 3.65). The results of research by Pratiwi et al., (2011) concerning the effect of fermentation time on the physical and chemical properties of kombucha from seaweed substrates yielded data, that is, the pH value decreased from day 0 to day 16, from pH 4.89 decreased to pH 3.09.

Nurhayati et al., (2020) conducted research on kombucha cascara tea (ripe coffee skin) and it can be concluded that the length of fermentation time affects the physical, chemical and sensory characteristics. The best results were 8 days of fermentation because the high total phenolic content, pH and total acid were still within safe levels for consumption. According to research Yanti et al., (2020) regarding the preparation of soursop leaf kombucha for 12 days had the highest antibacterial activity with an inhibition of Escherichia coli of 16.28 mm and Staphylococcus aureus of 17.08 mm.

The decrease in pH is caused by the formation of acetic acid. Yeast cells hydrolyze sucrose to glucose and fructose for ethanol production, while bacteria convert glucose to gluconic acid and fructose to acetic acid. In Acetobacter kombucha cultures, ethanol is oxidized to acetaldehyde and then to acetic acid (Puspitasari et al., 2017). The decrease in the pH of kombucha tea also occurs because during the fermentation process the yeast synthesizes sugar into ethanol and is broken down by acetate bacteria into organic acids, such as acetic acid and gluconic acid and some concentrations of organic acids result in a decrease in the pH of the fermentation medium.

According to Devita et al., (2019) the longer the fermentation time, the more lactic acid produced by lactic acid bacteria. These bacteria will break down lactose into lactic acid, causing free hydrogen ions to increase. Lactic acid bacteria are a group of probiotic bacteria that are non-pathogenic, belong to a group of Gram-positive bacteria, in the form of coccus (round) or bacillus (rod), do not form spores, catalase negative and oxidase positive, and produce lactic acid. The rapid production of lactic acid by lactic acid bacteria will inhibit the

growth of other unwanted microbes. In general, lactic acid bacteria come from the Lactobacillaceae family, namely Lactobacillus sp. and the Streptococcaceae family especially Leuconostoc, Streptococcus and Pediococcus (Princess et al., 2018).

The pH value is the degree of acidity which is commonly used to express the degree of acidity or degree of alkalinity of a solution (Sugeng & Sulardi, 2019). The acceptable and safe pH level for kombucha tea is between 3 and 5.5. If kombucha tea has a pH below this value, it needs to be diluted (Naland, 2008). The results of the pH level test in Table 1 can be seen that the pH level of kombucha tea decreases with the length of fermentation time. The decrease in pH occurs due to the growth and metabolic processes of acetic acid, lactic acid and yeast bacteria which produce organic acid compounds, causing a decrease in acidity during fermentation (Jayabalan et al., 2014).

Fermentation time also affects the antioxidant activity in kombucha tea. Antioxidants are compounds that have the ability to inhibit oxidative reactions, especially by binding to free radicals. New radical compounds are created from a chain reaction between free radicals attacking the surrounding molecules. Antioxidants have a molecular structure that can donate electrons to free radical molecules without disrupting their function and can interfere with the chain reaction of free radicals.(Azhar & Yuliawati, 2021). The fermentation process will increase the antioxidant activity because bacteria and yeast will increase the amount of phenol. The higher the phenolic compounds, the higher the antioxidant activity (Khaerah & Akbar, 2019).

Measurement activity antioxidants can use with DPPH method. DPPH method is method which simple, fast and easy for filter activity arrest radical free from a number of compound. DPPH method proven accurate, effective And practical (Samin et al., 2013). The mechanism for the radical scavenging reaction is shown in Figure 1.

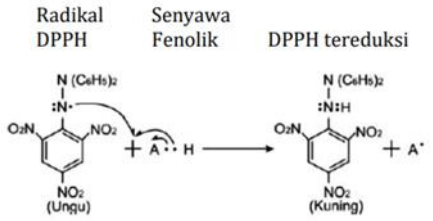


Figure 1: Mechanism of the color change reaction that occurs in the DPPH method

The maximum absorbance is given by the chromophore and auxochrome groups on the DPPH free radical. The response of antioxidants in capturing DPPH radicals can be seen from the color change in the sample. At first DPPH is purple, then gradually turns yellow. The yellow color change is due to the picryl group with the addition of electrons from the antioxidant to the DPPH radical (Husniati et al., 2021). The reduced color intensity of the DPPH solution is due to the reaction of the hydrogen atoms liberated from the test material with the DPPH radical molecules to form a yellow compound 1,1-diphenyl-2-picrylhydrazine. The greater the concentration value of a sample, the stronger the yellow color produced will be(Wijaya et al., 2014). The testing process with the DPPH method must be carried out in a dark room because DPPH is sensitive to light. A dark room will minimize the formation of radicals other than the DPPH free radicals which are added intentionally (Martiningsih et al., 2016).

The antioxidant activity of vanilla leaf kombucha tea for each treatment used the IC50 value parameter. IC50 (50% Inhibition Concentration) is the concentration value required for the sample solution to reduce or inhibit DPPH free radicals by 50%. The IC50 value is obtained from the % inhibition value, which is the value of free radical inhibition (Handayani et al., 2016). The inhibition value of each concentration in each treatment can be seen in table 2 below:

Table 2: Kombucha tea inhibition values on days 0, 4, 8 and 12

| | Concentration (%) | ln Concentration | Absorbance | % inhibition |
|---|---|---|---|---|
| 0 | 10 | 2.3025 | 0.3127 | 32.4598 |
| | 15 | 2.7080 | 0.2607 | 43.6898 |
| | 20 | 2.9957 | 0.2139 | 53.8031 |
| | 25 | 3.2188 | 0.2060 | 55.5098 |
| | 30 | 3.4011 | 0.1617 | 65.0649 |
| 4 | 10 | 2.3025 | 0.166 | 64.1525 |
| | 15 | 2.7080 | 0.157 | 66.0961 |
| | 20 | 2.9957 | 0.137 | 70.4150 |
| | 25 | 3.2188 | 0.126 | 72.7905 |
| | 30 | 3.4011 | 0.096 | 79.2689 |
| 8 | 10 | 2.3025 | 0.207 | 55.2987 |
| | 15 | 2.7080 | 0.147 | 68.2556 |
| | 20 | 2.9957 | 0.12 | 74.0862 |
| | 25 | 3.2188 | 0.096 | 79.2689 |
| | 30 | 3.4011 | 0.076 | 83.5879 |
| 12 | 10 | 2.3025 | 0.196 | 57.6741 |
| | 15 | 2.7080 | 0.186 | 59.8336 |
| | 20 | 2.9957 | 0.122 | 73.6543 |
| | 25 | 3.2188 | 0.11 | 76.2456 |
| | 30 | 3.4011 | 0.087 | 81.2125 |

After knowing the inhibition value of each concentration in each treatment, then look for the linear regression equation. The graph of percent inhibition can be seen in Figures 2, 3, 4 and 5 below:

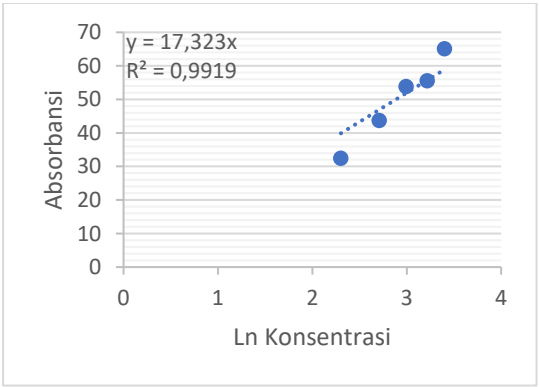


Figure 2: Calibration Curve of Inhibition Value of Kombucha P1 Tea (0 Days Fermentation)

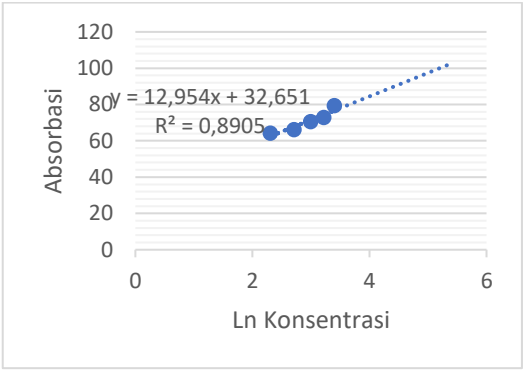


Figure 3: Calibration Curve of Inhibition Value of Kombucha P2 Tea (4 Days Fermentation)

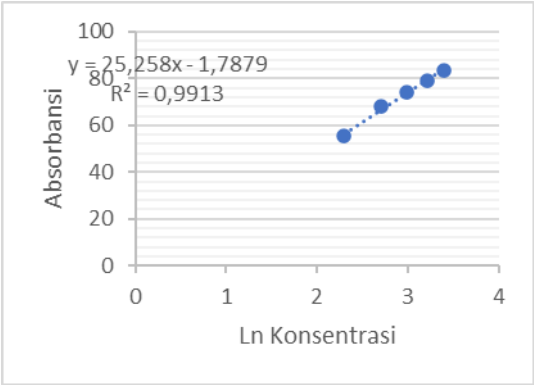


Figure 4: Calibration Curve of Inhibition Value of Kombucha P3 Tea (8 Days Fermentation)

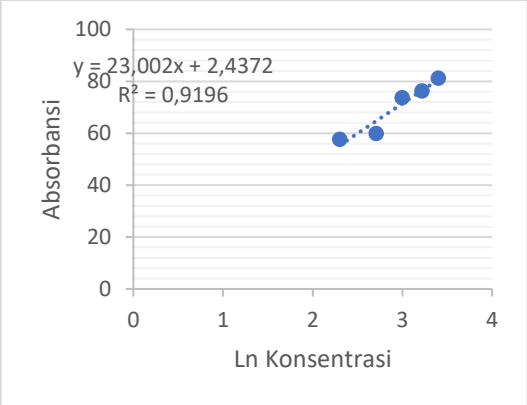


Figure 5: Calibration Curve of Inhibition Value of Kombucha P4 Tea (12 Days Fermentation)

The antioxidant activity of vanilla leaf kombucha tea for each treatment was expressed in IC50. IC50 value data for each treatment can be seen in table 3. From the calculated data, the lowest IC50 value was obtained at P2 (4th day of

fermentation) of 4%. The sample have a greater antioxidant activity if it has a smaller the IC50 value (Firdayani et al., 2015). Thus, the vanilla leaf kombucha tea on the 4th day of fermentation had the best antioxidant activity.

The IC50 value was obtained from calculating the concentration of the sample using the linear regression equation formula obtained from the linear regression graph of the relationship between concentration and % inhibition(Hani & Milanda, 2021). IC50 value data for each treatment can be seen in table 3. From the calculated data, the lowest IC50 value was obtained at P2 (4th day of fermentation) of 4%. The greater the antioxidant activity of a sample, the smaller the IC50 value (Firdayani et al., 2015). Thus, the vanilla leaf kombucha tea on the 4th day of fermentation had the best antioxidant activity.

Table 3. IC50 value

| Treatment | IC50 (%) |
|---|---|
| 1 | 18 |
| 2 | 4 |
| 3 | 7.7 |
| 4 | 7.9 |

Description: P1 (0 day fermentation time), P2 (4 days fermentation time), P3 (8 days fermentation time), P4 (12 days fermentation time).

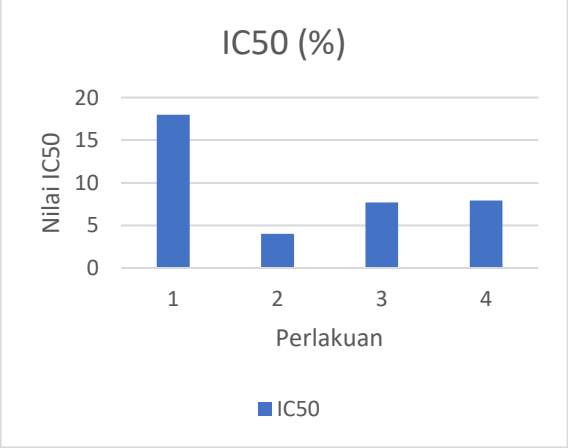


Figure 2. IC50 Value Diagram for Vanilla Leaf Kombucha Tea

According to research Nurikasari et al., (2017),The highest antioxidant activity (93.79%) was observed on the 7th day of fermentation. The antioxidant activity slightly decreased and reached 93.56% on the 11th day of fermentation by increasing the fermentation time. The pH of the samples decreased during fermentation (from 5.93 on day 3.65 on day 11). Agus et al., (2010) conducted a study on the antioxidant activity of kombucha tea on black tea substrate and it was found that the antioxidant activity of green tea kombucha had an optimum value on the 7th day of fermentation and the antioxidant activity decreased on the 10th day of fermentation. Suhardini and Zubaidah (2016) also conducted research on the analysis of the antioxidant activity of kombucha tea using various kinds of leaves with phenol content and obtained optimum antioxidant activity results of 88.24% to 92.97% on the 8th day of fermentation and the antioxidant activity decreased on 14 days (Puspitasari et al., 2017).

Antioxidant activity can increase because during the fermentation process it produces free phenolic so that a high phenolic concentration will cause high antioxidant activity. The increase in phenolic compounds was caused by the biotransformation of plant enzymes which led to an increase in microbial metabolism. The functional groups of chemical compounds are changed by biological transformation processes. In addition, phenolic compounds can be found in the leaves of the plant used as the basic ingredient for kombucha. The type of phenolic compound in plants that acts as a natural antioxidant is cinnamic acid (Suhardini & Zubaidah, 2016). Antioxidant activity decreases because the stability of phenolic compounds in acidic conditions makes it difficult to release protons that can bind to DPPH. This resulted in a decrease in antioxidant activity (Puspitasari et al., 2017).

## CONCLUSION

Based on the research that has been done, the IC50 value of vanilla leaf kombucha tea is obtained 18% on day 0, 4% on day 4, 7.7% on day 8, and 7.9% on day 12. The best antioxidant

activity was found on the 4th day of fermentation. Thus, it can be concluded that the length of time of fermentation affects the antioxidant activity in vanilla leaf kombucha tea.

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