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The Effect of Fermentation on the Characteristics and Antioxidant Activity of Wuluh Starfruit Leaf Kombucha Tea (*Avverhoa bilimbi* Linn.)

Fanny Fajrin Aulia Rosyada, Eva Agustina*, Hanik Faizah

Biology Deparment, Faculty Sains and Technology, UIN Sunan Ampel Surabaya, Dr. Ir, H. Seokarno Street Number 682, Surabaya, Indonesia.

*Corresponding Author: eva_agustina@uinsby.ac.id

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Abstract

Kombucha is a fermented drink that provides health effects. Wuluh starfruit leaves are one of the ingredients that can be used in making kombucha. This study was conducted to determine the effect of fermentation time on the physical, chemical, and antioxidant characteristics of wuluh starfruit leaf kombucha tea (*Avverhoa bilimbi* Linn.). This research is an experimental study with a completely randomized design (CRD) consisting of 4 treatments. Kombucha wuluh starfruit leaves are made with a fermentation time of 0, 4 8, and 12 days. The physical characteristics tested include an organoleptic test of scent, color, and taste. The chemical characteristics tested include pH, levels of titrated acids (tta), phenolic, and alcohols. The antioxidant activity is known by determining the value of IC_{50}. Organoleptic, pH, tat, and phenolic assessment data were statistically analyzed using Kruskal Wallis and Mann Whitney. Alcohol content and antioxidant activity were analyzed descriptively. The best result of physical, and chemical characteristics and antioxidant activity are on the 12^{th} day of fermentation with a pH of 3, TTA 0.11±0.070%, phenolic 87.33±1.140 mg/ml GAE and alcohol of 0.41% with an IC50 value of 3.65 ppm.

Keywords: Antioxidant, fermentation, Kombucha, total titrated acids, phenolic

INTRODUCTION

Health is a very valuable thing for a human that is generally often overlooked. The emergence of various health problems makes people aware of the importance of maintaining health to implement a healthy lifestyle by consuming healthy foods and drinks (Cahanar & Suhanda, 2006). One of the drinks that can improve body health is kombucha. Kombucha is one of the traditional drinks fermented from black tea containing sugar with tea mushrooms commonly known as Scoby (Symbiotic culture of bacteria and yeast). Kombucha has a taste like sparkling apple cider. During the fermentation period, kombucha will undergo a change in taste from refreshing sour to sour like vinegar (Goh et al., 2012; Sun, Li, & Chen, 2015). This happens because the bacteria and yeast in kombucha will convert sugar into several major compounds, namely acetic acid, ethanol, and glucuronic acid as well as minor compounds, namely lactic origin, phenolic acid, B vitamins, and enzymes. Organic acid compounds will increase during the fermentation process. This will affect the decrease in pH and increase in total acid in kombucha (Villarreal-Soto, Beaufort, Bouajila, Souchard, & Taillandier, 2018; Wistiana & Zubaidah, 2015).

Organic acids produced from fermentation are the main metabolites produced and can act as active ingredients to provide health effects such as antioxidants (Muhialdin et al., 2019; Purnami, Anom Jambe, & Wayan Wisaniyasa, 2018; Suhardini & Zubaidah, 2016). An antioxidant compound is defined as a compound that can delay, slow down, and prevent oxidation processes because it has the property of being easily oxidized or is a strong reductor compared to other molecules (Mahardika & Roanisca, 2018; Simanjutak, 2012). Kombucha tea is a drink that is known to have antioxidant activity, so consuming kombucha can have a fairly good effect on the activity of the intestines, stomach, and glands. The antioxidant effect on kombucha can also overcome diabetes, and aging problems, act as a laxative, and is known to relieve joint rheumatism, gout, and hemorrhoids (Firdaus, C, Isnaini, & Aminah, 2020; Goh et al., 2012).

In kombucha to produce a good taste, generally use black tea because black tea is a type of tea that has a fragrant scent compared to other types of tea, but for health purposes, the manufacturer of kombucha can use other herbal plants that contain

active substances in the form of natural antioxidants with higher levels (Naland, 2008). According to Nasir & Rahmani (2015), the scent and taste quality of a dry tea is influenced by the content of chemical compounds in it such as caffeine, tannins, and essential oils. The protection of several chemical compounds found in plants so that they have the potential to become a source of taste, aromatic compounds, and drugs that are very beneficial for human health (Fadeyi, Adeniran, & Akiode, 2022). Kombucha can be made from leaves that have a high content of polyphenols such as tannins and flavonoids. One of the plants that have the potential to be used as a material for making kombucha is star fruit leaves. The use of wuluh starfruit as a material for making kombucha has never been reported. Even star fruit leaves have several chemical compounds that have the potential to be utilized such as saponins, tannins, sulfur, formic acid, and phenol compounds, namely flavonoids. The total flavonoid content in star fruit leaves is not less than 0,7% (Misfadhila, Chandra, & Wahyuni, 2020). In addition to flavonoids, wuluh starfruit leaves also have a high tannin content of 10,92% compared to green tea of 1,44% and lime leaves of 1,8% (Andriani, Gde Mayun Permana, & Rai Widarta, 2019).

One of the factors that can affect when making kombucha is the fermentation time. Generally, the fermentation time of kombucha is 6-12 days. Based on research (Nurhayati, Yuwanti, & Urbahillah, 2020), the best treatment with the highest phenolic levels was obtained at a fermentation time of Kombucha 8 days. Research (Falahuddin, Apriani, & Nurfadilah, 2017) about kombucha soursop leaves are known to be the best treatment by producing the highest vitamin C value, namely at a fermentation time of 12 days.

Research about the effect of fermentation time on kombucha using wuluh starfruit leaves (Avverhoa bilimbi Linn.) to determine the physical, chemical, and antioxidant activity characteristics has never been carried out. Thus, it is necessary to research the effect of the treatment of long fermentation time on the kombucha of star fruit leaves (*Avverhoa bilimbi* Linn.).

METHODOLOGY

This study used an experimental method with a completely randomized design (CRD) consisting of 4 treatments with 3 replicates, that is 0, 4, 8, and 12 days of fermentation time at 10% sugar concentration.

Materials and Instrumentals

The ingredients used in this study were wuluh starfruit leaves (*Avverhoa bilimbi* Linn.), kombucha culture starter, black tea, granulated sugar, aquades, Na_2CO_3, gallic acid, methanol, ethanol 96%, Indicators pp, Folin-ciocalteu, DPPH, and NaOH. The instruments used in this study are glass containers, clean cloths, rubber bands, autoclaves, vortexes, ovens, UV-vis spectrophotometers, cameras, and label paper.

Methods

Tool sterilization

Sterilization of glass containers that have been covered with aluminum foil is carried out using an autoclave with a temperature of 121℃ and a pressure of 1 atm for 15 minutes.

Preparation of the kombucha starter

Water amount 500 ml boiled then 50 grams of 10% sugar (w/v) is added. After that, 2.5 grams of 0.5% (w/v) black tea is added. Then filtering is carried out and poured into sterile glass containers. After the tea has a temperature equal to room temperature, 50 ml (10% (w/v)) kombucha culture is added to the brewed black tea. Then cover the container with a clean cloth then tie it with a rubber band. Starter propagation of kombucha culture fermented for 14 days.

Preparation of the wuluh starfruit tea

The wuluh starfruit leaves separated by twigs. The part used for making tea is young leaves of wuluh starfruit. After that, the leaves are thoroughly washed under running water. Then, cut the leaves into small pieces and drying using the oven at a temperature of 55 ℃

Preparation of the wuluh starfruit leaves kombucha

Wuluh starfruit leaves tea of as much as 12 grams 0.5% (w/v) is brewed using 2400 ml boiling water. After that, poured 200 ml into each container and added sugar with as much as 10% (w/v). Then 20 ml of the kombucha liquid starter added, the glass container is covered with a clean cloth. After that, the brewed wuluh starfruit leaves tea is fermented for 0, 4, 8, and 12 days.

The test pH analysis of starfruit leaves kombucha tea

The measurement of pH in kombucha wuluh starfruit leaves tea samples is carried out by taking about 50 ml of the kombucha solution and then

putting it into a glass beaker. Then, the pH is measured using a universal pH, and the color changes on the pH paper are matched to the color standard listed in packaging.

Total levels of titrated acid

Total titrated acid was tested using the principle of acid titration by base referring to Cahyaningtyas (2018). The test was taking 10 ml of the sample into a 100 ml volumetric flask and adding distilled water until the boundary mark. After that, a sample was filtered. 10 ml of filtrate is taken and put into erlenmeyer and added indicator pp. After that, titration is carried out with a solution of NaOH 0.1 N. Titration is carried out until the solution changes color to pink. The calculation is carried out by the following Equation 1.

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

V_{NaOH} is volume NaOH for titration, N_{NaOH} is NaOH standard concentration, V_{sample} is the volume of the sample for titrate, and MW is the Molecular weight of acetic acid

The Phenolic Level Test

Measurement of phenolic levels in kombucha using the folin ciocalteu method refers to Pourmorad (2006). The test was carried out by making a standard solution of gallic acid with 5 concentration variations, namely 10 ppm, 20 ppm, 30 ppm 40 ppm, and 50 ppm. Then each concentration is taken 1 ml and put into a test tube. 0.5 ml of folin ciocalteu inserted. After that, 4 ml of Na_2CO_3 7% solution was added and homogenized using a vortex and allowed to stand for 8 minutes and calculated absorbance with a wavelength of 760 nm.

Measurements on the sample were carried out by taking 1 ml of the sample and putting it into a test tube and giving 0.5 ml of folin ciocalteu. Then 4 ml of Na_2CO_3 7% solution is added. It is homogenized using a vortex and allowed to stand for 8 minutes. Absorbance is calculated with a wavelength of 760 nm. Phenolic content can be calculated using equality and regression and using the following Equation 2.

$$\text{TPC} = \frac{c.v.fp}{g} \quad (2)$$

TPC is total phenolic content (mg/g GAE), c is concentration (value x) (ppm), v is extract volume (ml), fp is Dilution factor, g is sample weight (grams)

Alcohol test

The measurement of alcohol content using the Skoog (1985) by taking 25 ml of wuluh starfruit leaves kombucha solution. Then, the neutralization of the solution is carried out using sodium hydroxide (NaOH) 3 N. followed by the distillation process. Then the distillation results of 25 ml are put into a 25 ml pycnometer equipped with a thermometer. Before the treatment of pycnometer and thermometer tools are weighed first. After that, the pycnometer is put in cold water until the temperature has reached 28℃. Then, the pycnometer weighed the weight again, and the calculation of specific gravity can be done by the following Equation 3 (Bp is pycnometer weight, and) BM is molecular weight).

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$$\frac{V_{NaOH}\ X\ N_{NaOH} \times BM \times 100\%}{V_{sample} \times 1000} \frac{(Bp + destilat) - Bp\ empty}{(Bp + aquades - Bp\ empty} \quad (3)$$

Antioxidant activity test

Antioxidant activity was tested using the DPPH method referring to Hassmy et al. (2017). In testing the antioxidant activity added 1.1 ml of DPPH solution (10 mg/L) and 2 ml of Ethanol. Then 4 ml of kombucha sample solution was added with variations in concentrations of 50 ppm, 70 ppm, and 110 ppm. Then incubation is carried out for 30 minutes. Then the absorbance was measured at a wavelength of 516 nm. DPPH absorption inhibition calculation is as follows Equation 4 (A_0 is Absorbance control (DPPH +Aquades), and A_s and Sample absorbance and DPPH).

$$\text{\% Inhibition} = \frac{(A_0 - A_S)}{A_0} \times 100 \quad (4)$$

The determination of IC_{50} is determined by plotting the percentage of inhibition obtained into the regression equation y=ax + b where x is the sample volume and y is the value of % inhibition.

Organoleptic test

The process of collecting data on organoleptic tests was carried out using hedonic tests of scent, color, and taste conducted by 15 panelists. The hedonic scale ranges from 1-4 where 1: dislike, 2: somewhat like, 3: like, and 4: very like. Testing was carried out by pouring kombucha wuluh starfruit leaves as many as 3 samples on a container that has been coded to mark the identity of the kombucha tea according to the fermentation time and sugar concentration.

This section must also be able to provide complete procedural information so that it can be carried out by other researchers who will be returning.

All procedures that have been used must use international standard units and terms that are commonly used in scientific research.

Data Analysis

Research data including pH, total levels of titrated acid (tat), phenolic and organoleptic assessment were carried out non-parametric tests with a value of α <0.05. If there are differences, continue with the Mann Whitney test to find out the differences between treatments. Qualitative data, including alcohol content and antioxidant activity were analyzed descriptively.

RESULTS AND DISCUSSION

Characteristics of Physics: Organoleptic Test

The physical characteristics test on kombucha tea was known by conducting hedonic tests, including scent, color, and taste on 15 panelists. Hedonic tests are carried out to determine the acceptance of products in the community as drinks (Nainggolan, 2009). The results of organoleptic test research can be seen in Table 1

Table 1. The kombucha organoleptic test results

Treatment	Organoleptic Test and Standard deviation		
	Scent	Color	Taste
A	1.87±0.834	2.47±0.834	2.33±0.816
B	2.20±1.014	2.67±0.976	3.07±1.033
C	2.27±1.100	2.60±0.986	3.13±0.990

Note: A (fermentation time 4 days), B (fermentation time 8 days), C (fermentation time 12 days). Similar letter notation means there is no real difference in Mann Whitney's test

The results of the scent test statistically with the kruskal wallis test were found to have no significant differences. The results of the hedonic scent test in Table 1 the longer the fermentation time will also experience an increase in the hedonic value of the scent. Wuluh starfruit leaf kombucha tea has a strong sour scent and is quite pungent. The sour scent formed during the fermentation period is caused by the fermentation process of alcohol and acetic acid which causes the formation of volatile compounds that can be felt by the human sense of smell (Nurhidayah, 2018). Color is one of the factors of consumer acceptance of a product because it uses the sense of sight (Leal, Suárez, Jayabalan, Oros, & Escalante-Aburto, 2018; Rosita, Handito, & Amaro, 2021). The results color of wuluh starfruit leaf kombucha can be seen in Figure 1.

The results of the color test statistically with the kruskal wallis test were found to have no significant differences. The results of the color hedonic test in Table 1 the longer the fermentation time will make the hedonic value of the color increase. Wuluh starfruit leaf kombucha tea has a faded color from brownish yellow to clear yellow. According to (Pratiwi & Aryawati, 2012) the color fading in kombucha tea is caused by a change in pH to acid so that more fermentation time results in the decomposition of the components in the solution, namely tannins which are included in phenol compounds are damaged due to the presence of acids so that the concentration is reduced.

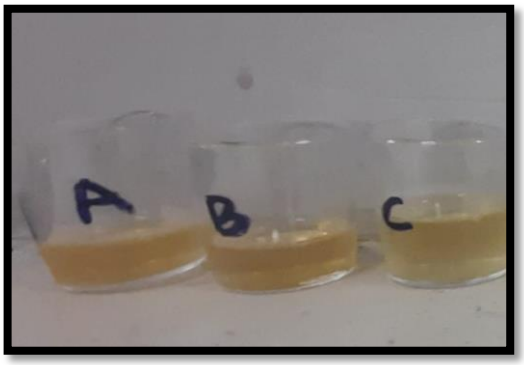

Figure 1. The result of wuluh starfruit leaves kombucha tea with fermentation time treatment A (4 days), B (8 days), and C (12 days)

The results of the taste test statistically with the Kruskal wallis test are known to have significant differences. The results of the color hedonic test in Table 1 the longer the fermentation time will also experience an increase in the hedonic value of the taste. The hedonic value of the most preferred taste is treatment C (fermentation time 12 days). Kombucha wuluh starfruit leaves wuluh treatment C has a slightly sweet and alcoholic sour taste. According to Nasir and Rahmani (2015) fermentation that is carried out long enough will make the sweetness of kombucha decrease because the existing sugar has been fermented. An overhaul of sugar during fermentation by fermentative microorganisms occurs by changing its chemical composition to acids, alcohols, and CO_2.

Chemical characteristics

This research was conducted to determine the effect of fermentation time on the chemical characteristics of kombucha wuluh starfruit leaves tea (*Avverhoa bilimbi* Linn.). Kombucha wuluh starfruit

tea has been observed parameters include pH value, total levels of total titrated acid (TTA), phenolic, and alcohol during the fermentation period of 0 days, 4 days, 8 days, and 12 days. The results of the kruskall wallis test on chemical characteristics are known to show differences in pH values, TTA levels, and phenolic levels of kombucha tea of star fruit leaves (*Avverhoa bilimbi* Linn.). The results of the chemical characteristics test can be seen in the following Table 2 (Note: P1 (fermentation time 0 days), P2 (fermentation time 4 days), P3 (fermentation time 8 days), P4 (fermentation time 12 days)).

Table 2. The kombucha chemical characteristics test results

Treatment	pH± SD	TTA (%) ±SD	Phenolic Levels (mg/ml GAE) ±SD	Alcohol (%)
P1	6a	0.010± 0.001a	58.67± 2.663a	0.11
P2	4b	0.018± 0.025bc	61± 4.52106ab	0.21
P3	3c	0.050 ± 0.025bc	92.53± 1.363bc	0.82
P4	3c	0.110 ± 0.070bc	87.33± 1.140c	0.41

*Similar letter notation means there is no real difference in Mann Whitney's test

The results of the Mann Whitney test at pH in Table 2 are shown by assigning different letters. The letter (a) notation indicates that the treatment of P1 is significantly different from P2, P3 and P4. The letter (b) notation indicates that P2 treatment is significantly different from P1, P3 and P4 treatments. The letter c notation indicates that the P3 treatment is significantly different from P1 and P2 but not significantly different from the P4 treatment.

The pH test results in Table 2 can be seen that the longer the fermentation time, the more the pH value of kombucha decreases. The decrease in pH occurs because during fermentation there are growth and metabolic processes of acetic acid bacteria, lactic acid, and yeast which can increase organic acids (Jayabalan, Malbaša, Lončar, Vitas, & Sathishkumar, 2014). According to Hapsari et al. (2021), the pH value in the fermentation process of kombucha is one of the most important environmental parameters because it is formed from several acid compounds such as acetic acid and gluconic. The acidity of kombucha drinks that are safe for consumption ranges from a pH value of 2.5-4.6 (Naland, 2008).

The effect of acidity estimation on taste and aroma in addition to using pH can be done with a TTA test. While the pH value only measure the total acid under dissociated conditions. It can be concluded that the measurement of TTA is better than pH (Angelia, 2017). The results of the Mann Whitney test at TTA in Table 2 shown by letter notation are known that the letter a notation shows that the treatment on P1 is significantly different from P2, P3 and P4. The letter (bc) notation indicates that P2 is significantly different from P1 and not significantly different from P3 and P4.

The results of the tat test in Table 2 are known that there is an increase in Total Titrated Acid (TTA) during the fermentation time take place. The highest total titrated acid in wuluh starfruit leaf kombucha was in the P4 treatment (fermentation time of 12 days) of 0.110±0,070%. This is comparable to the research that has been carried out by Pratiwi and Aryawati (2012) that there is an increase in total acid levels during the fermentation time in the seaweed kombucha *Saggarsum* sp. The increase in total acid levels is suspected to occur due to sugar in kombucha which will be overhauled in the fermentation process by bacteria and yeast into organic acids.

The results of the Mann Whitney test on phenolic levels in Table 2 shown by letter notation are known that the letter (a) notation indicates that the P1 treatment is significantly different from P3 and P4 treatment, but not significantly different from P2 treatment. The letter (ab) notation indicates that the treatment of P2 is significantly different from P4 but not significantly different from P1 and P3. The (bc) notation indicates that the treatment of P3 significantly different from P1 but not significantly different from P2 and P4. The letter (c) notation indicates that the P4 treatment is significantly different from the P1 and P2 treatments, but not significantly different from the P3 treatment.

Based on the phenolic test result in Table 2, it is known that with the longer fermentation time in kombucha wuluh starfruit leaves tea, it is known that phenolic levels increase until the 8^{th} day of fermentation and will decrease on the 12^{th} day. According to Suhardini and Zubaidah (2016), the increase in phenolic levels is suspected to be due to microbes of the bacterial and yeast groups that can metabolize to produce flavonoid compounds through enzymatic reactions, these conditions affect the total amount of phenols in kombucha tea. According to Ardheniati et al. (2009), the fermentation process causes polyphenol content to decrease due to the oxidation reaction. Decreased phenolic levels are also

associated with a decrease in the number of microbial cells because reduced sugar as an energy source in microbes also decreases.

The fermentation process of kombucha is also subject to oxidative chemical changes by microorganisms within the substrate. As a result, the microorganisms dissolve in the form of a more complex compound and enzymes convert the sugars into alcohols (Herwin, Kosman, & Siami, 2013). The results of the alcohol content test of wuluh starfruit leaf kombucha tea in Table 2 the longer fermentation time causes alcohol to increase. However, the alcohol content will decrease on the 12th day of fermentation. The increase in alcohol content is caused during the fermentation process of the yeast *Saccharomyces cerevisiae* produces alcohol anaerobically, and then alcohol stimulates the growth of *Acetobacter xylinum* to produce acetic acid aerobically, while acetic acid will stimulate the growth of *Saccharomyces cerevisiae*. Then alcohol will be used by *Acetobacter* bacteria for the formation of acetic acid. So kombucha tea has decreased alcohol levels (Pratiwi & Aryawati, 2012).

Antioxidant Activity

Antioxidant activity can be determined by a value of IC_{50}. The results of measuring the IC_{50} are known that all treatments produced kombucha tea with very strong antioxidants. In the antioxidant activity test, wuluh starfruit leaves kombucha tea was analyzed descriptively. The results of the antioxidant activity of wuluh starfruit leave kombucha tea are presented in Table 3.

Table 3. IC_{50} Value Result

Treatment	IC_{50} (ppm)	Information
P1	2.68	Very Strong
P2	4.26	Very Strong
P3	3.77	Very Strong
P4	3.65	Very Strong

Antioxidants are defined as compounds that have the role of delaying, slowing down, and preventing oxidation processes (Simanjutak, 2012). This study was tested for antioxidant activity by determining the IC_{50} value. In this study, it was known that there was a relationship between phenolic and Tat levels to antioxidant activity in wuluh starfruit kombucha. It is known that higher the phenolic and Tat levels make the IC_{50} values low which indicates that the activity on kombucha is getting stronger during the fermentation time. According to (Rustiah & Umriani, 2018) phenol content has a positive linear relationship with the content of antioxidant activity. Because phenol compounds such as phenolic acid are natural antioxidants in a plant. This is related to the Kombucha starter which produces several enzymes such as proteases, lipases, and polyphenol oxidase which can catalyze the biodegradation of the flavin and hydroxylates which are powerful antioxidants (Chu & Chen, 2006).

The IC_{50} value is the concentration of the extract needed to dampen 50% of the total DPPH. The results of the IC_{50} value test in Table 3 are known that the antioxidant activity in wuluh starfruit leaf kombucha tea has an IC_{50} value <50 so it can be said that in all treatments it produces strong antioxidant compounds. According to Tristantini et al. (2016), a compound is said to be a very strong antioxidant if the IC_{50} value is less than 50, strong (50-100), and weak (151-200). So it can be concluded that the smaller the IC_{50} value, the higher the antioxidant activity. In the figure, it is known that at the fermentation time of the 4th to the 8th day, it is known that there is a decrease in antioxidant activity marked by an increase in the value of IC_{50}. According to Hunandar (2017), the decrease in antioxidant activity can be caused because, during the fermentation process, new phenol compounds have been formed, but these compounds cannot inhibit free radicals. This happens because polyphenol compounds such as catechins are lost and further polymerized into higher molecular weight molecules leading to the detection of lower polyphenol content. At the time of fermentation on the 12th day, it is known that the antioxidant activity gradually increases again. The increase in antioxidant activity is due to the biotransformation process of several phenol compounds due to the presence of enzymes produced during the kombucha fermentation process and the release of catechins from microorganisms that are sensitive to acids (Hunandar, 2017). It can be concluded that the longer the fermentation of the IC_{50} value will reach the optimum point then decrease.

CONCLUSION

Fermentation time affects the physical, and chemical characteristics and antioxidant activity of wuluh starfruit leaf kombucha tea. Wuluh starfruit leaf kombucha tea which has the best physical, and chemical characteristics and antioxidant activity is in the P4 treatment (fermentation time 12 days) with a hedonic value of scent is 2.27 ± 1.100 with color value is 2.60, taste value is 3.13, pH value is 3, the total titrated acid level is 0.110 ± 0.070%, the phenolic

level is 87.33±1.140 mg/ml GAE, alcohol is 0.41%, and IC_{50} value is 3.65 ppm.

REFERENCES

Andriani, M., Gde Mayun Permana, I. D., & Rai Widarta, I. W. (2019). Pengaruh Suhu dan Waktu Ekstraksi Daun Belimbing Wuluh (*Averrhoa Bilimbi* L.) Terhadap Aktivitas Antioksidan dengan Metode Ultrasonic Assisted Extraction (UAE) Method. *Jurnal Ilmu Dan Teknologi Pangan*, *8*(3), 330–340.

Angelia, I. O. (2017). Kandungan Ph, Total Asam Tertitrasi, Padatan Terlarut dan Vitamin C Pada Beberapa Komoditas Hortikultura (pH Content, Total Acidified Acid, Dissolved Solids and Vitamin C in Some Horticultural Commodities). *Journal of Agritech*, *1*(2), 68–74.

Ardheniati, M., Andriani, M. A. M., & Amanto, B. S. (2009). Fermentation Kinetics in Kombucha Tea With Tea Kind Variation Based on its Processing. *Biofarmasi Journal of Natural Product Biochemistry*, *7*(1), 48–55.

Cahanar, P., & Suhanda, I. (2006). *Makan sehat hidup sehat*. Jakarta: Published by Kompas.

Cahyaningtyas, Y. D. W. (2018). *Pengaruh Lama Fermentasi Terhadap Total Asam Tertitrasi (TAT) dan Karakteristik Fisik (Uji Organoleptik) Pada Kombucha Serai (Cymbopogon citratus (DC.))*. Thesis, Sanata Dharma Yogyakarta.

Chu, S.C., & Chen, C. (2006). Effects Of Origins and Fermentation Time on the Antioxidant Activities of Kombucha. *Food Chemistry*, *98*(3), 502–507.

Fadeyi, A. E., Adeniran, O. I., & Akiode, S. O. (2022). Nutrients, Phytochemical, Antioxidant and Antimicrobial Analysis of Pterocarpus Osun Stem Bark and Leaf for Their Nutritional, Medicinal Capacity. *Indo. J. Chem. Res.*, *10*(1), 58–67. https://doi.org/10.30598/ijcr.2022.10-ade

Falahuddin, I., Apriani, I., & Nurfadilah. (2017). Pengaruh Proses Fermentasi Kombucha Daun Sirsak (*Annona muricata* L.) Terhadap Kadar Vitamin C. *Jurnal Biota*, *3*(2), 90–95.

Firdaus, S., C, A. I., Isnaini, L., & Aminah, S. (2020). "Review" Teh Kombucha Sebagai Minuman Fungsional dengan Berbagai Bahan Dasar Teh. *Prosiding Seminar Nasional Unimus*, *3*, 715–730.

Goh, W. N., Rosma A., Kaur, B., Fazilah, A., Karim, A. A., & Bhat, R. (2012). Fermentation of Black Tea Broth (Kombucha): I. Effects of Sucrose Concentration and Fermentation Time on The Yield of Microbial Cellulose. *International Food Research Journal*, *19*(1), 109–117.

Hapsari, M., Rizkiprilisa, W., & Sari, A. (2021). Pengaruh Lama Fermentasi Terhadap Aktivitas Antioksidan Minuman Fermentasi Kombucha Lengkuas Merah (Alpinia purpurata). *Agromix*, *12*(2), 84–87.

Hassmy, N. P., Abidjulu, J., & Yudistira, A. (2017). Analisis Aktivitas Antioksidan Pada Teh Hijau Kombucha Berdasarkan Waktu Fermentasi Yang Optimal. *PHARMACON: Jurnal Ilmiah Farmasi-UNSRAT*, *6*(4), 67–74.

Herwin, H., Kosman, R., & Siami, I. (2013). Produksi Sediaan Kombucha dari Daun Permot (*Passiflora foetida* L) Secara Fermentasi. *Jurnal Ilmiah As-Syifaa*, *5*(1), 20–27.

Hunandar, V. S. (2017). Penetapan Daya Antioksidan dan Kadar Total Fenol Kombucha Dibandingkan Teh Hijau Secara Spektrofotometri. *CALIPTRA: Jurnal Ilmiah Mahasiswa Universitas Surabaya (Maret)*, *5*(2), 435–445.

Jayabalan, R., Malbaša, R. V., Lončar, E. S., Vitas, J. S., & Sathishkumar, M. (2014). A review of Kombucha Tea-Microbiology, Composition, Fermentation, Beneficial Effects, Toxicity, and Tea Fungus. *Comprehensive Reviews in Food Science and Food Safety*, *13*(4), 538–550.

Leal, J. M., Suárez, L. V., Jayabalan, R., Oros, J. H., & Escalante-Aburto, A. (2018). A Review on Health Benefits of Kombucha Nutritional Compounds and Metabolites. *CYTA - Journal of Food*, *16*(1), 390–399.

Mahardika, R. G., & Roanisca, O. (2018). Aktivitas Antioksidan dan Fitokimia dari Ekstrak Etil Asetat Pucuk Idat (Cratoxylum glaucum). *Indo. J. Chem. Res.*, *5*(2), 69–74.

Misfadhila, S., Chandra, B., & Wahyuni, Y. (2020). Pengaruh Fraksi Air, Etil Asetat Dan N-Heksan Dari Ekstrak Etanol Daun Belimbing Wuluh (*Averrhoa bilimbii* L) Terhadap Kelarutan Kalsium Batu Ginjal Secara In Vitro. *Jurnal Farmasi Higea*, *12*(2), 115–123.

Muhialdin, B. J., Osman, F. A., Muhamad, R., Che Wan Sapawi. C. W. N. S., Anzian, A., Voon, W. W. Y., & Meor Hussin, A. S. (2019). Effects Of Sugar Sources and Fermentation Time on The Properties of Tea Fungus (Kombucha) Beverage. *International Food Research Journal*, *26*(2), 481–487.

Nainggolan, J. (2009). Kajian Pertumbuhan Bakteri Acetobacter sp. Dalam Kombucha-Rosela Merah (Hibiscus sabdariffa) pada Kadar Gula dan Lama Fermentasi yang Berbeda. *Tesis*, 1–103.

Naland, H. (2008). *Kombucha; Teh dengan Seribu Khasiat*. Jakarta Selatan: Agromedia.

Nasir, Muh., & Rahmani, St. (2015). Uji Organoleptik Teh Kombucha dari Berbagai Jenis Teh dan Waktu Fermentasi Yang Berbeda. *Oryza Jurnal Pendidikan Biologi*, *4*(1), 6–14.

Nurhayati, N., Yuwanti, S., & Urbahillah, A. (2020). Karakteristik Fisikokimia dan Sensori Kombucha *Cascara* (Kulit Kopi Ranum). *Jurnal Teknologi dan Industri Pangan*, *31*(1), 38–49.

Nurhidayah. (2018). *Pengaruh Lama Fermentasi Terhadap Mutu Kombucha Sari Buah Nanas*. Dowload from: http://eprints.unram.ac.id/7916/1/Artikel%20Kombucha%20Sari%20Buah%20Nanas.pdf

Pourmorad, F., Hosseinimehr, S. J., & Shahabimajd, N. (2006). Antioxidant Activity, Phenol, and Flavonoid Contents of Some Selected Iranian Medicinal Plants. *African Journal of Biotechnology*, *5*(11), 1142–1145.

Pratiwi, A., & Aryawati, R. (2012). Pengaruh Waktu Fermentasi Terhadap Sifat Fisik dan Kimia pada Pembuatan Minuman Kombucha dari Rumput Laut Sargasssum sp. *Maspari Journal*, *4*(1), 131–136.

Purnami, K. I., Anom Jambe, A. A. G. N., & Wayan Wisaniyasa, N. (2018). Pengaruh Jenis Teh Terhadap Karakteristik Teh Kombucha. *Jurnal ITEPA*, *7*(2), 1–10.

Rosita, Handito, D., & Amaro, M. (2021). Pengaruh Konsentrasi Starter Scoby (Symbiotic Culture Of Bacteria And Yeast) Terhadap Total Mikroba, Total Khamir dan Organoleptik Kombucha Sari Buah Apel. *Pro Food (Jurnal Ilmu Dan Tekonologi PAngan*, *7*(2), 12–22.

Rustiah, W., & Umriani, N. (2018). Uji Aktivitas Antioksidan Pada Ekstrak Buah Kawista (Limonia Acidissima) Menggunakan Spektrofotometer UV-Vis. *Indonesian Journal of Chemical Research*, *6*(1), 22–25.

Simanjutak, K. (2012). Peran Antioksidan Flavonoid Dalam Meningkatkan Kesehatan. *Bina Widya*, *23*(3), 135–140.

Skoog, D. A. (1985). *Principles of Instrumental Analysis*. Philadelphia: Saunder Collage Publishing.

Suhardini, P. N., & Zubaidah, E. (2016). Studi Aktivitas Antioksidan Kombucha Dari Berbagai Jenis Daun Selama Fermentasi Study of Antioxidant Activity on Various Kombucha Leaves During Fermentation. *Jurnal Pangan dan Agroindustri*, *4*(1), 221–229.

Sun, T.Y., Li, J.-S., & Chen, C. (2015). Effects of Blending Wheatgrass Juice on Enhancing Phenolic Compounds and Antioxidant Activities of Traditional Kombucha beverage. *Journal of Food and Drug Analysis*, *23*(4), 709–718.

Tristantini, D., Ismawati, A., Tegar Pradana, B., & Gabriel Jonathan, J. (2016). Pengujian Aktivitas Antioksidan Menggunakan Metode DPPH pada Daun Tanjung (Mimusops elengi L). *Prosiding Seminar Nasional Teknik Kimia "Kejuangan" Pengembangan Teknologi Kimia Untuk Pengolahan Sumber Daya Alam Indonesia*, 1–7. Yogyakarta.

Villarreal-Soto, S. A., Beaufort, S., Bouajila, J., Souchard, J. P., & Taillandier, P. (2018). Understanding Kombucha Tea Fermentation: A Review. *Journal of Food Science*, *83*(3), 580–588.

Wistiana, D., & Zubaidah, E. (2015). Karakteristik Kimiawi Dan Mikrobiologis Kombucha Dari Berbagai Daun Tinggi Fenol Selama Fermentasi. *Jurnal Pangan Dan Agroindustri*, *3*(4), 1446–1457.