

Chemical and Organoleptic Quality of Kombucha Tea at Different Fermentation Time and Concentration of Cherry Leaves (*Muntingia calabura* L.)

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Abstract. The leaves of the cherry plant (*Muntingia calabura* L.) contain vitamin C and phenolic compounds. High phenolic compounds have the potential to have antioxidants. Efforts to increase antioxidant activity can be carried out by fermentation using SCOBY. The purpose of this study was to determine the effect of cherry leaf concentration and fermentation time on the chemical and organoleptic quality of cherry leaf kombucha tea, and to determine the best treatment for cherry leaf kombucha tea. This study used a completely randomized design (CRD) which was arranged in a factorial manner with 2 factors. The first factor is the fermentation time with 3 levels (4 days, 8 days, 12 days). The second factor is the concentration of cherry leaves with 3 levels (1%, 2%, 3%). Each treatment combination was repeated 3 times. The chemical analysis of cherry leaf kombucha that will be tested is the total phenol content using the Folin Ciocalteau reagent with the spectrophotometric method, antioxidant activity with the DPPH method, and vitamin C with the iodine titration method. Analysis of organoleptic quality was carried out by testing the level of preference / hedonic test which included color, aroma, and taste. Determination of the best treatment is done by using the effectiveness test. K3L2 treatment with 3% cherry leaf concentration and 8 days of fermentation gave the best treatment results, namely 0.71, which means that this treatment is the best treatment with parameter criteria including antioxidant 109.77 ppm, phenol 9.42 mg GAE/g, vitamin C 22.92 ppm, taste 3.85 (neutral), scent 3.00 (rather dislike), and color 5.00 (rather like).

Keywords: Cherry Leaves, Fermentation, Kombucha, SCOBY

1 Introduction

The development of science and technology has made people increasingly aware that tea contains various compounds that are very beneficial for health. What is known as herbal tea is a tea drink that made from other plants beside the tea plant (*Camellia sinensis*) [1]. Herbal teas can be made from plant parts such as dried leaves and have healthful effects on the body, one of which is the cherry plant.

Kersen plant (*Muntingia calabura* L.) is a plant that is easy to find in Indonesia because it grows fast, spreads easily, is widely used as a shade and medicinal plant. The leaves have several benefits, including lowering cholesterol, lowering uric acid levels in the body, lowering high blood pressure, and anti-diabetic. This is because cherry leaves contain high levels of flavonoids, phenolics and vitamin C [2], so they can function as antioxidants. The high component of phenolic compounds in cherry leaves has the potential to be a strong antioxidant. Antioxidants are useful for neutralizing free radicals, inhibiting the growth of cancer cells, and lowering cholesterol levels [3].

Flavanols are phenolic compounds that are difficult to digest in the human body [4], therefore to facilitate absorption it is necessary to break them down by enzymes or microbes so that they are easily absorbed by the body, namely by a fermentation process using SCOBY/kombu mushrooms [5]. Kombucha fermentation is carried out for 7-14 days with temperatures between 22-30°C, during the fermentation time the biological activity can continue to increase [6]. The kombucha fermentation process begins when microorganisms convert glucose into ethanol and CO₂, then react with water to form carbonic acid. The fermentation process can produce new compounds, increase nutritional value, change the aroma and taste of these foodstuffs [7].

[8] showed that cherry leaf kombucha tea with different concentrations (2,4,6, and 8%) during seven days of fermentation experienced an increase in total acid 1.586%, vitamin C 6.725 mg/100 ml, reducing sugar 7.194 mg/ml, and antioxidant content of 98.251%, but decreased in the degree of acidity of 3.563 and organoleptic taste of 1.475. [9] showed that cherry leaf tea made from 20 mg of simplicia fermented for 10 days resulted in a total flavonoid content of 44.027 mg/g quercetin and an IC₅₀ value of 7.66 ppm in the very strong category. [10] showed

that cherry leaf kombucha at a concentration of 2% which was fermented for 10 days resulted in cherry leaf kombucha having vitamin C 123.16 mg/100g, total phenol 6.21 mg TAE/g and antioxidant levels of 44.13%. Based on the description above, the purpose of this study was to determine the effect of cherry leaf concentration and fermentation time on the chemical and organoleptic quality of cherry leaf kombucha tea, and to determine the best treatment for cherry leaf kombucha tea.

2 Research Method

2.1 Material

The main ingredient for making tea is fresh dark green cherry leaves obtained from the village of Pucanganom, Sidoarjo. The materials used for making kombucha are kombu mushroom and kombucha root obtained from wikikombucha.com. The supporting materials needed include water and Gulaku granulated sugar.

Materials used for chemical analysis include aquadest, Folin Ciocalteu reagent, NaCl, Na₂CO₃, FeCl₃, DPPH, ethanol solution, iodine solution, and starch solution.

2.2 Tool

The tools used for sample preparation include scissors, winnowing bowl, basin, measuring cup, digital scale, stainless steel knife, spatula, strainer, teapot, glass jar, cloth and rubber. The tools used for chemical analysis include dropper pipettes, measuring pipettes, erlenmeyer, test tubes, test tube racks, measuring flasks, and UV-Vis spectrophotometers.

2.3 Experimental Design

The experimental design used in this study was a completely randomized design (CRD) which was arranged in a factorial manner with 2 factors. Factor 1: Fermentation time/L consisting of 3 levels (L1 = 4 days, L2 = 8 days and L3 = 12 days) and factor 2: Cherry leaf tea concentration/K consisting of 3 levels (K1 = 1%, K2 = 2% and K3 = 3%) so that there were 9 treatment combinations, each treatment was repeated three times.

2.4 Chemical Analysis and Organoleptic Test

The chemical parameters measured in this study were total phenol levels [11], antioxidant activity [12], and vitamin C tests [13]. Organoleptic tests include color, taste, and aroma using 25 panelists with a scale of preference level, namely 1 = really dislike, 2 = dislike, 3 = somewhat dislike, 4 = neutral, 5 = rather like, 6 = like, 7 = very much Like.

2.5 Data Analysis

The parametric data (total phenol levels, antioxidant activity and vitamin C) obtained were analyzed using the Analysis of variance / two way ANOVA using the SPSS application. If the results of the analysis between treatments show significant or very significant differences, then a follow-up test is carried out with the Least Significant Difference (LSD) test if the Coefficient of Diversity/KK value is below 5% or can be carried out with the Honest Significant Difference test/BNJ if the CC value is 5-10%. or by Duncan's test if the KK value is above 10% [14].

Analysis of non-parametric data obtained from organoleptic tests including color, aroma, and taste was analyzed based on the preference level test/hedonic test. determine the best treatment [15].

3 Result and Discussion

3.1 Antioxidant Activity

The average results of the analysis of antioxidant activity tests can be seen in table 1 below. Based on the results of the analysis of variance, it was shown that differences in cherry leaf concentration and fermentation time had a very significant effect on the antioxidants of kombucha tea (counted F value > F table at $\alpha = 0.05$), so that it was continued with the Honest Significant Difference (BNJ) test to find out the differences between treatments.

Table 1. Average antioxidant activity of cherry leaf kombucha tea

Treatment		Average antioxidants
Concentration cherry leaves (K)	Long fermentation (L)	
1 % (1)	4 days (1)	330.52 ^{c,d}
	8 days (2)	135.32 ^a
	12 days (3)	281.80 ^{b,c}
2% (2)	4 days (1)	366.49 ^d
	8 days (2)	126.86 ^a
	12 days (3)	241.47 ^b
3% (3)	4 days (1)	312.48 ^{c,d}
	8 days (2)	109.77 ^a
	12 days (3)	234.53 ^b

Note: if there are the same letters behind the numbers, it indicates that there is no difference between treatments in the BNJ test $\alpha = 0.05$

Cherry leaf kombucha tea with 3% cherry leaf concentration and 8 days of fermentation gave the highest antioxidant activity level, 109.77 ppm, while 2% cherry leaf concentration with 4 days of fermentation gave the lowest antioxidant activity, 366.49 ppm. This shows that the more concentration of cherry leaf, the higher antioxidant activity in cherry leaf kombucha tea, but the longer the cherry leaf kombucha tea is fermented, the antioxidant activity will decrease.

Antioxidants have the ability to prevent free radicals. Free radical compounds can potentially damage DNA so that they can form cancer cells [16][17]. Fermented tea or kombucha tea has a higher antioxidant potential compared to regular tea [13]. The increase in antioxidant activity in cherry leaf kombucha tea was caused by the results of the metabolism of microorganisms in kombucha tea during the fermentation process. Antioxidant activity decreased on day 12, this was because the acids in kombucha tea made the phenolic compounds more stable and difficult to release protons that could bind to DPPH, so that antioxidant activity decreased [18].

3.2 Vitamin C

The mean results of the vitamin C test are presented in table 2 and the results of the analysis of variance show that differences in cherry leaf concentration, fermentation time, and the interaction of the two have a very significant effect on the kombucha tea's vitamin C (F count > F table at $\alpha = 0.05$). so that it is continued with the Honest Significant Difference (BNJ) test to find out the differences between treatments.

Table 2. Average Vitamin C for Kombucha cherry leaf tea

Treatment		Average vitamin C
Concentration cherry leaves (K)	Long fermentation (L)	
1 % (1)	4 days (1)	13.29 ^{a,b,c}
	8 days (2)	12.24 ^{a,b}
	12 days (3)	11.37 ^a
2% (2)	4 days (1)	15.56 ^c
	8 days (2)	21.16 ^{d,e}
	12 days (3)	14.51 ^{b,c}
3% (3)	4 days (1)	15.08 ^c
	8 days (2)	22.92 ^e
	12 days (3)	18.85 ^d

Note: if there are the same letters behind the numbers, it indicates that there is no difference between treatments in the BNJ test $\alpha = 0.05$

Cherry leaf kombucha tea with 3% cherry leaf concentration and 8 days of fermentation gave the highest vitamin C content, 22.92 ppm, while 1% cherry leaf concentration with 12 days of fermentation gave the lowest vitamin C content, 11.37 ppm. This shows that the more concentration of cherry leaves, the higher levels of vitamin C in cherry leaf kombucha tea, but the longer the cherry leaf kombucha tea is fermented, the vitamin C levels will decrease.

Vitamin C is a vitamin that can increase endurance. Vitamin C is a natural antioxidant. [19] states that one of the organic acids produced in the kombucha fermentation process is ascorbic acid or vitamin C. Vitamin C is

related to antioxidant activity, because vitamin C is a metabolite of kombucha fermentation [20]. Optimum vitamin C levels on the 8th day of fermentation had the best value in each treatment with different concentrations of cherry leaves and decreased levels of vitamin C on the 12th day. This is the same as [21] stating that kombucha fermentation for 4-12 days produces good vitamin C levels on the 8th day. The decrease in vitamin C levels on the 12th day was due to vitamin C being damaged due to the activity of bacteria capable of producing the enzyme L-gulonolactone oxidase which plays a role in converting L-gulonolactone oxidase to 2-keto-L-gulonolactone the final stage in the synthesis of vitamin C [22].

3.3 Phenol Compounds

Table 3 and the results of the analysis of variance (F count > F table $\alpha = 0.05$) shows that differences in cherry leaf concentration and fermentation time have a very significant effect on the phenolic compounds of cherry leaf kombucha tea, so it is continued with the Honest Significant Difference (BNJ) test to know the difference between treatments.

Table 3. Average total phenol levels of kombucha tea

Treatment		Average rate total phenol
Concentration cherry leaves (K)	Long fermentation (L)	
1 % (1)	4 days (1)	2.77 ^a
	8 days (2)	3.84 ^{b,c}
	12 days (3)	3.83 ^{b,c}
2% (2)	4 days (1)	2.80 ^a
	8 days (2)	6.55 ^d
	12 days (3)	4.43 ^c
3% (3)	4 days (1)	3.55 ^b
	8 days (2)	9.42 ^c
	12 days (3)	4.51 ^c

Note: if there are the same letters behind the numbers, it indicates that there is no difference between treatments in the BNJ test $\alpha = 0.05$

Cherry leaf kombucha tea with a concentration of 3% cherry leaves and a fermentation time of 8 days gave the highest value of phenolic compounds, namely 9.42 mg GAE/100 g, while the treatment with a concentration of 2% cherry leaves with a duration of 4 days of fermentation gave a low value of phenolic compounds, namely 2.80 mg GAE/100 g. This shows that the more concentration of cherry leaves, the higher levels of phenolic compounds in cherry leaf kombucha tea, but the longer cherry leaf kombucha tea is fermented, the levels of phenol compounds will decrease. Optimum levels of phenolic compounds on the 8th day of fermentation had the best value in each treatment with different concentrations of cherry leaves and decreased phenol levels on the 12th day. The increase in levels of phenolic compounds is due to the potential for microorganisms in kombucha to decarboxylate cinnamic acid components such as trans-4-hydroxy-methoxycinnamic acid (ferulic acid (FA)) and trans-4-hydroxynnamic acid (p-coumaric acid (PCA)) to synthesize phenolic compounds namely 4-vinyguaicol (4-VG) and 4-vinylphenol (4-Vp) [23].

Phenol compounds have antioxidant activity that can neutralize free radicals that damage body tissues and cells. The higher the phenolic content produced, the higher the antioxidant activity [24].

3.4 Taste Organoleptic Test Results

Table 4 shows that the treatment with a concentration of 3% cherry leaf with a 12-day fermentation time gave the highest preference value for taste, namely 6.05, which means that cherry leaf kombucha tea was considered liked by the panelists, while the 3% cherry leaf concentration treatment with 8 days of fermentation gave a preference value. for the lowest taste, namely 3.85, which means that cherry leaf kombucha tea is considered neutral by the panelists.

Table 4. Average organoleptic test results for taste, aroma and color of cherry leaf kombucha tea

Treatment		Average taste value	Average aroma value	Average color values
Concentration cherry leaves (K)	Long fermentation (L)			
1 % (1)	4 days (1)	4.68	5.15	5.15
	8 days (2)	4.55	3.77	4.88
	12 days (3)	5.07	4.52	4.93
2% (2)	4 days (1)	4.55	4.25	5.17
	8 days (2)	4.20	3.50	4.95
	12 days (3)	5.10	4.25	5.15
3% (3)	4 days (1)	3.90	3.85	5.32
	8 days (2)	3.85	3.00	5.00
	12 days (3)	5.35	3.83	6.05

The taste of a product is a determinant in the level of consumer and panelist acceptance [25]. Carbonic acid has a carbonating effect on kombucha tea so it will give it a fresh sour taste. The results of the Kruskal Wallis test showed that the value of $p = 0.003 < \alpha = 0.05$ indicated that the taste value was significantly different between each treatment. Differences in cherry leaf concentration and fermentation time affected the level of panelist acceptance of the organoleptic taste of cherry leaf kombucha tea. The longer the fermentation of cherry leaf kombucha tea caused by a decrease in pH that caused the sour taste, especially the distinctive and strong acetic acid taste in kombucha tea, to be less liked by the panelists.

3.5 Aroma Organoleptic Test Results

Table 4 shows that the treatment with a 1% concentration of cherry leaves with a 4-day fermentation time gave the highest preference value for aroma, namely 5.15, which means that the cherry leaf kombucha tea was considered somewhat favorable by the panelists, while the 3% cherry leaf concentration treatment with a fermentation time of 8 days gave a value the lowest preference for aroma is 3.00, which means that the cherry leaf kombucha tea is considered somewhat disliked by the panelists.

The aroma in kombucha tea is caused by the volatile compounds that are formed resulting in a distinctive and strong sour aroma. Cherry leaf kombucha tea has a strong, sour aroma. The fermentation process of cherry leaf kombucha tea gives rise to a strong sour aroma due to the formation of components of acetic acid, formic acid and other organic acids and the alcohol that is formed can have a refreshing aroma effect [24]. The results of the Kruskal Wallis test showed that the value of $p = 0.006 < \alpha = 0.05$ indicated that the aroma value was significantly different between each treatment. Differences in cherry leaf concentration and fermentation time affected the level of panelist acceptance of the organoleptic aroma of cherry leaf kombucha tea. The distinctive aroma caused some panelists to dislike cherry leaf kombucha tea because it was their first time drinking kombucha tea, so they were not familiar with the aroma.

3.6 Organoleptic Test Results Color

Table 4 above shows that the treatment with a concentration of 3% cherry leaf with a 12-day fermentation time gave the highest preference value for color, namely 6.05, which means that the cherry leaf kombucha tea was considered liked by the panelists, while the 1% cherry leaf concentration treatment with a fermentation time of 8 days gave a value the lowest preference for color is 4.88, which means that the cherry leaf kombucha tea is considered quite favorable by the panelists.

The duration of fermentation will affect the color of the cherry leaf kombucha tea. The longer fermentation and the less concentration of cherry leaves, the clearer color will be. [26] stated that color degradation occurs because the microorganisms in kombucha tea utilize dissolved solids as energy so that over time the solvent in the media will run out and the liquid will become clear or colorless. The results of the Kruskal Wallis test showed that the value of $p = 0.61 > \alpha = 0.05$ indicated that the color value was not significantly different between each treatment. Differences in cherry leaf concentration and fermentation time did not affect the level of panelist acceptance of the organoleptic color of cherry leaf kombucha tea.

3.7 Effectiveness Test Results

The results of the effectiveness test on all research parameters are presented in Table 5 below.

Table 5. The value of the effectiveness test results on the research parameters

Treatment	Result value (NH) Treatment								
	K1L1	K1L2	K1L3	K2L1	K2L2	K2L3	K3L1	K3L2	K3L3
Antioxidant	0.03	0.16	0.06	0	0.17	0.09	0.04	0.18	0.09
Vitamin C	0.03	0.01	0	0.07	0.15	0.05	0.06	0.18	0.12
Fenol	0	0.03	0.03	0.001	0.1	0.05	0.02	0.18	0.05
Taste	0.09	0.07	0.13	0.07	0.04	0.13	0.01	0	0.16
Aroma	0	0.1	0.05	0.07	0.12	0.07	0.1	0	0.1
Color	0.03	0	0.01	0.03	0.01	0.03	0.05	0.01	0.14
Total	0.18	0.38	0.27	0.24	0.59	0.41	0.27	0.71	0.65

The table above shows that the K3L2 treatment with 3% cherry leaf concentration and 8 days of fermentation gave the highest Yield Value (NH), namely 0.71, which means that this treatment is the best treatment with parameter criteria including antioxidants 109.77 ppm, phenol 9.42 mg GAE/ g, vitamin C 22.92 ppm, taste 3.85 (neutral), aroma 3.00 (rather dislike), and color 5.00 (rather like).

4 Conclusion

The results of the study on "Different Fermentation Time and Concentration of Cherry Leaves (*Muntingia calabura* L.) on the Chemical and Organoleptic Quality of Kombucha Tea" can be concluded as follows.

1. Treatment of different concentrations of cherry leaves and fermentation time had a significant effect on the activity of antioxidants, vitamin C and phenolic compounds.
2. The results of the Kruskal Wallis test on the organoleptic of cherry leaf kombucha tea showed that different concentrations of cherry leaf and fermentation time had a significant effect on taste and aroma, while color had no significant effect.
3. Based on the effectiveness test showed that the K3L2 treatment with 3% cherry leaf concentration and 8 days of fermentation time gave the highest Yield Value (NH), namely 0.71, which means that the treatment is the best treatment with parameter criteria including antioxidants 109.77 ppm, phenol 9.42 mg GAE/g, vitamin C 22.92 ppm, taste 3.85 (neutral), aroma 3.00 (rather dislike), and color 5.00 (rather like).

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