Agroteknika 8 (2): 276-287 (2025)

AGROTEKNIKA

ISSN: 2685-3450 (*Online*) www.agroteknika.id ISSN: 2685-3450 (*Print*)

Antioxidant Activity and Organoleptic Quality of Purple Chrysanthemum (*Chrysanthemum morifolium*) Kombucha on the Variation of Sugar Types and Fermentation Duration

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Abstract. *Kombucha is one of the probiotic beverages resulting from the fermentation of tea with a consortium of bacteria and yeast (Symbiotic Culture of Bacteria and Yeast/SCOBY) as the starter culture, requiring sugar as a source of nutrients and carbon. One innovation of kombucha raw materials is purple chrysanthemum flowers containing active compounds such as carotenoids, flavonoids, and anthocyanins. The aim of this study was to determine the antioxidant activity and organoleptic quality of kombucha from purple chrysanthemum flowers on the variation of sugar types and fermentation duration. This research method used an experimental method with 2 factors, Completely Randomized Design (CRD). Factor I: variation of sugar types (G): palm sugar and Javanese sugar. Factor II: fermentation duration (F): 5 days and 7 days, with green tea kombucha as a control. Data analysis of antioxidant activity and vitamin C used quantitative descriptive methods, while organoleptic quality used qualitative methods. It was concluded that the highest antioxidant activity and the best organoleptic quality of purple chrysanthemum kombucha was 76.37% with brown color, quite fragrant of kombucha, quite sour taste, and preferred by the panelists in the G1F2 treatment (200g of palm sugar with a fermentation duration of 7 days).*

Keywords: *antioxidant activity, fermentation duration, kombucha, organoleptic quality, purple chrysanthemum flower, sugar types.*

1. Introduction

Probiotic drinks are one of the functional and beneficial beverages for health, contain Lactic Acid Bacteria (LAB) that can produce lactic acid (Zubaidah *et al*., 2021). One of the potential probiotic drinks to be developed currently is kombucha. Kombucha resulted from tea fermentation with consortium of bacteria and yeast (Symbiotic Culture of Bacteria and Yeast / SCOBY) as starter culture, which requires sugar as source of nutrients and carbon. SCOBY bacteria will convert sugar into organic acids, while yeast will break down sugar into CO_2 and low levels of ethanol (Fadillah, 2022). Generally, the raw materials commonly used for kombucha are green tea,

Diterima: 9 April 2025; Disetujui: 5 Mei 2025; Diterbitkan: 25 Juni 2025
https://doi.org/10.55043/agroteknika.v8i2.523

black tea, and fruit-infused water. However, there have been many variations of kombucha raw materials, and the one potential flower to be developed for kombucha is purple chrysanthemum flower.

Purple chrysanthemum flowers (*Chrysanthemum morifilium*) belong to Asteracea Family, rich in anthocyanins and antioxidants (Safitri, 2023). Purple chrysanthemum flowers have the potential to be used as traditional medicine because of containing carotenoid compounds 0.99-3.12 µg/g, flavonoids 49.376 mg/g (Ryu *et al.*, 2019), and antioxidant activity showing inhibition properties ranging from 61%-76% with ATBS (Antioxidant Test by Spectrophotometry) and DPPH (2,2-difenil-1-pikrilhidrazil) tests (Han *et al.*, 2019). Purple chrysanthemum flower have been widely used as herbal tea bags (Sharma *et al.*, 2023) and can also be utilized as an ingredient of sunscreen lotion, containing potential antioxidants to neutralize free radicals from ultraviolet rays (Lumintang *et al*., 2022). But, there is not currently research on kombucha using purple chrysanthemum flower as the main ingredient.

One of the important factors in the process of making kombucha is sugar, which serves as a source of carbon and energy for SCOBY (Fadillah, 2022). The difference type of sugar can affect pH, antioxidants, and organoleptic qualities of kombucha yield (Verawati, 2019). Palm sugar processed from the sap of male flower clusters of sugar palm. Palm sugar has many benefits such as being natural food coloring, high content of fiber which is good for digestion, and inhibiting the absorption of cholesterol body (Assah, 2020). The sucrose content of palm sugar is 95%, reducing sugars 0.53%, protein 2.28%, minerals 3.66%, and calories 368 kcal (Yudho, 2021). Whereas, Javanese sugar is produced by tapping coconut sap. Javanese sugar or brown sugar contains 97.33 g of carbohydrates, 377 kcal of calories, 65-84% sucrose, 10-15% reducing sugars, vitamin A, vitamin E, and vitamin B12 (Liu *et al*., 2022). Palm sugar and Javanese sugar have a low glycemic index. The glycemic index of palm sugar is 35, while the glycemic index of Javanese sugar is 54. Sugar with a low glycemic index is better for blood sugar health (Framita et al., 2021). Duration of fermentation also affects the kombucha process. The fermentation period required to transform tea solution into kombucha product takes about 4-14 days (Gumanti *et al*., 2023). During the fermentation process, bacteria and yeast will break down sucrose into glucose and fructose. Glucose is used to form alcohol and organic acids, thereby increasing the acidity level of kombucha. Yeast activity uses glucose and produces alcohol. The standard alcohol level of kombucha is less than 0.5% (Suhardini & Zubaidah, 2016). The longer the fermentation duration, the higher the organic acids produced. This occurs because bacteria obtain food from the added sugar for growth and development (Dayanti, 2024). The increase of acetic acid in kombucha can affect antioxidant activity. In an acidic condition, phenolic compounds will become stable and will

have difficulty releasing protons associated with DPPH (2,2-difenil-1-pikrilhidrazil), leading to decrease of antioxidant activity. Therefore, the higher the phenolic content, the higher the antioxidant activity will be (Khaerah, 2019).

This research aimed to determine the antioxidant activity and organoleptic quality of kombucha of purple chrysanthemum (*Chrysanthemum morifilium*) flower on the variation of sugar types and fermentation duration.

2. Materials and Methods

2.1. Place and Time of Research

This study was conducted from September 2024 to March 2025 at the Industrial Microbiology Laboratory, Faculty of Teacher Training and Education, Muhammadiyah University of Surakarta. The assessment of antioxidant activity and vitamin C was conducted in Food Quality Analysis Laboratory, Faculty of Health Sciences, Muhammadiyah University of Surakarta. Meanwhile, the organoleptic tests were conducted around the UMS campus environment with 20 respondents, including students from the nutrition science program, biology education program, and students around the campus.

2.2. Tools and Materials

Sterilized jam jar 350 ml, analytical balance, UV-Vis spectrophotometer to measure antioxidant activity, and pH stick to measure the pH of kombucha.

The materials used in this research consist of 48 g of purple chrysanthemum flower from the Solo flower market, 200 g of palm sugar from the sari arenku store, 200 g of Javanese sugar bought in Sido Makmur market, 48 g of green tea, 5 mg of DPPH, 11.25 g of kombucha SCOBY starter from snackity kombucha store, 1500 ml of water.

2.3. Research Design

This research used an experimental method with two factors, Completely Randomized Design (CRD). Factor I was the variations of sugar types (G): palm sugar and Javanese sugar. Factor II was the fermentation duration (F): 5-days fermentation and 7-days fermentation, the control group was green tea kombucha. The treatments in this study include G1F1 (palm sugar 200 g with fermentation duration of 5 days), G1F2 (palm sugar 200 g with fermentation duration of 7 days), G2F1 (Javanese sugar 200 g with fermentation duration of 5 days), and G2F2 (Javanese sugar 200 g with fermentation duration of 7 days). Each treatment was with three repetitions.

2.4. The process of making Purple Chrysanthemum Kombucha Tea

2.4.1. Sterilization of Equipment

All tools used to make dried flowers of purple chrysanthemum were sterilized by soaking them in hot (boiling) water.

2.4.2. The process of Making Dried Purple Chrysanthemum Flower

All the petals of purple chrysanthemum were washed and placed in cabinet dryer lined with aluminium foil. The petals were dried at 60˚C for 30 minutes (Istiana, 2023).

2.4.3. The process of Making Tea Solution

As much 1000 ml of mineral water was boiled, then added 48 g of dried purple chrysanthemum petals for 3 minutes. Boiled dried petal was strained and divided into 250 ml for each treatment in jam jar. Each group was added 200 g of palm sugar and 200 g of Javanese sugar into 4 jars/treatment, stirred until the sugar dissolves and rested until it was lukewarm (Rindiani & Suryani, 2023).

2.4.4. The Process of Making Purple Chrysanthemum Kombucha

The SCOBY was added 2.25 into each jar containing purple chrysanthemum tea solution and covered with aluminium foil. Kombucha was fermented in a dark room at 25˚C - 27˚C for 5 days and 7 days (Rindiani & Suryani, 2023).

2.5. pH Testing

100 ml of kombucha was pipetted to test tube to measure pH with pH stick. The pH measurement was performed twice, once before fermentation and once after fermentation was done (kombucha harvesting) (Istiana, 2023).

2.6. Antioxidant Activity Testing

In this research, antioxidant activity was measured by using DPPH (2,2-difenil-1-pikrilhidrazil) radical method. 1 ml of DPPH was added into10 µL of kombucha sample, rested for 20 minutes, added 97% ethanol up to 5 mL, vortexed and measured at a wavelength of λ=517 using a UV-Vis spectrophotometer. The same treatment was applied to the blank solution (DPPH solution without the rest substance). The absorbance measurement result was analyzed for the percentage of antioxidant activity using the following equation (1) (Istiana, 2023).

$$\%\ \text{inhibition} = \frac{\text{A blanko} - \text{A sample}}{\text{A blanko}} \text{ x } 100\% \quad (1)$$

Explanation:
A = absorbance value

2.7. Vitamin C Testing

The vitamin C was assessed by using the iodometric titration method. 25 ml of filtrate, 5 ml of 10% H_2SO_4, and 20 drops of starch indicator were added into an Erlenmeyer flask titrated using iodine solution (I2) until a blue color forms. The repetition was performed three times, then calculated the average (2-4) (Santoso, 2021).

Calculation:

$$\text{water content } \% = \frac{Vt \times Nt}{0{,}1} \times equality = A \quad (2)$$

$$\text{In 100 g} = \frac{100\ g}{10\ ml} \times A = B \quad (3)$$

$$\text{Vitamin C concentration} = \frac{B}{100} \times 100\% = C \quad (4)$$

Description:
Vt: titration volume
Nt: normality of titration
C: vitamin C concentration

2.8. Organoleptic Quality Testing

Organoleptic testing was covered color, aroma, taste. Acceptability has been determined through acceptance tests. This assessment was involved 20 respondents.

2.9. Data Analysis

Organoleptic properties of testing (color, aroma, taste, and acceptance) using qualitative methods. Meanwhile, the testing of antioxidant activity and vitamin C used a quantitative descriptive method with a non-parametric test, Kruskal-Wallis test.

3. Result and Discussion

3.1. Result of Antioxidant Activity, pH, and Vitamin C

The study results of the antioxidant activity, pH, and vitamin C of purple chrysanthemum kombucha on the variation of sugar types and fermentation duration were described below:

Table 1. Results of antioxidant activity, pH, and vitamin C of purple chrysanthemum flower kombucha

Treatment	Antioxidant activity (%)	pH	Vitamin C (mg/100ml)
G1F1	68.50	4.5	56.11
G1F2	76.37**	4.0	72.52**
G2F1	55.17*	4.5	41.48*
G2F2	68.89	4.0	64.98

Description:
G1F1: palm sugar 200 g with fermentation duration of 5 days
G1F2: palm sugar 200 g with fermentation duration of 7 days
G2F1: Javanese sugar 200 g with fermentation duration of 5 days
G2F2: Javanese sugar 200 g with fermentation duration of 7 days
*) Lowest score
**) Highest score

Table 1 described that the acidity level (pH) of purple chrysanthemum kombucha was similar (4.5) with G1F1 treatment (200g palm sugar with fermentation duration for 5 days) and G2F1 treatment (200g javanese sugar with fermentation duration for 5 days), whereas the acidity level (pH) of purple chrysanthemum flower kombucha was similar (4) with G1F2 treatment (200g palm sugar with fermentation duration for 7 days) and G2F2 (200g Javanese sugar with fermentation duration for 7 days)

The highest antioxidant activity of purple chrysanthemum flower kombucha was 76.37% (G1F2 treatment), while the lowest antioxidant activity of purple chrysanthemum kombucha was

55.17% (G2F1 treatment). The comparison of antioxidant activity of purple chrysanthemum kombucha was viewed in Figure 1.

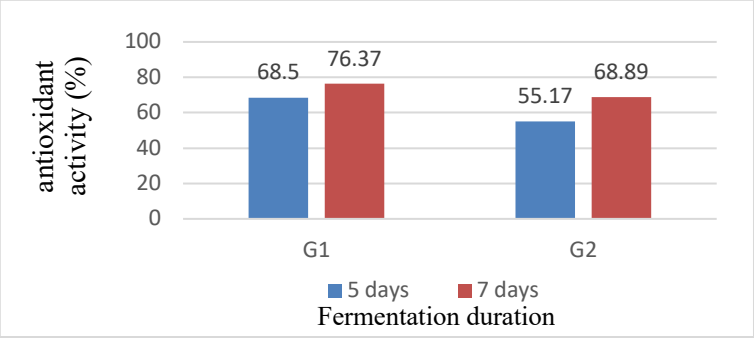

Figure 1. Antioxidant activity of purple chrysanthemum kombucha

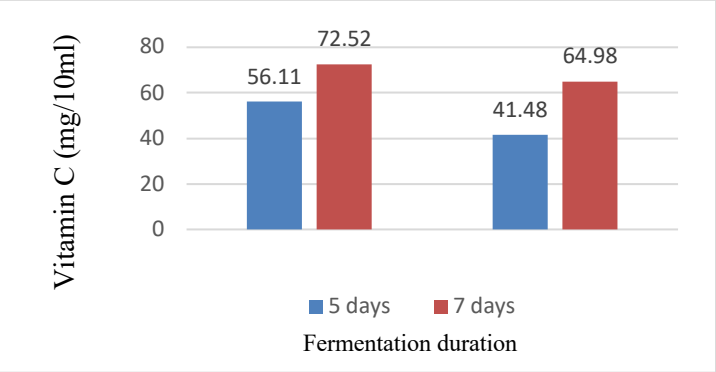

Figure 2. Vitamin C content of in purple chrysanthemum flower kombucha

The vitamin C content of purple chrysanthemum kombucha varied with each treatment (Figure 2). The highest vitamin C content of purple chrysanthemum kombucha was 72.54 mg/10ml in the G1F2 treatment, while the lowest vitamin C concentration in purple chrysanthemum kombucha was found in the G2F1 treatment (41.48 mg/100ml).

3.2. Organoleptic Quality Result

The organoleptic quality of purple chrysanthemum kombucha (color, aroma, taste, and acceptability) was as follows:

Table 2. The Result of the organoleptic quality of purple chrysanthemum flower kombucha

Treatment	Assessment aspect			
	Color	Aroma	Taste	Acceptability
G1F1	Brown	Quite fragrant of kombucha	Not sour	Like
G1F2	Brown	Quite fragrant of kombucha	Quite sour	Like
G2F1	Brown	Quite fragrant of kombucha	Quite sour	Like
G2F2	Brown	fresh vinegar aroma	Sour	Not really like

Table 2 showed that the color of purple chrysanthemum kombucha was brown in all treatments. The aroma of purple chrysanthemum kombucha was quite fragrant in all treatments, except for the aroma was fresh vinegar in G2F2 treatment. The taste of purple chrysanthemum kombucha was not sour in G1F1 treatment. The taste of purple chrysanthemum kombucha was quite sour in G1F2 and G2F1, while the taste of purple chrysanthemum kombucha was sour in

G2F2 treatment. The acceptability of purple chrysanthemum kombucha was liked in all treatments, except for G2F2.

3.3. Discussion

The result of the antioxidant activity, pH, vitamin C content, and organoleptic quality of purple chrysanthemum flower kombucha on the variation of sugar types and fermentation duration were described below:

3.3.1. Antioxidant activity

The highest antioxidant activity of purple chrysanthemum kombucha was 76.37% in the G1F2 treatment (200g palm sugar with fermentation duration of 7 days), it was depended on antioxidant activity of purple chrysanthemum kombucha increased along with the length of fermentation. The increase of antioxidant activity kombucha was caused by the higher phenolic content resulting from the biotransformation carried out microorganism during the fermentation process, the longer the fermentation time, the higher the antioxidant activity will be (Hassmy *et al*., 2017). Palm sugar has a higher sucrose content compared to Javanese sugar. During the fermentation process, bacteria and yeast will breakdown sucrose into glucose and fructose. Glucose is used to form alcohol and organic acids, thereby increasing rhe acidity level in kombucha. The increase in acetic acid in kombucha can affect its antioxidant activity (Budiandari et al., 2023). This result was in line with (Istiana, 2023), stated that the highest antioxidant activity of pegagan leaf kombucha was observed with 200 g of palm sugar and fermentation duration of 7 days. Conversely, the antioxidant activity of purple chrysanthemum kombucha was lowest (55.17%) in G2F1 treatment (200g Javanese sugar with fermentation duration of 5 days). It was because the organic acid compounds produced were not as much as during 7 days of fermentation. In line with (Nisak, 2023), revealed that the amount of acetic acid of black tea kombucha with fermentation duration of 3 days was lower than black tea kombucha fermented for 15 days. Compared to the antioxidant activity levels incontrol group, the antioxidant activity of purple chrysanthemum kombucha was higher (76.37%), while the control groupwith palm sugar treatment and fermentation duration of 7 days was 46.99%, and the antioxidant activity of green tea kombucha was 43.09% in the Javanese sugar treatment and fermentation duration of 7 days.

In the variation of fermentation duration, an Asymp. Sig value of 0.248>0.05 was obtained, indicating that H0 was accepted, that there was no significant effect of fermentation duration to antioxidant activity of purple chrysanthemum kombucha. Whereas in the variation of sugar types, an Asymp. Sig value of 0.004<0.05 was obtained, indicating that H0 was rejected, there was a significant effect of the variation in sugar types on the antioxidant activity of purple chrysanthemum kombucha. This study concluded that the variation in sugar types significantly

affects the antioxidant activity of purple chrysanthemum kombucha, but the fermentation duration did not significantly effect on antioxidant activity of purple chrysanthemum kombucha.

3.3.2. pH Kombucha

The acidity level (pH) of purple chrysanthemum kombucha was 4.5 in palm sugar and Javanese sugar with 5 days of fermentation duration, This condition explained that the pH decrease was slower in the early stages of fermentation, the yeast was still in the adaptation stage and the stage of breaking down sugar into glucose and fructose as a source of carbon and nutrients for the bacteria (Hafsari *et al*., 2021). Glucose is used to form alcohol and organic acids, thereby increasing the acidity level in kombucha (Suhardini & Zubaidah, 2016). In line with (Istiana, 2023), stated that the pH of pegagan leaf kombucha was 4 in the palm sugar with fermentation duration of 5 days, while the acidity level (pH) of pegagan leaf kombucha was 3 at Javanese sugar and granulated sugar treatment with fermentation duration of 7 days. This result showed that the longer fermentation process of kombucha, the lower the pH will be, resulted more acidic and the level was below the National Standard of Indonesian (4). The longer the fermentation process, the more acidic kombucha and the total acidity increase. It was caused by the activity of the *Acetobacter xylinum* bacteria producing acetic acid (Budiandari *et al*., 2023).

3.3.3. Vitamin C Content

The highest vitamin C content of purple chrysanthemum kombucha was 72.54 mg/100ml in G1F2 treatment (200g palm sugar with fermentation duration of 7 days). It was explained that the sugar in the kombucha fermentation process was utilized the SCOBY, allowing the vitamin C by the bacteria *Acetobacter xylinum.* Thus, the sugar was reduced to D-sorbitol and then D-sorbitol was converted into L-sorbose. L-sorbose will undergo chemical oxidation to become 2-keton-L-gulonic acid. In the fermentation process, it will be converted into L-ascorbic acid (vitamin C). The lowest vitamin C content of purple chrysanthemum kombucha was 41.48 mg/100ml in the G2F1 treatment (200g of Javanese sugar with fermentation duration of 5 days), this result was affected by fermentation duration to vitamin C content. In line with (Ruayati *et al*., 2019), the content of acetic acid, lactic acid, and pyruvic acid will increase with the fermentation duration. In this study, the highest vitamin C content and antioxidant activity of purple chrysanthemum kombucha were in the same treatment G1F2 (200g of palm sugar with fermentation duration of 7 days), while the lowest vitamin C content and antioxidant activity of kombucha were in the G2F1 treatment (200g Javanese sugar with fermentation duration of 5 days).

3.3.4. Organoleptic Quality

The organoleptic quality of purple chrysanthemum flower kombucha varied in each treatment. The color of the purple chrysanthemum kombucha was brown in all treatments G1F1,

G1F2, G2F1, and G2F2. This was because purple chrysanthemum flower contain an active compounds form of tannins (Lumintang *et al*., 2022). The compound of purple chrysanthemum flowers affects the formation of the infusion's color. The brown color produced from purple chrysanthemum flower kombucha was due to the high tannin content contained in the purple chrysanthemum flower. The higher the tannin content, the darker the color of the brew produced (Mawardi, 2016).

The aroma of purple chrysanthemum kombucha was quite fragrant of kombucha in treatments G1F1, G1F2, G2F1, whereas the aroma of purple chrysanthemum kombucha was fresh vinegar aroma at the treatment G2F2. During the kombucha fermentation process, bacteria and yeast convert sugar into organic acid compounds such as gluconic acid, acetic acid, and glucuronic acid, with the levels increasing over the fermentation duration. The higher acetic acid content produced, the stronger and more pungent aroma of kombucha. The organic acids form volatile compounds that can be detected by sense of smell (Rindiani & Suryani, 2023), the addition of sugar and the fermentation duration affect the aroma result of purple chrysanthemum kombucha.

The taste of purple chrysanthemum kombucha was not sour in the G1F1 treatment. The aroma of purple chrysanthemum kombucha was quite sour in the G1F2 and G2F1 treatments, whereas the aroma of purple chrysanthemum kombucha was sour in the G2F2 treatment. It was explained that during the fermentation process of kombucha with the SCOBY starter, organic acids such as acetic acid, lactic acid, gluconic acid, and glucuronic acid were produced (Fatonah *et al*., 2022). The longer fermentation process, the more acidic pH of kombucha, and total acidity increase, as will the taste of the resulting kombucha (Budiandari *et al*., 2023).

The acceptability of purple chrysanthemum kombucha in treatments G1F1, G1F2, and G2F1 was liked, whereas the acceptability of purple chrysanthemum kombucha was not really liked in treatment G2F2. it was caused by the taste of purple chrysanthemum kombucha in G2F2 treatment was more acidic compared to other three treatments. In line with (Istiana, 2023), stated that the longer fermentation process of pegagan leaf kombucha result was stronger sour taste. The strong taste tends to be less favored in purple chrysanthemum flower kombucha. This was because kombucha was new beverage for the panelists.

4. Conclusion

The highest antioxidant activity and the best organoleptic quality of purple chrysanthemum kombucha was 76.37% and brown color, quite fragrant of kombucha, quite sour taste, and favored by the panelists in the G1F2 treatment (200g palm sugar with fermentation duration of 7 days).

Abbreviations

SCOBY	Symbiotic Culture of Bacteria and Yeast
SNI	Standar Nasional Indonesia

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Aflah Ikromi: writing-original draft, resources, executor. **Titik Suryani:** conceptualization, supervision, writing-review and editing.

Declaration of Competing Interest

The authors of this manuscript declare that there are no conflicts of interest or competing interests.

Acknowledgement

The authors express gratitude to family, friends, and everyone who has always supported and prayed for the completion of this article.

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