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Antioxidant Activity and Organoleptic Quality of Yellow Marigold Flower (*Tagetes erecta* L.) Kombucha with Variations in Sugar Type and Fermentation Duration

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Abstract

Kombucha is a fermented beverage made from sweetened tea using kombucha microbes known as SCOBY (Symbiotic Colony of Bacteria and Yeast). One of the flowers with potential as a kombucha ingredient is the yellow marigold (*Tagetes erecta* L.). This study aimed to determine the antioxidant activity and organoleptic quality of yellow marigold flower kombucha with variations in sugar type and fermentation duration. An experimental method was employed using a Completely Randomized Design (CRD) with two factors: 1) Factor I (G): Type of sugar (palm sugar and Javanese sugar), and 2) Factor II (F): Fermentation duration (5 days and 7 days). The results showed that the highest antioxidant activity (71.44%) was observed in the G1F2 treatment (200 g palm sugar, 7 days of fermentation). The highest organoleptic quality, characterized by a brown color, moderately sour taste, mild kombucha aroma, and a moderate preference level, was found in the G2F2 treatment (200 g Javanese sugar, 7 days of fermentation).

Keywords: antioxidant activity; fermentation duration; kombucha; organoleptic quality; sugar type; yellow marigold flower

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Introduction

Probiotic beverages are fermented products containing lactic acid bacteria (Patty et al., 2021), serving to enhance nutritional value, protect the digestive tract from pathogenic bacteria, and provide various health benefits (Zubaidah et al., 2021). These beverages can inhibit the growth of harmful bacteria and aid in lactose metabolism, making them suitable for individuals with lactose intolerance. Additionally, regular consumption of probiotic beverages may help prevent digestive disorders such as typhoid, diarrhea, and dysentery (Yulia et al., 2022).

Kombucha is a functional beverage produced by fermenting a sweet tea solution using a kombucha starter known as SCOBY (Symbiotic Culture of Bacteria and Yeast) (Yusmita et al., 2023). It is a gelatinous biofilm formed through the fermentation of a mixture of *Acetobacter pasteurianus*, *Acetobacter aceti*, and yeast species, which coexist in a single colony. Kombucha is typically made from tea leaves or other plant-based ingredients with high bioactive content. These ingredients produce metabolites that contribute to the body's defense against infections (Rezaldi et al., 2022). The health benefits of kombucha include enhancing immune function, increasing energy, aiding digestion, and providing antioxidant effects (Sinaga et al., 2024). According to Majidah et al. (2022), the ethanol content of most kombucha products averages below 0.5%, which aligns with the LPPOM MUI 2018 standard. Recent developments in kombucha production have extended beyond traditional tea leaves to include edible flowers (Kushargina et al., 2023). Research by Choiriyah et al. (2020) indicates that edible flowers offer a range of aromas and unique flavors and are safe for consumption. In Indonesia, several edible flowers are known to contain antioxidants, such as phenolic compounds, anthocyanins, flavonoids, and carotenoids. Although numerous studies have explored kombucha made from tea leaves (*Camellia sinensis*) (Zubaidah et al., 2021), alternative raw materials are now being used, such as butterfly pea flowers (*Clitoria ternatea* L.) (Rahmawati et al., 2024), roselle flowers (*Hibiscus sabdariffa* L.) (Sa'diyah et al., 2024), and gardenia flowers (*Gardenia jasminoides*) (Rezaldi et al., 2022). One flower with promising potential as a kombucha raw material, yet unexplored in previous studies, is the yellow marigold (*Tagetes erecta* L.). The novelty of this study lies in the use of yellow marigold flowers for SCOBY kombucha, incorporating variations in sugar concentrations and fermentation durations. This study aims to expand knowledge regarding the use of yellow marigold flowers as a raw material in kombucha production.

Yellow marigold flowers are aromatic herbal plants rich in alkaloids, saponins, tannins, phenols, and flavonoids (Deshpande et al., 2019). They have demonstrated antioxidant activity of 257.65 mg/L, moisture content of 6.86%, total phenols of 83.88 mg GAE/g extract, and total flavonoids of 373.06 mg QE/g extract (Kusuma et al., 2020). In the creative industry, marigold flowers are commonly used as fresh decorative elements. Research by Wardana et al. (2024) has shown their effectiveness in eradicating mosquito larvae. In the culinary field, marigold flowers can be used as natural colorants and processed into food products such as tea (Wahyuni et al., 2024). Additionally, Tanago et al. (2023) reported the use of marigold flowers in the development of innovative jam products. However, there has been no research on the use of marigold flowers in kombucha production, making the present study an important contribution to the field.

One of the critical factors in the kombucha fermentation process is the type and concentration of sugar. Sugar serves as the primary energy source for SCOBY, supporting microbial growth and fermentation. The substrate concentration (sugar) is essential in the kombucha fermentation (Sinaga et al., 2024). Variations in sugar concentration can influence the development of kombucha microbes and the production of chemical compounds, including organic acids (Yanti et al., 2020). Palm sugar is a natural sweetener with a high caloric value, ranging from 2,704 to 3,617 calories per gram (Assah et al., 2020). In addition to being considered a healthier alternative to refined sugar, palm sugar has a high nutritional content and is believed to help regulate cholesterol levels (Musdalifah et al., 2022). Palm sugar contains approximately 84% sucrose, 0.53% reducing sugars, 2.28% protein, 1.37% calcium, and 1.37% phosphorus (Abdilah et al., 2022).

Fermentation duration is another key factor in kombucha production. The fermentation process influences the development of bioactive compounds, including phenolic compounds and antioxidant activity, while altering the beverage's physical and chemical properties, such as starch levels, alcohol content, antioxidant capacity, and pH (Gumanti et al., 2023). A significant decrease in pH during fermentation is attributed to microbial activity and the organic acids released by the SCOBY (Rachmawati et al., 2023).

Antioxidants play a crucial role in neutralizing free radicals, and kombucha's antioxidant activity is primarily derived from the production of free phenolics during the fermentation process. The higher the level of phenolics produced, the greater the antioxidant activity (Tunjungsari et al., 2024). Bioactive compounds such as polyphenols, flavonoids, and organic acids, generated during fermentation, are primarily responsible for the antioxidant properties of kombucha (Dewi et al., 2024). A study by Rindiani et al. (2023) found that the highest antioxidant activity in *Physalis angulata* (*ceplukan*) leaf kombucha occurred in the G1F2 treatment, which involved 225 g palm sugar and a fermentation duration of 7 days. This treatment also received the highest organoleptic scores, characterized by favorable consumer acceptance, a pleasant sour taste, a distinctive kombucha aroma, and a brown coloration.

This study aimed to determine the antioxidant activity and organoleptic quality of yellow marigold flower kombucha under different variations of sugar type and fermentation duration.

Research Methods

This research was conducted at the Biology Laboratory of Universitas Muhammadiyah Surakarta from February 1 to 12, 2025. The study employed a Completely Randomized Design (CRD) with two treatment factors and three replications, including 1) Factor 1: Type of sugar (200 g palm sugar and 200 g Javanese sugar), and 2) Factor 2: Fermentation duration (5 days and 7 days).

Tools and Materials

The tools used in this research included a stove, pans, spoons, strainers, basins, Erlenmeyer flasks, measuring cups, knives, pH sticks, jam bottles, chopping boards, a UV-Vis spectrophotometer, an analytical balance, rubber bands, aluminum foil, and label paper. Meanwhile, the materials used were 48 g yellow marigold flowers (purchased from a marigold flower farmer in Batu, Malang), 200 g palm sugar (purchased from Shopee, *Sari Arenku* store in Bandung), 200 g Javanese sugar, 75 g green tea (as control), 5 mg DPPH, 11.25 g SCOBY kombucha starter (purchased from Shopee, *Snackity Kombucha* store in Surakarta), and 1500 mL water.

Sterilization of Tools

Sterilization was carried out on jam bottles, spoons, strainers, measuring cups, glass jars, cutting boards, and knives by soaking them in hot water for 10 minutes (Istiana et al., 2023).

Kombucha Tea Preparation

Yellow marigold flowers were washed and air-dried in a clean container at room temperature. The petals were then wrapped in aluminum foil and placed in a cabinet dryer at 60°C for 30 minutes. The dried flowers were then ready for brewing (Rahmawati et al., 2024).

Preparation of Yellow Marigold Tea Solution

A total of 48 g yellow marigold flowers were boiled in 1000 mL of mineral water (250 mL × 4 treatment combinations) (Rindiani et al., 2023). The resulting infusion was steeped in boiling water for 3 minutes (Rahmawati et al., 2024), then filtered into four 250 mL jars. Each jar received either 200 g palm sugar or 200 g Javanese sugar. The mixtures were stirred and left to cool until lukewarm, allowing the sugar to fully dissolve (Istiana et al., 2023).

Preparation of Yellow Marigold Flower Kombucha

Each 250 mL yellow marigold tea solution was combined with 9 g SCOBY kombucha starter (2.25 g × 4 treatment combinations) (Rindiani et al., 2023). Fermentation was carried out based on the treatment duration (5 or 7 days) in room conditions (25°C–27°C), away from direct sunlight.

Analysis Stages

Antioxidant Activity Test (% Inhibition)

Antioxidant activity was measured using the DPPH method. A solution was prepared by dissolving 0.0394 g of 1,1-diphenyl-2-picrylhydrazyl (DPPH) in 97% ethanol to a final volume of 250 mL. A 10 µL sample was added to 1 mL of the DPPH solution and incubated for 20 minutes. Ethanol was then added to reach a total volume of 5 mL. The solution was vortexed, and absorbance was measured at 517 nm using a UV-Vis spectrophotometer. A blank solution was prepared using the same method. Antioxidant activity was calculated using the following formula (Istiana et al., 2023).

$$\%Inhibition = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100\,\%$$

Note:
A = Absorbance value

pH Testing

A 100 mL sample of yellow marigold kombucha was placed in a beaker glass and measured using a pH meter or pH stick (Rahmawati et al., 2024). pH measurements were conducted twice: before fermentation and after the kombucha was harvested.

Organoleptic Quality Testing

The organoleptic quality test assessed taste, aroma, color, and overall acceptability through a preference (hedonic) test. The test involved 20 untrained respondents from the Nutrition Science and Biology Education study programs, as well as other departments at Universitas Muhammadiyah Surakarta, using a hedonic scale.

Data Analysis

Antioxidant activity data were analyzed using quantitative descriptive methods with the Kruskal–Wallis test. Organoleptic data (color, aroma, taste, and acceptability) were analyzed using qualitative descriptive methods via Microsoft Excel.

Research Results and Discussion

Results

Kombucha is a functional beverage produced through the fermentation of a sweet tea solution using a SCOBY starter, with sugar serving as a nutritional source (Yusmita et al., 2023). Yellow marigold flowers, used as raw material for kombucha, contain 70% lutein, 619 ± 0.50 mg/kg carotene, 685 ± 0.30 mg/kg xanthophylls, and exhibit antioxidant activity that helps inhibit free radicals. Additionally, they possess antibacterial, antiepileptic, antipyretic, and terpenoid properties (Deshpande et al., 2019).

Antioxidant Activity

The results of the antioxidant activity, pH, and vitamin C content of yellow marigold flower kombucha are presented in **Table 1**.

Table 1. Antioxidant activity, pH, and vitamin C content of yellow marigold flower kombucha

Treatment	Antioxidant Activity (%)	pH	Vitamin C
G1F1	62.85	4.5	52.59
G1F2	70.89**	4	75.48**
G2F1	54.22*	4.5	41.64*
G2F2	64.17	4	63.03

Description: **Highest
*Lowest

Notes:
G1F1: 200 g palm sugar, 5 days of fermentation
G1F2: 200 g palm sugar, 7 days of fermentation
G2F1: 200 g Javanese sugar, 5 days of fermentation
G2F2: 200 g Javanese sugar, 7 days of fermentation

Table 1 displays that the highest antioxidant activity (70.89%) was observed in the G1F2 treatment, while the lowest (54.22%) was in the G2F1. The highest pH value (4.5) was recorded in the G1F1 and G2F1 treatments, whereas the lowest pH value (4.0) occurred in the G1F2 and G2F2 treatments. Vitamin C content was highest (75.48 mg/100 mL) in the G1F2 and lowest (41.64 mg/100 mL) in the G2F1.

Organoleptic Quality

The organoleptic quality of yellow marigold flower kombucha across different sugar types and fermentation durations is shown in **Table 2**.

Table 2. Organoleptic qualities of yellow marigold flower kombucha

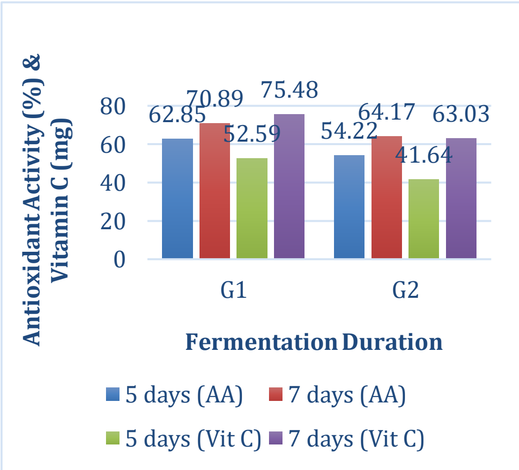
Treatment	Assessment Aspects			
	Color	**Aroma**	**Taste**	**Acceptability**
G1F1	Brown	Mild kombucha aroma	Moderately sour	Moderately liked
G1F2	Brown	Mild kombucha aroma	Moderately sour	Moderately liked
G2F1	Brown	Mild kombucha aroma	Moderately sour	Moderately liked
G2F2	Brown	Mild kombucha aroma	Moderately sour	Moderately liked

Explanation:
G1F1: 200 g palm sugar, 5 days of fermentation
G1F2: 200 g palm sugar, 7 days of fermentation
G2F1: 200 g Javanese sugar, 5 days of fermentation
G2F2: 200 g Javanese sugar, 7 days of fermentation

treatment. This difference could be attributed to the amount of organic acids produced; the 5-day fermentation period resulted in fewer organic acid compounds than the 7-day fermentation period. This finding aligns with the study by Khaerah et al. (2019), which stated that a longer fermentation process results in a more pronounced sour taste, indicative of increased organic acid content. The high antioxidant activity in the G1F2 treatment was also supported by the activity of microorganisms involved in the fermentation process.

According to Yanti et al. (2020), the presence of organic acids in kombucha is largely the result of microbial activity, particularly from acetic acid bacteria (*Acetobacter xylinum*) and yeast (*Saccharomyces* spp.). Compared to the control, the antioxidant activity of yellow marigold flower kombucha was 47.90% higher than that of green tea kombucha, which had antioxidant activities of 46.99% in both the G1F2 and G2F2 treatments. A histogram showing the antioxidant activity and vitamin C levels is presented in **Figure 1.**

Figure 1. *Histogram of antioxidant activity and vitamin C levels of yellow marigold flower kombucha*



Notes:
G1: 200 g palm sugar
G2: 200 g javanese sugar

A statistical analysis of antioxidant activity using the Kruskal–Wallis test showed that the variation in sugar type had a significant effect (Asymp. Sig = 0.005 < 0.05), leading to the rejection of H_0. This finding indicates that the type of sugar had a significant effect on the antioxidant activity of yellow marigold flower kombucha. However, the fermentation duration did not have a significant effect (Asymp. Sig = 0.172 > 0.05), as H_0 was accepted. Thus, the type of sugar played a more crucial role in determining antioxidant activity than fermentation duration.

Regarding sugar content, Assah et al. (2020) reported that palm sugar contains 84% sucrose, 2.28% protein, and mineral contents including iron (±0.83 ppm), sodium (±2466.13 ppm), potassium (±5281.97 ppm), magnesium (±272.59 ppm), and calcium (±204.22 ppm). Meanwhile, Javanese sugar consists of 87.86% sucrose, 4.64% glucose, and 3.70% fructose (Maryani et al., 2021). These compositional differences may contribute to the variation in antioxidant activity and vitamin C production.

The highest vitamin C content, 75.48 mg/100 mL, was also observed in the G1F2 treatment. This result could be attributed to the effective utilization of sugar by the SCOBY during fermentation, leading to increased vitamin C levels. In addition, the sugar fermentation process had a direct effect on the fluctuations in vitamin C content. This finding is consistent with Nurikasari et al. (2017), who reported that prolonged fermentation of green tea kombucha initially increases vitamin C levels, but eventually leads to a decline once sugar is depleted and microorganisms exhaust their nutrient supply. Compared to the control, the vitamin C content of yellow marigold flower kombucha was 47.90 mg higher than that of green tea kombucha, which had vitamin C levels of 46.99 mg/100 mL in both the G1F2 and G2F2 treatments.

The highest pH value of yellow marigold flower kombucha was 4.5 in the G1F1 and G2F1 treatments. Meanwhile, the lowest pH value was 4.0 in the G1F2 and G2F2 treatments. These findings indicate that the longer the fermentation process, the lower the pH level, making the kombucha more acidic. This result aligns with Rachmawati et al. (2023), who reported that the pH of *Eucheuma cottonii* seaweed kombucha decreased significantly not only due to the ongoing fermentation but also because of the release of acids from the SCOBY. Similar findings were reported by Gumanti et al. (2023), showing that extended fermentation of red dragon fruit peel (*Hylocereus polyrhizus*) kombucha increased phenolic content and antioxidant activity, and affected physical and chemical properties such as alcohol content, pH, and antioxidant levels. Therefore, a longer fermentation duration results in a lower (more acidic) pH, which aligns with the Indonesian National Standard (*SNI*) for kombucha pH values.

The color of the yellow marigold flower kombucha was brown across all treatments. This was due to the presence of active compounds in marigold flowers, especially tannins (Kurniati, 2021). The compounds in yellow marigold flowers affected the color of the infusion, with higher tannin levels producing a darker hue. According to Krisnadewi et al. (2023), yellow marigold flowers contain tannins ranging from 118.27 to 136.58 mg TAE (Tannic Acid Equivalent)/100 g dry weight, contributing to the brown coloration of the kombucha beverage.

The aroma of yellow marigold flower kombucha was consistently described as a typical aroma across all treatments. During fermentation, bacteria and yeast convert sugars into various organic acids, such as gluconic acid, acetic acid, and glucuronic acid, contributing to the beverage's characteristic aroma. Rindiani (2023) notes that these organic acids form volatile compounds that affect aroma perception. Therefore, sugar type and fermentation duration affect the aromatic intensity, with longer fermentations generally producing stronger and more pungent aromas due to increased acetic acid content.

The taste of yellow marigold flower kombucha was moderately sour in all treatments. This resulted from organic acid production during fermentation, including acetic, lactic, gluconic, and glucuronic acids. Fatonah et al. (2022) confirmed that these acids are natural byproducts of the kombucha fermentation process. Furthermore, according to Kushargina et al. (2023), the activity of lactic acid bacteria and yeast during fermentation imparts a sour taste and distinct kombucha flavor to the beverage.

The acceptability of yellow marigold flower kombucha was rated as "moderately liked" across all treatments. However, it was observed that a more extended fermentation period generally led to a more sour taste, which some panelists found less preferable. This finding corresponds with Istiana (2023), who stated that longer fermentation of *pegagan* leaf (*Centella asiatica*) kombucha results in a stronger sour taste, which may not be favorable for all consumers. The relatively low acceptability for more acidic samples might also be due to the novelty of kombucha among panelists, many of whom were tasting kombucha for the first time and were unfamiliar with its distinctive sour profile.

Conclusion

The highest antioxidant activity of yellow marigold flower kombucha was 70.89%, observed in the G1F2 treatment (200 g palm sugar, 7 days of fermentation). The best organoleptic quality was found in the G2F2 treatment (200 g palm sugar, 7 days of fermentation), characterized by a brown color, moderately aromatic scent, moderately sour taste, and a moderately favorable reception.

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