

THE EFFECT OF THE COMPARISON OF MORINGA OLEEFERA (*Moringa oleifera*) AND ROSELA (*Hibiscus sabdariffa*) LEAVES IN KOMBUCHA TISANE ON ANTIOXIDANT ACTIVITY, PH VALUE, AND ORGANOLEPTICS

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Abstract

Kombucha is a fermented beverage produced from bacteria and yeast. Moringa leaves are herbs that have long been known for their various benefits. Roselle is a plant with red petals and high anthocyanin content. This study aims to determine the effect of the ratio of moringa and roselle leaves on the antioxidant activity of kombucha and to determine the optimal ratio for kombucha antioxidant activity. The research method used was a laboratory experiment with a Completely Randomized Design (RUPS), which consisted of treatments with various concentrations of moringa and roselle leaves, namely 90%:10%, 80%:20%, 70%:30%, and 60%:40%. The variables observed were antioxidant activity using the DPPH method, pH levels using a pH meter, and organoleptic quality. Parametric data obtained were analyzed using ANOVA and if there were significant differences, continued with the DMRT test. Non-parametric data were analyzed using the Kruskal Wallis method. The results showed that increasing the concentration of moringa leaves significantly increased antioxidant activity, pH, and decreased taste. However, there were no significant differences in color and aroma. The De Garmo method identified 90% moringa leaves and 10% roselle flowers as the optimal formulation with an antioxidant inhibition value of 82.63%, a pH of 3.567, and organoleptic scores of color 3, taste 1, and aroma 2.

Keywords: *antioxidant; moringa leaves; kombucha; roselle*

INTRODUCTION

Kombucha is a fermented beverage produced through the fermentation of tea by bacteria and yeast. In Indonesia, the trend of kombucha consumption continues to increase along with increasing public awareness of a healthy lifestyle, as kombucha is considered a nutritious beverage containing probiotics that are beneficial for digestive health and immune function (Wibowo, 2020). Various innovations in kombucha raw materials have been developed, including the use of butterfly pea flowers to prevent stunting and boost immunity (Rezaldi et al., 2023), coffee to enhance functional properties through fermentation (Parhusip et al., 2022), and ginger, sappan wood, and butterfly pea flowers to improve organoleptic characteristics, pH, and nata thickness (Kusumaningrum et al., 2024). These findings indicate that modification of raw materials significantly affects the nutritional quality and organoleptic properties of kombucha.

Moringa oleifera leaves have strong potential as a raw material for kombucha due to their high flavonoid and phenolic compound content, which function as powerful antioxidants (Bortolemedi et al., 2022). The antioxidant activity of a 70% ethanol extract of moringa leaves has been reported at 50.595 µg/mL (Riskianto et al., 2021). Moringa leaves have also been applied in various food products, such as ice cream (Puspitasari et al., 2023), *dawet* (Dwiwana & Miftahul, 2023), and rolled crepes (Maharani et al., 2021), thereby increasing nutritional value and consumer acceptance. However, moringa leaves have the drawback of having a bitter taste and a strong grassy odor, which are generally unpopular with consumers (Sari & Ulilalbab, 2020). To overcome these limitations, roselle (*Hibiscus sabdariffa*) is included as an additional kombucha ingredient. Roselle is rich in anthocyanins, which function as antioxidants while providing a bright red color and a distinctive sour taste (Suharli et al., 2025). Roselle extract contains 10.74±0.14 mg/g of vitamin C with an IC₅₀ value of 202.47 µL/mL (Maksum

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& Purbowati, 2018), and its vitamin C content is reported to increase with higher roselle tea concentrations (Rusmarilin, 2018). In addition, roselle has been used in various food products, including cookies (Rahmah & Lastariwati, 2023), jam (Manik et al., 2017), and pudding (Gustiarani & Triastuti, 2021). Although numerous studies have investigated moringa and roselle leaves separately, their application as kombucha tea remains limited. Therefore, this study aimed to evaluate the antioxidant activity, pH value, and organoleptic characteristics of kombucha produced from moringa and roselle leaf tea as a locally sourced functional food innovation. Kombucha is a fermented beverage produced through the fermentation of tea by bacteria and yeast. In Indonesia, the trend of kombucha consumption continues to increase along with increasing public awareness of a healthy lifestyle, as kombucha is considered a nutritious beverage containing probiotics that are beneficial for digestive health and immune function (Wibowo, 2020). Various innovations in kombucha raw materials have been developed, including the use of butterfly pea flowers to prevent stunting and boost immunity (Rezaldi et al., 2023), coffee to enhance functional properties through fermentation (Parhusip et al., 2022), and ginger, sappan wood, and butterfly pea flowers to improve organoleptic characteristics, pH, and nata thickness (Kusumaningrum et al., 2024). These findings indicate that modification of raw materials significantly affects the nutritional quality and organoleptic properties of kombucha.

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LITERATURE REVIEW

Moringa (*Moringa oleifera*)

Moringa is a tropical-subtropical plant native to India and now widespread in Asia and Africa, including Indonesia, where it is commonly used as a hedge. Moringa grows as a shrub or tree reaching a height of 7–11 m, with a brittle woody stem, yellowish-white flowers, and triangular pods measuring 20–60 cm. This plant is highly adaptable and can grow in hot, dry environments and infertile soils (Tilong, 2012). Moringa is considered an economically valuable crop with high nutritional value and therefore has strong potential as an alternative food source (Marhaeni, 2021). Taxonomically, moringa belongs to the *Moringaceae* family, with *Moringa oleifera* Lam. as its main species (Isnani & Nurhaedah, 2017). Moringa leaves are rich in protein (27%), vitamins A and C, minerals, and essential amino acids (Fuglie, 2001; Prihatini, 2015). Their nutritional content exceeds that of some common food sources, containing approximately seven times more vitamin C than oranges and four times more vitamin A than carrots (Bruhns, 2011). Moringa leaves also contain antioxidant compounds such as flavonoids, polyphenols, tannins, saponins, alkaloids, and carotenoids (Gopalakrishnan et al., 2016), with higher polyphenol and flavonoid contents than chayote and fern leaves (Rajanandh et al., 2012). Phenolic and flavonoid contents were reported at 1.56 ± 0.04 mg GAE/g and 2.83 ± 0.09 mg QE/g, respectively (Saini et al., 2014). The antioxidant activity of moringa leaf extract in water showed an IC_{50} value of 87.54 ppm, which is classified as moderate (Muna, 2022). The nutritional composition of fresh and dried moringa leaves has been reported by Krisnadi (2015). Moringa leaves are widely used as a nutritional source for toddlers, pregnant women, and breastfeeding mothers due to their ability to increase iron levels and boost breast milk production (Isnani & Nurhaedah, 2017; Budury et al., 2022). Furthermore, moringa leaves exhibit pharmacological activities, including anti-inflammatory, antibacterial, antitumor, antifungal, and antioxidant effects, and have been used in the treatment of malaria, hypertension, and diabetes (Rudiana et al., 2020; Vergara-Jimenez et al., 2017).

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Roselle (*Hibiscus sabdariffa*)

Roselle is a tropical shrub native to India and Malaysia and propagated by seed. This plant can grow in a variety of environmental conditions, with flower color influenced by location, ranging from dark red to bright red (Susanti & Alfian, 2012). Roselle can reach a height of up to 3 m, has woody stems, and belongs to the *Malvaceae* family, with the species *Hibiscus sabdariffa* Linn. (Haidar, 2016). Roselle petals contain vitamin C, vitamin D, vitamins B1 and B2, amino acids, polysaccharides, dietary fiber, calcium, and organic acids that contribute to its distinctive sour taste (Lauren, 2014). Roselle also contains various fatty acids, including palmitic, oleic, and linoleic acids (Dharmawan, 2009). The nutritional composition of roselle petals has been reported by Maryani and Kristiana (2008). Roselle is rich in flavonoids and anthocyanins, which function as powerful antioxidants, natural colorants, and agents that prevent cardiovascular disease (Husni, 2023; Pujiyono et al., 2022). Furthermore, the phenolic and polyphenolic compounds in roselle exhibit antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, possess anti-inflammatory properties, and accelerate wound healing (Karmana, 2023). The high vitamin C content in roselle plays an important role in boosting the immune system and preventing infection (Pujiyono et al., 2022).

Kombucha

Kombucha is a fermented beverage produced from sweet tea through fermentation by bacteria and yeast using a starter culture known as SCOBY (*Symbiotic Culture of Bacteria and Yeast*) (Nurikasari, 2017; Siahaan, 2011). SCOBY consists of bacteria such as *Acetobacter* and *Gluconobacter*, and yeasts including *Saccharomyces* and *Zygosaccharomyces* (Greenwalt, 2016; Aloulou, 2012). During fermentation, organic acids, vitamins, and bioactive compounds are produced, with an alcohol content generally below 0.5% (Kumar & Joshi, 2016; Naland, 2008). The nutritional composition of kombucha has been reported by Ivanišová et al. (2020). Kombucha fermentation typically occurs over 7–14 days and results in a decrease in pH, an increase in organic acid concentration, cellulose formation, and increased antioxidant activity (Nyhan, 2022; Lea et al., 2018). The fermentation process involves the hydrolysis of sucrose by yeast, the formation of ethanol, and the oxidation of ethanol to acetic acid by bacteria (Wu et al., 2023; Jakubczyk et al., 2022). Antioxidant activity increases with the degradation of polyphenols during fermentation (Li et al., 2022; Sittisart et al., 2024; Phung et al., 2023).

Antioxidants

Antioxidants are compounds capable of neutralizing free radicals by donating electrons, thereby preventing cell damage and oxidative stress (Sofia, 2003; Fadlilah & Lestari, 2023). Antioxidants are classified into natural and synthetic types (Sari, 2017; Sayuti & Yenrina, 2020) and, based on their mechanism of action, into primary, secondary, and tertiary antioxidants (Risma, 2022). Antioxidants play an important role in maintaining immune function, slowing the aging process, and preventing degenerative diseases (Damayanti, 2025).

Analysis Methods (DPPH, pH, and Organoleptic Tests)

The DPPH method is used to measure antioxidant activity based on the compound's ability to reduce the purple DPPH radical to a pale yellow form at a wavelength of 517 nm (Molyneux, 2022; Phung et al., 2023; Sunami, 2005). This method is simple, rapid, and sensitive to phenolic and flavonoid compounds (Kedare & Singh, 2020; Wu et al., 2023), although it does not fully represent *in vivo* antioxidant activity (Li et al., 2023). The IC_{50} value is used to determine antioxidant strength (Li et al., 2022). The pH value indicates the acidity level of a solution and can be measured using litmus paper, a universal indicator, or a pH meter (Harvyandha, 2019; Wibowo, 2021; Zumdahl & Zumdahl, 2020). Organoleptic testing is conducted to evaluate consumer acceptance of sensory attributes such as taste, aroma, color, and texture using hedonic tests, which play an important role in food product development (Pratiwi & Ramadhan, 2020; Aprilia & Hidayat, 2021).

RESEARCH METHODS

This research was conducted from November to December 2025 at the Chemistry Laboratory, Faculty of Food and Fisheries Technology, Trunojoyo University, Madura. The main raw materials used were dried moringa leaves and roselle flowers obtained through an e-commerce platform, while analytical materials included distilled water, DPPH solution, and methanol. The equipment used consisted of an analytical balance, glassware, an electric stove, an autoclave, a UV-Vis spectrophotometer, a vortex stirrer, and a pH meter. This study used a laboratory experimental method to determine the effect of various concentration ratios of moringa leaves and roselle flowers

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on the antioxidant activity of kombucha. The experimental design used was a Completely Randomized Design (CRD) with a single factor, namely the ratio of moringa leaves to roselle flowers, consisting of four treatments (90:10, 80:20, 70:30, and 60:40), each with three replications, resulting in a total of 12 experimental units. The research procedure included the preparation of moringa leaf powder and roselle tea through grinding and packaging, followed by the production of kombucha using SCOBY culture. The kombucha preparation stages consisted of preparing a sugar solution, making herbal tea, adding a starter, fermenting for 7 days, and filtering. Observed variables included antioxidant activity (DPPH method), pH value, and organoleptic evaluation (color, aroma, texture, and taste) using a five-point hedonic scale with untrained panelists. Data were analyzed using SPSS software. Antioxidant activity data were analyzed using analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) if significant differences were found, while organoleptic data were analyzed using the Kruskal–Wallis test. Determination of the best treatment was carried out using the De Garmo effectiveness test by considering organoleptic results and overall product quality.

RESULTS AND DISCUSSION

1 Chemical Analysis

Antioxidant Activity

The results of the analysis of variance (ANOVA) for the %Inhibition of kombucha tisane from moringa leaves and roselle showed a significance value of 0.000. This value is smaller than 0.05, which means there is a difference in the concentration of the combination of moringa leaves and roselle on the %Inhibition. The average results of the %Inhibition of kombucha tisane from moringa leaves and roselle are presented in Table 1.

Table 1. Results of Antioxidant Activity Analysis

Treatment	Average %Inhibition
A1 (90:10)	82.63 ^c ± 0.72597
A2 (80:20)	81.89 ^c ± 0.37873
A3 (70:30)	73.17 ^b ± 0.05196
A4 (60:40)	69.11 ^a ± 0.90338

Description: The same letter notation behind the mean number indicates that there is no difference between the treatments using the Duncan test at 5% level.

Table 1 shows that the average % inhibition of kombucha differed significantly. Treatment A1 had the highest % inhibition at 82.63%, and treatment A4 had the lowest % inhibition at 69.11%. This indicates that higher concentrations of moringa tend to produce stronger antioxidant activity compared to higher proportions of roselle. The influence of bioactive compounds in Moringa leaves could be a factor. Moringa leaves are rich in flavonoids, phenolics, and vitamin C, which are relatively stable during the fermentation process. Roselle is rich in anthocyanins, but these compounds are less stable. In acidic fermentation, anthocyanins are easily degraded, thus decreasing antioxidant activity. Rodiatullah et al. (2023) stated that anthocyanins in roselle flowers are easily degraded due to the influence of pH and temperature, so antioxidant activity can decrease at high concentrations. This explains why treatments A3 and A4 with higher roselle concentrations showed a low % inhibition. The % inhibition value of kombucha ranged from 69.11 to 82.63%. This indicates that treatments A1-A3 exhibited effective antioxidant activity, indicating a strong ability to ward off free radicals. According to Molyneux (2004), a % DPPH inhibition value of >50% indicates potential as a free radical scavenger. Thus, a higher concentration of moringa leaves can be recommended as the optimal formulation to produce kombucha tisane with high antioxidant activity.

pH value

The results of the analysis of variance (ANOVA) on the pH value of the moringa and roselle leaf kombucha tisane showed a significance value of 0.001. This value is smaller than 0.05, which means there is a difference in the concentration of the combination of moringa and roselle leaves on the pH value. The results of the average pH value of the moringa and roselle leaf kombucha tisane are presented in Table 2.

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Table 2. Results of pH Value Analysis

Treatment	pH value
A1 (90:10)	3.567 ^c ± 0.30551
A2 (80:20)	3.133 ^b ± 0.05774
A3 (70:30)	2,900 ^{ab} ± 0.00000
A4 (60:40)	2,700 ^a ± 0.10000

Description: The same letter notation behind the mean number indicates that there is no difference between the treatments using the Duncan test at 5% level.

Table 2 shows that the pH values of the kombucha differ significantly. Treatment A1 had the highest pH value at 3.567, and A4 had the lowest at 2.7. This difference indicates that the higher the proportion of roselle, the lower the pH value of the kombucha. The difference in pH values is related to the organic acid content of roselle, namely citric, malic, and tartaric acids, which impart a sour taste and lower the pH. Rodiatullah et al. (2023) reported in their report that roselle flowers contain high levels of organic acids, which can significantly lower the pH of beverages. This is in line with the opinion of Tensiskam et al. (2017), who stated that the decrease in pH is due to the presence of citric and malic acids. Thus, the pH difference in the roselle moringa leaf kombucha tisane is due to the roselle organic acid content, which affects the pH value of the kombucha.

2 Organoleptic Evaluation Results

Color Attributes

The results of the organoleptic test using the *Kruskal Wallis method* on the color parameter of the kombucha tisane with roselle moringa leaves showed a significance value of 0.165. This value is >0.05, indicating there was no significant difference between the concentration treatments of the combination of moringa and roselle leaves on the color of the kombucha. The median color values of the kombucha tisane with roselle moringa leaves are presented in Table 3.

Table 3. Results of Organoleptic Color Evaluation

Treatment	Median Color
A1 (90:10)	3a ± 0.8668
A2 (80:10)	3a ± 1.0274
A3 (70:30)	3a ± 0.5841
A4 (60:40)	3a ± 1.1376

Description: The same letter notation behind the mean number indicates that there is no difference between the treatments using the Duncan test at 5% level.

Table 3 shows that The median color value for the roselle moringa leaf kombucha tisane across all treatments was 3, with no significant differences between treatments. This indicates that the base color of the main ingredient still dominates, so adding 10-40% roselle does not provide visual appeal to the kombucha. This neutral assessment is related to the anthocyanin pigments in roselle. Even though the concentration of roselle increases, the resulting color becomes darker, making it appear less fresh. Widyasari et al. (2020) reported that adding roselle extract does produce a strong purplish-red color, but excessively dark color intensity does not always increase panelists' preference. SCOBY fermentation can lose its brightness as the roselle concentration increases. The red color produced by anthocyanins is sensitive to pH and temperature. This aligns with the findings of Warni et al. (2025) who stated that during the fermentation process, the anthocyanin pigments in roselle kombucha will degrade, resulting in a color change. The main causes of degradation are low pH conditions due to the formation of organic acids and microbial activity in the SCOBY, which breaks down the anthocyanin structure. Based on Table 3, the median kombucha color was still preferred, with a score of 3 for all treatments. A median score of 3 indicates that panelists rated the product color neutrally, meaning neither attractive nor unfavorable. Therefore, the addition of roselle to kombucha did not significantly impact panelists' preference for color.

Flavor

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The results of the organoleptic test using the *Kruskal Wallis method* on the color parameter of the moringa roselle leaf kombucha tisane showed a significance value of 0.001. This value is <0.05 , which means there is a significant difference between the concentration treatments of the combination of moringa and roselle leaves on the color of the kombucha. The median taste results of the moringa roselle leaf kombucha tisane are presented in Table 4.

Table 4. Results of Organoleptic Taste Evaluation

Treatment	Median Taste
A1 (90:10)	1 ^a ± 1.1653
A2 (80:20)	2 ^a ± 0.7504
A3 (70:30)	3 ^b ± 1.0347
A4 (60:40)	2 ^{ab} ± 1.0706

Description: The same letter notation behind the mean number indicates that there is no difference between the treatments using the Duncan test at 5% level.

Table 4 shows significant differences in the taste parameters of the roselle moringa leaf kombucha tisane between treatments A1, A2, and A4 and A3. Moringa leaves contain *catechins*, a phenolic compound with a bitter taste. The higher the percentage of moringa leaves, the stronger the bitter taste produced, resulting in a striking difference between treatments A1 (90% moringa leaves) and A3 (70% moringa leaves). The higher the percentage of moringa leaves, the stronger the bitter taste. Conversely, the lower the percentage, the less bitter the taste and the more easily the product is accepted by the panelists. This is in line with research by Guspratiwi et al. (2025) that the higher the percentage of moringa leaves, the stronger the bitter taste.

Treatment A4 had a lower median value than A3, possibly due to the higher percentage of roselle, resulting in a strong sour taste. Roselle contains many organic acid compounds such as citric, malic, and tartaric acids, which impart a sharp sour taste. According to a report by Ibrahim et al. (2023), the distinctive sour taste of roselle comes from the citric, malic, and tartaric acids. Excessive use of roselle produces a sharp sour taste that is less favored by consumers. This is in line with the report by Takam Yari & Sonkar (2024) that high roselle concentrations produce a sharp sour taste, thus reducing consumer acceptance. Based on Table 4, treatment A3 with 70% moringa leaves and 30% roselle was the most preferred with a median score of 3 (neutral). Treatment A1 with 90% moringa leaves and 10% roselle was the least preferred with a median score of 1 (dislike). Thus, the addition of roselle up to 30% caused a significant difference in aroma parameters. Kombucha with 70% moringa and 30% roselle was a treatment that was still acceptable to panelists compared to kombucha with 90% moringa and 70% roselle.

Aroma

The results of the organoleptic test using the *Kruskal Wallis method* on the aroma parameters of kombucha tisane with rosella moringa leaves showed a significance value of 0.993. This value is >0.05 , indicating no significant difference between the moringa and roselle leaf concentration treatments on kombucha aroma. The median aroma values for each treatment are presented in Table 5.

Table 5. Results of Organoleptic Aroma Evaluation

Treatment	Median Aroma
A1 (90:10)	2 ^a ± 1.668
A2 (80:20)	2 ^a ± 0.7504
A3 (70:30)	2 ^a ± 0.7425
A4 (60:40)	2 ^a ± 1.0396

Description: The same letter notation behind the mean number indicates that there is no difference between the treatments using the Duncan test at 5% level.

Table 5 shows that the median aroma parameter for kombucha tisane with roselle moringa leaves across all treatments was 2, with no significant differences between treatments. The lack of significant differences in kombucha aroma across treatments is likely due to the acidic aroma produced during fermentation. The formation of excess acetic acid during fermentation produces a vinegar-like aroma. The fresh aroma of roselle is not strong enough to mask the acetic acid aroma. Hidayat et al. (2022) explained that panelists tend to reject fermented

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beverages if the acetic acid aroma is too dominant. This finding is supported by Warni et al. (2025) who reported that the fresh characteristics of raw materials tend to be lost during fermentation and replaced by a sharper final aroma. Based on Table 5, the median kombucha aroma score remained stable at 2 for all treatments. A median score of 2 indicates panelists tended to dislike the aroma of kombucha. Therefore, the aroma of the roselle moringa leaf kombucha tisane tended to be less favorable, although the product was still acceptable because aroma is not the sole factor in overall acceptability, but rather just one sensory aspect.

3 Determination of Best Treatment (Effectiveness Test)

The effectiveness value was calculated using the De Garmo method by calculating the index and yield values for all analyzed chemical and organoleptic parameters. Table 6 presents the weight, weight value, and yield value for each parameter for each concentration treatment of Moringa and Roselle leaves.

Based on the results of these calculations, the total effectiveness value (Ne) of each treatment was obtained, which was then used to determine the ranking of the best kombucha formulations.

Table 6. Effectiveness Test Calculation

Parameter	Weight	Weight Value	NH (A1)	NH (A2)	NH (A3)	NH (A4)
Antioxidant Activity	9	0.257	0.257	0.243	0.077	0
pH value	9	0.257	0.257	0.129	0.059	0
Color	7	0.2	0.2	0.2	0.2	0.2
Flavor	5	0.143	0	0.071	0.143	0.071
Aroma	5	0.143	0.143	0.143	0.143	0.143
Total	35	1	0.857	0.786	0.622	0.414

Based on the calculation results, treatment A2, with a ratio of 90% moringa leaves and 10% roselle, achieved the highest effectiveness score, at 0.857. This indicates that A1 is the most optimal formulation because it is able to balance various quality parameters, including antioxidant activity and pH, as well as organoleptics such as color, taste, and aroma. Although treatment A3 had the highest effectiveness value for the taste parameter (NH taste = 0.143), its total effectiveness was only 0.662, much lower than A1. This indicates that superiority in one parameter alone is not enough to guarantee overall product quality. Other parameters in A3 and A4 contributed little to antioxidant activity and pH, even reaching 0 in A4. In contrast, A1 showed a good balance with high contributions to the parameters of antioxidant activity (0.143) and pH (0.257), and stable color (0.2) and aroma (0.143). Although the taste aspect in A1 contributed minimally (NH taste = 0), the overall quality remained superior compared to the other treatments. Thus, treatment A1, which is 90% moringa leaves and 10% roselle, is recommended as the best formulation in making moringa roselle leaf kombucha tisane because it provides the most balanced product quality between chemical and sensory aspects .

CONCLUSION

Based on the results of this moringa-roselle tea kombucha study, variations in the concentration of moringa and roselle leaves significantly affected antioxidant activity, pH, and organoleptic properties. Higher concentrations of moringa leaves resulted in higher inhibition percentages, indicating stronger antioxidant activity, while increasing roselle concentrations resulted in lower pH values. From an organoleptic perspective, concentration variation significantly affected taste attributes but did not significantly affect color and aroma. Treatment A3 was the most preferred in terms of taste, while all treatments showed similar scores for color (median = 3, neutral) and aroma (median = 2, unfavorable). Based on the effectiveness test, formulation A1 consisting of 90% moringa leaves and 10% roselle achieved the highest effectiveness value (0.857) and was identified as the best treatment overall.

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