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Original Research

Differences in Sugar Concentration on Flavonoid Levels in Roselle Kombucha (*Hibiscus sabdariffa* L.) As An Antioxidant Beverage

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ABSTRACT

Background: Kombucha is a fermented tea beverage produced using a symbiotic culture of bacteria and yeast (SCOBY). Sugar concentration plays an essential role in the fermentation process as it influences microbial metabolism and the formation of bioactive compounds. Roselle (*Hibiscus sabdariffa L.*) is rich in flavonoids and anthocyanins, which act as natural antioxidants. **Objective:** This study aimed to determine the effect of different sugar concentrations on flavonoid levels in roselle kombucha. **Methods:** This was an experimental study using a completely randomized design (CRD) with one factor, namely sugar concentration: F1 (15%), F2 (25%), and F3 (35%). Fermentation was carried out with the addition of SCOBY for 14 days at room temperature. Flavonoid levels were analyzed using a spectrophotometric method with quercetin as the standard. Data were analyzed using the Kruskal-Wallis test followed by the Mann-Whitney test. **Results:** The highest average flavonoid level was found in F1 (sugar concentration 15%) at 0.015690%, while the lowest was in F3 (sugar concentration 35%) at 0.011364%. The Kruskal-Wallis test showed a significant difference among treatments ($p = 0.002$, $p < 0.05$). The Mann-Whitney test confirmed that all pairwise comparisons between treatments were significantly different. Sugar concentration significantly affects flavonoid levels in roselle kombucha. **Conclusion:** The 15% sugar concentration produced the highest flavonoid content, suggesting that low sugar formulation is preferable to maintain bioactive compounds and enhance antioxidant potential. Therefore, roselle kombucha with lower sugar concentration can be developed as a functional beverage to prevent degenerative diseases related to oxidative stress.

Keywords: Kombucha; roselle; flavonoid; sugar concentration; antioxidant

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1. INTRODUCTION

Degenerative diseases are chronic conditions that cause decreased tissue or organ function and impact a person's quality of life (Fatihaturahmi et al., 2023). Diseases included in the degenerative disease category include cataracts, diabetes mellitus, obesity, osteoporosis, rheumatoid arthritis, cancer, and cardiovascular disease (Fatihaturahmi et al., 2023). To date, degenerative diseases have become a leading cause of death worldwide. Nearly 17 million people die prematurely each year due to the global epidemic of degenerative diseases (Karwiti et al., 2023). The health problems currently facing Indonesia are influenced by lifestyle habits, diet, work environment conditions, physical activity, and stress factors. Changes in lifestyle, especially in large cities, have resulted in an increase in the incidence of degenerative diseases (Karwiti et al., 2023). Degenerative diseases are characterized by oxidative stress, a condition that occurs due to an imbalance between the number of free radicals and the body's antioxidant system (Rahayu, 2023).

Free radicals are formed as a result of metabolic processes, such as hydroxyl, peroxyl, and superoxide radicals. Excessive production of these radicals causes oxidative stress. This oxidative stress can cause direct and indirect oxidative damage to macromolecules such as proteins, lipids, carbohydrates, and nucleic acids. Furthermore, oxidative stress can also cause vasodilation, signal transduction, cell differentiation, and various degenerative diseases. Therefore, antioxidant compounds are needed to balance free radicals in the body (Agustiarini & Wijaya, 2022).

Antioxidants are compounds that can inhibit and slow oxidation reactions to counteract free radicals (Pambudi et al., 2021). Oxidative stress occurs due to excessive ROS production, so these endogenous antioxidants must receive additional antioxidants from outside the body (exogenous antioxidants) derived from food and beverage intake consumed daily (Yunita, 2021). Based on their source, exogenous antioxidants can be categorized into two, namely natural and artificial (Rahayu, 2023). Examples of artificial antioxidants include Butyl Hydroxyl Anisole (BHA), Butyl Hydroxyl Toluene (BHT), and Tetra Butyl Hydroxyl Quinone (TBHQ) (J Mbah et al., 2019). Meanwhile, natural antioxidants are usually obtained from natural sources such as vegetables and fruits. Natural antioxidants are generally considered safer than synthetic ones, because they have not been exposed to or mixed with chemicals, and are easily found in the surrounding environment. Examples of natural antioxidants include flavonoids, phenolic compounds, and folic acid (Adiprahara Anggarani et al., 2023). One drink that contains active antioxidant compounds is kombucha.

Kombucha is a beverage made through a fermentation process lasting between 7 and 21 days, using a mixture of tea and sugar, using various types of bacteria and fungi commonly called SCOBY (Symbiotic Culture of Bacteria and Yeast) (Priyono & Riswanto, 2021; Rindiani & Suryani, 2023). Kombucha contains several compounds, including various vitamins (B1, B2, B3, B6, B12, and C), polyphenols with antioxidant effects, and various acids (such as acetic acid, glucuronic acid, lactic acid, carbonic acid, folic acid, gluconic acid, chondroitin sulfate, and hyaluronic acid) (Cholidah et al., 2020). Roselle is a tea substitute for kombucha.

Roselle *(Hibiscus sabdariffa L.)* is an herbal plant with potential as a source of functional food ingredients, antioxidants, antibacterials, natural coloring agents, and health benefits (Shafirany et al., 2021). *Hibiscus sabdariffa L.* flowers contain various secondary metabolite components, including flavonoids, anthocyanins, organic acids, and phenolic acids (Montalvo-González et al., 2022). Flavonoids, one of the compounds that have been widely studied for their antioxidant activity, are reported to have anti-inflammatory, anti-cancer, anti-aging, cardioprotective, neuroprotective, immunomodulatory, antidiabetic, antiparasitic, and antiviral properties (Dias et al., 2021). An additional component needed to make kombucha is sugar. During the fermentation process, sugar is used by the kombucha starter culture or SCOBY as an energy source (Fadillah et al., 2022). Yeast will break down sucrose into glucose and fructose through the invertase enzyme, which is then fermented into ethanol and CO_2. Next, acetic acid bacteria oxidize ethanol to acetic acid and other organic metabolites. This bioconversion process not only produces acid but also influences the transformation of bioactive compounds contained in the basic ingredients of kombucha (tea, herbs, or fruit) (Guo et al., 2024). Yanti et al. (2020) reported that differences in sugar concentration can affect microbial growth in kombucha and the content of chemical compounds such as organic acids. Furthermore, Yanti et al. (2020) demonstrated that soursop leaf kombucha *(Annona muricata L.)* with varying sugar concentrations, showing that a 10% sugar concentration resulted in a higher degree of acidity (pH) and total lactic acid bacteria counts of 3.30 and 9.03×10^6 CFU/ml, respectively. Based on this background, this study was designed to examine the effect of different sugar concentrations on flavonoid levels as antioxidants in roselle kombucha *(Hibiscus sabdariffa L.)*.

2. METHODS

2.1 Study Design

This research was a quantitative experimental study. The research design used was a Completely Randomized Design (CRD) with one factor, three treatments, and five replications. The population in this study was roselle kombucha drinks. The sample in this study was 250 ml of roselle drinks from each treatment and replication. This research was conducted from June to July 2025 at the Ivet University Health Laboratory in Semarang for formula development and the Chem-Mix Pratama Laboratory in Yogyakarta for flavonoid content testing.

2.2 Roselle Kombucha Preparation

The procedure for making roselle kombucha drinks began by boiling 2000 ml of water for each formula, then adding sugar according to variations of 15%, 25%, and 35%, and stirring until the sugar was completely dissolved. Add 24 grams of dried roselle flowers to each formula and stir again until the solution turns red. Afterward, allow the solution to cool to room temperature. The next step was to transfer the solution into a sterile 3000 ml glass jar, then add 10 grams of SCOBY and 166 ml of SCOBY starter solution. Cover the jar with a clean cloth and secure it with a rubber band to allow limited air exchange while preventing contamination. Continue the fermentation process for 14 days under semi-aerobic conditions at room temperature (approximately 25–30°C).

2.3 Flavonoid Content Analysis

The flavonoid content test begins by weighing 5 grams of the sample. Then, dissolve it in 100 ml of ethanol. Filter or centrifuge to separate the solution. Take 1 ml of the clear solution and mix it with 2 ml of 5% $AlCl_3$ solution. Add ethanol to reach 10 ml. Measure the absorbance using a spectrophotometer at a wavelength of 415 nm. Flavonoid content was calculated based on a quercetin standard curve and expressed as a percentage (% w/v).

2.4 Statistical Analysis

The data obtained were then analyzed statistically using the nonparametric Kruskal-Wallis test. This was done to determine whether there were differences between three or more treatment groups. If the analysis results had a p-value <0.05, the Mann-Whitney test was used to further test for differences in treatment.

3. RESULTS

3.1 Flavonoid Content of Roselle Kombucha

Table 1. Results of Analysis of Average Flavonoid Content

Formula	N	Std.Dev	Mean (% w/v)
F1	5	0.0001430	0.015690
F2	5	0.0001539	0.013440
F3	5	0.0001882	0.011364

Based on table 1, the average flavonoid content of each sample was obtained with 5 replications, with the highest value being 0.015690% in sample F1 while the lowest average value was 0.011364% in sample F3.

3.2 The Effect of Sugar on Flavonoids in Roselle Kombucha

The Kruskal-Wallis statistical data analysis was conducted to determine whether there were differences between three or more treatment groups. The Kruskal-Wallis test yielded an Asymp. Sig. (2-tailed) value of

0.002, which is less than 0.05, indicating a significant difference among treatments.

Table 2. Kruskal-Wallis Test

	Chi-square	Df	Asymp. Sig. (2-tailed)
Value	12.500	2	0.002

Because the data analysis results had a p-value <0.05, the Mann-Whitney test was continued. The Mann-Whitney test in this study aims to determine which groups have differences and which do not.

Table 3. Results of Mann-Whitney Test

Treatment Comparison	p-value
F1-F2	0.008
F1-F3	0.008
F2-F3	0.008

The results of the study showed that for the F1F2 comparison, the significance value was p = 0.008 (p < 0.05), indicating a significant difference between the two groups. Similarly, the F1F3 comparison yielded p = 0.008 (p < 0.05), and the F2F3 comparison also yielded p = 0.008 (p < 0.05), both indicating significant differences. Taken together, all pairwise comparisons demonstrated statistically significant differences, confirming that each sugar concentration treatment produced a distinct effect on flavonoid levels in roselle kombucha.

4. DISCUSSION

Based on research results, sugar concentration significantly influences the flavonoid levels in roselle kombucha. This aligns with the theory that sugar concentration influences the metabolic processes of microbes in the SCOBY, which impacts the amount of bioactive compounds produced. At a sugar concentration of 15%, fermentation activity is optimal, allowing microbes to break down sucrose into glucose and fructose, which are then fermented to produce organic acids, polyphenols, and various other bioactive compounds, including flavonoids (Yanti et al., 2020). However, at higher sugar concentrations (25%35%), excess sugar can inhibit the growth of certain microbes, cause osmotic stress, and decrease metabolic efficiency, resulting in reduced flavonoid production (Rindiani & Suryani, 2023).

The decrease in flavonoid levels at high sugar concentrations may also be caused by the degradation of phenolic compounds due to excessively high osmotic conditions, which promote the oxidation and polymerization of flavonoid compounds (Zou et al., 2021). This is consistent with findings by Yanti et al. (2020), who reported that higher sugar concentrations in kombucha did not proportionally increase bioactive metabolite content, and that excessively high sugar concentrations were associated with reduced antioxidant activity due to osmotic inhibition of microbial metabolism. Furthermore, roselle flowers, the main ingredient in kombucha, are rich in flavonoids such as hibiscitrin, gossypitrin, and sabdaritrin, as well as anthocyanins (Montalvo-Gonzlez et al., 2022). These flavonoids act as powerful antioxidants by donating electrons to neutralize free radicals (Treml & mejkal, 2016).

In this study, optimal flavonoid levels were achieved at a sugar concentration of 15%, thus concluding that a low-sugar formulation better maintains the flavonoid content of roselle kombucha. A similar study conducted by (Nintiasari & Ramadhani, 2022) on cherry leaf kombucha tea also reported high flavonoid levels (44.026 mg/g quercetin) with very strong antioxidant activity, supporting the potential of this plant-based fermented beverage as a source of natural antioxidants. Furthermore, research (Winandari et al., 2022) also showed that roselle kombucha with different fermentation times produced varying levels of vitamin C and antioxidant activity, confirming that formulation factors (including sugar content) significantly influence bioactive compounds.

The flavonoids in roselle kombucha act as natural antioxidants that can ward off free radicals, potentially preventing oxidative stress, which triggers various degenerative diseases such as diabetes, cancer, and cardiovascular disease (Dias et al., 2021). Consuming kombucha with optimal flavonoid levels can be a safer functional beverage alternative compared to synthetic antioxidants, such as BHA and BHT, which carry toxic risks if consumed excessively (J Mbah et al., 2019). The results of this study also support the development of fermented beverage products based on local plants (roselle) with low-sugar formulations to support public health. Furthermore, these findings can serve as a basis for further research on combining other fermentation factors, such as fermentation time and starter variations, to increase flavonoid content in kombucha.

One limitation of this study is the lack of consistent monitoring and control of ambient temperature during the roselle kombucha fermentation process. Temperature is an environmental factor that significantly influences microbial growth in the SCOBY. Yeast and acetic acid bacteria have an optimal temperature range of approximately 2530C for metabolic activities, including the fermentation of sugars into ethanol and organic acids. When temperature is not adequately controlled, fermentation can become inconsistent, affecting fermentation rate, secondary metabolite production, anthocyanin pigment stability, and the final beverage profile. This condition may introduce variability into the results, as not all observed changes can be attributed solely to differences in sugar concentration. Sadok et al. (2025) demonstrated that temperature regulation during kombucha fermentation is critical, as it affects microbial community composition, total phenolic content, beverage pH, and the risk of fungal contamination and mycotoxin production, all of which underscore the necessity of temperature control for both product quality and safety. Additionally, future studies are recommended to further increase the number of replications and to implement standardized temperature control to improve the reliability and generalizability of results.

5. CONCLUSION

Based on the research results, the highest flavonoid content was obtained at a sugar concentration of 15% with an average value of 0.015690% (w/v), while the lowest flavonoid content was found at a sugar concentration of 35% with an average value of 0.011364% (w/v). There was a significant effect of sugar concentration variations (15%, 25%, and 35%) on flavonoid content in roselle kombucha drinks, as evidenced by the Kruskal-Wallis test results (p = 0.002), further confirmed by the Mann-Whitney test which showed significant differences between all pairwise treatment comparisons.

DECLARATIONS

Conflict of Interest

The authors declare no conflict of interest in relation to this study.

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