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Effect of Sucrose Addition on Viscosity, pH, Total Yeast, and Hedonic Quality of Water Kefir Roselle

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Abstract

Red Roselle (*Hibiscus sabdariffa* L.) is a plant known for its high antioxidant content, making it a suitable ingredient for probiotic water kefir beverages. This study aimed to investigate the impact of different sucrose concentrations in roselle water kefir on viscosity, pH, total yeast count, and hedonic quality. The experimental design used was a Completely Randomized Design. The treatments varied based on sucrose concentrations 6%, 9%, 12%, and 15% (w/v). The findings revealed that the addition of 12% sucrose resulted in a viscosity of 1.22 cP, pH of 2.93, total yeast count of 2.15 CFU/ml, and the highest hedonic quality. In conclusion, the addition of 12% sucrose (w/v) proved to be the optimal treatment, yielding a beverage with suitable viscosity, acceptable pH, favorable total yeast count, and an overall hedonic quality embraced by the panelists.

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Introduction

Red roselle (*Hibiscus sabdariffa* L.) is one of the popular plants among Indonesian society. The red-colored petals make this flower a decorative plant. One of the benefit of roselle flower petals is the red pigment commonly used as a natural dye in the food and cosmetic industries. Anthocyanin content as an antioxidant will be higher with the intensity of the red color in roselle petals (Hidayanti et al., 2014). Antioxidants play a role in protecting the body from free radicals that have an impact on various disease risks, such as cancer and degenerative diseases. Roselle flower petals also contain other compounds such as vitamin C, vitamin A, vitamin B, calcium, protein, fat, carbohydrates, phosphorus, β-carotene, organic acids, essential amino acids such as lysine and arginine, and omega-3. The utilization of red roselle, especially its flower petals in the food industry, not only as a dye but also can be utilized as beverages such as roselle tea and probiotic beverages such as yogurt and water kefir.

Water kefir is made from a liquid base containing sugar that is fermented by kefir grains. The liquid base for water kefir can be supplemented with fruit juice, leaf extracts, and flower petals. Water kefir has several health benefits such as lowering blood pressure, boosting the immune system, preventing allergies, aiding digestion, and acting as an antimicrobial against microbes that adversely affect health (Lestari et al., 2018). Further development of water kefir can serve as an alternative for people with lactose intolerance in obtaining probiotic compounds from fermented beverages commonly found in dairy products. The sour taste in roselle water kefir comes from the roselle flower petals, which have a sour taste due to citric acid content. Additionally, the sour taste of roselle water kefir can also come from the substrate breakdown by kefir grains. It is important to accurately measure the amount of sugar as the substrate for kefir grains to ensure that the resulting product has a balanced acidity level that is enjoyable for consumers. Research on the utilization of roselle in water kefir products with various concentrations of added sucrose has not been conducted, hence it is necessary to investigate the characteristics of roselle water kefir with varying concentrations of added sucrose concerning viscosity, pH, total yeast content, and hedonic quality to obtain the best flavor.

Materials and Methods

Materials

The materials used included dried red roselle

obtained from Manis Ratu (Semarang, Indonesia), mineral water (Le Minerale, Indonesia), sucrose obtained from Gulaku (PT. Sweet Indolampung, Lampung, Indonesia), water kefir grains obtained from Kefiree (Aracaki, East Jakarta, Indonesia), Potato Dextrose Agar (PDA) medium (Merck, German), chloramphenicol (Novapharin, Indonesia), and distilled water (aquades). The equipment used included a set of processing tools, petri dishes (Anumbra, Czech Republic), pycnometer (Iwaki, Indonesia), digital pH meter (Hanna Instrument, Indonesia), and Ostwald pipette (Pyrex, Indonesia).

Method

The research was conducted from October to December 2022 at Food Chemistry and Nutrition Laboratory and the Food Engineering and Agricultural Products Laboratory, Faculty of Animal and Agricultural Science, Diponegoro University.

Experimental Design

This research used a Completely Randomized Design (CRD). The treatments given included sucrose concentrations of 6%, 9%, 12%, and 15% (w/v).

Production of Roselle Water Kefir

The production of roselle water kefir was carried out according to the research procedure by Hastuti and Kusnadi (2016). Dried red roselle flowers weighted 2 grams were steeped in 200 ml of warm mineral water at a temperature of 80˚C for 15 minutes, then the roselle flowers are filtered. The next stage, sucrose was added according to the treatment concentration. The solution then stirred until homogenized and cooled to room temperature (± 25˚C), then transferred to sterilized glass jars. Kefir grains, 5% (w/v), were added to the glass jars, tightly sealed, and fermented at room temperature (25˚C) for 24 hours. Filtration of the roselle water kefir were performed after the fermentation period to separate the kefir grains from the product.

Analysis of Experiment Parameter

The analysis of experiment parameter including viscosity, pH, total yeast content, and hedonic quality. Analysis of viscosity was carried out with Ostwald pipette referring to Safitri et al. (2013), analysis of pH was carried out using digital pH meter referring to Kinteki et al. (2019), analysis of total yeast content was carried out using Total Plate Count (TPC) method referring to Aulia et al. (2019), and analysis of hedonic quality was carried out using organoleptic test referring to Meilgaard et al. (1999) procedure which conducted with 25 panelist to test samples for the attributes of colour, aroma, taste, and overall.

Statistical Analysis

The data obtained from the analysis viscosity, pH, and total yeast content were statistically analyzed using the one-way Analysis of Variance (ANOVA) parametric test at a significance level of 5% and if there is a significant effect on the results of the ANOVA test, it will be followed up with Duncan's Multiple Range Test (DMRT) analysis at a significance level of 5% to determine whether there is differences in the treatment given. Hedonic quality test data were analyzed using the Kruskal Wallis test at a significance level of 5% and if there was a significant effect, it would be followed by Mann-Whitney test analysis.

Results and Discussion

Viscosity

The viscosity of roselle water kefir when added to various concentrations of sucrose had a significant effect ($p<0,05$) with the value obtained in the range of 1.06 – 1.31 cP, as shown in Table 1. The increase value in the viscosity was due to the increase in total dissolved solids (Yanto et al., 2015). High concentrations of sucrose in roselle water kefir contain high degrees of brix, thereby increasing viscosity due to the presence of excess solids that bind water. The increase in viscosity is also influenced by the unconverted sugar solids by water kefir grains. Excessive sugar usage during the fermentation process of water kefir can result in yeast and bacterial cultures being unable to convert the substrate maximally, leading to an increase in total dissolved solids and viscosity (Kusmawati et al., 2020). Lactic acid bacteria found in water kefir only had metabolic activity to convert 30% of present sugar into lactic acid, while the remaining 70% will be extant in the form of sugar (Abdul et al., 2018).

pH Value

The data in Table 1 shows that the addition of different concentrations of sucrose had a significant effect ($p <0.05$) on the pH value of roselle water kefir in the range of 2.93 – 3.02. The decrease in pH in roselle water kefir is due to the addition of sucrose as a nutrient during fermentation and the use of roselle flowers as raw materials, which are classified as raw materials with low pH (Yusmarini et al., 2015). The low pH in roselle water kefir also indicates an increase in acidity due to the microbial metabolism in water kefir grains utilizing sugar as a nutrient source (Astuti et al., 2018). The availability of substrate during fermentation greatly influences the final pH of the product. The appropriate concentration of sucrose during fermentation enables lactic acid bacteria to convert sucrose into lactic acid optimally (Pranayanti et al., 2015). Conversely, excessively high sucrose

Table 1. Test results of viscosity, pH, and total yeast content of roselle water kefir with various sucrose concentration.

Parameter	Sucrose Concentration Treatment			
	6%	9%	12%	15%
Viscosity (cP)	1.06 ± 0.01^{a}	1.13 ± 0.01^{b}	1.22 ± 0.01^{c}	1.31 ± 0.01^{d}
pH	3.02 ± 0.03^{d}	3.01 ± 0.01^{c}	2.93 ± 0.01^{a}	2.97 ± 0.01^{b}
Total yeast (CFU/ml)	$6.40 \times 10^{4\,a}$	$1.36 \times 10^{5\,a}$	$2.15 \times 10^{5\,b}$	$1.03 \times 10^{5\,a}$

Note: Mean values within a row followed by the same letters are not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test.

Table 2. Organoleptic test result of roselle water kefir with various sucrose concentration.

Sucrose Concentration	Score ± Standard Deviation			
	Colour^{ns}	Aroma^{ns}	Taste	Overall
6%	2,92 ± 0,76	2,24 ± 0,66	2,20 ± 0,87^{a}	2,04 ± 0,98^{a}
9%	2,52 ± 0,96	2,40 ± 0,91	2,72 ± 0,84^{b}	2,84 ± 0,85^{b}
12%	2,44 ± 1,00	2,52 ± 0,96	3,00 ± 1,04^{b}	3,24 ± 0,60^{b}
15%	2,84 ± 0,94	2,72 ± 0,98	2,68 ± 0,95^{b}	2,76 ± 1,01^{b}

Note: Mean values within a column followed by the same letters are not significantly different at p<0.05 according to Duncan's Multiple Range Test.

concentrations can cause an increase in pH due to inhibitory effects by sucrose. Adding sucrose at certain concentrations can create osmotic pressure in the fermentation medium, affecting the growth of specific microorganisms (Taufik et al., 2020).

Total Yeast

The total yeast count among treatments as shown in Table 1 yielded the highest total yeast count was on 12% sucrose concentration at 2.15 x 10^5 CFU/ml. Yeast growth started at the concentration of 6% to 12% , then experienced a decrease at the concentration of 15%. Yeast will continue to grow until it reaches a saturation point, where stagnant conditions can lead to accelerated yeast death due to excessive CO_2 accumulation (Rohman et al., 2019). Yeast in water kefir works together with lactic acid bacteria in the fermentation process, obtaining nutrients from glucose breakdown. Typically, *Saccharomyces cerevisiae* is used in fermentation processes because yeast can convert sugar into alcohol through the action of zymase enzymes under anaerobic conditions, producing ethanol from glucose breakdown and flavor compounds (Rohman et al., 2019). Excessive CO_2 gas due to prolonged fermentation inhibits yeast growth. CO_2 saturated environments and acidic conditions are considered to hinder the growth of *Saccharomyces cerevisiae*, affecting fermentation performance by increasing fermentation lag phase and reducing ethanol yield and productivity (Cunha et al., 2019). The total yeast count in all treatments meets the CODEX 243 (2003) standard, which states that the total yeast count in kefir products should be at least 10^4 CFU/ml.

Hedonic Quality

a. Colour

The addition of different concentrations of sucrose did not have a significant effect ($p > 0.05$) on the panelists' preference for color. The appearance of the color in roselle water kefir with varying sucrose concentrations had almost the same value because the resulting color was consistently pink. There was no significant difference in the product's color due to the pigment in roselle, namely anthocyanin, which was water-soluble. This aligned with the findings of Simanjuntak et al. (2014), who stated that anthocyanins were a type of pigment classified as flavonoids that were generally soluble in water. Anthocyanins were a group of pigments responsible for red hues, located within the cell fluid and soluble in polar solutions (Nopiyanti et al., 2016). Color significantly influenced consumer acceptance and perception of food products. Bright colors could attract attention, convey meaning, and influence consumption (Rahardjo, 2016).

b. Aroma

The addition of different concentrations of sucrose also did not have a significant effect ($p > 0.05$) on the panelists' preference for aroma. Aroma played a crucial role in attracting consumers to consume a product. Aromatic compounds played a significant role in the food service industry, enhancing flavor and generally increasing the attractiveness of food products (Antara et al., 2014). The increasing addition of sucrose did not affect the panelists' preference for the aroma of roselle water kefir because granulated sugar did not have a distinct aroma (Atviolani, 2016). The aroma obtained tended to be acidic due to the process of sucrose conversion by water kefir grains into alcohol. The organic acids produced during the fermentation process were the result of the activity of lactic acid bacteria that converted substrates from sucrose into alcohol. Lactic acid provided sharpness of taste and determined the distinctive aroma of probiotic beverages (Utami, 2018).

c. Taste

The addition of different concentrations of sucrose had a significant effect ($p > 0.05$) on the panelists' preference for taste, particularly when comparing 6% to 9%, 12%, and 15% sucrose. This was likely due to the panelists' lower preference for beverages with an excessively sour taste as sucrose levels increased from 6% to 15%, resulting in more sucrose being converted into organic acids. The higher the concentration of added sucrose and the longer the fermentation time, the lower the pH, indicating a higher acidity level in the water kefir product (Wasilu et al., 2021). It was suspected that the sour taste was also caused by the use of roselle, which was naturally acidic due to its high anthocyanin content, giving it a deep red color and a low pH (Hidayanti et al., 2014).

d. Overall

The addition of different concentrations of sucrose had a significant effect ($p > 0.05$) on the overall acceptance of roselle water kefir in the preference test. The highest overall liking score for the product was 12% sucrose, with a score of 3.24 (like), while the lowest score was obtained by 15% sucrose, which was 2.04 (dislike). The overall liking score represented the panelists' overall acceptance of the tested samples. The overall score indicated the panelists' level of acceptance of the tested samples (Ginting et al., 2019). The results of the overall hedonic analysis were useful for determining the level of consumer liking for the product. Consumer preference mapping analysis was needed as a follow-up to understand the extent to which the product was accepted by consumers and to identify the sensory

Table 3. Determining the best treatment of roselle water kefir with various sucrose concentration.

Parameter	6%	9%	12%	15%	Standard, description
• Viscosity	√	-	-	-	± 0,55 – 1,16 cP (Kusmawati et al., 2020)
• pH	√	-	-	-	± 3,66 – 3,99 (Kusmawati et al., 2020)
• Total yeast	-	-	√	-	10^4 CFU/ml (CODEX 243-2003)
• Hedonic quality:					
▪ Color	√	√	√	√	Pink, all the panelists liked
▪ Aroma	√	√	√	√	Unique fermented aroma that panelists like
▪ Taste	-	-	√	-	Panelists liked the sour taste
▪ Overall	-	-	√	-	Most liked/accepted
Checklists total	4	2	5	2	

Note: Mean values within a row followed by the same letters are not significantly different at $p<0.05$ according to Duncan's Multiple Range Test.

attributes of the product that matched consumer expectations based on the overall evaluation (Afrianto et al., 2017).

d. Overall

The addition of different concentrations of sucrose had a significant effect ($p > 0.05$) on the overall acceptance of roselle water kefir in the preference test. The highest overall liking score for the product was 12% sucrose, with a score of 3.24 (like), while the lowest score was obtained by 15% sucrose, which was 2.04 (dislike). The overall liking score represented the panelists' overall acceptance of the tested samples. The overall score indicated the panelists' level of acceptance of the tested samples (Ginting et al., 2019). The results of the overall hedonic analysis were useful for determining the level of consumer liking for the product. Consumer preference mapping analysis was needed as a follow-up to understand the extent to which the product was accepted by consumers and to identify the sensory attributes of the product that matched consumer expectations based on the overall evaluation (Afrianto et al., 2017).

Best Treatment Analysis

The determination of the best treatment is aimed at identifying the best treatment outcome based on the number of checklists from the testing of viscosity, pH, total yeast, and hedonic quality parameters by comparing the closest evident result value to the standard or description. Based on Table 3, it is evident that 12% sucrose (Treatment 3) accumulates the best parameter values with checklists for total yeast and hedonic quality parameters such as color, aroma, taste, and overall. Roselle water kefir with sucrose addition variations also has the best overall liking score.

Conclusion

The addition of sucrose with different concentrations in the production of roselle water kefir leads to changes in viscosity, pH, total yeast, and hedonic quality, especially in terms of taste and overall liking. The variation in added sucrose will decrease pH but increase total yeast and viscosity. Based on the hedonic quality test results, roselle water kefir products are acceptable to the panelists. The best treatment, considering all parameters, was found in treatment 12% sucrose in the production of roselle water kefir.

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