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Physicochemical, Microbiological, and Organoleptic Characteristics of Rosella Water Kefir with Sucrose Sweetener

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ABSTRACT

Red rosella is one of the beneficial plants with its flower petals that have a distinctly sour taste. Using rosella in water kefir can enrich its functionality and taste. Rosella water kefir has acidic characteristics derived from rosella and sugar substrates. Sugars such as sucrose in the production of water kefir act as a carbon source for the metabolism of water kefir grains. The higher the added sugar causes the total of good microbes and organic acids in water kefir products to increase, but too high sugar can cause the death of these good microbes so it also affects the nutritional content and taste of the product. Therefore, the best concentration of sugar is needed. This study aims to obtain the best sucrose concentration in producing the best physicochemical, microbiological, and organoleptic characteristics of rosella water kefir. This study used the addition of sucrose at concentrations of 6%, 9%, 12%, and 15% (w/v) with the parameters observed in physicochemical characteristics including total dissolved solids, biomass, CO_2 content, and total acid, microbiological characteristics including total lactic acid bacteria and total microbes, and organoleptic characteristics including sweet taste, sour taste, soda sensation, and aroma. The results obtained in this study indicate that increasing the concentration of sucrose in rosella water kefir had a significant effect ($p<0.05$) on the physicochemical, microbiological, and organoleptic characteristics of sweet and sour tastes. The addition of 12% sucrose was the limit—tolerance for the addition of sugar in rosella water kefir which is also the best treatment.

Keywords: microbiological; physicochemical; rosella; sucrose; water kefir

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INTRODUCTION

Red rosella (*Hibiscus sabdariffa* L.) is a plant that is popular and has high selling value. The red flower petals are often used as a natural coloring agent in cosmetics and food products. The use of rosella in food products provides an attractive color where the more intense reddish color on the rosella petals indicates the presence of higher antioxidants. Rosella flower petals contain strong antioxidant activity with IC_{50} 69 – 102 ppm (Djaeni et al., 2017). Rosella flower petals also contain various compounds that are good for the body. Rosella contains vitamins B, C, and D, calcium, protein, fat, carbohydrates, phosphorus, β-carotene, organic acids, essential amino acids in the form of lysine and arginine, and omega-3 (Department of Health Republic Indonesia, 2005). The content of rosella can improve blood circulation and prevent cancer. Rosella in food products can be used in the form of drinks, either rosella tea or probiotic drinks, such as yogurt and kefir.

Kefir is a fermented drink made from kefir grains which contains a symbiosis between yeast and bacteria, such as lactic acid and acetic acid bacteria. The sugar substrate will be broken down into organic acids, alcohol and carbon dioxide by the microbes contained in the kefir grains. The type of kefir generally known by the public is kefir with milk fermentation medium. Milk kefir has a relatively high fat content, so not everyone can consume it, such as those with lactose intolerance (Hardiansyah, 2020). One solution so that people with lactose intolerance can still consume probiotic drinks which are generally found in fermented milk-based products is to consume water kefir. Water kefir is made through fermentation by water kefir grains in a liquid medium containing sugar. To make water kefir, fruit juice or leaf and flower petal extracts can also be added.

Rosella has been used in the production of rosella water kefir where various brands of red rosella tea have no significant effect on the organoleptic and physical properties of the product, but the Mahkota brand has quite a sour taste (Hastuti & Kusnadi, 2016). The sour taste of rosella water kefir comes from the substrate, namely the citric acid content in rosella and the breakdown of sugar into organic acids. Sucrose is a type of sugar that is easy to find and economical. The addition of sucrose to the fermented beverage serves as a carbon source for kefir grains to activate in metabolizing and multiplying so that the number of microbes and organic acids also increases which also affects the nutritional content and taste. The addition of sucrose or other types of sugar that are too high in concentration in fermented drinks can cause the death of the good bacteria contained therein, such as lactic acid bacteria which act as a functional property of probiotics. The death of the good bacteria is due to the high concentration of sugar causing hypertonic conditions in the fermentation medium.

The best sucrose concentration in the manufacture of sweet starfruit water kefir was obtained at 9% with a total lactic acid bacteria of 8.746 logs CFU/mL pH 4.32, and a preference score of 3.76 (Rizqiati et al., 2021), whereas in the production of fruit juice water kefir papaya obtained the best sucrose concentration of 15% with a total LAB of 11.6×10^7 CFU/mL, pH 6.97 and the color, aroma, and taste most preferred by the panelists (Wasilu et al., 2021). Thus the use of different water kefir ingredients produces different best sucrose concentrations and in water rosella kefir the sucrose concentration is unknown which can produce the best water rosella kefir drink characteristics with a taste that is acceptable to society and has a good impact on body health through its probiotic functional properties.

Therefore it is necessary to carry out research related to the concentration of sucrose which needs to be added in producing the physicochemical, microbiological and organoleptic characteristics of rosella water kefir so that it can be accepted by the public.

MATERIALS AND METHODS

Materials

The materials used for the production of rosella water kefir were dried red rosella obtained from Manis Ratu (Semarang, Indonesia), water kefir grains obtained from Kefiree (Aracaki, East Jakarta, Indonesia), mineral water (Le Minerale, Indonesia), and sucrose obtained from Gulaku (PT. Sweet Indolampung, Lampung, Indonesia). The chemicals, including pH 4 and 7 buffer solution (Merck, Germany), aquades, NaOH 0,1 N (Merck, Germany), NaCl (Refina, Indonesia), Na_2CO_3, phenolphthalein 1% (Merck, Germany), 70% and 96% alcohol (Onomed, Indonesia). The media for microbiological analysis, MRSa, and PCA (Merck, Germany).

Tools

The tools used in this study were analytical balance (Mettler Toledo, Switzerland), burette (Pyrex, France), refractometer (Atago, Japan), beaker glass (Pyrex, France), Erlenmeyer (Pyrex, France), hotplate (IKA, Indonesia), magnetic stirrer (IKA, Indonesia), conical tube (Dispolab, Indonesia), autoclave (Hirayama, Japan), vortex (Thermolyne, Germany), petri dishes (Anumbra, Europe), micropipette (Dragonlab, China), micropipette tip (Onomed, Indonesia), and incubator (Memmert, Germany).

Methods

Experimental Set-Up

The experimental design used in this study was a single factor Completely Randomized Design (CRD). The treatments given included sucrose concentrations of 6%, 9%, 12%, and 15% (w/v).

Production of Rosella Water Kefir

The production of rosella water kefir was carried out according to Hastuti and Kusnadi (2016) research procedure. Dried red rosella flowers as much as 2 grams are brewed with 200 ml of warm mineral water at 80˚C for 15 minutes then the rosella flowers are filtered. The next stage, giving treatment with the addition of sucrose concentration. The rosella solution was homogenized and cooled down to room temperature (±25˚C), then the solution was put into a glass jar that had been sterilized. Kefir grains of water as much as 5% (w/v) with a starter density of 3.70×10^8 CFU/g were added to a glass jar and tightly closed and fermented at room temperature (±25˚C) for 24 hours. Rosella water kefir is filtered after the boiling period is over to separate the kefir grains from the product.

Analysis of Physicochemical Characteristics

Analysis of physicochemical characteristics of rosella water kefir, including total dissolved solids, biomass, total acid, and CO_2 content. Analysis of total dissolved solids was carried out with a refractometer referring to the procedure carried out by Insani et al. (2018), analysis of biomass was carried out by weighing the initial and final weight before and after fermentation which referred to the research procedure carried out by Utami et al. (2018), total acid analysis was carried out by the titration method refers to the procedure carried out by Mulyani et al. (2021), and for the analysis of CO_2 levels it was carried out by titration referring to the research procedure carried out by Surja et al. (2019).

Analysis of Microbiological Characteristics

Analysis of the microbiological characteristics of rosella water kefir was carried out using the Total Plate Count (TPC) method which included total Lactic Acid Bacteria (LAB) referring to the research procedure of Rohman et al. (2019) and total microbes refer to the research procedure of Wiratna et al. (2019).

Analysis of Organoleptic Characteristics of Rosella Water kefir

Analysis of the organoleptic characteristics of rosella water kefir was carried out according to research procedures by Andaru et al. (2019), which was modified by using 25 panelists to test samples for the attributes of sweet taste, sour taste, sensation of soda, and aroma. Panelists were asked to sort intensity of sour taste, sweet taste, sensation of soda, and aroma from low to high intensity. Panelists were also asked to neutralize their sense of taste when changing samples. The score for sweet taste, namely (1) not sweet, (2) slightly sweet, (3) sweet, (4) very sweet. The score for sour taste, namely (1) not sour, (2) slightly sour, (3) sour, (4) very sour. The score for soda sensation, namely (1) not felt, (2) slightly felt, (3) felt, (4) strongly felt. The score for the aroma score, namely (1) not pungent, (2) slightly pungent, (3) pungent, (4) very pungent.

Statistical Analysis

The data obtained from the analysis of physicochemical and microbiological characteristics 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. Organoleptic characteristic 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.

RESULT AND DISCUSSIONS

Physicochemical Characteristics of Rosella Water Kefir

Total Dissolved Solids

Analysis of total dissolved solids is used to establish the amount of residual sugar that does not break down during the fermentation process. The data in Table 1 shows that the addition of different concentrations of sucrose had a significant effect ($p<0.05$) on the total dissolved solids of rosella kefir water, which ranged from 5.94 – 13.16˚ Brix. The sucrose addition contributed to the total dissolved solids value which also increased. Sucrose in the fermentation process will be overhauled by the kefir grain microbes, where the remaining sugar that is not broken will be included in the total dissolved solids (Ningsih et al., 2019). Lactic Acid Bacteria known as one of the microbes in water kefir grains, have an optimal ability to break down sugar during its process. Lactic Acid Bacteria only have metabolic activity converting about 30% of sugar into lactic acid, and the 70% will remain in the form of sugar (Abdul et al., 2018)

Biomass

Biomass analysis was used to determine the change in weight of water kefir grains after the fermentation process. The data in Table 1 shows that the addition of different concentrations of sucrose had a significant effect ($p<0.05$) on the biomass of water kefir grains ranging from 1.13 – 2.22g. There are several factors that affect the yield of biomass, including fermentation temperature, the type of bacterial strain in kefir grains, and the addition of nutrient concentrations correctly in the fermentation medium (Nursiwi et al., 2015).

Based on Table 1 the increase in biomass occurred up to the addition of 12% sucrose concentration, then at the addition of 15% sucrose concentration, there was a decrease in biomass. This increase in biomass is thought to have come from the optimal ability of microorganisms to produce exopolysaccharides. The exopolysaccharide in water kefir seeds consists mostly of insoluble dextran with the predominant α-1.6 bond glucan polysaccharide and a variable number of α-1, α-1.2, and α-1.3 4-branch linkages (Fels et al., 2018). While the decrease in biomass at the addition of a 15% sucrose concentration was caused by the high sucrose as a carbon source causing cell death. The high concentration of kefir grains carbon sources makes the condition hypertonic and results in cell plasmolysis so that the biomass will decrease (Utami et al., 2018). The decrease in the value of biomass in kefir grains can also be caused by the release of total polysaccharides including dissolved exopolysaccharides (EPS).

Table 1. The physicochemical characteristics of rosella water kefir

Physicochemical Characteristics	Sucrose Concentration Treatment (%)			
	6%	9%	12%	15%
Total dissolved solid (˚Brix)	5.94 ± 0.05^{a}	8.40 ± 0.12^{b}	11.06 ± 0.05^{c}	13.16 ± 0.15^{d}
Biomass (g)	1.38 ± 0.14^{b}	1.78 ± 0.15^{c}	2.22 ± 0.27^{d}	1.13 ± 0.06^{a}
Total acidity (% of lactic acid)	0.18 ± 0.01^{a}	0.23 ± 0.01^{b}	0.32 ± 0.01^{d}	0.28 ± 0.01^{c}
CO_2 content (%)	0.09 ± 0.01^{a}	0.10 ± 0.01^{b}	0.13 ± 0.01^{c}	0.14 ± 0.01^{d}

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.

Total Acidity

The total acids value of rosella water kefir when added to various concentrations of sucrose was obtained in the range of 0.18 – 0.32% (Table 1), which is in accordance with Indonesian National Standard but not in accordance with CODEX STAN standards. Water kefir does not yet have a set quality standard, so the quality standard refers to products similar to fermented milk probiotic drinks or milk kefir. Based on CODEX STAN 234 (2003), kefir products have a minimum value of total titrated acid as % lactic acid of 0.6%, while based on Indonesian National Standard with SNI number 2981 (2009) probiotic drinks have total acid with a value range of 0.2 – 0 .9%. The lactic acid in water kefir drinks is formed due to the activity of Lactic Acid Bacteria which break down sugar into lactic acid, therefore the total calculated acid product of water kefir is assumed to be the accumulation of lactic acid which is formed from the metabolism of Lactic Acid Bacteria.

The data in Table 1 shows that the addition of different concentrations of sucrose had a significant effect ($p < 0.05$) on the total acid value of rosella water kefir. The increase in total acid in rosella water kefir occurred with the addition of sucrose concentration up to 12% addition, then decreased at 15% sucrose concentration. Microbes of water kefir grains at a sucrose concentration of 12% were thought to be able to metabolize optimally resulting in the highest total acid value, whereas at a concentration of 15% a hypertonic condition occurred which disrupted microbial growth. According to Cahyanti et al. (2021), the total titrated acid decreased in the product by the treatment of adding ingredients with different sugar content indicating a decreased activity of microbial metabolism.

CO_2 Content

The CO_2 content of rosella kefir water in the addition of various sucrose concentrations was obtained in the range of 0.09 – 0.14% (Table 1). The data in Table 1 shows that the addition of different sucrose concentrations had a significant effect ($p<0.05$) on the CO_2 content of rosella water kefir. The higher the addition of the sucrose concentration up to a concentration of 15%, the higher the CO_2 content obtained in rosella water kefir. The sucrose is used by yeast as a microbe found in water kefir grains to grow and reproduce and to be broken down into metabolic products in the form of CO_2, alcohol, and organic acids (Hawusiwa et al., 2015). Carbon dioxide with alcohol presents characteristics similar to carbonated drinks, however, rosella water kefir cannot be categorized as a carbonated drink. The standard CO_2 content in carbonated drinks should have a level of 0.589 – 0.900% (SNI 3708-2015).

Microbiological Characteristics of Rosella Water Kefir

Total Lactic Acid Bacteria (LAB)

Based on Indonesian National Standard with SNI number 7552 (2018), the minimum number of Lactic Acid Bacteria in fermented milk drinks without heat treatment after fermentation is 10^6 CFU/mL. This rosella water kefir drink based on total LAB has met the requirements as a probiotic drink which has good benefits for health, especially for the. digestive tract.

Table 2. The microbiological characteristics of rosella water kefir

Microbiological Characteristics	Sucrose Concentration Treatment (%)			
	6%	9%	12%	15%
Total Lactic Acid Bacteria (CFU/mL)	1.28×10^{7a}	6.62×10^{7b}	7.42×10^{7d}	4.09×10^{7c}
Total microbes (CFU/mL)	3.39×10^{7a}	8.13×10^{7b}	7.24×10^{8c}	6.32×10^{7b}

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.

The minimum amount of probiotics in food products when consumed and active in digestion is 10^7 CFU/mL (Imelda et al., 2020).

The data in Table 2 shows that the addition of different concentrations of sucrose had a significant effect ($p<0.05$) on the total LAB of rosella water kefir with the highest total LAB occurring in the addition of 12% sucrose of 7.42×10^7 CFU/mL. The concentration of sugars can influence the growth and function of LAB (Abbasiliasi et al., 2017). The addition of sucrose will increase the total LAB as long as it can be tolerated by LAB, where LAB only has the ability to break down sugar into lactic acid by 30% (Abdul et al., 2018). The addition of sucrose with excess concentration will inhibit the growth of bacteria, such as the addition of a 15% sucrose concentration to rosella water kefir which decreases the total LAB. The addition of carbon as a nutritional source of excess Lactic Acid Bacteria will cause a change in enzyme activity in which cells lose water due to high osmotic pressure and then cells will experience plasmolysis (Effendi & Parhusip, 2021).

Total Microbes

The total microbes of rosella water kefir on the addition of various concentrations of sucrose obtained ranged 3.39×10^7 - 7.24×10^8 CFU/mL (Table 2), which met the minimum standard for total microbes in the reference CODEX STAN 234 (2003) where total microbes in the kefir starter culture were at least 10^7CFU/mL. The total microbial obtained increased with the addition of 12% sucrose, then decreased with the addition of 15% sucrose concentration. The more sugar added to fermented drinks, the more substrate for microorganisms will be so that the activity of microorganisms to reproduce increases. However, sugar concentrations that are too high can also make the microbial cell environment hypertonic so that microbial cells experience plasmolysis and there is a decrease in the total number of microorganisms in fermented beverage products. According to Nurainy et al. (2018) which states that sucrose added in excess of the microbial nutritional needs is thought to be able to cause the microbes to undergo plasmolysis because the external environment of the microbial cells becomes hypertonic so that the microbial intracellular fluids come out of the cells, then dehydration occurs which leads to microbial cell shrinkage and lysis.

Organoleptic Characteristics of Rosella Water Kefir

Sweet Flavor

Flavor is one of the important attributes of food products. The sweet flavor is a flavor attribute that is liked by most people. The data in Table 3 shows that the addition of different concentrations of sucrose had a significant effect ($p<0.05$) on the sweet taste of rosella water kefir with scores ranging from 1.40 to 3.16, that is, not sweet to sweet.

Table 3. The organoleptic characteristics of rosella water kefir

Organoleptic Characteristics	Sucrose Concentration Treatment (%)			
	6%	9%	12%	15%
Sweetness score**	1.40 ± 0.50^a	2.20 ± 0.71^b	2.88 ± 0.60^c	3.16 ± 0.80^c
Sourness score***	2.92 ± 0.95^a	2.36 ± 0.70^b	2.08 ± 0.70^{bc}	1.80 ± 0.71^c
Soda sensation score****	1.48 ± 0.71	1.52 ± 0.82	1.56 ± 0.71	1.60 ± 0.76
Aroma score*****	2.52 ± 1.00	2.44 ± 0.92	2.12 ± 0.67	2.36 ± 1.04

Note: Mean values within a column followed by the same letters are not significantly different at $p < 0.05$ according to Mann-Whitney Test.
**Sweetness score (1) not sweet, (2) slightly sweet, (3) sweet, (4) very sweet.
***Sourness score (1) not sour, (2) slightly sour, (3) sour, (4) very sour.
****Soda sensation score (1) not felt, (2) slightly tasted, (3) felt, (4) strongly felt.
*****Aroma score (1) not pungent, (2) slightly pungent, (3) pungent, (4) very pungent.

The addition of sucrose to fermented products will still add a sweet taste even though organic acids are produced during the fermentation process (Kusumawati et al., 2015). The sweet taste of rosella water kefir increased with the addition of sucrose concentration, but the addition of sucrose with a concentration of 12% had a sweetness score that was not significantly different from the addition of 15% sucrose.

Sour Flavor

The sour taste is a taste attribute that is generally considered in fermented products. The data in Table 3 shows that the addition of sucrose at different concentrations had a significant effect ($p<0.05$) on the sour taste of rosella water kefir with a score range of 1.80 – 2.92, from slightly sour to sour. The sour taste obtained in rosella water kefir comes from rosella raw materials which have a distinctive sour taste and from the metabolism of lactic acid bacteria and yeast in water kefir in the form of organic acids, such as lactic acid. The results of the sour taste in rosella water kefir indicate that the higher the concentration of sucrose added, the lower the sour taste produced. It is suspected that the increasing concentration of sucrose does not completely break down so that the sour taste will be covered by the sweet taste of the remaining sugar. The sour taste produced at the addition of 9% sucrose was not significantly different from the addition of 12% sucrose concentration and at the addition of 12% sucrose concentration was not significantly different from the addition of 15% sucrose concentration. The addition of sweeteners such as sucrose with a concentration of 8% or more in fermented products containing lactic acid bacteria will result in disruption of lactic acid production (Rumeen et al., 2018).

Soda Sensation

The sensation of soda is one of the factors that also determine the quality of the product. The data in Table 3 shows that the addition of different concentrations of sucrose had no significant effect ($p>0.05$) on the sensation of rosella kefir soda water with a score range of 1.48 – 1.60, that is, not felt to slightly tasted of soda. The soda sensation that is formed is the result of yeast metabolic activity. The difference in the concentration of added sucrose affects the formation of carbon dioxide and alcohol, where the higher concentration of sugar in the manufacture of water kefir can give a stronger soda sensation. The low sensation of soda in the water kefir product is due to the presence of microbial metabolites in the form of organic acids which can mask the sensation of the soda formed. The sensation of the taste of soda in water kefir made from green coconut is covered by the sour taste resulting from the fermentation process so that the soda flavor is not strong (Rohman et al., 2019).

Aroma

Fermented products generally have a distinctive aroma. The data in Table 3 shows that the difference in sucrose concentration had no significant effect ($p>0.05$) on the aroma of rosella water kefir with a score range of 2.12 – 2.52, which is slightly pungent. The strong aroma of kefir is influenced by the number of volatile compounds formed from the fermentation process (Lestari et al., 2018). The pungent aroma of roselle water kefir comes from volatile compounds produced by microbial activity, such as lactic acid bacteria in the form of lactic acid, acetic acid bacteria in the form of acetic acid, and yeast in the form of alcohol during the fermentation process.

The insignificant difference between the four sucrose concentration treatments could be due to the characteristics of lactic acid as the dominant organic compound in water kefir as well as being a volatile compound that has non-volatile characteristics at room temperature. According to Lumba et al. (2017) states that lactic acid does not evaporate at room temperature conditions. Lactic acid produced from lactic asthma bacteria has the ability to break down sugar by 30% so that any residual sugar that doesn't break down can also cover up the pungent smell.

According to Rahmah and Aulia (2022), the increasing addition of granulated sugar can cover up the presence of a sour aroma in a food product.

CONCLUSIONS

Based on the results of the study, the physicochemical, microbiological, and organoleptic characteristics of rosella kefir water were affected by the addition of sucrose. The physicochemical properties of rosella water kefir towards total dissolved solids, biomass, CO_2 content, and organoleptic sweetness increased with the addition of sucrose. Physicochemical properties of biomass and total acid and microbiological properties increased with the addition of up to 12% sucrose and decreased with 15% sucrose. The sour taste of rosella water kefir is reduced by the addition of sucrose. The best treatment was obtained with the addition of 12% sucrose.

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