Effect of Sucrose and Starter on the Chemical and Organoleptic Properties of Fig Leaf Tea Water Kefir

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ABSTRACT

Water kefir is a fermented drink made by adding water kefir grains to a water solution containing dried fruit or sugar. This research aims to determine the effect of sucrose, starter, and interactions on the chemical characteristics (antioxidant activity, total acid, medium pH) and organoleptics of fig leaf tea water kefir and to determine the interaction of antioxidant activity with total acid and medium pH. The experiment used variations in starter concentration (5% and 8%) and sucrose concentration (3%, 6%, 9% and 12%). Water kefir was analyzed through chemical analysis (antioxidants, total acid, medium pH, and sugar content) and organoleptic tests (flavor, aroma, color, and overall preference). The results showed that variations in the addition of sucrose, starter, and interactions influenced the metabolic results (primary and secondary metabolites) of microorganisms in water kefir grains and statically had a significant effect on changes in chemical and organoleptic properties (flavor, aroma, and overall preference). The best chemical characteristic parameters were obtained in the B2S3 treatment (8% starter + 9% sucrose) with antioxidant activity of 51.27%, total acid of 0.70%, and medium pH of 3.53. Meanwhile, the best organoleptic parameters were obtained in the B2S4 treatment (8% starter + 12% sucrose) with a taste value of 5.56 (liked), an aroma value of 4.80 (slightly liked), a color value of 4.97 (slightly liked), and the overall liking level was 5.45 (liked), and the antioxidant activity of fig leaf tea water kefir had a strong relationship with total acid and medium pH. Furthermore, the addition of sucrose and different starters during the water kefir fermentation process affects the chemical and organoleptic properties of fig leaf tea water kefir. It is hoped that the results of this research will make fig leaf tea water kefir an alternative probiotic drink for sufferers of lactose intolerance and allergies to milk-based drinks.

Keywords: Antioxidant activity; fig leaf tea; organoleptic; water kefir

INTRODUCTION

Kefir is a fermented milk drink well known to the public (Jaya, 2019) and produced using seeds as a starter containing probiotics. The drink has a unique taste that is slightly sour, fizzy, and alcoholic with beneficial effects on health (Nielsen et al., 2014; Rizqiati et al., 2021). The majority of probiotic drinks in circulation are milk-based. However, the development of lifestyles such as vegetarian groups underscores the need to produce probiotic drinks using non-dairy ingredients for individuals suffering from diseases such as lactose intolerance and those allergic to ingredients in milk including alpha lactabumin (Kandylis et al., 2016; Kumar et al., 2015). In this context, an effective solution is developing water kefir which uses a fermentation

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media in the form of sucrose solution, fruit or vegetable juice, and herbal tea with the addition of kefir seeds containing lactic acid bacteria (LAB), acetic acid bacteria (BAA), and yeast (Laureys et al., 2018).

Water kefir is a fermented drink that is rich in antioxidants, and according to Alsayadi et al., 2013 the antioxidants produced are obtained from intracellular-extracellular metabolites, as well as lysis products from LAB and yeast. Lathif (2016) using factors such as fermentation time and tea leaf concentration, reported that the fermentation time and concentration of tea leaves influenced the antioxidant activity of water kefir. The increase was dominated by antioxidant compounds contained in tea leaves. Fermentation time can also increase the antioxidant activity of water kefir through natural antioxidant content obtained from metabolites in the form of organic acids produced by microbes in water kefir seeds.

The fig plant (*Ficus carica* L.) is well-known and widely cultivated in the community. One part, namely leaves, is made into herbal tea which contains antioxidant compounds, including alkaloids, tannins, phenolics, organic acids, flavonoids, steroids, and quercetin derivatives (Li et al., 2021; Radwan et al., 2020). However, based on empirical experience, fig leaf tea produces a bitter taste which tends to be disliked by some groups of people, including children and teenagers. The use of herbal tea as a water kefir fermentation media has been widely applied in previous research, including using horsewhip leaf (Isrianto, 2019), ternate telang (Setiawati & Kusnadi, 2021), and ashitaba leaf tea (Kusumastuti et al., 2022). According to Serventi et al. (2020) using herbal tea as a fermentation medium for water kefir can optimally reduce the bitter taste of herbal tea, and based on organoleptic tests, water kefir using herbal tea can produce a taste acceptable to panelists. To produce a taste that is acceptable to all groups, the use of fig leaf tea as fermentation media for water kefir is a suitable approach.

The success of the fermentation process and the assurance of the physicochemical and microbiological quality of water kefir are dependent on the substrate and starter. The water kefir seeds' substrate serves as a source of nutrients and energy for the microorganisms' growth and development (Çevik et al., 2019). One kind of substrate that is frequently utilized in the manufacturing of water kefir is sucrose, which is said to be more effective in promoting the growth of microorganisms than glucose and fructose (Laureys et al., 2021). According to Rizqiati et al. (2021), adding 9% sucrose to star fruit water kefir resulted in the best media pH, alcohol concentration, total LAB, and organoleptic assessment. Effendi & Parhusip (2022) and Laureys & De Vuyst (2017) state that the minimum alcohol content for water kefir products is 0.5%, the maximum media pH is 4.5, the minimum total acid is 0.2-0.9%, and the total LAB must be at least 106 CFU/mL. These parameters indicate the direct role that the starter plays in the fermentation process. Using a starting concentration of 5% w/v and 8% w/v in water kefir fermentation produced treatment with the best chemical and organoleptic parameters, according to Lestari et al. (2018) and Utomo & Kurniawidi, (2021). Therefore, this research was conducted to determine the effect of adding sucrose, starter, and the interaction on chemical characteristics, including antioxidant activity, total acid, and media pH, as well as organoleptic properties of fig leaf tea water kefir. The relationship or correlation between antioxidant activity with total acid, media pH, and sugar content was also evaluated.

METHODS

Materials

The materials used in this research were fig leaves obtained from fig plantations in the Mount Arjuna area, distilled water, Gulaku brand granulated sugar, water kefir seeds obtained from water kefir activists in Malang City, 1% phenolphthalein (PP) indicator, Methanol Pro Analis (PA), SmartLab brand DPPH (1,1-diphenyl-2-picrylhydrazyl) powder, Sodium hydroxide (NaOH), anhydrous sodium carbonate (Na_2CO_3), Kna tatrate, sodium bicarbonate ($NaHCO_3$), copper sulfate pentahydrate ($CuSO_4 . 5H_2O$), concentrated sulfuric acid (H_2SO_4), diammonium molybdate $(NH_4)_2M_oO_4$, and anhydrous glucose powder.

The tools used were a pH meter (Hanna), analytical scales (OHAUS Pioneer & OHAUS Scout Pro), UV-Vis spectrophotometer (Shimadzu UV-1280), water bath shaker, 500 mL beaker, 10 mL beaker, 100 mL measuring flask, 10 mL measuring flask, burette, static, erlenmeyer, 10 mL measuring pipette (Iwaki Pyrex), micropipette, aluminum foil, tip, vortex, dropper pipette, and test tube.

Research Design

The research used was a randomized block design (RBD) arranged factorially with two treatment factors, namely sucrose concentration (3% w/v, 6% w/v, 9% w/v, and 12% w/v) and concentration starter (5% w/v and 8% w/v). The combination obtained was eight treatments, namely B1S1 (starter 5% + sucrose 3%), B1S2 (starter 5% + sucrose 6%), B1S3 (starter 5% + sucrose 9%), B1S4 (starter 5% + 12% sucrose), B2S1

(8% starter + 3% sucrose), B2S2 (8% starter + 6% sucrose), B2S3 (8% starter + 9% sucrose), and B2S4 (8% starter + 12 % sucrose). Each treatment was carried out two times with duplo testing which included total acid, sugar content, pH of the media, antioxidant activity, and organoleptic tests with parameters tested for taste, aroma, color, and overall preference.

Research Stages

Making fig leaf tea

Fresh fig leaves were washed with running water, and then dried in the sun for 10-15 minutes or until the water on the surface dried. Subsequently, the leaves were cut into pieces 3 – 5 cm in size, weighed at 10% w/v, and placed into a container, followed by brewing using hot water. Soaking was carried out until room temperature was achieved, and then the leaves were filtered. The filtrate was used to make fig leaf tea water kefir (Putri, 2018).

Making fig leaf tea water kefir

Fig leaf tea was poured into a container in the form of a 300 mL glass jar then 3% w/v, 6% w/v, 9% w/v, and 12% w/v sucrose were added to each glass jar and stirred until homogenous. Subsequently, the starter was added at 5% w/v and 8% w/v, then covered with a clean cloth and incubated for 48 hours in a dark room at room temperature. After 48 hours of fermentation, the seeds were filtered and fig leaf tea water kefir was obtained which was then tested using antioxidant activity, total acid, media pH, reducing sugar content, and organoleptic tests (Lathif, 2016).

Testing the antioxidant activity using the DPPH method

The DPPH method for testing antioxidant activity referred to Khotib (2018) with modification of the dilution volume. The sample was diluted by inserting 10 mL into a 100 mL measuring flask then adding PA methanol to the limit mark and homogenizing. The first dilution was taken as 5 mL, placed into a 10 mL measuring flask, and methanol PA was added, followed by homogenizing, and the dilution results were used as a test sample. Absorption measurements started by taking 4.5 mL of the test sample and placing in a test tube, then adding 1.5 mL of 50 ppm DPPH. The mixture was homogenized by vortexing and incubated for 30 minutes in the dark at room temperature. After incubation, the absorbance of the samples was measured using a spectrophotometer with a wavelength of 517 nm, and the % antioxidant activity was calculated using Equation 1.

$$\%\ \text{Antioxidant activity} = \frac{\text{Absorbance of the control-Absorbance of the sample}}{\text{Absorbance of the control}} \times 100\% \quad (1)$$

Total acid testing

Total acid testing referred to Mulyani et al. (2021) by placing 10 mL of the test sample in a beaker, then adding two to three drops of 1% PP indicator. The mixture was subsequently titrated using 0.1N NaOH until a pink color was formed. The volume of NaOH used was recorded and calculated using Equation 2.

$$\text{Total acid}\ (\%) = \frac{\text{Volume NaOH x N NaOH x 90}}{\text{Volume sampel x 1000}} \times 100\% \quad (2)$$

Media pH testing

Measurement of pH started by calibrating the pH meter on a pH 4 and 7 buffer solution. Subsequently, water kefir was placed into a 50 mL beaker and the pH was checked alternately for each treatment.

Testing for reducing sugar content

Testing for reducing sugar content referred to Haryanti and Mustaufik (2020) using the Nelson Somogyi method. Preparation of Nelson A reagent started by mixing 12.5 g of anhydrous Na_2CO_3, 10 g of $NaHCO_3$, and 100 g of Na_2SO_4 in 500 mL of distilled water. Nelson B reagent was prepared by dissolving 15 g $CuSO_4.5H_20$ in 100 mL distilled water and added with 1-2 drops of concentrated H_2SO_4. Furthermore, arsenomolybdate reagent was prepared by making ammonium molybdate solution (25 g ammonium molybdate to 400 mL distilled water add 21 mL H_2SO_4 concentrated) and arsenate solution (3 g disodium hydrogen arsenate in 25 mL distilled water). Both solutions were homogenized and incubated at 37°C for 24-28 hours using a water bath shaker. Subsequently, a glucose standard curve was constructed and the sugar content was tested by diluting the test sample and inserting 1 mL and 1 mL of C stock solution into a test tube. The test tube was heated in a beaker for 20 minutes, cooled to 25°C, added 1 mL of arsenomolybdate reagent, and 7 mL of distilled water. This was followed by vortexing until the Cu_2O precipitate dissolved. The absorbance of each sample was measured with a wavelength of 540 nm and the calculations were carried out using Equation 3.

$$\text{Reducing sugar}\ (\%) = \frac{\text{mg glucose x fp x v}}{\text{mg sample}} \quad (3)$$

Organoleptic test

Organoleptic test used the preference method (hedonic scale scoring) to determine the acceptance of taste, aroma, color, and overall liking using 40 untrained panelists who were Brawijaya University students. The hedonic scale used consisted of (1) dislike extremely, (2) dislike very much, (3) dislike, (4) like slightly, (5) like, (6) like very much, (7) like extremely (Astuti et al., 2018).

Data analysis

The data obtained from the total acid test, media pH, and antioxidant activity were analyzed using a two-way ANOVA test, while the organoleptic test data were assessed using a one-way ANOVA test through Minitab 19. When there were significant differences in the treatment factors in the form of sucrose, starter, and sucrose-starter interactions, the Tukey further test or Honest Significant Difference was used with a significance of 5%. Data on reducing sugar levels were analyzed using a non-parametric test in the form of the Wilcoxon test because the number of samples was small and not normally distributed. The objective was to determine differences between treatment samples. Pearson correlation testing used Minitab 19 to obtain the coefficient and the direction of the relationship for the test parameters including antioxidant activity, total acid, media pH, and reducing sugar content.

RESULTS AND DISCUSSION

Total Acid

The average total acid of fig leaf tea water kefir in the 5% starter treatment group ranged from 0.44% to 0.64%, while in the 8% treatment group, the value was between 0.53% to 0.70%. Figure 1 shows that the average total acid obtained from each sucrose and starter treatment group tended to increase.

Based on ANOVA analysis, each factor of sucrose, starter, and the interaction had a significant influence ($p<0.05$) on the average total acid produced by fig leaf tea water kefir. It shows that the 3% and 9% sucrose treatments produced significantly different average total acid values, yielding the lowest and highest average

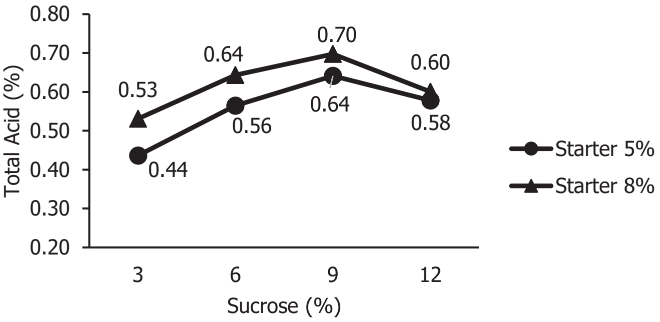

Figure 1. Graph of the total acidity of fig leaf tea water kefir

total acid respectively. The sucrose factor influences the organic acids produced during fermentation, as previously reported by Kinteki et al. (2019) and Lynch et al. (2021) stating that the total acid in water kefir increased along with a rise in sucrose concentration. The higher the concentration of sucrose or substrate added, the greater the amount of substrate broken down by yeast into monosaccharides (glucose and fructose) which are then consumed by LAB and BAA in water kefir seeds, thereby increasing the production of lactic acid and acetic acid during the fermentation process. On the other hand, the decrease in total acid in the 12% sucrose concentration treatment in each 5% and 8% starter concentration group could be attributed to the high sucrose concentration which interfered with the survival of the microorganisms in the water kefir seeds. One of the consequences of high sucrose concentrations is a disturbance in the balance of osmotic pressure in microbial cells which causes the loss of water, potentially triggering cell death (Rimadhini et al., 2020). This condition facilitates disturbances in bacteria growth and physiological activities such as acid production (Cai et al., 2021), hence, the amount of organic acid produced by microbes is not optimal. A similar incidence occurred in research on red rosella water kefir by Rizqiati et al. (2023) who obtained total acid of 0.32% at a sucrose concentration of 12% then experienced a decrease to 0.28% at a concentration of 15% followed by a decrease in the total amount of LAB.

Regarding the ANOVA analysis, it also shows that the 5% and 9% starter treatments produced significantly different average total acids. This implies that the addition of starter has a significant effect on the total production of water kefir acid in utilizing the substrate as energy and then converting into organic acid. Prastujati et al. (2018) and Laureys & De Vuyst (2016) explained that each increase in starter concentration allowed an increasing number of microbes to be added to the fermentation media. Therefore, more microbes can convert monosaccharides contained in the media into organic acids (lactic acid and acetic acid). Triwibowo et al. (2020) found that cow milk kefir experienced successive increases in total acid from a starter concentration of 3% (1.58%), 5% (1.89%), and 7% (2.18%). According to Anjliany et al. (2022) dragon fruit skin water kefir obtained a similar increase in total acid, namely at a starter concentration of 5% (0.18%), and 10% (0.21%).

In the Tukey test, treatment B1S1 (5% starter + 3% sucrose) and B2S3 (8% starter + 9% sucrose) had the lowest and highest average total acid respectively. This implies that the two treatments had an average total acid that was significantly different from the others. The

Table 1. Tukey total acid test of fig leaf tea water kefir

Sample	Total acid (%)	Notation
B1S1 (5% starter + 3% sucrose)	0.44	e
B1S2 (5% starter + 6% sucrose)	0.56	cd
B1S3 (5% starter + 9% sucrose)	0.62	b
B1S4 (5% starter + 12% sucrose)	0.59	bc
B2S1 (8% starter + 3% sucrose)	0.53	d
B2S2 (8% starter + 6% sucrose)	0.64	b
B2S3 (8% starter + 9% sucrose)	0.70	a
B2S4 (8% starter + 12% sucrose)	0.60	bc

B2S3 treatment (8% starter + 9% sucrose) produced an optimal fermentation process, yielding a higher total acid than the others. Meanwhile, the B1S1 treatment (5% starter + 3% sucrose) produced a fermentation process that was not optimal, resulting in less total acid. The differences observed in other treatments were not statistically significant.

Figure 1 shows that for each treatment combination, an increase in sucrose and starter concentration resulted in an elevated total amount of acid. This implies that increasing the concentration of sucrose and starter can optimize the fermentation process in producing total water kefir acid at a certain concentration limit. Previous research on walnut milk kefir by Cui et al. (2013) obtained an average increase in titratable acidity for each increase in starter concentration, namely 3% w/v (61%), 5% w/v (63%), 7% w/v (70%), 9% w/v (76%) but decreased when the sucrose concentration was 6% w/v (75%), 8% w/v (74%), 10% w/v (73%), 12% w/ v (73%). Effendi and Parhusip (2021) after reviewing several previous studies related to kefir and water kefir concluded that the interaction of substrate concentration factors with the starter or inoculum had a significant effect on the total acidity.

Reducing Sugar Levels

The average reducing sugar content of fig leaf tea water kefir in the 5% starter treatment group ranged from 0.67% to 1.19%, while in the 8% group, the value was between 0.87% and 1.39%. Changes in reducing sugar content occurred during the fermentation process in the treatment group. The concentration of sucrose and starter tended to increase during fermentation as shown in Figure 2.

Based on the Wilcoxon test analysis, there was a significant decrease in sugar content before and after fermentation in each combination treatment ($p<0.05$).

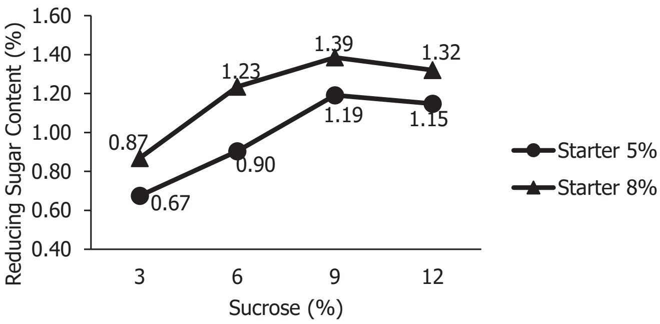

Figure 2. Graph of reducing sugar content in fig leaf tea water kefir

The highest reduction was obtained in the B2S3 concentration treatment (8% starter + 9% sucrose), yielding the most optimal fermentation process compared to other treatments and the total acid produced was 0.70%. On the other hand, the lowest reduction in sugar content was obtained in the B1S1 treatment (5% starter + 3% sucrose), with a less than optimal fermentation process and the lowest average total acid compared to others, namely 0.44%. In general, monosaccharides play an important role during the fermentation process, influencing homofermentative LAB in the glycolysis pathway, heterofermentative LAB in the phosphoketolase pathway, and yeast in ethanol fermentation. The metabolite products in the form of pyruvic acid and lactic acid, acetic acid, ethanol, carbon dioxide, and acetaldehyde accumulate in the fermentation media. Therefore, differences in the amount of acid accumulated in each treatment affect the total acid contained in fig leaf tea water kefir (Anggoro et al., 2022).

pH Media

The average pH value of the water kefir media for fig leaf tea in the 5% starter treatment group ranged

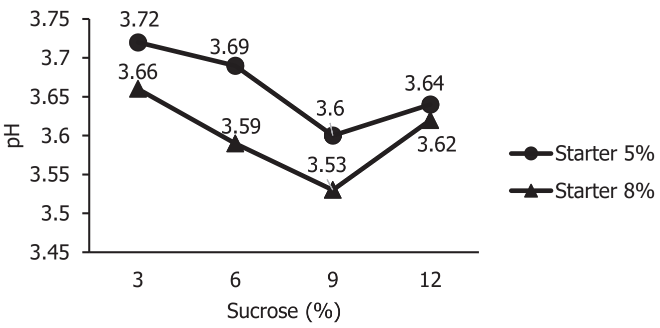

Figure 3. pH graph of water kefir media for fig leaf tea

from 3.60 - 3.72, while in the 8% group, the value was between 3.53 - 3.66. The pH value obtained met the standard set by FSANZ and Codex STAN 243-2003, namely a maximum of 4.50 (Effendi and Parhusip, 2021). Changes in the pH value of the media during the fermentation process in the sucrose and starter concentration treatment groups tended to decrease as shown in Figure 3.

Based on ANOVA analysis, each factor of sucrose, starter, and the interaction had a significant effect ($p<0.05$) on the average pH of the media. In the Tukey test, it shows that the 3% and 9% sucrose treatments produced significantly different values, yielding the highest and lowest average media pH respectively. This shows that the sucrose factor influences the acidity level produced by fig leaf tea water kefir. The acidity level obtained describes the amount of organic acid produced by LAB and BAA contained in kefir seeds during the fermentation process. The treatment that produces the most organic acids has the lowest acidity level and vice versa. Rizqiati et al. (2021) and Wasilu et al. (2021) explained that LAB and BAA used available sucrose and then converted into organic acids dominated by lactic and acetic acid, leading to a decrease in the acidity level or pH of the fig leaf tea water kefir media. Furthermore, a decrease in the pH of the media occurred at a concentration of 12% followed by a reduction in total acid. The high concentration of sucrose facilitates conditions detrimental to microorganisms in water kefir seeds, one of which is triggering an imbalance in osmotic pressure in microbial cells, resulting in the release of water and ultimately cell death (Rimadhini et al., 2020). This condition also triggers the disruption of bacteria growth and physiological activities such as acid production (Cai et al, 2021). In previous research by Rizqiati et al. (2021), water kefir with sweet star fruit experienced an increase in media pH from 4.32 at a sucrose concentration of 9% to 4.52 at 12%.

In the ANOVA analysis, it also shows that the 5% and 9% starter treatments produced significantly different average media pH, suggesting the starter concentration affected the pH of the media. The increase in pH produced in the 8% starter treatment compared to the 5% implied that the higher the amount of water kefir seeds added, the greater the level of organic acids formed during fermentation. This was supported by Effendi & Parhusip (2021) and Utomo & Kurniawidi (2021) stating that increasing the starter concentration directly raised the amount of LAB and BAA. Both play a role in fermentation by speeding up the breaking down of substrate and conversion into organic acids which are dominated by lactic and acetic acids, leading to a reduction in the pH of the fig leaf tea water kefir media.

In the Tukey pH media test on the sucrose-starter interaction, treatment B1S1 (5% starter + 3% sucrose) with the highest average media pH, and B2S3 (8% starter + 9% sucrose) with the lowest had different notations compared to others. This implied that the average media pH for the two treatments differed

Table 2. Tukey pH test of fig leaf tea water kefir media

Sample	pH value	Notation
B1S1 (5% starter + 3% sucrose)	3.720	a
B1S2 (5% starter + 6% sucrose)	3.685	b
B1S3 (starter 5% + sucrose 9%)	3.600	e
B1S4 (5% starter + 12% sucrose)	3.640	cd
B2S1 (8% starter + 3% sucrose)	3.655	bc
B2S2 (8% starter + 6% sucrose)	3.585	e
B2S3 (8% starter + 9% sucrose)	3.530	f
B2S4 (8% starter + 12% sucrose)	3.615	de

significantly from the others. Meanwhile, there were no significant differences in the other treatments, as indicated by similar notations. The high media pH in the B1S1 treatment was caused by the low amount of sucrose and starter added, which affected the fermentation process, producing metabolites in the form of organic acids (lactic acid and acetic acid). Furthermore, the high pH value in this treatment was in line with the low total acid, namely 0.44%. The results are in line with (Lestari et al., 2018) who obtained the smallest pH value of 4.32 in the treatment with a sucrose concentration of 9%. This suggests that the addition of 9% sucrose is the ideal concentration for the water kefir fermentation process. According to Jaya (2019), the optimal fermentation process in the water kefir fermentation media leads to increased conversion of substrate into various metabolites. Organic acids which are acidic in nature will increase and accumulate during the fermentation process. Therefore, B2S3 (8% starter + 9% sucrose) was considered the most optimal treatment for producing total acid. Coban (2020) and Lynch et al. (2021) explained that changes in the pH value of the media were caused by the presence of hydrogen ions (H^+) from the dissociation process of organic acids due to the metabolism of microorganisms contained in water kefir. The large number of H^+ ions contained in the media was measured by a pH meter electrode to quantify the degree of acidity.

Antioxidant Activity

The average antioxidant activity of fig leaf tea water kefir in the 5% starter treatment group ranged from 34.63% to 48.94%, while in the 8% group, the value was between 40.62% to 51.27. %. Figure 4 shows that the average antioxidant activity obtained from each sucrose and starter treatment group tended to increase.

Based on ANOVA analysis, each factor of sucrose, starter, and the interaction had a significant effect ($p < 0.05$) on the average antioxidant activity produced by fig leaf tea water kefir. It shows that the 3% and 9% sucrose treatments produced significantly different average values, yielding the lowest and highest average antioxidant activity respectively. This implied that the sucrose factor affected the antioxidant activity resulting from the breakdown of the substrate in the fermentation process by microbes in the water kefir seeds to produce primary and secondary metabolites. The decrease in antioxidant activity at a sucrose concentration of 12% was presumably due to the metabolic results of microorganisms, namely organic acids produced during the fermentation process that occurred in water kefir seeds. The high concentration of sucrose can trigger an increase in osmotic pressure on the microbes, particularly yeast which plays a role in breaking down sucrose into simple sugars utilized by LAB and BAA as substrates to produce lactic and acetic acid. Osmotic pressure on yeasts causes osmotic shock or stress which can reduce efficiency in the fermentation process and allow disruption of bacteria growth and physiological activities such as acid production (Cai et al., 2021). Non-optimal physiological activity, one of which is the production of organic acids, is thought to influence the level of antioxidant activity. This was supported by Alsayadi et al. (2013) and Jaya (2019) who explained that during the fermentation process LAB, BAA, and yeast produced primary and secondary metabolites with the potential to act as natural antioxidants.

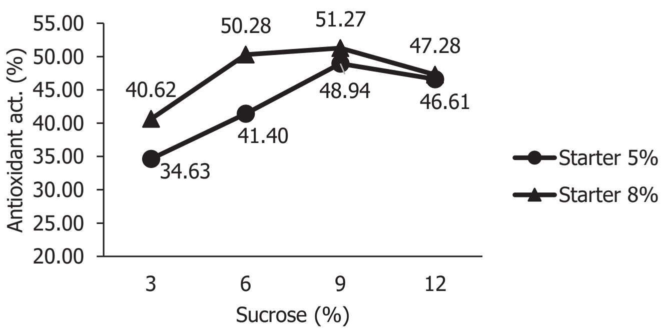

Figure 4. Graph of antioxidant activity of fig leaf tea water kefir

The ANOVA analysis shows that the 5% and 9% starter treatments produced significantly different average antioxidant activities, indicating that the starter concentration affected the antioxidant activity produced by fig leaf tea water kefir. The increase in antioxidant activity in line with a rise in starter concentration suggests that the microbes contained in water kefir seeds not only speed up the fermentation process but also use certain compounds as good antioxidants. Piekarska-Radzik & Klewicka (2021) explained that most of the lactic acid bacteria from the genus Lactobacillus sp. can produce several bioactive compounds with good antioxidant activity by using certain compounds in the fermentation media. For example, L. brevis, one of the LAB has the ability to convert furelic acid contained in fig leaf tea into Ethylphenol and Vinylguaiacol dihydrocafeic acid, which have been reported to possess strong antioxidant activity.

The Tukey test results in Table 3 showed that B1S1 (5% starter + 3% sucrose) with the lowest average total acid had a different notation compared to other treatments. Meanwhile, B2S3 (8% starter + 9% sucrose) which possessed the highest activity had a notation that was not significantly different from the

Table 3. Tukey test of antioxidant activity in fig leaf tea water kefir

Sample	Antioxidant activity	Notation
B1S1 (5% starter + 3% sucrose)	34.63	e
B1S2 (5% starter + 6% sucrose)	41.40	d
B1S3 (5% starter + 9% sucrose)	48.94	abc
B1S4 (5% starter + 12% sucrose)	46.61	c
B2S1 (8% starter + 3% sucrose)	40,62	d
B2S2 (8% starter + 6% sucrose)	50.28	ab
B2S3 (8% starter + 9% sucrose)	51.27	a
B2S4 (8% starter + 12% sucrose)	47.28	bc

B2S2 (8% starter + 6% sucrose) and B1S3 (5% starter + 9% sucrose) treatments.

The total acid average of B2S3 (8% starter + 9% sucrose) was 0.70, which was higher compared to the values for B2S2 (8% starter + 6% sucrose), and B1S3 (5% starter + 9% sucrose). Tukey's further tests on the average antioxidant activity showed that the three treatments had notations without significant differences. The presence of bioactive compounds in fig leaf tea affected the high antioxidant activity in water kefir. This was supported by Ahaddin (2014) and Mawa et al. (2013) who reported phenolic compounds, terpenoids, anthocyanins, flavonoids (flavones and flavonols), steroids, tannins, and alkaloids useful as antioxidants. In the B1S1 treatment (5% starter + 3% sucrose) with an average total acid of 0.44%, which is the lowest average total acid with the lowest average antioxidant activity and is significantly different from other treatments. It is suspected that there is a correlation between high antioxidant activity and total acid contained in the sample. This is in line with Purba et al. (2018) stating that the higher the total acid, the higher the antioxidant activity of water kefir and vice versa. Alsayadi et al. (2013) explain that the antioxidant activity of water kefir is related to intracellular-extracellular metabolites and lysis products from LAB and yeast which are of the same nature. This is confirmed by Jaya (2019) and Serventi et al. (2020) who explained that metabolites produced during the water kefir fermentation process such as polypeptides, organic acids, and glutathione have good antioxidant activity.

Pearson Correlation

Pearson correlation is a correlation technique used to measure the strength and direction of the relationship between two variables provided that the data is on an interval or ratio scale. The results of the analysis produce a Pearson coefficient (r) with a value of $-1 \leq r \leq 1$ which is divided into five interpretation groups, namely 0.000 to 0.199 (very weak), 0.200 to 0.399 (weak), 0.400 to 0.599 (moderate), 0.600 to 0.799 (strong) and 0.800 to 1.000 (very strong). The minus (-) and plus (+) signs of the coefficient determine the direction of the relationship, namely the (-) sign means the variable relationship is inversely proportional, while the (+) sign means the variable relationship is directly proportional (Bertan et al., 2016).

Based on the results of the Pearson correlation analysis, the antioxidant activity variable with the total acid (0.967) and the reducing (Δ) sugar content (0.962) had the same interpretation, namely a very strong relationship and directly proportional. This implies that the higher the antioxidant activity of the sample, the greater the total amount of acid and reducing sugar content. Meanwhile, the antioxidant activity variable and media pH (-0.931) had a very strong relationship and were inversely proportional. This implies that the higher the antioxidant activity of the test sample, the

Table 4. Results of Pearson correlation analysis

Variable	Pearson coefficient (r)	Interpretation
Act. antioxidants – total acids	0.967	The relationship is very strong and directly proportional
Act. antioxidants – pH of the media	-0.931	The relationship is very strong and inversely proportional
Act. antioxidant - Δ sugar	0.962	The relationship is very strong and directly proportional

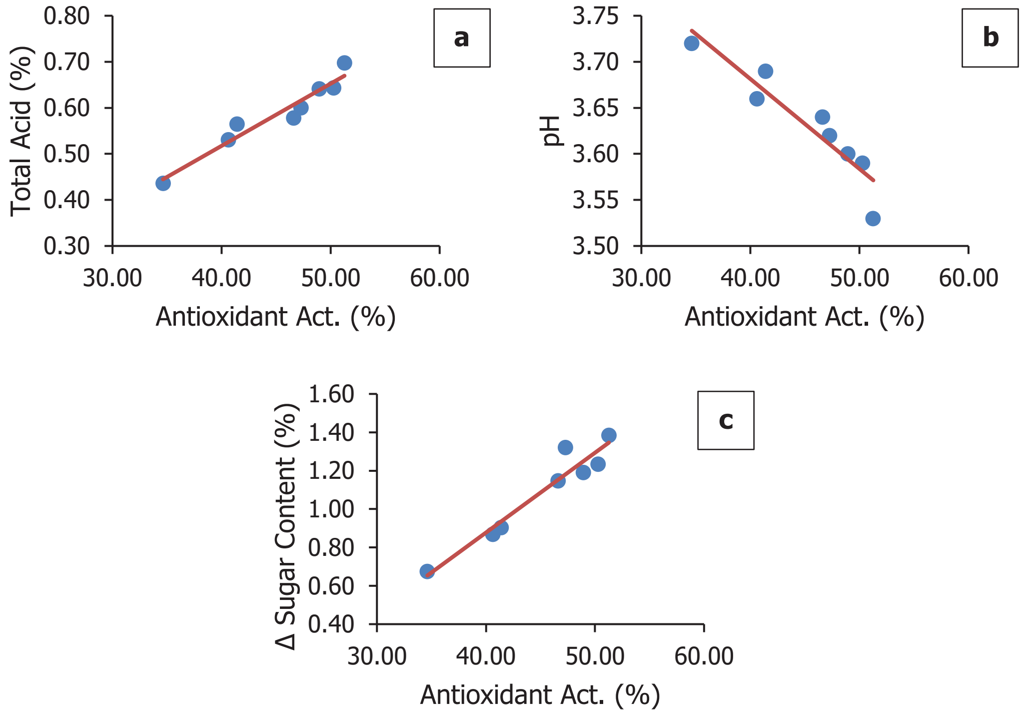

Figure 5. Graph of the Pearson correlation between antioxidant activity - total acid (a), antioxidant activity - media pH (b), and antioxidant activity - Δ sugar content (c)

lower the degree of acidity (pH) or the more acidic the condition of the solution. The interpretation results were in accordance with Alsayadi et al. (2013) who explained the relationship between intracellular-extracellular metabolites and the antioxidant activity of water kefir.

Organoleptic

Flavor

Based on Table 5, B2S4 treatment (8% starter + 12% sucrose) had the highest average score of 5.65 (Liked) but was not statistically significantly different from B1S3 (5% starter + 9% sucrose), B1S4 (5% starter + 12% sucrose) and B2S3 (8% starter + 9% sucrose). In the research conducted by Kinteki et al. (2019) the treatment with the highest sucrose concentration, namely 12%, produced the peak average taste rating of 3.56 (liked). Adding high levels of sucrose can produce a dominant sweet taste, thereby successfully eliminating or minimizing the bitter taste of fig leaf tea. Metabolites produced during the fermentation process including lactic and acetic acid characterized by a sour taste and carbon dioxide as well as ethanol which gives a unique fizzy and slightly alcoholic taste play a role in minimizing the bitter taste of fig leaf tea. Meanwhile, the lowest average score was obtained in the B2S1 treatment (8% starter + 3% sucrose) at 2.45 (disliked very much) which was not significantly different from the B1S1 treatment (5% starter + 3% sucrose). This result could be attributed to the small amount of sucrose which impacted the fermentation process in producing optimal metabolites including organic acids, carbon dioxide, and ethanol

Table 5. Organoleptic test results of fig leaf tea water kefir

Test Parameters	B1S1	B1S2	B1S3	B1S4	B2S1	B2S2	B2S3	B2S4
Flavor	2.47^{d}	4.25^{bc}	5.00^{ab}	5.02^{a}	2.45^{d}	4.05^{c}	4.92^{ab}	5.65^{a}
Aroma	3.52^{b}	4.15^{ab}	4.30^{ab}	4.52^{a}	4.20^{ab}	4.22^{ab}	4.52^{a}	4.80^{a}
Color	4.52	4.70	4.97	4.97	4.82	4.85	4.80	4.97
Overall liking	3.07^{d}	4.02^{c}	4.70^{bc}	4.90^{ab}	3.02^{d}	4.10^{c}	4.92^{ab}	5.45^{a}

Description: The notation on the data shows the results of Tukey's advanced test analysis which play a role in helping to eliminate or minimize the bitter taste of fig leaf tea (Irsalina et al., 2018).

Aroma

Based on Table 5, B2S4 treatment (8% starter + 12% sucrose) had the highest average score on aroma at 4.80 (slightly liked) which was statistically significantly different from B1S1 (5% starter + 3% sucrose) with the lowest average score of 3.52 (disliked). However, there was no significant difference from treatment B1S2 (5% starter + 6% sucrose), B1S3 (5% starter + 9% sucrose), B1S4 (5% starter + 12% sucrose), B2S1 (8% starter + 3% sucrose), B2S2 (8% starter + 6% sucrose), and B2S3 (8% starter + 9% sucrose). This implied that the aroma produced from fig leaf tea water kefir was not significantly different from one another, but certain combinations produced a contrasting aroma difference including B1S1 (starter 5% and sucrose 3%) and treatment B2S4 (starter 8 % + sucrose 12%). It was suspected that the B1S1 treatment (5% starter and 3% sucrose) produced fewer volatile compounds during the fermentation process compared to B2S4 (8% starter + 12% sucrose). Kinteki et al. (2019) and Patel et al. (2022) stated that volatile compounds produced by microbes in water kefir seeds during the fermentation process played a role in giving the sample a distinctive aroma, including acetaldehyde, diacetyl, acetone, esters, alcohol, ketones, and organic acids. Research by Hastuti & Kusnadi (2016) reported aroma ratings ranging from 2.30 (disliked) to 2.75 (disliked) for red rosella tea.

Color

Based on the one-way ANOVA analysis of variance test, the combination of treatments had no significant effect on the color assessment of fig leaf tea water kefir. This was evidenced in the average scores ranging from 4.52 to 4.97, included in the slightly favorable group, as well as from the results of the ANOVA analysis which obtained a p-value > 0.05. Similarly, Purba et al. (2018) in a 48-hour-long fermentation treatment produced a low average color assessment. The color of fig leaf tea water kefir tends to be the same or does not experience a significant change, due to the conversion of polyphenol compounds into thearubigin during the fermentation process (Ibrahim & Yulianto, 2021).

Overall liking

Table 5 shows that B2S4 treatment (8% starter + 12% sucrose) had the highest average score on overall liking at 5.45 (liked), but not significantly different from B1S4 (5% starter + 12% sucrose) and B2S3 (8% starter + 9% sucrose). The lowest average score was obtained in the B2S1 treatment (8% starter + 3% sucrose) with an average score of 3.02 (disliked) which was not significantly different from the B1S1 (5% starter + 3% sucrose). The low scores obtained in treatments B2S1 (8% starter + 3% sucrose) and B1S1 (5% starter + 3% sucrose) could be attributed to the inability of fermented metabolite compounds to minimize the bitter taste of fig leaf tea. This is supported by the opinion of Irsalina et al. (2018) who explained that the taste and aroma of a product are determining factors in the quality and level of consumer preference for food products. Research by Rizqiati et al. (2021) obtained an overall liking average in the 9% sucrose concentration treatment of 3.76 (liked). This is because the 9% sucrose treatment had a typical water kefir aroma and the strongest soda sensation, while the 12% sucrose treatment obtained an overall average liking of 2.92 (dislike), which is caused by the dominance of sweet taste compared to the typical taste of water kefir.

CONCLUSION

In conclusion, the concentration of sucrose and starter, as well as the sucrose-starter interaction, had a significant effect on chemical characteristics (antioxidant activity, total acid, and media pH), and organoleptic properties (flavor, aroma, and overall liking) of fig leaf tea water kefir. Based on the Pearson correlation test, total acid, pH of the media, and reducing sugar content had a very strong relationship with the antioxidant activity. The treatment identified to have the best chemical characteristics was B2S3 (8% starter + 9% sucrose) with the highest antioxidant activity of 51.27%, fulfilling the quality criteria for total acid and media pH parameters with values of 0.70% and 3.53 respectively. Meanwhile, the treatments with the best organoleptic analysis results were B1S4 (5% starter + 12% sucrose), B2S3 (8% starter + 9% sucrose), and B2S4 (8% starter + 12% sucrose), differing significantly in four test parameters compared to other treatments. Based on the results, B2S3 (8% starter + 9% sucrose) was selected as the best treatment, characterized by the highest antioxidant activity and good organoleptic properties.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest

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