

EFFECT OF PURPLE SWEET POTATO (*Ipomoea batatas*) EXTRACT AND FERMENTATION TIME IN WATER KEFIR CHEMICAL PROPERTIES

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ABSTRACT

As a bacteria starter, water kefir is commonly made from water, sugar, dried or fresh fruits, and kefir grains. Purple sweet potato (PSP) contains natural anthocyanins and carbohydrates as colouring agents and carbon sources in water kefir drinks production. This study determines the effect of the PSP extract addition, fermentation time, and their interaction in the water kefir. The study was based on chemical properties such as total anthocyanins, antioxidant activity IC₅₀, total acid, total sugar, degree of acidity (pH), and total dissolved solids (TDS). The method used Factorial Randomized Group Design with 0%, 10%, 30%, and 50% (v/v) of addition of PSP extract and 0, 24, and 48 hours of fermentation time as factors. The results were analyzed using Analysis of Variance (ANOVA) with a 5% confidence interval. The results showed that PSP extract addition and fermentation time significantly affected all chemical properties analysis and their interaction. The best treatment was retrieved with 50% PSP extract addition and 48 hours of fermentation time with total anthocyanin 193.197 ± 3.255 ppm, antioxidant activity IC₅₀ 460.620 ± 20.973 ppm, total acid $10.429 \pm 0.371\%$, total sugar $1.356 \pm 0.058\%$, pH 2.3 ± 0.058 , and TDS 2.1 ± 0.2 °Brix

Keywords: Anthocyanin; Chemical Properties; Fermentation; Purple Sweet Potato; Water Kefir

INTRODUCTION

Indonesia has the potential of carbohydrate sources for food availability. One of the sources is *Ipomoea batatas* L. or purple sweet potato (PSP) (Hardoko *et al.*, 2010). The purple colour of PSP is due to anthocyanin pigments. Anthocyanins are a group of pigments in the cell fluid, soluble in water, and cause a reddish colour (Francavilla and Joye, 2020). PSP anthocyanin components are mono or diacetyl 3-(2-glucosyl) glucosyl-5-glucosyl peonidin and cyanidin derivatives (Suda *et al.*, 2003). Anthocyanin functions as free radical scavengers and antioxidants that can prevent ageing, degenerative diseases, anticarcinogenic, antihypertensives, and reduce blood sugar levels (Qi *et al.*, 2022). PSP also contains 0.77% protein, 20% vitamins and minerals, 3.00% dietary fibre and non-fibre carbohydrates (Nintami and Rustanti, 2012; Saludung *et al.*, 2020).

With the development of the era, public awareness of the importance of healthy living is increasing. Foodstuffs that are now in great demand by consumers are not only those that have an attractive appearance and taste but also must have specific physiological functions for the body (Wahyono *et al.*, 2015). The existence of anthocyanin compounds as a source of natural antioxidants in purple sweet potatoes is quite interesting, considering the many benefits of anthocyanin content. One processing of PSP that can maintain its functional properties is extracting and adding it to a fermented drink. Water kefir is of fermented drink from a mixture of sugar in a water solution with various dried or fresh fruits fermented using kefir grains (Alsayadi *et al.*, 2013). Water kefir has low acid and alcohol content, which is 0.276% at a 24-hour fermentation time (Lestari *et al.*, 2018). In addition, it has very little fat

content compared to kefir made from milk (Supriono, 2008). However, in this study, the sugar used was stevia sugar which offers many health benefits, including it does not affect blood sugar levels, is safe for people with diabetes, prevents tooth decay by inhibiting the growth of bacteria in the mouth, helps improve digestion and relieves stomach pain (Raini and Ismawati, 2011). Based on the description above, stevia sugar is suitable as a substitute for sugar solution, so a low-calorie PSP extract water kefir fermented drink will be obtained.

In the previous research on water kefir using PSP with various concentrations, the best treatment was obtained at a concentration of 4% PSP flour with a fermentation time of 19 hours at a temperature of 28-30 °C which has a total lactic acid bacteria (LAB) 8.37×10^8 CFU/ml, total acid 1.13%, pH 3.87, antioxidant activity IC_{50} 70.28% and total sugar 3.42% (Rizky and Zubaidah, 2015). Based on this research, the concentration of PSP extract added to water kefir is not yet known. This study determines the effect of PSP extract addition and fermentation time on water kefir and their interaction. The chemical analysis used in this study are total anthocyanins, antioxidant activity IC_{50} , total acid, total sugar, degree of acidity (pH), and total dissolved solids (TDS).

METHOD

Materials

The PSP Antin 3-type was chosen from the Research Institute for Nuts and Tubers (Balitkabi) Malang, water kefir grains obtained from an online shop in Mojokerto city, stevia sugar obtained at Persada Supermarket Malang, mineral water, and citric acid.

Design Method

The factorial randomized group design was used in this study with two factors. Factor I consists of 4 levels, and factor II consists of 3 levels:

Factor I : PSP extract addition (P): P1 = 0% (v/v); P2 = 10% (v/v); P3 = 30% (v/v); and P4 = 50% (v/v).

Factor II : Fermentation time (T): T1 = 0 hours; T2 = 24 hours; and T3 = 48 hours.

The research was carried out in two steps. The first step was the extraction of PSP with mineral water and citric acid. The ratio of PSP and mineral water was 2:1. The second step was making water kefir.

Purple Sweet Potato Extract

PSP Antin 3 variety weighed as much as 180 g, was peeled, and washed with running water, and then grated. After grated, obtained 150 g of grated PSP slurry were. Prepare 10% citric acid in 300 ml of mineral water. Citric acid is dissolved in 300 ml mineral water or added until the pH value reaches 2. Then, 150 g of grated PSP slurry is put into the citric acid solution with the ratio of grated PSP slurry, and mineral water is 1:2. Next, macerated using a shaker for 24 hours at room temperature. Afterwards, the sample was filtered through a vacuum filter and centrifuged for 15 minutes at 5000 rpm (Maharani *et al.*, 2016). The PSP extract will be analyzed based on chemical properties, such as total anthocyanin (Husna *et al.*, 2013), antioxidant activity IC_{50} (Affandi and Handajani, 2011), total acid (Purwanti, 2013), total sugar (Rahmawati *et al.*, 2015), pH (Pratiwi *et al.*, 2018), and TDS (Ningsih *et al.*, 2019).

Water Kefir Production

Prepare 100 ml of mineral water into a bottle, add 6.5% stevia sugar, and PSP extract as much as 0%, 10%, 30% and 50% (v/v). After that, add 5% (w/v) of kefir grains. The mixture was then fermented at room temperature for 0, 24, and 48 hours. After fermentation, the product is filtered using filter paper to separate kefir grains from the product. Then water kefir will be analyzed based on chemical properties as same as PSP extract (Gulitz *et al.*, 2011).

Analysis Data

All data will be analyzed statistically using Analysis of Variance (ANOVA) with a 5% confidence interval. If the results show a significant difference, then Duncan's Multiple Range Test (DMRT) is carried out. The best treatment was selected by the Zeeleny method (Wibisanti, 2018).

RESULT AND DISCUSSION

Characteristics of PSP Extract

The raw materials analysis aims to determine the chemical characteristics of the PSP potato extract raw materials used in the water kefir. The analysis includes total anthocyanins, antioxidant activity, total sugar, total dissolved solids, total starch, and pH. The results analysis data of raw materials for PSP extract can be seen in Table 1.

From the analysis, the total anthocyanin content of the PSP extract was 99.57 mg/100 g. The total anthocyanin content in fresh PSP is

171.06 mg/100 g (Susanti *et al.*, 2018). The analysis and literature results differ due to differences in PSP's environment and place of life. The PSP in this study was from Balitkabi Malang, while the literature was from Bogor. Then the difference in the extraction method used, where the extraction method used in the study is the maceration method, while the method used in the literature is the distillation method. In addition, the amount of anthocyanin content also depends on the colour intensity of the PSP. The more purple in the PSP, the higher the anthocyanin content (Suda *et al.*, 2003).

Table 1. Chemical characteristics of PSP extract

Parameter Analysis	Value	Reference
Total Anthocyanin	99.57 mg/100 g	171.06 mg/100 g ^a
Antioxidant Activity IC ₅₀	938.02 ppm	322.08 ppm ^b
Total Sugar	7.23 %	0.38-5.64% ^c
TDS	8.6°Brix	5.0°Brix ^d
pH	3.0	3.2 ^e

Notes: (a) Susanti *et al.* (2018); (b) Dewi *et al.* (2014); (c) Saragih *et al.* (2017); (d) Husna *et al.* (2013); and (e) Wicaksono (2013)

The antioxidant activity IC₅₀ results in PSP extract was 938.02 ppm. The study results differ from the literature's 322.08 ppm (Dewi *et al.*, 2014). The results differ because the types of acids and solvents used in the extraction process differ. The type of acid and solvent used in this study were citric acid and mineral water, while in the literature, it was 96% ethanol and 1N HCl (85:15 v/v). Because the difference in acid and solvent causes the optimization of the extraction process for the flavonoid compounds to be different, the number of flavonoid compounds analyzed, namely the antioxidant activity of IC₅₀, will also be other. The antioxidant activity of sweet potatoes can also be caused by the presence of phenolic groups, tocopherols, and flavonoids (Huang *et al.*, 2004). This activity is indicated by the dense colour of sweet potato tubers, where the darker colour will affect the higher anthocyanin and carotene content (Teow *et al.*, 2007). The acid in the form of citric acid is added at the extraction process, which makes the colour of the PSP extract more concentrated and red so that the anthocyanin content is also higher and will be directly proportional to its antioxidant activity (Yang and Gadi, 2008).

The total sugar extract of PSP from the analysis was 7.23%. While in the literature, the

total sugar in PSP was 0.38-5.64% (Saragih *et al.*, 2017). The total sugar yield in the extract is greater than the literature result because the grated sweet potato used is ± 3x more than the literature, which is 100 g. However, sugar in the form of reduced sugar in the extract will decrease due to the addition of citric acid, which makes the flavour of the extract slightly acidic (Kunaepah, 2008).

From the analysis, results obtained a TDS of 8.6 °Brix. The TDS in the literature is 5.0°Brix (Husna *et al.*, 2013). The study results differ from the literature due to differences in solvent type and the ratio used between material and solvent. The solvent used in the literature is ethanol with a ratio of 1:10. The solvent used in this study is mineral water with a ratio of 1:2 material and the addition of citric acid until the pH reaches 2.

The pH of the PSP extract was 3. The results were similar to the literature, which was 3.2 because the extraction of PSP in the literature used tartaric acid, which decreased the sample's pH. Meanwhile, in this study, citric acid has the same function: to lower the sample's pH (Wicaksono, 2013).

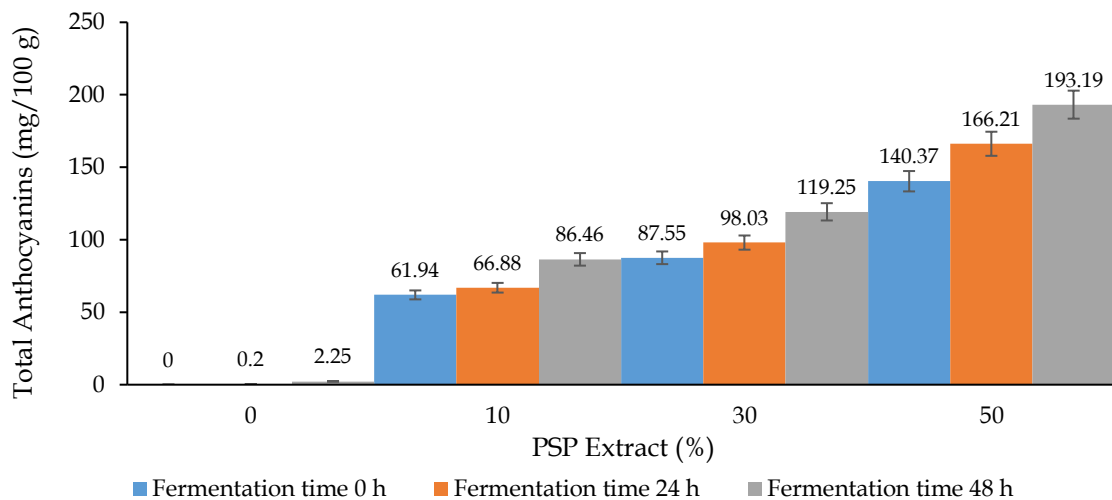
Chemical Characteristics of Water Kefir Treatment

1. Total Anthocyanins

The anthocyanins of water kefir ranged from 0 to 193.19 mg/100g. The ANOVA results showed that the concentration treatment of the PSP extract addition had a significant effect on the total anthocyanin of water kefir drinks. The fermentation length also significantly affected the total anthocyanins of water kefir. The interaction of the two treatments significantly impacted the total anthocyanins of the water kefir.

Figure 1 shows that each treatment’s total anthocyanins of water kefir were significantly different. The highest total anthocyanin was at a 50% PSP extract addition with 48 hours fermentation time of 193.19 mg/100g. In comparison, the lowest total anthocyanin was 0

mg/100g at a concentration of 0% PSP extract addition with a fermentation time of 0 hours (initial fermentation). The higher of the PSP extract concentration addition and the length of fermentation time, the higher the anthocyanins. The total anthocyanins will increase along with the addition of PSP in anthocyanin drinks. The longer fermentation time will cause a lot of organic acids, and the atmosphere of water kefir will be more acidic (Montilla *et al.*, 2011). The pH value affects anthocyanins’ stability, whereas anthocyanins are more stable under acidic conditions. According to Yang and Zhai (2010), the higher the use of PSP extract, the higher amount of the anthocyanin target compound extracted to a certain extent before it finally turns down because the solvent has started to saturate.



Notes: Each result of data analysis is the average of 3 replications ± standard deviation; Numbers with different notations indicate significantly different values ($\alpha=0.05$)

Figure 1. Average Total Anthocyanins Water Kefir with Various Concentrations of PSP Extract Addition and Fermentation Time

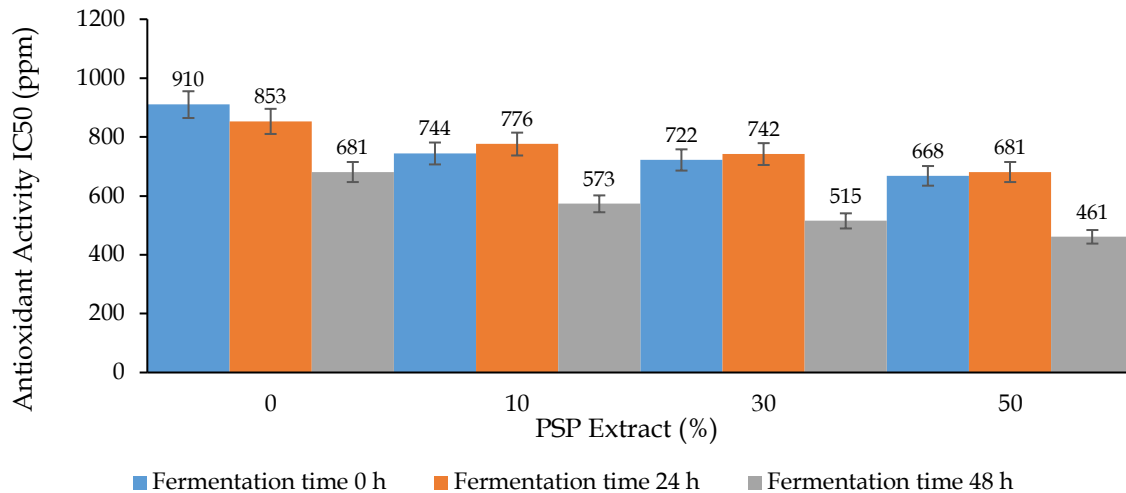
2. Antioxidant Activity IC₅₀

The antioxidant activity IC₅₀ analysis results in the water kefir ranged from 461–910 ppm. The ANOVA results showed that the concentration treatment of the PSP extracts addition significantly affected the antioxidant activity of IC₅₀ water kefir. The fermentation length also significantly affected the antioxidant activity IC₅₀ of water kefir. The interaction of the two treatments significantly impacted the antioxidant activity IC₅₀ of the water kefir.

Figure 2 shows that the antioxidant activity of IC₅₀ water kefir was significantly different for each treatment. The higher the concentration of the PSP extract and the longer the fermentation time, the lower the antioxidant activity value. The IC₅₀ value of DPPH indicates the concentration of the sample required to inhibit DPPH free radicals until 50% (Wicaksono, 2013). The highest antioxidant activity of IC₅₀ water kefir PSP extract (good activity) was at a concentration of 50% PSP extract addition with a 48 hours

fermentation time of 461 ppm. Meanwhile, the lowest antioxidant activity value (not good activity) was 910 ppm at 0% PSP extract addition with 0 hours fermentation time. The results follow the literature, which states that

the greater the addition of PSP concentration, the higher the antioxidant activity (Kano *et al.*, 2005).



Notes: Each result of data analysis is the average of 3 replications \pm standard deviation; Numbers with different notations indicate significantly different values ($\alpha=0.05$)

Figure 2. Average Antioxidant Activity of Water Kefir with Various Concentrations of PSP Extract Addition and Fermentation Time

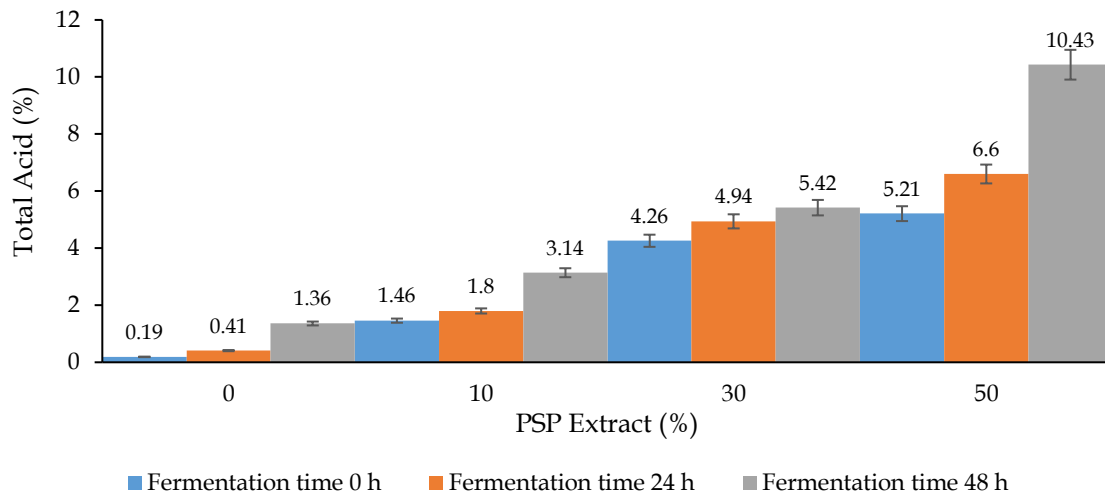
Affandi and Handajani (2011) stated that the more PSP extract added, the higher the antioxidant activity because PSP has solid antioxidant compounds derived from beta-carotene and anthocyanin. Beta carotene level in PSP reached 9000 g/100 g. In addition, organic acids produced during fermentation can have a synergistic effect on antioxidant compounds. In addition to acting as synergistic antioxidants, the resulting acid state can increase the antioxidant activity of primary antioxidants, namely phenol (Rizky and Zubaidah, 2015). Phenol is an antioxidant, the largest of which is in the form of esters, including quinic acid and caffeic acid. Synergistic antioxidants can act as hydrogen donors to phenoxyl radicals, regenerating primary antioxidants (Wu *et al.*, 2006).

3. Total Acid

The total acid analysis in water kefir ranged from 0.19 to 10.43%. The ANOVA results showed that the concentration treatment with the addition of PSP extracts

significantly affected the total acid of water kefir. The fermentation time treatment also significantly affected the total acid of water kefir. The interaction of the two treatments significantly impacted the total acid of the water kefir.

Figure 3 shows that the total acid was significantly different for each treatment: the longer the fermentation time, the higher the total acid in the water kefir. The highest total acid was at a concentration of 50% PSP extract addition with 48 hours fermentation time of 10.43%, while the lowest total acid was at a concentration of 0% PSP extract addition with a fermentation time of 0 hours is 0.19%. The longer fermentation is carried out, the more concentration of PSP extract is added, and the more acid is produced. The higher increase in acid is suspected because the bacteria have experienced a logarithmic growth phase. At the same time, the bacteria synthesizing alcohol into acid are increasing so that the total acid produced is also higher (Yoshinaga *et al.*, 2000).



Notes: Each result of data analysis is the average of 3 replications \pm standard deviation; Numbers with different notations indicate significantly different values ($\alpha=0.05$)

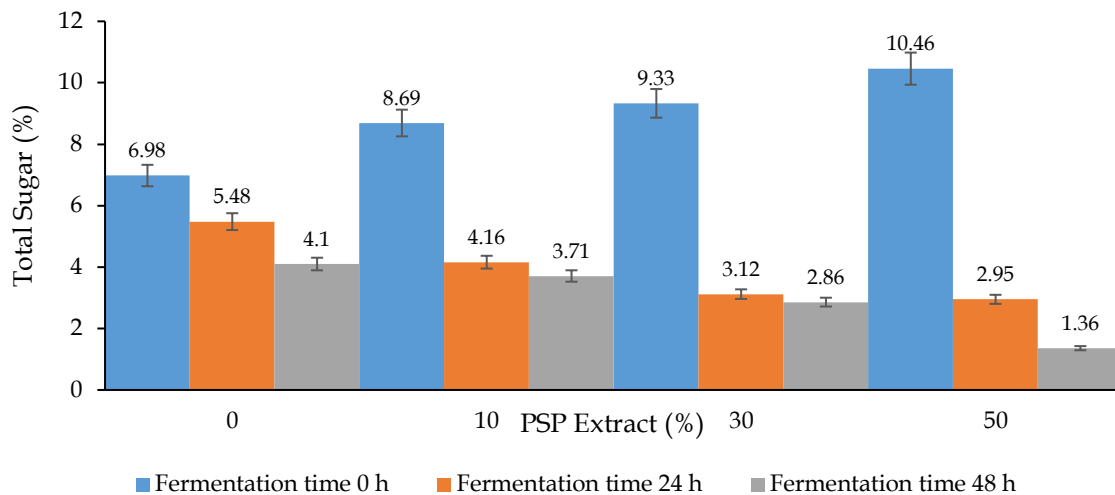
Figure 3. Average Total Acid of Water Kefir with Various Concentrations of PSP Extract Addition and Fermentation Time

The increase in total acid was also due to the length of fermentation. The results of the average total acid show that the longer the fermentation time, the more acid is produced. Afifah (2010) stated that the longer the fermentation time, the higher concentration of acetic acid in the water kefir extract of PSP extract because yeast and bacteria metabolize sucrose and produce organic compounds such as acetic and gluconic acid. The total acid yield is inversely proportional to the total sugar; the higher the total acid, the lower the total sugar. During the fermentation process, yeast will break down sucrose into glucose and fructose and use glucose for cell metabolism to produce ethanol and CO₂. Acetic acid bacteria oxidize ethanol to produce acetic acid (Afifah, 2010).

4. Total Sugar

The total sugar analysis in water kefir ranged from 1.23 to 10.46%. The ANOVA results showed that the concentration treatment of the addition of PSP extracts significantly affected the total sugar of water kefir. The fermentation length also significantly affected the total sugar of water kefir. The interaction of the two treatments significantly impacted the total sugar of the water kefir. Figure 4 shows that the total sugar is significantly different for each

treatment. The longer the fermentation time and the higher concentration of the addition of PSP extract, the total sugar decreased. The highest total sugar was at a concentration of 0% PSP extract addition with an initial 24-hour fermentation time of 5.48%. The sugar yield at 0% PSP extract concentration had the highest total sugar value because the sugar measured was sugar from the addition of stevia sugar, one of which was steviol sugar. The lowest total sugar was at the concentration of 50% PSP extract addition with 48 hours of fermentation time, which was 1.36%. The addition of PSP extract will add nutrients to the microorganism in the kefir grains to grow. LAB will remodel the sugar as a substrate used by microorganisms for metabolism into organic acids (Rizky and Zubaidah, 2015). The total sugar yield will be inversely proportional to the total acid, where the longer fermentation time, the total sugar will decrease. In contrast, the total acid will increase as the fermentation time increases. The same thing was also stated by Mubin and Zubaidah (2016), where during the fermentation process, microorganisms could multiply and remodel sugar as a carbon source into lactic acid so that total sugar would decrease while total acid would increase.



Notes: Each result of data analysis is the average of 3 replications \pm standard deviation; Numbers with different notations indicate significantly different values ($\alpha=0.05$)

Figure 4. Average Total Sugar of Water Kefir with Various Concentrations of PSP Extract Addition and Fermentation Time

The longer the fermentation time, the microorganisms contained in the starter will have the opportunity to break down the sugar contained in the PSP extract water kefir longer. According to Sampurno and Cahyanti (2015), the length of fermentation treatment affects the total sugar produced during fermentation. Every microbe requires sugar as a carbon source, so there is a decrease in sugar content as fermentation time increases. The sugar in the media will be used as nutrients and then converted into alcohol and CO₂. Then CO₂ gas reacts with water vapour and forms carbonic acid (Sampurno and Cahyanti, 2015).

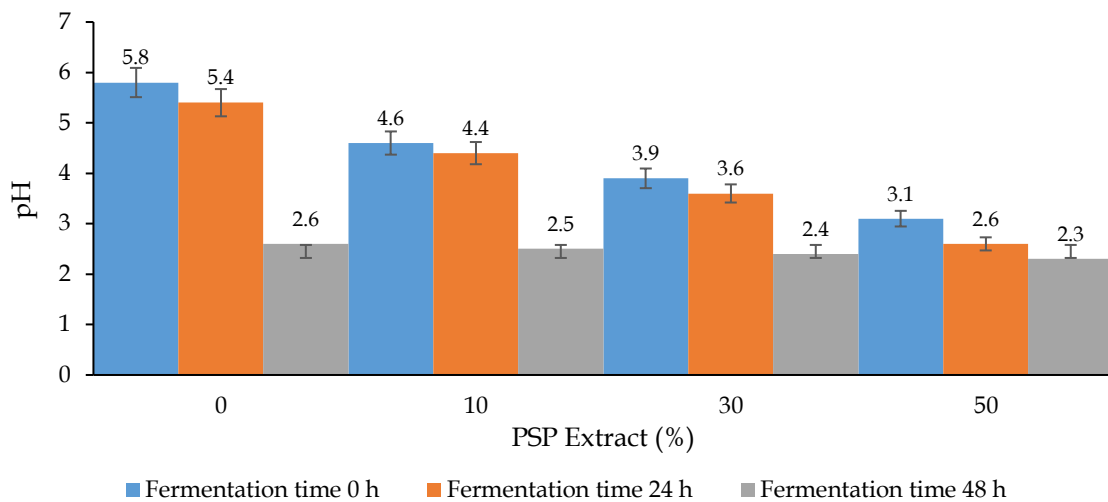
5. Acidity Degree (pH)

The pH analysis of water kefir ranged from 5.8-2.3. The ANOVA results showed that the concentration treatment with the addition of PSP extracts significantly affected the pH of water kefir. The length of fermentation also significantly affected the pH of water kefir. The interaction of the two treatments significantly impacted the pH of the water kefir.

Figure 5 shows that the pH of water kefir was significantly different for each treatment. The longer the fermentation time and the higher the PSP extract concentration, the lower the water kefir pH. The highest pH was at the concentration of 0% PSP extract

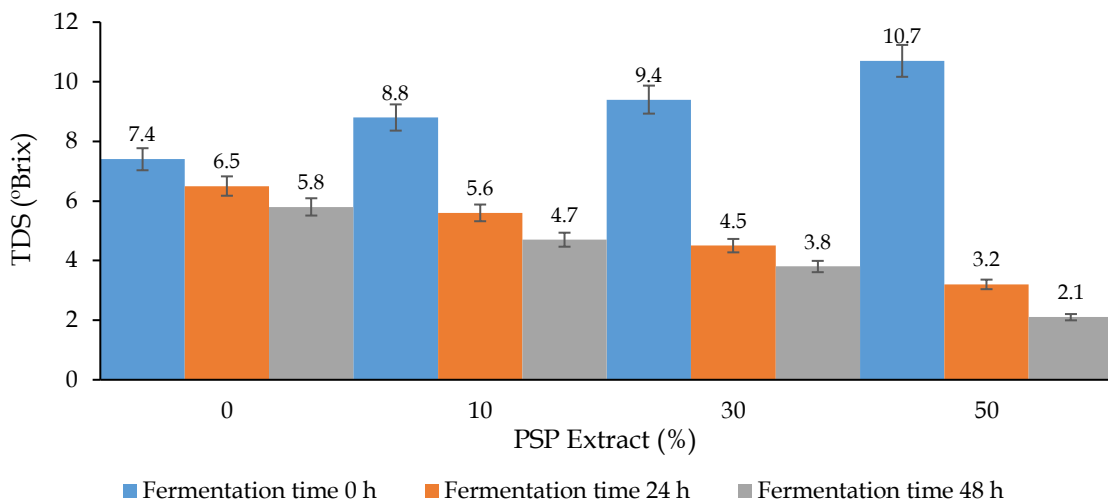
addition with 0 hours of fermentation time 5.8, while the lowest pH was 2.3 at 50% PSP extract added concentration with 48 hours of fermentation time. The higher the concentration of PSP extract, the lower the product's pH. It is because the pH condition of the extract was already low or in an acidic environment. The low pH of the extract is influenced by the addition of citric acid, which stabilizes the anthocyanin components. According to Umam *et al.* (2012), pH decreased because of the amount of LAB product. The breakdown of sugar will produce energy for LAB, which produces lactic acid. The formation of lactic acid will lower the pH and give a sour taste to the product (Umam *et al.*, 2012).

The longer the fermentation time, the longer microbiology in the starter will continue to actively break down sugar so that it will produce acid contained in the water kefir. The continuous breakdown of sugars will increase the total acid, whereas a high total acid will lower the pH due to an increase in the amount of ion H⁺. According to Hambali *et al.* (2014), the longer the fermentation, the maximum ionization process for lactic acid and other organic acids, resulting in more H⁺ ions being released and causing the pH of the product to decrease.



Notes: Each result of data analysis is the average of 3 replications \pm standard deviation; Numbers with different notations indicate significantly different values ($\alpha=0.05$)

Figure 5. Average Ph of Water Kefir with Various Concentrations of PSP Extract Addition and Fermentation Time



Notes: Each result of data analysis is the average of 3 replications \pm standard deviation; Numbers with different notations indicate significantly different values ($\alpha=0.05$)

Figure 6. Average TDS of Water Kefir with Various Concentrations of PSP Extract Addition and Fermentation Time

6. Total Dissolved Solids (TDS)

The TDS of water kefir ranged from 2.1 to 10.7 °Brix. The ANOVA results showed that the concentration treatment of the addition of PSP extracts significantly affected the TDS value. The fermentation time also significantly affected the TDS. The interaction of the two treatments significantly affects the TDS value.

Figure 6 shows that each treatment's TDS of water kefir was significantly

different. The longer the fermentation time, the lower the TDS value. The highest TDS was at 50% PSP extract addition with 0 hours fermentation time of 10.7 °Brix. At the initial time of 0 hours, the TDS increased with the concentration of PSP potato extract, whereas in the PSP, there were various components of soluble solids in carbohydrate form. This treatment has the shortest fermentation time, so the sugar converted by *Saccharomyces cerevisiae* will be less than the

other treatments. The smallest TDS of 50% PSP extract addition with 48 hours of fermentation time has 5.1 °Brix. The results follow the literature, which explains that the longer the fermentation time, simple sugars will be converted by LAB in the water kefir. According to Ismawan (2003), TDS increased because complex components such as carbohydrates and proteins break down into simpler compounds.

CONCLUSION

Water kefir from the addition of PSP extract concentration and fermentation time significantly affected chemical properties such as total anthocyanins, antioxidant activity IC₅₀, total sugar, total acid, degree of acidity (pH), and total dissolved solids (TDS), as well as their interaction. The best treatment was the addition of 50% PSP extract and 48 hours of fermentation with chemical analysis results of total anthocyanins 193.197 ± 3.255 ppm; antioxidant activity IC₅₀ 460.620 ± 20.973 ppm; total sugar 1.356 ± 0.058%; total acid 10.429 ± 0.371 %; pH 2.3 ± 0.058; and TDS 2.1 ± 0.2 °Brix.

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