ANTIOXIDANT ACTIVITY AND PSYCHOCHEMICAL OF CASCARA KOMBUCHA FROM ROBUSTA COFFEE

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ABSTRACT

Coffee husks are one of the waste products from coffee processing. Most of the coffee husk waste is just thrown away. Some have used coffee husk waste to make coffee husk tea (cascara). Cascara is obtained from the dried husk of coffee berries and brewed to make tea. Antioxidants found in cascara act as a barrier against free radicals. Cascara can be fermented and is often referred to as cascara kombucha. The cascara kombucha fermentation process will affect the quality of the cascara kombucha tea produced. Factors that influence the fermentation process include sugar concentration and fermentation time. This research aims to determine the effect of sugar concentration and fermentation time on color, Total Dissolved Solids (TDS), pH, and antioxidant activity of cascara kombucha. The design used in this research was a factorial, Completely Randomized Design (CRD) with two factors. The factors used were sugar concentration (5%, 10%, and 15%)

and fermentation time (6 days, 8 days, 10 days, and 12 days). Each treatment was repeated three times. The results showed that low sugar concentration and long fermentation time showed a bright color of cascara kombucha. Sugar concentration and fermentation time significantly affect TDS and antioxidant activity. However, sugar concentration and fermentation time had no significant effect on the pH of cascara kombucha.

Keywords: cascara, coffee robusta, fermentation, kombucha

1. Introduction

Indonesia is the fourth-largest coffee-producing country in the world. According to data from Badan Pusat Statistik (2022) coffee production in Indonesia in 2021 reached 774.6 thousand tons. The increase in coffee production is accompanied by several problems, including waste from coffee husks, where 45% of the coffee fruit is part of the coffee husk. Based on coffee production in 2021, coffee husk waste reached 348,570 thousand tons. Most Indonesians do not know the benefits of coffee husks and cannot process them; in the end, their waste is thrown away and burned. Processed coffee husk waste can increase community finances because of its high economic value. Coffee husk waste can be used as animal feed (Nuraini et al., 2015) and made into coffee husk tea (cascara) (Muzaifa et al., 2021; Muzaifa et al., 2023; Rohaya et al., 2022; Supeno et al., 2018).

Cascara comes from coffee husks that are dried and made into tea. Coffee husks are processed into cascara tea because they taste very distinctive. Cascara contains antioxidants as a moisturizer for free radicals entering our body. Apart from processing cascara into tea, cascara can be processed into a functional drink, which is processed through a fermentation process called kombucha. Cascara kombucha has more benefits than directly brewed cascara (Nurhayati et al., 2020).

Cascara kombucha is cascara tea produced through fermentation, requiring a starter as a microbe that will be grown in the substrate. The length of time in the fermentation process will determine the ability of an organism to grow. In the research of Puspaningrum et al. (2022), long fermentation can affect the pH of kombucha due to the metabolic activity of bacteria and yeast. Likewise, different sugar concentrations can affect the growth of kombucha microbes and the content of chemical compounds such as organic acids. The formation of organic acids causes high acid levels. Other research conducted by Wulandari (2018), shows that the kombucha fermentation process is influenced by the length of fermentation time, starter, and the amount of sugar used, with the total titrated acid content showing results at a fermentation time of 4 days of 5.33% mg/mL and the third day of 13.89% mg/mL.

Fermentation time dramatically influences the content produced from the fermentation process. In the research of Nurhayati et al. (2020) with fermentation times of 4 days, 8 days, 10 days and 12 days with a sugar concentration of 10% (constant) and cascara content of 1% and 2%, the selected kombucha characteristic results were obtained, namely with a cascara content of 1% and fermentation time. 8 days. Other research also states that fermentation times of 4 days, 8 days, 10 days, 12 days and 14 days with a sugar concentration of 10% produce an increase in total acid and alcohol, and the total sugar and pH value of cascara kombucha produced decreases during the fermentation process (Puspaningrum et al. 2022). Sugar plays a very active role in fermentation because, during fermentation, microbes degrade substrates such as sucrose (sugar) and other dissolved substances (fructose, glucose, protein and fat). Apart from that, during the fermentation process, sucrose is converted into alcohol and yeast and bacteria also metabolize sucrose to produce organic acids Nurhayati et al. (2020). According to Putri (2022), the optimal pH and highest antioxidant activity of cocoa leaf kombucha tea were produced from 14 days of fermentation and a sugar concentration of 10%. Therefore, it is necessary to conduct further research with different sugar concentrations and varying fermentation times for each

treatment in making cascara kombucha to obtain a more optimal product.

2. Research Methodology

2.1 Materials

The main ingredients used in this research were cascara tea, Symbiotic Culture of Bacteria and Yeast (SCOBY), white granulated sugar, and water. The supporting materials used are phenolphthalein (PP) indicator, 0.1 N NaOH, DPPH powder (1,1 – diphenyl -2-picrylhydrazyl), methanol and distilled water.

The tools used in this research were a gas stove (Rinai), a pan, a 400 ml glass jar, a stainless steel tea strainer, a cotton cloth covering the jar, a rubber band, an Erlenmeyer (Pyrex), a stirrer, an analytical balance (Kern 440-35N), measuring cup (Iwaki), pH meter (ATC), burette (Germany), dropper pipette, label paper, distillation flask (Duran), distillation condenser (Pyrex), distillation connector, three-burner stove, 5 ml pycnometer (Pyrex), Munsell color chart for plant tissues, hand refractometer (ATC), and Genesys 10S UV-Vis spectrophotometer.

2.2 Experimental design

The research design used in this study was a factorial, completely randomized design (CRD) consisting of two factors. The first factor is sugar concentration, namely 5% sugar content (G1), 10% sugar concentration (G2), and 15% sugar concentration (G3); the second factor is fermentation time, namely 6 days fermentation time (F1), fermentation time 8 days (F2), fermentation time 10 days (F3) and fermentation time 12 days (F4). Based on these two factors, 12 treatment combinations were obtained, each repetition three times to obtain 36 experimental units.

2.3 Experimental Procedure

2.3.1 Production of Cascara Tea

Cascara tea is made by pouring 1% of the tea into a pot containing 1 liter of water (Nurhayati et al., 2020). Then boil until it boils, and the tea dissolves. The tea was filtered using a tea strainer, and sugar was added according to the concentration used, namely 5% (experiment 1), 10% sugar concentration (experiment 2), and 15% sugar concentration (experiment 3), then the stirring process until the sugar dissolved. After the sugar dissolves, the tea is cooled to room temperature.

2.3.2 Production of Cascara Kombucha

Cascara kombucha made with the tea solution has cooled and then put it in 36 clean glass containers according to the treatment, namely container 1 with a sugar concentration of 5% and a fermentation time of 6 days, container 2 with a sugar concentration of 5% and a fermentation time of 8 days, container 3 with a sugar concentration of 5% and a fermentation time of 10 days, container 4 with a sugar concentration of 5% and a fermentation time of 12 days, container 5 with a sugar concentration of 10% and a fermentation time of 6 days, container 6 with a sugar concentration of 10% and a fermentation time of 8 days , container 7 with a sugar concentration of 10% and a fermentation time of 10 days, container 8 with a sugar concentration of 10% and a fermentation time of 12 days, container 9 with a sugar concentration of 15% and a fermentation time of 6 days, container 10 with a sugar concentration of 15% and a fermentation time 8 days, container 11 with a sugar concentration of 15% and a fermentation time of 10 days, container 12 with a sugar concentration of 15% and a fermentation time of 12 days. Then add 10% kombucha starter (SCOBY). The top of the container is covered with cotton cloth tied with a rubber band to provide small amounts of oxygen (microaerophilic), with an empty volume ranging between 25 and 50 mL per container.

Next, it is fermented according to the treatment at room temperature. The optimal temperature is 30-35 °C, protected from sunlight and free from shock or vibration (Indriyani, 2018).

2.4 Parameters

2.4.1 Analysis of Color

Color testing used the Munsell color chart for plant tissue by comparing the sample color with the Mussell color chart for plant tissue (Ferguson, 2012).

2.4.2 Total Dissolved Solids

Testing for total dissolved solids was carried out with the help of a hand refractometer. The refractometer prism is rinsed with distilled water and then wiped with a tissue. Then, the sample is dripped approximately two drops onto the refractometer prism, which is then measured in degrees Brix (Wahyudi & Dewi, 2017).

2.4.3 Antioxidant Activity

The blank solution was prepared by taking 2 mL of DPPH solution plus 1 mL of methanol solution. The sample solution was made with a concentration of 1000 µg/mL and then diluted to a concentration of 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL and 50 µg/mL. 2 ml of solution at each concentration was taken, 1 ml of DPPH

solution was added, then incubated for 30 minutes, followed by absorption using a UV-Vis spectrophotometer with a maximum wavelength of 519 nm. Calculation of % inhibition using the formula (Bakti *et al*., 2017).

$$\% \text{ Antioxidant} = \frac{(\text{A blanko} - \text{A sample})}{\text{A blanko}} x\ 100$$

Next, the calculated values are entered into a linear equation with concentration (µg/mL) as the abscissa (X-axis) and the percent antioxidant value as the ordinate (Y-axis). From the equation obtained, the IC50 price is determined.

2.4.4 pH

The pH of the kombucha coffee sample was measured by taking about 20 ml of the cascara kombucha solution, then putting it in a glass beaker, then measuring the pH of the kombucha coffee solution using a pH meter (Hassmy *et al*., 2017).

3. Results

3.1 Color of Cascara Kombucha

Table 1 showed that the color of kombucha cascara is at a value of 2.5Y 8/6, 5YR 2/6 to 10YR 8/8. The

brightest color was found in the 5% sugar concentration treatment with a fermentation time of 12 days, namely 10YR 8/8. Meanwhile, the most intense color is at a sugar concentration of 15% at a fermentation time of 6 days, namely 5YR 2/6. The higher the hue value (5YR-10YR), the brighter the color. The longer the fermentation time, the brightness level of cascara kombucha increases (chroma and value), while the color (hue) decreases (Nurhayati et al., 2020).

Table 1. Color of *Cascara* Kombucha

Sample	6 days	8 days	10 days	12 days
Sucrose 5%	7,5YR 5/8 	7,5YR 6/8 	10 YR 7/8 	10YR 8/8 
Sucrose 10%	5YR 4/8 	7,5YR 5/8 	7,5 YR 7/8 	2,5YR 8/8 
Sucrose 15%	5YR 2/6 	5 YR 3/6 	5YR 4/8 	2,5Y8/6 

note: 7,5 YR showed *hue*, 5 *value*, and 8 *chroma*

3.2 *Total Dissolved Solids*

ANOVA results showed that the interaction of sugar concentration and fermentation time significantly affected the total dissolved solids of kombucha cascara ($p < 0.05$). DMRT showed that the effect of sugar concentration and fermentation time on the total dissolved solids of kombucha cascara can be seen in Figure 1.

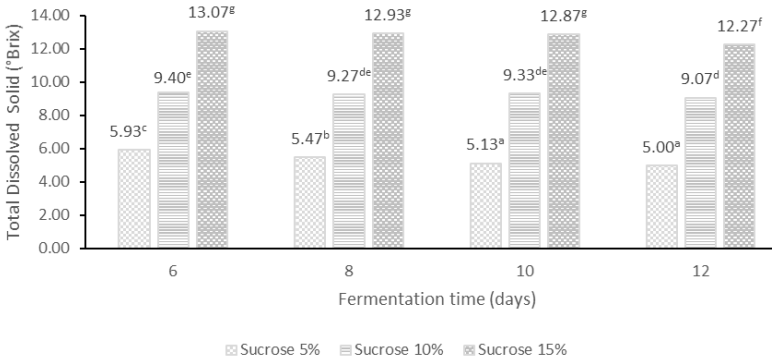

Figure 1. Effect of Sucrose and Fermentation time on Total Dissolved Solids

3.3 Antioxidant Activity

The interaction between sugar concentration and fermentation time had a significant effect on the antioxidant activity of kombucha cascara ($0.000 < 0.05$). DMRT results on the effect of sugar concentration and

fermentation time on the antioxidant activity of kombucha cascara can be seen in Figure 2.

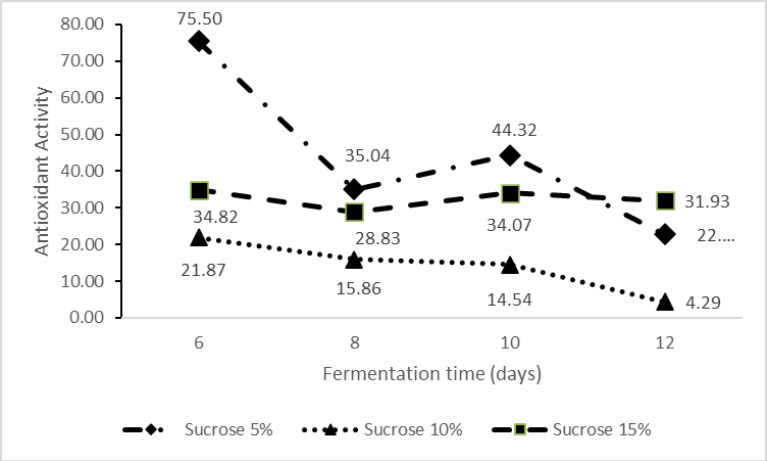

Figure 2. Effect of Sucrose and Fermentation time on Antioxidant Activity

3.4 pH

The results of research on the pH of cascara kombucha are in the range of 3.03-2.63. The highest pH was found in 6-day fermentation with a sugar concentration of 5%, namely 3.03, while the lowest pH was found in 12-day fermentation with a sugar concentration of 15%, namely 2.63. The ANOVA results showed that the interaction between sugar concentration and fermentation time had no significant effect on the pH of Cascara kombucha ($p > 0.05$).

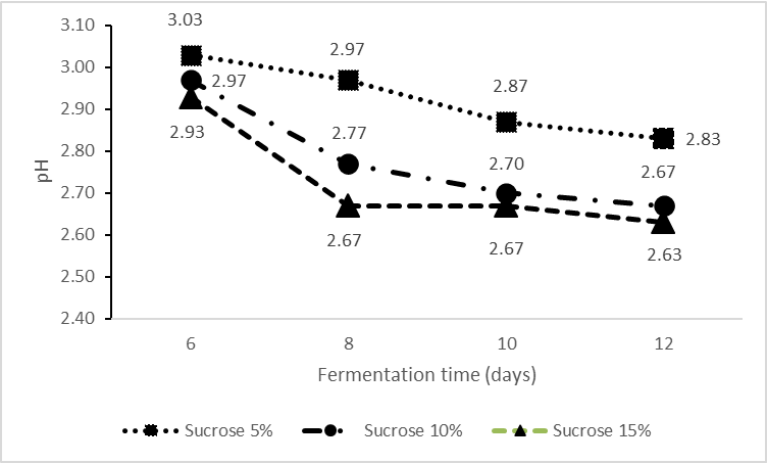

Figure 3. Effect of Sucrose and Fermentation time on pH

4. Discussion

4.1 Color of Cascara Kombucha

The compound that plays a role in changing the color of cascara kombucha is the tannin content, which gives it a dark yellow color (Mawardi, 2016). According to Wistiana & Zubaidah (2015), tannin compounds are damaged because, during the fermentation process, microbes degrade the color of the kombucha so that the resulting color fades further. Color degradation occurs because microbes utilize energy sugars so that over time, the solvent in the medium will run out and result in the kombucha becoming clearer or fading. This is in line with research by Puspitasari et al. (2017) and Nurhayati et al.

(2020) regarding kombucha drinks, where the longer the fermentation time for kombucha, the brighter the kombucha produced.

4.2 Total Dissolved Solid

Figure 1 showed that the more sugar concentration added, the greater the total dissolved solids produced, namely between 5.3830 Brix - 12.7830 Brix. This research's results align Simanjuntak *et al*., (2017) that the addition of granulated sugar produces a higher total dissolved solids value. This is because sugar (sucrose) is composed of glucose and fructose, which dissolve quickly in water—in this study, the longer fermentation time decreased the total dissolved solids of kombucha. The total dissolved solids produced based on fermentation time range from 9.4670Brix to 8.7780Brix. The increase in microbes is accompanied by a decrease in the amount of substrate such as sugar because it is consumed for the metabolism of cell microorganisms so that the total dissolved solids decreases (Nurhayati *et al*., 2020; Bayu *et al*., 2017; Napitupulu & Setyohadi, 2015).

4.3 Antioxidant Activity

During the fermentation process, the pH decreases, resulting in unstable IC_{50} values (Puspaningrum *et al*., 2022). According to Pratama *et al*.

(2015), the instability of antioxidants in acidic conditions is due to the presence of compounds that have active groups, namely hydroxyl groups, which can act as anti-free radicals, by donating unpaired electrons to radical compounds so that free radicals become stable. A compound can be said to have very strong antioxidant activity if it is less than 50 µg/mL, strong for IC_{50} 50-100 µg/mL, medium for IC_{50} 100-150 µg/mL and weak if the IC_{50} value is 151-200 µg/mL (Tristantini *et al*., 2016). Based on Figure 2, the antioxidant activity produced was very strong in all treatments because it had a small IC_{50} value of 50 µg/mL except for the 5% sugar concentration treatment with a fermentation time of 6 days which had an IC_{50} value of 75.50 µg/mL, which means it had strong antioxidant activity.

4.4 pH

Figure 3 showed a graph of the pH results for cascara kombucha. The more sugar concentration given, the lower the pH of the kombucha produced. The pH of cascara kombucha produced in this study based on sugar concentration was 3.02 to 2.8. During fermentation there is a breakdown of sucrose into alcohol and other organic acids. This research is in line with research conducted by Putri (2022), where the more sugar concentration used, the more acid is produced and the more protons are

released, causing the pH to drop. Based on the fermentation time, the pH of cascara kombucha is in the range of 3.07-2.8. The longer the fermentation time, the more the pH value will decrease because the organic acids in the kombucha increase. The organic acids in kombucha dissolve and release protons, so the pH drops. The metabolic activity of bacteria and yeast also causes a decrease in pH. This research is in line with research conducted by Puspaningrum et al. (2022) and Hassmy et al. (2017) where the pH of kombucha produced in the research of Puspaningrum et al. (2022) is in the range of 1.46 to 2.98, while in research Hassmy et al. (2017) the pH of kombucha is in the range of 2.75-3.11.

5. Conclusion

The interaction between sugar concentration and longer fermentation time causes the color of cascara kombucha to become brighter. The interaction between sugar concentration and fermentation time significantly affects total dissolved solids and antioxidant activity. However, it did not significantly affect the pH of cascara kombucha.

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