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Physicochemical Characteristics Of Robusta Coffee Cascara Tea From Temanggung (Coffea Canephora) With Varying Levels Of Coffee Cherry Ripeness And Fermentation Time.

Titisari Juwitaningtyas*[1], Kevin Bagus Pamukti[1]

[1] Departement of Food Technology, Faculty of Industry Technology, Ahmad Dahlan 8 University, Yogyakarta, Indonesia

Abstract

Cascara tea is a beverage made from the dried skin of coffee beans. Cascara contains polyphenol compounds such as chlorogenic acid, catechin, rutin, and ferulic acid. It also contains active compounds such as tannins, pectin, caffeine, chlorogenic acid, caffeic acid, and total anthocyanins. This study aims to determine the effect of variations in the maturity level of coffee cherries and the duration of fermentation of robusta coffee cascara tea on the physical and chemical properties of cascara tea. This study used a completely randomized design with two factors, the first factor being the maturity level of coffee cherries (green, yellow, and red). The second factor is the duration of fermentation with three variations, namely 24 hours, 36 hours, and 48 hours. The results showed that the lowest moisture content of coffee cascara tea was found in sample C3, which was 6.4117%. The lowest pH value of cascara tea was found in sample C3, which was 4.3467. The highest reducing sugar content was found in sample C1, which was 1.5206%. The highest total phenol value was found in sample C1, which was 23.14594 mgGAE/g. The highest antioxidant activity was found in sample C1 with an IC_{50} value of 21.2203 µg/mL. The lowest caffeine content was found in sample A3, which was 0.3195%. The maturity level of coffee cherries and the duration of fermentation significantly affected the pH value, total phenol, reducing sugar, antioxidant activity, and caffeine content, while the moisture content did not significantly affect the results.

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1. Introduction

Indonesia produces coffee as one of the commodities from its plantations. However, Indonesia is not the largest coffee producer in the world. Some countries that produce the largest amount of coffee are Brazil, Vietnam, Colombia, and Indonesia (1). As the coffee production in Indonesia increases, the coffee waste also increases. When coffee is processed, about 40-45% of the yield consists of husks. Coffee skins have a high water content, around 75-80%, making them wet and prone to damage (2). Cascara tea has a sweet taste and a distinctive aroma, similar to herbal tea. This was stated by Carpenter (2015) as cited by Nafisah and Widyaningsih (3). According to Pandey et al. (4), cascara contains polyphenolic compounds such as chlorogenic acid (42.2%), catechin (2.2%), rutin (2.1%), and ferulic acid (1%). On the other hand, coffee skin also contains active compounds such as tannins (1.8-8.56%), pectin (6.5%), caffeine (1.3%), chlorogenic acid (2.6%), caffeic acid (1.6%), and total anthocyanins (43%).

Cascara tea is a beverage made from coffee skins, named so because it has a color and taste resembling tea. Although cascara tea has been known in other countries for a long time, it is still rare to find in Indonesia. The word "cascara" itself comes from Spanish, meaning

* Correspondence : **Titisari Juwitaningtyas** titisari.jwitaningtyas@tp.uad.ac.id

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"skin" (5). The production of cascara tea typically uses Arabica and Robusta coffee beans. Both of these coffee varieties have distinct flavors, especially the acidic taste derived from chlorogenic acid and caffeic acid compounds. During the process of making cascara tea, the compounds in coffee skins are processed to give unique characteristics to the tea, such as a sweet taste, a reddish-yellow infusion color, and a distinctive aroma. According to Rahayu et al. (6), cascara tea has characteristics marked by a strong flavor and aroma, as well as containing polyphenolic compounds.

Harvesting time is an environmental factor that influences the compound content in plants. The level of fruit maturity greatly affects the harvesting time, which is usually marked by changes in the fruit's skin color. These color changes indicate changes in the chemical composition of the fruit. Coffee is also a fruit crop that requires the right harvesting time and level of maturity. According to Abdullah et al. (7) cited in Srikandi (8), Robusta coffee has a green color when young, slightly yellowish to reddish when half-ripe, and bright red to dark red when fully ripe. The level of fruit maturity in coffee greatly affects the chemical content in the fruit, especially caffeine. The caffeine content in coffee fruits varies depending on the maturity level when the fruit is harvested. Not all coffee cherries will ripen at the same time, so it is important to pay attention to the color of each coffee cherry that will be harvested.

Fermentation is one of the methods that can be used to reduce the caffeine content in coffee. This is because during the fermentation process, microorganisms break down the caffeine compounds present in coffee beans. Research conducted by Kristianto et al. (9) showed that the longer the fermentation time, the lower the caffeine content in coffee beans. Therefore, fermentation can be a viable method to decrease the caffeine content in coffee beans. Wet fermentation significantly reduces the caffeine content in coffee (10). According to Oktadina et al. (11), wet fermentation aims to aid in the breakdown of mucilage components and degrade compounds present in the mucilage attached to the coffee beans. This potentially lowers the caffeine content in coffee skins. The addition of yeast can expedite the fermentation process, resulting in low-caffeine coffee in a shorter time (12). Based on the aforementioned information, a research study will be conducted on the Physicochemical Characteristics of Robusta Coffee (Coffea Canephora) Cascara Tea with Variations in Coffee Cherry Maturity and Fermentation Duration.

2. Materials and Methods

2.1 Materials

The materials used in this study are Robusta coffee cherries with green, yellow, and red cherry maturity levels, obtained from coffee farmers in Brujulan Village, Gemawang, Temanggung. The materials used for testing include tape yeast, distilled water, methanol PA, DPPH solution, Folin-Ciocalteu solution, gallic acid solution, Na_2CO_3, chloroform, aluminum foil, ascorbic acid powder, H_2SO_4, MgO powder, 1% KOH solution, Nelson solution. The equipment used in this study includes a pH meter (Ohaus), UV-Vis spectrophotometer, measuring glass (iwaki), analytical balance (iwaki), test tubes (iwaki), beakers (iwaki), vortex, measuring pipettes (1 ml, 5 ml, and 10 ml), Erlenmeyer flask (iwaki), dropper pipette, cabinet dryer, oven.

2.2. Method

The research will be conducted from March 2022 to July 2022. The study includes the process of making coffee cascara tea and the analysis of the physicochemical characteristics of coffee cascara tea. The physicochemical analysis includes the determination of moisture content (13), pH value (13), antioxidant activity (14), total phenols (15), reducing sugars (16), and caffeine content (13).

2.3 Process of making cascara tea

The process of making cascara coffee tea refers to the modified research by Nailasari (17). The first step in the cascara tea-making process is sorting the coffee cherries to separate them from foreign objects. Next, the coffee cherries are washed to remove any dirt or impurities. Afterward, the washed cherries undergo a wet fermentation process by placing them in a jar and adding 1% yeast starter and water. The jar is then sealed and stored for 24 hours, 36 hours, and 48 hours. Subsequently, the fermented coffee cherries are separated into the coffee fruit skin and coffee beans, either manually or without using a coffee depulper machine. This is done to obtain coffee fruit skin that is minimally damaged. Next, the coffee fruit skin is dried using a cabinet dryer for 24 hours at a temperature of 50°C. The grinding process follows, which is carried out after the drying process is complete using a blender. The finely ground samples are then stored in aluminum foil containers to protect them from contaminants that could degrade the sample's contents.

2.4 Procedure for Moisture Content Analysis

The moisture content of coffee beans is measured using the gravimetric method, following the AOAC (13) guidelines. A sample weighing 2 grams is first taken and then dried in an oven (105ºC; 24 hours). The dried sample is then placed in a desiccator and weighed.

2.5 Procedure for pH Value Analysis

The acidity of coffee is measured using the pH meter method. A 5 g sample is weighed and diluted with distilled water (1:5 ratio). The sample solution is stirred for 30 minutes and its acidity is measured using a pH meter, following the AOAC (13) guidelines.

2.6 Procedure for Antioxidant Activity Analysis

The antioxidant analysis of cascara tea is conducted using the DPPH method. The antioxidant activity testing is carried out by taking samples of 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, and 1 ml to create a series of measurements at concentrations of 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm. Methanol for analysis purposes is then added to each sample, bringing the total volume to 10 ml. The mixture is vortexed, and 5 ml of each solution is taken and combined with 5 ml of 0.1 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution. The resulting mixture is vortexed again and incubated in a dark place for 30 minutes. After incubation, the absorbance of the samples is measured at a wavelength of 517 nm, following the AOAC (14) guidelines.

2.7 Procedure for Total Phenol Analysis

The total phenolic compound content in cascara tea is measured using the Folin-Ciocalteu method. The total phenol test involves measuring a 0.2 ml sample, adding 0.8 ml of 7.5% Na_2CO_3 solution and 1 ml of Folin-Ciocalteu reagent, followed by vortexing. The mixture is then incubated at room temperature for 30 minutes. After incubation, the absorbance is

measured at a wavelength of 753 nm. Calibration is performed using a gallic acid standard curve to determine the total phenol content in µgGAE/ml, according to AOAC (15) guidelines.

2.8 Procedure for Reducing Sugar Analysis

The measurement of reducing sugar content in cascara tea is performed using the Nelson-Somogyi method. A 1-gram sample of cascara tea is weighed and placed in a 100 ml Erlenmeyer flask. The sample is then diluted with 100 ml of distilled water, centrifuged, and filtered. Clear filtrate (1 ml) is taken and combined with 1 ml of Nelson C reagent (a mixture of Nelson A and Nelson B in a 25:1 ratio). The mixture is heated in a water bath at 100℃ for 30 minutes. After cooling, 1 ml of arsenomolybdate is added, followed by thorough mixing. Distilled water is added to bring the total volume to 10 ml. The solution is vortexed, and the absorbance is measured using a spectrophotometer at a wavelength of 540 nm, following the AOAC (16) guidelines.

2.9 Procedure for Caffeine Content Analysis

The caffeine content is determined using the High Performance Liquid Chromatography (HPLC) method. The procedure involves weighing 5 grams of the sample and adding 1 gram of MgO. The sample is then dissolved in distilled water. Next, the sample is heated using a reflux condenser for 2 hours and diluted to a final volume of 250 ml. The solution is filtered, and 100 ml of the filtered sample is taken. To this, 10 ml of H_2SO_4 is added and the mixture is boiled until the volume reduces to 25 ml. The resulting liquid is transferred to a separating funnel, and 10 ml of H_2SO_4 (1:9 ratio) is added. The solution is vigorously shaken with the addition of chloroform in increasing amounts (10 ml, 15 ml, 20 ml, 25 ml). Then, 5 ml of 1% KOH is added to the solution, followed by another rinse with chloroform. The solution forms two layers, with the bottom layer being the chloroform solution that binds to caffeine, while the top layer consists of water and other substances. The bottom layer is heated in an oven at 100℃ until its weight remains constant.

2.10 Statistical Analysis

The statistical analysis used in this research is Two-Way Analysis of Variance (ANOVA). If the results of the Two-Way ANOVA show a significant value of $p<0.05$, a post-hoc test, such as Tukey's test, will be conducted. The data analysis is performed using SPSS (Statistical Package for the Social Sciences) version 25.

3. Results and Discussion

3.1. Water Content

Water content is an important component in food materials as it can influence the shelf life of a food product. High water content in a food material can lead to spoilage. Conversely, low water content in a food material can prolong its shelf life (18).

The average water content of the measured cascara tea, with different treatments of coffee cherry ripeness and fermentation duration, ranged from 6.4117% to 7.8713% as shown in Figure 1.

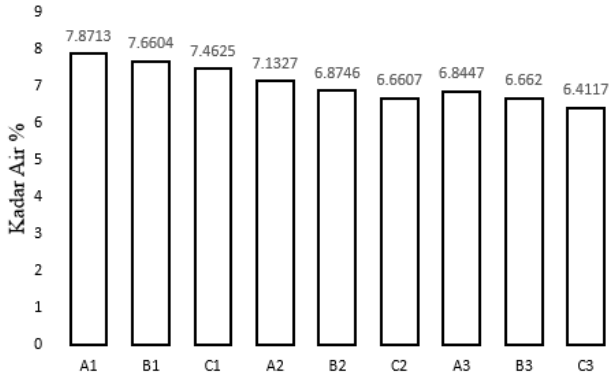

Figure 1. Average water content

Note :

A1: Green Coffee Cherry Ripeness Level and 24-Hour Fermentation Duration.
B1: Yellow Coffee Cherry Ripeness Level and 24-Hour Fermentation Duration.
C1: Red Coffee Cherry Ripeness Level and 24-Hour Fermentation Duration.
A2: Green Coffee Cherry Ripeness Level and 36-Hour Fermentation Duration.
B2: Yellow Coffee Cherry Ripeness Level and 36-Hour Fermentation Duration.
C2: Red Coffee Cherry Ripeness Level and 36-Hour Fermentation Duration.
A3: Green Coffee Cherry Ripeness Level and 48-Hour Fermentation Duration.
B3: Yellow Coffee Cherry Ripeness Level and 48-Hour Fermentation Duration.
C3: Red Coffee Cherry Ripeness Level and 48-Hour Fermentation Duration.

This study demonstrates that the maturity level of coffee cherries and the duration of fermentation significantly affect the moisture content of coffee cascara. The results of the post-hoc test on the influence of the maturity level of coffee cherries and fermentation duration on the moisture content of coffee cascara can be seen in Table 1.

Table 1. Effect of coffee cherry maturity level and fermentation duration on the moisture content of coffee cascara

Varying Levels Of Coffee Cherry Ripeness	Fermentation Time (hours)		
	24	36	48
Green	7.8713 ± 0.0112^{a1}	7.6604 ± 0.0192^{a2}	7.4625 ± 0.0533^{a3}
Yellow	7.1327 ± 0.0216^{b1}	6.8746 ± 0.0158^{b2}	6.6607 ± 0.0092^{b3}
Red	6.8447 ± 0.0033^{c1}	6.6620 ± 0.0143^{c2}	6.4117 ± 0.0355^{c3}

Note: The letter symbols indicate significant differences among the samples.

The data in Table 1 shows the results of the analysis of water content that has been conducted, indicating that the water content of tea cascara coffee ranges from 6.4117% to 7.8713%. The two-way ANOVA analysis with a significance level of $p<0.05$ shows that the maturity level of coffee cherries significantly affects the water content of tea cascara coffee, with a significance value of 0.000. The fermentation duration of tea cascara coffee also shows a significant difference with a significance value of 0.000. The interaction between the maturity level of coffee cherries and fermentation duration does not show a significant difference, with a significance value of 0.075. This indicates that the maturity level of coffee cherries and fermentation duration have a significant difference in each treatment. The more mature the coffee cherries and the longer the fermentation, the lower the water content in tea cascara. The maturity level of coffee cherries also affects the water content of tea cascara coffee. Riper coffee cherries have a lower water content compared to younger cherries. This

is because the ripening process causes organic matter and water within the fruit to evaporate, resulting in a decrease in water content.

The length of fermentation also affects cascara tea, as it involves changes in the biochemical compounds present in the coffee cherry's skin during fermentation. Specifically, the transformation of tannin compounds into derivative compounds occurs, causing water to condense along with the condensation of tannin compounds. According to Yulia (19) cited in Kusumaningrum et al. (20), enzymatic oxidation leads to the condensation of tannins, transforming them into derivative compounds known as theaflavins and thearubigins. Tannins in plants are typically found in condensed and hydrolyzed forms, with condensed tannins being the most abundant (21). Additionally, air humidity also affects the decrease in moisture content of the coffee cherry's skin, with suboptimal humidity leading to water loss from the coffee cherry's skin.

3.2 pH Value

The acidity level (pH) is a measurement of the acidity or alkalinity of a solution. The higher the pH value, the more alkaline it is. Conversely, the lower the pH value, the more acidic it is. The pH values obtained from measuring cascara tea can be seen in Figure 2.

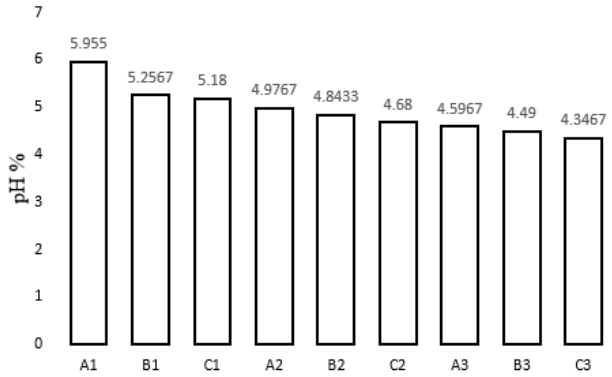

Figure 2. Average pH value

Note :

A1: Green Coffee Cherry Ripeness Level and 24-Hour Fermentation Duration.
B1: Yellow Coffee Cherry Ripeness Level and 24-Hour Fermentation Duration.
C1: Red Coffee Cherry Ripeness Level and 24-Hour Fermentation Duration.
A2: Green Coffee Cherry Ripeness Level and 36-Hour Fermentation Duration.
B2: Yellow Coffee Cherry Ripeness Level and 36-Hour Fermentation Duration.
C2: Red Coffee Cherry Ripeness Level and 36-Hour Fermentation Duration.
A3: Green Coffee Cherry Ripeness Level and 48-Hour Fermentation Duration.
B3: Yellow Coffee Cherry Ripeness Level and 48-Hour Fermentation Duration.
C3: Red Coffee Cherry Ripeness Level and 48-Hour Fermentation Duration.

This study demonstrates that the degree of coffee cherry ripeness and the duration of fermentation significantly affect the pH value of cascara tea. The results of further analysis on the influence of coffee cherry ripeness and fermentation duration on the pH value of cascara tea can be seen in Table 2.

Table 2. The Influence of Coffee Cherry Ripeness and Fermentation Duration on the pH of Cascara Tea

Varying Levels Of Coffee Cherry Ripeness	Fermentation Time (hours)		
	24	36	48
Green	5.9550 ± 0.0071^{a1}	5.2567 ± 0.0153^{a2}	5.1800 ± 0.0265^{a3}
Yellow	4.9767 ± 0.0252^{b1}	4.8433 ± 0.0306^{b2}	4.6800 ± 0.0400^{b2}
Red	4.5967 ± 0.0308^{c1}	4.4900 ± 0.0173^{c2}	4.3467 ± 0.0351^{c3}

Note: The letter symbols indicate significant differences among the samples.

Table 2. The pH Value/Acidity Level of Cascara Coffee Tea ranges from 4.3467 to 5.9550. The pH values of cascara tea also show significant differences for each treatment. The two-way ANOVA analysis with a significance level ($p<0.05$) reveals that the ripeness level of coffee cherries in cascara tea significantly differs with a significance value of 0.000. The fermentation duration of cascara tea shows a significant difference with a significance value of 0.000. The interaction between the ripeness level of coffee cherries and fermentation duration shows a significant difference with a significance value of 0.000.

The riper the coffee cherries and the longer the fermentation duration, the lower the pH of cascara tea. Fermentation duration leads to a decrease in the pH of cascara tea. This is because as the coffee cherry skin undergoes longer fermentation, the formation of thearubigins increases while the content of theaflavins decreases. As a result, cascara tea becomes more acidic due to the strong acidic nature of thearubigins, which also impart a brownish color. Additionally, acid contents in the coffee cherry skin, such as chlorogenic acid and caffeic acid, can also influence the pH of cascara tea. This indicates that the pH of cascara tea is influenced by various factors such as fermentation duration, thearubigin content, and acid contents in the coffee cherry skin. This aligns with the opinion of Yusianto and Widyotomo (22), who state that during fermentation, microbial activities, particularly lactic acid bacteria, transform mucilage layers into organic acids, making the mucilage layer more acidic. The ripeness level of coffee cherries also significantly affects the pH value of cascara tea, and it can be concluded that mature coffee cherries have lower pH levels compared to younger ones. This is because as the coffee cherries mature, they contain a higher amount of organic acids. These organic acids contribute to a decrease in the pH of coffee cherries, indicating increased acidity. These findings indicate that the maturity level of coffee cherries is one of the factors influencing the pH level in coffee cherries.

3.3 Antioxidant Analysis

Antioxidant activity refers to the ability of compounds to bind free radicals. In this study, the DPPH radical scavenging method was used to determine the IC_{50} value for each tested sample. The IC_{50} value indicates the concentration of the sample (ppm) required to inhibit 50% of the free radicals. If the IC_{50} value falls within the range of 50-100 ppm, the antioxidant activity is classified as strong. If the IC_{50}value falls within the range of 100-150 ppm, the antioxidant activity is classified as moderate. If the IC_{50} value falls within the range of 150-200 ppm, the antioxidant activity is classified as weak. If the IC_{50}value exceeds 200 ppm, the antioxidant activity is classified as very weak (23).

The IC_{50} values obtained from measuring cascara tea with different treatments of coffee cherry ripeness and fermentation duration can be seen in Figure 3.

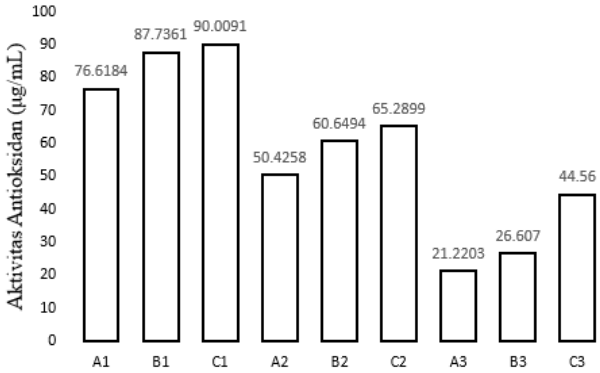

Figure 3. Average IC_{50} value

Note :

A1: Green Coffee Cherry Ripeness Level and 24-Hour Fermentation Duration.
B1: Yellow Coffee Cherry Ripeness Level and 24-Hour Fermentation Duration.
C1: Red Coffee Cherry Ripeness Level and 24-Hour Fermentation Duration.
A2: Green Coffee Cherry Ripeness Level and 36-Hour Fermentation Duration.
B2: Yellow Coffee Cherry Ripeness Level and 36-Hour Fermentation Duration.
C2: Red Coffee Cherry Ripeness Level and 36-Hour Fermentation Duration.
A3: Green Coffee Cherry Ripeness Level and 48-Hour Fermentation Duration.
B3: Yellow Coffee Cherry Ripeness Level and 48-Hour Fermentation Duration.
C3: Red Coffee Cherry Ripeness Level and 48-Hour Fermentation Duration.

This research shows that the ripeness level of coffee cherries and the fermentation duration significantly affect the IC_{50} value of cascara tea. Teh result of further analysis on the influence of coffee cherry ripeness and fermentation duration on the IC_{50} value of cascara tea can be seen in Table 3.

Table 3. The Influence of Coffee Cherry Ripeness and Fermentation Duration on the Antioxidant Activity of Cascara Coffee Tea

Table 3. The Influence of Coffee Cherry Ripeness and Fermentation Duration on the Antioxidant Activity of Cascara Coffee Tea

Varying Levels Of Coffee Cherry Ripeness	Fermentation Time (hours)		
	24	36	48
Green	76.6184 ± 0.5758^{a1}	87.7361 ± 0.2042^{a2}	90.0091 ± 1.3609^{a3}
Yellow	50.4258 ± 0.3636^{b1}	60.6494 ± 0.2921^{b2}	65.2899 ± 0.3199^{b3}
Red	21.2203 ± 0.1494^{c1}	26.6070 ± 0.2651^{c2}	44.5600 ± 0.1702^{c3}

Note: The letter symbols indicate significant differences among the samples.

The data in Table 3 shows that the IC_{50} values of cascara coffee tea range from 21.2203 to 90.0091 µg/mL. The antioxidant activity values of cascara coffee tea show significant differences for each treatment. The two-way ANOVA analysis with a significance level ($p<0.05$) indicates that the ripeness level of coffee cherries in cascara coffee tea significantly differs with a significance value of 0.000. The fermentation duration of cascara coffee tea shows a significant difference with a significance value of 0.000. The interaction between the ripeness level of coffee cherries and fermentation duration shows a significant difference with a significance value of 0.000.

The maturity level of coffee cherry affects the antioxidant activity value. The older the coffee cherry, the higher the antioxidant activity. On the other hand, the duration of fermentation affects the antioxidant activity value in coffee cascara tea. The longer the fermentation, the more the antioxidant activity value of coffee cascara tea decreases. Acidic

conditions can reduce antioxidant activity because phenolic compounds, which have antioxidant activity, become more stable and have difficulty releasing protons that can bind with DPPH. This causes a decrease in antioxidant activity, as explained by Villarreal et al. (24).

The fermentation process in coffee skins causes tannin compounds, which function as antioxidants, to undergo enzymatic oxidation, resulting in a decrease in antioxidant activity in cascara tea. This is consistent with the research conducted by Rohdiana (25), which states that during the tea fermentation process, bioactive compounds such as tannins undergo a decrease due to enzymatic oxidation, leading to a decrease in antioxidant activity. This process is caused by the oxidation of tannins, transforming them into derivative compounds such as theaflavins and thearubigins.

3.4 Total Phenol

Total phenolic analysis is a method used to determine the phenolic content present in a sample. The Folin-Ciocalteu method is the method used in this study. The total phenolic values obtained from measuring the coffee cascara tea samples with different treatments of coffee cherry maturity levels and fermentation durations can be seen in Figure 4.

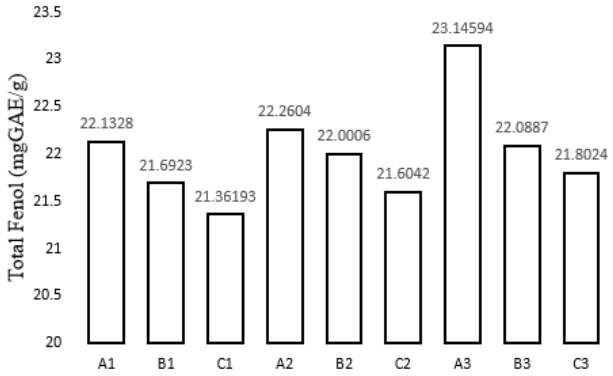

Figure 4. Average total phenol value

Note :

A1: Green Coffee Cherry Ripeness Level and 24-Hour Fermentation Duration.
B1: Yellow Coffee Cherry Ripeness Level and 24-Hour Fermentation Duration.
C1: Red Coffee Cherry Ripeness Level and 24-Hour Fermentation Duration.
A2: Green Coffee Cherry Ripeness Level and 36-Hour Fermentation Duration.
B2: Yellow Coffee Cherry Ripeness Level and 36-Hour Fermentation Duration.
C2: Red Coffee Cherry Ripeness Level and 36-Hour Fermentation Duration.
A3: Green Coffee Cherry Ripeness Level and 48-Hour Fermentation Duration.
B3: Yellow Coffee Cherry Ripeness Level and 48-Hour Fermentation Duration.
C3: Red Coffee Cherry Ripeness Level and 48-Hour Fermentation Duration.

This study shows that the maturity level of coffee cherries and fermentation duration have a significant effect on the total phenolic values of coffee cascara tea. The results of further tests on the effects of coffee cherry maturity level and fermentation duration on the total phenolic values of coffee cascara can be seen in Table 4.

Table 4. The Influence of Coffee Cherry Maturity Level and Fermentation Duration on the Total Phenolic Values of Coffee Cascara Tea

Varying Levels Of Coffee Cherry Ripeness	Fermentation Time (hours)		
	24	36	48
Green	22.1328 ± 0.0763^{a1}	21.6923 ± 0.0661^{a2}	21.36193 ± 0.0661^{a3}
Yellow	22.2604 ± 0.0762^{b1}	22.0006 ± 0.0381^{b2}	21.6042 ± 0.0381^{b3}
Red	23.14594 ± 0.0762^{c1}	22.0887 ± 0.1144^{C2}	21.8024 ± 0.1009^{c3}

Note: The letter symbols indicate significant differences among the samples.

The data in Table 4 shows that the total phenolic values of coffee cascara tea range from 21.36193-23.14594 mgGAE/g. The total phenolic values of coffee cascara tea show significant differences in each treatment. The results of the two-way ANOVA analysis with a significance level of ($p<0.05$) indicate that the maturity level of coffee cherries in cascara tea significantly influences the total phenolic values with a significance value of 0.000. The fermentation duration in cascara tea also shows significant differences with a significance value of 0.000. The interaction between the maturity level of coffee cherries and fermentation duration shows significant differences with a significance value of 0.000.

Based on the analysis results, the fermentation duration affects the total phenolic values of coffee cascara tea, and the longer the fermentation duration, the lower the total phenolic values of coffee cascara tea. Rohdiana (26) stated that during the fermentation process, phenolic compounds present in the leaves undergo changes and transform into theaflavins and thearubigins. Theaflavins and thearubigins are derivatives of catechin compounds, which are types of polyphenolic compounds (27). This finding is consistent with the statement by Lelita et al. (28), who mentioned that black tea extract has a low content of phenolic compounds due to undergoing full fermentation. In this context, the longer the fermentation process, the lower the total content of phenolic compounds in black tea.

3.5 Reducing Sugar

Reducing sugars are a type of sugar that can be reduced by an enzyme into alcohol or organic acid. In the coffee industry, reducing sugars can affect the taste and aroma characteristics of coffee beans after processing. Controlling the level of reducing sugars is crucial to ensure the quality and consistency of the harvested produce. The measurements of reducing sugars in coffee cascara tea samples with different treatments of coffee cherry maturity levels and fermentation durations can be seen in Figure 5.

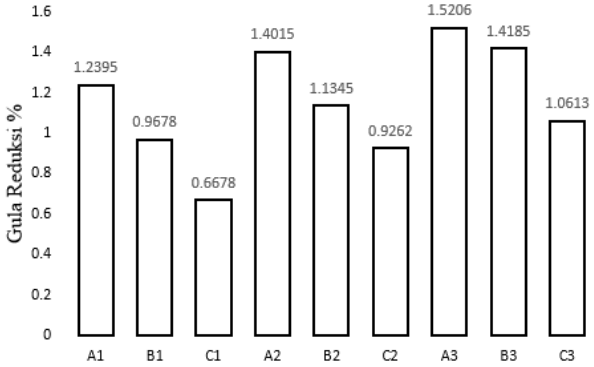

Figure 5. Average reducing sugar value

Note :

A1: Green Coffee Cherry Ripeness Level and 24-Hour Fermentation Duration.
B1: Yellow Coffee Cherry Ripeness Level and 24-Hour Fermentation Duration.
C1: Red Coffee Cherry Ripeness Level and 24-Hour Fermentation Duration.
A2: Green Coffee Cherry Ripeness Level and 36-Hour Fermentation Duration.
B2: Yellow Coffee Cherry Ripeness Level and 36-Hour Fermentation Duration.
C2: Red Coffee Cherry Ripeness Level and 36-Hour Fermentation Duration.
A3: Green Coffee Cherry Ripeness Level and 48-Hour Fermentation Duration.
B3: Yellow Coffee Cherry Ripeness Level and 48-Hour Fermentation Duration.
C3: Red Coffee Cherry Ripeness Level and 48-Hour Fermentation Duration.

This research shows that the maturity level of coffee cherries and fermentation duration significantly affect the reducing sugars in coffee cascara tea. Further analysis of the effects of coffee cherry maturity level and fermentation duration on the reducing sugars in cascara tea can be seen in Table 4.

Table 4. The Influence of Coffee Cherry Maturity Level and Fermentation Duration on the Reducing Sugars in Coffee Cascara Tea

Varying Levels Of Coffee Cherry Ripeness	Fermentation Time (hours)		
	24	36	48
Green	1.2395 ± 0.0085^{a1}	0.9678 ± 0.0067^{a2}	0.6678 ± 0.0069^{a3}
Yellow	1.4015 ± 0.0033^{b1}	1.1345 ± 0.0051^{b2}	0.9262 ± 0.0070^{b3}
Red	1.5206 ± 0.0034^{c1}	1.4185 ± 0.0050^{c2}	1.0613 ± 0.0050^{c3}

Note: The letter symbols indicate significant differences among the samples.

Based on the data in Table 5, coffee cascara tea has reducing sugar content ranging from 0.6678% to 1.5206%. The values of reducing sugars in coffee cascara tea also show significant differences in each treatment. The results of the two-way ANOVA analysis with a significance level of ($p<0.05$) indicate that the maturity level of coffee cherries in coffee cascara tea significantly influences the reducing sugar values with a significance value of 0.000.

Based on the research results, it is evident that fermentation duration reduces the level of reducing sugars in coffee cascara tea. According to Rahayu and Kuswanto (29), the decrease in reducing sugar content is attributed to yeast (S. cerevisiae) breaking down glucose into alcohol, thereby increasing the alcohol content in kombucha tea. According to Azizah and Wijaya (30), the value of reducing sugars is influenced by the fermentation duration. During the fermentation process, ethanol is produced as a result of the breakdown of sugars by Saccaromyces cerevisiae in cassava tape fermentation. During fermentation, the reducing sugars present in the mucilage are degraded by Saccaromyces cerevisiae, leading to the production of enzymes that convert glucose into ethanol. As time progresses, the ethanol content increases, causing the reducing sugar values to decrease. This indicates that the longer the fermentation duration, the lower the reducing sugar content, as more glucose is converted into ethanol by the enzymes produced by Saccaromyces cerevisiae. In addition to fermentation duration, the maturity level of coffee cherries also affects the reducing sugar content in coffee cascara. Older coffee cherries have higher sugar content compared to younger ones.

3.6 Caffeine Content

Caffeine is a crystalline alkaloid compound belonging to the xanthine group, known for its bitter taste. It functions as a psychoactive stimulant and a mild diuretic. Caffeine can affect the central nervous system, muscles, and kidneys. In the central nervous system, caffeine plays a role in preventing drowsiness, enhancing sensory perception, speeding up thought processes, and reducing fatigue. The safe daily consumption limit for caffeine is approximately 100-150 mg.

The analysis results of caffeine levels in coffee cascara tea can be seen in Figure 6.

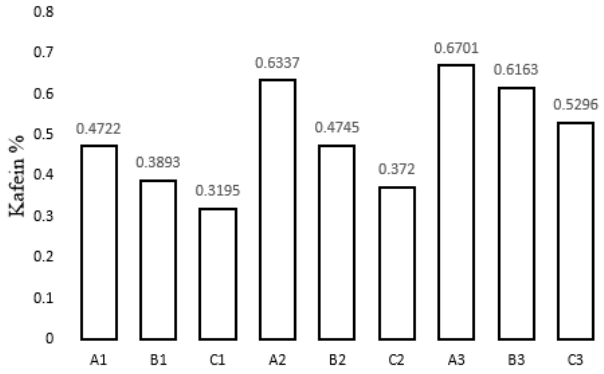

Figure 6. Average caffeine content value

Note :

A1: Green Coffee Cherry Ripeness Level and 24-Hour Fermentation Duration.
B1: Yellow Coffee Cherry Ripeness Level and 24-Hour Fermentation Duration.
C1: Red Coffee Cherry Ripeness Level and 24-Hour Fermentation Duration.
A2: Green Coffee Cherry Ripeness Level and 36-Hour Fermentation Duration.
B2: Yellow Coffee Cherry Ripeness Level and 36-Hour Fermentation Duration.
C2: Red Coffee Cherry Ripeness Level and 36-Hour Fermentation Duration.
A3: Green Coffee Cherry Ripeness Level and 48-Hour Fermentation Duration.
B3: Yellow Coffee Cherry Ripeness Level and 48-Hour Fermentation Duration.
C3: Red Coffee Cherry Ripeness Level and 48-Hour Fermentation Duration.

This research shows that the ripeness level of coffee cherries and the fermentation duration significantly affect the caffeine content of coffee cascara tea. Further analysis on the influence of coffee cherry ripeness and fermentation duration on the caffeine content of coffee cascara tea can be seen in Table 6.

Table 6. The Influence of Coffee Cherry Ripeness and Fermentation Duration on the Caffeine Content of Coffee Cascara Tea

Varying Levels Of Coffee Cherry Ripeness	Fermentation Time (hours)		
	24	36	48
Green	0.4722 ± 0.0232^{a1}	0.3893 ± 0.0074^{a2}	0.3195 ± 0.0327^{a3}
Yellow	0.6337 ± 0.0387^{b1}	0.4745 ± 0.0190^{b2}	0.3720 ± 0.0138^{b3}
Red	0.6701 ± 0.0158^{c1}	0.6163 ± 0.0422^{c2}	0.5296 ± 0.0200^{c3}

Note: The letter symbols indicate significant differences among the samples.

Based on Table 6, coffee cascara tea has a caffeine content ranging from 0.3195% to 0.6701%. The results of the two-way ANOVA analysis with a significance level of $p<0.05$ indicate that the ripeness level of coffee cherries in coffee cascara tea shows a significant difference with a significance value of 0.000. The fermentation duration in coffee cascara tea also shows a significant difference with a significance value of 0.000.

The ripeness level of coffee cherries and fermentation duration both affect the caffeine content in coffee cascara tea. The more mature the coffee cherries, the higher the caffeine content. This is because more mature coffee cherries undergo more complete metabolism compared to younger ones, resulting in higher caffeine levels in mature coffee cherries.

Fermentation duration has an influence on the caffeine content of coffee cascara tea, where a longer fermentation duration leads to a decrease in caffeine content. This decrease in caffeine content is caused by the activity of lactic acid bacteria. The reduction in caffeine content is also influenced by the duration of fermentation. The presence of proteolytic bacteria with high protease enzyme activity results in a decrease in caffeine content during the fermentation process (31). Additionally, the presence of S. cerevisiae bacteria in the tape fermentation further contributes to this process. This is because the absence of mucilage facilitates the entry of proteolytic enzymes derived from S. cerevisiae into the cytoplasm, leading to the degradation of caffeine in coffee (32).

4. Conclusions

Based on the obtained research results, the lowest water content of coffee cascara tea was found in sample C3, which was 6.4117%. The lowest pH value of cascara tea was found in sample C3, which was 4.3467. The highest reducing sugar content was found in sample C1, which was 1.5206%. The highest total phenol value was found in sample C1, which was 23.14594 mgGAE/g. The highest antioxidant activity was found in sample C1 with an IC_{50} value of 21.2203 µg/mL. The lowest caffeine content was found in sample A3, which was 0.3195%. Coffee cherry ripeness and fermentation duration significantly affect the pH value, total phenol, reducing sugar content, antioxidant activity, and caffeine content. However, they do not have a significant effect on the water content.

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Author Contributions

T.J were responsible for experiments design and coordinating all the research processes; K.B.P performed the experiments, analyzed the data, and wrote the paper.

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The available data are indicated in the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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