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EFFECT OF FERMENTATION DURATION ON CAFEIN CURRENCY IN ROBUSTA COFFEE (*Coffea canephora*) FROM PTPN XII JEMBER

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ABSTRACT

Background: Indonesia is one of the largest coffee producing and exporting countries in the world. Jember Regency is one of the areas that develop robusta coffee, especially PT. Perkebunan Nusantara XII in the city of Jember has a coffee plantation area of 10,715 Ha. Coffee contains caffeine compounds. Caffeine is a xanthine alkaloid compound where most people believe that caffeine contained in coffee functions against drowsiness and fatigue. The fermentation process of coffee beans is a process in which the decomposition of complex compounds present in coffee beans, into simple compounds by adding some microorganisms to help release the mucus layer that still covers the coffee beans. BAL is a bacteria that can reduce the caffeine content in coffee. **Methods:** This research design is true experimental with UV-Vis Spectrophotometry method to determine the caffeine content. The sample used was robusta coffee beans (*Coffea canephora*) from PTPN XII Jember. Robusta coffee was fermented for 8, 18, 24 hours. Absorbance measurement was performed at a wavelength of 273 nm. **Result :** Caffeine levels in Robusta coffee that underwent BAL (*Lactobacillus plantarum*) fermentation process decreased due to the activity of proteolytic acid bacteria that produce protaseae enzymes high enough to cause caffeine levels in coffee to decrease. The results of caffeine levels can be seen in non-fermentation of 0,5212 mg/g, and 8 hours of fermentation time of 0,4437 mg/g, 18 hours of 0,3340 mg/g, and 24 hours of 0,1183 mg/g. **Conclusion:** The longer the fermentation time, the lower the caffeine content.

Key words: BAL (*Lactobacillus plantarum*), robusta coffee, determination of levels, fermentation process, UV-Vis spectrophotometry.

INTRODUCTION

Indonesia is one of the world's major coffee producers and exporters. After palm oil, rubber and cocoa, coffee is Indonesia's fourth largest export in terms of foreign exchange earnings. Indonesian coffee has a unique aroma and flavor and as one of the world's top coffee producing and exporting countries, Indonesia is one of the world's top

coffee exporters. (Parnadi & Loisa, 2018). Indonesia ranks fourth in the world for coffee production, and because Indonesian coffee is highly competitive, it can compete with other major coffee exporters, such as Brazil, Colombia, and Vietnam.(Alexander & Nadapdap, 2019).

Jember Regency is one of the areas that develop robusta coffee, especially PT. Perkebunana Nusantara XII in Jember City has a coffee plantation area of 10,715 Ha. Robusta coffee production in Jember in 2019 produced 3,125 tons with a plantation area of 3,967 Ha. (Ditjenbun, 2021).

Coffee is one of the plantation products used as a refreshing drink with a very distinctive taste. Coffee is now the most popular drink in the world after water and tea. (Afriliana, 2018). Coffee contains caffeine compounds. Caffeine is a xanthine alkaloid compound where most people believe that the caffeine contained in coffee works against drowsiness and fatigue. (Zarwinda & Sartika, 2019).

Various plants or fruits, energy drinks, chocolate, coffee and tea all contain chemicals. The caffeine content contained in coffee varies depending on the type of coffee, arabica coffee has a caffeine content of 1g/100 grams, robusta coffee 2g/100 grams. (Maria Ulfa, 2018). According to Aryadi *et al.*, (2020) The highest caffeine content is found in robusta coffee, which is 2.15%.

Decreasing caffeine levels can be done by fermenting coffee beans (Wiraputra damar, 2020). Fermentation is one of the ways that can be done to reduce caffeine levels and high flavor. The fermentation process of coffee beans is a process in which the decomposition of complex compounds present in coffee beans, adding some microorganisms to help remove the mucus layer that still covers the coffee beans. Based on the results of the study, the fermentation treatment of coffee beans was able to reduce caffeine levels from 1.6% to 0.047%.(Poerwanty, 2021). The fermentation process can be done using Lactic Acid Bacteria (BAL).

Bacteria called BAL can reduce the amount of caffeine in coffee. The caffeine concentration of 1.11% in Robusta coffee can be reduced to 0.28%. BAL is responsible for this reduction in caffeine levels. The amount of caffeine in coffee beans decreases the longer they are cooked due to the increased activity of proteolytic bacteria that produce protease enzymes. (Wiraputra damar, 2020).

MATERIAL AND METHODS
Fermentation Process

Robusta coffee beans that have no skin were weighed 1 kg, separated 100 grams unfermented and 300 grams for fermentation, BAL (lactobacillus plantarum) as much as 10 ml was put into a fermentation container (glass jar) containing coffee beans and added distilled water as much as 500 ml, then checked the pH and closed the jar and covered it with plastic wrap then put into an incubator (37oC). The fermentation process is adjusted to the treatment length of 8, 16, and 24 hours, after the fermentation process is complete, the final pH is checked and then the coffee beans are dried. (Adrianto, 2020).

Extraction Manufacturing

The powdered beans were then extracted; 2 grams of coffee powder was put into a beaker glass and mixed with 100 ml of hot distilled water; the mixture was then filtered; the filtrate was then mixed with 2 Na_2CO_3 salts; then placed in a separating funnel; and finally, successively extracted with 25 ml chloroform three times, the filtrate was collected in a beaker glass. To obtain caffeine extract, the chloroform phase was evaporated using a water bath or waterbath.

Preparation of Caffeine Parent Standard Solution

A solution with a concentration of 1000 ppm was prepared by weighing 50 mg of caffeine, putting it into a 50 ml volumetric flask, dissolving it with hot distilled water, adding it with distilled water until the limit mark, and shaking it until homogeneous.

Wave Length Optimization

Determination of the maximum absorption wavelength is done by checking the 50 mg caffeine mother solution with a concentration of 1000 ppm. Determined the maximum absorption wavelength with a wavelength range of 200-400 nm. (Fatoni, 2015).

Preparation of Caffeine Standard Curve

Caffeine standard solution was pipetted 10 µL, 20 µL, 30 µL, 40 µL, 50 µL into a 1 ml cuvette to obtain concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm, then add distilled water until the limit mark was homogenized. After that, the absorbance was measured at the maximum wavelength achieved, the regression equation was calculated. and $Y = bx + a$.

Determination of Caffeine Content

The caffeine extract obtained from the previous extraction was dissolved with 50 ml of hot distilled water. Then dilution was carried out by pipetting 600 µL for non-fermentation, 800 µL for 8 and 18 hours fermentation, and 500 µL for 24 hours fermentation, the solution was put into a 1 ml cuvette with 3x replication. Then the levels were determined by UV-Vis Spectrophotometry at the maximum wavelength. (Tjahjani, 2021).

Data Analysis

The research data were processed and presented in tabular form after data collection of the caffeine content of fermented and non-fermented Robusta coffee. To determine the lowest and highest caffeine levels. All used IBM SPSS Statistic 26 analysis to determine the normality test of the data, then the normality test was carried out using Saphiro Wilk, followed by a homogeneity test, then the last one used the One Way Anova test.

RESULT AND DISCUSSION

Analisis data

Data on caffeine levels in robusta coffee beans with non-fermentation and fermentation treatments with a length of time of 8, 18, 24 hours show the results of the normality test with a sig value of 0.0594>0.05, 0.334>0.05, 0.297>0.05 and 0.142>0.05, it can be concluded that the data is normally distributed. Furthermore, the homogeneity test was carried out with a sig value of 0.055>0.05, so the data can be said to be homogeneous,

and continued with the One Way Anova test which showed a sig value of 0.000 <0.05 from the test results it can be concluded that the data on caffeine levels in non-fermentation and fermentation with a length of time of 8, 18, 24 hours is said to have a difference.

Fermentation Result

Based on the results of fermentation shows the results in table 1.

Table 1. Results of pH measurements

Fermentation time	Temperature	pH (before fermentation)	pH (after fermentation)	Caffein levels
Non fermentasi	37 ºC	6,1	6,1	0,5212 mg/g
8 jam	37 ºC	6,1	5,5	0,4337 mg/g
18 jam	37 ºC	6,1	5,3	0,3340 mg/g
24 jam	37ºC	6,1	4,8	0,1183 Mg/g

From the observation data above, it shows that the use of BAL (Lactobacillus plantarum) can reduce caffeine levels in coffee. The caffeine level in robusta coffee of 0.5212 can be reduced to 0.1183. The decrease in levels is due to the activity of proteolytic bacteria that produce protaseae enzymes high enough to cause caffeine levels in the beans to decrease and with the longer fermentation process (Ristanti, 2013).

The fermentation process influenced by microbes can not only reduce caffeine levels but can also reduce ph levels, the decrease in ph is influenced by the accumulation of organic acids and an increase in the number of H + protons (Adrianto, 2020).
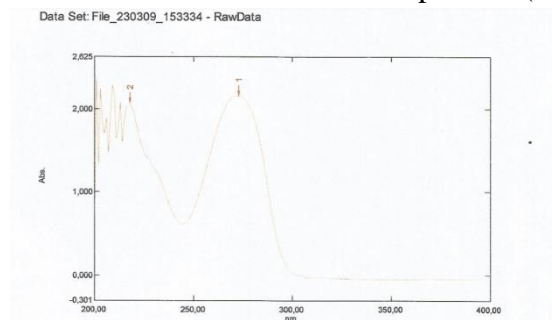

Figure 1. The wavelength

Based on the optimization results, the wavelength obtained is 273 nm with a range of 200-400 nm. According to the results of the wavelength obtained theoretically the maximum wavelength of caffeine in aqueous solution is 273nm (Gandjar dan Rohman, 2012).
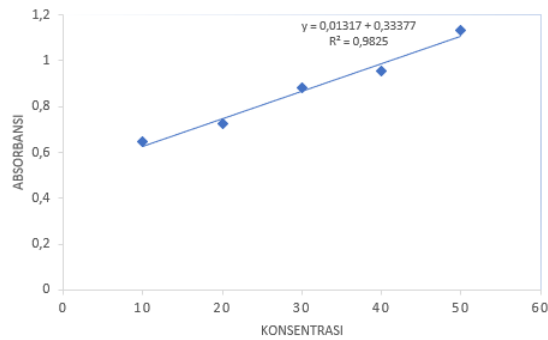
Figure 2. Linearity curve

Based on the results of the curve formation, it can be seen that the resulting absorbance value increases in line with the concentration, with a correlation coefficient of 0.9825 and the equation y = 0.01317x + 0.33377 derived from the curve. The correlation coefficient (r) value which is close to 1 indicates that the calibration curve is qualified.

The results of determining the caffeine content obtained from the calculation using the formula:

$$\text{Kadar Kafein (mg/g)} = \frac{\text{Konsentrasi}\left(\frac{\text{mg}}{\text{L}}\right) \text{X Volume total sampel (L) X FP}}{\text{Berat sampel (g)}}$$

Waktu fermentasi	Replikasi sampel	Y Absorbansi	X konsentrasi (ppm)	Kadar kaein (mg/g)	Rata-rata Kadar kafein	Sd	Rsd
Non fermentasi	1	0,878	41,2970	0,6195			
	2	0,751	31,6601	0,4679	0,5212	0,0696	0,1335
	3	0,748	31,4324	0,4746			
8 (jam)	1	0,603	20,4295	0,4106			
	2	0,623	21,9472	0,4246	0,4437	0,0314	0,0708
	3	0,654	24,2995	0,4860			
18 (jam)	1	0,541	15,7248	0,3616			
	2	0,530	14,8901	0,2978	0,3340*	0,0389	0,1166
	3	0,592	19,5948	0,3880			
24 (jam)	1	0,403	5,2532	0,0647			
	2	0,499	12,5378	0,1575	0,1183*	0,0392	0,3317
	3	0,475	10,7167	0,1326			

Figure 3. Calculation of caffeine rate determination

Based on the table above, the results of caffeine levels with non-fermentation and fermentation treatments with a length of time of 8, 18, 24 hours show that the results of 24-hour fermentation have the lowest caffeine levels of 0.1183 mg/g. and also from the table shows that caffeine levels in non-fermentation and fermentation groups with a length of time of 18, 24 have significant differences.

CONCLUSION

The conclusion of this study is that the longer the fermentation time of robusta coffee beans, the smaller the caffeine content in robusta coffee with non-fermentation

results of 0.5212%, fermentation with a length of time of 8 hours of 0.4337%, 18 hours of 0.3340%, 24 hours of 0.1183%.

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