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PAPER · OPEN ACCESS**Effect of decaffeination and re-fermentation on level of caffeine, chlorogenic acid and total acid in green bean robusta coffee**

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Effect of decaffeination and re-fermentation on level of caffeine, chlorogenic acid and total acid in green bean robusta coffee

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Abstract. Caffeine, chlorogenic acid, and total acid are important compounds affecting the taste of coffee. The purpose of this study is to determine the effect of decaffeination and re-fermentation on levels of caffeine, chlorogenic acid, and total acid in green beans of robusta coffee. This study consisted of several treatments, namely: green beans without treatment, green beans re-fermentation without decaffeination, decaffeinated green beans without fermentation, and decaffeinated green beans of coffee by re-fermentation with the addition of mucilage analogs. The decaffeination process used the Swiss Water Process (SWP) method, and re-fermentation used a mucilage analog of the blend of purple sweet potato and passion fruit pulp. The caffeine and chlorogenic acid content were determined using UV-VIS spectrophotometry, and total acid was determined using acid-base titration methods. The results were showed that the decaffeination and re-fermentation processes affected the levels of caffeine, chlorogenic acid, and total acid of green bean coffee. Caffeine concentration ranged from 1.04% (green bean coffee without fermentation treatment) to 2.6% (green beans coffee without treatments). The results of the chlorogenic acid analysis showed that the highest amount of green beans coffee without treatment (2.13%) and the lowest found in advanced fermented green beans coffee without decaffeination (1.07%). According to the result of total acid, the highest total acid was green beans coffee without treatment (0.22%), and the lowest was decaffeinated green beans coffee without fermentation (0.16%).

1. Introduction

Robusta coffee is one type of coffee that is widely cultivated in Indonesia [4], which is supported by a large area of environment and resistance to the environment and pests. In Indonesia, robusta coffee production, is higher than Arabica coffee [22]. The consumption of robusta coffee in Indonesia is around 82.49% [2]. However, the quality of robusta coffee is lower than arabica coffee due to its bitter taste, less acidity, and has a higher caffeine content [3-4].

The post-harvest coffee process to obtain green beans can be done in various ways, namely the natural process, the wet process, and the honey process. The natural process is one of the simplest methods in which the freshly harvested coffee cherries are dried directly under the sun to a moisture content of 11-12%, and the fermentation occurs during the drying process [5]. The wet process, a processing method by mechanically peeling the coffee cherries, immersing them in water in a fermentation tank, and fermentation lasts for 6-72 hours, then drying it [6]. The honey Process is a processing method where the coffee cherries are peeled without washing and then dried with the



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mucilage that still covers the coffee beans. During the drying process, the mucilage layer undergoes natural fermentation which produces compounds that play a role in giving the coffee bean a honey flavor [7].

Robusta coffee contains bioactive compounds, especially caffeine, trigonelline and chlorogenic acid [8]. Caffeine is an alkaloid group derived from xanthine which is produced from secondary metabolic processes. The caffeine content of robusta coffee is two times higher than arabica coffee [18]. It tastes bitter because caffeine contributes 10-30% of the bitter taste [4]. Caffeine compounds can have both positive and negative effects on the body if consumed in excess. The positive effects of caffeine consumption can increase psychomotor work, stimulate the nervous system and gastric acid secretion [9], while adverse negative effects can cause nervousness, anxiety, insomnia, nausea, hypertension, and seizures [10]. One way that can be done to reduce caffeine levels is by decaffeinating green bean robusta.

One of the decaffeination methods is through the Swiss Water Process (SWP), using water as a solvent. Water is safe for health, environmentally friendly, inexpensive, and can be used by small industries. However, water can dissolve caffeine at high temperatures, so heating is needed to optimize the decaffeination process. In addition, the heating of the Swiss water process can result in a decrease in flavor due to the degradation of flavor-forming compounds such as chlorogenic acid [11].

In addition, the quality of taste, especially the acidity component in coffee brewing, is influenced by the total acid. The primary organic acids found in green coffee beans are formic acid, oxalic acid, lactic acid, acetic acid and citric acid [12]. However, the high content of organic acids can be harmful to the health of coffee drinkers [13].

A development strategy that can be done to increase the flavor-forming compounds of green bean robusta is by re-fermentation using mucilage analogs that are sourced from passion fruit pulp and purple sweet potato. Passion fruit pulp contains sugars such as glucose, fructose and sucrose. In addition, the passion fruit pulp is rich in phenolic compounds [14-15] and purple sweet potato which is rich in phenolic compounds and endogenous sugars. The content of these compounds is almost the same as natural mucilages in coffee beans, which can provide a sweet taste and enhance the coffee's flavor.

The purpose of this study was to determine the effect of decaffeination and re-fermentation on levels of caffeine, chlorogenic acid and total acid in green bean robusta.

2. Materials and methods

2.1. Materials

The materials and tools used in this research are green bean robusta, passion fruit pulp, purple sweet potato, alpha-amylase enzyme, distilled water, sodium hydroxide, phenolphthalein indicator, caffeine standard, gallic acid, ethanol, activated carbon, plastic samples, tissue, aluminum foil, Whatmann No.1 filter paper, UV-visible spectrophotometer, analytical scale, oven, test tube, test tube rack, horn spoon, beaker glass, hotplate, measuring cup and stirring rod.

2.2. Methods

2.2.1. Preparation of a Caffeine-Free Saturated Solution. Green bean robusta 300 grams is boiled for 2 hours at 80°C which aims to increase the width of the green bean pores so that it is easy to extract caffeine. After that, the green beans are separated from the cooking water and dried to a moisture content of 25-30%. Boiled water is added with 10% activated carbon and stirred for 1 hour at room temperature to obtain caffeine-free saturated water.

2.2.2. *Decaffeination Process.* Green bean robusta that has reached a moisture content of 25-30% is put in caffeine-free saturated water, and the boiling process is carried out for 3 hours at a temperature of 750C. After that, the green beans are dried to a water content of <12%.

2.2.3. *Re-Fermentation.* First, analog mucilage was made by peeling and grating purple sweet potato first. Then measured the sweet potato moisture content using a moisture analyzer. Based on the analysis, it was found that the sweet potato content was 57%. So that the water content needs to be increased because based on the water content of coffee mucilage is 85-91% [16]. So it needs to be included in the following equation:

$$(100\% - KA1) \times BS1 = (100\% - KA2) \times BS2$$

KA1 = Moisture content of the sample at initial conditions
 KA2 = The desired moisture content of the sample
 BS1 = Initial condition sample weight
 BS2 = Sample weight after reaching KA2

KA1 = Water content of purple sweet potato 57%

KA2 = Natural mucilage moisture content 85%

$$\begin{aligned} (100\% - 57\%) \times 100 &= (100\% - 85\%) \times BS2 \\ 43\% \times 100 &= 15\% \times BS2 \\ 43 &= 15\% \times BS2 \\ BS2 &= 286.66 \end{aligned}$$

The amount of water added = BS2 – BS1
 = 286.66 – 100
 = 186.66

Based on the calculation of the equalization of water content above, to get a water content of 85% it is necessary to add a liquid of as much as 186.66 mL. This is the basis for the making of analog mucilages. So that if you weigh as much as 100 grams, 0.28 mL of alpha-amylase enzyme and 186 mL of distilled water are added to achieve levels of 85% of the total weight of the material, namely 286.66 grams. After the ingredients are blended according to their respective dosages, they are heated to a temperature of 80°C to activate the performance of the alpha-amylase enzyme. After that it is cooled and then added 100 g passionfruit pulp which has been mashed, stirred until blended. Then the mucilage analog is mixed with green beans that have been decaffeinated and fermented for 24 hours and then dried to a water content of <12%.

2.2.4. *Determination of the caffeine.* One gram of coffee beans in 100 ml of 70% ethanol extracted for 24 hours in dark conditions. The extracted sample was then filtered with filter paper and then the caffeine content was determined using a UV-vis spectrophotometer at a wavelength of 273 nm.

2.2.5. *Determination of the Chlorogenic Acid.* One gram of coffee beans are added to 100 ml of 70% ethanol and then shaken for 24 hours in dark conditions. The extracted sample was then filtered with filter paper and then the levels of chlorogenic acid were determined using a UV-vis spectrophotometer at a wavelength of 329 nm.

2.2.6. *Determination of the Total Acid.* A powder sample of 5 grams was dissolved in 100 ml of distilled water and filtered with filter paper. The resulting filtrate was put in a 100 ml measuring flask and diluted with distilled water to mark the mark. The sample was then piped 5 ml and dropped 2-3

17 drops of 1% phenolphthalein indicator (pp indicator) then titrated with 0.1 NaOH to form pink. The titration is calculated using the following formula:

$$\text{Acid levels} = \frac{\text{ml NaOH} \times \text{N NaOH} \times \text{BM} \times \text{FP}}{\text{sample weight (gram)} \times 1000} \times 100\%$$

12 2.2.7. Data Analysis. Data statistical analysis was performed using Analysis of Variance (ANOVA). The significant differences (p<0.05) were evaluated by analysis, and the mean separation was conducted using the Duncan post hoc test.

3. Results and discussion

3.1. Caffeine

Caffeine is a class of alkaloids derived from xanthines found in almost all parts of coffee [17]. Therefore, each coffee has a different amount of caffeine, such as 2.47% robusta coffee and 1.99% arabica [18]. The results of the caffeine test on green coffee beans from the four treatments can be seen in the image below.

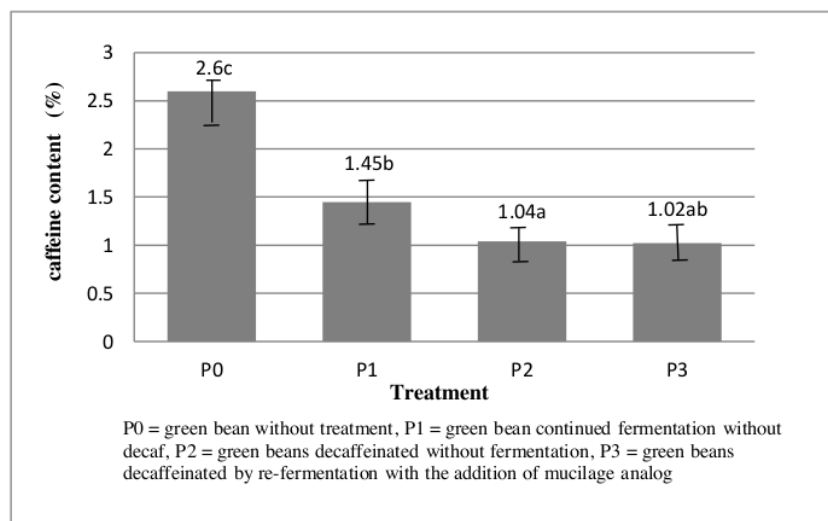


Figure 1. Effect of decaffeination and re-fermentation on caffeine levels in green beans

The results of testing the caffeine content of green coffee beans with four treatments ranged from 1.04 to 2.60%. Green beans without treatment were 2.60%, re-fermented green beans without decaffeination were 1.45%, decaffeinated green beans without fermentation were 1.04% and decaffeinated green beans by re-fermentation were added mucilage analog was 1.02%. ANOVA test results showed that decaffeination and re-fermentation significantly affected the caffeine of green coffee beans produced (p<0.05%). The lowest caffeine content found in green beans is decaffeinated by re-fermentation. This is due to the treatment of decaffeination and re-fermentation so that the caffeine level decreases. The decrease in caffeine is caused by boiling in the decaffeination process which can widen the pores of the coffee network so that the solvent diffuses quickly into the tissue and dissolves the caffeine. Kuncoro *et al.*, 2018 stated that heat treatment could break the bonds of caffeine complex compounds so that caffeine compounds are free with smaller sizes and are easy to

move. In addition, it is caused by the capture of caffeine by activated carbon [19]. Mazzafera, 2012 states that caffeine will be captured by activated carbon to obtain saturated water without caffeine [30]. This is in accordance with the ability of activated carbon to absorb caffeine compounds in coffee. The higher the adsorbent concentration used, the more caffeine is absorbed [11]. Green coffee beans fermented with alpha-amylase enzymes (P1 and P3) have decreased caffeine levels. During the fermentation process the enzymes released by microorganisms during the fermentation process break down caffeine compounds into simpler compounds, caffeine is converted into dimethyl xanthine (theobromine, paraxanthine, theophylline) and during the fermentation process, uric acid, 7-methyl xanthine, and uric acid are produced during the fermentation process xanthin [21].

3.2. Chlorogenic acid

Chlorogenic acid is an important component in coffee because it has properties as an antioxidant classified as a phenolic group [17]. The most common chlorogenic acids are caffeic acid, ferulic acid, and p-cumaric acid, which form esters with quinic acid [21]. The amount of chlorogenic acid in coffee varies, such as arabica coffee around 4.0-8.0% while robusta coffee is around 6.0-11.5%.

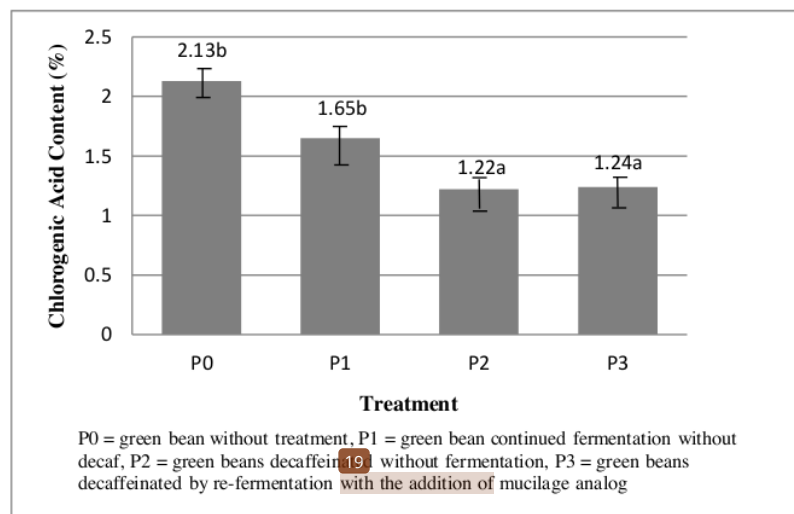


Figure 2. Effect of decaffeination and re-fermentation on chlorogenic acid levels in green beans.

Figure 2 shows that the amount of chlorogenic acid from the four treatments is around 1.22 - 2.13%. Green beans without treatment were 2.13%, re-fermented green beans without decaffeination were 1.65%, decaffeinated green beans without fermentation were 1.22% and green beans were decaffeinated by re-fermentation with the addition of mucilage analog 1.24%. ANOVA test results showed that decaffeination and re-fermentation had a significantly affected the chlorogenic acid of green coffee beans produced ($p < 0.05\%$). This shows that the caffeine content decreased after re-fermentation while the chlorogenic acid increased slightly. However, the increase is not significant. The increase in chlorogenic acid is thought to be due to microorganisms that grow during the fermentation process [21] while in the decaffeination process, there is the degradation of chlorogenic acid during the boiling process [22].

3.3. Total Acid

Total acid is the number of organic acids found in green coffee beans. The primary organic acids found in green coffee beans are formic acid, oxalic acid, lactic acid, acetic acid and citric acid [12]. Robusta coffee contains 0.5-3.5% organic acid.

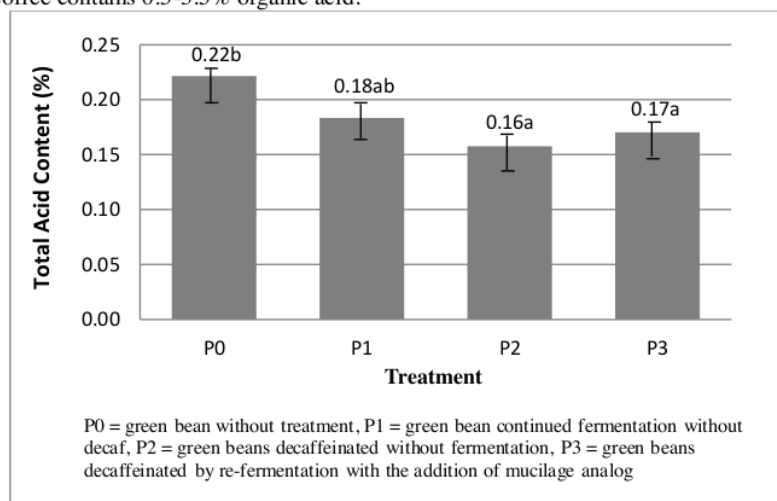


Figure 3. Effect of decaffeination and re-fermentation on total acid levels in green beans.

The results of testing the caffeine content of green coffee beans with four treatments ranged from 0.16 to 0.22%. Green beans without treatment were 0.22%, re-fermented green beans without decaffeination were 0.18%, decaffeinated green beans without fermentation were 0.16% and decaffeinated green beans by re-fermentation were added the addition of 0.17% mucilage analog. ANOVA test results showed that decaffeination and re-fermentation had no significant effect on the total acid yield of green bean coffee ($p > 0.05\%$). However, re-fermentation occurred, which was not too significant. The longer the fermentation process, the more acidic the coffee will be decreased. The formation of aliphatic acids causes the high acidity of coffee during the fermentation process. This is in accordance with Sulistyowati and Sumartono, 2002 who state that the acids formed will escape into the environment, causing changes in acidity levels [23]. The total acid value correlates with the pH value. The higher the total acid, the lower pH [24].

4. Conclusions

This research concludes that the decaffeination and fermentation processes affect the content of green beans including caffeine, chlorogenic acid and total acid. The amount of caffeine in the decaffeination and fermentation process decreased, chlorogenic acid decreased after the decaffeination process and increased after re-fermentation was almost the same as the total acid increased after fermentation.

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