Biochemical content of Robusta coffees under fully-wash, honey, and natural processing methods

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Abstract. Robusta coffee processors in Indonesia apply three methods of processing cherry coffee into green coffee beans, which can affect the quality of the coffee produced. Therefore, this study aims to determine the effect of those various processing methods on the coffee beans by finding out differences in Robusta coffee samples' biochemical content, both as a pulped and green bean. Red-picked Robusta coffee samples were collected from the Kepahiang Bengkulu, one of Indonesia's highest Robusta coffee producer areas. The primary biochemical contents of coffee, such as moisture, caffeine, chlorogenic acid, sucrose, and lipids, were tested using standard methods on cherry, pulped, and green bean samples. We observed that both pulped and green bean samples showed significant differences (p<0.05) in their biochemical content. Therefore, selecting the right processing method is necessary to get green beans with biochemical content that will produce coffee with preferable sensory attributes.

1. Introduction

Kepahiang Regency is the largest producer of Robusta coffee [1]. The area of the Robusta Kepahiang coffee plantation is 24,606 hectares wider than other districts so that it is designated as a coffee development area in Bengkulu Province and has begun to develop a pattern for processing premium grade coffee beans, with the full wash, honey, and natural process. The stages of the coffee processing process may determine the quality of the coffee beans produced. The quality of coffee is primarily determined by handling during harvest and postharvest. The fruit was picked in appropriate maturity, fully ripen, indicated by full red color. On the other hand, it will result in less aroma and taste due to the coffee cherries' incomplete maturity. The mixing between old and young coffee is often done by traders, which will cause a decrease in the quality of the coffee produced [2].

The processes that occur in coffee processing affect the physical properties of the coffee beans and affect their chemical properties. Physical characteristics include performance (smell, color, and taste), bean size, grain weight, and bean hardness. Chemical properties include proximate (moisture, ash, lipid, protein, and carbohydrate content [3]. Coffee characteristics are properties that can be directly observed, measured and are essential quality elements. Biochemical components that affect coffee quality include caffeine, chlorogenic acid (CGA), sucrose, and lipid [4]. The caffeine content of coffee beans varies depending on the type of coffee and the geographical conditions from which the coffee is grown [5].
The largest acid component in coffee is chlorogenic acid, which has antibacterial, antiviral, and anticancer activity. However, too much CGA can reduce the taste quality of coffee [6]. The main carbohydrate compound in coffee beans is sucrose. Meanwhile, fat is the largest constituent of coffee beans. The method of obtaining coffee beans also varies. Each processing method has the advantage of getting good quality commercial coffee [7]. Dry processing method, the coffee cherries that have been harvested are dried in the sun. After drying, the coffee cherries are removed mechanically using a coffee bean pulping machine. The wet processing stages that differentiate it from dry processing, are the stages of peeling the skin of coffee (pulping), fermentation, and washing to remove mucus (washing) [8].

Author [9] reported that altitude affects the physical and biochemical quality characteristics of Arabica Gayo as a geographic indication product, then rainfall is also positively correlated with physical and biochemical qualities in explaining the flavor profile that is formed. Besides, [10] conducted research on the effect of elevation and processing on the chemical content and taste of Lampung Robusta coffee, the higher the elevation and the place where Robusta coffee grows, the caffeine and fat levels tend to increase, the processing also affects the taste quality results. Lampung Robusta coffee [11] changes in caffeine content of Arabica coffee beans resulting from semi-wet processing of various types of containers and fermentation time to obtain variations in the type of container that have no effect on decreasing caffeine content, while time variations affect decreasing caffeine levels. Treatment of different varieties and pre-process (natural-honey) affects the quality characteristics of robusta coffee beans [12].

Based on the explanation above, the purpose of this study is to determine the effect of various processing methods of premium red-picked robusta coffee from the area of Bandung Jaya Kabutapeten Kepahiang, Bengkulu Province, namely full wash, honey, and natural processes on the biochemical content and sensory evaluation of coffee beans. The differences in biochemical characteristics range from cherry coffee, the washing process, and fermentation to the dry coffee beans after processed as green beans. The tested biochemical content was moisture content, caffeine, sucrose, lipid, and chlorogenic acid (CGA).

2. Methodology
2.1. Sample preparation
The material used was 450 kg cherry Robusta coffee from Bandung Jaya, Kepahiang Regency, Bengkulu Province, which was purchased directly from farmer groups.

2.2. Processing steps of cherry to green bean coffee samples
Processing red-picked premium cherry coffee into green bean was conducted with three processing methods, as developed by [13] namely: full wash, honey, and natural processes, where each method has its own stages as illustrated in Figure 1. Full wash process of red coffee fruit when received by the coffee processing house, must be immersed. Only those that sink was processed later to be peeled by a pulpi machine. After that, it was soaked for twenty-four hours. Then the samples were dried in the sun. In the fermentation or honey methods, after separating in a soaking tub, the coffee beans were peeled using a pulpi machine and put in a sack to stand for twenty-four hours. After that, the samples were dried in the drying house.

Meanwhile, in the natural method, red cherry coffee sample did not need to be peeled, it can directly have dried in the sun. At the stage after washing in the full wash process and fermentation in the honey process, coffee beans that still have epidermis and have not undergone a drying process were carried out the analysis of biochemical content, observation or testing parameters were the same as for cherry coffee, namely: moisture, caffeine, sucrose, lipid, and chlorogenic acid contents.
2.3. Biochemical content analysis

Testing of biochemical content in cherry coffee consisting of caffeine was analyzed using the Balley-Andrew method, sucrose with the Nelson Somogyi method. Lipid test with the Soxhlet method (association of Official Analytical Chemistry, 2005) and chlorogenic acid using the Naegele method (2012). HPLC conditions: column, reverse phase - ODS, 250X4.6 MM, rate of 1 ml / min, detector, photodiode array set at 278 nm, pressure 150 KHF / cm 2, water motion, acetic acid, methanol (799.1 and 200 ml) and a sample volume of 20 ml. Peak area calibration curve with standard concentrations in the plot. The sample is calculated using the best line regression equation. Chlorogenic acid levels of samples were obtained from comparison standard chromatography with sample chromatography obtained. Moisture content was also observed using the oven method, which previously determined the moisture content of the base material.

The sample was calculated using the best line regression equation. Chlorogenic acid levels of samples were obtained from comparison standard chromatography with sample chromatography obtained. Water content was also observed using the oven method, which previously determined the moisture content of the base material. The parameters for testing the biochemical content of green bean were the same as for cherry coffee, namely moisture, caffeine, sucrose, lipid, and chlorogenic acid using the same method.

2.4. Sensory analysis of green bean sample

Sensory evaluation was conducted on green bean samples using hedonic test (preference level). A number of 64 panelists were asked to fill in the sensory attribute scores listed on the questionnaire. Color, aroma, bean dryness, and overall appearance were chosen as sensory attributes. The hedonic test (favorite level) on the test consists of 1-5 Likert scale on each of the attributes [14][15]. The sample was presented in a plastic standing pouch with a uniform size and was coded as follows: A for the sample of green bean under fullwash process, B for the sample of green bean produced under honey process, and C for the sample of green bean produced under natural process.

2.5. Statistical analysis

For biochemical content data, one-way ANOVA was carried out to compare means between sample treatments and Tukey's HSD post-hoc test was used to separate the means. Differences were statistically significant at p < 0.05. Meanwhile, data from sensory test results were analyzed using the ANOVA.
3. Result and Discussion

3.1. Biochemical content of sample

Biochemical content testing starts with cherry coffee, pulped, and green beans. In cherry coffee, the average content of moisture content (172.89%), caffeine (3.57%), chlorogenic acid (0.71%), sucrose (1.29%) and lipids (29.30%) on a dry basis (db). Biochemical content of pulped samples was presented in Table 1, while the content of green bean samples, was presented in Table 2.

Table 1. Biochemical content of pulped bean Robusta coffee from three processing methods

| Biochemical content | Full wash | Honey |
|---------------------|-----------|-------|
| Moisture content    | 170.96 ± 0.05 b | 168.49 ± 0.56 b |
| Caffeine            | 3.58 ±0.00 a    | 3.37 ±0.01 b    |
| Chlorogenic acid    | 0.13 ± 0.00 c   | 0.37 ±0.01 c    |
| Sucrose             | 6.84 ± 0.02 a   | 5.62 ±0.01 a    |
| Lipid               | 28.48 ± 0.01 b  | 32.27 ± 0.07 a  |

Mean values followed by the same letter per variable were not significantly different from each other at (p> 0.05). Results are shown as average value of green coffee on a dry basis (db) percent (%).

Table 1. Biochemical content of green bean Robusta coffee from three processing methods

| Biochemical content | Full wash | Honey | Natural |
|---------------------|-----------|-------|---------|
| Moisture content    | 12.02±0.03 a | 9.46±0.07 b | 11.95±0.06 a |
| Caffeine            | 1.82 ±0.02 a | 1.54±0.00 b | 1.81±0.01 a |
| Chlorogenic acid    | 5.84±0.00 c | 10.91±0.01 b | 9.57±0.01 b |
| Sucrose             | 0.19±0.02 b  | 0.23±0.01 ab | 0.24±0.00 a |
| Lipid               | 6.11±0.00 a  | 2.79±0.00 c | 3.21±0.00 b |

Mean values followed by the same letter per variable were not significantly different from each other at (p> 0.05). Results are shown as average value of green coffee on a dry basis (db) percent (%).

3.1.1. Moisture content.

The moisture content of green beans is one of the physical properties that will affect the quality of coffee, because it will be related to the storage capacity to prevent discoloration, fungi and other microorganisms [8]. The water content of coffee beans depends on the type of coffee, the origin of the region and in what form it is stored (cherry or green bean coffees) [9]. Based on the Table 2, moisture content of the samples ranged from 9.46 to 12.02%. The results had met the SNI and SCAA standards (less than 12.5%).

The results of statistical tests showed that the moisture content of coffee beans in the form of cherries, pulpers and green beans had a significant difference (Table 1 and Table 2). Under fullwash method, moisture content of cherry, pulped, and green bean coffee samples were 172.89%, 170.96%, and 12.04%, respectively. Under honey processing method, moisture content of cherry, pulped, and green bean coffee samples were 172.89%, 168.49%, and 9.46%, respectively, while under natural processing method, they were 172.89%, 11.95%, for cherry and green bean samples, respectively. One of the causes of lower water content is postharvest handling and incomplete drying of coffee beans. Drying aims to reduce the water content in coffee beans from 60-65% to 20%. Author [8] stated that the water content of robusta coffee should be cultivated to a maximum of 11% for the stability of the storage process.
3.1.2. Caffeine.
Caffeine is a common biochemical component of coffee. In coffee, where it is grown and the variety affects the caffeine in the coffee beans. Authors [16][9] stated that differences in caffeine content are triggered by complex reactions that occur during the processing process, and the loss of water in the material affects the percentage of caffeine content. The caffeine content in coffee beans in the study was 1.54 -1.81% (db). Authors [17][18] stated that the caffeine content of green robusta coffee ranged from 1.5 to 2.5%

From Table 1 and Table 2, caffeine green bean robusta coffee based on processing methods and forms were significantly different (p <0.05). The caffeine content in this study was related to processing methods and forms. Caffeine for honey-treated coffee was different from full wash and natural, while for the form of green bean caffeine, it was significantly different from cherries and pulpers. Authors [19][20] found that there is no significant variation in caffeine levels between wet and semi-dry processing methods. Studied the effects of wet and dry coffee processing on chemical composition and found no significant variation in caffeine content. The processing method that uses excess water and the fermentation process is not different from the method with less water and without fermentation seen from the levels of biochemical compounds [21].

Wet processing contains less protein and caffeine than dry processing [10]. In wet processing, there is a fermentation process to remove the mucous layer on the surface of the coffee bean horn skin. However, in the results of this study, both wet and dry processing have high caffeine content, which is suspected occurred caused by unsuitable washing and soaking time.

3.1.3. Chlorogenic acid (CGA)
Robusta green coffee beans contain the highest content of chlorogenic acid [17]. The chlorogenic acid content in Robusta coffee beans reaches 6.1-11.3 g per gram of coffee beans. Author [19] reported that higher chlorogenic acid was observed on the samples produced under the washed method compared to the semi-washed and dry methods. Author [21], also reported a significant effect of postharvest processing methods on total chlorogenic acid and its subclasses. Not only based on type, heating and roasting of green coffee beans are also different factors in the content of chlorogenic acid.

Chlorogenic acid levels in the results of this study were in the range 5.84-10.9% (db). Chlorogenic acid was reported as a water-soluble component that can be removed from coffee extract (Nigam and Singh, 2014). Statistical analysis showed that there was a difference (sig <0.05) between the chlorogenic acid content with the processing method and the sample form. The interaction of full wash processing methods was different from honey and natural processes. For full wash, honey and natural processing form samples, the content of chlorogenic acid increased. In general, [19] stated that coffee beans processed using the washed method provide higher CGA content than those processed using the semi washed and dry methods for all varieties.

3.1.4. Sucrose
The sucrose in each processing process (Figure 1) increases and decreases. The sucrose content in the test results of red-picked robusta cherry coffee was 0.48%, after fermentation in a bucket soaked with water and fermented in sacks, respectively 2.53%, and 2.10%. The sucrose content of green bean coffee under various processing methods (full wash, honey, and natural) were 0.17%, 0.21%, and 0.21%, respectively. The sucrose content in honey and natural processed green beans was higher than that of a full wash. Similar findings [19] for hybrid coffee types and [21] also reported that higher sucrose content in dry-processed coffee beans was reported compared to washed processed coffee. The reduction in sucrose content observed in washed processed coffee beans may be due to the high water solubility of this compound during washing and soaking. Sucrose can be depleted as a result of anaerobic fermentation during the washed processing method.

Table 2, sucrose in green beans was susceptible to 0.185-0.240% (db). The lowest sucrose was the result of full wash processing, and the highest was natural processing. This is in line with the previous
statement, [19] for hybrid types of coffee and [21] also reported that a higher sucrose content in dry processed coffee beans was reported compared to ground coffee.

3.1.5 Lipid Author
Showed a decrease in lipid content after mucus fermentation. It has been reported that most of the lipids are present in the endosperm oil fraction of the coffee beans and, to a lesser extent, coffee wax, located in the outer layer of the coffee beans [22]. Hence those in the outer layer are affected by processing or metabolic activity [20] The washing and soaking process for 24 hours using full wash water, the fermentation process of the coffee beans in the pulpier using a honey bag for 24 hours, and drying directly after mining, causes a decrease in the lipid content of these results. The chemical components in coffee beans can then be reduced or lost due to processing. Variations in levels of chemical composition due to the effect of metabolic activity on coffee beans have also been reported by other authors such as [23]

3.2. Sensory evaluation of green bean sample
Based on the results of data analysis, it was known that the variant of premium red-picked robusta coffee processing technology which was processed in the full wash, honey, and natural processes has an effect on the sensory attributes of color, aroma, shape, and size of the coffee beans, but does not affect the attributes of dryness of the beans and their overall appearance. Sensory evaluation was carried out for panelists who did not know the characteristics of the biochemical content of each coffee processing. Sensory evaluation of green bean coffee aims to link marketing with a visual view of green bean quality.

Table 3. Sensory test results from each variant of processing technology

| Process       | Color     | Aroma     | Dryness   | Overall   |
|---------------|-----------|-----------|-----------|-----------|
| Full wash     | 3.5000\(^b\) | 3.3750\(^b\) | 3.7188\(^a\) | 3.6406\(^a\) |
| Honey         | 3.5313\(^b\) | 3.6094\(^ab\) | 3.8125\(^a\) | 3.7031\(^a\) |
| Natural       | 3.8750\(^a\) | 3.8438\(^a\) | 3.8906\(^a\) | 3.8281\(^a\) |

Mean values followed by the same letter per variable were not significantly different from each other at \((p> 0.05)\).

3.2.1. Color
Color is a factor that determines the quality and also an indicator of freshness and maturity. The color of coffee beans can vary from bluish-gray, brownish-yellow to black (Hayati et al., 2012). Table 3 shows that the color attribute most preferred by the panelists was the result of natural processing with a score of 3.87 then continued with honey and full wash at a score of 3.53 and 3.50. The color of the coffee which was naturally processed was the green color of the coffee beans and the combination with the brown from the bran of the green bean coffee so that the brightness level was more than the full wash and honey processing. Author [24] stated that the combination treatment of container types and fermentation time affects the color of the coffee beans.

The longer fermentation causes the dissolution of pigments in the coffee beans, and the decrease in brightness that occurs was thought to have an excess of between acids produced in coffee. The full wash process undergoes a process of immersion for 24 hours and washing to remove mucus after the coffee was stripped of red skin with a pulper machine so it was suspected that this process causes a decrease in the color pigment in coffee so that it affects brightness, for coffee that is honey-processed, the coffee process after pulping. It was fermented for 24 hours using a sack were during the pulper process to make it easier to peel the cherry skins it was assisted with water then put into the sack so that there was still a lot of water in the coffee beans during curing which causes the pigment to dissolve so that it reduces the brightness of the coffee color. Meanwhile, in the natural process of
cherry coffee, after mining for sorting, it was immediately dried, the color pigments found in cherry coffee penetrate the coffee beans during the drying process.

3.2.2. Aroma
Sucrose accounts for up to 11% of the dry matter of green Arabica beans and acts as a precursor to aroma [21]. If it was correlated with the sucrose content with the results of sensory green bean aroma testing, it can be seen that dry processing, namely the natural process, gives the highest aroma score, indicating that the natural process smells strongly as well, so that it appears more favored by panelists.

3.2.3. Other sensory attributes
Dryness of beans is a synchronization of organoleptic measurements by measuring the moisture content of beans using a tester. Respectively, these scores indicated that the dryness level of the honey process was preferred, when compared to the results of measurements of moisture content from the laboratory carried out with the respective moisture content. Each coffee bean under full wash, honey, and natural processing methods. The results of the measurement of process honey moisture content also have a better quality.

The results of the overall appearance of coffee beans that were processed in a natural process have the most preferred score by the panelists. In the assessment, the appearance is entirely a combination of color, of sensory parameters previously observed. This result was also shown by the panelists who score the results of the natural processing also has the highest score. From some comments and reasons expressed by the panelists, the overall appearance of samples under natural processing methods was more preferred.

4. Conclusion
There was an effect of the full wash, honey, and natural processing method on the moisture, caffeine, chlorogenic acid, sucrose and lipid content in green bean coffees (p <0.05). The difference in sensory evaluation on premium red-picked robusta coffee based on fullwash, honey and natural process technology variants affected the sensory attributes of color and aroma, but did not affect the attributes of dryness of the beans and overall appearance.

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