Non-volatile compounds of unwashed Gayo Arabica coffee (Coffea arabica) with anaerobic fermentation process

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Abstract. Coffee is a hot drink made from the roasted and ground seeds (coffee beans) of a tropical shrub with a preferred flavour, which is consumed by many people. Furthermore, its flavour is influenced by the chemical composition of green beans that goes through series of processing such as fermentation. The chemical composition of green beans with fermentation process in the process without washing is not well-known in Gayo coffee. This study aims to analyse the effect of anaerobic fermentation on chemical composition of green beans and to enhance its quality. The coffee fruit was processed and fermented for 0 to 48 hours. Citric and malic acids varied and showed positive quality in the process of Pulp Natural (PN) and Black Honey (BH), while propionic acid was only discovered in the process of Drying (D), which indicated a negative quality. Sucrose tends to increase in PN and BH. Glucose and fructose were lower than sucrose, and chlorogenic acid increased, while trigonelline tends to decrease from all processes. Caffeine had very low levels after the fermentation process. In conclusion, this research indicated that the anaerobic fermentation process in PN and BH produced generally desirable flavour precursors.

Keywords: Coffea arabica, Fermentation, Non-Volatile Compounds, HPLC, Coffee Processing, Coffee Flavor

1. Introduction

Coffee is a kind of beverage widely consumed throughout the world for several centuries. Factors that influence flavor are determined by the content of precursors discovered in green coffee beans [1]. Non-volatile compounds such as sugars, proteins, free amino acids, chlorogenic acid, and trigonelline are compounds contributing to aroma and taste [2].

Gayo Arabica coffee has many varieties and complex flavors. The most common postharvest process is semi-washed. [3–5]. This process leads to a fermentation process which when compared to other processes, it can achieve better acidity and quality [6]. Unwashed processes such as dry and semi-dry do not carry out fermentation and washing during processing. Furthermore, It has been reported that the processes can produce undesirable traits such as bitterness, the presence of propionic acid and isobutyric acid, medicinal flavor, earthiness and powdery mildew [7,8,9]. These negative characteristics can could
be as a result of improper processing. The anaerobic fermentation process is recommended to improve the quality of green coffee beans in the non-washed process.

This study aims to analyze the composition of non-volatile green coffee bean compounds from the unwashed postharvest process through the fermentation process in Gayo Arabica coffee.

2. Material and Method

2.1. Materials
Arabica coffee cherries (Ateng super variety), located at an altitude of 1800 meters above sea level in Bener Meriah Regency, Aceh, Sumatra, Indonesia, were manually harvested (handpicked). The standard organic acids (citric, malic, succinic, lactic, acetic, propionic) and perchloric acid were purchased from Sigma Aldrich. Ultrapure water (Onelab), Methanol (Merck), distilled water, and syringe filter 0.45 µm were also used. All chemicals and reagents used were pro-analysis and analytical grades.

2.2. Sample Preparation
The coffee cherries were manually sorted to separate the stones, branches, leaves, and impurities, then the sorting process was then completed by flotation using a water tub. The floating coffee cherries were separated and only the non-floating cherries were used. The fermentation treatment was applied to samples of Dry (D), Pulped Natural (PN), and Black Honey (BH) with a length of 0, 24, and 48 hours fermentation time. Furthermore, 13 kg of coffee cherries were used in each treatment and the process was repeated three times. The anaerobic fermentation was carried out using polyethylene plastic (75 cm x 50 cm, thickness 200 microns). Each treatment in plastic was immersed in a 60-liter bucket containing water.

2.3. Drying Process
The fermented coffee cherries were spread over drying racks with size 1 x 0.6 m² made from wood and coated iron. These samples were spread with 2 cm thickness and stirred 4-5 times during the day, and was dried for 48 hours under the sunlight. The second stage of drying was performed using a drying house with a dimension of 8 x 4 x 6, 5 m³. The drying process in the drying house was carried out until the water content reached below 17%. The dried cherries were then peeled using a dry huller to obtain green coffee beans.

2.4. Organic Acid Analysis
The organic acids were analyzed according to the method of Ribeiro [10] using the HPLC system (Shimadzu LC-20, autosampler). A total of 10 mL of aqua pro was added to 1.5 grams of coffee samples in a 15 mL Falcon tube. The sample was shaken and left for 10 minutes. The pH of the supernatant was adjusted to 2 using 2 mL perchloric acid 0.2 M added into a falcon tube. The sample was then centrifuged at 10,000 g at 4 °C for 10 minutes. The supernatant was filtered using a 0.45 µm cellulose filter. The filtrate was stored at 4 °C before being injected into the HPLC system. The column used was AMINEX HPX-87H (300×7.8 mm) with a mobile phase of 0.1 M perchloric acid. The flow rate used was 0.6 mL/min with an oven temperature of 30 °C. The detector used a PDA (Photometric Diode Array) with a wavelength of 215 nm.

2.5. Sugar Analysis
The analysis of sugars includes the study of the content of fructose, glucose, and sucrose content following the method of Sesta [11]. Sample preparation begins with grinding and weighing of about 10 g of green coffee beans and then transferred to a 125 mL Erlenmeyer and dissolved with 50 mL of distilled water. The sample solution was then sonicated for 10 minutes and filtered using filter paper. The filtrate was then concentrated using a rotary evaporator and dissolved again using volumetric distilled water into a 10 mL measuring flask. Similarly, the filtrate was filtered using a syringe filter and injected as much as 5 µL at HPLC. The HPLC used was the SHIMADZU 10 series using the Restriction
The Range Test (DMRT) was used for statistical significance of differences (p<0.05). Duncan’s Multiple Range Test (DMRT) was used for post-hoc analysis.

3. Result and Discussion

3.1. Organic acids

The content of organic acids in D, PN, and BH is shown in Table 1. Citric and malic acid can provide good acidity characteristics in brewed coffee [9,12]. Sivetz [13] stated that the content of citric acid and malic acid of more than 1 mg/mL can provide good acidity to the coffee drink.

Table 1. The organic acid content of D, PN, and BH with different fermentation times

| Processing       | Fermentation Time (hours) | Organic Acids (mg/g d.b) |       |       |       |       |
|------------------|---------------------------|--------------------------|-------|-------|-------|-------|
|                  |                           | Citric                   | Malic | Succinic | Lactic | Acetic | Propionic |
| Dry (D)          | 0                         | 2.23 ± 0.48              | 5.32 ± 2.12 | 0.05 ± 0.00 | nd | nd | 0.56 ± 0.75 |
|                  | 24                        | 2.54 ± 0.52              | 3.08 ± 0.39 | 0.28 ± 0.32 | nd | nd | nd |
|                  | 48                        | 2.31 ± 0.67              | 3.51 ± 0.13 | 0.41 ± 0.50 | nd | nd | nd |
| Pulp Natural (PN)| 0                         | 2.56 ± 0.80              | 4.64 ± 0.88 | 0.51 ± 0.53 | nd | nd | nd |
|                  | 24                        | 2.08 ± 0.58              | 3.13 ± 1.16 | 0.33 ± 0.12 | nd | nd | nd |
|                  | 48                        | 2.93 ± 0.26              | 3.31 ± 0.00 | 0.34 ± 0.14 | nd | nd | nd |
| Black Honey (BH) | 0                         | 3.52 ± 1.17<sup>a</sup>  | 1.49 ± 0.21 | 0.41 ± 0.48 | nd | nd | nd |
|                  | 24                        | 2.46 ± 1.18<sup>b</sup>  | 2.06 ± 0.66 | 0.43 ± 0.10 | nd | nd | nd |
|                  | 48                        | 2.85 ± 1.18<sup>b</sup>  | 2.48 ± 0.55 | 0.37 ± 0.15 | nd | nd | nd |

Data are presented as mean. Means followed by the different letter for black honey are significantly different at p=0.05 according to Duncan Test. nd = not detected.

The increase in the levels of citric and malic acid in green coffee beans was caused by the diffusion process that takes place during the fermentation and drying process. Acid diffusion processes such as citric and malic acid were discovered in previous studies. The increased amounts of citric and malic acid in the dry process without fermentation have been reported by Silva [14].
The citric acid in the BH process decreased, and this may be due to the release of mucilage containing organic acids from the surface of fermented coffee beans in such a way that the diffused acid was lower. Furthermore, this is unlike the case of D and PN processes. The fermentation in the D and PN processes takes place when the condition of the coffee fruit was intact, diffusion of citric acid occurs from the mucilage and pulp of each coffee cherry into each coffee bean that was intact or not depulped yet.

Propionic acid was only found in the D-0 treatment of 0.56 ± 0.75 mg/g. Silva [14] stated that propionic acid was a type of acid produced by microorganisms metabolites that caused off-flavor and could be diffused into coffee beans.

### 3.2. Sugars

The results of the analysis of green coffee bean sugar in the dry and semi-dry processes are shown in Figure 1.

The fermentation treatment can increase the sucrose content in the PN-24 process (24 hours of fermentation) to reach 23.08% w/w and the BH-48 process (48 hours of fermentation) to about 21.35% w/w. Fructose decreased in all processes (D, PN, BH), in contrast, glucose tends to increase in D and PN but decreased in BH.

The rise in sucrose in the semi-dry process (PN, BH) was caused by enzyme activity. Ribeiro [10] stated that enzymes such as sucrose phosphate synthase present in coffee beans are involved in the synthesis of sucrose. Glucose as reducing sugar also increased with the fermentation treatment in the D and PN processes. Joët [6] stated that reducing sugars could be due to the degradation of sucrose and cell wall polysaccharides. Lee [11] stated that the reduced sugar concentration obtained through fermentation is due to the deterioration of the mucous layer and has a positive effect on the aroma of coffee, for which sugar is an important precursor in caramelization and Maillard reaction during roasting. In addition, sweet taste can effectively inhibit the formation of bitter taste [15].

![Figure 1. Sugar content (fructose, glucose, and sucrose) green bean process D, PN, and BH with different fermentation times](image)

### 3.3. Chlorogenic acid, Caffeine, and Trigonelline

The contents of chlorogenic acid of green bean in the D, PN, and BH process are shown in Figure 2. Fermentation showed a significant difference in the chlorogenic acid of PN and BH (p <0.05). Furthermore, the fermentation treatment tends to increase the amount of chlorogenic acid in PN but decrease in BH. Changes in chlorogenic acid levels were also reported in previous studies. The research conducted by Martinez [16] in arabica coffee (Var. Catuai-amarelo) showed that fermentation for 16
hours in a polystyrene bucket in the semi-dry process increased the levels of chlorogenic acid to 18.39 g/kg compared to non-fermentation, which was only 14.91 g/kg.

Selmar [17] stated that the high-temperature factor during fermentation and drying causes degradation and isomerization of chlorogenic acid.

Figure 2. The content of chlorogenic acid, caffeine, and trigonelline in D, PN, and BH processes with different fermentation times. The different letters of each coffee bean condition show a significant difference between fermentation times based on the Duncan test at a level of 5.0%.

The caffeine levels tend to increase in PN and tend to decrease in D and BH. The caffeine content of all processes was relatively low. The caffeine levels in Arabica coffee generally range between 10-12 mg/g, but some arabica species such as Mokka and Laurina ranges between 5-6 mg/g [18]. In addition, caffeine is a thermostable compound with minor changes or unchanged levels after roasting. A decrease in caffeine content after roasting is due to its thermal stability properties [19], while an increase in caffeine content can be due to weight loss during roasting [8].

The fermentation treatment has a significant effect on the content of trigonelline. The trigonelline content tends to decrease during the fermentation of all treatments, and the average trigonelline content of all treatments is 1.5%. According to the research conducted by Wamuyu [20] on the dry fermentation of arabica coffee cherries in a bucket showed a trigonelline content of 0.91 ± 0.43%, while in wet fermentation, the trigonelline content produced was 1.01 ± 0.24%.

4. Conclusion
The fermentation treatment significantly affects the non-volatile compounds in Gayo Arabica coffee beans. Malic and citric acid were discovered to be dominant in Gayo Arabica coffee and tends to decrease during fermentation. Propionic acid can cause off-flavor and is only discovered in the D (Dry). Sucrose is a type of sugar that dominates the sample and tends to increase during fermentation in PN and BH due to enzyme activity. Chlorogenic acid, trigonelline, and caffeine were significantly affected by the fermentation process. The increase of chlorogenic acid and the decrease of trigonelline levels have been previously investigated and can occur in the fermentation process without washing, while caffeine content was very low and essentially stable during the process.

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