The Impacts of Traditional Fermentation Method on the Chemical Characteristics of Arabica Coffee Beans from Bondowoso District, East Java

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Received 11 February 2020; Accepted 31 August 2020

ABSTRACT

Bondowoso district is the predominant supplier of coffee beans, and also known as Republik Kopi. However, there was still insufficient data about the chemical characteristics of coffee from Bondowoso. This research has main aims to characterize the chemical characteristic of Coffea arabica L. from Bondowoso, and determine the impact of traditional fermentation on them. Coffee beans were naturally-fermented through soaking in water for less than 12 hours. Unfermented coffee beans were used as a control. Both unfermented and fermented coffee beans were subjected to chemical analysis. Results show that total nitrogen and lipid contents of both fermented and unfermented beans decreased after fermentation into 0.19%±0.03 and 10.03%±0.14, respectively. LCMS analysis of coffee beans revealed that the majority of amino acid contents in fermented beans were higher than that found in unfermented beans, while caffeine and derivatives to be lower in fermented coffee beans than in unfermented beans. One exception for this was 7-methylxanthine, which was found only in fermented beans. Other metabolites, including procyanidines A and B, were found to decrease through fermentation. Interestingly, 3-flavanol was found only in fermented coffee beans. This research results would benefit on improving the quality of coffee through fermentation step.

Keywords: coffee, fermentation, total nitrogen, lipids, amino acids, caffeines

INTRODUCTION

In 1985, the world coffee market dominated by Arabica coffee, 75% of all traded coffee was Arabica [1]. Robusta coffee, by contrast, is known to have a weak flavor, tasting strongly acid and bitter [2]. Though Arabica coffee is known for its superior quality, but it is also susceptible to disease, drought, and temperature. Arabica coffee is indigenous, grows wild in the forest [3,4].

Compounds correlated to the quality of coffee include lipids, proteins and chlorogenic acids. These compounds contribute to the formation of aromas, flavors, pigmentation and astringency [5,2,6]. Lipids aid in flavor-retention and in the trapping of volatile compounds, which play an important role in taste and aromatic stability. Proteins and amino acids react with sugar during roasting to form aromatic compounds. Coffee compounds that are correlated to human health are cafestol and kahweol [6]. Lipid content in coffee is typically
around 10-15%, which may be broken down into two parts: composition of 75% of triglycerides and 20% of fatty acid diterpene esters [7].

Previous research has explored the content of hydrosoluble compounds including amino acids, metals, sucrose, lipid fractions such as kahweol and 16-O-methyl cafestol, trigonelline, caffeine, CGAs (mostly 5-CQA) and nicotinic acid in coffee. Dias and Benassi [8] found that caffeine is the main compound that responsible to discriminate C. arabica and C. canephora in blends. Caffeine is a major alkaloid group present in coffee beans instead of trigonelline. Najib et al [9] found the caffeine concentration in ethanol extract from soxhletation of Arabica coffee to be 120.12 μg/mL. Fox et al. [10] found that caffeine contents in most coffee ranges from 10.0 mg/g to 12.0 mg/g, with Italian coffee as an exception at 19.9 mg/g, and only 0.01 mg/g in decaffeinated coffee. Kharpe et al [2] found that caffeine levels in Arabica green coffee beans in Kenya range from 1.05% to 1.34%. Vinecky et al [11] found that caffeine and chlorogenic acid content in Arabica coffees from continously irrigation fields are higher than those from non-irrigated, at a difference of 11.1 g/kg and 77.0 to 82.5 g/kg respectively. However, the trend of lipid content from these coffees is inversed from caffeine and chlorogenic acid content which is in a range of 121.2 to 135.8 g/kg.

Trigonelline is the second most common alkaloid in coffee, taking the form of methylated alkaloid of nicotinic acid. It is beneficial for health because it improves memory retention and is an anticancer agent [12]. Arabica green beans contain trigonelline concentration in the range of 0.88 – 1.77%, while Robusta contain 0.75 – 1.24% [13]. Yisak et al. [14] found Ethiopian green coffee beans to be composed of 0.83 – 1.13% (w/w) of trigonelline, with a caffeine content of 0.84 – 1.15% (w/w). Taguchi et al [15] found that the trigonelline content in coffee was extremely high (up to 1% on the wet basis) through microbiological assay. Sridevi and Giridhar [16] found that trigonelline occurring with nicotinic acid is typically found in higher amounts when coffee plants are grown at higher altitude with concentrations as high as 975.8 ± 7.24 mg/100 g, and 5.164 ± 0.131 mg/100 g, respectively.

Phenolic compounds are related to the stress of a plant, typically deriving from the surrounding environment. In coffee pulp, phenolic compounds are rich in tannins, while in coffee seed, ester of hydroxycinnamic acids and quinic acid, in the form of chlorogenic acid (CGA) and its derivatives which commonly known as chlorogenic acids (CGAs), are the major one. However, tannin, lignin and anthocyanin still present in a minor level [17,18]. The red skin of fruit cultivars also contains anthocyanins [19-22]. Those phenolic compounds, specifically CGAs and caffeic acid (CA) have been found to have bioactive functionality against a number of diseases related to cell oxidation [23], or commonly known as scavenger of reactive oxygen species, since the presence of vicinal hydroxyl groups on its aromatic residues [24].

In coffee, CGAs contribute to acidity, astringency, and bitterness to beverages by releasing caffeic acid (CA) and supporting the formation of lactones and phenol derivatives [25]. Through these processes, CGAs confer health benefits to consumers through their antiviral properties and antioxidant content [26]. According to Clifford and Wight [27] and Carelli et al. [28], CGAs content varies from 4 to 8.4% in C. arabica, and can reach level up to 14% of dry mass based. CGAs content in Arabica coffee beans from Kenya have been found to be in concentrations ranging from 7.11% to 7.94% [2].

3-Flavanols compounds are generally were formed as a result of the Maillard reaction that occurs during coffee roasting [29]. Ramirez-Coronel et al. [30] identified flavan-3-ols (monomers and procyanidins), hydroxycinnamic acids (caffeoylquinic acid and its
derivatives, and p-coumaroyl-quinic acid), flavonols and anthocyanidins in the pulp of Arabica coffee. Flavan-3-ols were a major component of the pulp for this study, representing over 58.3% of total phenolic compounds in their sample [30]. Geremu et al. [31] suggested that red coffee pulp of Coffea arabica L. can be used as an alternative source of polyphenols and antioxidant compounds. It is interesting to noted that coffee leaves have also been found to show antioxidant activity when consumed due to the phenolic compound content [32].

Generally, free amino acid content in germinated coffee seeds is lower than in ungerminated seeds, while protein content remains the same throughout the germination process. Shimizu and Mazzafera [33] found that tyrosine content was the only of all the amino acids to increase over the first week of germination, then decrease during the following week, the sixth. In comparing amino acid content in both coffee varieties, Arabica generally contain smaller concentrations of amino acids than Robusta, respectively. In terms of specific content, Murkovic and Derler [34] found the highest concentration of amino acids in Arabica to be alanine at 800 μg/g, followed by asparagine at 360 μg/g.

Since coffee from specific regions will produce specific taste and quality [35,36], then it would be great to explore the quality of coffee from specific region such as Bondowoso, to investigate whether its quality could fetch higher price than other local commodities. Bondowoso as the predominant supplier of coffee beans in East Java is also known as Republik Kopi (Republic of Coffee). Providing biochemical characteristic data of coffee beans from Bondowoso district would be benefit on suggesting some efforts on improving coffee bean quality.

EXPERIMENT

Materials and instrumentation

Green Coffea arabica L. beans were obtained from traditional coffee farmers in the Bondowoso district. Fermentation was done through soaking the green coffee beans in water for 24 hours. After fermentation, beans were sun dried. Once dry, the fleshy pulp and mucilage were removed. Dried green beans were ground into powder and then stored in a dry container.

Instrumentation applied for analysis included Shimadzu GCMS-QP2010S with column of Rtx 5 MS, and Shimadzu LCMS-8040LC/MS with column of Shim Pack FC-ODS (2mm D x 150mm, 3μm).

Lipid extraction.

To extract lipids, first a thimble containing a sample of green coffee powder was inserted into a soxhlet extractor was assembled with condenser and a round bottom flask containing n-hexane. The solvent in the flask was heated using a heating mantle until a boil was reached. The extraction occurred over 3 hours, or until the solvent in soxhlet became clear and colorless. After removing the extraction unit from mantle and condenser, the solvent was evaporated using rotavapour. The flask was then put in an oven at 100°C and the contents were dried until a constant weigh was obtained. The flask was then cooled in a desiccatar, and then the flask and contents were weighed. Percent lipid yield was calculated as the amount of lipid extracted in current number of samples multiplied by 100%.

Nitrogen determination

Nitrogen content was determined based on Kjeldahl method, which involves three steps: digestion, distillation and titration. The digestion step converts organic nitrogen in coffee bean powder into ammonium sulfate through catalyzing a reaction with sulfuric acid
using copper sulfate at a temperature between 350-380°C until white fumes are given off. Heating was then continued for another 180 minutes and vapors were transferred into sodium hydroxide until the sample was transparent. The material was then allowed to cool to room temperature, at which point 100 mL of water was added. The solution was then transferred into a flask distillation unit and the ammonium vapour was entrapped in boric acid. Distillation occurred over 5-10 minutes. The condensate was then titrated with HCl 0.25 M until a slightly violet color was obtained. The amount of HCl was used to calculate the number of mol of nitrogen atoms in the sample. The resulting number was then converted to obtain total protein content using a factor of 6.25.

**Extraction of volatile compounds**

Volatile compounds in coffee were extracted by hydrodistillation. Green coffee bean powder was boiled in water inside a round bottom flask connected to Clevenger. The sample was distilled for 5 hours to produce a distillate in two layers, where the upper layer contained non-polar groups, including essential oil, while lower layer contains aqueous phase. Both phases were separated, and the upper layer was subjected to GCMS analysis.

**LCMS analysis.**

Sample preparation for LCMS began with extraction from green coffee beans (unfermented and fermented) in a solution of methanol 95% in a 1:5 proportion. Extracts were subject to a protein precipitation process by 8000 rpm centrifugation for 30 seconds in acetonitrile and formic acid. The supernatant was then purified using a solid phase extraction method using a Sep-Pak C18 Cartridge (1 cc, 100 mg) in 1 mL of acetonitril and water/methanol/ammonium formic buffer. The resulting pure extract was filtered by a cellulose acetate filter membrane of 0.45 µm and followed by degassing. LCMS analysis was carried out using Shimadzu LCMS – 8040 LC/MS with a column of Shimadzu, Shim Pack FC-005 (2 mm D x 150 mm, 3µm). LCMS condition for analysis included an injector volume of 1 µL; a column temperature 35°C; a flow gradient of 0/0 at 0 min, 15/85 at 5 min, 20/80 at 20 min, 90/10 at 24 min; a flow rate 0.5 mL/min; a sampling cone 23.0 V; with methanol 90% in water as solvent. MS focused ion mode was ion type [M]+, ionization ESI, and a fragmentation method that employed low energy CID, with a collision energy of 5.0 Volt, a desolvation gas flow in 6L/h, a desolvation temperature at 350°C, a scanning of 0.6 sec/scan, with a source temperature of 100°C over a 50 minute run time.

**RESULT AND DISCUSSION**

The quality of coffee beans can be determined using the following considerations: (1) characteristic of its coffee beans prior to and after roasting, including shape, size, weight and physical appearance; (2) organoleptic assessment of coffee brew (liquor or cup quality) by panels of experienced coffee liquors. Additional quality traits considered in coffee assessment are lipids, proteins, amino acids, as well as caffeine and chlorogenic acids contents.

Farah [37] found that coffee beans contain a total nitrogen content equivalent to that of protein, about 9-16%. However, this number was excluded of alkaloid content in coffee such as trigonelline and caffeine. To calculate protein content, nitrogen content was multiplied by 6.25, resulting in a calculation indicating that unfermented coffee has a protein content of 11.75%, and fermented coffee has a protein content of 1.19% (Table 1). Previous studies have found that lipid content in green beans ranges from 7-15% [38,7]. The results of this study indicate that lipid content in the study beans was in the range of 10.03% - 12.14% of dry mass.
Table 1. Chemical contents of Arabica coffee from Bondowoso district

<table>
<thead>
<tr>
<th>Components</th>
<th>Unfermented coffee beans (%)</th>
<th>Fermented coffee beans (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total nitrogen</td>
<td>1.88</td>
<td>0.19</td>
</tr>
<tr>
<td>Lipids</td>
<td>12.14</td>
<td>10.03</td>
</tr>
<tr>
<td>Volatile compounds</td>
<td>0.12</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Extraction of volatile compounds from coffee was done using hydrodistillation. This process resulted in a wax form of oil, and was found to be ineffective overall for the extraction of volatile compounds from coffee because many aromatic compounds were lost during extraction. This research obtained a yield of 0.12% for volatile compound from unfermented coffee, and 0.03% from the fermented one. GCMS analysis of this revealed the presence of long chain hydrocarbons and minor components including patchoulol, farnesene and citronellol, though these were obtained only from fermented coffee beans.

From both forms of coffee, all amino acids were found in greater levels following fermentation. Arabica coffee beans (fermented and unfermented) from Bondowoso contained glutamic acid in the greatest concentrations and proline as the amino acid in the lowest concentration (Table 2). These results support those of Arnold and Ludwig [39], which showed that notified glutamic acid was the amino acid with the concentration that varied the most widely during the postharvest storage phase. Shimizu and Mazzafera [33] also observed that glutamic acid/glutamine and glycine were the amino acids of the greatest concentration within coffee seed proteins. By contrast, Murkovic and Derler [34] found alanine and asparagine to be the major amino acid of green coffee beans.

Table 2. Amino acids content within Arabica coffee from Bondowoso district

<table>
<thead>
<tr>
<th>No</th>
<th>Compounds</th>
<th>Unfermented coffee beans (%)</th>
<th>Fermented coffee beans (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glycine</td>
<td>1.444</td>
<td>1.836</td>
</tr>
<tr>
<td>2</td>
<td>Alanine</td>
<td>1.569</td>
<td>2.239</td>
</tr>
<tr>
<td>3</td>
<td>Serine</td>
<td>1.079</td>
<td>1.463</td>
</tr>
<tr>
<td>4</td>
<td>Proline</td>
<td>0.705</td>
<td>0.989</td>
</tr>
<tr>
<td>5</td>
<td>Valine</td>
<td>1.591</td>
<td>1.828</td>
</tr>
<tr>
<td>6</td>
<td>Aspartic acid</td>
<td>2.352</td>
<td>2.635</td>
</tr>
<tr>
<td>7</td>
<td>Glutamic acid</td>
<td>3.282</td>
<td>3.64</td>
</tr>
<tr>
<td>8</td>
<td>Histidine</td>
<td>2.665</td>
<td>3.105</td>
</tr>
<tr>
<td>9</td>
<td>Arginine</td>
<td>2.327</td>
<td>2.769</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>17.014</strong></td>
<td><strong>20.504</strong></td>
</tr>
</tbody>
</table>

Caffeine and some associated derivatives, such as hypoxanthine, xanthine, guanosine and xanthosine, were found in lesser concentrations following fermentation (Table 3). Other caffeine derivatives, such as 3-methylxanthine, theophylline and adenine, were found in greater concentrations after fermentation. According to Augur et al [24], theophylline and theobromine are typically formed during caffeine degradation. During fermentation, fungal growth occurs while caffeine degrades into dimethylxanthine as a product of bacteria.
activity inside coffee pulp. Additionally, Augur et al [24] found that caffeine degradation by fungi undergoes demethylation reaction in position of 1 and 7, where 7-demethylation is predominantly used by the fungus to form xanthine. A notable observation is that 7-methylxanthine was also found be present in fermented coffee beans.

### Table 3. Caffeine and associated derivatives of Arabica coffee from Bondowoso district

<table>
<thead>
<tr>
<th>No</th>
<th>Compounds</th>
<th>Unfermented coffee beans (%)</th>
<th>Fermented coffee beans (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hypoxanthine</td>
<td>2.109</td>
<td>1.673</td>
</tr>
<tr>
<td>2</td>
<td>Xanthine</td>
<td>3.529</td>
<td>3.104</td>
</tr>
<tr>
<td>3</td>
<td>7-Methylxanthine</td>
<td>-</td>
<td>0.838</td>
</tr>
<tr>
<td>4</td>
<td>3-Methylxanthine</td>
<td>1.861</td>
<td>2.08</td>
</tr>
<tr>
<td>5</td>
<td>Theophylline</td>
<td>1.589</td>
<td>1.321</td>
</tr>
<tr>
<td>6</td>
<td>Caffeine</td>
<td>2.838</td>
<td>2.161</td>
</tr>
<tr>
<td>7</td>
<td>Guanosine</td>
<td>1.616</td>
<td>1.346</td>
</tr>
<tr>
<td>8</td>
<td>Adenine</td>
<td>1.885</td>
<td>2.159</td>
</tr>
<tr>
<td>9</td>
<td>Xanthosine</td>
<td>2.376</td>
<td>1.923</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>17.803</strong></td>
<td><strong>16.605</strong></td>
</tr>
</tbody>
</table>

One alkaloid compounds in coffee, instead of caffeine, is trigonelline. This compound is a precursor for volatile compounds formation during roasting. In this research, trigonelline was found to increase in concentration over fermentation from 1.442% to 1.673% of coffee extract. Because nicotinic acid was not found within coffee extract in this research, researchers hypothesize that nicotinic acid was converted into trigonelline. This is a phenomenon that is supported by the findings of Hughes and Smith [40].

Phenolic compounds in coffee include tannin, lignin, flavonoid, and CGAs. Moreira et al. [41] found that decaffeinated coffee contains 10% less CGA content than caffeinated coffee. Generally, coffee of lower quality has higher levels of 5-CQA and FQA [25]. This finding is supported by that of Mazzafera [42] which showed that immature defective beans contained 5-CQA and phenolic substances in higher concentrations when compared to good quality beans. Farah et al [43] found that all CGA isomers were higher in immature-black defective beans, and that the increase in concentrations of 3-CQA, 4-CQA, and 4-FQA observed in sour and black defective beans were a result of isomerization of 5-CQA and 5-FQA, and hydrolysis of di-CQAs (isochlorogenic acids) during fermentation.

This research found that most CGAs decreased in concentration after fermentation, though 5-CQA and 3-FQA increased after fermentation (Table 4). These results differ from isochlorogenic acids (di-CQAs) which were linier to phenomenon of chlorogenic acid (3-CQA).

Ky et al. [13] found that C. arabica in Kenya contained CGA in a concentration of 11.3% of the total dry mass, with component compounds: CQA, 3-CQA and FQA in concentrations of 67%, 20% and 13% of total CGA, respectively. Mullen et al [44] found 5-CQA accounting for 50% of the total CGAs in green coffee beans. In this research, we obtained found that CGAs comprised 50% of purified coffee bean extract mass. The CGAs component in the greatest concentration was 3-CQA, followed by 5-CQA and 3-FQA, as a result of isomerization of 3-CQA and hydrolysis of di-CQAs during fermentation process.
The concentrations of free forms of caffeic, p-coumaric, and quinic acids in green coffee beans were normal, consistent with the findings of Clifford [17].

Table 4. Chlorogenic acid and related compounds (CGAs) content in Arabica coffee from Bondowoso district

<table>
<thead>
<tr>
<th>No</th>
<th>Compounds</th>
<th>Unfermented coffee beans (%)</th>
<th>Fermented coffee beans (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>p-Coumaric acid (p-CoA)</td>
<td>4.045</td>
<td>3.771</td>
</tr>
<tr>
<td>2</td>
<td>Quinic acid (QA)</td>
<td>1.861</td>
<td>1.352</td>
</tr>
<tr>
<td>3</td>
<td>p-Coumaroylquinic acid (p-CoQA)</td>
<td>5.143</td>
<td>4.737</td>
</tr>
<tr>
<td>4</td>
<td>Chlorogenic acid (3-CQA)</td>
<td>8.092</td>
<td>7.679</td>
</tr>
<tr>
<td>5</td>
<td>Neochlorogenic acid (5-CQA)</td>
<td>6.522</td>
<td>7.289</td>
</tr>
<tr>
<td>6</td>
<td>Quinic acid-4-O-caffeate (4-CQA)</td>
<td>2.420</td>
<td>2.775</td>
</tr>
<tr>
<td>7</td>
<td>3-O-Feruloylquinic acid (3-FQA)</td>
<td>5.982</td>
<td>6.674</td>
</tr>
<tr>
<td>8</td>
<td>Caffeic acid (CA)</td>
<td>4.293</td>
<td>4.609</td>
</tr>
<tr>
<td>9</td>
<td>Isochlorogenic acid A (3.5-diCQA)</td>
<td>4.569</td>
<td>4.179</td>
</tr>
<tr>
<td>10</td>
<td>Isochlorogenic acid B (3.4-diCQA)</td>
<td>5.115</td>
<td>4.771</td>
</tr>
<tr>
<td>11</td>
<td>Isochlorogenic acid C (4.5-diCQA)</td>
<td>4.801</td>
<td>4.488</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>50.423</strong></td>
<td><strong>52.324</strong></td>
</tr>
</tbody>
</table>

Table 5. 3-Flavanols compound content in Arabica coffee from Bondowoso district

<table>
<thead>
<tr>
<th>No</th>
<th>Compound names</th>
<th>Unfermented coffee beans (%)</th>
<th>Fermented coffee beans (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3-Flavanol</td>
<td>-</td>
<td>1.462</td>
</tr>
<tr>
<td>2</td>
<td>Procyanidin A1</td>
<td>3.775</td>
<td>2.572</td>
</tr>
<tr>
<td>3</td>
<td>Procyanidin A2</td>
<td>2.666</td>
<td>1.927</td>
</tr>
<tr>
<td>4</td>
<td>Procyanidin B1</td>
<td>2.104</td>
<td>1.249</td>
</tr>
<tr>
<td>5</td>
<td>Procyanidin B2</td>
<td>2.349</td>
<td>1.680</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>10.894</strong></td>
<td><strong>8.890</strong></td>
</tr>
</tbody>
</table>

The flavanoid group is another important phenolic compound in coffee. Most procyanidins, flavonoid derivatives, were found to decrease after fermentation (Table 5). It is likely that some parts of procyanidins were hydrolyzed during fermentation, which resulted in the production of the monomer, 3-flavanol. It is interesting that 3-flavanol was found only in fermented coffee bean, at a concentration of approximately 1.462%. This data suggests that some procyanidins were hydrolyzed to produce 3-flavanol. Flavan-3-ol is the monomer of procyanidins linked by carbon-carbon bonds (C₄-C₆, or C₄-C₈) to form condensed tannin [45,24].

CONCLUSION
Arabica green coffee beans from Bondowoso district were characterized for their component chemical contents. Specifically, nitrogens, lipids, and volatile compound contents were investigated. Soluble parts which were investigated included amino acids, caffeine,
trigonelline and phenolic substances, CGAs and 3-flavanols. The effect of fermentation on both fermented and unfermented coffee beans have also been studied. Further research on utilization and investigation of the treatment effect on this green coffee bean, such be a source of decaffeinated coffee, would be suggested.

CONFLICT OF INTEREST
Authors declare that published this manuscript no competing interest.

ACKNOWLEDGMENT
This research was been conducted with support from the Islamic Development Bank research grant scheme Hibah Penelitian Pendukung IDB in 2018.

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