Biochemical compounds of Arabica coffee (Coffea arabica L.) varieties grown in northwestern highlands of Ethiopia

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Abstract: The study was initiated to evaluate biochemical components of Arabica coffee varieties grown in the northwestern highlands of Ethiopia. For that purpose, green beans of eight highland Arabica coffee varieties, namely, Merdacheriko, Yachi, Wush Wush, Buno wash, 741, 7440, Ababuna and Ageze were harvested from coffee trees. Coffee varieties were grown in RCBD with three replications in Adet and Woramit ARC. Biochemical contents of green beans were analyzed in the Food Science and Nutrition Laboratory of the Ethiopian Institute of Agricultural Research in Addis Ababa, following the standard procedures of the specific chemicals. The results depicted the presence of significant variations for trigonelline, chlorogenic acid, and caffeine contents of beans of coffee varieties grown at a different location. The trigonelline content of the coffee varieties grown at AARC was generally higher than coffee grown in WARC where Buno wash grown in AARC recorded the highest (1.20%) while Wush Wush grown in WARC recorded the lowest (0.93%) trigonelline content. Since CGA content is inversely related to the coffee beverage quality, Merdacheriko variety with the lowest CGA content (2.95%) at WARC and 741 with relatively low CGA content (4.06%) in AARC may have high beverage quality compared to other coffee varieties. The caffeine contents of the tested coffee varieties in both locations were at normal ranges which are common for most Arabica coffee varieties. Further researches on the yielding potential and cup quality of the coffee

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PUBLIC INTEREST STATEMENT
Amhara Region has suitable agroecology for the production of coffee. The crop is produced in by small-scale farmers in different parts of the region including in Yilmana Denssa and Bahir Dar Zurua districts. It is the most important cash crop and contributing as a major source of foreign exchange earnings of the country. Despite its economic and social importance, the production and productivity of coffee in Ethiopia in general and in Amhara Region in particular are low. Moreover, the quality of coffee produced in the country is low. Therefore, evaluating the biochemical contents that contribute to the beverage quality of Arabica coffee varieties is critical to enhance, production, productivity and quality of coffee and thus the livelihoods of smallholder farmers in Amhara Region.
varieties are recommended to identify the suitable coffee varieties for coffee growing areas of Amhara Region.

Subjects: Agriculture & Environmental Sciences; Botany; Plant & Animal Ecology; Soil Sciences; Entomology

Keywords: Arabica coffee; coffee varieties; caffeine; chlorogenic acid; trigonelline

1. Introduction

Coffee is the world’s favorite beverage and the second most traded commodity worldwide after oil in the international market both in terms of volume and value (Alemseged & Yeabsira, 2014). Over 60 tropical and subtropical countries produce and export coffee. It is the leading global beverage after water and its trade exceeds 10 billion USD worldwide (Vega, 2008). Coffee is also representing a significant source of foreign exchange earnings to several developing countries in Africa, Asia, and Latin America (Mishra & Slater, 2012). Ethiopia is also the largest coffee producer in Africa and ranks fifth in the world where the contribution of Ethiopia to the world coffee market is about 4.2% (ICO, 2015).

Ethiopia is the center of origin for Arabica coffee (Weinberg & Bonnie, 2001) which is produced throughout the country in four major production systems, namely forest coffee, semi-forest coffee, garden coffee, and plantation coffee production systems (Woldetsadik & Kebede, 2000). Its production is an increasing trend where the current production is estimated to be about 449,229.8 tons with the productivity of 0.612 t ha$^{-1}$ clean coffee (Central Statistic Agency (2018)). Amhara Region which is found in the northwestern part of Ethiopia is not a traditional coffee-growing area in the country. In recent years, however, coffee production in the region is increasing from time to time. According to Central Statistic Agency (2018), currently, about 3,006.8 tons of green coffee beans are produced on 9,961.2 hectares of land with 0.302 t ha$^{-1}$ productivity.

The quality of coffee is strongly influenced by environmental factors including altitude, daily temperature fluctuations, amount, and distribution of rainfall as well as the physical and chemical properties of soil (Decasay et al. (2003)). Moreover, the genetic make-up of genotype also influences the quality of coffee beans (Silva et al., 2005). In this regard, Yigzaw (2005) and Leroy et al. (2006) reported that genetic origins may influence the coffee quality by affecting genes of chemical compounds and aroma precursors which are expressed during the coffee roasting process.

Green coffee beans contain a wide range of biochemical compounds that have greater diversity and complexity. Such compounds include Caffeine, Trigonelline, Chlorogenic Acid (CGA), and lipids among others which react and interact at all stages of coffee processing to produce the final product (Kathurima et al., 2010). Moreover, green coffee beans are composed of nonvolatile fractions including water, carbohydrates, fiber, free amino acids, lipids, and minerals. CGAs, caffeine, trigonelline, soluble fiber, and diterpenes from the lipid fraction are more likely bioactive and contribute to the beverage flavor of the beans after roasting where anthocyanins and lignans are reported to be residues of the beans (Farah & Donangelo, 2006). In addition, theophylline and theobromine that are identified in coffee beans are traces and reported as caffeine metabolites (Mazzafera et al., 2009).

Although different coffee varieties are growing in Amhara Region, the extent of their biochemical compounds that have impacts on adaptability and quality of coffee is not yet analyzed. In this regard, Yigzaw (2005) reported that biochemical compounds confer the adaptive capacity of coffee varieties in a given production area. According to him, the compounds may also contribute to resistance of the crop towards diseases and insect pests and give a characteristic odor or taste to the crop. The present study was therefore initiated to assess the biochemical contents of Arabica coffee varieties which were grown in northwestern highlands of the Amhara Region.
2. Material and methods

2.1. Study site
Coffee beans were collected from coffee trees grown in research sites of Adet, and Woramit Agricultural Research Centers which were planted in 2010 with Randomized Complete Block Design and three replications. The study site which was covered by coffee trees in each experimental location is 880 m² with a length of 55 m and width of 16 m. The geographical locations of the two research sites are presented in Table 1 where the altitude of the Adet Agricultural Research Center (AARC) is relatively higher than that of Woramit Agricultural Research Center (WARC). The soil of both experimental sites was clay. The minimum and maximum temperatures of AARC were 9°C and 25.5°C, respectively, while those of WARC was 13°C and 30°C, respectively. The available phosphorus, total nitrogen and organic matter contents of the soil of WARC were relatively higher compared to the soil of AARC. On the other hand, the average annual rainfall of AARC was relatively higher compared to that of WARC (Table 1).

2.2. Description of coffee varieties
Eight coffee varieties were grown in Adet and Woramit Agricultural Research sites which were planted in 2010 for adaptation trail. Seven coffee varieties (Merdacheriko, Yachi, Wush Wush, Buno wash, 741, Ababuna and 7440) were sourced from Jimma Agricultural Research Center and planted in each research site while Ageze variety was a local check. The origin, year of release, yield potential and suitable environmental conditions of each variety are presented in Table 2. Coffee trees are grown under the Sesbania sesban tree as a temporary shade tree. Moreover, the agronomic practices including watering, weeding, disease, and insect pest control measures, fertilization and others were conducted uniformly for all varieties in each research site as described by Bertrand et al. (2012), Cheng et al. (2016), and Bote and Jan (2017).

2.3. Experimental procedures and data collection

2.3.1. Harvesting of coffee cherries and sample coffee bean preparation
Well-ripened red coffee cherries of each variety were hand-harvested and dried on a raised bed constructed from local materials and mesh wise. Two harvests were made to collect the required amount of beans necessary for the experiment which were dried separately. The dried cherries from each butch were mixed together thoroughly and the cherries were pulped with hand. From the pulped beans, a total of 100 g clean coffee beans from each variety was weighed and taken to the Food Science and Nutrition Laboratory of Ethiopian Institute of Agricultural Research (EIAR) in Addis Ababa for biochemical analysis.

2.3.2. Method of analysis for biochemical components of coffee beans
Caffeine, chlorogenic acids, and trigonelline were extracted and purified according to the method of Ky et al. (2001). Half a gram of each ground coffee sample (8 x 3 replications) was accurately weighed in 100 ml Erlenmeyer flask and 50 ml of double-distilled water boiled at 90°C was added, followed by heating at 121°C in a heating plate for 20 min. Extracts were filtered using Whitman filter paper and then finally filtered with a microfilter size of 0.45 mm and were maintained for caffeine, chlorogenic acids, and trigonelline determination.

Biochemical analysis was conducted in the food science and nutrition laboratory at the Ethiopian Institute of Agricultural Research (EIAR), Addis Ababa. Simultaneous analysis of trigonelline, chlorogenic acids, and caffeine was carried out according to the modified method of Vignoli et al. (2014) using the HPLC machine consisting of a solvent reservoir, a quaternary pump, autosampler, a 250 × 4.6 mm column with a 5 μm pore size. Compounds were eluted with a mobile phase consisting of 5% acetic acid and acetonitrile and elution were carried out at 0.7 ml/min by injecting 10 μl of a sample into the chromatographic system.
| Study site | Altitude (m) | Geographical location | Soil type | pH | Weather data | Nutrients | OM (%) |
|------------|--------------|-----------------------|-----------|----|--------------|-----------|--------|
| AARC       | 2240         | 11°17'N 37°43'E       | Clay      | 5.17 | MXT (°C) 25.5 | MIT (°C) 9 | RF (mm) 1257 | AvP (mg/kg) 1.68 | TN (%) 0.09 | OM (%) 1.89 |
| WARC       | 1800         | 11°38'N 37°10'E       | Clay      | 6.4  | MXT (°C) 30.5 | MIT (°C) 13 | RF (mm) 800 - 1250 | AvP (mg/kg) 6.30 | TN (%) 1.16 | OM (%) 3.90 |

MXT, Maximum Temperature; MIT, Minimum Temperature; AvP, Available phosphorous; TN, Total Nitrogen; OM, Organic Matter.

Source: Bahir Dar Meteorology Agency (2018) and Habtamu et al. (2016)
| Variety          | Origin/Zone       | Year of release | Yield potential (t/ha) | Suitable conditions |
|------------------|-------------------|-----------------|------------------------|---------------------|
|                  |                   |                 | Research field | Farmers Field | Altitude (m) | Rainfall (mm) | T (°C) |
| Merdacheriko     | Gera/Jimma        | 2006            | 1.9           | 1.71          | 1750-2100   | ≥1500        | 10-26  |
| Yachi            | Yachi/Jimma       | 2006            | 1.64          | 1.52          | 1750-2100   | ≥1500        | 10-26  |
| Wush Wush        | Wush Wush/Kaffa   | 2006            | 2.35          | 1.62          | 1750-2100   | ≥1500        | 10-26  |
| Buno wash        | Washi/Kaffa       | 2006            | 1.22          | 1.56          | 1750-2100   | ≥1500        | 10-26  |
| 741              | Gera/Jimma        | 1977/78         | 1.62          | 0.6-0.7      | 1900        | >1400        | 11-28  |
| 7440             | Washi/Kaffa       | 1979/80         | 2.38          | 0.8-0.9      | 1700        | >1400        | 11-28  |
| Ababuna          | Hybrid coffee     | 1978/79         | 2.38          | 1.5-1.6      | 1000-1752   | >1400        | 15-30  |
| Ageze            | Local             |                 |              |              |             |              |        |

Source: Demelash and Kifle (2015)
2.3.3. Determination of biochemical components of coffee beans

Caffeine, trigonelline, and chlorogenic acid contents of the sample coffee beans were determined using the HPLC system consisting of the Discovery C18 column with an Isocratic flow of 1 ml/min. A calibration curve was made using the standard concentration and area of the sample. Subsequently, the area was used to calculate the concentration of the respective biochemical component using the area generated and eluted at different retention times for Caffeine (CAF), Chlorogenic acid (CGA), and Trignolleine (TRG) standards as described by Gichimu et al. (2014). TRG and CAF were determined using the standard solution of 5, 10, 20, 40, 50, 100, and 200 μg/ml concentrations, whereas CGA was evaluated over the range of 5, 20, 100, 200, and 500 μg/ml. The concentrations of the respective metabolites were then calculated from peak areas using the following calibration equations which were done at the laboratory. Calibration curves for standards of trigonelline, chlorogenic acid, and caffeine were obtained by injecting increasing concentrations of mixed standards. TRG and CAF were detected at 272 nm, while CGA was detected at 320 nm.

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Y = 0.0606 \times \text{Peak area} + 0.3778 \quad \text{(for Trignollene)}
\]

\[
Y = 0.0274 \times \text{Peak area} - 3.0404 \quad \text{(for Chlorogenic Acid)}
\]

\[
Y = 0.0266 \times \text{Peak area} + 0.8036 \quad \text{(for Caffeine)}
\]

2.4. Data analysis

The collected data were subjected to Analysis of Variance (ANOVA) using the general linear model (GLM) Statistical Software Program (SAS 9.4). Whenever ANOVA showed significant variation, the mean separation was conducted using LSD at 5%, 1%, and 0.1% probability level depending on the ANOVA results.

3. Results and discussion

3.1. Trigonelline content of Arabica coffee varieties

The statistical analysis showed that there were very highly significant differences (P ≤ 0.001) for trigonelline (TRG) concentration among coffee varieties at both locations and across the locations. At Woramit, the highest TRG content was obtained from Ageze local coffee (1.11%) variety followed by Yachi (1.07%) while the lowest value was recorded from the variety 741 (0.87%) as indicated in Table 3.

At Adet, the highest value of TRG was recorded from variety Buno wash (1.20%) followed by Ageze local (1.17%) variety while the lowest TRG concentration was recorded from Ababuna variety (Table 4). On the other hand, Ageze local coffee (1.14%) and Ababuna varieties (0.95%) gave the highest and lowest TRG values across the locations, respectively (Table 5).

Trigonelline is a bitter alkaloid and one of the precursors of aroma in coffee that degrades up to 90% during roasting and forming mainly niacin, pyridines, and some pyrroles: important aroma compounds (Ky et al., 2001). The results of the present study are generally in line with the findings of other researchers where they reported the trigonelline content of coffee varieties ranges from 0.39% to 1.77% depending on the coffee species (Campa et al., 2004). As indicated in Tables 3 and 4, the concentration of trigonelline in Adet grown coffee varieties was relatively greater than those coffee varieties grown in Woramit which might be associated with the difference in environmental conditions, pH value and soil fertility status as indicated in Table 1. The lower the trigonelline content the lower is also the quality of the coffee beverage which can be however influenced by the roasting process (Monteiro & Trugo, 2005). The trigonelline concentration of local Ageze variety was relatively high in both locations and across the location which indicates the high beverage quality of this variety.
3.2. The Chlorogenic acid content of Arabica coffee varieties

The statistical analysis showed that there were very highly significant differences ($P \leq 0.001$) for chlorogenic acid (CGA) concentration among coffee varieties grown in both locations and across the location. In Woramit, the highest CGA concentration was obtained from a variety of 7440 (4.96%) followed by Ababuna (4.95%) variety while the lowest was recorded from Merdacheriko (2.95%) variety (Table 3). In the Adet research site, however, the highest CGA concentration was obtained from Merdacheriko (5.05%) variety followed by Ageze (4.85%) while the lowest CGA content (4.06%) was obtained from variety 741 (Table 4). On the other hand, a relatively higher concentration of CGA was obtained from a variety of 7440 (4.64%) followed by Ababuna (4.61%) and the lowest value was obtained from variety Merdacheriko (4.00%) across the locations as indicated in Table 5.

| Table 3. Biochemical contents of Arabica coffee varieties grown in Woramit Research Center in the 2018 cropping season |
|---|---|---|
| Coffee varieties | Trigonelline (%) | Chlorogenic acid (%) | Caffeine (%) |
| Merdacheriko | 0.97$^d$ | 2.95$^g$ | 1.13$^g$ |
| Yachi | 1.07$^a$ | 4.23$^b$ | 1.30$^d$ |
| Wash Wush | 0.93$^f$ | 4.30$^a$ | 1.23$^f$ |
| Buno Wash | 0.98$^c$ | 4.55$^d$ | 1.30$^d$ |
| 741 | 0.87$^b$ | 4.66$^c$ | 1.44$^c$ |
| 7440 | 0.973$^d$ | 4.96$c$ | 1.50$^b$ |
| Ababuna | 0.953$^e$ | 4.95$a$ | 1.37$^a$ |
| Ageze | 1.113$^a$ | 4.31$^d$ | 1.60$^a$ |
| Mean | 0.982 | 4.365 | 1.362 |
| CV (%) | 0.30 | 0.11 | 0.23 |
| P- values | *** | *** | *** |
| LSD ($P \leq 0.01\%$) | 0.005 | 0.0084 | 0.0056 |
| SE± | 0.03 | 0.12 | 0.03 |

CV, Coefficient of variance; LSD, Least significance difference; ***Very highly significant SE, Standard error.

| Table 4. Biochemical contents of coffee varieties grown in Adet Agricultural Research Center in 2018 cropping season |
|---|---|---|
| Coffee varieties | Trigonelline (%) | Chlorogenic acid (%) | Caffeine (%) |
| Merdacheriko | 1.14$^d$ | 5.05$^g$ | 1.51$^a$ |
| Yachi | 1.10$^a$ | 4.52$^d$ | 1.38$^g$ |
| Wash Wush | 1.16$^c$ | 4.75$^d$ | 1.37$^e$ |
| Buno Wash | 1.20$^a$ | 4.38$^a$ | 1.30$^d$ |
| 741 | 1.05$f$ | 4.06$^d$ | 1.20$c$ |
| 7440 | 0.97$^g$ | 4.32$^f$ | 1.27$f$ |
| Ababuna | 0.95$^h$ | 4.27$^g$ | 1.26$^g$ |
| Ageze | 1.17$^b$ | 4.85$^d$ | 1.38$^d$ |
| Mean | 1.095 | 4.52 | 1.33 |
| CV (%) | 0.32 | 0.045 | 0.15 |
| P- values | *** | *** | *** |
| LSD ($P \leq 0.01\%$) | 0.006 | 0.0036 | 0.0036 |
| SE± | 0.02 | 0.06 | 0.02 |

CV, Coefficient of variance; LSD, Least significance difference; ***Very highly significant; SE, Standard error.

3.2. The Chlorogenic acid content of Arabica coffee varieties

The statistical analysis showed that there were very highly significant differences ($P \leq 0.001$) for chlorogenic acid (CGA) concentration among coffee varieties grown in both locations and across the location. In Woramit, the highest CGA concentration was obtained from a variety of 7440 (4.96%) followed by Ababuna (4.95%) variety while the lowest was recorded from Merdacheriko (2.95%) variety (Table 3). In the Adet research site, however, the highest CGA concentration was obtained from Merdacheriko (5.05%) variety followed by Ageze (4.85%) while the lowest CGA content (4.06%) was obtained from variety 741 (Table 4). On the other hand, a relatively higher concentration of CGA was obtained from a variety of 7440 (4.64%) followed by Ababuna (4.61%) and the lowest value was obtained from variety Merdacheriko (4.00%) across the locations as indicated in Table 5.
CGA plays an important role in the formation of the flavor of roasted coffee and has a marked influence in coffee cup quality (Farah & Donangelo, 2006). They are known to be responsible for coffee pigmentation, aroma formation, bitterness, and astringency of coffee beverages (De Maria et al., 1995). Chlorogenic acids are present in the range of 6-12% of the dry weight of the green coffee bean (Crozier et al., 2009). The CGA concentration of a given coffee variety has inversely correlated with its beverage quality where higher CGA content was observed in coffee varieties that had lower beverage quality (Silva, 1999). Accordingly, Merdacheriko variety (2.95%) grown at Woramit and 741 varieties (4.06%) at Adet as well as Merdacheriko variety (4.00%) across the locations had relatively highest cup quality compared to other coffee varieties. As indicated in Table 4, Merdacheriko variety grown in Adet recorded the highest CGA concentration. However, the same variety grown in Woramit recorded the lowest CGA concentration (Table 3) which indicates that the CGA concentration of a given variety increased with the increasing altitude and thus reduced average temperatures. The general higher concentration of CGA in Adet Agricultural Research Center grown coffee varieties compared to those grown in Woramit Agricultural Research Center in the present study (Tables 3 and 4) also supports this fact. The results of the present study are generally in agreement with the findings of Bertrand et al. (2006) who reported higher chlorogenic acid and fat contents of beans of Arabica coffee varieties with increasing growing elevation.

### 3.3. The Caffeine content of Arabica coffee varieties

The statistical analysis revealed that there were very highly significant variations (P ≤ 0.001) for their caffeine (CAF) concentration of coffee varieties grown in both locations and across the location. In Woramit, the highest caffeine concentration was obtained from Ageze local coffee variety (1.60%) while the lowest was from Merdacheriko (1.13%) variety (Table 3). On the other hand, the highest caffeine concentration in Adet grown coffee varieties was obtained from Merdacheriko (1.51%) while the lowest value was obtained from variety 741 (1.20%) as indicated in Table 4. Relatively higher CAF concentration across the location was obtained from a variety of Ageze local coffee (1.49%) and the lowest values were obtained from Buno wash and Wush Wush (1.30%) varieties (Table 5).

The caffeine content of green beans significantly influences the mildness and thus the cup quality of a given coffee species. Generally, the higher the caffeine content the bitter is its taste.

| Coffee varieties | Trigonelline (%) | Chlorogenic acid (%) | Caffeine (%) |
|------------------|------------------|----------------------|--------------|
| Merdacheriko     | 1.055±           | 4.00±                | 1.32±        |
| Yachi            | 1.086±           | 4.37±                | 1.34±        |
| WushWush         | 1.04±            | 4.52±                | 1.30±        |
| Buno wash        | 1.09±            | 4.66±                | 1.30±        |
| 741              | 0.96±            | 4.36±                | 1.32±        |
| 7440             | 0.97±            | 4.64±                | 1.38±        |
| Ababuna          | 0.95±            | 4.61±                | 1.31±        |
| Ageze            | 1.14±            | 4.58±                | 1.49±        |
| Mean             | 1.03             | 4.45                 | 1.35         |
| CV (%)           | 0.30             | 0.08                 | 0.19         |
| P- values        | ***              | ***                  | ***          |
| LSD (P ≤ 0.01%)  | 0.0038           | 0.0042               | 0.0031       |
| SE±              | 0.01             | 0.07                 | 0.018        |

CV, Coefficient of variance; LSD, Least significance difference; ***Very highly significant; SE, Standard error
and thus the lower is its cup quality (Lipchuck et al., 2017; Tanimura & Mattes, 1993). In this regard, Arabica coffee has generally low caffeine content and thus has high cup quality than that of Robusta coffee (Ky et al., 2001; Silvarolla et al., 2004). As indicated in the present study, caffeine content of Arabica coffee varieties grown in both locations ranged from 1.13% to 1.60% which is at optimum level and in line with the findings of Silvarolla et al., (2000) and Viani (1993) who reported the caffeine content of Arabica green coffee varieties can reach up to 1.45%. Similarly, Farah (2012) reported that caffeine contents of Arabica coffee beans are ranging from 0.90% to 1.3% while that of Robusta coffees are in the range of 1.51–3.33% (Ky et al., 2001). Although all the tested coffee varieties have relatively low caffeine contents, Merdacheriko (1.13%), Wush Wush (1.23%), Yashi (1.30%), and Buno Wash (1.30%) varieties grown at Woramit and 741 (1.20%), Ababuna (1.26%) and 7440 (1.27%) varieties which were grown at Adet Agricultural Research Center had relatively the highest quality in terms of caffeine content.

The altitude at which coffee is grown plays a major role in determining the quality of green coffee beans since beans of coffee plants grown at higher altitudes require a longer time to mature than those grown at lower altitudes. Similarly, the fertility and pH values of the soil influence the quality of coffee beans. In this regard, the development and maturity of coffee trees, as well as their cherries at higher altitudes like Adet Agricultural Research Center, may take a longer time compared to those grown in relatively low altitudes like Woramit that may in turn influence the caffeine content of the beans. Generally, beans of coffee trees grown at higher altitudes usually have a higher density than beans of coffee trees grown at lower altitudes (Sridevi & Giridhar, 2014).

3.4. Correlation of biochemical components of Arabica coffee varieties

The simple correlation analysis showed that trigonelline was positively associated with caffeine ($r = 0.34$) but negatively correlated with chlorogenic acid ($r = -0.20$) at Woramit (Table 6) while at Adet it was positively associated with chlorogenic acid ($r = 0.40^*$) and caffeine ($r = 0.62^*$) contents. In this regard, Ky et al. (2001) reported that trigonelline is a pyridine derivative and precursor of the volatile compounds known to contribute indirectly to the formation of appreciated flavor products including furans, pyrazine, alkyl-pyridines, and pyrroles during coffee roasting. Caffeine and trigonelline are major nitrogenous compounds, whose contents are also closely related to the quality of coffee beverage (Farah, 2009; Santos et al. (2015)).

Chlorogenic acid was positively associated with caffeine ($r = 0.60^*$) but negatively correlated with trigonelline ($r = -0.20$) (Table 6) at Woramit while at Adet it was positively correlated with trigonelline ($r = 0.40^*$) and caffeine ($r = 0.75^{***}$) (Table 7). Bicchi et al. (1995) were, however, unable to correlate the beverage quality or a specific sensory attribute such as astringency with the presence of specific chlorogenic acid(s).

Generally, the cup quality of coffee is increased when the CGA content decreased (Ky et al., 1999). In this regard, various researchers have been reported the influence of CGA on cup quality of coffee including coffee pigmentation, aroma formation, bitterness, and astringency (Barbosa et al., 2012; Farah, 2009; Farah & Donangelo, 2006; Koshiro et al., 2007; De Maria et al., 1995; Ribeiro et al., 2014).

| Table 6. Correlation between biochemical components of Arabica Coffee varieties grown at Woramit in 2018 cropping season |
|-------------|-------|-------|-------|
| Trait | TRG | CGA | CAF |
| TRG | 1 | | |
| CGA | -0.20 | 1 | |
| CAF | 0.34 | 0.60^{***} | 1 |

*** Significant at $p < 0.001$; TRG, Trignolline; CGA, Chlorogenic acid; CAF, Caffeine.
Caffeine was positively correlated with trigonelline ($r = 0.34$) and chlorogenic acids ($r = 0.60$) contents at Woramit (Table 6). Similarly, it was positively correlated with trigonelline ($r = 0.62$) and chlorogenic acids ($r = 0.75$) at Adet (Table 7). Caffeine, a xanthenes derivative is responsible for the bitter taste of coffee beverages which affects the coffee flavor (Farah, 2009; Santos et al., 2015; Trugo, 1984).

4. Conclusion
The biochemical contents of Arabica coffee are influenced by the varieties in both locations. The trigonelline content of the coffee varieties grown at AARC Center was generally higher than that of coffee grown in WARC where Buno wash variety grown in AARC recorded the highest (1.20%) while Wush Wush grown in WARC recorded the lowest (0.93%) trigonelline content. Since CGA content is inversely related with the coffee beverage quality, Merdacheriko variety with the lowest CGA content (2.95%) may have high beverage quality compared to other varieties grown at WARC while the variety 741 with relatively lowest CGA content (4.06%) may have high beverage quality in AARC. Although all the tested Arabica coffee varieties have relatively low caffeine contents at normal range, Merdacheriko (1.13%), Wush Wush (1.23%), Yashi (1.30%), and Buno Wash (1.30%) varieties grown at WARC and 741 (1.20%), Ababuna (1.26%), and 7440 (1.27%) varieties grown at AARC had relatively the lowest caffeine content and could be mild and best in their beverage quality. Further research on yield potential and cup quality of the coffee varieties are recommended to identify suitable coffee varieties for coffee-growing areas of Amhara Region.

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Competing interest
The authors declare no competing interest.

Authors’ contributions
Melese Wale conceived and designed the experiments and analyzed the data and wrote the manuscript. Melkamu Alemayehu has also designed the study as well as commented and edited the manuscript. Abrar Sulaeh commented and edited the manuscript. All authors approved the manuscript.

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