Influence of genotype and processing on bioactive compounds of Ethiopian specialty Arabica coffee

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ABSTRACT

Ethiopian specialty coffee is exported to the international market based on the cup quality classification; however, there is limited information on the chemical characteristics. In this study, dry and wet-processed sixteen Ethiopian specialty coffee genotypes were investigated for total polyphenols, chlorogenic acids, caffeine, and trigonelline at different stages using UV-Vis spectrophotometric and Rapid Separation Liquid Chromatographic methods. Cup qualities of green coffee beans and brewed coffee were also assessed by professional cuppers. The results indicated that the bioactive compounds and cup qualities were significantly influenced by genotypes, green bean processing methods, and medium roasted-ground and brewed coffees. It was shown that the percentage of reduction in total polyphenols, chlorogenic acids, caffeine, and trigonelline concentrations were 77.3%, 82.5%, 47.8%, and 70.6%, respectively as the beans are transformed to brew. This study also fills the gap of information on the influence of processes and correlation to cup quality with bioactive compounds.

Introduction

Coffee is one of the most important crops as it grows in over 80 countries in the tropical and subtropical regions of the world. It is also traded commodity in the globe next to petroleum and the most popular beverage. Coffee is mostly consumed as a beverage for its stimulant effect and it is globally the second most widely consumed beverage next to water. Ethiopia is one of the five top Arabica coffee-producing countries in the world and the country itself consumes half of its annual production. Ethiopian coffee grows in many parts of the country but the most commercially available coffees grow in the eastern, southern, and southwest parts of the country. 37 commercialized coffee genotypes are produced in Ethiopia for the local as well as international markets.

Coffee has a profound taste and aroma characteristics as well as potential beneficial health effects for humans. Different researchers reported that coffee has important bioactive compounds. The bioactive compounds in coffee include total polyphenols (TP), chlorogenic acids (CGA), caffeine (CAF), and trigonelline (TRG). These bioactive compounds in coffee may be influenced by coffee species, genotypes, geographical origins, processing methods, and roasting conditions.
Cup quality, one of the most used methods in identifying the market acceptability of coffee beverages, desirability for consumption, price determination, and for the release of new genotypes, plays a vital role in the coffee industry.\textsuperscript{[13,15,18–22]} Ethiopian coffee industry suffers from adulteration\textsuperscript{[23]} as only cup quality is used to classify it for the national and international market destinations.

Despite the presence of many reports worldwide that growing regions, red cherry processing methods, roasting, and brewing processes influence bioactive compounds in coffee. There are limited research endeavors on Ethiopian specialty coffee genotypes coupled with different processing methods and preparation stages. Most research on Ethiopian coffees has focused on breeding\textsuperscript{[24]}, cup analysis,\textsuperscript{[23,25,26]} and physical parameters,\textsuperscript{[27–29]} as affected by altitude irrespective of genotypes\textsuperscript{[30]} and on biochemical characteristics of a few genotypes from particular coffee growing areas.\textsuperscript{[31,32]} The current work is the first of its kind in comprehensively dealing with known genotypes in Ethiopia concerning processing methods and different stages of preparation. The objectives of this work were specifically to investigate the influence of dry and wet green bean processing methods, coupled with medium roasting levels and brewing on total polyphenol (TP), total chlorogenic acids (CGA), caffeine (CAF), and trigonelline (TRG) of Ethiopian specialty coffee genotypes. The correlation of the four bioactive compounds with the cup quality attributes was also assessed. The research is important in generating relevant information for future breeding programs and the classification of Ethiopian specialty coffee.

Materials and methods

Descriptions of sample sources

Sixteen Ethiopian specialty coffee genotypes were collected from four (Awada, Mechara, Jimma, and Haru) agricultural centers. These centers are described as follows. Awada (6°3′N, 38°3′E, 1740 masl altitude, 11–28°C temperature and 1335 mm mean annual rainfall), Mechara (40°19.114’N, 08°35.589’E, 1760 masl altitude, 16°C annual average temperature and 963 mm annual average rainfall), Jimma (7°40′9″N, 36°47′6″E, 1753 masl altitude, 9–28°C temperature and 1561 mm annual rainfall) and Haru (7°40′9″N, 36°47′6″E, 1750 masl altitude, 18.9–26.8°C and 1561 mm annual rainfall) that comprise five major coffee Arabica growing regions of the country. Coffee fruits were harvested in the cherry maturation stage during the harvesting season of October to December 2016. The genotypes with their respective Agriculture Research Centers were Feyate, Odicha, Angefa and Qoti from Awad (Sidama & Yirgachefe); Arusa, Bultum, Mechara – 1, and Mocha from Mechara (Harar); 744, 741, 7487, and 74110 from Jimma (Southwest); and Menesibu, Haru – 1, Challa, and Sende from Haru (Wollega).

Green bean preparation

The red cherries of the sixteen genotypes were divided into two lots each and converted into green beans using dry and wet processing methods at the respective agricultural centers\textsuperscript{[33]}. Parts of the green beans were ground to 1 mm mesh size using a Thomas – Wiley Laboratory Mill (Model 4, USA) and packaged in airtight plastic bags, and held at 4°C until required for further analysis.

Roasting, grinding, and cupping of the samples

Samples of 100 g (about 32 green beans above 14” screen) were medium roasted and ground for cup quality at Jimma Agricultural Research Center (JARC) laboratory using a six-cylinder roaster machine (Probat BRZ6, Welke, Von Gimborn Gmbhan Co. KG) and electrical grinder (MahlKonig, Germany), respectively. Cup analysis of green beans and brew was done by three professional cuppers using standard method.\textsuperscript{[34]}
Chemicals
Gallic acid, sodium carbonate, sodium phosphate dibasic, sodium hydroxide, and sodium bicarbonate were acquired from the Nutritional Sciences Lab at Oklahoma State University. CGA, CAF, and TRG standards were purchased from VWR International, USA. All HPLC grade solvents (methanol, acetonitrile, triethylamine, and acetic acid) as well as Folin-Ciocalteu’s, and Carrez I & II reagents were purchased from VWR International, USA. Ultrapure water (Milli-Q) was obtained from a MilliPore purification and filtration system (Billerica, MA, USA).

Determination of total polyphenols
TP for dry and wet processed coffee samples of green, medium roast, and the brew was determined as follows following standard methods. The detailed protocols for the analysis are presented in the sections that follow.

Aqueous extraction of total polyphenols
Coffee sample extracts were prepared described in the previous reports\[^{35}\] with slight modifications. The roasted coffee samples were ground using a BRAUN Aromatic coffee grinder, Model KSM2. One gram of ground coffee was weighed and mixed with 10 mL of Milli-Q water. The mixture was heated at 98°C for 15 min while shaking at 120 rpm in a water bath (VWR). The extract was cooled to 20°C and centrifuged at 3600 rpm for 30 min at 4°C (Eppendorf Centrifuge 5810 R). The extracts were filtered using a 0.45 µm syringe filter into a 15 mL centrifuge tube. The extracts were then stored at 4°C until used for analyses.

TP was determined using the Folin–Ciocalteu method with some modifications 36. Extracts (20 µL) were mixed with Milli-Q water (1,580 µL). From this mixture, an aliquot of 160 µL was mixed with 10 µL of 2 N Folin–Ciocalteu’s phenol reagent and held for 8 min. Next 30 µL of Na2CO3 was added to the mixture and kept at room temperature for two hours. A blank sample was prepared by substituting the reagent with Milli-Q water. The absorbance values of samples were measured with a microplate reader at 765 nm against the blank sample after incubation. Quantification of TP was carried out using a standard calibration curve of gallic acid solution (0–1 mg/mL; \(R^2 = 0.99\)). The concentration of TP was presented as g of gallic acid equivalent/100 g of coffee (mg GAE/100 g).

Determination of CGA, CAF, and TRG
CGA, CAF, and TRG were identified based on the combined results of the following parameters: elution order on reversed-phase column and characteristics of UV-visible optical density as compared with those obtained with the standards analyzed under the same conditions.

Extraction of CGA, CAF, and TRG
About 0.2 g roasted ground coffee was weighed in a 50 mL Sarstedt-capped tube. For the extraction of CGA 5 mL of 0.5% NaHSO3 was added to each sample. CAF and TRG were extracted from the green and roasted coffee powders as well as the coffee brew by using some modifications of method \[^{36,37}\]. The extraction was done twice by shaking for 30 min, rather than once for 5 min by stirring. A 0.200-g portion of the coffee powder was weighed directly into a centrifuge tube, and the coffee powder and the brew samples were extracted with 5.00 mL of boiling Milli-Q water by shaking for 30 min on a platform shaker at 200 rpm. The mixture was centrifuged for 5 min at 3600 rpm, after which the supernatant was decanted into a second centrifuge tube. The residue was returned to the tube and extracted a second time with another 5.00 mL of boiling water. After a combination of the supernatants, the volume was adjusted to 10.0 mL. A 1.50-mL portion of the extract was treated with 30 µL of 20% aqueous lead acetate solution to precipitate polysaccharides, proteins, and other colloidal material from the extract.
solution. After centrifuging the mixture for 5 min at 12,000 rpm, the supernatant was filtered directly into a chromatographic vial through a 0.2-μm syringe filter (Acrodisc; Sigma-Aldrich) for HPLC analysis.

**HPLC determination of CGA, CAF, and TRG**

CGA, CAF, and TRG concentrations in the aqueous extract of coffee samples were determined using some modification of the method of Vignolli and colleagues. A high-performance liquid chromatography (Dionex UPLC 3000 system with SRD-3400 Integrated Solvent and Degasser Rack, HPG-3200RS Binary Pump, and Variable Wavelength Detector (VWD-3400RS), Thermo-Fisher, U.S.A.) with a 25 cm × 4.6 mm, 5 μm particle C18 Discovery column (SUPLECO, Bellefinte, USA) was used. The data from the chromatograph was analyzed using a Dionex Chromleon PC workstation.

After the preparation of coffee brews, as previously described, the extracted samples were filtered using a 0.2 μm nylon syringe filter for automatic injection into the chromatographic system. CGA was eluted with linear gradient containing A: 2 mM H₃PO₄ with 5% methanol (pH 2.7) and B: methanol with 5% 2 mM H₃PO₄ (pH 3.9), as follows: 0–2 min: 4% B; 2–12 min: 100% B; 12–18 min: 100% B; 30–40 min: 0% B; and 40–50 min: 4% B. CAF and TRG were eluted using 0.1% triethylamine (TEA) (A) and acetonitrile (B) as follows 0 min: 15% B; 5 min: 30% B; 7.5 min: 30% B; 10 min: 15% B; 12 min: 4% B; and 14 min: 3% B. The injection volume and the flow rate were 10 μL and 0.5 mL/min respectively for both cases. CGA, CAF, and TRG were detected at 263, 272, and 325 nm respectively. Quantifications of CGA, CAF, and TRG were carried out by a 5-point external standard calibration curve over the range of 20 to 120 mg/L with triplicate measurements. All results were expressed as % on a dry basis (DB) of the bioactive compound of soluble solids in the original coffee.

**Statistical design and analysis**

The research was a factorial design of processing methods (dry, wet), and stages of preparation (green beans, medium roasted ground, and brewing) for 16 commercialized Ethiopian specialty coffee genotypes. All measurements and analyses were carried out in triplicate. The results were analyzed statistically using the JMP Pro 13 program to determine the average value and standard error. Sigma Plot 14.0 software was used to generate figures. Variance analysis, with a significance level of α = 0.05%, was performed to determine the differences in the bioactive compounds between the dry and wet processing methods, among stages of preparation, and the genotype differences. Pairwise correlation of bioactive compounds and cup quality attributes were also analyzed using JMP Pro 13 statistical software of the SAS Company.

**Results and discussion**

**Influence of genotype and processing on bioactive compounds**

**Influence of genotypes on TP, CGA, CAF, and TRG**

Certain bioactive compounds (CAF and TRG) of Arabica coffee were significantly (p < .05) influenced by genotype (Table 1). Coffee genotype Feyate from Awada Agricultural Research Center showed a lower percentage of CAF (1.37% [db]) while genotype 744 from Jimma Agricultural Research Center showed a statistically higher percentage (1.77%. [db]). On the other hand, genotypes Qoti and Mechara – 1 from different agricultural research centers exhibited a higher concentration of TRG (1.06% [db] for both) while the 7487 genotype showed a lower value (0.88% [db]). The result of Arabica coffee indicated that CAF and
Table 1. Bioactive compounds of sixteen coffee genotypes (main effect of genotypes).

| Genotypes    | TP, mg GAE/100 g | CGA, % [db] | CAF, % [db] | TRG, % [db] |
|--------------|------------------|-------------|-------------|-------------|
| Feyate       | 9.49±            | 4.27±       | 1.37±       | 0.99±c      |
| Odicha       | 10.19±           | 4.07±       | 1.649±c     | 0.988±c     |
| Angfa        | 10.44±           | 4.11±       | 1.51±bc     | 1.00±c      |
| Qoti         | 9.91±a           | 3.59±       | 1.63±ab     | 1.06±a      |
| Arusa        | 9.85±a           | 3.82±       | 1.66±c±a    | 0.95±c      |
| Bultum       | 10.38±a          | 3.88±       | 1.63±ab     | 0.99±c      |
| Mechara-1    | 10.58±a          | 4.15±       | 1.54±c      | 1.06±a      |
| Mocha        | 10.63±a          | 4.31±       | 1.55±c      | 0.98±c      |
| 744          | 10.33±a          | 4.28±       | 1.77±a      | 0.94±c      |
| 741          | 9.92±a           | 3.78±       | 1.53±bc     | 0.93±c      |
| 7487         | 9.92±a           | 3.63±       | 1.62±ab     | 0.88 ±c     |
| 74110        | 9.86±a           | 3.73±       | 1.48±bc     | 0.91±c      |
| Menesibu     | 10.32±a          | 3.80±       | 1.61±ab     | 0.91±bc     |
| Haru-1       | 9.66±a           | 4.22±       | 1.53±bc     | 1.03±ab     |
| Chalía       | 9.69±a           | 3.73±       | 1.48±bc     | 0.97±c      |
| Sende        | 10.99±a          | 4.35±       | 1.46±bc     | 1.05±b      |
| Std Err       | 0.33             | 0.16        | 0.05        | 0.03        |

Values are least-square means of triplicate measurements; levels not connected by the same superscript letter are significantly (p < 0.05) different. [TP = total polyphenol; CGA = chlorogenic acid; CAF = caffeine; TRG = trigonelline]

TRG were significantly (p < .05) varied by the growing location. TP and CGA were not significantly (p > .05) influenced by genotypes (Table 1). These results were in agreement with previous reports 22,39,40. CAF concentration dependence on origin was reported by previous researcher 7. The dependence of bioactive compounds on coffee genotype was also reported 40. The results implied that various bioactive compounds (TP, CGA, CAF, and TRG) significantly varied by the genotypes and centers, presenting a breeding potential to choose genotypes for specific locations for optimal concentration of the chemicals for health-promoting functionality.

**Processing influence on TP concentration**

The total polyphenol of Arabica coffee was significantly (p < .05) influenced by the processing methods (Figure 1 [a]). Coffee samples from the wet-processing method exhibited significantly higher concentration (10.79 mg GAE/100 g) than the dry-process counterparts (9.48 mg GAE/100 g). The wet processing method retained more TP compared to the dry processing technique. The results of the present work agree with previous reports. The uncontrolled handling and processing steps of dry processing under traditional practices in different parts of Ethiopia might have resulted in the degradation of the bioactive compounds. Coffee farmers dry red cherries by spreading them on the sidewalk, on the asphalt road, or just on the soil, which might increase the contamination of beans with dirt, causing the loss of aroma and flavor components.

**Processing influence on CGA concentration**

The CGA of Arabica coffee was significantly (p < .05) influenced by the processing methods (Figure 1 [b]). Coffee sample from the wet-processing method exhibited higher concentration (4.44% [db]) than the dry-processed process versions (3.52% [db])). The wet processing method might have retained larger concentrations of CGA due to the mild heat after soaking the cherries in water. Similar trends of higher biochemical concentrations of coffee beans and brews were reported by previous researchers. The present work indicated across 16 genotypes that wet-processed green coffee beans and their brews have higher concentrations of natural antioxidants having greater
health promotion functionality for consumers compared to the dry-processed equivalents of the same genetic materials.

**Processing influence on CAF concentration**
The CAF of Arabica coffee was also significantly (p < .05) influenced by the processing methods (Figure 1 [c]). Coffee samples from the wet processing method exhibited higher concentration (1.60% [db]) than the dried process equivalents (1.52% [db])). The wet processing methods resulted in a higher concentration of CAF, which gives the best stimulant in coffee. The ranges of CAF concentrations were less comparable to those reported by previous researchers .

**Processing influence on TRG concentration**
TRG concentration of Arabica coffee also varied significantly (p < .05) by the processing method (Figure 1 [d]), where samples from the wet processing method exhibited higher concentration (1.05% [db]) than the dried process counterpart (0.91% [db]). The wet processing method helps maintain TRG in the beans as it did for total phenolic compounds and CGA. Corresponding results were reported by previous authors . The overall observation is that the bioactive components of coffee beans are significantly varied, which presents a chance to choose

![Figure 1. Bioactive compounds as influenced by the processing methods; box plots of the same style (parameter) with different letters are significantly (p < .05) different.](image-url)
Table 2. Bioactive compounds of sixteen coffee genotypes by processing method.

| Processing* genotypes | TP (mg GAE/100 g) | CGA (% [db]) |CAF (% [db]) | TRG (% [db]) |
|-----------------------|-------------------|--------------|--------------|--------------|
| Dry, Feyate           | 8.45 f           | 3.66 c-h     | 1.46 d-j     | 0.86 e-f     |
| Dry, Odicha           | 9.51 d           | 3.63 d-h     | 1.52 c-i     | 0.87 d-f     |
| Dry, Angefa           | 9.96 f           | 3.56 f-h     | 1.58 b-i     | 0.93 f-i     |
| Dry, Qoti             | 8.97 f           | 3.45 g-h     | 1.75 a-e     | 0.95 f-i     |
| Dry, Anura            | 9.39 d           | 3.37 g-h     | 1.82 ab      | 0.87 d-f     |
| Dry, Bultum           | 10.08 d          | 3.37 g-h     | 1.42 d-i     | 0.92 f-i     |
| Dry, Mechara-1        | 9.75 d           | 3.49 f-h     | 1.67 a-h     | 0.94 f-i     |
| Dry, Mocha            | 10.22 d          | 3.37 g-h     | 1.49 b-i     | 0.98 f-i     |
| Dry, 744              | 9.61 f           | 3.72 c-h     | 1.74 a-f     | 0.86 f-e     |
| Dry, 741              | 8.84 d           | 3.47 g-h     | 1.42 d-i     | 0.93 f-i     |
| Dry, 7487             | 9.02 d           | 3.45 g-h     | 1.56 a-i     | 0.81 f-i     |
| Dry, 74110            | 9.46 d           | 3.82 b-h     | 1.40 e-i     | 0.89 d-f     |
| Dry, Menesibu         | 9.50 d           | 3.79 c-h     | 1.45 c-i     | 0.84 f-i     |
| Dry, Haru-1           | 9.49 d           | 3.59 e-h     | 1.35 g-i     | 1.03 f-i     |
| Dry, Challa           | 9.29 d           | 3.04 b-h     | 1.33 d-i     | 0.88 d-f     |
| Dry, Sende            | 10.21 d          | 3.65 c-h     | 1.37 g-i     | 0.93 f-i     |
| Wet, Feyate           | 10.53 d          | 4.88 a-c     | 1.29 ab      | 1.12 a-b     |
| Wet, Odicha           | 10.87 d          | 4.51 d-g     | 1.76 a-e     | 1.08 d-f     |
| Wet, Angefa           | 10.91 d          | 4.71 e-f     | 1.45 c-i     | 1.08 d-f     |
| Wet, Qoti             | 10.86 d          | 3.74 g-h     | 1.51 a-b     | 1.18 a-i     |
| Wet, Anura            | 10.31 d          | 4.27 a-g     | 1.39 f-i     | 1.02 f-i     |
| Wet, Bultum           | 10.69 d          | 4.46 a-g     | 1.83 a-i     | 1.07 e-a     |
| Wet, Mechara-1        | 11.41 a-b        | 4.80 a-e     | 1.41 a-i     | 1.17 a-i     |
| Wet, Mocha            | 11.04 a-c        | 5.24 a       | 1.61 a-i     | 0.98 f-i     |
| Wet, 744              | 11.05 a-c        | 4.83 d-d     | 1.79 c-c     | 1.03 f-i     |
| Wet, 741              | 11.00 a-c        | 4.09 e-h     | 1.65 a-i     | 0.93 f-i     |
| Wet, 7487             | 10.83 d          | 3.82 b-h     | 1.68 h-h     | 0.95 b-f     |
| Wet, 74110            | 10.26 d          | 3.63 g-h     | 1.55 a-i     | 0.94 c-f     |
| Wet, Menesibu         | 11.14 a-c        | 3.81 c-h     | 1.78 a-d     | 0.99 f-f     |
| Wet, Haru-1           | 9.83 d           | 4.85 a-d     | 1.71 a-g     | 1.02 f-e     |
| Wet, Challa           | 10.09 d          | 4.43 a-g     | 1.63 a-i     | 1.07 e-e     |
| Wet, Sende            | 11.78 a          | 5.04 ab      | 1.56 a-i     | 1.16 a-i     |
| Std Error             | 0.46             | 0.23         | 0.23         | 0.04         |

Values are least-square means of triplicate measurements; levels not connected by the same superscript letter are significantly (p < .05) different.

[TP = total polyphenol; CGA = chlorogenic acid; CAF = caffeine; TRG = trigonelline]

a processing method for coffee beans and brews that retain more of the desired health functionality.

**Influence of the interaction of processing method by genotype on bioactive compounds**

TP, CGA, CAF, and TRG were significantly (p < .05) influenced by the interaction of processing methods with genotypes (Table 2). The interactions of wet-processing for TP concentration with the Sende genotype (Haru Agricultural Research Center) exhibited higher TP (11.78 mg GAE/100 g). The interactions of the dry-processing with the Feyate genotype (from Awada Agricultural Research Center) exhibited a lower concentration (8.45 mg GAE/100 g sample) than the rest of the processing and genotype combinations. The interactions of the wet-processing with Mocha genotype (from Mechara Agricultural Research Center) had a higher concentration of CGA (5.24% [db]). The interactions of the dry-processing with Challa genotype of Haru Agricultural Research Center exhibited a lower concentration (3.04% [db]) than the rest of the combinations.

For CAF concentrations, Bultum genotype of Mechara Agricultural Research Center was recorded higher (1.83% [db]) and Feyate genotype of Awada Agricultural Research Center resulted in a lower (1.29% [db]) concentration. Higher TRG concentration (1.18% [db])
corresponded to the wet-processed Qoti genotype of Awada Agricultural Research Center, whereas lower TRG (0.81% [db]) corresponded to the dry-processed 7487 genotype from Jimma Agricultural Research Center than the remaining interactions. The wet-processed samples of all genotypes have generally higher bioactive concentrations except for CAF (Table 2). Similar trends with wet-processed samples providing better biochemical quality than the dry-processed equivalents were reported by previous researcher \[46\].

**Influence of preparation stages on bioactive compounds**

**Preparation stage influence on TP**

The TP of Arabica coffee was significantly (p < .05) influenced by the stages of coffee preparation (Figure 2 [a]). The preparation stages of coffee samples showed a declining trend in total polyphenol as the coffee passes from green beans to roast and then brewed. The concentration declined from green bean (15.24 mg GAE/100 g) to medium roasted bean (10.75 mg GAE/100 g), to brew (4.42 mg GAE/100 g). The declining trend in total polyphenol was also observed by different researchers \[47,48\]. The results of the current trial are slightly lower than previous reports \[49\] which might be due to differences in the coffee origins, processing, and sample preparations or perhaps due to the reaction of melanoids with Folien-Ciocalteu’s reagent.

![Figure 2. Bioactive compounds as influenced by the stages of preparation; box plots of the same style (parameter) with a different letter are significantly (p < .05) different.](image-url)
Preparation stage influence on CGA
The CGA of Arabica coffee was significantly (p < .05) influenced by the stage of preparations (Figure 2 [b]). Samples showed a declining trend in chlorogenic acids as the beans were roasted and converted to brews. CGA concentrations from green bean (6.88% [db]) reduced on medium roasting (3.35% [db]) to the final brew (1.72% [db]). The trends observed in the present work were similar to those from previous reports[42] and similar ranges of concentrations were reported in the literature [31,38,43,50]

Preparation stage influence on CAF concentration
CAF concentrations of Arabica coffee genotypes fluctuated significantly (p < .05) over the different stages of preparation (Figure 2 [c]). The preparation stages of coffee samples exhibited a different trend than the other bioactive components. CAF levels increased on medium roasting (1.92% [db]) from the levels in green bean samples (1.59% [db]) and then decreased in the brew (1.78% [db]) but remained higher than that of green. The observed increase in roasting might be due to loss of moisture and volatile fractions that might have resulted in increased concentration of the stable CAF on heating. A similar trend of CAF increment on roasting reported in the literature[42,43,49,51,52] with a slight difference in the increment rate. The differences in the rate of fluctuation might be due to differences in genotypes, geographic locations, processing methods, and the degree of roasting.

Preparation influence on TRG concentration
TRG concentrations of Arabica coffee were significantly (p < .05) influenced by the stage of preparations (Figure 2 [d]). Similar to total phenolic compounds and CGA, the preparation stages of coffee samples were characterized by a declining trend in TRG concentrations from green bean (1.32% [db]) to the roasted beans (0.96% [db]) and the brew (0.66% [db]). A constant reduction of TRG was reported during coffee roasting.[41]

Influence of the interaction of genotype by stage of preparation on bioactive compounds
In this study, the reduction of bioactive compounds was statistically significant (p < .05). The percentage of decline for TP, CGA, CAF, and TRG was 77.30%, 82.47%, 47.78%, and 70.58% respectively. The results implied that various bioactive compounds, such as TP, CGA, CAF, and TRG, declined over the roasting and brewing stages (Table 3). It established that bioactive compounds decline in concentrations at all coffee processing stages and brew preparation .[38,41,53] The decline reason might be the compounds are highly volatile and escape on heating (dry roasting and wet brewing).

Cup quality of coffee genotypes
The eight-cup quality attributes evaluated for the sixteen coffee genotypes include aromatic intensity, aromatic quality, acidity, astringency, bitterness, body, flavor, and overall cup quality. The evaluated cup quality attributes showed significant differences among genotypes except for astringency and bitterness. A similar result reported on cup quality differences of genotypes by researchers.[31] The interactions effect of genotype by processing methods also indicated a significant difference in cup quality attributes except for astringency, bitterness, and body.

The quality scores for dry and wet processing methods of green beans and brew ranged from 33.59 to 37.00 and 41.33 to 50.50, respectively. The quality scores for both processing methods of green beans and the coffee brew were significantly (p < .05) different (Figure 3). Processing methods and practices result in varying cup quality scores of coffee brews.[46] The scores of
green beans quality in the present study ranged from 31.67 to 38.00 for the dry and wet-processed Arusa and Feyate genotypes, respectively. Similarly, the score range of brew quality was from 39.17 to 51.00 for dry and wet-processed Menesibu and 74110 genotypes, respectively. The total scores for both processing methods ranged from 75.17 (dry processed Menesibu) to 87.00 (wet processed 74110) samples. Mocha genotype exhibited the highest brew and total scores among the tested genotypes. A similar range of scores was reported in the literature. \[^7\]
The green bean and the brew scores were not clearly and statistically segregated (Figure 3). A similar report was presented by other researchers. The cup quality data presented in the present work is the first of its kind in terms of comprehensiveness for the majority of the recently released genotypes of Ethiopia and are in line with the bioactive concentrations reported previously. There is a good contribution of these bioactive compounds to coffee quality in terms of flavor and health functionality. Higher concentrations of bioactive compounds result in a better flavor profile of the coffee beverage.

**Correlation of bioactive compounds and cup quality**

The concentrations of the bioactive compounds were significantly associated with the cup quality attributes (Table 4). Significant positive correlations (r ranging between 0.204 and 0.879) were observed between the TP and CGA with aromatic intensity; CAF with aromatic quality; CGA, CAF, and TRG with acidity, body, and overall cup quality. CAF (r = 0.204) and TRG (r = 0.264) had weak but significant positive correlation with flavor. CAF greatly contributes to the rich flavor formation of coffee brews. It was also observed that there was a strong positive correlation between the overall cup quality and the flavor of the brew, implying that flavor is the most important attribute for the overall quality of coffee brew. Similar associations between the different bioactive components and the cup quality of coffee have been reported in the literature. Increasing degradation of CGA in the brew is an indication of aroma formation. Other bioactive components also are reported to contribute to the cup quality of coffee.
Table 4. Correlation of bioactive compounds with cup quality of Ethiopian specialty coffee.

| Variable        | by Variable                  | Correlation | Count | Signif Prob |
|-----------------|------------------------------|-------------|-------|-------------|
| CGA, %          | TP, mg GAE/100 g             | 0.4089      | 96    | <.0001      |
| CAF, %          | CGA, %                       | 0.4755      | 96    | <.0001      |
| TRG, %          | TP, mg GAE/100 g             | 0.49        | 96    | <.0001      |
| Aromatic Intensity | CGA, %                      | 0.2642      | 96    | 0.0093      |
| Aromatic Intensity | TP, mg GAE/100 g           | 0.266       | 96    | 0.0088      |
| Aromatic Intensity          | CAF, %                       | 0.2672      | 96    | 0.0085      |
| Aromatic Quality          | Aromatic Intensity        | 0.2081      | 96    | 0.0418      |
| Aromatic Quality          | Aromatic Intensity        | 0.6736      | 96    | <.0001      |
| Acidity          | CGA, %                       | 0.2163      | 96    | 0.0343      |
| Acidity          | CAF, %                       | 0.2324      | 96    | 0.0227      |
| Acidity          | TRG, %                       | 0.236       | 96    | 0.0206      |
| Acidity          | Aromatic Intensity          | 0.3077      | 96    | 0.0023      |
| Acidity          | Aromatic Quality            | 0.4054      | 96    | <.0001      |
| Astringency     | Acidity                      | 0.2385      | 96    | 0.0193      |
| Bitterness      | Astringency                 | 0.6905      | 96    | <.0001      |
| Body            | CGA, %                       | 0.265       | 96    | 0.0091      |
| Body            | CAF, %                       | 0.2844      | 96    | 0.005       |
| Body            | TRG, %                       | 0.208       | 96    | 0.042       |
| Body            | Aromatic Intensity          | 0.245       | 96    | 0.0161      |
| Body            | Aromatic Quality            | 0.292       | 96    | 0.0039      |
| Body            | Acidity                      | 0.6078      | 96    | <.0001      |
| Body            | Astringency                 | 0.2305      | 96    | 0.0239      |
| Flavor          | CAF, %                       | 0.2041      | 96    | 0.046       |
| Flavor          | TRG, %                       | 0.2636      | 96    | 0.0095      |
| Flavor          | Aromatic Intensity          | 0.3401      | 96    | 0.0007      |
| Flavor          | Aromatic Quality            | 0.4592      | 96    | <.0001      |
| Flavor          | Acidity                      | 0.7651      | 96    | <.0001      |
| Flavor          | Astringency                 | 0.3843      | 96    | 0.0001      |
| Flavor          | Bitterness                  | 0.2875      | 96    | 0.0045      |
| Flavor          | Body                         | 0.6496      | 96    | <.0001      |
| Overall Cup Quality | CGA, %                       | 0.2364      | 96    | 0.0204      |
| Overall Cup Quality | CAF, %                       | 0.225       | 96    | 0.0275      |
| Overall Cup Quality | TRG, %                       | 0.2649      | 96    | 0.0091      |
| Overall Cup Quality | Aromatic Intensity         | 0.3488      | 96    | 0.0005      |
| Overall Cup Quality | Aromatic Quality           | 0.4643      | 96    | <.0001      |
| Overall Cup Quality | Acidity                      | 0.858       | 96    | <.0001      |
| Overall Cup Quality | Astringency                 | 0.3422      | 96    | 0.0006      |
| Overall Cup Quality | Bitterness                  | 0.2321      | 96    | 0.0229      |
| Overall Cup Quality | Body                         | 0.6988      | 96    | <.0001      |
| Overall Cup Quality | Flavor                       | 0.8794      | 96    | <.0001      |

**Conclusion**

Genotype, dry and wet processing methods, medium roasting, and brewing processes showed significant influences on the concentrations of bioactive compounds of specialty Arabica coffee genotypes. The processing stages observed on varying concentrations of the bioactive components, where the levels steadily decreased for most, except for caffeine, which increased on roasting and it decreased on brewing. There were significant positive associations between the different bioactive components and the cup quality attributes of the brews, indicating that the bioactive concentrations are responsible for the difference in flavor notes and their intensities.

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Author’s contributions statements

Yishak Worku Wondimkun: initiated the research concepts, conducted experiments, collected data, analyzed them, and write the manuscript; Shimelis Admassu Emire: supervised the activities, edited the concepts and drafts, and shaped the overall outlines; Tadesse Fikre Tefera: has supervised to design the experiment, to generate the graphics and edited the final version of the manuscript; Barbara Stoecker has supervised the experimental activities and commented on the drafts. Tarekeng Berhanu Esho: has contributed to the concept, and commented on the drafts.

Data availability statement

The data used for this research can be made available by email request to the first or correspondent authors.

Ethical reviews

The ethical aspect of the research was reviewed by the institutional review committee of the Ethiopian Public health Institute (EPHI). Informed consent was obtained from the coffee cuppers.

Additional information

There is no additional information regarding this manuscript.

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