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Impact of Conventional Processing Techniques and Growing Conditions on the Phenolic Content and Bioactive Compounds of Ethiopian Coffee Beans

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ABSTRACT

Background and Objective: Growing conditions, roasting, and brewing methods are some of the factors and a sequence of procedures determining brewed coffee's bioactive compounds and phenolic content. This work aimed to evaluate the impact of growing locations and efficient traditional coffee processing methods on better bioactive components and phenolic content of Ethiopian coffee. **Materials and Methods:** The efficient Ethiopian traditional coffee processing procedures with the commonly used medium particle size of coffee powder and widely used water type (surface water) were used for brewing by the Ethiopian traditional coffee brewing method (190°C of roasting, 16 min of brewing time). The HPLC was used to assess the phenolic content and determine the bioactive chemicals. The study used a two-factor factorial design with three treatment levels, considering roasting methods and coffee-producing areas (A and B). Data from five coffee origins were analyzed using ANOVA in SPSS (version 26) at a 5% significance level. **Results:** The caffeine content of Ethiopian Coffee Arabica origins ranged from 7.55 to 10.38 mg/mL, with Yirgachefe having the highest and Hararge the lowest ($p<0.05$). Chlorogenic acid levels were highest in Jimma, Sidama, and Nekemte (45 mg/mL) and lowest in Hararge (36.78 mg/g), while trigonelline was also highest in these three origins ($p>0.05$). Roasting significantly reduced total phenolic content (TPC) by an average of 27% and chlorogenic acid by 81% ($p<0.05$). Trigonelline decreased by 40% on average, with significant variation among coffee varieties ($p<0.05$). **Conclusion:** In general, the overall number of bioactive components and phenolic contents in processed coffee is influenced by growing regions and coffee processing. Further research may be necessary to determine how brewing temperature and extraction methods affect coffee's phenolic content and bioactive components.

KEYWORDS

Bioactive compounds, growing location, green coffee, traditional processing, roasting

INTRODUCTION

Coffee is becoming increasingly popular as a daily beverage and as a crop crucial to Ethiopian and international trade. In Ethiopia and around the world, coffee is one of the most commercially significant

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crops and one of the most popular daily beverages. Its consumption is also rising these days. According to a survey by the International Coffee Organization (ICO), 4 billion cups of coffee, or 9.4 million tons, were consumed worldwide in 2016-2017. These cups are made using various brewing techniques. Coffee is composed of several chemical constituent combinations that influence its flavor and aroma. These consist of alkaloids, phenolic compounds, vitamins, minerals, carbohydrates, lipids, and nitrogenous chemicals. In addition, coffee contains a lot of health benefits and is rich in bioactive substances, including trigonelline, caffeine, and chlorogenic acid[1]. Drinking coffee has been linked to a lower risk of hepatocellular carcinoma and vascular diseases[2], anti-proliferative effects on certain human cancer cells[3] and potential therapeutic benefits against Alzheimer's disease, liver disease, and diabetes[4-6].

The entire coffee process, from selecting coffee beans to choosing the best water for brewing to serving the coffee, affects the end product's quality and sensory attributes. To achieve a higher sensory score, coffee must have at least five distinct flavor notes in harmony[7,8]. The Specialty Coffee Association of America[9], criterion analysis procedure states that HPLC is used to analyze the bioactive compounds in roasted and brewed coffee. The coffee's ability to pass the aspect or preliminary grading and cupping tests can be used to assess its sensory and cup quality[9]. Important steps that affect the quality and quality of the final coffee product include sorting and grading green coffee beans, roasting, grinding, and different extraction methods[10].

Depending on the desired qualities of the coffee cup, dry coffee beans are roasted by heating them to temperatures ranging from 200-240°C for varying amounts of time[11]. Green beans lose a lot of water, which makes them fragile. Numerous metabolic pathways, including Strecker and Maillard, produce over 1000 distinct kinds of aromatic chemicals[12]. Alcohols, aldehydes, amines, carboxylic acids, Di carbonyls, enols, esters, furans, furanone, hydrocarbons, imidazole's, indoles, ketones, lactones, oxazole's, phenols, pyrazines, pyridines, pyrroles, quinoxalines, sulfur compounds, terpenes, and thiazoles are among the volatile substances found in roasted coffee beans[13,14]. Depending on the heat profile used during roasting, these chemicals may experience significant modifications. For this reason, roasting is the most crucial process in identifying the coffee bean's distinct flavor and color. The different roasting conditions have a big impact on the physical and chemical properties of roasted coffee beans.

After roasting, the coffee beans are ground to maximize their surface area and regulate humidity for effective extraction. While the caffeine content does not significantly drop after roasting, 20-40% of the polysaccharides that store cell walls are broken down[15,16]. Trigonelline's primary metabolites, nicotinic acid and N-methylpyridinium, are helpful markers of roasting level[17]. Metabolites created by microbes may infiltrate the beans after roasting to escape the heat process. The metabolites with the greatest ability to affect the final coffee beverage's quality are flavor-active esters.

Following roasting, complex physical and chemical changes, including caramelization, are produced by the Maillard and Strecker process, which involves hundreds of biological components[13]. The Maillard reaction, which transforms sugar into color, flavor, and fragrance, is catalyzed by amino acids. Acetic and formic acids are primarily responsible for the potent scent during the initial roasting stages. As quinic acid concentration rises, the breakdown of chlorogenic acids results in astringent and bitter flavors[18]. Roasting significantly lowers trigonelline and CGA while leaving caffeine unchanged[19]. Green beans' trigonelline, carbs, and some proteins also decompose into volatile chemicals, claim.

Coffee beans get their distinctive dark color from melanoidins, which are byproducts of the Maillard reactions and may help preserve the flavor components[20]. While trigonelline helps produce both acceptable and disagreeable smell molecules during roasting, caffeine accounts for more than 30% of the bitter taste[18]. Asparagine and glucose-fructose can promote the Maillard reactions, which result in the

production of unwanted substances like furan and acrylamides[20]. The Strecker degradation, a component of the Maillard reaction network, contributes volatile aldehydes to the coffee's aroma spectrum, including notes of honey, sulfur, malt, and potato.

Coffee origin, grinding size, and brewing time all affect the production of coffee aromas, the composition of bioactive components, and the antioxidant potential of brewed coffee. Coffee's flavor and aroma can change during the preparation process due to its biochemical makeup[21].

Ethiopian coffee is processed using very healthy methods, employing locally produced elements that are very tasty to consumers and quite beneficial after intake. The quality of Ethiopian coffee beans is therefore influenced by a variety of factors, including the growing environment, processing methods, and particularly the roasting process. Though there are some gaps in the current research on efficient coffee processing methods and the effects of coffee origin on the phenolic and bioactive compounds of brewed coffee, the coffee's quality and taste can also be greatly influenced by the coffee's origin and brewing materials. The majority of current studies, for instance, have concentrated on how coffee origin and processing methods affect the biochemical composition and cup quality of coffee beans from other nations. Furthermore, the majority of previous studies have employed rather small sample sizes, additionally, most of the existing research has used relatively small sample sizes, which makes it difficult to generalize the findings to the wider population.

Ethiopian traditional coffee processing techniques and their efficacy, in addition to the growing environment, affect the physico-chemical characteristics and cup quality of the brewed coffee. Not all of the main Ethiopian coffee beans have had their bioactive components and phenolic contents thoroughly examined for the effects of cultivation circumstances and efficient processing methods. This study was carried out to ascertain how the bioactive composition and physicochemical characteristics of Ethiopian coffee that is traditionally roasted and brewed, were affected by the growing environment and processing stages. This study aimed to evaluate the effects of processing procedures and growing locations on the bioactive composition, physicochemical properties, and sensory acceptability of Ethiopian brewed coffee.

MATERIALS AND METHODS

Study area and duration: In this study, five *Coffee arabica* varieties (Jimma/Limu, Sidama, Hararge, Nekemte, and Yirgachefe) were selected because they are the top highly produced and consumed varieties in Ethiopia. Furthermore, these varieties grow in different agro-ecological areas, which is the core to address the objective of the study to evaluate their influence on the biochemical composition of the beans. From each variety, a composite sample was collected from the major producing sites of the agro-ecological regions. The sampling was replicated in time and space three times in the harvest year of 2021/22. The samples were collected unwashed or dry-processed for wholesome coffee quality. The raw coffee bean varieties were physically examined for defects, odor quality, size, and shape, then packed and stored in a polyethylene bag.

The Hararge Zone is located in Ethiopia's eastern highlands and ranges in elevation from 974 to 3,264 m above sea level (mm.a.s.l.) (average 2047 m). The region receives 820.01 mm of rain on average each year. The average yearly temperature is between 15 and 20°C. Sand predominates in the sandy clay loam soil type found in this study area[22].

Sidama Zone: This research area is situated in the Southern Region of the country, in the Sidama Regional State. The average elevation is 1,694 m, with a range of 560 to 2300 mm.a.s.l. The average annual temperature is 27.2°C. Rainfall averages between 700 and 1,200 mm/year. In this study region, clay, silt, and sand are the most common soil types. In the region, there is a bimodal rainfall pattern that happens in the spring and summer[23].

In Ethiopia's Southern Nations, Nationalities, and Peoples' Region (SNNPR), the Yirgachefe zone is situated in the Gedeo Zone. The elevation of this town ranges from 200 to 1,919 mm.a.s.l. (average: 1,570 m). The zone's average annual temperature is between 18 and 25°C, and its annual precipitation ranges between 150 and 1,000 mm. In this region, nitisols are the predominant soil type[24].

Jimma Zone: Jimma lies in the western region of the country, with an average elevation of 1,657 m and an altitude range of 1200 to 3,020 mm.a.s.l. Rainfall is 1,500 mm annually. The maximum daily temperature in Jimma is between 24 and 27°C, making it extremely warm all year round. The predominant soil types in the zone are nitisols[25,26].

East Wollega Zone: The region includes a variety of topographic features, and the research area's elevation spans from 1,300 to 3,140 mm. a.s.l. (average 2,123 m). The region experiences 291.8 to 1325.6 mm of rainfall annually, with an average temperature of 21.5°C. Nitisol is the predominant soil type in the region[27,28].

Coffee beans: Ethiopian coffee beans were gathered from the Ethiopian Coffee and Tea Authority Coffee Quality Inspection and Certification Center (ECTACQICC) in Addis Ababa, Ethiopia, representing various Jimma, Hararge, Sidama, Nekemte, and Yirgachefe origins[29]. Three kilograms of each type of coffee were gathered. After cleaning and washing the coffee beans, any damaged seeds were taken out.

Standardizing the roasting and brewing process for Ethiopian traditional coffee: In ten Addis Ababa sub-cities, the traditional Ethiopian coffee-processing method was surveyed. Addis Ketema, Arada, Bole, Gullele, Kirkos, Kolfe Keranio, Ledeta, Knife's Silk Lafto, Akaky Kaliti, and Yeka were the ten sub-cities from which ten homes were chosen at random to participate in the study. The brewing process was then interviewed using questionnaires in each home. Actual measurements (weighed records) were also taken when needed[30]. A coffee processing flowchart was constructed based on the survey and simulated in a lab. This consistent procedure was used to prepare all of the brewed and roasted coffee powder samples.

Roasting: The coffee beans were roasted in a metal roasting pan measuring 16 cm in diameter and 76 mm in thickness. Charcoal cooking required 10 to 12 min, whereas electric roasting took 6 to 9 min at 190°C. The beans were roasted and then allowed to cool at room temperature for 11 min before being ground using a traditional wooden mortar and pestle to a sieve size of 1.2 mm.

Coffee brewing: The coffee bean was roasted for 16 min at a maximum temperature of 190°C, which is the standard and advised level of roast[31]. The exothermic reaction was then terminated by leaving the air-cooled coffee beans overnight to allow for full taste development and CO_2 degassing. The roasted coffee beans were ground into fine (2 mm), medium (4 mm), and coarse (6 mm) powder sizes using an electronic laboratory-scale coffee grinder (VTA6S, MAHLKÖNIG GmbH and Co., 2009)[29]. Following that, the ground coffee samples were placed in airtight, odor-proof polyethylene bags. Before brewing and analysis, the sample was kept at 4°C and 70% relative humidity. Coffee was made using surface water and 1:17 (w/w) ratios of coffee powder to water[32].

Sample extraction for bioactive compounds: To extract the total phenolic, flavonoid content, and bioactive components present in green and roasted coffee, the methodology of Iriondo-DeHond *et al.*[33] was adopted. In summary, 3 g of ground green coffee was mixed with 20 mL of 98% methanol. Each coffee sample was mixed with 20 mL of 98% methanol. The samples were placed in Erlenmeyer flasks in a shaking incubator (Lucadema, Brazil) for 30 min at 40°C and 120 rpm and then centrifuged for 15 min at 3200 rpm in an Excelsa Baby II centrifuge (Flamen, model 206-R). The supernatant's phenolic compounds were detected. Three extractions were performed.

Determination of total polyphenol content (TPC): According to Stalmach *et al.*[34] the Folin-Ciocalteu method was used to estimate the TPC. Gallic acid was used to generate the TPC measuring standard curve. Gallic acid equivalent (GAE)/g of the dry matter of coffee samples was used to express the results. One milliliter of 99% ethanol was added to standard solutions of gallic acid, which included 2, 4, 6, 8, 10, and 12 mg of gallic acid. After that, three milliliters of distilled water were added. Next came the addition of 0.1 mL of Folin-Ciocalteu Reagent. Three min later, distilled water was added to the volumetric flasks to bring them up to the mark, and then 0.3 mL of a 2% sodium carbonate solution was added. The entire material was stored for two hours at 25°C. The gallic acid solution was then substituted with 100 mL of ethanol. The spectrophotometer's wavelength was set to 765 nm after two hours. The absorbance of each solution was measured three times, and the average was recorded after the device was initially zeroed with the control (gallic acid) solution. Following the development of a standard curve that plotted concentration (µg) against absorbance, the line equation was applied to the quantification.

Ten milligrams of the green bean extract were weighed, placed in a test tube, and then completely dissolved with 2 mL of Dimethyl Sulfoxide (DMSO) solution to create the coffee samples. Triplicate assays were performed on each extracted sample using three 5 mL volumetric flasks. There were 0.20 mL of each extract and 3 mL of distilled water in each volumetric flask. The volumetric flasks were then filled with 100 µL of Folin-Ciocalteu Reagent, and 3 min later, 0.3 mL of sodium carbonate (2%) was added. Distilled water was added to the volumetric flasks to bring them to the mark.

Instead of the extract, 0.20 mL of DMSO was added to a 5 mL control volumetric flask. The mixture was homogenized and then stored for 2 hrs at 25°C. After two hours, the absorbance of each solution was measured three times at 765 nm using a UV-Vis spectrophotometer (PerkinElmer, Model CF728YW-950, and UK). The TPC was expressed as µg of GAE/g of extract by Stalmach *et al.*[34] using Eq. 1:

$$TPC = \frac{C \times V}{M} \times D \quad (1)$$

Where:

C = Gallic acid equivalent concentration obtained from the calibration curve (g/mL)
V = Volume of the extract's stock solution (mL)
M = Dry weight of the extract in the stock solution (g)
TPC = Phenolic content expressed as (mg of GAE/g dry extract)
D = Dilution factor

Bioactivity analysis

Caffeine, trigonelline, and chlorogenic acid extraction: A significantly modified technique was used to extract CAF, CGA, and TRG. A 50 mL Erlenmeyer flask containing roughly 0.5 g of finely ground coffee grounds was carefully weighed. Stir on a hot plate for 20 min after adding 50 mL of heated (95°C) pure water. About 10 µL of the extract was placed in an Agilent 1260 Infinity, Germany, High-Performance Liquid Chromatography (HPLC) after It had been filtered through No. 4 Whatman filter paper and a 0.22 µm pore size filter.

Measurement of caffeine, trigonelline, and chlorogenic acids: Using a method adapted from TRG, CGA, and CAF were measured concurrently using an HPLC system with a C18 column and an isocratic flow of 0.7 mL/min. A 4.6×250 mm column with a 5 µm particle size was used by Waters, Taunton, USA. At a flow rate of 0.7 mL/min, the following elute compounds were present in gradients with 5% acetic acid (A) and acetonitrile (B): 4% B for 0-4 min, 10% for 4-8 min, 90% for 8-12 min, 0% for 12-15 min, and 4% for 15-17 min. A three-minute post-run was held. The TRG and CAF were measured at 272 nm, but CGA was

detected at 320 nm. TRG and CAF test concentrations were 5, 10, 20, 40, 50, 100, and 150 µg/mL. The ranges for CGA, on the other hand, were 5, 10, 20, 100, 150, and 200 µg/mL. The identities of CAF, TRG, and CGA were ascertained by comparing the retention durations of the TRG (Sigma Aldrich), CGA (Acros Organics), and CAF (Fischer Scientific) standards (99%), as well as their concentrations derived from peak regions using calibration equations. Following a time of retention, the area was utilized to determine the composition of the related biological component, which was then determined using calibration curves that plotted the area against standard concentration[34]. The limit of detection (LOD) for caffeine, trigonelline, and chlorogenic acid was 0.9, 1.02, and 1.5 µg/g, respectively, and the limits of quantification (LOQ) for caffeine, trigonelline, and chlorogenic acid were 1.7, 2.3, and 3.0 µg/g, respectively.

Statistical analysis: The study employed two-factor factorial experimental designs with three treatment levels. Roasting and coffee-producing areas (A and B) were the chosen criteria. Five coffee origins-Jimma, Sidama, Yirgachefe, Hararge, and Nekemte coffee-were employed for brewing purposes, and green and roasting methods. At least three observations were made during the fully randomized experimental design. Using SPSS software version 26, the collected experimental findings were examined and interpreted using Analysis of Variance (ANOVA) at a 5% significance level.

RESULTS AND DISCUSSION

A standardized Ethiopian coffee brewing method was developed in the lab using survey data from Addis Ababa. The process involves roasting 30-35 g of beans using charcoal or electric heat, grinding to 1.2 mm, and brewing 25-30 g in 750-800 mL of water across three rounds-Abol, Tona, and Baraka. Acrylamide levels were then measured in the brewed coffee. This approach helps assess dietary acrylamide exposure from traditionally prepared coffee[35,36].

Impact of Ethiopian traditional coffee roasting on the biochemical contents: The impact of roasting Ethiopian coffee on the biochemical makeup of coffee beans from various agroecological origins is documented in Table 1. The caffeine concentration ranged from 7.55 to 10.38 mg/mL for the five Ethiopian Coffee Arabica cultivars. Yirgachefe coffee beans had the highest caffeine content, while Hararge coffee beans had the lowest ($p<0.05$). All cultivars have a substantial variation in caffeine concentration ($p<0.05$), except for Nekemte and Yirgachefe. As evidence for this work, several investigations have reported varying quantities of caffeine in coffee beans[37]. For instance, used HPLC techniques to find that the caffeine concentrations in 42 Ethiopian coffee samples ranged from 9.6±0.01 to 12.3±0.06 mg/mL, with an average value of 10.10 mg/mL. Other studies have shown that the average caffeine content of Arabic coffee is less than 15 mg/mL[37]. The caffeine concentration of this study is higher than other study[38] investigations, which discovered that the caffeine content of various coffee varieties ranges from 9 to 25 mg/g. The highest levels of chlorogenic acid ($p>0.05$) were found in the Jimma, Sidama, and Nekemte coffee varietals (45 mg/mL) in comparison to the other two. With 36.78 mg/g of chlorogenic acid, the Hararge cultivar had the lowest levels. Chlorogenic acid levels in several coffee bean varietals ranged from 4.1 to 11.3 mg/g[38]. This number is greater than the one found in the current study. Trigonelline levels were also highest in the Jimma, Sidama, and Nekemte coffee varietals, with 12.88, 13.56, and 13.46 mg/mL, respectively ($p>0.05$). Hararge and Yirgachefe kinds had the lowest concentrations, with respective values of 11.65 and 11.78 mg/mL ($p>0.05$).

Every home uses Ethiopian coffee, which is roasted and brewed distinctively. Reports on its impact on the biochemical makeup of the regional coffee types are, nevertheless, few. As seen in Table 1, roasting significantly decreased the TPC ($p<0.05$) for all coffee beans. The percentage reduction was: Jimma (24%), Sidama (26%), Nekemte (30%), Yirgachefe (23%), and Hararge (29%), had the lowest percentage of TPC. On average, there was a 27% drop. The caffeine content of the all-growing regions of Sidama, Nekemte, Hararge, Jimma, and Yirgachefe coffee beans has not significantly varied after roasting ($p<0.05$). Among

Table 1: Impact of traditional ethiopian coffee roasting on caffeine, trigonelline, chlorogenic acid, and total phenols in green and roasted coffee across agro-ecological zones

Coffee origin	Coffee type	Total polyphenols (mg GAE/g)	Caffeine (mg/mL)	Chlorogenic acid (mg/mL)	Trigonelline (mg/mL)
Jimma	Green coffee	46.52±0.82^{a}	8.85±0.00^{b}	45.95±0.01^{a}	12.88±0.01^{ab}
	Roasted	35.56±1.08^{cd}	8.84±0.00^{b}	7.84±1.08^{de}	5.88±1.00^{f}
idama	Green coffee	44.31±4.68^{ab}	8.23±0.00^{c}	45.29±0.01^{a}	13.56±0.02^{a}
	Roasted	32.87±0.05^{d}	8.11±0.01^{d}	9.38±0.91^{d}	9.60±0.66^{cd}
Nekemte	Green coffee	44.55±3.28^{ab}	10.18±0.00^{a}	46.39±0.01^{a}	13.46±0.01^{a}
	Roasted	31.36±0.19^{de}	9.88±0.10^{b}	8.43±0.69^{de}	7.33±0.67^{de}
Yirgachefe	Green coffee	34.25±3.83^{c}	10.38±0.00^{a}	39.54±0.01^{b}	11.78±0.00^{b}
	Roasted	26.56±0.91^{f}	9.92±0.07^{b}	7.66±0.84^{ef}	7.27±1.19^{de}
Hararge	Green coffee	39.02±2.93^{bc}	7.55±0.00^{e}	36.78±0.02^{c}	11.65±0.00^{b}
	Roasted	27.86±1.01^{ef}	7.45±0.03^{e}	6.46±0.89^{f}	7.64±0.64^{de}
Average	Green coffee	41.73±0.43*	9.51±0.66*	42.79±0.67*	12.67±0.47*
	Roasted	30.84±0.75	8.84±0.45	7.95±0.46	7.54±1.34

On a dry basis, the data are presented as Mean±SD (n = 3). Significant differences at $p<0.05$ in mean comparison using the Duncan's multiple range test are shown by mean values with different superscript letters within a column and *Uses the paired t-test to show a significant difference between green and roasted coffee beans ($p<0.05$)

all coffee varietals, the chlorogenic acid content had the largest significant percentage decrease ($p<0.05$). Jimma (83%), Sidama (79%), Nekemte (82%), Yirgachefe (81%), and Hararge (82%) were the countries with the largest percentage decreases. The average reduction was 81% overall. Furthermore, upon roasting, there were significant differences in the trigonelline content across all coffee kinds ($p<0.05$). With Jimma (54%), Sidama (29%), Nekemte (45%), Yirgachefe (38%), and Hararge (34%), the average reduction was 40%.

The average CGA level in the current study ranged from 45.95 to 36.78 mg/g on a dry weight basis, while the average caffeine content ranged from 10.38 to 8.23 mg/mL. According to Illy and Navarini[37], these values are often in the lower range of values previously observed for green Arabica coffee beans. Our caffeine measurements were marginally higher for both CAF and CGA[39].

Chlorogenic acid content in brewed and roasted coffee ranged from 16 to 33%. This is in line with the range discovered by Farah[40], who demonstrated that spent and roasted coffee still had 23-68% chlorogenic acid. About 23-36% of the caffeine was still present in the wasted coffee. Retention rates for Gallic and protocatechuic acids were lower in comparison, at 4-13 and 4-24%, respectively. These findings show that the retention rates of each component differ according to its water solubility and high-temperature stability.

Chlorogenic acids are the primary phenolic components found in green coffee. According to Patil[41], they are esters of quinic acid and trans-hydroxycinnamic acid. It is known that these chemical groups are responsible for the color, flavor, bitterness, and astringency of coffee beverages[42]. About 6-12% of green coffee beans' dry weight is composed of chlorogenic acids. When compared to the other two coffee kinds in this investigation, the Jimma, Sidama, and Nekemte varietals had the greatest levels of chlorogenic acid ($p>0.05$), at 45 g/100 g. With 36.78 g/100 g of chlorogenic acid, the Hararge variety exhibited the lowest levels (Table 2). There is an inverse relationship between a coffee variety's chlorogenic acid concentration and beverage quality; coffee types with lower beverage quality had higher chlorogenic acid concentrations[43]. The green coffee beans gathered from nine districts in Southwest Ethiopia have a chlorogenic acid level ranging from 2.80 to 5.42 g/100 g[38,44]. According to Illy and Navarini[37]. the amount of chlorogenic acids in green coffee beans gathered from Northern Ethiopia ranged from 3.29 to 7.73 g/100 g. According to Faran[40], the amounts of chlorogenic acid in various coffee types ranged from 4.1 to 11.3 g/100 g. Compared to the current study, the chlorogenic acid content was lower in all of these earlier investigations. According to Mengistu *et al.*[45], chlorogenic acids exhibit potent antiviral, antidiabetic, antioxidant, and neuroprotective properties.

Table 2: Total phenolic, flavonoid, trigonelline, chlorogenic acid, and caffeine content in Ethiopian Green Arabica Coffee varieties from different agro-ecological zones

Bioactive compounds	Jimma coffee	Sidama coffee	Yirgachefe coffee	Nekemte coffee	Hararge coffee
TPC (mg GAE/100 g)	46.52±0.82^{a}	44.31±4.68^{ab}	34.25±3.83^{c}	44.55±3.28^{ab}	39.02±2.93^{bc}
TFC (mg QE/100 g)	45.10±0.36^{ab}	37.30±0.75^{bc}	37.80±0.55^{bc}	50.10±0.23^{a}	31.50±0.57^{c}
Caffeine (g/100 g)	8.85±0.00^{b}	8.23±0.00^{c}	10.38±0.00^{a}	10.18±0.00^{a}	7.55±0.00^{e}
Chlorogenic acid (g/100 g)	45.95±0.01^{a}	45.29±0.01^{a}	39.54±0.01^{b}	46.39±0.01^{a}	36.78±0.02^{c}
Trigonelline (mg/100 g)	12.88±0.01^{ab}	13.56±0.02^{a}	13.46±0.01^{a}	11.78±0.00^{b}	11.65±0.00^{b}

On a dry basis, the data are presented as Mean±Standard Deviation (n = 3). In mean comparisons using Duncan's multiple range test, mean values within a row with different superscripts indicate a significant difference at $p<0.05$ using One-way Analysis of Variance (ANOVA), GAE: Gallic acid equivalent, QE: Quercetin equivalent, TPC: Total phenolic content and TFC: Total flavonoid content

Accordingly, the highest trigonelline levels were found in the Jimma, Sidama, and Yirgachefe coffee varietals, with 13.56, 13.46, and 12.88 mg/100 g, respectively ($p>0.05$). The Nekemte and Hararge types had the lowest concentrations, measuring 11.78 and 11.65 mg/100 g, respectively ($p>0.05$) (Table 2). The amount of trigonelline in coffee beans gathered from nine districts in Southwest Ethiopia varied significantly (from 0.80 to 1.08 g/100 g), according to Illy and Navarini[37]. Trigonelline levels in green coffee beans gathered from the country's north ranged from 0.53 to 1.27 g/100 g, according to Illy and Navarini[37]. Trigonelline levels were lower in these earlier investigations than in the current one, suggesting that it is dependent on agro-ecological variation. After caffeine, trigonelline, often referred to as N-methylpyridinium-3-carboxylate, is the second most prevalent alkaloid in coffee[41]. According to Gichimu *et al.*[46] Coffee is the primary source of trigonelline; however, it can also be found in barley, corn, tomatoes, peas, onions, and soybeans.

Overall, differences in the growing environment circumstances (i.e., altitude, soil type, rainfall, and other agricultural techniques) can be associated with variations in the TPC, TFC, caffeine, chlorogenic acid, and trigonelline content among coffee regions[32]. For instance, compared to Hararge and the Southern regions, the Southwestern, Western, and Northwest regions experience more rainfall overall and a longer rainy season[47]. This greatly impacts the TPC[48]. Climate adaptation measures and other management circumstances may counteract these environment-driven variations in crop quality. Effects on crop quality, however, have been the subject of fewer studies[49].

For example, the volatiles 2, 3-butanedione, 2, 3-pentanedione, 2-methylbutanal, and 2, 3-dimethylpyrazine were among the significant odor-active indicators of coffee that changed as a result of these regional differences[49-51]. Therefore, to anticipate coffee quality using evidence-based innovations of climate adaptation, it is required to evaluate the biochemical composition of coffee beans throughout time. In addition to assessing the impact of geographic origins on biochemical composition, this study is designed to add to the database.

CONCLUSION

Geographic origin and processing technique are thought to affect coffee's quality and biological makeup. The current study's findings demonstrated the significant bioactive chemicals and phenolic contents found in coffee from Ethiopia's five main production regions: Jimma/Limu, Sidama, Hararge, Nekemte, and Yirgachefe. Additionally, it was confirmed that roasting the coffee beans decreased their bioactive chemicals. The total TPC and other bioactive components have been considerably decreased after roasting all of the coffee beans. The amount of chlorogenic acid was reduced by the largest percentage after roasting. Nevertheless, roasting did not significantly alter the caffeine level of the various coffee varietals. For evidence-based climate change adaptation aimed at producing high-quality coffee, updated data on the biochemical makeup of coffee varietals throughout time can be utilized. This study proved the efficient methods and techniques of Ethiopian traditional coffee processing, starting with the collection, roasting, grinding, brewing, and serving of coffee, together with the whole range of materials utilized in the operations. The overall green and roasted coffee's total phenolic and bioactive chemical content was compared with the growth area and roasting influence.

SIGNIFICANCE STATEMENT

This study discovered the superior coffee origins and varietals with enhanced biochemical composition, cup quality, and bioactive ingredient levels that can be beneficial for breeders, growers, processors, and consumers seeking high-value and nutritionally rich coffee. It further identified the optimal processing and brewing techniques, including water quality and powder size, ensuring the production of safe and premium-quality coffee. The most suitable Ethiopian sites for coffee cultivation were also recommended based on production benefits. Additionally, the efficiency and effectiveness of traditional Ethiopian coffee processing methods were critically assessed. This study will help the researchers to uncover the critical areas of traditional processing impacts on coffee quality that many researchers were not able to explore. Thus, a new theory on indigenous processing optimization may be arrived at.

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