Hindawi
Journal of Food Processing and Preservation
Volume 2023, Article ID 8831024, 5 pages
https://doi.org/10.1155/2023/8831024

Research Article

Analysis of Caffeine and Antioxidant Content of Ethiopian Coffee Varieties from Different Growing Areas

Beatrix Sik, Erika Lakatos, Henrietta Bura, and Rita Székelyhidi

Department of Food Science, Széchenyi István University, Lucsony Street 15-17, 9200 Mosonmagyaróvár, Hungary

Correspondence should be addressed to Rita Székelyhidi; szekelyhidi.rita@sze.hu

Received 6 July 2023; Revised 22 August 2023; Accepted 2 September 2023; Published 11 September 2023

Academic Editor: Miguel Rebollo-Hernanz

Quality coffee consumption is increasing nowadays; however, quality can only be achieved through appropriate cultivation and processing techniques. The study is aimed at using high-performance liquid chromatography (HPLC) and spectrophotometric techniques for evaluating different coffee varieties grown in Ethiopia based on caffeine, antioxidant, and polyphenol content. Despite coming from various coffee-growing regions, all of the tested coffee varieties (heirloom, forest coffee, heirloom 2, bourbon, mixed heirloom, and catuai) underwent wet processing. The data revealed that the caffeine content ranged from 0.14 to 0.37 mg/g, total antioxidant content from 63.75 to 78.05 mg AAE/g, and total polyphenol content from 27.08 to 31.04 mg GAE/g in the selected varieties. The findings show that the amount of the identified compounds is significantly ($p \leq 0.05$) influenced by the variety type, growing area, and processing (intensity of roasting). The different coffee varieties were also tested for sensory quality, and the findings suggest that the typical coffee drinker prefers fruity flavors in their coffee, along with medium acidity and roasting intensity levels. The cultivation and processing technologies used in coffee production have a significant impact on the quality and sensory enjoyment of coffee.

1. Introduction

The world's fifth-largest producer of coffee is Ethiopia. Coffee is the primary source of income for countries that grow coffee, which includes more than 80 nations, including Ethiopia [1]. Coffee is the second most traded good after oil [2].

Harar, Southern, Southwestern, and Northwestern are the four main regions of Ethiopia where coffee is grown, and in each of these regions, different environmental factors and coffee cultivation techniques are used. In Ethiopia, coffee is grown in four different ways: in the forest, semiforest, gardens, and plantations [3]. Ethiopia has expanded its coffee-growing areas in recent years, but no interventions have been made to increase yield or quality [4]. However, the climate significantly impacts the characteristics of the coffee plant and product [5, 6].

Coffea arabica L. is a perennial plant species from the *Coffea* genus that belongs to *Rubiaceae* family. It typically grows in tropical and subtropical regions [7, 8] and is the most widely grown coffee variety in Ethiopia. This variety has high levels of heterogeneity [9], which allows the nation to produce and market coffees with distinctive qualities. The genetic component and cultivation and processing practices significantly impact the physical characteristics and cup quality of coffee [10]. Recent research has shown that the yield, quality, and chemical makeup of Ethiopian coffees are also influenced by the cultivation region [11], environmental factors [12], harvest time [13], and postharvest processing techniques [14, 15].

The study is aimed at comparing the sensory qualities and caffeine, antioxidant, and polyphenol content of coffee on the basis of varieties and growing regions in Ethiopia.

2. Materials and Methods

2.1. Chemicals. For the HPLC analysis, a caffeine (>99%, Merck, Germany) standard was used for the calibration curve, and a mixture of acetonitrile (Merck, Germany), phosphoric acid (LiChrosolv, Merck, Germany), and high-purity water was used as eluent. For the determination of

total antioxidant content with the FRAP assay, sodium acetate (Merck, Germany), 2,4,6-tripyridyl-s-triazine (TPTZ) (Sigma-Aldrich, USA), anhydrous iron chloride (Merck, Germany), ascorbic acid (Sigma-Aldrich, USA), acetic acid (Reanal, Hungary), and 37% hydrochloric acid (Reanal, Hungary) were used. Anhydrous sodium carbonate (Riedel-de Haën, Germany), gallic acid (Sigma-Aldrich, USA), and Folin-Ciocalteu reagent (Fischer Chemical, USA) were used for the determination of total polyphenol content with the Folin-Ciocalteu method.

2.2. Coffee Samples. The coffee samples were obtained by ordering them from an online store which provides the consumer with all the cultivation and processing characteristics of the sold coffees. In the study examined only Arabica coffee varieties that are grown in Ethiopia. All investigations were performed in three replicates. The green coffee beans were roasted for 5 to 15 minutes at 200°C. The characteristic features and growing regions of the coffee under investigation are listed in Table 1.

2.2.1. Examination of the Sensorial Properties of Coffee Varieties. Sensorial testers were average consumers with average sensory sensitivity. Testers used a questionnaire to assess the samples. The study involved 21, with 16 women and 5 men participating. The respondents were 37 years old on average. 20 g of roasted and ground coffee samples was combined with 500 mL of water to create the coffee drinks using an espresso coffee maker [16]. The Food Industry Manual's Part 7 served as the basis for creating the assessment sheet [17].

2.2.2. Sample Preparation for HPLC Analysis. Based on the methodology of Nhan and Phu [18], caffeine extraction and HPLC determination were carried out, with some adjustments made by Shrestha et al. [19]. The coffee beans were finely ground using a coffee grinder (Bosch), and 0.3 g of the sample was weighed into 250 mL Erlenmeyer flasks with an analytical balance (TE 214S, Sartorius). The samples were added to 200 mL of ultrapure water (Zener Power I Scholar-UV, Human Corporation) and then submerged for 30 minutes in a 100°C water bath. After the sample solutions had cooled, they were centrifuged (Z206A, Hermle) at 6000 RPM for 20 minutes. 1 mL of the supernatant was then pipetted into volumetric flasks marked at 10 mL, and the remaining used eluent was added to the mark. About 1 mL of the diluted solutions was filtered through a 0.22 μm hydrophilic syringe filter and placed into 1.8 mL HPLC vials.

2.2.3. Sample Preparation for Spectrophotometric Analysis. The active compounds were extracted from the matrix using solvent extraction to calculate the levels of polyphenols and antioxidants in coffee samples [20]. 3.5 g of the ground coffee beans was weighed and put into 100 mL Erlenmeyer flasks on an analytical balance. The samples were centrifuged (Z206A, Hermle) at 6000 RPM for 20 minutes after being centrifuged for 5 minutes in 60 mL of high-purity water. In order to calculate the antioxidant content, 1 mL of the supernatant was pipetted into 10 mL volumetric flasks and completely filled with high-purity water. The samples did not require dilution after centrifugation to determine the total polyphenol content.

2.3. Determination of Caffeine Content with HPLC-UV Equipment. Reverse-phase liquid chromatography was used to determine the amount of caffeine in the coffees. The stationary phase was a LiChrospher 100 C-18 column (5 μm), which had the following specifications: a length of 250 mm, an inner diameter of 4 mm, and a temperature of 40°C. Acetonitrile-0.05% phosphoric acid solution (10 : 90 V/V%) was the mobile phase, flowing at a rate of 1 mL/min. The amount of the injected sample was 5 μL, and the detection was done using a UV detector at a wavelength of 275 nm. As a standard, caffeine solutions (0.2–30 g/mL) were used.

2.4. Determination of Total Antioxidant and Polyphenol Content

2.4.1. FRAP Assay. The process used for the FRAP assay is based on the approach outlined by Benzie and Strain [21]. 100 mL of water, 3 mL of FRAP solution, and 50 μL of the extracted coffee sample were pipetted into a test tube. After being left in the dark for 5 minutes, the finished solutions were compared to a blank solution made up only of water and FRAP solution using a Spectroquant Pharo 100 spectrophotometer (Merck, Germany). The results were expressed as ascorbic acid equivalent (AAE)/g dry matter using ascorbic acid as standard (40-500 mg/L).

2.4.2. Folin-Ciocalteu Assay. Total polyphenol content was determined using a modified version of the Folin-Ciocalteu method as described by Singleton et al. [22, 23]. 1.5 mL of ultrapure water was pipetted into test tubes with 50 μL of coffee extract before adding the reagents. 2 mL of a 7.5% Na_2CO_3 solution should be added after 2.5 mL of 10% Folin-Ciocalteu reagent. The mixture-containing tubes were left in a dark location for 90 minutes, after which the absorbance at 725 nm was compared to the blank, which contained the reagents and water. The used standard solutions were made with gallic acid (25–1000 mg/L).

2.5. Data Analysis. By using the equation of the second-order least squares analytical curve fitted to the measurement solutions by the nonlinear least squares method, the absorbance values measured for samples were converted into the caffeine, total antioxidant, and polyphenol contents. This information was then entered into Microsoft Office Excel to be calculated. In order to compare the significant difference in the data ($p < 0.05$), analyses of variance (ANOVA) were used. All results are expressed as means $(n = 3) \pm$ standard deviation.

3. Results and Discussion

3.1. Sensorial Test Results. The results of the sensorial test are shown in Figure 1. Due to its dark color, heirloom coffee had the lowest popularity and catuai coffee had the highest. Customers favor chocolate brown, medium-roasted coffees over black, heavily roasted samples.

Table 1: Cultivation and physicochemical parameters of the examined coffee varieties ($n = 3$).

Coffee varieties	Region	Altitude (m)	Taste	Processing	Acidity	Roasting
Heirloom	Kaffa	1500	Fruity, winey	Wet	High	Very dark
Forest coffee	Kaffa	1500	Floral, grapey	Wet	High	Medium
Heirloom 2	Oromia	1800	Floral, fruity	Wet	Low	Dark
Bourbon	Sidamo	1500-2200	Chocolate	Wet	Medium	Medium
Mixed heirloom	Yirgacheffe	2200	Floral	Wet	Medium	Dark
Catuai	Sidamo	2150-2500	Blueberry, pineapple, plum	Wet	Medium	Medium

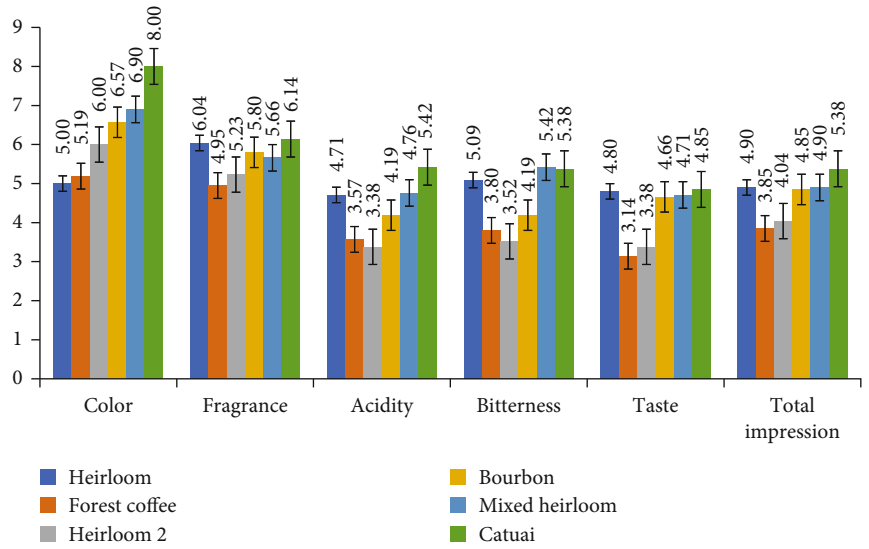

Figure 1: Results of the sensorial examination of coffee samples.

The coffee samples from catuai and heirloom were rated as having the best aromas and a mildly fruity flavor. Forest coffee, which some respondents said smelled like tobacco, was the least preferred coffee scent.

Catuai coffee was the most popular coffee in terms of acidity, while heirloom 2 was less well liked due to its low acid content and forest coffee was less well liked due to its high acidity.

In terms of bitterness, reviewers rated mixed heirloom and catuai coffees as the best. In this regard, heirloom 2 and forest coffee performed less well. There are similarities in how coffee's acidity and bitterness are rated.

The most well-liked flavors were fruity-tasting catuai and heirloom coffee. The least preferred coffee flavor was forest coffee, which many tasters claimed had a tobacco flavor, possibly explaining its floral undertones.

The catuai coffee was the most well liked overall. The sensorially evaluated coffees that received the least favorable ratings from the participants were forest coffee and heirloom 2.

3.2. Caffeine, Antioxidant, and Polyphenol Content of Ethiopian Coffee Samples. The findings from the analysis of samples of Ethiopian coffee's caffeine, antioxidant, and polyphenol content are displayed in Table 2.

According to the findings, the heirloom coffee variety stands out because its caffeine content is significantly ($p \leq 0.05$) lower than that of the other coffee varieties at 0.14 mg/g. This might be a result of the coffee being chosen to come from a particularly low altitude (1500 m), where it is likely that it did not need to be protected from insects in the growing area, unlike the other coffee plants. Because coffee beans contain more caffeine before roasting, the low caffeine content is also a result of the very dark roasting. The amounts of caffeine in the coffee varieties forest coffee, bourbon, mixed heirloom, and catuai were all 0.36 mg/g, while those in heirloom 2 were 0.37 mg/g. The investigated caffeine level values for the various species are consistent with Miłek et al. [24] published data.

The FRAP technique was successful in detecting the total antioxidant content. With the exception of bourbon-type coffee, which had a value of 64.75 mg AAE/g, the antioxidant content of the coffee samples decreased with altitude. The bourbon (64.75 mg AAE/g) and catuai (63.75 mg AAE/g) coffee varieties from the Sidamo and heirloom (78.05 mg AAE/g) and forest coffee (71.84 mg AAE/g) from the Kaffa region showed the difference in TAC value, which can be attributed to their region of cultivation. According to the article by Tasew et al. [25], the degree of roasting is another factor that influences the total antioxidant content in

Table 2: Results of caffeine, total antioxidants (TAC), and total polyphenol content (TPC) of coffee samples ($n = 3$). Letters a, b, c, and d indicate significant differences ($p \leq 0.05$).

Coffee varieties	Caffeine content (mg/g dw)	TAC (mg AAE/g dw)	TPC (mg GAE/g dw)
Heirloom	0.14 ± 0.018^{b}	78.05 ± 2.25^{a}	$27.08 \pm 1.41^{a,c}$
Forest coffee	0.36 ± 0.001^{b}	71.84 ± 1.97^{b}	$28.09 \pm 1.68^{a,c}$
Heirloom 2	0.37 ± 0.001^{b}	68.38 ± 1.41^{c}	31.04 ± 0.68^{b}
Bourbon	0.36 ± 0.005^{b}	64.75 ± 2.44^{d}	28.93 ± 1.46 a,b,c
Mixed heirloom	0.36 ± 0.003^{b}	66.68 ± 1.17^{c}	29.83 ± 1.68 a,b,c
Catuai	0.36 ± 0.003^{b}	63.75 ± 1.55^{d}	27.78 ± 0.55^{c}

addition to the region of origin. The presented results, with the exception of the catuai variety, also support the literary data of Daniel and Workneh [26] that the antioxidant content of Ethiopian coffees ranges from 64 to 97 mg AAE/g, depending on the region.

Plants needed to produce a lot of polyphenols to protect themselves from strong UV radiation in the desert, so next to the lowest acidity the Heirloom 2 coffee variety had the highest polyphenol content (31.04 mg GAE/g), this may be explained with the origin- Oromo region. The lowest polyphenol content (27.08 mg GAE/g) was found in the heirloom coffee variety, which may be attributed to the very dark roasting that reduced the amount of phenolic compounds. The results are further supported by the study of Dybkowska et al. [27], which found that the amount of total polyphenols differed between the light and dark roasts of the same Ethiopian coffee sample by up to 14 mg GAE/g.

4. Conclusion

The panelists preferred coffee drinks with a medium level of acidity, softer fruit flavors, and lighter chocolate brown coffee, which is influenced by the degree of roasting. The evaluation of the bitter taste's results showed a strong correlation with acidity. Coffee drinks with floral notes did not fare well in the taste test; they were compared to the smell of tobacco.

Five grams of caffeine is the lethal dose. The findings show that the examined coffee varieties do not contain enough caffeine to harm the body when consumed in the amount of 3-4 cups per day. The caffeine levels in the tested coffee varieties were typical of the region where they were grown.

The samples contained a significant amount of antioxidants and polyphenols, which can be used to refute the myth that coffee is unhealthy. The degree of roasting determines the concentration of these compounds. With altitude, antioxidant content exhibited a trend toward decreasing. The results also reveal significant differences, which might be brought on by variations, growing regions, and altitude.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors have declared no conflict of interest.

Acknowledgments

This study is funded by the Széchenyi István University. Open access funding is enabled and organized by the Electronic Information Service National Programme.

References

[1] T. W. Gole, *Coffee: Ethiopia's Gift to the World. In: The Traditional Production Systems as Living Examples of Crop Domestication, and Sustainable Production and an Assessment of Different Certification Schemes*, Environment and Coffee Forest Forum (ECFF), Addis Ababa, Ethiopia, 2015.

[2] M. Worku, "Production, productivity, quality and chemical composition of Ethiopian coffee," *Cogent Food & Agriculture*, vol. 9, no. 1, 2023.

[3] M. Worku, H. R. Upadhayay, K. Latruwe et al., "Differentiating the geographical origin of Ethiopian coffee using XRF- and ICP-based multi-element and stable isotope profiling," *Food Chemistry*, vol. 290, pp. 295–307, 2019.

[4] A. Ayele, M. Worku, and Y. Bekele, "Trend, instability and decomposition analysis of coffee production in Ethiopia (1993-2019)," *Heliyon*, vol. 7, no. 9, article e08022, 2021.

[5] J. Moat, J. Williams, S. Baena, T. Wilkinson, S. Demissew, and Z. K. Challa, *Coffee Farming and Climate Change in Ethiopia: Impacts, Forecasts, Resilience and Opportunities. –Summary. The Strategic Climate Institutions Programme (SCIP)*, Royal Botanic Gardens, 2017.

[6] J. Moat, J. Williams, S. Baena et al., "Resilience potential of the Ethiopian coffee sector under climate change," *Nature Plants*, vol. 3, no. 7, p. 17081, 2017.

[7] T. Benti, E. Gebre, K. Tesfaye et al., "Genetic diversity among commercial arabica coffee (*Coffea arabica* L.) varieties in Ethiopia using simple sequence repeat markers," *Journal of Crop Improvement*, vol. 35, no. 2, pp. 147–168, 2021.

[8] Y. Y. Melese and S. A. Kolech, "Coffee (*Coffea arabica* L.): methods, objectives, and future strategies of breeding in Ethiopia-review," *Sustainability*, vol. 13, no. 19, article 10814, 2021.

[9] M. Bart Bart, T. Seneshaw, K. Tadesse, and N. Yaw, *Structure and Performance of Ethiopia's Coffee Export Sector*, International Food Policy Research Institute, Washington, DC, USA, 2014.

[10] M. W. Mengistu, M. A. Workie, and A. S. Mohammed, "Physical and cup quality attributes of Arabica coffee (*Coffea arabica* L.) varieties grown in highlands of Amhara region, northwestern Ethiopia," *International Journal of Agronomy*, vol. 2020, Article ID 6420363, 9 pages, 2020.

[11] K. F. Wiersum, T. W. Gole, F. Gatzweiler, J. Volkmann, E. Bognetteau, and O. Wirtu, "Certification of wild coffee in Ethiopia: experiences and challenges," *Forests, Trees and Livelihoods*, vol. 18, no. 1, pp. 9–21, 2008.

[12] M. Getachew, K. Tolassa, P. De Frenne, K. Verheyen, A. J. M. Tack, and K. Hylander, "The relationship between elevation, soil temperatures, soil chemical characteristics, and green coffee bean quality and biochemistry in Southwest Ethiopia," *Agronomy for Sustainable Development*, vol. 42, no. 4, 2022.

[13] K. Tolessa, J. D'heer, L. Duchateau, and P. Boeckx, "Influence of growing altitude, shade and harvest period on quality and biochemical composition of Ethiopian specialty coffee," *Journal of the Science of Food and Agriculture*, vol. 97, no. 9, pp. 2849–2857, 2017.

[14] M. Worku, T. Astatkie, and P. Boeckx, "Shade and postharvest processing effects on arabica coffee quality and biochemical composition in lowland and midland coffee-growing areas of southwestern Ethiopia," *Journal of Food Composition and Analysis*, vol. 115, article 105027, 2023.

[15] E. Firdissa, A. Mohammed, G. Berecha, and W. Garedew, "Coffee drying and processing method influence quality of Arabica coffee varieties (*Coffee arabica* L.) at Gomma I and Limmu Kossa, Southwest Ethiopia," *Journal of Food Quality*, vol. 2022, Article ID 9184374, 8 pages, 2022.

[16] U. Çakılcıoğlu, "An ethnobotanical field study; traditional foods production and medicinal utilization of *Gundelia* L. species in Tunceli (Turkey)," *Indian Journal of Traditional Knowledge*, vol. 19, no. 4, pp. 714–718, 2020.

[17] Élelmiszeripari kézikönyv 7, *Érzékszervi vizsgálatok (Organoleptic tests)*, Agrárminisztérium. Nemzeti Agrárgazdasági Kamara, 2020.

[18] P. P. Nhan and N. T. Phu, "Effect of time and water temperature on caffeine extraction from coffee," *Pakistan Journal of Nutrition*, vol. 11, no. 2, pp. 100–103, 2012.

[19] S. Shrestha, P. Pokhrel, S. K. Rijal, and K. P. Rai, "A simple HPLC method for the determination of caffeine content in tea and coffee," *Journal of Food Science and Technology Nepal*, vol. 9, pp. 74–78, 2016.

[20] A. Bobková, M. Hudáček, S. Jakabová et al., "The effect of roasting on the total polyphenols and antioxidant activity of coffee," *Journal of Environmental Science and Health. Part. B*, vol. 55, no. 5, pp. 495–500, 2020.

[21] I. F. F. Benzie and J. J. Strain, "The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay," *Analytical Biochemistry*, vol. 239, no. 1, pp. 70–76, 1996.

[22] V. L. Singleton, R. Orthofer, and R. M. Lamuela-Raventos, "Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent," *Methods in Enzymology*, vol. 299, pp. 152–178, 1999.

[23] F. J. Barba, Z. Zhu, M. Koubaa, A. S. Sant'Ana, and V. Orlien, "Green alternative methods for the extraction of antioxidant bioactive compounds from winery wastes and by-products: a review," *Trends in Food Science and Technology*, vol. 49, pp. 96–109, 2016.

[24] M. Miłek, Ł. Młodecki, and M. Dżugan, "Caffeine content and antioxidant activity of various brews of specialty grade coffee," *Acta Scientiarum Polonorum Technologia Alimentaria*, vol. 20, no. 2, pp. 179–188, 2021.

[25] T. Tasew, Y. Mekonnen, T. Gelana et al., "In vitro antibacterial and antioxidant activities of roasted and green coffee beans originating from different regions of Ethiopia," *International Journal of Food Science*, vol. 2020, Article ID 8490492, 8 pages, 2020.

[26] A. Daniel and M. Workneh, "Determination of total phenolic content and antioxidant activities of five different brands of Ethiopian coffee," *Nutrition Research*, vol. 1, p. 2, 2017.

[27] E. Dybkowska, A. Sadowska, R. Rakowska, M. Dębowska, F. Świderski, and K. Świąder, "Assessing polyphenols content and antioxidant activity in coffee beans according to origin and the degree of roasting," *Roczniki Państwowego Zakładu Higieny*, vol. 68, no. 4, pp. 347–353, 2017.