Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article OPEN ACCESS

Variability of Chlorogenic Acids in Roasted Coffee: Influence of Botanical and Geographical Origin

Terézia Švecová, Alica Bobková, Alžbeta Demianová*, Andrea Mesárošová, Lukáš Jurčaga, Ivana Timorácká, Marek Bobko, Judita Lidiková, Ľubomír Belej, Natália Čeryová

Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Food Sciences, Slovakia

Terézia Švecová: https://orcid.org/0009-0007-2612-6962
Alica Bobková: https://orcid.org/0000-0002-6798-7204
Alžbeta Demianová: https://orcid.org/0000-0002-3615-5747
Andrea Mesárošová: https://orcid.org/0000-0003-2657-4582
Lukáš Jurčaga: https://orcid.org/0000-0001-9693-4796
Ivana Timorácká: https://orcid.org/0009-0001-2387-7652
Marek Bobko: https://orcid.org/0000-0003-4699-2087
Judita Lidiková: https://orcid.org/0000-0001-9922-4300
Ľubomír Belej: https://orcid.org/0000-0001-8523-6650
Natália Čeryová: https://orcid.org/0000-0002-1865-5131

Article Details:
Received: 2025-04-08
Accepted: 2025-05-20
Available online: 2025-05-31

DOI: https://doi.org/10.15414/ainhlq.2025.0016

Chlorogenic acids (CQAs) are among the most important phenolic compounds found in plant-based foods, and coffee is recognized as one of their richest natural sources. This study aimed to evaluate the profile of CQAs in medium-roasted *Coffea arabica* L. and *Coffea canephora* Pierre ex A.Froehner beans originating from diverse geographical regions. High-performance liquid chromatography (HPLC) was used to quantify three main CQA isomers: 5-CQA, 4-CQA, and 3-CQA. Among them, 5-CQA was found to be the predominant compound across all samples analyzed. The results demonstrated that *C. canephora* samples contained significantly higher and more variable levels of CQAs (20.43 ±5.49 mg·g^{-1}) compared to *C. arabica* samples (12.02 ±0.98 mg·g^{-1}). Statistical analysis using ANOVA, combined with Duncan, Tukey, and Dunn post hoc tests, confirmed species-related differences in CQAs content. Additionally, violin plots provided a clear visualization of these distinctions. Principal Component Analysis (PCA) further indicated that the geographical origin of the samples may influence the accumulation of chlorogenic acids. These findings highlight the influence of both botanical species and environmental factors on the chemical composition of coffee. Understanding such variability is essential for ensuring product quality, maintaining authenticity, and guiding the development of value-added coffee-based products tailored to consumer preferences and health-related expectations.

Keywords: Chlorogenic acid, *Coffea arabica*, *Coffea canephora*, HPLC

*Corresponding Author: Alžbeta Demianová, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Food Sciences, Trieda Andreja Hlinku 2, 94976 Nitra, Slovakia
alzbeta.demianova@uniag.sk

Introduction

Coffee is one of the most widely consumed beverages worldwide. The genus *Coffea* comprises more than 124 species, and this number continues to grow as new ones are still being discovered. Among the species described so far, only two – *Coffea arabica* L. (*Arabica coffee*) and *Coffea canephora* Pierre ex A. Froehner (Robusta coffee) – dominate global trade (Mishra et al., 2022). Coffee is valued for its distinctive aroma, pleasant taste, and potential health benefits (Kim et al., 2024). Among its bioactive compounds are phenolic substances, particularly chlorogenic acids (CQAs), which play a key role in the antioxidant activity, flavour profile, and stability of coffee (Ayseli et al., 2021; Wu et al., 2022). CQAs are a group of esters formed by the esterification of *trans*-cinnamic acids with quinic acid. They naturally accumulate in coffee cherries during ripening and may account for as much as 6–12% of the dry weight of green coffee beans (Colomban et al., 2020; Yeager et al., 2023). The major CQAs in coffee include caffeoylquinic acids, feruloylquinic acids, and dicaffeoylquinic acids, with caffeoylquinic acids and dicaffeoylquinic acids being the most abundant in nature (Yeager et al., 2023).

During roasting, CQAs undergo significant transformations, leading to the formation of lactones and volatile compounds that contribute to the bitterness and aroma of coffee (Yeager et al., 2023). Although their overall content decreases with heat treatment, a substantial proportion of CQAs can remain in roasted beans, depending on the degree of roasting and processing conditions (Sagu et al., 2024). The geographical origin of coffee significantly influences the CQAs composition. Notably, *Coffea arabica* (*C. arabica*) and *Coffea canephora* (*C. canephora*) species exhibit distinct CQAs patterns, which affect the quality and flavour of both the beans and the resulting beverages. Understanding these geographical and species-specific variations is a crucial aspect of coffee research and sensory evaluation (Viencz et al., 2023).

CQAs not only influence flavour but also contribute to health promotion, attracting interest across analytical chemistry, nutrition, and the coffee industry (Hall et al., 2022; Viencz et al., 2023; Oussou et al., 2025). Studies have demonstrated their antiviral, antidiabetic, antioxidant, and neuroprotective effects (Awwad et al., 2021). They may regulate glucose metabolism by slowing intestinal absorption and reducing hepatic output (Tunnicliffe et al., 2015), and support tissue regeneration by enhancing cell proliferation and reducing oxidative damage (Chiang et al., 2015).

Although the metabolism and health-promoting effects of chlorogenic acids (CQAs) have been studied, continuous comparative data on the influence of botanical and geographical origin are needed. This study addresses variability of CQA composition in medium-roasted coffee beans from *Coffea arabica* and *Coffea canephora*, with an emphasis on species-specific and geographical distinctions.

Material and Methodology

The coffee beans samples used in this study were obtained from ManuCafe coffee roastery (Ludgeřovice, Czech Republic). The samples were sourced from two different botanical varieties: *C. arabica* L. and *C. canephora* Pierre ex A. Froehner, from various origins (Table 1). Both varieties were purchased in 250 g packages and were pre-roasted to a medium roast level. Altogether, we analyzed 10 samples from 4 batches (n = 40 samples).

Table 1 List of analysed samples

Samples	Botanical origin	Country of origin	Variety	Altitude	Processing	Roast level
1 B	100% *C. arabica*	Peru	Typica	1,900 mamsl	Wet	medium light
2 B		Kenya	SL 34	1,800 mamsl		medium
3 B		Colombia	Bourbon	1,750–1,900 mamsl		medium light
4 B		Cuba	Bourbon	900 mamsl		medium light
5 B		Vietnam	Typica	1,000–1,600 mamsl		medium
6 B	100% *C. canephora*	Indonesia	Robusta	1,500 mamsl		medium light
7 B		Brazil		540–820 mamsl		medium light
8 B		Guatemala		1,100 mamsl		medium
9 B		India		1,000–1,600 mamsl		medium light
10 B		Uganda		1,500 mamsl		medium light

Notes: Samples: B – roasted coffee beans; mamsl – meters above mean sea level

Extracts Preparation

Prior to analysis, 30 g of each coffee bean sample was homogenized using an electric grinder (Grindomix GM 200, Retsch, Haan, Germany) at 10,000 rpm for 60 seconds to obtain a representative sample. Then, 7 g of the ground coffee was weighed on a laboratory scale (Kern PCB 1000-1, Kern & Sohn GmbH, Germany) and extracted with 120 mL of hot distilled water heated to 98 °C. The extraction was carried out for 5 minutes with occasional stirring. Afterward, the samples were filtered using Sartorius filter paper (Grade 390, Germany) to obtain an extract for analysis.

Qualitative and Quantitative Determination of CQAs in Samples Using HPLC

For the determination of CQAs content, HPLC analysis was performed following Bobková et al. (2021) using an Agilent Infinity 1260 HPLC-DAD chromatograph. Before analysis, the coffee extracts were re-filtered through a Frisenette syringe microfilter (25 mm, 0.45 µm) and transferred to HPLC vials. Separation was carried out on a C-18 Poroshell 120 column (150 mm × 3 mm × 2.7 µm), with acetonitrile (A) and 0.1% H_3PO_4 in water (B) as mobile phases. The gradient elution program was as follows: 0–1 min (20% A + 80% B), 1–5 min (25% A + 75% B), 5–15 min (30% A + 70% B), and 15–25 min (40% A + 60% B). The flow rate was 1 $mL.min^{-1}$, the injection volume was 10 µL, and the separation temperature was maintained at 30 °C. Detection was performed at 320 nm, with a wavelength range of 210–400 nm. The CQAs content was expressed as $mg.g^{-1}$ of dry matter.

Statistical Analysis

Statistical analyses were conducted using a suite of specialized Python libraries to ensure robust and reproducible data evaluation. Independent *t*-tests, implemented via the SciPy package, were applied to determine statistically significant differences in CQA concentrations between *Coffea arabica* and *Coffea canephora*. To further explore group-level differences, one-way analysis of variance (ANOVA) was performed at a significance level of $\alpha = 0.05$. Data visualization was achieved through violin plots generated with Matplotlib and Seaborn library. Basic numerical computations, including the calculation of means and standard deviations, were facilitated by the NumPy library during data preprocessing and statistical assessment.

Results and Discussion

The main phenolic compounds in coffee are chlorogenic acids (CQAs), which are highly bioavailable (Farah and Lima 2019; Ayseli et al., 2021; Wu et al., 2022). Using HPLC, we determined the content of the three major CQAs derivatives measured in our study, which belong to the group of caffeoylquinic acids: chlorogenic acid (5-CQA), cryptochlorogenic acid (4-CQA), and neochlorogenic acid (3-CQA). Among these, chlorogenic acid (5-CQA) was the most abundant in all coffee samples, with concentrations ranging from 10.79 to 28.08 $mg{\cdot}g^{-1}$. This was followed by cryptochlorogenic acid (4-CQA), which ranged from 5.93 to 15.43 $mg{\cdot}g^{-1}$, while the lowest concentrations were observed for neochlorogenic acid (3-CQA), with values ranging from 3.34 to 8.42 $mg{\cdot}g^{-1}$. As shown in Table 2, *C. canephora* samples generally exhibited a higher content of chlorogenic acids (CGA) compared to *C. arabica*. The mean concentration in *C. canephora* samples was 20.43 $mg{\cdot}g^{-1}$, with a standard deviation of 5.49 $mg{\cdot}g^{-1}$, indicating a higher variability in CGA profiles.In contrast, *C. arabica* samples showed a lower mean concentration of 12.02 $mg{\cdot}g^{-1}$ and a standard deviation of only 0.98 $mg{\cdot}g^{-1}$, reflecting more consistent CQAs levels. These findings are consistent with previous studies. Seninde and Chambers (2020) reported that both green and roasted *C. canephora* beans typically contain higher levels of CQAs than *C. arabica*. Similarly, Clifford et al. (2017) and Viencz et al. (2023) noted that *C. canephora* generally contains higher concentrations of various cinnamic acid isomers, although some exceptions may occur. The observed higher CQAs levels and greater variability in *C. canephora* may be attributed to its broader genetic diversity, greater environmental adaptability, or differences in post-harvest processing. In contrast, the more uniform CQAs content in *C. arabica* may reflect its narrower genetic base and more standardized cultivation practices.

Table 2 ANOVA results of statistical differences between botanical origin of coffee

Botanical origin	Chlorogenic acid (5-CQA)	Cryptochlorogenic acid (4-CQA)	Neochlorogenic acid (3-CQA)
Coffea canephora	20.430^{a}	10.656^{a}	6.193^{a}
Coffea arabica	12.024^{b}	7.205^{b}	3.856^{b}

Notes: Columns with different upper indices are significantly different at α=0.05

Statistical analysis confirmed that the difference in CQAs concentration between the two species was significant.

Pedan et al. (2020) found in their study that prolonged roasting time, or higher roasting temperatures, led to CQAs degradation, with a loss of approximately 60% under mild roasting conditions and nearly 100% after dark roasting. They reported that the most abundant CQAs in both green and roasted coffee beans are caffeoylquinic acids, specifically 5-CQA, followed by 4-CQA and 3-CQA. Bicho et al. (2013) analyzed the CQAs composition in green coffee beans of *C. arabica* and *C. canephora* and reported the same order of CQA isomers as observed in our study. Jeszka-Skowron et al. (2023) observed that the levels of 4-CQA and 3-CQA increased in medium-roasted coffees compared to green beans. According to Belviso et al. (2014), the total CQAs content in *C. arabica* significantly decreased from green coffee to roasted coffee due to increasing roasting intensity (from 52.87 to 2.09 $mg \cdot g^{-1}$), with the lowest values associated with dark roast, which undergoes longer roasting times. Badmos et al. (2020) analyzed 68 roasted coffee samples from various regions of Brazil, cultivated using organic, conventional, and biodynamic agricultural practices, using HPLC. The highest average concentration was recorded for 5-CQA (9.12 $mg \cdot g^{-1}$), followed by 4-CQA (8.26 $mg \cdot g^{-1}$) and 3-CQA (4.21 $mg \cdot g^{-1}$). This order was consistent across all three farming systems, with the highest CQAs content observed in conventionally grown coffee. These values are comparable to our findings, except for 5-CQA, for which they reported a slightly lower mean concentration than observed in our study. This discrepancy may be attributed to differences in the geographical origin of the samples or longer storage times. Król et al. (2020) found that during 12 months of storage, the CQAs content decreased to 0.02 $mg \cdot g^{-1}$ due to enzymatic and non-enzymatic oxidation, pointing to stability of these compounds.

Fujioka and Shibamoto (2008) measured the amounts of 5-CQA in commercial coffee blends ranging from 2.13 to 7.06 $mg \cdot g^{-1}$, 4-CQA from 1.44 to 4.56 $mg \cdot g^{-1}$, and 3-CQA from 1.32 to 3.95 $mg \cdot g^{-1}$. Differences within these and our results could be attributed to varying proportions of *C. arabica* and *C. canephora* species in the blends or to the degree of roasting.

As shown in Figure 1, the CQAs distribution in *C. canephora* is wider, indicating greater variability in CQAs concentrations. The median concentration is higher compared to *C. arabica*, as seen from the thicker portion of the violin. On the other hand. *C. arabica* showed a narrowed distribution, reflecting more consistent chlorogenic acid profiles. The median concentration is lower than *C. canephora*, with less spread in the data. The broader variability in *C. canephora* may be due to its genetic diversity and adaptability to different environments. This is supported by Junior et al. (2024), who reported significant differences in bioactive compound content among various *C. canephora* genotypes. Similarly,

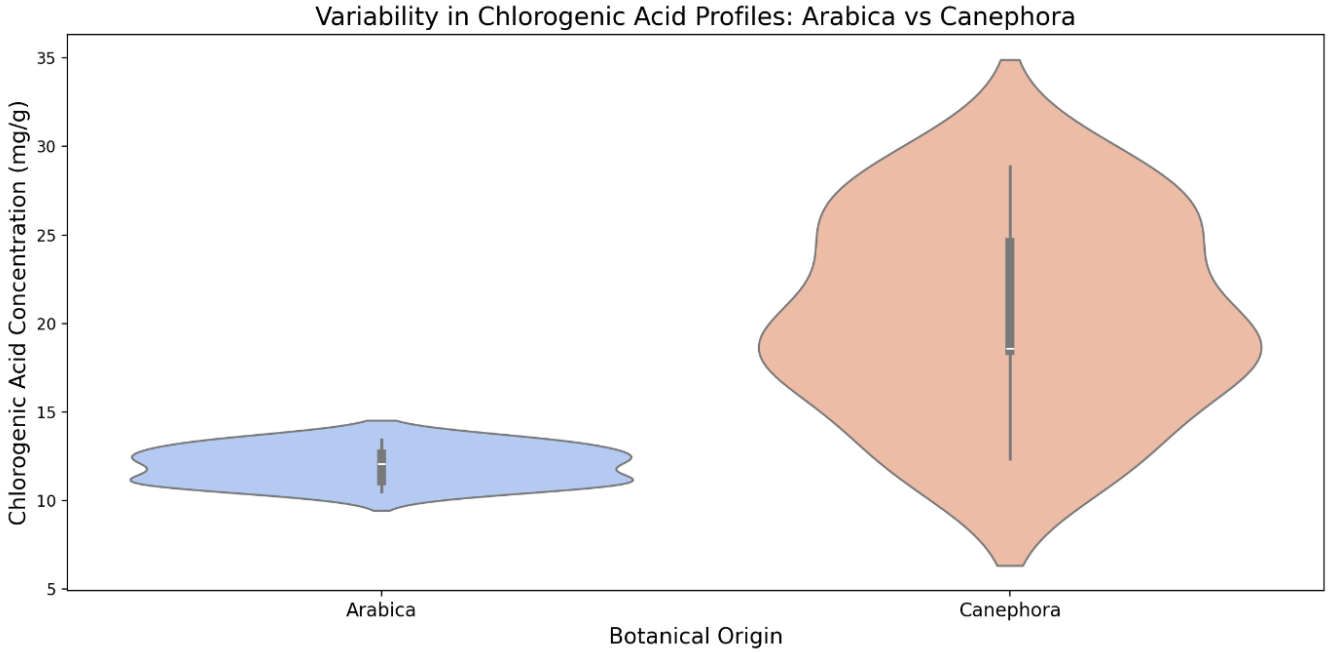

Figure 1 Violin plot illustrating the variability in CQAs profiles of *Coffea arabica* L. and *Coffea canephora* Pierre ex A.Froehner

Badmos et al. (2019) identified notable differences in the concentrations of key CQAs derivatives in green coffee beans between *C. canephora* and *C. arabica*, with *C. canephora* showing stronger correlations among certain isomers, indicating species-specific variability in its chemical profile. In addition, Kiwuka et al. (2021) demonstrated high genetic diversity among wild and cultivated *C. canephora* coffee populations in Uganda, shaped by environmental factors across different climatic zones. Such genetic structuring could partly explain the observed chemical variability. *C. arabica*'s narrower distribution suggests more uniform growing and processing conditions. This may be influenced by environmental factors such as altitude, which Bertrand et al. (2012) described influence of these factors on the biochemical composition and quality of *Arabica coffee* beans.

The CQAs content in coffee is influenced by its geographical origin (Abubakar et al., 2024). Principal Component Analysis (PCA) visualizes the first two principal components (PC1 and PC2), which together explain the majority of the variance in the data. PC1 (Principal Component 1) accounts for approximately 98.80% of the total variance, meaning that most of the variability in CQAs profiles is captured along this axis. PC2 (Principal Component 2) explains around 1.10% of the variance, reflecting secondary differences in the dataset.

The plot shows distinct groups based on geographical origin, with each group represented by a different colour. These groupings suggest that coffee samples from different regions possess unique CQAs profiles, likely influenced by environmental factors such as soil composition, climate, agricultural practices, and altitude. We can observe an overlay in samples from South and Central America, except Colombia. These findings are consistent with those of Rocha et al. (2023), who reported that while caffeine content is primarily under genetic control, CQAs are more susceptible to environmental variation, resulting in greater differences in genotype performance across different conditions. This reinforces the importance of evaluating CQAs profiles when comparing coffee from distinct botanical origins. Different coffee-growing regions offer diverse environmental conditions that shape the chemical composition of coffee beans, including CQAs content. Demianová et al. (2021) described the impact of geographical origin on chemical composition of coffee. However, analyses done in harvesting year of 2020 did not show statistical differences in CQAs concentrations in samples from South America, Central America, and Africa. Several

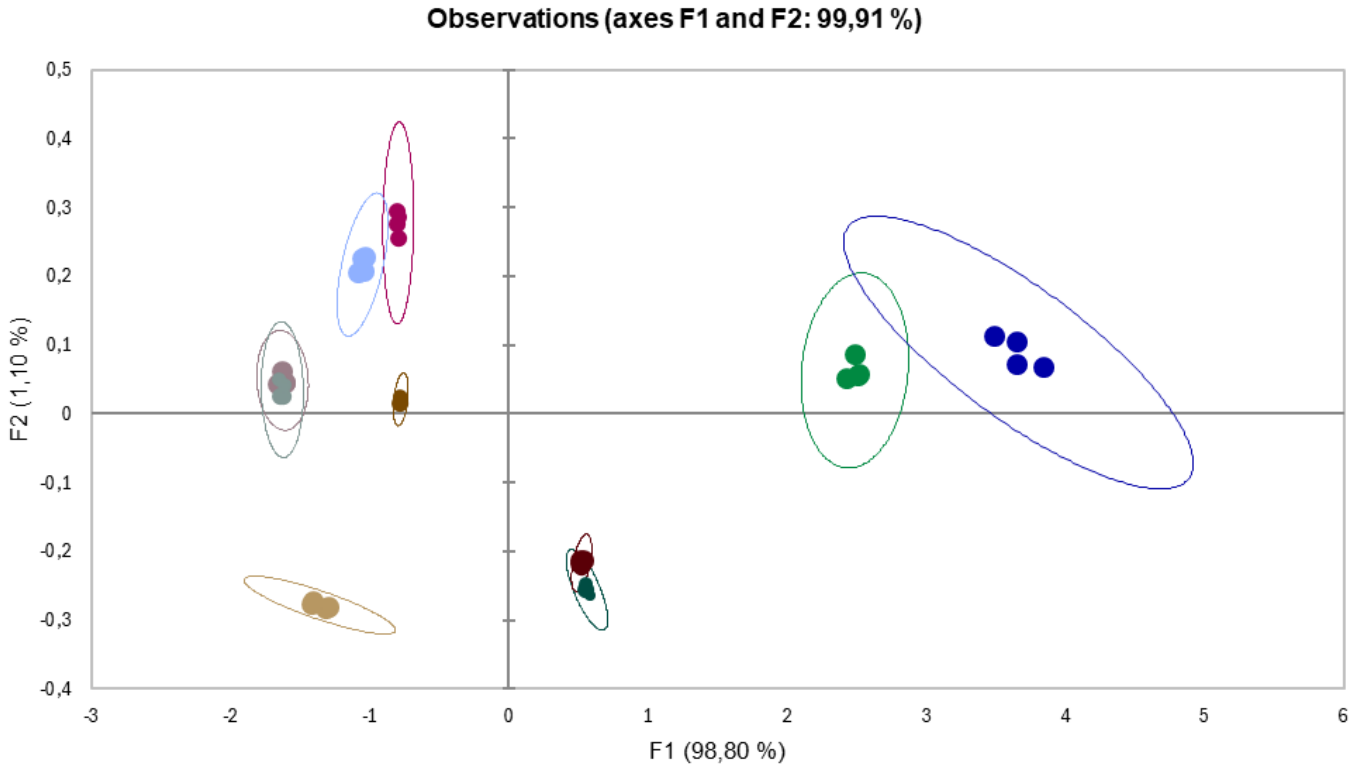

Figure 2 PCA Plot of CQAs composition according to geographical origin

other studies have shown that increasing altitude is associated with a decrease in CQAs concentrations, possibly due to lower temperatures and changes in plant metabolic activity (Chen et al., 2024). Abubakar et al. (2024) studied the effects of coffee variety and growing altitude on chemical composition, particularly CQAs, caffeine, and antioxidant activity. Their results confirmed a decreasing trend in CQAs content with higher altitude, while caffeine levels and antioxidant capacity tended to increase.

Sample of *Coffea canephora* from Guatemala exhibits the highest mean concentration of chlorogenic acid (~28.08 $mg.g^{-1}$), with a relatively larger standard deviation of 0.84 $mg.g^{-1}$, indicating variability in the samples. Brazil and Indonesia also show high concentrations (~24.38 $mg.g^{-1}$ and ~18.62 $mg.g^{-1}$, respectively), with Brazil having a slightly higher variability. On the other hand, the lowest concentrations were recorded in the following samples: Peru (~10.79 ±0.19 $mg.g^{-1}$), followed by Vietnam (~11.18 ±0.11 $mg.g^{-1}$), and Kenya (~12.08 ±0.10 $mg.g^{-1}$). These regions show relatively lower variability in their chlorogenic acid profiles. Regions like Colombia, Cuba, and India exhibit moderate concentrations, ranging between ~12.5 ±0.04 $mg.g^{-1}$ and ~13.4 ±0.02 $mg.g^{-1}$, with small variability, indicating consistent profiles. It needs to be emphasized that regions such as Guatemala and Brazil show higher variability in chlorogenic acid concentrations, suggesting diverse environmental or processing factors influence the samples. In contrast, regions like Cuba and India have lower variability, indicating more uniform profiles.

These findings are consistent with observations by Girma et al. (2020), who reported variations in 5-CQA levels in *C. arabica* grown at different altitudes in southwestern Ethiopia. Similarly, in our study, coffee from Peru (sample 1 B), grown at a high elevation of 1900 meters, exhibited the lowest mean concentration of 5-CQA (10.79 $mg{\cdot}g^{-1}$) compared to samples from lower-altitude regions. Worku et al. (2018) investigated the individual and interactive effects of altitude, shade, and postharvest processing on caffeine, chlorogenic acids, and sucrose contents in green *Arabica coffee* beans from southwestern Ethiopia. The study also found that both caffeine and CQAs contents decreased significantly with increasing altitude, while sucrose content increased.

The spread of each group along the principal components suggests variability within each geographical origin. Groups that are farther apart indicate more distinct differences in CQAs profiles, while groups that are closer together may share similar CQAs compositions.

PC1 captures the largest variance in the data and likely reflects a gradient of overall CQAs concentration, while PC2 captures the second-largest variance and may reflect differences in the relative proportions of specific CQAs (e.g., chlorogenic acid vs. cryptochlorogenic acid). Kuhnert et al. (2011), also demonstrated that PCA can effectively differentiate coffee samples based on variety and processing conditions, and highlighted the importance of selecting appropriate PCA parameters to reveal meaningful chemical variation between sample groups.

Conclusions

Coffea arabica and *C. canephora* are the most consumed coffee species globally, with coffee being a key dietary source of chlorogenic acids (CQAs). This study reveals marked variability in CQA profiles of medium-roasted beans, shaped by both botanical and geographical origin. HPLC-DAD analysis identified 5-CQA as the dominant isomer, followed by 4-CQA and 3-CQA. *C. canephora* exhibited significantly higher and more variable CQAs concentrations than *C. arabica*, reflecting differences in genetics, environmental resilience, and agronomic practices. Principal Component Analysis (PCA) further demonstrated that CQAs composition can be closely linked to geographical origin. A deeper understanding of CQAs variability can guide optimized processing, robust quality control, and the innovation of health-oriented coffee products. Future studies should investigate how post-harvest and roasting conditions modulate CQAs profile across origins and species.

Conflicts of Interest

The authors declare no conflict of interest.

Ethical Statement

This article doesn't contain any studies that would require an ethical statement.

Funding

The study was supported by Grant Agency of the Slovak University of Agriculture (GA SPU), project No. 03-GA-SPU-2024 and by the Slovakia's recovery and resilience plan, project No. 09I03-03-V05-00018 – Early Stage Grants at SUA in Nitra.

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