

IDENTIFICATION OF GEOGRAPHICAL ORIGIN BASED ON QUANTITATIVE ANALYSIS OF BIOACTIVE COMPOUNDS AND TRACE ELEMENTS IN 100% COFFEA ARABICA GREEN BEANS

Alica Bobková, Alžbeta Demianová*, Terézia Švecová, Marek Bobko, Lukáš Jurčaga, Andrea Mesárošová, Judita Lidiková, Natália Čeryová, Katarína Poláková

Address(es):

Institute of Food Sciences, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Trieda Andreja Hlinku 2, 94976 Nitra.

*Corresponding author: alzbeta.demianova@uniag.sk

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ABSTRACT

This study investigates green coffee beans' physicochemical properties and trace metal content from three major coffee-growing regions: America, Africa, and Asia. Results revealed that American beans had the highest dry matter content (92.00%), followed by African (91.58%) and Asian (91.06%) beans, with variations attributed to environmental factors and post-harvest practices. Water activity was lowest in African (0.47) and American (0.47) samples, suggesting microbial stability compared to Asian beans (0.544). Based on Anova we can see significantly higher differences in parameters water activity in Asian samples. The pH remained stable across all regions (5.75-5.76), indicating that pH is more influenced by genetic factors than environmental ones. Concentrations of nine trace elements were measured, with Pb and Cd being below detection limits in all samples, emphasizing coffee safety. Trace elements analysis using Linear Discriminant Analysis (LDA), and hierarchical clustering demonstrated (AHC) distinct regional differentiation based on metal profiles, particularly with Cu, Zn, and Cr. Water-soluble compound analysis revealed regional distinctions, with African samples showing higher variability. The correlation between bioactive compounds and trace metals indicated that elements such as Zn and Mn may enhance antioxidant activity, whereas excessive Cu could inhibit the biosynthesis of key antioxidants. These findings underscore the potential of using chemical profiles for origin authentication, quality control, and traceability in the coffee industry.

Keywords: green coffee beans, geographical origin, heavy metal compounds, bioactive compounds

INTRODUCTION

Coffee is one of the most widely consumed beverages globally, cherished not only for its stimulating effects but also for its rich bioactive composition and appealing sensory profile. As a significant source of antioxidants and essential nutrients, coffee has been linked to numerous health benefits (Liang and Kitts, 2014). Its chemical composition is influenced by multiple factors, including geographical origin, roasting degree, and preparation methods, which collectively shape its flavor, quality, and health attributes (Anderson and Smith, 2002; Farah *et al.*, 2006). Understanding these factors is crucial for optimizing quality, ensuring consumer safety, and enhancing the overall coffee experience.

Geographical origin is important role in determining the physicochemical properties and traces metal content of green coffee beans. Understanding these factors is important for producers, traders, and consumers because it affects cultivation practices to quality assessment and marketability. Differences in climate, soil composition, and agricultural practices across regions contribute to variations in dry matter content, water activity, and trace metal concentrations. Studies have shown that these parameters not only influence the roasting behavior and sensory properties of coffee but also serve as reliable indicators for authenticating the geographical origin of coffee products (da Silva *et al.*, 2017; Berego *et al.*, 2023). Such knowledge is invaluable for quality control and traceability, especially in an industry where origin and quality are closely linked. The presence of essential and potentially toxic metals in coffee has been a growing area of research due to concerns about food safety. Metals like lead (Pb), cadmium (Cd), and nickel (Ni) can be introduced into coffee beans through environmental factors, soil contamination, and post-harvest practices (González-Rodríguez *et al.*, 2011; Berego *et al.*, 2023). While essential metals like copper (Cu) and zinc (Zn) contribute to the nutritional value of coffee, excessive levels of toxic metals pose significant health risks. Recent studies have emphasized the importance of monitoring these contaminants to ensure consumer safety (da Silva *et al.*, 2017; Mousavi Khaneghah *et al.*, 2022).

In addition to metal contamination, the bioactive profile of coffee is of significant interest. Compounds such as chlorogenic acids and phenolics are known for their antioxidant properties, which might contribute to coffee's health benefits and influence its flavor (Alnsour *et al.*, 2022; Jeszka-Skowron *et al.*, 2016). These compounds vary significantly based on the geographical origin and processing methods. The roasting process alters the concentration of these bioactive

compounds, impacting both the antioxidant capacity and the overall sensory profile of the beverage (Farah *et al.*, 2006; Hall *et al.*, 2022; Tieghi *et al.*, 2024). Understanding these variations is crucial for optimizing roasting techniques and enhancing the quality of the final product.

To address these multifaceted aspects, advanced analytical techniques such as chemometrics and metabolomics have been employed to differentiate coffee samples based on their elemental and chemical profiles (Mannino *et al.*, 2023; Mendes *et al.*, 2022). These methods not only enable origin authentication but also provide insights into how environmental and processing factors influence the chemical composition and bioactivity of coffee.

This study aims to evaluate the influence of geographical origin on the physicochemical properties, trace metal content, and bioactive compound profiles of green coffee beans from three major regions America, Africa, and Asia. By integrating findings from recent research, this study will contribute to a deeper understanding of the impact of origin on coffee quality, safety, and traceability, thereby supporting better quality control measures and enhanced consumer trust in coffee products.

MATERIAL AND METHODS

Material

For this research were used 15 samples from different geographical origins (Africa, America, Asia). Post harvest processing of these samples was a wet method. Detailed information about the samples is presented in the following Table 1.

Methodology

Extract preparation

The samples were initially homogenized using an electrical grinder (Grindomix GM 200, Retsch, Haan, Germany) at a rotational speed of 10,000 min⁻¹ for a duration of 60 s. Subsequently, 7 g of each sample were subjected to extraction with 120 mL of deionized water maintained at a temperature of 95 °C. The extraction process was carried out over a 5 min. Post-extraction, the samples were filtered using Sartorius filter paper (Sartorius Lab Instruments GmbH & Co. KG, Goettingen, Germany). The resulting filtrates were then employed for subsequent

analytical procedures. The amount of coffee homogenized to create an average sample was 250 g.

Table 1 List of samples analyzed.

Samples	Continent	Country	Variety	Processing	Altitude (m)
1A	Africa	Rwanda	B	W	1400 - 1500
2A		Ethiopia	H		1900 - 2200
3A		Tanzania	C		1200 - 1400
4A		Burundi	B		1800 - 1950
5A		Malawi	C		1100 - 1300
1B	America	Panama	T, C1	W	1400 - 1500
2B		El Salvador	B		1400
3B		Nicaragua	C, C1, B		1100 - 1400
4B		Honduras	C, C1, B		1300 - 1500
5B		Mexico	C, B		1200 - 1600
1C	Asia	Indonesia	B	W	1000 - 1500
2C		Indonesia	T		1100 - 1600
3C		Nepal	T		1100 - 1300
4C		Papua New Guinea	T, B		1200 - 1600
5C		India	C		1100 - 1200

Note: B – Bourbon, H- Heirloom, C- Catimor, C1 – Catuai, T -Typica; W – washed; m - height above sea level

Determination of dry matter

The dry matter content of the coffee powder samples was determined using a KERNDAB 100 laboratory analyzer (KERN & SOHN GmbH, Balingen, Germany). A specialized drying protocol was applied, maintaining a constant temperature of 110 °C throughout the procedure. The dry matter content was subsequently expressed as a percentage (%).

Determination of pH

The pH of the extracts was assessed at 20 °C utilizing a portable pH meter (model pH 70, XS Instruments, Italy). The pH meter was calibrated with Hamilton Buffer Solutions (Hamilton Bonaduz AG, Bonaduz, Switzerland) at pH levels of 4.01, 7.00, and 10.01.

Determination of water activity

The water activity of the samples was analyzed using a Meter Fast-Lab instrument (SI Analytics Lab 845, Germany). Measurements were performed in triplicate for each individual sample to ensure accuracy and reproducibility.

Determination of total antioxidant capacity using the DPPH radical

The total antioxidant capacity of the samples was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, following the protocol established by **Brand-Williams et al. (1995)**. To prepare the DPPH stock solution, 0.025 g of the DPPH radical was accurately weighed and dissolved in 100 mL of 96% ethanol (Centralchem, Bratislava, Slovakia). The stock solution was subsequently diluted with ethanol in a 1:9 ratio. A volume of 3.9 mL of the diluted DPPH solution was then transferred into glass cuvettes, and the initial absorbance (A_0) was recorded at 515.6 nm using a T80 UV/VIS Spectrometer (PG Instruments, Ltd., Lutterworth, UK). Following this, 100 µL of the extract was introduced into the cuvette, and the mixture was homogenized using a glass rod. After a 10 min incubation period, the final absorbance (A_t) was measured at 515.6 nm. The percentage of DPPH radical inhibition by the extract was calculated using the following equation.

Where:

- A_0 is the initial absorbance of DPPH solution,
- A_s is the absorbance of ethanol (blank), and
- A_t is the absorbance after 10 min.

$$\% \text{ inhibition DPPH} = \frac{(A_0 - A_s) - (A_t - A_s)}{(A_0 - A_s)} \times 100$$

This parameter indicates the antioxidant activity of the extract samples by measuring the percentage of DPPH radicals inhibited.

Determination of total polyphenols content

The sample preparation for analysis involved pipetting 50 µL of each extract into 50 mL volumetric flasks. The Folin–Ciocalteu reagent was diluted with distilled water at a 1:2 volume ratio, and 2.5 mL of the diluted reagent was then added to each flask. Subsequently, 5 mL of a 20% aqueous Na_2CO_3 solution was added. 20% aqueous Na_2CO_3 solution was prepared from anhydrous sodium carbonate (Centralchem, Bratislava, Slovakia). The flasks were brought to a final volume of 50 mL with distilled water and left to stand at room temperature for two hours to facilitate the formation of the blue-colored complex. The absorbance of the developed complex was measured at a wavelength of 765 nm (**Fu et al., 2011**).

Determination of caffeine and chlorogenic acids

The quantification of chlorogenic acids and caffeine content was performed using high-performance liquid chromatography (HPLC) as described by **Bobková et al. (2021)**. Separation was achieved using a C-18 Poroshell 12 column (150 mm × 3 mm × 2.7 µm; Agilent Technologies, Waldbronn, Germany) with a mobile phase comprising acetonitrile (solvent A) and 0.1% H_3PO_4 in distilled water (solvent B) (v/v). The gradient elution was programmed as follows: isocratic elution with 20% A and 80% B for the first minute, followed by a linear gradient transitioning from 25% A + 75% B to 40% A + 60% B over the course of 1–25 min. A 3 min equilibration period was maintained between injections. The mobile phase flow rate was set at 1 L·min⁻¹, and the injection volume was 10 µL, with separation conducted at a column temperature of 30 °C. Detection wavelengths were set at 276 nm for caffeine and 320 nm for chlorogenic acids, with spectral data recorded over a wavelength range of 210–400 nm. The standards used for calibration included caffeine (purity 98%), neochlorogenic acid (purity 99%), chlorogenic acid (purity 99%), cryptochlorogenic acid (purity 98%), 4,5-dicaffeoylquinic acid (purity 99%), and 3,5-dicaffeoylquinic acid (purity 99%) (all from Sigma-Aldrich GmbH, Steinheim, Germany). The solvents used were deionized water (resistivity 18.2 MΩ·cm at 25 °C) and HPLC-grade acetonitrile (purity ≥ 99.8%, Sigma-Aldrich GmbH, Steinheim, Germany).

Determination of trace elements

Precisely 1 g of the homogenized sample was accurately weighed using analytical balances (Sartorius, TE214S-oCE, Germany), and transferred into a mineralization cartridge. Subsequently, 5 cm³ of redistilled water and 5 cm³ of concentrated nitric acid were added to the cartridge. The closed cartridge was subjected to mineralization in a MARS X-press microwave digestion system (USA). Following mineralization, the sample was filtered through MUNKTELL quantitative filter paper (grade 390.84 g/m²) into a 50 mL volumetric flask, and the volume was adjusted to 50 mL using distilled water. The prepared sample was then analyzed using a VARIAN AA 240FS atomic absorption spectrometer (Varian Inc., Mulgrave, VIC, Australia) under the specific mineralization parameters (Table 2). A multi-element standard for graphite furnace AAS (16 elements) obtained from Merck ((Munktel&Filtrak, GmbH, Bearenstein, Germany) was used, ensuring a maximum analytical deviation of 3%. The gas flow rates were maintained at 13.5 L·min⁻¹ for air and 2.0 L·min⁻¹ for acetylene. Mercury was directly quantified in the sample without further preparation using the AMA 254 instrument (Czech Republic) via atomic absorption spectrometry with mercury vapor generation. The detection wavelength for mercury was set to 253.65 nm, with a detection limit of 1.5 ng·kg⁻¹ in dry matter. The specific operating conditions for the AAS analysis are provided in Table 3.

Table 2 Parameters of the mineralization process

Phase	Power (W)	Power (%)	Building Up Time (min)	Temperature (°C)	Hold-Off (min)
Initialization (Achieving the specified conditions)	800	90	15	160	0
Mineralization (Maintaining the specified conditions)	800	90	0	160	20
Cooling	–	–	–	–	20

Table 3 Condition of Atomic Absorption Spectroscopy measurements of trace elements

Heavy Metal	Detection Limit (mg·L ⁻¹)	Sensitivity (mg·L ⁻¹)	Wavelength (nm)
Cd	0.001	0.01	228.8
Pb	0.02	0.1	217.0
Cu	0.002	0.03	324.8
Zn	0.006	0.008	213.9
Co	0.005	0.05	240.7
Cr	0.003	0.04	357.9
Ni	0.008	0.06	232.0
Mn	0.003	0.02	279.5
Fe	0.005	0.04	241.8

Notes: Cd – cadmium, Pb – lead, Cu – copper, Zn – zinc, Co – cobalt, Cr – chrome, Ni – nickel, Mn – manganese, Fe – iron,

Statistical analysis

To summarize and interpret our findings, descriptive analysis was employed, which included calculating arithmetic means, minimum and maximum values of the measured parameters. Statistical tests such as ANOVA with Duncan's test and REGWQ, LDA (Linear discriminant analysis) were conducted to assess potential differences among samples across the determined parameters (bioactive compounds TAC, TPC, and trace elements in samples). All statistical analyses were performed using Microsoft Office Excel 365 for Windows with the XLSTAT Addinsoft (2021) and Microsoft Office Excel 365 for iOS with the XLSTAT (2022)

RESULTS AND DISCUSSION

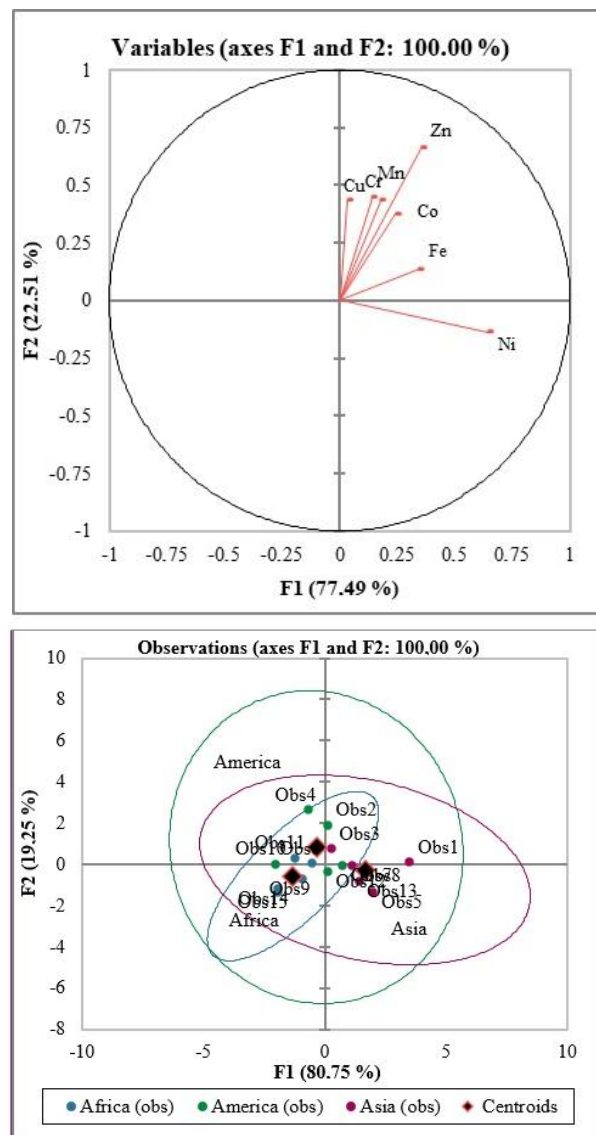
The differences in parameters regarding geographical origin water activity and dry matter and pH are shown in **Table 4**. Samples were divided in 3 geographical groups (Africa, Asia, America). Using ANOVA, we can determine whether there are significant differences between groups based on the variability in the data.

Table 4 ANOVA of dry matter, water activity (a_w) and pH

Category	Dry matter (%)	a_w	pH
Africa	91.58 ^b	0.47 ^a	5.76 ^a
Asia	91.06 ^a	0.54 ^b	5.76 ^a
America	92.00 ^b	0.47 ^a	5.75 ^a
Significant	Yes	Yes	No

Notes: a, b = groups within a column with different superscripts differ significantly at $p \leq 0.05$; ANOVA Duncan test

American samples showed the highest dry matter content (92.00%), followed by African (91.58%) and Asian (91.06%) beans. This variation could influence roasting behavior, as observed by *Belay et al. (2008)*, who attributed differences in dry matter to environmental factors and post-harvest practices. African and American beans had lower water activity (0.47 and 0.47, respectively) compared to Asian beans (0.54), indicating better microbial stability and shelf life. Similar findings were reported by *Pramudya and Seo (2019)*, who linked a_w variations to climatic conditions affecting moisture retention. The pH remained stable around 5.75–5.76 across all regions, consistent with *Hall et al. (2022)*, who found pH values are more influenced by genetic factors than environmental ones. These results emphasize the role of geographical origin in defining the physicochemical properties of green coffee beans, aligning with research by *da Silva et al. (2017)* on the impact of soil and climate. Such insights are crucial for quality control and traceability in the coffee industry.

**Figure 1** LDA of trace elements compound in samples

Some elements in certain samples were below the detection limit (ND Not Detected) meaning concentrations were below 0.001 mg.kg⁻¹. Specifically, Lead (Pb) and Cadmium (Cd) were the elements where ND was recorded. Similar findings were reported by *Berego et al. (2023)*, who emphasized that Pb and Cd levels in coffee beans are often below detection limits, especially when beans are grown in uncontaminated soils. The absence of these toxic metals is crucial for ensuring coffee safety, as noted by *Mollakhalili-Meybodi et al. (2022)*, who highlighted the importance of monitoring these contaminants due to their potential health risks. The trace metal composition in coffee beans has been studied widely by *Anderson and Smith (2002)*, who noted that heavy metal profiles can vary significantly based on soil composition and local agricultural practices. These variations serve as reliable indicators for differentiating between coffee-growing regions. The heavy metal analysis using Linear Discriminant Analysis (LDA) and hierarchical clustering demonstrates that green coffee beans exhibit distinct regional differentiation based on their heavy metal profiles. American samples are particularly distinguishable, primarily due to higher concentrations of metals such as Cu, Zn, and Cr, while African and Asian samples show more overlap but still exhibit separability, especially with metals like Ni and Mn. These results highlight the potential of using heavy metal composition for geographic origin authentication, which is valuable for quality control and traceability in the coffee industry. The use of Linear Discriminant Analysis (LDA) and hierarchical clustering to distinguish regional profiles based on trace metal content has also been supported by *Gure et al. (2017)*. They demonstrated that elements like Cu and Zn are effective markers for geographical origin, supporting the present study's findings. This differentiation could be beneficial for coffee traceability and authenticity, contributing to enhanced quality control measures in the coffee industry.

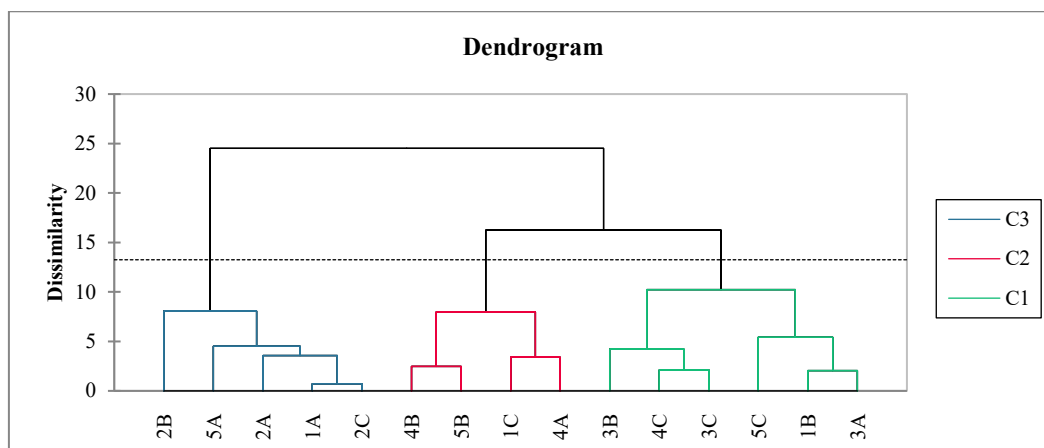


Figure 2 AHC of heavy metal compounds in samples

The dendrogram provides a visual representation of how the green coffee bean samples cluster based on their trace metal content. Each sample is grouped according to the dissimilarities in their heavy metal profiles. The dendrogram provides a comprehensive view of how green coffee bean samples cluster according to their trace metal content, grouping them based on chemical similarities and differences in their heavy metal profiles. Each of the three clusters C1 (Green), C2 (Red), and C3 (Blue) represents varying degrees of similarity among the samples' heavy metal content, reflecting potential influences of geographical origin, environmental conditions, and agricultural practices. The samples are grouped based on their geographical origin: samples labeled with A originate from Africa, B from America, and C from Asia.

Cluster C1 (Green) this cluster primarily includes samples that are more similar in their heavy metal composition. Cluster C1 includes sample from Africa, America, Asia (3B, 4C, 3C, 5C, 1B, 3A). The samples in this group are relatively similar in their heavy metal composition, indicating minimal variation within this cluster. This could suggest that, despite originating from different regions, these samples share certain environmental or processing factors that contribute to a similar trace metal profile (Ikenaka *et al.*, 2010). Cluster C2 (Red) samples like 4B, 5B, 1C, 4A in this middle cluster demonstrate moderate dissimilarity, suggesting a more diverse but still related heavy metal profile among these coffee beans. The presence of samples from different continents suggests that although these beans vary more significantly than those in C1, they still share commonalities in trace metal content. This moderate dissimilarity could be attributed to specific regional factors unique to each continent, such as differences in agricultural inputs, slight variations in soil chemistry, or different processing methods. (Tchounwou *et al.*, 2012). Cluster C3 (Blue) this cluster exhibits the highest level of dissimilarity among samples, indicating significant variance in heavy metal content. These Cluster C3 include samples 2B, 5A, 2A, 1A, 2C. This cluster exhibits the highest level of dissimilarity, indicating significant variance in heavy metal content across these samples. The wide range in trace metal profiles suggests that these beans may have been grown in distinctly different environmental conditions or were subject to unique agricultural or processing practices that led to their unique chemical compositions. High dissimilarity in Cluster C3 could be influenced by factors such as specific soil types with unique mineral compositions, varying altitudes that affect nutrient uptake, or distinct industrial or natural sources of trace elements in the regions where these coffees were cultivated (Anderson and Smith, 2002). These findings can be significant for coffee producers and consumers alike, as understanding the trace metal content in coffee beans could aid in identifying environmental or agricultural practices that minimize undesirable metal absorption, ensuring both quality and safety in coffee production (Berego *et al.*, 2023).

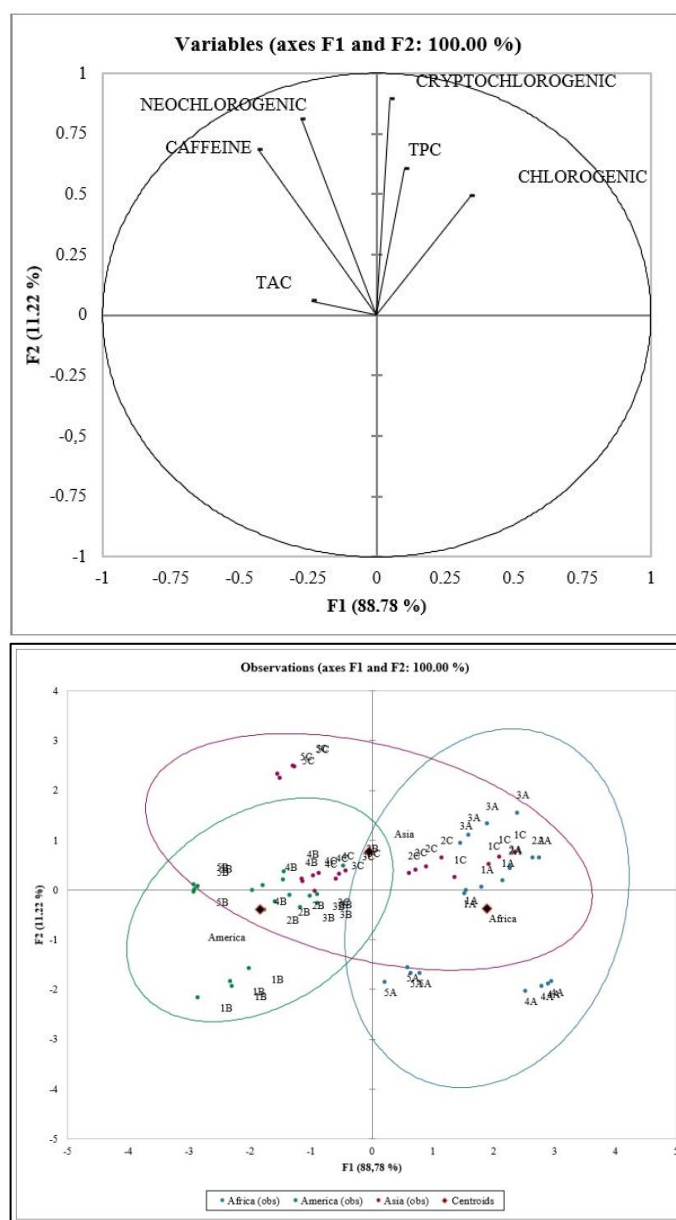


Figure 3 LDA of water- soluble compounds in samples

The Linear Discriminant Analysis (LDA) presented in Figure 3 highlights the distinct patterns in water-soluble compound profiles among green coffee beans from three geographical regions: Africa, America, and Asia. The LDA plot illustrates a clear separation of samples, indicating that geographical origin significantly influences the chemical composition of water-soluble compounds such as chlorogenic acids, total phenolic content (TPC), total antioxidant capacity (TAC), and caffeine. These findings are consistent with

previous research by *Jeszka-Skowron et al. (2016)* and *Alnsour et al. (2022)*, who reported that environmental factors, soil composition, and post-harvest processing techniques contribute to the variability in bioactive compound profiles in coffee. The primary axis (F1), which accounts for 88.78% of the total variance, is primarily influenced by variables like chlorogenic acids, TPC, and caffeine, suggesting that these compounds are the main discriminating factors among the regions. This high degree of separation along the F1 axis aligns with the findings of *Farah et al. (2006)*, who noted that chlorogenic acids are key indicators of coffee quality and are influenced by geographical and environmental conditions. Furthermore, the grouping of Asian samples in the upper left quadrant, characterized by lower levels of TPC and caffeine, corroborates the research of *Hall et al. (2022)*, who identified region-specific trends in these bioactive compounds. The secondary axis (F2), explaining 11.22% of the variation, contributes to distinguishing certain outlier samples, such as those from Africa high exhibit distinct profiles. This observation might be attributed to unique cultivation practices or microclimatic conditions that influence the biosynthesis of secondary metabolites in these beans. Similar variability within African coffee samples was also reported by *Gure et al. (2017)*, emphasizing that intra-regional differences can be significant due to localized environmental factors and farming methods. The LDA results also reveal a partial overlap between samples from America, Asia and Africa, suggesting that some common factors, such as similar soil compositions or processing methods, might be at play. *Anderson and Smith (2002)* noted that shared agricultural practices and environmental conditions could result in overlapping chemical profiles, which may complicate origin authentication efforts. Despite this overlap, the centroid positioning of American samples indicates that they have distinct overall profiles, primarily due to higher concentrations of certain bioactive compounds such as caffeine and neochlorogenic acid. This finding is supported by *Mendes et al. (2022)*, who found that coffee samples from the Americas typically show elevated levels of these compounds due to favorable growing conditions and soil mineral content. The distinct clustering of Asian samples further supports the hypothesis that geographical origin significantly impacts coffee chemistry. Asian samples tend to exhibit lower antioxidant capacity and different chlorogenic acid profiles compared to African and American beans, which is in line with the work of *da Silva et al. (2017)*, who highlighted the influence of geographical and environmental factors on antioxidant properties in coffee. In conclusion, the LDA analysis demonstrates that water-soluble compound profiles can effectively differentiate green coffee beans based on their geographical origin. These findings are consistent with existing literature and underscore the potential of using chemical profiling for origin authentication, quality control, and traceability in the coffee industry. Future studies could build upon this research by integrating additional variables such as soil mineral composition and climatic data to provide a more comprehensive understanding of the factors driving these regional differences.

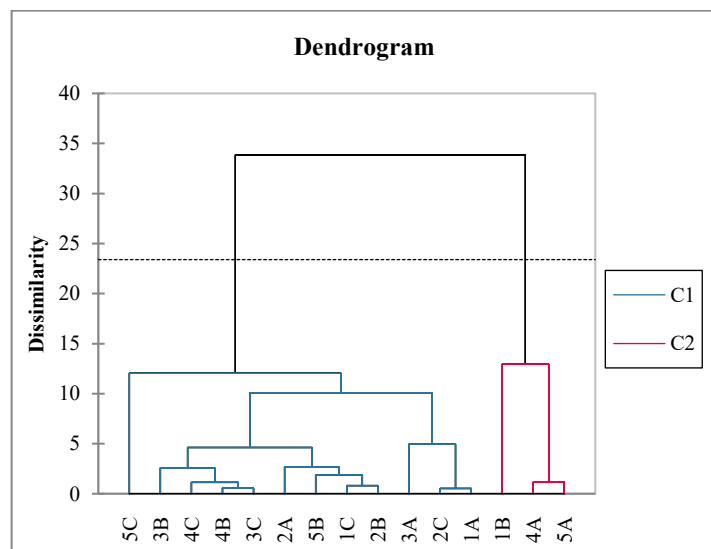


Figure 4 AHC of water-soluble compounds in samples

The dendrogram visualizes relationships between coffee bean samples from Africa, America, and Asia based on their water-soluble compound content, such as chlorogenic acids, total phenolic content (TPC), total antioxidant capacity (TAC), and caffeine. Cluster 1 (C1) Blue Color includes the majority of samples from all three continents, indicating similar profiles of water-soluble compounds. Predominantly consists of African samples (3A Tanzania, 4A Burundi) and Asian samples (1C Indonesia, 2C Indonesia), along with some American samples (3B Nicaragua, 4B Honduras). This grouping suggests that these samples have comparable levels of chlorogenic acids, caffeine, and overall antioxidant activity, potentially due to similar growing conditions or processing methods (*da Silva et al., 2017*). Cluster 2 (C2) red color contains samples from Africa (4A Burundi, 5A

Malawi) and America (5B Mexico). The clustering patterns in this dendrogram align with previous research on coffee bean composition and its dependence on geographical origin and processing. Studies indicate that chlorogenic acid and antioxidant levels in coffee beans are highly influenced by altitude, sunlight exposure, and temperature during growth, as well as post-harvest processing methods like drying and roasting. For example, coffee beans from high-altitude regions tend to have higher chlorogenic acid levels, contributing to greater antioxidant activity (*Barbosa et al., 2019; Mendes et al., 2022; Badmos et al., 2019; Wan et al., 2024*).

Research by *da Silva et al. (2017)* also supports the observation that coffee beans from different continents can exhibit similar water-soluble compound profiles when grown under comparable environmental conditions or subjected to similar processing techniques.

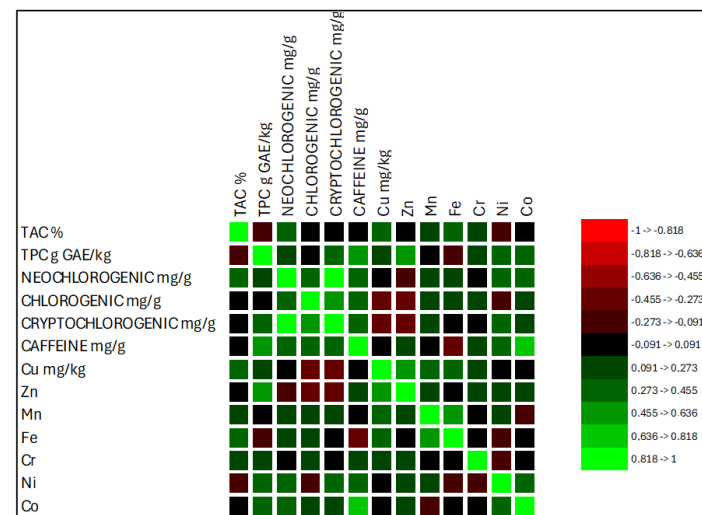


Figure 5 Correlation map for trace elements and water-soluble compounds in samples

Finally, the correlation analysis between bioactive compounds (chlorogenic acids and caffeine) and trace metals highlights important interactions. Positive correlations between metals such as Zn, Mn, and antioxidant compounds suggest that these elements may enhance antioxidant activity in green coffee beans. Conversely, negative correlations, particularly between Cu and certain bioactive compounds, indicate that excessive trace metals may inhibit the biosynthesis of key antioxidants. These findings align with prior research and underscore the impact of environmental and soil conditions on the chemical composition of coffee beans (*Brzezicha et al., 2021; Semen et al., 2017; Socala et al., 2020*).

CONCLUSION

The study confirms that geographical origin significantly influences green coffee beans' physicochemical properties and trace metal composition. American beans exhibited higher dry matter content, whereas African and American beans demonstrated better microbial stability due to lower water activity. Consistent pH levels across regions suggest that environmental conditions play a key role in pH determination. Trace metal analysis revealed that toxic metals like Pb and Cd were below detection limits, ensuring coffee safety. Distinct regional differentiation was observed through heavy metal and water-soluble compound profiling, with LDA and hierarchical clustering effectively classifying coffee samples based on their chemical compositions. Positive correlations between trace elements (e.g., Zn, Mn) and antioxidant compounds suggest that these metals may enhance bioactivity, while excessive Cu content could negatively impact antioxidant levels. These findings are valuable for enhancing quality control, traceability, and origin authentication in the coffee industry, contributing to improved coffee safety and marketability.

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