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Volatolomics Profiling of Specialty Green Coffee Beans from South and Central American Harvesting Sites

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Understanding and analyzing coffee volatiles is essential for quality control, authenticity verification, and product development. To qualify as specialty coffee, the beans must not only achieve this score but also meet specific quality standards throughout the supply chain, which includes factors such as the growing conditions, processing methods, and overall flavor profile. This study investigates volatile organic compound (VOCs) profiles for further geographical authentication of green specialty coffee (*Coffea arabica* L.) samples using GC-MS, hierarchical cluster analysis, and principal component analysis (PCA). The dissimilarity dendrogram revealed distinct clustering patterns with intra-regional distances ranging from 13.97 to 16.10 units for Brazilian samples and inter-regional distances of 40.03–44.17 units between geographical groups. PCA of ten VOC classes demonstrated that the first two principal components account for 51.93% of the total variance. The clustering effectiveness was supported by a cophenetic correlation coefficient of 0.55, indicating moderate reliability in geographical discrimination. PCA revealed that furans, hydrocarbons, and heterocyclic compounds contributed positively to PC1, while alcohols showed strong positive loading (0.518) on PC2. The plot indicates that five principal components (factors) explain 87.48% of the total variance, suggesting complex VOC interactions in geographical differentiation. The significant overlap observed in the PCA analysis suggests shared characteristics between regions, which may be attributed to similar agricultural practices or cultivars

Keywords: *Coffea arabica*, geographical origin, volatolomics, GC-MS, quality

Introduction

Specialty coffee, the premium segment within the global coffee industry, is characterized by exceptional quality, distinct flavor profiles, and traceable origins, both botanical and geographical. The Specialty Coffee Association (SCA) defines specialty coffee as green coffee beans scoring 80 points or above on a 100-point scale, evaluated through standardized cupping protocols (Rhinehart, 2017; Giacalone et al., 2019). This advanced classification considering selected

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quality parameters, including the absence of primary defects, exact moisture content, but most of all distinctive sensory attributes. The growing demand for specialty coffee has led to increased focus on origin-specific characteristics and the need for application and development of sophisticated analytical methods to authenticate origin of such products, both geographical and botanical (Dong et al., 2022, Demianová et al., 2022).

Volatile organic compounds (VOCs) in coffee beans represent a complex chemical constituents that significantly contribute to the sensory experience of coffee. These compounds, including furans, aldehydes, ketones, alcohols, esters, pyrazines, and various heterocyclic compounds, are formed through multiple biochemical pathways during bean development and are further modified during post-harvest processing (Toledo et al., 2020; Sunarharum et al., 2019, Capporaso et al., 2018). The volatile profile of green coffee beans reflects both the genetic predisposition of the coffee variety and the environmental conditions of growing and harvesting area. Over 1,000 volatile compounds can be found in coffee, but only a few (approx. 40-50) compounds represent key aroma and flavor contributors (Yeretzian et al., 2019).

The application of volatolomics presents a promising approach for authenticating the geographical origin of specialty coffee beans (Freire et al., 2020, Demianová et al., 2022). This analytical approach makes use of the idea that the coffee volatile metabolome influenced by environmental factors such altitude, soil composition, climate, and regionally specific farming practices. Developments in analytical techniques, especially gas chromatography-mass spectrometry (GC-MS) accompanied with multivariate statistical analysis represent a powerful tool for food authentication (Chin et al., 2021; Martinez-Saez et al., 2019). In addition to supporting quality assurance in the specialty coffee sector, the ability to confirm geographical origin through volatile compound analysis also helps preserve the integrity of origin-specific coffee designations and the premium prices they fetch on the global market.

There is an urgent need to systematically study these compounds in specialty coffees from various well-known regions. Thus, this research focused on a comprehensive description of VOCs in specialty coffee from renowned regions of Central and South America, with the aim to emphasize possible differences caused by environmental conditions.

Table 1 List of analyzed samples.

Origin	Harvesting site	Post-harvest processing	Cultivar	Altitude
Brazil 1	Conillon Esprito Santos	pulped natural	Bourbon	1200–1500
Brazil 2	Minas Gerais	pulped natural	Bourbon	940–1120
Brazil 3	Cerrado	natural	Bourbon	940–1120
Brazil 4	Cerrado	pulped natural	Bourbon	1150–1350
Brazil 5	São Sebastião da Grama,	pulped natural	Bourbon	900–1500
Colombia	Huila - Tello	washed	Cattura, Typica	1400–1700
Colombia	Santander - Bucamaranga	washed	Cattura, Castillo	1200–1500
Peru	Amazonas Province	washed	Bourbon, Catimor, Typica	1400–1800
Bolívia	Altiplano-Hochebene	washed	Poca, Cattura	1500–1700
Guatemala	Rainforest Coban	washed	Bourbon	1300–1500
Guatemala	Sacatepequez	washed	Bourbon	1450–1800
Nicaragua	Nueva Segovia	washed	Caturra	1000–1300
Nicaragua	Jalapa, Nueva Segovia	washed	Cattura, Catimor	1150–1400
Costa Rica	San Rafael Tarrazu	washed	Cattura	1200–2000
Costa Rica	Tarrazu - Leon Cortes Llano Bonito	pulped natural	Cattura	1200–2000
El Salvador	San José La Majada	washed	Bourbon	900–1200
El Salvador	Apaneca	washed	Cattura,Catuai	1250
Honduras	La Paz, Marcala	natural	Bourbon, Typica	1200–1800
Mexiko	Huatusco, Veracruz	washed	Catimor, Bourbon	1200–1600
Cuba	Sierra Maestra	washed	Typica	1000
Panama	Boquete	washed	Typica, Catuai	1400–1500

Materials and methodology

The study analyzed volatile organic compounds (VOCs) from coffee (*Coffea arabica* L.) samples (Table 1) originating from South America (Brazil, Colombia, Peru, Bolivia) and Central America (Guatemala, Nicaragua, Costa Rica, El Salvador, Mexico, Cuba, and Panama). The dataset comprised measurements of ten volatile compound classes: furans and derivatives, aldehydes, alcohols, organic acids and esters, hydrocarbons (alkanes), terpenoids, hydrocarbons (alkenes), other heterocyclic compounds, ketones, and aromatic hydrocarbons. All samples (n=21) were specialty quality and obtained from Café ORO (Zvolen, Slovakia).

Determination of volatile compounds using gas chromatography (GC-MS)

Ten grams of homogenized green coffee beans were put into 40 mL glass vials with ptfe/sil septum Archon caps (Agilent Technology, Palo Alto, USA). The samples were warmed to 35 °C for 15 min in Metaltermoblock (Liebisch Labortechnik GmbH & Co., Bielefeld, Germany). Volatiles were collected with a 2 cm Carboxen®/PDMS (CAR/PDMS) SPME fiber (Sigma-Aldrich GmbH, Steinheim, Germany), exposed for 30 min at 35 °C, followed by GC-MS analysis. Volatiles were determined according to the method described in Sádecká et al. (2014) with modifications, using an Agilent Technologies 6890 gas chromatograph (Agilent Technology, Palo Alto, USA) equipped with an Agilent Technologies 5973 selective inertial detector (MSD) and a J&W 122–7333 DB-WAXetr 30 m × 0.25 mm x 0.5 µm capillary column (Agilent Technology, Palo Alto, USA). The carrier gas was helium, and the injector temperature was 250 °C, using splitless mode with an initial temperature of 250 °C. The pressure was 88.9 kPa, the flow rate was 20.0 mL min^{-1}, the cleaning time was 1.00 min, and the total flow rate was 24.6 mL min^{-1}. The oven temperature was programmed to be isothermal at 50 °C for 1 min, then heated to 250 °C at 5 °C.min^{-1}. The transfer line temperature and ion source temperature were 280 °C. Electron ionization (EI) was set to 70 eV and the mass spectrometer collected data in full scan mode. Identification was performed by comparing the collected mass spectra and chromatography data with reference materials and the NIST 14 library.

Statistical analysis

Statistical analysis was performed using Python 3.x software tool with scikit-learn library for PCA, scripy for statistical calculation, and seaborn and matplotlib for visualization. Data normalization was performed using Z-score using formula $z = \frac{x-\mu}{\delta}$ where x is the raw value, µ is the mean, and δ is the standard deviation. Significant differences were calculated using ANOVA Tukey's HSD test at the p-value=0.05. For visualization results, we used a Z-score heat map, principal component analysis, and hierarchy clusters (AHC).

Results and discussions

Using GC-MS we were able to identify more than 185 volatile compounds (VOCs). Based on the chemical structure we divide them into the following categories: furan derivates, aldehydes, alcohols, organic acids and their esters, alkanes, terpenoids, alkenes, heterocyclic compounds, ketones, and aromatic hydrocarbons. Tsegay et al., (2020) pointed out that about 80% of the total volatile compounds are alcohols, furans, aldehydes, and esters and very few heterocyclic compounds are identified in the green coffee beans, which is in accordance with our findings (Table 2). Poyraz et al. (2016) stated that even though high concentrations of other heterocyclic compounds are present, furan derivates, mainly furfural, furfuryl acetate, and furfurylalcohol are relatively abundant. The authors further described that significantly, the most abundant were organic acids and esters, alcohols, terpenoids, and furan derivates. However, Marek et al. (2020) and Kulapichitr (et al., 2018) stated that changes that occurred during the roasting process are significant regarding the VOCs. Our preliminary findings align with previous research indicating that environmental factors such as soil composition, altitude, and climate significantly influence the volatile composition of coffee beans (DeVivo et al., 2023; Marwani et al., 2024).

One-way ANOVA results proved significant differences ($p < 0.05$) in VOCs groups across geographical origins - organic acids and esters and aromatic hydrocarbons. This suggests that the geographical origin of the coffee has a potential impact on the concentration of specific volatiles, while other VOCs remain relatively consistent across different origins. Vezzulli et al., (2023) observed that aromatic compounds are linked to the geographical origin. Authors further stated that samples from Brazil, Panama, and Colombia were discriminated using ethanol, methyl nonane, n-decan, D-limonene, and tributyl phosphate. However, it is necessary to add that we did not identify tributyl phosphate in either of our samples. Min et al. (2022) pointed out the need for fingerprinting profiling, given VOCs can differ within the harvesting year.

Table 2 ANOVA results of statistical differences between analysed samples

Origin group	Furan + derivates	Aldehydes	Alcohols	Organic acids + esters	Alkanes	Terpenoids	Alkenes	Heterocyclic compounds	Ketones	Aromatic hydrocarbons
Brazil	9.28^{a}	9.67^{a}	19.70^{a}	20.65^{ab}	4.03^{a}	9.23^{a}	2.62^{a}	9.02^{a}	0.75^{a}	2.62^{b}
Nicaragua	10.67^{a}	5.14^{a}	8.91^{a}	14.46^{a}	7.73^{a}	7.27^{a}	4.40^{a}	8.67^{a}	2.10^{a}	9.49^{c}
Honduras	9.07^{a}	9.21^{a}	9.91^{a}	27.30^{abc}	0.98^{a}	9.74^{a}	5.29^{a}	6.43^{a}	1.20^{a}	0.72^{a}
Panama	4.12^{a}	6.73^{a}	16.41^{a}	34.96^{abc}	2.86^{a}	7.28^{a}	0.58^{a}	10.47^{a}	2.12^{a}	1.36^{a}
Peru	4.12^{a}	6.73^{a}	16.46^{a}	34.96^{abc}	2.86^{a}	7.28^{a}	0.58^{a}	10.47^{a}	2.12^{a}	1.36^{a}
Mexiko	6.89^{a}	9.56^{a}	19.74^{a}	36.78^{abc}	0.00^{a}	6.08^{a}	5.99^{a}	2.88^{a}	0.00^{a}	2.90^{b}
El Salvador	11.57^{a}	10.76^{a}	19.76^{a}	26.09^{abc}	9.24^{a}	3.43^{a}	0.29^{a}	4.32^{a}	0.32^{a}	0.56^{a}
Costa Rica	4.80^{a}	7.80^{a}	26.47^{a}	31.15^{abc}	3.00^{a}	8.54^{a}	0.35^{a}	3.01^{a}	1.41^{a}	0.38^{a}
Guatemala	13.49^{a}	5.93^{a}	15.79^{a}	27.55^{abc}	2.85^{a}	2.14^{a}	6.26^{a}	6.67^{a}	0.58^{a}	0.50^{a}
Colombia	4.44^{a}	3.49^{a}	20.84^{a}	53.48^{c}	0.85^{a}	3.05^{a}	1.13^{a}	1.99^{a}	2.42^{a}	0.90^{a}
Bolivia	4.60^{a}	5.68^{a}	9.65^{a}	36.24 ^{abc}	5.99^{a}	8.22^{a}	0.00^{a}	3.09^{a}	0.00^{a}	8.29^{c}
Cuba	2.61^{a}	6,37^{a}	15.77^{a}	47.58^{bc}	3.23^{a}	8,11^{a}	1,69^{a}	4.64^{a}	0.00^{a}	0.00^{a}
Pr > F(Model)	0.37	0.95	0.75	0.01	0.42	0.84	0.15	0.80	0.33	0.02
Significant	No	No	No	**Yes**	No	No	No	No	No	**Yes**

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

For more complex data expression, we performed Tukey's HSD. Regarding the organic compounds and esters, results showed that there are significant differences between certain geographical areas, thus between Bolivia and Nicaragua, Brazil and Colombia, Brazil and Peru, and El Salvador. Considering aromatic hydrocarbons, significant differences were observed between Bolivia, Nicaragua, and the rest of the dataset, alongside Brazil and Mexico samples and the rest of the analyzed samples.

For better profiling visualization we used z-score normalization and Heatmap visualization (Figure 1).

Yulia et al. (2023) pointed out the necessity for the geographical authentication of green coffee. Our results showed a substantial overlap between regions suggesting shared volatile profiles between South and Central American coffees. The wider dispersion of South American samples indicates more diverse volatile profiles. Quan et al. (2023) focused on Vietnamese coffees and UV-VIS analysis accompanied by a PLS-DA prediction model. The authors reached a relatively high prediction ability of the model. Given the number of samples in our dataset (n=12) we have performed the principal component analysis for a comprehensive study of geographical origin on VOCs (Figure 2).

There has been various research focused on the possibility of using volatile fingerprinting to differentiate between coffee from various regions, thereby ensuring product authenticity and quality (Bertrand et al., 2012; Dippong et al., 2022). Our results showed that organic acids + esters were particularly effective in distinguishing between South and Central American coffees. However, PCA revealed that furans, hydrocarbons, and heterocyclic compounds contributed positively to PC1, while alcohols showed strong positive loading (0.518) on PC2. The scree plot indicates that five principal components (factors) explain 87.48% of the total variance, suggesting complex VOC interactions in geographical differentiation. The significant overlap observed in the PCA analysis suggests shared characteristics between regions, which may be attributed to similar agricultural practices or cultivars. The tighter clustering of Central American samples suggests more consistent volatile profiles within this region. The total explained variance using Factors 1 and 2 (51.9%) indicates that the PCA captures approximately half of the total variation in the dataset. This level of explained variance suggests that while the PCA provides useful insights, there are additional dimensions of variation not captured in the first two components (factors). This PCA analysis reveals both regional patterns and significant overlap in volatile profiles between South and Central American coffees, suggesting that while geographic origin influences volatile composition, other factors also play important roles in determining coffee characteristics. Therefore, for proper origin prediction, it is necessary to employ more advanced analysis such as linear or quadratic discriminant analysis, and examine the results of the bigger dataset.

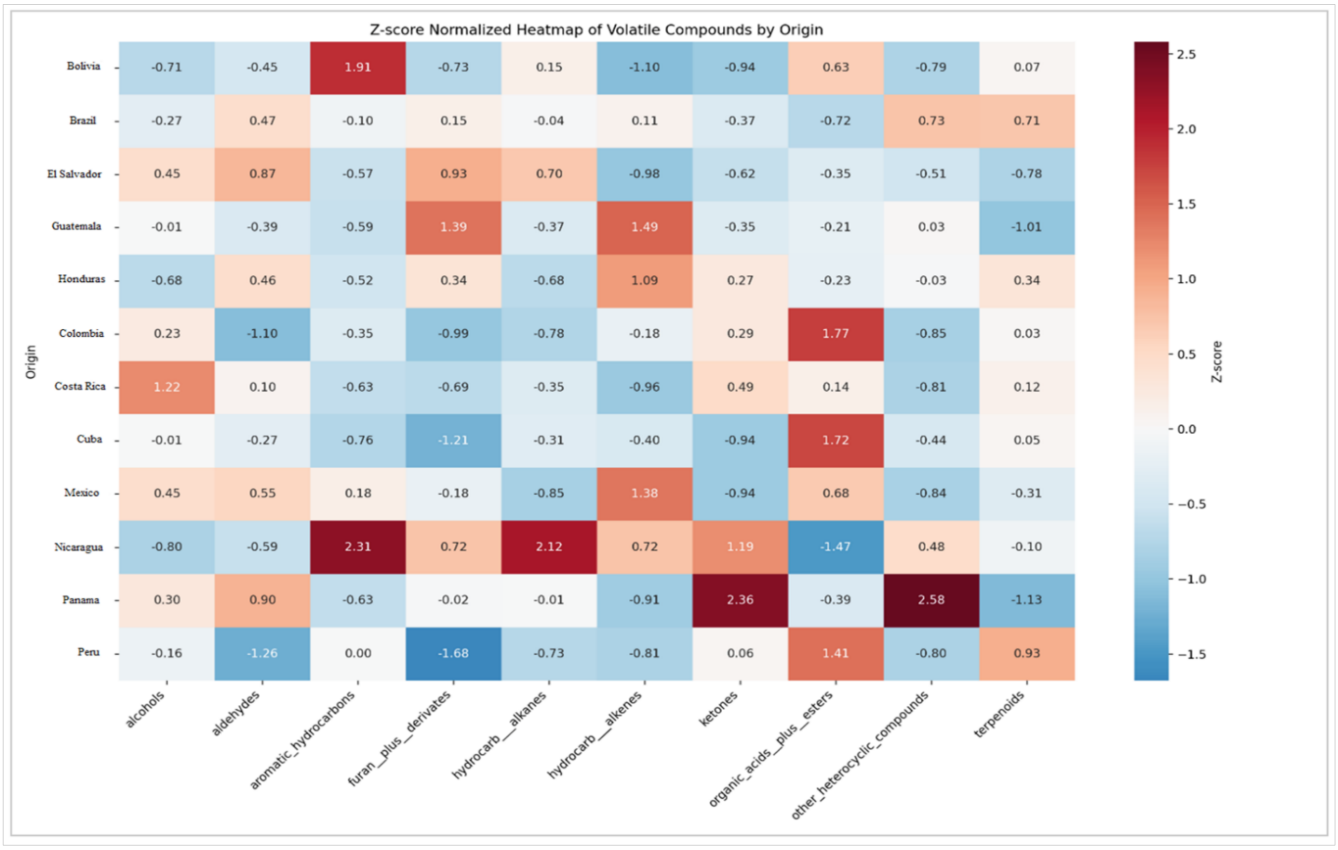

Figure 1 Z-score normalization and Heatmap visualization of VOCs concerning harvesting sites.

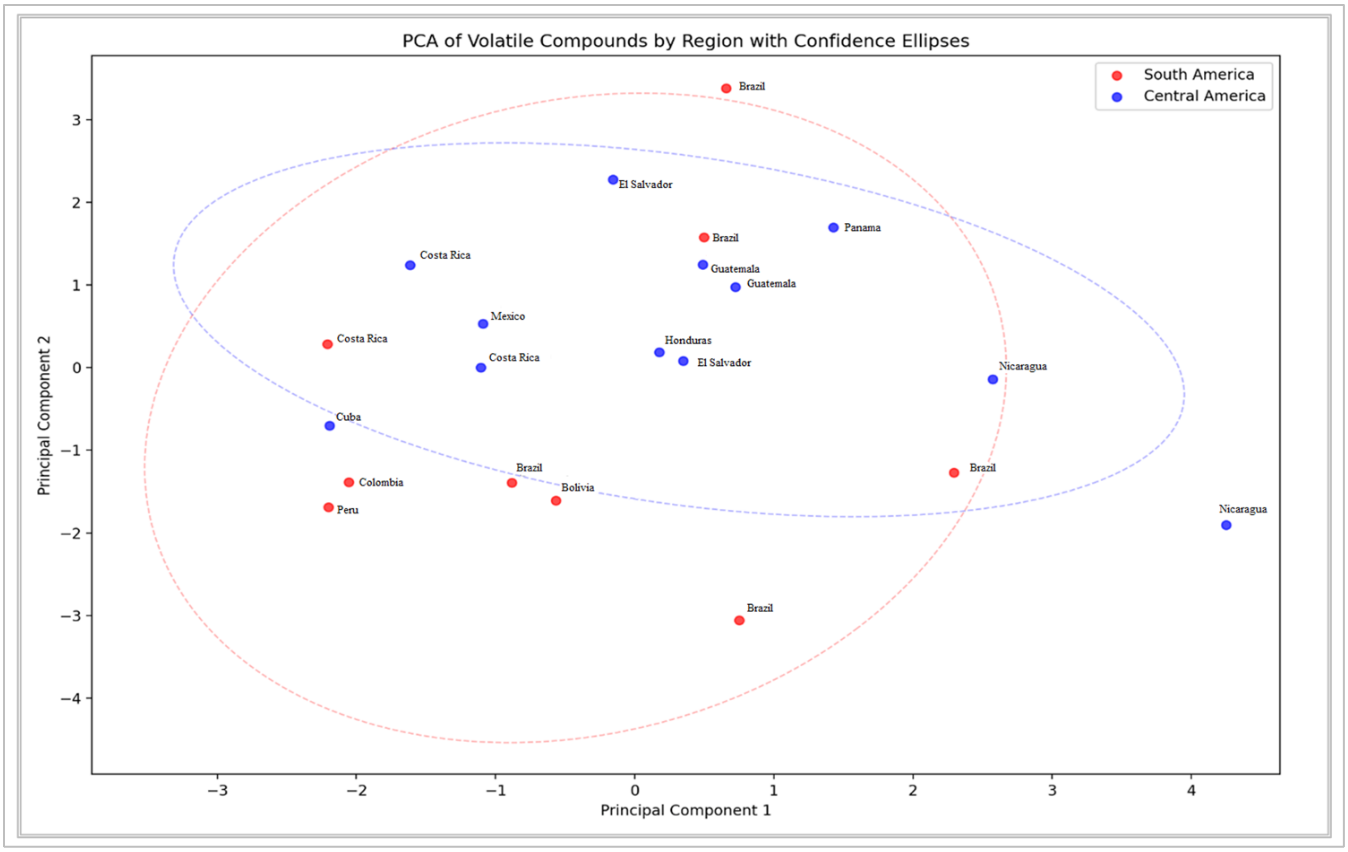

Figure 2 PCA map of geographical distinguishing based on VOCs

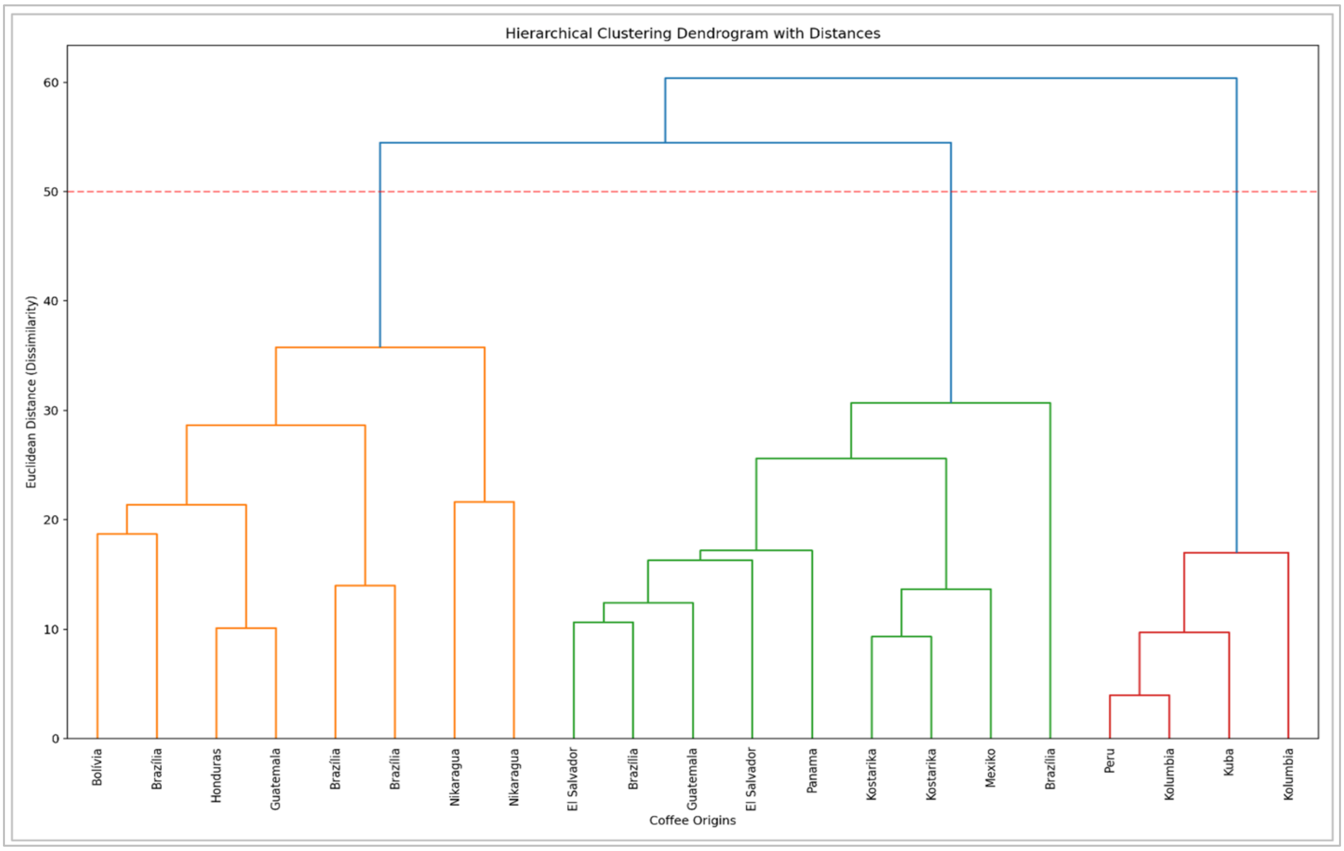

Figure 3 AHC dendrogram of Euclidean Distance.

The dendrogram visualizes dissimilarities between samples, with closer branches indicating more similarity (Figure 3). The cluster assignments reveal three main clusters, highlighting which origins are closer to each other in terms of their volatile profiles, with the following group cluster assignments: Cluster 1 Colombia, Peru, and Cuba. Cluster 2: Brazil, Guatemala, Costa Rica, El Salvador, Mexico, Panama. Cluster 3: Brazil, Bolivia, Guatemala, Nicaragua, and Honduras. However, it is essential to emphasize that selected Brazilian samples, representing South America, correlated with Guatemala and Nicaragua (Central America). This can be explained by the same cultivars Bourbon and Typica of the samples, which suggest that using VOCs for geographical authentication can have limitations caused not only by harvesting years but also by botanical aspects.

The dissimilarity dendrogram analysis of volatile organic compounds (VOCs) in coffee samples reveals patterns in geographical differentiation and processing influences, with a cophenetic correlation coefficient of 0.55. The dendrogram demonstrates distinct clustering based on geographical origins, with Brazilian samples showing closer relationships (dissimilarity distances 13.97–16.10 units). Similar origin patterns were described by DeVivo et al. (2023) and Demianová et. al. (2022), who demonstrated that regional environmental factors significantly influence VOCs profiles. The clear separation between Brazilian and other origins (distances 40.03–44.17 units) supports Bressanello et al. (2021)'s conclusions about terroir-specific volatile signatures.

The clustering patterns could reflect processing method variations, particularly evident in the Brazilian samples' grouping. This corresponds with review research by Toledo et al. (2020) and Boo et al (2021), who found that post-harvest processing significantly affects volatile compound development. Therefore, is essential to systematically perform profiling analysis to obtain as valid and robust data as possible, considering both geographical, environmental, and botanical factors.

Conclusions

Coffee is one of the most traded commodities worldwide. Based on the recent market evolution the necessity arises to develop methods suitable for geographical origin authentication, especially for specialty quality coffees. This research was focused on the description of selected VOCs groups measured in specialty green coffee beans from renowned South and Central America harvesting sites. Preliminary volatolomics accompanied by statistical analysis so far shows a potential to be used for origin authentication purposes. However, it is essential to emphasize the need to systematically study these compounds in coffees harvested from significantly more regions and various harvesting years to assess possible variances caused by environmental and botanical factors.

Concentrations of Pb and Cd remain within safe limits for consumption, as determined by the Provisional Maximum Tolerable Daily Intake (PMTDI) values set by the WHO.

Conflict of interest

The authors declare no conflict of interest.

Ethical statement

This article does not include any studies necessitating an ethical statement.

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