

ANALYSIS OF CHEMICAL ATTRIBUTES BASED ON IDENTIFICATION MARKERS TO DIFFERENTIATE MEDIUM ROASTED *COFFEA ARABICA* REGARDING DIFFERENT GEOGRAPHICAL ORIGIN

Katarína Poláková, Alica Bobková, Marek Bobko, Ľubomír Belej, Andrea Mesárošová

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: xpolakovak1@uniag.sk

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ABSTRACT

Traceability in the coffee supply chain is crucial for ensuring transparency and authenticity. It helps safeguard the interests of both producers and consumers by minimizing the risk of fraudulent practices and ensuring fair trade. Different geographical origins can lead to variances in coffee taste, quality of product and economic value. Just because controlling the authenticity of the geographical origin of coffee beans is of great importance for producers and consumers worldwide. This study determined parameters (caffeine, chlorogenic acids, total antioxidant capacity (TAC), total polyphenols content (TPC) volatile compounds, pH, water activity a_w , and dry matter) based on which was testing classification of geographical origin of coffee beans. For this research, six samples of 100% *Coffea arabica* medium roasting coffee beans, from the two major growing country of America and Africa were studied. As part of the research, variability in chemical composition based on geographical origin was confirmed. For America samples were significant parameters TAC, 4,5-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, caffeine and chlorogenic acids. Second group was sample from Africa. For these samples was determine parameter like TPC, cryptochlorogenic acid, neochlorogenic acid and pH. Volatile substances, caffeine, pH, chlorogenic acids were considered the most important parameters that can help to identify the geographical origin.

Keywords: geographical origin, chemical composition, medium roast coffee, *Coffea arabica*

INTRODUCTION

Coffee beans are extremely important agricultural and industrial commodity. Coffee cultivation represents is of great importance in the world economy. Due to the growing consumer demand for commodities with higher quality and geographic certification, the topic is relevant (Babova et al., 2016). The coffee tree we include to the family Rubiaceae. Green coffee beans are produced from the plant *Coffea L.*, of which nowadays there are more than 70 species. However, only two of these species are economically used and scientific explored worldwide: *Coffea arabica* (Arabica), considered to be acid but coffee disease resistant, and provides 25% of world's production. These species present a very different chemical composition. The most preferred is Arabica coffee in comparison to Robusta coffee due to its superior sensory properties (Quan et al., 2023). The most producing countries of coffee are South America with around 43%, Asia (24%), Central America (18%), and Africa (16%) (Mussato et al., 2011; Link et al., 2014). In the coffee industry, sustainability, quality, and origin have become hot topics (Krishnan, 2017). Coffee beans are rich in different chemical compounds that influence factors like botanical and geographical origin, environmental conditions, or roasting process (Mendes et al., 2022). The most important factor for quality end products cup of coffee is the highest quality raw green beans of *Coffea arabica* (Demianová et al., 2022). Verification and declaration of geographical origin can help in the coffee industry because the product with certification of geographical origin has twice the market value of a similar product without certification (Markos et al., 2023). Furthermore, the interest in local and quality coffee beans, with certificates of geographical origin and production environment, are becoming more and more coveted in the coffee sector. The geographical origin is one of the most relevant factors that determine the quality and commercial value of coffee beans (Mendes et al., 2022; Yang et al., 2021). The availability of reliable verification and authentication based on markers based on chemical composition as a tool to assess the geographical origin or relative amounts of different origins of coffee beans in a mixture would be highly desirable and could represent a useful tool to prevent possible fraud in the coffee industry (Romano et al., 2014). The aim of these studies was based on determined parameters to confirm geographical origin.

MATERIAL AND METHODS

Material

For this research was used samples *Coffea arabica* obtained from different geographical origins (Africa, America). Samples were roasted through a medium roasting process. Samples were distributed from Barzzuz Ltd. (Banská Bystrica, Slovakia). Detailed description of samples is shown in the Table 1.

Table 1 List of analyzed samples

Samples	Country	Variety	Processing	Altitude (mamsl)
1A	Burundi	B		1700 - 1900
2A	Rwanda	B, K	W	1400 - 1900
3A	Ethiopia	B, H		1900 - 2200
1B	Brazil	B		900 - 1100
2B	Panama	T, C1	W	1500 - 1700
3B	Columbia	B, C1,T		1200 - 1500

Note: Samples: A - Africa, B - America, Variety: B - Bourbon, C1 - Caturra, H - Heirloom, K - Kent, T - Typica; W - wet (fully washed); mamsl - meters above mean sea level

Methodology

Extract preparation

All samples of medium roasting coffee were first homogenized by milling using electrical equipment Grindomix GM 200 (Retsch, Haan, Germany) for 60 s at 10,000 min⁻¹. The extraction process involved utilizing 120 mL of deionized water at a temperature of 95 °C to extract 7 g coffee samples. This extraction, lasting 5 minutes with occasional stirring, was followed by filtration through Sartorius filter paper for further refinement. (Sartorius Lab Instruments GmbH & Co. KG, Nottingham, Germany). Final extracts were used in individual analyses.

Determination of water activity

For the determination of water activity of roasted coffee samples parameter, a measurement through the lab instrument Meter Fast-Lab (Germany). The

measurement was conducted three times independently for each sample, ensuring consistency and reliability in the results.

Determination of dry matter

The dry matter content of medium-roasted coffee powder samples was assessed using the KERNDAB 100 - 3 lab instrument (KERN & SOHN GmbH, Balingen, Germany) with a specific drying program set at 110 °C. The results were expressed as a percentage.

Determination of pH

The pH was determined in coffee extracts at a temperature of 20 °C. For the determination, equipment pH 70 portable pH-meter (XS Instruments, Italy) was used (Bobková et al., 2022).

Determination of total antioxidant capacity

The total antioxidant capacity was determined using the DPPH radical scavenging activity assay, following the methodology by Brand-Williams et al. (1995). Initially, 0.025 g of DPPH radical was weighed and dissolved in ethanol (Centralchem, Bratislava, Slovakia, 96%). The resulting stock solution was prepared by filling a volumetric flask with a volume of 100 mL, followed by dilution with ethanol (1:9). Subsequently, 3.9 mL of the diluted DPPH solution was transferred into glass cuvettes, and the initial DPPH absorbance (A_0) was measured at a wavelength of 515.6 nm. For the next step, 100 μ L of the sample extract was pipetted into a cuvette, and the mixture was stirred with a glass rod. The absorbance (A_t) was then measured after 10 minutes at 515.6 nm using the T80 UV/VIS Spectrometer (PG Instruments, Ltd.; Lutterworth, UK). The scavenging capacity in coffee extract samples was determined as a percentage of the inhibition of DPPH radicals, with the calculation performed using a specific equation. The scavenging capacity was calculated using the following equation:

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

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.

Determination of polyphenols content

Before the measurement, sample preparation involved pipetting 50 μ L of extracts into 50 mL volumetric flasks. The Folin-Ciocalteu reagent, diluted with distilled water (1:2 v/v), was added with a volume of 2.5 mL to the flask containing the extract. Following this, 5 mL of Na₂CO₃ (20% water solution) was introduced. The flasks were then filled with distilled water up to 50 mL and left for two hours at room temperature to allow the development of the blue-colored complex. The absorbance of the samples was measured at a wavelength set to 765 nm according to the method described by Fu et al. (2011).

Determination of caffeine and chlorogenic acids

HPLC analysis of the content of chlorogenic acids and caffeine was carried out using the methodology described in Bobková et al., (2021). Separation was performed via a C-18 Poroshell 12 colony (150 mm * 3 mm * 2.7 μ m; Agilent Technologies, Waldbronn, Germany) acetonitrile (A) and 0.1% H₃PO₄ in ddH₂O (v/v) (B) was used as a mobile phase. The gradient elution program was set as follows: 0 - 1 min isocratic elution using 20% A + 80% B; 1 - 25 min linear gradient elution: 1 - 5 min using 25% A + 75% B, 5 - 15 min using 30% A + 70% B and 15-25 min using 40% A + 60% B. The equilibration time was 3 min before the next injection. The flow rate of the mobile phase was 1 mL.min⁻¹, the injection volume 10 μ L and the separation temperature 30 °C. The detection wavelengths were 276 nm for caffeine and 320 nm for chlorogenic acids. The total wavelength of data collection was set to 210 - 400 nm. The standards used were as follows: caffeine standard (HPLC standard, purity 98%, Sigma-Aldrich GmbH, Steinheim, Germany), standard neochlorogenic acid (HPLC standard, purity 99%, Sigma-Aldrich GmbH, Steinheim, Germany), standard chlorogenic acid (HPLC standard, purity 99%, Sigma-Aldrich GmbH, Steinheim, Germany), standard crypto chlorogenic acid (HPLC standard, purity 98%, Sigma-Aldrich GmbH, Steinheim, Germany), 4,5 dicaffeoylquinic acid (HPLC standard purity 99%, Sigma-Aldrich GmbH, Steinheim, Germany), 3,5 dicaffeoylquinic acid (HPLC standard purity 99%, Sigma-Aldrich GmbH, Steinheim, Germany), deionize water ddH₂O (18.2 M Ω .cm⁻¹; 25 °C); HPLC Acetonitrile (Chromasolv Gradient, purity \geq 99.8%, Sigma-Aldrich GmbH, Steinheim, Germany).

Determination of volatiles

For GC-MS analysis, 10 g of homogenized roasted coffee beans were placed in 40 mL glass vials with septum Archon caps pte/sil. The coffee samples were warmed up to 35 °C for 15 min in a Metaltermoblock Liebig Labortechnik. Self-sorption was carried out with Fiber: Carboxen® / PDMS (CAR/PDMS) for 2 cm, at a temperature of 35 °C, for a duration of 30 min, followed by GC-MS analysis. The determination of volatile compounds followed the methodology outlined by Sádecká et al. (2014) with modifications. An Agilent Technologies 6890 gas chromatograph equipped with an Agilent Technologies 5973 selective inertial detector (MSD) was used. Separation of volatiles utilized a J&W 122-7333 DB-WAXetr 30 m x 0.25 mm x 0.5 μ m capillary column. Helium served as the carrier gas, and the injector temperature was set at 250 °C. The oven temperature was programmed at 50 °C for 1 min, then ramped to 250 °C at a rate of 5°C.min⁻¹. Specific parameters included splitless mode for coffee, an initial temperature of 250 °C, pressure at 88.9 kPa, flow rate of 20.0 mL.min⁻¹, cleaning time of 1.00 min, and a total flow rate of 24.6 mL.min⁻¹. Electron ionization (EI) was set to 70 eV, with the transfer line and ion source temperatures set at 280 °C. The mass spectrometer collected data in full scan mode, and identification was performed by comparing mass spectra and chromatography data with reference materials and the NIST 14 library.

Statistical analysis

For summarizing and interpretation our results was used descriptive analysis, including arithmetic means, minimum, maximum and standard deviation. ANOVA Duncan test and REGWQ were used to evaluate any possible differences between samples and determined parameters. LDA was used to visualize differences between the chemical content base on geographical origin in samples. All statistical analyses were performed using Microsoft Office Excel 365 for Windows (XLSTAT Addinsoft, statistical and data analysis solution, 2021, New York, NY, USA) Microsoft Office Excel 365 pre iOS a Addinsoft 2022 (XLSTAT New York, USA) (Demianová et al., 2022).

RESULTS AND DISCUSSION

The differences in parameters regarding on geographical origin water activity and dry matter are shown in Table 2.

Table 2 Average value of water activity (a_w) and dry matter (DM %) of samples considering groups of geographical origin.

	a_w	DM %
Africa	0.199 ^a	97.762 ^b
America	0.173 ^a	98.585 ^a

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

Coffee beans are a highly hygroscopic commodity and could readily take up moisture due to incorrect exposure to environment conditions during storage. That is why is water activity one of the parameters determining the quality of the coffee bean and is subject to legislative control (Pittia et al., 2007 and Baqueta et al., 2019). Based on our results we can see statistically significant differences between groups of our samples in parameter dry matter. Water activity for Africa samples was determine to value 0.199 and for America samples 0.173. Value of dry matter was higher for America samples (98.585 %) than samples from Africa (97.762 %). Flambeau et al. (2017), Bertrand et al. (2008) and Link et al. (2014) have applied measuring physicochemical parameters, to discriminate the geographical origins of coffee beans they focused especially caffeine, and chlorogenic acids. Throughout the next part of our research, we used Linear Discriminant Analysis we focused on aqueous soluble compounds and their properties (pH, total antioxidant capacity, total polyphenolic content, caffeine, and chlorogenic acids). These results are shown in Figure 1 (Demianová et al., 2022). We determined 5 specific chlorogenic basic and that, chlorogenic acid, neochlorogenic acid, 4,5 dicaffeoylquinic acid, 3,5 dicaffeoylquinic acid. Based on this analysis LDA specified parameters typical for American and Africa samples. Total polyphenol content was parameter the most indicated for Africa samples and for America samples it was content of 3,5 dicaffeoylquinic acid. We can say that based on these parameters is a possible difference in geographical origin. Bicho et al. (2013) explained that coffee's chemical composition depends on the geographic origin of the green coffee beans and the post-harvest processing. No significant differences (ANOVA; $p \geq 0.05$) between geographic origins were found for parameters pH, neochlorogenic acid, cryptochlorogenic acid caffeine and 4,5 dicaffeoylquinic acid however some differences were observed and therefore this attribute was included in a linear discriminant analysis (LDA). Bobkova et al. (2021) defined method preparation of the final drink is one factor affecting the content of caffeine and chlorogenic acid. These values are in accordance with Demianová et al. (2022). American coffee samples have the lowest pH on average, followed by African samples with a higher pH (Rune et al., 2023). This fact we can confirm based on our results (pH samples from Africa 5.390 and pH samples from America 5.385). The studies states that content of caffeine in *C. arabica* beans can differently

depends on the geographical origin, environmental conditions (Hagos et al., 2018; Jeszka-Skowron et al., 2016; Farah, 2012) or variety (Mazzafera and Silvarolla, 2010). Average value of caffeine content in our research was 7.404 mg.(100g)⁻¹ (America samples) and 7.349 mg.(100g)⁻¹ (Africa samples). The Figure 1 shows how the initial variables are correlated with the two factors of F1 and F2.

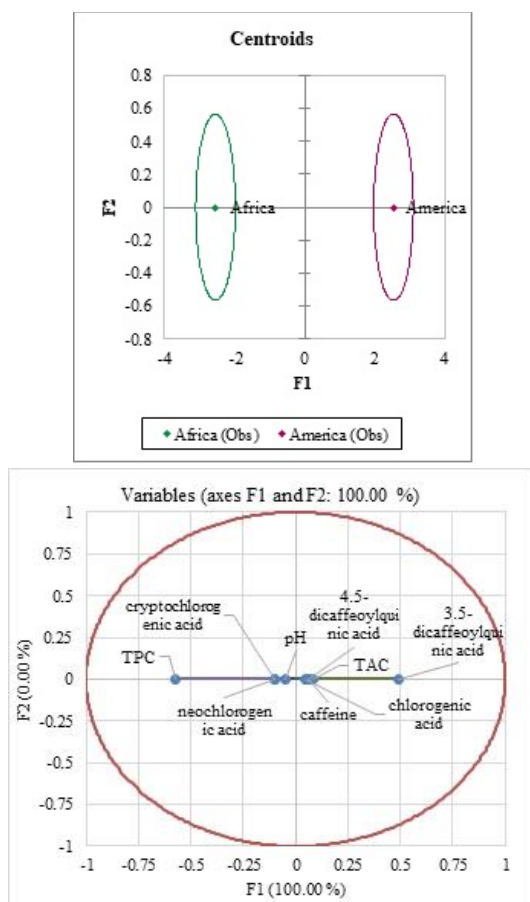


Figure 1 LDA of water-soluble chemical compounds
 Notes: TPC total polyphenol content g GAE. (100 g)⁻¹, TAC total antioxidant capacity in %, chlorogenic acids and caffeine mg.(100g)⁻¹

In the subsequent phase of our study, we aimed to analyze volatile compounds. The sensory attributes of coffee delineate the volatile organic compounds found in roasted coffee, encompassing diverse chemical classes like alcohols, aldehydes, esters, furans, ketones, phenols, pyrazines, pyridines, pyrroles, and sulfur compounds. There was a notable disparity in the volatile compound compositions between roasted and green coffee beans. The roasting process is instrumental in generating key aromatic compounds from green coffee beans, a phenomenon absent in their unroasted state, as highlighted by Dippong et al. (2022). Our research focused on identifying and characterizing groups of compounds, including furans and their derivatives, aldehydes, alcohols, organic acids and their esters, alkanes, terpenoids, alkenes, other heterocyclic compounds, ketones, amines, aromatic hydrocarbons, nitriles, and alkynes. Groups of furans and their derivatives, alcohols, organic acids and their esters was identification in significant amount for Africa samples. For America samples was indicate group of aldehydes, other heterocyclic compounds, ketones, aromatic hydrocarbons, and nitriles. Dippong et al. (2022) state that furans are the most abundant group of volatiles present in the roasted coffee samples and the second prevalent group of compounds identified in coffee samples was ketones. This claims we can confirm this. Content of furan in our research was for Africa samples 39.886 % and for America samples 27.604 %. Next group was ketones (America samples 26.330 % and Africa samples 10.745 %). Vezzuli et al. (2023) affirmed that the origin exerts a more significant influence on the volatile profile compared to the post-harvesting process. According to Dippong et al. (2022), roasting conditions, coffee variety, climate, and terroir play a crucial role in shaping the volatile compounds in coffee. Seninde and Chambers (2020) discovered that the coffee origin (location) significantly affected the volatile compounds in the final coffee product. Figure 2 illustrates the correlation between the initial variables and the two factors, F1 and F2.

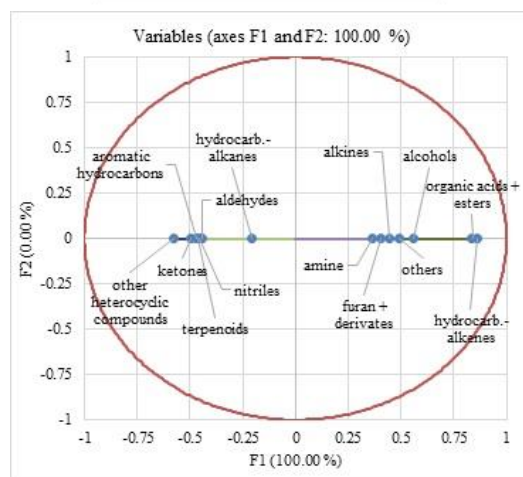
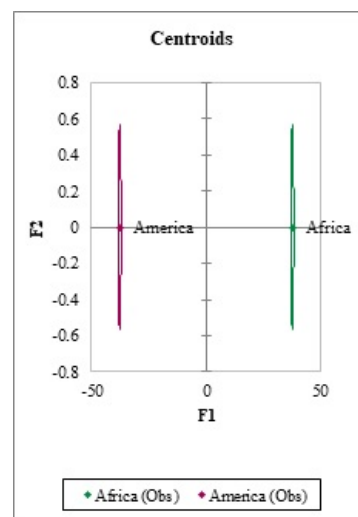


Figure 2 LDA map volatile compounds.

CONCLUSION

The primary factor influencing the quality and commercial value of coffee beans is their geographical origin. Our study indicated that incorporating certain soluble compounds as input parameters, along with volatile compounds, enhances the precision of identifying the geographical origin. Groups of furans and their derivatives, other heterocyclic compounds, ketones, and aromatic hydrocarbons appeared to be important chemical parameters used to determine the geographical origin. pH, TPC and 3,5-dicaffeoylquinic from soluble compounds demonstrated significance. For America samples were significant parameters TAC, 4,5-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, caffeine and chlorogenic acid. Second group was sample from Africa. For these samples was determine parameter like TPC, cryptochlorogenic acid, neochlorogenic acid and pH. Further studies are needed to identify authenticity of geographical origin based on the chemical parameters of roasted coffee. Nevertheless, external factors can exert an influence on the individual chemical profiles and concentrations of these compounds. One of the most significant factors is geographical origin of coffee, second factor including impact of botanical origin, coffee variety impact of environment and impact of post-harvest processing.

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