Research Article

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Specialty and high-quality coffee: discrimination through elemental characterization via ICP-OES, ICP-MS, and ICP-MS/MS of origin, species, and variety

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Abstract

BACKGROUND: This study aimed to establish the elemental profiling and origin combined with the genetic asset of coffee samples collected from major coffee-producing countries. A total of 76 samples were analysed for 41 elements using inductively coupled plasma-optical emission spectroscopy (ICP-OES), inductively coupled plasma-mass spectrometry (ICP-MS), and inductively coupled plasma-triple quadrupole mass spectrometry (ICP-MS/MS). The mineral composition of the silver skin detachment during the roasting process was also evaluated to verify the loss of minerals during roasting, differences in composition with beans, and between species.

RESULTS: Application of linear discriminant analysis provided models with an accuracy of 93.3% for continents, 97.8% for countries of cultivation, and 100% for species. Discrimination between Arabica, Canephora coffee, and Eugenoides, and different varieties of Arabica species were identified in both models with calcium (Ca), barium (Ba), cadmium (Cd), rubidium (Rb), and strontium (Sr) as significant discriminant elements. Rb, Sr, sulphur (S), and thulium (Tm) were significant discriminant elements in both models for geographical distinction at different scales. Most of the elements had significantly higher values in silver skin than those in roasted coffee at different magnitudes, with exceptions of P and Rb.

CONCLUSION: In summary, determination of mineral elements, processed by multivariate statistical analysis, was demonstrated to be discriminant for different coffee species. Linear discriminant analysis of the elemental analysis of samples from the seven major producing countries provided a reliable prediction model. Elemental analysis of major and minor elements is relatively easy and can be used together with other traceability systems and sensory evaluations to authenticate the origin of roasted coffee, different species, and varieties.

Supporting information may be found in the online version of this article.

Keywords: elemental analysis; traceability; green coffee; roasted coffee; silver skin

INTRODUCTION

Coffee is the accumulation of roasted beans from the green seeds of drupes produced by a bush belonging to the family *Rubiaceae* and genus *Coffea*.[1] The two main species cultivated in the tropical area between the two tropics were *Coffea arabica* and *Coffea canephora*.[2]

The genetic differences between the two species and several related varieties ascribable to them are reflected in tree behaviour when housed at diverse latitudes, longitudes, altitudes, and soils. This is one of the main reasons why the origin of coffee lots is one of the main features that influence the cup sensory profile, together with the roasting process and non-defective nature.

As for other crops and food products whose origin and varieties are linked to different levels of quality and price,[3-5] coffee is of major importance to assure the downstream players of the supply chain on the traceability of lots and to guarantee to final consumers that the financial outlay paid for a specific cup of coffee is directly proportional to the intrinsic quality in terms of the sensory profile and origin of the raw material.

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In addition, it is necessary to identify a reliable method to guarantee that the origin and variety declared on roasted coffee packages conform to the true ones to prevent fraud. Considering the role of roasting on the physical features of green coffee and its impact on molecular composition, it is challenging to identify systems that track the origin using these features.[6–8]

As provided by many authors, the elemental composition of coffee beans, being roasted or green, is helpful to discriminate the quality of the raw material, identifying if the seed is defective or non-defective and whether it is already roasted. In addition,[9] it can differentiate between different regions of cultivation when considering Arabica coffee from a specific country and, in the same context, seeds of other subspecies.[10–12]

In addition, elemental characterization conducted via different analytical approaches has already been applied to trace the origin of coffee; however, to the best of our knowledge, there are only a few studies[13,14] based on a comprehensive approach to traceability, linking green coffee to the origin and roasted to the related green, considering a vast range of minerals, such as macroelements, microelements, and rare-earth elements, in samples of specialty or premium specialty coffee.

Therefore, this study evaluated the elemental composition of different lots of coffee via inductively coupled plasma-optical emission spectroscopy (ICP-OES), inductively coupled plasma-mass spectrometry (ICP-MS), and inductively coupled plasma-triple quadrupole mass spectrometry (ICP-MS/MS) to initially verify whether the elemental profile of coffee is stable once green coffee is roasted, considering the losses caused by the detachment of silver skin during the roasting process. In addition, this study investigated whether the differences in geographical origin, combined with the genetic assets of coffee trees cultivated in different countries, provide differences in the elemental composition of the seeds.

MATERIALS AND METHODS

Coffee samples

From 75 samples of coffee from species Arabica, Canephora, and Eugenoides (Supporting Information, Table S1), 1 kg was drawn as a representative sample from GrainPro bags of 60 kg each containing Arabica coffees recognized as ‘Specialty’ or ‘Premium’ coffee according to the Specialty Coffee Association. These coffees, representatives of three continents and seven different geographical nations, scored at cupping 80/100 or more, and beans were free from primary defects (only a small number of unripe or broken beans were allowed). The selected Canephora samples were obtained from the higher quality standards for each country (Supremo NV, 2021) involved in the sample list. Coffee samples also represent the main varieties cultivated worldwide, considering the variability available in the coffee market. Finally, all post-harvesting processes applied to the coffee chain were included in our sample set.

Green coffee samples (500 g) were frozen and milled using a cyclone hammer mill (1 mm sieve, Pulverisette; Fritsch GmbH, Idar-Oberstein, Germany) and then homogenized. Thereafter, 300 g of the sample was collected and stored at −20 °C until analysis.

Roasting

The samples were roasted using an IKAWA Model V2-PRO (IKAWA Ltd, London, UK). This equipment is a convective roaster that applies airflow into the roasting chamber that agitates and roasts simultaneously. The silver skin was removed using a cyclone system and collected in a jar. Each roasting batch weighed 50 g (±0.5 g). The roasting profile applied was settled as follows: the chamber was preheated at 174–175 °C before the coffee inlet, and the roasting ended at 215 °C for 6.46 min. Once roasted, all samples were accurately weighted and, both for roasted coffee and silver skin, stored at −20 °C. At the time of analysis, the frozen seeds were ground using a Moulinex blender (Model AR110830).

The relative stability of the elemental composition between green and roasted coffee was verified by analysing the silver skin detached during roasting and calculating the mass balance dry-based.

Roasted coffee analysis

In this study, 0.5 g of milled roasted coffee was mineralized in a Teflon tube with 5 mL of ultrapure nitric acid (HNO_3) 65% Carlo Erba (Milan, Italy) and 1 mL of hydrogen peroxide (H_2O_2) 30% Carlo Erba using a microwave system (Mars 5 Express, CEM) at 800 W, following two temperature steps: one at 140 °C for 20 min hold time and the next one at 200 °C for 20 min hold time. After cooling, the mixtures were added to a final volume of 50 mL of ultrapure water (18.2 MΩ cm, ELGA PURELAB flex; Veolia Water Solutions and Technologies, Ontario, Canada) in polypropylene tubes (DigiTUBES; SCP Science, Champlain, NY, USA). The extracts were filtered using a 0.45 μm Teflon filter (DigiFILTER, SCP Science).

The digests were diluted with distilled water, and the microelements were analysed using an inductively coupled plasma-mass spectrometer (ICP-MS 7850; Agilent Technologies, Santa Clara, CA, USA). The operating conditions are listed in Table S2. The macroelements were determined using an inductively coupled plasma-optical emission spectrometer (ICP-OES 5100; Agilent Technologies), and the operating conditions are listed in Table S3. The analysis of rare-earth elements was performed by an inductively coupled plasma-triple quadrupole mass spectrometer (ICP-MS/MS 8900; Agilent Technologies) (Table S4).

The method detection limit (MDL) for coffee treatments was calculated as three times the standard deviation of six analytical blanks prepared and diluted in the same manner as the samples5.

The calibration solutions were prepared in the same acid matrix used for roasted coffee and measurements were in triplicate. Analytical quality control was periodically carried out in triplicate with two certified reference materials: tea (NCS DC 73351) and rice flour (NIST 1568a).

Statistical analysis

Statistical analysis of the elemental composition was carried out using the IBM SPSS statistics package (version 27, SPSS Inc., Chicago, IL, USA) and Microsoft Excel. The homogeneity of variance was checked. A *t*-test was conducted to evaluate whether significant differences were present between Robusta and Arabica coffee compositions. One-way analysis of variance (ANOVA) and discriminant analysis were applied to assess significant differences in elemental composition among continents and countries of origin. Tukey's *post hoc* test was applied. A *t*-test and principal component analysis (PCA) were performed on the silver skin composition data to discriminate between coffee species.

RESULTS AND DISCUSSION

Validation of quality control procedure

In every mineralization run, a blank and a triplicate of tea (NCS DC 73351) and rice flour (NIST 1568a) were digested and then analysed. Recoveries for certified elements concentrations were

Table 1. Validation parameters for the determinations of macroelements, microelements, and rare-earth elements in roasted coffee: method detection limits (MDLs), values of certified reference materials (NCS DC 73351 and NIST 1568a), recovery on certified matrices, and relative standard deviation (RSD)

Element	MDL (mg kg^{-1})	NCS DC 73351 (mg kg^{-1}) $N = 3$	Recovery (%)	RSD (%)	NIST 1568a (mg kg^{-1}) $N = 3$	Recovery (%)	RSD (%)
ICP-OES							
Ba	0.05	58	97	0.9			
Ca	7.53	4300	88	1.1	118	94	0.9
Fe	0.69	264	81	6.4	7	84	6.4
K	34.4	16 600	88	1.6	1280	96	3.0
Mg	0.35	1700	85	2.6	560	85	3.0
Na	0.23						
P	0.56	2840	96	0.3	1530	94	1.7
S	2.00	2450	97	0.4	1200	89	1.3
Zn	2.96	26	95	1.8	19	92	1.1
ICP-MS							
Li	0.014						
Be	0.0051						
Al	0.25				4.40	88	15
V	0.0054						
Cr	0.013	0.80	80	8			
Mn	0.017	1240	100	10	20	88	8
Co	0.0070	0.180	99	9			
Ni	0.0095	4.60	99	10			
Cu	0.44	17.30	99	7	2.40	88	14
As	0.0060	0.280	93	11	0.290	95	14
Se	0.011				0.38	88	18
Rb							
Rb	0.036	7.4	97	4	6.14	95	5
Sr							
Sr	0.020	15.2	95	8			
Mo	0.013	0.038	104	12	1.46	91	10
Ag	0.13						
Cd	0.0054	0.057	107	6	0.022	102	5
Sb	0.0058	0.056	80	7			
Pb	0.098	4.40	94	10			
ICP-MS/MS (µg kg^{-1})							
La	2.40	600	96	9.1			
Ce	2.55	1000	91	9.2			
Pr	2.12						
Nd	3.12						
Sm	3.99	85	96	8.5			
Eu							
Eu	3.93	18	110	12			
Gd	3.71						
Tb	3.25						
Dy	3.94						
Ho	2.79						
Er	2.66						
Tm	2.23						
Yb	3.82	44	86	7.1			
Lu	2.46						

Note: *N* is the number of the independent replicates for each certified material.

calculated as the ratio: (Measured concentration/Certified concentration) × 100. The concentrations and recoveries of the elements determined in tea and rice flour are listed in Table 1. The actual experimental values of these contents were in line with the specified concentrations in the certified reference materials, with recoveries ranging between 80% and 110%. The detection limits for this method are listed in Table 1. Linearity was satisfactory; R^2 was above 0.9995 for all analysed elements. The precision

Table 2. Distribution of most concentrated elements in roasted coffee from different nations

Nation	Samples	Element distribution
Brazil	$N = 9$	K > Mg > P > S > Ca > Fe > Mn > Rb
Colombia	$N = 8$	K > Mg > P > S > Ca > Mn > Fe > Rb
Costa Rica	$N = 3$	K > Mg > P > S > Ca > Rb > Fe > Mn
Ethiopia	$N = 13$	K > P > Mg > S > Ca > Rb > Fe > Mn
India	$N = 5$	K > P > Mg > S > Ca > Fe > Na > Rb
Indonesia	$N = 6$	K > P > Mg > S > Ca > Rb > Fe > Mn
Panama	$N = 5$	K > Mg > P > S > Ca > Fe > Rb > Mn

Note: *N* is the number of the investigated samples for each group.

was evaluated through the relative standard deviation (RSD) and the values obtained were below 18%.

Distribution of mineral elements

The elements analysed in this study were classified as macro, micro, and trace based on their concentration levels, as previously reported in the literature.[11,16–26]

Brazilian and Ethiopian are the most investigated roasted coffee, both as an elementary composition and for geographical discrimination (Table S4(a)). However, roasted coffees, ground, or beans from Costa Rica, Panama, and India have been less studied or differentiated (Table S4(b)). Therefore, it can be inferred that the basic composition of these coffees is unknown and that there is no information on some fundamental elements, such as phosphorus (P), sulphur (S), and sodium (Na).

The magnitude distributions of essential mineral concentrations, including rubidium (Rb), in roasted coffee divided by nation, are presented in Table 2.

The same distribution was described by other authors in roasted coffee from the Brazilian region, except for Bitter *et al.*,[16] who published a different order of magnitude between manganese (Mn) and iron (Fe). Debastiani *et al.*[18,20,21] found a lower concentration of S in all samples (Table S4(a)).

In the Colombian database, only Cloete *et al.*[24] reported a different distribution of macroelements, with higher values of calcium (Ca) and Rb and lower values of P (Table S4(b)). The same author wrote about the lower distribution of P in the Ethiopian group. However, Feleke *et al.*[11] wrote about three times higher Ca concentration and an extremely high value of Fe (Table S4(a)).

The macroelement with the highest concentration was potassium (K), followed by magnesium (Mg) or P, with some differences from different origins. Central and southern American coffee show higher concentrations of Mg than P, and the opposite behaviour is shown by samples from Africa and Asia.

The elements in Tables 3–7 are reported as the concentrations of macroelements, microelements, and rare-earth elements, as already defined by Habte *et al.*[10] Through the determination of the method detection limit, elements that were near or below the limit of detection in the sample were suppressed from multivariate analysis, like lithium (Li) and beryllium (Be) and different rare earth elements, such as samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), holmium (Ho), thulium (Tm), ytterbium (Yb), and lutetium (Lu). No significant differences depending on the origin were detected by Tukey's *post hoc* test (0.05) for the concentrations of barium (Ba), Na, lithium, beryllium, copper (Cu), arsenic (As), selenium (Se), molybdenum (Mo), silver (Ag), and antimony (Sb), and different rare-earth elements, such as samarium, gadolinium, terbium, holmium, erbium (Er), thulium (Tm), ytterbium, and lutetium.

There are non-essential elements that have no functional effects on the body. Lead (Pb), cadmium (Cd), and As are of particular concern because of their adverse health effects. To avoid toxic effects, the European Union (EU) Commission has set maximum permitted levels for Pb, Cd, and As in a number of foods in its Regulation No. 1881/2006. The maximum levels for As, Cd and Pb are 0.1–0.25, 0.05–1, and 0.02–1.5 mg kg^{-1}, respectively. The reported concentrations of heavy metals were below the maximum levels set for other foodstuffs or food supplements as set in Commission Regulation (EC) No 1881/2006[27] and food supplements as set in Regulation (EU) No 488/2014,[28] except for a few exceptions in the Ethiopian batch for As and Pb, with higher values in the South American group.

Calcium was higher in Brazilian and Indian coffee than that in Panama, which, in contrast, peaked in the concentration of Fe. Potassium was significantly higher in Brazilian and Indian coffee samples than that in Colombian, Costa Rican, and Ethiopian samples. Magnesium was higher in American (especially Brazilian) coffee than that in Asian coffee, whereas the opposite was true for P. African coffee was the least concentrated in S, zinc (Zn), and cadmium, together with Panama. Costa Rican coffee had an average higher concentration of aluminium (Al), vanadium (V), chromium (Cr), Mn, and strontium (Sr), together with Panamanian and Colombian coffee. Indian coffee peaked at concentrations of cobalt (Co) and nickel (Ni), similar to Indonesian coffee, for rubidium. Brazilian and Panamanian coffee had lower concentrations of Pb, and Ethiopian coffee had lower concentrations of Mn.

The Al, Ba, Ca, Cr, Cu, Fe, K, Mg, Mn, Ni, Rb, and Sr concentrations found in our sample of Ethiopian coffee are consistent with those reported by Worku *et al.*[12]

Regarding rare-earth elements, Indian coffee peaked for lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), europium (Eu), and dysprosium (Dy). No significant differences were found in the concentrations of the other rare-earth elements; this also depends on the fact that the concentrations were generally as low as the MDL for all elements.

Discrimination of species, variety, continent, and county of origin

The result of the discriminant analysis carried out among the species is shown in Fig. 1. As provided by many authors,[29–33] Arabica and Robusta coffee seeds profoundly differ in terms of macromolecular concentration, such as proteins, sugars, trigonelline, chlorogenic acids, fatty acids, and alkaloids. In addition, a deep genetic difference, namely diploid genome for Robusta and tetraploid for Arabica, is used to identify the two species and their presence in blends;[34] that feature also reflects in the characteristic physiology of the Coffea species and a different elemental composition of the seeds.[7] Using canonical discriminant analysis on elemental composition, it is possible to discriminate between Arabica and Canephora coffee and the Eugenoides. This ancestor species concurred in the speciation process with Canephora coffee to create Arabica coffee.[35]

The three subgroups were clustered via functions based on the concentrations of Ba, Ca, K, Mg, P, Zn, Mn, Co, Rb, Sr, Cd, and Pb. Almost 100.0% of the 75 original grouped cases were classified correctly by the two discriminant functions, explaining the 85.3% and the 14.7% of the variance, respectively.

Table 3. Means ± standard deviation (SD) of the macroelements contents in roasted coffee from different nations.

Continent	Nation		Ba (mg kg^{-1})		Ca (mg kg^{-1})		Fe (mg kg^{-1})		K (mg kg^{-1})		Mg (mg kg^{-1})		Na (mg kg^{-1})		P (mg kg^{-1})		S (mg kg^{-1})		Zn (mg kg^{-1})	
America	Brasil	Mean	3.32	a	1075	b	29.6	ab	19 548	b	1922	c	13.8	a	1609	ab	1524	c	5.64	ab
	$n = 9$	SD	1.60		161		2.8		1251		55		23.6		173		67		0.61	
		Maximum	7.08		1467		34.4		21 391		1992		67.0		2019		1609		6.92	
		Minimum	2.14		913		26.0		18 053		1829		1.1		1438		1409		4.86	
	Colombia	Mean	7.27	a	990	ab	27.1	ab	17 077	a	1769	ab	5.8	a	1754	abc	1411	b	6.39	b
	$n = 8$	SD	2.07		208		2.9		1229		117		4.0		109		62		1.51	
		Maximum	9.57		1463		30.4		17 961		1938		12.9		1885		1494		9.60	
		Minimum	3.06		777		24.1		14 503		1586		1.4		1542		1311		5.10	
	Panama	Mean	6.55	a	909	a	25.5	a	18 329	ab	1780	ab	7.8	a	1557	a	1401	ab	5.28	ab
	$n = 5$	SD	2.46		246		2.6		785		117		8.4		144		39		1.00	
		Maximum	9.03		1319		28.4		19 246		1943		21.9		1720		1464		6.58	
		Minimum	3.70		683		21.7		17 373		1660		0.9		1405		1364		4.46	
	Costa Rica	Mean	4.71	a	1023	ab	35.4	ab	16 891	a	1882	bc	5.3	a	1625	ab	1420	b	4.90	ab
	$n = 3$	SD	1.89		145		13.8		579		53		5.5		145		64		0.77	
		Maximum	6.43		1170		51.3		17 560		1941		11.7		1719		1460		5.57	
		Minimum	2.68		880		26.6		16 554		1840		2.0		1457		1347		4.06	
Africa	Ethiopia	Mean	3.75	a	884	ab	23.4	a	17 227	a	1720	ab	8.9	a	1780	bc	1313	a	4.62	a
	$n = 13$	SD	0.83		66		2.9		429		48		6.0		98		49		0.36	
		Maximum	5.67		1000		28.8		17 727		1820		21.3		1959		1391		5.41	
		Minimum	2.16		786		19.5		16 473		1628		2.7		1647		1235		4.13	
Asia	India	Mean	3.59	a	1142	b	35.9	b	19 288	b	1683	a	28.0	a	1869	c	1471	bc	6.08	ab
	$n = 5$	SD	0.71		69		13.5		422		163		12.4		202		15		0.88	
		Maximum	4.52		1208		59.4		19 715		1909		48.2		2092		1492		7.62	
		Minimum	2.76		1030		24.9		18 580		1545		17.9		1625		1451		5.51	
	Indonesia	Mean	4.21	a	1072	ab	30.2	ab	18 499	ab	1701	a	9.6	a	1758	abc	1405	ab	5.71	ab
	$n = 6$	SD	2.47		66		5.1		840		89		4.5		120		36		0.58	
		Maximum	8.56		1176		37.9		19 695		1794		15.0		1912		1455		6.59	
		Minimum	1.15		994		22.8		17 125		1553		3.6		1656		1351		5.04	

Note: Different letters indicate significant differences in element content (ANOVA, Tukey's test, $P < 0.05$) between nations.

Table 4. Means ± standard deviation (SD) of the microelements contents in roasted coffee from different nations.

Continent	Nation		Li* (μg kg^{-1})	Be* (μg kg^{-1})	Al (μg kg^{-1})		V (μg kg^{-1})		Cr (μg kg^{-1})		Mn (μg kg^{-1})		Co (μg kg^{-1})		Ni (μg kg^{-1})		Cu (μg kg^{-1})	
America	Brasil	Mean	<14	<5	3153	abc	<5.4	a	78	a	27 594	abc	180	a	276	a	13 671	a
	n = 9	SD			1575				148		8076		113		102		1715	
		Maximum	18	5	5126		7.8		470		44 504		397		383		15 798	
		Minimum	<14	<5	<250		<5.4		<13		14 851		35		123		10 167	
	Colombia	Mean	<14	<5	2097	ab	5.7	a	86	a	32 773	bc	143	a	424	a	12 954	a
	n = 8	SD			1578		3.9		85		9439		129		185		2093	
		Maximum	26	14	4314		9.8		209		49 021		368		702		15 117	
		Minimum	<14	<5	<250		<5.4		<13		22 014		17		57		9132	
	Panama	Mean	<14	<5	2719	a	<5.4	a	90	a	16 615	ab	48	a	400	a	13 903	a
	n = 5	SD			4474				123		1821		10		202		875	
		Maximum	11	<5	7884		<5.4		273		18 795		58		716		14 538	
		Minimum	<14	<5	<250		<5.4		<13		14 892		31		171		12 366	
	Costa Rica	Mean	<14	<5	8822	c	37.2	b	1475	b	32 241	c	92	a	840	a	14 367	a
	n = 3	SD			6591		3.5		2381		3893		8		925		868	
		Maximum	17	<5	13 257		39.7		4224		36 155		98		1899		15 196	
		Minimum	<14	<5	1248		<5.4		78		28 369		83		188		13 465	
Africa	Ethiopia	Mean	<14	<5	2991	abc	6.8	a	35	a	14 012	a	35	a	305	a	12 600	a
	n = 13	SD			2484		3.9		36		1008		12		251		987	
		Maximum	22	10	7588		9.5		116		15 451		51		1078		14 673	
		Minimum	<14	<5	<250		<5.4		<13		12 650		14		104		10 471	
Asia	India	Mean	<14	<5	6049	bc	54.0	ab	73	a	20 040	abc	311	b	2379	**b**	13 998	a
	n = 5	SD			6164				38		9099		37		813		1234	
		Maximum	18	5	16 589		54.0		127		33 062		340		3295		15 520	
		Minimum	<14	<5	1817		<5.4		31		13 092		250		1531		12 256	
	Indonesia	Mean	14	<5	6180	abc	<5.4	a	161	a	21 790	abc	59	a	232	a	12 798	a
	n = 6	SD	1		2881				250		11 078		61		124		2824	
		Maximum	14	<5	9552		11.6		668		37 735		178		401		15 873	
		Minimum	<14	<5	1944		<5.4		16		11 864		16		114		8207	

Note: Different letters indicate significant differences in element content (ANOVA. Tukey's test. $P < 0.05$) between nations.
*elements excluded by ANOVA.

Table 5. Means ± standard deviation (SD) of the microelements contents in roasted coffee from different nations.

Continent	Nation		As (µg kg^{-1})		Se (µg kg^{-1})		Rb (µg kg^{-1})		Sr (µg kg^{-1})		Mo (µg kg^{-1})		Ag (µg kg^{-1})		Cd (µg kg^{-1})		Sb (µg kg^{-1})		Pb (µg kg^{-1})	
America	Brasil	Mean	<6.0	a	63	a	26 264	a	5435	ab	73	a	<134	a	<5.4	ab	6.6	a	<98	a
	n = 9	SD			138		13 029		1987		72						8.0			
		Maximum	<6.0		375		44 037		8732		248		283.5		6.7		20.4		461	
		Minimum	<6.0		<11		9856		3252		<13		<134		<5.4		<5.8		<98	
	Colombia	Mean	<6.0	a	17	a	24 005	a	8559	bc	102	a	<134	a	8.5	ab	13.7	a	<98	ab
	n = 8	SD			8.9		8480		1490		114				7.0		9.6			
		Maximum	9.0		25		34 114		11 088		306		<134		22.9		20.5		367	
		Minimum	<6.0		<11		11 452		6471		<13		<134		<5.4		<5.8		<98	
	Panama	Mean	<6.0	a	<11	a	20 283	a	12 889	c	106	a	<134	a	<5.4	a	8.5	a	<98	a
	n = 5	SD					2453		3354		88						0.0			
		Maximum	<6.0		14.1		22 502		16 014		236		<134		<5.4		8.5		133	
		Minimum	<6.0		<11		16 167		9054		28		<134		<5.4		<5.8		<98	
	Costa Rica	Mean	15.9	a	<11	a	38 578	a	9389	bc	316	a	<134	a	7.5	b	12.7	a	129	b
	n = 3	SD	21.3				7644		2897		426				6.9		0.0		202	
		Maximum	31.0		<11		47 232		12 498		618		<134		15.4		12.7		362	
		Minimum	<6.0		<11		32 749		6765		<13		<134		<5.4		<5.8		<98	
Africa	Ethiopia	Mean	20.1	a	101	a	29 024		3882	a	106	a	1068	a	<5.4	a	<5.8	a	<98	ab
	n = 13	SD	42.0		69		8153		505		79		1763							
		Maximum	105.9		213		46 932		4998		263		3104		<5.4		8.3		196	
		Minimum	<6.0		<11		19 716		3024		<13		<134		<5.4		<5.8		<98	
Asia	India	Mean	<6.0	a	34	a	23 996	a	3831	a	51	a	<134	a	7.0	b	6.4	a	<98	ab
	n = 5	SD			7		4694		560		19				3.5		3.1			
		Maximum	<6.0		41		29 325		4470		79		<134		9.9		8.9		105	
		Minimum	<6.0		25		16 557		3300		<13		<134		<5.4		<5.8		<98	
	Indonesia	Mean	<6.0	a	21	a	71 253	b	6799	ab	102	a	<134	a	<5.4	ab	<5.8	a	<98	ab
	n = 6	SD			20		22 927		2722		91									
		Maximum	<6.0		58		88 558		11 825		223		<134		9.0		<5.8		160.5	
		Minimum	<6.0		<11		28 446		3725		<13		<134		<5.4		<5.8		<98	

Note: Different letters indicate significant differences in element content (ANOVA, Tukey's test, $P < 0.05$) between nations.

Table 6. Means ± standard deviation (SD) of the rare-earth elements contents in roasted coffee from different nations.

Continent	Nation		La (μg kg^{-1})		Ce (μg kg^{-1})		Pr (μg kg^{-1})		Nd (μg kg^{-1})		Sm* (μg kg^{-1})	Eu* (μg kg^{-1})	Gd* (μg kg^{-1})
America	Brasil	Mean	11.1	ab	11.7	ab	<2.12	ab	5.1	a	<3.99	<3.93	<3.71
	n = 9	SD	7.4		6.7				3.9				
		Maximum	23.1		23.2		2.74		11.9		<3.99	<3.93	<3.71
		Minimum	<2.40		<2.55		<2.12		<3.12		<3.99	<3.93	<3.71
	Colombia	Mean	<2.40	a	<2.55	a	<2.12	ab	<3.12	a	<3.99	<3.93	<3.71
	n = 8	SD											
		Maximum	4.9		4.0		<2.12		<3.12		<3.99	<3.93	<3.71
		Minimum	<2.40		<2.55		<2.12		<3.12		<3.99	<3.93	<3.71
	Panama	Mean	10.0	ab	28.1	ab	4.85	ab	9.6	ab	<3.99	<3.93	<3.71
	n = 5	SD	14.3		32.4		5.82		16.3				
		Maximum	34.8		64.2		8.96		38.2		9.26	<3.93	7.57
		Minimum	<2.40		<2.55		<2.12		<3.12		<3.99	<3.93	<3.71
	Costa Rica	Mean	3.1	a	3.7	a	<2.12	ab	<3.12	a	<3.99	<3.93	<3.71
	n = 3	SD	2.0		2.8								
		Maximum	5.3		6.8		<2.12		3.5		<3.99	<3.93	<3.71
		Minimum	<2.40		<2.55		<2.12		<3.12		<3.99	<3.93	<3.71
Africa	Ethiopia	Mean	4.5	a	6.8	ab	<2.12	ab	3.4	a	<3.99	<3.93	<3.71
	n = 13	SD	4.1		8.2				3.9				
		Maximum	15.2		28.5		2.71		13.2		<3.99	<3.93	<3.71
		Minimum	<2.40		<2.55		<2.12		<3.12		<3.99	<3.93	<3.71
ASIA	India	Mean	22.0	b	27.7	b	5.17	b	18.1	b	<3.99	<3.93	<3.71
	n = 5	SD	35.7		48.6		7.31		32.8				
		Maximum	85.9		114.5		13.61		76.8		8.8	4.3	6.9
		Minimum	4.5		3.1		<2.12		<3.12		<3.99	<3.93	<3.71
	Indonesia	Mean	4.4	a	5.5	ab	<2.12	ab	<3.12	a	<3.99	<3.93	<3.71
	n = 6	SD	2.0		2.1								
		Maximum	7.1		9.4		<2.12		3.9		<3.99	<3.93	<3.71
		Minimum	<2.40		4.2		<2.12		<3.12		<3.99	<3.93	<3.71

Note: Different letters indicate significant differences in element content (ANOVA. Tukey's test. $P < 0.05$) between nations.
*elements excluded by ANOVA.

Table 7. Means ± standard deviation (SD) of the rare-earth elements contents in roasted coffee from different nations

Continent	Nation		Tb* (μg kg^{-1})	Dy (μg kg^{-1})		Ho* (μg kg^{-1})	Er (μg kg^{-1})		Tm* (μg kg^{-1})	Yb* (μg kg^{-1})	Lu* (μg kg^{-1})
America	Brasil	Mean	<3.25	<3.94	ab	<2.79	<2.66	a	<2.23	<3.82	<2.46
	n = 9	SD									
		Maximum	<3.25	<3.94		<2.79	<2.66		<2.23	<3.82	<2.46
		Minimum	<3.25	<3.94		<2.79	<2.66		<2.23	<3.82	<2.46
	Colombia	Mean	<3.25	<3.94	a	<2.79	<2.66	a	<2.23	<3.82	<2.46
	n = 8	SD									
		Maximum	<3.25	<3.94		<2.79	<2.66		<2.23	<3.82	<2.46
		Minimum	<3.25	<3.94		<2.79	<2.66		<2.23	<3.82	<2.46
	Panama	Mean	<3.25	7.4	ab	<2.79	4.0	a	<2.23	<3.82	<2.46
	n = 5	SD									
		Maximum	<3.25	7.4		<2.79	4.0		<2.23	<3.82	<2.46
		Minimum	<3.25	<3.94		<2.79	<2.66		<2.23	<3.82	<2.46
	Costa Rica	Mean	<3.25	<3.94	a	<2.79	<2.66	a	<2.23	<3.82	<2.46
	n = 3	SD									
		Maximum	<3.25	<3.94		<2.79	<2.66		<2.23	<3.82	<2.46
		Minimum	<3.25	<3.94		<2.79	<2.66		<2.23	<3.82	<2.46
Africa	Etiopia	Mean	<3.25	<3.94	ab	<2.79	<2.66	a	<2.23	<3.82	<2.46
	n = 13	SD									
		Maximum	<3.25	<3.94		<2.79	<2.66		<2.23	<3.82	<2.46
		Minimum	<3.25	<3.94		<2.79	<2.66		<2.23	<3.82	<2.46
Asia	India	Mean	<3.25	<3.94	b	<2.79	<2.66	a	<2.23	<3.82	<2.46
	n = 5	SD									
		Maximum	<3.25	9.9		<2.79	3.4		<2.23	<3.82	<2.46
		Minimum	<3.25	<3.94		<2.79	<2.66		<2.23	<3.82	<2.46
	Indonesia	Mean	<3.25	<3.94	ab	<2.79	<2.66	a	<2.23	<3.82	<2.46
	n = 6	SD									
		Maximum	<3.25	<3.94		<2.79	<2.66		<2.23	<3.82	<2.46
		Minimum	<3.25	<3.94		<2.79	<2.66		<2.23	<3.82	<2.46

Note: Different letters indicate significant differences in element content (ANOVA. Tukey's test. $P < 0.05$) between nations.
*elements excluded by ANOVA.

Applying leave-one-out validation to verify the power of the discriminant model, we reached 97.3% of cases classified correctly.

Since only one sample of Eugenoides coffee beans was collected at state of art development, it is not possible to conclude that Eugenoides species had a significantly different composition from Arabica and Canephora. However, compared to previous studies, we can confirm that the discrimination is possible due to the molecular compositions.[6,36]

Using a *t*-test ($P < 0.05$, Cohen's $D > 2$), it was also possible to state that Robusta coffee had significantly higher concentrations of La, Ce, Nd, P, S, Co, and Ni than Arabica, and that, in contrast, Arabica had higher concentrations of Ba, K, and Mg than Robusta.

The differences in the elemental composition of soils from different continents have widely been reported, and this, together with the characteristic deposition of the element in coffee beans, allowed, via canonical discriminant analysis, to obtain an efficient clustering (93.3%) of samples based on the continent of origin (Fig. 2).

The discriminant model identified Ce, Mg, Nd, Rb, S, Se, Sr, Tm, and Zn as the significant discriminant elements. The first two discriminant functions explained the 73.9% and the 26.1% of the variance, respectively. Leave-one-out validation was applied to verify the power of the discriminant model, which showed that 90.7% of cases were classified correctly.

As already provided by Liu *et al.*,[37] Sr and Rb are powerful elements in the discrimination of macroareas of coffee origin.

A higher level of detail was reached only by selecting samples from a country of origin represented in the sample set by a few samples of five or more coffee lots.

Figure 3 shows the graphical result of the canonical discriminant analysis conducted to investigate whether common trends in the elemental composition of coffees from the same country of origin but different farms of area are present and if these can provide clustering of them.

As shown, Ethiopian coffee is the most unique in the set due to the presence of indigenous varieties in Ethiopia, many of which are not listed in the taxonomic records responsible for the expression of an incomparable product of variety × terroir. Statistically, no misclassifications were detected during the leave-one-out validation of the model for this origin. The same was true for Indian coffee, even if samples representing the origin were from Arabica, Robusta, and Panamanian species.

Misclassification occurred during the validation between Brazilian and Indonesian coffees, and Colombian coffee was identified as Panamanian. All that considered, the two discriminant functions explaining the 42.8% and the 24.9% of the total variance had correctly classified 97.8% of the 46 original grouped cases. In addition, 93.5% of the cases were correctly

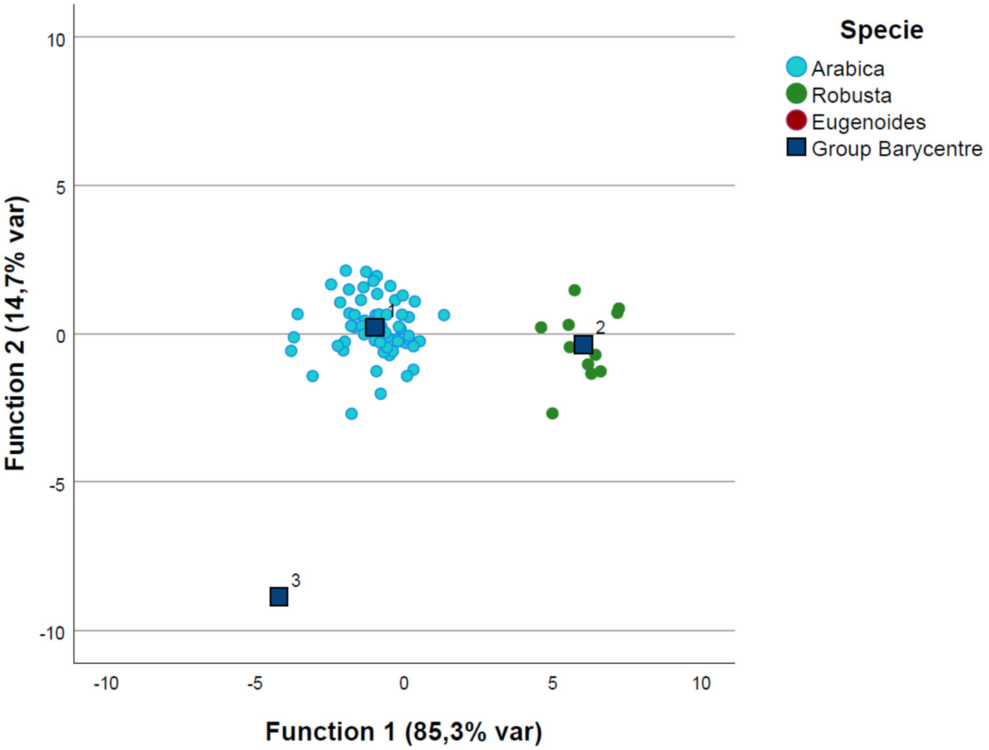

Figure 1. Graphical outcome of discriminant analysis of Coffea species.

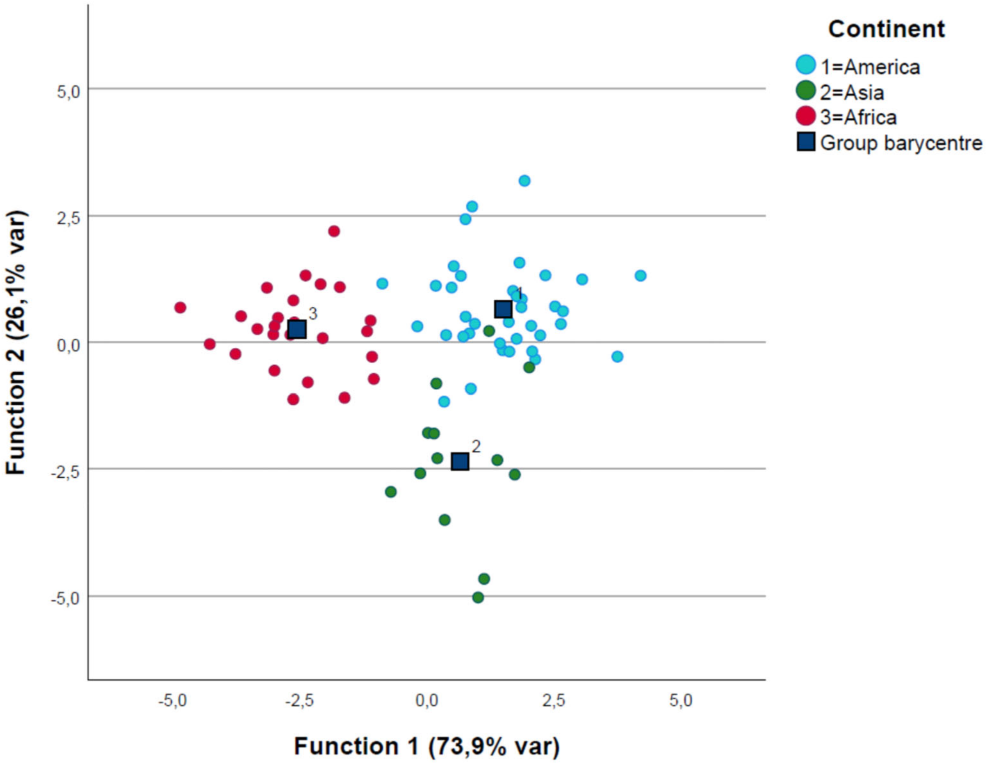

Figure 2. Graphical outcome of discriminant analysis of continent of origin.

classified using leave-one-out validation. In this context, discrimination was built on the Tm, Ca, Fe, K, P, S, Mn, Ni, Rb, and Sr concentrations.

Four identified elements, namely Ca, Mn, Rb, and Sr, have already been identified as discriminants for the different growing regions of Ethiopian coffee by Worku *et al.*[12]

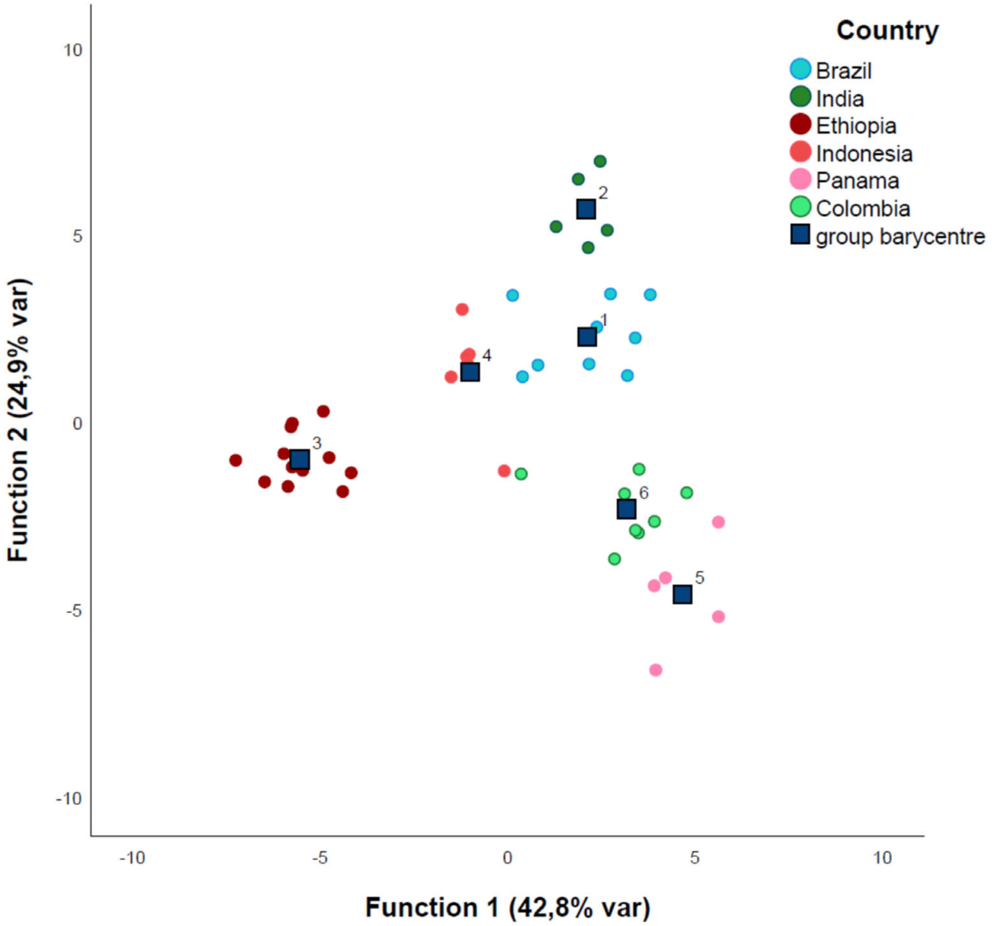

Figure 3. Graphical outcome of discriminant analysis of country of origin.

By analysing the elemental composition data, considering only monovarietal lots of coffee, canonical discriminant functions could classify samples by *C. arabica* varieties (Fig. 4), even if they were from different countries. The varieties used in our study were bourbon, caturra, geisha, and heirloom. For the heirloom variety, the only one represented by Ethiopian samples because it is a group of indigenous genotypes, it is possible to state that the phenotype and terroir were discriminants. Indeed, Sr and Rb were strongly discriminated for this cluster. Bourbon samples were from Burundi, Colombia, Rwanda, and El Salvador; Caturra was from Panama, Bolivia, and Nicaragua; and Geisha was from Panama, Colombia, Costa Rica, and Guatemala.

The discriminant elements leading to the setting of the two functions explaining 73.8% and 23.8% of the total variance were Ba, Ca, Cd, Lu, Mg, Rb, and Sr.

Silver skin

Since the concentration of macroelements and microelements slightly changed from green to roasted coffee (dry-based measurements), the silver skin composition was analysed to verify the loss of minerals during roasting and whether differences in the composition of this layer were present when compared with roasted beans and between coffee species. The elemental composition of this by-product confirmed the stability of the elements considered in our study during the thermal process. Differences between green and roasted beans are explained by the detachment of silver skin (0.5–1% of green coffee weight) (data not shown).

We verified the correlation between coffee seed composition and silver skin using Pearson's test. Concentrations of Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, S, and Sr were directly correlated ($P < 0.05$) in the two matrices; no correlation was found for Ca and Zn, which seemed to be influenced by parameters independent of the genetic deposition of elements in the different tissues of the fruit and seed. Most elements had higher values in silver skin than in roasted coffee. Lithium, V, and some rare-earth elements, such as La, Ce, Pr, Nd, Sm, and Gd, had much higher values, even higher than 20 times those of the roasted samples. Ba, Ca, Na, Be, Cr, and Sr had concentrations 10 and 20 times greater than those in the roasted samples. In contrast, P and Rb were more concentrated in roasted coffee than in silver skin; even with only silver skin from Robusta coffee, these differences were reduced.

From our quantification, the most concentrated elements were K > Ca > Mg > S > P > Fe > Al > Na > Ba > Sr > Cu > Mn > Rb > Zn in all silver skins analysed. This mineral distribution changes only if the Arabica variety is considered: Ca > K > Mg > S > P > Fe > Na > Ba > Al > Sr > Mn > Cu > Zn > Rb. The following trend was observed in the Robusta silver skin: K > Ca > Mg > S > P > Fe > Al > Na > Cu > Sr > Ba > Rb > Mn > Zn. Using *t*-test and PCA, we also identified different compositions of the silver skin. Robusta coffee silver skin showed significantly ($P < 0.05$) higher concentrations of Al, Fe, K, Mg, and S

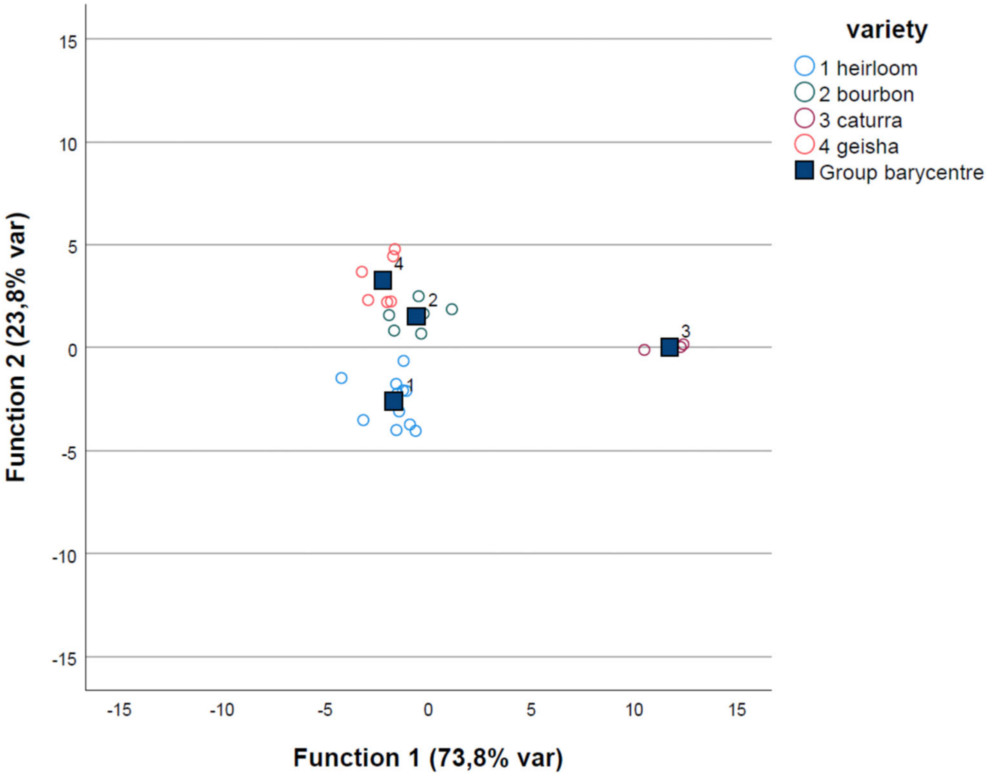

Figure 4. Graphical outcome of discriminant analysis of *Coffea arabica* varieties.

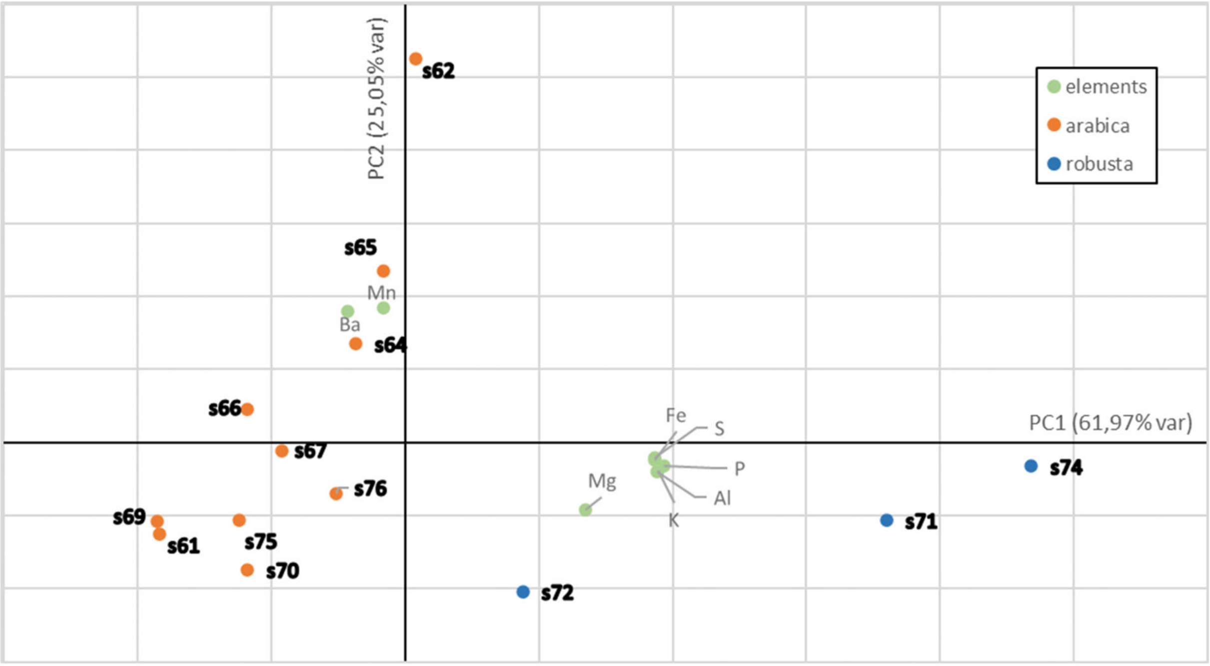

Figure 5. Graphical distribution of silver skin samples, based on Coffee specie, via PCA (87.02% cumulate explained variance).

when compared to Arabica coffee, which, in contrast, had higher concentrations of Ba and Mn. Nzekoue *et al.*[38] published similar distribution but with a lower concentration of P and particularly of S. In addition, Gottstein *et al.*[39] reported a similar order of magnitude of the analysed elements, except for Fe, Al, Mn, and Cu, with concentrations two to five times higher than the values of our study (Table S5). The same author described the distribution inside silver skin from the Robusta variety with a recorded value of Na approximately three times greater, Al and Rb at three or two times greater than the concentrations found in our study (Table S6).

Considering the earlier-mentioned elements, it was possible to build two principal components to explain 87.02% of the variance via PCA (61.97% and 25.05%, respectively). Figure 5 shows a graphical representation of the PCA and its sample distribution.

Because of the novelty of the topic and interest in the reuse of this by-product, to the best of our knowledge, it is not possible to compare the results presented here with similar work in the literature.

CONCLUSION

The elemental composition profiles of the coffee samples collected from the main producing countries, representing the variability in terms of origin and variety present in the market, were assessed for the first time. Macro, micro, and trace element analyses were performed with an adequate number of samples to represent the variability of coffee world production. For each roasted coffee sample, K had the highest concentration, and Mn or Rb had the lowest concentration levels. The contents of trace toxic elements (Pb, Cd, and As) were below the maximum levels set for other foodstuffs or food supplements as set in the Commission Regulations.

Linear discriminant analysis of the elemental analysis of samples from different continents provided a reliable prediction model with 93.3% accuracy and 90.7% prediction ability. The model constructed, based on the elemental compositions divided by countries, was found to be effective in classifying the coffee samples into their respective production zones with 93.5% prediction ability, even if the Panamian group influenced misclassification. In both models, Rb, Sr, S, and Tm are significant discriminant elements for geographical distinction at different scales.

Using canonical discriminant analysis on elemental composition, it is possible to discriminate between Arabica and Canephora coffee and tentatively also the Eugenoides and different varieties of Arabica species identified in both Ca, Ba, Cd, Rb, and Sr as significant discriminant elements.

The chemical analysis and statistics of data from silver skin have helped to determine the distinctive characteristics of coffee species and between this by-product and roasted coffee.

Elemental analysis of major and minor elements is relatively easy and can be used together with the existing paper-based traceability system and sensory evaluation to reliably authenticate the origin of roasted coffee, different species, and varieties. Further studies are ongoing to verify the potential of our model for more accurate discrimination (varieties and regions of origin) and to develop the knowledge of silver skin composition and possible applications.

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DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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