Comparison of the Effectiveness of Fatty Acids, Chlorogenic Acids, and Elements for the Chemometric Discrimination of Coffee (*Coffea arabica* L.) Varieties and Growing Origins

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The objective of this work was to compare the effectiveness of three chemical families, namely, chlorogenic acids, fatty acids, and elements, for the discrimination of Arabica varieties (traditional versus modern introgressed lines) and potential *terroirs* within a given coffee-growing area. The experimental design included three Colombian locations in full combination with five (one traditional and four introgressed) Arabica varieties and two field replications. Chlorogenic acids, fatty acids, and elements were analyzed in coffee bean samples by HPLC, GC, and ICP-AES, respectively. Principal component analysis and discriminant analysis were carried out to compare the three methods. Although elements provided an excellent classification of the three locations studied, this chemical class was useless for Arabica variety discrimination. Chlorogenic acids gave satisfactory results, but fatty acids clearly offered the best results for the determination of both varieties and environments, with very high percentages of correct classification (79 and 90%, respectively).

KEYWORDS: Coffee; fatty acids; chlorogenic acids; elements; authenticity; variety; geographical origin; environment; introgression

INTRODUCTION

Coffee is one of the most important commodities around the world. Two types of coffee, produced by two distinct species, are consumed worldwide: Robusta (*Coffea canephora* Pierre) and Arabica (*C. arabica* L.). It is commonly accepted that the beverage quality of Arabica coffee is higher than that of Robusta, leading to a significant difference in price. However, the species *C. arabica* is characterized by a low genetic diversity, which is reflected in its susceptibility to numerous diseases (*1*). By contrast, *C. canephora* exhibits a considerable diversity and represents a valuable source of disease-resistance genes. Therefore, most breeding programs worldwide have focused their efforts on the transfer of genes of resistance from *C. canephora* to *C. arabica* via an interspecific hybrid, named the Timor hybrid. Almost all new varieties of Arabica are introgressed lines that contain a substantial amount of *C. canephora* genetic material (*2*). Although some introgressed varieties of Arabica

show a beverage quality similar to that of traditional varieties (reviewed in ref *3*), most coffee buyers claim that they frequently possess a poorer beverage quality. Introgression could thus carry not only resistance genes but also other undesirable genes involved in a drop in quality. There is therefore a crucial need for efficient methods to ascertain the authenticity of beans of traditional Arabica varieties.

Together with the discrimination of traditional and modern introgressed varieties, there is also an increasing demand for the determination of coffee origin (*4*). This includes not only the authentication of the country of origin, as carried out by Anderson and Smith (*5*) using bean element composition, but also the differentiation of small *terroirs*, the emergence of which on the market is expanding rapidly. The environmental factors most frequently mentioned in terroir effects are altitude and, to a certain extent, rainfall (*6*). Guyot et al. (*7*) demonstrated that coffee from higher elevations in Guatemala showed a better beverage quality. This feature was later confirmed in several countries of Central America (*6, 8*). Accordingly, within national markets of most mountainous countries, coffee originating from high-elevation areas fetches a higher price than coffees from low-altitude localities. Therefore, it seems also timely to develop appropriate methods for the determination of bean origin within a country area.

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Table 1. Agroclimatic Characteristics of the Three Colombian Coffee-Growing Regions Studied: Altitude, Latitude, Longitude, Mean Monthly Temperature, Sunlight, Precipitation, and Relative Humidity for the Year of Study and the Past 30 Years (*40*)

location	altitude (m)	latitude (deg)	longitude (deg)	year	temperature (°C)			sunlight (h)	precipitation (mm)	relative humidity (%)
					av	min	max			
Naranjal	1381	4° 58′ N	75° 39′ W	year of study	21.4	17.3	27.4	151.3	240.6	74.3
				30-year mean	21.1	16.8	26.8	143.6	228.0	76.1
Paraguaicito	1203	4° 24′ N	75° 44′ W	year of study	22.1	17.0	28.5	144.9	153.7	81.0
				30-year mean	21.9	17.0	28.2	141.0	175.7	77.9
Rosario	1635	5° 58′ N	75° 42′ W	year of study	20.5	16.6	25.5	166.9	214.5	71.1
				30-year mean	20.3	16.2	25.0	167.4	207.3	73.9

The chemometric discrimination of the two coffee species *C. arabica* and *C. canephora* has been an active area of research during the past 10 years. In addition to near-infrared (NIR) spectrometric approaches (*9*), various chemical families such as amino acids, chlorogenic acids (CGA), lipids, elements, purine alkaloids, and sugars have been tested for this purpose (*10–20*). In this respect, the lipid fraction of coffee beans has been shown to be of great interest, because almost all components of the coffee oil were successfully employed for differentiating the two cultivated coffee species: fatty acids (FA) (*15, 20*), sterols (*16*), triacylglycerols (*17*), tocopherols (*17*), and diterpene esters (*18, 19*), as analyzed by chromatographic (*15–20*) or spectroscopic (*19*) methods.

By contrast, very little has been done for the authentication of Arabica varieties and geographic growing origins. Only the study of Bertrand et al. (*21*) based on NIR spectral treatment investigated the means of discriminating modern introgressed varieties from traditional ones. These authors demonstrated that about 90% of varieties could be successfully classified in these two categories through combined principal component analysis (PCA) and discriminant analysis of spectral data. Similarly, although an increasing demand for tools to determine geographic coffee-growing origins exists, the work undertaken in that field remains scarce (*5, 22*). However, using combined PCA and discriminant analysis, Anderson and Smith showed that 70–80% of coffee samples analyzed for their element composition could be correctly classified in their country of origin.

To our knowledge the present study is the first to carry out a chemometric analysis of traditional (Caturra as the standard) and new introgressed Arabica lines based on the chemical profile of beans harvested in different environments, chosen to mimic potential terroirs within a given country. Among all chemical classes of interest for this purpose in coffee, elements, FA, and CGA were chosen because of (i) their well-established efficiency for discriminating the two coffee species *C. arabica* and *C. canephora* (*10, 14, 15, 20*) or for determining the country of origin of green beans (elements) (*5*), (ii) the absence of common metabolic pathways or roles between these three chemical families in coffee seed development physiology, and (iii) the very high reliability of current HPLC, GC, and ICP-AES analytical procedures employed for the determination of CGA, FA, and elements, respectively. The objective of this work was not only to test the possibility to discriminate Arabica varieties and their growing areas on the basis of their chemical profile but also to compare, for the first time, the effectiveness of different chemical families for this purpose.

MATERIALS AND METHODS

Materials. The experimental design employed in this study included three Colombian locations (Naranjal, Paraguaicito, and Rosario) in full combination with five *C. arabica* L. genotypes (four introgressed lines and Caturra) and two field replications (total of 30 coffee bean samples). The variety Caturra was selected for representing high-quality traditional

varieties. The four introgressed lines (BGB.1033, BGB.1044, BGB.1076, and BGB.1040) are advanced lines (at least generation F5) derived from crosses between Caturra and the Timor hybrid accession CIFC-1343. They were selected for their high yield, quality, and resistance to rust. These lines belong to different genealogies, except the lines BGB.1040 and BGB.1044, which are derived from the same plant at generations F_1, F_2, and F_3. The three locations studied represent the main coffee-growing regions in Colombia. They exhibit contrasting agroclimatic characteristics (**Table 1**). Samples were collected during the harvest peak, using healthy ripe cherries. For each sample, 1 kg of cherries was processed by the wet method (pulping, fermentation and drying) to obtain approximately 250 g of green coffee beans. The samples of green coffee were screened through a size 17 sieve ($^{17}/_{64}$ in.), and the most defective beans were discarded.

Storage and Preparation of Coffee Samples. For each sample, coffee beans were dried and stored over silica gel in a hermetic plastic box placed at room temperature in the dark. Then the coffee beans were reduced to a fine powder using an analytical grinder (IKA A15). Coffee powder was stored over silica gel in a hermetic plastic box placed at 4 °C. The water content of powders was estimated after complete drying of 0.2 g aliquots in an oven at 105 °C overnight. The water content was measured in triplicate using a totally random experimental design.

Fatty Acid Determination. Total lipids were extracted from 2 g samples of dried powder using a modified Folch method (*23*) with methylene chloride replacing chloroform. Roughly, the bean material was homogenized for 30 s in 10 mL of methylene chloride/methanol (2:1) using an IKA (Staufen, Germany) T25 Ultraturrax prior to filtration onto a glass filter cup (pore size 4). The residue was extracted again with 10 mL of methylene chloride/methanol (2:1) under the same conditions. The filtrate was transferred in a 60 mL glass separatory funnel and washed with 4 mL of 0.73% NaCl solution by vigorous hand-shaking. After the resulting mixture had separated into two phases, the lower phase was recovered. All steps were performed at room temperature. Extracted lipids were dried under nitrogen at 40 °C, then dissolved in 1 mL of methylene chloride/methanol (2:1), and stored at −20 °C until further analysis. Fatty acid methyl esters (FAMEs) were prepared according to the ISO-5509 standard (*24*). Lipid extracts were first saponified with 4 mL of a 0.5 M methanolic solution of sodium hydroxide at 90 °C for 10 min and then methylated with 5 mL of 14% BF$_3$ methanolic solution at 90 °C for 3 min. GC analyses were performed using an HP 6890 system with flame ionization detection (FID). A Famewax capillary column (RESTEK), 30 m × 0.25 mm × 0.25 µm, was used. The analyses were carried out in program temperature mode from 185 to 225 °C at 4 °C/min and then in the isothermal mode for 10 min at 225 °C. Helium was used as carrier gas at 40 cm s^{-1}. Both injector and detector were at 230 °C. FAMEs were identified by comparing their retention times with those of the fatty acid methyl ester standards (Supelco) and were quantified as percentages over total FA (w/w). For each genotype−environment combination studied, the fatty acid composition was analyzed in triplicate (from three different lipid extracts).

Element Determination. Nitrogen was determined according to the Dumas method (*25*). Roughly, about 150 mg of dry powder was weighed precisely in a tin foil and analyzed using a Leco nitrogen determinator (model FP-528, Leco Corp., St. Joseph, MI). The nitrogen content (milligrams per gram) was determined using calibration curves set up with commercial standards of EDTA and glycine. The other

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Table 2. Effects of the Genotype, the Environment, and Their Interaction, on the Element Composition of Green Beans: Means and Probability of Significance (*P*) As Determined by Two-Way Analysis of Variance over Three Different Locations (Naranjal, Rosario, and Paraguaicito) and Five Different Genotypes (Caturra, BGB.1033, BGB.1076, BGB.1040, and BGB.1044)[a]

variety/location		N (mg/g)	P (mg/g)	K (mg/g)	Ca (mg/g)	Mg (mg/g)	Cu (ppm)	Fe (ppm)	Zn (ppm)	B (ppm)
Caturra		23.01a	1.52bc	14.53	1.22	0.180a	16.07	45.60	11.88	10.68
BGB.1033		22.58a	1.62a	15.14	1.21	0.165b	17.12	42.70	10.07	11.72
BGB.1044		21.51b	1.45c	14.68	1.24	0.166b	16.62	41.95	13.02	11.60
BGB.1076		22.61a	1.56ab	14.12	1.29	0.172ab	20.47	51.42	19.12	12.05
BGB.1040		21.99ab	1.46c	14.39	1.26	0.169ab	17.58	53.73	13.18	11.65
F probability		0.00	0.00	0.68	0.76	0.00	0.13	0.81	0.58	0.56
Naranjal		21.65b	1.47b	14.27	1.07b	0.148c	18.55a	45.46	21.83a	9.76a
Paraguaicito		22.81a	1.53a	15.20	1.36a	0.168b	19.75a	44.07	9.52b	12.75b
Rosario		22.52a	1.57a	14.24	1.30a	0.196a	14.41b	51.71	9.01b	12.11b
F probability		0.00	0.00	0.17	0.00	0.00	0.00	0.72	0.01	0.00
Naranjal	× Caturra	22.51	1.45	13.19	1.01	0.158	18.75	59.35	21.30	10.30
	× BGB.1033	21.38	1.57	14.57	1.07	0.144	16.25	32.60	11.10	9.35
	× BGB.1044	20.45	1.41	14.98	1.08	0.147	17.00	45.30	19.60	9.45
	× BGB.1076	21.96	1.50	13.58	1.17	0.144	22.70	53.55	36.15	9.70
	× BGB.1040	21.95	1.45	15.02	1.03	0.147	18.05	36.50	21.00	10.00
Paraguaicito	× Caturra	23.27	1.54	15.91	1.32	0.180	15.10	40.60	6.85	10.55
	× BGB.1033	23.25	1.61	15.68	1.22	0.161	21.50	55.85	10.65	13.60
	× BGB.1044	22.67	1.49	15.00	1.40	0.160	19.05	41.65	8.95	12.50
	× BGB.1076	22.54	1.55	14.43	1.38	0.170	22.85	42.65	12.00	14.40
	× BGB.1040	22.32	1.46	15.01	1.49	0.169	20.25	39.60	9.15	12.70
Rosario	× Caturra	23.51	1.59	14.49	1.34	0.204	14.35	36.85	7.50	11.20
	× BGB.1033	23.12	1.68	15.18	1.35	0.189	13.60	39.65	8.45	12.20
	× BGB.1044	21.42	1.47	14.06	1.25	0.192	13.80	38.90	10.50	12.85
	× BGB.1076	23.33	1.64	14.34	1.31	0.201	15.85	58.05	9.20	12.05
	× BGB.1040	21.71	1.49	13.14	1.26	0.193	14.45	85.10	9.40	12.25
F probability		0.23	0.73	0.64	0.41	0.53	0.60	0.41	0.79	0.42

[a] The following full-factorial experimental design was employed: 3 locations × 5 varieties × 2 field replications. Means followed by the same letter are not significantly different at *P* = 0.05 as determined by the Newman and Keuls' test.

elements (P, K, Ca, Mg, Fe, Cu, Zn, and B) were determined by inductively coupled argon plasma atomic emission spectrometry (ICP-AES) after dry mineralization. About 500 mg of dried powder was placed in a platinum capsule and progressively heated to 200 °C, maintained at this temperature until the completion of smoke release, then heated to 500 °C, and maintained at this temperature for 2 h. The mineralized sample was digested in 2 mL of 6 N HCl, dried, and filtered with 2 mL of 0.5 N HCl. The residue was calcinated again for 2 h at 500 °C. Silica was then eliminated by addition of hydrofluoric acid prior to evaporation on a heating plate. The residue was filtered again with 2 mL of HCl, and the two filtrates were combined and diluted to 50 mL with pure water. Samples were then analyzed by ICP-AES using a Varian-Vista MPX (Varian Inc., Palo Alto, CA), equipped with a CCD detector. All elements were determined in triplicate and expressed in milligrams per gram or micrograms per gram on a dry weight basis.

Chlorogenic Acid Measurement. Chlorogenic acids, namely, caffeoylquinic (3-CQA, 4-CQA, 5-CQA), feruloylquinic (3-FQA, 4-FQA, 5-FQA), dicaffeoylquinic (3,4-diCQA, 3,5-diCQA, 4,5-diCQA), caffeoylferuloylquinic (CFQA), and caffeic acids (CA), were determined by HPLC. CGA-like components, caffeoyl-tyrosine (CT) and caffeoyl-tryptophane (CTR), where the caffeic unit is coupled with an amino acid, were also measured in the same chromatogram. About 250 mg of dried powder precisely weighed was placed with 80 mL of aqueous methanol (70% w/w) and 1 mL of aqueous acetic acid (50:50 v/v) in a 100 mL flask and shaken for 16 h at 20 °C in darkness on a stirring table at 125 rpm. The solution was adjusted to 100 mL with 70% methanol and diluted 5 times with 70% methanol prior to filtration through a 0.45 μm filter and injection. The LC equipment comprised a Varian 9010 pump, a Rheodyne valve with a 20 μL loop, and a UV detector (Shimadzu SPD 10AV) recording at 327 nm. CGA separation was achieved on an Uptisphere ODB 5μ column (250 mm × 4.6 mm) from Interchim. Solvent A was methanol, and solvent B was 2 mM phosphoric acid. The flow rate was 1 mL min⁻¹. Sample was analyzed

at room temperature using the following gradient profile: 5% A to 75% A linearly in 35 min, a linear increase to 100% A at 40 min, followed by 5 min isocratic and a return to 5% A at 50 min. CGA isomers, as well as CGA-like components, were identified by comparison of their retention time at 327 nm with those of commercial standards (Sigma). All samples were analyzed in triplicate (from three different extractions).

Statistics. Data analyses were carried out using SAS (SAS, Cary, NC) for two-way ANOVAs and Statistica (Statsoft, Tulsa, OK) for PCA and discriminant analysis. For each chemical class studied, discriminant analysis was performed using principal components having eigenvalues > 1. All samples were used to establish the classification rule.

RESULTS AND DISCUSSION

Chemical Composition of Beans: Effects of Location and Variety. The overall bean chemical composition obtained in the present study (**Tables 2−4**) is in full agreement with previous reports dealing with the composition in elements (*5, 14, 26*), CGA (*10, 11*), and FA (*15, 20, 27*) of *C. arabica* and *C. canephora* beans. Because many reports have presented in detail the overall coffee bean composition for these three chemical families, they are not further described in the present study.

A significant effect of the location was observed for almost all compounds measured, as inferred from two-way ANOVA, revealing the potential of the three chemical classes studied for discriminating coffee terroirs within a given country (**Tables 2−4**). Compounds exhibiting no differences among locations were potassium and iron, 5-CQA, caffeic acid, and caffeoyl-tyrosine, and oleic (18:1n-9) and lignoceric (24:0) acids for elements, chlorogenic, and fatty acids, respectively. All other

Table 3. Effects of the Genotype, the Environment, and Their Interaction, on the Chlorogenic Acid Composition (Milligrams per Gram) of Green Beans: Means and Probability of Significance (*P*) As Determined by Two-Way Analysis of Variance over Three Different Locations (Naranjal, Rosario, and Paraguaicito) and Five Different Genotypes (Caturra, BGB.1033, BGB.1076, BGB.1040, and BGB.1044)[a]

variety/location		3-CQA	4-CQA	5-CQA	3-FQA	4-FQA	5- FQA	CFQA[b]	3,4-diCQA	3,5-diCQA	4,5-diCQA	CA	CTR	CT
Caturra		3.70	5.37a	39.15b	0.06a	0.46a	3.52b	0.19a	1.16b	5.52a	2.96b	0.17	0.31b	0.11
BGB.1033		3.83	5.71a	38.72b	0.01ab	0.32bc	3.42b	0.14b	1.83a	4.83b	3.34a	0.19	0.52a	0.13
BGB.1044		3.54	5.78a	44.94a	0.00b	0.35b	3.83a	0.10d	0.99b	4.47b	2.75b	0.12	0.55a	0.11
BGB.1076		3.65	5.54a	42.21ab	0.03ab	0.30c	3.22c	0.12c	1.28b	4.98b	2.89b	0.32	0.49a	0.11
BGB.1040		3.70	5.79a	46.51a	0.01ab	0.34b	3.50b	0.10d	0.94b	4.42b	2.56c	0.25	0.54a	0.10
F probability		0.76	0.03	0.00	0.05	0.00	0.00	0.00	0.01	0.00	0.00	0.59	0.00	0.20
Naranjal		3.45b	5.37b	41.60	0.01b	0.32b	3.61a	0.13b	1.19b	5.18a	3.10a	0.16	0.47b	0.11
Paraguaicito		4.09a	6.05a	43.04	0.03ab	0.42a	3.43b	0.14a	1.62a	3.74b	2.78b	0.13	0.42b	0.12
Rosario		3.49b	5.50b	42.62	0.05a	0.30b	3.45b	0.10c	0.89b	5.63a	2.80b	0.35	0.58a	0.11
F probability		0.00	0.00	0.54	0.03	0.00	0.02	0.00	0.00	0.00	0.00	0.17	0.00	0.39
Naranjal	× Caturra	3.29	5.09	37.61	0.01	0.43	3.67	0.19	0.40	6.90	3.21	0.14	0.29	0.11
	× BGB.1033	3.78	5.46	39.43	0.02	0.29	3.68	0.14	1.70	5.14	3.51	0.27	0.49	0.12
	× BGB.1044	3.07	5.50	43.48	0.00	0.30	3.68	0.10	1.12	4.52	2.93	0.14	0.55	0.11
	× BGB.1076	3.49	5.09	40.34	0.00	0.25	3.34	0.11	1.50	5.06	3.23	0.12	0.43	0.10
	× BGB.1040	3.63	5.74	47.15	0.00	0.34	3.68	0.11	1.23	4.28	2.66	0.13	0.57	0.11
Paraguaicito	× Caturra	4.24	5.77	40.11	0.15	0.53	3.32	0.21	1.83	4.03	2.79	0.22	0.27	0.11
	× BGB.1033	3.68	6.13	39.43	0.00	0.39	3.21	0.16	1.96	4.03	3.14	0.12	0.48	0.15
	× BGB.1044	4.45	6.21	44.48	0.00	0.39	4.06	0.11	1.42	3.60	2.74	0.12	0.51	0.11
	× BGB.1076	3.92	6.09	44.49	0.02	0.37	3.16	0.13	1.61	3.66	2.73	0.10	0.43	0.12
	× BGB.1040	4.06	6.05	46.35	0.00	0.41	3.39	0.12	1.30	3.38	2.48	0.12	0.45	0.09
Rosario	× Caturra	3.43	5.12	40.32	0.00	0.38	3.64	0.16	1.37	5.77	2.82	0.13	0.45	0.11
	× BGB.1033	4.03	5.53	37.30	0.13	0.28	3.36	0.11	1.84	5.34	3.38	0.19	0.58	0.11
	× BGB.1044	3.01	5.63	46.51	0.00	0.35	3.76	0.07	0.44	5.29	2.57	0.10	0.61	0.11
	× BGB.1076	3.55	5.44	41.81	0.06	0.28	3.14	0.12	0.75	6.24	2.72	0.73	0.60	0.10
	× BGB.1040	3.42	5.58	46.01	0.03	0.29	3.44	0.07	0.30	5.61	2.55	0.50	0.60	0.11
F probability		0.84	0.11	0.78	0.00	0.10	0.43	0.36	0.09	0.01	0.36	0.02	0.02	0.46

[a] The following full-factorial experimental design was employed: 3 locations × 5 varieties × 2 field replications. Means followed by the same letter are not significantly different at *P* = 0.05 as determined by the Newman and Keuls' test. [b] Likely to be CFQA according to Perrone et al. (*41*).

constituents showed significant variations, ranging quantitatively from slight [e.g., 3–5% for nitrogen, 5-FQA, or palmitic (16:0) acid] to moderate [e.g., about 10% for 4,5-di CQA or linoleic (18:2) acid] to very high variations (up to 142% for Zn) (**Tables 2−4**).

By contrast with the effect of the location, most of the minerals and metals analyzed showed no significant differences among varieties (**Table 2**), suggesting that these elements offer a poor discriminating capacity of varieties. Conversely, the effect of the variety was highly significant with most of the chlorogenic and fatty acids measured, with the exception of 3-CQA, 3-FQA, caffeic acid, and caffeoyl-tyrosine and vaccenic, behenic, and lignoceric acids (**Tables 3** and **4**). Again, the degree of variations over varieties differed considerably among the various constituents studied, ranging from low (e.g., palmitic acid) to intermediate (e.g., oleic acid) to high (e.g., 3,4-diCQA) variability. It is worth mentioning that for various fatty and chlorogenic acids, the effect of introgression was monodirectional; that is, the value of Caturra beans was significantly lower—or higher—than those measured in the four introgressed varieties, as assessed by post hoc Newman and Keuls' tests (**Tables 3** and **4**). For instance, introgression was always associated with a decrease in 3,5-diCQA or stearic acid and an increase in oleic acid. In some cases, the direction of the effect coincided with the difference reported in previous works between *C. arabica* and *C. canephora*. For example, the bean content in oleic acid of *C. canephora* is generally higher than that of *C. arabica* (*15, 20, 27*). However, these occurrences were not observed with all FA and CGA, emphasizing that introgression of *C. canephora* genes in *C. arabica* may lead to complex regulations of metabolic pathways.

The level of significance of the interaction between the variety and the location can also constitute a useful indicator of the possible discriminating value of each compound studied. Indeed, it is expected that chemical classes showing no—or a low proportion of—significant interactions exhibit a high discriminating value for both variety and origin determinations. It is therefore worth noting that most FA and CGA exhibited no interaction, suggesting again that these two chemical families could present valuable chemometric ability for distinguishing varieties and origins (**Table 3** and **4**).

PCA of Element, Chlorogenic Acid, and Fatty Acid Data. For each of the three chemical families studied, the same statistical approach was performed. PCA was employed to set up noncorrelated—a prerequisite for discriminant analysis achievement—variables that contain the maximum of the initial variance. For all chemicals studied, PCA provided a similar pattern for the cumulative percentage of variance explained by the first principal components. In all cases, the first, the first two, and first three factors explained about 35, 60, and 75% of the total variance, respectively. This homogeneity in PC-explained variance offers here the opportunity to compare these three chemical classes for coffee chemometrics without any speculation about the impact of PCA results.

Discriminant Analysis of Varieties. For the three chemical classes studied, the same approach was carried out. Factorial scores of PCs showing an eigenvalue >1 were used to calculate the discriminant function models. When the variety was employed as the criterion, significant classifications were obtained with FA and CGA, as estimated by the *P* value associated with the Wilk's lambda coefficient, whereas varieties could not be significantly discriminated according to their bean

Table 4. Effects of the Genotype, the Environment, and Their Interaction, on the Fatty Acid Composition (Percent of Total Fatty Acids) of Green Beans: Means and Probability of Significance (*P*) As Determined by Two-Way Analysis of Variance over Three Different Locations (Naranjal, Rosario, and Paraguaicito) and Five Different Genotypes (Caturra, BGB.1033, BGB.1076, BGB.1040, and BGB.1044)[a]

variety/location		16:0	17:0	18:0	18:1n-9	18:1n-7	18:2	18:3	20:0	20:1	22:0	24:0
Caturra		33.31b	0.11a	7.90a	8.94c	0.47	43.60b	1.48c	2.80a	0.33c	0.76	0.25
BGB.1033		32.66c	0.11a	6.88c	9.32b	0.48	45.20a	1.56a	2.52b	0.35ab	0.63	0.25
BGB.1044		33.91a	0.10b	7.26b	9.83a	0.46	43.01e	1.52b	2.64ab	0.34bc	0.62	0.25
BGB.1076		34.04a	0.11a	7.25b	10.15a	0.48	42.39d	1.45d	2.74a	0.35a	0.72	0.28
BGB.1040		33.32b	0.10b	7.42b	9.96a	0.47	43.12c	1.56a	2.73a	0.34bc	0.66	0.25
F probability		0.00	0.00	0.00	0.00	0.46	0.00	0.00	0.03	0.00	0.20	0.49
Naranjal		33.92a	0.10b	7.24b	9.65	0.47b	43.20b	1.43c	2.67ab	0.35ab	0.68ab	0.25ab
Paraguaicito		32.77b	0.11a	7.74a	9.74	0.46b	43.38b	1.58a	2.80a	0.33a	0.76a	0.28a
Rosario		33.69a	0.11a	6.94c	9.61	0.49a	43.84a	1.55b	2.55b	0.34b	0.59b	0.24b
F probability		0.00	0.00	0.00	0.36	0.00	0.00	0.00	0.00	0.00	0.01	0.06
Naranjal	× Caturra	33.82	0.11	7.82	8.90	0.47	43.22	1.40	2.82	0.34	0.80	0.26
	× BGB.1033	33.35	0.11	6.78	9.36	0.47	44.75	1.44	2.48	0.35	0.62	0.24
	× BGB.1044	34.17	0.10	7.19	9.69	0.45	43.18	1.43	2.58	0.34	0.58	0.23
	× BGB.1076	34.94	0.10	7.06	10.18	0.49	41.61	1.37	2.79	0.36	0.77	0.29
	× BGB.1040	33.32	0.10	7.36	10.11	0.47	43.23	1.48	2.69	0.35	0.60	0.24
Paraguaicito	× Caturra	32.55	0.11	8.28	8.97	0.45	43.89	1.54	2.82	0.32	0.75	0.25
	× BGB.1033	31.85	0.11	7.11	9.42	0.47	45.50	1.63	2.56	0.34	0.68	0.27
	× BGB.1044	33.23	0.10	7.65	10.17	0.47	42.50	1.58	2.85	0.34	0.75	0.30
	× BGB.1076	33.26	0.10	7.75	10.23	0.45	42.47	1.50	2.81	0.34	0.76	0.27
	× BGB.1040	32.98	0.10	7.92	9.91	0.47	42.55	1.62	2.95	0.32	0.84	0.29
Rosario	× Caturra	33.81	0.11	7.28	8.96	0.50	43.77	1.51	2.70	0.34	0.70	0.25
	× BGB.1033	32.77	0.11	6.74	9.19	0.49	45.34	1.62	2.51	0.35	0.60	0.24
	× BGB.1044	34.32	0.10	6.92	9.64	0.48	43.36	1.55	2.49	0.33	0.53	0.23
	× BGB.1076	33.93	0.11	6.93	10.05	0.50	43.09	1.48	2.61	0.36	0.62	0.26
	× BGB.1040	33.67	0.10	6.99	9.88	0.48	43.60	1.59	2.54	0.34	0.54	0.24
F probability		0.03	0.42	0.20	0.86	0.17	0.01	0.39	0.57	0.17	0.53	0.33

[a] The following full-factorial experimental design was employed: 3 locations × 5 varieties × 2 field replications. Means followed by the same letter are not significantly different at *P* = 0.05 as determined by the Newman and Keuls' test.

Table 5. Discriminant Analysis of the Five Genotypes Studied (Caturra, BGB.1033, BGB.1076, BGB.1040, and BGB.1044) Based on Bean Element, Fatty Acid, and Chlorogenic Acid Composition: Probability of Significance, As Assessed by Wilks's Lambda and Corresponding *F* Value, and Classification Efficiency, As Determined by Percentages of Correct Classification[a]

chemical	λ Wilk	F	P	% correct classification					
				total	Caturra	BGB.1044	BGB.1076	BGB.1033	BGB.1040
elements	0.712	0.66	0.77	34.5	0	50	50	50	17
fatty acids	0.049	6.84	0.00	79.3	100	67	67	100	67
chlorogenic acids	0.095	6.97	0.00	69.0	60	67	67	83	67

[a] The following full-factorial experimental design was employed: 3 locations × 5 varieties × 2 field replications.

elemental composition (**Table 5**). The nonsignificance of the discriminant analysis performed with elements is congruent with the nonsignificance of the effect of the variety on these traits, as analyzed by ANOVA (**Table 2**).

The percentage of correct variety classifications was satisfactory with both FA and CGA (ca. 70–80%), revealing for the first time the high potential of these two chemical families for the chemometric discrimination of *C. arabica* varieties (**Table 5**). However, the proportion of well-classified samples was slightly higher with FA and, moreover, some Caturra (the quality standard employed here) samples were not appropriately classified with CGA, suggesting that the bean FA composition could be the best candidate among the three families tested for Caturra authentication. It is worth mentioning that the overall proportion of correct classification obtained with FA (79%) is similar to that (76%) obtained by Bertrand et al. (*21*) on the basis of the NIR spectra of traditional and new introgressed varieties.

Two significant canonical functions were obtained using the discriminant model obtained with FA, whereas the first canonical function only was significant with CGA (**Table 6**). Scatterplots

Table 6. Eigenvalue and χ^2 Significance (*P*) of the First Two Canonical Functions Resulting from the Discriminant Analysis of the Five Genotypes Studied (Caturra, BGB.1033, BGB.1076, BGB.1040, and BGB.1044) Based on Bean Fatty Acid and Chlorogenic Acid Composition[a]

chemical	canonical function	eigenvalue	χ^2	P
fatty acids	1	5.20	71.1	0.00
	2	1.34	28.2	0.00
chlorogenic acids	1	7.12	56.4	0.00
	2	0.26	6.1	0.41

[a] The following full-factorial experimental design was employed: 3 locations × 5 varieties × 2 field replications.

presented in **Figure 1**, based on canonical scores of the 30 samples analyzed, illustrate that the bean FA composition allowed a better discrimination of Caturra and BGB.1033 bean samples than the CGA composition. Interestingly, the two introgressed lines, which are derived from the same plant at generations F_1, F_2, and F_3, BGB.1044 and BGB.1040, could not be satisfactorily discriminated on the basis of either their FA or CGA profiles.
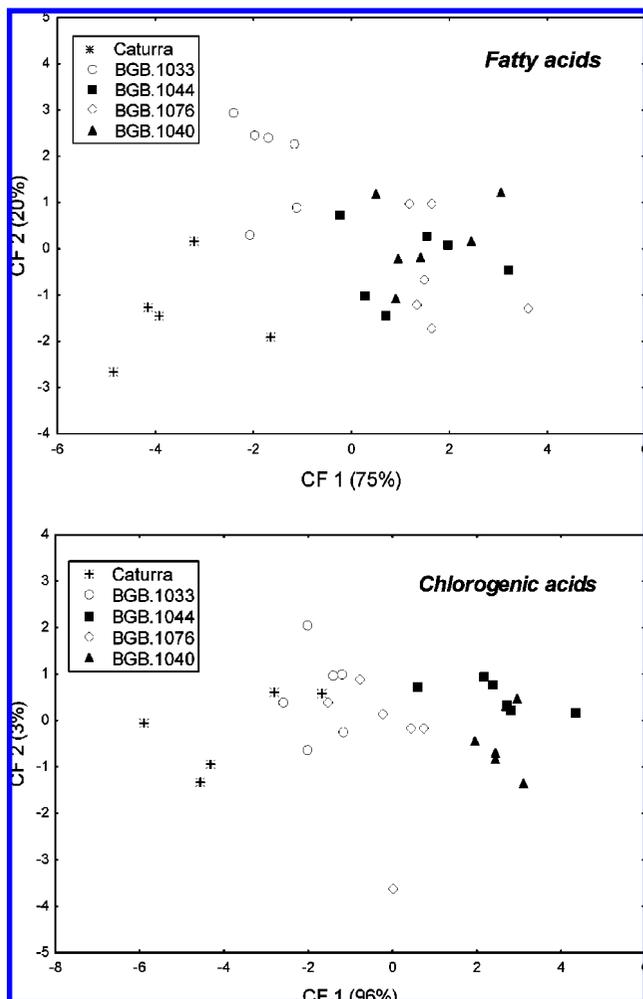
Figure 1. Scatterplot of canonical scores for the first two canonical functions resulting from the discriminant analysis of the five genotypes studied (Caturra, BGB.1033, BGB.1076, BGB.1040, and BGB.1044) based on bean fatty acid and chlorogenic acid composition. The following full-factorial experimental design was employed: 3 locations × 5 varieties × 2 field replications.

Table 7. Discriminant Analysis of the Three Locations Studied (Naranjal, Paraguaicito, and Rosario) Based on Bean Element, Fatty Acid, and Chlorogenic Acid Composition: Probability of Significance, As Assessed by Wilks's Lambda and Corresponding F Value, and Classification Efficiency, As Determined by Percentages of Correct Classification[a]

chemical	λ Wilk	F	P	% correct classification			
				total	Naranjal	Paraguaicito	Rosario
elements	0.080	19.42	0.00	100.0	100	100	100
fatty acids	0.092	13.24	0.00	89.7	80	100	90
chlorogenic acids	0.139	13.43	0.00	82.8	100	100	44

[a] The following full-factorial experimental design was employed: 3 locations × 5 varieties × 2 field replications.

Discriminant Analysis of Locations. The bean elemental composition provided an excellent classification of the 30 samples studied, because 100% of samples analyzed were correctly classified through discriminant analysis (**Table 7**). The proportion of well-classified samples was also very satisfactory with FA (ca. 90%) and acceptable with CGA (ca. 83%).

With elements, the first canonical function allowed an excellent separation of Naranjal samples, on one side, and Paraguaicito and Rosario samples, on the other side (**Figure**
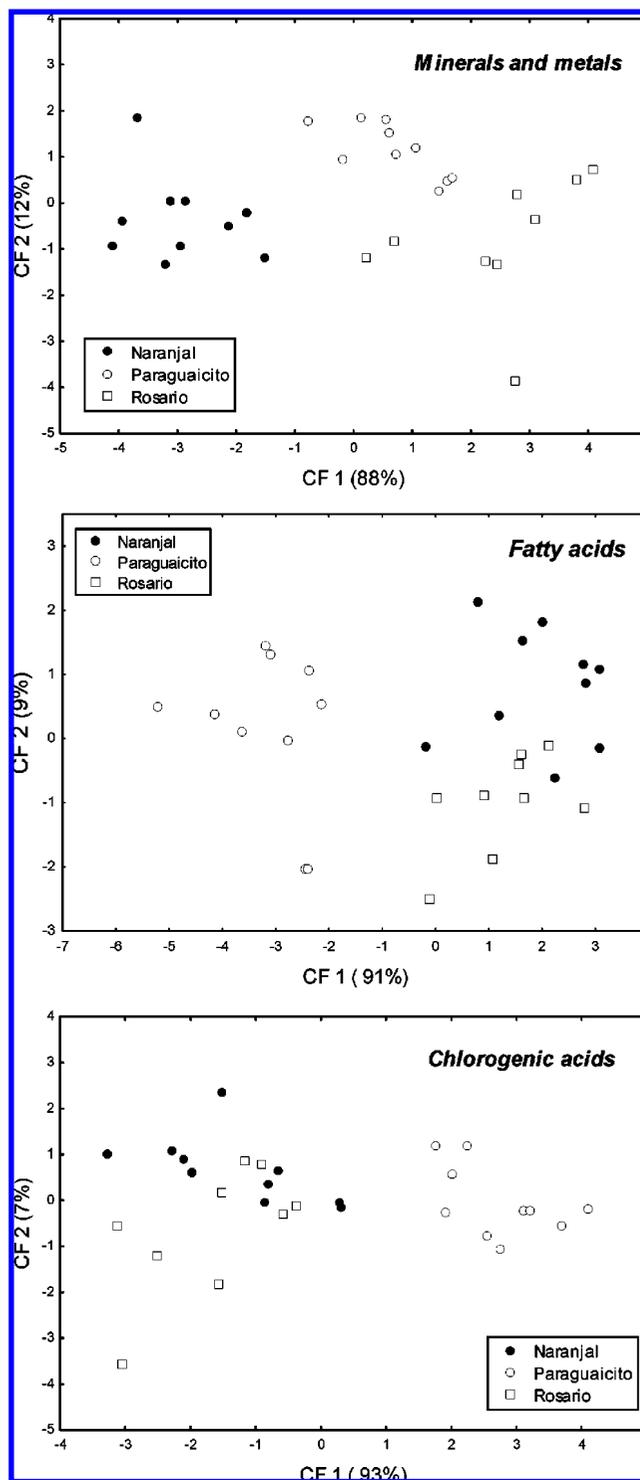
Figure 2. Scatterplot of canonical scores for the first two canonical functions resulting from the discriminant analysis of the three locations studied (Naranjal, Paraguaicito, and Rosario) based on bean element, fatty acid, and chlorogenic acid composition. The following full-factorial experimental design was employed: 3 locations × 5 varieties × 2 field replications.

2). By contrast, with FA and CGA, the first axis offered a very good partition of Paraguaicito samples from samples collected in the two other locations. In all cases, the second canonical function separated the two locations that could not be discriminated by the first canonical function. However, although this partition on the second axis was satisfactory with elements and FA, an overlapping of Naranjal and Rosario samples was observed with CGA.

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These results reveal for the first time the potential value of elements and FA and, to a lower extent, CGA for coffee terroirs determination. The excellent location classification obtained here with elements confirms the very high proportion of coffee samples correctly classified according to their environment using this chemical class (*5*). The effectiveness of elements for origin determination is very likely associated with the fact that the mineral and trace metal composition of beans reflects the composition of the soil in which the plants grow, which certainly varies significantly among locations. Whereas the utility of FA has been unambiguously shown for the discrimination of Robusta and Arabica green coffees (*15, 20*), to our knowledge, the present study establishes for the first time their value for determining coffee varieties as well as environments. Such efficiency for origin determination has, however, been demonstrated in other fruits and beans, for instance, pistachio (*28*), hazelnut (*29*), and olive (*30*).

Overall Comparison of the Effectiveness of FA, CGA and Elements for the Chemometric Discrimination of *C. arabica* Varieties and Terroirs. As mentioned by Anderson and Smith (*5*), the choice of a technique relies not only on discrimination performances but also on the time of analysis, the price of analytical equipment, and the possibility of automation. Although based on very different procedures, the time required for sample preparation was roughly the same with the three methods tested, allowing the preparation of about 20–30 samples per day. The prices of the three analytical devices used (GC, ICP-AES, and HPLC) are of similar orders of magnitude, and all equipment can be easily automated. By contrast, the analytical time once the sample is injected varied considerably among the three chemical classes studied, ranging from ca. 3 min for elements to 25 min for FA to about 50 min for CGA. Together with our results of discrimination efficiency, element profiling is thus a method of choice for origin determination. However, elements were useless for variety determination and, therefore, if only a single technique can be developed in a given laboratory, bean FA represents the best candidate among the three chemical families tested for an efficient discrimination of both varieties and terroirs. Their high discriminating value is certainly associated with the highly significant effects of both genetic and environmental factors on these traits, as previously observed in other lipid-rich seeds such as soybean (*31*) or sunflower (*32*). The influence of climatic conditions during the development of seeds, especially temperature and, to a lesser extent, precipitation, on their final FA composition has been reported in many oilseed crops (*32–35*) and model plant species (*36*). For instance, one common feature is an increase in polyunsaturated FA with increasing temperature. In coffee, even if the degree of unsaturation of leaf polar lipids was already shown to be affected by environmental temperature regimes (*37*), how climatic parameters influence the bean FA composition remains a key issue to investigate. Besides, one can reasonably question whether FA profiling alone may be sufficient to discriminate a very high number of environments or terroirs, considering that climatic variations within coffee-growing areas are rather restrained. One can logically consider that, when technically feasible, combining FA and element profiling should provide a higher potential for terroir characterization than FA alone. It is indeed generally recognized that the mineral and metal compositions of plants reflect the bioavailable nutrients present in the soils on which they grow (*5, 38, 39*). To take advantage of both climatic and soil diversity, one major practical recommendation that can be drawn from the present work would be thus to undertake the simultaneous analysis of FA and elements for coffee origin authentication.

Table 8. Eigenvalue and χ^2 Significance (*P*) of the First Two Canonical Functions Resulting from the Discriminant Analysis of the Three Locations Studied (Naranjal, Paraguaicito, and Rosario) Based on Bean Element, Fatty Acid, and Chlorogenic Acid Composition[a]

chemical	canonical function	eigenvalue	χ^2	*P*
elements	1	5.59	61.6	0.00
	2	0.78	14.5	0.00
fatty acids	1	5.83	58.5	0.00
	2	0.60	11.5	0.01
chlorogenic acids	1	4.46	49.3	0.00
	2	0.32	6.9	0.03

[a] The following full-factorial experimental design was employed: 3 locations × 5 varieties × 2 field replications.

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