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Volatile Compound Analysis to Authenticate the Geographical Origin of Arabica and Robusta Espresso Coffee

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Abstract: The traceability of the geographical origin of coffee is a challenging issue to protect producers and consumers from the risk of fraud. A total of 162 Arabica from Peru, Colombia and Brazil, and Robusta from India, Vietnam and Uganda, espresso coffee (EC) samples of different degrees of roasting (light, medium and dark) were characterized for physico-chemical features (lipids, solids, and chlorogenic acids) and analyzed via SHS-GC/MS analysis, with the aim of discriminating the samples according to their geographical origin. Linear discriminant analysis (LDA), performed on the data of the chemical classes of the volatile organic compounds (VOCs), was able to correctly identify 97.53% of the tested samples through cross-validation. The dark roasting of the coffee beans implied a higher quantity of volatile compounds in the headspace of the EC, belonging to chemical classes of furans, esters, N-heterocyclic and sulfur compounds, reducing the differences by geographical origin. Light- and medium-roasted Robusta EC showed a major contribution of pyrazines and pyrimidines, while aldehydes, alcohols and ketones were generally more representative in Arabica samples. The quantitative distribution of volatile compounds proved to be a useful tool to discriminate samples by geographical origin.

Keywords: VOCs; physico-chemical properties; coffee authentication; coffee geographical origin; discriminant analysis; different roasting degree

1. Introduction

The formation of volatile organic compounds (VOCs) during coffee roasting is a key process that significantly impacts the aroma and flavor of the resulting espresso coffee (EC). The profile of VOCs can be influenced by several factors, ranging from the genetics of the coffee plant to the brewing method used to prepare the final cup [1–5]. Among these factors, coffee varieties (Arabica and Robusta) directly connected with their cultivars, geographical origin and climate environment, as well as roasting conditions, have been widely investigated in previous literature [6–9]. Although the different sugar, lipid, caffeine and chlorogenic acid content of the Arabica and Robusta coffee fruits make the aroma of Arabica more complex and nuanced, the growing conditions (soil type, climate, and agricultural practices) affect the chemical composition of the coffee beans. Consequently, the VOC profile of espresso coffee could help control the geographical origin of coffee fruits. The degree of roasting and the condition of the roasting process are significant factors in determining the final overall VOC profile of EC, resulting in a fruity aroma for the lighter roast evolving toward caramel, dark chocolate, and smokiness notes for the darker roast [10].

Gas chromatography coupled with mass spectrometry (GC/MS), proton-transfer-reaction mass spectrometry (PTR-MS) and electronic nose (EN) are the main analytical techniques reported in the literature, which have allowed for the identification of almost 850 VOCs in EC. Within the GC/MS, the sampling by static headspace extraction (SHS) without using sorbents or solvents leads to a more realistic representation of EC aroma as

experienced by consumers [11], limiting the production of artifacts. Earlier studies have developed statistical models demonstrating the potential of using VOCs for the geographical origin determination of Arabica and Robusta green and roasted coffee beans [12–14]. However, the profile of volatile compounds in EC has never been used for the geographical discrimination of coffee beans. Secondly, the role of different roasting levels in the discrimination of coffee geographical origin by VOC analysis of EC has never been explored. Previously, Vezzulli et al. [15] demonstrated the discrimination ability of the physico-chemical parameters of roasted beans, such as acrylamide, caffeine, acidity and total phenolics, to cluster samples of different origins.

Therefore, the traceability of geographical origin of coffee beans using VOCs of EC as chemical markers can be a challenging issue for producers and consumers eager for reliable criteria of authenticity. This study uses SHS-GC/MS analysis to discriminate a total of 162 samples of Arabica and Robusta EC subjected to three different levels of roasting (light, medium, and dark), of different origins (India, Vietnam, Uganda, Brazil, Colombia and Peru). A linear discriminant analysis (LDA) was performed on the major VOC chemical classes to classify coffee samples according to their geographical origin.

2. Materials and Methods

2.1. Chemical and Coffee Samples

Six green coffee samples (three Arabica and three Robusta) were provided by Kimbo S.p.a. (Melito di Napoli, Italy). As far as Robusta is concerned, 2 samples came from Asia (India *Cherry* and Vietnam) and 1 from Africa (Uganda), whereas the Arabica samples came from South America (Peru, Colombia and Brazil). Reference chemical compounds were obtained from Sigma-Aldrich (Steinheim, Germany).

2.2. Experimental Design

The green coffee was roasted using a rotating drum Probatino roaster (Probat, Emmerich am Rhein, Germany). A preliminary phase involved tuning 3 roasting curves to obtain light-, medium- and dark-roasted coffee. The degree of the roast was measured using a polychromatic LED reflection colorimeter (https://my-tonino.com/shop/en/tonino; accessed on 27 February 2023) and a "Gourmet" Agtron scale. An average of two readings, one for the whole-bean and the other for the ground, gave a score of 38, 50 and 65 for dark-, medium- and light-roasted coffee, respectively. The roasting curve resulting from the preliminary analysis was carried out on 1 kg of green coffee for each roasting and geographical origin. The Arabica coffee species required lower roasting temperatures and a shorter roasting time compared to the Robusta coffee species (Figure 1), as reported in the literature [16]. Robusta beans are denser than Arabica beans, and thus require more heat to be roasted. The roasted coffee beans were stored in triple-layer bags (PET/AL/PE) for 48 h, to allow for the residual gases to completely evaporate. Subsequently, the coffee was ground using a typical grinder with flat burrs (Promac, Milan, Italy). The degree of grinding was adjusted based on the typical extraction speed of an EC, which is 1 mL/s, to obtain a well-extracted and high-quality espresso [17]. Espresso coffee was prepared for each sample from 15 g of coffee powder, through which purified water of 90 °C was forced at 7–9 bar of pressure and a double-holder filter diameter of 38 mm (Rancilio, Model: Rancilio Classe 11 USB). A fixed volume of EC (8 mL) was introduced in 5 vials (20 mL), immediately closed and sealed. Three aliquots of EC (40 mL) were extracted for each roasting degree and geographical origin to be analyzed, in triplicate.
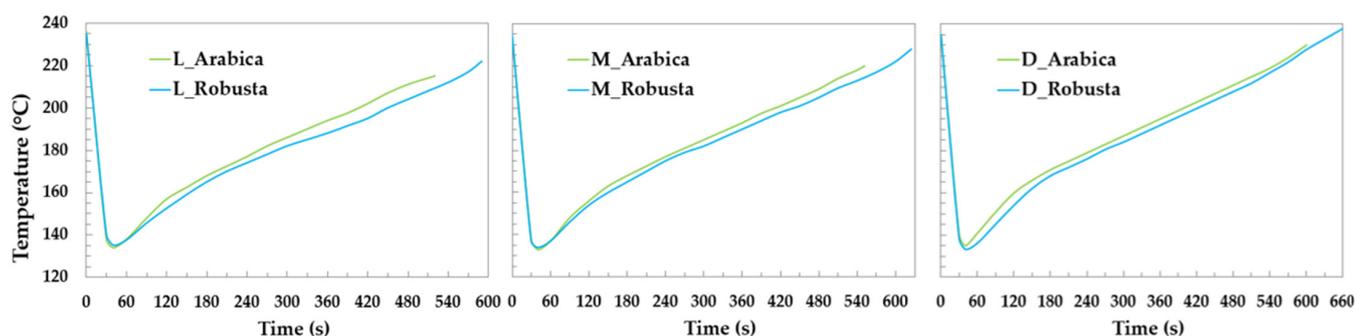
Figure 1. Roasting profile of Arabica (green) and Robusta (blue); L = light; M = medium; D = dark.

2.3. Lipid, Solid and Chlorogenic Acid Determination

Lipids, total solids and chlorogenic acids (3-CQA, 4-CQA and 5-CQA) were determined as described in De Vivo et al. [2]. Roughly, the total amount of lipids was estimated by liquid–liquid extraction using hexane. Solids were determined by oven-drying 5 mL of EC to a constant weight (102 °C). Caffeoylquinic acids (CQAs) were detected via an UHPLC (Thermo Scientific Dionex UltiMate 3000) equipped with a *Kinetex EVO C18* analytical column (100 × 4.6 mm I.D., 2.6 μm particle size; Phenomenex) and a diode array detector. The mobile phase consisted of 10 mM acetic acid in water (v/v) (solvent A) and methanol (solvent B). The elution conditions applied were as follows: 0–2 min, linear gradient to 15% B; 2–10 min, linear gradient from 15 to 30% B; 10–11 min, linear gradient from 30 to 95%; 11–15 min, 95% B isocratic; 15–16 min, linear gradient from 95 to 5% B; 16–20 min, 5% B isocratic. The flow rate was 0.7 mL min^{-1}. CQAs were quantified at 325 nm. All metabolites were analyzed in triplicate from 3 different extractions.

2.4. Analysis of VOCs by SHS–GC/MS

The profiles of volatile compounds were analyzed using the method described by De Vivo et al. [2], using a gas chromatograph (TRACE 1310) equipped with mass spectrometry (TSQ 8000 Evo triple quadrupole, Thermo Fisher Scientific, Waltham, MA, USA). Each vial was equilibrated at 70 °C for 30 min, and 1 mL of the coffee headspace sample was injected into the ZB-FFAP capillary column (Length: 60 m; internal diameter: 0.25 mm; film thickness: 0.25μm) (Phenomenex, Torrance, CA, USA). The injector temperature was set at 180 °C and the carrier gas (helium) flow rate was set at 1 mL/min. The GC oven program was set as follows: 40 °C held for 6 min, followed by an increase to 220 °C at a rate of 3 °C min^{-1} (maintained for 2 min), continuing with an increase of 3 °C min^{-1} until the temperature reached 250 °C, which was held for 5 min. The mass spectrometer operated with electron ionization (70 eV), with a scan range of 33–300 amu. The ion source temperature was set at 260 °C.

The identification of compounds was confirmed by comparing the experimental spectra with the spectra from NIST14 libraries with a match factor higher than 97% and by comparison of the retention times with those of standard compounds when reference compounds were available. GC/MS analysis in selective ion monitoring (SIM) was applied to quantify the volatile compounds. The results were expressed as relative percentage of each peak area of quantifying ion to the total GC-MS peak area. Data were processed using the software TraceFinder™ (version 5.1 SP1; Thermo Fisher Scientific, Waltham, MA, USA).

2.5. Statistical Analysis

Results were expressed as the mean ± standard deviation. Statistical analysis and visualization were carried out in XLStat (Version 2019 v.2.2), an add-in software package for Microsoft Excel (Addinsoft Corp., Paris, France). The differences between the physico-chemical and VOC variables for the EC samples were assessed by an ANOVA with Tukey's HSD test, for a significance level set at $p < 0.05$. The data of the chemical classes of VOCs for the 162 EC samples were processed by linear discriminant analysis (LDA), in order

to classify samples by geographical origin. Principal component analysis (PCA) was performed on the average of the chemical classes of VOCs of the EC samples to assess the distribution of the chemical classes of VOCs among the samples.

3. Results and Discussion

3.1. Chemical Analysis

Arabica and Robusta EC samples, obtained by using coffee coming from different geograhycal origin, were compared in terms of chemical characterization by quantification of solid content, lipids and caffeoylquinic acids. As shown in Table 1, higher levels of CQAs, 3-CQA, 4-CQA and 5-CQA were found in Arabica ECs compared to Robusta, in agreement with the previous studies [18–20]. This result can be explained, since during the roasting process, the CGAs present in the Robusta samples are degraded more rapidly than in those of Arabica, resulting in low levels of CGAs in Robusta EC [21]. Furthermore, the results have shown no significant differences among different cultivars of Robusta in acid content. The degradation of CQAs during the roasting process, as a consequence of the breakage of carbon–carbon bonds, has been extensively reported in previous literature [18,22–24], as also demonstrated by the findings of this study. The major lipid fraction was found in Arabica EC samples, whatever the geographical origin and roasting degree [25]. It is known that VOCs are trapped in oil droplets and are released during extraction, and finally reach the nasal receptor of the consumers. Thus, the oil fraction explains the difference between Arabica and Robusta flavor. Additionally, the lipid content has shown a decrease with increasing the roasting degree, as reported in previous literature [26]. Solid content of EC samples seemed unable to discriminate coffee varieties, as well as geographical origin, as given in Table 1. However, the stronger the roasting conditions, the higher the amount of total solids, even if no significant differences were found between the medium and dark roast degree [26].

Table 1. Influence of geographical origin and roasting treatment on chemical parameters of EC samples [1].

		Robusta			Arabica		
	RD [2]	INDIA Cherry	VIETNAM	UGANDA	PERU	COLOMBIA	BRAZIL
3-CQA (mg/L)	L	34.156 ± 3.127 cA	33.682 ± 0.381 cA	34.644 ± 4.791 cA	50.585 ± 3.913 bA	62.483 ± 4.806 aA	48.756 ± 3.704 bA
	M	14.233 ± 0.193 cB	15.83 ± 2.04 cB	17.845 ± 0.829 cB	32.712 ± 0.905 aB	30.872 ± 1.007 aB	26.219 ± 2.755 bB
	D	2.492 ± 0.052 cC	2.865 ± 0.024 cC	3.438 ± 0.188 cC	6.274 ± 0.231 bC	10.402 ± 1.261 aC	9.211 ± 0.343 aC
4-CQA (mg/L)	L	30.025 ± 2.843 bA	30.24 ± 0.144 bA	31.469 ± 4.826 bA	37.892 ± 3.146 bA	46.25 ± 2.078 aA	37.683 ± 2.493 bA
	M	15.049 ± 0.225 bB	16.683 ± 1.656 bB	18.436 ± 0.799 bB	23.869 ± 2.41 aB	25.825 ± 0.745 aB	23.894 ± 0.757 aB
	D	3.323 ± 0.115 dC	3.737 ± 0.059 dC	4.348 ± 0.284 dC	6.94 ± 0.294 cC	11.262 ± 1.488 aC	9.433 ± 0.424 bC
5-CQA (mg/L)	L	22.595 ± 1.997 cA	24.254 ± 2.126 cA	23.694 ± 3.612 cA	31.97 ± 2.635 abA	38.086 ± 3.506 aA	28.625 ± 2.137 bcA
	M	11.506 ± 0.144 bB	13.38 ± 2.336 bB	14.172 ± 0.737 bB	21.266 ± 0.226 aB	20.878 ± 1.247 aB	19.701 ± 1.464 aB
	D	1.884 ± 0.05 cC	2.139 ± 0.029 cC	2.754 ± 0.208 cC	5.531 ± 0.234 bC	8.096 ± 1.008 aC	7.608 ± 0.188 aC
Lipids (mg/mL)	L	4.371 ± 0.122 bA	2.182 ± 0.224 cA	4.184 ± 0.15 bA	4.48 ± 0.425 abA	5.739 ± 1.017 aA	4.016 ± 0.416 bA
	M	3.145 ± 0.13 bcB	1.645 ± 0.066 dB	2.027 ± 0.168 cdB	4.066 ± 0.841 abA	5.416 ± 0.839 aA	3.339 ± 0.268 bcAB
	D	2.167 ± 0.272 bcC	1.335 ± 0.087 cB	1.28 ± 0.107 cC	3.024 ± 0.353 bB	4.785 ± 0.793 aA	2.655 ± 0.538 bcB
Total solids (mg/mL)	L	81.914 ± 1.345 abB	78.23 ± 2.488 bC	92.659 ± 7.202 aB	74.091 ± 4.093 bB	83.427 ± 7.901 abA	70.924 ± 2.094 bB
	M	85.607 ± 2.342 bAB	86.571 ± 1.142 bB	103.207 ± 8.502 aAB	88.602 ± 5.677 bAB	82.617 ± 5.086 bA	76.375 ± 5.117 bB
	D	89.833 ± 3.575 bA	100.831 ± 4.335 abA	120.208 ± 9.338 aA	108.269 ± 15.711 abA	101.161 ± 12.132 abA	90.394 ± 0.594 bA

[1] All values are shown as the means ± standard deviations (n = 3). In each row, lowercase letters indicate significant differences between geographical origins within the same roasting treatment. Capital letters indicate significant differences between the roasting profiles within the same coffee origin ($p < 0.05$). [2] RD = roast degree; L = light; M = medium; D = dark.

3.2. Discriminant Analysis of EC Samples

Linear discriminant analysis (LDA, after log transformation) was performed on the data of the chemical classes of volatile compounds quantified in the 162 total coffee samples, with the aim of discriminating them by geographical origin. The results of the analysis are shown in Figure 2.
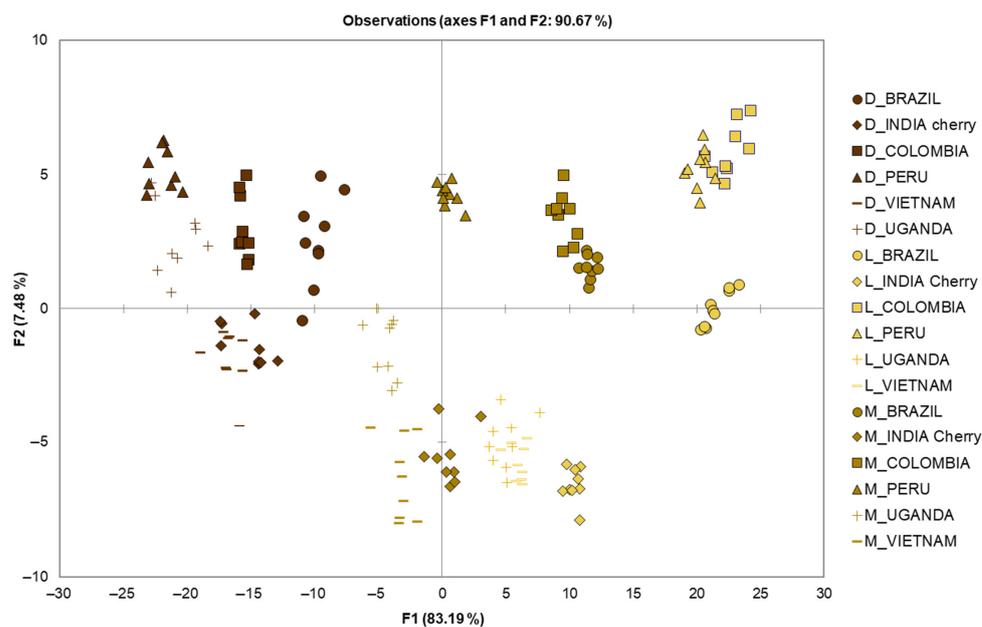
Figure 2. Identification of geographical origin by LDA using the chemical classes of volatile compounds quantified in the 162 EC samples. L = light; M = medium; D = dark.

The chemiometric model obtained by LDA showed a λWilks value 0.000, suggesting that the group means were different. The LDA (probability of F to remove 0.10 and to enter 0.05) selected all chemical classes as discriminant of two discriminant factors, based on standardized canonical discriminant function coefficients, for FD1 (83.19%): aldehydes (2.149), esters (−1.536), sulphur compounds (−1.054), N-heterocycle (−0.892), phenols (0.549), pyrazines (0.516), alcohols (−0.386), ketones (0.359), furans (−0.208), pyrimidine (0.190) and acids (0.091); and for FD2 (7.48%): ketones (2.093), N-heterocycle (−1.609), aldehydes (−1.460), pyrazines (−0.699), esters (0.697), phenols (0.522), alcohols (−0.391), sulphur compounds (0.370), furans (0.121), pyrimidine (−0.071) and acids (0.066).

The LDA showed 98.15% correctly classified samples. The model was leave-one-out cross-validate, obtaining 97.53% correctly validated samples, and the confusion matrix for the cross-validated results is shown in Table 2. Apart from samples D_VIETNAM and D_COLUMBIA, all other samples were correctly classified, leading to a total correct classification of 97.53%. Specifically, D_VIETNAM was misclassified as D_COLUMBIA twice, and vice versa. Previously, Demianová et al. [13] reported the correct classification of coffee samples according to geographical origin of 82.61%. The authors focused on nine green coffee Arabica samples from Africa, South and Central America, for a total of 23 samples, using the chemical classes of volatile compounds as model parameters for LDA analysis.

In our study, we examined nine samples from Brazil, Peru, Colombia, India, Vietnam and Uganda, also investigating the effect of three different roasting levels. The score plot (Figure 2) showed a clear separation not only for the origin of the samples, but also for the different roasting degree, suggesting that the volatile compounds were effective in discriminating both origin and roasting level. Interestingly, the lower the level of roasting, the more evident the separation of the samples according to their geographical origin. Therefore, the light roasting showed a better separation than dark roasting, where the latter was the cause of misclassification for the D_VIETNAM and D_COLUMBIA samples. The increase in the level of roasting of the coffee beans led to the development of new volatile compounds, such as furans and sulfur compounds, which reduced the differences originally present in the green coffee beans, and higher classification errors occur in ECs with higher roasting levels [14], corroborating our findings of better classification for light- and medium-roasted samples.

Table 2. Confusion matrix of the EC samples.

	D_BRAZIL	D_INDIA *Cherry*	D_COLOMBIA	D_PERU	D_UGANDA	D_VIETNAM	Total	Correct Response (%)
D_BRAZIL	9	0	0	0	0	0	9	100.00
D_INDIA *Cherry*	0	7	0	0	0	2	9	77.78
D_COLOMBIA	0	0	9	0	0	0	9	100.00
D_PERU	0	0	0	9	0	0	9	100.00
D_UGANDA	0	0	0	0	9	0	9	100.00
D_VIETNAM	0	2	0	0	0	7	9	77.78
	M_BRAZIL	M_INDIA *Cherry*	M_COLOMBIA	M_PERU	M_UGANDA	M_VIETNAM		
M_BRAZIL	9	0	0	0	0	0	9	100.00
M_INDIA *Cherry*	0	9	0	0	0	0	9	100.00
M_COLOMBIA	0	0	9	0	0	0	9	100.00
M_PERU	0	0	0	9	0	0	9	100.00
M_UGANDA	0	0	0	0	9	0	9	100.00
M_VIETNAM	0	0	0	0	0	9	9	100.00
	L_BRAZIL	L_INDIA *Cherry*	L_COLOMBIA	L_PERU	L_UGANDA	L_VIETNAM		
L_BRAZIL	9	0	0	0	0	0	9	100.00
L_INDIA *Cherry*	0	9	0	0	0	0	9	100.00
L_COLOMBIA	0	0	9	0	0	0	9	100.00
L_PERU	0	0	0	9	0	0	9	100.00
L_UGANDA	0	0	0	0	9	0	9	100.00
L_VIETNAM	0	0	0	0	0	9	9	100.00
Total	9	9	9	9	9	9	162	97.53

L = light; M = medium; D = dark. Values in bold indicate misclassifications.

3.3. Volatile Compound Analysis

The results of the analysis of volatile compounds were reported in Table 3. In general, volatile compounds belonging to aldehyde, ketone and alcohol chemical classes discriminated the geographical origin of EC samples, especially for light and medium roasting conditions. However, the development of furans and sulfur compounds was pronounced in dark roasting EC samples [27,28], reducing the differences attributable to the geographical origin. N-heterocyclic compounds, e.g., pyrroles, increase as the degree of roasting increases (D treatment), while pyrazines decrease [29].

In order to summarize the results and reduce the dataset dimensionality [30], PCA was conducted. Figure 3 shows the biplot of the PCA, carried out on the chemical classes quantified in the EC samples. As previously stated, the dark roasting treatment caused a higher abundance of volatile compounds, in agreement with the work of Wu et al. [31] and Charles et al. [32]. In particular, a greater quantity of furan, ester, N-heterocyclic and sulfur compounds were present in the dark-treated samples. Moreover, the dark roasting of coffee beans reduced differences in VOCs by variety and provenance, corroborating the results obtained in the LDA. Esters were more abundant in the coffee Arabica varieties, as previously found by Abdelwareth et al. [33]. Alcohols, ketones and aldehydes, as well as pyrazines, phenols and pyrimidines, were distributed between the light and medium treatments. Furthermore, the Arabica coffee samples had a higher quantity of phenols, contributing to spicy and smoky odors [33]. The roasted light and medium Robusta varieties, i.e., INDIA Cherry and VIETNAM, showed higher amounts of pyrazines and pyrimidines, while aldehydes, alcohols and ketones were generally more abundant in the Arabica variety samples. These results confirm the findings of Procida et al. [14], who reported higher amounts of alcohols in Arabica green coffee varieties. Furthermore, Sanz et al. [34] reported a higher quantity of sulfur compounds in coffee with 80% of Robusta variety, while aldehydes, ketones and alcohols were present in higher quantities in samples with 80 % of Arabica variety.

Table 3. Changes in the VOCs identified in headspace of ECs resulting from different geographical origin and different roasting treatments.

Chemical Classes	RD [2]	Peak Area ($\times10^6$) [1]					
		Robusta			Arabica		
		INDIA Cherry	VIETNAM	UGANDA	PERU	COLOMBIA	BRAZIL
Acids	L	0.512 ± 0.107 bA	0.458 ± 0.112 Bb	0.474 ± 0.079 bA	1.21 ± 0.126 aA	1.252 ± 0.104 aA	1.278 ± 0.179 aA
	M	0.469 ± 0.096 dA	0.608 ± 0.141 cdA	0.557 ± 0.14 cdA	0.704 ± 0.076 cB	1.332 ± 0.219 aA	1.066 ± 0.112 bB
	D	0.294 ± 0.058 cB	0.258 ± 0.06 cC	0.51 ± 0.211 bA	0.413 ± 0.12 bcC	0.5 ± 0.133 bB	0.731 ± 0.066 aC
Alcohols	L	2.454 ± 0.17 abB	2.483 ± 0.409 abA	2.279 ± 0.275 abA	2.15 ± 0.131 bB	2.525 ± 0.176 aC	2.591 ± 0.199 aB
	M	2.719 ± 0.239 bcA	2.864 ± 0.376 bcA	2.706 ± 0.344 cA	2.836 ± 0.193 bcA	3.377 ± 0.254 aA	3.114 ± 0.263 abA
	D	1.741 ± 0.221 cC	1.781 ± 0.265 cB	2.303 ± 0.474 bA	2.652 ± 0.394 abA	3.059 ± 0.346 aB	2.952 ± 0.1 aA
Aldehydes	L	66.106 ± 11.094 bcA	63.369 ± 7.956 bcA	57.905 ± 7.222 cA	66.902 ± 3.09 bcA	74.287 ± 8.791 bA	91.029 ± 9.319 aA
	M	56.369 ± 3.895 cB	58.024 ± 2.734 cA	51.85 ± 5.095 cA	66.012 ± 5.234 bA	67.976 ± 6.397 bA	87.828 ± 7.189 aA
	D	39.839 ± 5.141 cC	40.998 ± 7.295 cB	40.308 ± 2.894 cB	44.326 ± 0.957 cB	50.501 ± 2.36 bB	58.348 ± 4.653 aB
Esters	L	16.246 ± 2.71 bC	19 ± 2.578 abB	18.247 ± 1.636 abC	16.721 ± 0.714 abC	16.857 ± 2.101 abC	19.192 ± 2.143 aC
	M	19.627 ± 1.106 dB	22.299 ± 1.703 cAB	24.054 ± 2.605 bcB	28.3 ± 2.236 aB	21.559 ± 1.37 cdB	25.296 ± 1.704 bB
	D	22.309 ± 1.668 cA	25.524 ± 4.044 cA	32.877 ± 3.278 bA	38.558 ± 2.584 aA	31.866 ± 2.8 bA	33.64 ± 2.052 bA
Furans	L	119.361 ± 18.804 aB	132.468 ± 9.161 aB	83.643 ± 13.825 bcB	71.351 ± 5.592 cC	72.168 ± 17.713 cC	98.727 ± 8.867 bB
	M	136.409 ± 29.636 abB	155.639 ± 11.933 aA	87.077 ± 12.152 cB	145.992 ± 13.196 abB	124.623 ± 16.829 bB	131.645 ± 22.953 abA
	D	199.046 ± 40.927 aA	175.625 ± 29.609 abA	106.4 ± 19.774 cA	158.82 ± 9.254 bA	181.232 ± 26.18 abA	150.911 ± 22.294 bA
Ketones	L	24.457 ± 4.188 cA	22.562 ± 3.502 cA	20.934 ± 2.872 cA	30.672 ± 1.802 bA	35.963 ± 4.541 aA	33.77 ± 3.861 abA
	M	21.926 ± 1.466 bA	22.402 ± 1.172 bA	22.078 ± 2.229 bA	31.208 ± 2.763 aA	30.131 ± 3.036 aB	32.918 ± 3.238 aA
	D	18.106 ± 1.712 bB	18.71 ± 2.853 bB	19.936 ± 0.924 bA	23.359 ± 1.122 aB	25.484 ± 1.525 aC	24.68 ± 2.408 aB
N-Heterocycle	L	10.04 ± 2.24 aB	9.92 ± 1.727 aC	8.92 ± 1.606 aB	4.637 ± 0.792 bC	5.54 ± 1.574 bB	6.257 ± 1.513 bB
	M	10.544 ± 1.061 bB	14.203 ± 1.372 aB	10.054 ± 1.226 bB	9.595 ± 1.547 bB	7.118 ± 1.402 cB	6.508 ± 1.345 cAB
	D	16.045 ± 2.355 abA	17.989 ± 3.846 aA	15.204 ± 2.182 abA	13.309 ± 0.898 bA	14.585 ± 1.846 bA	8.064 ± 1.588 cA
Phenols	L	1.438 ± 0.23 abA	1.341 ± 0.204 abB	1.065 ± 0.271 bB	1.185 ± 0.247 bAB	1.598 ± 0.329 aA	1.234 ± 0.325 abA
	M	1.167 ± 0.128 bcB	1.439 ± 0.106 abA	1.177 ± 0.164 bcB	1.287 ± 0.195 abA	1.493 ± 0.274 aA	1.023 ± 0.163 cA
	D	1.504 ± 0.154 aA	1.634 ± 0.253 aA	1.558 ± 0.106 aA	0.993 ± 0.037 bB	1.424 ± 0.17 aA	0.715 ± 0.092 cB
Pyrazines	L	5.17 ± 0.716 aA	4.249 ± 0.912 bAB	3.02 ± 0.313 cA	2.767 ± 0.222 cB	2.773 ± 0.383 cA	3.805 ± 0.359 bA
	M	4.572 ± 0.99 abA	4.819 ± 0.939 aA	3.629 ± 1.07 bcdA	3.025 ± 0.195 cdA	2.769 ± 0.287 dA	3.817 ± 0.446 abcA
	D	2.955 ± 0.482 cdB	3.651 ± 0.68 aB	3.062 ± 0.241 cdA	2.554 ± 0.162 dB	3.094 ± 0.248 bcA	3.625 ± 0.198 abA
Pyrimidine	L	0.432 ± 0.046 aA	0.367 ± 0.101 aB	0.253 ± 0.021 bcB	0.214 ± 0.028 cB	0.184 ± 0.022 cC	0.29 ± 0.038 bC
	M	0.445 ± 0.113 aA	0.581 ± 0.118 aA	0.344 ± 0.123 bcA	0.286 ± 0.024 cA	0.265 ± 0.03 cB	0.366 ± 0.034 bcB
	D	0.401 ± 0.069 bA	0.484 ± 0.083 aAB	0.376 ± 0.035 bA	0.294 ± 0.026 cA	0.362 ± 0.031 bcA	0.476 ± 0.037 aA
Sulfur Compounds	L	10.7 ± 1.436 aB	10.047 ± 1.2 aC	8.211 ± 1.287 bC	3.806 ± 0.282 dC	3.716 ± 0.629 dC	5.221 ± 0.45 cC
	M	12.995 ± 2.574 bB	16.846 ± 2.463 aB	12.99 ± 3.205 bB	9.522 ± 0.868 cB	6.339 ± 0.459 dB	8.202 ± 0.568 cdB
	D	22.404 ± 4.214 abA	24.991 ± 4.31 aA	25.954 ± 4.939 aA	19.883 ± 2.112 bcA	21.691 ± 1.295 abA	16.956 ± 1.308 cA

[1] All values are shown as the means ± standard deviations (n = 3). In each row, lowercase letters indicate significant differences between geographical origins within the same roasting treatment. Capital letters indicate significant differences between roasting profiles within the same coffee origin ($p < 0.05$). [2] RD = roast degree; L = light; M = medium; D = dark.
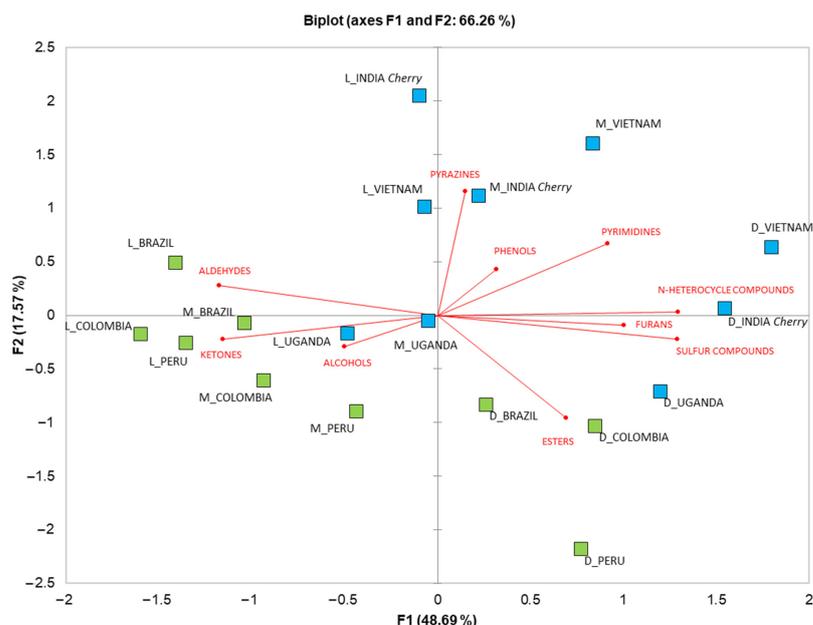
Figure 3. Principal component analysis biplot of VOC classes detected in Arabica (in green) EC from Peru, Colombia and Brazil (in green), and Robusta (in blue) EC from India *Cherry*, Uganda and Vietnam (in blue) at three different roasting levels (L = light; M = medium; D = dark).

4. Conclusions

The present study has examined the role of volatile compounds in ECs brewed using Arabica and Robusta coffee, in discriminating the geographical origin of coffee at three different roasting levels. Using linear discriminant analysis to process VOC chemical class data, the coffee samples were 97.53% correctly classified in cross-validation. Classification errors occurred for coffee samples with a higher degree of roasting (dark), due to the formation of volatile compounds, such as furans and sulfur compounds, which reduced the differences in the VOC profiles. A second major finding was the possibility to discriminate the samples according to the Arabica or Robusta variety, as particular chemical classes of VOCs are more representative of one or the other variety. The evidence from this study implies that volatile compounds in espresso coffee may be potential markers of coffee origin, variety, and roasting, providing the possibility to check several quality parameters in a single analysis.

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Institutional Review Board Statement: No ethical approval was required for this study, since the animals were reared under actual farm conditions, and no distress, pain, suffering or lasting harm was caused to them. The animals were managed in compliance with the European requirements concerning the protection of animals used for scientific purposes (Dir. 2010/63/UE), as implemented by the Italian legislation (DL n. 26, 4 March 2014).

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