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Chemical and sensory profile of new genotypes of Brazilian *Coffea canephora*

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ABSTRACT

The study of Brazilian Conilon coffee genotypes with unknown chemical composition and sensory quality is extremely important since these data may contribute to the launching of new coffee cultivars in the international market with high cup quality. The present study aimed to investigate the metabolic profile of 3 genotypes of Conilon and compared them to Robusta Tropical and Arabica coffees, all collected at 3 different levels of ripeness. The extracts were analysed by ESI-LTQ-ORBITRAP, and 11 attributes were evaluated by sensory analysis. To correlate sensory, composition and maturation, chemometric analysis was used. The metabolites trigonelline, caffeine, caffeoylquinic acid and sugars revealed higher concentrations in genotypes 105 and 108. According to the sensorial analysis, genotype 108 showed the highest final score (82), which was even higher than the Arabica coffees. Among the new coffees studied, genotype 108 presented promising characteristics, sparking interest in its national and international commercialization.

1. Introduction

Coffee is one of the most appreciated and consumed beverages in the world. It has different aromas and flavours and is an important commodity and a source of economic revenue for many developing countries (ICO, 2019c). There are many species of the genus *Coffea* (Rubiaceae); however, only two species are commercially distributed for consumption: *Coffea arabica* L. and *Coffea canephora*, more commonly known as Arabica and Robusta coffee, respectively. Arabica beans are known to have a better-quality brew with an intense aroma, providing a beverage of higher commercial value. On the other hand, Robusta beans, also known as Conilon, is a species with a reduced commercial price that has a stronger and more bitter taste than Arabica. Therefore, Arabica beans are more desired by consumers because of their taste (Ferrão, da Fonseca, Ferrão, & De Muner, 2019).

With the purpose of developing new coffees with better agronomic and sensorial characteristics while still maintaining a good commercial price, the Capixaba Institute of Research, Technical Assistance and Extension (INCAPER, Espírito Santo, Brazil) started a genetic improvement programme for Conilon coffees that develops new genotypes by increasing the productivity, resistance to pests and droughts, uniformity of the coffee beans, as well as a better taste and quality in general. For the engineering of these genetic materials, INCAPER took advantage of the genetic variability of the Conilon coffee and used strategies of asexual clonal breeding through vegetative propagation (Ferrão et al., 2019), developing new genotypes with unknown macro and micronutrient profiles.

According to data from the International Coffee Organization, there are approximately 72 coffee bean producers in the world, with Brazil being the largest, producing approximately 37% of world's production (ICO, 2019b). In addition, the country occupies the position of the world's largest exporter of coffee, accounting for 32% of coffee exports in the world market according to 6 months of the year (November 2018 to April 2019) (ICO, 2019a). These data show that the study of new Brazilian Conilon coffee genotypes with unknown chemical compositions or sensory quality is extremely important since these coffees may soon be released to international markets as commercial coffee cultivars, contributing to their consumption in different parts of the world.

It is known that diversity in the environments may influence the chemical composition of coffee (Cheng, Furtado, Smyth, & Henry,

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2016), not only due to the differences between the genetic variability of the species but also from the agronomic conditions, such as harvesting at different levels of maturation (Amorim et al., 2009; Smrke, Kroslakova, Gloess, & Yeretzian, 2015; Souza et al., 2019).

The harvest is an important phase that exerts a total influence on the composition of the coffee beans and should be performed at its ideal point of maturation because it is at that moment that the important chemical precursors are aggregated into the coffee, providing a beverage of higher quality. However, many production farms perform coffee fruit collection before all of the fruits are fully ripe; that is, the fruits are picked with different degrees of ripeness (cherry, green or dried in the plant). This practice may damage the final beverage, resulting in a lower cup quality (Smrke et al., 2015).

The chemistry of coffee quality is highly complex, with numerous compounds that may change during fruit maturation. Among them, there are more than 700 compounds that contribute to the aroma and flavour. The most important metabolites involved in regulating the quality and taste are caffeine, trigonelline, sugars, lipids and chlorogenic acids (Souza et al., 2019; Wintgens, 2012).

The current literature makes it clear that the relationship between the chemical composition, coffee bean maturation and coffee quality is still unknown. Considering the economic importance of Conilon coffee, the objective of the present study was to investigate the chemical and sensory profile of three new genotypes of Conilon coffee, as well as to compare with commercial cultivar of Robusta Tropical and Arabica coffees (Arabica Catuai 81, Obata and Topazio) harvested across three stages of bean ripeness (60%, 80% and 100%).

2. Materials and methods

2.1. Green coffee bean samples

Green coffee beans were collected from the experimental farms of INCAPER in Espírito Santo State, Brazil, during 2017. Three cultivars of green Arabica coffee beans (Catuai 81: AC; Obata: AO; Topazio: AT) (Lat: 20°22′38.56″S Long: 41°11′54.24″W), three genotypes of green Conilon coffee beans (genotypes Diamante C101, C105 and C108) and one cultivar of green Robusta coffee beans (RT) (Lat: 20°45′21.30″S Long: 41°17′4.33″W) were collected. All species were harvested at three different levels of maturity, namely, 60%, 80% and 100% of cherry-stage fruits, for a total of 21 green coffee bean samples. Approximately 5 kg of each coffee was collected and naturally sun-dried (greenhouse), processed and cleaned. Only non-defective and 16 sieve-sized beans (Brasil, 2003) were selected. The samples were stored in the dark at room temperature until analysis.

2.2. . Chemicals

Methanol (HPLC grade), formic acid (reagent grade), ammonium hydroxide (reagent grade), deuterated caffeine (standard grade) and salicylic acid (standard grade) were all purchased from Sigma-Aldrich (St. Louis, USA).

2.3. . Extract preparation

Before the preparation of the methanolic extracts, the green coffee beans were stored for 3 days at –80 °C to facilitate grinding. The samples were ground to a powder with a coffee grinder (Hamilton Beach), and 1.0 g of the powder was extracted with 10 mL of methanol. The samples were placed in an ultrasonic water bath (Branson 2510MT) for 40 min. The samples were centrifuged for 10 min at 5000 rpm (2800 *g*) using an Allegra 25R centrifuge (Beckman Coulter), and the supernatant was collected and kept at –80 °C until MS analysis.

2.4. ESI-LTQ-ORBITRAP: sample preparation

A fast and easy direct infusion ESI-HRMS quantitative method was proposed to investigate the selected metabolites: caffeine, trigonelline, phenolic acids, and sugars. Prior to the ESI-HRMS analysis, 10.0 µL aliquots of each extract were transferred to 1.5 mL Eppendorf tubes, and 988 µL of methanol was added. The samples were analysed in positive and negative ion modes. For positive mode, the solutions were acidified with 1.0 µL of concentrated formic acid, and 10.0 µL of deuterated caffeine at a concentration of 1 mg/mL was spiked into the sample for a final standard concentration of 48 µmol/L caffeine in each extract. In negative mode, 1.0 µL of concentrated ammonium hydroxide and 2.0 µL of the standard salicylic acid solution at a concentration of 1 mg/mL were added for a final concentration of 14.4 µmol/L salicylic acid in each extract. Before injection in the ESI-LTQ-Orbitrap Elite mass spectrometer, samples were stirred for 10–15 s using a vortex apparatus (Bench Mixer™).

2.5. ESI-LTQ-ORBITRAP: Analysis

Coffee bean extracts were injected using a 2 µL valve injection into the mass spectrometer (LTQ-Orbitrap Elite, Thermo Scientific) equipped with an electrospray ionization (ESI) source operating in positive and negative ion mode. The optimized parameters for positive ion mode were as follows: flow rate 3 µL/min; spray voltage 3.5 kV; 1 microscan; injection volume 10 µL, capillary temperature 275 °C; source heat temperature 75 °C; tube lens 64 V; and resolution 240,000. The mass spectra were acquired over the range of m/z 120–500. In negative ion mode, the spray voltage was set to 3.0 kV, the tube lens was set to 50 V, and the full scan mass spectra were obtained over the range of m/z 120–600. A method was created in Xcalibur™ software (version 3.0, Thermo Fischer Scientific Inc.) for data dependent acquisition (DDA), by which the top 100 ions were selected for MS/MS by high energy collision dissociation (HCD) using a collision energy of 100 arbitrary units. The DDA method was performed once on the same day for 21 green coffee bean samples across three stages of maturity (60%, 80% and 100%).

2.6. Data calibration

The LTQ-Orbitrap mass spectrometer was calibrated online and offline in positive and negative ion mode, respectively. The mass calibration was performed offline in negative ion mode using the program mMass. Xcalibur™ mass spectra files were converted from .raw files with ProteoWizard to XML format, and these files were uploaded onto mMass. mMass was used to automatically subtract blanks from the samples. A compound list was created with the exact and accurate masses of all of the ions of interest, including the internal standard (salicylic acid). The entire negative ion mode data set of 21 mass spectral files was successfully calibrated. The calibrated mass data achieved a mass error between 1 and 2 ppm for all ions.

2.7. Sensory analysis

The coffee beans were preprocessed prior to the sensory analysis. Defective beans were removed, and the coffees were subjected to the roasting process according to the protocol of the Uganda Coffee Development Authority (UCDA, 2010). The sensory analyses were carried out in the Laboratory of Research and Analysis in Coffee, LAPC, of the Federal Institute of Espírito Santo, Venda Nova do Imigrante campus, and the cupping was performed by 06 Q-Graders, according to the method proposed by (Pereira et al., 2018). Five cups of each coffee sample were prepared. To analyse uniformity, the samples were prepared with a proportion of 8.25 g of ground coffee to 150 mL of water at 94–95 °C. The sensory parameters of the coffee consisted of different attributes: fragrance/aroma, flavour, acidity, sweetness, balance,

aftertaste, mouthfeel, body, uniformity, clean cup, defects and overall.

2.8. Multivariate analysis of data

MATLAB software was used for principal component analysis (PCA). Self-escalation, centralization and multiplicative scattering correction (MSC) were used in the data treatment to aid in the extraction of chemical information. In addition, the correlation between the properties used in the construction of the PCA model with the sensory note and maturation properties was analysed.

3. Results

3.1. Analysis of trigonelline, caffeine and carbohydrates by (+)ESI-HRMS

Green coffee bean extracts from three species of Arabica (Catuai 81, Obata, and Topazio), three Conilon genotypes (Diamante 101, 105, 108) and Robusta Tropical at three different stages of maturity each (60%, 80%, 100%) were analysed by (+)ESI-HRMS. The coffee metabolites were identified by MS/MS via comparison of the experimental *m/z* values with the theoretical *m/z* values from previous works with coffee (Alonso-Salces, Claude, & Berrueta, 2009; Clifford, Johnston, Knigh, & Kuhnert, 2003; Rodrigues & Bragagnolo, 2013), and the mass accuracy ranged between 0.2 and 3.8 ppm, as shown in Table 1.

A semi-quantitative (+)ESI-HRMS analysis of the metabolites was performed by spiking caffeine-D9 into the green coffee bean extracts to compare the changes in the responses (Table 2). Although basically the same set of ions was detected in all coffee species, the distinction between the studied coffee cultivars was achieved due to significant differences in the relative abundance of the ions. Trigonelline (and its potassium adduct), caffeine and sucrose (sodium and potassium adducts) were present in all 21 samples (supplementary material).

The peaks with *m/z* 138.05547 and *m/z* 176.01166 were identified as trigonelline and its potassium adduct, respectively. The sum of the concentration ($[M+H]^+ + [M+K]^+$ of the mentioned metabolites ranged from 21.9 to 61.4 μmol/L. Higher concentrations were observed in the Arabica samples and in genotypes Diamante 105 and 108. In contrast, genotype Diamante 101 presented the lowest concentrations, as observed in Robusta Tropical.

Caffeine was the other identified ion (*m/z* 195.08840) within a range from 7.7 to 48.5 μmol/L. In this study, the highest caffeine concentrations were found in genotypes Diamante 105 and 108 at 41.7 – 48.5 μmol/L and 36.6 – 47.2 μmol/L, respectively, which were higher than in the Robusta Tropical and Arabica coffees (Catuai, Obata, Topazio) (Table 2). Genotype Diamante 101 more closely resembles Arabica species based on caffeine content.

The carbohydrate metabolites are also present in high amounts in green coffee beans. The ions *m/z* 365.10739 and *m/z* 381.08034 were identified by MS/MS experiments and literature comparison as the $[M+Na]^+$ and $[M+K]^+$ adducts of sucrose, and the same was observed for the sodium adduct of glucose *m/z* 203.05375 $[M+Na]^+$ and the potassium adduct *m/z* 219.02778 $[M+K]^+$ (Garrett, Rezende, & Ifa, 2013, 2016). Sucrose content was found in all coffee cultivars within a range of 4.2 to 12.7 μmol/L, with the highest concentration in Arabica samples and in genotypes Diamante 105 and 108. In addition to sucrose, glucose was also present in the green coffee beans, but at a much lower concentration (0.1 – 3 μmol/L).

3.2. Analysis of malic acid, phenolic acids and chlorogenic acids by (−) ESI-HRMS

Green coffee bean extracts from three species of Arabica (Catuai 81, Obata, and Topazio), three Conilon genotypes (Diamante 101, 105, 108) and Robusta Tropical at three different stages of maturity (60%, 80%, 100%) were also analysed by (−)ESI-HRMS. The major constituents were phenolic acids: caffeic acid (*m/z* 179.03455), quinic acid (*m/z* 191.05577) and ferulic acid (*m/z* 193.05023); many chlorogenic acids such as coumaroylquinic acid (*m/z* 337.09299), caffeoylquinic acid (*m/z* 353.08701), feruloylquinic acid (*m/z* 367.10241), dicaffeoylquinic acid (*m/z* 515.11906), and feruloylcaffeoylquinic acid (*m/z* 529.13474); and organic acids such as malic acid (*m/z* 133.01440) as listed in Table 1. Fig. 1 shows the ESI-HRMS fingerprints in negative ion mode of the Robusta Tropical 100% mature coffee fruits.

Salicylic acid (*m/z* 137.02411) was used to compare increases/decreases in the above-mentioned compounds in the genotypes Diamante 101, 105 and 108 versus Arabica (Catuai 81, Obata, Topazio) and Robusta Tropical species across three stages of maturity. The ion intensity ratios between phenolic acid/salicylic acid, chlorogenic acid/salicylic acid or malic acid/salicylic acid were used to obtain and compare the concentrations of these metabolites (μmol/L) across green coffee bean species and stages of maturity, as presented in Table 3.

Chlorogenic acids are known as the major group of phenolic

Table 1
High-resolution MS/MS characterization of green coffee bean metabolites identified in Arabica (Catuai 81, Obata, Topazio), Genotypes Diamante 101, 105 and 108 and Robusta Tropical at three stages of maturity.

Ion Mode	Compounds	Type of Ion	Molecular Formula	Exact Mass (*m/z*)	Accurate Mass (*m/z*)	Mass Error (ppm)	MS/MS Fragments (*m/z*)		
Positive							Fragments Ions		
	Trigonelline	$[M+H]^+$	$C_7H_8NO_2$	138.05550	138.05547[a]	0.2	110.06015		
		$[M+K]^+$	$C_7H_7NO_2K$	176.01139	176.01166[a]	−1.6	138.05471		
	Caffeine	$[M+H]^+$	$C_8H_{10}N_4O_2$	195.08820	195.08840[a]	−1.0	123.04293	138.06660	110.07145
	Standard- Caffeine-D9	$[M+H]^+$	$C_8H_1N_4O_2D_9$	204.14469	204.14470[a]	−0.03	126.06179	144.10418	116.10911
	Glucose	$[M+Na]^+$	$C_6H_{12}O_6Na$	203.05316	203.05375[a]	−2.9			
		$[M+K]^+$	$C_6H_{12}O_6K$	219.02710	219.02778 [a]	−3.1			
	Sucrose	$[M+Na]^+$	$C_{12}H_{22}O_{11}Na$	365.10599	365.10739 [a]	−3.8			
		$[M+K]^+$	$C_{12}H_{22}O_{11}K$	381.07992	381.08034 [a]	−1.1			
Negative									
	Caffeic acid	$[M-H]^-$	$C_9H_8O_4$	179.034435	179.03455[a]	−0.6	135.05084	105.49029	
	Quinic Acid	$[M-H]^-$	$C_7H_{12}O_6$	191.055565	191.05577[a]	−1.1	110.02035	173.04821	132.42753
	Ferulic Acid	$[M-H]^-$	$C_{10}H_{10}O_4$	193.050085	193.05023[b]	−0.7	134.04546	161.02859	136.09716
	Coumaroylquinic acid	$[M-H]^-$	$C_{16}H_{18}O_8$	337.092345	337.09299[a]	−1.9	191.05560	173.04779	163.04378
	Caffeoylquinic acid	$[M-H]^-$	$C_{16}H_{18}O_9$	353.087260	353.08701[a]	0.7	191.05551	135.05296	179.03640
	Feruloylquinic acid	$[M-H]^-$	$C_{17}H_{20}O_9$	367.102910	367.10241[a]	1.4	191.05552	173.04771	134.04515
	Dicaffeoylquinic acid	$[M-H]^-$	$C_{25}H_{24}O_{12}$	515.118955	515.11906[a]	−0.2	173.04774	179.03630	191.05560
	Feruloylcaffeoylquinic acid	$[M-H]^-$	$C_{26}H_{26}O_{12}$	529.134605	529.13474[a]	−0.2	173.02081		
	Standard - Salicylic Acid	$[M-H]^-$	$C_7H_6O_3$	137.023870	137.02411[a]	−1.7			

[a] MS/MS data reported from Robusta Tropical at 100% maturity.
[b] MS/MS data reported from Arabica Obata at 100% maturity. High-resolution MS/MS by HCD using a collision energy of 100 arbitrary units.

Table 2
Caffeine, trigonelline and sugar contents (μmol/L) in different coffee species by (+)ESI-HRMS.

Coffee	Caffeine-D9 [C-D9] in Extract	Caffeine	Trigonelline [M+H] + [M+K] Sum	Glucose $[M+Na]^+$ + $[M+K]^+$ Sum	Sucrose $[M+Na]^+$ + $[M+K]^+$ Sum	Total Sugar*	Final score#
AC.60	48.7	23.7	59.2	0.1	8.4	8.5	75
AC.80	48.7	20.6	51.0	1.5	11.6	13.1	78
AC.100	48.7	19.1	48.9	0.8	12.5	13.3	78
AO.60	48.7	23.5	55.5	0.5	12.7	13.2	32
AO.80	48.7	16.3	39.0	1.3	8.1	9.4	76
AO.100	48.7	7.7	21.9	0.3	4.2	4.6	81
AT.60	48.7	20.8	47.5	0.2	7.8	8.0	68
AT.80	48.7	18.5	52.0	1.3	10.4	11.7	76
AT.100	48.7	19.6	52.3	1.0	11.7	12.7	78
C101.60	48.7	18.8	29.6	0.3	7.0	7.3	49
C101.80	48.7	25.4	33.8	3.0	7.2	10.3	73
C101.100	48.7	26.1	46.3	1.6	8.5	10.2	77
C105.60	48.7	41.7	53.1	0.2	8.8	9.0	77
C105.80	48.7	41.8	48.9	0.8	8.4	9.2	73
C105.100	48.7	48.5	50.7	1.3	8.1	9.5	75
C108.60	48.7	43.8	55.2	0.4	11.7	12.1	77
C108.80	48.7	36.6	39.8	1.1	11.0	12.1	82
C108.100	48.7	47.2	61.4	1.7	14.7	16.4	81
RT.60	48.7	26.0	33.1	0.2	6.4	6.6	77
RT.80	48.7	34.7	40.6	0.3	7.5	7.8	79
RT.100	48.7	36.3	43.4	0.9	7.2	8.1	76

* Sum of the total sugar content (glucose + sucrose).
\# Final score by the sensory analysis.

compounds in green coffee beans. The total amount of chlorogenic acids (CGAs) in the present work was characterized by the majority presence of caffeoylquinic acid (3.41–13.59 μmol/L) and the presence of other chlorogenic acids, such as feruloylquinic acid (0.52–4.96 μmol/L), coumaroylquinic acid (0.01–0.12 μmol/L), dicaffeoylquinic acid (0.05–0.47 μmol/L) and feruloylcaffeoylquinic acid (0.0–0.19 μmol/L), as shown in Table 3. Robusta species showed a total content of chlorogenic acids between 11.67 and 18.65 μmol/L, while Arabica (Catuai 81, Obata and Topazio) presented a range of 4.02–10.38 μmol/L. Regarding the studied genotypes, the total amount of chlorogenic acids in genotype Diamante 101 (6.32–8.59 μmol/L) resembled that of the Arabica species, while genotypes Diamante 105 (15.66–18.11 μmol/L) and Diamante 108 (11.21–14.64 μmol/L) more closely resembled that of Robusta Tropical.

Concerning the principal chlorogenic acids, the highest caffeoylquinic acid concentration was found in Robusta Tropical (8.4–13.6 μmol/L), genotypes Diamante 105 (11.8–13.5 μmol/L) and Diamante 108 (7.4–9.3 μmol/L), and increased across the stages of maturity. Compared to the Arabica species (approximately 4.5 μmol/L), genotype Diamante 105 and Robusta Tropical contained nearly three times as much caffeoylquinic acid. However, genotype Diamante 101 (6.3 μmol/L) contained a slightly higher caffeoylquinic acid concentration than the Arabica species. Like caffeoylquinic acid, feruloylquinic acid concentrations were lower in all Arabica species (0.5–1.3 μmol/L) and higher in Robusta Tropical, genotypes Diamante 105 and 108. Once again, feruloylquinic acid remained low in genotype Diamante 101, similar to the Arabica species (Table 3).

Other metabolites, such as caffeic acid, ferulic acid, coumaroylquinic acid, dicaffeoylquinic acid and feruloylcaffeoylquinic acid, were observed at low and constant concentrations across green coffee bean species at all stages of maturity.

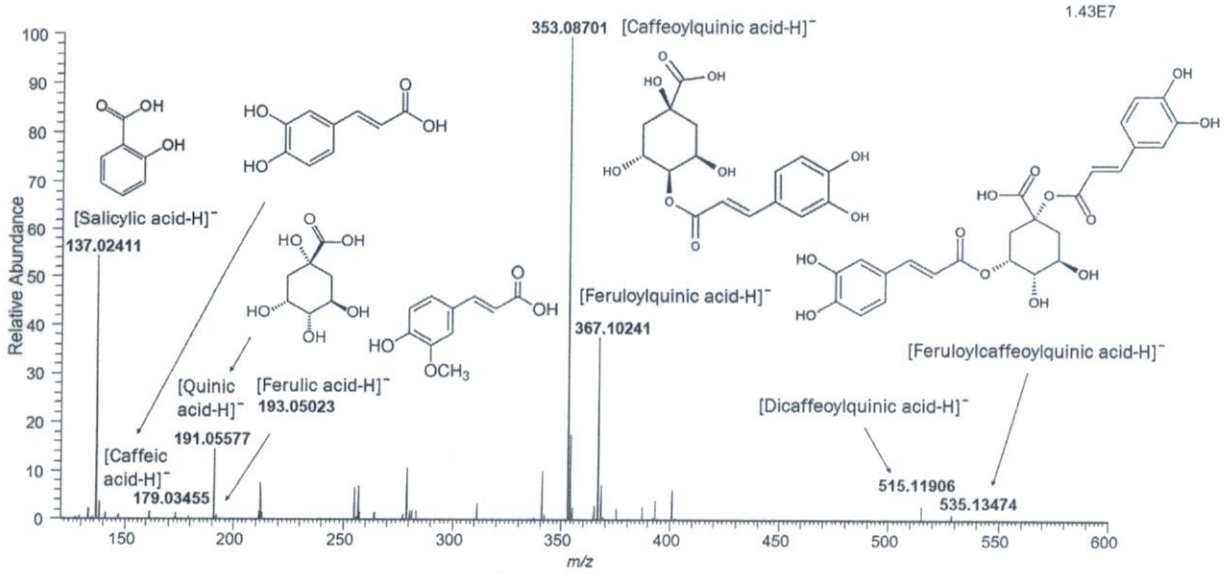

Fig. 1. Phenolic and chlorogenic acids in negative ion mode. HRMS LTQ-Orbitrap spectrum of Robusta Tropical at 100% maturity.

Table 3
Chlorogenic acid and phenolic acid contents (μmol/L) in different coffee species by (−)ESI-HRMS.

Coffee	Salicylic Acid	Malic acid	Caffeic acid	Quinic acid	Ferulic acid	Coumaroyl quinic acid	Caffeoyl quinic acid	Feruloyl quinic acid	Dicaffeoyl quinic acid	Feruloyl Caffeoyl quinic acid	Total CGA*
AC.60	14.6	0.05	0.01	1.68	0.09	0.01	3.41	0.54	0.15	0.00	4.11
AC.80	14.6	0.23	0.01	1.36	0.01	0.01	3.42	0.52	0.07	0.00	4.02
AC.100	14.6	0.56	0.01	1.60	0.00	0.01	4.60	0.65	0.09	0.00	5.37
AO.60	14.6	0.31	0.01	1.58	0.02	0.01	4.85	0.81	0.09	0.00	5.76
AO.80	14.6	0.56	0.01	1.73	0.05	0.02	4.95	0.85	0.07	0.00	5.89
AO.100	14.6	0.35	0.01	1.32	0.02	0.01	4.09	0.66	0.06	0.00	4.82
AT.60	14.6	1.05	0.04	2.72	0.04	0.12	8.80	1.33	0.13	0.00	10.38
AT.80	14.6	0.65	0.05	2.62	0.06	0.12	8.39	1.21	0.11	0.00	9.84
AT.100	14.6	0.47	0.01	1.43	0.02	0.05	4.85	0.71	0.05	0.00	5.65
C101.60	14.6	0.13	0.02	1.00	0.01	0.02	6.10	1.70	0.12	0.04	7.97
C101.80	14.6	0.14	0.12	0.85	0.02	0.01	4.81	1.37	0.09	0.03	6.32
C101.100	14.6	0.14	0.02	1.12	0.06	0.01	6.29	2.13	0.11	0.05	8.59
C105.60	14.6	0.42	0.28	1.28	0.01	0.05	11.78	3.36	0.37	0.10	15.66
C105.80	14.6	0.35	0.28	1.60	0.02	0.09	13.52	3.87	0.47	0.15	18.11
C105.100	14.6	0.34	0.25	1.42	0.01	0.06	12.97	3.86	0.46	0.16	17.52
C108.60	14.6	0.36	0.04	1.65	0.17	0.01	9.27	4.96	0.21	0.19	14.64
C108.80	14.6	0.23	0.03	1.22	0.01	0.01	7.40	3.52	0.18	0.11	11.21
C108.100	14.6	0.37	0.06	1.62	0.10	0.01	8.96	4.60	0.19	0.16	13.92
RT.60	14.6	0.15	0.20	0.88	0.00	0.02	8.41	2.96	0.21	0.07	11.67
RT.80	14.6	0.11	0.17	1.03	0.00	0.04	10.63	3.47	0.32	0.12	14.58
RT.100	14.6	0.14	0.28	1.44	0.01	0.08	13.59	4.36	0.44	0.18	18.65

CGA: chlorogenic acids.
* Sum of the content of chlorogenic acids (Coumaroylquinic Acid + Caffeoylquinic Acid + Feruloylquinic Acid + Dicaffeoylquinic Acid + Feruloylcaffeoylquinic Acid).

3.3. Comparison of maturation levels

The present study investigated the relative increases and decreases of metabolites influenced by the harvest time of the coffee fruit. The collection was performed across three stages of bean ripeness: 60%, 80% and 100% mature coffee fruits.

The concentration of trigonelline in the Arabica Catuai 81 and Obata varieties decreased from the 60% mature beans to the 100% mature beans. In contrast, trigonelline increased in Arabica Topazio, genotype Diamante 101 and Robusta Tropical (Table 2). Additionally, it was noticed that in genotypes Diamante 101, 105, 108 and Robusta Tropical, the caffeine content increased across the stages of maturity. However, in Arabica species (Catuai 81, Topazio and Obata), the caffeine content decreased or remained constant throughout the stages of maturity, with concentrations ranging from 23.7 to 19.1 μmol/L, 23.5–7.7 μmol/L and 20.8–18.5 μmol/L, respectively (Table 2). Carbohydrate content showed significant alterations during coffee maturity. Sucrose content increased in Arabica Catuai 81, Arabica Topazio, genotype Diamante 101 and genotype Diamante 108 throughout the maturation time. Nonetheless, it was observed that sucrose decreased in Arabica Obata, genotype Diamante 105 and Robusta Tropical (Table 2).

A decrease in the malic acid content was observed in Arabica Obata and Arabica Topazio, while an increase was observed in Arabica Catuai 81 from 60% to 100% maturity. In contrast, in genotypes Diamante 101, 105, 108 and Robusta Tropical, the malic acid concentration remained constant throughout all stages of maturity (Table 3).

Among the evaluated phenolic and chlorogenic acids, only quinic acid, caffeoylquinic acid and feruloylquinic acid were influenced by the maturation time, and these results are presented in Table 3. Quinic acid increased from 0.9 to 1.4 μmol/L in Robusta Tropical and in Arabica Obata, whereas quinic acid decreased from 2.7 to 1.4 μmol/L as the bean matured in Arabica Topazio. On the other hand, quinic acid remained constant throughout all stages of maturity in Arabica Catuai 81, Arabica Topazio, and genotypes Diamante 101, 105 and 108.

The caffeoylquinic acid content in Arabica Catuai 81, Topazio, Obata and in genotype Diamante 101 was found to be the lowest among all species across all stages of maturity. It was also observed that in Arabica Obata and Topazio, caffeoylquinic acid decreased across all stages of maturity and slightly increased in Arabica Catuai 81. The caffeoylquinic acid concentration in genotype Diamante 101 (6.1–6.3 μmol/L) and genotype Diamante 108 (9.3–9.0 μmol/L) remained constant throughout the stages of maturity, while in genotype Diamante 105 (11.8–13 μmol/L) and Robusta Tropical (8.4–13.6 μmol/L), a slight increase was observed as the bean ripened.

Similar to caffeoylquinic acid, feruloylquinic acid concentrations were the lowest in all Arabica species, Arabica Catuai 81, Obata and Topazio contained 0.5–0.7 μmol/L, 0.7–0.8 μmol/L and 1.3–0.7 μmol/L, respectively (Table 3). It was also observed that in Arabica Catuai 81 and Topazio, feruloylquinic acid remained constant across all stages of maturity and slightly decreased in Arabica Obata. Genotype Diamante 101 contained slightly higher feruloylquinic acid content at 1.7–2.1 μmol/L across all stages of maturity compared to the Arabica species. On the other hand, higher feruloylquinic acid concentrations of 3.0–4.4 μmol/L were obtained for Robusta Tropical and it was also observed that this metabolite increased in concentration as the bean matured. Regarding genotypes Diamante 105 and 108, feruloylquinic acid remained constant across all stages of maturity.

3.4. Sensory analysis

The sensory analysis of the 21 coffee samples was performed by certificated cuppers, and sensory testing was performed to determine the sensory differences between the coffee samples and to describe their flavours. The quality of specific flavour attributes was analysed, and the samples were rated on a numeric scale. The final score was calculated by first summing the individual scores given for each of the primary attributes (fragrance/aroma, flavour, aftertaste, acidity, body, balance, uniformity, clean cup, sweetness, mouthfeel and overall) and then the defects were subtracted from the attributes (data not shown). Coffees that received higher scores should be noticeably better than coffees that received lower scores, and according to the final score, coffees were classified as outstanding (90–100), excellent (85–89.99), very good (80–84.99) and below specialty quality (< 80.0).

Table 2 presents the final scores for each sample. The final sensory scores of the different species were very similar; most of the species were classified as below specialty quality (32–79), except for genotype

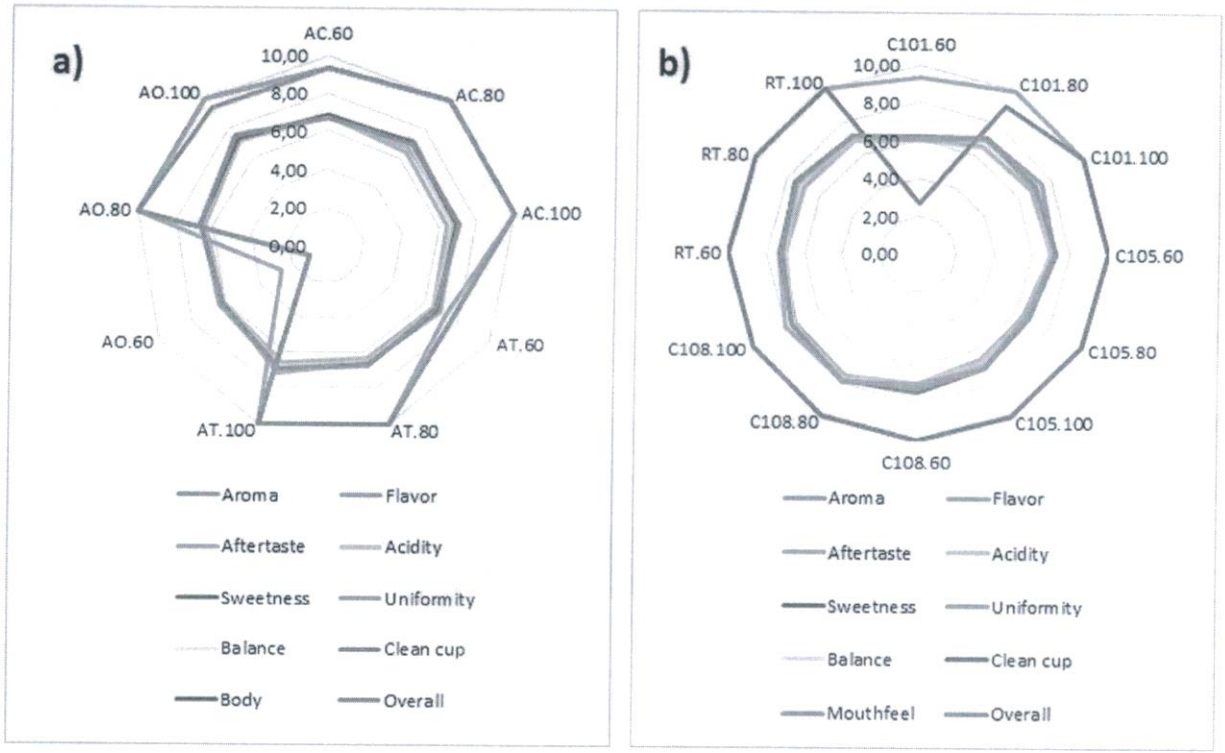

Fig. 2. (A) Numeric scale of the specific flavour attributes of *Coffea arabica* species and (B) Numeric scale of the specific flavour attributes of *Coffea canephora* species.

Diamante 108 80%, genotype Diamante 108 100% and Arabica Obata 100%, which showed scores of 82, 81 and 81, respectively, and were all classified as very good coffees.

Fig. 2 shows the results of the individual parameters classified on a numeric scale that ranged from a minimum value of 0 to a maximum value of 10 points. Most of the analysed attributes received a score between 6.00 and 7.92, which justified the overlapping data, except for sweetness, uniformity and clean cup, which ranged from 1.00 to 10.00.

Fig. 2A shows the sensory score for the individual attributes of the *Coffea Arabica* samples. Arabica Obatã 100% received the highest score for all attributes analysed, while Arabica Obatã 60% received the lowest score. According to the cuppers, Arabica Obatã 100% presented caramel and sweet flavours, while Arabica Obatã 60% presented unpleasant flavours, such as those of straw, green and astringent. Fig. 2B represents the sensory score for the individual attributes of *Coffea canephora* samples. Diamante 108 80% and 100% received the highest scores for all attributes analysed. Diamante 108 80% was described as having honey, caramel and fruity notes, and Diamante 108 100% was described as having different flavours, such as woody, fruity, sweet and liquor. In contrast, genotype Diamante 101 60% received the lowest score for all attributes and had the bad tastes of land, phenolic, and old.

The results clearly showed that the sensory scores were influenced by the maturation time. Although basically all of the coffees were classified as below specialty quality, the coffees harvested with 60% mature green coffee beans showed the lowest scores and the highest prevalence of defects, highlighting Arabica Obatã, Arabica Topázio and Genotype Diamante 101, which presented scores of 32, 68 and 49, respectively. This suggests that beverages prepared with a great quantity of immature coffee beans might provide a low cup quality.

4. Discussion

Coffee is a complex natural product that contains over 1000 compounds that are responsible for its pleasant aroma and flavour. It is known that coffee undergoes several steps (postharvest processing, roasting, storage, transportation and seed maturation) to reach its human destination, and, in addition to all these steps, genetic factors also have an impact on its chemical composition (Abreu, Borém, Oliveira, Almeida, & Carvalho Alves, 2019; Souza et al., 2019; Wintgens, 2012).

Coffea arabica and *Coffea canephora* are genetically different species for the chemical composition and quality. While Arabica is known to have a higher sensory quality, Robusta/Conilon presents a lower quality, with strong and pronounced bitterness (Bertrand, Guyot, Anthony, & Lasherme, 2003). Due to the complex chemical composition of coffee beans, different metabolites (caffeine, trigonelline, carbohydrates, chlorogenic and phenolic acids) were analysed to contribute to the characterization of new coffee genotypes and then to correlate the metabolites with the quality of the beverage.

Trigonelline and carbohydrates are present in coffee beans, and both favour good coffee quality. In addition to contributing to sweetness, sugars contribute to the colour and aroma formation of the coffee brew (da Rosa, Freitas-Silva, Rouws, & da Moreira, 2016). Previous findings make it clear that Arabica species have more trigonelline and sugars than Robusta (Ayed et al., 2019; Ciaramelli, Palmioli, & Airoldi, 2019; Tran, Lee, Furtado, Smyth, & Henry, 2016), and this trend was also noticed in the observed results. However, these metabolites were found with the highest concentrations in genotype Diamante 108 harvested with 100% mature cherry fruit, which were even higher when compared to the Arabica species.

Another relevant group that may influence the quality of coffee is the chlorogenic acids. These metabolites are important markers to differentiate Arabica and Robusta species, and the literature makes it clear that Robusta has higher concentrations than Arabica beans (Alonso-Salces, Serra, Reniero, & Héberger, 2009; Ciaramelli et al., 2019; Ky et al., 2001). Previous studies have investigated the metabolic profile of Robusta coffee silverskin, revealing nearly six times more chlorogenic acids than Arabica (Panusa, Petrucci, Lavecchia, & Zuorro, 2017). These observations are in agreement with the present work, since the highest concentration was found in Robusta Tropical, followed by genotype Diamante 105 and genotype Diamante 108, increasing across the stages of maturity. Compared to Arabica species,

genotype Diamante 105 and Robusta Tropical contained nearly three times as much caffeoylquinic acid.

Although many studies have often associated high levels of chlorogenic acids with a low-quality coffee beverage (Farah, Monteiro, Calado, Franca, & Trugo, 2006), it is possible to suggest that different concentrations of chlorogenic acids in green coffee beans can generate coffees with good sensory qualities, affirming that cup quality is associated not only with one specific metabolite but also with possible interactions between the metabolites (Scholz, Kitzberger, Prudencio, & Silva, 2018).

Similar to the chlorogenic acids, caffeine content is also higher in Robusta species than Arabica beans (Caporaso, Whitworth, Grebby, & Fisk, 2018), and both metabolites are related to the astringency and bitterness in coffee brews (Farah et al., 2006). The highest caffeine concentration was found in genotypes Diamante 105 and 108, increasing across the stages of maturity, and reaching a level that was nearly twice as high as Robusta and Arabica. On the other hand, genotype Diamante 101 contained comparable caffeine concentrations to Arabica species.

Acidity is also an important attribute of coffee quality in combination with the sweetness, bitterness and aroma profile (da Rosa et al., 2016). A previous study has shown that coffee fruits contain malic acid, citric acid, quinic acid and tartaric acid. Together, these organic acids influence coffee fruit acidity, which favours the quality of the beverage (Kitzberger, Scholz, Pereira, & de Benassi, 2013). In the present study, only malic and quinic acid were identified in green coffee beans collected at the different maturation stages. The malic acid content changed between the species and studied genotypes, with higher concentrations in Arabica species than in Robusta Tropical. Compared with the new genotypes, Diamante 105 and 108 were similar to Arabica species, while genotype Diamante 101 was similar to Robusta Tropical. Quinic acid content did not change between the species and studied genotypes.

Regarding the results of the above mentioned metabolites, curiously, genotype Diamante 108 showed the highest quality cupping score, even higher when compared to the Arabica species. This elevated final score tended to be related to its metabolic profile: the highest concentrations of trigonelline, sugars, and caffeine and considerable levels of chlorogenic and malic acids. Certainly, trigonelline and sugars contributed to the sweetness and aroma profile of genotype Diamante 108, but more importantly, although caffeine and chlorogenic acid contribute to the bitter and astringent taste in coffee, the high concentrations of these metabolites present in genotype Diamante 108 did not negatively affect the quality of the beverage. These results may suggest that the sensory information was positively influenced by the combination or interaction of the metabolites present in the coffee beans. However, to understand the complexity of coffee flavour, the sensory information of coffee aroma properties alone is not enough to explain what is causing specific sensory attributes. Similarly, compositional data alone is not enough to explain the importance of the key compounds either, or importantly, the nature of their contribution to the coffee flavour.

To better understand the correlation between sensory analysis and chemical composition data and the influence of maturation time, the present research used a multivariate data analysis tool. PCA was applied using a matrix of 21 samples and 17 variables. The score plot is shown in Fig. 3A: PC1 explained 38.09% and PC2 explained 20.57% of the total data variation, summing up to 59% total. The coffee species tended to group according to the similarity in their composition, and separation was achieved with the use of PC1: Arabica coffees and genotypes Diamante 101 were distributed in the PC1 > 1 area, while Robusta Tropical and genotypes Diamante 105 and 108 were distributed in PC1 < 1. In Fig. 3A, no clear trends between the degrees of ripeness were observed, but it is possible to affirm that the maturation time affected the composition of the coffee samples, since one coffee between the three levels of maturation was always an outlier from the

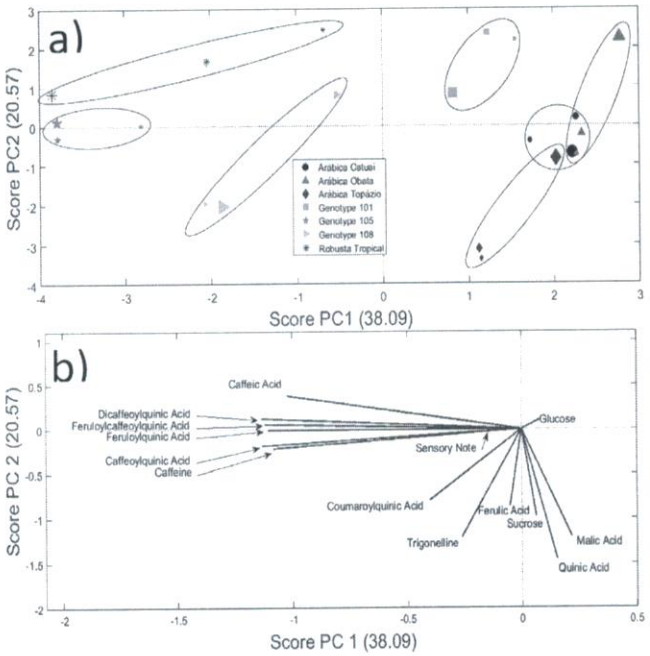

Fig. 3. (A) Score plots where the marks are in maturity ratios of 60, 80 and 100; and (B) Loading plots for different types of coffee beans.

others.

Fig. 3B shows the loadings plot, and the variables selected as being the most important for the separation of the coffee species were caffeoylquinic acid, dicaffeoylquinic acid, feruloylcaffeoylquinic acid, quinic acid, trigonelline and malic acid. The sensory note is almost completely perpendicular to PC2 and is overlapped by feruloylcaffeoylquinic acid. It was noted that quinic acid was the variable with the greatest influence on PC2, while caffeoylquinic acid and dicaffeoylquinic acid had the same effect on PC1. In this way, these latter two compounds are positively correlated with the grouping of Robusta Tropical and genotypes Diamante 105 and 108; that is, these coffee beans presented high concentrations of these metabolites.

To extract possible correlations between the chemical and the sensory profiles and the correlation between the maturation time, the various data sets were analysed (supplementary material). For the chemical and sensory profiles, it was observed that the variables did not influence systematically. Feruloylquinic acid had the highest coefficient (0.2609), followed by feruloylcaffeoylquinic acid (0.2553) and glucose (0.2060). Regarding the correlation between the chemical profile and maturation time, glucose (0.4805) and sensory note (0.4681) had the highest coefficient.

Despite the low correlations found in this work, it is important to note that the chemical analyses were carried out on green beans, whereas the sensorial analyses were conducted with roasted beans, making it clear that it is not possible to establish a correlation of sensorial quality with the chemical composition of green beans. However, it was affirmed that glucose was the metabolite with the highest influence on maturation, since it increased in mature beans in almost all samples. Similarly, the sensory note was correlated with the maturation time since the coffees harvested with 60% mature coffee beans received the lowest scores, and defects were identified in the sensory analyses. In contrast, the coffees with 80% or 100% mature beans showed better scores. Although caffeine contents presented very significant alterations during seed maturation, caffeine was not one of the first 5 variables used to discriminate ripeness in this research.

5. Conclusion

Genotypes 105 and 108 contained higher trigonelline, sugars and caffeine content and considerable levels of chlorogenic and malic acids when compared to Arabica (Catuai, Obata, Topazio) and Robusta species. The PCA showed a grouping of the coffees according to the similarity of the chemical composition, indicating that the variables with the highest influence on this differentiation were acids and trigonelline. It was observed that the variables studied did not influence the sensorial note or maturation time in a systematically relevant way, showing uncorrelated data. This low correlation can be explained by the sensorial analyses performed using roasted beans and the chemical analyses of green beans. However, it is important to understand the relationship between the chemical composition and the sensorial profile of green coffee since this represents the largest portion of coffee exports. It is possible to affirm that maturation affected the chemical and sensory profile of coffee beans. Among the three new genotypes studied, it was possible to conclude that genotype Diamante 108 presented promising aroma and flavour characteristics, sparking interest in its national and international commercialization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.125850.

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