

Coffee brews composition from *coffea canephora* cultivars with different fruit-ripening seasons

Coffee brews
composition

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Abstract

Purpose – The purpose of this paper is to evaluate the contents of bioactive compounds and/or that of interest for the brew quality (trigonelline, caffeine, total chlorogenic acids and melanoidins), acidity and antioxidant activity (AA) of roasted coffee brews produced with *Coffea canephora*.

Design/methodology/approach – Coffee samples corresponded to three cultivars – Diamante ES8112, ES8122 “Jequitibá,” and Centenária ES8132 – with different fruit-ripening seasons (early, medium and late, respectively). The study evaluated five genotypes from each cultivar and coffees were cultivated in two sites, a total of 30 samples.

Findings – The average contents on the coffee brews varied from 1,176 to 1,452 $\mu\text{g mL}^{-1}$ for caffeine; from 206 to 413 $\mu\text{g mL}^{-1}$ for trigonelline; from 528 to 942 $\mu\text{g mL}^{-1}$ for total chlorogenic acids; from 6.8 to 7.8 mg mL^{-1} for melanoidins; showing total titratable acidity between 1.15 and 1.79 mL of NaOH 0.1 mol L^{-1} by 20 mL of the brew. AA varied from 6.78 to 8.80 mg of TROLOX mL^{-1} , correlating positively with the contents of caffeine, total chlorogenic acids, melanoidins. Fruit-ripening seasons had no effect on coffee brew composition and AA.

Originality/value – The results presented provide not only a unique analysis of coffee brew from genotypes developed to improve the good agricultural practice and brew quality, but also relevant information that can be extended for research in coffee composition and for the coffee industry.

Keywords Bioactive compounds, Centenária ES8132, Conilon, Diamante ES8112, ES8122 “Jequitibá”

Paper type Research paper



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1. Introduction

Brazil is the second-largest *Coffea canephora* producer in the world, with an estimation of 18.3m 60 kg bags for the 2019/2020 harvest (USDA, 2019). *C. canephora* is one of the main commercialized species, used directly in the production of instant coffee and blends with *Coffea arabica* for roasted coffee (Clarke and Macrae, 1985; Farah and Santos, 2015).

Coffee brew quality is associated with the content of water-soluble compounds extracted during the preparation such as caffeine and chlorogenic acids (Gloess *et al.*, 2013), aliphatic acids (Ginz *et al.*, 2000), carbohydrates degradation compounds formed during roasting and carbohydrates remaining in the brew (Leloup and Liardon, 1993; Oosterveld *et al.*, 2003). In addition to their importance to the cup quality, chlorogenic acids and its degradation products, caffeine and its metabolites and trigonelline and melanoidins have been associated with antioxidant activity (AA) and chemo-protective activity on the prevention, development, and progression of cancer (Gaascht *et al.*, 2015). They also act on cirrhosis risk reduction (Kennedy *et al.*, 2016), cardiovascular diseases prevention, Type 2 diabetes and Parkinson (Ludwig *et al.*, 2012), death by chronic diseases reduction (Freedman *et al.*, 2012) and other factors (Ding *et al.*, 2015).

The composition of beverages originated from roasted coffee depends on the coffee species and cultivars used in the blend, harvesting processes, post-harvesting, roasting and grinding processes and preparation methods (Alves *et al.*, 2009; Nilsson, 2015; Nogueira and Trugo, 2003). Thus, this study emphasizes the importance of knowing the profile of constituents of coffee brews – final consumption form of the product – with impact on the quality and/or bioactive effect.

The literature provides more information on the overall composition of *C. arabica* compared to *C. canephora*, and data availability on brews composition is still scarce. In general, *C. canephora* brews are characterized by low acidity and strong body, and by a lower sensory quality compared to *C. arabica* (Clarke and Macrae, 1985; Farah and Santos, 2015). However, the high caffeine and melanoidin content associated with the high AA contributes to the great functionality of *C. canephora* brews (Vignoli *et al.*, 2014).

The *C. canephora* genetic breeding program from Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (Incaper, Espírito Santo, Brazil) developed a wide variability of genotypes with different agronomic characteristics (Ferrão *et al.*, 2009). Nine cultivars were developed and recommended for the State of Espírito Santo, the largest grower of *canephora* in Brazil (CONAB, 2019), including eight clonal cultivars (formed by the grouping of at least nine compatible clones) and one of seed multiplication. Among clonal cultivars, Diamante ES8112, ES8122 “Jequitibá” and Centenária ES8132 stand out for showing distinct fruit-ripening seasons (early, medium and late, respectively).

Considering the limited data on *C. canephora* brew and the importance of Brazil as producer of this coffee species, this research aimed to evaluate the content of bioactive compounds and/or that of quality interest (trigonelline, caffeine, total chlorogenic acids and melanoidins), acidity and AA in coffee brews of 15 *C. canephora* genotypes. The study used five genotypes from each cultivar (Diamante ES8112, ES8122 “Jequitibá” and Centenária ES8132) grown at two sites in the State of Espírito Santo.

2. Materials and methods

2.1 Genetic material and preparation

In total, 15 genetic materials (genotypes) from *C. canephora* originated from the breeding program of INCAPER (Espírito Santo, Brazil) were studied. These genotypes are agronomic divergent and are correspondent to three clonal cultivars that have distinct fruit-ripening seasons. Five of the nine genotypes were studied for each clonal cultivar: Diamante ES8112 – early-maturing (genotypes 101E, 103E, 105E, 106E, and 108E), ES8122 “Jequitibá” – medium-maturing (genotypes 201M, 202M, 203M, 207M and 209M) and Centenária

ES8132 – late-maturing (genotypes 301L, 302L, 303L, 306L and 307L). Thus, the material studied allows obtaining data representative of the genetic variability of *C. canephora* coffee in the State of Espírito Santo, the largest producer of this coffee species in Brazil.

Cherry stage fruits were collected (500 g of each genotype), in the year 2014 (May, June and July), from demonstration crops grown at 36 months under two distinct experimental conditions in Espírito Santo State (Table I). Coffees were naturally sun-dried and processed; non-defective and 16 (6.5 mm) sieve-size beans (Brasil, 2003) were selected. The green beans were stored in plastic bags at room temperature until roasting.

Coffees (100 g) were roasted in a Rod Bel (Rod-Bel, São Paulo, Brazil) gas pilot roaster at temperatures from 210°C to 230°C for 17 to 29 min (Mendes *et al.*, 2001). Differences in process time were due to diversity in the size and characteristics of each coffee. The degree of roasting was standardized to achieve a weight loss of $16.5 \pm 0.4 \text{ g } 100 \text{ g}^{-1}$, described by (Mendes *et al.*, 2001) as an optimal roasting degree for *C. canephora*.

Samples were grounded using a Burr bench grinder GXV 2 (Krupps, Shanghai, China) at a fine granulometry (70 percent retained in sieve size 0.60 mm, and 30 percent passing in sieve size 0.60 mm – pan), according to the Brazilian Coffee Roasters Association (ABIC) (ABIC, 2018). Roasted and grounded coffees had L^* of 25.3 ± 1.4 , a^* of 8.2 ± 0.5 , b^* of 10.6 ± 1.9 ; analyses were performed using a Minolta CR-410 colorimeter (Konica Minolta Sensing Inc., Osaka, Japan) with standard illuminant C and 10° observer. Average moisture of $0.8 \pm 0.1 \text{ g } 100 \text{ g}^{-1}$ was observed; the analyses were performed using a gravimetric moisture analyzer MB 45 (Ohaus, Barueri, Brazil) with a halogen lamp. Samples were stored in plastic bags and kept under refrigeration at 8°C until the brews were prepared.

The coffee brews were prepared through percolation with Melita paper filter (Melita, Guaíba, Brazil) following the proportion of 50 g of roasted and ground coffee for 500 mL of mineral water (Ouro Fino, Campo Largo, Brazil) at 92°C, according to ABIC (2018) and Mendes *et al.* (2001). The coffee brews have an average soluble solids content of $1.7 \pm 0.2 \text{ g } 100 \text{ mL}^{-1}$. The analyses were carried out according to AOAC – Official Methods of Analysis of AOAC International (2003) and Sivetz and Desrosier (1979), using a refractometer Atago PAL-3 (Atago, Tokio, Japan) The brews were stored in plastic bottles of 100 mL (Inplavel, Joinville, Brazil) at 18°C until analyses.

2.2 Caffeine, trigonelline, 5-caffeoylquinic acid (5-CQA) and total chlorogenic acids determination

Caffeine, trigonelline, and 5-CQA were determined by high-performance liquid chromatographic based on Corso *et al.* (2016).

One milliliter of the coffee brew was diluted to five milliliters using ultrapure water Purelab Option-Q (Elga, High Wycombe, UK) and the solution was filtered at $0.45 \mu\text{m}$ nylon filter membrane (Millipore, São Paulo, Brazil). An aliquot of $20 \mu\text{L}$ was injected into the liquid chromatograph ThermoFischer® – Dionex (Dionex, Sunnyvale, CA, USA) equipped with an automatic sample injector, quaternary pump, column oven and diode array detector, controlled by

Experimental farm of Marilândia Experimental farm of Bananal do Norte

County	Marilândia	Cachoeiro do Itapemirim
Region of Espírito Santo	Northwest	South
Latitude/Longitude	19°24'/40°31'	20°75'/41°29'
Altitude (m)	104	146
Average annual temperature (°C)	24.2	23.8
Annual rainfall (mm)	1,129	1,086
Type of soil	Red-yellow latosol	Red-yellow latosol

Table I.
Experimental conditions of crops of *Coffea canephora* genotypes grown in two sites

Chromeleon 7.0 software. A Spherisorb ODS-1 column (250 mm × 4.6 mm, 5 μm) (Waters, Milford, MA, USA), HPLC grade methanol (Fischer Scientific, Bridgewater, NJ, USA), HPLC grade acetic acid (purity ≥ 99.8 percent, Merck, Darmstadt, Germany) and chromatographic standards of caffeine, trigonelline, 5-CQA (Sigma-Aldrich, Saint Louis, MO, USA) was employed. It was used a flow rate of 1 mL·min⁻¹ of the mobile phase of acetic acid/ultrapure water (0.2:99.8 v/v) (A) and methanol (B) using the following gradient elution: 1 to 10 min, 92.5 to 80 percent of A; 10 to 12 min, 80 to 70 percent of A; 12 to 20 min, 70 to 65 percent of A; 20 – 28 min, 65 to 60 percent of A; 30 min, 92.5 percent of A. Detection was set at 260 nm for trigonelline, 272 nm for caffeine and 320 nm for chlorogenic acids. Analyses were carried out at 40°C in duplicate.

The identification of the compounds was based on retention times and UV spectra. Quantification was performed by external standardization using five-point analytical curves with triplicate measurements. The concentration ranges from 4.5 to 60.0 μg mL⁻¹ for trigonelline ($r = 0.999$, $p < 0.001$); 4.5 to 120.0 μg mL⁻¹ for caffeine ($r = 0.999$, $p < 0.001$); and 4.5 to 60.0 μg mL⁻¹ for 5-CQA ($r = 0.998$, $p < 0.001$). The results were expressed in μg·mL⁻¹ of coffee brew. The total chlorogenic acids were estimated by the sum of the compounds detected at 320 nm using the 5-CQA as a standard quantifier (Corso *et al.*, 2016).

2.3 pH and total titratable acidity determination

The pH was determined directly in coffee brews, in duplicate, using a pHmeter HI 2,212 pH/mv meter (Hanna, São Paulo, Brazil).

The total titratable acidity (TTA) was determined according to Scholz *et al.* (2013) and Buenaventura-Serrano and Castaño-Castrillón (2002). In total, 20 milliliters of coffee brews were titrated with NaOH 0.1 mol·L⁻¹ until pH 8.2. The analysis was performed in duplicate, and the results were expressed as mL de NaOH 0.1 mol·L⁻¹ in 20 mL of coffee brew.

2.4 Melanoidins estimation

Melanoidins were analyzed by diluting 400 μL of the coffee brew with 7,600 μL of ultrapure water to achieve a concentration of 5 mg of coffee mL⁻¹. Samples were read in a spectrophotometer Libra S22 (Biochrom, Cambourne, UK) at a wavelength of 420 nm (Pérez-Hernández *et al.*, 2012). The melanoidins content was estimated based on the absorptivity value of 1.1289 L g⁻¹ cm⁻¹ proposed by Tagliazucchi *et al.* (2010). A coffee brew was used as a source of melanoidins to obtain an analytical curve (six points in triplicate, $r = 1$; $p < 0.001$) in the concentration from 0.06 to 0.97 mg mL⁻¹, corresponding to an absorbance range of 0.067 to 1.095 AU. The analysis was performed in duplicate, and the results were expressed in mg of melanoidins mL⁻¹ of coffee brew.

2.5 Antioxidant activity determination

The ABTS radical scavenging activity was estimated according to Vignoli *et al.* (2014). A 2.5 mL aliquot of the coffee brew was transferred to a 50 mL flask, and the volume filled with water to a concentration of 5 mg·mL⁻¹. Briefly, ABTS radical cations (ABTS+•) were produced by reacting 7 mmol·L⁻¹ of ABTS 98 percent (Sigma-Aldrich, Saint Louis, MO, USA) stock solution with 2.45 mmol·L⁻¹ of potassium persulfate 99 percent (Merck, Darmstadt, Germany). The mixture stood in a dark flask at room temperature for 12–16 h before use. The ABTS+• solution was then diluted with phosphate buffered saline 5 mmol·L⁻¹ (pH 7.4) to an absorbance of 0.70 ± 0.2 at 730 nm. 10 μL of the sample or Trolox standard 95 percent (Sigma-Aldrich, Saint Louis, MO, USA) was added to 4 mL of the diluted ABTS+• solution and readings were taken at 730 nm in a spectrophotometer Biochrom Libra S22 (Biochrom, Cambourne, UK) after a reaction time of 6 min. A six-point analytical curve with triplicate measurements in the concentration range from 1.0 to 8.0 mmol L⁻¹ ($r = 0.999$, $p < 0.001$) in ethanol (Êxodo Científica, Hortolândia, Brazil) was

used. Samples were analyzed in duplicate, and the results were expressed as the Trolox equivalent antioxidant capacity in mg Trolox mL⁻¹ of coffee brew.

2.6 Statistical treatment

The results were submitted to ANOVA and Tukey Test ($p \leq 0.05$) using the free software SISVAR version 5.6 (Sisvar, 2016) in order to evaluate the effect of growing site and genetic variability. Growing site/Experimental farm (main plot) and genotype (subplot) treatments were considered in a split-plot design. If a significant main X subplot interaction ($p \leq 0.05$) was observed, the effect of genotype was independently studied for each experimental farm.

AA and chemical composition data were analyzed by principal component analysis using the "Multivariate Exploratory Techniques – Principal Components and Classification Analysis" procedure of the Statistics 7.1 package software (Statsoft, Inc., 2006). The composition parameters (trigonelline, total chlorogenic acids, caffeine, titratable acidity and melanoidins) were used as active variables for the derivation of the principal components; the supplementary variable (AA) was projected onto the factor space.

3. Results and discussion

Tables II and III show the contents of the alkaloids trigonelline and caffeine in coffee brews obtained from 15 different *C. canephora* genotypes in two cultivation sites. Beverages from the Diamante, Jequitibá, and Centenária cultivars showed average trigonelline contents varying from 206 to 413 $\mu\text{g}\cdot\text{mL}^{-1}$ (Table II) and caffeine contents, from 1,176 to 1,452 $\mu\text{g}\cdot\text{mL}^{-1}$ (Table III).

Caffeine contents were similar to those described for *C. canephora* brews originated from Vietnam and prepared in a 0.6:10 coffee:water ratio (1,153 $\mu\text{g}\cdot\text{mL}^{-1}$) (Ludwig *et al.*, 2012). However, these values (Table II) were higher than those reported by Rodrigues *et al.* (2007) (between 314 and 762 $\mu\text{g}\cdot\text{mL}^{-1}$), for *C. canephora* brews originated from different countries and prepared in a 0.7:10 coffee:water ratio.

Cultivar	Genotypes	Site/experimental farm	
		Marilândia	Bananal do Nortea
Diamante (early maturing)	101E	178 ^{Bi} ± 25	297 ^{Ad} ± 2
	103E	378 ^{Ade} ± 26	257 ^{Bdef} ± 9
	105E	370 ^{Ade} ± 8	181 ^{Bfgh} ± 16
	106E	410 ^{Acd} ± 19	421 ^{Abc} ± 19
	108E	469 ^{Abc} ± 58	379 ^{Bc} ± 19
	Mean** ± SD (CV%)	361 ± 109 (39)	307 ± 96 (31)
Jequitibá (medium maturing)	201M	330 ^{Aef} ± 1	145 ^{Bh} ± 16
	202M	261 ^{Afgh} ± 32	234 ^{Adefg} ± 5
	203M	583 ^{Aa} ± 44	280 ^{Bde} ± 13
	207M	333 ^{Adef} ± 21	160 ^{Bgh} ± 37
	209M	328 ^{Aef} ± 18	210 ^{Befgh} ± 2
	Mean** ± SD (CV%)	367 ± 125 (34)	206 ± 55 (27)
Centenária (late maturing)	301L	241 ^{Aghi} ± 5	143 ^{Bh} ± 13
	302L	209 ^{Bhi} ± 7	489 ^{Aab} ± 61
	303L	528 ^{Aab} ± 47	427 ^{Bbc} ± 11
	306L	315 ^{Befg} ± 9	535 ^{Aa} ± 17
	307L	286 ^{Bfg} ± 1	473 ^{Aab} ± 49
	Mean** ± SD (CV%)	316 ± 126 (40)	413 ± 155 (38)

Notes: *Mean (duplicates) ± SD (standard deviation) for each genotype. **Average content for each cultivar ± SD (standard deviation) and CV (coefficient of variation) between genotypes of the same cultivar. Means followed by the same capital letter in the same line show no significant difference between growing sites (Tukey, $p \leq 0.05$). Means followed by the same lower case letter in the same column show no significant difference between genotypes (Tukey, $p \leq 0.05$)

Table II. Trigonelline content* ($\mu\text{g}\cdot\text{mL}^{-1}$ of brew) in *Coffea canephora* genotypes grown in two sites

Cultivar	Genotypes	Site/experimental farm	
		Marilândia	Bananal do Norte
Diamante (early maturing)	101E	941 ^{Bd} ± 95	1,289 ^{Abcd} ± 22
	103E	1,365 ^{Aabc} ± 68	1,236 ^{Acde} ± 98
	105E	1,479 ^{Aab} ± 91	1,290 ^{Bbcd} ± 33
	106E	1,470 ^{Bab} ± 21	1,850 ^{Aa} ± 196
	108E	1,297 ^{Bbc} ± 119	1,597 ^{Bab} ± 15
	Mean** ± SD (CV%)	1,310 ± 220 (17)	1,452 ± 264 (18)
Jequitibá (medium maturing)	201M	1,311 ^{Abc} ± 32	1,152 ^{Ade} ± 4
	202M	1,397 ^{Aabc} ± 75	1,345 ^{Abcd} ± 1
	203M	1,678 ^{Aa} ± 103	1,223 ^{Bcde} ± 41
	207M	1,249 ^{Abcd} ± 102	951 ^{Be} ± 198
	209M	1,329 ^{Abc} ± 11	1,209 ^{Acde} ± 39
	Mean** ± SD (CV%)	1,393 ± 168 (12)	1,176 ± 144 (12)
Centenária (late maturing)	301L	1,515 ^{Aab} ± 63	1,130 ^{Bde} ± 67,52
	302L	1,371 ^{Abc} ± 119	1,434 ^{Abcd} ± 104,41
	303L	1,327 ^{Abc} ± 89	1,357 ^{Abcd} ± 16,24
	306L	954 ^{Bd} ± 11	1,400 ^{Abcd} ± 190
	307L	1,080 ^{Bcd} ± 49	1,501 ^{Abc} ± 99
	Mean** ± SD (CV%)	1,249 ± 228 (18)	1,364 ± 141 (10)

Table III. Caffeine content* ($\mu\text{g}\cdot\text{mL}^{-1}$ of brew) in *Coffea canephora* genotypes grown in two sites

Notes: *Mean (duplicates) ± SD (standard deviation) for each genotype. **Average content for each cultivar ± SD (standard deviation) and CV (coefficient of variation) between genotypes of the same cultivar. Means followed by the same capital letter in the same line show no significant difference between growing sites (Tukey, $p \leq 0.05$). Means followed by the same lower case letter in the same column show no significant difference between genotypes (Tukey, $p \leq 0.05$)

The average contents of the two compounds were correspondent to 7.7 g-of caffeine and 1 g-of trigonelline in 100 g⁻¹ of soluble solids. The results are in the range of those described by Vignoli *et al.* (2014) (7.2 g of caffeine and 0.2 to 2.2 g of trigonelline 100 g⁻¹ of soluble solids) for beverages originated from Brazilian *C. canephora* submitted to different roasting degrees and using the same coffee to water ratio of this study. The wide ranges on trigonelline contents found in the literature are justified by the high degradation of the trigonelline during the roasting process compared to the thermal stability of the caffeine (Dias and Benassi, 2015; Vignoli *et al.*, 2014),

There was a difference among genotypes for trigonelline and caffeine contents ($p < 0.000$) as well as an interaction between site and genotype ($p < 0.000$), showing that caffeine and trigonelline contents in each genotype were influenced by cultivation site, but this effect was genotype-dependent.

In regards to caffeine content, there was no difference among cultivation sites ($p = 0.722$). However, the difference among sites was significant for trigonelline ($p = 0.010$). In general, higher trigonelline values were observed for genotypes grown at Marilândia, being the highest value observed for the medium-maturing Jequitibá cultivar (genotype 203M) (Table II). There was also greater variability among genotypes of the same cultivar for trigonelline contents (CVs from 27 to 40 percent) than for caffeine (CVs from 10 to 18 percent) (Tables II and III).

The average contents of total chlorogenic acids for the Diamante, Jequitibá and Centenária cultivars varied from 528 to 942 $\mu\text{g}\cdot\text{mL}^{-1}$ (Table IV), corresponding to a range of 3.1 to 5.3 g·100 g⁻¹ of soluble solids.

Total chlorogenic acids contents in the brews differed among genotypes ($p < 0.000$) but not between cultivation sites ($p = 0.056$). There was an interaction between site and genotype ($p < 0.000$), showing that the site influenced the content of total chlorogenic acids in each genotype, but the effect was genotype-dependent.

Cultivar	Genotypes	Site/experimental farm	
		Marilândia	Bananal do Norte
Diamante (early maturing)	101E	455 ^{Bg} ± 46	738 ^{Ab} ± 33
	103E	919 ^{Ad} ± 75	664 ^{Bbc} ± 61
	105E	910 ^{Ad} ± 49	468 ^{Bde} ± 6
	106E	958 ^{Acd} ± 45	1,000 ^{Aa} ± 43
	108E	1,103 ^{Abc} ± 71	997 ^{Ba} ± 40
	Mean** ± SD (CV%)	869 ± 244 (28)	773 ± 228 (30)
Jequitibá (medium maturing)	201M	820 ^{Ade} ± 22	416 ^{Bde} ± 18
	202M	657 ^{Aef} ± 57	587 ^{Abcd} ± 42
	203M	1,363 ^{Aa} ± 34	721 ^{Bb} ± 13
	207M	853 ^{Ad} ± 56	400 ^{Be} ± 81
	209M	835 ^{Ade} ± 11	515 ^{Bcde} ± 21
	Mean** ± SD (CV%)	906 ± 268 (30)	528 ± 132 (25)
Centenária (late maturing)	301L	621 ^{Afg} ± 41	367 ^{Be} ± 17
	302L	625 ^{Bfg} ± 101	1,108 ^{Aa} ± 66
	303L	1,155 ^{Ab} ± 30	1,107 ^{Aa} ± 43
	306L	814 ^{Bde} ± 45	1,124 ^{Aa} ± 20
	307L	671 ^{Bef} ± 37	1,007 ^{Aa} ± 42
	Mean** ± SD (CV%)	777 ± 225 (29)	942 ± 325 (35)

Notes: *Mean (duplicates) ± SD (standard deviation) for each genotype. **Average content for each cultivar ± SD (standard deviation) and CV (coefficient of variation) between genotypes of the same cultivar. Means followed by the same capital letter in the same line show no significant difference between growing sites (Tukey, $p \leq 0.05$). Means followed by the same lower case letter in the same column show no significant difference between genotypes (Tukey, $p \leq 0.05$)

Table IV. Total chlorogenic acids content* ($\mu\text{g}\cdot\text{mL}^{-1}$ da bebida) in *Coffea canephora* genotypes grown in two sites

The coffee brew from the medium-maturing Jequitibá cultivar (203M) grown at Marilândia showed higher total chlorogenic acids content ($583 \mu\text{g}\cdot\text{mL}^{-1}$ of the brew) (Table IV). A wide range on total chlorogenic acids content among genotypes within each cultivar was observed (CVs from 25 to 35 percent) (Table IV).

There are no reports on total chlorogenic acids content in *C. canephora* brews, but there are data on the contents of 5-ACQ, the main chlorogenic acid isomer. 5-ACQ represented 31 to 40 percent of the brew's total chlorogenic acid (Tables IV and 1S), in agreement to the values (from 31 to 39 percent) reported by Perrone *et al.* (2012) for *C. canephora* under different roasting times. Average contents of 5-ACQ in the brews varied from 146 to $344 \mu\text{g}\cdot\text{mL}^{-1}$ (Table 1S, Supplementary Material), corresponding to a range of 0.9 to $2.0 \text{ g}\cdot 100 \text{ g}^{-1}$ of soluble solids. The results are in accordance with those described by Herawati *et al.* (2019) (0.54 g to $2.54 \text{ g } 100 \text{ g}^{-1}$ of soluble solids) for *C. canephora* beverages (from light to dark roasting degrees, coffee to water ratio 0.5:10). The content of 5-ACQ (Table 1S) were within the range described in the literature for *C. canephora*: from $218 \mu\text{g}\cdot\text{mL}^{-1}$ (1:10 coffee: water ratio) (Perrone *et al.*, 2012) to $529 \mu\text{g}\cdot\text{mL}^{-1}$ (0.6:10, coffee: water ratio) (Ludwig *et al.*, 2012). In addition to preparation variability, the chlorogenic acid degradation during the roasting process – more noticeable in *C. canephora* matrix (Dias and Benassi, 2015; Vignoli *et al.*, 2014) – contributes to the high variability observed in literature data.

Coffee brews from the Diamante, Jequitibá, and Centenária cultivars showed TTA from 1.15 to 1.79 mL of $\text{NaOH } 0.1 \text{ mol}\cdot\text{L}^{-1}$ by 20 mL of the brew (Table III) and average pH value of 5.75 ± 0.23 .

Scholz *et al.* (2013) described pH values of 5.12 to 5.24 and TTA of 2.73 to 3.21 mL of $\text{NaOH } 0.1 \text{ mol}\cdot\text{L}^{-1}$ by 20 mL for brews obtained from several *C. arabica* cultivars. Lowest TTA and highest pH for *C. canephora* brews have been reported in the literature (Belitz *et al.*, 2009).

The TTA of the brews differed among genotypes ($p < 0.000$) and cultivation sites ($p = 0.037$). In general, greater TTA values were observed in brews from genotypes grown

at Marilândia, the highest value (2.95 mL of NaOH 0.1 mol·L⁻¹ by 20 mL) was observed for late-maturing Centenária cultivar (302L) (Table V).

There was also an interaction between cultivation site and genotype ($p < 0.000$), showing that TTA values in each genotype were influenced by cultivation site, but this effect was genotype-dependent. The variability on TTA values among genotypes within each cultivar was quite noteworthy (CVs from 9 to 39) (Table V).

The Diamante, Jequitibá and Centenária cultivars showed melanoidin contents varying from 6.8 to 7.8 mg·mL⁻¹ of the coffee brew (Table VI), equivalent to a range of 39 to 46 g·100 g⁻¹ of soluble solids. These values are in agreement to those reported by Fogliano and Morales (2011) – from 2.5 to 8.1 mg·mL⁻¹ – for filtered brews with different in coffee to water ratio. In regards to soluble solids content, the melanoidin contents were higher than those reported by Vignoli *et al.* (2014) (from 18.50 to 27.30 g·100 g⁻¹ of soluble solids) using the same coffee to water ratio for *C. canephora* brews from coffees submitted to different roasting degrees.

It is important to highlight that, although melanoidins are the only group of compounds studied formed during processing, as the roasting process was standardized, the variation found can be attributed to the differences between genotypes and cultivation sites. Low variability (CVs from 9 to 16 percent) (Table VI), lower to that observed for most of the other compounds, reinforces the good standardization of the roasting process.

There was a difference in melanoidin content among genotypes ($p < 0.000$) but not between cultivation sites ($p = 0.076$), and interaction between site and genotype ($p < 0.000$), showing that the cultivation site influenced the content of melanoidin in each genotype, but the effect was genotype-dependent. High average melanoidin values were observed in the early-maturing Diamante cultivar, the highest value (9.71 mg·mL⁻¹) was correspondent to genotype 101E from Bananal do Norte (Table VI).

Cultivar	Genotypes	Site/experimental farm	
		Marilândia	Bananal do Norte
Diamante (early maturing)	101E	1.05 ^{Af} ± 0.07	1.55 ^{Ab} ± 0.07
	103E	1.55 ^{Accd} ± 0.07	1.05 ^{Accd} ± 0.07
	105E	1.25 ^{Aef} ± 0.07	0.85 ^{Ad} ± 0.07
	106E	1.25 ^{Aef} ± 0.07	1.65 ^{Ab} ± 0.07
	108E	1.45 ^{Ade} ± 0.07	1.75 ^{Aab} ± 0.07
	Mean** ± SD (CV%)	1.31 ± 0.19 (15)	1.37 ± 0.40 (29)
Jequitibá (medium maturing)	201M	1.95 ^{Ab} ± 0.07	1.05 ^{Bcd} ± 0.07
	202M	1.35 ^{Ade} ± 0.07	1.25 ^{Ac} ± 0.07
	203M	1.75 ^{Abc} ± 0.07	1.25 ^{Ac} ± 0.07
	207M	1.35 ^{Ade} ± 0.07	1.15 ^{Ac} ± 0.07
	209M	1.45 ^{Ade} ± 0.07	1.05 ^{Accd} ± 0.07
	Mean** ± SD (CV%)	1.57 ± 0.27 (17)	1.15 ± 0.10 (9)
Centenária (late maturing)	301L	1.25 ^{Aef} ± 0.07	1.05 ^{Accd} ± 0.07
	302L	2.95 ^{Aa} ± 0.07	1.95 ^{Ba} ± 0.07
	303L	1.95 ^{Ab} ± 0.07	1.55 ^{Ab} ± 0.07
	306L	1.45 ^{Ade} ± 0.07	1.55 ^{Ab} ± 0.07
	307L	1.35 ^{Ade} ± 0.07	1.25 ^{Ac} ± 0.07
	Mean** ± SD (CV%)	1.79 ± 0.70 (39)	1.47 ± 0.34 (23)

Table V.

Titratable acidity* (mL of NaOH 0.1 mol·L⁻¹ by 20 mL) in *Coffea canephora* genotypes grown in two sites

Notes: *Mean (duplicates) ± SD (standard deviation) for each genotype. **Average content for each cultivar ± SD (standard deviation) and CV (coefficient of variation) between genotypes of the same cultivar. Means followed by the same capital letter in the same line show no significant difference between growing sites (Tukey, $p \leq 0.05$). Means followed by the same lower case letter in the same column show no significant difference between genotypes (Tukey, $p \leq 0.05$)

Cultivar	Genotypes	Site/experimental farm	
		Marilândia	Bananal do Norte
Diamante (early maturing)	101E	5.9 ^{Bh} ± 0.0	9.7 ^{Aa} ± 0.1
	103E	8.4 ^{Ab} ± 0.0	7.5 ^{Bc} ± 0.1
	105E	6.6 ^{Ae} ± 0.1	6.5 ^{Afg} ± 0.1
	106E	7.0 ^{Bd} ± 0.2	7.4 ^{Acd} ± 0.0
	108E	6.5 ^{Bef} ± 0.1	7.7 ^{Ac} ± 0.0
	Mean** ± SD (CV%)	6.9 ± 0.9 (14)	7.8 ± 1.2 (15)
Jequitibá (medium maturing)	201M	6.1 ^{Bgh} ± 0.1	7.0 ^{Ade} ± 0.1
	202M	6.6 ^{Bef} ± 0.0	6.8 ^{Aef} ± 0.3
	203M	7.2 ^{Bd} ± 0.0	7.6 ^{Ac} ± 0.1
	207M	6.3 ^{Aefg} ± 0.1	6.1 ^{Bh} ± 0.2
	209M	8.8 ^{Aa} ± 0.1	6.4 ^{Bgh} ± 0.0
	Mean** ± SD (CV%)	7.0 ± 1.1 (16)	6.8 ± 0.6 (9)
Centenária (late maturing)	301L	7.8 ^{Ac} ± 0.1	6.4 ^{Bgh} ± 0.1
	302L	6.1 ^{Bgh} ± 0.1	7.5 ^{Ac} ± 0.1
	303L	7.6 ^{Ac} ± 0.0	6.7 ^{Befg} ± 0.1
	306L	6.3 ^{Befg} ± 0.0	8.9 ^{Ab} ± 0.3
	307L	6.3 ^{Afg} ± 0.1	6.3 ^{Agh} ± 0.2
	Mean** ± SD (CV%)	6.8 ± 0.8 (12)	7.2 ± 1.1 (15)

Notes: *Mean (duplicates) ± SD (standard deviation) for each genotype. **Average content for each cultivar ± SD (standard deviation) and CV (coefficient of variation) between genotypes of the same cultivar. Means followed by the same capital letter in the same line show no significant difference between growing sites (Tukey, $p \leq 0.05$). Means followed by the same lower case letter in the same column show no significant difference between genotypes (Tukey, $p \leq 0.05$)

Table VI. Melanoidin content* (mg·mL⁻¹ da bebida) in *Coffea canephora* genotypes grown in two sites

In summary, the composition of coffee brews from the three cultivars was significantly affected by genotype, and an effect of the interaction genotype and cultivation site was also observed. In regard to the cultivation site, higher trigonelline contents and TTA values were found in brews from genotypes grown at Marilândia.

The AA of the 15 *C. canephora* genotypes in two cultivation sites is shown in Table VII. The Diamante, Jequitibá and Centenária cultivars showed average values from 6.78 to 8.80 mg of TROLOX mL⁻¹ of the brew (Table VII) equivalent to a range from 39.9 to 51.8 g TROLOX·100 g⁻¹ of soluble solids, similar to the described by Vignoli *et al.* (2014) (36.4 to 48.2 g TROLOX·100 g⁻¹ of soluble solids).

The AA values (Table VII) were higher than those reported by Vignoli *et al.* (2016) for a *Coffea arabica* brew (6.40 mg of TROLOX mL⁻¹) prepared under the same conditions of this research. This is in agreement with the literature that describes higher AA for *C. canephora* products compared to *C. arabica* ones (Vignoli *et al.*, 2011; Vignoli *et al.*, 2014).

The AA of the coffee brews was affected by both the genotype ($p < 0.000$) and by the cultivation site ($p = 0.024$). In general, beverages from genotypes grown at Bananal do Norte showed higher AA, being the highest value obtained from the early-maturing Diamante cultivar genotype 101E (9.78 mg of TROLOX mL⁻¹) (Table VII). There was an interaction between cultivation site and genotype ($p < 0.000$), showing that the AA of each genotype was influenced by cultivation site, but this effect as genotype-dependent.

It is important to emphasize that, despite the variation on the AA of the beverages (CVs from 7 to 15 percent) (Table VII), this variability was lower than that found for the coffee brews composition (Tables III to VI and 1S, supplementary material). Kitzberger *et al.* (2014), studying seven *C. arabica* coffee genotypes grown at the same edaphoclimatic conditions, showed that the genetic variability had a more significant effect on the coffee bioactive compound profile than on the AA. Similar behavior has been reported in the literature for roasted and instant coffee produced by different processes (Corso *et al.*, 2016; Vignoli *et al.*,

Cultivar	Genotypes	Site/experimental farm	
		Marilândia	Bananal do Norte
Diamante (early maturing)	101E	5.73 ^{Bi} ± 0.10	9.78 ^{Aa} ± 0.30
	103E	8.50 ^{Bab} ± 0.30	9.29 ^{Aab} ± 0.50
	105E	8.18 ^{Aabc} ± 0.15	7.37 ^{Befg} ± 0.30
	106E	7.22 ^{Bdef} ± 0.10	8.54 ^{Abcd} ± 0.35
	108E	7.58 ^{Bcde} ± 0.30	9.00 ^{Aabc} ± 0.40
	Mean** ± SD (CV%)	7.44 ± 1.08 (15)	8.80 ± 0.92 (10)
Jequitibá (medium maturing)	201M	6.51 ^{Afghi} ± 0.20	6.48 ^{Agh} ± 0.25
	202M	7.15 ^{Adefg} ± 0.20	7.22 ^{Afg} ± 0.10
	203M	7.79 ^{Abcde} ± 0.30	7.15 ^{Bfg} ± 0.30
	207M	7.01 ^{Aefgh} ± 0.10	6.19 ^{Bh} ± 0.15
	209M	8.01 ^{Aabcd} ± 0.00	6.83 ^{Bgh} ± 0.25
	Mean** ± SD (CV%)	7.30 ± 0.61 (8)	6.78 ± 0.44 (7)
Centenária (late maturing)	301L	7.33 ^{Acdef} ± 0.05	7.26 ^{Afg} ± 0.45
	302L	7.30 ^{Bcdef} ± 0.30	8.43 ^{Abcd} ± 0.30
	303L	8.86 ^{Aa} ± 0.30	8.26 ^{Bcde} ± 0.15
	306L	6.30 ^{Bghi} ± 0.10	7.79 ^{Adef} ± 0.40
	307L	6.16 ^{Bhi} ± 0.20	8.61 ^{Abcd} ± 0.35
	Mean** ± SD (CV%)	7.19 ± 1.08 (15)	8.07 ± 0.55 (7)

Table VII.

Antioxidant activity* (mg·TROLOX mL⁻¹ da bebida) in *Coffea canephora* genotypes grown in two sites

Notes: *Mean (duplicates) ± SD (standard deviation) for each genotype. **Average content for each cultivar ± SD (standard deviation) and CV (coefficient of variation) between genotypes of the same cultivar. Means followed by the same capital letter in the same line show no significant difference between growing sites (Tukey, $p \leq 0.05$). Means followed by the same lower case letter in the same column show no significant difference between genotypes (Tukey, $p \leq 0.05$)

2011, 2014, 2016). Taking into account that AA is the result of joint activity of some bioactive components – such as total chlorogenic acids, trigonelline, caffeine and melanoidins – specific variations in the content of each bioactive causes lower impact on the coffee brew's global antioxidant potential.

The principal component analysis was used to characterize and discriminate the beverages. A bi-dimensional solution (PC1 and PC2) accounted for 75 percent of the variance. PC1 was characterized mainly by caffeine, trigonelline and total chlorogenic acids, while PC2 was characterized mainly by melanoidins and TTA (Figure 1(a)). The AA (supplementary variable) was positively correlated to the contents of caffeine ($r = 0.58$), trigonelline ($r = 0.51$), total chlorogenic acids ($r = 0.53$) and melanoidins ($r = 0.66$), in agreement to the described by Perrone *et al.* (2012) and Vignoli *et al.* (2014).

Genotypes from the medium-maturing Jequitibá cultivar – located at the right side of the plot (second and third quadrant) (Figure 1(b)) – were characterized by a lower content of caffeine, trigonelline and total chlorogenic acids, and AA. Genotype 203M, grown at Marilândia, was an exception, showing an opposite behavior and high contents of these bioactive compounds (Figure 1, Tables II, III and IV).

In general, most genotypes of the late-maturing Centenária cultivar were located in the upper region of the plot (first and second quadrant), while the genotypes from the early-maturing Diamante cultivar, were in the central region of the plot (Figure 1(b)). It is interesting to verify that, by considering the whole set of components of the coffee brew, there was no clear discrimination among cultivars. This behavior pointed that fruit-ripening seasons (early, medium and late maturing) has less impact on the chemical composition and AA of the coffee brew, being the variation among genotypes of the same cultivar more relevant. This is highly positive information for the grower, industry, and consumer, since it is possible to obtain coffee brews with similar composition profile and AA the using coffee cultivars with different fruit-ripening seasons.

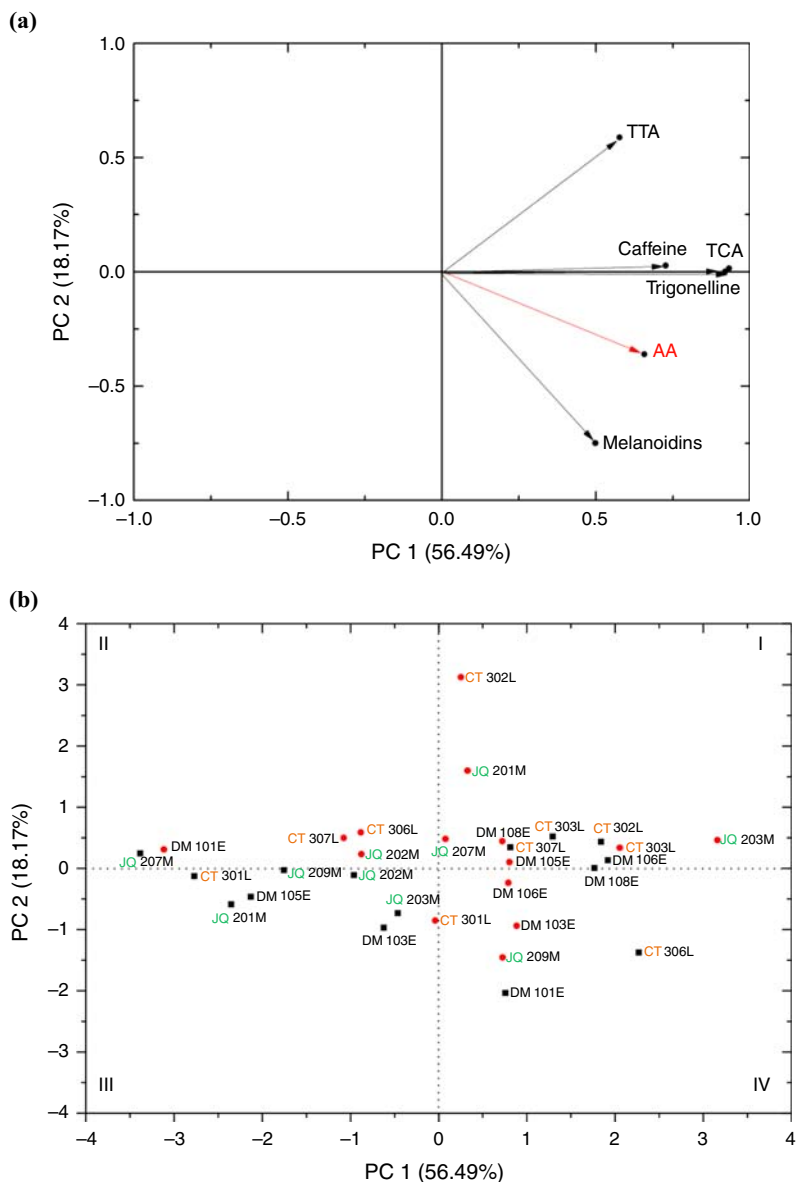


Figure 1. PCA considering the chemical composition and the antioxidant activity of brews of *Coffea canephora*: (a) variables; and (b) samples

In relation to the cultivation site, there was no discrimination of the coffee brews grown at different locations. Some genotypes showed great similarity considering composition profile of the beverages from the two sites (such as Centenária 303L and Diamante 106E) while for a site effect could be observed for others (Diamante 101E and Jequitibá 207M and 203M) (Figure 1(b)). Further studies with some consecutive harvests are necessary for an adequate evaluation.

4. Conclusions

The composition of the coffee brews from cultivars Diamante ES8112, ES8122 “Jequitibá,” and Centenária ES8132 was significantly affected by genotype, and an effect of the interaction of genotype and cultivation site was also observed. Higher contents of trigonelline and higher values of TTA were found in coffee brews for genotypes grown at Marilândia. Fruit-ripening seasons (early, medium and late maturing) has little impact on the chemical compositions and AA of the beverages, being the variation within genotypes of the same cultivar more relevant. Coffee brews from the three cultivars showed high AA associated with the contents of caffeine, trigonelline, total chlorogenic acids, and melanoidin.

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