Physico-chemical properties and sensory profile of *Coffea canephora* genotypes in high-altitudes

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Abstract

In Brazil, *Coffea canephora* coffee is generally cultivated in hot climate regions and at altitudes below 400 - 450 m. There is little information on *C. canephora* cultivation at higher altitudes. Thus, the objective of this work was to determine the physicochemical properties and to perform the sensorial analysis of 21 different *Coffea canephora* coffee genotypes, grown at 720 m altitude in the state of Espírito Santo, Brazil. The field experiment was implemented in 2011 at the Incaper, Experimental Farm of Venda Nova using randomized block design, with four replications, eight plants per plot and spacing of 3.0 x 1.0 m. Thirteen clones of the clonal cultivar Vitória Incaper 8142 (V1 to V13) and eight clones of the clonal cultivar Robustão Capixaba Emcapa 8141 (R1, R2, R3, R6, R7, R8, R9 and R10) were studied. Grain samples were obtained from the third harvest in 2016. The harvest was performed when more than 80% of the fruits were ripe (August) and the freshly harvested coffee was processed using the conventional terrace drying method (natural processing). After the coffee was dried and processed, the four replicates were of each treatment were combined for the physicochemical analyses. The physicochemical analyses were performed (total titratable acidity, pH at 25°C and 96°C), reducing, non-reducing and total sugars were determined, chlorogenic acid (5-CQA), trigonelline and caffeine levels were determined by HPLC using the external standard method. Chlorogenic acid contents were found in the range of 2.60 to 3.65%. Caffeine levels ranged from 2.06 to 2.89%. There was no statistical difference in the final scores of the sensory analysis of the *C. canephora* coffees and the average value was 77.44 points, the same score for high-quality/premium coffee. Cultivation of *C. canephora* at high altitudes can be promising to obtain higher quality coffees from *C. canephora* species.

Keywords: *Conilon* coffee, Robusta coffee, chemical composition, genotypes, altitude, quality.

Introduction

Brazil is the largest producer and the second largest consumer of coffee in the world, with an average consumption of 20 million bags per year.The species of coffee of higher economic importance in Brazil are *Coffea* *arabica* and *Coffea canephora*, which account for about 74% and 26%, respectively, of national production (Conab, 2017). The state of Espírito Santo (ES) is the leader in the production of *C. canephora* in Brazil. The cultivation of this coffee on *capixaba* lands (lands in the state of Espírito Santo) began in the 1970s, but it was from 1985 onwards that the research and rural extension work in the state were

intensified (Ferrão *et al*., 2017). In this context, many genetic materials with superior agronomic characteristics were selected and developed by INCAPER's *conilon* coffee breeding program, resulting in the launch and recommendation of 10 cultivars for Espírito Santo, nine clonal cultivars and one obtained by seed propagation.These cultivars constitute the basis of the coffee state park: 'Emcapa 8111', 'Emcapa 8121' and 'Emcapa 8131' (Bragança *et al*., 2001); 'Emcapa 8142 - Robustão Capixaba' (Ferrão *et al*., 1999); 'Emcaper 8151 - Tropical Robusta' (Ferrão *et al*., 2000); 'Vitoria Incaper 8142' (Fonseca *et al*., 2004); 'Diamante ES8112', 'Jequitibá ES8122', 'Centenary ES8132' (Ferrão *et al*., 2013) and 'Marilândia ES8143 (Ferreira *et al*., 2017). Despite the great evolution in coffee production, quality has been a factor of growing concern for researchers and for the coffee agribusiness (Lima Filho *et al*., 2013).

Factors that influence coffee quality have been researched and are quite complex. The ones that stand our among them are planting site; soil type; coffee species; genetic aspects (Ramalho *et al*., 2016); climate (Dandendo *et al*., 2014; Martins *et al*., 2015); harvesting (Fagan *et al*., 2011); drying; storage (Coradi, 2008); dry and wet processing (Giomo, 2012); roasting and milling (Schmidt *et al*., 2008). Among the environmental factors, the planting site may have a particular influence on the production of higher quality coffees, due to differences in temperature and rainfall regime. According to Laviola *et al*. (2007), at higher altitudes the coffee tree may have greater accumulation of photoassimilates in leaves and fruits, due to the slower maturation influenced by lower temperatures. Following this approach, Fritzsons *et al*. (2008) reported that at each 180-meter altitude increase, the temperature decreases around 1 degree Celsius and there is higher average rainfall. *C. canephora* in Brazil is generally cultivated at altitudes below 400-450 m, in hot climate regions (Matiello, 1991; Dadalto and Barbosa, 1997). Worldwide, coffee quality is determined by a qualitative method, the cup test (sensorial analysis), carried out by trained professionals, where the aroma, acidity, bitterness, sweetness, astringency and body of the beverage are evaluated. The aspects evaluated in the sensorial analysis are directly related to the chemical constituents present in the coffee beans (Figueiredo *et al*., 2018). The physicochemical parameters of the coffee can be used to determine desirable coffee quality factors such as: pH and total titratable acidity (Borém *et al*., 2008), as well as the constituents that influence coffee flavor, such as reducing, non-reducing and total sugars, as well as the levels of chlorogenic acids, trigonelline and caffeine (Farah *et al*., 2006). The objective of this work was to quantitatively determine the physicochemical characteristics and to perform the sensory analysis of different genotypes of *C. canephora*, grown at 720 m altitude, in order to investigate the potential quality of this species.

Results and Discussion

Coffee acidity (C. canephora)

Table 1 shows the values of total titratable acidity (TTA), pH at 25 and 96°C for *C. canephora* coffee samples. There was no significant difference for the TTA values of the 21 *C. canephora* samples and the mean value was 178.09 (mL of

NaOH0.1 mol L^{-1}/100g). Pinheiro *et al*. (2012) found TTA values above 200 (mL of NaOH 0.1 mol L^{-1}/100g) for raw *C. canephora* coffee beans dried by fire or sun-dried on concrete or brick patios. Values below 142 (mL NaOH 0,1 mol L^{-1}/100g) were obtained for higher quality coffees dried in a greenhouse, indicating that the higher the TTA value, the higher the acidity, the lower the coffee quality. Partelli *et al*. (2014) found total titratable acidity equal to 213 (mL of NaOH 0.1 mol L^{-1} / 100g) for a sample of *conilon* coffee with 13% humidity and 75.50 points of sensory analysis.

The pH values at 25°C were very close for all samples analyzed (mean value of 5.99), and pH values at 96°C did not show a significant difference (P < 0.05), with an average of 5.86. Leroy *et al*. (2006) found a pH range from 5.27 to 6.13 for *C. canephora* by reviewing the literature. A pH value of 5.71 at 25°C was found for *C. canephora* (Bicho *et al*., 2013).

In the sensorial analysis, the acidity of the coffee is an attribute of great importance. In terms of quality, the increase in acidity can be associated to inferior coffee quality. Coffee acidity may vary according to the stage of fruit maturation, place of origin, harvest and processing method, climatic conditions of the crop, harvest and drying (Clifford *et al*., 1987; Lima Filho *et al*., 2013). The *C. canephora* coffee samples in the present work were cultivated in the same place, had standard harvesting, processing and drying methods. These facts can explain the uniformity of the acidity of these coffees.

Coffee sugars (C. canephora)

The reducing (RS), non-reducing (N-RS) and total (TS) sugars found for *C. canephora* samples ranged from 0.66 to 0.39%; 1.32 to 2.31%; and 2.02 to 2.83%, respectively. There was a statistical difference for these sugar contents among the samples, but the values found were similar. Non-reducing sugars, in particular, sucrose, are found in larger quantities in coffee, and are of great sensory importance. Pinheiro *et al*. (2012) found values of 2.04 to 2.82% of TS, 0.62 to 0.87% of RS and 1.40 to 2.49% of N-RS for samples of *conilon* coffee in Espírito Santo, Brazil.

Higher sugar contents in coffee give the beverage a sweeter taste. During the coffee roasting process, reducing sugars mainly react with amino acids (Maillard reaction), giving rise to desirable color compounds, responsible for the brown color of the coffee. Volatile compounds are produced in these reactions, which have a great effect on the aroma of the final product, resulting in better quality (Wang and Lim, 2017).

The sugars are precursors of the characteristic flavor and aroma of coffee, giving rise to substances belonging to the classes of furans, aldehydes and carboxylic acids that influence the quality of the final product (Farah *et al*., 2006).

Bioactive constituents that influence the quality of C. canephora

Chlorogenic acids (CA), trigonelline (Tr) and caffeine (Cf) are biologically active compounds present in coffees, which impact the quality of the beverage (Abrahão *et al*., 2008). The levels of chlorogenic acid (5-ACQ) found for *C. canephora* coffee samples presented a statistical difference. The values found ranged from 2.60 to 3.65%. Lower levels of

Table 1. Average of total titratable acidity (TTA), expressed as mL of NaOH 0.1 mol L^{-1}/100 g of dry coffee, pH at 25°C and 96°C for 21 samples of *C. canephora* cultivated at 720 m of altitude obtained from Incaper clones: Vitória (V1 to V13) and Robustão Capixaba (R1-R3, R6-R10).

C. canephora(Clones)	TTA	pH (25ºC)	pH(96 ºC)
V1	201.67a	5.92b	5.74a
V2	146.67a	5.92b	5.82a
V3	146.67a	5.99b	5.83a
V4	183.33a	6.02a	5.82a
V5	201.67a	5.94b	5.86a
V6	143.67a	5.93b	5.75a
V7	165.00a	6.11a	5.75a
V8	183.33a	6.07a	5.99a
V9	146.67a	5.87b	6.00a
V10	128.33a	5.91b	5.87a
V11	183.33a	6.08a	6.05a
V12	183.33a	6.11a	5.93a
V13	183.33a	6.17a	5.84a
R1	183.33a	6.03a	5.92a
R2	201.67a	6.10a	5.92a
R3	165.00a	6.00b	5.90a
R6	201.67a	6.00b	5.83a
R7	146.67a	6.06a	5.79a
R8	238.33a	5.89b	5.76a
R9	183.33a	5.83b	5.75a
R10	220.00a	5.98b	5.73a
Average	178.09	5.99	5.86
CV (%)	21.31	1.23	1.97
Significance Test F (0.05)	ns	*	ns

Means followed by the same letter belong to the same group according to the *Scott-Knott* test (P <5%).

Table 2. Average of reducing sugars (RS), non-reducing sugars (N-RS) and total sugars (TS) for 21 samples of *C. canephora* cultivated at 720 m altitude obtained from Incaper clones: Vitória (V1 to V13) and Robustão Capixaba (R1-R3, R6-R10).

C. canephora (Clones)	RS(%)	N-RS (%)	TS (%)
V1	0.66a	2.00a	2.66a
V2	0.52d	1.70b	2.21b
V3	0.44f	1.64b	2.09b
V4	0.44f	1.91a	2.34b
V5	0.47f	1.54b	2.02b
V6	0.73b	1.65b	2.38b
V7	0.47e	1.93a	2.40b
V8	0.55d	1.9a	2.45a
V9	0.85a	1.32b	2.15b
V10	0.62a	1.58b	2.21b
V11	0.43f	1.97a	2.39b
V12	0.52d	2.31a	2.83a
V13	0.46e	2.20a	2.67a
R1	0.54d	1.96a	2.50a
R2	0.68c	1.59b	2.26b
R3	0.46e	1.69b	2.15b
R6	0.65c	1.99a	2.64a
R7	0.43f	1.9a	2.33b
R8	0.73b	1.89a	2.61a
R9	0.57d	2.05a	2.62a
R10	0.39f	1.64a	2.03b
Average	0.55	1.83	2.38
CV(%)	4.9	9.82	7.33
Significance Test F (0.05)	*	*	*

Means followed by the same letter belong to the same group according to the *Scott-Knott* test (P <0.05).

Table 3. Average of chlorogenic acid (CA: 5-caffeoylquinic acid, 5-CQA), trigonelline (Tr) and caffeine (Cf) expressed as g 100g^{-1} of coffee on a dry basis for 21 samples of *C. canephora* cultivated at 720 m altitude obtained from Incaper clones: Vitória (V1 to V13) and Robustão Capixaba (R1-R3, R6-R10).

C. canephora (Clones)	CA (%)	Tr (%)	Cf (%)
V1	3.13b	1.02a	2.46b
V2	2.94b	0.94a	2.228c
V3	3.46a	0.96a	2.60b
V4	3.02b	0.95a	2.49b
V5	2.60c	0.90a	2.06c
V6	3.58a	0.96a	2.89a
V7	3.43a	0.85a	2.49b
V8	3.65a	0.88a	2.75a
V9	3.44a	0.91a	2.48b
V10	3.38a	0.91a	2.51b
V11	3.20a	1.01a	2.43b
V12	3.37a	0.96a	2.59b
V13	3.05b	0.94a	2.32c
R1	3.33a	0.95a	2.51b
R2	3.51a	0.87a	2.51b
R3	3.08b	0.89a	2.45b
R6	2.65c	0.89a	2.21c
R7	3.06b	0.84a	2.35c
R8	3.19a	0.96a	2.31c
R9	2.83c	1.02a	2.41c
R10	3.42a	0.85a	2.35c
Average	3.21	0.93	2.45
CV (%)	7.62	8.55	5.76
Significance Test F (0.05)	*	ns	*

Means followed by the same letter belong to the same group according to the *Scott-Knott* test (P <0.05).

chlorogenic acids are desirable for better coffee quality, as these substances are degraded during the roasting phase, producing the compounds caffeic acid, lactones and different phenols, through Maillard and Strecker reactions, resulting in increased bitterness and astringency (Shan *et al*., 2017). There was no significant difference in the values of trigonelline found for the 21 samples of *C. canephora* coffee. The total average value found was 0.93%, which is in accordance with the literature. In raw coffee beans the trigonelline content is around 1%. Trigonelline is responsible for the formation of degradation products in the roasting process. Among these products, pyrroles and pyridines stand out, since they are highly important for coffee aroma (Monteiro and Trugo, 2005; Vignoli *et al*., 2014). In addition to these compounds, trigonelline is a precursor of niacin, a vitamin which has nutritional importance (Farah *et al*., 2006). The average caffeine content found in *C. canephora* coffee samples was around 2.20%, almost double the value found for *C. arabica*, which presents on average 1.40% of this substance (Leroy *et al*., 2006). In the present work, the caffeine contents presented significant differences among the samples of *C. canephora*, with values ranging from 2.06 to 2.89%. Ky *et al*. (2001) found an average value of 2.54% caffeine in *conilon* coffee from different African countries.

Caffeine is an alkaloid present in teas, soft drinks and coffee, which acts on the human body in the central nervous system, having a stimulant and diuretic effect. During the roasting process, caffeine is very stable, and although it is an odorless substance, it is bitter and can contribute to this sensorial characteristic of coffee (Monteiro and Trugo, 2005).

Sensory analysis of C. canephora

The 21 samples of *C. canephora* were submitted to sensory analysis and there was no statistical difference in the scores obtained for any of the attributes tested. The final mean value was 77.44 points (Table S3, Supplementary). By the UCDA protocol (2010), this score ranks in the 'good quality' range. This result suggests that at 720 m altitude the *C. canephora* of the clonal cultivars 'Vitoria' (V1-V13) and 'Robustão Capixaba' (R1-R3, R6-R10) were obtained with similar flavors and aroma and had good drink acceptance (superior/premium). Sturm *et al*. (2010), investigating the relationship between altitude and quality of *C. canephora*, used crops with different genotypes of this species in order to avoid interactions of genotypes with specific environments. The crops were located in the cities of Alegre and Mimoso do Sul in the state of Espírito Santo, Brazil. The coffees from seven rural properties were planted at different altitudes: below 250 m, from 250 to 500 m and above 500 m, and were submitted to sensory analysis. Based on the results and statistical analysis, there was an influence of the altitude on the quality of the *conilon* coffee drink; the higher the altitude, the higher the beverage quality.

Materials and methods

Genetic materials and grain sample preparation

Twenty-two genetic materials of *Coffea canephora* from the Incaper breeding program were analyzed, grown at the Experimental Farm of Venda Nova (FEVN), at 720 meters

above sea level. The genetic materials were the clones of the cultivars 'Vitória Incaper 8142' (V1 to V13) and 'Emcaper 8141 Robustão Capixaba' (R1-R3, R6-R10).

Grain samples, obtained in the 2016 harvest, were prepared at Incaper, from an experiment conducted at the Experimental Farm of Venda Nova do Imigrante (FEVN) in a randomized block design with 21 treatments, 4 replications, 8 plants per plot with spacing of 3.0x1.0 m.

During the harvest, in July-August 2016, 3.0 kg of cherry coffee were harvested from each plot for post-harvest evaluations regarding sensory and physicochemical analysis. The samples of cherry coffee were dried on covered ground until reaching 11-12% humidity (natural processing). They were then stored in closed bags and processed in February 2017. The four replicates were grouped and 200 grams of the 21 treatments were transported to the Analytical Central and Chemistry Laboratory of the Exact, Natural and Health Sciences Center (CCENS) of the Federal University of Espírito Santo (UFES), in Alegre-ES, for the physicochemical analysis.

Total Titratable Acidity (TTA)

Total titratable acidity (TTA) was determined by titration with 0.1 mol L^{-1} NaOH (expressed as mL of NaOH 0.1 mol L^{-1}/100 g dry coffee) according to procedures described in AOAC (1990).

pH at 25°C and after heating at 96°C

The pH measurements were made at 25°C and after heating at 96°C and were performed on a Digimed DM-22pHmeter according to procedures described by IAL (1985).

Analysis of sugars

Total and reducing sugars were extracted by the Lane-Enyon method, cited by AOAC (1990) and determined using the Somogy technique, adapted by Nelson (1944). Non-reducing sugars were obtained by calculating the difference between total and reducing sugars. Values were expressed as percentages.

Chlorogenic acid, trigonelline and caffeine

For the simultaneous determination of chlorogenic acid, trigonelline and caffeine, 0.5 g of ground coffee was dissolved in 100 mL of Mili-Q water at 80°C under magnetic stirring for 15 minutes. After this time, simple filtration was carried out and the filtrate was collected in a 100 mL volumetric flask. After the filtrate cooled to room temperature, it was filtered through a syringe membrane filter (0.45µm pore size) and the aqueous coffee extracts were placed in 1-mL vials. These extracts were analyzed by high-performance liquid chromatography (HPLC) using a Shimadzu chromatograph (Prominence model) with a Shimadzu Shim-pack VP-ODS reverse phase C-18 column (250 mm long x 4.6 mm ID). The system was coupled to a Shimadzu UV-Visible spectrophotometric detector (SPD-20A model), with a CBM-20A system controller.The analysis conditions used were: mobile phase composed of HPLC grade methanol, Mili-Q water and HPLC grade acetic acid in the ratio of 20:80:1; flow of 1 mL min^{-1}; column temperature

was kept at 40°C and wavelength detector was set at 272 nm (Abrahão *et al.*, 2008).

The external standard method was used in the simultaneous quantification of chlorogenic acid, trigonelline and caffeine contents in *C. canephora* coffee samples. For this, standard substance (Sigma-Aldrich) solutions of known concentrations were prepared and analyzed under the conditions mentioned above. The calibration curves were obtained with $R^2 > 0.99$ from the peak areas obtained in the chromatograms for each standard substance at different concentrations. The equations obtained were used to calculate the amount of the target compounds present in the coffee extracts.

The chlorogenic acid solutions (5-caffeoylquinic acid) used to establish the calibration curve were prepared at concentrations of 25, 50, 100, 150 and 300 µg mL^{-1} (ppm); trigonelline solutions (3-carboxy-1-methylpyridinium chloride) were prepared at 12.5, 25, 50, 100 and 150 µg mL^{-1}; and caffeine (1,2,7-trimethylxanthine) solutions were prepared at concentrations of 40, 60, 80, 100 and 200 µg mL^{-1}.

Sensorial analyses

Sensorial analyses were performed according to the methodology proposed by the Uganda Coffee Development Authority (UCDA, 2010). The samples of *C. canephora* coffees of the cultivars 'Vitória Incaper 8142' (V1 to V13) and 'Emcaper 8141 Robustão Capixaba' (R1-R3, R6-R10) were left standing for 45 days and subsequently classified by type and by sieving. For the roasting process, coffees with 100% sieve 15 and above were admitted. The roaster used was Laboratto TGP-2 with the Agtron-SCA disc set. The roasting point of these samples was between the colors determined by the discs nº 65 and nº 55 for specialty coffees (SCAA, 2013). Roasting was executed 24 hours in advance and grinding respected the time of 8 hours of rest after roasting. The roasting was carried out for 9 to 10 minutes and, after roasting and cooling, the samples remained sealed.

The Bunn G3 electric crusher was used to grind the coffees to obtaina medium to coarse particle sizes. Five cups of each coffee batch were used and the ideal proportion of 8.25 g of ground coffee per 150 mL of water (SCAA, 2013) was adopted. After the water reached 92-95°C, infusion was performed. When the cup temperature reached 55ºC the Q-Graders started the evaluation after 4 minutes of infusion. The sensory analysis of the coffees was carried out by a panel of six (6) tasters, all of them Q Certified Robusta Graders (skilled and credible Robusta coffee cuppers, certified by the Coffee Quality Institute, CQI). This minimum number of evaluators in the sensorial analysis was initially proposed by Pereira *et al*. (2016), in order to reduce the subjectivity of the sensory analysis of coffees.

Statistical analysis

Data was submitted to analysis of variance for each response variable and, in significant cases (P <5%), the Scott-Knott averages group test (P <5%) was applied. Analyses were performed using the GENES software (Cruz, 2016).

Conclusion

Based on the results, it can be inferred that the cultivation of *C. canephora* at an altitude of 720 m can result in higher quality coffees, as the chemical composition of the *conilon* and robust genotypes present similarities and are compatible with data obtained for higher-value coffee aggregates. The 21 samples of *C. canephora* coffee grown at 720 m of altitude had similar mean values of total titratable acidity (TTA), pH at 96°C and trigonelline contents, which did not present significant differences (P<5%). The values of reducing, non-reducing and total sugars were different for the genotypes. The levels of chlorogenic acid (5-CQA) found for all *C. canephora* coffee samples were lower than those reported in the literature. The percentages of caffeine found were close to the average value for this species.

From a sensory standpoint, the coffees were similar. There was no statistical difference in the final score for the 21 analyzed samples and the average of 77.44 points indicates good drinking quality for these coffees. Thus, the cultivation of *C. canephora* coffee at an altitude of 720 m can be viable for obtaining superior coffee.

Acknowledgments

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