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.

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 3.89%. 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 (Fandendo et al., 2014; Martins et al., 2015); harvesting (Fagan et al., 2011); drying; storage (Coradi, 2008); dry and wet processing (Giomio, 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 (Figueirêdo 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 (Borêm 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 (mL) | pH (25°C) | pH (96°C) |
|--------------------------|----------|-----------|-----------|
| 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

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

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): ns ns *

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).

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 ‘Vitória’ (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 'Emcapер 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 R² > 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 'Emcapер 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 Laboratîto 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 gridding 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 obtain 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 55°C 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. 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

The authors are grateful for the financial support and the productivity grant to PF Pinheiro granted by the Foundation for Support to Research and Innovation of Espirito Santo (FAPESP) and the National Council of Scientific and Technological Development (CNPq). "This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code 001".

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