Antioxidant activity and stable free radicals in robusta Green Coffee Genotypes

Atividade antioxidante e radicais estáveis livres em Genótipos de Café Verde Robusta

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ABSTRACT

This study reports on physicochemical properties (total soluble solids, titratable acidity plus caffeine, trigonelline, 5-CQA, and total phenols contents) of five genotypes of Robusta coffee beans; Bamburral, Beira Rio, Clementino, Coringa, and Pirata. Green bean of Clementino presents the highest concentration of total soluble solids in dry basis (18.0%) and the lowest titratable acidity (154.0 mL-NaOH). Moreover, green beans of Clementino and Pirata show intermediate values of caffeine and trigonelline contents while presenting the highest yield index ofstable free radical formation after roasting, respectively equal to 26 and 23 (electron paramagnetic resonance-EPR data), and the highest content of 5-CQA (around 42 mg/g). Green beans of Bamburral, Beira Rio and Coringa show the highest concentration of total phenols (53 - 56 mg/g), meaning the highest antioxidant activities (IC50 test) but reduced yield index of formation of stable free radicals after roasting, respectively equal to 13.0, 5.9, and 13.0 (EPR data).

Keywords: Coffee genotypes, phenolic compounds, EPR, stable free radicals, antioxidant activity.

RESUMO

Este estudo relata as propriedades físico-químicas (sólidos solúveis totais, acidez titulável mais cafeína, trigonelina, 5-CQA e teor total de fenóis) de cinco genótipos de grãos de café Robusta; Bamburral, Beira Rio, Clementino, Coringa e Pirata. O grão verde de Clementino apresenta a maior concentração de sólidos solúveis totais em base seca (18,0%) e menor acidez titulável (154,0 mL-NaOH). Além disso, o feijão verde de Clementino e Pirata apresenta valores intermediários de teor de cafeína e trigonelina, apresentando o maior índice de rendimento da formação de radicais livres estáveis após a torrefação, respectivamente igual a 26 e 23 (dados de ressonância paramagnética de elétrons -EPR), e o maior teor de 5-CQA (cerca de 42 mg/g). Os grãos verdes de Bamburral, Beira Rio e Coringa apresentam a maior concentração de fenóis totais (53 - 56 mg/g), significando a maior atividade antioxidante (teste IC50), mas índice de rendimento reduzido de formação de radicais livres estáveis após a torrefação, respectivamente igual a 13,0, 5,9 e 13,0 (dados EPR).

Palavras-chave: Genótipos do café, compostos fenólicos, EPR, radicais livres estáveis, atividade antioxidante.

1 INTRODUCTION

The world coffee production reached 170.94 million 60-kilogram bags as of 2018/2019, up from 158.07 million 60-kilogram bags in 2017/2018 [1]. The cultivar Arabica (C.arabica) accounts for 59.7% and the cultivar Robusta (C.canephora) for 40.3%

of this total[2]. The annual income from the entire coffee chain is around USD 173,000 million [3]. The majority of the world's coffee is produced by South America; Brazilian coffee provides approximately 32% of the global production [4].Within Brazil, the macroeconomic region producing the Robusta species is the Southeast with 9.82 million bags in 2019; the state of Espirito Santo (Brazil) reaching productivity of 39.25 bags per hectare while producing 9.49 million bags, which represents 96.6% of the volume produced in the region [5]. The climatic conditions of the Brazilian Southeastregion provide good flow for both the Arabica and Robusta species.

Assessment of coffee quality is usually focused on factors that influence consumers' preference, being assessed using three characteristics: physical, sensorial and physicochemical aspects [6,7, 8]. Coffee quality varies across different genotypes. Arabica coffee is considered of higher quality, with lower caffeine content while producing a more aromatic brew compared withRobusta coffee [7, 10]. Robusta coffee has been characterized as neutral and, occasionally, presenting strong acid and pronounced bitterness [9, 11]. On the other hand, Robusta coffee is less susceptible to pests than Arabica coffee [12] and tolerates temperatures of up to 37 oC, based on the maintenance or reinforcement of photo protection and antioxidant mechanisms [13, 14].

The presence of specific biochemical components could impact favourably the aroma and flavor of thecoffee beverage, like trigonelline and sugars, or unfavourably, if containing chlorogenic acids (CGA) and caffeine [9, 15]. These compounds turn to be important for the consumers' health. Caffeine, for instance, is known to have positive health effects by lowering cancer, diabetes mellitus, Alzheimer's disease, Parkinson's disease, and cardiovascular risks [16, 17, 18]. Trigonelline, on the other hand, has hypoglycemic, hypolipidemic, neuroprotective, antimigraine, sedative, memory-improving, antibacterial, and antiviral actions [19].Worth mentioning that phenolic compounds are secondary metabolites of plants involved in adapting to environmental stress conditions. In these compounds, CGA are present in higher amounts. Phenolic compounds are natural antioxidant that has antihypertensive effects, prevents diabetes, improves glucose control in normal, pre-diabetic and diabetic human beings [20]. Furthermore, phenolic compoundsare chemical sensitizers responsible for human respiratory allergy agains tcertain types of plant materials [18, 20].

Many chemical components found in coffee are correlated with stable free radicals, associated with molecules containing at least one unpaired electron [21, 22]. The quantitative assessment of these radicals is carried out to determine the oxidation of the

coffee bean during storage and roasting [23]. Recently, ALVES et al. [24] showed that the incidence of microorganisms presents in Robusta coffee grown under shade with high humidity increases the amount of these radicals with respect to the coffee grown in the sun. Thus, monitoring the evolution of the content of these stable free radicals has provided a way to assess the quality of the coffee beans during and after cultivation.

The natural reproduction of the Robusta species generates highly heterozygous individuals and populations with high genetic variability. In this regard, the characterization and exploitation of genetic variability within this species may reveal valuable genetic resources for both production systems and their use in breeding programs. Currently, five genotypes of the Robusta species, namely Bamburral, Beira Rio, Clementino, Coringa and Pirata ,belonging to the cv. Tributun [25] and Coringa [26], have shown remarkable adaptations to adverse situations in tropical weather. In the State of Espirito Santo (Brazil), during the 2014 and 2015 seasons, the above-mentioned varieties presented highlights among others, with productivity of around 70 bags/ha [27]. Thus, in order to obtain information about the quality of the coffee produced by those genotypes, the contents of caffeine, trigonelline, 5-caffeoylquinic acid (5-CQA), titratable acidity and total soluble solids is herein evaluated. Moreover, the antioxidant activity is also assessed, aiming to correlate the chemical components of the coffee bean that present stronger affinity in capturing DPPH radicals from the samples. Finally, the content of stable free radicals present in the above-mentioned genotypes of green and roasted coffees is also evaluated in the present study.

2 MATERIALS AND METHODS

2.1. GROWTH AND PROCESSING

Five genotypes of Coffea sp., C.canephora Pierre ex A. Froehner were used. Moreover, these genotypes were propagated by cutting according to GILES et al. [26]. Plants were installed in May 2012, with 2.7m line spacing and 1.2m row spacing (density of 3,086 plants per hectare), in the county of Vila Valerio, State of Espirito Santo, Brazil (latitude 18°58′05″ S; longitude 40° 20′ 02″ O), at an average altitude of 150mwith annual mean temperature of 23°C. The region presents tropical climate, characterized by hot humid summers and dry winters, classified as Aw according to ALVARES et al.[28]. The local average annual precipitation is 1,200mm, although the entire area benefits from irrigation by sprinklers.

After harvest, the beans of all genotypes were placed in soil drying ovens for 5 days at 50 oC, characterizing the green beans. A sample of the beans (0.5 kg) was placed within a metal recipient with a lid and stirrer at an average temperature between 200 and 240oC, which characterizes the clear roast beans. All samples were ground in a knife mill for 1 min and then sieved to obtain powder around 30 mesh. For better storage, the samples were vacuum packed and kept refrigerated at temperatures of around 5 oC.

2.2 CHEMICAL ANALYSIS

Caffeine, Trigonelline and 5-CQA

Caffeine, trigonelline and 5-CQA content were assessed by high performance liquid chromatography (HPLC) using a Beckman chromatograph (USA) equipped with a Spherisorb ODS2 C18 reverse phase column (250 mm long × 4.6 mm internal diameter, Waters, USA). The hot extraction method was applied to 0.5 g of each ground coffee sample, in 100 mL of ultrapure water (Milli-Q), under constant stirring for 20 min, at 80˚C. HPLC operation conditions were 1 mL/min flux, mobile phase with methanol, water and acetic acid (20:80:1), column temperature set at 40˚C, and reading wavelength of 272 nm[29]. Quantification was performed by the external standard method using a calibration line obtained from chromatogram peak areas at 272 nm from the CGA standard solution 5-caffeoylquinic (5-CQA), caffeine (1,3,7-trimetilxantina) and trigonelline (1-methylpyridinium-3-carboxylate hydrochloride) from Sigma-Aldrich [30]. Recorded data are presented as composite mass (in milligrams) for each gram of coffee beans (dry bases).

Total Phenol Content

The testing sample (1 g fresh weight) was extracted with 10 mL acetone/water solvent (70:30, v/v) according to GU et al.[31]. After addition of solvent, the sample was stirred for 2 h, centrifuged (2,000 g, 15 min, 4 oC) and the supernatant was filtered (Whatman No. 3, protected from light). Total phenolic content in the extracts from each coffee blend was determined using the Folin-Ciocalteau method as described by SINGLETON&ROSSI [32]. The extracts were properly diluted in order to obtain an absorbance in the range of the prepared calibration curve. Then, the Folin-Ciocalteau reagent plus saturated Na2CO3 solution and deionized water were added sequentially. After standing for 1 h at room temperature in darkness, the absorbance was recordedat

750 nm using a UV 1203 spectrophotometer (Shimadzu, Tokyo, Japan). The results are expressed as mg of gallic acid equivalents per g of blends.

Titratable Acidity

Total titratable acidity was determined by titration with NaOH, expressed as mL of NaOH(0.1 mol/L) per 100 g of dry coffee, according to procedures described in the AOAC protocol[33]. The pH of the filtrate was measured at room temperature, after calibration of the electrode with pH 4.0 and 7.0 buffer solutions.

Total Soluble Solids

Soluble solids were measured according to the AOAC protocol [34]. Ground green coffee (10± 0.1) g mixed with water (200 mL) was boiled for 5 min, cooled at room temperature, and the weight adjusted by adding water. After filtration (Whatman No. 1),and forthe quantification of soluble solids, 25 mL of the filtrate was dried in water bath until dryness, after which the residue was placed in an oven at 105◦C, cooled in a desiccator, and weighted.

2.3 EPR MEASUREMENTS: QUANTIFICATION OF STABLE FREE RADICALS AND ANTIOXIDANT ACTIVITY IN DPPH

Stable free radicals were analyzed by EPR measurements, performed on ground samples, using a Bruker spectrometer (Bruker EMXplus, Germany), equipped with an X-band bridge (□9 GHz), high sensitivity resonator (Bruker ER 4119HS, Germany), 20 mW microwave power, 1 G modulation amplitude, 100 kHz modulation frequency, 14 G sweeping width, 5 s sweep time, 336mTcentral field in 4 scans. All spectra were collected in air atmosphere, at 25 oC, using a temperature and gas controller (NoxygenNOX-E.4-TGC, Germany),in the field range from 312 to 360 mT.

Concentration of stable free radicals was assessed using total microwave absorption data evaluated by double integration of the first derivative of the EPR spectra [24, 36]. The g-Factor values were obtained from the resonance condition according to KRAKOWIAN et al. (2014) [36].

The coffee extracts were prepared as described by the Institute Adolfo Lutz protocol [37],with minor modification. A volume of 100 mL of distilled water at 96 oC was added into 10.0 g of ground coffee, infused for 1 min and filtered using paper filter.

For solid content quantification in the extracts, aliquots of 1.0 mL were dried in a tared flask at 105oC, for 6 h, cooled in a desiccator and weighted.

The free radical scavenger effect of coffee genotypes on DPPH(2,2-diphenyl-1-picrylhydrazyl) was performed on two sets of samples; labelled green and roasted coffee. Different volumes of green coffee extract (50, 100, 200, and 300 μL) or roasted coffee extract (25 μL, 50 μL, 75 μL, and 100 μL) were added to 50 mL of distilled water. 500 microliters of each extract sample is mixed with the same volume of 250 μM DPPH, kept in the dark for one hour, then frozen in liquid nitrogen while waiting for EPR spectra recording. The thawed extract samples were placed within precision 50 μL glass capillaries (Blaubrand, Germany) and measured in triplicates.

The antioxidant activity of the coffee samples was monitored by the radical concentration of the oxidants after addition of the coffee brews. The concentrations were calculated by the intensities of the EPR spectra, obtained by double integrating the spectrum using the WIN EPR program (Bruker).

For calculation of the inhibitory concentration (IC50), which represents the concentration of the sample required to sequester 50% of the DPPH radicals, plots of the DPPH content not reacted with the antioxidants, labelled relative concentration (crel), were registered according to the concentrations of the extracts (mg/mL) from each sample. The crel values were calculated according to:

$$c_{rel}(\%) = \frac{DI_{EPR}}{DI_{EPR}^{ref}} \times 100$$

Where DI_{EPR} and DI_{EPR}^{ref} are the double integrals of the coffee/DPPH solution and reference samples, respectively. DI_{EPR} represents the concentration of DPPH, after reacting with the extract (each reaction lasts for 1 h) and DI_{EPR}^{ref} represents the reference DPPH concentration, i.e. at the beginning of the reaction (zero time).

2.4 STATISTICAL ANALYSES

All collected data were expressed as mean ± standard deviation. Means were compared by One-way Analysis of Variance (ANOVA), followed by Tukey's pair wise comparisons [38]. The confidence level required for significance was selected at $p \leq 0.05$.

3 RESULTS AND DISCUSSION

3.1 CHEMICAL CHARACTERIZATION

Table 1 collects the contents of all measured compounds in green beans of all genotypes analysed. The soluble solids contents comprise values ranging between 16.5% and 18.0% in dry basis. These contents are lower than those reported in the literature for green beans of Arabica and Robusta species, which present values between 26% and 34% of dry basis [39, 41]. The Clementino genotype is slightly superior in soluble solids, whereas others genotypes did not show statistical differences ($p \leq 0.05$). For titratable acidity, the contents are in the range of 154.0 – 196.0 mL-NaOH/100 g, which corresponds to a similar range of 128.3 – 238.3 mL-NaOH/100 g obtained by PINHEIRO et al. [30] while analysing 21 genotypes of the Robust species. However, a larger value range of titratable acidity, namely 200 - 291 mL-NaOH/100 g, was reported by RAMALAKSHMI et al. [40]. The Coringa genotype presents the highest concentration of titratable acidity. The Pirata genotype shows an intermediate value of titratable acidity and the others genotypes, with lower concentrations, do not differ statistically ($p \leq 0.05$).

The composition of soluble solids includes sugars (mainly glucose, fructose, and sucrose), organic acids, vitamins, minerals and other minor constituents, presenting a direct relationship with the degree of "sweetness" of the end product. In roasted coffee, soluble solids can increase the body of the beverage and mask the bitterness associated with caffeine [41, 42]. Levels of soluble solids increase with plant maturation [43], being indicated as one of the parameters for the appropriate point of harvest. On the other hand, factors such as defects generated during cultivation and processing tend to decrease the mass of the bean, leading to low levels of soluble solids [40]. As for titratable acidity, higher values among a variety of samples can be attributed to defective beans and/or to inadequate fermentation during drying processes and even harvested at inadequate stage of development [40, 44]. Taking these facts into account, among the genotypes herein investigated, the Clementino has the highest quality factor regarding the concentration of soluble solids. On the other hand, special care must be taken with the Coringa and Pirata genotypes, from cultivation to the stages of bean processing, as they present higher concentrations of titratable acidity.

Caffeine content values are between 25.9 and 31.4 mg/g (Table 1), thus comprising a range of acceptable values for Robusta species. According to MACRAE [45], the content of caffeine for this species varies between 17.2 and 27.6 mg/g. CAPORASO et al. [46] obtained caffeine contents values respectively of 15.7 mg/g and 19, 9 mg/g, for

the Arabica and Robusta species, using hyper spectral imaging. Efficient extraction methods allow to obtaincaffeinecontents in the range of 70.4 - 81.7 mg/g [47]. From the results herein obtained, there is a slight variation in regard to the caffeine content between the evaluated genotypes: Beira Rio presenting the highest content of caffeine whereas Coringa and Bamburral show lower contents, although not differing statistically (p ≤ 0.05). The others genotypes revel intermediate contents. With respect to trigonelline, the values shown in Table 1 are between 8.1 and 9.3 mg/g, being consistent with the values reported by MACRAE [45] in the range of 7.0 and10.0 mg/g. Recently, CAPORASOet al. [46] obtained values of trigonelline for Robusta and Arabica species equal to7.3 mg/g and 8.6 mg/g, respectively. The Pirata genotype shows the highest content of trigonelline among all samples analyzed. The others genotypes herein investigated did not show statistical differences (p ≤ 0.05) with respect to this compound.

The chlorogenic acids (CGA) are the main components of the phenolic fraction of green coffee, reaching levels of up to 14% (in dry weight) [48]. The main subgroups of CGA, namely feruloilquinico (FQA), caffeoylquinico (CQA) and Dicaffeoylquinic (di-CQA)], make up about 98% of the total CGA in coffee, with 5-CQA being the most representative with about 56 – 62% of the total CGA fraction in green beans [39, 48]. Taking this into account, the measured contents of 5-CQA (Table 1),for the studied genotypes, are in the range of47.2 – 56.9 mg/g. BICHO et al. [24, 39] found values of 44.3 mg/g and 47.0 mg/g, respectively for the species Arabica (Brazil) and Robusta (India), where as ALVES et al. [39] obtained values in the range of 44.8 – 49.3 mg/g for Brazilian Robusta beans. From the results herein obtained, the genotypes Bamburral and Coringa present lower contents of this compound, whereas the others genotypes, with higher fractions, did not present statistical differences (p ≤ 0.05).

Caffeine, trigonelline and CGA levels have been used as parameters to distinguish genetic variations between species. For example, it is known that the Robusta species is characterized by presenting higher contents of caffeine and CGA and lower trigonelline content than the Arabian species [39, 49, 50]. On the other hand, for genetic variations within the same species, the subgroups of CGA have been better suited to distinguish between genotypes [47, 49]. Among genetic variations, a reduction in caffeine or CGA may reduce bitterness in the brew, however, this might be a problem for the coffee plant if caffeine or CGA contents are too low to allow the plants to resist from pests and diseases [7]. Also, considering the pharmacological properties which are beneficial to consumers, the Beira Rio, Clementino and Pirata genotypes stand out for presenting

slightly higher values of caffeine and 5-CQA. The Clementino genotype is noteworthy for presenting simultaneously higher contents of soluble solids and lower titratable acidity, which could in principle provide more body to the drink. However, professional drink evaluations should be performed to better confirm this aspect.

3.2 ANTIOXIDANT ACTIVITY IN DPPH

The antioxidant capacity of the coffee genotypes was measured by the in vitro DPPH (2,2-diphenyl-1-picryl-hydrazil) assay based on a single electron transfer reaction. Figures 1 (a) and (b) show the antioxidant activity of the Beira Rio and Clementino genotypes, measured by the relative concentration (crel, %) as a function of the coffee extract concentration (mg/mL) for green and roasted beans, respectively. The average linear fitting factor ($R \approx 0.97 \pm 0.01$) indicates the linear dependence between crel and the extract concentration for the ranges of measured values. This linear dependence is also observed for all others samples (green and roasted). RABAet al. [50] observed a linear behaviour when using the DPPH test to analyse the antioxidant activity of oils extracted from coffee samples.

The slope of the straight lines in Fig.1,indicates the rate of inhibition of free radicals from coffee extracts in DPPH. However, for better interpretation of the results, the IC50 values that express the concentration of sample required to scavenge 50% of DPPH radicals are calculatedand collected in Fig. 2.Considering that the content of phenolic compounds in green beans are the main responsible for the antioxidant activity in several assays with free radicals [52, 53], the IC50 values (olive color) were plotted in Fig. 2 (a), together with the total phenol content (gray color) for all samples. After roasting, the phenolic compounds are drastically reduced and the products of the Maillard reaction contribute more to the antioxidant activity [36, 53].Thus, Fig. 2 (b) shows only the IC50 values for the roasted samples.

The levels of total phenols (Fig. 2(a)) are in the range of 47.2 – 56.9 mg/g. These values are consistent with those obtained by RIBEIRO et al. [18] and BREZOVÁ et al.[54]. The genotypes Bamburral, Beira Rio and Coringa show higher content of total phenols, with no statistical differences ($p \leq 0.05$). Regarding the IC50, these genotypes show lower values, indicating higher efficiency as antioxidants in DPPH. On the other hand, the genotype Clementino, with intermediate content of total phenols, and the Pirate with lower content, exhibit higher IC50 values, indicating lower efficiency as antioxidants. Using these data, it was possible to observe a direct relationship between

the content of total phenols and the antioxidant activity in the genotypes. After roasting, the IC50 values are considerably reduced for all samples; only the Beira Rio genotype appears to have a slightly lower value among the genotypes, indicating slightly higher antioxidant power.

In the present study, there is no direct relationship between the contents of caffeine, trigonelline and 5-CQA (Table 1) and the antioxidant activity for all green beans. Studies have reported that the kinetics of inhibition of free radicals by these compounds depends on the assay used. LIANG et al. [53] using PCA observed that caffeine and CQA isomers have a low correlation in chemical tests with ORACFL, nitric oxide, and ABTS. However, the author reported that these isomers showed a high correlation in assays with cell culture of the Caco-2 intestinal cells, indicating higher affinity with in vivo assays. On the other hand, JESZKA-SKOWRON et al. [47] observed that there is intense antioxidant activity in several Robusta genotypes, when compared to Arabica, due to the notable difference between the amount of total CGA between these two species. Therefore, in the present study, slight differences observed in the contents of caffeine, trigonelline and 5-CQA, added to their low inhibition kinetics in DPPH, are not significant to discriminate the efficacy of these compounds in antioxidant activity among the investigated genotypes.

3.3 STABLE FREE RADICALS

Stable free radicals are associated with different chemical components, such as sugars, carbohydrates and semiquinones that are important in ranking the quality of the beans. Studies indicate that these stable radicals are due to unpaired electrons in carbon atoms in organic molecules [21, 22]. Stable free radicals have been used as markers for checking stress factors during plant cultivation and processing. Recently, LABANOWSKA et al. [22] reported higher amounts of stable radicals in the husks and beans of wheat in plants that are not tolerable to water stress. In this study, it was observed that the accumulation of stable radicals works as a defence/resistance mechanism of the plant to water stress. In another study, it was reported that during storage of the roasted bean, exposure to O2 is the main mechanism for generation of stable radicals through oxidation of the grain surface [24]. It is also known that stable free radicals increase continuously during the bean roasting and are associated with chemical components arising from oxidation reactions and melanoidin formation through the Maillard and caramelization reactions [55].

Figure 3 shows the mass normalized EPR spectra of the ground coffee beans: (a) green and (b) roasted. Assessed g-factors were in the range of 2.0043 –2.0045, with a peak-to-peak line width of about 0.65 mT; these parameters are characteristic of stable free radical species previously described in coffee beans [22, 24].The quantitative assessment of stable free radicals was carried out by double integrating the EPR spectra (intensity) and the results are collected in Table 2. Relative values vary in the range of 0.032 – 0.083 a.u. and 0.49 – 1.21 a.u., respectively for green beans and roasted beans. These ranges of values are in agreement with those obtained by KRAKOIAN et al. [36], when reporting that green and roasted beans from Peru presenthigher contents of stable free radicals when compared with beans from Brazil, Ethiopia, India, and Colombia.

In the study of formation of stable free radicals, it is possible to conclude that: (i) in green beans(Fig. 3 (a)) the genotypes Pirata and Clementino present lower intensities ofEPR signals and therefore lower contents of stable free radicals; the level of stable free radicals for the Beira Rio genotype exceeds that of Pirata by about 110% (Table 2); (ii) as expected, the roasting process resulted in an increased EPR signal for stable free radicals due to oxidation and melanoidin formation (Fig. 3 (b)); (iii) inspection of Fig.3 (b) and Table 2 allows us to observe that the intensity of the EPR signals for the roasted beans do not follow the same trend as for green beans, indicating that the same roasting condition affects differently the content of stable radicals in the investigated genotypes.

Finally, to evaluate the increase in the formation of stable free radicals after roasting, the Iroasted/Igreen fraction (yield index) was defined, where Igreen and Iroasted are the intensities ofthe EPR signals before and after roasting the beans, respectively. Thus, the Pirata and Clementino genotypes, which initially presented lower stable free radicals EPR intensities, are the ones showing higher EPR intensitiesafter roasting, indicating improving kinetics in the stable free radicals formation. It is suggested that these genotypes are more susceptible to formation of oxidation products and/or formation of melanoidins. On the other hand, the low stable free radical yield index value is obtained for the Beira Rio genotype, indicating higher resistance of this genotype to thermal-induced formation of stable free radicals.

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FIGURES AND TABLES

Table 1 - Average values of soluble solids, titratable acidity (n = 4), caffeine, trigonelline, 5-CQA (n = 3) contents in green beans measured for all coffee genotypes examined. All values are shown as mean ± standard deviation. Different letters (a, b, c) indicate significant differences by Tukey's test (p ≤ 0.05) between genotypes.

Genotype →	Bamburral	Beira Rio	Clementino	Coringa	Pirata
Soluble solids (% in dry basis)	16.5 ± 0.3^a	17.1 ± 0.3^a	18.0 ± 0.7^b	16.9 ± 0.3^a	16.7 ± 0.9^a
Titratable acidity (mLNaOH/100g)	166 ± 5^a	167 ± 10^a	154 ± 10^a	181 ± 10^b	174 ± 10^{ab}
Caffeine (mg/g)	25.9 ± 0.6^a	31 ± 1^b	28.5 ± 0.3^c	26.2 ± 0.5^a	28.6 ± 0.2^c
Trigonelline (mg/g)	8.7 ± 0.2^a	8.1 ± 0.5^a	8.5 ± 0.2^a	8.3 ± 0.3^a	9.3 ± 0.1^b
5–CQA (mg/g)	37 ± 3^a	41.1 ± 0.3^b	42.2 ± 0.7^b	35 ± 1^a	42 ± 1^b

Table 2– Relative intensity values (a.u.) for ground coffee genotypes. All relative intensities are shown as mean ± standard deviation (n = 3). Different letters (a, b, c) indicate significant differences (p ≤ 0.05

Genotype→	Pirata	Clementino	Coringa	Bamburral	Beira Rio
Green beans	0.039 ± 0.005^a	0.048 ± 0.005^a	0.075 ± 0.005^b	0.070 ± 0.005^b	0.083 ± 0.005^c
Roasted beans	0.88 ± 0.05^a	1.27 ± 0.05^b	0.97 ± 0.05^c	0.89 ± 0.05^c	0.49 ± 0.05^d
Fraction	23 ± 4^a	26 ± 4^a	13 ± 2^b	13 ± 2^b	5.9 ± 0.9^c
($I_{roasted}/I_{green}$)					

Figure 1 – Relative concentrationc_{rel}(%)versuscoffee extract concentration (mg/mL) for (a) green and (b) roasted coffee.
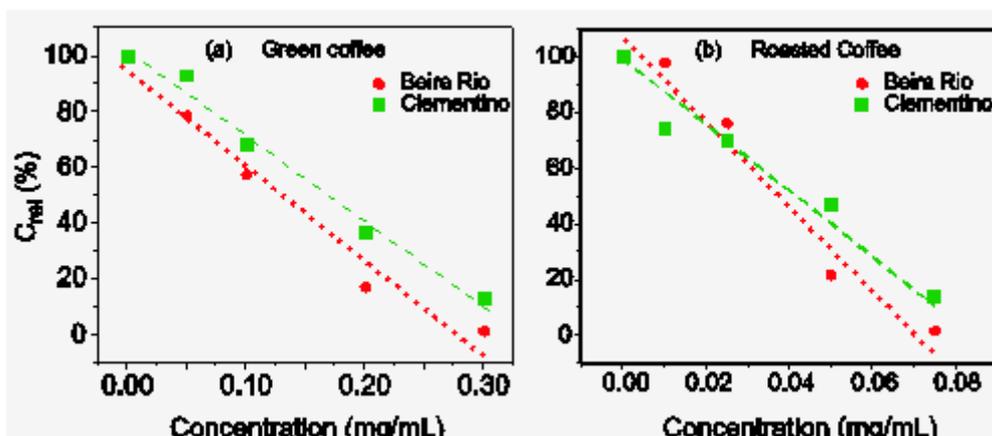
Figure 2 –Total phenol content (gray vertical bars) and antioxidant activity (olive vertical bars), determined by IC_{50} values of coffee genotypes measured in the DPPH assay. In (a) green and (b) roasted beans. All values represent the mean ± S.D. (n ≤ 6). Different letters (a, b, c) indicate significant differences (p ≤ 0.05) between coffee genotypes.
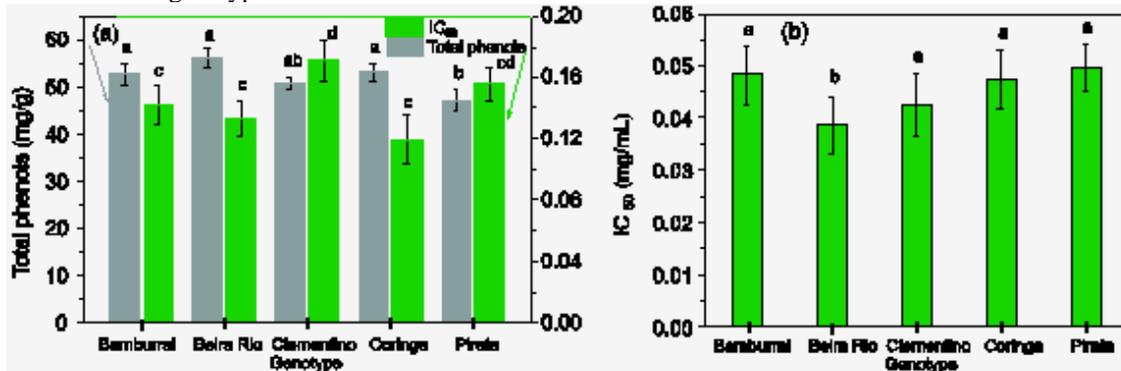

Figure3 - EPR spectra of the coffee genotypes: (a) green and (b) roasted.