

Microparticles of golden coffee from Angola (Amboim) in coffee blends: chemical and prophylactic implications

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Article history

Received: 16 October 2015

Received in revised form:

8 March 2016

Accepted: 17 March 2016

Abstract

The search for new ingredients with nutritional and prophylactic characteristics for incorporation into functional foods parallels with an increasing demand for sustainable and economically viable blends. Pursuing these aims, green *C. canephora* cv. Robusta coffee beans from Angola were processed to obtain golden coffee or, alternatively, submitted to a medium roasting. It was found that the chromatic parameters CIEL*a*b*, CIEL*C*H° and coordinate L* can assess and differentiate golden coffee and Robusta roasted coffee. Higher amounts of total soluble solids, caffeine, trigonelline and polyphenols (i.e., total chlorogenic acids) also prevailed in golden coffee, as volatilization and degradation rises during roasting. Additionally, as Angola I (N-β-Caffeoyl-tyrosine) and Angola II (p-Coumaroyl-N-tyrosine) was found to be characteristic of Robusta coffee from Amboim – Angola, it is concluded that incorporation of microparticles of golden coffee allow an extend of the antioxidant capacity of coffee blend formulations, which might add relevant prophylactic characteristics to human health.

Keywords

Antioxidant Activity

Caffeine; Chlorogenic Acids

Golden Coffee

Robusta Green Coffee

Trigonelline

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Introduction

World coffee yield is currently somewhat above 8 million tonnes per year (ICO, 2014). However, the recognized positive effects on human health (Florián *et al.*, 2013) points to an increase of the trade market in the near future. However, impacts on this crop are expected from estimated future climate changes, with the potential to decrease the world coffee supply and coffee bean quality, although with a lower extent than initially envisaged due to a mitigating effect provided by enhanced air [CO₂] against heat negative impacts (Martins *et al.*, 2014; Santos *et al.*, 2015; Rodrigues *et al.*, 2016). The chemical composition of green coffee beans depends, namely, on genotype, geographic area of origin, cultural practices, maturation, and post-harvest conditions, namely storage (Smith, 1989). Nevertheless, green coffee is a rich source of antioxidants for human ingestion (Esquivel and Jiménez, 2012; Murthy and Madhava-Naidu, 2012) and has other beneficial bioactive compounds (namely, caffeine, trigonelline and chlorogenic acids) for human health, with potential to be considered a functional food product (Dórea and da Costa, 2005; Farah *et al.*, 2006; Bonita *et al.*, 2007; Esquivel and Jiménez, 2012; Murthy and Madhava-Naidu, 2012).

Caffeine is a methylxanthine which has a positive action on cancer, diabetes mellitus, Alzheimer's, Parkinson's disease, cardiovascular diseases and on neurotransmitters, such as norepinephrine, acetylcholine, dopamine, serotonin and glutamate (Smith, 2002; Thompson and Keene, 2004; Nkondjock, 2009; Arendash and Cao, 2010; Hasan *et al.*, 2010; Zhang *et al.*, 2011; Cao *et al.*, 2012). Caffeine also has anti-inflammatory properties, mostly due to the similarity of the molecular structure to adenosine (Ribeiro *et al.*, 2014). Trigonelline, an alkaloid formed by the methylation of the nitrogen atom of niacin, has hypoglycemic, hypolipidemic, neuroprotective, antimigraine, sedative, memory-improving, antibacterial, antiviral, and anti-tumor activities, and it also has been shown to reduce diabetic auditory neuropathy and platelet aggregation (Zhou *et al.*, 2012). It acts by affecting β cell regeneration, insulin secretion, activities of enzymes related to glucose metabolism, reactive oxygen species, axonal extension and neuron excitability (Zhou *et al.*, 2012). CGA, the ester of caffeic acid and quinic acid, accumulates (Bicho *et al.*, 2013) in the cuticular layer, cytoplasm of epidermal and parenchyma cells (where it prevails). Besides, caffeine is also associated with chlorogenic acid (forming a potassium chlorogenate

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complex). CGA is a natural antioxidant that has antihypertensive effects, prevents diabetes, improves glucose control in normal, pre-diabetic and diabetic people and is a chemical sensitizer responsible for human respiratory allergy to some plant materials (Freedman *et al.*, 1964; Zhao *et al.*, 2011).

During roasting, green coffee beans are usually subjected to temperatures ranging between 180-190°C and 220-230°C for about 12-15 minutes (Bicho *et al.*, 2011b). Tissue structure of coffee beans starts changing at ca. 50°C, and with a continued temperature elevation protein denaturation and water evaporation increase. Above 100°C, beans undergo browning related to a series of reactions (Maillard and Strecker mechanisms) giving rise to various substances, including melanoidins. Around 150°C, gaseous substances (water vapor, carbon dioxide, and carbon monoxide) are released, and the bean volume increases. At 180-200°C, with the disruption of the endosperm, bean cracking occurs, bluish smoke and aroma appears, and caramelization develops (Belitz and Grosch, 1988). In this context, the amount of caffeine, trigonelline and CGAs decreases during roasting. Trigonelline degradation further triggers nicotinic acid, pyridine, 3-methyl-pyridine and methyl ester of nicotinic acid synthesis. Roasting mediates the decrease of chlorogenic acids contents due to isomerization, hydrolysis, oxidation, fragmentation, polymerization and association of these compounds with denatured proteins. A large number of aromatic compounds, namely phenol esters, carbonyl compounds and esters, and polycyclic compounds also result due to thermal decomposition of chlorogenic acids (Bicho *et al.*, 2014b). Hydroxycinnamic acids, especially chlorogenic acid in the double form of caffeine and potassium chlorogenate (Clifford, 1987), are significantly destroyed with the release of alkaloids (Correia, 1995).

It is known that green coffee extracts show high levels of antioxidant compounds (Suzuki *et al.*, 2002; Thom, 2007; Madhava *et al.*, 2008), which decreased along the roasting process, namely in Robusta type of coffees (Bicho *et al.*, 2011a,b; 2014b). Besides, chlorogenic acids derivatives such as N- β -caffeoyl-tyrosine and p-coumaroyl-N-tyrosine, are known to occur solely in Robusta coffee from Angola - Amboim (Clifford *et al.*, 1989; Clifford and Knight, 2004), being dependent of the roast extend (Correia *et al.*, 1995). Accordingly, this study aimed to chemically assess golden coffee and medium roasted Robusta coffee from Angola - Amboim in order to develop blend formulations incorporating microparticles of golden coffee with added nutritional / prophylactic

value for human health (i.e., incorporating chlorogenic acids derivatives).

Materials and Methods

Material preparation

Sampling of *C. canephora* Pierre ex A. Froehner cv. Robusta coffee beans from Angola (Amboim), supplied by NovaDelta company (Portugal), was carried out according to ISO 4072 (1982), as recommended by the ICO for sampling green coffee in bags. The sampling process began with the selection of green coffee bags, at random (a minimum of 10% of the lot). The selected bags were separated from the lot and, with a probe, 30 \pm 6 g of coffee were collected in triplicate from three different points in the bag (top, middle and bottom). After collection and standardization of the portions, these were mixed, for an overall take of green coffee, with a minimum mass of 1.5 kg. Foreign bodies and defects were analyzed according to ISO 4149 (2005). Green coffee beans (300.0 \pm 0.1 g) were spread over an orange surface, being observed under diffuse natural light and therefore black beans, green, burnt, stained, wrinkled, spongy, broken and brocades were removed. Green coffee was grinded in a hammer mill MIAG (Germany) and sieved to particle size lesser than 425 μ m (which constitute the microparticles to be used in coffee blends, after roasting). The grinded green coffee sample was thereafter dried in a Heraeus oven (140°C for 10 minutes), a process that changed the samples into a golden tone (GC - Golden Coffee). Robusta roasted coffee (RRC) was obtained by a medium roasting level (220 \pm 10°C, for 9 minutes) of Robusta green coffee (Bicho *et al.*, 2011).

Colour analysis

A Minolta colorimeter CR-300 was used to assess the coffee colour, using a D65 illuminant (Bicho *et al.* 2014a,b). White tile, used as standard, with the coordinates: Y=93.10, x=0.3161, y=0.3326 or L* = 97.27, a* = - 0.01, b* = 1.98, for the illuminant D65. The colorimeter was first calibrated to white Yxy coordinates. According to McGuire (1992), the coordinate L* represents the lightness; a* indicates the contribution of red or green; b* the contribution of blue or yellow. The coordinate L* is perpendicular to the plane containing the chromaticity coordinates a* and b* (McGuire, 1992). Considering the coordinates L*a*b*, the colour is expressed through L*C*H°, being: L* the lightness; C* the chroma or saturation (Chervin *et al.*, 1996); H° the tone (or hue angle, which indicates colour variation in the plane formed

by the coordinates a^* and b^*) (Chervin *et al.*, 1996). These parameters were determined considering (McGuire, 1992; Chervin *et al.*, 1996): $C^*=(a^2+b^2)^{1/2}$; $H^\circ=(\arctg(b/a)/6.2832) \times 360$ (if $a > 0$ and $b \geq 0$), or $H^\circ=180+(\arctg(b/a)/6.2832) \times 360$ (if $a < 0$ and $b \geq 0$ or $b < 0$), or $H^\circ=360+(\arctg(b/a)/6.2832) \times 360$ (if $a > 0$ and $b < 0$). The overall colour difference, ΔE , was determined using the equation $\Delta E=[(\Delta L)^2+(\Delta a)^2+(\Delta b)^2]^{1/2}$ (Chervin *et al.*, 1996). Data is the average of five replicates for each coffee sample.

Soluble solids and pH evaluation

Soluble solids and pH were measured according to Bicho *et al.* (2013), at 25°C, after calibration of the electrode with pH 4.0 and 7.0 buffer solutions. Grinded green coffee (10 g \pm 0.1 mg) mixed with water (200 mL) was boiled for 5 min, cooled at room temperature, and the weight was adjusted by adding water. After paper filtration with Whatman No 1, the pH was measured at room temperature. For quantification of soluble solids, 25 mL of the filtrate was dried in a water bath, after which the residue was placed in an oven at 105°C, cooled in a desiccator, and weighed. Data is the average of triplicate for each coffee sample.

Caffeine and trigonelline determination

Caffeine and trigonelline contents were measured as previously reported (Bicho *et al.*, 2011a,b). Coffee samples (1 g \pm 0.1 mg) and magnesium oxide (4.5 \pm 0.5 g) were homogenized in water (100 mL) and placed in a water bath at 90°C for 20 min, with continuous stirring. After cooling, the volume was restored to the original level and the mixture was filtered (Whatman no 1 paper) without washing the solid residue. A 2-mL aliquot of the mixture was added to 10 mL of distilled water and filtered through a 0.45- μ m filter. Caffeine and trigonelline were quantified in an integrated HPLC system (Beckman Coulter, Inc., USA, equipped with a diode-array detector, model 168, column Lichrosorb 100 RP-18 from Merck, 5 μ m particle size, 4 mm x 250 mm), using a 32 Karat Software (version 7.0, Beckman Coulter, Inc., USA). The elution of 20- μ L aliquots was performed with 20 mM phosphate buffer (pH 4.3) and acetonitrile solution (9:1), using a 1 mL min⁻¹ isocratic flow rate and a column temperature of 25°C. Detection was performed at 254 nm, and the retention times for trigonelline and caffeine were recorded at 2 and 13 min, respectively.

For identification and quantification, standard curves were constructed for both caffeine and trigonelline having concentrations of (8 to 1,000 μ g

mL) and (8 to 500 μ g mL) respectively. All extractions and chromatographic analysis were performed in triplicate.

Total phenol content and antioxidant activity evaluation

Total phenol content and antioxidant activity followed Ribeiro *et al.* (2014). Samples (1 g fresh weight) were extracted with 10 mL acetone/water solvent (70:30, v/v). After addition of solvent, each sample was stirred for 2 h, centrifuged (2000g, 15 min., 4°C) and the supernatant was filtered (Whatman no 3, protected from light). Total phenolic content in the extracts from each coffee was determined using the Folin–Ciocalteu method. The extracts were diluted in order to obtain an absorbance in the range of the prepared calibration curve. Then, the Folin–Ciocalteu reagent, the saturated Na₂CO₃ solution and deionized water were added sequentially. After standing for 1 h at room temperature (ca. 20°C) in darkness, the absorbance was measured at 750 nm using a UV 1203 spectrophotometer (Shimadzu, Tokyo, Japan). Each sample data was expressed as mg of GAE per g.

Determination of the antioxidant capacity was carried out as previously detailed by Gao *et al.* (2007). The vitamin C equivalent antioxidant capacity method was used for the antioxidant analysis. This is a reaction of free radicals with blue–green 2,2-azinobis (3-ethylbenzo-thiazoline-6-sulphonic acid) diammonium salt using ascorbic acid as the standard with the resulting antioxidant activity being equivalent to a specific ascorbic acid concentration. This method is based on decolorization of radical cation of 2,2-azinobis (3-ethylbenzo-thiazoline-6-sulphonic acid) diammonium salt, measured as percent reduction of absorbance at 734 nm. The stock solution of the radical cation of 2,2-azinobis (3-ethylbenzo-thiazoline-6-sulphonic acid) diammonium salt (ABTS) was prepared via addition, at 1:1 (v/v), of 7 mM 2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt to a solution of 2.45 mM K₂S₂O₈. The developing reaction took place in the dark, for 16 h. This solution was adjusted with ultra-pure water to absorbance ca. 0.700 \pm 0.020 at 734 nm, measured with a spectrophotometer. For the analysis 1 mL of diluted 2,2-azinobis (3-ethylbenzo-thiazoline-6-sulphonic acid) diammonium salt solution was mixed with 20 μ L of sample and was assayed for inhibition percentage, after 6 min of reaction; triplicates of each sample were averaged to generate each datum point (which implies a total of twelve replicates per sample). The final result was

expressed as equivalent concentration of ascorbic acid (g L^{-1}), using a calibration curve previously prepared.

The determination of the scavenging effect on 2,2-diphenyl-1-picrylhydrazyl free radical by the coffee samples was carried out following Brand-Williams *et al.* (1995) with some modifications. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) stock solution was prepared adding 3.9 mg of 2,2-diphenyl-1-picrylhydrazyl to 100 mL of 70% acetone and then diluted, at 1:1 (v/v). In order to obtain an absorbance of 0.565 ± 0.025 at 515 nm, measured against the blank of acetone at 70% in a spectrophotometer, the above mentioned extract solution was diluted. A 100 μL aliquot of the sample was assayed for inhibition percentage (between 20% and 80%, in order to ensure a linear response of the analytical method), after 30 min of reaction with 3.9 mL of diluted 2,2-diphenyl-1-picrylhydrazyl in the dark; duplicates of each sample were averaged to generate each datum point (which gave rise to four replicates per sample). The final result was expressed as an equivalent concentration of ascorbic acid (g L^{-1}), using a calibration curve previously prepared. The results were expressed as mg of vitamin C equivalent antioxidant capacity per g.

Chlorogenic acids quantification

Chlorogenic acids were analyzed as described by Bicho *et al.* (2011). After mixing $2.0 \text{ g} \pm 0.1 \text{ mg}$ of grinded coffee samples with 10 mL of methanol/water (40:60), followed by mechanical agitation for 30 min, the mixture was centrifuged (9400g, 5 min, 25°C). The supernatant was decanted, 1 mL of Carrez solutions I (aqueous solution of zinc acetate dihydrate and glacial acetic acid, 10.95 g and 1.5 mL, respectively, to a final volume of 50 mL) and II (aqueous solution of 5.3 g of potassium hexacyanoferrate II trihydrate in a final volume of 50 mL) was added for clearing, and thereafter, a methanol/water (40:60) solution was added to a final volume of 100 mL. After 15 min, the mixture was filtered (Whatman no 1 paper) and an aliquot of 10 mL was taken and filtered (PVDF filter, 0.45 μm) prior to a reversed-phase HPLC analysis (Beckman System Gold) using an end-capped, C18, 5- μm Spherisorb S5 ODS-2 column (250 x 4.6 mm) and 32 Karat Software (version 7.0, Beckman Coulter, Inc.). The elution of 20 μL aliquots was performed at ca. 25°C, over 45 min with a 1 mL min^{-1} flow rate, using a linear gradient of 20-70% methanol in 100% tripotassium citrate buffer solution (10 mM, pH 2.5). The column was allowed to re-equilibrate for 10 min. Detection was carried out at 325 and 330 nm using a

diode-array detector (mod. 168, Beckman).

For the isomerization of 5-CQA, in order to obtain 3-CQA and 4-CQA standards, 200 mg of 5-CQA standard was solubilized in 20 mL of distilled water and the pH was adjusted to 8 with ammonium hydroxide (4 M). Then, the solution was boiled for 30 min in a water bath and cooled and the pH adjusted to 2.5 with HCl (4 M). After filtration, samples were used for quantification. The identification of chromatographic peaks and quantification of results were carried out using standard solutions of 5-CQA. To identify the isomers 3-CQA and 4-CQA, the standard 5-CQA isomer was subjected to isomerization, as already described. The peaks appeared with the following sequence: 3-CQA, 3-FQA, 4-CQA, 5-CQA, 4-FQA, 5-FQA, 3,4-diCQA, 3,5-diCQA, and 4,5-diCQA. The calibration curve was obtained from 5-CQA with readings at 325 and 330 nm. The quantification was done using the peak areas as a reference and comparing them with the standard 5-CQA. To quantify each compound, the following equation was used, according to Trugo and Macrae (1984): $c = [(Fr \times \epsilon_1 \times Mr_2 \times A) / (\epsilon_2 \times Mr_1)]$ (being: c , the concentration of the isomer to quantify, in mg L^{-1} ; Fr , the response factor of the standard 5-CQA in mg L^{-1} per unit area; ϵ_1 , the molar absorption coefficient of the standard 5-CQA in $\text{L mol}^{-1} \text{cm}^{-1}$; ϵ_2 , the molar absorption coefficient of the isomer to quantify, in $\text{L mol}^{-1} \text{cm}^{-1}$; Mr_2 , the molecular weight on the isomer under study—CQA = 354.31 g mol^{-1} , FQA = 368.28 g mol^{-1} , diCQA = 516.44 g mol^{-1} ; Mr_1 , the molar mass of acid 5-CQA; A , the peak area of the isomer to quantify). The molar absorption coefficients (3-CQA = 18,400, 4-CQA = 18,000, 5-CQA = 19,500, 3,4-diCQA = 31,800, 3,5-diCQA = 31,600, 4,5-diCQA = 33,200, with $\lambda = 330 \text{ nm}$; 3-FQA = 19,000, 4-FQA = 19,500, 5-FQA = 19,300, with $\lambda = 325 \text{ nm}$), in $\text{L mol}^{-1} \text{cm}^{-1}$, were used. Data were within the detection limits of the method. All extractions and chromatographic analysis were performed in triplicate.

Statistical analysis

Data were statistically analyzed using one-way ANOVA ($P \leq 0.05$) to compare golden coffee to medium roasted coffee. Based on the ANOVA results, a Tukey's test was performed for mean comparison. Different letters in the figures indicate significant differences in a multiple range analysis for 95% confidence level.

Results and Discussion

The predominant colour parameters of green

coffee might vary with the botanical source, nature of soil, agricultural techniques applied and duration and conditions of storage (Clifford, 1987; Coste, 1992). In roasted coffee beans these parameters additionally depend on temperature and time of roasting (Bicho *et al.*, 2014b). In this context, according to CIE, it is possible to express not only overall variations in colour, but also in relation to one or more of the parameters L^* , a^* and b^* , which include all perceived colours. Thus, following the CIELAB space, the collection of chromatic CIEL^{*} a^*b^* and CIEL^{*} C^*H^* , as well as coordinate L^* , revealed significant differences between RRC and GC, with higher values in the latter for all parameters, except a^* (Figure 1).

Relatively to RRC, the chromatic parameters of GC showed a greater contribution (2.52 fold) of yellow component (b^*), whereas lightness (L^*) pointed a higher contribution of white (1.79 fold), with chroma / saturation (C^*) and tone (H^*) prevailing (1.85 and 1.80 fold, respectively). As previously reported by Bicho *et al.* (2014a,b), these data also revealed that colour (green or yellow) differences can be used by the coffee industry as a quick way to identify the type of post-harvest processing of green coffee beans, since differences in coordinate a^* are linked to the processing of green coffee beans. Nevertheless, it also must be pointed that the bean age can also affect the value of a^* (Mendes *et al.*, 2001).

Generation, volatilization and degradation of organic acids occur simultaneously during the roasting process (Bicho *et al.*, 2011a,b). In this context, it was found that (Figure 2) pH was slightly (although significant) reduced in RRC, possibly resulting from the relative lesser proportion between total aliphatic acids and the minimum processed green bean mass (Woodman, 1985). Besides, organic acids losses and chemicals volatilization during pyrolysis might trigger soluble solids losses (Bicho *et al.*, 2011), mostly due to increased cell breakage (thus, celluloses/carbohydrates breakdown and protein denaturation), justifying the lower soluble solids in RRC, as compared to GC (Figure 2).

During coffee roasting, bean temperature surpasses 200°C, mostly due to a combination of external with internal heating (as temperature changes inside the roaster between 210-230°C). Therefore, as the temperature of caffeine sublimation (178°C) is exceeded, considerable losses of this compound occurred in RRC (Figure 2), as also observed in Bicho *et al.* (2011a,b; 2013). Moreover, relatively to GC, caffeine was reduced only to 84% in RRC (Fig. 2), eventually because during roasting the sublimation point also increases due to the internal pressure rise,

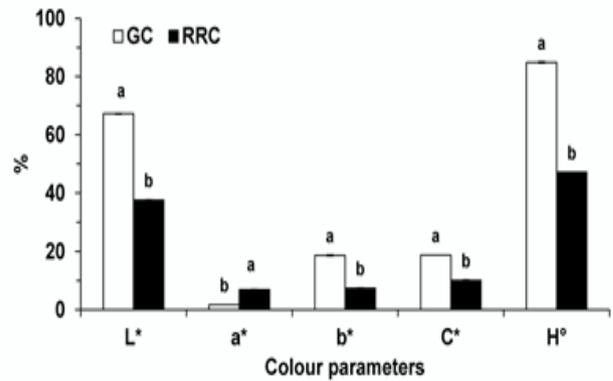


Figure 1. Chromatic parameters of golden coffee (GC) and Robusta roasted coffee (RRC) samples. For each parameter, the mean values \pm SE ($n = 5$) followed by different letters express significant differences between GC and RRC, after a mean comparison by the Tukey's test at a 95% confidence level

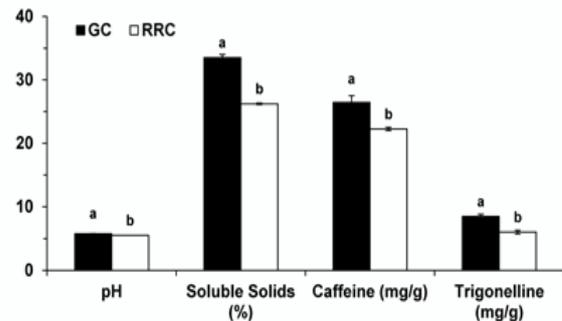


Figure 2. Values for of pH, as well as for soluble solids, caffeine and trigonelline contents in golden coffee (GC) and Robusta roasted coffee (RRC) samples. For each parameter, the mean values \pm SE ($n = 3$) followed by different letters express significant differences between GC and RRC, after a mean comparison by the Tukey's test at a 95% confidence level

triggered by carbon dioxide synthesis and low vapor diffusion produced through the outer layers of the grain (Bicho *et al.* 2011a,b; 2013). Nevertheless, in RRC the amounts of caffeine remained quite similar to other roasted coffee types and above the minimum (0.7%) required for marketing (Bicho *et al.* 2011a,b; 2013).

The content of trigonelline followed a similar pattern to caffeine (Figure 2), significantly decreasing in RRC to about 71%. Trigonelline reduction leads to pyridines, N-methylpyrrole and, due to demethylation and niacin synthesis (Bicho *et al.*, 2011a,b; 2013), being the amounts similar to other roasted coffees from different origins (Clifford, 1985; ICAB, 2007; Bicho *et al.*, 2011a,b; 2013). Considering the chemical specificity for determination of the polyphenol content and antioxidant activities (Ikawa *et al.*, 2003; Sharma and Bhat, 2009; Walker *et al.*, 2009),

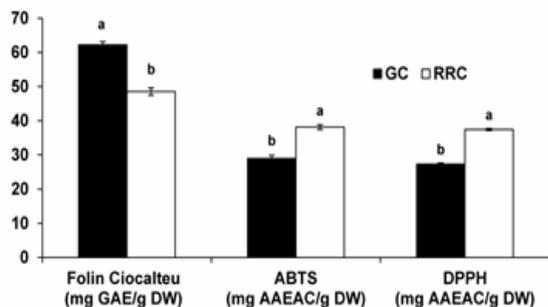


Figure 3. Total polyphenol content (given by Folin Ciocalteu method, expressed in mg gallic acid equivalent g^{-1}) and antioxidant activity (given by ABTS and DPPH methods, expressed in mg vitamin C equivalent antioxidant capacity g^{-1} in golden coffee (GC) and Robusta roasted coffee (RRC). For each method, the mean values \pm SE ($n = 12$) followed by different letters express significant differences between GC and RRC, after a mean comparison by the Tukey's test at a 95% confidence level

it was found that, according to the Folin Ciocalteu method, the total polyphenol content (Figure 3), was significantly higher in GC (ca. 1.28 fold), with 62.21 mg of GAE g^{-1} .

On the other hand, the ability of RRC to scavenge radicals of ABTS and DPPH (Figure 3) was significantly higher than that of GC (about 31.7% and 37.2%, respectively), which was consistent with similar data obtained by Brezová *et al.* (2009), as increased antioxidant activity can be observed after roasting (Sánchez-González *et al.*, 2005). Although some natural antioxidants are eliminated during the heating, the antioxidant properties can be therefore maintained or improved by the formation of new antioxidants (Ribeiro *et al.*, 2014) what agrees with our results (Figure 3).

Within the total polyphenol content of coffee beans, the most abundant phenolic compounds are chlorogenic acids (CGA) (Bicho *et al.*, 2011a,b; 2013; Trugo and Macrae, 1984). With roasting, the contents of 3-CQA and 4-CQA did not vary significantly between GC and RRC (Figure 4), whereas 5-CQA significantly decreased in RRC. Therefore, 5-CQA was the CQA with higher reduction rates triggered by roasting, what agrees with other reports (Farah *et al.*, 2005). Due to such large decrease, total CQA significantly decreased as well in RRC (Figure 4). This CQA reduction is related to roasting that triggers various reactions, such as acyl migration, hydrolysis, oxidation, fragmentation, polymerization, and association with denatured proteins that led to

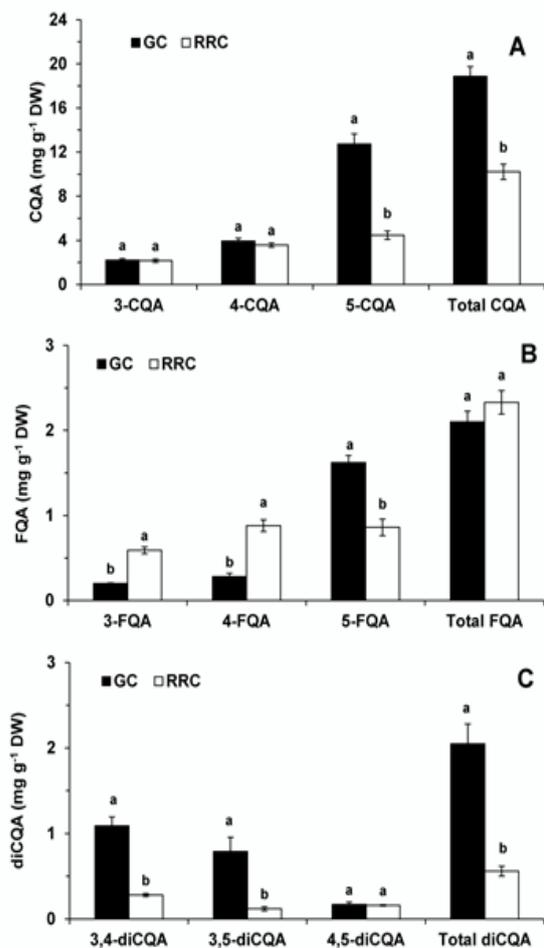


Figure 4. Content of A) 3-CQA, 4-CQA, 5-CQA, Total CQA, B) 3-FQA, 4-FQA, 5-FQA, Total FQA, and C) 3,4-diCQA, 3,5-diCQA, 4,5-diCQA and Total diCQA in golden coffee (GC) and Robusta roasted coffee (RRC) samples. For each parameter, the mean values \pm SE ($n = 3$) followed by different letters express significant differences between GC and RRC, after a mean comparison by the Tukey's test at a 95% confidence level

lactone synthesis, due to the dehydration of quinic acid residue, with the consequent formation of a ring, resulting from the participation of acid substituent groups at position C1 and alcohol in position C5.

Relatively to GC, in RRC the contents of 3-FQA and 4-FQA were significantly increased (by 195% and 214% respectively). In opposition, 5-FQA was reduced to about half of the GC value (Figure 5). Due to this opposite behavior of FQAs, the Total FQA was only slightly increased (11%) in RRC (Figure 4). As regards diCQAs, the 3,4-diCQA, 3,5-diCQA, and Total diCQA contents were significantly higher in GC (3.89, 6.58 and 3.66 fold, respectively). Only 4,5-diCQA content did not vary with roasting (Figure 4). Moreover, among the CGA compounds, Total diCQA are the most sensitive to roasting, whereas Total FQA (due to 3-FQA and 4-FQA) slightly increased.

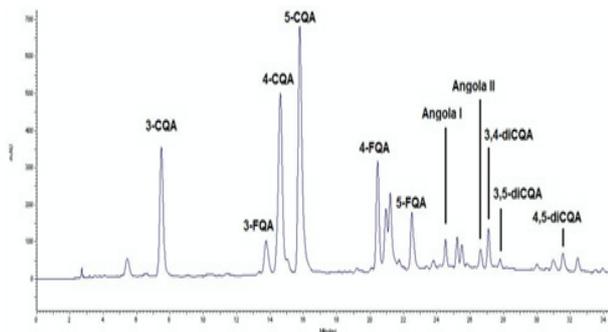


Figure 5. Typical chromatogram (at 325 nm) of CGAs in Robusta medium roasted coffee (RRC), including, namely, chlorogenic acids derivatives such as N- β -caffeoyl-tyrosine (Angola I) and p-coumaroyl-N-tyrosine (Angola II) that are still present

Notably, although without quantification, it was noted that the genetic markers of Robusta coffee beans from Angola – Amboim, the chlorogenic acids derivatives Angola I (N- β -Caffeoyl-tyrosine) and Angola II (ρ -Coumaroyl-N-tyrosine), were detected both in minimally processed green coffee (GC) and after medium roasting (RRC) (Figure 5). This agrees with previous reports that identified these compounds even after a medium roasting (Correia *et al.*, 1995).

Conclusion

The chromatic parameters, which allow a fast, reliable, low cost and non-destructive analysis, easily differentiate GC from RRC samples within the established range of the international market, since the spectral composition of incident light leads to different colour perception at the collection of chromatic CIEL*a*b*, CIEL*C*H° and coordinate L*.

As roasting in RRC prompts generation, volatilization and degradation of organic acids, decreased soluble solids, caffeine and trigonelline is cross linked to polyphenol content and antioxidant activity deviation. Quantitatively, among polyphenols, total CQA and diCQA prevail in GC and, similarly to Angola I (N- β -Caffeoyl-tyrosine) and Angola II (ρ -Coumaroyl-N-tyrosine). Total FQAs was similar in both GC and RRC. Therefore, GC microparticles presents the highest phenolic contents of the studied compounds and relevant antioxidant activity.

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