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Potential antioxidant of brazilian coffee from the region of *Cerrado*

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Abstract

Coffee is one of the most consumed beverages in the world. Its chemical composition may have varied according to the planting site, degree of roasting, and method of preparation. This work aimed to evaluate the antioxidant activity of coffee from the region of Cerrado in the State of Minas Gerais, Brazil. The evaluation was performed with samples roasted at two different levels (traditional and extra dark) and using two different preparation methods (decoction and infusion) that reflect the conditions of preparing coffee. *In vitro* antioxidant activity by ABTS and DPPH radical methods and the concentration of total phenolic compounds and caffeine were determined. Samples made by decoction showed a higher content of phenolic compounds and no significant difference was observed between the degrees of roasting. However, the antioxidant activity and caffeine concentration of the extra dark samples were higher than those of the traditional samples for both preparation methods. The decoction preparation method was better for extracting phenolic compounds and the extra dark roast showed a higher concentration of caffeine and antioxidant activity. The samples showed a high antioxidant activity, indicating the coffee from Cerrado is an important source of antioxidants.

Keywords: decoction; infusion; roasting degree; phenolic compounds; caffeine.

Practical Application: In our study, we used conditions of preparation of the coffee beverage and we tried to establish correlations with the possible ingestion of these compounds in recommended amounts of consumption, comparing different degrees of roasting and methods of preparation. We observed that coffee from the Cerrado region presents as an important source of compounds with antioxidant activity and that the studied variables were significant in different ways in different responses. Being these results pioneering and important for the knowledge and valorization of Brazilian coffee.

1 Introduction

Coffee is considered the most important alimentary raw material in the world and is produced extensively in about 60 tropical and subtropical countries (Lashermes et al., 2008; Vieira, 2008). Brazil is the largest coffee producer in the world, accounting for 30%of the international market, and the second largest coffee consumer, just behind the United States (Brasil, 2014).

The region of Cerrado is located in the northwest of the state of Minas Gerais, which produces 53% of Brazilian coffee (Ensei, 2010; Associação Brasileira da Indústria de Café, 2014a; Sindicato da Indústria de Café do Estado de Minas Gerais, 2014). This region was a pioneer in implementing the denomination of origin for coffee, indicating that the product has characteristics related with aspects of the geographical environment such as climate, altitude, and soil (Região do Cerrado Mineiro, 2014).

The roasting process involves the application of heat to the beans ranging from 200 to 240 °C for a period of time that depends on the desired final product. There are three main levels in coffee roasting, light, medium, and dark. The main difference in the process is the time that the coffee remains in the roaster and this process can affect the composition of the coffee indifferent ways (Lerici & Nicoli, 1990).

The coffee beverage has unique characteristics, which are caused by the partial degradation of phenolic compounds and the development of other bioactive compounds that occur during the roasting process. The antioxidant activity of coffee is produced by components present in the green bean such as chlorogenic acid, and compounds formed during the roasting process such as melanoidins formed by the Maillard reaction (López-Galilea et al., 2006; Santos et al., 2007; Daglia et al., 2008; Babova et al., 2016). Components such as caffeine produce high antioxidant activity and are relatively unaffected during the roasting process (Rodarte et al., 2009; Bicchi et al., 1995). Because of these particularities, the influence of process in the composition of coffee is a subject of interest in many studies.

The antioxidant potential of both coffee beans and beverages has been widely studied. Coffee drink consumption has been associated with several health benefits such as decreased risk of chronic diseases as cancer and diabetes and a reduction of the oxidative damage caused by free radicals (Olthof et al., 2001; Roginsky & Lissi, 2004; Silva et al., 2007; Stelmach et al., 2015; Oliveira-Neto et al., 2016; Ballesteros et al., 2017).

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In this context, the objective of this work was to determine the influence of roasting levels and preparation methods on the antioxidant activity of Brazilian coffee from the region of Cerrado in the State of Minas Gerais.

2 Materials and methods

2.1 Obtaining coffee samples and preparation of the drink

Two samples (2 kg each) of roast arabica coffee beans were collected at a company located in the city of São Gotardo, Minas Gerais, Brazil in the region of Cerrado, the first being traditional coffee (medium level of roasting) and the second extra dark coffee (dark level of roasting). Samples were ground to an average particle size (20 mesh), suitable for preparation in a paper filter and espresso machine (Associação Brasileira da Indústria de Café, 2014b).

To prepare the beverage, 12 g of grounded sample (equivalent to three to four tablespoons) was diluted in one liter of mineral water. Using the method of decoction, water was boiled with the ground coffee until reaching a temperature of 95 °C and after this was filtrated on filter paper. Using the infusion method, the water was boiled at a temperature of 95 °C and brought into contact with the ground coffee on the filter paper. After preparing the beverage, the samples were stored in plastic tubes and frozen at -18 °C until the moment of analysis.

The four samples of different degrees of roasting and preparation methods were annotated in this way:

CTI: Coffee traditional by infusion;

CTD: Coffee traditional by decoction;

CEI: Coffee extra dark by infusion;

CED: Coffee extra dark by decoction.

2.2 Determination of phenolic compounds

The content of phenolic compounds in the beverage was determined by the Folin-Ciocalteu method, using gallic acid as standard (Singleton et al., 1999).

A sample of 20 μL of the diluted coffee at a concentration of 1:20 (v/v) was transferred to a test tube and 100 μL of the Folin-Ciocalteau solution at 10% were added to it. After five minutes, 75 μL of potassium carbonate solution at 7.5% were added and the mixture was kept at room temperature and protected from light for 40 minutes. The reading of absorbance was done in the UV-1230 spectrophotometer (Shimadzu UV 1203 model) at 740 nm.

The results were calculated from a standard curve with known concentrations (5 to 80 $\mu g \cdot mL^{-1}$) of gallic acid and the results were expressed in mg of Gallic Acid (GA) per mL of beverage of coffee and per 100 g of ground coffee grounds taking into account the preparation of the samples (Al-Duais et al., 2009).

2.3 Evaluation of antioxidant activity

Determination by the DPPH radical method

The antioxidant activity was determined in triplicate using the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) (Brand-Williams et al., 1995).

In test tubes were added 66 μL of the diluted coffee at a concentration of 1:100 and 134 μL of DPPH radical 0.5 mM diluted in ethanol solution. Then the tubes were stirred and incubated for 45 minutes at room temperature and protected from light. The reading of absorbance was done in UV-1230 spectrophotometer (Shimadzu UV 1203 model) at 517 nm.

The results were calculated from a standard curve with known concentrations (from 0.01 to 0.10 $\mu Mol \cdot 0.5\ mL^{-1}$) of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and expressed as the Trolox equivalent antioxidant capacity (μMol of TEAC per mL of coffee and per 100 g of ground coffee) (Al-Duais et al., 2009).

Figure 1 shows the stabilization of the DPPH radical by an antioxidant.

Determination by the ABTS radical method

The antioxidant activity was determined in triplicate using the free radical ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) (Re et al., 1999).

The ABTS radical was formed by the reaction of ABTS solution 7 mM with a potassium persulfate solution 140 mM, and incubated for 16 hours at room temperature and protected from light. Once formed, the radical was diluted with ethanol 99% at a concentration of 1:100.

In test tubes were added 20μL of the diluted coffee at a concentration of 1:40 and 220 μL of ABTS radical solution. The reading

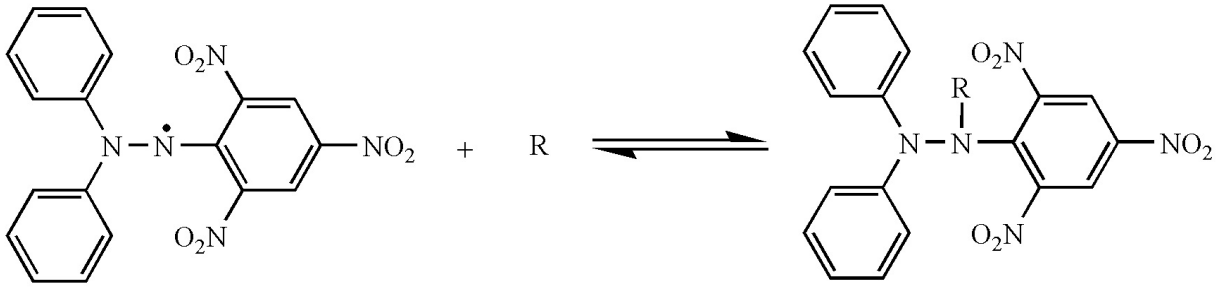

Figure 1. Structure of free and stable DPPH radicals.

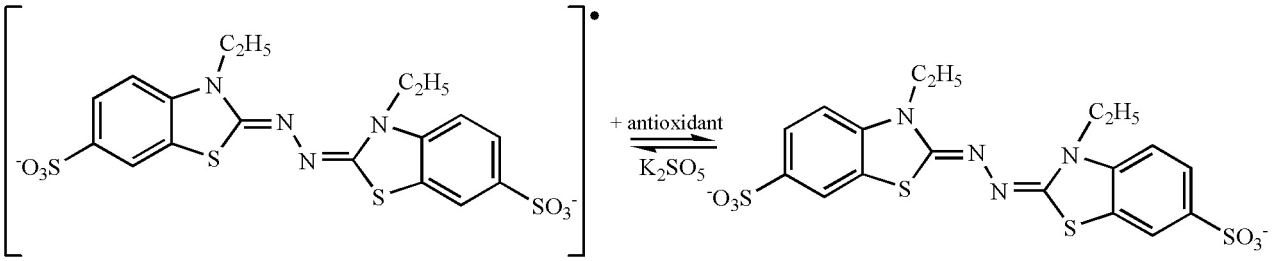

Figure 2. Structure of free and stable ABTS radicals.

of absorbance was done in the UV-1230 spectrophotometer (Shimadzu UV 1203 model) at 730 nm after six minutes of reaction. The results were calculated from a standard curve with known concentrations (from 0.04 to 0.1 μmol·mL^{-1}) of Trolox and expressed as the Trolox equivalent antioxidant capacity (μmol of TEAC per mL of coffee and per 100 g of ground coffee) (Al-Duais et al., 2009).

Figure 2 shows the stabilization of the ABTS radical by an antioxidant.

2.4 Quantification of caffeine

The coffee samples were filtered on a Millipore membrane made of cellulose ester, with 0.45 μm pore and a 47 mm diameter, white, smooth, 100/CX and then diluted in the proportion of 1:50 before being injected into the HPLC. A curve was done with a caffeine standard of 10 to 100 ppm. The chromatography was performed on an Agilent HP series 1100 (UV/Vis) with diode array detector (DAD), quaternary pump and a UV detector with a wavelength of multiple waves (MWD), a cooled column compartment (30 °C), and a Phenomenex column (4.6 × 250 mm - 5μm). The mobile phase consisted of 20% of water and 80% of methanol for 25 minutes and the gradient was isocratic elution. The flow rate was 1 mL·min^{-1}, the injection volume was 20 μL. The DAD was set to collect the signal at 254 nm.

2.5 Statistical analysis

The experiment was completely randomized with three repetitions and analyses were performed in triplicate. An analysis of variance (ANOVA) and Tukey test were performed at 5% of significance.

3 Results and discussion

Coffee is one of the most consumed drinks in the world, and is known for the presence of several compounds with antioxidant activity. Among the chemical classes responsible for this activity are mainly phenolic compounds, which are considered important antioxidants. These compounds present an aromatic ring bearing one or more hydroxyl groups and their structures may vary from that of the simple phenolic molecule of a complex high-molecular weight polymer. Their mechanism of antioxidant activity is linked with their ability to act as Lewis acids stabilizing free radicals (Balasundram et al., 2006).

The results of total phenolic compounds for the coffee samples are shown in Table 1.

Table 1. Total phenolic compounds of coffee traditional and extra dark subjected to two preparation methods: decoction and infusion.

Samples	mgGA·mL^{-1} of beverage	mgGA·100g^{-1} of ground coffee
CTD	1.2**0** ± 0.02^{a}	2946 ± 3^{a}
CTI	1.0**0** ± 0.03^{b}	2364 ± 3^{b}
CED	1.1**0** ± 0.04^{ab}	2696 ± 5^{ab}
CEI	1.0**0** ± 0.02^{b}	2449 ± 2^{b}

Legend: Means followed by different letters in the same column indicate significant differences (p <0.05) by Tukey test. CTD: Coffee Traditional Decoction; CTI: Coffee Tradicional Infusion; CED: Coffee Extra dark Decoction; CEI: Coffee Extra dark Infusion.

The results showed a significant difference (p<0.05) between the two methods of preparation (decoction and infusion). Traditional coffee prepared by decoction presented a larger amount of total phenolic compounds than the samples prepared by infusion and for extra dark sample the results were higher but not statistically different.

However comparing the amount of total phenolic compounds in the coffee did not change significantly (p>0.05) with different degrees of roasting (traditional and extra dark) using the same preparation method (decoction or infusion).

The traditional coffee submitted to the decoction method had a higher concentration of phenolic compounds in the beverage and grain when compared to the coffees submitted to the infusion method, suggesting that this fact occurred by the contact of the coffee with the water.

Morais et al. (2008) observed that the phenolic compound content in coffee from Cerrado decreased with an increase in the degree of roasting, and the quantity of phenolic compound was higher than the current study reaching values up to 3.000 mgGA·100g^{-1} of coffee. However, Morais et al. (2008) used an extraction with methanol to conduct the analysis, and this does not reflect the method of preparing coffee in real-world circumstances. This can be a problem because the results can be overestimated when using organic solvents that more readily extract compounds but do not represent the conditions of intake in the real world.

On the other hand. **S**ome studies showed difference in phenolic compound content between different degrees of roasting. It was observed that when comparing the concentration of phenolic compounds in green coffee grains and in dark roasts, there was a linear decrease in total phenolic compounds (Sacchetti et al., 2009). Farah et al. (2005) reported that some phenolic compounds present in coffee, such as chlorogenic acids,

are degraded into low molecular weight compounds through the roasting process and are partly transformed into quinolactones due to dehydration and the formation of intermolecular bonds.

The coffee is one of the most consumed drinks of the world, studies comparing the influence of decoction and infusion preparation methods were not found in the literature, perhaps because the decoction method is not popular in many countries. Sometimes researchers have not used methods for extracting phenolic compounds that represent the preparation of the beverage.

Traditional coffee, using both methods of preparation (decoction and infusion), presented lower caffeine content ($p < 0.05$) than the extra-strong coffee, both in the beverage and ground coffee (Table 2).

Caffeine is the main component with psychoactive action in coffee. Among its effects is an increase in cognitive performance, alertness, ability to concentrate, auditory vigilance and visual retention time. It also decreases drowsiness and fatigue and acts as an aid in weight loss due to its thermogenic properties (Nehlig, 2004). The main mechanism of action of caffeine is caused by its structural similarity with the molecule of adenosine (potent endogenous neuromodulator). Thus caffeine can bind to adenosine receptors by blocking them, and the inhibitory action of adenosine is prevented by providing a stimulating effect of caffeine (Biaggioni et al., 1991; Dunwiddie & Masino, 2001).

Higher degree of roasting produced higher results ($p<0.05$) for the samples. Our results were similar to those found by Morais et al. (2008), where the amount of caffeine was around 1.600 to 2.000 mg·100g^{-1} of ground coffee, and the higher degree of roasting produced more caffeine than light roasting.

Caffeine is a stable molecule when subjected to high temperatures and because of that the concentration is not affected by the roasting process. The lower amounts in the sample with a low degree of roasting can be explained by the fact that caffeine does not stay totally free of coffee structure and when it is submitted to the roasting process, more intense caffeine can be more available to be extracted from the coffee (Monteiro & Trugo, 2005).

There was no statistical difference ($p > 0.05$) for the antioxidant activity by the DPPH method for both groups and types of samples (beverage and coffee powder), as described in Table 3. However, by the ABTS method, traditional coffee in both groups Preparation (decoction and infusion) presented lower antioxidant activity ($p < 0.05$) than extra dark coffee.

Camargo & Toledo (1998) used the same methodology to prepare the coffee and found a low amount of caffeine when they analyzed 14 brands of Brazilian coffees, concluded that the caffeine concentration can be affected by many factors such as the type of beans and preparation methods, but the authors didn't evaluate coffee from Cerrado.

Several analyses have been used to quantify the antioxidant activity in fresh fruits, vegetables, and others raw foods using radical methods including 2,2′-Azino-bis(3-ethylbenzothia zoline-6-sulfonic acid) (ABTS), diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and oxygen radical absorption capacity (ORAC) (Thaipong et al., 2006; Liang et al., 2016).

DPPH and ABTS results presented different results that can be related to the different radical structures applied (Figures 1 and 2). These structures have different types of affinities that involve electrostatic, steric hindrance, and polar interactions among others, which promote different response by several methods of analysis for antioxidant activity, showing the importance of using different methodologies. These results show the importance of utilizing different methodologies to quantify antioxidant activity.

Del Castillo et al. (2002) found an increase in the antioxidant activity of Arabica coffee in the reaction with ABTS in accordance with the degree of roasting when compared with green coffee beans. A higher antioxidant activity in coffee with darker as opposed to lighter roasting was found, and this effect to the increase of products like melanoidins formed during the Maillard reaction in the roasting process (Sánchez-González et al., 2005).

According Nicoli (1998), the formation of melanoidins, which have high antioxidant potential, occurs only at certain stages of roasting which would explain the superiority of antioxidant activity for the sample with a higher degree of roasting. Melanoidins are molecules with important antioxidant activity in coffee and they can represent 25 to 47% of the total activity depending on the assay used (Perrone et al., 2012). Using the ABTS and DPPH methods melanoidins account for about 40% of the antioxidant activity, in addition, it has also been

Table 2. Amount of caffeine of coffee traditional and extra dark subjected to two preparation methods: decoction and infusion.

Samples	mg caffeine·mL^{-1} of beverage	mg caffeine·100g^{-1} of ground coffee
CTD	0.7**0** ± 0.01^{b}	1644 ± 1^{b}
CTI	0.6**0** ± 0.02^{c}	1445 ± 2^{c}
CED	0.8**0** ± 0.01^{a}	1898 ± 1^{a}
CEI	0.8**0** ± 0.01^{a}	1879 ± 1^{a}

Legend: Means followed by different letters in the same column indicate significant differences ($p < 0.05$) by Tukey test. CTD: Coffee Traditional Decoction; CTI: Coffee Tradicional Infusion; CED: Coffee Extra dark Decoction; CEI: Coffee Extra dark Infusion.

Table 3. Antioxidant activity of coffee traditional and extra dark subjected to two preparation methods: decoction and infusion, by the DPPH and ABTS method.

Samples	µMol TEAC·mL^{-1} of beverage (DPPH)	µMol TEAC·100g^{-1} of ground coffee (DPPH)	µMol TEAC·mL^{-1} of beverage (ABTS)	µMol TEAC·100g^{-1} of ground coffee (ABTS)
CTD	7.0 ± 0.9^{a}	16633 ± 9^{a}	4.4 ± 0.3^{b}	10460 ± 3^{b}
CTI	6.6 ± 0.7^{a}	15816 ± 7^{a}	6.6 ± 0.2^{a}	15816 ± 2^{a}
CED	5.6 ± 0.5^{a}	13345 ± 6^{a}	5.7 ± 0.2^{a}	13520 ± 2^{a}
CEI	6.7 ± 0.9^{a}	16032 ± 9^{a}	5.6 ± 0.4^{a}	13309 ± 4^{a}

Legend: Means followed by different letters in the same column indicate significant differences ($p < 0.05$) by Tukey test. CTD: Coffee Traditional Decoction; CTI: Coffee Tradicional Infusion; CED: Coffee Extra dark Decoction; CEI: Coffee Extra dark Infusion.

Table 4. Intake of phenolic compounds and equivalents in TEAC and caffeine for the recommended daily amount of coffee.

Samples	mg of GA·300mL^{-1}	µMol TEAC (DPPH)·300mL^{-1}	µMol TEAC (ABTS)·300mL^{-1}	mg of caffeine·300mL^{-1}
CTD	371.2^{a}	2095.7^{a}	1318.0^{b}	207.1^{b}
CTI	297.8^{b}	1992.9^{a}	1437.0^{b}	182.1^{c}
CED	339.7^{ab}	1681.4^{a}	1703.6^{a}	239.1^{a}
CEI	308.6^{b}	2020.0^{a}	1677.0^{a}	236.8^{a}

Legend: Means followed by different letters in the same column indicate significant differences ($p < 0.05$) by Tukey test. CTD: Coffee Traditional Decoction; CTI: Coffee Tradicional Infusion; CED: Coffee Extra dark Decoction; CEI: Coffee Extra dark Infusion.

suggested that chlorogenic acids in green coffee beans produce antioxidants under heat treatment, suggesting that the roasting conditions for coffee play an important role in the formation of antioxidants (Kamiyama et al., 2015).

The daily intake of coffee is divided into three categories, namely low (≤2 cups), moderate (3 to 4 cups), and high (≥5 cups) (Van Woudenbergh et al., 2008). It is considered that a cup contains 75mL of coffee; therefore, moderate coffee consumption would be a maximum of 300mL per day (in 4 cups).

Moderate coffee consumption (3 to 4 cups per day) has shown benefits in preventing cardiovascular disease in men and women from South Korean. This intake was associated with a lower prevalence of subclinical coronary atherosclerosis in a large sample of men and women in South Korea apparently free of clinical evident of cardiovascular disease (Choi et al., 2015).

Table 4 shows the relationship between intake of phenolic compounds and equivalents in TEAC and caffeine for the moderate amount of coffee (300 mL or 4 cups per day).

Coffee is a significant source of compounds with antioxidant activity (Passos et al., 2017) even if there is no ingestion daily intake recommended for these compounds. For example, Falcão et al. (2007) found values ranging from 2.6 to 9.1 µMol TEAC·g^{-1} (determined by the DPPH and ABTS method) and a total phenolic content raining from 63.4 to 235.4 mg GAE·100g^{-1} in grape juice, which is considered an important source of antioxidant compounds.

In addition coffee is considered the most important source of antioxidant compounds in Brazilian's diet when compared with mate, acai and beans. Confirming the importance of this beverage (Torres & Farah, 2017).

In vitro studies in microsomes, erythrocytes, monocytes, and oxidized LDL designs afforded significant inhibition of lipid peroxidation, mainly to the phenolic compounds like chlorogenic acids and in particular caffeic acid, indicating the coffee drink is a source of natural antioxidants (Smith, 2002).

In our work the results obtained for the antioxidant activity of coffee with two roasting degrees and preparation methods were higher than values for exotic fruits from Brazil considered good sources of antioxidants such as passion fruit, *Araça* and *Jaracatia* (Genovese et al., 2008). In addition. The amount of caffeine did not exceed the daily limit (250 mg.day^{-1}) indicated by some authors (Grobbee et al., 1990).

Coffee consumption is recommended with a healthy diet and can elicit many health benefits by decreasing the risk of chronic diseases and reducing the oxidative damage caused by free radicals (Olthof et al., 2001; Roginsky & Lissi, 2004; Silva et al., 2007; Van-Woudenbergh et al., 2008).

4 Conclusions

Different degrees of roasting and preparation methods influenced antioxidant properties of the Brazilian coffee from Cerrado. It was conclude that in the conditions of this study the coffee extra dark prepared by decoction had more phenolic compounds and higher antioxidant activity, being this degree of roasting and method of preparation the most indicated in this aspect. Coffee from Cerrado showed high concentrations of these compounds that are proven to be beneficial to human health.

Acknowledgements

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