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Influence in the Drink Preparation Mode Associated Coffee the Antioxidant Capacity of Different Brands

[1]Ázula Narayama Malacco Ferreira, [2]Mariana Mirelle Pereira Natividade, [1]Roberto Gonçalves Junqueira, [1]Scheilla Vitorino Carvalho de Souza and [1]Renata Adriana Labanca

[1]Department of Food, Faculty of Pharmacy, Federal University of Minas Gerais, Av. Antônio Carlos 6627, Campus da UFMG, Pampulha, CEP 31.270-010, Belo Horizonte, MG, Brasil
[2]Department of Nutrition, Federal University of Lavras, Campus Universitário, Caixa Postal 3037, CEP 37200-000, Lavras, MG, Brasil

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Corresponding Author:
Renata Adriana Labanca
Department of Food, Faculty of Pharmacy, Federal University of Minas Gerais, Av. Antônio Carlos 6627, Campus da UFMG, Pampulha, CEP 31.270-010, Belo Horizonte, MG, Brasil
Tel: +55 (31) 3409-6928
Email: renata@bromatologiaufmg.com.br

Abstract: Coffee is one of the most consumed beverages worldwide and commonly present on Brazilian tables. It is known for its health benefits, mainly ascribed to its antioxidant compounds. Because of a lack of standardized analytical methods to evaluate these compounds in coffee, the variety of products sold, as well as the influence of the form of preparation of the beverage upon the content of the antioxidants, this work is intended to evaluate the reducing capacity of beverages prepared with samples of roasted and ground coffee, as well as the influence of the preparation, employing the Folin Ciocalteu (FC) spectrophotometric method. After the validation of the method, the reducing capacity in commercial coffee beverages was evaluated as well as the influence of different ways of preparation, including infusion and decoction. The optimization steps and validation of spectrophotometric method FC allowed to obtain more reliable results, facilitating the analysis of the evaluated coffee samples and ensuring greater security in the analysis of the results. The preparation technique also influenced the reductive capacity of drinks, as different preparation methods resulted in different values and the decoction the most efficient. This information can be valuable to consumers, who can choose to domestic preparation techniques that result in a coffee drink with better content of antioxidant compounds.

Keywords: Antioxidant Activity, Coffee, Influential of Preparation Technique, Phenolic Compounds, Reference Measurement

Introduction

Coffee is the most consumed beverage in the world and a valuable product in the international market. The Brazilian domestic coffee consumption is growing year by year. Among the reasons for this increased consumption, we highlight the improvement in the quality of Brazilian production and public perception regarding the benefits of coffee comsuption (Farah and Donangelo, 2006). *Coffea Arabica* (Arabica) and *Coffea canephora var. Robusta* (Robusta) are the two main species of the genus Coffea that are cultivated for commercial production. Arabica is considered to be superior to Robusta because of its milder and more flavorful taste (Zuorro and Lavecchia, 2013). Several compounds in coffee have been studied for their antioxidant potential, such as the melanoidins, formed during the roasting process, caffeine and various phenolic compounds. Due to the presence of these substances, coffee has been ascribed effects on human health (Borrelli *et al*., 2002).

Phenolic substances are the most commonly found active compounds in fruits and vegetables, known as potent antioxidants and natural antagonists of pathogens. They are either found free in plants or bound to sugars and proteins forming esters or, more often, glycosides. Reducing properties of these compounds, leads to the neutralization of free radicals and to chelation of transition metals, both of which acting as the initiation step in the process of oxidative propagation (Naczk and Shahidi, 2004). In this perspective, the coffee is a major source of antioxidants and may contribute markedly to

the intake of phenolic compounds. Other foods such as chocolate and teas also contain phenolic compounds, but coffee has a higher concentration of some compounds, such chlorogenic acid, caffeic acid and coumaric acid compared to others sources (Crozier *et al*., 2009).

The overall objective of this study was to optimize and validate a method for investigating reducing capacity in coffee, to implement this method to compare the reducing capacity of beverages prepared from roasted and ground coffee samples commercially available in Brazil, as well as the influence of the preparation method, using the spectrophotometric method Folin Ciocalteu. The objective was also to optimize the extraction conditions and spectrophotometric quantification of reducing compounds in coffee and to validate the spectrophotometric method. Then the influence of the beverage preparation, infusion and decoction, the reducing capacity.

Materials and Methods

Chemicals

Methanol reagent was supplied by Vetec Química Fina Ltda (Rio de Janeiro, Brazil). Folic Ciocalteu and gallic acid standard were purchased from Sigma Chemical Co. (St. Louis, MO). The solutions were prepared with ultra pure-water (Milli-Q Plus system; Millipore Corp., Billerica, MA).

Samples

For the characterization of reducing capacity, roasted and ground coffee samples were purchased in supermarkets in the city of Belo Horizonte – Minas Gerais - Brazil, in 2012, within the period of validity and stored properly. In total 38 samples were purchased, comprising 14 different brands and various degrees of roasting (Table 1). For the optimization steps and method validation one of the commercial samples was selected at random. All samples were kept in their original packaging and stored under refrigeration, between 4 and 10°C (±1°C) until the time of analysis. Since the analyzes with coffee beverage, made with these extracts and gallic acid standard solution, were done due to the need to test for potential interferences from matrix components in the spectrophotometric responses to tests using the standard analyte added to the matrix. The absorption data were submitted to analysis of variance and mean test Tukey test with significance $\alpha = 0.05$ and the selected wavelength of the largest response.

Extraction Conditions

The extraction process was optimized by means of a full factorial design (3×3×2, in triplicate). The following factors were evaluated: Type of solvent (deionized water, methanol: Deionized water (1+1, v/v) methanol: Deionized water (1+4, v/v)), standing time (20, 40 and 60 min) and number of extractions (1 or 2). The extracts (250 µL) were made up to 1 mL with deionized water and subjected to spectrophotometric analysis by the Folin-Ciocalteu method at 760 nm as described above in random order.

Analytical Methods

An aliquot of 500 µL of the drink was diluted with 9.5 mL of deionized water, this being the best evaluated in the previous extraction step. The solution was stirred and then 250 µL was transferred to a tube and added 750 µL of deionized water and 5 mL of Folin-Ciocalteu reagent at 10%. The tube was stirred in vortex. After 3-8 min, 4 mL of 7.5% sodium carbonate was added. The samples were stirred again and were placed to rest for two h, protected from light, at a temperature from 16 to 18.5°C, approximately. The spectrophotometric reading was taken at 760 nm in a glass cuvette. The matrix matched curves was constructed with gallic acid (20.0; 30.0; 40.0; 50.0; 60.0 mg mL). The straight line equation was used to calculate the reductive capacity of each sample, expressing the values as mg gallic acid equivalents per 100 mL of beverage.

In-House Validation of Spectrophotometric Method Folin-Ciocalteu

The inicial sample was the coffee beverage prepared according to the recommendations of ABIC (2017). We used 3 g of coffee powder, weighed directly into the strainer with filter paper. It was heated in 30 mL of deionized water to reach 90°C (±1°C) and proceeded to the infusion beverage, with 5 min of filtration time. After reaching the temperature 18°C (±1°C), each validation step began, evaluating the parameters. The validation parameters evaluated were: Linearity, matrix effect, accuracy and selectivity (Souza and Junqueira, 2005).

Evaluation of the Beverage Preparation of Influence in the Reducing Capacity

For this evaluation a random sample is selected and all preparations were tested with the same sample. The amount of powder used was 3 g in 30 mL water, keeping the percentage of 10% as recommended by ABIC (2017).

The preparation of the drink was made in 2 different ways: Decoction and infusion varying the water temperature to 90°C and boiling water (approximately 96-97°C). The preparation was made according to the recommendations of ABIC, with modifications in relation to temperature and contact time with the water. Analyses were performed in triplicate and the difference among the various forms of preparation was analyzed by t test with a 0.05 significance level.

Table 1: Identification of the brands of commercial samples and their names and degrees of roasting

Brand	Names	Degree of roasting informed in label
1	Gourmet	Average
	Traditional	Average
	Extra strength	Average
	Organic	Average
	Decaffeinated	Average
	Soluble	N.I.*
2	Traditional	N.I.
	Extra strength	N.I.
	Mais strength	N.I.
	Estrada real	N.I.
3	Flavor and lightness	Average
	Brazil strong coffee	Strength
	Strong	Strength
	Organic	Severe
	Special reserv	Strength
	Soluble	Severe
4	Traditional	Classical
	Strength	Dark
	Extra strength	Very dark
	Decaffeinated	Average
	Soluble	N.I.
5	Traditional	Dark
6	Traditional	N.I.
	Extra strength	N.I.
7	Traditional	N.I.
	Extra strength	N.I.
	Strength demais	N.I.
8	Traditional	N.I.
9	Traditional	Average
	Extra strength	Deeper
10	Traditional	Average
	Extra strength	Average
11	Traditional	N.I.
12	Traditional	N.I.
	Extra strength	N.I.
13	Traditional	N.I.
	Extra strength	N.I.
14	Traditional	N.I.

*N.I. - Not information in label

Results

Optimization

The spectrophotometric reading between 640 and 800 nm was carried out in coffee beverage extract rates, drink extract added gallic acid and gallic acid only. The response of both aliquots analyzed was similar, with the peak occurring at approximately 750-770 nm reading. According to the ANOVA, there was no difference between the absorbance values obtained within the range ($p>0.05$), so we set up the wavelength of the visual analysis of the curves obtained in each case. It was established at 760 nm for this study, corroborating with Singleton *et al*. (1999).

Extraction Conditions

The factorial design ensures the study of different variables, with a small number of experiments without compromising the work, as well as being a simple statistical tool, providing savings in time and financial resources and allowing to avoid the method of trial and error (Silva *et al*., 2008).

According to the factorial design done, there was no significant difference between treatments, considering the extraction of factors that were evaluated in 18 treatments ($p>0.05$). Factors rest time, solvent and number of extractions had no significant influence ($p>0.05$) in the extraction process, according to ANOVA and Tukey's test. It was therefore concluded that, an additional extraction step is not necessary.

The use of water as solvent for the extraction of phenolic compounds may be observed in some studies. Vignoli (2009) used the aqueous extract for quantitation of phenolic compounds, it was prepared in the same 50 mL water at 95°C (±1°C) in contact with 0.15 g of ground coffee for five min with stirring, followed by

filtration with Whatman filter paper no. 4. added 100 mL of distilled water at 90 to 10 g of roasted coffee beans, followed by filtration through filter paper (Whatman No. 3). It can be seen that in all these mentioned techniques, the method of extraction was very similar to the beverage preparation process as done in this study.

Depending on the results, we evaluated the influence of centrifugation step and rest time in the extraction of compounds with reducing capability present in coffee drink, as this centrifugation step was contemplated. The centrifugation step preceded by 20 min of rest of the extracts, created final absorbance values below the pre centrifugation condition without rest (t student test, $p<0.05$). It follows therefore that it is not necessary to centrifuge the extract or leave them at rest, after preparation of the beverage, guaranteeing therefore greater flexibility of the method.

It was also decided that no extraction step would be necessary after the preparation of the drink. Only dilution of the finished drink was required to achieve the absorbance values between 0.1 and 1.0 nm. Therefore, a 500 µL aliquot was pipetted into the beverage falcon tube and add 9.5 mL of deionized water. Aliquot of this diluted solution was pipetted to be used for the spectrophotometric analysis.

In-house Validation of Spectrophotometric Method Folin-Ciocalteu

The linear range was 20.0 and 60.0 mg mL gallic acid aqueous solution (20.0; 30.0; 40.0; 50.0; 60.0 mg mL). Standard curve three outliers were removed under test and any residue Jacknife linear regression assumptions were confirmed, so that the waste followed standard showed no auto-correlation and were homoscedastic. Finally, regression and no significant deviation from linearity. These results confirm therefore the linearity. In the study Vignoli (2009) the linearity of the Folin-Ciocalteu method was studied in the range of 0.5 to 7.0 mmol of gallic acid, considering the linear $R^2 \geq 0.99$ for presenting values for $p \leq 0$ 01. The assumptions of linearity have not been tested in this study.

The introduced coffee beverage matrix effect ($p<0.05$), based on a comparison of the slopes of the curves. Assuming water as the solvent might have been responsible for extracting the interfering compounds, we tested the matrix effect using methanol as the solvent, in order to assess if there was no matrix effect. However, the interference remained ($p<0.05$), concluding that the matrix has interferences, which were extracted in both solvents.

In view of this, the other validation parameters were assessed using matrix matcheds curves. Whereas the spectrophotometric analysis was performed on several commercial samples of coffee, we tested the effect of additional matrix samples randomly selected to demonstrate the necessity of using matrix matched curve analysis in the reductive capacity of all samples (Table 2). No studies evaluated the matrix effect for the methodology of Folin-Ciocalteu coffee. Vignoli (2009) pointed out that the absence of the matrix effect of the coffee matrix for the study is due to the fact that the antioxidant activity has been evaluated in a complex array rather than a single compound.

There was no difference in absorbance measured in the beverage prepared with and without sugar, under the same conditions of temperature and concentration. For sugar-free drink, the mean absorbance values was 0.449±0.006 nm and to drink with sugar 0.454±0.007 nm (t student test; $p>0.05$). Assessing the accuracy under conditions of repeatability and reproducibility part, from the calculation of the respective standard deviations (DPRr and DPRR) and considering the critical limits calculated by the equation of Horwitz (1982), only the level 0 showed accuracy within acceptable limits.

The evaluation of the accuracy level 0 (no addition of gallic acid) was made for three different brands of coffee (1, 23, 29), which had reductive capacity values at three different levels of calibration curve corresponding to levels 3, 4 and 5. For sample 1, the critical values calculated by the Horwitz equation were 12.34 and 8.22 for DPRR to DPRr. The DPRR and DPRr values were 5.52 and 1.86. For sample 23, the critical values were 13.58 and 9.05 to DPRR to DPRr. The DPRR and DPRr values were 7.49 and 2.01. For sample 29, the critical values calculated by the Horwitz equation were 18.37 and 12.24 for DPRR to DPRr. The DPRR and DPRr values were 2.58 and 1.90 (Table 3).

For the addition of level 1, tested only for sample 1, the standard deviation of repeatability and reproducibility partial met above the established limits. The critical values calculated by the Horwitz equation were 2.88 and 1.92 for DPRR to DPRr. The DPRR and DPRr values were 26.83 and 26.31.

Considering the discrepancy of the values obtained for level 1, additional tests were performed, reducing the room temperature to 17°C (±1°C) in order to assess whether the discrepancy of values reduce. After undertaking all analytical battery refrigerated and the DPRR and DPRr obtained for level 1 still were above the calculated limits. The critical values calculated by the Horwitz equation were 3.69 to 2.46 for DPRR and DPRr. The DPRR and DPRr values were 25.27 and 15.18. This discrepancy values could be explained by a possible synergy of gallic acid with the matrix, which occurs differently between samples according to the specific composition of each. This reaction is probably influenced by time, temperature and agitation, among other unknown factors. The influences of these variables have not been tested separately, given the range of factors possibly responsible for the antagonism or agonism with respect to reaction with Folin-Ciocalteu reagent. The use of another pattern may be suggested in order to find the least disparity values where a less reactive pattern of the sample components, however, these tests were performed in this study.

Table 2: Tilt the usual curves and matrix matched prepared with different coffee drinks

Sample (No ID)	Slopes of the curves and their standard errors		$t_{calculated}$	$t_{critical}$
4	0.1204±0.0009	0.1020±0.0016	6.36	2.20
16	0.1204±0.0009	0.1040±0.0011	11.17	2.10
18	0.1204±0.0009	0.1046±0.0011	10.80	2.07
24	0.1204±0.0009	0.1026±0.0012	11.99	2.12
25	0.1204±0.0009	0.1044±0.0009	12.34	2.09
26	0.1204±0.0009	0.1045±0.0006	14.71	2.08
27	0.1190± 0.0005	0.1086±0.0005	17.69	2.08
31	0.1204±0.0009	0.0942±0.0063	4.13	2.23
32	0.1204±0.0009	0.1091±0.0001	7.56	2.15
33	0.1204±0.0009	0.1078±0.0007	10.73	2.09
39	0.1190±0.0005	0.1068±0.0011	9.71	2.11
40	0.1190±0.0005	0.1040±0.0012	11.54	2.09
41	0.1190±0.0005	0.1123±0.0005	8.98	2.06

t = t statistic for contrasts between slopes, significance level $p<0.05$

Table 3: Standard Deviation of Repeatability (DPRr) and Partial Reproducibility (DPR_R) obtained in the evaluation of the accuracy of the level 0, without the addition of three commercial samples

		DPR_R (%)		DPR_r (%)	
Samples	Level	Critic (Horwitz, 1982)	Obtained	Critic (Horwitz, 1982)	Obtained
1	3	12.34	5.52	8.22	1.86
23	4	13.58	7.49	9.05	2.01
29	5	18.37	2.58	12.24	1.90

Intermediate precision was also evaluated in the Vignoli (2009) from the inter-day analysis, analysis done on different days with different analysts. The accuracy was assessment performing five repetitions (independent preparations) at three different concentrations for roasted coffee (2.0, 3.0 and 5.0 mmol/l ac. gallic/mg.mL^{-1}) for 5 consecutive days. The medium obtained variation coefficient was 2.89% and is therefore suitable under Resolution 899 of 23 May 2003 ANVISA (ANDVS, 2003).

The average recovery of Level 1 was 123.03±32.28%, appearing above acceptable values (between 80 and 110%) (EC, 2002). Several additional tests were performed with the aim of improving the values obtained, achieving optimal recovery range. The first assumption for the outliers was related to the reactivity of gallic acid with the coffee beverage. It was speculated that the reduction in temperature would reduce this reactivity and generate appropriate values. To test the effect of temperature on recovery test, the tests were all done under refrigeration (about 17°C) gave 73.25±17.40. The average recovery showed relatively lower (123.03±32.28% Vs 73.25±17.40) and presented itself below the recommended range.

Determination of the Reducing Ability of Beverages made from Roasted and Ground Coffee Samples Commercialized in Belo Horizonte – MG

By comparing the reductive capacity values of all commercial coffee samples was observed that the samples differ from each other ($p<0.05$). The reductive capacity values found for the 38 samples analyzed ranged from 217.09 to 459.51 mg gallic acid equivalents/100 mL. It was not any trend of values to explain some similarity between the names of each analyzed brand. This can be explained by the wide variety of blends obtained by the manufacturers, as well as different conditions of roasting, with unknown time and temperature variables. Souza (2007), analyzed some bioactive compounds in market commercial coffees with different names. It was observed, despite the small variation of roasting, great diversity in the composition, indicating probable differences in raw materials and species of coffee used in blends. This is likely to entail heterogeneity in composition also diversity in antioxidant activity.

Since this great variability and lack of direct relationship between reducing capacity and the names used for cafes (traditional, strong, extra strong, gourmet, etc.), each brand was analyzed separately as a way to find some similar variation within each group. Still, it is noteworthy that, for each brand, the corresponding blend can be variable, even comparing different denominations within the same brand, as the manufacturer's information does not emphasize the percentage of each variety of coffee in the final product.

For the brand 1, two of its six denominations of cafes, showed higher values when compared to other samples of other brands. The strong and gourmet extra name showed the highest values of the group, followed by decaffeinated, organic and traditional name, in descending order of reducing capacity. According to the package, the brand names that have been subjected to medium roast (Fig. 1a).

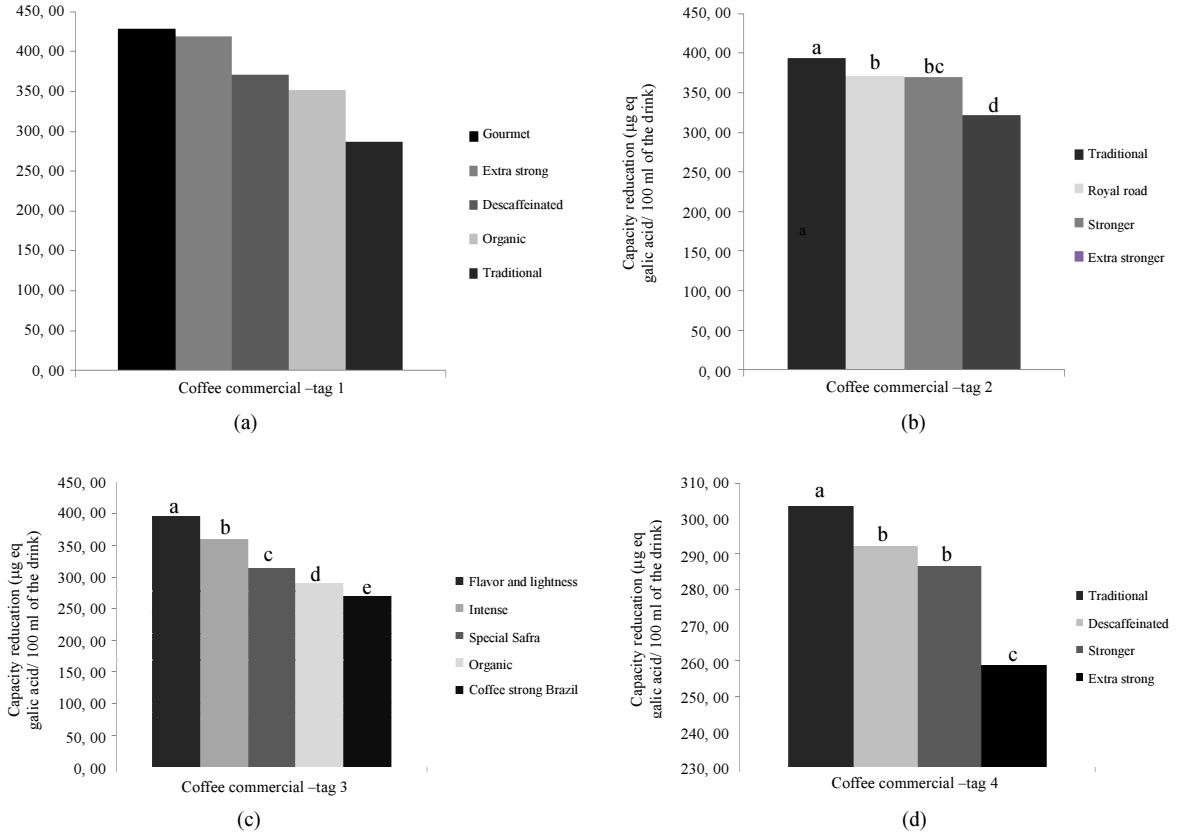

Fig. 1: Ability of Reductive commercial coffee samples from the tag 1 (a) tag 2 (b) tag 3 (c) and tag 4 (d) ($p<0.05$). Same letters indicate no difference by ANOVA test at 5% probability

The trade name of roasting informed by the manufacturer for the gourmet coffees and organic is the same. Because of this similarity of the degree of roasting informed, was expected converging values of these two names, as both comprise the variety of Arabica coffee, according to the manufacturer. However, the gourmet denomination had the highest value of the sample in question, while the organic designation had lower values. This fact should be given by the difference in the cultivation of both denominations, as the gourmet comes from a conventional farming and organic an organic cultivation. It is suggested in the literature that organic farming results in foods with higher levels of polyphenols, which may be explained by the greater exposure of the plant to stress, leading to increased production of natural substances defenders such as phenolic compounds (Winter and Davis, 2006). However, an inverse relationship was found in this study.

Evaluation of the Reductive Capacity of the Coffee Beverage Prepared in Different Ways

The larger reduction capacity values were obtained by decoction with boiling water, followed by 90°C decoction, infusion at 90°C and infused with boiling water. In filtration, the passage of water between 92 and 96°C accommodated in the coffee powder in the filter causes a stirring of the powder, preventing the water saturation. Can be explained, so that the decoction greater contact of the powder particles with the water led to the lower water saturation, resulting in increased extraction of reducing compounds.

The decoction with boiling water still provided higher values, probably due to increased turbulence in the water by boiling and a lower saturation thereof. Another explanation is the largest powder contact time with water compared with the decoction infusion, because the powder was mixed with water and only after about five sec gave up continuing preparing the drink with the filtration step (Lingle, 1996).

Pérez-Martínez *et al*. (2010) compared the antioxidant capacity, measured by method of Folin-Ciocalteu reagent, the coffee beverage prepared with different machines (filtration, pressing, mocha and espresso). The preparation technique that was extracted higher antioxidant content of the espresso machine, followed by filtered coffee, mocha and the beverage prepared by the machine piston. The pressure applied in espresso preparation and in relation to the filtered coffee, hot water contact time with the coffee powder probably facilitated the extraction of antioxidants compounds according to the authors.

Other preparation techniques have also been studied in the literature, for example, Turkish and instant coffee. Niseteo *et al*. (2012) determined the content of compounds derived from chlorogenic acid of coffee drink prepared in different ways (espresso, Turkish, instant cappuccino and filtered). For filter coffee, the total phenolic content found was 2967 mg/L of gallic acid equivalents, lowest value found among the studied preparations. Instant coffee showed the highest values, explained by the concentration of water-soluble components in the product dehydration process. The lowest values for the filtered coffee can be explained by the use of filter paper, which retains some of bioactive substances.

Therefore, it can be assumed that the filtered coffee, has a content of reducing compounds intermediary between the techniques tested by above authors. As other preparation methods are more efficient in the extraction of antioxidant compounds, they are not widely consumed, which is important to evaluate extraction of other ways within the filtration technique. This study provides new to this literature, since the evaluation of different ways to filter the coffee, with variations in temperature and contact the powder with water to decoction showed the best alternative with regard to the optimized extraction reducing compounds.

Discussion

A reduction of the recovery trend with the decrease in the temperature was observed ones. However, the standard deviation under repeatability conditions and reproducibility of all analyzes presented above critical values, which indicates a failure of the gallic acid interaction with initial sample with sequential reduction in temperature.

Some factors may account for this divergence of results: (1) The Folin-Ciocalteu reagent is reduced when in contact with reactive hydroxyl of the matrix in question. The coffee drink contains many reactive compounds, by having free OH groups, citing the phenolic compounds, melanoidins, caffeine, trigonelline and reducing sugars. Gallic acid is also the Folin-Ciocalteu reactive, but may also interact with the coffee matrix in a manner not assessed in this study. Thus, the reaction of reducing compounds coffee, as well as gallic acid, the reagent is impaired, which leads to different results. (2) The complexity of the coffee and your drink, can also be responsible for the divergence of results, since the interaction of the compounds in coffee may influence the reactivity of these with gallic acid. (3) Analytical parameters such as time, temperature, dilution and agitation may influence differently each replicate analysis, because small differences can occur in these parameters along the analytical battery, but that can influence intensively the reaction in each sample aliquot generating conflicting results. (4) Gallic acid is a phenolic little presence in the coffee matrix. The use of a phenolic predominance could lead to better results, since the reactivity of the same could be lower when compared to gallic acid. However, this is not actually tested in this study, it is necessary to study the recovery using other analytical standards. No studies were found that evaluated the recovery parameter for coffee samples analyzed by the spectrophotometric method Folin-Ciocalteu.

Carvalho *et al*. (2011) reported a higher content of chlorogenic acids, analyzed by high performance liquid chromatography efficiency, organic Arabica coffee when compared to conventional, grown in the same soil and climate, varying only the fertilizer. Moreover, studies show little or inconsistent differences in the nutritional composition of organic and conventional food products. Faller and Fialho (2010) evaluated the difference in polyphenol content between various organic and conventional fruits and found no significant differences. However, the shells of organic fruit showed higher polyphenol content compared to their conventional counterparts. It is suggested, therefore, an increase in production of protective substances only in the outer layers, emphasizing that this response was not like all fruits, suggesting specific effects of certain foods. Therefore, considering the difference between the gourmet name and organic 100% Arabica mark 1, in this study, may or may not be due to the difference in the form of cultivation. According to the results, the reductive organic farming generated lower capacity values for the analyzed coffee. However, differences in soil, climate and even in commercial roasting conditions may have been responsible for this difference, instead of the cultivation medium alone.

The species of Robusta coffee is known for its high content of chlorogenic acids compared to Arabica (Vignoli, 2009) and these compounds contribute to reductive capacity of the samples. The extra strong names, decaffeinated and traditional consist of blends of coffee, probably Arabica and Robusta, since failure to provide this information by the manufacturer. Due to the high amount of chlorogenic acids Robusta coffee, it was hoped a different order of values found for the brand 1 samples and the gourmet name, which has no Robusta coffee in its composition, should not present the largest capacity values reductive. It is assumed that there was some difference in the roasting process, a ratio of Arabica and Robusta coffee divergent between samples or even differences in the process of filling and storage that may have preserved the content of reducing compounds of gourmet name. Another explanation for this discrepancy would be the transformations that occur in the roasting process, leading to degradation of chlorogenic acids in derivatives or their incorporation into melanoidins by changing the profile end of each sample reducer (Clifford, 2000; Farah *et al*., 2005). For the 2 brand, the traditional name had the highest value, followed by the names royal road, stronger and extra strong. Packaging of coffee of this brand, there was no information on the degree of roasting (Fig. 1b).

It is assumed that, for this brand, the roasting degree of reduction has led to increased capacity reduction, if we take into account that the traditional name showed the greatest amount of capacity reduction. However, previous studies have found an inverse relationship found for these samples brand 2. It is speculated that there is formation of phenolic along the toasting from phenolic compounds other (Bekedam *et al*., 2008) as well as high temperature of the coffee roasting process leads to the destruction of some phenolic compounds and favors the development of other antioxidants, high lighting the melanoidins (Daglia *et al*., 2000). Opposing the aforementioned authors state that very pronounced roasts can lead to degradation of bioactive compounds, which can contribute to reducing the final reducing capability. Taking into account this discrepancy between the studies, it is speculated that, for the brand 2, minors under reductive capacity values for the actual road names, stronger and extra strong, with lower brightness than traditional (L = 20 15±0.19, 18.45±0.21 and 20.15±0.11 respectively) is given by the divergence of the commercial roasting process between them, leading to degradation of some compounds as well as the formation of others with reducing potential. How not evaluated the profile of reducing compounds in this study, but the final reductive capacity, one cannot argue with regard to training and/or degradation of specific compounds.

For the 3 brand, the flavor name and lightness showed the highest reducing capacity values, followed by intense and soluble denominations and after special reserve, organic and strong coffee from Brazil, the latter showed a lower value than most commercial coffees. According to the packaging, "flavor and lightness" designation suffered medium roast, the intense denominations and strong coffee from Brazil - strong roasting, soluble - marked roasting, special reserve - strong and organic - sharp (Fig. 1c).

The highest value found for the name "flavor and lightness" can also be explained by the degree of roasting, indicated as average by the manufacturer and therefore less intense than other denominations and finally, for the brand 4, soluble denomination had the highest value, followed by the traditional, decaffeinated, strong and extra strong and the latter name showed lower value than most commercial coffees. According to the package, the brand names that have been subjected to medium roast (Fig. 1d).

Regarding the brightness, there is a positive correlation to reducing capability (R^2 = 0.90, $p<0.05$). Traditional coffee to extra strong decreasing of reducing capacity and brightness. The L value of the traditional name was 19.74±0.10; decaffeinated 19.72±0.23; strong 19.52±0.11 and 19.44±0.18 extra strong, speculating that the increase of the degree of roasting led to a reduction of the reducing capacity, a measure of antioxidant capacity, which corroborates with others studies. There are, however, a lack of relationship between the reducing capacity and roasting degree of names of coffees according to the manufacturers.

Whereas samples of 100% Arabica coffee toast in three different conditions - 10 min at 24°C (light roast coffee); 11 min 24°C (medium roast); 12 min at 24°C (dark roast) - the light roasts and had lower average reduction ability of the dark, so the more intense was the roast, the greater the reduction capacity thereof. The high temperature of the coffee roasting process leads to the destruction of some phenolic compounds and favors the development of other antioxidants and highlighting the melanoidins (Daglia *et al*., 2000). Moreover, it also shows the formation of lactones, during the roasting process and these, as well as chlorogenic acid, can be incorporated into the formed melanoidins (Bekedam *et al*., 2008). These substances also have formed reducing properties and is also measured by the spectrophotometric method Folin-Ciocalteu. It is speculated that there is formation of phenolic along the toasting from phenolic compounds other than (Bekedam *et al*., 2008). Thus, increasing the degree of roasting may have led to increased formation of reducing compounds in the samples in question Arabica coffee, reflecting the increase in measured capacity reduction.

Conclusion

This study is relevant from a methodological point of view, because in the scientific literature, there is no spectrophotometric coffee analysis studies that addressed all validation parameters as studied in the present study (linearity, matrix effect, precision, recovery, interfering). From the analysis of the prepared coffee drinks, one can note the great variability of reducing compounds present in different samples of coffee, because of possible influences in the composition of blends, different roasting techniques practiced in hulling and even due to defective possible to use coffee beans for obtaining commercial coffees. Thus, it is difficult to guide the consumer at the time of purchase because the selection of a coffee with higher antioxidant profile is not possible. Products from the same commercial coffee brand are obtained with different antioxidant profile.

The preparation technique also influenced the reductive capacity of drinks, as different preparation methods resulted in different values and the decoction the most efficient. This information can be valuable to consumers, who can choose to domestic preparation techniques that result in a coffee drink with better content of antioxidant compounds.

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Author Contributions

Each author have participated sufficiently in the work to take public responsibility for appropriate portions of the content.

Conflict of Interest

There is no conflict of interest.

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