Adding Adulterants to Coffee Reduces Bioactive Compound Levels and Antioxidant Activity

Fernanda Paola de Pádua Gandra¹, Adriene Ribeiro Lima², Eric Batista Ferreira³, Michel Cardoso De Angelis Pereira⁴*, Rosemary Gualberto Fonseca Alvarenga Pereira¹

¹Department of Food Science, Federal University of Lavras, Lavras, Brazil
²Institute of Health and Biological Sciences, University Center-UNA, Belo Horizonte, Brazil
³Institute of Exact Sciences -Federal University of Alfenas, Alfenas, Brazil
⁴Department of Nutrition, Federal University of Lavras, Lavras, Brazil
*Corresponding author: deangelis@dnu.ufla.br

Abstract The present study aimed to evaluate the effect of coffee adulteration on antioxidant activity in vitro. Coffee beverages were adulterated with different concentrations of coffee hulls, coffee straw, and corn (0%, 10%, 20%, 30%, 40%, 50%, and 100%) and tested separately. Each coffee beverage was prepared according to the same methods in all of the treatments. Phenolic compound, caffeine, trigonelline, and chlorogenic acid levels were determined in the beverages. Antioxidant activity in vitro was evaluated using the DPPH radical scavenging activity, reducing power, iron chelating activity, and lipoperoxidation inhibition methods. Phenolic compound, caffeine, and chlorogenic acid levels of the samples decreased with increasing adulterant concentration. Adding adulterant reduced the antioxidant capacity tested using all of the methods. The results show that adding coffee hulls, coffee straw, and corn affect the antioxidant capacity of the coffee beverages, reducing protection against oxidative stress.

Keywords: food, oxidative stress, health

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1. Introduction

Ground coffee is quite vulnerable to tampering since it has physical characteristics that are easily reproduced by roasting and grinding various materials. In Brazil, largest producer and second largest consumer, the most common adulterants used in roasted and ground coffee comprise residues from processing dried fruit, coffee itself, corn, and many others. Corn is considered the most widely used adulterant due to its significantly lower cost compared to coffee.

Coffee processing byproducts such as hulls and straw are also often used in adulteration due to the high volume produced and because they are very similar to roasted and ground coffee. In dry coffee processing, the fruit are preliminarily washed, separated by different maturation stages, and then dried. These cleaning and dehulling procedures generate similar proportions of coffee and solid residues. These two residues are separated by different densities using mechanical ventilation. Coffee straw is defined as coffee hulls without endocarp (parchment), comprising mesocarp (mucilage) and epicarp (hulls). Coffee hulls comprise the endocarp, mesocarp, and epicarp [1].

Coffee adulteration has existed for decades in Brazil and peaked around 1980, causing a decline in the quality of coffee sold in Brazil at that time. Concern about coffee quality arose in 1989, when the Brazilian Coffee Industry Association (ABIC) created the ABIC Roasted and Ground Coffee Control / Purity Seal Program. Despite the purity seal mitigating adulteration, it is still common, where many commercial coffees contain intentionally added substances. The ABIC considers adulteration to be one of the most serious problems affecting Brazilian coffee quality [2].

Food adulteration not only compromises product quality but also nutritional value. Coffee’s antioxidant capacity is due to caffeine, phenolic compounds, and other compounds that form by roasting [3,4].

Studies related to biological activity of roasted coffee have contributed to increased coffee consumption among Brazilians [5]. However, little is known about the antioxidant potential of coffee available for the majority of the population. There are analytical methods for determining numerous antioxidant capacities, which may be subjected to interference and are based on different foundations. Thus it is recommended that two or more techniques be used since no test alone to determine the antioxidant capacity will accurately reflect a sample’s "total antioxidant capacity" [6].

The following methods are most commonly used to measure antioxidant activity in vitro: antioxidant’s ability to reduce free radicals by donating hydrogen or electrons (ORAC, TRAP, DPPH, and ABTS); reducing power; ability to chelate iron ions; and ability to inhibit lipid oxidation [6].
The adulterated coffees can have lower quality and altered chemical composition, even compromising bioactive compound content that is responsible for coffee's antioxidancy. This study aimed to evaluate the effects of coffee adulteration on bioactive compounds and antioxidant activity in vitro, tested using different analytical methods.

2. Material and Methods

2.1. Samples

We used “Rio” coffee (Coffea arabica L.) beverage samples, from the same batch assigned by a coffee roaster located in southern Minas Gerais, Brazil. Coffee hulls and straw were given to us by a coffee estate. The corn was purchased at a local market.

2.2. Preparing the Samples

Coffee beans, coffee hulls, coffee straw, and corn were roasted in a 12-Kg Rototec® roaster, (RT-12 model) at final temperatures of 263°C (29 minutes); 247°C (8 minutes 45 seconds); 270°C (23 minutes); and 262°C (14 minutes), respectively. The coffee roasting process was observed to determine when the dark roast point was reached. The adulterants were roasted to resemble roasted coffee. Next, the roasted coffee beans and adulterants were finely ground (70% retention) in a Probat® electric grinder.

The roasted ground coffee samples were adulterated with 10%, 20%, 30%, 40%, and 50% roasted and ground coffee hulls, coffee straw, or corn. Positive (100% adulteration) and negative controls (0% adulteration) were used. The samples were vacuum packed in 500g metallized packaging.

2.3. Preparing the Coffee Beverage

The coffee beverage was prepared according to that recommended by the ABIC. Ten grams of each sample was placed on Whatman no. 3 filter paper and then 100 mL of distilled water (at 90°C) was poured on top. The beverages were prepared at the time of use in all of the experiments performed.

2.4. Determining Caffeine, Trigonelline, and Chlorogenic Acid Levels

The extraction for determination of caffeine, trigonelline and chlorogenic acids procedures with hot water diluted to 0.5 mL aliquot of the beverage in 100 mL of distilled water and high performance liquid chromatography (HPLC) analysis by Schimadzu chromatograph with a C-18 reverse phase column were used to determine caffeine, trigonelline, and chlorogenic acid (5-caffeoylquinic acid) levels according to Vitorino et al. [7].

2.5. Determining Total Phenolic Compound Content

The total phenolic content of each sample was quantified according to the methods described by Souza et al. [8] and Dias et al. [9], with modifications. The beverages were diluted in water, obtaining the concentration: 0.2mg/mL and 0.5 mL aliquot of each dilution was added to 2.5 mL of 10% Folin-Ciocalteau aqueous solution. After 8 minutes of rest was added 2.0 mL of 20% sodium carbonate. The mixture was kept in the dark for two hours and then absorbance was measured by a spectrophotometer at 740 nm using a white reference. The results were expressed as gallic acid (100g/g of sample).

2.6. DPPH free Radical Scavenging Activity

DPPH free radical scavenging activity was determined according to the method described by Yen et al. [10]. To analyze DPPH free radical (1,1-diphenyl-2-picrilhidrazil) scavenging activity, the beverages were diluted in ethanol (0.2mg/mL). One mL of DPPH (0.5 mmol/L), also diluted in ethanol, was added to a 4 mL sample. The mixture was stored in an amber vial and stirred. After 30 minutes the reading was recorded at 517 nm. The lower absorbance indicates free radical scavenging activity. The tests were performed in triplicate. Free radical scavenging activity (FRSA) was expressed in percentage compared to the white reference (0% inhibition). BHT was used as control.

2.7. Reducing Power

Reducing power was evaluated according to Yildirim et al. [11]. The coffee beverages (0.01 mL aliquots) were diluted in 1 mL of absolute ethanol and transferred to test tubes containing 2.5 mL of 0.2M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide (w/v). The mixture was incubated in a water bath at 50°C for 30 minutes. Aliquots of 2.5% trichloroacetic acid at 10% (w/v) were added to test tubes and subsequently stirred. The mixture (2.5 mL aliquots) was transferred another set of test tubes, in which 2.5 mL of distilled water and 0.5 mL of 0.1% FeCl₃ (w/v) were added, then subsequently stirred. The absorbance reading (700nm) indicated increased reducing power. Reducing activity of the coffee beverages was expressed as percentage inhibition compared to the standard (BHT).

2.8. Evaluating Fe²⁺ chelating activity

Fe²⁺ chelating activity was evaluated according to the method described by Tang et al. [12], modified by Lima et al. [13]. A 1 mL aliquot of the sample was transferred to 25 mL amber test tubes. Next, 3.7 mL of deionized water; 0.1 mL (2mM) of FeSO₄ (Fe²⁺), 0.2 mL (5mM) Ferrozine (chromogenic reagent) were added. The mixture was stirred and after 20 minutes the reading was recorded at 562 nm hitting the apparatus with the white reference (in this case replacing the sample with 2% EDTA). Lower absorbance indicates Fe²⁺ chelating activity.

2.9. Assessing Ability to Inhibit Lipid Peroxidation (ex vivo)

Lipid peroxidation was determined in rat brain homogenate by evaluating the reaction between the oxidation products formed and thiobarbituric acid
(TBARS) as described by Buege and Aust [14]. These compounds are colored and their levels can be determined by spectrophotometry.

Brains from ten male Wistar rats weighing 250 ± 20 g were used. The brain homogenate was prepared according to Paula et al. [15] with modifications. The brain was collected, weighed, and homogenized into three volumes of 0.1M PBS, pH 7.4. After centrifuging the sample at 10,000 g for 15 minutes, the supernatant was collected and the protein concentration of the samples was determined by the Peterson method [16]. Aliquots of homogenate containing 6 mg protein were placed in test tubes with the coffee beverage samples. The coffee beverage was diluted resulting in a final concentration of 20,0 mg/mL. The sample volume comprised 1 mL of PBS and these samples were incubated in a water bath at 37°C for 30 minutes. Then 0.5 mL of 25% hydrochloric acid (v/v) and 0.5 mL of 1% thiobarbituric acid (w/v) were added. The mixture was heated in boiling water for 15 minutes and cooled in an ice bath for 10 minutes. Two mL of butanol was added to the test tubes and then they were vortexed. After centrifugation at 10,000 g for 15 minutes, the samples were measured using TBARS (535nm). The mixture without coffee samples was used as a control, representing 100% oxidation. The results were expressed as follows (Eq. 1):

\[
% \text{ PI} = \frac{Cs - Ts}{Cs} \times 100
\]

where PI is peroxidation inhibition, Cs: control sample, Ts: coffee samples.

2.10. Statistical Analysis

A completely randomized design was considered for the experiment, under a factorial combination of the factors (three different adulterants and seven concentrations). The data were subjected to analysis of variance (ANOVA) followed by Tukey test or regression model fit, both at 5% of significance. Further, the results were subjected to principal component analysis (PCA).

3. Results and Discussion

3.1. Bioactive Compound

Bioactive compound (total phenolic, chlorogenic acid, caffeine, and trigonelline) levels of the coffee beverages were affected by adding coffee hulls, coffee straw, and corn. The variables analyzed were compared at each adulterant concentration. (Table 1).

Coffee contains the following phenolic compounds: caffeic acid, chlorogenic acid, and other derivatives of caffeolquinic acid [17]. Chlorogenic acid and its isomers are the main components of the phenolic fraction in raw coffee beans [3,18] and 5-caffeoylquinic acid (5-CQA) is the most abundant [19].

Adding adulterants altered the 5-caffeoylquinic acid levels in the samples. Adulterant-free coffee had higher 5-CQA values compared to the other coffee beverages.

In comparing the adulterants at different concentrations, there is similar chlorogenic acid content in coffee hulls and straw, contrasting that reported by Andrade et al. [20] who did not find 5-CQA in the straw.

Souza et al. [21] reported that defects in coffee due to adding different materials such as corn, wheat, rye, and barley to coffee beverages could result in coffee products with different composition. According to the authors, adding an adulterant would result in decreasing compound levels, such as 5-CQA, as shown in this study. However, despite the effect of adding adulterants on the 5-CQA levels, dark roasting can more expressively affect it at low 5-CQA levels. According to Budryn et al [22], samples that have lower total chlorogenic acid levels have undergone a more drastic roasting process. Bekedam et al. [5] claim that dark roasting reduces the 5-8% chlorogenic acid levels in green Arabica coffee beans to below 0.2%, as observed in this study.

Table 1. Total phenolic compound, 5-cafeoylquinic acid, caffeine, and trigonelline levels in coffees adulterated with different percentages of coffee hulls, coffee straw, and corn

| Adulterant concentration (%) | Adulterants | Bioactive compounds | Total Phenolic | 5-Caffeoylquinic acid | Caffeine | Trigonelline |
|-----------------------------|------------|--------------------|---------------|----------------------|---------|-------------|
| 0                           | Hulls      | 4.92 a             | 0.21 a        | 1.83 a               | 0.35 a  |
|                             | Straw      | 4.92 a             | 0.21 a        | 1.83 a               | 0.35 a  |
|                             | Corn       | 4.92 a             | 0.21 a        | 1.83 a               | 0.35 a  |
| 10                          | Hulls      | 3.31 a             | 0.14 a        | 1.57 a               | 0.27 b  |
|                             | Straw      | 3.16 a             | 0.15 ab       | 1.58 a               | 0.31 a  |
|                             | Corn       | 3.29 a             | 0.16 a        | 1.53 a               | 0.28 b  |
| 20                          | Hulls      | 3.54 a             | 0.14 b        | 1.56 a               | 0.42 a  |
|                             | Straw      | 3.04 b             | 0.15 b        | 1.48 b               | 0.37 b  |
|                             | Corn       | 3.13 b             | 0.17 a        | 1.37 c               | 0.21 c  |
| 30                          | Hulls      | 3.20 b             | 0.14 b        | 1.47 a               | 0.41 a  |
|                             | Straw      | 3.45 a             | 0.15 a        | 1.40 b               | 0.37 b  |
|                             | Corn       | 3.00 c             | 0.16 a        | 1.22 c               | 0.22 c  |
| 40                          | Hulls      | 3.18 a             | 0.14 a        | 1.32 a               | 0.46 a  |
|                             | Straw      | 3.10 a             | 0.14 a        | 1.23 b               | 0.39 b  |
|                             | Corn       | 2.46 b             | 0.14 a        | 1.10 c               | 0.19 c  |
| 50                          | Hulls      | 3.19 a             | 0.14 a        | 1.25 a               | 0.54 b  |
|                             | Straw      | 3.18 a             | 0.15 a        | 1.24 a               | 0.57 a  |
|                             | Corn       | 2.52 b             | 0.10 b        | 0.92 b               | 0.13 c  |
| 100                         | Hulls      | 2.70 a             | 0.14 a        | 0.90 a               | 0.84 a  |
|                             | Straw      | 2.61 a             | 0.10 b        | 0.79 b               | 0.83 a  |
|                             | Corn       | 0.64 b             | 0.04 c        | 0.00 c               | 0.00 b  |

Means followed by the same letter for each adulterant concentration do not differ (p<0.05) by Tukey test.
Several studies have shown the protective effects of phenolic components in coffee against oxidative stress [23,24,25,26]. Adulterated coffee beverages may have reduced antioxidant capacity due to the lower phenolic compound content found in these samples.

Adding coffee hulls, coffee straw, and corn also reduced the caffeine levels in the coffee beverages analyzed. In this study, we found no caffeine in the 100% corn sample. Thus, the corn-adulterated coffee beverages had lower caffeine levels than those adulterated with the two other adulterants at levels higher than or equal to 20%.

Caffeine values obtained in pure coffee were approximately 1.8g/100g, contrasting caffeine values described in the scientific literature for roasted Arabica coffee, ranging from 1.0 to 1.3g/100g [27]. The lower caffeine levels in the adulterated samples may interfere with the beverages' antioxidant activity since it is a coffee constituent with antioxidant activity via hydroxyl radical uptake and singlet oxygen reaction with electrons [28].

The trigonelline content in pure coffee in this study corroborates the values between 0.2 and 0.5g/100g obtained by Monteiro and Trugo [27] in quantifying trigonelline content in medium to extra strong roasted Brazilian coffee. Alves et al. [29] reported lower values (0.297g/100g) for dark roasted arabica coffee.

Trigonelline content in roasted coffee samples depends on the species and binomial roasting duration and temperature used in bean processing [27]. Trigonelline losses may vary from 50 to 100% depending on the degree of roasting [30].

The 100% corn sample did not contain trigonelline and thus the acrylamide levels in corn-adulterated coffee decrease with increasing adulterant concentration. Conversely, there were higher trigonelline levels in the coffee residues (hulls and straw) compared to pure coffee. These results corroborate those obtained by Andrade et al. [20], who found higher trigonelline levels in coffee straw compared to pure coffee.

During roasting, trigonelline degrades into niacin, a vitamin belonging to the B vitamin group. Niacin is considered as pellagra-preventive factor and is precursor to NAD and NADP coenzymes, which are important enzymes in various oxidation reactions [31].

Our results show that coffee hulls and coffee straw have higher trigonelline levels, which might generate higher niacin levels. However, levels of trigonelline were quantified in raw coffee, coffee hulls, and coffee straw to infer the levels present and effect of roasting. The mean trigonelline values were similar in raw coffee, coffee hulls, and coffee straw (1.7g/100g, 1.8g/100g, 1.6g/100g, respectively), revealing that less trigonelline degraded and most likely less niacin formed when the hulls and straw were roasted compared to coffee.

We must note that as there are no data published on the chemical composition of coffee hulls and the alterations that occur when they are roasted, it is impossible to infer which factors were responsible for lower trigonelline degradation in these residues.

Souza et al. [21] found highly altered trigonelline levels in commercial coffee roasted to the same degree as the coffee beverages they analyzed, reporting that they could hide the mixtures (blends and/or adulteration), thus interfering in the quantity of bioactive compounds such as trigonelline. Our study confirmed the hypothesis that adulterated coffee beverages have varied trigonelline content.

3.2. Antioxidant Activity in vitro

3.2.1. DPPH Free Radical Scavenging Activity

Free radical scavenging activity decreased with increasing adulterant concentration. We evaluated the compounds' DPPH free radical scavenging activities, preventing oxidation. Altered bioactive compound content affected antioxidant activity in the coffee beverages.

A study has shown a positive correlation between chlorogenic acid content and DPPH scavenging activity in coffee beverages [32].

Component analysis (Figure 5) shows no close relationship between total phenolic compounds and 5-CQA with FRSA. Caffeine did not expressively affect the ability to scavenge free radicals (Figure 5). These results indicate that other compounds in coffee also have antioxidant power. Liu and Kitts [33] showed that antioxidant activity of coffee beverages was due to compounds formed during roasting (melanoidins). The authors state that the
antioxidant action mechanism associated with the melanoidins involves the transfer of hydrogen atoms and electrons. Formation of these compounds would compensate for the degradation of chlorogenic acid caused by dark roasting. Thus, the likely reduction in melanoidin content in adulterated samples may have more significantly contributed in reducing radical scavenging activity in the adulterant-free drinks analyzed.

Note that coffee hulls, coffee straw, and corn (100%) have lower scavenging activity than pure coffee, which led to reduced FRSA in adulterated coffee beverages containing these materials. In comparing FRSA between pure coffee and coffee straw using the same methodology, Andrade et al. [21] concluded that coffee straw had lower FRSA, similar to the results we obtained in this study.

3.2.2. Reducing Power

The reducing power test assesses an antioxidant's ability to reduce the ferricyanate ion, in the presence of an iron ion (derived from FeCl₃) in Prussian blue form. The higher the absorbance, the higher the antioxidant power [34] due to the increased ferricyanate formation.

The reducing power of the coffee beverages was affected by adding adulterants (Figure 2). In a study by Santos et al. [34], the authors found that roasting green coffee increased beverage reducing power, however, the reducing capacity decreased with increasing degree of roasting, possibly due to degradation of chlorogenic acid. Lima et al. [13] concluded reduced coffee decaffeination lowers the reducing capacity due to reduced caffeine and chlorogenic acids.

The results of this study corroborate the findings of the aforementioned study. In analyzing the PCA, it is possible to conclude that reduced caffeine, and especially phenolic compounds and chlorogenic acid, levels affected the reducing power of the coffee beverage samples.

3.2.3. Iron Chelating Activity (ICA)

Similar to in the previous tests, there was no effect of the altered iron chelating activity on the samples. As adulterant concentration increases, ICA decreases (Figure 3). Based on the results shown in the PCA, we infer that reduced bioactive compound content, mainly phenolic compounds, lowers ICA. Santos et al. [34] found that green coffee samples had higher chelating activity, probably due to functional phenolic compounds. Lima et al. [13] evaluated the antioxidant potential of samples from different coffee beverages and concluded that the roasting and decaffeination processes reduced the samples' chelating activities. The authors reported reduced phenolic compound and caffeine levels induced by these processes, which led to reduced iron chelating activity.

In contrast, Bekedam et al. [5] reported that melanoidins form by roasting coffee, which are responsible for the coffee beverage's metal chelating activity. The results of these studies indicate that reduced caffeine, phenolic compound and melanoidin levels likely interfere with iron chelating ability. The first defense mechanism against free radicals is to prevent their formation, mainly by inhibiting chain reactions with iron and copper. Thus, the coffee beverages adulterated with coffee hulls, coffee straw and corn have reduced ability to protect against oxidative stress compared with pure coffee.

3.2.4. Lipid Peroxidation Inhibition (ex vivo)

Our study showed that adulteration altered coffee's ability to inhibit lipid peroxidation (Figure 4). This change probably occurred due to less compounds with antioxidant activity in the adulterated samples as aforementioned. Duarte et al. [35] evaluated the ability of natural dehulled coffee to inhibit lipid peroxidation and obtained the highest values (58.7) for medium roasted dehulled coffee (20,000 ppm). These values are similar to those obtained for the pure coffee beverages (58%) analyzed in this study. The authors stated that the higher chlorogenic acid content found in dehulled coffee was responsible for greater ability to inhibit lipid peroxidation compared with the natural coffee.

An in vitro study with human cells using Apocynum venetum leaf extract demonstrated that the chlorogenic acid in the Apocynum venetum leaves almost completely inhibited formation of thiobarbituric acid reactive substances (TBARS) [36]. According to the PCA, reduced phenolic compound content more significantly interferes with malonaldehyde formation, which reduced caffeine.

3.2.5. Principal Component Analysis (PCA)

Increased coffee hull, coffee straw, and corn concentrations were responsible for variation in individual factors compared to pure coffee. Corn-adulterated coffee had higher discrepancy for all of the variables regarding
pure coffee compared to coffee adulterated with coffee hulls and coffee straw. These results were expected since the coffee hulls and coffee straw are by-products and can be similar to coffee's composition. However, parchment, present in coffee straw and absent in coffee hulls, may have been affected, albeit discreetly, by altering some variables. In analyzing the variable factor map (Figure 5), it is possible that the reduced caffeine, phenolic compound, and 5-caffeoylquinic acid levels caused by presence of the adulterants affect the beverages' antioxidant capacity. Conversely, we cannot conclude that the altered trigonelline content of the samples affected the beverages' antioxidant power evaluated.

Figure 5. Principle Component Analysis (PCA)- Individual Factor Map and Variable Factor Map of coffee beverages adulterated with different concentrations of coffee hulls, coffee straw, and corn

4. Conclusion

Our study showed that substances added to coffee alter its composition and interfere with the synergism between these compounds, thus reducing antioxidant activity and interfering in protection against oxidative stress.

Future studies are required to evaluate the effect of adding adulterants on compounds that form when roasting coffee, such as melanoidins, and the antioxidant activity exerted by these compounds in adulterated coffee beverages.

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