



Total phenolic content and primary antioxidant capacity of aqueous extracts of coffee husk: chemical evaluation and beverage development

Jorge Vitório Gomes das NEVES¹, Marília Viana BORGES¹, Daniel de Melo SILVA²,
Cristina Xavier dos Santos LEITE², Mariana Romana Correia SANTOS¹, Neuma Gonçalves Barbosa de LIMA¹,
Suzana Caetano da Silva LANNES³, Marcondes Viana da SILVA^{1*} 

Abstract

This study aimed to evaluate the efficiency of aqueous extraction to obtain bioactive phytochemicals from grains and residual husk of organic Arabica coffee, as well as to develop a beverage with high antioxidant capacity and assess its sensorial acceptability. Aqueous extracts were obtained from dried and crushed coffee beans and husk. Various extraction methods were used to select the one capable of extracting the most amount of total phenolic constituents. The decoction without mechanical agitation was highlighted as the best method, from which the chemical characterization, antioxidant capacity and the presence of antinutrients were investigated. Three beverage formulations were prepared with coffee husk extract and added in different proportions to concentrated pineapple juice. The beverages were analyzed for antioxidant capacity, microbiological properties and sensorial acceptance. No hemagglutinins and low oxalate content were found in the samples. The antioxidant capacity of the aqueous husk extract was higher than that of the grains. The beverage made with the addition of concentrated pineapple juice was sensorially preferred by the tasters. In addition, it contributed to raise the antioxidant capacity of the beverage. It was concluded that the aqueous extract of coffee husk appears as a new alternative for the beverage industry.

Keywords: bioactive phytochemicals; by-products; coffee growing.

Practical Application: Mixed drink with functional attributes from the coffee husk.

1 Introduction

Coffee is an agricultural product of great economic importance for Brazil, currently, the world's largest coffee producer and exporter. *Coffea arabica* (Arabica) and *Coffea canephora* (Robusta) are the most commercially important species, accounting for 70% and 30% of the global production, respectively (United States Department of Agriculture, 2017).

According to data from the National Supply Company (Companhia Nacional de Abastecimento, 2017), coffee production in Brazil is estimated to be between 54.4 and 58.5 million bags (60 kg bags) in 2018, representing a growth of up to 30.1% when compared to the 2017 harvest, a 20% increase in the harvest is also expected in 2018 compared to 2019. The International Coffee Organization (2018) estimates an increase of 0.8% in world production, rising from 157.69 to 158.93 million bags (60 kg bags) between 2016/17 to 2017/18.

The economic importance of coffee is mainly due to the drink, an infusion prepared from roasted and ground beans. This beverage contains bioactive phytochemicals, such as flavonoids (catechins and anthocyanins), caffeic and ferulic acid (Meletis, 2006), as well as nicotinic acid, trigonelline, quinolinic acid, tannic acid, pyrogallol acid and caffeine (Minamisawa et al., 2004).

In this context, the coffee industry produces considerable amounts of waste at all stages of its production chain, from the harvest to the final product (husk, lees, straws and pulp), by-products still little explored in the food industry, despite representing sources of bioactive phytochemicals with potential use as functional ingredients or for the development of new products. In addition, they are value-added renewable resources (Murthy & Madhava Naidu, 2012). One of the most common ways to obtain bioactive constituents of agroindustrial residues, such as coffee husk, is through the solid-liquid extraction process. According to the Brazilian Pharmacopoeia of Phytotherapeutics (Brasil, 2018), extracts are preparations of a liquid consistency, solid or intermediate, obtained from animal or vegetable material, using water, ethyl alcohol or other suitable solvents.

There is growing interest in recovering bioactive phytochemicals from agroindustrial by-products rich in secondary metabolites, especially those with antioxidant capacity, to add value to these residues. Thus, the present study intended to evaluate the efficiency of various aqueous-based extraction methods to obtain bioactive phytochemicals from residual organic Arabica coffee husk, as well as to develop a beverage with abundant natural antioxidants and analyze its sensory acceptability.

Received 01 Dec., 2018

Accepted 09 May, 2019

¹Departamento de Ciências Exatas e Naturais – DCEN, Universidade Estadual do Sudoeste da Bahia – UESB, Itapetinga, BA, Brasil

²Departamento de Química e Exatas – DQE, Universidade Estadual do Sudoeste da Bahia – UESB, Jequié, BA, Brasil

³Departamento de Tecnologia Bioquímico-Farmacêutica, Universidade de São Paulo – USP, São Paulo, SP, Brasil

*Corresponding author: mviaana@uesb.edu.br

2 Materials and methods

Coffee beans (*C. arabica* L.), produced under the biodynamic management of the 2014 crop, were used in the municipality of Ibicoara, Bahia State, located in the southern region of Chapada Diamantina (latitude 13°24'50.7"S, longitude 41°17'7.4"W of Greenwich, approximate altitude of 1,027 m asl). The raw material is certified by the Biodynamic Institute (Inspeções e Certificações Agropecuárias e Alimentícias, 2017) with seal Demeter CA 9302/17 Substitute CA 9263/17. The coffee fruits were randomly collected at four different farm locations, from two plants in the northern (husk 1 and grains 1), southern (husk 2 and grains 2), eastern (husk 3 and grains 3), western (husk 4 and grains 4) and central region (husk 5 and grains 5), respectively. The coffee fruits were washed, separated and sun-dried to a 10% moisture. The dried materials were transferred to the laboratory of the Nucleus of Studies in Food Science, the State University of Southwest of Bahia, where they were ground in a Willy MSSL-031 type (Solab, São Paulo, Brasil) mill and packed in polyethylene containers, sealed with a lid, identified and kept in a dry place at room temperature (25 ± 2 °C) until analysis.

2.1 Aqueous extraction of coffee husk: preliminary tests

The aqueous extracts were obtained from a coffee husk:water ratio of 1:75 (parts per volume). In this way, five extraction methods were tested: 1. Infusion (immersion of the sealed container of the sample in water at 100 °C for 10 min); 2. Decoction with mechanical agitation; 3. Decoction without mechanical agitation (plate-heating at 100 °C for 10 min); 4. Ultrasound-assisted extraction (Ultracleaner, USC-1400, Indaiatuba, São Paulo, Brazil) operating at room temperature (25 °C) at a frequency of 40 kHz for 10 min); 5. Extraction with the aid of a Philco electric coffee maker (Amazonas, Brasil) (100 °C). The resultant extracts were filtered, packed in amber bottles and refrigerated (4 ± 2 °C) until analysis. The total phenolic constituents (TPC) of the extracted extracts were determined, and the one with the highest concentration of TPC was selected for chemical analysis and beverage preparation.

2.2 Chemical characterization of extracts of husk and coffee beans

Determination of Total Phenolic Constituents (TPC)

The concentration of TPC was determined using the Folin-Ciocalteu reagent, according to ISO 14502-1 (International Organization for Standardization, 2005). Gallic acid was used as the standard for the calibration curve (0.2, 0.4, 0.6, 0.8 and 1.0 mg mL⁻¹), and the results were expressed as gallic acid equivalents (GAE) per 100 g of extract.

Determination of total flavonoids

The total flavonoids were determined according to Woisky & Salatino (1998). The assay is based on the formation of acid-stable complexes between aluminum chloride with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. Also, aluminum chloride forms acid-labile complexes with the *o*-dihydroxyl groups in the A- or B-ring of flavonoids,

with absorbance at 425 nm. Quercetin was used to construct the calibration curve (0.025, 0.05, 0.1, 0.2 and 0.4 mg mL⁻¹), and the results were expressed as milligrams of quercetin equivalents per 100 g sample (dry basis).

Determination of proanthocyanidins (condensed tannins): Vanillin method

The proanthocyanidins (condensed tannins) were assayed using the chromogen method described by Julkunen-Tiitto (1985). The absorbance was measured at 500 nm. Catechin was used to establish the calibration curve (0.02-0.60 mg mL⁻¹), and the results were expressed as milligrams of catechin equivalents per 100 g sample (dry basis).

Evaluation of antioxidant capacity

Reduction of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical

The antioxidant capacity of the aqueous extracts of the coffee beans and shells was determined using the procedure proposed by Brand-Williams et al. (1995). The method is based on the reduction of the DPPH radical by antioxidants present in the extract, resulting in a decrease in absorbance measured at 515 nm. The results were expressed in EC₅₀ efficient concentration.

The EC₅₀ values were calculated by linear regression generated from graphs where the abscissa axis (x) represents the concentration in µg.mL⁻¹ and the (y) axis, the average percentage of the antioxidant activity of the triplicates according to with Equation 1.

$$EC_{50} \text{ (g mL}^{-1}\text{)} = a \frac{Abs_{DPPH \ 60 \ \mu M}}{2} - b \quad (1)$$

where: EC₅₀ = is the concentration of extract capable of sequestering 50% of the DPPH radical; a = slope coefficient of the decreasing line, constructed from five solutions with known concentrations of each extract; Abs_{DPPH 60 µM} = half of the absorbance of the 60 µM DPPH solution; b = linear coefficient of the descent line, constructed from five solutions of known concentrations of each extract.

Co-oxidation of β-carotene and linoleic acid

The antioxidant capacity of the extracts was determined according to the protocol described by Marco (1968) and modified by Hammerschmidt & Pratt (1978). The results were expressed as a percentage of inhibition of the oxidation, calculated in relation to the decay of the control absorbance ($Abs_{control}$). The decrease in absorbance of the samples (Abs_{sample}) was correlated with the decrease in $Abs_{control}$ and converted to a percentage of the antioxidant capacity using Equation 2:

$$\text{Antioxidant capacity (\%)} = \frac{(Abs_{control}) - (Abs_{sample})}{(Abs_{control})} \quad (2)$$

where: ($Abs_{control}$) = $Abs_{initial} - Abs_{final}$; and (Abs_{sample}) = $Abs_{initial} - Abs_{final}$.

2.3 Coffee husk antinutrients

Determination of oxalic acid

Oxalic acid was extracted by heating in an acidic medium (6 N HCl), according to Loures & Jokl (1990). The precipitate was quantitated by titration with 0.02 M KMnO₄ solution, and the results were expressed as milligrams per gram of the dry sample.

Determination of hemagglutinating activity

The hemagglutinating activity was estimated by adding erythrocyte suspension (human blood type A) to microtiter plates, which contained the diluted series of the husk extract (Figueroa & Lajolo, 1997). The reading was performed visually, to verify the formation or non-agglutination of the blood cells. As the negative control, only erythrocyte suspension was used, and as a positive control, black bean extract was used.

2.4 Obtaining concentrated pineapple juice

Pineapples (*Ananas comosus*) at the mature maturation stage were purchased from the Itapetinga retail market. The fruits were sanitized in chlorinated water and peeled manually. The fruit pulp was disintegrated in an industrial blender and vacuum-filtered to obtain the juice, which was concentrated on a rotary evaporator at 50 °C until a soluble solids content corresponding to 25 °Brix was reached.

2.5 Manufacture of beverages containing coffee husk extract and concentrated pineapple juice

To obtain the beverages, three formulations with the following proportions (v/v) of coffee husk extracts and concentrated pineapple juice were developed: 80:20 (F1); 90:10 (F2) and 100:00 (F3). The drinks were packed in plastic containers and stored under refrigeration (approximately 7 ± 2 °C) until microbiological analysis (molds, yeasts and *Salmonella* sp.), which was performed according to Silva et al. (2010), and sensory testing (see section 2.6).

2.6 Sensory evaluation

The acceptance of the drink was assessed according to Stone & Sidel (2004), using a structured 9-point hedonic scale (1 = "I greatly disliked" to 9 = "I enjoyed it very much"). Non-trained judges (52 people) evaluated the flavor, aroma and general impression of the beverages in the sensory analysis laboratory of the State University of Southwest of Bahia. The study was approved by the research ethics committee of the State University of Southwest of Bahia (CAAE: 79093717.1.0000.0055).

3 Results and discussion

3.1 Aqueous extraction of coffee husk: preliminary tests

The results of the TPC contents obtained from the five different extraction methods (decoction with mechanical agitation, decoction without mechanical agitation, infusion, coffee pot and ultrasonic bath) revealed decoction with mechanical agitation and decoction without agitation stood out from the others (Table 1).

Therefore, it was decided to select the most economical and simple method for industry (i.e., decoction without mechanical agitation) to conduct the physical-chemical analyzes, beverage preparation, microbiological analysis and sensory evaluation.

Several studies have been conducted to determine the best conditions (Boeira et al., 2018; Souza et al., 2009) and methods of extraction (Codevilla et al., 2018; Cruz et al., 2017; Santos et al., 2016) to obtain bioactive constituents, antioxidants and antimicrobials from different raw materials.

3.2 Chemical characterization of aqueous extracts of husk and coffee beans

In the chemical characterization, aqueous extracts of coffee husk obtained by decoction without mechanical agitation showed a high TPC content (Table 2). The results were higher than those observed by Andrade et al. (2012), who quantified the TPC of *C. arabica* coffee husk from extracts obtained using several different extractive solvents (hexane, dichloromethane, ethyl acetate and ethanol) and extraction methods (ultrasound, Soxhlet, supercritical fluid extraction with CO₂), and obtained results varying between 16.1 and 151 mg g⁻¹ of sample. In that study, the best antioxidant activity was demonstrated by the coffee husk (husks) extracted by ultrasound or Soxhlet, whereas, here,

Table 1. Total phenolic contents obtained from coffee husk by various aqueous-based extraction techniques.

Extraction technique	Total phenolic content (mg GAE 100 g ⁻¹)
Decoction with mechanical agitation	382.97 ± 0.03 ^a
Decoction without mechanical agitation	345.72 ± 0.02 ^a
Infusion	276.28 ± 0.04 ^b
Electric coffee maker	270.95 ± 0.01 ^b
Ultrasonic bath	184.79 ± 0.03 ^c

Means followed by different letters differ from each other (P < 0.05), by the Tukey test. GAE = gallic acid equivalents.

Table 2. Chemical characterization and antioxidant capacity of aqueous extracts of organic arabica husk and grains, total phenolic constituents (TPC), total flavonoids and condensed tannins.

Sample	TPC (mg GAE 100 g ⁻¹)	Total flavonoids (mg QE 100 g ⁻¹)	Condensed tannins (mg CE 100 g ⁻¹)
Husk 1	383.47 ± 0.35 ^e	7.10 ± 0.20 ^f	1.15 ± 0.56 ^b
Husk 2	417.76 ± 0.86 ^e	7.66 ± 0.63 ^e	1.79 ± 0.89 ^b
Husk 3	404.00 ± 1.72 ^e	7.18 ± 0.12 ^{ef}	2.37 ± 0.27 ^a
Husk 4	396.82 ± 0.08 ^e	7.43 ± 0.35 ^{ef}	2.14 ± 0.20 ^{ab}
Husk 5	454.59 ± 0.20 ^{de}	8.47 ± 0.11 ^d	2.12 ± 0.74 ^{ab}
Grains 1	662.15 ± 0.83 ^a	13.88 ± 0.87 ^a	Not analyzed
Grains 2	606.40 ± 0.78 ^{ab}	13.44 ± 0.80 ^a	Not analyzed
Grains 3	514.66 ± 0.29 ^{cd}	10.72 ± 0.35 ^c	Not analyzed
Grains 4	545.74 ± 0.56 ^{bc}	11.63 ± 0.87 ^b	Not analyzed
Grains 5	568.97 ± 0.40 ^{bc}	11.53 ± 0.20 ^b	Not analyzed

Mean values of three replicates; Means followed by different letters in the same column differ statistically (P < 0.05) by the Tukey test. GAE = gallic acid equivalents; QE = quercetin equivalents; CE = catechin equivalents.

the grains had significantly higher TPC contents and antioxidant activity (see section 3.3) when compared to the coffee husk.

Using ether, alcohol and water as solvents in the extraction of phenolic compounds from coffee powder generated during the dry processing of husking Arabica coffee, Baggio et al. (2007) observed TPC levels in the range 288.6-424.5 mg GAE 100 g⁻¹ of dry sample; results similar to those found in the present study.

Studies related to the solubility of organic compounds indicate that TPCs tend to be more soluble in polar organic solvents (Kim & Lee, 2002; Martins et al., 2013). Therefore, in the literature, methanol and ethanol are solvents most indicated and considered as most efficient in the extraction of these compounds (Chirinos et al., 2007; Jamal et al., 2010). However, the present study highlighted the efficacy of aqueous extraction for obtaining the phenolic constituents. In addition, water stands out as being a non-polluting solvent.

The coffee beans had a greater total flavonoids content when compared to the coffee husk (Table 2). However, even if the aqueous extracts of the grains are considered superior to the husk extracts, meaningful results were obtained for the extracts of the husk.

3.3 Evaluation of antioxidant capacity

The DPPH antioxidant capacity of aqueous extracts of coffee husk at the different dilutions ranged from 81.37% to 86.29%, demonstrating that the aqueous extract of the coffee husk is effective in inhibiting the DPPH radical, especially when compared to the coffee beans data. From the percentage of DPPH inhibition, the EC₅₀ of the grains and coffee husk samples was determined (Table 3), with the best antioxidant potential (lowest EC₅₀ value) identified as the extract labeled husk 4.

For the β-carotene and linoleic acid co-oxidation test, the synthetic antioxidant 2,6-bis(1,1-dimethylethyl)-4-methylphenol (BHT) was used as a reference because it is widely used in the food industry. In this assay, 95.5% inhibition of BHT was observed. Among the samples, the extracts of grains 1, 2 and 3, presented the greatest percentage of inhibition of co-oxidation of β-carotene and linoleic acid (Table 3). The percentage inhibition obtained in this assay demonstrates good antioxidant activity by aqueous extracts of organic arabica husk, thereby indicating that the extracts contain phytochemicals capable of inhibiting the oxidation of β-carotene and linoleic acid. This result corroborates the high TPC level determined in the aqueous extracts since these compounds are described as biomolecules capable of inhibiting lipid peroxidation (Haslam, 1996; Soares, 2002).

3.4 Microbiological and antinutrient analysis of coffee husks

In the analyzed samples, the absence of *Salmonella* sp., bacteria indicative of hygienic-sanitary quality was observed, thereby meeting the standards established by RDC 12 of the National Agency of Sanitary Surveillance (Brasil, 2001). In addition, the absence of molds, yeasts and total fecal coliforms was verified for the analyzed samples, and the beverage could be recommended for human consumption.

In relation to the tests used to investigate antinutrients, the absence of hemagglutinating activity was verified while oxalic acid was detected at 3.31 mg/100 g dry sample. The oxalic acid content in the coffee husk is considered low when compared to results found in the literature for various food products. Teixeira dos Santos (2006) evaluated the effect of boiling on some antinutritional factors in leaves of broccoli, cauliflower and cabbage, and found average contents of 60.53, 49.66 and 38.09 mg oxalic acid 100 g⁻¹ of dry matter, respectively. In an investigation into the oxalate content of fruit and vegetable juices, nectars and beverages, Siener et al. (2016) noted 54 and 65 mg oxalic acid/100 mL beet juice and mentioned that the high values of oxalate in juice containing organic agriculture might be associated with the possible role of oxalate in plant tissues. The consumption of foods or beverages containing oxalates should be avoided, owing to the formation of calcium oxalate stones. Therefore, the coffee husk presented as the best consumption option because they gave the lowest value.

3.5 Sensory analysis

The sensorial analysis verified that among the developed beverages, F2 and F3, produced with the addition of concentrated pineapple juice (10 and 20%), received better acceptance among the tasters (Table 4). Therefore, the addition of concentrated

Table 3. Antioxidant capacity of aqueous extracts of coffee husk by the free DPPH radical sequestration method and inhibition of co-oxidation of β-carotene and linoleic acid (%).

Sample	DPPH EC ₅₀ (mg mL ⁻¹)	Inhibition of co-oxidation of β-carotene and linoleic acid (%)
Husk 1	4.71 ^f	40.78 ^{bc}
Husk 2	3.57 ^h	34.88 ^c
Husk 3	4.44 ^g	43.74 ^{abc}
Husk 4	2.73 ^j	44.55 ^{abc}
Husk 5	3.44 ⁱ	40.80 ^{bc}
Grains 1	15.09 ^a	68.58 ^a
Grains 2	11.48 ^b	66.43 ^{ab}
Grains 3	10.44 ^c	58.22 ^{abc}
Grains 4	10.10 ^d	64.65 ^{ab}
Grains 5	7.53 ^e	68.22 ^a

Means in the same column followed by different letters differed from each other (P < 0.05), by the Tukey test.

Table 4. Flavor, aroma and overall appearance of the beverage developed with the rinds of coffee with different concentrations of concentrated pineapple juice.

Sample	Flavor	Aroma	Overall impression
F1*	2.96 ± 1.88 ^b	4.25 ± 2.06 ^b	3.75 ± 1.90 ^b
F2**	4.86 ± 2.02 ^a	5.44 ± 1.91 ^a	5.05 ± 2.01 ^a
F3***	5.48 ± 2.11 ^a	5.44 ± 1.95 ^a	5.48 ± 2.05 ^a

Means in the same column followed by different letters differ from each other (P < 0.05), by the Tukey test. *100% coffee husk extract; **90% coffee husk extract; ***80% coffee husk extract.

Table 5. DPPH antioxidant capacity (EC_{50}) of the least and most accepted drinks in the sensory test.

Sample	DPPH EC_{50} (mg mL ⁻¹)
F1*	3.44 ^a
F3**	0.94 ^b

Means in the same column followed by different letters differ from each other ($P < 0.05$), by the Tukey test. *100% coffee husk extract; **80% coffee husk extract.

pineapple juice in the formulations was a determinant factor to increase product acceptance, as it contributed significantly to the flavor, aroma and overall impression characteristics of the beverage.

3.6 Potential antioxidant activity of the beverage developed from extracts of the coffee husk (comparison between the least and most sensorially accepted drinks)

F1 and F3 (corresponding to the least and most sensorially accepted drinks, respectively), developed from residual organic coffee husk, showed good DPPH antioxidant activity (Table 5). F1, with 100% of the coffee husk extract, highlighted the coffee husk as a valuable source of phytochemicals with antioxidant potential. In F3, with 80% of the coffee husk extract in its composition, the EC_{50} value was even better since it was lower than that observed in F1. The best antioxidant potential presented in F3 can be directly related to the addition of concentrated pineapple juice, which was used with the purpose of benefiting the sensorial characteristics of the developed beverage. However, it is evident that the addition of the juice contributed significantly to the chemical composition and antioxidant potential of the beverage.

4 Conclusion

Aqueous extracts of coffee husk constitute a promising natural source of bioactive phytochemicals, in addition to being considered safe for consumption, owing to the low levels of antinutrients. It was noticed that the beverage incorporated with concentrated pineapple juice presented the greatest acceptability, besides increasing the antioxidant capacity of the product. Thus, the formulated beverages constitute a promising alternative for the beverage market, given the meaningful content of phenolic constituents derived from coffee husk.

Acknowledgements

The authors thank the Bahia Research Foundation – FAPESB, for the financial research assistance (Process n. BOL2060/2014).

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