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Optimization of the extraction of bioactive compounds from Arabica coffee husk using ultrasound-assisted and their potential as a source of antioxidant, and aromatic substances

Otimização da extração de compostos bioativos da casca do café arábica por ultrassom e seu potencial como fonte de substâncias antioxidantes e aromáticas

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This work aimed to optimize the process of polyphenolic compounds extraction from Arabica coffee industrial residue (pulp, skin, mucilage, and parchment) using the ultrasound-assisted technique, evaluate the antioxidant potential of the extracts and determine the volatile profile of the extract with the highest antioxidant activity. In the experiments, the ethanol concentration, solvent/solid ratio and extraction time were varied through a central composite rotational design. Mathematical models were obtained for the extraction of total phenolic, total flavonoid and antioxidant activity were obtained, where the ethanol concentration was the parameter with the greatest influence on the extraction. In the extract with the highest antioxidant potential by the DPPH and FRAP methods, 43 compounds were detected, with higher concentrations of diethyl acetal, isoamyl acetate, ethyl hexadecanoate, and limonene, compounds known as fragrance aromas. Arabica coffee industrial residue showed the potential to be used in future applications as a source of bioactive and aroma compounds in food, pharmaceutical or cosmetic products.
Keywords: by-products, response surface, bioactive compounds.

Este trabalho teve como objetivo otimizar o processo de extração de compostos polifenólicos do resíduo industrial do café arábica (polpa, casca, mucilagem e pergaminho) por meio da técnica assistida por ultrassom, avaliar o potencial antioxidante dos extratos e determinar o perfil volátil do extrato com maior atividade antioxidante. Nos experimentos, a concentração de etanol, a razão solvente/sólido e o tempo de extração foram variados pelo delineamento rotacional composto central. Foram obtidos modelos matemáticos para extração de fenólicos totais, flavonoides totais e atividade antioxidante, sendo a concentração de etanol o parâmetro que mais afetou a extração. No extrato com maior potencial antioxidante pelos métodos DPPH e FRAP, foram detectados 43 compostos, com maiores concentrações de dietil acetal, acetato de isoamila, hexadecanoato de etila e limoneno, compostos conhecidos como aromas fragrantes. O resíduo industrial de café arábica apresentou potencial para futuras aplicações como fonte de substâncias bioativas e aromatizantes em produtos alimentícios, farmacêuticos ou cosméticos.
Palavras-chave: subprodutos, superfície de resposta, compostos bioativos.

1. INTRODUCTION

The coffee plant belongs to the genus Coffea and the species belonging to this genus are: *Coffea liberica*, *Coffea brevipes*, *Coffea racemosa*, *Coffea sessiflora*, *Coffea stenophylla*, *Coffea salvatrix*, *Coffea kapakata*, *Coffea eugenioides*, *Coffea canephora* or "Robusta coffee" and Coffea Arabica or "Arabica coffee" [1]. In the years 2021 and 2022 the estimated total coffee production was of 167.2 million bags, being South America's production of 77.5 million bags. The production of Arabica and Robusta coffee was approximately 93.97 and 73.2 million bags, respectively [2].

The parts of coffee fruit are shown in Figure 1. Various by-products are produced from coffee processing such as coffee husk, pulp, mucilage, and parchment are produced. The beans are initially harvested, selected and cleaned. After that, the beans can be processed to dry, semi-dry or wet.

Coffee husk can be obtained as by-products of dry processing consisting of skin, pulp, mucilage and parchment. Wet processing generates coffee pulp as a solid residue as well as wastewater. In semi-dry processing, mucilage, pulp and parchment are obtained as by-products. There is also spent ground coffee, a residue generated by coffee consumers in coffee shops and through the industrial production of soluble coffee [3]. These residues may possess properties that make them attractive to various industrial sectors as antioxidants, antimicrobials and a source of natural flavours, among others.

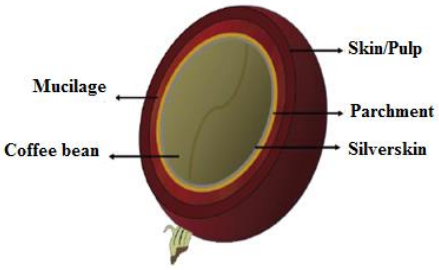

Figure 1. Parts of coffee fruit in natura (Adapted of Duran et al., 2017).

Some researchers have found compounds such as chlorogenic, caffeic, ferulic and coumaric acids in coffee pulp; chlorogenic acid, vanillic acid, protocatechuic acid, p-coumaric acid and caffeine in parchment and chlorogenic acid and flavonoids in the silver skin [4-6]. Regarding the identification of volatile aromatic compounds, only studies with robusta coffee or roasted arabica coffee and spent coffee grounds were found [7-10].

To obtain extracts from coffee and its by-products, conventional techniques such as maceration and Soxhlet extraction or unconventional techniques such as ultrasound, supercritical fluid, subcritical water, and pulsed electric field are used [3]. The ultrasound-assisted method used to extract bioactive compounds is based on the application of mechanical waves, which have a frequency greater than 20 kHz. These act by breaking the cell wall of the plant matrix, facilitating the penetration of the extracting solution and, consequently, the contact of the solute-solvent, releasing the compounds of interest. [11]. The ultrasound-assisted technique has been used to obtain bioactive extracts from coffee silver skin, spent coffee grounds and coffee pulp [12-17]. Regarding coffee husk, Rebollo-Ernandez et al. (2021) [18] optimized the extraction of phenolic compounds from coffee husk in a temperature-controlled water bath with constant stirring, and Andrade et al. (2012) [19] described the chemical composition and antioxidant activity of coffee husk extracts obtained by supercritical fluid extraction, ultrasound and Soxhlet extraction. However, no reports have been found on the optimization the ultrasound-assisted extraction of bioactive compounds from coffee husk and their volatile compound composition. Considering the commercial demand for aroma compounds, the discovery of new natural sources of these is of great value. Hence, the present study aimed to optimize the process of ultrasound-assisted extraction of polyphenolic compounds from coffee husk by evaluating the antioxidant of the extracts and determining the profile of volatile aroma compounds.

2. MATERIALS AND METHODS

2.1 Materials

The industrial coffee residue (pulp, skin, mucilage and parchment), known as coffee husk, was obtained from an industry located in Sergipe state (Brazil) after roasting of the Arabica coffee beans at 256℃ for 5 min. A total of approximately 1 kg of residue (coffee husk), from several batches of coffee processing, was collected in january of 2020 and used for all experiments. Folin-Ciocalteau phenol reagent, gallic acid, quercetin, 6-hydroxy-2,5,7,8- tetramethylchromo -2-carboxylic acid (Trolox); ABTS (2,2′-azino-di-(3-ethylbenzthiazoline sul phonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (ferric reduction antioxidant power) reagent were acquired from Sigma

Aldrich and Fluka Analytica (St Louis, MO, USA). Ethanol was obtained from Neon (São Paulo, Brazil). Aluminium chloride and sodium carbonate were obtained from Dinâmica (Indaiatuba, São Paulo, Brazil). The alkanes (C7-C30) and 1-octanol (purity≥99.7%) were acquired from Sigma-Aldrich (San Luis, Missouri, USA).

2.2 Optimization of extraction of bioactive compounds from coffee husk by the assisted-ultrasound technique

The coffee husk was dried in an air circulating dryer with at 37°C for 25 h, until a final humidity content of 10% was obtained. The extraction of polyphenolic compounds was performed in aqueous ethanol solutions of varying concentration between 20% and 90%, a solvent/solid ratio from 10 to 50 (mL/g) and a time of extraction between 20 and 120 min, according to a central composite rotational design (CCRD) 2^3 with six axial points and three central points [20]. The extractions were performed in an ultrasonic bath (USC Ultrasonic Bath – 1400A, UNIQUE) with a frequency and ultrasonic power of 40 kHz and 135 watts, respectively. After the extractions, the samples were centrifuged at 129.1 x g at 4°C (refrigerated centrifuge model 5810R Eppendorf – Hamburg, Germany) for 10 min and filtered. The supernatants obtained were analysed for total phenolic and flavonoid contents and antioxidant activity.

A CCRD was performed to obtain a second-order model (Eq. 1) to predict the bioactivity of coffee residue extract in terms of total phenolic and flavonoid contents.

$$y = \beta 0 + \sum_j \beta_j x_j + \sum_{i<j} \beta_{ij} x_i x_j + \sum_j \beta_{jj} x_j^2 + \varepsilon \quad (1)$$

where y is the predicted response (total phenolic content, total flavonoid content and antioxidant activity), β0 is the global mean, βj is the linear coefficient, βij is the interaction coefficient, βjj is the quadratic coefficient, ε is the error of the model, and xi and xj are the encoded values of the independent variables.

The experimental data from CCRD were analysed using Statistica software, version 8.0 (StatSoft, Inc., Tulsa, OK, USA). The analysis of variance (ANOVA) and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined at the 5% significance level. The quality of the model equation was expressed as the coefficient of determination (R^2) and its statistical significance was determined using the F-test. For validation of the statistical results, the observed values of total phenolic and total flavonoid content were compared with the predicted values obtained by the experimental models.

2.3 Determination of total phenolic content

Total phenolic compounds (TP) were determined according to the Folin-Ciocalteau method described by Moo-Huchin et al. (2015) [21]. Aliquots of the extracts (50 μL) were transferred to test tubes, 3 mL of distilled water and 250 μL of 1N Folin-Ciocalteau reagent were added, ansd the mixture was homogenized and incubated for 8 min. Then, 750 μL of 20% (w/v) sodium carbonate solution and 950 μL of distilled water were added and homogenized in a vortex. The test tubes were kept in a darkroom for 30 min. The absorbances of the samples were measured at a wavelength of 765 nm.

The same procedure was performed, replacing the sample with the solvent to obtain the blank. For the quantification of these extracts, a calibration curve was constructed based on a range of gallic acid concentrations (0–1000 mg/L) ($y = 0.0008x + 0.0045$; $R^2 = 0.9993$). The results were expressed in mg equivalent of gallic acid per gram of solid on a dry basis (mg GAE/g of solid in d.b.).

2.4 Determination of total flavonoids content

The total flavonoids (TF) were determined by the aluminium chloride colorimetric method, with some modifications [21]. Aliquots of the extracts (1 mL) were mixed with 4 mL of distilled water and 300 μL of 5% sodium nitrite in test tubes. The samples were agitated in a vortex and incubated in the dark for 5 min. After that, 300 μL of 10% aluminium chloride was added and allowed to rest

for 1 min. Subsequently, 2 mL of 1 M NaOH and 2.4 mL of distilled water were added and homogenized again in a vortex. The absorbances of the samples were measured at 415 nm. For the blank, the sample was replaced with solvent. A calibration curve was constructed using a range of quercetin concentrations (0-700 mg/L) ($y= 0.0014x + 0.0046$; $R^2 = 0.9979$). The results were expressed as mg quercetin per gram solid on a dry basis (mg QCE/g solid d.b.).

2.5 Antioxidant activity (AA)

The *in vitro* AA was assayed via the ABTS [22], DPPH [23] and Ferric Reducing antioxidant Power (FRAP) [24] methods. For the ABTS method, the extract (30 μL) was mixed with 3.0 mL of ABTS reagent and homogenized in a vortex. After 6 min the absorbance was read at 734 nm. A calibration curve was constructed using different Trolox concentrations ranging from 200 to 2400 μmol Trolox/mL ($y= -0.0002x + 0.6662$; $R^2 = 0.9853$). The results were expressed in μmol Trolox per gram of solid. For the DPPH method, 100 μL of extract was mixed with 3.9 mL of DPPH. After 5 min, the absorbance was measured at 515 nm. Different concentrations of Trolox (between 500 and 1000 μmol Trolox/L) were used to construct the calibration curve ($y= 0.0011x-0.5225$; $R^2 = 0.9794$). The blank was performed by replacing the extract with 95% ethanol. The results were expressed as μmol Trolox/g solid. The FRAP method was performed by mixing 90 μL of extract with 270 μL of distilled water and 2.7 mL of the FRAP reagent. This solution was homogenized in a vortex and incubated at 37°C in a water bath for 30 min. After that, the absorbance was determined at 595 nm. The calibration curve was obtained with concentrations ranging from 100 to 1200 μmol Trolox/mL ($y=0.001x + 0.0307$; $R^2 = 0.9952$). The results were expressed in μmol Trolox per gram of solid.

2.6 Profile of volatile compounds

The extract, which showed the highest antioxidant activity was evaluated by gas chromatography in order to identify and quantify volatile compounds.

2.6.1 Extraction of volatile compounds by Stir Bar Sorptive Extraction (SBSE)

Stir bars 10 mm in length and coated with 0.5 mm of polydimethylsiloxane (PDMS) were used. Prior to use, stir bars were conditioned for 3 hours to 300°C under helium flow using a TC2 tube conditioner (Gerstel, Muelheim an der Ruhr, Germany), according to the manufacturer's recommendations. For extraction, 5 mL of the sample was placed in a 20 mL vial sealed with septum (Gerstel, Germany); the PDMS stir bar was immersed in the sample for adsorption of analytes and stirred at 750 rpm at room temperature (25°C) for 60 min. After that, the bar was washed with deionized water and dried with a clean, lint-free tissue and then transferred to a thermal desorption tube (Gerstel, Germany) for further analysis by GC-MS. The extraction was performed in triplicate. Desorption of volatile compounds in SBSE was performed in a Thermal Desorption Unit (TDU) equipped with a cold injection system (CIS-4) (Gerstel, Mülheim an der Ruhr, Germany), installed on an Agilent 7890B gas chromatograph. (Agilent Technologies, Santa Clara, USA). The TDU temperature was programmed from 30°C (held for 0.5 min) to 250°C at 120°C/min (held for 5 min); then, the analytes were desorbed and concentrated in the CIS-4 with a schedule of 45°C to 250°C (5 min) with a flow rate of 12°C/s to transfer the volatiles to the analytical column. The TDU was operated in splitless desorption mode. The PDMS stir bars were reconditioned after each analysis.

2.6.2 Gas chromatography-mass spectrometry (GC-MS) analysis

The samples were analysed in a 7890B gas chromatograph equipped with a 5977A mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Volatile components were separated using a non-polar HP-5MS capillary column (30 m × 0.25 mm, 0.25 μm; Agilent Technologies, USA). Helium (> 99.99%) was used as carrier gas at a constant flow rate of 1.3 mL/min. Injection was in vent mode (purge flow to split vent 30 mL/min, vent 60 mL/min and pressure 16.40 psi). The GC oven temperature was programmed to 40°C (held for 3 min), raised to 130°C (3°C/min,

held for 5 min) and then raised to 250°C (10°C/min, held for 3 min). The transfer line, quadrupole and source temperatures were maintained at 260°C, 180°C and 280°C, respectively. For mass spectrometry was used in 70 eV electron impact mode with a scanning range of 35–350 m/z.

Volatile compounds were identified by comparing their mass spectra with those obtained from the NIST14 database, and from the comparison of calculated retention indices with those in open-access data of the NIST Mass Spectral Search Program library (NIST, Washington, DC). The retention index of the compounds was calculated based on the retention time of a series of alkanes (C7-C30) (Sigma-Aldrich, San Luis, Missouri, USA). To quantify volatiles, the integrated areas, based on the total ion chromatogram, were divided by the peak of the internal standard, assuming a response factor equal to 1. The relative concentrations of volatiles were determined by comparison with the concentration of the internal standard (1-octanol) (≥ 99.7%, Sigma-Aldrich, San Luis, Missouri, USA).

3. RESULTS AND DISCUSSION

3.1 Effect of parameters on the extraction of bioactive compounds from coffee residue using the ultrasound-assisted technique

The effect of the ethanol concentration, solvent/solid ratio and extraction time on the recover of polyphenolic compounds from coffee husk by ultrasound-assisted extraction was evaluated. Total phenolic and total flavonoids contents ranged from 14.25 to 94.56 mg GAE/g solid d.b.; 23.12 and 68.08 mg QCE/g solid d.b., respectively (Table 1). In this study, the quantification of polyphenolic compounds using the Folin-Cicalteau method, is subject to the presence of other non-phenolic compounds or seldom thought of as phenols substances, as proteins, which can also react under the same conditions of the method [25]. However, researchers have reported that ethanolic and aqueous extracts from coffee husk likely contain polyphenolic compounds such as vanillic acid, chlorogenic acid, protocatechuic acid, kaempferol-3-ogalactoside and gallic acid [18, 19]. Rebollo-Hernanz et al. (2021) [18] obtained lower values of TP (3.47 to 5.93 mg/g) in aqueous coffee husk extract using heat-assisted extraction. Shang et al. (2017) [26] optimized the extraction of TP from spent coffee grounds by liquid extraction under pressure. The authors obtained TP between 7.87 and 22.64 mg/g, values much lower than those obtained in this work. Araujo et al. (2022) [27] optimized the extraction of total phenolics and total flavonoids from spent coffee grounds using maceration with stirring. The authors varied the temperature and ethanol concentration and also obtained lower values of TP (between 3.77 and 9.36 mg GAE/g dry residue) and TF (between 0.38 and 0.66 mg QCE/g dry residue).

The antioxidant activity of the extracts ranged from 42.06 to 619.93; 5.60 to 196.29 and 55.64 to 620.24 µmol Trolox/g solid by ABTS, DPPH and FRAP methods, respectively (Table 1). Extract 2 (obtained with 34.17% ethanol, 41.91 mL of solvent/g solid and 99.76 min extraction) showed higher AA by DPPH (196.29 µmol of Trolox/g solid) and FRAP (620.24 µmol of Trolox/g solid) methods. This result may have been due to the high content of TP (84.87 mg GAE/g solid) and the presence of antioxidants such as chlorogenic acids [19] in the extract. Also, considering that the extract has a higher water content than alcohol, this means that the antioxidant compounds have a polar character and thus a greater affinity for water. By contrast, extract 5 (obtained with 75.83% ethanol, 41.91 mL/g and 40.24 min) showed higher AA by ABTS, indicating the presence of less polar compounds with a greater affinity for ethanol. Shang et al. (2017) [26] optimized the extraction of TP from spent coffee grounds by liquid extraction under pressure. The authors obtained TP between 7.87 and 22.64 mg/g and AA by ABTS between 6.72 and 17.19 mg/g, values much lower than those obtained in this work.

Table 1: A central composite rotatable design with 2^3 plus 4 axial points and 3 central points and the responses of total phenolic, total flavonoid compounds and antioxidant activity by ABTS, DPPH and FRAP methods of coffee husk extracts.

Extract	X_1	X_2	X_3	TP (mg GAE/g solid d.b.)	TF (mg QCE/g solid d.b.)	ABTS (µmol Trolox/g solid)	DPPH (µmol Trolox/g solid)	FRAP (µmol Trolox/g solid)
1	75.83 (+1)	41.91 (+1)	99.76 (+1)	39.55 ± 0.02	54.45 ± 0.02	51.50 ± 0.89	112.46 ± 1.02	456.24 ± 29.63
2	34.17 (-1)	41.91 (+1)	99.76 (+1)	84.87 ± 0.01	68.08 ± 0.01	97.10 ± 0.61	196.29 ± 1.42	620.24 ± 10.67
3	75.83 (+1)	18.09 (-1)	99.76 (+1)	16.39 ± 0.01	41.08 ± 0.03	42.86 ± 0.28	54.86 ± 0.60	132.03 ± 1.53
4	34.17 (-1)	18.09 (-1)	99.76 (+1)	56.84 ± 0.01	67.63 ± 0.01	70.44 ± 0.34	112.90 ± 0.42	451.21 ± 22.52
5	75.83 (+1)	41.91 (+1)	40.24 (-1)	57.89 ± 0.00	43.83 ± 0.01	616.93 ± 5.67	112.25 ± 0.74	361.52 ± 22.52
6	34.17 (-1)	41.91 (+1)	40.24 (-1)	94.56 ± 0.01	62.99 ± 0.02	79.01 ± 0.56	115.65 ± 0.21	408.88 ± 21.93
7	75.83 (+1)	18.09 (-1)	40.24 (-1)	56.08 ± 0.05	37.33 ± 0.04	42.06 ± 0.39	48.98 ± 0.64	169.48 ± 0.76
8	34.17 (-1)	18.09 (-1)	40.24 (-1)	72.02 ± 0.05	65.43 ± 0.01	169.42 ± 4.88	57.33 ± 1.87	55.64 ± 0.25
9	90 (+1.68)	30 (0)	70 (0)	14.25 ± 0.01	23.12 ± 0.04	354.70 ± 11.06	15.82 ± 0.07	93.18 ± 0.84
10	20 (-1.68)	30 (0)	70 (0)	69.56 ± 0.02	70.26 ± 0.00	62.77 ± 0.23	83.03 ± 0.26	424.38 ± 32.24
11	55 (0)	50 (+1.68)	70 (0)	70.00 ± 0.04	65.32 ± 0.02	425.75 ± 8.07	26.73 ± 0.17	444.30 ± 35.35
12	55 (0)	10 (-1.68)	70 (0)	69.25 ± 0.04	58.81 ± 0.03	254.30 ± 1.04	5.60 ± 0.01	84.69 ± 0.42
13	55 (0)	30 (0)	120 (+1.68)	70.00 ± 0.01	53.01 ± 0.02	72.34 ± 0.89	18.71 ± 0.03	385.98 ± 28.84
14	55 (0)	30 (0)	20 (-1.68)	70.50 ±0.02	52.91 ±0.00	52.05 ± 4.26	85.31 ± 0.30	391.38 ± 8.48
15	55 (0)	30 (0)	70 (0)	82.87 ± 0.07	59.55 ± 0.05	61.57 ± 0.38	85.50 ± 1.05	449.28 ± 3.82
16	55 (0)	30 (0)	70 (0)	76.50 ± 0.03	48.94 ± 0.02	61.74 ± 1.15	85.71 ± 2.41	476.58 ± 21.21
17	55 (0)	30 (0)	70 (0)	69.19 ± 0.03	40.26 ± 0.01	50.43 ± 0.30	81.81 ± 0.85	503.28 ± 25.88

X_1= Concentration of ethanol aqueous solution; X_2= Solvent/solid ratio; X_3= extraction time; TP = Total phenolic content; TF = Total flavonoid content.

The influence of various parameters on the extraction of polyphenolic compounds and in antioxidante activity is shown in Table 2. In the extraction of TP the parameters considered significant were the linear and quadratic ethanol concentration (X1), the linear solvent/solid ratio (X2) and the linear extraction time (X3). The parameters X1 (linear and quadratic) and X3 (linear) showed a negative effect, meaning that the lower the values of ethanol concentration and time, the greater the efficiency the TP extraction. And the positive effect of X2 indicated that the higher the value of solvent/solid ratio, the greater TP extraction. For the extraction of TF, the significant parameters were the linear ethanol concentration (X1), with a negative effect, and the quadratic solvent/solid ratio (X2), with a positive effect. The ethanol concentration was the parameter with the greatest influence on the extraction of these compounds: the extraction was more efficient at lower ethanol solvent concentrations. Some researchers have reported that alcohol can cause precipitation of high-molecular-weight melanoidins, complicating the extraction of these compounds and phenolic compounds linked to them [28]. On the other hand, water facilitates the extraction of low-molecular-weight melanoidins associated with phenolic compounds, increasing the antioxidant activity of the extracts [28].

Table 2: Regression coefficients (β) of the predicted second-order polynomial models for total phenolic, total flavonoid compounds and antioxidant activity by ABTS, DPPH and FRAP methods of coffee husk extracts

	Total phenolic	**Total flavonoid**	**ABTS**	**DPPH**	**FRAP**
Intercept/X_0	76.2711*	49.5242 *	66.0648^{ns}	82.0155**	478.6410*
Linear					
X_1	-16.9518*	-12.2123*	60.6680***	-26.1147***	-74.6669**
X_2	5.6287***	2.1114^{ns}	59.2021***	15.2626 ^{ns}	129.9819*
X_3	-5.1514***	1.6004^{ns}	-44.8104***	8.8117 ^{ns}	39.2990***
Quadratic					
X_1	-12.4258**	-0.8192^{ns}	37.2982^{ns}	-0.8069 ^{ns}	-69.9207**
X_2	-2.6049^{ns}	4.6283***	83.8153 ^{ns}	-12.5899 ^{ns}	-65.4511**
X_3	-0.9662 ^{ns}	1.4015^{ns}	-14.6201 ^{ns}	0.1108 ^{ns}	-22.7799 ^{ns}
Interaction					
X_1X_2	-3.1999 ^{ns}	2.7342^{ns}	80.9073***	-13.8288 ^{ns}	-15.6153 ^{ns}
X_1X_3	-4.1438 ^{ns}	0.8865^{ns}	-60.4672 ^{ns}	-5.0389 ^{ns}	-53.9179***
X_2X_3	3.3542 ^{ns}	1.2220^{ns}	-56.1455 ^{ns}	13.6501 ^{ns}	-13.5416 ^{ns}

X_1= Ethanol concentration. X_2= solvent/solid ratio. X_3 =extraction time. Significance level= *p<0.001. **p<0.05. ***0.05≤p≤0.15^{fcs} (factor considered significant). ^{ns}p>0.15 (not significant).

To obtain the mathematical models, the non-significant coefficients were excluded, and the values of the coefficients were recalculated to obtain the fitted model coefficients. The ANOVA (Table 3) shows the coefficients of determination (R^2) of the models, F-values, and p-values of the lack-of-fit test for each fitted model. The F-values of the models of TP (14.64) and TF (34.83) extraction were much higher than the F-values tabulated (3.26 and 3.74, respectively) and the R^2 of 83% for both models meant that they were well adjusted to the experimental data.

In relation to AA by the ABTS method, only the liquid/solid ratio in the quadratic function was significant at the 90% confidence level (p<0.10). The linear ethanol concentration, quadratic liquid/solid ratio and interaction between ethanol concentration and liquid/solid ratio were significant when p<0.15 was considered. However, the coefficient of determination of 0.52 indicated that the model was not adjusted to the experimental data. Also, the optimization to AA by the DPPH method was not possible due to low value of R^2 of 0.50. For this analysis, the linear ethanol concentration was significant when p<0.15 was considered, however the value of R^2 was

kept at 0.50, not being possible to optimize the model. For AA by FRAP method, ethanol concentration (linear and quadratic), liquid/solid ratio (linear and quadratic), extraction time (linear) and interaction between ethanol concentration and extraction time were the significant parameters at 85% significance ($p<0.15$). The F-value of the model (11.86) was much higher than the F-value tabulated (3.22) and R^2 of 87 % indicated that the model was well adjusted to the experimental data. Therefore, the following fitted models were obtained according Equations 2-4:

$$\text{TP (mg GAE/g solid)} = 72.75 - 16.95\,X_1 - 11.61\,X_1^2 + 5.63\,X_2 - 5.15\,X_3 \quad (2)$$

$$\text{TF (mg QCE/g solid)} = 50.09 - 12.21\,X_1 + 4.49\,X_2^2 \quad (3)$$

$$\text{AA (μmol trolox /g solid)} = 449.59 - 74.66\,X_1 - 63.21\,X_1^2 + 129.98\,X_2 - 58.74\,X_2^2 + 39.29\,X_3 - 53.91\,X_1\,X_3 \quad (4)$$

Table 3 ANOVA for a response surface quadratic polynomial model for significant independent variables of total phenolic and total flavonoid compounds and antioxidant activity by FRAP method of coffee husk extracts.

	Total phenolic		**Total flavonoid**		**FRAP**	
Parameter	F-value	*p-value*	F-value	*p-value*	F-value	*p-value*
	14.64	0.000145	34.83	0.000004	11.86	0.000480
Linear model						
X_1	35.51550	0.000066*	61.7099465	0.000002***	12.72363	0.005121**
X_2	3.91559	0.071255***	ns	ns	38.56060	0.000100*
X_3	3.27976	0.095228***	ns	ns	3.52484	0.089899***
Quadratic model						
X_1	15.85782	0.001819**	ns	ns	8.22754	0.016715**
X_2	ns	ns	7.95645	0.013613**	7.10522	0.023672**
X_3	ns	ns	ns	ns	ns	ns
Interaction						
X_1X_2	ns	ns	ns	ns		
X_1X_3	ns	ns	ns	ns	3.89006	0.076847***
X_2X_3	ns	ns	ns	ns	ns	ns
Lack of Fit	2.62388	0.307362^{ns}	0.24553	0.955331^{ns}	7.5857	0.121642***
R^2	0.83		0.83		0.87	

X_1=Ethanol concentration, X_2= solvent/solid ratio, X_3 = time of extraction. R^2= Coefficient of determination; Significance level= *p<0.001, **p<0.05, ***0.05≤p≤0.15 (factor considered significant), ^{ns}p>0.15(not significant), F-value$_{tabulated}$ (p≤0.05) to TP (4;12) = 3.26; to TF (2;14) = 3.74; to FRAP (6;10) = 3.22

The response surfaces of the extraction conditions of total phenolic and total flavonoid compounds and antioxidant activity of extracts are shown in Figures 1-3. Higher extraction of TP and TF were obtained when ethanol concentrations of 27.0 to 50.0% and 20.0 to 27.0%, solvent/solid ratio of 42.0 to 50.0 mL/g and 10 to 12 mL/g and extraction time of 20 to 35 min and 20 to 120 min, respectively were used (Figures 1-2). To obtain higher AA, ethanol concentration between 35 and 46%, solvent/solid ratio between 41 and 48 mL/g and extration time between 115 and 120 min can be used (Figure 3). The optimized operational conditions to bioactive compounds extraction and antioxidant activity of extracts from coffee husk are shown in the desirability function graphics (Figure 4). Maximum yields of TP and TF and higher antioxidant activity by

FRAP method are obtained with 41% ethanol, 41.91 mL of solvent/g of solid and 75 min of extraction. Zuorro (2015) [29] obtained a higher polyphenol extraction efficiency from spent coffee grounds (solid waste from espresso coffee) when a temperature of 47.1°C, a time of 150 min, a solvent/solid ratio of 47.98 mL/g and an ethanol concentration of 57.7% were used. These values were close to those obtained in the present work.

In order to validate the mathematical models, the experimental values (observed values) were compared with predictive values calculated by Eqs. 1-3 (Figure 5). The degree of replication, assuming tolerable deviation from the calculated model ≤15%, was satisfactory, since about two thirds of the data fell within the 15% deviation boundaries [30].

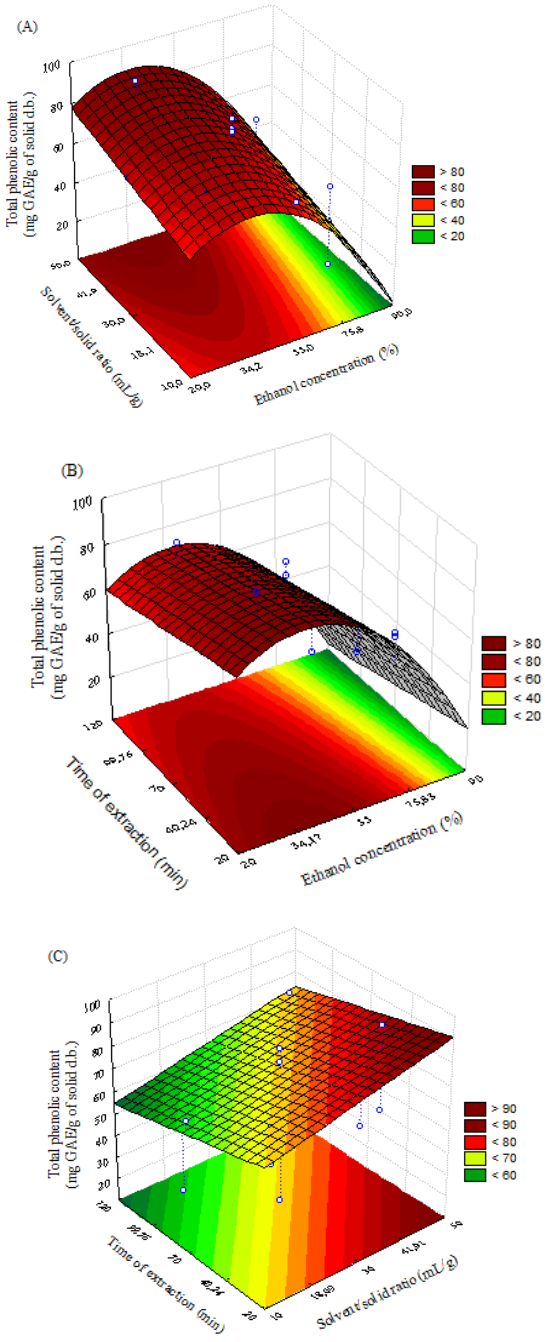

Figure 1: Response surfaces for extraction of total phenolic compounds by the ultrasound-assisted method. (A) solvent/solid ratio versus ethanol concentration, (B) Time of extraction versus ethanol concentration, (C) time of extraction versus solvent/solid ratio.

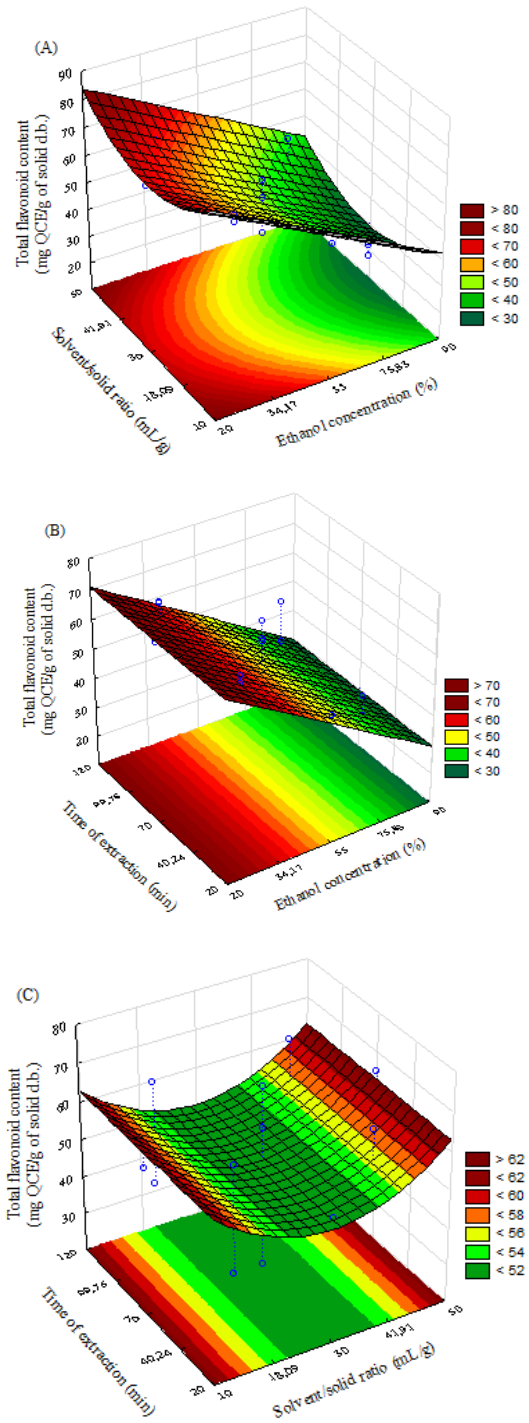

Figure 2: Response surfaces for extraction of total flavonoids by the ultrasound-assisted method. (A) solvent/solid ratio versus ethanol concentration, (B) Time of extraction versus ethanol concentration, (C) time of extraction versus solvent/solid ratio.

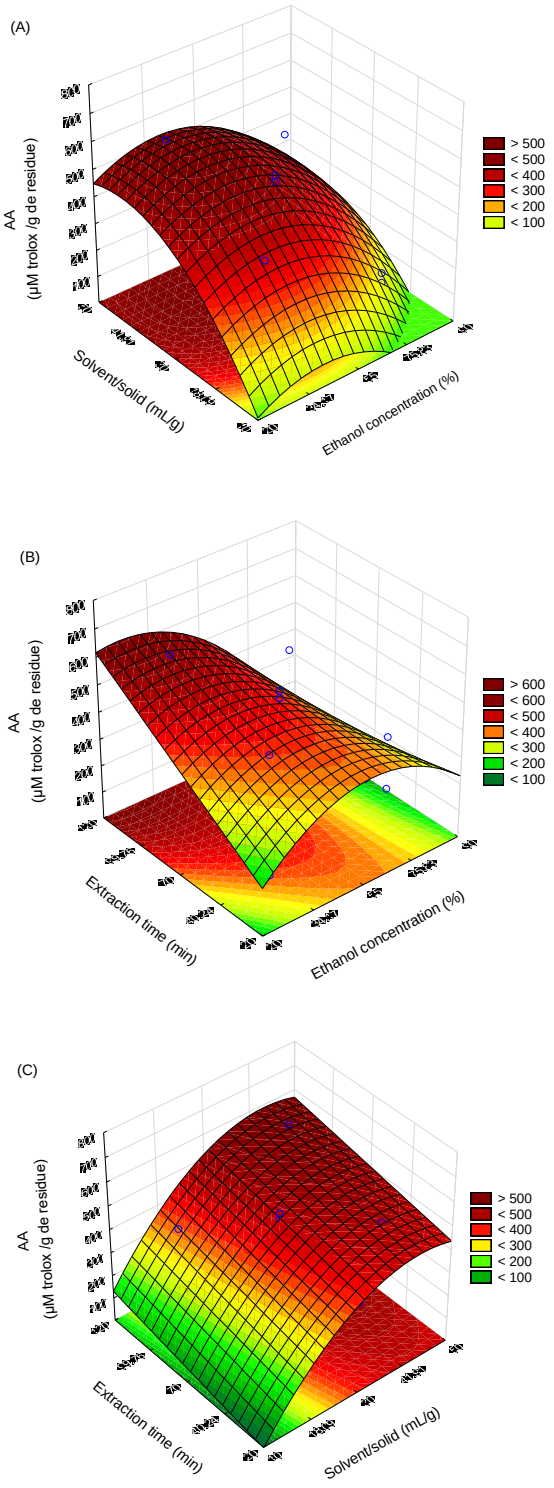

Figure 3: Response surfaces for antioxidant activity by FRAP method. (A) solvent/solid ratio versus ethanol concentration, (B) time of extraction versus ethanol concentration, (C) time of extraction versus solvent/solid ratio.

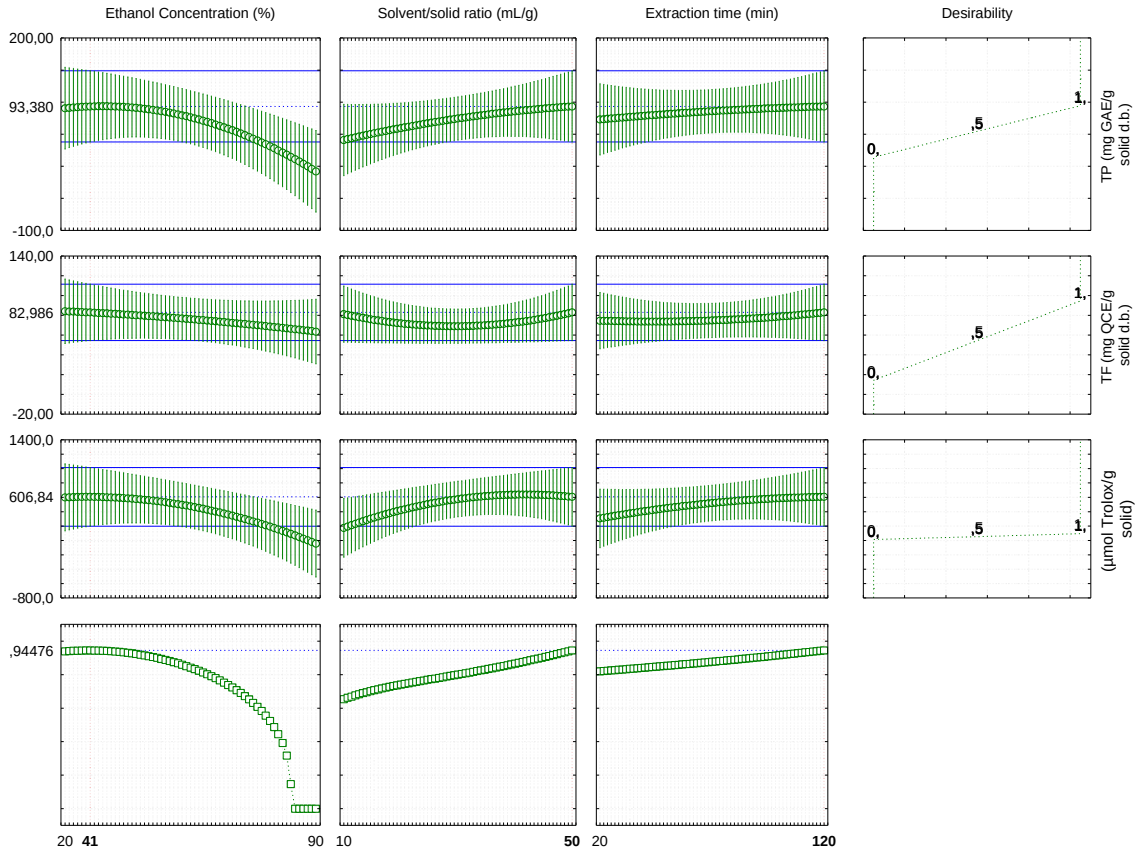

Figure 4: Desirability function for total phenolic and total flavonoid content and antioxidant activity of coffe husk extracts as a function of ethanol concentration (%), solvent/solid ratio (mL/g) and time of extraction (min).

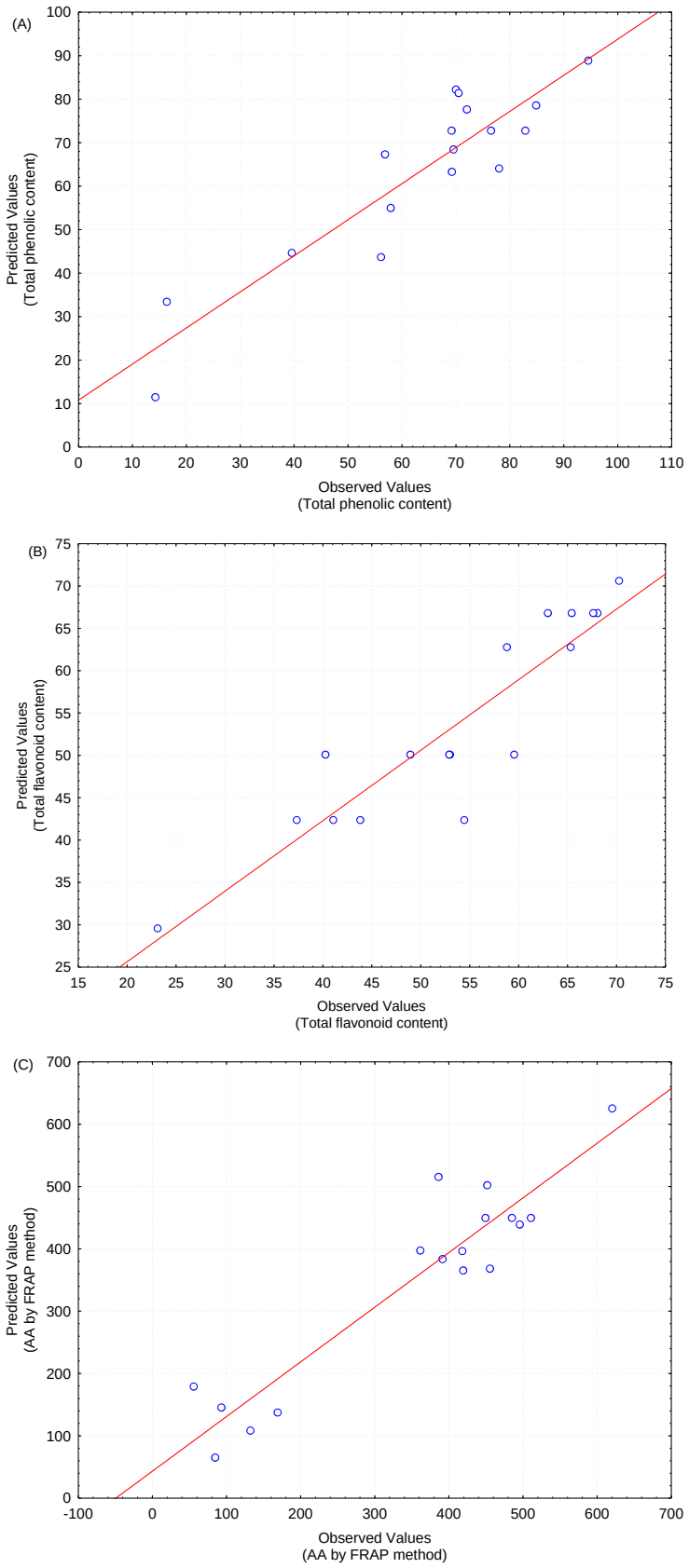

Figure 5: Comparison between the observed values and those calculated using Eqs. (1), (2) and (3) (A) For total phenolic compounds, (B) For total flavonoid compounds, (C) Antioxidant activity.

3.3 Profile of volatile compounds

The profile of volatile compounds was determined in extract 2 (higher antioxidant activity by DPPH and FRAP methods), where 43 compounds were detected: 20 esters, 9 aldehydes. 5 hydrocarbons, 1 alcohol, 2 terpenes, 3 monoterpenes, 2 sesquiterpenes and 1 ketone (Table 4). Diethyl acetal was the main compound (1238.63 µg/L) and can be found in distilled beverages such as cognac [31]. Second, isoamyl acetate (869.09 µg/L), known as the aroma sweet fruity banana flavour and ethyl hexadecanoate (519.24 µg/L), with the aroma of mild, waxy, fruity, creamy, milky, balsamic, fatty and oily can be found in distilled beverages [32]. Among the terpenes, limonene (88.18 µg/L) was the most common compound. Limonene has been used as an aroma and fragrance additive in fruit juices, candies, chewing gums, soft drinks, ice cream, cleaning products and cosmetic formulations. Also, this compound has therapeutic effects such as anti-inflammatory, antioxidant, antinociceptive, anticancer, antidiabetic, antihyperalgesic, antiviral and gastroprotective [33]. Alamri et al. (2022) [10] also identified hydrocarbons, alcohols, and esters as the most prevalent compounds in roasted Arabica coffee. On the other hand, Haile et al. (2020) [9] obtained higher levels of furans, pyrazines and carboxylic acids in dry-processed cherry coffee. No reports were found about the volatile compound profile of coffee husk.

Table 4: Volatile compounds identified in coffee husk extract by Stir-Bar-GC-MS.

Class	Compound	Formula	RI[a]	RI[b]	C (µg/L)	Odor description
Ester	Ethyl Acetate	$C_4H_8O_2$	748	634	15.46 ± 0.25	Ethereal, fruity, sweet, weedy, green
	Ethyl propanoate	$C_5H_{10}O_2$	768	715	16.51 ± 0.23	Sweet, fruity, rum, juicy, fruit, grape, pineapple
	Isobutyl acetate	$C_6H_{12}O_2$	790	781	9.05 ± 0.05	Sweet, fruity, ethereal, banana
	Ethyl butanoate	$C_6H_{12}O_2$	803	803	84.51 ± 2.63	Fruity, juicy, cognac
	Isoamyl acetate	$C_7H_{14}O_2$	877	877	869.09 ± 15.59	Sweet, fruity, banana, solvent
	Ethyl pentanoate	$C_7H_{14}O_2$	902	902	5.14 ± 0.12	Sweet, fruity, apple, pineapple, green, tropical
	Ethyl octanoate	$C_{10}H_{20}O_2$	1198	1198	53.10 ± 1.22	Fruity, wine, waxy, sweet, apricot, banana, brandy,pear
	Ethyl nonanoate	$C_{11}H_{22}$	1297	1297	0.87 ± 0.01	Fruity, rose, waxy, rum, wine, natural, tropical
	Ethyl 9-decenoate	$C_{12}H_{22}$	1386	1387	6.75 ± 0.26	Fruity, fatty
	Ethyl decanoate	$C_{12}H_{24}$	1395	1394	85.59 ± 0.10	Sweet, waxy, fruity, apple, grape, oily, brandy
	Ethyl tetradecanoate	$C_{16}H_{32}O_2$	1795	1795	192.74 ± 5.86	Sweet, waxy, violet, orris
	Isopropyl myristate	$C_{17}H_{34}O_2$	1827	1827	17.45 ± 0.40	Faint, oily, fatty
	Methyl hexadecanoate	$C_{17}H_{34}O_2$	1928	1928	14.80 ± 0.08	Oily, waxy, fatty, orris
	Ethyl 9-hexadecenoate	$C_{18}H_{34}O_2$	1976	1977	66.39 ± 1.14	-
	Ethyl hexadecanoate	$C_{18}H_{36}O_2$	1995	1995	519.24 ± 17.18	Mild, waxy, fruity, creamy, milky, balsamic, greasy, oily
	Isopropyl palmitate	$C_{19}H_{38}O_2$	2025	2026	13.81 ± 0.74	Bland, oily
	Ethyl linoleate	$C_{20}H_{36}O_2$	2168	2169	505.79 ± 12.75	Mild, fatty, fruity, oily
	Ethyl linolenate	$C_20H_{34}O_2$	2172	2169	221.55 ± 21.77	-

RI[a] – Linear retention index calculated with alkane standard (C_7-C_{30}). Non-polar column with temperature ramp; RI[b] – Retention index of linear alkanes according to the literature (https://webbook.nist.gov/); C= concentration of the compound (µg/L); Odor description – (http://www.thegoodscentscompany.com/).

Continued Table 4: Volatile compounds identified in extract of coffee husk by stir-bar-GC-MS.

Class	Compound	Formula	RI[a]	RI[b]	C (µg/L)	Odor description
	Diethyl acetal	$C_6H_{14}O_2$	774	734	1238.63 ± 34.28	Ether, green, nut, earthy, sweet, vegetable
	1.1-Diethoxy-3-methylbutane	$C_9H_{20}O_2$	955	955	15.86 ± 0.17	Fruity, fatty
	1.1-Diethoxyhexane	$C_{10}H_{22}O_2$	1095	1092	16.70 ± 0.65	Cognac, pear, floral, hyacinth, apple, fruity
Aldehyde		$C_9H_{18}O$				Waxy, aldehydic, rose, fresh, orris, orange, peel, fatty,
	Nonanal		1104	1104	4.29 ± 0.16	peely
	Decanal	$C_{10}H_{20}$	1205	1205	4.98 ± 0.16	Sweet, aldehydic, waxy, orange, peel, citrus, floral
	Pentadecanal	$C_{15}H_{30}O$	1716	1716	2.22 ± 0.15	Fresh, waxy
	p-Xylene	C_8H_{10}	867	867	1.71 ± 0.08	-
	o-Ethyltoluene	C_9H_{12}	978	976	2.69 ± 0.27	-
	m-Cymene	$C_{10}H_{14}$	1023	1023	7.59 ± 0.25	-
	2-Methyldecane	$C_{11}H_{24}$	1064	1065	1.30 ± 0.02	-
Hydrocarbon	3-Methyldecane	$C_{11}H_{24}$	1070	1070	1.38 ± 0.12	-
	Pentylcyclohexane	$C_{11}H_{22}$	1133	1130	7.42 ± 0.11	-
	α-Copaene	$C_{15}H_{24}$	1374	1374	1.51 ± 0.13	Woody, spicy, honey
	Cyclohexane	C_6H_{12}	756	688	11.16 ± 0.52	-
Alcohol	1-Hexadecanol	$C_{16}H_{34}O$	1883	1883	25.43 ± 1.76	Waxy, clean, greasy, floral, oily
Terpene	α-Terpinene	$C_{10}H_{16}$	1015	1015	3.48 ± 0.07	Woody, terpene, lemon, herbal, medicinal, citrus
	Limonene	$C_{10}H_{16}$	1027	1027	88.18 ± 1.82	Citrus, herbal, terpene, camphor
	(E)-Geranylacetone	$C_{13}H_{22}O$	1447	1447	5.08 ± 0.08	Fresh, green, fruity, waxy, rose, woody, magnolia, tropical
Monoterpene	β-cis-Ocimene	$C_{10}H_{16}$	1048	1048	4.21 ± 0.19	Warm, floral, herb, flower, sweet
	y-Terpinene	$C_{10}H_{16}$	1058	1058	4.89 ± 0.15	Oily, woody, terpene, lemon/lime, tropical, herbal
Sesquiterpene	β-Caryophyllene	$C_{15}H_{24}$	1416	1416	1.23 ± 0.12	Sweet, woody, spice, clove, dry
	β-Caryophyllene oxide	$C_{15}H_{24}O$	1587	1587	3.29 ± 0.21	Sweet, fresh, dry, woody, spicy
Ketone	2-Tridecanone	$C_{13}H_{26}O$	1494	1495	3.75 ± 0.09	Fatty, waxy, dairy, milky, coconut, nutty, herbal, earthy

RI[a] – Linear retention index calculated with alkane standard (C_7-C_{30}). Non-polar column with temperature ramp; RI[b] – Retention index of linear alkanes according to the literature (https://webbook.nist.gov/); C= concentration of the compound (µg/L); Odor description – (http://www.thegoodscentscompany.com/).

4. CONCLUSIONS

In this work, the ultrasound-assisted extraction of polyphenolic compounds from Arabica coffee husk was evaluated. Mathematical models of the extraction of these compounds were obtained as a function of the ethanol concentration, solvent/solid ratio and extraction time, and the ethanol concentration was the parameter with the greatest influence on the process. Extract 2 (obtained with 34.17% ethanol, 41.91 mL of solvent/g of solid and 99.76 min of extraction) showed higher AA (by the DPPH and FRAP methods) and the presence of various compounds with aroma characteristics. Arabica coffee husk, not yet well explored, has proven potential to be used as a source of bioactive and aroma compounds of interest to the food, pharmaceutical and cosmetic industries.

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