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Supercritical fluid extraction from spent coffee grounds and coffee husks: Antioxidant activity and effect of operational variables on extract composition

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ABSTRACT

The present study describes the chemical composition and the antioxidant activity of spent coffee grounds and coffee husks extracts, obtained by supercritical fluid extraction (SFE) with CO_2 and with CO_2 and co-solvent. In order to evaluate the high pressure method in terms of process yield, extract composition and antioxidant activity, low pressure methods, such as ultrasound (UE) and soxhlet (SOX) with different organic solvents, were also applied to obtain the extracts. The conditions for the SFE were: temperatures of 313.15 K, 323.15 K and 333.15 K and pressures from 100 bar to 300 bar. The SFE kinetics and the mathematical modeling of the overall extraction curves (OEC) were also investigated. The extracts obtained by LPE (low pressure extraction) with ethanol showed the best results for the global extraction yield (X_0) when compared to SFE results. The best extraction yield was $15 \pm 2\%$ for spent coffee grounds with ethanol and $3.1 \pm 04\%$ for coffee husks. The antioxidant potential was evaluated by DPPH method, ABTS method and Folin–Ciocalteau method. The best antioxidant activity was showed by coffee husk extracts obtained by LPE. The quantification and the identification of the extracts were accomplished using HPLC analysis. The main compounds identified were caffeine and chlorogenic acid for the supercritical extracts from coffee husks.

1. Introduction

Brazil is currently the world's largest producer of coffee, representing near 30% of the global market, with a volume equivalent to the sum of production of the other six countries with the highest production [1]. The quality of coffee, in addition to the sensory attributes can be accessed through the identification and quantification of the components present in this product, such as caffeine, which is associated to human health, trigonelline and chlorogenic acids, which are compounds responsible for the formation of flavor during roasting [2].

The large production and consumption of coffee also leads to the generation of a huge amount of waste. According Badocha et al. [3], the amount of coffee husks generated during processing is equivalent to the total amount of beneficiated grains. In a soluble coffee industry, for every ton of coffee produced, 4.5 tons of coffee grounds are generated, with approximately 80% moisture. Despite the large amount of waste generated by the agricultural and agribusiness, only a small percentage is utilized because of the lack of knowledge related to its energy potential and also to the lack of appropriate equipment for their use [4]. The coffee husk is the main residue from the processing of coffee and is normally used in ruminant feed. However, it is considered anti-nutritional due to the presence of toxic substances for these animals, such as caffeine (1.2%), tannins (6.3%) and polyphenols [5].

Because of the importance of the different compounds present in the coffee waste, the extraction of these substances appears as an important alternative to increase the aggregated value of the agro-industrial residues. The quality of extracts obtained from a raw material is strongly related to the extraction technique employed, and the quality of the extracts is measured by the chemical profile of the product. Supercritical technology is then a modern technique for extraction that seeks to increase quality by exploiting the selectivity of the process, one of its main characteristics [6,7].

Therefore, this study proposes to obtain extracts from coffee husks and from coffee grounds (*Coffea arabica*) in order to evaluate the application of supercritical technology in obtaining compounds of high added value by analyzing the composition profile and the biological activity of the extracts. The determination of the kinetic parameters of the process was also object of investigation.

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2. Materials and methods

2.1. Raw material and sample preparation

The spent coffee grounds were supplied by "Cantina do CCS", a coffee shop located at the Federal University of Santa Catarina, UFSC, SC, Brazil. The raw material was dried at 318.15 K for 5 h with air circulation (De Leo, Porto Alegre/RS, Brazil) up to a final content of 14 ± 1% (w/w) of moisture and volatile content, determined according to the 950.46B method of A.O.A.C. [8].

Coffee husks were supplied by "Fazenda Tulha", placed in Guaxupé, MG, Brazil. The coffee husks were supplied with a moisture content of 13.04 ± 0.02% w/w. Then, the material was ground in knife mill (De Leo, Porto Alegre/RS, Brazil) and characterized by classification in a vertical vibratory sieve shaker (Bertel Metalurgic Ind. Ltda., Caieiras/SP, Brazil). The mean particle diameter of both samples (spent coffee grounds and coffee husks) was determined through the micrographs from the Scanning Electron Microscopy (SEM), performed in microscope (JEOL JSM-6390LV, USA), by means of the software Size Meter, version 1.1 [9]. The dried spent coffee grounds and the grounded coffee husks were stored at 255.15 K in a domestic refrigerator until the extractions were performed.

2.2. Supercritical fluid extraction (SFE)

The supercritical extraction unit was used to obtain the spent coffee grounds and coffee husks extracts and was previously described by Zetzel et al. [10]. The equipment contain a pressurized CO_2 reservoir, a thermostatic bath (Microquímica–MQBTZ99–20, SC, Brazil) kept at 278.15 K, an air driven pump (Maximator M111, Germany) and a stainless steel jacketed column with 2 cm inner diameter, 32 cm long and 100 cm^3 capacity. The extraction temperature was additionally controlled by a thermostatic bath (Microquímica–MQBTZ99–20, SC, Brazil), while the solvent flow was adjusted and monitored by high-pressure valves, regulators and manometers. The high-pressure equipment was modified according to Campos et al. [11] by the insertion of a co-solvent pump (Constametric, 3200, EUA) connected to the extraction line in order to supply the modifier (organic solvent at high-pressure). The modifier was supplied at pre-established flow rate and mixed with CO_2 before entering the extraction vessel. The co-solvent pump works with flow rate from 0.01 mL min^{-1} to 9.99 mL min^{-1}. Ethanol (EtOH) was used as co-solvent in concentrations of 4% and 8% (w/w) for the coffee husks, and 8% and 15% (w/w) for the spent coffee grounds, related to the CO_2 amount.

The SFE was performed to obtain the global yield (X_0) according to extraction conditions of 313.15 K, 323.15 K and 333.15 K, and pressures from 100 bar to 300 bar and constant solvent flow rate of 11 ± 2 g min^{-1}, during 4.30 h extraction, for the coffee husks, and 2.30 h extraction for the spent coffee grounds. The co-solvent (CS) assays with coffee husks were performed at 200 bar and 323.15 K at constant solvent flow rate of 11 ± 2 g min^{-1}. The CS assays for spent coffee grounds were performed at 100 bar and 333.15 K at constant solvent flow rate of 11 ± 2 g min^{-1}. The co-solvent was separated from the extract according to the procedure described for the low pressure methods (Section 2.3). For all SFE assays, 15 g of raw material was used to pack the extraction vessel and the CO_2 used was 99.9% pure, delivered at pressure up to 60 bar (White Martins, Brazil). The solvent density values were obtained according to Angus et al. [12].

Kinetics assays were also performed by SFE method to obtain the overall extraction curves (OEC). The experiments were carried out with coffee husks at 100 bar and 313.15 K. The operational conditions of CO_2 flow rate and particle diameter were analyzed in 3 levels in order to evaluate their effect on the kinetics of the extraction and the mass transfer mechanisms involved, as described by the mathematical models applied (Section 2.4). The solvent flow rates and the diameter particles used were 6.6, 11.6, 16.6 g CO_2 min^{-1} and 0.03 cm, 0.05 cm and 0.06 cm, respectively.

2.3. Low pressure extractions (LPE)

The low pressure extraction methods used in this study were Soxhlet (SOX) and ultrasound (UE) methods, which were applied using four different solvents: hexane (HX), dichloromethane (DCM), ethyl acetate (EtOAc) and ethanol (EtOH), with ascending polarity of 0, 3.1, 4.4 and 5.2, respectively [13]. The solvents were provided by Merck (Brazil) and by Lafan (Brazil).

The SOX extraction was performed according to 920.39C method of A.O.A.C. [8]. The procedure consists of 150 mL of solvent recycling over 5 g of dried sample, in a Soxhlet apparatus for 6 h extraction at the boiling temperature of the solvent used.

The UE was conducted according to Freitas [14]. Briefly, 7 g of raw material and 210 mL of solvent were used for each assay, placed inside an evaporation flask connected to a condenser. The extraction time was 2 h, for both raw materials (spent coffee ground and coffee husk), conducted at room temperature. The equipment used was an ultrasonic cleaner bath (Unique Ultracleaner, USC-700), which operates in a frequency of 55 kHz and potency of 220 V.

The extracts obtained by each extraction method with different solvents were submitted to the solvent elimination in a rotary evaporator (Fisatom, 802, Brazil), supplied with cooling and vacuum control. The evaporation temperatures were adjusted to a level below the boiling point of the solvent in order to avoid thermal degradation of the extracts. The vacuum was adjusted at 650 mmHg.

The global yield (X_0) for all method of extraction was obtained by the mean value from the duplicate experiments considering the ratio between mass of extract and mass of raw material.

2.4. Mathematical modeling

The OECs of SFE kinetics for coffee husks were obtained by the extraction yield (as accumulated mass) *versus* extraction time. For the kinetic evaluation, the modeling of the OEC was performed using the following models: Sovová [15], logistic model of Martínez et al. [16] and diffusion model of Crank [17]. The model equations were described by Campos et al. [18]. The subroutine BOBYQA, which eliminates the need for the use of derivatives, was used for the application of the mass transfer models tested [19]. The values for the total extraction yield (X_0), necessary for the curves modeling, were determined considering the experimental data obtained at 270 min of extraction, because after this time, the extraction reaches the *quasi-null* extraction rate, according to preliminary assays.

For the application of the Sovová model [15], several data are requested, such as: solid density (ρ_s), determined by helium pycnometer (model Accu Pyc II 1340, Micromeritics); bed diameter and height; apparent density (ρ_a), calculated by the ratio between the raw material mass and bed volume; solvent density (ρ_{CO_2}) and bed porosity (ε), determined by the ratio $(\rho_s - \rho_a)/\rho_s$. The Sovová's model [15] also uses the value of extract solubility (Y^*) in the supercritical CO_2, a parameter which is a function of the extraction condition of temperature and pressure. The solubility values obtained by Gupta and Shim [20] for caffeine in supercritical CO_2 at the operational conditions equivalent to the ones used in the present work were used for the curve modeling.

2.5. Antioxidant activity

The antioxidant activity was determined for the extracts of coffee husk and spent coffee ground obtained by SFE, by SOX and by

UE. The results were compared with the synthetic compound BHT (butylated hydroxytoluene). All reagents used in the antioxidant activity analysis were purchased from Sigma–Aldrich Co. (USA).

2.5.1. Free radical scavenging activity (DPPH)

The free radical scavenging of coffee husks and spent coffee ground extracts was evaluated using 1,1-diphenyl-2-picrylhydrazil (DPPH) as described by Mensor et al. and Benelli et al. [21,22]. Briefly, each extract was mixed with a 0.3 mM DPPH ethanol solution, to give final concentrations of 5, 10, 25, 50, 125, 250 and 500 μg extract mL^{-1} DPPH solutions. After 30 min at room temperature, the absorbance values were measured at 517 nm in spectrophotometer (FEMTO, 800 XI, São Paulo, SP) and converted into percentage of antioxidant activity (%AA). This activity was also presented as the effective concentration at 50% (EC_{50}), i.e., the concentration of the test solution required to give 50% decrease in the absorbance of the test compared to that of a blank solution, and expressed in μg of extract mL^{-1} DPPH. The EC_{50} values were calculated from the linear regression of the % AA curves obtained for all extract concentrations. The %AA and EC_{50} for all extracts were obtained considering the mean value of triplicate assays.

2.5.2. $ABTS^{\bullet+}$ radical scavenging assay

This assay was carried out according to the procedure described by Re et al. and Michielin et al. [23,24]. The radical monocationic pre-formed $ABTS^{\bullet+}$ [2,2_-azino-bis-(3-ethylbenzotiazoline-6-sulfonic acid)] is generated by chemical oxidation of the ABTS, and is reduced in the presence of an antioxidant compound hydrogen donor. The synthetic vitamin E, Trolox (6-hidroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma–Aldrich Co, St. Louis, EUA), was used as antioxidant reference, which was prepared in ethanol and stored as a standard solution. The ABTS was dissolved in water to a concentration of 7.0 mM, and submitted to reaction with 2.45 mM potassium persulfate for the formation of the radical ABTS. The absorbance was measured at 754 nm in spectrophotometer 6 min after the mixture of the samples to the solution of ABTS. Results were expressed as trolox equivalent antioxidant capacity (TEAC). TEAC is defined as the mM concentration of a trolox solution whose antioxidant activity is equivalent to the activity of 1.0 mM test solution. In order to find TEAC values, a separate concentration response curve for standard trolox solutions was prepared.

2.5.3. Total phenolic content (TPC)

The TPC was determined according to the Folin–Ciocalteau method [25]. Briefly, the reaction mixture was composed by 0.1 mL of extract (concentration of 1667 mg L^{-1}), 7.9 mL of distilled water, 0.5 mL of Folin–Ciocalteau reagent (a mixture of phosphomolybdate and phosphotungstate) and 1.5 mL of 20% sodium carbonate, placed in opaque flasks. The flasks were agitated, held for 2 h, and the absorbance was measured at 765 nm. The TPC was calculated according to a standard curve, prepared previously with chlorogenic acid as standard. The results (mean value of the triplicate assays) were expressed as milligrams of chlorogenic acid equivalent (CAE) per gram of the extract (mg CAE g^{-1}) [24].

2.6. Chemical profile

The identification and the relative quantification of the phenolic compounds and methylxanthines present in the coffee husk and spent coffee ground extracts were achieved by a high performance liquid chromatography in reverse phase (RF-HPLC) (Shimadzu LC-10, Kyoto, Japan) using a Shim-pack C18 column (internal diameter 4.6 mm and length 250 mm). In order to determine the composition of the phenolic compounds, 5 mg of extract were diluted in 1 mL of ethanol, in the case of extracts obtained by SFE, or diluted in 1 mL of the respective solvent, for the LPE methods (Soxhlet and ultrasound) with different solvents. An aliquot of 10 μL of each solution was injected into the HPLC column maintained at 40 °C using a mobile phase consisting of acetonitrile/0.1% formic acid (15:85, v/v) flowing at a flow rate of 0.8 mL min^{-1}. The same procedure was used for the quantification of the methylxanthines of coffee husk and spent coffee ground extracts, with the exception of extracts obtained by SFE, which were dissolved in dichloromethane. The quantification was based on external standard method by comparison with the retention time of pure standards of phenolic compounds and methylxanthines. For all samples, the final concentration of the compounds was determined by averaging the results of three consecutive injections [26].

2.7. Statistical analysis

The global yield (X_0) and the % AA results were evaluated statistically by software SAS for Windows version 6.0, at 5% level of significance, in order to identify significant differences between values of global yield, as a function of temperature, pressure, solvent/extraction type, and percentage of antioxidant activity.

3. Results and discussion

3.1. Global yield (X_0) of SFE and LPE

The yield results obtained for the different extraction methods and solvents (Soxhlet, UE and SFE) are presented in Table 1, together with the polarity index and the solvent density for SFE.

The results presented in Table 1 indicate that the best yields were obtained by the Soxhlet extraction using ethanol as solvent, for both materials studied (coffee husk and spent coffee ground). For the coffee husk, no significant differences among the results were detected for the different solvents and low pressure methods used (soxhlet and ultrasound). The only exception was the result for UE with hexane, a low polarity solvent, which achieved the lowest overall yield. This same behavior was observed for the spent coffee ground, where also the lowest yield was obtained for the UE using the lower polarity solvent.

Comparing the extraction methods for the same solvent, it was observed that the Soxhlet extraction had higher yields compared to ultrasound. The operating temperature of the recycle solvent and the interactions between solvent and plant matrix, characteristic of Soxhlet extraction, may contribute to increase the solubility of compounds of different types, raising the extraction yield [22,24,27].

The results also indicate that solvents of higher polarity lead to higher extraction yields, suggesting that compounds present in plant matrix have intermediate to high polarity. However, it is important to note that the Soxhlet extraction with hexane for coffee husk and spent coffee ground, produced yield values very close to the ones obtained by more polar solvents such as ethyl acetate and dichloromethane, also suggesting the presence of compounds with lipophilic characteristic, more easily dissolved in nonpolar solvents such as hexane.

Couto et al. [28] showed a yield of 5.8% for oil extraction from coffee grounds with hexane, a result superior to the ones achieved in this work. The differences in the results may be associated with the coffee variety, the conditions of preparation and the pretreatment of the raw materials. Lipids tend to stay in the coffee grounds after brewing, but the amount can vary according to the method used, such as hot water or steam [29]. The results presented in Table 1 for the LPE with different organic solvents show that ethanol is an appropriate solvent to be used as co-solvent in SFE.

Table 1
Global yield (X_0) of coffee husks and spent coffee grounds extracts obtained by low pressure extractions (LPE) and supercritical fluid extraction (SFE).

LPE	Solvent	SPI[1]	X_0 (%)[2]	
			Coffee husk	Spent coffee
SOX	Hx	0	$3.94^{ab} \pm 0.54$	$12^{abc} \pm 1$
	DCM	3.1	$2.7^{ab} \pm 0.3$	$10.8^{bc} \pm 0.2$
	EtOAc	4.4	$3.4^{ab} \pm 0.2$	$11.8^{abc} \pm 0.1$
	EtOH	5.2	$4.8^{a} \pm 0.1$	$15^{a} \pm 2$
UE	Hx	0	$1.45^{b} \pm 0.01$	$9^{c} \pm 1$
	DCM	3.1	$2.3^{ab} \pm 0.1$	$9.9^{bc} \pm 0.1$
	EtOAc	4.4	$2.1^{ab} \pm 0.1$	$9.7^{bc} \pm 0.1$
	EtOH	5.2	$3.1^{ab} \pm 0.4$	$12.2^{ab} \pm 0.5$

SFE	Solvent	ρ_{CO_2} (g/cm^3)	X_0 (%)[2]	
			Coffee husk	Spent coffee
313.15 K/100 bar	CO_2	0.629	$1.24^{ab} \pm 0.02$	$5.1^{b} \pm 0.5$
313.15 K/200 bar	CO_2	0.840	$1.65^{ab} \pm 0.08$	$9.1^{a} \pm 0.2$
313.15 K/300 bar	CO_2	0.911	$1.56^{ab} \pm 0.11$	$10.5^{a} \pm 0.2$
323.15 K/100 bar	CO_2	0.385	$1.03^{ab} \pm 0.07$	$1.33^{c} \pm 0.63$
323.15 K/200 bar	CO_2	0.785	$1.71^{a} \pm 0.11$	$9.7^{a} \pm 0.1$
323.15 K/300 bar	CO_2	0.871	$1.97^{a} \pm 0.56$	$9.38^{a} \pm 0.01$
333.15 K/100 bar	CO_2	0.295	$0.55^{b} \pm 0.02$	$0.43^{c} \pm 0.09$
333.15 K/200 bar	CO_2	0.724	$1.86^{a} \pm 0.01$	$9.1^{a} \pm 0.5$
333.15 K/300 bar	CO_2	0.830	$1.55^{ab} \pm 0.11$	$9.8^{a} \pm 0.3$

[1] Solvent polarity index.
[2] Same letters indicated no significant difference at level of 5% ($p < 0.05$).

3.1.1. SFE with pure CO_2

In the extraction with supercritical CO_2, the evaluation of global yield extraction, obtained under different conditions of temperature and pressure, indicates the effect of solubility of the solvent and consequently the influence on the process yield.

According to Table 1, the highest yield for the coffee husk extracts was $2.0 \pm 0.6\%$, obtained for the condition of 300 bar and 323.15 K. This value show no significant difference, at level of 5%, to the results obtained at 200 bar and at temperatures of 323 K and 333.15 K. The lower yield was obtained under the condition of 100 bar and 333.15 K. The results achieved by the other extraction conditions also show no significant difference among each other.

At 323.15 K it is possible to observe the increase in yield with increasing pressure. This behavior is explained by the increase in solvent density with enhancing pressure, increasing the solvation power of CO_2 [7]. The increased pressure can lead to disruptions in the plant cells, facilitating the release of compounds that were not previously available, and thus increasing the yield of the process [18,27].

The effect of temperature on extraction yield, at constant pressure, occurs by two mechanisms: the increase in process temperature increases the solubility due to increased vapor pressure of the solute and on the other hand reduces the solubility due to the decrease in density the solvent. These two opposite effects result in the crossover of the isotherms, a phenomenon known as retrogradation [7,27,30].

Fig. 1 shows the yield isotherms of the SFE for spent coffee ground extracts. The isotherms show an inversion in the region between 200 bar and 250 bar, depending on the pair of temperatures compared, indicating a possible region of retrogradation. Bellow 200 bar, the yield increases with decreasing of temperature, where the predominant effect is of the density of the solvent. After the crossover (above 250 bar), the effect of the solute vapor pressure is dominant. The same behavior was detected for the coffee husk extracts, with a retrogradation pattern between 150 bar and 200 bar.

According to Table 1, the highest yield of spent coffee grounds extracts was achieved at 300 bar and 313.15 K ($10.5 \pm 0.2\%$), a result that shows no significant difference from the extracts obtained at 200 bar and the three temperatures studied, and also from those obtained at 300 bar and at 323.15 K and 333.15 K. The lowest yields were found using the pressure of 100 bar at temperatures of 313.15 K, 323.15 K and 333.15 K.

It is also possible to observe the yield increase with the pressure enhancement, keeping the temperature constant, a behavior justified by the increased in the supercritical solvent density with pressure, as explained earlier. The reduction in yield with increasing temperature, at constant pressure, is easily observed at pressure of 100 bar. Such behavior is due to the fact that an increase in temperature reduces the solubility of the solute due to reduction in the density of the supercritical solvent [27].

Couto et al. [28] obtained 13% yield in oil extraction from spent coffee ground, at pressures of 200 bar and 300 bar and temperatures of 313.15 K and 323.15 K for 3 hrs of extraction. In this work it was also observed an increase in yield with the pressure, at constant temperature. The effect of temperature on extraction yield, at constant pressure, was similar to the ones from the present study, probably due to the reduced density of the supercritical solvent with increasing temperature, decreasing their solvation ability.

Comparing the yields obtained by supercritical CO_2 with the results obtained by conventional extraction (Soxhlet and ultrasound), for both raw materials used, it is observed that the low pressure extractions using ethanol as a solvent produced yield values superior to those achieved by SFE. These results can be

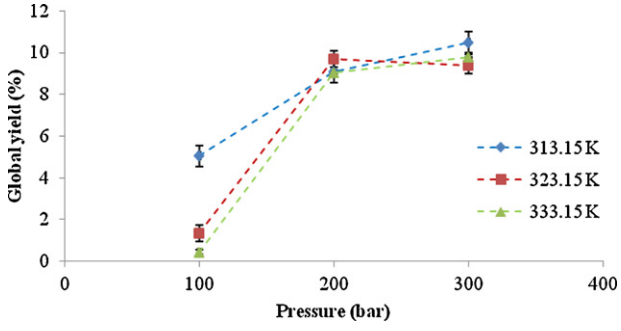

Fig. 1. Crossover isotherms from spent coffee ground extracts by SFE.

Table 2
Global yield (X_0) of coffee husks and spent coffee extracts obtained by SFE using CO_2 with co-solvent.

Raw material	Pressure (bar)	Temperature (K)	% Co-solvent	X_0[1](%)
Coffee husk	200	323.15	4	$2.1^a \pm 0.7$
			8	$2.2^a \pm 0.2$
Spent coffee	100	333.15	8	$6.3^b \pm 0.5$
			15	$14^a \pm 2$

[1] Same letters indicate no significant difference at level of 5% ($p < 0.05$).

explained by the extraction of more polar compounds, not soluble in CO_2, a nonpolar solvent. Additionally, for the Soxhlet extractions, the solvent recycling, the extended extraction period and the high amount of solvent aid the yield enhancement, compared to other procedures.

3.1.2. SFE with CO_2 plus co-solvent (CS)

According to yield results for Soxhlet and ultrasound methods, ethanol presented the best solvent performance; therefore it was selected to be used as a co-solvent for the supercritical extraction, in order to improve the technique efficiency. The experimental conditions of temperature and pressure for the SFE with CO_2 and co-solvent (ethanol) were defined based on the yield obtained by pure CO_2 and also by the biological activity shown by the extracts (results in Section 3.3). Consequently, the condition selected were: 200 bar and 323.15 K for coffee husks, using ethanol concentrations of 4% and 8% (w/w) and 100 bar and 333.15 K for coffee grounds, using ethanol in concentrations of 8% and 15% (w/w). The results obtained by the SFE with co-solvent for both raw materials are presented in Table 2.

The addition of the co-solvent in the extraction process with supercritical CO_2, increased the values of yields, for both the extracts, in all concentrations and conditions tested. This increase was more evident in extracts of spent coffee grounds, which reached a yield of $0.45 \pm 0.09\%$ at 100 bar and 333.15 K using pure CO_2, whereas with 8% ethanol as co-solvent the yield was up to $6.3 \pm 0.5\%$ and $14 \pm 2\%$ when using 15% co-solvent. The increase in the yield of SFE from spent coffee grounds, with 6% ethanol as co-solvent, was also verified by Couto et al. [28]. This behavior is explained by the increase in the solubility of polar compounds in the mixture ethanol/CO_2, compared to the solubility in pure CO_2. Furthermore, not only the solubility of a certain component increases with the use of co-solvent, but also the number of components solubilized by the solvent, which reduces the process selectivity and increases the yield.

3.2. Mathematical modeling

For the modeling of the overall extraction curves, conducted as described in Section 2.4, three mass transfer models were applied: the Sovová model [15] and the logistic model of Martínez et al. [16], based on differential mass balance, and the model of Crank [17], based on analogy to heat transfer.

The results, expressed by the values of the adjustable parameters and the sum of squared errors (SSE) between experimental and modeled data, for the different models used, are presented in Table 3. The effect of the solute geometry (particle size) and the process kinetics (solvent flow rate) was evaluated by the different operational conditions used to construct the overall extraction curves.

According to results from Table 3, the Sovová model [15] was, in general, the best tool to fit the experimental data. This model provides good adjustment when the experimental curves present a well defined constant extraction rate period. It also presents the advantage to interpret the transfer phenomenon that occurs in the process of supercritical fluid extraction, conferring a physical meaning to the adjustable parameters. Although, its application is limited to systems where the solute solubility in the supercritical solvent is available for the conditions of pressure and temperature employed [31]. The good fit achieved in this study suggests that the consideration made in Section 4.7, assuming the extract solubility as the literature data for solubility of pure caffeine in supercritical CO_2 [20], was adequate.

The values for the solid phase mass transfer coefficient ($k_x a$) were lower than the values obtained for the fluid phase mass transfer coefficient ($k_y a$), for all curves evaluated. These results indicate that the diffusion mechanism is less representative when compared to the convection mechanism, for the SFE of coffee husks [16,27,29,32].

The Crank model [17] considers that the extraction process is controlled only by diffusion, not taking into account the convection as a mass transfer mechanism, which probably caused the less accurate adjustment provided by this model, comparing with the results from the Sovová model.

The model of Martínez et al. [16] showed the worst fit among the models tested. This model is based on the differential mass balance in the bed of extraction, considering the mass transfer phenomena that occur in the fluid phase and solid phase. The t_m parameter represents the time when the extraction rate reaches its maximum. From Table 3 we detected that, for all the modeled curves, this parameter showed a negative value, losing their physical meaning. This behavior indicates that the extraction rate is decreasing, i.e., it reaches its maximum value at the initial time of extraction, time zero [16,27].

3.3. Antioxidant activity

The results for the antioxidant activity, performed according to the analyses of TPC, DPPH and ABTS, are presented in Table 4, obtained for all extract samples analyzed, and compared to the results presented by the synthetic product BHT, as standard sample. The extracts were obtained from coffee husk and spent coffee ground using different extraction methods (SFE and LPE).

The values of total phenolic content from coffee husk extracts obtained by SFE show no variation behavior with changes in pressure or temperature. Although no significant differences were detected among the TPC values, the highest data was obtained at 200 bar and 323.15 K using 8% ethanol as co-solvent (36 ± 1 mg CAE g^{-1}). For the LPE methods, the best results were obtained with ethanol and with ethyl acetate as solvents, for the Soxhlet extraction method, reaching 151 ± 11 mg CAE g^{-1} and 106 ± 4 mg CAE g^{-1}, respectively. The ultrasonic extraction with ethanol produced TPC of 133.4 ± 0.6 mg CAE g^{-1}. Nevertheless, the low yields observed for these extracts are limiting factors to the viability of the process of obtaining phenolic compounds by the techniques tested here.

For the spent coffee ground extracts the higher TPC value was obtained by ultrasound extraction with ethanol (587 ± 46 mg CAE g^{-1}). This value was higher compared to the one detected for the synthetic antioxidant BHT (423 ± 13 mg CAE g^{-1}), showing the antioxidant potential of extracts evaluated in the present work. Among the extracts obtained by SFE, the highest

Table 3
Adjustable parameters and sum of squared errors (SSE) obtained by the modeling of SFE curves for coffee husks.

Curves		1	2	3	4	5	6	7	8	9
P (bar)/T (°C)/D_p (cm)/Q_{co2} (g min^{-1})		100/313/0.06/11.6	100/313/0.06/16.6	100/313/0.06/6.6	100/313/0.03/16.6	100/313/0.03/11.6	100/313/0.03/6.6	100/313/0.05/16.6	100/313/0.05/11.6	100/313/0.05/6.6
Crank	D (m^2/s)	2.57×10^{-12}	1.23×10^{-13}	2.57×10^{-13}	3.63×10^{-14}	2.79×10^{-14}	9.09×10^{-14}	4.18×10^{-14}	1.34×10^{-13}	1.37×10^{-13}
	SQR	5.1×10^{-10}	9.6×10^{-10}	6.3×10^{-10}	3.7×10^{-10}	6.6×10^{-10}	4.8×10^{-10}	4.5×10^{-10}	7.3×10^{-10}	8.8×10^{-10}
Martínez	b (s^{-1})	0.00008	0.00005	0.00008	0.00006	0.00005	0.00011	0.00004	0,00007	0,00008
	t_m (s)	−99846.2	−99846.2	−99846.2	−99846.2	−99846.2	−99846.2	−99846.2	−99846.2	−99846.2
	SQR	2.9×10^{-9}	3.8×10^{-9}	3.7×10^{-9}	2.1×10^{-9}	1.2×10^{-9}	2.2×10^{-9}	7.1×10^{-10}	1.1×10^{-9}	1.1×10^{-9}
Sovová	t_{CER} (s)	1908.1	1682.2	1649.4	1841.5	3257.1	1612.6	2447.6	1740.5	3246.3
	X_k	0.0089	0.0095	0.0087	0.0097	0.0093	0.0083	0.0105	0.0099	0.0084
	$k_y a$ (s^{-1})	0.0027	0.0025	0.0032	0.0021	0.0013	0.0037	0.0011	0,0021	0.0017
	$k_x a$ (s^{-1})	0.00004	0.000009	0.00003	0.00003	0.00002	0.00005	0.00002	0.00005	0.00003
	SQR	1.6×10^{-10}	6.1×10^{-12}	5.4×10^{-11}	1.4×10^{-10}	5.8×10^{-11}	1.0×10^{-10}	3.7×10^{-11}	1.6×10^{-10}	7.5×10^{-11}

P, pressure (bar); T, temperature (K); D_p, particle diameter (m); Q_{CO_2}, flow rate solvent (g min^{-1}); D, difusion coeficient; b,e,t_m, adjustable parameters for Martínez et al. [16] model; t_{CER}, time of CER periode; X_k, mass ratio for the easily accessible solute in the solid phase; $k_x a$, mass transfer coefficient in the solid phase; $k_y a$, mass transfer coefficient in the fluid phase.

Table 4
Antioxidant activity for coffee husks extracts and for spent coffee ground extracts.

Extraction	Solvent	TPC (mgCAE g^{-1} extract)		%AA[1]		EC_{50} [2] (μg mL^{-1})		TEAC [3] (μMTEAC/g)		%Inibition[3]	
		Coffee husk	Spent coffee	Coffee husk	Spent coffee	Coffee husk	Spent coffee	Coffee husk	Spent coffee	Coffee husk	Spent coffee
EU	Hx	$61^{e} \pm 3$	$264.1^{c} \pm 18.1$	28.3^{f}	14.4^{k}	286.7^{n}	1972.23^{c}	$128^{c} \pm 6$	$68.1^{f} \pm 9.6$	$10.9^{c} \pm 0.4$	$6.6^{e} \pm 0.7$
	DCM	$71^{e} \pm 2$	$221.5^{cd} \pm 18.3$	34.3^{e}	47.3^{c}	732.5^{l}	532.5^{i}	$156^{b} \pm 19$	$98.3^{e} \pm 0.3$	$12.9^{c} \pm 1.4$	$9.5^{d} \pm 1.2$
	EtOAc	$67^{e} \pm 9$	$553.4^{a} \pm 59.8$	79.6^{c}	29.1^{g}	286.7^{n}	814.57^{f}	$128^{c} \pm 8$	$115.4^{d} \pm 8.7$	$11.1^{c} \pm 0.5$	$10^{d} \pm 1$
	EtOH	$133.4^{c} \pm 0.6$	$587.7^{a} \pm 46.6$	91.5^{a}	32.2^{f}	235.1^{p}	787.63^{fg}	$161^{b} \pm 3$	$123.6^{d} \pm 9.8$	$13.3^{b} \pm 0.2$	$10.6^{cd} \pm 0.7$
SOX	Hx	$65^{e} \pm 6$	$177.5^{de} \pm 25.2$	25.4^{g}	19.6^{h}	1029.5^{j}	1421.53^{e}	$98^{d} \pm 9$	$75^{f} \pm 3$	$8.7^{d} \pm 0.6$	$7.1^{e} \pm 0.3$
	DCM	$65^{e} \pm 2$	$173.7^{de} \pm 17.3$	37.5^{d}	38.8^{d}	684.3^{m}	659.43^{h}	$168^{b} \pm 9$	$154^{c} \pm 10$	$13.4^{b} \pm 0.6$	$12.9^{c} \pm 0.7$
	EtOAc	$106^{d} \pm 4$	$182.6^{de} \pm 28.2$	81.5^{b}	93.5^{a}	242.1^{o}	202.23^{j}	$381^{a} \pm 16$	$160^{c} \pm 13$	$29.3^{a} \pm 1.2$	$13^{c} \pm 1$
	EtOH	$151^{b} \pm 12$	$119.5^{ef} \pm 2.1$	90.3^{a}	46.5^{c}	235.4^{p}	537.37^{i}	$375^{a} \pm 6$	$137^{d} \pm 10$	$28.9^{a} \pm 0.5$	$11.6^{cd} \pm 0.7$
SFE 100 bar/313.15 K	CO_2	$23^{f} \pm 2$	$46.1^{g} \pm 3.5$	11.2^{kl}	17.6^{i}	2808.2^{d}	1380.1^{e}	$46^{e} \pm 8$	$116.1^{d} \pm 7.7$	$5.1^{e} \pm 0.6$	$10.9^{cd} \pm 1.6$
SFE 100 bar/323.15 K	CO_2	$16.1^{f} \pm 0.5$	$56.7^{g} \pm 2.3$	16.5^{i}	34.9^{e}	21298.1^{a}	724.1^{gh}	$44^{e} \pm 2$	$225.1^{b} \pm 14.6$	$4.8^{e} \pm 0.1$	$18^{b} \pm 1$
SFE 100 bar/333.15 K	CO_2	$20.6^{f} \pm 0.8$	$30.9^{g} \pm 0.8$	10.5^{l}	51.5^{b}	3183.6^{c}	478.2^{i}	$50^{e} \pm 5$	$275.1^{a} \pm 14.4$	$5.3^{e} \pm 1.1$	$22^{a} \pm 1$
SFE 200 bar/313.15 K	CO_2	$20.6^{f} \pm 0.8$	$38.6^{g} \pm 5.6$	15.7^{i}	10.3^{m}	1706.2^{i}	2264.3^{b}	$46^{e} \pm 6$	$50.7^{g} \pm 5.1$	$5.1^{e} \pm 0.5$	$5.3^{e} \pm 0.3$
SFE 200 bar/323.15 K	CO_2	$20.9^{f} \pm 0.9$	$24.1^{g} \pm 0.8$	10.6^{l}	11.7^{l}	2711.7^{e}	2369.5^{a}	$54^{e} \pm 3$	$48.7^{g} \pm 3.8$	$5.5^{e} \pm 0.2$	$5.9^{e} \pm 1.2$
SFE 200 bar/333.15 K	CO_2	$19.8^{f} \pm 0.1$	$42.1^{g} \pm 13.6$	23.6^{h}	15.3^{j}	3310.2^{b}	1748.1^{d}	$47^{e} \pm 7$	$81^{f} \pm 3$	$5.2^{e} \pm 0.5$	$7.4^{e} \pm 0.1$
SFE 300 bar/313.15 K	CO_2	$21.5^{f} \pm 0.8$	35.7g ± 1.9	23.7^{h}	15.2^{j}	1713.5^{h}	1962.5^{c}	$38^{e} \pm 3$	$77.3^{f} \pm 0.1$	$4.8^{e} \pm 0.6$	$7.3^{e} \pm 0.1$
SFE 300 bar/323.15 K	CO_2	$28.1^{f} \pm 1.3$	$36.1^{g} \pm 0.8$	13.5^{j}	14.3^{k}	2294.8^{g}	2185.2^{b}	$35.9^{e} \pm 0.8$	$59.7^{g} \pm 5.3$	$4.3^{e} \pm 0.1$	$6.9^{e} \pm 1.5$
SFE 300 bar/333.15 K	CO_2	$17.2^{f} \pm 0.8$	$37.2^{g} \pm 1.9$	12.1^{k}	18.9^{h}	2442.7^{f}	1949.4^{c}	$123^{c} \pm 40$	$56^{g} \pm 5$	$9.2^{d} \pm 3.3$	$6.3^{e} \pm 0.7$
SFE 200 bar/323.15 K	CO_2 + 4% ethanol	$26^{f} \pm 2$	$57^{g} \pm 3$	25.4^{g}	47.9^{c}	961.1^{j}	516.2^{i}	$104^{d} \pm 3$	$169^{c} \pm 3$	$9.2^{d} \pm 0.2$	$13.9^{c} \pm 0.2$
SFE 200 bar/323.15 K	CO_2 + 8% ethanol	$36^{ef} \pm 1$	$42^{g} \pm 2$	39.6^{d}	33.1^{e}	630^{m}	746.7^{gh}	$141^{b} \pm 1$	$99.2^{e} \pm 7.8$	$11.8^{d} \pm 0.1$	$9^{d} \pm 1$
BHT	–	$423^{a} \pm 13$	$423^{b} \pm 13$	–	–	–	–	–	–	–	–

[1] Antioxidant activity evaluated by free radical scavenging activity (DPPH).
[2] Effective concentration at 50%.
[3] Antioxidant activity evaluated by ABTS method.

Table 5
Profile of phenolic compounds from coffee husk and coffee ground extracts.

Extraction method	Raw material	Conditions/solvents	Epicatechin (μgGAE/g)[1]	Gallic acid (μgGAE/g)	Tannic acid (μgGAE/g)	Protocatechuic acid (μgGAE/g)	Chlorogenic acid (μgGAE/g)	*p*-Hydroxybenzoic acid (μgGAE/g)	Vanilic acid (μgGAE/g)	Caffeic acid (μgGAE/g)
			R_t[2] = 4.3 min	R_t = 5.8 min	R_t = 6.2 min	R_t = 7.5 min	R_t = 8.9 min	R_t = 10.4 min	R_t = 11.7 min	R_t = 12.6 min
Ultrasound	Spent coffee	Ethanol	0.3	–	0.7	33.1	–	0.8	–	–
	Spent coffee	Ethyl acetate	0.2	14.3	–	–	0.3	–	–	–
	Coffee husk	Ethanol	–	–	–	–	–	–	75.7	–
Soxhlet	Coffee husk	Ethyl acetate	1.4	113.8	–	–	0.5	–	–	–
	Coffee husk	Ethanol	–	–	80.3	–	–	–	–	–
SFE	Spent coffee	200 bar/333.15 K	–	–	–	0.6	41.3	–	–	0.1
	Spent coffee	300 bar/333.15 K	–	–	–	–	27.3	3.1	–	–
CO_2	Coffee husk	200 bar/313.15 K	–	0.9	–	–	174.5	–	–	–
	Coffee husk	300 bar/333.15 K	2.1	–	–	0.8	942.8	–	–	–
SFE	Spent coffee	100 bar/333.15 K/15%	–	–	–	–	19.6	2.2	–	–
CO_2 + ethanol	Coffee husk	200 bar/323.15 K/8%	–	–	–	–	232.3	–	–	–

[1] Phenolic compound in μg galic acid equivalent/g extract.
[2] R_t, retention time (min).

TPC value was found for the extract obtained using 8% ethanol as co-solvent, 57 ± 3 mg CAE g^{-1}. This value shows no significant difference to other extract samples obtained using the same technique (SFE).

The coffee husk extracts that showed the higher values of antioxidant activity from the DPPH method were obtained using ethanol and ethyl acetate as solvents for Soxhlet and ultrasound methods. These values were higher compared to the result found for the synthetic antioxidant BHT, as determined by Benelli et al. [22], which was 89.7 ± 0.5% of antioxidant potential.

The EC_{50} results, calculated by means of the DPPH data, for extracts obtained by SFE were above the 250 μg mL^{-1}. Bellow this value the extract is considered a good antioxidant product [11]. The low antioxidant activity may be associated to the low amount of phenolic compounds with intermediate to high polarity present in extracts, since CO_2 as a non polar solvent does not favor the solubilization of such components. According to Table 4, the extract of spent coffee ground obtained by Soxhlet using ethyl acetate showed the best value of antioxidant activity (93.5%) and consequently the lower EC_{50} value (202.23 μg mL^{-1}).

The results from Table 4 also show that Soxhlet extraction with ethyl acetate and with ethanol, for coffee husk extracts, presented the highest AA% behavior by the ABTS method, with values of 381.2 ± 16.1 μMTEAC/$g_{extract}$ and 375.2 ± 6.3 μMTEAC/$g_{extract}$, respectively. The SFE extract at 200 bar and 323.15 K with 8% ethanol, showed antioxidant capacity of 140.4 ± 1.1 μMTEAC/$g_{extract}$. Additionally, the best antioxidant capacities for supercritical extracts of spent coffee were found for pressure of 100 bar and temperatures of 333.15 K and 323.15 K, probably because the components responsible for antioxidant characteristics detected by the ABTS method were present in higher concentrations.

3.4. Composition profile

The phenolic compounds identified in extracts from coffee husk and from spent coffee ground are presented in Table 5 and the results are expressed as gallic acid equivalents. The phenolic compound identified in higher concentration in the extracts was the chlorogenic acid. The gallic acid, p-hydroxybenzoic, protocatechuic, vanillic and tannic were also detected, but in lower concentrations. The phenolic compounds present in coffee samples, mostly discussed in the literature, are chlorogenic acids and their metabolites, which represent the main phenolic fraction of the grains. The main groups of chlorogenic acids are caffeoylquinic acids, dicaffeoylquinic, and feruloyquinic and coumaroylquinic. The other phenolic compounds, although they represent biological potential, are not as exploited as chlorogenic acids, known to have numerous medicinal properties, strong antioxidant activity, in addition hepatoprotective, hypoglycemic and antiviral functions [33–35].

The concentrations of methylxanthines (theobromine, caffeine and theophylline) found for all extracts analyzed by HPLC are also listed in Table 6. Caffeine was detected for all samples, except for those obtained by ethyl acetate. The concentrations ranged from 0.734 μg mg^{-1} to 684.2 μg mg^{-1} extract. The extracts from coffee husks presented the higher caffeine concentrations, compared to extracts from spent coffee ground. The concentration of caffeine in the coffee husk extract obtained by SFE at 300 bar and 333.15 K represents about 70% of the total composition of the extract. Caffeine can be considered the most consumed stimulant for the central nervous system, either as a drink of coffee or tea, soft drinks and chocolate, and is widely used as co-adjuvant or agent in pharmaceutical formulations. The consumption in moderate doses has a stimulating effect, reduces fatigue, without causing harm [36].

Table 6
Profile of methylxanthines from coffee husk and coffee ground extracts.

Extraction method	Raw material	Variables of process/solvents	Theobromine ($\mu g/mg_{extract}$) R_t[1] = 4.7 min	Caffeine ($\mu g/mg_{extract}$) R_t[1] = 7.9 min
UE	Spent coffee	Hexane	–	0.734
	Spent coffee	Dichlorometane	–	38.2
	Spent coffee	Ethanol	–	25.7
	Coffee husk	Hexane	–	5.54
	Coffee husk	Dichlorometane	0.66	139.2
	Coffee husk	Ethanol	–	71.1
Soxhlet	Spent coffee	Hexane	–	3.27
	Spent coffee	Dichlorometane	–	25.9
	Spent coffee	Ethanol	–	11.8
	Coffee husk	Hexane	–	2.1
	Coffee husk	Dichlorometane	0.745	189.9
	Coffee husk	Ethanol	–	129.6
SFE CO_2	Spent coffee	200 bar/333.15 K	–	27.2
	Spent coffee	300 bar/333.15 K	–	41.3
	Coffee husk	200 bar/313.15 K	–	185.7
	Coffee husk	300 bar/333.15 K	1.13	684.2
SFE CO_2 + ethanol	Spent coffee	100 bar/333.15 K/15%	–	23.4
	Coffee husk	200 bar/323.15 K/8%	0.655	87.8

[1] R_t, retention time (min).

The supercritical extraction from coffee husks was presented as an appropriate technique for obtaining extracts containing chlorogenic acids and caffeine. However, the low extraction yields obtained for this raw material suggest the study of alternatives that facilitate and enhance the efficiency of extraction, in order to obtain a higher concentration of the compounds of interest.

4. Conclusions

The use of coffee wastes as raw material for different extraction methods is promising due to the high quality of the substances that remain in this industrial residue. When comparing the different extraction methods, besides the estimation of the process yield, it is also necessary to estimate the antioxidant potential of the product (extract) by diverse procedures and also evaluate the chemical composition of the extracts. SOX and UE presented highest process yield when using ethanol as solvent. SFE extracts presented lowers yields when compared to SOX and UE, although good results of antioxidant activity by ABTS method were detected from the supercritical extracts. The use of ethanol as co-solvent in SFE increased the yield extraction, mainly for the extraction from spent coffee grounds. Extracts from coffee husks obtained by SFE had relevant concentrations of chlorogenic acids and caffeine. The mathematical modeling showed the best fit to the OEC was presented by Sovová [15] model, with the lowest SSE values, indicating the suitability of the considerations made. The combination of the results suggests the potential of the SFE to increase the aggregated value from coffee industry residues.

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