Ultrasound-assisted extraction of bioactive compounds from dedo de moça pepper (*Capsicum baccatum* L.): Effects on the vegetable matrix and mathematical modeling

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ABSTRACT

Extracts with bioactive compounds were obtained from dried and milled dedo de moça pepper (*Capsicum baccatum* L.) using two different extraction techniques: Soxhlet with four different solvents (hexane, ethyl acetate, methanol and ethanol), and ultrasound-assisted extraction (UAE) using methanol and ethanol. In Soxhlet, the solvent reflux was kept for 6 h, whereas the UAE temperatures were 40, 50 and 60 °C with ultrasonic intensities (UI) of 150, 300 and 450 W/cm². Analyses of global yield, total phenolics, antioxidant capacities (DPPH and FRAP) and total capsaicinoids were performed. UAE raised the global extraction yield up to 26%. The concentration of phenolics and antioxidants increased with ultrasound, although higher intensities reduced their recoveries. However, capsaicinoids yield fell down to 50% with ultrasound. A phenomenological model provided good adjustment of the UAE kinetic curves. FESEM images showed that ultrasound did not change the sample morphology and did not damage the sample surface.

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1. Introduction

Chili peppers (*Capsicum* sp.) are among the most important plants cultivated around the world. Phenolics, flavonoids, carotenoids, antioxidants and capsaicinoids found in these peppers have industrial interest due to their medical and nutritional properties (Aguiar et al., 2013; Santos et al., 2015). Moreover, some works assigned anti-inflammatory (Bicas et al., 2011) and antioxidant capacities (Deppa et al., 2007) to chili peppers. Capsaicinoids are responsible for flavor and pungency of *Capsicum* peppers. Besides, some studies have proven their medical benefits, such as analgesic (Menéndez et al., 2004), cardiovascular and respiratory system stimulation (Tremarin et al., 2014).

Ultrasound-assisted extraction (UAE) appears as an alternative to extract bioactive compounds from plants, since it increases the extraction yield, working in lower temperatures and shorter time than in conventional techniques, such as Soxhlet extraction or maceration (Gogate and Kabadi, 2009). UAE also requires less solvent and is less pollutant than traditional methods, being considered environmentally friendly (Chemat et al., 2011). Among the advantages of sonication, the reduction of particle size, rupture of cell walls and enhancement of mass transfer may be highlighted (Paniwnyk et al., 2001). The propagation of ultrasound pressure waves through the solvent has been related to the intensification of extraction processes, by producing cavitation phenomena. The consequences of the implosion of cavitation bubbles are inter-particle collisions, micro-turbulence and perturbation in microporous particles, which accelerate eddy and internal diffusion. Erosion and particle breakdown are also observed when cavitation occurs on the surface of the sample (Shirsath et al., 2012).

Efforts have been made to increase the concentration of extracted bioactive compounds and to keep the process costs low. Some operational variables should be optimized to enhance the

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extraction from plant materials, such as extracting solvent, time, temperature and solvent to solid ratio.

Solid-liquid extractions consist on two steps: 1) dissolution of bioactive compounds in the extracting solvent; and 2) diffusion of the extractable compounds (Veljković and Milenović, 2002). Mathematical models can predict the extraction kinetics, contributing to further improvements in the process. Different models can describe the variation of extraction yield with time or evaluate the effects of operating conditions on extraction yield.

Although UAE has been widely applied to quantify bioactive compounds in vegetable matrices, few published works deal with the use of ultrasound (Barbero et al., 2008; Boonkird et al., 2008; Paduano et al., 2014; Santos et al., 2015) in the extraction from Capsicum peppers, and mathematical models and FESEM analysis have been applied only to supercritical carbon dioxide extraction from these fruits (Santos et al., 2015; Dias et al., 2016), but not to low pressure extractions. This work evaluated two extraction processes (Soxhlet and UAE) from dedo de moça pepper (Capsicum baccatum L.), a widely consumed pepper variety native from Brazil. Ultrasound was applied during the extraction to enhance its velocity and yield. The concentration of phenolics, capsaicinoids and the antioxidant capacities of the extracts were determined. A phenomenological model based on thermodynamics and mass transfer was adjusted to the UAE curves. Furthermore, images were obtained from the pepper samples through field emission scanning electron microscopy (FESEM) in order to assess the influence of ultrasound in the extraction process.

2. Material and methods

The material used in this work was the pepper "dedo de moça" (Capsicum baccatum L.), obtained from a local market in Campinas/SP, Brazil.

2.1. Sample preparation

Peppers were prepared according to Aguiar et al. (2013). The fruits were selected based on their physical integrity, sanitized in a sodium hypochlorite solution (10 mL/L) for 20 min and washed with water. The samples were oven-dried at $70 \pm 2\ °C$ during 24 h and milled in a knife mill (Marconi, model MA 340, Piracicaba, Brazil) to be homogenized and intensify the mass transfer during the extractions. The dried and milled peppers were stored in a freezer at $-18\ °C$.

Moisture was determined after 24 h of drying at $70 \pm 2\ °C$. The total lipid concentration in the samples was obtained according to the AOAC method 963.15 (1999), by Soxhlet with hexane.

The dried and milled peppers were classified by size in a vibratory sieve system with sequential openings (Tyler, Wheeling, USA), and the mean particle diameter (d_{mg}) was determined according to the A.S.A.E. model (1998), using Eq (1).

$$d_{mg} = \exp\left\{ \frac{\sum_{i=1}^{n}\left[\sqrt{w_i \cdot \log(d_i \cdot d_{i+1})} \right]}{\sum_{i=1}^{n} w_i} \right\} \tag{1}$$

where d_{mg} = mean particle diameter (mm); d_i = diameter of the sieve opening i (mm); d_{i+1} = diameter of the sieve opening above sieve i (mm); w_i = retained mass (g); n = total number of fractions.

The bulk density (ρ_a) was calculated by weighing a known volume of the dried and milled peppers, whereas the particle density (ρ_r) was determined by helium pycnometry (Quantachrome Instruments, Ultrapyc 1200e, Boynton Beach, USA). The bed porosity (ε) was calculated from the ratio between bulk and particle densities, according to Eq (2).

$$\varepsilon = 1 - (\rho_a/\rho_r) \tag{2}$$

2.2. Soxhlet extraction

Soxhlet was chosen as a conventional extraction technique to determine the total phenolic content, antioxidant capacity and total capsaicinoids in the extracts from dedo de moça pepper. The technique was carried out with four solvents with different polarities: hexane, ethanol, methanol and ethyl acetate. Each extraction was performed with 150 mL of solvent and 5 g of dried and milled samples inside a filter paper. The reflux of boiling solvent was kept for 6 h. After extraction, the solvent was evaporated under vacuum (at $25\ °C$), the extracts were weighed and stored under freezing ($-18\ °C$). All experiments were performed in triplicates.

2.3. Ultrasound-assisted extraction (UAE)

The UAE system is composed of a jacketed glass beaker (100 mL), with a 5 cm internal diameter, a heating bath that controls the extractor temperature and an ultrasound system (Unique Group, DES500, Campinas, Brazil), which is regulated with a 13 mm diameter (D) titanium probe coupled to a transducer. The transducer operates from 20 to 99% of its total power (800 W) with frequency of 20 kHz. The distance (h) between the ultrasonic probe and the beaker bottom was kept 5 cm for all the experiments. Fig. 1 shows the diagram of the UAE system with its components.

The measurement of the ultrasonic energy transferred to the samples was based on the calorimetric method, which assumes that there is an amount of energy introduced in the UAE system (Tiwari, 2015). The ultrasonic intensity (UI in W/cm^2) is defined as the energy emitted in the sample through the radiating surface area of the transducer, according to Eq (3).

$$UI = (P/A) \tag{3}$$

where P is the ultrasonic power (W) and A is the surface area of the transducer (cm^2).

UAE experiments were performed using methanol and ethanol as solvents. Prior the UAE experiments, kinetic runs were carried out to determine the extraction time for each solvent. The kinetics were performed at $150\ W/cm^2$ and $40\ °C$ and the extracts were collected at 5, 10, 20, 30, 60, 90 and 120 min. The solvent was evaporated under vacuum (at $25\ °C$), and the dry extract was weighed and stored under freezing ($-18\ °C$). Based on the kinetic profiles, extraction times of 20 min and 60 min were defined for UAE with methanol and ethanol, respectively. The UAE experiments were performed with a sample to solvent ratio of 1:20 (w/v). The effects of temperature and UI on global yield, total phenolic content, antioxidant capacity and total capsaicinoids of the extracts were investigated.

The experimental plan was a full factorial design of two independent variables (temperature and UI) with two levels in triplicate, added of three central points, totaling 15 experiments for each solvent.

2.4. Global yield (X_o)

The global yield (X_o) was determined as the ratio between the dry extract (m_{ext}) and the sample mass (F) in dry basis, according to Eq (4).
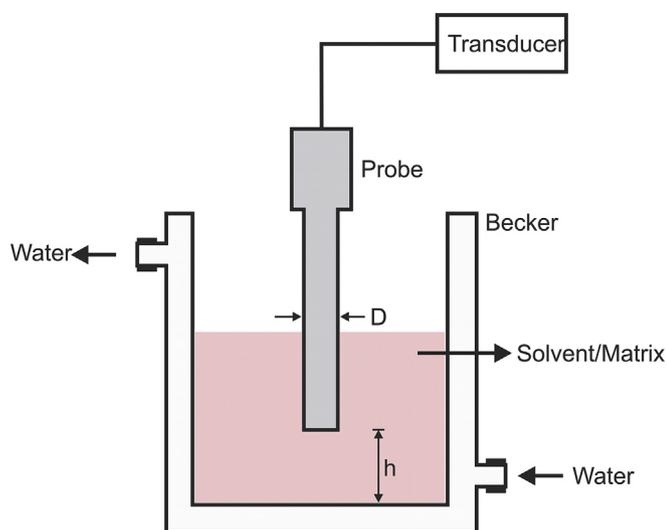
Fig. 1. Diagram of the UAE system; D — Ultrasonic probe diameter; h — Ultrasonic probe height.

$$X_0 = (m_{ext}/F) \times 100 \qquad (4)$$

The total phenolic content, antioxidant capacity and total capsaicinoids were determined according to the methods described in Sections 2.5, 2.6 and 2.7, respectively.

2.5. Total phenolic content (TPC)

The total phenolic content of the extracts was determined by UV-VIS spectrophotometry using the Folin-Ciocalteu method, based on Singleton et al. (1999) with modifications, in which 2.5 mL of diluted Folin-Ciocalteu reagent (1:10; v/v) were added to 0.5 mL of an extract solution diluted in methanol. After 5 min, 2.0 mL of sodium carbonate (7.5%) were added and the mixture reacted for 2 h in the dark at room temperature. Absorbance was measured at 760 nm and gallic acid was used as standard. The results were expressed in milligrams of gallic acid equivalent/gram of raw material (mg GAE/g RM).

2.6. Antioxidant capacity

The *in vitro* antioxidant capacities of the extracts were determined through the DPPH (Rufino et al., 2007) and FRAP (Benzie and Strain, 1996) methods with modifications.

2.6.1. DPPH (2,2-Diphenyl-1-picryl-hidrazil) method

Ethanol as control (0.1 mL) or diluted sample (1.0 mg/mL in ethanol) was mixed with 3.9 mL of ethanolic DPPH solution (0.024 mg/mL), stirred for 30 s and let reacting for 40 min in the dark at room temperature. Absorbance was measured at 516 nm. A Trolox solution (2 mM) was prepared dissolving 25 mg of Trolox reagent in 50 mL of ethanol. A calibration curve ($R^2 = 0.9909$) was prepared with concentrations of Trolox varying from 50 to 1000 μM. The results were expressed in μM of Trolox/gram of raw material (μM Trolox/g RM).

2.6.2. FRAP (ferric reducing antioxidant power) method

Solutions of ferric chloride (20 mM), acetate buffer (0.3 M), standard solution of ferrous sulfate (2 mM) and hydrochloric acid (40 mM) were prepared. All solutions were diluted in distilled water. A TPTZ solution (2,4,6-tripyridyl-S-triazine) was prepared by

dissolving 3.12 g of TPTZ in 5 mL of HCl (40 mM) and completing the volume with distilled water in a 1 L volumetric balloon.

The calibration curve ($R^2 = 0.9954$) was prepared using ferrous sulfate solutions ranging from 0.5 to 2 mM. A 90 μL aliquot of the diluted sample was added to 270 μL of distilled water, mixed with 2.7 mL of FRAP reagent (25 mL of acetate buffer, 2.5 mL of TPTZ solution and 2.5 mL of aqueous solution of ferric chloride), homogenized in a tube stirrer and kept at 37 °C for 30 min. Absorbance was measured at 595 nm. The results were expressed in grams of ferrous sulfate/gram of raw material (g $FeSO_4$/g RM).

2.7. Analysis of capsaicinoids

The capsaicinoid profile was determined according to Barbero et al. (2008) through Ultra High-Performance Liquid Chromatography coupled with Diode Arrangement Disposition (UHPLC-DAD). The reagents used in chromatography separation were capsaicin (97%) and dihydrocapsaicin (90%) standards, water Milli-Q, methanol and glacial acetic acid.

The capsaicinoid analyses were carried out on an Acquity UPLC system (Waters, Milford, USA) equipped with a photodiode detector array (Model 2996 PDA) and data acquisition by Empower 2 software. The capsaicinoids were separated using a Waters — BEH C18 column (50 mm × 2.1 mm I.D., particle size 1.7 μm). The detection wavelength was 280 nm and the separation temperature was 50 °C.

The method used the gradient of two solvents: acidified water (0.1% of acetic acid, solvent A) and acidified methanol (0.1% of acetic acid, solvent B), as follows (min, % of solvent B): 0.85 min, 55%; 1.60 min, 55%; 1.95 min, 60%; 2.45 min, 63%; 2.80 min, 70%; 3.00 min, 70%; 4.00 min, 100%. The UHPLC-DAD was used to prepare the regression equations of capsaicin (C) ($y = 2660.63x + 139.36$) and dihydrocapsaicin (DHC) ($y = 2865.97x + 89.83$), which are the commercially available capsaicinoid standards, where "y" refers to the peak area and "x" is the capsaicinoid (C or DHC) concentration. The detection and quantification limits were calculated to validate the capsaicinoids analyses. The limits of detection (0.242 mg/L for C and 0.137 mg/L for DHC) and quantification (0.801 mg/L for C and 0.457 mg/L for DHC) were determined as the analyte concentration giving a signal to noise ratio (S/N) of 3:1 and 10:1, respectively (Aguiar et al., 2013). All these values were determined through the ALAMIN software (Campana et al., 1997).

Nordihydrocapsaicin (n-DHC), capsaicin (C), dihydrocapsaicin (DHC), homocapsaicin (h-C) and homodihydrocapsaicin (h-DHC) were the five major capsaicinoids identified in the extracts. C and DHC were quantified from the respective standard solutions and calibration curves. The concentrations of n-DHC, h-C and h-DHC were determined from the calibration curves of DHC (for n-DHC and h-DHC) and C (for h-C), based on their structural similarities and molecular weights (Aguiar et al., 2013; Santos et al., 2015). The results were expressed in mg capsaicinoids/g raw material.

2.8. Mathematical modeling

A mathematical model was adjusted to each extraction curve in order to evaluate the influence of temperature and UI on the extraction kinetics. The model was developed by Veljković and Milenović (2002) and divides the extraction kinetics in two steps: extraction of bioactive compounds on or near the surface of the samples (washing process) and extraction of the compounds located inside the cells of the vegetable matrix (slow diffusion). Eq (5) describes the mathematical model.

$$y = y_\infty[1 - f \exp(-k_1 t) - (1 - f)\exp(-k_2 t)] \qquad (5)$$

where y and y_∞ are the extract yield and the extract yield at saturation, respectively, f is the extract fraction washed from broken cells on the particle surface, t is the extraction time, and k_1 and k_2 are the rate constants for washing and diffusion, respectively. The fraction of extract dissolved by washing is assumed as constant. The model also assumes that the washing step is faster than the diffusion step, i.e. k_1 is greater than k_2. The values of k are functions of the Arrhenius equation (Eq (6)).

$$k = A \exp\left(-\frac{E_a}{RT}\right) \qquad (6)$$

where k = constant, A = pre-exponential factor, E_a = activation energy, R = gas constant and T = temperature. This model is derived from that developed by Sovová (1994) to recover bioactive compounds from essential oils.

Since the washing process is much faster than diffusion ($k_1 \gg k_2$), Eq (5) can be reduced to a simpler model where the instantaneous washing is followed by diffusion:

$$y = y_\infty[1 - (1 - f)\exp(-k_2 t)] \qquad (7)$$

Veličković et al. (2006) applied Eq (7) to adjust the extraction kinetics of essential oils from garden sage (*Salvia officinalis* L.) and glutinous sage (*Salvia glutinosa* L.) through UAE and observed that ultrasound was more effective than maceration to extract oils.

Considering that the washing process does not occur (f = 0), the extraction yield increases exponentially due to diffusion, as shown in Eq (8), namely pseudo-first order model:

$$y = y_\infty[1 - \exp(-k_2 t)] \qquad (8)$$

The fitting quality of each extraction curve was statistically evaluated by the mean relative percent deviation (MRPD) and the coefficient of determination (R^2), according to Eqs. (9) and (10), respectively:

$$\text{MRPD} = \frac{100}{n} \sum_{i=1}^{n} \left| \frac{y_{p,i} - y_{a,i}}{y_{a,i}} \right| \qquad (9)$$

$$R^2 = \frac{\sum_{i=1}^{n} \left(y_{p,i} - y_{a,i} \right)^2}{\sum_{i=1}^{n} \left(y_{p,i} - y_m \right)^2} \qquad (10)$$

where $y_{p,i}$ and $y_{a,i}$ are the predicted and experimental yields, y_m is the mean yield, and n is the number of experiments.

2.9. Field emission scanning electron microscopy (FESEM)

FESEM images were obtained from different parts of the pepper pericarp before and after extraction, using a scanning electron microscope equipped with a field emission gun (Quanta 650, FEI, Hillsboro, Oregon, USA). The pericarp of the samples was previously separated from the seeds and peduncles, using tweezers. Before the analysis, the samples were coated with gold in an SCD 050 sputter coater (Oerlikon-Balzers, Balzers, Liechtenstein). The analyses of the sample surfaces were carried out under vacuum with a 5 kV acceleration voltage. At least 20 images of each sample were obtained to guarantee the reproducibility of the results.

2.10. Statistical analysis

The effects of temperature and UI on global yield, total phenolics, antioxidant capacities (DPPH and FRAP) and total capsaicinoids were evaluated at the level of 5% ($p < 0.05$) to detect significant differences in the UAE extracts, using the software Statistica 7.0 (Statsoft Inc., USA).

3. Results and discussion

3.1. Sample characterization

Table 1 shows the characteristics of the dried and milled pepper samples. The lipid content is influenced by the pepper genotype and the possibility of cross-species (Shahidi and Naczk, 2003), which may explain the difference between the present results with those previously published using the same technique. Aguiar et al. (2014) obtained total lipid content of 2.60± 0.03% from biquinho pepper (*Capsicum chinense*), and Santos et al. (2015) obtained 9.70± 0.10% from malagueta pepper (*Capsicum frutescens* L.).

Table 2 presents the global yields, total phenolics and antioxidant capacities (DPPH and FRAP) of the extracts obtained by Soxhlet and UAE.

3.2. Global yield (X_o)

As noted on Table 2, Soxhlet extraction was favored by polar solvents (ethanol and methanol), which indicates that most extracted compounds are polar. In Soxhlet the reflux of boiling solvent may have reduced its viscosity and surface tension, thus reaching soluble substances inside the vegetable matrix (Markom et al., 2007). Moreover, temperature influences the solvent recirculation and the solvent/solute interaction, enhancing the solubilization of compounds (Mezzomo et al., 2009). On the other hand, the high extraction yields achieved with ethanol and methanol result from the low selectivity of these solvents, leading to the extraction of a large variety of compounds.

Despite the high extraction yield achieved with methanol, this solvent is not adequate to obtain extracts for application in food and pharmaceutical industries. According to the FDA (2012), the use of methanol (Class 2—3000 ppm residual concentration limit) must be restricted due to its inherent toxicity (Aguiar et al., 2014). However, ethanol may be a better choice from the industrial viewpoint, since it can be reused and generates less waste at the end of the process.

For UAE by methanol, the effect of the interaction between temperature and UI was significant (p-value < 0.05). Higher temperature and UI increased the extraction yields. According to Purohit and Gogate (2015), temperature influences the global yield by changing the solubility of the solute in the extraction solvent and by modifying its viscosity and density, which may have a remarkable effect on the cavitational activity. Moreover, Vinatoru (2001) observed that ultrasound intensifies the oleoresin extraction and cavitational effects. On the other hand, none parameter had significant effect for UAE by ethanol (p-value > 0.05). One possible

Table 1
Characterization of the dried and milled samples.

Characteristic	
Mean particle diameter	0.68 ± 0.03 mm
Real density	1.41 ± 0.01 g/cm^3
Porosity	0.69
Moisture content	$4.32 \pm 0.10\%$
Total lipid	$5.11 \pm 0.36\%$

Table 2
Global yields, total phenolic contents and antioxidant capacities (DPPH and FRAP) of the extracts from dedo de moça pepper obtained by Soxhlet and UAE by methanol and ethanol.

Method	Solvent	Polarity*	X_o (%)	TPC	DPPH	FRAP
Soxhlet	Hexane	0.0	5.11 ± 0.36	0.82 ± 0.09	0.34 ± 0.08	0.07 ± 0.00
	Ethyl acetate	5.3	5.87 ± 0.33	0.99 ± 0.09	4.28 ± 0.65	0.05 ± 0.00
	Ethanol	8.8	28.93 ± 2.15	2.46 ± 0.47	10.97 ± 0.57	0.96 ± 0.27
	Methanol	12.3	53.62 ± 0.58	4.93 ± 0.23	8.52 ± 0.27	2.52 ± 0.16

Method	Temperature (°C)	Ultrasonic intensity (W/cm^2)	Polarity*	X_o (%)	TPC	DPPH	FRAP
UAE/Methanol	40	150	12.3	37.84 ± 0.67	5.89 ± 0.18	22.30 ± 1.98	1.53 ± 0.43
	40	450	12.3	32.95 ± 9.15	4.43 ± 1.35	21.69 ± 1.20	0.99 ± 0.29
	60	150	12.3	22.73 ± 6.83	2.58 ± 0.65	26.43 ± 6.40	0.67 ± 0.13
	60	450	12.3	40.05 ± 0.81	3.54 ± 0.37	23.66 ± 2.70	1.24 ± 0.30
	50	300	12.3	38.69 ± 4.06	6.33 ± 0.63	21.14 ± 2.52	1.23 ± 0.17
UAE/Ethanol	40	150	8.8	31.73 ± 4.45	0.34 ± 0.04	13.35 ± 4.43	0.92 ± 0.18
	40	450	8.8	29.94 ± 4.95	5.42 ± 0.79	16.74 ± 4.41	0.65 ± 0.16
	60	150	8.8	26.82 ± 2.76	7.19 ± 1.34	31.62 ± 4.76	0.48 ± 0.09
	60	450	8.8	26.57 ± 7.16	7.62 ± 1.86	21.75 ± 6.95	0.53 ± 0.16
	50	300	8.8	33.47 ± 5.81	7.89 ± 1.05	23.19 ± 2.19	0.71 ± 0.16

Results are expressed as mean ± standard deviation of the analyses; UAE – Ultrasound-assisted extraction; *Hansen (2000); TPC – Total phenolic content expressed as mg GAE/g RM; DPPH – antioxidant capacity expressed as μM Trolox/g RM; FRAP – antioxidant capacity expressed as g FeSO$_4$/g RM.

reason for this behavior is that, for UAE by ethanol, the clumping of pepper samples reduced the interfacial area between the plant material and solvent phase, resulting in no significant differences between the operational conditions. A similar result was found by Vinatoru et al. (1997), who extracted bioactive compounds from carrot powder using ultrasound. Besides, comparing both techniques, UAE increased the global extraction yield up to 26%.

3.3. Total phenolic content (TPC)

Table 2 shows that the concentrations of phenolic compounds obtained by Soxhlet were higher in the extractions with methanol and ethanol, which was expected, since phenolics are generally polar (Casas et al., 2009). The ability of phenolic compounds to scavenge oxidation-initiating radicals is related to the polar phase of the extracts. Moreover, phenolic compounds have an electron donating group, such as hydroxyl, which makes most recovered phenolic compounds polar (Duan et al., 1998).

Temperature had statistically significant effect (p-value < 0.05) on the recovery of phenolic contents through UAE by methanol, whereas by ethanol both temperature and ultrasound were significant. The use of ultrasound to extract bioactive compounds from inside the vegetable matrix can promote cell disruption and microstreaming effects, enhancing the solute/solvent contact (Purohit and Gogate, 2015). Rabelo et al. (2016) extracted phenolics from artichoke wastes through UAE by ethanol, which increased the solubility and diffusion of these compounds. According to Carrera et al. (2012), the energy provided by ultrasound releases phenolic compounds from the vegetable matrix, increasing their extraction. However, higher UI accelerates their degradation. Purohit and Gogate (2015) observed that UI increased the cavitation effect and energy dissipation as heat, which was directly related to the degradation of thermolabile compounds.

3.4. Antioxidant capacity

For Soxhlet, the highest antioxidant capacities were detected in the extracts obtained with methanol and ethanol, due to the polarity of these solvents. A similar trend was observed by Andrade et al. (2012), who extracted antioxidant compounds from coffee husk (Coffea arabica). However, the lower antioxidant capacity obtained with hexane and ethyl acetate may be related to the lower amount of phenolic compounds with intermediate to high polarities in the extracts.

DPPH results showed that none parameter was statistically significant (p-value > 0.05) for UAE by methanol, while for UAE by ethanol the effect of the interaction between temperature and UI was significant (p-value < 0.05). For FRAP, the effect of the interaction between temperature and UI was significant (p-value < 0.05) for UAE by methanol. In this case, lower temperature and UI promote higher solubilization of antioxidants. Soria and Villamiel (2010) noted that sonication enhances the mass transfer of solutes in the solvent, increasing the extraction of antioxidant compounds. However, for UAE by ethanol none parameter was significant (p-value > 0.05).

3.5. Analysis of capsaicinoids

Table 3 shows the concentrations of capsaicinoids (n-DHC, C, DHC, h-C and h-DHC) in the extracts obtained through Soxhlet and UAE by methanol and ethanol.

The use of hexane and ethyl acetate as solvents in Soxhlet resulted in higher capsaicinoid yields when compared to methanol and ethanol. The non-polar characteristic of capsaicinoids may explain their affinity to low polarity solvents, increasing their yields. The higher vapor pressure of hexane and ethyl acetate may also explain the higher capsaicinoid yields. Moreover, the lower diffusivity and higher viscosity of ethanol and methanol can reduce the efficiency of these solvents to extract capsaicinoids. Barbero et al. (2008) extracted capsaicinoids from hot Cayenne peppers with different solvents and observed that polar solvents, such as water, have poor capacity to extract capsaicinoids, especially the less polar ones: DHC, h-C and h-DHC.

The statistical analysis of UAE by methanol showed that the effects of temperature and UI were significant (p-value < 0.05). Table 3 indicates that the increase of temperature and UI reduced the extraction of capsaicinoids. Boonkird et al. (2008) extracted capsaicinoids from malagueta pepper (Capsicum frutescens L.) and obtained higher yields at 45 °C than at 60 °C. According to the authors, the collapse of bubbles was more intense at 45 °C, when cavitation was more efficient. At lower temperatures, there are less cavities and the intensity of the bubble collapse is higher, whereas at higher temperatures more cavities are formed but the bubble collapse is less intense due to higher vapor pressure (Tiwari et al., 2010). However, none parameter was statistically significant (p-value > 0.05) for UAE by ethanol. Besides, it was noted that UAE reduced capsaicinoid yields down to 50% in comparison to the Soxhlet extraction.

Table 3
Capsaicinoids yields of extracts from dedo de moça pepper obtained by Soxhlet and UAE by methanol and ethanol.

Method	Solvent	Polarity*	n-DHC	C	DHC	h-C	h-DHC	Total capsaicinoids
Soxhlet	Hexane	0.0	0.09 ± 0.00	1.44 ± 0.07	0.60 ± 0.02	0.07 ± 0.00	0.02 ± 0.00	2.22 ± 0.10
	Ethyl acetate	5.3	0.11 ± 0.01	1.77 ± 0.21	0.73 ± 0.09	0.09 ± 0.01	0.03 ± 0.00	2.73 ± 0.33
	Ethanol	8,8	0.02 ± 0.01	0.33 ± 0.14	0.14 ± 0.06	0.02 ± 0.01	nd	0.51 ± 0.22
	Methanol	12.3	0.01 ± 0.01	0.18 ± 0.03	0.07 ± 0.01	0.01 ± 0.00	nd	0.28 ± 0.04

Method	Temperature (°C)	Ultrasonic intensity (W/cm^2)	n-DHC	C	DHC	h-C	h-DHC	Total capsaicinoids
UAE/Methanol	40	150	0.01 ± 0.00	0.14 ± 0.01	0.06 ± 0.00	0.01 ± 0.00	nd	0.22 ± 0.02
	40	450	0.01 ± 0.00	0.12 ± 0.01	0.05 ± 0.00	0.01 ± 0.00	nd	0.18 ± 0.01
	60	150	0.01 ± 0.00	0.12 ± 0.01	0.05 ± 0.00	0.01 ± 0.00	nd	0.18 ± 0.02
	60	450	0.01 ± 0.00	0.11 ± 0.00	0.05 ± 0.00	0.01 ± 0.00	nd	0.17 ± 0.00
	50	300	0.01 ± 0.00	0.12 ± 0.01	0.05 ± 0.00	0.01 ± 0.00	nd	0.19 ± 0.02
UAE/Ethanol	40	150	0.01 ± 0.00	0.11 ± 0.02	0.05 ± 0.01	0.01 ± 0.00	nd	0.18 ± 0.03
	40	450	0.01 ± 0.00	0.12 ± 0.01	0.05 ± 0.00	0.01 ± 0.00	nd	0.18 ± 0.02
	60	150	0.01 ± 0.00	0.17 ± 0.07	0.07 ± 0.03	0.01 ± 0.00	nd	0.26 ± 0.11
	60	450	0.01 ± 0.00	0.10 ± 0.00	0.04 ± 0.00	0.01 ± 0.00	nd	0.16 ± 0.10
	50	300	0.01 ± 0.00	0.13 ± 0.02	0.05 ± 0.01	0.01 ± 0.00	nd	0.19 ± 0.04

Results are expressed as mean ± standard deviation of the analyses; UAE – Ultrasound-assisted extraction; *Hansen (2000); n-DHC - nordihydrocapsaicin, C - capsaicin, DHC - dihydrocapsaicin, h-C – homocapsaicin and h-DHC – homodihydrocapsaicin. All capsaicinoids expressed as mg capsaicinoids/g RM. nd – not detected.

Table 4
Parameters of pseudo-first order model and model based on instantaneous washing followed by diffusion for extraction of oleoresin from dedo de moça pepper through UAE by ethanol.

Method	Condition	Pseudo-first order model			Model based on instantaneous washing followed by diffusion			
		$k_2 \times 10^2$ (min^{-1})	R^2 (%)	MRPD (%)	f	$k_2 \times 10^2$ (min^{-1})	R^2 (%)	MRPD (%)
UAE	150 W/cm^2/40 °C	8.930	87.35	13.696	0.146	5.580	88.89	11.654
	150 W/cm^2/50 °C	15.590	95.09	7.6975	0.030	14.880	95.18	7.541
	150 W/cm^2/60 °C	13.830	94.87	10.904	0.040	12.940	95.02	7.913
	450 W/cm^2/40 °C	15.600	94.22	8.174	0.003	14.830	94.31	7.927
	450 W/cm^2/50 °C	14.160	91.49	9.529	0.037	13.200	91.60	9.635
	450 W/cm^2/60 °C	17.470	81.80	16.994	0.199	5.690	82.39	14.191

Table 5
Phenomenological model parameters for extraction of oleoresin from dedo de moça pepper through UAE by ethanol.

Method	Condition	y(%)[a]		f	$k_1 \times 10$ (min^{-1})	$k_2 \times 10^2$ (min^{-1})	R^2 (%)	MRPD (%)
		y_{exp}(%)	y_{cal}(%)					
UAE	150 W/cm^2/40 °C	15.94 ± 1.21	15.98 ± 0.02	0.311	2.968	2.290	99.86	0.906
	150 W/cm^2/50 °C	17.72 ± 0.06	17.75 ± 0.07	0.591	4.292	2.480	99.76	1.438
	150 W/cm^2/60 °C	19.80 ± 1.03	19.68 ± 0.23	0.548	4.587	2.840	99.36	2.192
	450 W/cm^2/40 °C	18.46 ± 0.25	18.71 ± 0.15	0.355	3.061	0.240	99.87	2.228
	450 W/cm^2/50 °C	21.00 ± 1.21	21.20 ± 0.07	0.289	3.188	0.240	99.80	1.467
	450 W/cm^2/60 °C	25.69 ± 0.54	26.23 ± 0.93	0.338	3.138	0.520	99.87	0.939

[a] Value ± standard deviation.

3.6. Mathematical modeling

Two simple models (a pseudo-first order model (Eq (8)) and a model based on instantaneous washing followed by diffusion (Eq (7))) were applied and compared to the phenomenological model (Eq (5)). Table 4 presents the results obtained from these models and Table 5 presents the adjusted parameters of the phenomenological model for UAE by ethanol. The pseudo-first order model and the model based on instantaneous washing followed by diffusion achieved MRPD-values higher than the phenomenological model in all conditions, being thus inadequate to fit the extraction curves. Therefore, the phenomenological model was the best to describe the extraction kinetics, indicating that both washing and diffusion steps must be considered. Furthermore, the phenomenological model had a good adjustment to the experimental values, which is confirmed by R^2 close to unity and the reduced MRPD. Fig. 2 shows the experimental and modeled extraction curves obtained for UAE by ethanol.

As can be seen in Fig. 2, the extraction yield increased with temperature and ultrasound application. According to Stanisavljević et al. (2007), higher extraction temperatures increase the oleoresin solubility and reduce its viscosity, increasing the diffusive and convective coefficients, and thus increasing the extraction yield. Furthermore, the effect of ultrasound can be related to the rupture of cavitational bubbles promoted by ultrasonic waves near the vegetable matrix, which may help breaking cell walls and enhance mass transfer (Vilkhu et al., 2008). In addition, it can be noted in Fig. 2 that the extraction yield increased with ultrasonic intensity. For instance, at 40 °C the yield raised from 15.9% to 18.4% at 150 W/cm^2 and 450 W/cm^2, respectively. Therefore, more energy was applied per time, enhancing the cavitation effects and therefore increasing the extraction yield.

The fast washing and slow diffusion steps of the extraction kinetics are also noted in the kinetics shown in Fig. 2. In the fast washing, the samples located near the particle surfaces were easily extracted, possibly due to the rupture of the cell walls or to the increase of agitation promoted by ultrasound. On the other hand, the particles located inside the vegetable matrix slowly diffused to
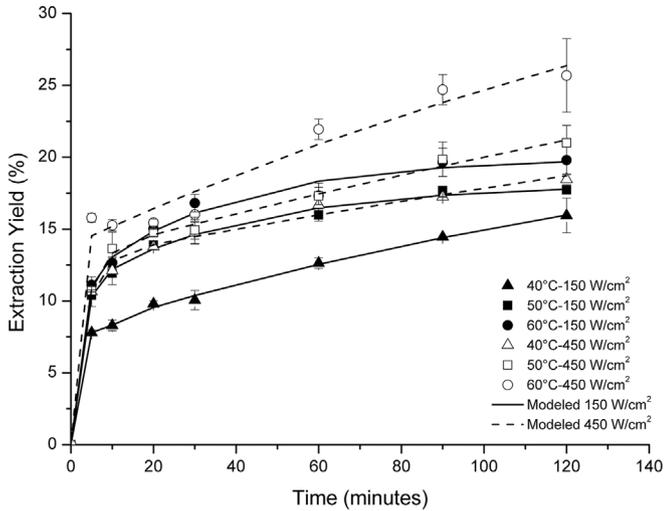
Fig. 2. Experimental and modeled extraction kinetics of oleoresin from dedo de moça pepper obtained through UAE by ethanol. Temperatures from 40 to 60 °C and ultrasonic intensities from 150 to 450 W/cm².

Table 6
Parameters from Arrhenius equation.

UAE	Step	A (min^{-1})	E_a (kJ/mol)
150 W/cm²	washing process	4.6×10^2	18.60
	slow diffusion	0.13×10^1	11.77
450 W/cm²	washing process	9.0×10^{12}	79.10
	slow diffusion	5.9×10^2	31.92

the surface. This behavior can also be deduced from the values of constant rates of first and second step of the phenomenological model, which are exposed in Table 5.

Table 6 shows the parameters calculated from Arrhenius equation. From the values of A and E_a it can be affirmed that ultrasound affected both extraction steps, washing and slow diffusion. For the washing process, the activation energy increased from 18.6 kJ/mol at 150 W/cm² to about 79 kJ/mol at 450 W/cm², and for slow diffusion it increased from 11.7 kJ/mol at 150 W/cm² to about 31.9 kJ/mol at 450 W/cm². Chemat et al. (2004) compared the extraction rates and kinetics of conventional and ultrasound-assisted extraction of carvone and limonene from caraway seeds. The authors affirmed that the high activation energy in the ultrasound experiment can be explained by the weak extraction selectivity of supersonics on a specific compound. So, the high activation energy in the ultrasound experiment at 450 W/cm² can elucidate the reason why oleoresin was better extracted at higher temperatures. Besides, at higher UI other components than capsaicinoids and phenolics are extracted. Milić et al. (2013) observed that ultrasound acted only in the washing step during the UAE of bioactive compounds from *Galium mollugo* L. Moreover, mass transfer mechanisms, such as convection and diffusion, can be enhanced by ultrasound. The activation energy obtained at 150 W/cm² for the two steps was approximately that reported by Qu et al. (2010) in the extraction of antioxidants from dry peel powders of pomegranate (14.5 kJ/mol).

3.7. Field emission scanning electron microscopy (FESEM)

Fig. 3 shows the FESEM images obtained on the surface of the

(a) Unextracted dry raw material   (b) After Soxhlet   (c) After UAE
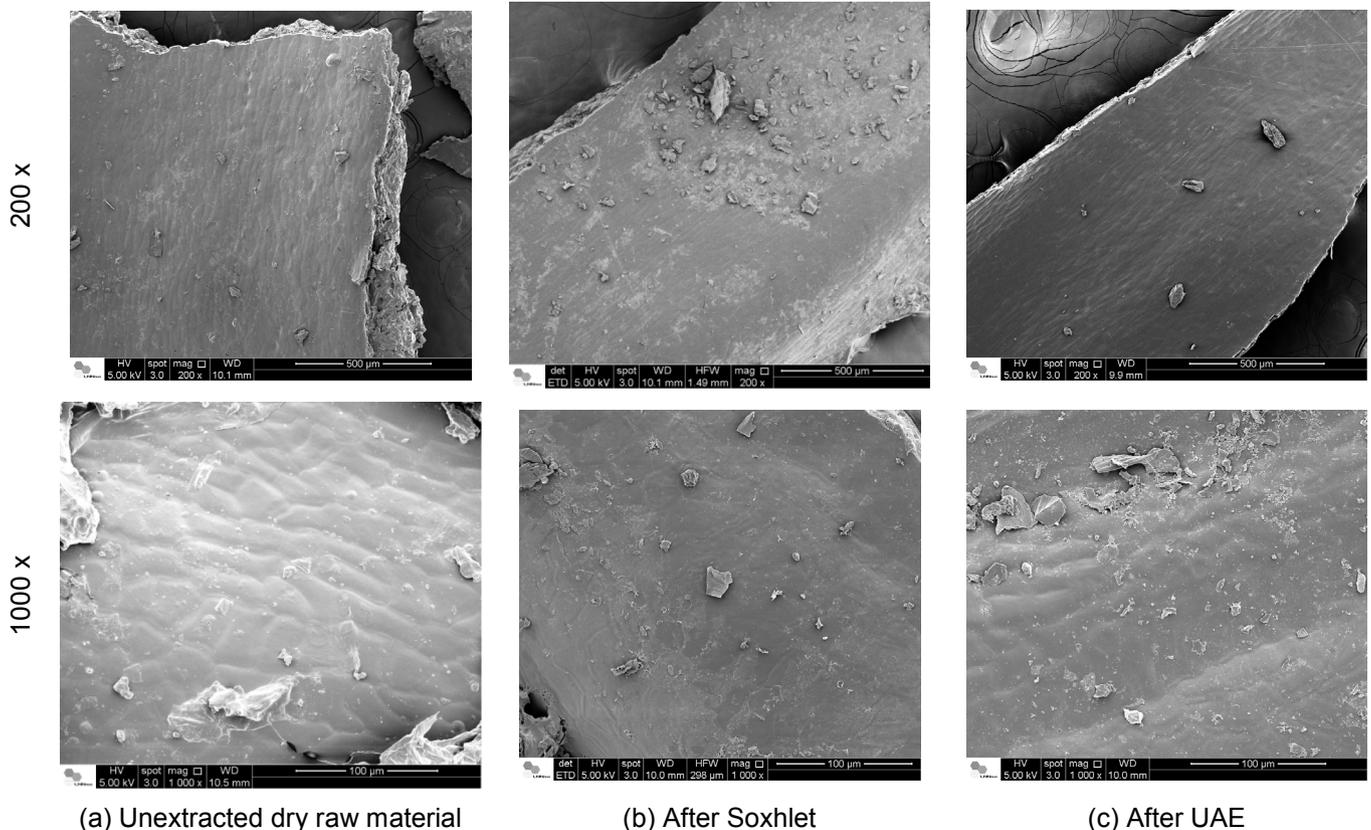
Fig. 3. FESEM images obtained on the surface of the sample pericarp: unextracted dry raw material (a), after Soxhlet (b) and after UAE (c). 200× - Scale bar - 500 μm. 1000× - Scale bar - 100 μm.

sample pericarp, showing the unextracted dry raw material (a), the samples after Soxhlet using ethanol (b) and after UAE (60 °C and 450 W/cm^2) using ethanol (c). As can be noted by the FESEM images (Fig. 3a–c) (scale bar = 500 μm), ultrasound did not change the sample morphology, since the same amount of particles is observed on the surfaces of the extracted peppers (Fig. 3b–c) and of the unextracted material (Fig. 3a). Fig. 3 also presents a magnified view of the samples (scale bar = 100 μm). These micrographs show that the surfaces below the particles did not present any sign of fissure, cracks or rupture, keeping their integrity after the extractions. According to Dias et al. (2016), Santos et al. (2015), Barrales et al. (2015) and Reátegui et al. (2014), the application of ultrasound waves in supercritical fluid extraction of vegetal materials can lead to an enhanced deposition of particles on the surface of the extracted samples. According to these authors, ultrasound promotes mechanical vibration of the sample, releasing internal particles of the pericarp cell walls through lateral cracks formed by knife milling, and the released particles are deposed on the surface. However, by comparing the FESEM images obtained in this work, this increased particle deposition was not observed in the samples that underwent UAE.

Opposite results were obtained by Ma et al. (2015), Nowacka et al. (2012), Zhang et al. (2008) and Sharma and Gupta (2006), who found that ultrasonic treatment resulted in the change of the physical structure of plant materials due to cavitation energy. This different behavior suggests that sonication promoted only the intensification of the mixture between vegetal material and solvent, which enhanced the solute/solvent contact and intensified mass transfer effects, thus increasing the extraction yields (Vilkhu et al., 2008).

4. Conclusions

Soxhlet and UAE were effective to obtain extracts with bioactive compounds from dedo de moça pepper (*Capsicum baccatum* L.). UAE appears as an alternative to conventional extraction, since it works at lower temperatures and shorter time. From the global yields, phenolic contents and antioxidant capacities, a direct relation was observed between the polarity of the solvents and the concentration of these compounds. However, low polarity solvents increased the capsaicinoid extraction. Ultrasound enhanced the extraction of oleoresin up to 26%. The recoveries of phenolics, antioxidants and capsaicinoids also increased with ultrasound, but higher ultrasonic intensities reduced their yields.

The phenomenological model provided a good fit of the extraction kinetics in all performed conditions and revealed that ultrasound influences both washing and slow diffusion steps. The FESEM analyses showed that ultrasound did not cause any damage to the surface of the pepper pericarp particles. Also, the images did not show any change in the morphological structure between both UAE and Soxhlet. To sum up, the use of ultrasound has potential to increase the added value of bioactive compounds from dedo de moça pepper and to replace conventional extraction techniques. For further works, the scale-up of the UAE process and the study of its economic viability should be addressed.

Acknowledgments

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