Journal of Food Engineering 204 (2017) 38–45

Contents lists available at ScienceDirect

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng

Sustainable extraction and encapsulation of pink pepper oil

Kátia S. Andrade [a], Denis Poncelet [b], Sandra R.S. Ferreira [a, *]

[a] Chemical and Food Engineering Department, Federal University of Santa Catarina, EQA/UFSC – C.P. 476, CEP 88040-900, Florianópolis, SC, Brazil
[b] Génie des Procédés Environnement et Agroalimentaire, GEPEA/ONIRIS, Rue de la Géraudière, B.P. 82225-44332 Nantes Cedex 3, France

ARTICLE INFO

Article history:
Received 25 October 2016
Received in revised form
11 January 2017
Accepted 19 February 2017
Available online 20 February 2017

Keywords:
Pink pepper
Antioxidant activity
Supercritical fluid
Encapsulation

ABSTRACT

Pink pepper (*Schinus terebinthifolius R.*) is a native tree from Brazilian coast and presents important biological activities, such as antitumor, anti-inflammatory, antioxidant, among others. The objective of this work was to explore the use of the supercritical fluid extraction (SFE) for pink pepper fruits, a sustainable method for a novelty material, comparing with different techniques in terms of process yield, total phenolic content (TPC), and antioxidant activity of the recovered extracts. The SFE was performed at pressures from 150 to 300 bar and temperatures of 40, 50 and 60 °C, with CO_2 as solvent. Pink pepper extracts were also obtained by Soxhlet (SOX) and by Ultrasound accelerated extraction (UE) using hexane, ethanol (EtOH), and ethyl acetate (EtOAc) as solvents. The extracts were evaluated as antioxidant potential (ABTS and DPPH methods), total phenolic content (TPC) and chemical profile (GC-MS). The encapsulation process was evaluated using the extract obtained at 300 bar and 60 °C, through the emulsification and solvent extraction technique, using PLA as encapsulating agent. The best extraction yields were obtained by SOX-EtOH and UE-EtOH (44 ± 1% and 21 ± 2%, respectively), followed by SFE at 300 bar and 60 °C (5.9 ± 0.3%). Pink pepper extracts obtained by SOX-EtOH and SFE 300 bar/60 °C presented the best DPPH value. The major compounds identified in the pink pepper extracts were germacrene D, sabinene, β and α-phellandrene. The microencapsulation by the emulsification and solvent extraction technique led the formation of micrometer-sized particles with spherical shape and morphology of the microspheres. The encapsulation efficiency of pink pepper extract in polylactic acid (PLA) ranged from 34.3% to 74.1%. The results from this work suggest the importance of this raw material as a potential for generating high value products with therapeutic applications.

1. Introduction

Schinus terebinthifolius Raddi (Anacardiaceae) is a perennial tree native to the Brazilian coast and spread to other regions of South America, Central America, Europe, Asia and Africa. The use of the fruits from *Schinus terebinthifolius* R. as food seasoning in homely and industrial products is rather extensive. The pink pepper has been used as a substitute for black pepper and, according to chemical analysis reported in the literature, there are similarities in chemical composition between both species (Cláudio et al., 2007).

Different therapeutic properties are attributed to pink pepper, such as antioxidant, antitumor and antimicrobial. Brazilian's folk medicine uses it as anti-inflammatory, astringent, tonic and stimulant. These properties have been related to the presence of polyphenols such as apigenin, ellagic acid, and naringin. In addition, antimicrobial activity have been associated to substances like terebinthona, hidroximasticadienoic acid, terebinthifolic acid and ursolic acid, present in the plant extract. The main monoterpenes found in ripe fruits extracts are α-pinene, β-phellandrene and *trans*-ocimene, followed by the sesquiterpene germacrene-D (Bendaoud et al., 2010; Bertoldi, 2006; Santos et al., 2007).

The quality of natural extracts, related to composition and biological activity, is strongly associated to the extraction process, the solvent used, the characteristics of the vegetal matrix, its storage condition and pre-treatment applied. The extraction techniques and the solvents used must be carefully chosen to optimize the balance between maximizing yields and selectivity (Azmir et al., 2013; Louli et al., 2004; Moure et al., 2001).

Several studies involving the extraction of pink pepper leaves are reported in the literature, and very few works with pink pepper fruits. However, the methods employed are basically hydrodistillation and Soxhlet extraction with ethanol (Bendaoud et al., 2010; Bertoldi, 2006; Lloyd et al., 1977; Santana et al., 2012).

* Corresponding author.
E-mail address: s.ferreira@ufsc.br (S.R.S. Ferreira).

http://dx.doi.org/10.1016/j.jfoodeng.2017.02.020
0260-8774/

The supercritical fluid extraction (SFE) is an alternative extraction method which enables the solvating power modulation by controlling the process temperature and pressure, with consequent selectivity adjustment. Besides, SFE employs green solvents, like carbon dioxide, and not requires a step for solvent removal. Then, SFE is a viable alternative for extraction and fractionation of natural products, especially for food and pharmaceutical industries (Michielin et al., 2009; Reverchon and De Marco, 2006).

The use of high quality natural extracts, obtained by environment friendly technologies, is the focus of countless studies in different fields of research. Furthermore, it is also important to preserve the integrity of these compounds against degradation, for instance by means of encapsulation in biopolymers by equally friendly methods. The encapsulation process provides protective effect of the core material, preventing losses of volatile compounds, masking undesirable flavors and odors and enhances the solubility of hydrophobic compounds in a aqueous media that increases its range of application (Gomes et al., 2011). The microencapsulation by solvent extraction technique is widely used in pharmaceutical industries and consists of four steps: *(a)* dissolution or dispersion of the bioactive compound in an organic solvent containing the polymer; *(b)* emulsification of this organic phase in a second continuous phase immiscible with the first one; *(c)* extraction of the organic solvent from the dispersed phase by the continuous phase, in order to transforming the droplets into solid microspheres; *(d)* harvesting and drying of the microspheres.

In this method, a careful selection of the encapsulation conditions and the materials used aid the control of the particle size, within *nano*metric to *micrometer* range (Freitas et al., 2005; Li et al., 2008). Polylactic acid (PLA), widely used in encapsulation process, is an aliphatic polyester with adjusted hydrolyzability, approved by the Food and Drug Administration (FDA) and by European regulatory authorities, and is *Generally Recognized As Safe* (GRAS) (Costa Lima et al., 2012).

Studies related to biological activities and encapsulation process of SFE from *Schinus terebinthifolius* fruits are new and suggest a distinct valorization of this unique raw material. Therefore, the objective of the present work aimed to compare different extraction methods and solvents to obtain pink pepper fruit extracts, for encapsulation in biopolymers to extend its use by means of green processes. The results from this work are presented comparing the extraction yields, and also evaluating the extracts quality in terms of total phenolic content, antioxidant activity and chemical profile. With this research, different method to obtain and protect bioactive extracts from pink pepper fruit were investigated to aid the development of therapeutic formulations.

2. Materials and methods

2.1. Raw material and sample preparation

Pink pepper samples (*Schinus terebinthifolius* R.) were collected in the campus of the Federal University of Santa Catarina. The plant was identified and a voucher specimen (number 54104) was deposited at “Herbário Flor” of the Federal University of Santa Catarina. After harvest, the samples were cleaned to remove leaves, branches, debris, dust and other undesired materials. The pepper grains were then stored in transparent polyethylene bags at 2 °C until the drying process. The raw material presented 20.8 ± 0.4% (w/w) of moisture and volatile content, determined according to the 950.46B method of AOAC (Latimer, 2012). After the drying process, the pepper grains were ground in a domestic blender and the grounded material was stored at − 18 °C until the extractions were performed. All solvents used in the experiments were of analytical grade (P. A.) purchased from Lafan Química Fina LTDA. Carbon dioxide (99.9% purity) was purchased from White Martins S/A (São Paulo, Brazil) and delivered at pressure up to 60 bar. NaturePlast (France) kindly provided the PLA pellets (PLE 003).

2.2. Extraction methods

2.2.1. Soxhlet (SOX)

The SOX extraction was performed according to 920.39C method of AOAC (Latimer, 2012). Three different solvents were used: hexane (Hx), ethyl acetate (EtOAc) and ethanol (EtOH), with ascending polarity of 0, 4.4 and 5.2, respectively (Ritchie, 2000). The residual solvent from all extracts (SOX and UE with three solvents) was eliminate in a rotatory evaporator (Fisatom, 802, Brazil), supplied with cooling and vacuum control. Next, the extracts were stored in amber glass flasks at −18 °C. The extraction yields of all method/solvent systems were determined by the ratio between the mass of extract obtained and the mass of raw material (wet basis) and the results are presented by average ± standard deviation.

2.2.2. Ultrasound-assisted extraction (UE)

The ultrasound-assisted extractions were carried out in an ultrasonic cleaner bath, which operates at a frequency of 55 kHz and potency of 100 W (Vinatoru, 2001). The method consisted of placing 7 g of raw material and 210 mL of solvent inside a covered glass balloon. The extractions were performed in duplicate at room temperature for 45 min using the same solvents described in Section 2.2.1.

2.2.3. Supercritical fluid extraction (SFE)

A high-pressure unit, previously described by Zetzl et al. (2007), was used for the SFE with CO_2 as solvent by following the extraction procedure from Michielin et al. (2005) to obtain the pink pepper extracts. Briefly, the extraction consisted of placing a fixed mass of 15 g of the dried raw material (grounded pink pepper) inside the extractor cell to form the fixed bed of particles, followed by the control of the process variables (temperature and pressure). The extraction was then performed and the solute collected in amber flasks and weighed on an analytical balance (OHAUS, Model AS200S, NJ, USA). The SFE with CO_2 was conducted at temperatures of 40, 50 and 60 °C and pressures of 150, 200 and 300 bar, at constant solvent flow rate of 8 ± 2 g/min. The extraction time was set at 3 h according to the kinetic extraction curve performed at 200 bar, 50 °C (intermediary conditions of extraction) and 8 ± 2 g CO_2/min. The solvent density values were obtained according to Angus et al. (1976). The results were expressed in terms of extraction yield (X_0).

2.3. Determination of total phenolic content (TPC)

The TPC was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton and Rossi, 1965). Briefly, the reaction mixture was composed by 0.1 mL of extract (concentration of 1667 mg/L), 7.9 mL of distilled water, 0.5 mL of Folin–Ciocalteu reagent (a mixture of phosphomolybdate and phosphotungstate) and 1.5 mL of 20% sodium carbonate, placed in opaque flasks. The flasks were agitated and allowed to rest for 2 h, then the absorbance measured at 765 nm in a spectrophotometer (Femto, 800XI, Brazil). The TPC value was calculated according to a standard curve ($y = 0.0011x + 0.0352$; $R^2 = 0.9951$), prepared previously with gallic acid as standard (galic acid equivalent: GAE). The analysis was performed in triplicate and the results expressed as mg GAE/g extract as mean ± standard deviation.

2.4. Antioxidant activity

The antioxidant activity of the pink pepper extracts, obtained by different extraction methods (SOX, UE and SFE), was evaluated by two scavenging activity procedures, DPPH and ABTS methods. The results were compared with the antioxidant activity from the synthetic compound BHT (butylated hydroxytoluene).

2.4.1. Free radical scavenging activity by DPPH assay

The free radical scavenging of pink pepper extracts was evaluated using 1,1-diphenyl-2-picrylhydrazil (DPPH) as described by Mensor et al. (2001). 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 DPPH solutions. After 30 min at room temperature, the absorbance values were measured at 517 nm and converted into percentage of antioxidant activity (% AA). The final DPPH results were also represented 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 DPPH. Thus, the lower its value the better is the antioxidant activity. 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.

2.4.2. $ABTS^{+}$ radical scavenging assay

The ABTS•+ [2,2-azino-bis-(3-ethylbenzotiazoline-6-sulfonic acid)] radical scavenging assay was performed for the pink pepper extracts according to the procedure described by Re et al. (1999). The synthetic vitamin E (Trolox) (6-hydroxy-2,5,7,8 − tetramethylchroman-2-carboxylic acid, Sigma-Aldrich Co, St. Louis, USA), was used as antioxidant reference. The ABTS was dissolved in water to a concentration of 7.0 mMol, and submitted to reaction with 2.45 mMol potassium persulfate for the formation of the radical ABTS•+. The ABTS solution was diluted with ethanol to absorbance of 0.70 ± 0.05 at 734 nm. Solutions were prepared with 20 μL of the extract at concentrations of 100, 250, 500 and 1000 mg/mL, in triplicate. Then 980 μL of ABTS•+ solution was added to the test tubes and after 6 min the absorbance was measured at spectrophotometer (Femto, 800XI, Brazil). In order to find TEAC values, a concentration response curve for standard Trolox solutions was prepared. The results were expressed in Trolox Equivalent Antioxidant Capacity (TEAC) per gram of extract (MTEAC/g) ± standard deviation.

2.5. Chemical profile of volatile compounds

The identification and relative quantification of the volatiles compounds present in the pink pepper extracts were performed by a gas chromatograph equipped with a mass spectrophotometer (GC/MS, model 7890 A, mass detector 5975C, Agilent Technologies, USA), attached to a HP-5MS column (30 m × 0.25 mm internal diameter x 0.25 μm film thickness, Agilent Technologies, USA). The carrier gas was helium with flow rate of 1 mL/min, split ratio of 1:50, injector temperature of 250 °C and Thermal Aux 2 (MSD Transfer Line) 250 °C, while column temperature programmed from 70 to 280 °C at a rate of 3 °C/min. The main components of pink peppers extracts were identified by comparing the mass spectra and the retention time with *NIST 11 Mass Spectral Library* available on equipment.

2.6. Microencapsulation by emulsification and solvent extraction technique

The microencapsulation of pink pepper extract in PLA (polylactic acid) was based on the methodology described by Sah (1997) and it is basically divided in two steps: first the formation of a primary emulsion and then the extraction of the organic solvent. In the primary emulsion, the organic phase consists of polymer PLA, ethyl acetate and pink pepper extract obtained by SFE at 300 bar and 60 °C, according to the methodology described in Section 2.2.3. The SFE extract was selected by combining high extraction yield and antioxidant activity. The aqueous phase was 1% polyvinyl alcohol solution, saturated with ethyl acetate, in order to prevent rapid solidification of the microparticles at the time of formation of the primary emulsion. The organic phase is then transferred to the aqueous phase and stirred at 600 rpm for 2 min to form an oil in water emulsion. Then, the emulsion was quickly transferred to a vessel with distilled water (250 mL), maintaining the agitation at 150 rpm for 45 s.

The experiments were performed using two formulations. In the first, named Formulation 1, was added 10 mL of ethyl acetate, and in the second formulation (Formulation 2) 15 mL of solvent were employed. The quantities of pink pepper extract and PLA were kept constant in both formulations, being 50 or 100 mg and 700 mg respectively. The recovery of microparticles was carried out in a filter with pore size 63 μm, followed by extensive washing with distilled water to remove the remaining solvent and to avoid the particles agglomeration on the filter surface. The drying takes place at room temperature, about 20 °C for 24 h, after which the particles are recovered and stored in a closed flask.

2.6.1. Particles morphology and size

Encapsulated particles were analyzed by a scanning electron microscope (SEM, JEOL, model JSM-6390LV) to determine particle morphology and shape. The average particle diameter and size distribution of the microparticles was evaluated by laser diffraction (Mastersizer, Malvern Instruments, Royaume-Uni), with a monochromatic red light from a helium-neon laser. This technique relies on the fact that diffraction angle of the incident laser beam is inversely proportional to the size of the particles to be measured. Thus, this becomes an indirect measure, which depends on the optical properties of the material to be studied (Zeng, 2011). The measurement range of equipment is from 0.5 to 880 μm.

The results were expressed as the volume average diameter ($D_{4,3}$), which corresponds to the average diameter of a sphere with the same volume of analyzed microparticles, and size distribution, called *Span*, which is the size distribution of the microparticles relative to the mean diameter, represented by Equations (1) and (2), respectively.

$$D_{4,3} = \frac{\sum n_i D_i^4}{\sum n_i D_i^3} \tag{1}$$

$$Span = \frac{D_{0.9} - D_{0.1}}{D_{0.5}} \tag{2}$$

where $D_{0.9}$, $D_{0.1}$ and $D_{0.5}$ represents the diameter of 90%, 10% and 50% of the analyzed particles, respectively.

2.6.2. Encapsulation efficiency

The encapsulation efficiency of the pink pepper extract in PLA was carried out according to the method described by Boschetto et al. (2014). The extract encapsulated mass was determined by comparing the results with a pattern curve of absorbance versus

extract concentration. The encapsulation efficiency was expressed as the percentage of encapsulated extract related to the initial amount used in each formulation, according to Equation (3):

$$EE\% = \frac{m_{encapsulated}}{m_{initial}} \quad (3)$$

where $m_{encapsulated}$ correspond to mass of extract encapsulated and $m_{initial}$ is a mass of extract used in the encapsulation process.

2.7. Statistical analysis

The extraction yield (X_0), the TPC values and the antioxidant activity results were evaluated statistically by software SAS for Windows *version* 6.0. All these analyses were carried out in triplicate and the results were expressed as means ± standard deviation (SD). Statistical significance was determined at the $p < 0.05$ level.

3. Results and discussion

3.1. Extraction yield (X_0)

The extraction yield indicates the process efficiency, and it is defined as the amount of solute extractable by the solvent at the established extraction conditions. The yield results obtained for the different extraction methods and solvents (SOX, UE and SFE with CO_2) for pink pepper are presented in Table 1.

The extractions carried out by SOX and by UE with ethanol provided the highest yields (44 ± 1% and 21 ± 2%), respectively. The good performance of the ethanol suggest the presence of components with medium to high polarity, like phenolic acids, at pink pepper fruits (*Schinus terebinthifolius*), due to the polarity of the solvent. The lowest yields were found for hexane and ethyl acetate as solvents, for UE method (8.7 ± 0.4% and 13.1 ± 0.1%), respectively. Also, it is possible to observe a yield increase trend due to the increase in solvent polarity, suggesting that compounds present in plant matrix have intermediate to high polarity (Markom et al., 2007).

The SOX yield results with hexane and with ethanol (14.1 ± 0.2% and 44 ± 1%, respectively) are similar from the literature (10.6 ± 0.4% and 48.6 ± 1.9% by hexane and ethanol). These differences can be explained by the variation in place of cultivation, degree of ripeness, variety of the plant, among others (Bertoldi, 2006).

Comparing yield results obtained by LPE methods, using the same solvents, the SOX could be considered more advantageous than UE method. The operating temperature, the recycle solvent and the interactions between solvent and plant matrix, characteristic of Soxhlet extraction, may contribute to increase the solubility of different components, raising the yield (Andrade et al., 2012; Benelli et al., 2010; Mezzomo et al., 2009).

The SFE results from Table 1 indicate the maximum yield value of 5.9 ± 0.3% obtained at 60 °C/300 bar, with solvent specific mass of 830 kg CO_2/m^3, while the lowest yield was 2.6 ± 0.2% at 60 °C/150 bar, with solvent specific mass of 606 kg CO_2/m^3.

The pressure increase at constant temperature enhances the extraction yield, due to the increase in the CO_2 specific mass and consequently in the solvent power. However, the temperature effect is more complex and affected by two simultaneous mechanisms. The increase in temperature rises the solute vapor pressure, which favors its extraction. On the other hand, the increase in temperature reduces the solubility due to decrease in solvent density. At 150 bar, raising temperature from 50 °C to 60 °C the yield reduces from 4.6 ± 0.3% to 2.6 ± 0.2%, due to the effect of solvent specific mass (from 701 kg CO_2/m^3 to 606 kg CO_2/m^3). The vapor pressure effect can be observed at 300 bar, when the increase in temperature also promotes the increase in yield, while the solvent specific mass decreases (Campos et al., 2005; Mezzomo et al., 2013).

Comparing the yields obtained by supercritical CO_2 with the results obtained by conventional extraction (Soxhlet and ultrasound-assisted extraction), it is observed that the extractions using ethanol as solvent produced yield values superior to those achieved by SFE. These results can be explained by the extraction of more polar compounds, not soluble in CO_2, a nonpolar solvent. Furthermore, not only the solubilities of certain components increase with the use of ethanol, but also the number of components solubilized by this solvent, which reduces the process selectivity and increases the yield (Pereira and Meireles, 2010).

Pink pepper (*Schinus terebinthofolius* R.) is an important native tree with recognized therapeutic application and many biological activities and, to the best of our knowledge, no studies related to the supercritical extraction from this plant were found in the literature, showing the novelty related to this raw material and limiting the comparison of results.

Table 1
Extraction yield (X_0) of pink pepper extracts obtained by SOX, UE and SFE.

Extraction Method	Parameters or solvent		ρCO_2[(1)] (kg/m^3)	X_0 (%)[(2)]
SFE-CO_2	40 °C	150 bar	781	3.7 ± 0.4^{d}
		200 bar	840	4.1 ± 0.5^{cd}
		300 bar	911	3.6 ± 0.1^{d}
	50 °C	150 bar	701	4.6 ± 0.3^{bc}
		200 bar	785	4.2 ± 0.4^{cd}
		300 bar	871	5.1 ± 0.2^{ab}
	60 °C	150 bar	606	2.6 ± 0.2^{e}
		200 bar	724	4.2 ± 0.2^{cd}
		300 bar	830	5.9 ± 0.3^{a}
LPE[(3)]	Solvents		SPI[(4)]	X_0 (%)[(2)]
SOX	Hx		0	14.1 ± 0.2^{C}
	EtOAc		4.3	15 ± 1^{C}
	EtOH		5.2	44 ± 1^{A}
UE	Hx		0	8.7 ± 0.4^{D}
	EtOAc		4.3	13.1 ± 0.1^{C}
	EtOH		5.2	21 ± 2^{B}

[(1)] CO_2 density (Angus et al., 1976).
[(2)] Same letters indicated no significant difference at level of 5% ($p < 0.05$).
[(3)] Low pressure extraction.
[(4)] Solvent polarity index.
Hx, hexane; EtOAc, ethyl acetate; EtOH, ethanol.

3.2. Total phenolic content (TPC) and antioxidant activity

The TPC results and the antioxidant activity (AA) values, determined by DPPH and ABTS methods, for all extract samples are presented in Table 2. Both radical scavenging methods are widely used for determination of the antioxidant activity of foodstuff due to the simple, rapid and reproducible procedures. The results are compared to the values presented by the synthetic product BHT, as standard sample.

The results for TPC and AA provided by the SFE samples show no variation with changes in solvent density (pressure and/or temperature), and low values compared to that found from BHT, the commercial antioxidant. In general, the oily extracts obtained by nonpolar solvents such as CO_2 presented low antioxidant activity and TPC values, mostly due to the polarity of these compounds (Mazzutti et al., 2017). Same behavior was observed by do Prado et al. (2014) in the extractions of pecan nut oil. Although no trends were detected among the TPC values, the highest data was obtained at 300 bar and 40 °C (16 ± 1 mg GAE/g). Oliveira et al. (2016) found a similar result

Table 2
TPC and antioxidant activity of pink pepper extracts.

Extraction Methods	Process Parameters	TPC[1,5](mgGAE/g $_{extract}$)	%AA[2,5]	EC_{50} [3](μg/mL)	TEAC[4](μMTEAC/g)	%inhibition[4,5]
UE	Hx	39 ± 2^{c}	18^{f}	>1000	75 ± 10	7 ± 1^{c}
	EtOAc	16 ± 2^{d}	7^{h}	>1000	341 ± 15	26 ± 1^{b}
	EtOH	14.2 ± 0.3^{de}	66^{d}	339	297 ± 5	23.2 ± 0.4^{de}
SOX	Hx	ND	6^{l}	>1000	51 ± 7	5.3 ± 0.5^{cd}
	EtOAc	65 ± 1^{a}	23^{e}	>1000	168 ± 18	14 ± 1^{b}
	EtOH	60 ± 1^{b}	94^{a}	112	403 ± 41	31 ± 3^{cde}
SFE	150 bar/40 °C	4.1 ± 0.6^{g}	2.5^{j}	>1000	60 ± 5	6 ± 1^{de}
	150 bar/50 °C	13 ± 1^{e}	ND	>1000	106 ± 9	9.3 ± 0.6^{c}
	150 bar/60 °C	2.9 ± 0.4^{h}	1.3^{k}	>1000	342 ± 38	26 ± 3^{de}
	200 bar/40 °C	9 ± 1^{f}	10^{g}	>1000	75 ± 4	7.1 ± 0.3^{cde}
	200 bar/50 °C	13 ± 1^{e}	ND	>1000	70 ± 9	6.6 ± 0.6^{c}
	200 bar/60 °C	14.4 ± 0.4^{de}	1.3^{k}	>1000	100 ± 6	9 ± 1^{e}
	300 bar/40 °C	16 ± 1^{d}	1.4^{k}	>1000	98 ± 42	9 ± 3^{c}
	300 bar/50 °C	15 ± 1^{de}	10^{g}	>1000	82 ± 8	7.6 ± 0.6^{c}
	300 bar/60 °C	4.1 ± 0.5^{g}	60^{c}	188	87 ± 6	7.9 ± 0.5^{de}
BHT[6]	–	268 ± 13	–	44	–	93.1 ± 0.2

[1]Total phenolic content.
[2]Antioxidant activity evaluated by free radical scavenging activity (DPPH).
[3]Effective concentration at 50%.
[4]Antioxidant activity evaluated by ABTS method.
[5]Same letters at the same column indicated no significant difference at level of 5% ($p < 0.05$).
[6]Benelli et al. (2010).
Hx, hexane; EtOAc, ethyl acetate; EtOH, ethanol. UE, ultrasound extraction; SOX, soxhlet; SFE, supercritical fluid extraction.

for SFE of passion fruit seed cake at the same conditions of pressure and temperature (13.3 ± 0.6 mg GAE/g).

For the LPE methods, the best TPC results were obtained with ethyl acetate and ethanol as solvents, for the Soxhlet method, reaching 65 ± 1 mg GAE/g and 60 ± 1 mg GAE/g, respectively. Uliana et al. (2016) reported TPC values of 69.66 ± 3.3 mg GAE/g for the essential oil of *S. terebinthifolius* leaves obtained by hydrodistillation. Considering the polar characteristic of the solvents used (water, ethanol and ethyl acetate) in these cited works and whereas different parts of the plant were evaluated, we can observe a similarity between the results obtained.

Although the phenolic compounds are the main responsible for the antioxidant activity of natural products, the determination of this class of compounds by the Folin-Ciocalteu method, expressed in terms of gallic acid content, not characterized fully the antioxidant activity, representing only a good estimate of this property (Roginsky and Lissi, 2005).

The pink pepper extracts that showed the higher values of antioxidant activity from the DPPH assay were obtained using ethanol as solvent for Soxhlet (94%), SFE 300 bar/60 °C (60%) and ultrasound (66%) methods, respectively. According to de Campos et al. (2008), EC_{50} values below 250 mg/mL characterize a product with high antioxidant potential, then, the EC_{50} value (112 mg/mL) of the SOX-EtOH extract represents a strong antioxidant potential of the sample.

The extracts obtained by SFE at 150 and 200 bar, for all tested temperatures had EC_{50} values exceeding 1000 g/mL. In this case, we can assume that in these specified pressure and temperature conditions, the extracts showed no detectable antioxidant activity by DPPH method. This behavior is due to the nonpolar aspect of the CO_2 and the lipid characteristic of the SFE extracts, suggesting that the antioxidant components are mainly present in polar fractions.

Differences in the results from each antioxidant method may be explained by the variety of the components present in the pink pepper extracts, with different chemical characteristics. Each extraction method may recover different compounds that react with DPPH or ABTS radical. Considering the results from the antioxidant activity methods used in this study, the best extracts were the ones obtained by Soxhlet with ethanol, followed by ultrasound with ethanol (Salvador et al., 2016).

3.3. Chemical profile of volatile compounds

The chemical profile of the volatile fraction of the extract samples, result from the chromatographic analysis, are presented in Table 3, with the compounds identified and the relative composition (integrated composition) for the pink pepper extracts obtained by supercritical fluid extraction (SFE) and low pressure extraction (SOX and UE).

The major compounds identified in terms of area percentage relative and/or impact in different extracts were germacrene D, followed by sabinene, β and α-phellandrene.

Germacrene D belongs to a class of volatile organic hydrocarbons typically produced in a high variety of plant species, and presents antimicrobial and insecticidal properties (Adio, 2009; Flamini et al., 2005). Sabinene, a bicyclic monoterpene, is one of the chemical compounds related to the characteristic flavor of certain extracts and also exhibit antibacterial activity (Rashid et al., 2013). The sesquiterpene caryophyllene presents anticarcinogenic and anti-inflammatory activities (Zheng et al., 1992).

In the chemical composition of essential oil of *Schinus terebinthifolius* leaves, presented by Uliana et al. (2016), 32 compounds were identified, between them: δ-3-carene, the major compound, followed by caryophyllene and α-pinene. Santana et al. (2012) identified forty-nine compounds, such as germacrene D, bicyclogermacrene, β-pinene, and β-longipinene in *Schinus terebinthifolius* extracts.

A greater variety of compounds was identified in extracts obtained by SFE when compared to LPE methods. This could be attributed to the presence of a larger number of compounds with affinity with the CO_2, and which are identifiable by GC-MS analysis. For the SFE, only in the extracts obtained at 300 bar was verified the presence of α-phellandrene, suggesting that high pressures are required for recovery of this compound.

Among the compounds identified, no trends were detected considering the effects of pressure and temperature on composition of extracts obtained by SFE. No correlation was verified between the chemical profiles of the extracts analyzed and the antioxidant activity, which may suggest that the GC–MS technique was not effective to identify components with antioxidant activity from the *Schinus terebinthifolius* extracts and other methods such as

Table 3
Relative composition profile of pink pepper extracts obtained by low pressure (LPE) and supercritical fluid extractions (SFE).

t_r (min)	Identified compound	Relative area (%)														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
4.15	α-pinene	3.054	10.55	–	6.539	7.659	–	–	–	–	–	0.549	0.335	0.826	0.268	3.378
4.90	β-terpinene	–	1.357	–	1.829	–	–	–	–	–	–	–	–	–	–	1.432
5.20	β-pinene	–	0.894	–	–	0.792	–	–	–	–	–	–	–	–	–	0.990
5.56	α-phellandrene	3.483	19.04	–	4.507	12.55	–	–	–	–	–	–	–	0.672	0.207	1.415
6.05	*p*-cymene	1.191	5.702	–	1.289	–	1.836	–	–	–	–	–	–	–	–	1.401
6.18	β-thujene	–	–	–	–	–	–	0.551	–	–	–	–	–	–	–	–
6.19	sabinene	11.55	20.25	–	–	18.78	7.362	–	–	–	–	–	0.581	–	0.827	–
6.19	β-phellandrene	–	–	1.635	22.31	1.18	–	–	–	–	–	0.594	–	2.530	–	13.31
16.9	δ-elemene	–	–	–	0.738	–	–	1.157	–	–	1.197	1.132	1.299	1.100	1.087	0.718
17.4	2-carene	–	–	–	–	–	–	–	–	–	–	–	0.619	–	0.294	–
18.4	copaene	–	–	–	0.929	0.885	3.248	–	–	–	–	5.201	0.748	1.218	1.153	1.544
19.1	α-farnesene	–	–	–	–	–	–	–	–	–	1.057	–	0.684	1.081	1.113	–
20.1	caryophyllene	1.884	1.079	–	–	1.700	–	4.588	1.239	0.846	3.323	1.648	5.358	2.468	2.504	1.055
21.5	humulene	–	–	–	–	–	–	–	–	–	–	–	–	0.582	0.555	–
21.7	*cis*-beta-farnesene	1.491	1.383	–	–	1.963	–	1.342	2.517	1.769	2.177	1.883	1.920	2.369	2.278	0.969
22.4	longipinene	–	–	–	–	–	3.687	–	–	–	–	–	1.172	1.354	1.322	1.274
22.6	germacrene D	65.27	26.55	83.98	56.71	28.70	70.21	71.69	31.33	20.21	54.29	57.84	64.74	60.40	55.11	48.88
23.2	bicyclogermacrene	–	–	–	–	–	–	3.532	–	–	5.730	1.304	3.565	1.356	1.402	–
23.3	α-muurolene	–	–	–	–	–	–	–	1.089	0.777	1.033	–	0.846	0.700	0.685	–
23.9	4-*epi*-cubedol	3.761	2.327	–	1.592	3.156	–	5.210	9.114	7.876	6.978	–	4.988	3.810	4.013	2.054
24.2	δ-cadinene	3.001	1.816	2.482	1.657	2.494	6.578	3.811	4.618	–	4.302	3.310	3.546	3.450	3.060	1.907
25.4	germacrene B	–	0.772	–	0.986	1.009	–	–	8.434	1.672	3.354	1.654	0.671	1.516	1.539	1.390
26.2	γ-muurolene	2.604	–	–	–	–	–	2.951	–	–	1.812	–	–	–	1.104	–
26.2	spatulenol	–	–	–	–	–	–	–	–	8.119	–	4.601	–	–	–	1.140
28.2	γ-eudesmol	–	–	–	–	–	–	–	–	–	–	–	–	0.915	1.086	0.973
28.9	β-eudesmol	–	–	–	–	–	–	–	–	–	1.362	1.918	–	1.365	1.737	1.623
29.0	β-guaiene	–	0.866	–	–	1.415	–	–	0.869	0.861	1.667	3.365	0.861	–	–	–
29.5	eremophylene	–	–	–	–	–	–	–	–	–	–	–	–	–	0.531	0.657
73.7	β-amyrin	–	–	–	–	–	–	–	–	3.263	2.263	1.173	1.057	–	0.671	–

1: UE Hex; **2**: UE EtOAc; **3**: UE EtOH; **4**: SOX Hex; **5**: SOX EtOAc; **6**: SOX EtOH; **7**: SFE 150 bar/40 °C; **8:** SFE 150 bar/50 °C; **9**: SFE 150 bar/60 °C; **10**: SFE 200 bar/40 °C; **11**: SFE 200 bar/50 °C; **12**: SFE 200 bar/60 °C; **13**: SFE 300 bar/40 °C: **14**: SFE 300 bar/50 °C; **15**: SFE 300 bar/60 °C.
t_r: retention time.

liquid chromatography may be more adequate to quantify phenolic compounds and flavonoids from the extracts.

3.4. Microencapsulation of pink pepper oil in PLA

Table 4 presents the values of the yield of encapsulation process, the average particle diameter, *Span* and encapsulation efficiency.

In this work, the maximum process yield was 93 ± 2%. In the research developed by Sah (1997) the average yield achieved in the formation of PLGA particles by the same method was 86.3%, similar to that found in the present study.

According to Astete and Sabliov (2006) a controlled and balanced release of the encapsulated compounds occurs when the particles are uniform, spherical and small. The values of the average size of the particles of pink pepper extract in PLA and *Span* are presented in Table 4. In all cases, the *Span* value was close to 1, and, according to Mogi et al. (2000), *Span* values equals or smaller than a unit indicates a low dispersion of the microparticles diameter. The particle size depends on several factors such as solvent used and type of surfactant, the emulsion stirring speed, viscosity of the organic phase, among others. Thus, the particle size can be adjusted according needs of the desired application.

In both formulations tested (1 and 2), it is possible to observe a reduction in the encapsulation efficiency with increasing mass of extract encapsulated. According to de Paz et al. (2012), process efficiency is related to the concentration of encapsulation agent, which means, when the ratio between the concentration of polymer and the extract is high, the droplets of solute (extract) are more easily coated with polymer.

The external morphology of particles analyzed by scanning electron microscopy (SEM) is shown in Fig. 1. As can be seen in the micrographs, all particles are spherical, for both formulations, and presents a slightly porous surface.

According O'Donnell and McGinity (1997) the porosity of the particle can accelerate the release of the active principle and

Table 4
Yield process, average particle diameter, *Span* and encapsulation efficiency of particle synthesis of pink pepper oil in PLA.

Experiment	Yield (%)(1)	Average particle diameter (μm)	*Span*	Encapsulation efficiency (%)
PLA (F1)	86 ± 4^{ab}	148.6^{f}	1.28	–
50 mg extract (F1)	82.4 ± 3.5^{ab}	182.2^{b}	1.04	74 ± 2^{a}
100 mg extract (F1)	78.7 ± 5.5^{b}	233.6^{a}	0.97	54 ± 5^{b}
PLA (F2)	88 ± 3^{ab}	173.9^{d}	1.17	–
50 mg extract (F2)	88 ± 4^{ab}	177.6^{c}	1.21	67 ± 2^{a}
100 mg extract (F2)	93 ± 2^{a}	173.1^{e}	1.34	34 ± 1^{c}

F1: formulation 1–700 mg PLA; 10 mL ethyl acetate.
F2: formulation 2–700 mg PLA; 15 mL ethyl acetate.
(1)Same letters at the same column indicated no significant difference at level of 5% ($p < 0.05$).

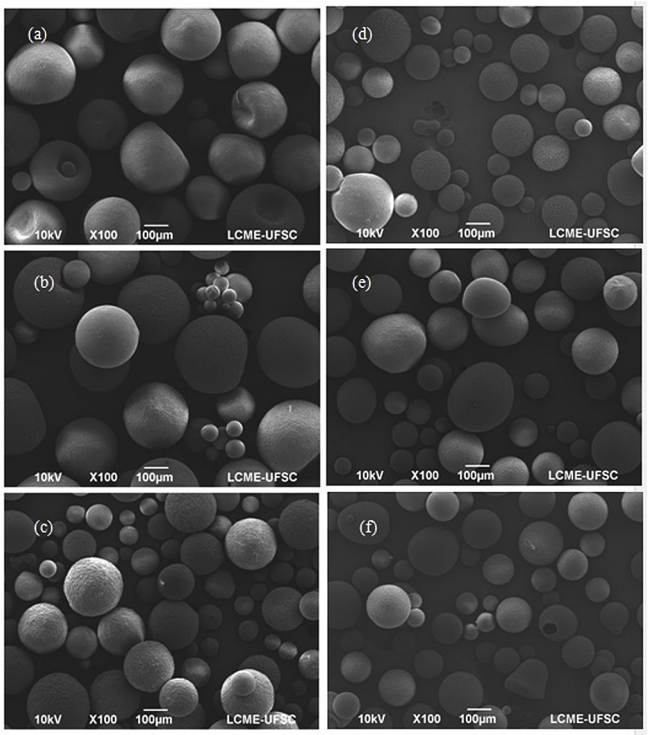

Fig. 1. Micrographs obtained by SEM of the particles formed by emulsification and solvent extraction technique. (a) PLA (700 mg) – F1; (b) PLA (700 mg) + 50 mg extract – F1; (c) PLA (700 mg) + 100 mg extract – F1; (d) PLA (700 mg) – F2; (e) PLA (700 mg) + 50 mg extract – F2; (f) PLA (700 mg) + 100 mg extract – F2.

indicates a low encapsulation efficiency, because the presence of pores may allow the migration and loss of the active compound during the encapsulation process (Martins, 2015). However, each system has its own peculiarities, which depend on the employed polymer and the material to be encapsulated. Yu et al. (2014) observed an increase in the gentamicin sulfate encapsulation efficiency with increasing porosity of the particles formed. In the present study, it is not possible to observe the presence of hollow cores inside the particles, indicating the formation of microspheres, morphology commonly found in the particles obtained by the emulsification and solvent extraction technique. This behavior may be related to the presence of PVA, the emulsifier agent, which avoided the coalescence of the emulsion droplets (Yang et al., 2001).

Until the end of this work had not been reported in the literature any study involving encapsulation of pink pepper extracts. Therefore, considering the important biological activities associated with the compounds presents in the pink pepper extracts and their antioxidant potential, we suggest the relevance of this research that aims the valorization of a natural extract with numerous therapeutic applications.

4. Conclusions

SFE showed to be an interesting method to obtain pink pepper (*Schinus terebinthifolius)* extracts, providing a good yield extraction and free-solvent extracts. Chemical profile analysis of LPE and SFE extracts presented many compounds with recognized biological activities. The encapsulation method employed in this work showed satisfactory results in terms of particle diameter and encapsulation efficiency. Most studies on *Schinus terebinthifolius* uses aerial parts of the plant, such as leaves and branches, and researches using pink pepper fruits are still scarce, highlighting the importance of this research in the raw material valorization.

Acknowledgments

The authors wish to thank CAPES (Coordination for the Improvement of Higher Level Personnel, Brazil), project number 99999.002515/2014-08, for the scholarship, and CNPq (National Council of Technological and Scientific Development, Brazil), Project number 473153/2012-2, for the financial support. The authors also acknowledge **LCME-UFSC** for the SEM analysis.

References

Adio, A.M., 2009. Germacrenes A–E and related compounds: thermal, photochemical and acid induced transannular cyclizations. Tetrahedron 65, 1533–1552. http://dx.doi.org/10.1016/j.tet.2008.11.050.

Andrade, K.S., Gonalvez, R.T., Maraschin, M., Ribeiro-Do-Valle, R.M., Martínez, J., Ferreira, S.R.S., 2012. Supercritical fluid extraction from spent coffee grounds and coffee husks: antioxidant activity and effect of operational variables on extract composition. Talanta 88, 544–552. http://dx.doi.org/10.1016/j.talanta.2011.11.031.

Angus, S., Armstrong, B., De Reuck, K.M., 1976. International Thermodynamic Tables of the Fluid State: Carbon Dioxide. Pergamon Press, Oxford.

Astete, C.E., Sabliov, C.M., 2006. Synthesis and characterization of PLGA nanoparticles. J. Biomater. Sci. Polym. Ed. 17, 247–289. http://dx.doi.org/10.1163/156856206775997322.

Azmir, J., Zaidul, I.S.M., Rahman, M.M., Sharif, K.M., Mohamed, A., Sahena, F., Jahurul, M.H.A., Ghafoor, K., Norulaini, N.A.N., Omar, A.K.M., 2013. Techniques for extraction of bioactive compounds from plant materials: a review. J. Food Eng. 117, 426–436. http://dx.doi.org/10.1016/j.jfoodeng.2013.01.014.

Bendaoud, H., Romdhane, M., Souchard, J.P., Cazaux, S., Bouajila, J., 2010. Chemical composition and anticancer and antioxidant activities of Schinus Molle L. and Schinus Terebinthifolius raddi berries essential oils. J. Food Sci. 75, 466–472. http://dx.doi.org/10.1111/j.1750-3841.2010.01711.x.

Benelli, P., Riehl, C.A.S., Smânia, A., Smânia, E.F.A., Ferreira, S.R.S., 2010. Bioactive extracts of orange (Citrus sinensis L. Osbeck) pomace obtained by SFE and low pressure techniques: mathematical modeling and extract composition. J. Supercrit. Fluids 55, 132–141. http://dx.doi.org/10.1016/j.supflu.2010.08.015.

Bertoldi, M., 2006. Atividade antioxidante in vitro da fração fenólica, das oleorresinas e do óleo essencial de pimenta rosa (Schinus terebinthifolius Raddi). Univ. Fed. Viçosa, Viçosa 96, 96f.

Boschetto, D.L., Aranha, E.M., de Souza, A.A.U., Souza, S.M.A.G.U., Ferreira, S.R.S., Priamo, W.L., Oliveira, J.V., 2014. Encapsulation of bixin in PHBV using SEDS technique and in vitro release evaluation. Ind. Crops Prod. 60, 22–29. http://dx.doi.org/10.1016/j.indcrop.2014.05.050.

Campos, L.M., A. S, Michielin, E.M.Z., Danielski, L., Ferreira, S.R.S., 2005. Experimental data and modeling the supercritical fluid extraction of marigold (Calendula officinalis) oleoresin. J. Supercrit. Fluids 34, 163–170. http://dx.doi.org/10.1016/j.supflu.2004.11.010.

Cláudio, L., Barbosa, A., Demuner, A.J., Clemente, A.D., Paula, V.F. De, Ba, J., 2007. Artigo 30, 1959–1965.

Costa Lima, S., Rodrigues, V., Garrido, J., Borges, F., Kong Thoo Lin, P., Cordeiro Da Silva, A., 2012. In vitro evaluation of bisnaphthalimidopropyl derivatives loaded into pegylated nanoparticles against Leishmania infantum protozoa. Int. J. Antimicrob. Agents 39, 424–430. http://dx.doi.org/10.1016/j.ijantimicag.2012.01.003.

de Campos, L.M.A.S., Leimann, F.V., Pedrosa, R.C., Ferreira, S.R.S., 2008. Free radical scavenging of grape pomace extracts from Cabernet sauvingnon (Vitis vinifera). Bioresour. Technol. 99, 8413–8420. http://dx.doi.org/10.1016/j.biortech.2008.02.058.

de Paz, E., Martin, Angel, Duarte, C.M.M., Cocero, M.J., 2012. Formulation of β-carotene with poly-(β-caprolactones) by PGSS process. Powder Technol. 217, 77–83. http://dx.doi.org/10.1016/j.powtec.2011.10.011.

do Prado, A.C.P., da Silva, H.S., da Silveira, S.M., Barreto, P.L.M., Vieira, C.R.W., Maraschin, M., Ferreira, S.R.S., Block, J.M., 2014. Effect of the extraction process on the phenolic compounds profile and the antioxidant and antimicrobial activity of extracts of pecan nut [Carya illinoinensis (Wangenh) C. Koch] shell. Ind. Crops Prod. 52, 552–561. http://dx.doi.org/10.1016/j.indcrop.2013.11.031.

Flamini, G., Cioni, P.L., Morelli, I., 2005. Composition of the essential oils and in vivo emission of volatiles of four Lamium species from Italy: L. purpureum, L. hybridum, L. bifidum and L. amplexicaule. Food Chem. 91, 63–68. http://dx.doi.org/10.1016/j.foodchem.2004.05.047.

Freitas, S., Merkle, H.P., Gander, B., 2005. Microencapsulation by solvent extraction/evaporation: reviewing the state of the art of microsphere preparation process technology. J. Control. Release 102, 313–332. http://dx.doi.org/10.1016/j.jconrel.2004.10.015.

Gomes, C., Moreira, R.G., Castell-Perez, E., 2011. Poly (DL-lactide-co-glycolide) (PLGA) nanoparticles with entrapped trans-cinnamaldehyde and eugenol for antimicrobial delivery applications. J. Food Sci. 76, N16–N24. http://dx.doi.org/

10.1111/j.1750-3841.2010.01985.x.
Latimer, G.W., 2012. Official Methods of Analysis of AOAC International.
Li, M., Rouaud, O., Poncelet, D., 2008. Microencapsulation by solvent evaporation: state of the art for process engineering approaches. Int. J. Pharm. 363, 26–39. http://dx.doi.org/10.1016/j.ijpharm.2008.07.018.
Lloyd, H.A., Jaouni, T.M., Evans, S.L., Morton, J.F., 1977. Terpenes of Schinus terebinthifolius. Phytochemistry 16, 1301–1302. http://dx.doi.org/10.1016/S0031-9422(00)94384-X.
Louli, V., Ragoussis, N., Magoulas, K., 2004. Recovery of phenolic antioxidants from wine industry by-products. Bioresour. Technol. 92, 201–208. http://dx.doi.org/10.1016/j.biortech.2003.06.002.
Markom, M., Hasan, M., Daud, W.R.W., Singh, H., Jahim, J.M., 2007. Extraction of hydrolysable tannins from Phyllanthus niruri Linn.: effects of solvents and extraction methods. Sep. Purif. Technol. 52, 487–496. http://dx.doi.org/10.1016/j.seppur.2006.06.003.
Martins, M.N., 2015. Aprimoramento da metodologia de encapsulamento com poliestireno visando a escala industrial.
Mazzutti, S., Riehl, C.A.S., Ibañez, E., Ferreira, S.R.S., 2017. Green-based methods to obtain bioactive extracts from Plantago major and Plantago lanceolata. J. Supercrit. Fluids 119, 211–220. http://dx.doi.org/10.1016/j.supflu.2016.09.018.
Mensor, L.L., Menezes, F.S., Leitão, G.G., Reis, A.S., Santos, T.C. dos, Coube, C.S., Leitão, S.G., 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phyther. Res. 15, 127–130. http://dx.doi.org/10.1002/ptr.687.
Mezzomo, N., Martínez, J., Ferreira, S.R.S., 2009. Supercritical fluid extraction of peach (Prunus persica) almond oil: kinetics, mathematical modeling and scale-up. J. Supercrit. Fluids 51, 10–16. http://dx.doi.org/10.1016/j.supflu.2009.07.008.
Mezzomo, N., Martínez, J., Maraschin, M., Ferreira, S.R.S., 2013. Pink shrimp (P. brasiliensis and P. paulensis) residue: supercritical fluid extraction of carotenoid fraction. J. Supercrit. Fluids 74, 22–33. http://dx.doi.org/10.1016/j.supflu.2012.11.020.
Michielin, E.M.Z., Bresciani, L.F.V., Danielski, L., Yunes, R.A., Ferreira, S.R.S., 2005. Composition profile of horsetail (Equisetum giganteum L.) oleoresin: comparing SFE and organic solvents extraction. J. Supercrit. Fluids 33, 131–138. http://dx.doi.org/10.1016/j.supflu.2004.07.004.
Michielin, E.M.Z., Salvador, A.A., Riehl, C.A.S., Smânia, A., Smânia, E.F.A., Ferreira, S.R.S., 2009. Chemical composition and antibacterial activity of Cordia verbenacea extracts obtained by different methods. Bioresour. Technol. 100, 6615–6623. http://dx.doi.org/10.1016/j.biortech.2009.07.061.
Mogi, T., Ohtake, N., Yoshida, M., Chimura, R., Kamaga, Y., Ando, S., Tsukamoto, T., Nakajima, T., Uenodan, H., Otsuka, M., Matsuda, Y., Ohshima, H., Makino, K., 2000. Sustained release of 17β-estradiol from poly (lactide-co-glycolide) microspheres in vitro and in vivo. Colloids Surfaces B Biointerfaces 17, 153–165.
Moure, A., Cruz, J.M., France, D., Dominguez, J.M., Sineiro, J., Dominguez, H., Al, E., 2001. Natural Antioxidants from residual source. Food Chem. 72, 145–171. http://dx.doi.org/10.1016/S0308-8146(00)00223-5.
O'Donnell, P.B., McGinity, J.W., 1997. Preparation of microspheres by the solvent evaporation technique. Adv. Drug Deliv. Rev. 28, 25–42. http://dx.doi.org/10.1016/S0169-409X(97)00049-5.
Oliveira, D.A., Angonese, M., Gomes, C., Ferreira, S.R.S., 2016. Valorization of passion fruit (Passiflora edulis sp.) by-products: sustainable recovery and biological activities. J. Supercrit. Fluids 111, 55–62. http://dx.doi.org/10.1016/j.supflu.2016.01.010.
Pereira, C.G., Meireles, M.A.A., 2010. Supercritical fluid extraction of bioactive compounds: fundamentals, applications and economic perspectives. Food Bioprocess Technol. 3, 340–372. http://dx.doi.org/10.1007/s11947-009-0263-2.
Rashid, S., Rather, M.A., Shah, W.A., Bhat, B.A., 2013. Chemical composition, antimicrobial, cytotoxic and antioxidant activities of the essential oil of Artemisia indica Willd. Food Chem. 138, 693–700. http://dx.doi.org/10.1016/j.foodchem.2012.10.102.
Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26, 1231–1237. http://dx.doi.org/10.1016/S0891-5849(98)00315-3.
Reverchon, E., De Marco, I., 2006. Supercritical fluid extraction and fractionation of natural matter. J. Supercrit. Fluids 38, 146–166. http://dx.doi.org/10.1016/j.supflu.2006.03.020.
Ritchie, M., 2000. QTL Mapping a User's Guide.
Roginsky, V., Lissi, E.A., 2005. Review of methods to determine chain-breaking antioxidant activity in food. Food Chem. 92, 235–254. http://dx.doi.org/10.1016/j.foodchem.2004.08.004.
Sah, H., 1997. Microencapsulation techniques using ethyl acetate as a dispersed solvent: effects of its extraction rate on the characteristics of PLGA microspheres. J. Control. Release 47, 233–245. http://dx.doi.org/10.1016/S0168-3659(97)01647-7.
Salvador, A.A., Podestá, R., Block, J.M., Ferreira, S.R.S., 2016. Increasing the value of pecan nut [Carya illinoinensis (Wangenh) C. Koch] cake by means of oil extraction and antioxidant activity evaluation. J. Supercrit. Fluids 116, 215–222. http://dx.doi.org/10.1016/j.supflu.2016.05.046.
Santana, J.S., Sartorelli, P., Guadagnin, R.C., Matsuo, A.L., Figueiredo, C.R., Soares, M.G., da Silva, A.M., Lago, J.H.G., 2012. Essential oils from Schinus terebinthifolius leaves – chemical composition and in vitro cytotoxicity evaluation. Pharm. Biol. 50, 1248–1253. http://dx.doi.org/10.3109/13880209.2012.666880.
Santos, A.C. Dos, Rossato, M., Agostini, F., Santos, P.L. Dos, Serafini, L.A., Moyna, P., Dellacassa, E., 2007. Avaliação química mensal de três exemplares de Schinus terebinthifolius Raddi. Rev. Bras. Biociências 5, 1011–1013.
Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16, 144–158.
Uliana, M.P., Fronza, M., da Silva, A.G., Vargas, T.S., de Andrade, T.U., Scherer, R., 2016. Composition and biological activity of Brazilian rose pepper (Schinus terebinthifolius Raddi) leaves. Ind. Crops Prod. 83, 235–240. http://dx.doi.org/10.1016/j.indcrop.2015.11.077.
Vinatoru, M., 2001. An overview of the ultrasonically assisted extraction of bioactive principles from herbs. Ultrason. Sonochem 8, 303–313. http://dx.doi.org/10.1016/S1350-4177(01)00071-2.
Yang, Y.-Y., Chung, T.-S., Ping Ng, N., 2001. Morphology, drug distribution, and in vitro release profiles of biodegradable polymeric microspheres containing protein fabricated by double-emulsion solvent extraction/evaporation method. Biomaterials 22, 231–241. http://dx.doi.org/10.1016/S0142-9612(00)00178-2.
Yu, M., Zhou, K., Zhang, F., Zhang, D., 2014. Porous HA microspheres as drug delivery: effects of porosity and pore structure on drug loading and in vitro release. Ceram. Int. 40, 12617–12621. http://dx.doi.org/10.1016/j.ceramint.2014.04.100.
Zeng, X., 2011. Particulate Analysis – Particle Size. http://dx.doi.org/10.1002/9780470656792.ch11.
Zetzl, C., Lozano, G.A., Brunner, G., 2007. Compilation of batch SFE-Models for natural products. In: I Iberoamerican Conference on Supercritical Fluids (PROSCIBA). Foz do Iguaçu, Paraná, Caderno de Resumos do PROSCIBA, 2007.
Zheng, G.Q., Kenney, P.M., Lam, L.K., 1992. Sesquiterpenes from clove (Eugenia caryophyllata) as potential anticarcinogenic agents. J. Nat. Prod. 55, 999–1003.