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Supercritical fluid extraction from guava (*Psidium guajava*) leaves: Global yield, composition and kinetic data

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ABSTRACT

The aim of this work was to obtain guava leaf extracts using supercritical fluid extraction. The use of ethanol and isopropyl alcohol as co-solvents was evaluated. In addition, mass transfer kinetics experiments were carried out and overall extraction curves were modeled. The global yield and extract composition from supercritical fluid extraction were compared with those from conventional extractions. The extracts were analyzed by thin layer chromatography (TLC) and gas chromatography–mass spectrometry (GC–MS). The results indicated the presence of essential oils, flavonoids and anti-oxidant compounds in all extracts. The highest global yield was observed with the Soxhlet extraction process. However, the extracts from supercritical fluid extraction had higher levels of functional compounds. Overall, the Sovová model best described the overall extraction curves.

1. Introduction

Psidium guajava L. (Mirtaceae), commonly known as the guava tree, is native to the Mesoamerican geographical area and widely distributed in tropical and sub-tropical countries. Besides the tasty and highly nutritive fruit produced by this tree, pharmacological studies on leaf extracts from *P. guajava* L. have demonstrated potentially useful biological activity.

In traditional medicine, guava leaf infusion is used for the treatment of gastrointestinal disorders and hypoglycemic disease [1,2]. More recent pharmacological studies have shown that guava leaf extract can be used for the treatment of many diseases through anti-cough, anti-microbial, anti-inflammatory and anti-oxidant effects [3–8]. Guava leaves have also been shown to have hepatoprotective and anti-nociceptive effects [9,10]. Given this array of medicinal properties, guava can be consumed as a nutraceutical or functional food. Gutiérrez et al. [11] have reviewed the traditional applications and pharmacological studies of *P. guajava* from a range of extracts, including infusions, decoctions and tinctures. Almost 20 pharmacological effects were detailed in the review in addition to the results of five clinical trials. Different functional compounds and biological activities have been attributed to different parts of the plant, including the leaves and fruit.

Natural product functional activities are attributed to different classes of compounds, such as essential oils, alkaloids, flavonoids and carotenoids. Therefore, these classes of compounds are fundamental in the formulation of diverse pharmaceutical or cosmetic products. Extracts from leaves of *P. guajava* contain mainly essential oils [12], tannins [13], triterpenoids [14,15], carotenoids and flavonoids [16,17], phenolic compounds [18] and vitamin C [19].

The amount of functional compounds recovered from raw plant materials depends on the method used for extraction. For this reason, optimization of the extraction process is essential when preparing extracts for potential pharmaceutical use. Supercritical fluid extraction (SFE) has been used as an alternative to conventional methods for extracting and/or fractionating functional compounds, mainly for use in food and pharmaceutical products. SFE is highly selective, the solvent is easily removed from the final extract and process temperatures are moderate. Carbon dioxide is the most used solvent in SFE because it is nontoxic, safe, and generally accessible at a reasonable cost. The addition of modifiers or co-solvents to CO_2 can also improve the extraction efficiency by increasing the solubility of the solute. Literature reports several studies showing the application of CO_2 plus co-solvents for the extraction bioactive compounds [20–25]. There are different possible reasons for the enhancement of the process when co-solvent is used: the solute–co-solvent interaction, the matrix swelling, and/or

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Abbreviations: CER, constant extraction rate; DCR, diffusion-controlled rate; FER, falling extraction rate; GC–MS, gas chromatography–mass spectrometry; LPSE, low pressure solvent extraction; M_{CER}, extraction rate for the CER period; X_k, fraction of hardly extractable solute; k_{XA}, mass transfer coefficient in the solid phase; k_{YA}, mass transfer coefficient in the fluid phase; t_{CER}, duration of the CER period; t_{mi}, time of the maximum extraction rate in the Martinez et al. model; OEC, overall extraction curves; SFE, supercritical fluid extraction; TLC, thin layer chromatography; Y_{CER}, mass ratio of solute in the supercritical phase.

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the increasing of the solvent polarity [26]. The type of co-solvent to be used is determined by taking into account the affinity between the co-solvent and the target compounds. Among the organic solvents, ethanol has been largely used as a co-solvent in extraction of bioactive compounds due to its low toxicity compared with other options [27–29].

Extraction of phenolic compounds and other constituents from guava seeds has previously been demonstrated using supercritical fluid extraction [18,30]. Sagrero-Nieves et al. [31] have also used supercritical CO_2 extraction to obtain volatile constituents from guava leaves. However, in this study the use of co-solvent was not evaluated and the extraction of bioactive compounds from guava leaves was not examined. Therefore, the objectives of this study were to obtain extracts from guava leaves using SFE with co-solvents, determine global yield isotherms and model overall extraction curves. In addition, the global yield of conventional techniques, including Soxhlet extraction, low pressure solvent extraction, ultrasound and hydrodistillation, were compared with the global yield of SFE.

2. Materials and methods

P. guajava leaves were collected from the Lagoa Nova region in Natal, Brazil. The leaves were dried at ambient conditions (17–20 °C) in the shade with free air circulation for a period of 25 days and subsequently packed in plastic bags before storage in a refrigerator (Cônsul, VU28A0 model – Santa Catarina, Brazil).

2.1. Characterization of the leaf particle size and extraction bed density

P. guajava leaves were triturated using a knife mill (Tecnal, Model TE-631/2- Piracicaba, Brazil). The particle size distribution was determined using a mechanical agitator (Bertel, Model NOVO 110/220 – São Paulo, Brazil) with 24, 28, 32 and 48 mesh sieves (Tyler series). The real density of the particles was measured by helium picnometry by the Analytical Facilities of the Institute of Chemistry (IQ/Unicamp). The apparent bed density was calculated using the mass of feed and volume of the extractor cell. The bed plus particles porosity was calculated as the ratio of the apparent bed density and real density.

2.2. Extraction methods

2.2.1. Supercritical fluid extraction

SFE was carried out using $8.7 \pm 0.1 \times 10^{-3}$ kg of raw leaf material using a cell column of approximately 221 mL (37.5 cm long, 2.74 cm inner diameter). The experimental runs were conducted in two steps: (i) to evaluate the use or not of co-solvent at temperature and pressure constants (45 °C, 200 bar). In this step, ethanol (Lot 17697, Ecibra) and isopropyl alcohol (Lot 16256, Ecibra) were considered as co-solvent at 5% (m/m) due to it application in other studies [20–25]; (ii) to optimize the process through factorial design. In this second step, the extraction process was then optimized through a factorial experimental design using $2^2 + 3$ central points where temperature and pressure were adjusted. The ranges for the factorial design were 35–55 °C for temperature and 100–300 bar for pressure. The choice of the limits of temperature and pressure used in the factorial design were based on literature [26]. The extractions were carried out over approximately 2 h using $3.6 \pm 0.1 \times 10^{-6}$ kg/s as the solvent flow rate.

2.2.2. Low pressure solvent extraction (LPSE)

$15.05 \pm 0.05 \times 10^{-3}$ kg of raw leaf material was suspended in ethanol (99.8% PA-ACS, Lot 070706, QEEL) and this solution was inserted in a shaker (Tecnal, TE- 422 model, São Paulo, Brazil) for 2 h. The solution was then filtered and the solvent was evaporated in a vacuum evaporator (Heidolph, Rotavapor Laborota 4000 OB – Schwabach, Germany). The extraction was carried out at 40 °C using a solid:solvent ratio of 1:10.

2.2.3. Soxhlet extraction

$10.03 \pm 0.01 \times 10^{-3}$ kg of raw leaf material was extracted in 100 mL of ethanol for 3 h. The extraction was carried out at boiling temperature of the solvent. After the extraction, the solvent was evaporated using a vacuum evaporator.

2.2.4. Ultrasound extraction

$1.002 \pm 0.001 \times 10^{-3}$ kg of raw leaf material was extracted in 10 mL of ethanol using an ultrasound apparatus (Unique, Metasom 14, São Paulo, Brazil). The extraction was carried out at 40 °C for 60 min. After that, the material was filtered through filter paper using a vacuum system. The solvent was then evaporated using a vacuum evaporator.

2.2.5. Hydrodistillation

25.0×10^{-3} kg of raw leaf material was distilled in 250 mL of water (TE-2761, Tecnal – São Paulo, Brazil) for 3 h. The process was performed at boiling temperature of the solvent. After the extraction, the volatile oil and water fractions were collected and separated using methyl chlorate (PA, Lot 0608285, Vetec).

2.3. Extract analysis

2.3.1. Thin layer chromatography

The extracts were analyzed by thin layer chromatography (TLC) using different eluents and detection solutions. TLC analyses were performed using 5 mg of extract diluted in 1 mL of ethyl acetate (PA, Lot 0804680, Vetec). Samples were applied to silica gels for separation (5 × 20 cm, Silica gel 60 com UV254, Lot 711331, Macherey-Nagel). Essential oils, flavonoids and anti-oxidants were separated using 80:20 hexane (PA, Lot K3828037 Merck; Lot 0722.03/08 CRQ):ethyl acetate (Lot K38466423, Merck) as the eluent. Essential oils were detected using an anisaldehide solution. Flavonoids were detected using a solution of NP/PEG or boric acid (PA, Lot 0805119, Vetec) and oxalic acid in ethanol (PA, Lot 0801084, Vetec). Anti-oxidants were detected using DPPH dissolved in ethanol. All plates were heated at 100 °C and spots were observed with UV 366 nm light. Carotenoids were separated using 85:20:3 hexane:ethyl acetate:formic acid (Lot 044755, Vetec) as the eluent. The plates were then directly heated for detection (without application of solution in the plates for revelation). Quercetin (Lot Q0125-10G, Sigma), hydrated quercetin (95%, Lot S43521-367, Aldrich), *trans*-caryophyllene (Lot 38H2503, Sigma), α-pinene (Lot 2437-95-8, Aldrich), β-carotene (Lot 1319424, Fluka) were used as standards.

2.3.2. Gas chromatography–mass spectrometry (GC–MS)

SFE extracts were analyzed by GC–MS (Hewlett-Packard, Model 5970) using a fused silica capillary column (30 m × 0.25 mm × 0.25 m, HP-5MS, DB5 equivalent). The carrier gas was helium (1.1 mL/min.; 99.9995% purity). The injector and detector temperatures were 200 and 300 °C, respectively. The initial column temperature was 60 °C, followed by 3 °C/min to 246 °C with a 20 min hold. The sample (10×10^{-6} kg of extract diluted in 1×10^{-6} m^3 ethyl acetate, chromatographic grade, 99.9% purity) injection volume was 1 μL (split ratio 1:20). Identification of the major compounds was based on comparison of mass spectra with a GC–MS databank (Wiley 275 and NIST05 Libraries).

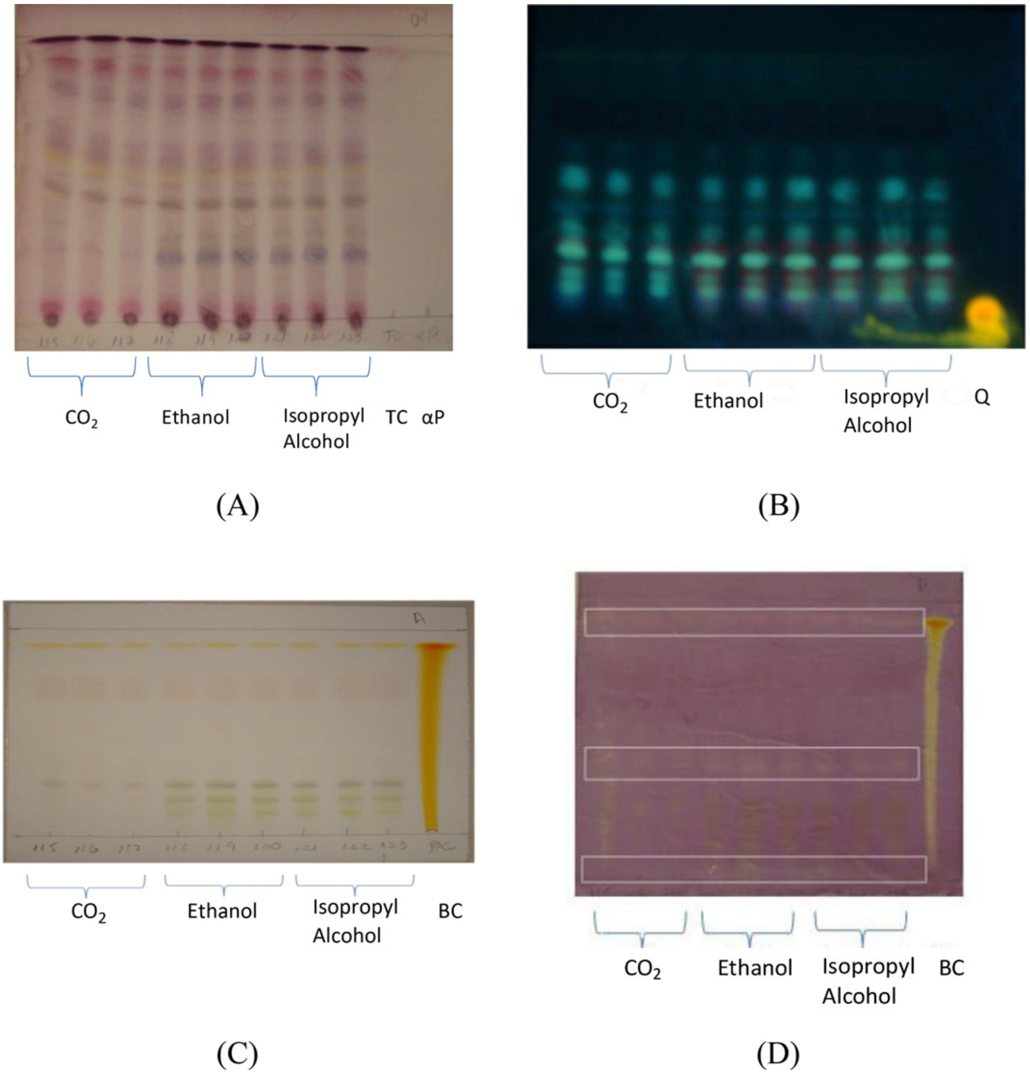

Fig. 1. TLC analysis of the guava extracts obtained by SFE at 200 bar/45 °C, using CO_2, CO_2 + ethanol (5%); or CO_2 + isopropyl alcohol (5%), using the standards: TC = *trans*-caryophyllene; αP = alfa-pinene, Q = quercetin, BC = β-carotene, for identification of: (A) essential oils, (B) flavonoids, (C) carotenoids, (D) anti-oxidant compounds.

2.4. Overall extraction curves (OEC) for SFE

Mass transfer kinetics experiments were carried out at the conditions that maximized the SFE global yield based on the results of the factorial design described in Section 2.2.1.

The experimental runs were conducted using an SFE unit with a cell column volume of approximately 221 mL and a maximum pressure of 400 bar as previously described [32]. $47.1 \pm 0.6 \times 10^{-3}$ kg of raw leaf material and a total solvent flow rate of 1.12×10^{-4} kg/s were used, as previously described [23]. The overall extraction curves (OEC) were generated by considering the mass of extract obtained as a function of the extraction duration.

2.5. Mass transfer rate at constant extraction rate (CER)

Using the experimental data, the overall extraction curves were fitted to a spline using two or three straight lines. Spline fitting was done using the procedures described by Carvalho et al. [33]. The first line was identified from the constant extraction rate (CER) period. From the spline of the extraction rate for the CER period (M_{CER}), the time corresponding to the interception of the first two lines or the duration of the CER period (t_{CER}), and the mass ratio of solute in the supercritical phase (Y_{CER}) were evaluated. Using these parameters, the fluid-phase mass transfer coefficients were calculated [34].

2.6. Mathematical modeling

The overall extraction curves were fitted to the models of Crank [35], Sovová [36] and Martinez et al. [37]. For the Crank model, the curves were fitted to the mass coefficient of diffusion (D). The fraction of hardly extractable solute (X_k), mass transfer coefficient in the solid phase (k_{XA}), and mass transfer coefficient in the fluid phase (k_{YA}) were used for the Sovová model. For the Martinez model, the Martinez parameter (b) and the time of the maximum extraction rate (t_{mi}) were fitted.

3. Results and discussion

3.1. Extraction methods

Results from the experimental SFE runs first conducted to evaluate the use of ethanol or isopropyl alcohol as co-solvents showed that the global yields of the extracts obtained with use of co-solvent

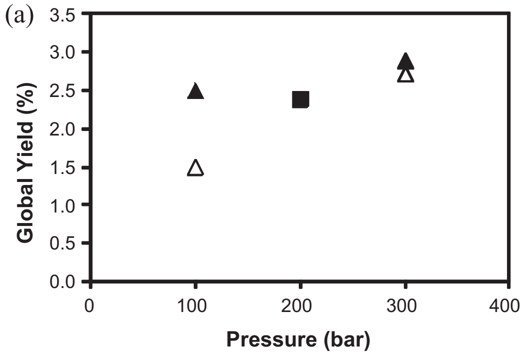

Fig. 2. Global yield of SFE at 35 °C (△), 45 °C (■) and 55 °C (▲) as a function of (a) pressure and (b) solvent density. Ethanol (5%) was used as a co-solvent.

were higher (2.4 ± 0.1%, for both) than when no co-solvent was used (1.96 ± 0.02%). In addition, the results indicated that there was no significant difference in the chemical composition of the SFE extracts obtained with co-solvent. Fig. 1 shows the TLC analysis of the extracts obtained from SFE using CO_2 and CO_2 + co-solvent (5%). According to Fig. 1, the extracts obtained with CO_2 + co-solvent present similar bands; in other words, it presented basically the same composition. Moreover, although it has not been observed the presence of compounds used as standard in some SFE extracts, as quercetin, higher number of functional compounds (essential oils, flavonoids, carotenoids, anti-oxidant compounds) were detected in the extracts obtained with supercritical CO_2 + co-solvent than that extracts obtained with only supercritical CO_2. Then, with these results, the optimization of the SFE extraction process using a factorial experimental design was carried out using ethanol (5%) as a co-solvent. Ethanol was chosen over isopropyl alcohol as it costs less and is easier to use.

Fig. 2 shows the global yield obtained for each factorial design condition as a function of pressure and solvent density. The highest global yields were obtained at 300 bar due to the high solvent density. The solubility of solutes in supercritical fluids is influenced by temperature and pressure, and is generally a function of the density of the solvent. However, the effect of temperature on solubility can be complex due to the opposing factors of solute vapor pressure and solvent density. Solute vapor pressure increases with increasing temperature resulting in increased solubility while solvent density decreases resulting in decreased solubility. At 100 bar, increasing the temperature from 35 °C ($\rho_S = 712.81$ kg/m^3) to 55 °C ($\rho_S = 325.07$ kg/m^3) increased the global yield from 1.50% to 2.49%, while at 300 bar, the same temperature increase increased the global yield from 2.72% to 2.89%. These results indicate that the solute vapor pressure effect is more pronounced than the solvent density effect.

A factorial analysis was performed in order to determine the region of optimum extraction conditions and a surface response was generated from the various temperatures and pressures examined (Fig. 3). Higher global yields were obtained at higher temperatures and pressures. Statistical analysis indicated that pressure has a significant effect on the global yield ($p < 0.05$).

The global yields of a number of conventional extraction processes were determined in order to evaluate the relative efficiency of the SFE extraction process (Table 1). The global yields of the conventional processes, with the exception of hydrodistillation, were higher than SFE. However, the global yield does not differentiate between the extraction of functional and non-functional compounds. Further characterization of the compounds present in the extracts was carried out in order to determine the most effective method for the extraction of functional compounds.

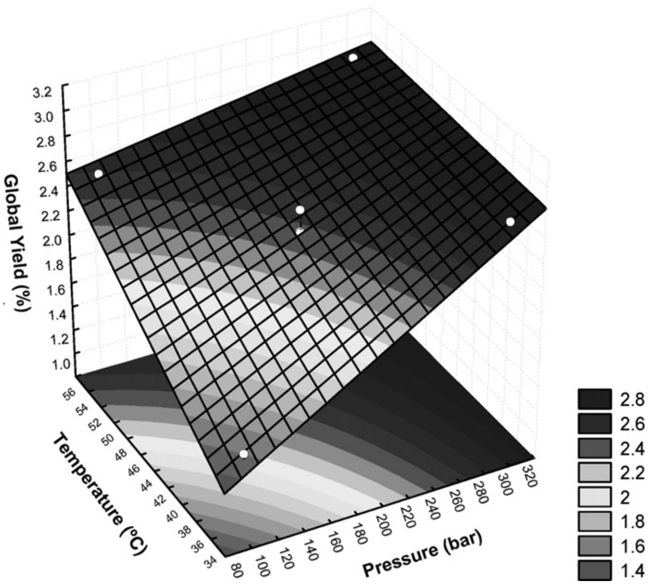

Fig. 3. Response surface for global yield of SFE extracts as a function of temperature and pressure.

3.2. Extract analysis

TLC was used to identify the presence and estimate the relative abundance of different classes of compounds in the extracts in terms of compounds detected and intensity of the band (Table 1). Essential oils were detected in extracts from all extraction methods used in this study. Flavonoids were detected in SFE and organic conventional extracts.

Anti-oxidant compounds were also detected in the TLC analysis. Interestingly, the highest level of anti-oxidants was detected in the SFE extracts, where high levels of β-carotene were measured. Bands from compounds not related to β-carotene were detected, indicating that the presence of anti-oxidant compounds in the extract could be due to a range of different bioactive compounds. Besides, the higher level of flavonoids presented in the SFE extracts can also have contributed for the higher level of anti-oxidant compounds.

Although higher global yields were obtained with conventional extraction methods, SFE resulted in better recovery of functional compounds. This is one of advantages of supercritical fluid technology. Generally, conventional processes, like Soxhlet or LPSE, needs extensive fractionation steps with organic solvents to provide an active fraction or an isolate active compound. This not needed for SFE, since the solvation power of the supercritical fluid can be easily modified by manipulating the operating pressure and temperature.

Table 1
Global yield and identification of classes of compounds present in extracts of *P. Guajava* leaves from different extraction processes.

Extraction method	Global yield (%)	Compound class			
		Essential oils	Flavonoids	Antioxidant compounds	β-Carotene
SFE, 35 °C/300 bar[a]	2.7	+++	+++	+++	++
SFE, 55 °C/300 bar[a]	2.9	+++	+++	+++	++
LPSE	12.0	+	+	+	–
Soxhlet	21.0	+	+	+	–
Ultrasound	5.9	+	+	+	–
Hydrodistillation	0.2	++	–	–	–

[a] With ethanol (5%) as co-solvent.
+/++/+++ presence/relative intensity of compound class.
– absence of compound class.

Table 2
Compounds present in *P. Guajava* leaf extracts obtained by SFE at 35 °C/300 bar, with ethanol (5%) as co-solvent as detected by GC–MS.

Retention time (min)	Compound	% Area	Quality (%)[a]
23.35	β-Caryophyllene	15.09	99
26.15	β-Selinene	21.28	99
26.53	α-Selinene	19.29	99
32.14	β-Caryophylla-4(12),8	8.59	99
32.86	Globulol	18.50	86
40.67	Trimethylcyclo dodecanodiol	6.38	AMDIS[b]
61.05	Diochyl phtalate	10.86	91

[a] The "Quality (%)" of the analysis indicates that there is an % certainty in the identification of that compound.
[b] AMDIS (Automated Mass Spectral Deconvolution Mass and Identification System) program used for mass spectrum interpretation when there was a significant difference between the mass spectrums obtained and the library spectrum.

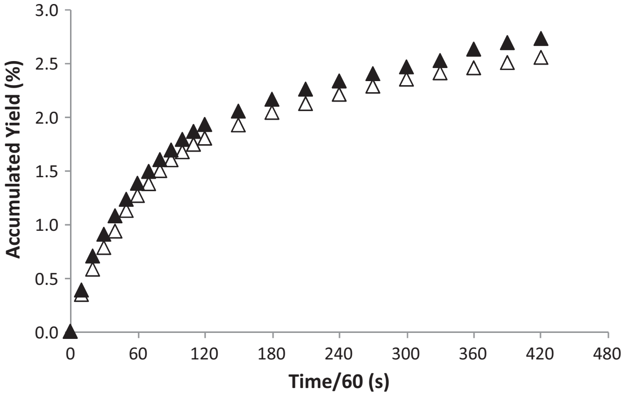

Fig. 4. Overall extraction curves for the extracts obtained with CO_2 + ethanol (5%): 35 °C/300 bar (△), 55 °C/300 bar (▲).

Table 4
Experimental conditions and parameters of the SFE extractions of *P. guajava* leaves.

Parameter	Condition	
	1	2
Temperature (°C)	35	55
Pressure (bar)	300	300
Density of CO_2 (kg/m^3)	929.11	850.22
Density of the solid phase (kg/m^3)	1480	1480
X_0 (kg solute/kg feed)	0.0280	0.0306
Flow rate of solvent (×10^4 kg/s)	1.12	1.12
Mass of feed (kg)	0.0474	0.0469
Height of the bed (m)	0.111	0.111
Diameter of the bed (m)	0.034	0.034
Bed porosity	0.035	0.035
Solubility of the solute in CO_2 (kg/kg)	0.003	0.003
Diameter of the particle (m)	0.0657	0.0657
Porosity of the particles	0.54	0.54

For all classes evaluated, SFE showed to be the best technique to obtain higher number of bioactive compounds. It has been observed that the conventional processes had reduced ability to extract functional compounds mainly because: (i) the conventional methods are not selective, with exception for hydrodistillation. Most of time, these processes need further stages of fractionation to obtain the desired compound. Consequently, lost of compounds can occur along these fractionation steps, (ii) the high temperature used in the Soxhlet method can degrade thermolabile compounds, (iii) the solvating power of supercritical fluids is higher that organic and water solvent.

In order to identify the compounds present in the SFE extracts, GC–MS analysis was performed on SFE extracts obtained at 35 °C and 300 bar with ethanol as the co-solvent (Table 2, with quantification defined in terms of % area). The major compounds detected were β-selinene (21.28%), α-selinene (19.29%) and globulol (18.50%). The types of compounds found in the SFE extract in this study are similar to those reported in the literature, but vary in the quantity of the main compounds. For example, α-selinene (23.70%), β-caryophyllene (18.77%) and δ-selinene (18.28%) were the major compounds identified by Sagrero-Nieves et al. [31] in extracts obtained by CO_2-SC at 50 °C and 137.8 bar without any co-solvent.

The compounds found in the SFE extracts are known to have bioactivities. β-selinene has anti-malarial activity [38] and globulol has anti-fungal activity [39]. α-selinene has been found in other species used for medicinal purposes, such as *Hypericum linarioides* [40], *Litsea mushaensis* and *L. linii* [41].

3.3. Overall extraction curves (OEC) for SFE

Mass transfer kinetic experiments were carried out using ethanol (5%) as the co-solvent at the conditions that maximized the global yield (35 °C, 300 bar (2.7%); 55 °C, 300 bar (2.9%)) Fig. 4 shows the overall extraction curves at these conditions.

Table 3
OEC kinetic parameters for SFE of *P. guajava* leaves using CO_2 with ethanol (5%) as co-solvent.

T (°C)	P (bar)	Solvent flow ×10^4 (kg/s)	t_{CER}/60 (s)	M_{CER} ×10^7 (kg/s)	Y_{CER} ×10^3 (kg/kg)	X_{CER} (%)	X_{final} (%)
35	300	1.12	36.19	1.91	1.71	0.87	2.55
55	300	1.12	34.84	2.22	1.99	0.99	2.75

Table 5
Model parameters for extraction of *P. guajava* leaves by SFE using CO_2 and ethanol (5%) as co-solvent.

T (°C)	P (bar)	Model								
		Crank (7)		Sovová (8)				Martinez et al. (9)		
		D ($\times 10^{12}$ m^2/s)	SSD[a] ($\times 10^8$)	X_k ($\times 10^2$)	k_{ya} ($\times 10^3$)	k_{xa} ($\times 10^5$)	SSD[a] ($\times 10^8$)	b ($\times 10^4$)	t_{mi} ($\times 10^{-3}$ min)	SSD[a] ($\times 10^8$)
35	300	0.77	5.53	1.51	0.99	4.39	1.21	1.38	−1.60	12.86
55	300	0.75	4.00	1.57	1.26	4.18	1.87	1.36	−1.60	20.41

[a] Sum of squares deviation.

In the extraction process, the modeling is an important step for optimize and development of new procedures. The overall extraction curve of SFE is not a linear function of time. In general, a typical extraction curve can be divided in three regions: (i) constant extraction rate, where the convection in the fluid phase is the main mass transfer mechanism; (ii) falling extraction rate (FER), where both diffusion into vegetal particle and convection in the fluid phase are important to the mass transfer; and (iii) diffusion-controlled rate (DCR), where the mass transfer is limited by the diffusion inside of the particles. Generally, from 50% to 90% of the total extractable material is obtained at the CER step, thus in order to reduce the costs of the process, the process optimization should be focused on this region. In this work, the kinetic behavior was similar for both conditions. The OECs were typical with CER, FER and DCR period. The OEC kinetic parameters are given in Table 3. A comparison of the OEC curves at the two conditions over 34–36 min (t_{CER}) shows that the process at 55 °C/300 bar entered the FER period at 34.8 min, while the process at 35 °C/300 bar was still in the CER period until 36.2 min. Although, the process at 35 °C/300 bar had remained for longer in the CER period (t_{CER} = 36.19 min), the extraction rate during the CER period at 55 °C/300 bar was higher (M_{CER} = 2.22×10^{-7} kg/s) than at 35 °C/300 bar, resulting in a higher final yield (2.75%).

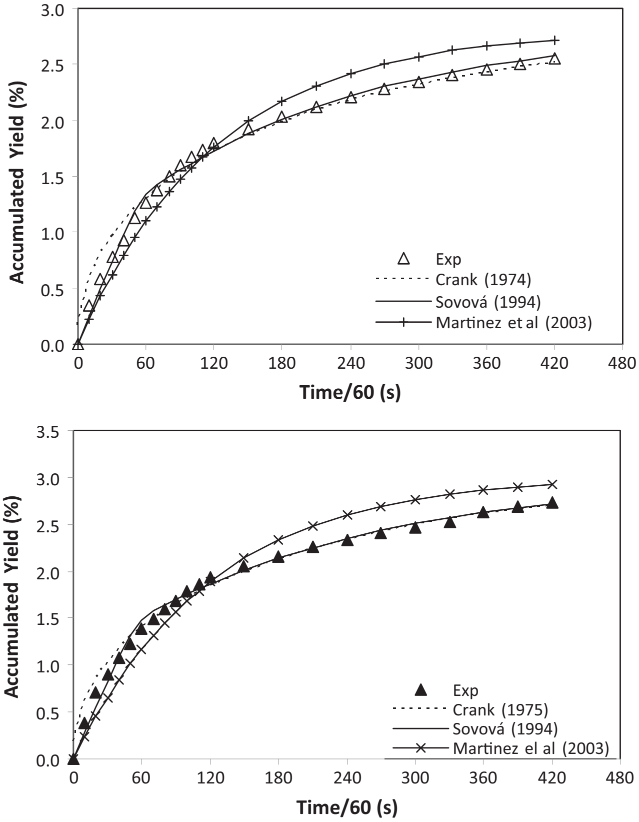

Fig. 5. Comparison between experimental and fitted OECs for SFE process using CO_2 + ethanol (5%) at 35 °C/300 bar (△), 55 °C/300 bar (▲).

For process design, one of the important parameters to describe the extraction process is the mass transfer coefficient. This parameter is fundamental to the scale-up of columns operating under supercritical conditions. There are many models proposed in the literature that can be since simple empirical models to complex phenomenological models used to fit experimental data [26]. The models used in our study were chosen for a variety of reasons. The Crank model [35] was chosen for the simplicity of the model while the Sovová model [36] was selected for the physical meaning of the model parameters. The Martinez model [37] was selected for the capability of the model to describe various OEC shapes.

The OECs were modeled using the parameters given in Table 4. The initial solute mass ratio (X_0) was set at 10% above the total amount of extract for each experimental condition [34]. Fig. 5 shows a comparison of the experimental and modeled OECs and Table 5 shows the parameters fitted to the various models.

The model of Sovová [36] provided the best fit with the experimental data as shown by the sum of squares deviation (SQDs) (Table 5). The Sovová model [36] has previously been applied to model kinetic curves from the extraction of different natural products, such as chamomile, aniseed, fennel and rosemary [34,42–44]. Although the extractions were obtained from different matrices (flower, seeds and leaves), all of the extractions had similar kinetic behaviors. The Sovová model assumes that the solute extractible content can be divided into accessible solutes (from the broken solid particles) and hardly accessible solutes (from intact solid particles). Furthermore, the Sovová model also defines three phases to the SFE process. The first phase is the easy extraction of accessible solute from superficial structures, followed by a decrease in the accessible solute and then extraction of the hardly accessible solute. To model the OEC, the mass balance for each step is used, resulting in adjustment of the mass transfer coefficients for both fluid and solid phases.

Another interesting observation is that in this study, the values of t_{mi} for the Martinez model [37] are negative, indicating that the rate of extraction is always decreasing with a maximum value at the initial instant.

4. Conclusions

In this study, it were determined the global yield and chemical profile of extracts from *P. guajava* leaves obtained by SFE and conventional extraction processes. A higher global yield was observed for SFE extraction at higher temperatures and pressures, and with a co-solvent than without a co-solvent. The highest global yield was obtained with the Soxhlet extraction process. However, a greater number of functional compounds were obtained using the SFE process. Essential oils, flavonoids, carotenoids and anti-oxidant compounds were all found in SFE extracts. The OECs were typical with CER, FER and DCR periods. The values of t_{CER} and M_{CER} were determined and shown to have a direct effect on the OEC shape and final yield of each extraction. The OECs were best fit by Sovova's model for all study conditions.

Acknowledgments

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