Optimization and economic evaluation of pressurized liquid extraction of phenolic compounds from jabuticaba skins

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ABSTRACT

The optimization of the extraction of anthocyanins and other phenolic compounds from jabuticaba skins, a promising Brazilian source of these compounds, was studied using pressurized liquid extraction (PLE). An optimization study was performed using ethanol as a solvent and with extraction pressure (5–10 MPa), temperature (313–393 K) and static extraction time (3–15 min) as independent variables. The optimum PLE conditions for all response variables were estimated; however, PLE conditions resulting in the highest recovery of anthocyanins (5 MPa, 553 K and 9 min of static extraction time) were chosen for comparison with a conventional low-pressure solvent extraction (LPSE). The attributes compared were yield, content of anthocyanins and phenolic compounds and economic feasibility. Similar extraction yields were obtained by LPSE and PLE under optimized conditions; however 2.15 and 1.66-fold more anthocyanins and total phenolic compounds, respectively, were extracted using PLE, while the cost of manufacturing (COM) obtained for the PLE extract was 40-fold lower.

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1. Introduction

Anthocyanins belong to the phenolic compound class, an important class of natural pigments found in flowers, fruits and berries. Anthocyanins are useful as colorants (red and blue colors) and in human health because of their roles as antioxidants and free radical scavengers (Santos and Meireles, 2009).

Conventional analytical anthocyanin extraction methods depend on solid–liquid extraction, where organic solvents such as methanol, ethanol or acetone are normally used. However, classical extraction methods are time- and solvent-consuming and may promote anthocyanin degradation during the extraction process (Santos et al., 2010).

Elevated temperatures are reported to improve the efficiency of extraction because of enhanced diffusion rates and solubilities of analytes in solvents. Nevertheless, elevated extraction temperatures may simultaneously increase the rate of anthocyanin degradation. The conventional extraction and purification/concentration of anthocyanins is typically conducted at temperatures ranging from 20 to 50 °C (293–323 K) because temperatures >70 °C (343 K) have been shown to cause rapid anthocyanin degradation (Ju and Howard, 2003).

Because the degradation rate of anthocyanins is time- and temperature-dependent, high-temperature, short-duration extraction conditions have been used successfully to obtain an anthocyanin-rich extract (Gizir et al., 2008).

The use of a pressurized liquid extraction (PLE) technique is an attractive alternative because it allows for fast extraction and reduced solvent consumption. Sometimes referred to as pressurized solvent extraction (PSE) and accelerated solvent extraction (ASE), PLE has been successfully used for the extraction of thermolabile anthocyanins from various plants (Petersson et al., 2010).

PLE enables the rapid extraction (less than 30 min) of analytes in a closed and inert environment under high pressures [no higher than 200 bar (20 MPa)] and temperatures (25–200 °C). A major advantage of PLE over conventional solvent extraction methods conducted at atmospheric pressure is that pressurized solvents remain in a liquid state well above their boiling points, allowing for high-temperature extraction. These conditions improve analyte solubility and the kinetics of desorption from matrices (Richter et al., 1997). Hence, extraction solvents such as ethanol and water, which are inefficient in extracting anthocyanins and other phenolic compounds at low temperatures, may be much more efficient at the elevated temperatures used in PLE (Ju and Howard, 2003).

The increasing interest in the health benefits of phenolic compounds such as anthocyanins has prompted researchers to screen plants for phenolic content and antioxidant capacity. It was recently reported by our research group that jabuticaba (*Myrciaria cauliflora*) skins appear to be a promising source of antioxidant compounds, including anthocyanins (cyanidin-3-glucoside and delphinidin-3-glucoside). Jabuticaba fruit has a dark purple to almost black skin color that covers a white gelatinous flesh (Santos et al., 2010).

The low stability during extraction, formulation, purification and storage of this class of phenolic compounds has influenced all these steps, which are being studied by different researchers interested in novel forms for processing these compounds with minimum degradation. Extracts rich in anthocyanins have been extracted (Ghafoor et al., 2010; Seabra et al., 2010), formulated with carrier material (Vatai et al., 2008; Santos et al., 2011) and purified (Bleve et al., 2008) using mild operation conditions of temperature.

Besides temperature, anthocyanin extraction step is also enhanced by other different factors such as acid pH and presence of water. On the other hand, higher degradation of the extracted anthocyanins during storage may be observed (Vatai et al., 2009; Seabra et al., 2010). Thus, in this study, no acidified extraction media without any addition of water were employed.

Therefore, this research was undertaken to evaluate the PLE of anthocyanins and other phenolic compounds from jabuticaba skins and to investigate how extraction pressure, temperature and static extraction time influence extraction yield, content and degradation of anthocyanins and phenolic compounds and the economic feasibility of the PLE process with ethanol as the solvent.

2. Materials and methods

2.1. Plant material

Jabuticaba fruits (M. cauliflora) harvested from a plantation in the State of São Paulo, Brazil, were acquired from a fruit and vegetable market centre (CEASA-Campinas, Brazil). Immediately after they were acquired, the fruits were manually peeled and the jabuticaba skins were dried for a few hours at 45 °C in an oven with air circulation (Marconi, MA 035/1, Piracicaba, São Paulo, Brazil). The dried skins (66.52% moisture) were cut into approximately 5-mm cubes and stored in the dark in a domestic freezer (−10 °C) (Double Action, Metalfrio, São Paulo, Brazil) until extraction.

2.2. Extraction procedures

2.2.1. Pressurized liquid extraction (PLE)

The pressurized liquid extraction setup is shown in Fig. 1. The solvent was pumped by a HPLC pump (Thermoseparation Products, Model ConstaMetric 3200 P/F, Fremoni, USA) into the extraction cell, which was placed in an electrical heating jacket at a desired temperature, until the required pressure was obtained. All connections within the system were made using stainless steel tubes (1/16″ and 1/8″).

Dried pieces of jabuticaba skin (4.5 g) were placed in a 6.57-cm^3 extraction cell (Thar Designs, Pittsburg, USA) containing a sintered metal filter at the bottom and upper parts. The cell containing the sample was heated, filled with extraction solvent and then pressurized. The sample was placed in the heating system for 5 min to ensure that the extraction cell would be at the desired temperature (313–393 K) during the filling and pressurization procedure. After pressurization, the sample with pressurized solvent was kept statically at the desired pressure (5–10 MPa) for the desired time (3–15 min). Thereafter, the blocking and micrometric valves were carefully opened, keeping the pressure at an appropriate level for the desired flow (1.67 cm^3/min), to rinse the extraction cell with fresh extraction solvent for 12–13 min (dynamic extraction time). After PLE, the extracts were rapidly cooled to 5 °C in ice water using amber flasks to prevent anthocyanin degradation. The cell was then exhaustively purged with carbon dioxide 99.9% at a rate of 0.71 kg/h (Gama Gases Especiais Ltda., Campinas, Brazil) for 8–9 min to ensure that no residual extract solution would be left in the extraction cell. The extraction solvent was ethanol 99.5% (Ecibra, Santo Amaro, Brazil). After extraction, the solvent was evaporated using a rotary evaporator (Laborota, model 4001, Vertrieb, Germany) with vacuum control (Heidolph Instruments Gmbh, Vertrieb, Germany) and a thermostatic bath held at 40 °C. All extracts were stored (−10 °C) in the dark prior to analysis.

To determine the effects of extraction pressure, temperature and static extraction time on extraction yield, recovery of anthocyanins and phenolic compounds, 13 experiments (Table 1) were performed. All extractions were performed in duplicate. Experiments 9 and 13, the central points of the 2^3 and complementary 2^2 full factorial designs, respectively, were performed in triplicate.

2.2.2. Conventional low-pressure solvent extraction (LPSE)

Conventional solid–liquid extraction was performed in the percolation regime at room temperature (22–23 °C), with the solvent/solution pumped continuously through the biomass to increase the efficiency of intraparticle mass transfer. Ten grams of dried jabuticaba skin pieces was packed into a bed column, and 100 cm^3 of ethanol 99.5% (Ecibra, Santo Amaro, Brazil)/solution (solvent plus extract) was passed through the packed bed slowly under gravity for 2 h, making sure that the plant material to be extracted was always covered with extraction solvent (flow rate of 28.02 cm^3/min). After extraction, the solvent was evaporated and the extract was stored as described before.

2.3. Extract characterization

2.3.1. Anthocyanin content

The total monomeric anthocyanin (TMA) content was determined using the pH differential method described by Giusti and Wrolstad (2001), which relies on the structural transformation of the anthocyanin chromophore as a function of pH. A UV–Vis spectrophotometer (Hitachi, model U-3010, Tokyo, Japan) was used for spectral measurements at the maximum absorbance wavelength (approximately 512 nm) and 700 nm using distilled water as a blank. This procedure is completely described in a previous work (Santos et al., 2010).

2.3.2. Total phenolic compounds content

The total phenolic content was estimated using the Folin–Ciocalteau method for total phenolics, which is based on the colorimetric oxidation/reduction reaction of phenols (Singleton et al., 1965). This procedure is completely described in a previous work (Santos et al., 2010).

2.4. Statistical analysis

First 'Statistica' software (release 7, StatSoft, Tulsa, USA) was used to calculate the effects of the extraction conditions (pressure, temperature and static extraction time) using a 2^3 full factorial design for extraction yield, recovery of anthocyanins and phenolic compounds, employing pressure, temperature and static extraction time ranges of 5–10 MPa (50–100 bar), 313–353 K (40–80 °C) and 3–9 min, respectively (experiments 1–9 in Table 1). As the optimum extraction conditions were not achieved, a complementary 2^2 full factorial design at a constant pressure (50 bar) employing a larger temperature (80–120 °C) and static extraction time (9–15 min) ranges (experiments 10–13 in Table 1) was performed. The selected optimum extraction process conditions were estimated through three-dimensional response surface plots of the independent variables and each dependent variable. Response surface methodology (RSM) analysis was also applied to the data to predict the optimum conditions of PLE for extraction yield, anthocyanins and total phenols from jabuticaba skins.
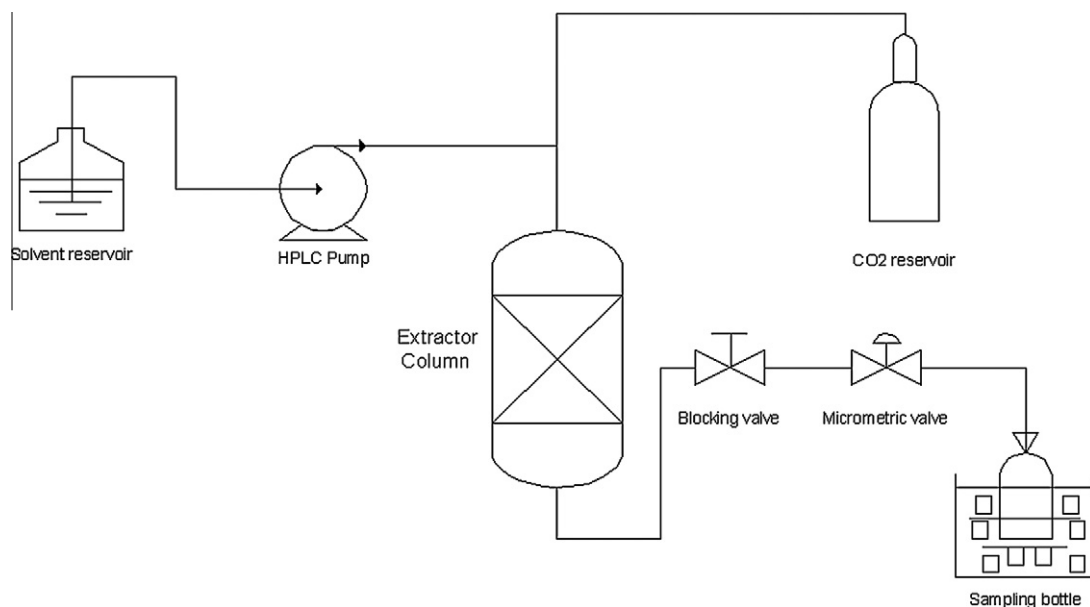
Fig. 1. Pressurized liquid extraction set-up.

Table 1
Summary of the process conditions and the main experimental results obtained in each experiment.

Experiment	Pressure (bar)	Temperature (°C)	Static extraction time (min)	Extraction yield (%)	Anthocyanins (mg Cy-3-glucoside/g dry material)	Total phenols (mg of GAEs/g dry material)
1	50	40	3	6.4 ± 0.3	0.7 ± 0.1	2.8 ± 0.3
2	100	40	3	7.3 ± 0.3	0.8 ± 0.1	3.4 ± 0.2
3	50	80	3	12.7 ± 0.4	2.1 ± 0.1	7.6 ± 0.3
4	100	80	3	13.3 ± 0.3	2.0 ± 0.2	6.3 ± 0.3
5	50	40	9	8.0 ± 0.5	0.9 ± 0.1	3.5 ± 0.3
6	100	40	9	7.1 ± 0.3	0.6 ± 0.1	2.9 ± 0.1
7	50	80	9	13.3 ± 0.4	2.5 ± 0.1	8.0 ± 0.1
8	100	80	9	12.8 ± 0.2	2.0 ± 0.3	8.1 ± 0.2
9	75	60	6	11.7 ± 0.1	1.5 ± 0.1	5.5 ± 0.1
10	50	80	15	9.3 ± 0.1	2.6 ± 0.4	11.5 ± 0.1
11	50	120	9	11.1 ± 0.1	1.9 ± 0.1	17.1 ± 0.3
12	50	120	15	11.2 ± 0.1	1.3 ± 0.1	18.7 ± 0.4
13	50	100	12	8.6 ± 0.1	2.4 ± 0.2	13.7 ± 0.5

2.5. Process simulation

To simulate the extraction processes of PLE and LPSE, SuperPro Designer 6.0® was used. This software allows for mass and energy balance estimation for all streams of the process; it also estimates purchase costs and reports stream and equipment data as well as capital and manufacturing costs.

The PLE process developed in Fig. 2 consists of a solvent storage tank (ethanol), pump, two extractors (while one of the vessels is under operation, the other goes through the cleaning and recharging processes) that operate semi-continuously, a flow controller and a distiller.

2.5.1. Economical evaluation

The cost of manufacturing (COM) was estimated for the crude extract, the phenolic compounds fraction and the anthocyanin-rich fraction of the extracts obtained by PLE and LPSE.

The main costs are similar to those described by Turton et al. (2003), which are the total capital investment cost and operating cost. The total capital investment cost represents the fixed capital investment (FCI), working capital and start-up cost. The first involves expenses related to equipment, installation, territorial taxes, engineering, etc., while the second represents the operating liquidity available to a business and finally, the start-up cost is associated with the beginning of operation and the validation of the process. The operating cost represents the costs that are directly dependent on the production rate; it consists of the cost of raw materials (CRM) and the cost of solvent lost during the process, cost of utilities (CUT), which represents the demand for steam and cooling water required for the evaporator and condenser, electricity and cost of operational labor (COL).

According to Pereira and Meireles (2007), the estimation of the COM for the phenolic compound fraction and anthocyanin-rich fraction was performed by taking into account the fact that the percentage of these fractions in the extracts can affect their specific cost.

2.5.2. Scale-up

The scale-up procedure assumed that the industrial-scale unit has the same performance as the laboratory-scale unit when the ratio between the mass of solid and solvent, the porosity of the substrate and the operating conditions are kept constant. The process was designed to run 7920 h per year, which corresponds to 330 days per year of continuous 24-h-per-day shifts.

This study considered an industrial setup of extractors with volumes of 0.05, 0.1 and 0.3 m^3. The amount of jabuticaba skins required for each industrial batch was determined for each capacity. The solvent loss was taken to be 2% of the total ethanol
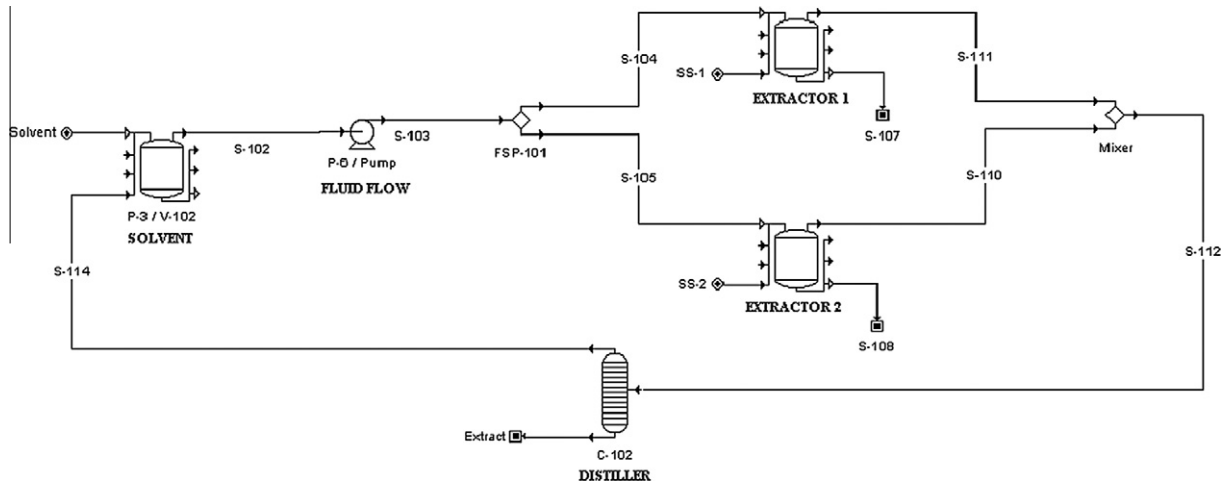
Fig. 2. Flowchart of pressurized liquid extraction developed in SuperPro Designer®.
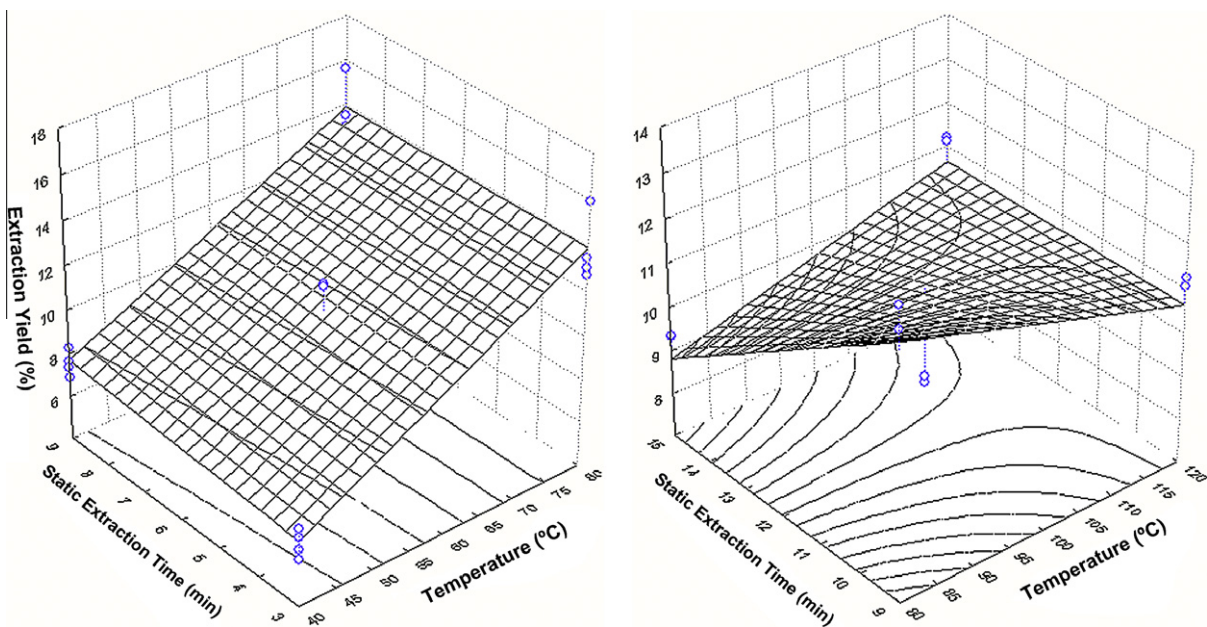

Fig. 3. Three-dimensional response surfaces of the influence of extraction temperature and static extraction time on the extraction yield.

involved in the process. The vegetable material's true density was 1450.5 kg/m³, and the specific cost of ethanol was US\$ 0.75/kg. For a better comparison with literature data, the commercialization price of jabuticaba fruit employed in this study was the same as that reported recently by Santos et al. (2010) (US\$ 1.80/kg).

3. Results and discussion

3.1. Effects of process variables on the extraction yield

The effects of extraction pressure, temperature and static extraction time on the extraction yield were evaluated. In the variable ranges of 5–10 MPa (50–100 bar), 313–353 K (40–80 °C) and 3–9 min, the extraction yield variable was significantly (95% confidence level, $p = 0.05$) affected only by the extraction temperature. On the other hand, at variable ranges of 353–393 K (80–120 °C) and 9–15 min and fixed extraction pressure of 50 bar (5 MPa), both temperature and pressure were not statistically significant (95% confidence level, $p = 0.05$) with respect to the extraction yield.

The relationship between the extraction yield, extraction temperature and static extraction time is depicted in Fig. 3 and Table 1.

An increase in extraction temperature is reported to improve the efficiency of extraction because of enhanced diffusion rate and solubility of analytes in solvents; nevertheless, high extraction temperatures may simultaneously increase the degradation rate of some extracted compounds; anthocyanins are easily degraded because of their high thermolability (Ju and Howard, 2003), thus reducing the overall extraction yield.

3.2. Effects of process variables on the recovery of anthocyanins

The effects of extraction pressure, temperature and static extraction time on the recovery of anthocyanins from jabuticaba skins were also evaluated. At a range of variables of 50–100 bar, 40–80 °C and 3–9 min, similar to the extraction yield, the extraction of anthocyanins was significantly (95% confidence level, $p = 0.05$) affected only by the extraction temperature. In the range: 50–100 bar, 40–80 °C and 3–9 min, the extraction temperature had a significant positive effect on this response variable, while at
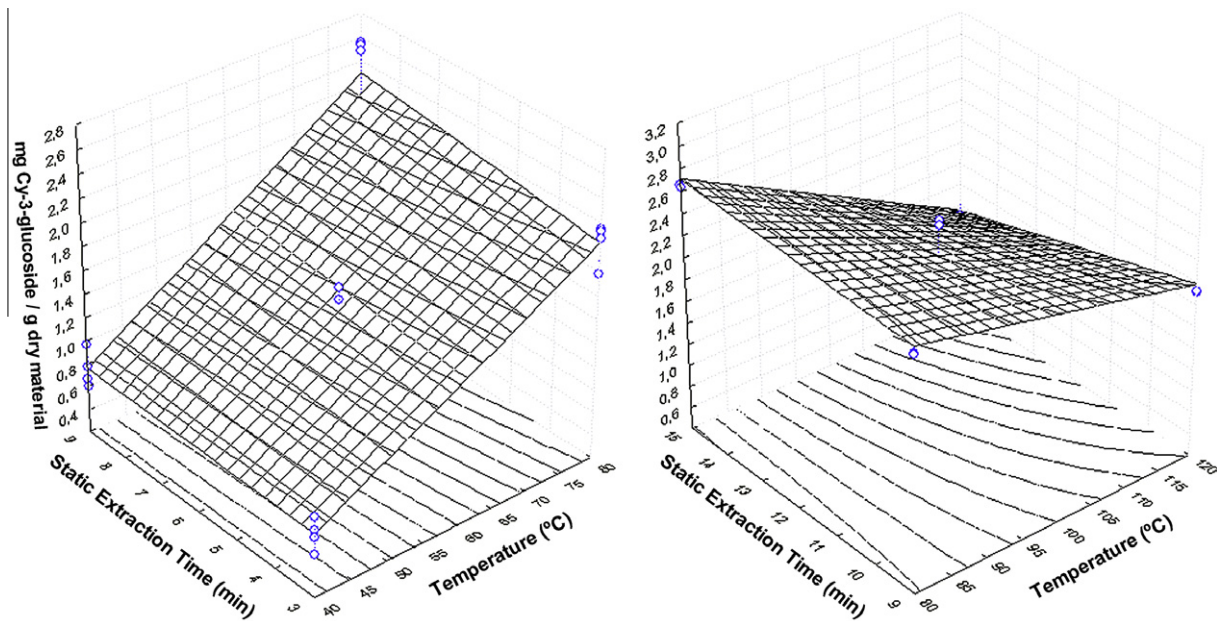
Fig. 4. Three-dimensional response surfaces of the influence of temperature and static extraction time on recovery of anthocyanins.

80–120 °C and 9–15 min (at a fixed extraction pressure of 50 bar), the extraction temperature and the interaction between the extraction temperature and static extraction time negatively influenced the extraction of anthocyanin pigments. The relationship between anthocyanin recovery, extraction temperature and static extraction time is depicted in Fig. 4 and Table 1.

Arapitsas and Turner (2008) observed similar results for the extraction of anthocyanins from red cabbage using pressurized liquid extraction (PLE). An extraction temperature and static extraction time range of 80–120 °C and 6–11 min (at a fixed pressure of 50 bar), respectively, were employed in their study because the optimal anthocyanin extraction was achieved in a short extraction time (7 min) and at medium temperature (99 °C). As the degradation rate of anthocyanins is also time-dependent, high-temperature-short-duration extraction conditions have been successfully used to obtain an anthocyanin-rich extract (Gizir et al., 2008).

Corroborating our results, Pompeu et al. (2009) have also concluded that extraction temperature should not be increased indefinitely because of the degradation of heat-sensitive phenolic compounds, such as anthocyanins, present in vegetable sources. Thus, a maximum temperature limit should be fixed and should depend on other factors, particularly time.

Usually, in studies on the recovery of anthocyanins (for LPSE as well as PLE), a compromise is sought between high temperatures and long durations, which would increase the extraction yield, and low temperatures and short durations, which would avoid the possibility of thermal degradation of anthocyanins.

Based on the achievements of Petersson et al. (2010) in studying the extraction/degradation of anthocyanins from red onion, we can elucidate what may have occurred in our study. Petersson et al. (2010) proposed that during all of the extraction process, anthocyanin degradation and extraction occur simultaneously. Thus, we suggest that in our work, after a certain static extraction time (depending on the extraction temperature this time can be very short), a maximum level of anthocyanins will be reached; thereafter, degradation effects will overcome the extraction, decreasing the anthocyanin level and consequently the overall extraction yield.

Besides temperature and static extraction time, anthocyanin degradation is also related to processing conditions including pH,

light, presence of copigments, self-association, metallic ions, enzymes, oxygen, ascorbic acid, sugar, among others (Cavalcanti et al., 2011), thus in order to evaluate the effect of temperature and static extraction time only, in this study all these other conditions were assumed be constant. Although, water was not added into the extraction media, the residual moisture after raw material drying may have affected the extraction of anthocyanins acting as a co-solvent. Future studies will be done in order to evaluate this effect.

3.3. Effects of process variables on the recovery of phenolic compounds

The effects of extraction pressure, temperature and static extraction time were also studied in the extraction of other phenolic compounds. At 50–100 bar, 40–80 °C and 3–9 min the extraction temperature, again, exerted a significant influence. Moreover, the extraction pressure, static extraction time, the interaction between the extraction pressure and static extraction time, the interaction between extraction pressure and temperature and the interaction between all three variables were also statistically significant. In the range: 50–100 bar, 40–80 °C and 3–9 min, the extraction temperature, static extraction time, interaction between extraction pressure and static extraction time and the interaction between all three variables had a significant positive effect on yield; meanwhile, the extraction pressure and the interaction between extraction pressure and temperature negatively affected the extraction of all phenolic compounds, as was the case with anthocyanin pigments. At 80–120 °C and 9–15 min (at a fixed extraction pressure of 50 bar) the extraction temperature and static extraction time increasingly influenced the recovery of total phenolic compounds positively, but the interaction between the two negatively affected this response variable. The relationship between the recovery of total phenolic compounds, extraction temperature and static extraction time is depicted in Fig. 5 and Table 1.

The results obtained in this work are consistent with current scientific literature. Herrero et al. (2010), who extracted phenolic compounds from rosemary using PLE and ethanol as solvent, also verify that higher extraction temperatures promote higher phenolic compound extraction. Four temperatures (50, 100, 150 and 200 °C) were investigated at a constant extraction time and pres-
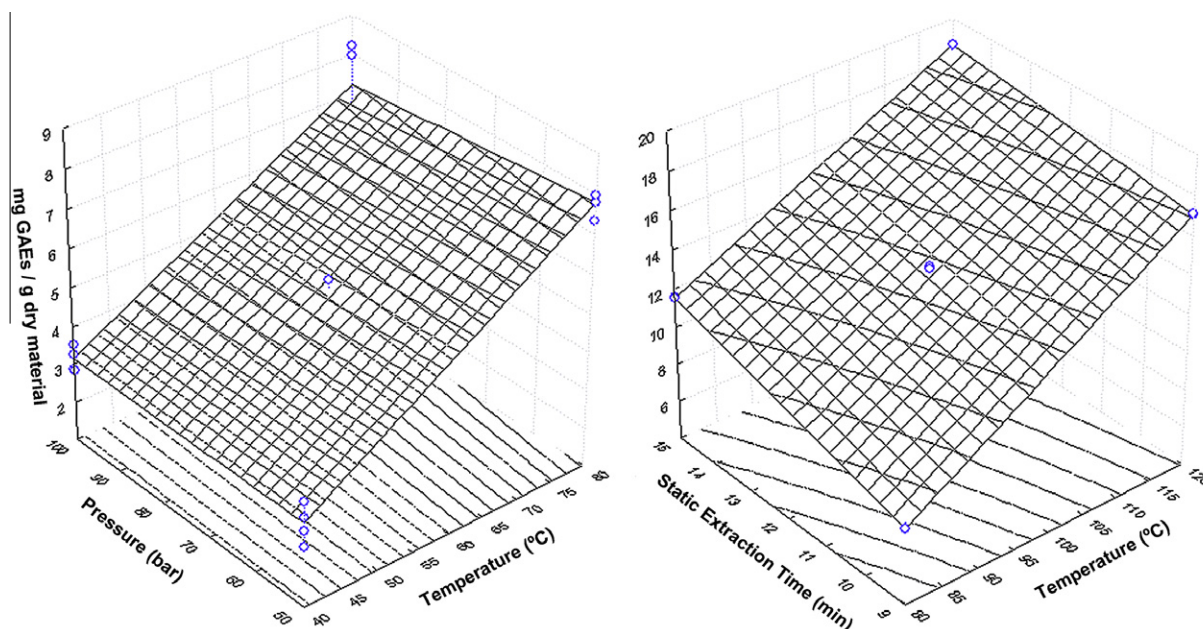
Fig. 5. Three-dimensional response surfaces of the influence of temperature and static extraction time on recovery of total phenolic compounds.

Table 2
Optimum PLE conditions for extraction yields, anthocyanins and total phenols from jabuticaba skins.

Response variables	Optimum PLE conditions		
	Pressure (bar)	Temperature (°C)	Static extraction time (min)
Extraction yield (%)	48–52	75–87	8–11
Anthocyanins (mg Cy-3-glucoside/g dry material)	48–51	78–82	8–10
Total phenols (mg of GAEs/g dry material)	48–50	118–120	14–15

sure. However, no significant difference was observed between the total phenols extracted at 150 and 200 °C. It looks like the maximum amount of phenolic compounds that can be extracted is already reached at 150 °C; increasing the temperature up to 200 °C resulted in the degradation of some of these components. Indeed, Howard and Pandjaitan (2008) found that the extraction temperature should be set near 150 °C, to effectively extract phenolic compounds from spinach leaves with the PLE method using an ethanolic solvent (mixture of ethanol and water; 70:30 v/v).

The difference in behavior between the total phenolic and anthocyanin content could be explained by a higher susceptibility of anthocyanins, a specific class of phenolic compounds, to high temperature (Cacace and Mazza, 2003). As in our work, the main objective in the study by Cacace and Mazza (2003) was to determine the PLE conditions that are most effective for the extraction of thermolabile anthocyanins; no extraction temperature higher than 120 °C was employed in order to achieve the optimal extraction temperature for total phenolic compounds, which may also be near 150 °C.

3.4. Optimization of the extraction process

The optimum PLE conditions for the extraction yields of anthocyanins and total phenols from jabuticaba skins within the experimental variable ranges employed are presented in Table 2.

Considering the results in terms of the extraction yield and anthocyanin extraction, the use of a PLE extraction pressure of 50 bar (5 MPa), temperature of 80 °C (353 K) and static extraction time of 9 min gave the optimal extraction yield (13.3%) and anthocyanin content (2.139 mg Cy-3-glucoside/g dry material). On the

other hand, under these conditions the total phenols was 7.976 mg of gallic acid equivalent (GAEs)/g dry material, 2.34-fold lower than the maximum total phenols (obtained at PLE extraction pressure of 50 bar, temperature of 120 °C and static extraction time of 15 min). These PLE conditions could be the most appropriate processing conditions to obtain a large amount of extract with a high content of anthocyanins from jabuticaba skins.

It is well known that the performance of each technique in terms of anthocyanin and other phenolic compounds extraction and yield could be effectively compared in terms of the minimization of the possible strong effects of the origin, year of production, pre-processing storage conditions and treatments of the sample (Al-Farsi et al., 2005; Dourtoglou et al., 2006; Gizir et al., 2008). Thus, the selected optimum PLE conditions were compared to conventional LPSE using the same raw material and solvent. The experimental results demonstrate that PLE procedure is much more effective in extracting anthocyanins and other phenolic compounds from jabuticaba skins (Table 3). Similar extraction yields were obtained by both extraction methods; however, 2.15- and 1.66-fold more anthocyanins and phenolic compounds, respectively, were extracted using PLE compared to conventional LPSE. In both extraction processes, the solid is stationary and the solvent flows through the bed containing jabuticaba skins; hence, for a better comparison, we preferred to use conventional LPSE. Even though the solvent/solution is pumped continuously through the biomass to increase the efficiency of intraparticle mass transfer in the LPSE process, the phenolic compounds extraction efficiency was lower than that achieved using the PLE process. This fact can be attributed to the use of combined pressure and temperature during the PLE extraction process. Several authors have

Table 3
Predicted and experimental values of responses under optimum PLE conditions (50 bar, temperature of 80 °C and static extraction time of 9 min) and experimental values responses obtained by conventional low pressure solvent extraction (LPSE).

Response variables	PLE values		LPSE values
	Predicted	Experimental (mean ± SD)	Experimental (mean ± SD)
Extraction yield (%)	13.3	13.0 ± 0.9	12.1 ± 0.8
Anthocyanins (mg Cy-3-glucoside/g dry material)	2.1	2.4 ± 0.5	1.2 ± 0.2
Total phenols (mg of GAEs/g dry material)	8.0	7.8 ± 0.4	4.8 ± 0.1

demonstrated that this combination enhances the extraction of anthocyanins and other phenolic compounds from different sources (Ju and Howard, 2003; Seabra et al., 2010; Corrales et al., 2009; Xu et al., 2010).

According to our previous studies, which featured the same extraction solvent and raw material, the recovery of anthocyanins and other phenolic compounds using other extraction methods, such as soxhlet, ultrasound-assisted and agitated solvent extraction, were lower than those obtained in this work (Santos et al., 2010). These extraction yields might be due to the adverse effects of drying (pre-processing treatment) on jabuticaba skin samples containing anthocyanins and other phenolic compounds. Gizir et al. (2008), in comparing the anthocyanin contents of dried black carrot samples, verified that the anthocyanin contents of these dried samples were lower than those of fresh samples, indicating that significant anthocyanin degradation occurred during the drying process. Because this variation was identified, Gizir et al. (2008) also focused on comparing their PLE extracts with extracts obtained by other extraction methods using the same dried carrot sample. For similar reasons, Herrero et al. (2010) adopted identical procedures in their studies.

In a previous study by our research group, anthocyanins and other phenolic compounds were extracted from fresh jabuticaba skins using the same LPSE apparatus and solvent used in this work, although the results in terms of anthocyanin and total phenolic compounds extraction were 88.2% and 58.8%, respectively, higher than those obtained here (Santos et al., 2009); this validates our hypothesis that dried jabuticaba skins present lower anthocyanin and phenolic compounds content than fresh samples.

The promising use of pressurized ethanol for the extraction of anthocyanins and phenolic compounds from jabuticaba skins offers the possibility of using a faster, more efficient and more environmentally friendly technique. In fact, the optimized PLE extraction was carried out in less than 21 min using small amounts of solvent (20 cm^3), while the other low pressure conventional extraction methods took at least 2 h and used 2.25 times more ethanol in each run. By analyzing the experimental extraction kinetics curves (Fig. 6), we can further reduce the PLE extraction time (reducing the dynamic extraction time) and consequently reduce the amount of solvent used.

Fig. 6 shows that at the beginning of the extraction procedure, the amount of anthocyanin and total phenolic compounds extracted increases with increasing extraction time, reflecting the faster solubilization of anthocyanins and other phenolic compounds in the unsaturated extraction solutions. After a period of time, the extraction was maximized to a plateau indicating that the mobility of anthocyanins and other phenolic compounds from jabuticaba skins into the extraction solution approaches values near zero at later times. From an industrial point of view, the operating time plays an important role in the manufacturing cost estimate because it is related to the number of extraction cycles that can be performed by an extraction unit (Rosa and Meireles, 2005).
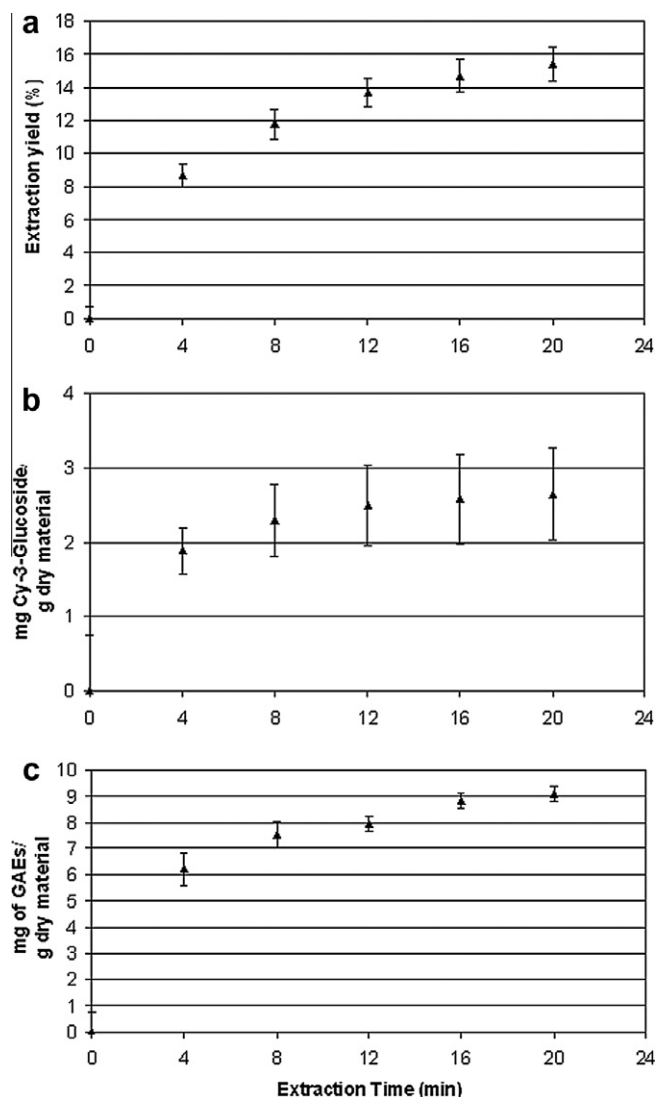

Fig. 6. Kinetics curves: (a) for the overall extraction yield; (b) for the recovery of anthocyanins; (c) for the recovery of total phenolic compounds under optimum PLE conditions (50 bar, temperature of 80 °C and 9 min of static extraction time).

4. Economic evaluation of the extraction processes

The change in the COM of PLE extracts with time, estimated by the SuperPro Designer® with 0.3 m^3 extractor for the extract, anthocyanin and phenolic compounds and fractions, is shown in Fig. 7. It is known that by increasing the extractor's volume the productivity increases, lowering the COM. Therefore, the use of extractors with higher capacities is more profitable. It can be observed that the COM for the longer times (more than 9 min), were similar with respect to yield, fraction of anthocyanins and phenolic
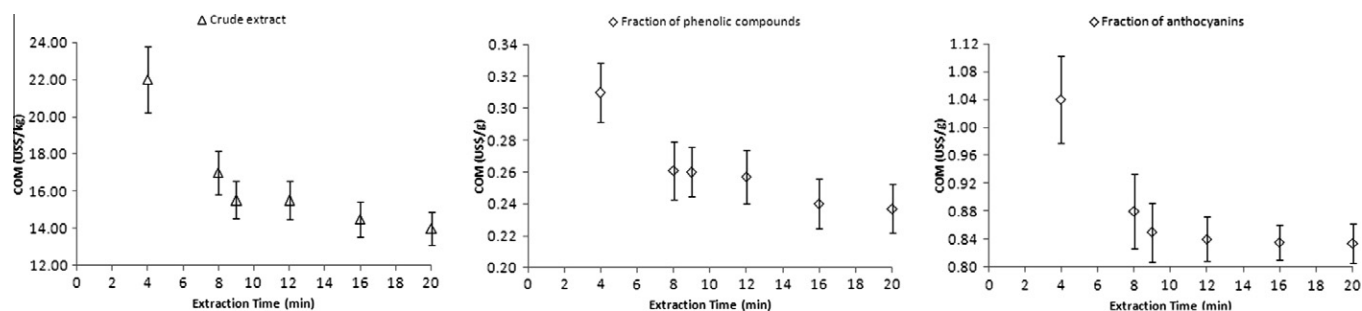
Fig. 7. COM for crude extract (△), fraction of anthocyanins (◇) and phenolic compounds (○) estimated for PLE at 9 min using 0.3-m^3 extractor.

Table 4
COM for crude extract, fraction of phenolic compounds and anthocyanins estimated for PLE using different extractor capacities at 9 min.

Extractor capacity (m^3)	Global yield (%)	COM for crude extract (US$/kg)	Content of phenolic compounds (mg/g)	COM for phenolic compounds fraction (US$/g)	Content of anthocyanins (mg/g)	COM for anthocyanin fraction (US$/g)
0.05	13.0	19.26	7.8	0.32	2.4	1.06
0.10		17.24		0.29		0.95
0.30		15.53		0.26		0.85

Table 5
Contribution of each component in COM of PLE extract's at 0.3 m^3 extractor.

Extraction time (min)	Global yield (%)	FCI (%)	COL (%)	CUT (%)	CRM (%)
4	8.7	12.0	1.5	1.5	85.0
8	11.7	14.5	3.0	1.5	81.0
9	13.0	15.3	3.3	1.4	80.0
12	13.7	17.5	4.2	1.3	77.0
16	14.7	18.9	5.3	1.3	74.5
20	15.4	20.8	6.4	1.2	71.6

compounds. Moreover, as was discussed before, at 9 min we have a higher amount of extracts with the highest contents of anthocyanins; thus, it is economically viable to stop the extraction at this time.

Table 4 shows the behavior of the COM with different extractor capacities and 9 min of extraction. As expected, a low COM was obtained for phenolic compounds because their percentage is higher as they include other classes of compounds, such as anthocyanins. When the extractor capacity was increased from 0.05 to 0.1 and 0.3 m^3, the COM for the crude extract decreased by 10% and 23%, respectively. This variation was essentially the same for the COM estimated for the anthocyanin and phenolic compound fractions.

The COM obtained for the extraction employing the PLE process for 9 min compared to that for extraction employing conventional LPSE for 2 h, as expected, is much lower. An approximately 40-fold decrease in the COM is observed for the optimized PLE process.

Santos et al. (2010) compared the COM of four different extraction methods (ultrasound-assisted extraction (UAE), agitated bed extraction (ABE), combined UAE + ABE and soxhlet extraction (acidified solvent or not)) also employing an extraction time of 2 h. They found that the COM was 30–110-fold higher than that for the optimized PLE process. The time required for the PLE process was relevant for the COM; because with the use of high pressure with moderately high temperature the extraction time was considerably reduced, leading to a low COM for PLE extracts. Thus, shorter extraction times lead to the increased productivity of the industrial equipment.

Table 5 shows the behavior of the COM with respect to the extraction time using an extractor capacity of 0.3 m^3. It can be confirmed that the extraction time is an important parameter in the process; an increase in the fixed cost of investment (FCI) and cost of labor (COL) is observed at higher times. The COL is fixed because

it depends only on the extractor capacity; thus, it increases proportionally with FCI. The FCI is directly related to the raw material expense (US$/year) and the total extracts obtained (kg/year) at the end of process. Therefore, when the extraction time is longer, fewer batches and smaller masses of extracts are obtained, leading to a higher FCI. On the other hand, the CRM increases with shorter extraction times because there are more batches, requiring a greater amount of jabuticaba. The SuperPro Designer® estimated about 55% more mass of extract produced per year employing 9 min of extraction time compared to a 20-min extraction. The variation of the CUT with time was less pronounced, probably due to the small variation required by utilities in the process, which consequently diluted the total costs.

Extracts rich in phenolic compounds from jabuticaba skins have not been commercially available until now. On the other hand, one can find glycolic extracts from jabuticaba, which cost US$ 9.90/kg (Sarfarm, Sao Paulo, Brazil). A better comparison would require information regarding the concentration of phenolic compounds and anthocyanins in glycolic extracts – data that are not available. Taking into account the fact that the compositions of both extracts are similar, the price per kilogram of jabuticaba skins in this study was considered the price of the total fruit, including pulp, skin and seed (US$ 1.8/kg of fruit); this price and, consequently, the price of the final extract could be even smaller if the skins were residues taken from the production of jabuticaba wine, jabuticaba pulp, etc., which would result in a competitive extract price.

5. Conclusions

The results presented in this research contribution show the possibility of obtaining phenolic compounds, such as anthocya-

nins, from jabuticaba skins using an environmentally clean extraction technique. The results show that the extraction yields of anthocyanin and other phenolic compounds are most affected by extraction temperature. Anthocyanin extraction is also affected by the static extraction time, specifically that higher temperatures [>80 °C (553 K)] and higher static extraction time (>9 min) cause anthocyanin degradation, and lower temperatures and static extraction times resulted in poor extraction efficiency of the pigments. Total phenolic compounds extraction is also affected by other variables and the interactions between them; nevertheless, the optimum variable conditions were not achieved using the variable ranges employed in this work. The use of PLE conditions set at 50 bar (5 MPa) and 80 °C (553 K) under a static extraction time of 9 min was selected for further economical evaluation analysis to produce the highest amount of extract with the highest content of anthocyanins. Similar extraction yields were obtained by LPSE and PLE under optimized conditions; however 2.15 and 1.66-fold more anthocyanins and total phenolic compounds, respectively, were extracted using PLE. The experimental values agree with the values predicted, thus indicating the suitability of the model employed and the success of response surface methodology (RSM) in optimizing the extraction conditions. The COM obtained for extracts employing the PLE process under optimized conditions was 40-fold lower than the COM obtained for the conventional LPSE operating for 2 h, corroborating our previously reported results from studies employing other conventional, time-consuming extraction methods. Thus, the PLE process appears to be a technically promising and economically viable technique in extracting anthocyanins and other phenolic compounds from jabuticaba skins.

Acknowledgments

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