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## Chemical and economic evaluation of natural antioxidant extracts obtained by ultrasound-assisted and agitated bed extraction from jussara pulp (*Euterpe edulis*)



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### ABSTRACT

This work aimed to evaluate the influence of ultrasonic and agitated bed extractions on the chemical composition and manufacturing costs of extracts obtained from jussara (*Euterpe edulis*) pulp. The effects of extraction time (5–180 min), temperature (25–55 °C), ethanol concentration (0–90% in acidified water) and solvent/pulp ratio (5–30 mL/g) on the extraction yield, phenolic content, anthocyanin content, antioxidant capacity and manufacturing costs were assessed. The yields provided by the ultrasound-assisted and agitated bed extractions were not significantly different. The anthocyanins and phenolic compound yields were significantly affected by the extraction time, the ethanol concentration in water and the solvent/feed ratio, but not by the temperature. In general, the antioxidant capacity of the extracts displayed tendencies similar to the anthocyanin and phenolic compound yields. The production of crude extracts obtained by ultrasound and agitated bed extraction incurred greater manufacturing costs compared to the market prices of assai extracts.

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### 1. Introduction

The current trend of preferred consumption of natural foods, coupled with numerous epidemiological studies that have reported possible deleterious effects of synthetic additives to health, has contributed to the search for new sources of natural ingredients. In this context, phenolic compounds, specifically anthocyanins, have attracted attention due to their antioxidant properties and their great potential as a natural food colorant (Tsuda et al., 2003; Melo et al., 2009). The anthocyanins, which belong to the flavonoid group, represent an attractive source of pigments that are responsible for the cyanic colours, ranging from salmon pink to through red and from violet to dark blue, that are observed in most of the flowers, fruits and leaves of angiosperms commonly found in nature (Cavalcanti et al., 2011).

The demand for natural colorants has increased by almost 35% from 2005 to 2009, and this demand is expected to keep rising. By the middle of the next decade, the global food colouring market is expected to reach 1.6 billion USD, up 10% from its present level (Prepared Foods, 2011). Grape skins remain the primary source of anthocyanins in the industrial production of natural colours, marketed as enocianina or colorant E163, due to their ability to obtain large amounts from the wine production industry at extremely low

costs. However, the use of grape pomace reveals several drawbacks, including the seasonal nature of grapes and irregularities in their quality and quantity (Melo et al., 2009). Therefore, the agri-food industry has become increasingly interested in identifying new sources of stable and economically viable anthocyanins (Melo et al., 2009).

Brazil is known worldwide for its large vegetal and animal biodiversity and has a high potential for the affordable production of various raw materials due to its vast breadth in and tradition of agricultural production. The jussara palm (*Euterpe edulis* Martius) is widely distributed throughout in the Brazilian Atlantic Forest and produces edible palm hearts and spherical fruits known as jussara. These fruits contain only one light brown seed that is covered by a thin and dry skin that is shiny and dark purple and, due to its high anthocyanin content, appears almost black in colour when ripe (Borges et al., 2011a). The two major anthocyanins in jussara fruits were identified by Brito et al. (2007) as cyanidin 3-glucoside and cyanidin 3-rutinoside, which are the same anthocyanins present in assai fruit (*Euterpe oleracea*) (Schauss et al., 2006).

As the extraction process is extremely important for the production of natural colorants, different research groups have put forth great efforts to develop efficient extraction processes (Santos et al., 2010). An efficient solid-liquid extraction (the most commonly used technology) of antioxidant compounds, predominantly anthocyanins, from plant materials should maximise target compound extraction with minimal degradation while using

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environmentally friendly technologies yield result in anthocyanin-rich products with high antioxidant capacities. In this way, ultrasonic-assisted extraction (UAE) has been used by some research groups to extract pigments such as anthocyanins (Santos et al., 2010; Corrales et al., 2008; Ghassempour et al., 2008; Chen et al., 2007), carotenoids (Sun et al., 2011) and betalains (Sivakumar et al., 2009).

Ultrasonic extraction can improve mass transfer, thereby providing more processing time and reducing solvent consumption as compared to using conventional methods (Vilkhu et al., 2008). Ultrasound promotes the solvent penetration into the product by disrupting of the cell walls via acoustical cavitation (Rastogi, 2011; Vilkhu et al., 2008).

Recently, emerging techniques such as sub and supercritical fluid extraction (Serra et al., 2010; Ghafoor et al., 2010) and microwave-assisted extraction (Li et al., 2012; Liazid et al., 2011) have been employed to extract anthocyanins from different fruits. However, relative to those techniques, the use of UAE is recognised as being more economically viable. Additionally, UAE can be easily implemented by local industries (Boonkird et al., 2008). However, we identified only a few studies that demonstrated manufacturing cost and economic feasibility of extracting anthocyanins from new sources, such as jussara.

The aims of this work were (i) to investigate the efficacy of ultrasound on antioxidant compound extraction, mainly anthocyanins, from jussara and to compare with the results from a conventional solvent extraction using an agitated bed; (ii) to evaluate the influence of extraction conditions, including the solvent composition, ratio of solvent to feed, time and temperature, on phenolic and anthocyanin yields; and (iii) to estimate the manufacturing cost of the crude extracts.

## 2. Materials and methods

### 2.1. Chemicals

Ultrapure water was supplied by Milli-Q Direct-Q 3 (Billerica, MA, USA). Methanol, ethanol and citric acid were purchased from Synth (Diadema, SP, Brazil). Standards of gallic acid; 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ); 2,2-diphenyl-1-picryl-hidrazil (DPPH); and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The Folin–Ciocalteu reagent was obtained from Dinâmica (São Paulo, SP, Brazil). All reagents were analytical grade.

### 2.2. Jussara pulp

Frozen jussara pulps were supplied by farmers from the rural communities of Ubatuba, São Paulo, Brazil (Projeto Juçara, 2012). The use of frozen pulp was necessary because these fruits are extremely perishable. The pulp, even when refrigerated, has a maximum shelf life of 12 h, which is similar to assai (Tonon et al., 2010). The completely ripe fruits were used to produce pulp and were harvested during April 2011. The frozen product was taken to the University of Campinas (Campinas, SP, Brazil), stored in a freezing chamber and thawed according to the quantity required for each trial. The composition of the jussara pulp used in the trials is displayed in Table 1.

### 2.3. Equipment

The ultrasound-assisted extraction (UAE) experiments were performed in a stainless steel ultrasonic cleaning bath (USC-2800-A model, Thorton, São Paulo, Brazil) at 40 kHz frequency and 154 W of power. The equipment consisted of a

**Table 1**

Moisture content, centesimal composition and total acidity of jussara pulp.

Analysis	Mean $\pm$ SD <sup>a</sup>	Method
Moisture (%)	86.42 $\pm$ 0.02	AOAC (2002)
Total protein (%)	1.15 $\pm$ 0.03	AOAC (2002)
Fat (%)	5.86 $\pm$ 0.35	Bligh and Dyer (1959)
Ash (%)	0.41 $\pm$ 0.01	AOAC (2002)
Total Sugar (%)	2.46 $\pm$ 0.05	By difference
Fibre (%)	3.41 $\pm$ 0.31	AOAC (2002)
Total acidity (g citric acid/100 g)	0.27 $\pm$ 0.03	AOAC (2002)

<sup>a</sup> Percentage in wet basis; All data are presented as the mean  $\pm$  standard deviation (SD) of three replicates ( $n = 3$ ).

rectangular tank (0.30  $\times$  0.24  $\times$  0.15 m) with a useful volume of 9 L and a digital panel for inputting the time and temperature settings. The agitated bed extraction (ABE) experiments were conducted in an orbital shaker (0.50  $\times$  0.60  $\times$  0.52 m) (TE-420 model, Tecnal, Piracicaba, Brazil) with 600 W of power. The stirring speed was 100 rpm.

### 2.4. Experimental protocol and extraction design

Jussara pulp was thawed and homogenised, 5 g were weighed directly into 250-mL Erlenmeyer flasks. Extraction solutions were prepared with different ethanol concentrations in distilled water and adjust to a pH of 3.0 with 0.35% citric acid (w/v). Then, different solvent volumes were added into the flasks, generating a solvent to feed ratio ranging from 5 to 30 mL/g. The flasks were sealed by Parafilm M and covered using aluminium foil to prevent solvent loss by evaporation. Next, they were immersed in the ultrasonic bath or in an orbital shaker. After extraction, the flasks were immediately cooled by immersion in an ice bath, the samples were centrifuged for 10 min at 20 °C at 10,000 rpm in an Allegra 25R Centrifuge (Beckman Coulter, Brea, CA, USA), and the supernatant was collected and filtrated through a filter paper Qualy (J Prolab, SP, Brazil), with 14- $\mu$ m-diameter pores to eliminate the remaining fat droplets and suspended solids. The extracts were stored in amber glass bottles at  $-18$  °C until the analyses were performed. All extraction experiments were performed in duplicate. A single factor experimental design was used to evaluate the whether the extraction yields of antioxidant compounds from jussara were affected by the time (5, 20, 40, 60, 80, 100, 120, 140 and 180 min), temperature (25, 35, 45 and 55 °C), ethanol concentration in water (0, 30, 50, 70 and 90% (v/v)) and solvent to feed ratio (5, 10, 15, 20, 25 and 30 mL/g (v/w)). The following fixed parameters were used for the extraction processes for all treatments: an extraction time of 20 min, a temperature of 25 °C, a solvent to feed ratio of 20 mL/g and an extraction solvent of 50% (v/v) ethanol in water. All process conditions were selected based on literature data (Sun et al., 2011; Kim and Lee, 2009; Patil et al., 2009; Cacace and Mazza, 2003).

### 2.5. Analytical methods

#### 2.5.1. Extract yield

The extraction yield (EY) was calculated as the ratio between the total extract mass and the dry feed mass of raw material loaded into the extraction tank. The extract mass was obtained by drying the samples in a vacuum oven at  $70 \pm 1$  °C until a constant weight was reached. The results were expressed as g of dry extract/100 g dry pulp. Sampling was performed in triplicate.

#### 2.5.2. Total monomeric anthocyanins

Total monomeric anthocyanins (TMA) were measured with the pH-differential method, described by Giusti and Wrolstad (2001). A

Unico SQ2800 UV–Vis spectrophotometer (Unico, New Jersey, USA) was used to measure the absorbance at the visible wavelengths of 510 nm and 700 nm. The anthocyanin content from liquid extracts was calculated as cyanidin-3-glucoside equivalents using the molar absorption ( $\epsilon$ ) of 26,900 L/mol.cm and a molecular weight of 449.2 g/mol. The results were expressed as mg of cyanidin-3-glucoside (Cyd-3-GluE)/g dry pulp. Disposable cuvettes of 1-cm-path lengths were used to obtain the measurements. This assay was performed in triplicate.

### 2.5.3. Total phenolic compounds

The phenolic compounds (TPC) were characterised by the Folin–Ciocalteu method described by Waterhouse (2001). Briefly, in a series of glass tubes, 40  $\mu$ L of extract were mixed with 3.16 mL distilled water and 200  $\mu$ L of Folin–Ciocalteu reagent. After 5 min, 600  $\mu$ L of 20% sodium carbonate solution were added to each test tube, mixed and let stand in the dark at room temperature for 120 min. Next, the samples were measured at 765 nm using a Unico SQ2800 UV–Vis spectrophotometer. Distilled water was used for the blank sample. The total phenolic compound content was expressed as mg of gallic acid equivalent (GAE)/g of dry pulp. Each sample was evaluated in triplicate.

### 2.5.4. Antioxidant capacity

**2.5.4.1. DPPH (free radical scavenging capacity) assay.** The free radical scavenging capacity of the extracts was analysed using the DPPH (2,2-diphenyl-1-picryl-hydrazil) radical according to Brand-Williams et al. (1995) with some modifications. Briefly, in the dark, 0.1 mL of the extract (diluted 1:10 with ethanol) was mixed with 3.9 mL of a DPPH ethanolic solution (60  $\mu$ mol/L). Based on preliminary results (data not shown), we required 60 min to obtain DPPH readings, which was the same amount of time employed by Rufino et al. (2010) with jussara extracts. The readings were taken at a wavelength of 515 nm. The scavenging ability (SA) of the extracts was determined by Eq. (1), as follows:

$$SA(\%) = ((Abs_c - Abs_s)/Abs_c) \times 100 \quad (1)$$

where  $Abs_c$  and  $Abs_s$  are the absorbance values of the control and sample after 60 min, respectively.

The DPPH inhibition percent values of the extracts were fitted to a standard curve of ethanolic solutions of Trolox that ranging from 50 to 400  $\mu$ mol/L. The results were expressed as  $\mu$ mol of Trolox equivalent (TE)/g dry raw material, and the samplings were performed in triplicate.

**2.5.4.2. FRAP (Ferric Reducing Antioxidant Power) assay.** The FRAP assay was performed according to Benzie and Strain (1996) with some modifications. The FRAP reagent was prepared by mixing of 300 mM acetate buffer (pH 3.6), 10 mM/L TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) solution in 40 mM/L HCl, and 20 mM/L FeCl<sub>3</sub>.6 H<sub>2</sub>O at a ratio of 10:1:1 (v/v/v). Jussara extracts (90  $\mu$ L in an appropriate water-dilution) were allowed to react with 2700  $\mu$ L of the FRAP solution and 270  $\mu$ L water for 30 min at 37 °C in the dark. The readings were taken at a wavelength of 595 nm. Trolox was used as standard for further comparisons. Aqueous solutions of known Trolox concentrations between 50 and 500  $\mu$ M/L were used for the calibration. The results were expressed as  $\mu$ mol Trolox equivalent (TE)/g dry raw material, and the samplings were performed in triplicate.

## 2.6. Economical evaluation

### 2.6.1. Process simulation and scale-up

The process, scale-up and economic simulation were performed for UAE and ABE using the software SuperPro Designer 6.0 (Intelligen Inc., Scotch Plains, NJ, USA). The reactors for both extraction

methods were considered to be similar, differing only in the required power for stirring or ultrasonic trials. We assumed that the ultrasonic transducers are bonded to the external walls of the tank (Santos et al., 2010). According to Bravi et al. (2002), at least two extraction cells must be operating intermittently to simulate a batch process in a semi-continuous mode, where one cell is active (extracting) if the other cell extraction is inactive (unloading, cleaning, refilling, etc.). The necessary number of extraction tanks can be determined according to Eq. (2), as proposed by Bravi et al. (2002).

$$n_e = 2 \times ((t_e + t_i)/t_e) \quad (2)$$

where  $n_e$  is the amount of extractor units,  $t_e$  is the active extraction time and  $t_i$  is the inactive extraction time.

This work required an estimated 10 min of inactive operation because only loading/unloading procedures were needed. The scale-up procedure assumes that the industrial-scale unit performs as well as the laboratory-scale unit when the solvent volume to feed mass ratio (S/F), temperature, pressure, extraction time, porosity and apparent bed density are kept constant (Rosa and Meireles, 2005).

Thus, the active stage of extraction was fixed at 20 min. Hence, according to Eq. (2), the UAE and ABE processes were simulated using three extractor units of 1000 L. The processes were designed to run 24 h per day, 330 days per year, which corresponds to 7920 h per year (Rosa and Meireles, 2005). The flowchart of the industrial plant designed for performing the UAE and ABE processes using ethanol/water mixture is comprised of three extraction tanks, one solvent extraction reservoir tank, three centrifugal pumps, one falling film evaporator for concentrating the solvent mixture, one spray drier for producing the extract powder, and two condensers for recycling the solvent mixture (Fig. 1).

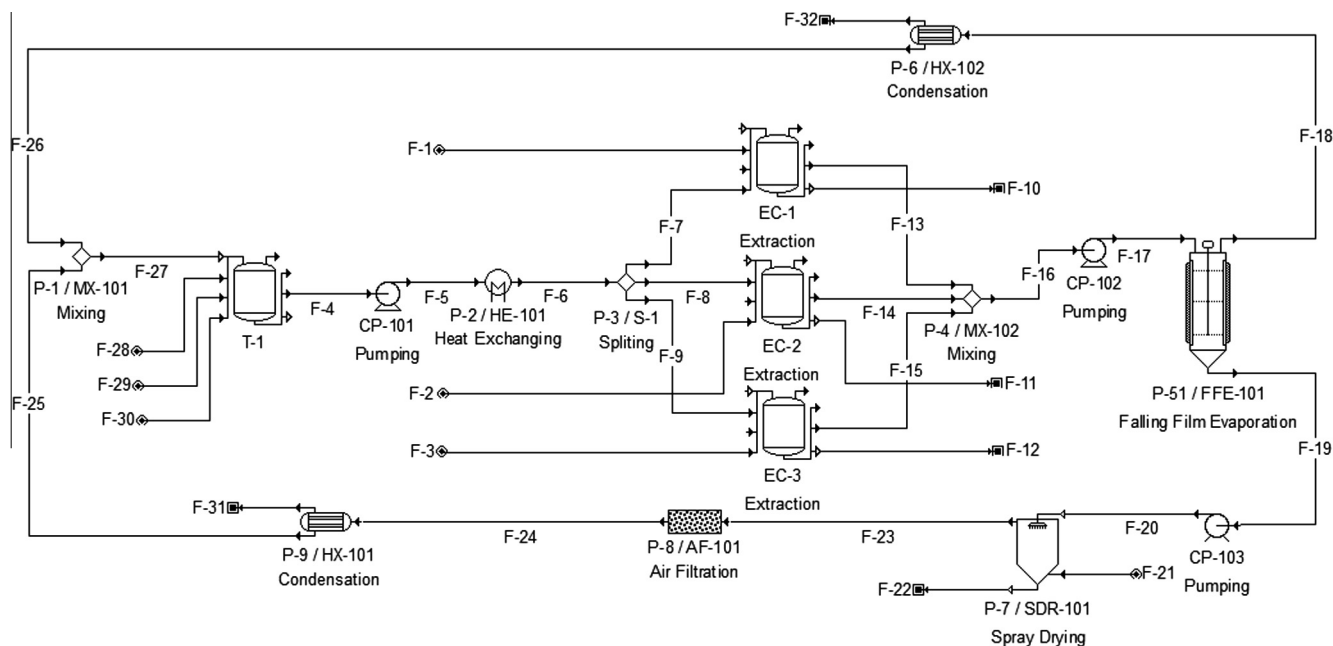
### 2.6.2. Manufacturing cost estimation

The cost of manufacturing (COM) of crude jussara extracts was estimated using the methodology described by Turton et al. (2009), where the COM is a function of five main costs: fixed capital of investment (FCI), cost of operational labour (COL), cost of utilities (CUT), cost of waste treatment (CWT) and cost of raw material (CRM), according to Eq. (3).

$$COM = 0.280 \times FCI + 2.37 \times COL + 1.23 \times (CUT + CWT + CRM) \quad (3)$$

The COM was expressed as unit production cost (USD/kg) by calculating the ratio between the cost of manufacturing (COM, USD/year) and production rate (kg/year) obtained per year. The COM (USD/kg) was estimated for all of the crude extracts produced by ABE and UAE procedures.

The plant had an estimated useful life of 15 years. All economic data fed into the simulator are presented in Table 2. To estimate the FCI the equipment prices were considered (Table 2) as a fraction of the initial investment cost according to the flowchart showed in Fig. 1. The equipment prices were budgeted from Chinese manufacturers. The annual equipment depreciation rate was considered to be 10%. Labour charges and work-people not directly associated with production were estimated by the simulator. The number of operators needed per shift was estimated according to Turton et al. (2009). The cost of utilities required for the operation of each equipment as well operational conditions, efficiency, mass and energy balances were estimated by the simulator. The temperature and pressure used in the vacuum falling film evaporator were tentatively determined by the simulator using a Peng–Robinson cubic equation of state (Peng and Robinson, 1976) to achieve a solution with a solid content ranging from 20% to 40%. The spray drier was set to achieve a final solid content of 95% in the extract powder. The electricity cost used in this study was based on the price charged by the industries (USD 0.138 kW h<sup>-1</sup>) in São Paulo, Brazil (CPFL,



### Streams

F-1; F-2; F-3: Jussara pulp mass.

F-4; F-5; F-6; F-7; F-8; F-9; F-25; F-26; F-27: Liquid Solvent mixture.

F-10; F-11; F-12: Jussara pulp residue.

F-13; F-14; F-15; F-16; F-17; F-19; F-20: Liquid solvent + extract mixture.

F-18; F-23; F-24: Vapor solvent mixture.

F-21: Air input.

F-22: Jussara powder extract.

F-25; F-26; F-27: Liquid solvent mixture recycled.

F-28; F-29; F-30: Ethanol, water and citric acid replacer

F-31; F-32: Ethanol, water and citric acid losses.

Fig. 1. Flowchart of the ultrasound-assisted extraction (UAE) and agitated bed extraction (ABE) simulated by the SuperPro Designer.

Table 2

Economic parameters used in the process simulation performed by SuperPro Designer 6.0 to estimate the cost of manufacturing the crude extracts produced by ultrasound-assisted extraction (UAE) and agitated bed extraction (ABE).

	UAE	ABE
<i>FCI (fixed capital of investment)</i>		
3 Extraction tanks (USD)	84400.00	18600.00
1 Reservoir solvent tank (USD)	5000.00	5000.00
3 Centrifuge pumps (USD)	30000.00	30000.00
1 Falling film evaporator (USD)	99000.00	99000.00
1 Spray drier (USD)	167000.00	167000.00
Depreciation rate (%/year) <sup>a</sup>	10	10
<i>COL (cost of operational labour)</i>		
Basic rate (USD/hr) <sup>b</sup>	11.65	11.65
Number of operators <sup>a</sup>	3	3
<i>CRM (cost of raw material)</i>		
Pulp of jussara (USD/kg)	1.80	1.80
Ethanol (USD/kg)	0.85	0.85
Water (USD/kg)	0.04	0.04
Citric acid (USD/kg)	0.55	0.55
<i>CUT (cost of utility)</i>		
Electricity (USD/kWh <sup>-1</sup> )	0.138	0.138
Chilled water (USD/ton)	0.34	0.34
Steam (USD/ton)	4.20	4.20
<i>Profitability</i>		
Selling price (USD/kg)	25.00	25.00

<sup>a</sup> Turton et al. (2009).

<sup>b</sup> US Department of Labor (2011).

2012). The CWT was neglected because it produces a major residue that is a nontoxic solid plant material and may be incorporated into the soil or commercialised as a by-product. The CRM is related to the cost of all start-up raw materials as well as their loss during the

process (Table 2). The total ethanol and water losses during of the processes were estimated to be 2%. For citric acid, an 80% loss was experimentally determined.

### 2.7. Statistical analysis

The analysis of variance (ANOVA one-way) and Tukey's test at  $p < 0.05$  were used to verify the statistical significance of the results using the statistical free software R version 2.14.1 (R Development Core Team, 2011). To analyse the results, the multcomp R package was used.

## 3. Results and discussion

### 3.1. Influence of process variables on extraction

#### 3.1.1. Effect of extraction time

Fig. 3 demonstrates the influence of extraction time on the yields of phenolic compounds and anthocyanin content from jussara pulp using ultrasound-assisted extraction (UAE) and agitated bed extraction (ABE). High yields were obtained, ranging from 29.97 to 38.12 g of extract/100 g dry pulp, 7.12 to 15.72 mg anthocyanin/g dry pulp and 42.09 to 63.97 mg GAE/g dry pulp. The extract yield increased within the time range of 5–20 min and did not vary significantly until the end of the UAE process. However, the extraction yield did not vary with increased of extraction time when ABE was used ( $p = 0.187$ ). The anthocyanin yield significantly increased ( $p < 0.001$ ) up to 20 min and increased gradually thereafter, reaching an extraction equilibrium at 120 min for both processes. For the phenolic compound content, a significant

increase in the first 40 min was noted ( $p < 0.001$ ). Using an ultrasound did not reveal an additional benefit to the extraction kinetics process when compared to the ABE. However, the estimated volumetric energy density of UAE (0.02 W/mL) was smaller than ABE (0.06 W/mL), precluding a precise evaluation of efficiency using an ultrasound extraction. In addition, the used frequency (40 kHz) was not adequate for this raw material. Conversely, the efficiency of UAE can depend on the raw material. Sun et al. (2011) did not observe any statistically significant differences in extraction yields of  $\beta$ -carotene from citrus peel particles between the UAE and maceration extraction when particles smaller than 0.28 mm were used. The jussara pulp used in this work contains small particles (approximately 0.17 mm), that are produced by an initial pulping process, with large superficial areas that promote a contact between the extraction solvent independently of the process (UAE or ABE). Chen et al. (2007) reported that UAE efficiently increased the anthocyanin extraction yield from red raspberries. Conversely, Santos et al. (2010) observed that a conventional extraction using an agitated bed removed more anthocyanins from jaboticaba skins than an ultrasound extraction.

The impact of the extraction procedures on the antioxidant capacity of the extracts was determined by the DPPH free radical scavenging capacity assay and the Ferric reduction antioxidant power (FRAP) assay. Fig. 3 presents the results. Following similar tendencies observed for the content of anthocyanin and phenolic compounds both the UAE and ABE processes yielded jussara extracts with high antioxidant capacity values. Depending on the extraction condition, the values of the antioxidant capacities ranged from 405.87 to 786.30  $\mu\text{mol TE/g}$  dry pulp and from 104.35 to 144.17  $\mu\text{mol TE/g}$  dry pulp according to the FRAP and DPPH assays, respectively. However, sonicated extracts yielded smaller antioxidant capacities than extracts produced by agitated bed process. The methodologies displayed similar trends but different magnitudes due to the use of distinct assays.

### 3.1.2. Effect of ethanol concentration

Table 3 presents the effect of ethanol concentration in water on the extract yields, total anthocyanins and total phenolic content of the jussara extract using UAE and ABE processes. The highest extraction yield was obtained using water acidified with citric acid (pH 3.0) as a solvent, which provided values of approximately 30%.

Nevertheless, the increase ethanol concentration promoted the decreased the extraction yields for both UAE and ABE ( $p < 0.05$ ). The use of a 30–70% (v/v) ethanol–water solution promoted the biggest anthocyanin yields, for both UAE and ABE ( $p < 0.05$ ). The increased ethanol proportion reduces the dielectric constant of the solution and consequently reduces the interaction energy between the solute and solvent molecules (Cacace and Mazza, 2003). According to Pompeu et al. (2009) and Cacace and Mazza (2003), 74% and 60% ethanol–water solutions most effectively enhanced the extraction of anthocyanins from assai fruits and black currant, respectively.

A larger ethanol concentration increased the phenolic compound yield ( $p < 0.01$ ), mimicking the tendency observed for anthocyanins (Table 3). At the extreme ethanol concentration levels (0% and 90%), the smallest yields of phenolic compounds and anthocyanins have been observed. The antioxidant capacities of the extracts produced by UAE and ABE followed similar trends that were independent of the extraction method (Table 4), confirming the relationship between anthocyanin content and antioxidant capacity in foods that contain these pigments.

### 3.1.3. Effect of the process temperature

The effect of the temperature was evaluated on the extraction processes and effects on the extract, anthocyanins and phenolic compounds are presented in Table 3. The temperature increase only increased the extraction yield for the ABE process from 25 to 35 °C ( $p = 0.016$ ). However, the temperature did not display any statistically significant influence on the anthocyanin and phenolic compound yields ( $p > 0.05$ ). The extract antioxidant activities measured by the DPPH assay did not differ with a temperature rise for both processes ( $p > 0.05$ ). However, the extract values measured by FRAP exhibited a slight decrease from 35 °C to 55 °C ( $p = 0.03$ ). This behaviour may be due to the extraction of non-phenolic compounds from jussara that may also have also contributed to the antioxidant capacity and were degraded at this temperature range.

### 3.1.4. Effect of the solvent to feed ratio

Table 3 displays the extraction yield, anthocyanins and total phenolic contents of the extracts obtained by UAE and ABE at different solvent to feed ratios (S/F). The evaluated responses were dependent on the S/F but did not vary significantly ( $p > 0.05$ ) as a

**Table 3**  
Extraction yield (EY), total monomeric anthocyanins (TMA) and total phenolic compounds (TPC) of the extracts obtained by ultrasound-assisted extraction (UAE) and agitated bed extraction (ABE) from jussara at different ethanol concentrations in water (% v/v), extraction temperature (°C) and solvent to feed ratio (mL/g) levels.

Process variable	Level	EY (g extract/ 100 g d.p. <sup>a</sup> )		TMA (mg Cyd-3-gluE <sup>b</sup> /g d.p.)		TPC (mg GAE <sup>c</sup> /g d.p.)	
		UAE	ABE	UAE	ABE	UAE	ABE
Ethanol concentrations in water (%)	0	30.64 ± 0.80 <sup>Aa</sup>	29.60 ± 0.85 <sup>Aa</sup>	8.32 ± 0.72 <sup>Aa</sup>	7.98 ± 0.43 <sup>Aa</sup>	46.58 ± 1.25 <sup>ACa</sup>	48.84 ± 2.14 <sup>ACa</sup>
	30	26.10 ± 0.14 <sup>Ba</sup>	27.75 ± 0.35 <sup>ABa</sup>	12.71 ± 0.13 <sup>Aa</sup>	12.82 ± 0.13 <sup>Ba</sup>	55.23 ± 3.40 <sup>Ba</sup>	55.71 ± 3.01 <sup>Ba</sup>
	50	25.07 ± 0.23 <sup>Ba</sup>	26.40 ± 0.57 <sup>Ba</sup>	12.23 ± 0.15 <sup>Aa</sup>	12.45 ± 1.89 <sup>Ba</sup>	53.31 ± 3.85 <sup>BCa</sup>	52.28 ± 0.69 <sup>BCa</sup>
	70	23.82 ± 0.96 <sup>BCa</sup>	25.89 ± 0.16 <sup>Ba</sup>	11.56 ± 0.22 <sup>Aa</sup>	11.59 ± 1.32 <sup>BCa</sup>	52.66 ± 2.03 <sup>BCa</sup>	48.06 ± 0.08 <sup>ACa</sup>
	90	21.55 ± 0.78 <sup>Ca</sup>	21.25 ± 0.35 <sup>Ca</sup>	3.89 ± 0.17 <sup>Ca</sup>	8.43 ± 0.16 <sup>ACb</sup>	43.74 ± 1.59 <sup>Aa</sup>	45.40 ± 3.33 <sup>Aa</sup>
Temperature (°C)	25	28.02 ± 0.70 <sup>Aa</sup>	28.19 ± 1.02 <sup>Aa</sup>	13.16 ± 0.19 <sup>Aa</sup>	12.91 ± 0.24 <sup>Aa</sup>	53.51 ± 1.42 <sup>Aa</sup>	55.43 ± 3.74 <sup>Aa</sup>
	35	28.46 ± 0.80 <sup>Aa</sup>	30.72 ± 0.46 <sup>Ba</sup>	13.17 ± 1.17 <sup>Aa</sup>	12.52 ± 0.22 <sup>Aa</sup>	49.93 ± 0.71 <sup>Aa</sup>	53.10 ± 2.02 <sup>Aa</sup>
	45	29.91 ± 0.86 <sup>Aa</sup>	31.80 ± 0.42 <sup>Ba</sup>	12.88 ± 0.10 <sup>Aa</sup>	12.85 ± 0.10 <sup>Aa</sup>	50.42 ± 2.62 <sup>Aa</sup>	53.87 ± 0.97 <sup>Aa</sup>
	55	29.31 ± 0.56 <sup>Aa</sup>	30.75 ± 0.21 <sup>Ba</sup>	12.38 ± 0.21 <sup>Aa</sup>	12.52 ± 0.68 <sup>Aa</sup>	52.88 ± 0.86 <sup>Aa</sup>	53.20 ± 0.78 <sup>Aa</sup>
Solvent/feed ratio (mL/g)	5	19.55 ± 0.10 <sup>Aa</sup>	17.30 ± 0.15 <sup>Aa</sup>	9.82 ± 0.22 <sup>Aa</sup>	9.23 ± 0.19 <sup>Aa</sup>	38.45 ± 0.64 <sup>Aa</sup>	39.05 ± 0.64 <sup>Aa</sup>
	10	21.56 ± 0.28 <sup>Aa</sup>	23.25 ± 0.10 <sup>ABa</sup>	10.37 ± 0.14 <sup>ABa</sup>	10.83 ± 0.61 <sup>ABa</sup>	43.75 ± 0.35 <sup>Ba</sup>	45.90 ± 1.27 <sup>Ba</sup>
	15	26.93 ± 2.19 <sup>Ba</sup>	25.51 ± 0.31 <sup>BCa</sup>	11.78 ± 0.29 <sup>ABCa</sup>	11.79 ± 0.33 <sup>ABa</sup>	45.75 ± 1.06 <sup>Ba</sup>	48.10 ± 0.85 <sup>Ba</sup>
	20	28.39 ± 0.77 <sup>Ba</sup>	27.14 ± 2.88 <sup>BCa</sup>	12.62 ± 0.23 <sup>Ca</sup>	13.12 ± 1.58 <sup>ABa</sup>	48.80 ± 1.13 <sup>Ca</sup>	48.80 ± 1.13 <sup>BCa</sup>
	25	40.24 ± 0.49 <sup>Ca</sup>	31.40 ± 2.95 <sup>CDa</sup>	12.54 ± 1.15 <sup>BCa</sup>	13.24 ± 1.76 <sup>ABa</sup>	52.45 ± 0.64 <sup>Da</sup>	52.80 ± 1.27 <sup>CDa</sup>
	30	34.03 ± 0.57 <sup>Da</sup>	34.60 ± 1.48 <sup>Da</sup>	12.59 ± 0.59 <sup>BCa</sup>	13.35 ± 0.49 <sup>Ba</sup>	52.85 ± 0.21 <sup>Da</sup>	54.05 ± 0.78 <sup>CDa</sup>

All data is presented as means ± standard deviation of three replicates ( $n = 3$ ). Different letters (small: amongst effect of different processes variables for the same extraction process; capital: amongst different extraction processes for the same process variable) indicate statistically significant differences ( $p < 0.05$ ).

<sup>a</sup> dp = dry pulp.

<sup>b</sup> Cyd-3-GluE = cyanidin-3-glucoside equivalent.

<sup>c</sup> GAE = gallic acid equivalent.

**Table 4**

Antioxidant capacity determined by DPPH and FRAP methods at a different ethanol concentration, extraction temperature and solvent to feed ratio level of the jussara extracts obtained by ultrasound-assisted extraction (UAE) and agitated bed extraction (ABE).

Process variable	Level	DPPH ( $\mu\text{mol TE}^a/\text{g d.p.}^b$ )		FRAP ( $\mu\text{mol TE}^a/\text{g d.p.}^b$ )	
		UAE	ABE	UAE	ABE
Ethanol concentration in water (%)	0	115.53 $\pm$ 4.34 <sup>Aa</sup>	111.94 $\pm$ 3.96 <sup>Aa</sup>	384.80 $\pm$ 7.34 <sup>Aa</sup>	380.73 $\pm$ 38.34 <sup>Aa</sup>
	30	115.82 $\pm$ 2.5 <sup>Ba</sup>	109.47 $\pm$ 2.00 <sup>Aa</sup>	421.51 $\pm$ 2.87 <sup>Ba</sup>	346.60 $\pm$ 30.60 <sup>Aa</sup>
	50	126.95 $\pm$ 2.06 <sup>Ca</sup>	117.94 $\pm$ 4.54 <sup>Aa</sup>	452.20 $\pm$ 28.42 <sup>Ca</sup>	471.45 $\pm$ 28.61 <sup>Ba</sup>
	70	111.80 $\pm$ 1.56 <sup>ABa</sup>	117.14 $\pm$ 1.88 <sup>Aa</sup>	323.69 $\pm$ 9.92 <sup>ABa</sup>	361.11 $\pm$ 4.31 <sup>Ab</sup>
	90	85.88 $\pm$ 0.17 <sup>Da</sup>	91.93 $\pm$ 1.09 <sup>Bb</sup>	81.33 $\pm$ 19.01 <sup>Da</sup>	74.22 $\pm$ 9.58 <sup>Ca</sup>
Temperature ( $^{\circ}\text{C}$ )	25	89.33 $\pm$ 4.54 <sup>Aa</sup>	90.79 $\pm$ 6.13 <sup>Aa</sup>	598.82 $\pm$ 74.83 <sup>Aa</sup>	638.94 $\pm$ 3.16 <sup>Aa</sup>
	35	94.16 $\pm$ 10.82 <sup>Aa</sup>	92.34 $\pm$ 12.67 <sup>Aa</sup>	672.64 $\pm$ 13.37 <sup>Aa</sup>	627.39 $\pm$ 3.02 <sup>ACb</sup>
	45	85.44 $\pm$ 4.93 <sup>Aa</sup>	85.52 $\pm$ 4.36 <sup>Aa</sup>	598.23 $\pm$ 17.89 <sup>Aa</sup>	572.94 $\pm$ 11.85 <sup>Ba</sup>
	55	87.70 $\pm$ 2.39 <sup>Aa</sup>	89.38 $\pm$ 4.94 <sup>Aa</sup>	634.67 $\pm$ 11.20 <sup>Aa</sup>	587.11 $\pm$ 18.10 <sup>BCa</sup>
Solvent/Feed ratio (mL/g)	5	123.83 $\pm$ 2.36 <sup>Aa</sup>	109.72 $\pm$ 5.54 <sup>Aa</sup>	521.16 $\pm$ 4.87 <sup>Aa</sup>	498.63 $\pm$ 8.64 <sup>Ab</sup>
	10	156.82 $\pm$ 8.14 <sup>Ba</sup>	165.47 $\pm$ 3.64 <sup>Ba</sup>	551.29 $\pm$ 4.06 <sup>Ba</sup>	636.67 $\pm$ 4.22 <sup>Bb</sup>
	15	199.19 $\pm$ 2.20 <sup>Ca</sup>	206.03 $\pm$ 6.94 <sup>CDa</sup>	634.12 $\pm$ 11.85 <sup>Ca</sup>	661.13 $\pm$ 8.22 <sup>Cb</sup>
	20	205.19 $\pm$ 2.49 <sup>Ca</sup>	191.56 $\pm$ 3.72 <sup>BDa</sup>	635.03 $\pm$ 14.31 <sup>Ca</sup>	619.47 $\pm$ 7.03 <sup>Da</sup>
	25	204.98 $\pm$ 16.01 <sup>Ca</sup>	222.65 $\pm$ 2.67 <sup>Ca</sup>	758.73 $\pm$ 5.51 <sup>Da</sup>	721.39 $\pm$ 9.19 <sup>Eb</sup>
	30	267.82 $\pm$ 2.34 <sup>Da</sup>	277.18 $\pm$ 8.69 <sup>Ea</sup>	708.21 $\pm$ 11.86 <sup>Ea</sup>	671.13 $\pm$ 5.12 <sup>Cb</sup>

DPPH = free-radical scavenging capacity; FRAP = ferric reducing antioxidant power. All data is presented as means  $\pm$  standard deviation of three replicates ( $n = 3$ ). Different letters (small: amongst effect of different processes variables for the same extraction process; capital: amongst different extraction processes for the same process variable) indicate statistically significant differences ( $p < 0.05$ ).

<sup>a</sup> TE = trolox equivalent.

<sup>b</sup> d.p. = dry pulp.

function of the extraction process. In general, to maintain the mass constant and increase solvent volume, the release of target compounds from the matrix is facilitated by a larger concentration gradient. The crude extract, anthocyanin and phenolic compound yields increased with an increasing solvent to feed ratio for both the UAE and ABE process. The mass transfer plateaued at a solvent to feed ratio of 15 mL/g pulp for anthocyanins and 20 mL/g pulp for phenolic compounds. Pompeu et al. (2009), while working with whole assai fruits, observed that the total phenolic and anthocyanin yields were maximised at a 1:4 solid-to-liquid ratio, which is approximately five times less than the one identified in our study. Conversely, Borges et al. (2011b) employed methanol/1.5 M HCl as a solvent for 24 h, to extract anthocyanins from jussara pulp and reported that the optimum condition for the solid to solvent ratio varied from 1:30 to 1:50. The antioxidant capacity of the extract increased significantly with the rise of S/F (Table 4), as expected. The extracts obtained from UAE displayed FRAP values than those obtained with the agitated bed method, when an S/F greater than 20 was used. The UAE and ABE extracts displayed similar DPPH values.

### 3.2. Manufacturing costs

Table 5 presents the estimated cost of manufacturing jussara crude extracts by ultrasound assisted extraction (UAE) and agitated bed extraction (ABE) while evaluating effects on the following process variables: the extraction time, ethanol concentration in water, temperature and solvent volume to feed mass ratio. The scale-up criterion assumes that the extraction yields obtained by industrial-scale units are experimentally similar to the yields obtained by laboratorial-scale units when operational conditions are kept constant. The components CRM, COL, FCI and CUT represent the percentage of costs of raw material costs, operational labour, investment and utilities, respectively, which compose the manufacturing cost obtained for each operational condition.

Jussara crude extracts obtained by UAE garnered higher manufacturing costs for all variables studied than with using ABE, except for the solvent volume to feed mass ratio. As displayed in Table 5, the extraction time did not significantly influence the manufacturing cost of the extracts, which exhibits a slightly oscillatory profile along the extraction kinetic time. The cost of manufacturing is ob-

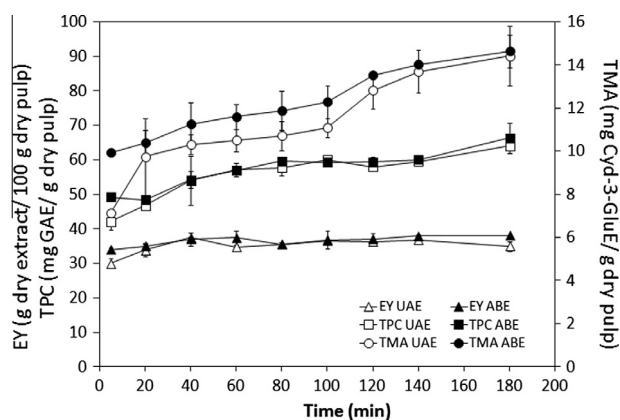
tained by the ratio of the annual operating cost and the production rate. Although the solvent amount used in the extraction kinetic is kept constant due to the use of a static mode of extraction, the operating cost linearly grows with time due to the continuous consume of electricity and heat exchange agents during the process. Conversely, the extraction yield (Fig. 2) displays the same oscillatory behaviour as the COM. This observations is likely due to the use of static extraction in which the same amount of solvent is used along the extraction kinetic. Thus, the amount of solute present in the solvent increases during the extraction time thereby inhibiting the process of mass transfer by diffusion. These results differ from those obtained by Albuquerque and Meireles (2012), who studied the dynamic supercritical fluid extraction of annatto to produce bixin-rich extracts. These authors achieved parabolic profiles in which the manufacturing cost decreased until a minimum value and ascended continuously until the end of the extraction kinetic. This behaviour is likely associated with the use of a dynamic extraction, which consists of a continuous flow of free-solute solvent through a raw material packed into an extraction column thereby facilitating mass diffusion. The authors demonstrated that an extraction time greater than 40 min caused an expressive increase in the COM. Mezzomo et al. (2011) also observed a significant influence of time on peach almond oil production. As expected the kinetic time did not affect the COM composition of the UAE and ABE processes because the operational conditions were kept constant.

The concentration of ethanol in water remarkably affected the manufacturing costs. Extracts produced with solutions lacking ethanol provided the lowest COM independently of the extraction method. Although a 30% alcohol solution exhibited the highest extraction yield, the cost of manufacturing continuously increased with the incorporation of ethanol into the solvent solutions. This behaviour may be associated with the cost of ethanol (0.85 USD/kg), which had a major influence on the COM when compared to the cost of water (0.04 USD/kg).

The vacuum pressure and temperature of the falling film evaporator varies with the ethanol concentration, and its values were tentatively determined by the simulator using the cubic equation of state of Peng-Robinson (Peng and Robinson, 1976). The equipment was set to operate for 60 min to concentrate the mixture solutions received from the three extractors. The operational

**Table 5**  
Cost of manufacturing (COM, USD/kg of crude extract) and COM composition (%) of cost of raw material (CRM), cost of operational labour (COL), fixed capital of investment (FCI) and cost of utility (CUT) for ultrasound-assisted extraction (UAE) and agitated bed extraction (ABE).

Process variables	Level	UAE				ABE					
		COM (USD/kg of crude extract)	COM composition (%)				COM (USD/kg of crude extract)	COM composition (%)			
			CRM	COL	FCI	CUT		CRM	COL	FCI	CUT
Time (min)	5	110.39 ± 2.55	41.97	42.26	8.22	7.54	98.74 ± 2.42	42.9	43.19	6.27	7.63
	20	97.70 ± 3.19	41.97	42.26	8.22	7.54	94.27 ± 0.76	42.9	43.19	6.27	7.63
	40	90.57 ± 0.40	41.97	42.26	8.22	7.54	89.68 ± 0.34	42.9	43.19	6.27	7.63
	60	96.56 ± 0.42	41.97	42.26	8.22	7.54	88.02 ± 0.99	42.9	43.19	6.27	7.63
	80	95.55 ± 1.90	41.97	42.26	8.22	7.54	92.56 ± 0.55	42.9	43.19	6.27	7.63
	100	92.07 ± 0.10	41.97	42.26	8.22	7.54	92.13 ± 2.76	42.9	43.19	6.27	7.63
	120	93.76 ± 2.82	41.97	42.26	8.22	7.54	88.14 ± 2.18	42.9	43.19	6.27	7.63
	140	92.32 ± 2.66	41.97	42.26	8.22	7.54	87.32 ± 0.32	42.9	43.19	6.27	7.63
Ethanol concentration in water (% v/v)	0	103.95 ± 2.70	42.01	47.01	5.94	5.04	107.50 ± 3.10	42.04	47.05	5.94	4.97
	30	125.91 ± 0.67	42.87	45.00	5.68	6.45	118.34 ± 1.51	42.91	45.03	5.69	6.38
	50	134.13 ± 1.27	43.32	43.62	5.51	7.55	127.27 ± 2.74	43.35	43.65	5.51	7.48
	70	146.01 ± 5.87	43.28	41.86	6.08	8.78	134.11 ± 0.79	43.31	41.89	6.09	8.71
	90	165.34 ± 5.94	42.23	40.84	5.93	11.00	167.48 ± 2.80	42.26	40.87	5.94	10.93
Temperature (°C)	25	122.55 ± 3.09	42.43	42.72	7.23	7.62	119.51 ± 4.31	43.25	43.56	5.50	7.69
	35	143.24 ± 4.01	35.72	46.91	10.99	6.38	128.98 ± 1.94	36.77	48.29	8.44	6.51
	45	136.80 ± 3.93	35.61	46.76	10.95	6.68	124.92 ± 1.64	36.65	48.13	8.41	6.81
	55	139.99 ± 2.66	35.50	46.62	10.92	6.97	129.61 ± 0.92	36.53	47.98	8.39	7.10
Solvent/feed ratio (mL/g)	5	98.57 ± 0.47	70.49	21.85	4.25	3.40	110.14 ± 0.29	71.29	22.1	3.21	3.40
	10	112.52 ± 1.45	57.34	31.88	5.39	5.39	102.84 ± 0.01	58.17	32.34	4.08	5.42
	15	109.26 ± 8.90	48.54	38.29	6.48	6.69	113.04 ± 1.36	49.39	38.96	4.92	6.74
	20	120.97 ± 3.27	42.43	42.72	7.23	7.62	124.78 ± 13.25	43.25	43.56	5.5	7.69
	25	98.14 ± 1.21	37.72	46.20	7.81	8.28	123.74 ± 11.63	38.51	47.16	5.95	8.37
	30	130.67 ± 2.19	34.22	48.71	8.24	8.82	125.80 ± 5.39	34.98	49.81	6.29	8.93



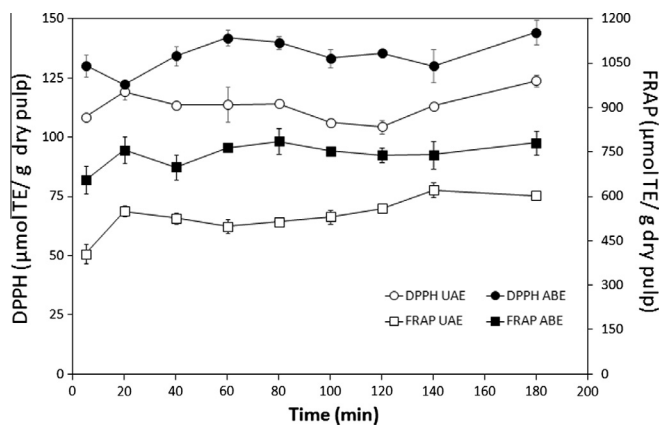
**Fig. 2.** Total extract yield (EY), total phenolic compounds (TPC) and total monomeric anthocyanins (TMA) of the jussara extracts obtained by ultrasound-assisted extraction (UAE) and agitated bed extraction (ABE) at different processing times. Data are presented as the means ± SD ( $n = 3$ ).

conditions used to achieve an output mixture with solid concentration ranging from 20% to 40% were fixed at a temperature of 65 °C and at vacuum pressure values of 170.7, 201.6 and 239.8 mmHg for 0%, 30% and 50% of ethanol in water (v/v), respectively. For 70% and 90% ethanol, a fixed temperature of 60 °C and pressures of 271.4 and 405.0 mmHg, respectively, were used. This decrease in temperature and vacuum pressure should reduce the costs, but the opposite effect was observed, corroborating the major contribution of ethanol to the cost of manufacturing.

The percentage of CRM and FCI did not vary the COM significantly with increasing ethanol concentrations in water. However, the cost of utilities (CUT) has increased with ethanol concentration while the COL decreased. The same behaviour was observed for both extraction methods.

Increasing temperature appeared to slight increase the cost of manufacturing even though this variable did not significantly affect the extraction yield. The COM components did not vary significantly between 35 and 55 °C. However, at 25 °C the CRM and CUT displayed the highest percentage values while the lowest were observed for COL and FCI.

The increase in the solvent volume to feed mass ratio (S/F) caused an oscillatory variation in the manufacturing costs, which kept them almost constant although the extraction yield increased with S/F, as is demonstrated in Tables 3 and 5. This behaviour may be explained by the fact that the increase in the S/F occurred concurrently with the increase in the solvent volume and a decrease in the jussara pulp mass fed to the extractor to fill the total volume due to the use of the static extraction mode. This double effect decreased the cost of jussara pulp cost (1.80 USD/kg) and increased the ethanol/water (1:1) solvent mixture cost (0.85 USD/kg of ethanol plus 0.04 USD/kg of water), which may be attributable to the oscillatory variation in the cost of manufacturing.



**Fig. 3.** Antioxidant capacity of extracts produced by ultrasound-assisted extraction (UAE) and agitated bed extraction (ABE) measured at different processing times. Data are presented as the means ± SD ( $n = 3$ ).

The analysis of the effects of the process variables on the COM components demonstrated that the cost of operational labour had the most influence on the final manufacturing costs, followed by the cost of raw material, fixed capital of investment and cost of utilities for all operational conditions evaluated, except for the solvent volume to feed mass ratio, which demonstrated that CRM had a more significant impact on the manufacturing cost than the COL. The featureless effects of the FCI and CUT on the COM may be due to the low costs of the equipment, electricity and heat exchange agents. Conversely, the high values of the COL and CRM indicate that the labour charge and raw materials costs were key factors on defining the COM in all situations evaluated in this work.

As presented in Table 5, the COM component percentages obtained by the UAE and ABE processes, where the CRM, COL and CUT yielded almost the same values that varied only with the operational condition evaluated but not with the extraction method used. However, the fraction of the fixed capital of investment (FCI) percentage of UAE displayed higher values than those obtained for ABE, which demonstrated an incremental effect of the cost of the ultrasonic system of the UAE as compared to the stirring used in the ABE.

Because jussara extract is not available on the market, the estimated selling prices were based on the assai extract commercial price, which is the most similar product available in the market. In Brazil, the prices of assai extract powder vary from 9.50 to 18.00 USD/kg (Biotae, 2012; Blue Macaw Flora, 2013), and in international markets, these prices change to 25.00 USD/kg (Phyto Nutraceutical, 2012). Nonetheless, a better comparison requires information about the anthocyanin content in assai extract powder, which is not available.

Jussara pulp is extremely costly (1.80 USD/kg of pulp); therefore, the manufacturing costs of the crude extracts obtained by UAE (90.57–165.34 USD/kg) and ABE (87.32–167.48 USD/kg) were higher when the assai selling prices budget is compared to this project (25.00 USD/kg).

The extracts produced by UAE garnered slightly higher manufacturing costs than those obtained by ABE. However, the cost of an ultrasonic extractor unit was 4.5-fold higher than the unit that relies exclusively on stirring and an extremely small difference was detected between the UAE and ABE processes for FCI. These results agree with previous results demonstrated by Santos et al. (2010).

#### 4. Conclusions

The extracts obtained by the UAE and ABE are natural products rich with phenols and anthocyanins and harbour significant antioxidant capacities. The anthocyanin and phenolic compound yields were statistically equal between the UAE and ABE extraction methods. However, anthocyanin and phenolic contents were significantly affected by the time, ethanol concentrations in water and solvent/feed ratios, but not by the extraction temperature. In general, the antioxidant capacities of the extracts were measured by FRAP and DPPH assays, which displayed similar tendencies as the anthocyanin and phenolic compound yields. The economic evaluation of using the UAE and ABE processes to obtain natural crude extracts from jussara pulp revealed high COM values, due mainly to high charge labour and the costs of raw materials. Further studies are needed to optimise the process conditions for an economically viable extraction process.

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