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ORIGINAL ARTICLE

Determination of anthocyanins and non-anthocyanin polyphenols by ultra performance liquid chromatography/electrospray ionization mass spectrometry (UPLC/ESI–MS) in jussara (*Euterpe edulis*) extracts

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Abstract This work aimed to propose two analytical methods for the quantitative and qualitative analysis of major anthocyanins and non-anthocyanin phenolic compounds in jussara (*Euterpe edulis*) extracts, using ultra performance liquid chromatography-mass spectrometry. These methods were evaluated for selectivity, precision, linearity, detection and quantification limits. The complete separation of 5 anthocyanins and 22 non-anthocyanins polyphenols was achieved in 4.5 and 7 min, respectively. Limits of detection ranged from 0.55 to 9.24 µg/L, with relative standard deviation for concentration up to 7.0%. In jussara extract, 13 of the 27 analytes were characterized. The dominant compound was cyanidin-3-O-rutinoside, representing about 73% of the total phenolic compounds content (approximately 23 mg/g of extract in dry weight). Other phenolic compounds found in the extract were: cyanidin-3-O-glucoside, pelargonidin-3-O-glucoside, quercetin, rutin, myricetin, kaempferol, kaempferol-3-O-rutinoside, luteolin, apigenin, catechin, ellagic acid and 4,5-dicaffeoylquinic acid.

Keywords Red pigments · Color · Flavonoid · Cyanidin-3-rutinoside · Keracyanin · Oenin

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Introduction

Phenolic compounds represent a wide group of secondary metabolites of plant tissues, which are regarded as important bioactives molecules mainly due to their antioxidant properties. They can play a potential role on preventive control of certain types of cancer, cardiovascular diseases, autoimmune disorders and the process of aging. This group comprises well-known subgroups like phenolic acids, flavonoids, specially anthocyanins, stilbenes, tannins, tocopherols, tocotrienols, lignans, and lignins (Shahidi and Naczk 2006). Anthocyanins are water-soluble pigments, responsible for the cyanic colors, of most plant species and their fruits, ranging from salmon pink to through red and from violet to dark blue (Delgado-Vargas and Paredes-López 2003). These pigments have been commercially used as nutraceuticals, antioxidants, and red natural colorants in different foodstuffs, cosmetics and medicines.

The jussara palm belongs to the Arecaceae family, genus Euterpe, and specie *Euterpe edulis* Martius. This is a native fruit tree widely distributed in Brazilian Atlantic Forest, with erect and solitary stems that measure from 5 to 12 m in height, and from 10 to15 cm in diameter (Henderson 2000). Theses palms produce an edible palm heart, recognised as a noble product of economic relevance in Brazil. Jussara palm also produces spherical berries commonly known as jussara. These fruits contain only one light brown seed that accounts for approximately 90% of the fruit diameter (1–2 cm) and up to 90% of its weight (0.7–1.9 g) (Borges et al. 2011).). The seeds are covered by a thin, shiny, dark purple dry skin that appears almost black in color when ripe due to its high anthocyanin content (Vieira et al. 2013). The completely ripened fruits are used to produce pulp by a process of maceration in water. After extraction, the seeds are separated, and then a thick pulp

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A critical factor for quality evaluation of polyphenolic-rich products is the quantification and identification of their compounds. Advanced separation techniques such as high performance liquid chromatography (HPLC), coupled to photodiode array detection (PDA) and or to mass spectrometry (MS) have been used to analyze anthocyanins and non-anthocyanin phenolic compounds that always co-exist in plant extracts, wine, fruit juices, and other products (Pradhan and Saha 2016; Loypimai et al. 2016; Choi et al. 2016; Borges et al. 2013; Huang et al. 2009). The ultraviolet/visible (UV/Vis) molecular absorbance measured by PDA is commonly used to distinguish anthocyanins from non-anthocyanin phenolics, since anthocyanins have maxima absorption within 480–540 nm range and the other phenolics only absorb at wavelengths below 400 nm. Nevertheless, PDA has much poorer selectivity and sensitivity than MS (Sun et al. 2012). Thus, detection of small quantities and the analysis of co-eluting anthocyanins and non-anthocyanin phenolics can be very difficult using PDA (Sun et al. 2012). Therefore, MS detection is considered a valuable tool for the characterization and identification of anthocyanins and other polyphenols (Pradhan and Saha 2016, Borges et al. 2013; Anastasiadi et al. 2012; Gordon et al. 2012; Sun et al. 2012; Boros et al. 2010; Huang et al. 2009).

Recently, the development of ultra-high pressure pump systems and sub-2 µm packing columns has allowed significant improvement in the separation, speed, and efficiency of traditional HPLC analysis of complex sample matrices. In addition, the major advantages of ultra performance liquid chromatography (UPLC) over conventional HPLC are improved resolution, shorter retention times and reduced solvent consumption. Hence it can also be considered more cost-effective than HPLC (Motilva et al. 2013; Wang et al. 2012).

In the current study, analytical methods were proposed to characterize the main anthocyanins and non-anthocyanins phenolics in jussara extracts using ultra performance liquid chromatography/electrospray ionization mass spectrometry.

Materials and methods

Chemicals

Ultrapure water was supplied by Milli-Q Direct-Q 3 (Billerica, MA, USA). Ethanol and citric acid were purchased from Synth (Diadema, SP, Brazil). Anthocyanins and other phenolic compounds standards were obtained from several sources. Cyanidin-3-O-rutinoside, delphinidin-3-O-glucoside, kaempferol-3-O-glucoside, and kaempferol-3-O-rutinoside were obtained from extrasynthese (Genay, France). Pelargonidin-3-O-glucoside; malvinidin-3-O-glucoside; cyanidin-3-O-glucoside; gallic acid; protocatechuic acid; *p*-hydroxybenzoic acid; sinapic acid; chlorogenic acid; ellagic acid; ferrulic acid; caffeic acid; *p*-coumaric acid; (+)-catechin, 4,5-dicaffeoylquinic acid; myricetin; quercetin; rutin; kaempferol; luteolin; resveratrol; crisin; apigenin; hydroxycinamic acid; 2,4,6-tris-(2-pyridyl)-s-triazine (TPTZ), and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Methanol (MeOH), acetonitrile (ACN), formic acid and ethyl acetate were purchased from J.T. Baker (Mexico City, Mexico). All reagents were analytical grade.

Raw material

Frozen jussara pulp was supplied by farmers from the rural communities of Ubatuba, Sao Paulo, Brazil (Projeto Juçara, 2012). Completely ripened jussara fruits, harvested in May 2012, were used to produce the pulp. The frozen product was taken to the University of Campinas (Campinas, SP, Brazil), stored in a freezing chamber at −18 °C, and thawed, according to the quantity required for each trial.

Extraction design

Sample preparation was done as reported previously by Vieira et al. (2013). Briefly, approximately 5 g of jussara pulp were weighed directly into 250 mL Erlenmeyer flasks. Extraction solutions were prepared with solutions containing 70% of ethanol in distilled water and adjusted to a pH of 3.0 with 0.35% citric acid (w/v). The ratio solvent to feed mass was fixed at 20 mL/g. For trials, a temperature of 30 °C, for a period of 20 min, was used. The flasks were sealed and immersed in an orbital shaker (TE-420 model, Tecnal, Piracicaba, Brazil) with 100 rpm stirring speed. After extraction, the samples were centrifuged for 10 min at 20 °C at 10,000 rpm and the supernatant was collected and filtered through a qualitative filter paper. The extracts were stored in amber glass bottles at −18 °C until the analyses were performed. All extraction experiments were performed in duplicate.

Chromatographic analysis

Purification and fractionation

To remove several interfering compounds present in the crude jussara extracts (organic acids, sugar, etc.), the Sep-Pak solid phase octadecyl (C18) cartridges (Applied Separations, PA, USA) were used to purify and to fraction the extracts, following the method described by Rodriguez-Saona and Wrolstad (2001). This step was very important to improve the resolution of the peaks and the separation efficiency of the chromatographic run. Briefly, the C18 cartridges were pre-conditioned with ethyl acetate, methanol and water. Then, the extract previously concentrated under vacuum stream was loaded into the cartridges and washed with 6 mL of ultrapure water to remove interfering molecules. Next, non-anthocyanic phenolic fraction (phenolic acids, flavonoids, etc.) was eluted with 40 mL of ethyl acetate. The anthocyanin-rich fraction was eluted with 10 mL of methanol acidified with 0.01% chloride acid. Both fractions were concentrated to dryness under vacuum using a rotoevaporator. Finally, samples were redissolved in ultrapure water, filtered through a 0.22 μm PTFE microfilter (Millipore, Billerica, MA, USA) and then analysed by UPLC coupled with a single quadrupole mass spectrometer with electrospray ionization (ESI) source.

UPLC-ESI–MS analysis

An ACQUITY UPLC/MS system (Waters Corp., Milford, MA, USA) was used to perform the chromatographic analysis. The equipment consisted of a binary solvent manager, sample manager, column heater, PDA diode array detector (DAD), interfaced with a mass spectrometer equipped with an ESI source, operating in either positive or negative mode. Instrument control and data processing were performed using MassLynx[TM] Software (4.1 version, Waters Corp.). Separations were performed on an Acquity BEH C18 column, 1.7 μm, 2.1 mm × 50 mm, connected to a BET C18 VanGuard pre-column 1.7 μm, 2.1 mm × 5 mm (Waters Corp., Ireland). The autosampler and analytical column oven were maintained at 10 and 30 °C, respectively.

The mobile phase used to elute anthocyanins fraction consisted of water (A) and methanol (B) both acidified with 2% formic acid, in a ratio specified by the following gradient with linear interpolation (curve 6): (0–3 min, 90% A; 3–4.5 min 40% A; 4.5–5.5 min, 40% A; 5.5–6.5 min, 90% A; 6.5–7.0 min, 90%). For non-anthocyanin polyphenols, water (A) and acetonitrile (B) acidified with 0.01% formic acid were used as mobile phase, with a linear gradient: (0–2.4 min, 95% A; 2.4–8.0 min, 40% A; 8.0–9.0 min, 10% A; 9.0–11.0 min, 95% A; 11.0–12.0 min, 95% A). The flow rate was 0.5 mL/min in all trials. Mass spectrometric data of the eluted compounds from the column were acquired in one single run, for both positive and negative ion mode for anthocyanins and non-anthocyanin polyphenols, respectively. Ultrapure nitrogen was used as both drying and nebulizing gas, and the flow conditions were 900 and 20 L/h for desolvation and at the cone, respectively (Peak Scientific, Billerica, MA, USA). The source block temperature and desolvation temperature were set at 150 and 450 °C, respectively. Optimization of ionization conditions was based on the intensity of the mass signals of protonated/deprotonated molecules and performed for each individual peak/compound detected. Mass spectrometer parameters were optimized as follow: capillary voltage at 3.0 kV and cone voltage ranged from 30 to 40 V for positive/negative ionization, respectively. From the purified phenolic and anthocyanins extracts, 5 μL were analyzed on UPLC-ESI–MS system.

Identification, quantification and evaluation of methods

Monomeric anthocyanins and other non-anthocyanin phenolics were tentatively identified by comparing mass spectrometric patterns and retention times to those of authentic standards. The quantification was performed using the external calibration method. Standard stock solutions of individual compounds were prepared in methanol at concentrations around 500 mg/L and were subsequently diluted to working concentrations. Calibration standard solutions at six concentrations levels ranging from 65 to 2000 μg/L were then prepared in water. The evaluation of the methods was carried out from three series of experiments in different days. For each trial the mobile phases were renewed and six injections were performed. From the acquired data, selectivity, precision, linearity, limits of detection (LOD), and limits of quantification
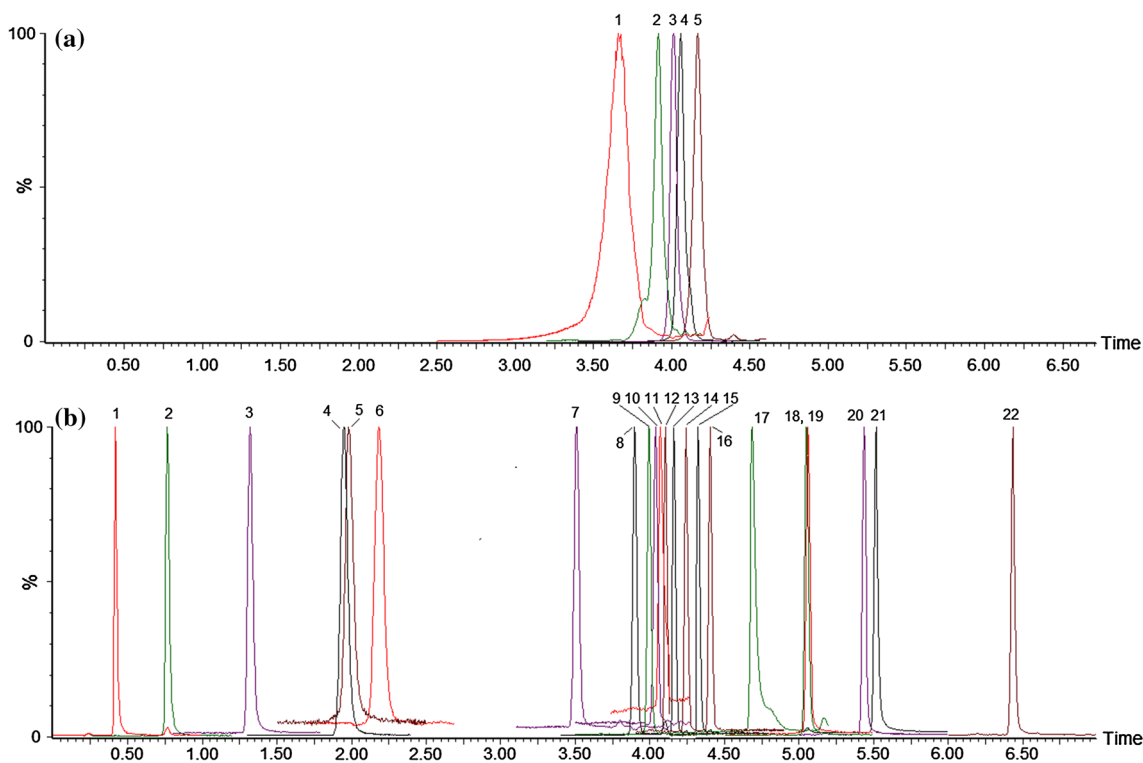
Fig. 1 Overlayed total ion UPLC/MS chromatograms of mixture of the standards. Anthocyanins (**a**): dephinidin-3-O-glucoside (*1*), cyanidin-3-O-glucoside (*2*), cyanidin-3-O-rutinoside (*3*), perlagonidin-3-O-glucoside (*4*), malvinidin-3-O-glucoside (*5*). Non-anthocyanic phenolic compounds (**b**): gallic acid (*1*), protocatechuic acid (*2*), *p*-hydroxybenzoic acid (*3*), catechin (*4*), chlorogenic acid (*5*), caffeic acid (*6*), *p*-coumaric acid (*7*), ferrulic acid (*8*), sinapic acid (*9*), hydroxycinamic acid (*10*), ellagic acid (*11*), rutin (*12*), kaempferol-3-O-rutinoside (*13*), kaempferol-3-O-glucoside (*14*), 4,5-dicaffeoylquinic acid (*15*), myricetin (*16*), resveratrol (*17*), luteolin (*18*), quercetin (*19*), apigenin (*20*), kaempferol (*21*), crisin (*22*)

(LOQ) were evaluated to determine suitability of the method for these analyses. LOD and LOQ were based on 3 and 10 times the signal-to-noise ratio, respectively. These parameters were determined by repeated injections of diluted standard solutions. Results were expressed in mg/g of dry mass extract.

Statistical analysis

The one sample *t* test was applied for verification of statistical significance, with a confidence interval of 95%, for phenolic compounds identified in jussara extract, with the assumption that data follow a normal distribution. The free statistical software R version 2.14.1 (R Development Core Team 2011) was used for every analysis.

Results and discussion

Development of the UPLC methods

Analysis of phenolic compounds from anthocyanin-rich extracts is rather laborious due to their great diversity and to the interfering compounds. In general, an initial purification step is added to minimize peak overlap that is apparent when crude extracts are directly injected in UPLC system. Therefore, an analytical method for anthocyanins and another one for non-anthocyanin polyphenols fractions have been proposed.

The first UPLC method developed allowed the complete separation of five anthocyanins in 4.5 min within a total run time of 7 min (Fig. 1a). All analyzed anthocyanins are found in many other fruits, for example black raspberry, assai, mulberry, strawberry and others (Choi et al. 2016, Wu et al. 2011, Silva et al. 2007). Dias et al. (2012) developed an UPLC method for anthocyanins analysis from assai, using a HSS C18 column (from Waters), that provided a separation of seven anthocyanins within 17 min of total run. These authors obtained the separation of major anthocyanins from assai in approximately 6 min. Typical HPLC methods to analyze anthocyanins can take up to 80 min (Naczk and Shahidi, 2004; Schauss et al. 2006; Brito et al. 2007). Therefore, the proposed method offers substantial improvements over the methods available in the literature.

Table 1 Performance parameters for different analytes evaluated by UPLC–MS

Compound	Retention[a] time (min)	RSD[b] (%) for retention time	Cone voltage (V)	MS[c] (m/z)	LOD[d] (μg/L)	LOQ[e] (μg/L)	RSD[f] (%) for concentration	Linearity[g] (μg/L)	R^2
Anthocyanins									
Dephinidin-3-O-glucoside	3.67	1.36	35	465.2	5.60	18.50	1.44	65–2000	0.9964
Cyanidin-3-O-glucoside	3.91	1.60	35	449.2	4.40	14.80	3.57	65–2000	0.9958
Cyanidin-3-O-rutinoside	4.01	1.69	35	595.2	1.40	4.70	1.37	65–2000	0.9997
Perlagonidin-3-O-glucoside	4.06	1.00	35	433.2	4.10	13.50	1.84	65–2000	0.9979
Malvinidin-3-O- glucoside	4.18	1.54	35	493.2	4.20	14.10	2.59	65–2000	0.9998
Non-anthocyanin polyphenols									
Gallic acid	0.43	0.20	30	169.0	0.80	2.66	1.76	65–2000	0.9999
Protocatechuic acid	0.78	0.53	30	153.1	0.55	2.82	0.62	65–2000	0.9997
p-Hydroxybenzoic acid	1.35	1.36	30	137.1	2.02	6.72	1.05	65–2000	0.9992
(+)-Catechin	2.04	0.28	35	289.0	1.19	3.95	1.05	65–2000	0.9952
Chlorogenic acid	2.10	2.20	30	353.0	3.95	13.16	1.82	65–2000	0.9994
Caffeic acid	2.29	3.10	30	179.1	3.98	13.26	1.10	65–2000	0.9994
p-Coumaric acid	3.54	2.05	30	163.1	5.30	15.32	1.31	65–2000	0.9977
Ferrulic acid	3.92	1.71	30	193.1	3.03	10.11	2.67	65–2000	0.9998
Sinapic acid	4.01	1.02	30	223.0	2.03	6.78	3.92	65–2000	0.9988
Hydroxycinamic acid	4.05	0.45	30	163.0	4.66	15.54	1.55	65–2000	0.9992
Ellagic acid	4.07	8.49	40	301.0	4.64	15.47	7.00	65–1000	0.9828
Rutin	4.11	0.45	40	609.0	3.43	11.43	0.99	65–1500	0.9985
Kaempferol-3-O- rutinoside	4.33	0.44	40	593.0	2.23	7.43	1.42	65–1500	0.9956
Kaempferol-3-O- glucoside	4.39	0.97	35	447.0	1.52	5.08	1.11	65–1500	0.9869
4,5- Dicaffeoylquinic acid	4.42	1.42	35	515.1	0.35	2.17	1.19	65–1500	0.9995
Myricetin	4.57	4.08	40	317.1	4.03	13.45	2.31	65–1500	0.9899
Resveratrol	4.69	0.64	35	227.0	1.26	4.19	1.77	65–1500	0.9983
Luteolin	5.05	1.84	40	285.0	2.66	8.85	1.02	65–1500	0.9975
Quercetin	5.06	0.33	40	301.0	9.24	30.79	2.22	65–1500	0.9983
Apigenin	5.43	2.95	40	269.0	7.68	25.61	1.60	65–1500	0.9939
Kaempferol	5.51	3.28	40	285.0	6.70	22.32	1.26	65–1500	0.9950
Crisin	6.43	2.46	40	253.1	5.13	17.11	1.31	65–1500	0.9945

[a] Mean value

[b,f] Relative standard deviation (n = 6) for experiments performed on different days

[c] $[M]^+$ for anthocyanins and $[M–H]^-$ for non-anthocyanins phenolic compounds

[d] Limit of detection

[e] Limit of quantification

[g] At least five standard solutions were used

The separation of anthocyanins was achieved with MeOH as the organic phase and 2% formic acid as additive in both aqueous and organic phase. The mobile phase had a low pH (2.0) to keep the anthocyanins in the flavylium form and thus enhance the separation, as reported previously by Gomez-Alonso et al. (2007). Furthermore, the flavylium cation form is easier to be detected in positive ionization mode.

In the second UPLC method, conditions were optimized to allow the separation of 22 non-anthocyanin phenolic compounds (Fig. 1b). The mixture of standard analytes included three pairs (p-coumaric acid and hydroxycinnamic acid; ellagic acid and quercetin; luteolin and kaempferol) of same molecular weight that were well distinguished. Concerning the organic mobile phase, MeOH and ACN were tested using the same gradient of anthocyanins analysis. However, the optimal separation was achieved using ACN instead of MeOH as the organic phase and 0.01% formic acid as additive, in both aqueous and organic phase, using a modified gradient. These mobile phases provided a better resolution and a fast elution of the peaks, resulting in a chromatography separation of non-anthocyanic polyphenols in 12 min. The combination of ACN, water and formic acid has been previously used to profile the polyphenols of E. oleracea (Gordon et al. 2012) in HPLC equipment. The developed method in this work is many times faster than some reported for polyphenolic elution by HPLC that can vary from 35 to 70 min (Anastasiadi et al. 2012; Gordon et al. 2012; Mulabagal and Calderon 2012).

Non-anthocyanic polyphenols standards were well solubilized in the initial acidified mobile phase of H_2O:ACN (95:5), mainly due to the low concentration of the working solutions, once the utilized instrument showed high sensitivity. Both negative and positive ionization modes were tested, but most of the analytes provided better MS detection in negative ionization mode, showing higher sensitivity. Therefore, the negative mode was applied to ionize these analytes. For both methods, the cone voltage parameter was estimated for each molecule to obtain a better peak area response for the analytes. To increase the method's sensitivity, the chromatographic runs were divided into individual acquisition channels based on the retention time of each molecular ion ±0.5 min around the peak. The dwell time was adjusted to ensure at least 12 data points per peak.

Performance parameters of methods

Assuring that the UPLC–MS methods had performances compatible with those required for routine analysis of polyphenolic compounds from berry fruits like E. edulis, E. oleracea, and others, the following parameters were considered for the evaluation of each method: selectivity, precision, linearity, detection and quantification limits. These parameters were calculated for each analyte from standard solutions, used to obtain the corresponding calibration curves, using the UPLC method developed (Table 1).

All five anthocyanins analyzed showed good peaks resolution. Concerning the non-anthocyanin polyphenolics, the lowest resolution was observed for the peaks of chlorogenic acid and catechin; hydroxycinnamic acid and ellagic acid; luteolin and quercetin (Table 1). However, the suggested method is sufficiently selective for monitoring these compounds, taking into account that each of their ions is entirely distinct. The repeatability of the methods was evaluated by assaying six replicate injections of each standard of the polyphenolic compound (250 μg/L), during three consecutive days. The responses measured on each chromatogram were the retention time of each peak and the corresponding concentration of each one. A small variation among the retention times has been observed, except for the ellagic acid, which showed the largest relative standard deviation (RSD), of 8.49%. The RSD values for concentration of the analytical standards ranged from 0.62 to 7.0%. The method's precision was not significantly affected by the amount of analytes used.

Limits of detection (LOD) and quantification (LOQ) are based on 3 and 10 times the signal-to-noise ratio, respectively. The LOD of the anthocyanins and other phenolic compounds were found within the range of 1.40–5.60 μg/L and 0.55–9.24 μg/L, respectively. The LOQ ranged from 4.7 to 18.50 μg/L and from 2.17 to 30.79 μg/L for anthocyanins and non-anthocyanins polyphenols, respectively. These LOD and LOQ were comparable to values previously reported for phenolic compounds, using HPLC–MS methods (Anastasiadi et al. 2012; Boros et al. 2010). Concerning the anthocyanins, these parameters were relatively lower when compared to those found in a different method developed for E. oleracea extracts (Dias et al. 2012). The method linearity was determined by repeated injections of standard solutions within a concentration range of 65–2000 μg/L. The correlation coefficients (R^2), LOD and LOQ values are summarized in Table 2.

Phenolic compounds identified in jussara extract

Extracts obtained from jussara pulp were used for chromatographic analyses, as presented in "Development of the UPLC methods" section. Table 2 displays the content of polyphenolic compounds on dry-weight basis tentatively identified in the jussara extract. Because of both high selectivity and high separation efficiency of UPLC-MS methods, thirteen phenolic compounds were found in the jussara extract (Figs. 2, 3) with concentrations ranging from 0.01 to 23 mg/g dry weight (Table 2). These 13

Table 2 Content of polyphenolic compounds on dry-weight basis in the jussara extract

Compound	Jussara extract (mg/g dry weight)	95% Confidence interval of the difference	
		Lower limit	Upper limit
Anthocyanins			
Cyanidin-3-glucoside	2.81 ± 0.14	2.45	3.17
Cyanidin-3-rutinoside	23.07 ± 0.27	22.37	23.76
Perlagonidin-3-glucoside	0.006 ± 0.001	0.003	0.009
Non-anthocyanin polyphenolics			
Catechin	Trace amounts[a]		
Ellagic acid	1.40 ± 0.15	1.05	1.78
Rutin	1.50 ± 0.02	1.45	1.55
Kaempferol-3-rutinoside	0.25 ± 0.01		
4,5-Dicaffeoylquinic acid	Trace amounts[a]		
Myricetin	0.66 ± 0.01	0.63	0.68
Luteolin	Trace amounts[a]		
Quercetin	1.02 ± 0.01	0.99	1.05
Apigenin	0.25 ± 0.01	0.22	0.27
Kaempferol	0.44 ± 0.01	0.41	0.46
Sum of the identified phenolic compounds	31.40 ± 0.62	30.68	32.10

[a] Below the limit of quantification. All data is presented as mean ± standard deviation of six replicates (n = 6)

compounds consisted of 3 anthocyanins (cyanidin-3-O-rutinoside, cyanidin-3-O-glucoside, and pelargonidin-3-O-glucoside), 5 flavonols (quercetin, rutin, myricetin, kaempferol, and kaempferol-3-rutinoside), 2 flavones (luteolin, and apigenin), 1 flavanol (catechin), and 2 phenolic acids (ellagic acid, and 4,5-dicaffeoylquinic acid).

The confidence interval was set at a confidence level of 95% (Table 2). Except for catechin, 4,5-dicaffeoylquinic acid and luteolin, that were bellow quantification limit, amounts quantified in the jussara extract are statistically significant. The sum of the concentrations of cyanidin-3-O-rutinoside, cyanidin-3-O-glucoside, and pelargonidin-3-O-glucoside obtained in this work (26 mg/g dry weight) was similar to the one results found by Brito et al. (2007) (29 mg/g dry weight) for jussara fruit. Corroborating our results, Bicudo et al. (2014) and Cardoso et al. (2015) also reported the presence of cyanidin-3-O-rutinoside and cyanidin-3-O-glucoside in different samples of jussara fruit. Cyanidin-3-O-rutinoside (peak 2, Fig. 2) was the major anthocyanin found in the jussara extract, representing in average 89% and 73% of the total anthocyanin and total phenolic compounds content, respectively. Cyanidin-3-O-glucoside (peak 1, Fig. 2) represented 11% of total anthocyanin content, followed by pelargonidin-3-O-glucoside, which was detected as <0.1% (peak 3, Fig. 2). These anthocyanins are the same as those reported in the literature for *E. edulis* (Brito et al. 2007) and *E. oleracea* (Rosso et al. 2008; Dias et al. 2012; Gordon et al. 2012).
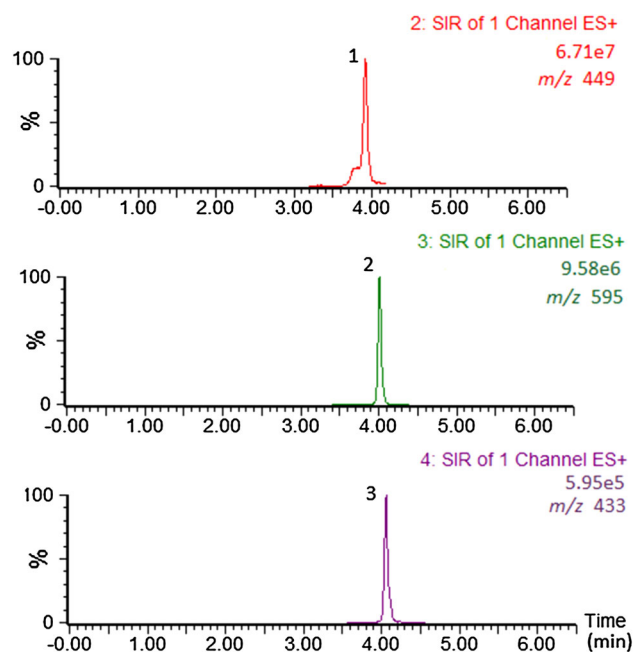
Fig. 2 Selected ion recording (SIR) chromatograms of the anthocyanins found in jussara extract, detected in positive ionization mode (ES+): cyanidin-3-O-glucoside (*1*), cyanidin-3-O-rutinoside (*2*) and perlagonidin-3-O-glucoside (*3*)

The jussara extract presented high amounts of rutin, ellagic acid and quercetin (>1 mg/g in dry weight), which were quantitatively the major non-anthocyanin phenolic in
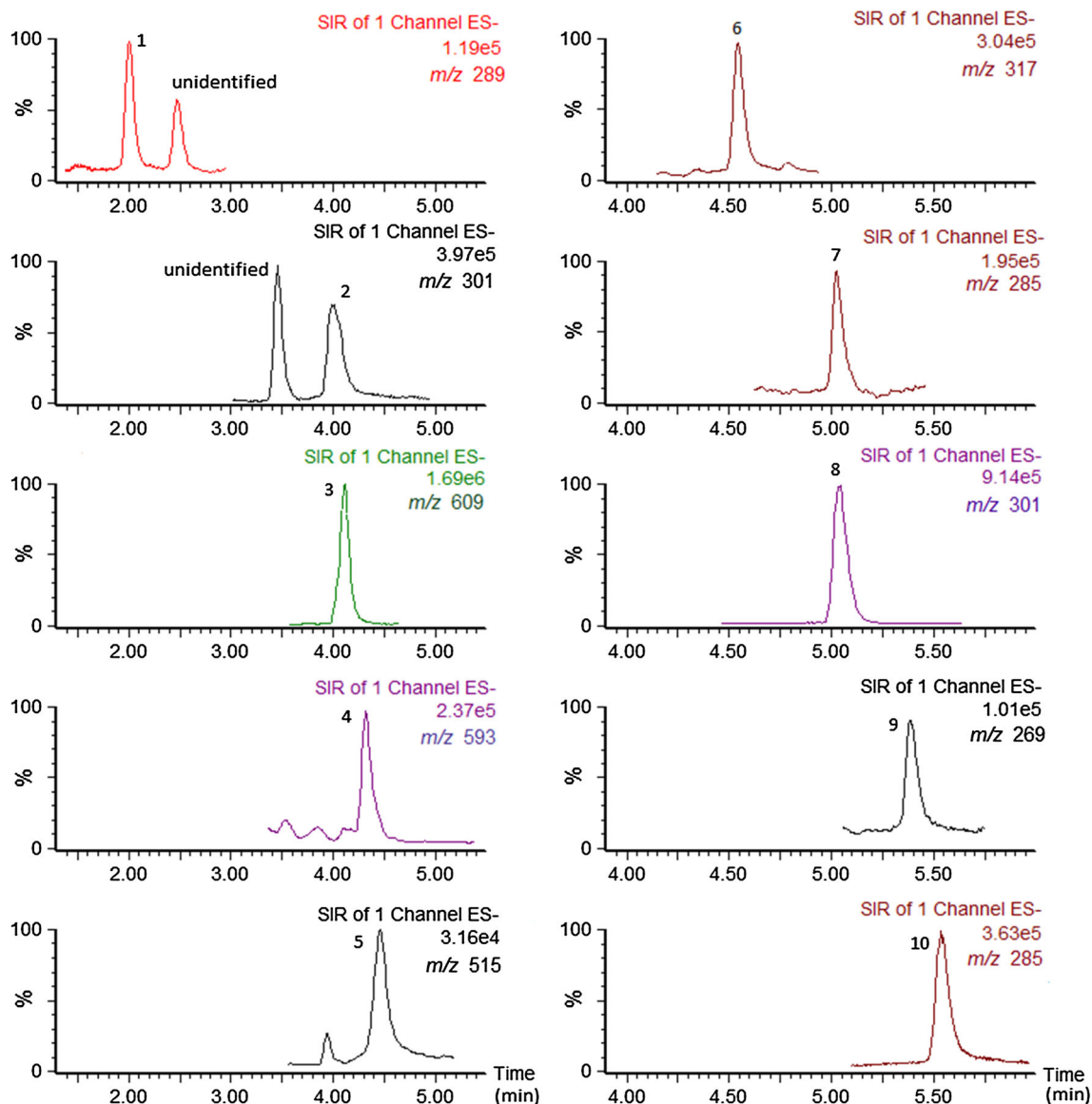
Fig. 3 Selected ion recording (SIR) chromatograms of non-anthocyanins polyphenolics found in jussara extract, detected in negative ionization mode (ES−): catechin (*1*), ellagic acid (*2*), rutin (*3*), kaempferol-3-rutinoside (*4*), 4,5-dicaffeoylquinic acid (*5*), myricetin (*6*), luteolin (*7*), quercetin (*8*), apigenin (*9*), kaempferol (*10*)

this extract (Fig. 3). Kaempferol-3-rutinoside, myricetin, apigenin and kaempferol were found in lower amounts (<1 mg/g in dry weight). Catechin, 4,5-dicaffeoylquinic acid and luteolin were detected in traces. Kaempferol-3-rutinoside, apigenin, ellagic acid, 4,5-dicaffeoylquinic acid and luteolin were identified for the first time in jussara extract.

However, in contrast to the results found in our study, Borges et al. (2013) have detected by HPLC–ESI–MS/MS benzoic, caffeic, chlorogenic, ferulic, protocatechuic, syringic, vanillic and *p*-coumaric acids in methanolic extracts of jussara. Besides these, Schulz et al. (2015) have evaluated the effect of ripening on phenolic composition of

jussara fruit and also detected aromadendrin, hispidulin, resveratrol and taxifolin in extracts. These changes in phenolic acids profile may be dependent on the growth region, climate conditions and maturity stage and on the parameters selected for the extract production as well as the analytical method used.

Conclusion

Rapid UPLC–ESI–MS methods for determination of 5 anthocyanins and 22 non-anthocyanin phenolic compounds were developed. These methods demanded short run times,

allowing the separation of polyphenolics that are dominant in other berry fruits. Moreover, they showed high sensitivity, precision, and reproducibility. Thirteen compounds, out of 27, were tentatively identified and quantified in jussara extracts. Extracts presented a high amount of anthocyanins, approximately 26 mg/g of dry weight extract from a total of 31 mg of phenolics compounds/g of dry weight extract. Cyanidin-3-O-rutinoside was the most abundant anthocyanin followed by cyanidin-3-O-glucoside and perlagonidin-3-O-glucoside. Rutin, ellagic acid and quercetin were the major non-anthocyanin phenolics found in the extract. The analysis of jussara extracts has demonstrated the presence of valuable phenolic compounds. This renders the jussara fruit a promising source of polyphenolics, mainly anthocyanins.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest

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