Food Chemistry 126 (2011) 1571–1578

Contents lists available at ScienceDirect

Food Chemistry

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

Identification of bioactive compounds from jambolão (*Syzygium cumini*) and antioxidant capacity evaluation in different pH conditions

Adelia F. Faria, Marcella C. Marques, Adriana Z. Mercadante *

Department of Food Science, Faculty of Food Engineering, University of Campinas (UNICAMP), Rua Monteiro Lobato 80, P.O. Box 6121, Campinas, SP 13083-862, Brazil

ARTICLE INFO

Article history:
Received 29 June 2010
Received in revised form 28 October 2010
Accepted 1 December 2010
Available online 7 December 2010

Keywords:
Syzygium cumini
Myrtaceae
Anthocyanins
Carotenoids
Flavonoids
HPLC-DAD-MS/MS
Antioxidant capacity
ORAC
Singlet oxygen
CIELAB colour

ABSTRACT

The composition of carotenoids and phenolic compounds from jambolão fruits (*Syzygium cumini*) was determined by HPLC-DAD-MS/MS. Two main carotenoids were found in the fruits, all-*trans*-lutein (43.7%) and all-*trans*-β-carotene (25.4%). The anthocyanin composition was characterised by the presence of 3,5-diglucosides of five out of six aglycones commonly found in foods. This pattern was also observed for the other flavonoids, since diglucosides of dihydromyricetin, methyl-dihydromyricetin and dimethyl-dihydromyricetin, along with myricetin glucoside and a galloyl-glucose ester were identified. Furthermore, the antioxidant capacity of a functional extract rich in anthocyanins was evaluated through the scavenging capacities of $ABTS^{\cdot+}$ and peroxyl radical (ORAC) and the protective effect against singlet oxygen ($^1O_2$). The TEAC values indicated that the hemiacetals/chalcones and quinonoidal bases species ($pH \geqslant 5$) possess higher scavenging capacity as compared to the flavylium cation ($pH < 3$). The functional extract also showed 60% of dimethylanthracene protection against $^1O_2$ and an ORAC value of 16.4 μmol Trolox/g fruit.

1. Introduction

Syzygium cumini fruit, known as jambolão, black plum, jambolan, Java plum or jamun, is a plant from the Myrtaceae family, originated in tropical Asia, specifically India. Its synonym names are *Eugenia jambolana* and *Eugenia cumini* (Veigas, Narayan, Laxman, & Neelwarne, 2007). Jambolão fruits are small, with 2–3 cm long, ovoid form with a purple-red to black colour when ripe, containing a fleshy pink or almost white pulp with astringent taste (Benherlal & Arumughan, 2007).

Due to the popular use of jambolão leaves and fruits to assist in the treatment of diabetes, the antioxidant properties of extracts from different parts of the plant were evaluated in recent years. For example, the seed kernel of the jambolão fruits showed high activity against the superoxide anion and hydroxyl radical when compared to standards, such as catechin and Trolox (Benherlal & Arumughan, 2007). In addition, a jambolão fruit extract showed antiproliferative and pro-apoptotic effects against breast cancer cells, but not toward the normal breast cells (Li et al., 2009a). Compared to other fruits, extracts from jambolão fruit showed high antioxidant activity induced by copper acetate in liposomes, while in the β-carotene-linoleic acid system, this activity was intermediate (Hassimotto, Genovese, & Lajolo, 2005). These beneficial effects are most probably related to the presence of bioactive compounds, such as carotenoids and phenolic compounds.

The major anthocyanins identified in jambolão were reported to be 3,5-diglucosides of delphinidin, petunidin and malvidin (Brito et al., 2007; Li et al., 2009a; Veigas et al., 2007). However, no information was found in the literature regarding the identification of non-anthocyanic phenolic compounds or of carotenoids in jambolão fruits. Therefore, the purpose of this study was to quantify some bioactive compounds present in jambolão fruits, and to identify the phenolic compounds and carotenoids by high performance liquid chromatography coupled to the diode array and mass

Abbreviations: AAPH, α,α′-azodiisobutyramidine dihydrochloride; ABTS, 2,2′-azinobis(3-ethylbenzthiazoline-6-sulphonic acid); APCI, atmospheric pressure chemical ionisation; BSA, bovine serum albumin; CA, slope of the linear fit obtained for the calibration curve (LOD determination); CE, catechin equivalent; cyd, cyanidin; DAD, diode array detector; DMA, dimethylanthracene; dpn, delphinidin; ESI, electrospray ionisation; FE, functional extract; GAE, gallic acid equivalent; glu, glucose; HPLC, high performance liquid chromatography; LOD, limit of detection; MA, monomeric anthocyanins; MB, methylene blue; MS, mass spectrometry; mvd, malvidin; NMR, nuclear magnetic resonance; ORAC, oxygen radical absorbance capacity; pnd, peonidin; ptd, petunidin; SD, standard deviation; SDS, sodium dodecyl sulphate; TAE, tannic acid equivalent; TEAC, Trolox-equivalent antioxidant capacity.

* Corresponding author. Tel.: +55 19 35212163; fax: +55 19 35212153.
E-mail address: azm@fea.unicamp.br (A.Z. Mercadante).

0308-8146
doi:10.1016/j.foodchem.2010.12.007

spectrometer detectors (HPLC-DAD-MS/MS). Furthermore, the antioxidant activity of a functional extract rich in anthocyanins was evaluated in different conditions of pH, through the scavenging capacity of both 2,2′-azinobis(3-ethylbenzthiazoline-6-sulphonic acid) ($ABTS^{\cdot+}$) and peroxyl radicals, along with the protective effect against singlet oxygen ($^1O_2$).

2. Materials and methods

2.1. Materials

Jambolão mature fruits, harvested in 2008, were directly obtained from producers in the region of Pelotas, Rio Grande do Sul, Brazil. The fruits were stored at −36 °C, and only the edible portion (pulp and peel) was homogenised before the extraction.

Standards of cyanidin 3-glucoside, cyanidin 3-galactoside, cyanidin 3-rutinoside, cyanidin 3,5-diglucoside, cyanidin 3-rhamnoside, malvidin 3-glucoside, malvidin 3,5-diglucoside, pelargonidin 3-glucoside, cyanidin, pelargonidin, quercetin 3-galactoside, quercetin 3-rhamnoside, epicatechin, and gallic, p-hydroxybenzoic, caffeic, coumaric, ferulic and ellagic acids were obtained from Extrasynthèse (Genay, France). Standards of rutin, quercetin 3-glucoside, quercetin, naringenin, luteolin, tannic and ascorbic acids were purchased from Sigma–Aldrich (Munich, Germany). Standards of naringin, myricetin, apigenin, kaempferol and catechin were obtained from Fluka (Steinheim, Germany). Standards of all-*trans*-lutein, all-*trans*-zeaxanthin, all-*trans*-β-cryptoxanthin, all-*trans*-β-carotene and all-*trans*-α-carotene, as well as the isomers 9-*cis*-, 13-*cis*- and 15-*cis*-β-carotene were provided by DSM Nutritional Products (Basel, Switzerland). All standards showed at least 95% purity, determined by HPLC-DAD.

The reagents 2,2′-azinobis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), potassium persulphate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), methylene blue (MB), dimethylanthracene (DMA), α,α′-azodiisobutyramidine dihydrochloride (AAPH, PM = 271.19 g/mol), fluorescein as sodium salt (MW = 376.27 g/mol) and bovine serum albumin (BSA) were purchased from Sigma–Aldrich and the Folin–Ciocalteau reagent was supplied by Merck (Darmstadt, Germany). Solvents, acids and salts used were pro analysis grade purchased from Labsynth (Diadema, Brazil). Solvents for HPLC were obtained from Merck or Mallinckrodt Baker (Philipsburg, USA). The water was purified by the Milli-Q system (Millipore, Billerica, USA).

For chromatographic analysis, samples and solvents were filtered using, respectively, membranes of 0.22 and 0.45 μm, both from Millipore.

2.2. Equipments

The quantitative analysis of total phenolics, flavonoids, monomeric anthocyanins, and tannins, as well as some antioxidant tests were performed on a spectrophotometer Agilent 8453 (Santa Clara, USA).

The CIELAB colour parameters (L^*, a^*, b^*) of the functional extract, diluted at 0.35%$_{v/v}$ in different buffer solutions (pH 1.0, 3.0, 5.0 and 7.0), were obtained in a spectrocolorimeter (Hunter, Color Quest XE model, Reston, VA, USA) using total transmittance, D65 illuminant and observation angle of 10°. The h_{ab} (hue) and C^*_{ab} (chroma) values were calculated according to Eqs. (1) and (2), respectively.

$$h_{ab} = \arctan\left(\frac{b^*}{a^*}\right) \quad (1)$$

$$C^*_{ab} = \sqrt{(a^*)^2 + (b^*)^2} \quad (2)$$

Steady-state illumination was utilised for the excitation of the photosensitizer MB and formation of $^1O_2$, the excitation source being a 150 W filament xenon lamp coupled to a red cut-off filter, allowing only the passage of light with wavelengths longer than 600 nm.

The method of oxygen radical absorbance capacity (ORAC) for the measurement of peroxyl radical scavenger capacity was carried out in a microplate reader Synergy Mx (Bio-Tek Instruments, Winooski, USA).

All chromatographic analysis were carried out on a Shimadzu HPLC (LC-20AD model, Kyoto, Japan) equipped with quaternary pump system, on line degasser and *Rheodyne* injection valve of 20 μl, connected in series to a diode array detector (DAD) (Shimadzu, SPD-M20A model) and a mass spectrometer (MS) with ion trap analyzer, equipped with electrospray (ESI) and atmospheric pressure chemical ionisation (APCI) interfaces (Bruker Daltonics, Esquire 4000 model, Bremen, Germany).

2.3. Extracts preparation

Anthocyanins were exhaustively extracted from 3.0 g of homogenised fruit using ethanol containing 1% HCl, while the other phenolic compounds were exhaustively extracted from 10.0 g, with methanol/water (8:2, v/v). Besides these two extracts, a third extract rich in anthocyanins was obtained with ethanol containing 5% H_3PO_4 as acidifying agent, called functional extract (FE), which was used to evaluate the antioxidant properties. This solvent combination was chosen due to its extractability capacity and/or acceptability for use in food products. All extracts (anthocyanins, phenolic compounds and FE) were obtained by stirring in a Metabo GE700 homogenizer (Nürtingen, Germany), followed by vacuum filtration. The extracts were concentrated in a rotary evaporator (T < 35 °C) and stored under nitrogen, at −36 °C. All extraction procedures were performed in duplicate. Before HPLC-DAD-MS/MS analysis, the anthocyanin extract was partially purified on a XAD-7 column (Sigma) in order to remove sugars.

The carotenoids were exhaustively extracted from 15.0 g of homogenised fruit (De Rosso & Mercadante, 2007a). The carotenoids present in the FE were isolated using liquid–liquid extraction with ethyl acetate. Both extracts were submitted to complete solvent evaporation in rotary evaporator (T < 40 °C), and stored under nitrogen at −36 °C.

Ascorbic acid extraction was carried out with 10.0 g of fruit or 10 mL of FE stirring with 30 mL of 1% oxalic acid aqueous solution, filtering, and additional washing of the sample with 10 mL of the extraction solution. The extract was transferred to a 50 mL volumetric flask, the volume was completed with the same solution used for extraction, and immediately submitted to HPLC-DAD analysis.

2.4. Quantitative analysis of phenolic compounds

The determination of total phenolic compounds was performed in both the FE and fruit extracts, by the reaction with Folin-Ciocalteau reagent (Singleton & Rossi, 1965). The quantification was based on the calibration curve of gallic acid (2.0–8.0 mg/L), and the results were expressed in mg gallic acid equivalent (GAE)/100 g sample.

The total flavonoid contents were determined in both the FE and fruit extracts, by reaction with $AlCl_3$ according to Zhishen, Mengcheng, and Jianming (1999). Briefly, the extracts were added to an aqueous solution of $NaNO_2$ 21.7 mM (final concentration). After 5 min, $AlCl_3$ 22.5 mM (final concentration) was added to the extract, and after 6 min, NaOH 0.2 M (final concentration) was added followed by measurement at 510 nm. The quantification was carried out with a calibration curve of catechin

(5.0–20.0 mg/L), and the results were expressed in mg catechin equivalent (CE)/100 g sample.

The monomeric anthocyanin (MA) contents were determined in both the FE and fruit extracts, through the differential pH method (Lee, Durst, & Wrolstad, 2005). MA content was calculated as equivalent of cyanidin 3-glucoside (cyd 3-glu), considering the molecular weight (MW) of 449.2 g/mol and molar absorption coefficient (ε) of 26,900 L/mol cm.

To determine the contents of tannins, the phenolic extract and FE were initially precipitated with BSA. After 15 min, the precipitate was collected and re-dissolved in an aqueous solution containing 34.7 mM of sodium dodecyl sulphate (SDS), 5%$_{v/v}$ triethanolamine and 20%$_{v/v}$ isopropanol. This solution was added to an acidic solution (HCl 2 mM final concentration) of $FeCl_3$ (final concentration of 2 mM), kept for 15–30 min, followed by an absorbance measurement at 510 nm (Waterman & Mole, 1994). The quantification was based on the calibration curve of tannic acid (0.2–1.2 mg/L), and the results expressed as mg tannic acid equivalent (TAE)/100 g sample.

2.5. Analysis by HPLC-DAD-MS/MS

The anthocyanins from the fruit extract and FE were separated on a C_{18} Shim-pack CLC-ODS column (5 μm, 250 × 4.6 mm i.d.) (Shimadzu, Canby, USA), using as mobile phase a linear gradient of water/methanol, both with 5%$_{v/v}$ formic acid, from 90:10 to 60:40 in 20 min, passing to 20:80 in 15 min and keeping this proportion for 5 min. The other phenolic compounds were separated on a C_{18}(2) Luna column (5 μm, 250 × 4.6 mm i.d.) (Phenomenex, Torrance, USA), using as mobile phase a linear gradient of water/acetonitrile, both with 2%$_{v/v}$ formic acid, from 93:7 to 86:14 in 25 min, passing to 80:20 in 10 min, to 70:30 in 7 min, and to 20:80 in 13 min, and keeping this proportion for 3 min. In both analyses, the flow rate was set at 0.9 mL/min and the column temperature was maintained at 29 °C. The UV–Vis spectra were acquired between 200 and 600 nm and the chromatograms were processed at 280, 320, 360 and 520 nm. After passing through the cell of the DAD, the flow from the column was split, allowing only 0.15 mL/min into the ESI source. For MS detection, the ESI ionisation source was used in either positive (ESI^+) or negative (ESI^-) modes, with capillary voltage at 2.5 and 1.5 kV, respectively. The output voltage of the capillary was 95.2 V for anthocyanins (ESI^+) and 120 V for the other phenolic compounds (ESI^+ and ESI^-). The other conditions in both modes were: end plate offset −500 V, drying gas (N_2) temperature of 325 °C and flow of 8 l/min, nebulizer at 30 psi. The MS/MS was acquired in automatic mode, applying fragmentation energy of 1.2 V. The scan range was from m/z 100 to 1000.

The carotenoids were separated on C_{30} YMC column (5 μm, 250 × 4.6 mm id) (Waters, Wilmington, USA), using the APCI ionisation source, according to the method previously described by De Rosso and Mercadante (2007a). Carotenoids were quantified by HPLC-DAD based on calibration curves obtained for all-*trans*-lutein, all-*trans*-zeaxanthin, all-*trans*-β-cryptoxanthin, all-*trans*-α-carotene and all-*trans*-β-carotene. The *cis* isomers, when present, were estimated using the calibration curve of the corresponding all-*trans* isomer.

The ascorbic acid was analysed by HPLC-DAD, using a C_{18} Shim-pack column described above and as mobile phase an aqueous solution of sulphuric acid at pH 2.5, in isocratic condition at a flow rate of 0.7 mL/min and a column temperature set at 25 °C. The chromatograms were processed at 254 nm. The limit of detection (LOD) of 0.01 mg/100 g was calculated from Eq. (3), using the parameters obtained from the calibration curve for ascorbic acid (5–60 μg/mL).

$$\mathrm{LOD} = 3.3 \times \left(\frac{\mathrm{SD}}{\mathrm{CA}}\right) \quad (3)$$

where SD is the standard deviation of the response for peak area and CA is the slope of the linear fit obtained for the calibration curve.

The identification of all compounds was performed using the combined data of the following parameters: elution order on reversed-phase column, co-chromatography with standards, and characteristics from the UV–Vis and mass spectra, compared with standards analysed in same conditions and with data available in the literature (Britton, Liaaen-Jensen, & Pfander, 2004; Cuyckens & Claeys, 2004; De Rosso & Mercadante, 2007a, 2007b; De Rosso et al., 2008; Fabre, Rustan, Hoffmann, & Quetin-Leclercq, 2001; Lin & Harnly, 2007; Wu & Prior, 2005).

2.6. Antioxidant activity

The $ABTS^{\cdot+}$ scavenging capacity test was carried out according to the method described by Re et al. (1999), under pH 1.0, 3.0, 5.0, 7.0 and 9.0 conditions, using the appropriate buffer for each pH. The FE was diluted in each buffer at a proportion of 0.7%$_{v/v}$. The diluted extract was added to the $ABTS^{\cdot+}$ solution (1:1$_{v/v}$ proportion) to achieve an initial $ABTS^{\cdot+}$ absorbance (at 734 nm) of 0.80 ± 0.02, and absorbance was immediately monitored at 734 nm for 15 min. The results were calculated based on a calibration curve of Trolox (3–20 μM), obtained for each condition of pH, and TEAC (Trolox-equivalent antioxidant capacity) values were expressed as μmol Trolox/g fruit.

The protection against $^1O_2$ was only performed under pH 1.0 and 3.0, using DMA (0.1 mM, final concentration) as actinometer and MB (10 μM, final concentration) as sensitiser, both dissolved in ethanol. The FE was diluted in each buffer at a proportion of 5%$_{v/v}$. The diluted extract was mixed with the MB solution at 1:1 (v/v) proportion, followed by addition of DMA. The reaction was monitored by spectrophotometric measurements in the range of 190–900 nm during 21 min. The percentage of protection was determined according to Eq. (4).

$$\%\mathrm{Protection} = \left(\frac{k_{\mathrm{DMA}} - k_{\mathrm{DMA+FE}}}{k_{\mathrm{DMA}}}\right) \times 100 \quad (4)$$

where k_{DMA} and k_{DMA+FE} were the first-order decay constants for DMA absorbance at 375 nm, in absence and presence, respectively, of functional extract.

In the other pH values, methylene blue became not soluble when mixed to the FE in different types of buffers; therefore, these experiments were not carried out under pH values from 5.0 to 9.0.

The peroxyl radical scavenging capacity of FE was determined by the oxygen radical absorbance capacity (ORAC) method (Huang, Ou, Hampsch-Woodill, Flanagan, & Prior, 2002). Briefly, the FE was 100 times diluted in phosphate buffer pH 7.4 (75 mM), mixed with a fluorescein solution (61.2 nM final concentration) and kept at 37 °C for 30 min. AAPH (19.1 mM final concentration) was added to the system, and the fluorescence at 528 nm ($\lambda_{excitation}$ = 485 nm) was monitored for 60 min at 37 °C. The results were calculated using a calibration curve of Trolox (16–128 μM), obtained under the same conditions, and expressed as TEAC (Trolox equivalent antioxidant capacity).

3. Results and discussion

3.1. Bioactive compounds

As can be seen in Table 1, all bioactive compounds in the functional extract are present in lower concentrations when compared to those found in the fruit. Considering that the functional extract was obtained with a solvent compatible to be added to foods, this difference can be attributed to two factors. One is related to the

Table 1
Contents of bioactive compounds in jambolão.

Bioactive compound	Fruit[a]	Functional extract (FE)[a,b]
Total phenols (mg GAE[c]/100 g sample)	148.3 ± 32.4	86.3 ± 3.9
Total flavonoids (mg CE[d]/100 g sample)	91.2 ± 15.7	20.8 ± 3.3
Monomeric anthocyanins (mg cyd 3-glu[e]/100 g sample)	210.9 ± 9.1	158.3 ± 26.7
Tannins (mg TAE[f]/100 g sample)	3.9 ± 0.8	0.2 ± 0.0
Ascorbic acid (mg/100 g)	<0.01	<0.01
Total carotenoids (μg/100 g sample)	89.2 ± 5.4	0.7 ± 0.1

[a] Average and standard deviation of duplicate analysis.
[b] FE – functional extract.
[c] GAE – gallic acid equivalent.
[d] CE – catechin equivalent.
[e] cyd 3-glu – cyanidin 3-glucoside.
[f] TAE – tannic acid equivalent.

exhaustive extraction carried out with the most appropriate solvent in order to quantify each compound in the fruit. The second factor is related to the extraction capacity of ethanol acidified with 5% of H_3PO_4 used to obtain the FE. This solvent has a polar character and consequently provides a worse extraction of apolar compounds, such as carotenoids. In addition, ethanol is less efficient than methanol/water for extraction of non-anthocyanic phenolic compounds, and the use of phosphoric acid as acidifying agent is less efficient for anthocyanin extraction as compared to hydrochloric acid.

The lower tannin content found in the FE as compared to the fruit one is a favourable feature to the FE application in food products, since, as a general trend, high tannin contents are not desirable in foods due to the astringent flavour, among other effects, that these compounds may cause. No other studies in the literature applied BSA precipitation and ferric chloride reaction for tannin determination in jambolão fruits.

Higher contents of phenolic compounds in both the fruit and FE were expected, even though a reasonable variability was observed in the levels found in the literature for this fruit, 236–390 mg GAE/100 g of fruit (fresh weight) (Benherlal & Arumughan, 2007; Luximon-Ramma, Bahorun, & Crozier, 2003).

On the other hand, the content of flavonoids found in jambolão fruits in this study was about 7–13 times higher than those previously reported, 13.5 mg/100 g (Luximon-Ramma et al., 2003) and 7 mg/100 g (Benherlal & Arumughan, 2007). This difference can be attributed to the inherent variability of the raw material, as well as to differences in methodology or standard used.

The monomeric anthocyanins content (211 mg/100 g) was in the same range as those found in literature for jambolão fruits, 134 mg cyd 3-glu/100 g (fresh weight) (Benherlal & Arumughan, 2007) and 230 mg cyd 3-glu/100 g (dry basis) (Veigas et al., 2007).

When compared to other fruits from the Myrtaceae family, jambolão showed a high content of monomeric anthocyanins (211 mg/100 g), as compared to those reported for camu–camu (*Myrciaria dubia*), 30–54 mg/100 g (Zanatta, Cuevas, Bobbio, Winterhalter, & Mercadante, 2005) and *Eugenia myrtifolia*, 33 mg/100 g (Longo, Scardino, Vasapollo, & Blando, 2007). On the other hand, the content of total carotenoids present in camu–camu, 355–1095 μg/100 g (Zanatta & Mercadante, 2007) was higher than what was found in jambolão fruits (Table 1).

3.2. Profile of phenolic compounds

Table 2 presents the chromatographic, UV–Vis and mass spectrometry characteristics of anthocyanins from jambolão fruit. The chromatogram obtained for these pigments and their structures are shown, respectively, in Fig. S1 and S2 from Supplementary data.

The composition of anthocyanins from jambolão was marked by the presence of different aglycones diglucosides. Five of the six aglycones most commonly found in foods were identified by tandem-MS: delphinidin (dpn, *m*/*z* 303), cyanidin (cyd, *m*/*z* 287), petunidin (ptd, *m*/*z* 317), peonidin (pnd, *m*/*z* 301) and malvidin (mvd, *m*/*z* 331). In all anthocyanins, the hexose was assigned as glucose considering the standards available and that glucose was the only monosaccharide previously found after acid hydrolysis of an anthocyanin extract obtained from fruits of *S. cumini* (Veigas et al., 2007). For diglycosilated anthocyanins (peaks 1, 2, 3, 4a, 5 and 6a), the presence of two glucose unities glycosylated at different positions (probably 3 and 5) rather than a disaccharide at position 3 was assigned considering the presence of two fragments derived from two consecutive losses of 162 u, instead of a fragment resulting from a single loss of 324 u, as reported in previous studies (De Rosso et al., 2008; Wu & Prior, 2005). Moreover, the presence of 3,5-diglucosides of dpd, cyd, ptd, pnd, and mvd in jambolão was recently confirmed by nuclear magnetic resonance (NMR) (Li, Zhang, & Seeram, 2009b). The identification of cyd 3-glucoside (peak 6b), cyd 3,5-diglucoside (peak 2), mvd 3-glucoside (peak 8) and mvd 3,5-diglucoside (peak 5) was confirmed by co-chromatography with the respective standards.

Two anthocyanins co-eluted in peak 6. The presence of delphinidin acetyl-diglucoside (peak 6a) together with cyanidin 3-glucoside (peak 6b) was verified by the different molecular ions. The

Table 2
Chromatographic and spectroscopic characteristics, and composition of anthocyanins from jambolão, obtained by HPLC-DAD-MS/MS.

Peak[a]	Anthocyanin	t_R (min)[b]	λ_{max} (nm)[c]	$[M]^+$ (*m*/*z*)	MS/MS fragment ions (*m*/*z*)	Fruit (mg cyd 3-glu[e]/100 g)[f]	FE[d]
1	Delphinidin 3,5-diglucoside	10.3–10.4	274, 523	627	465[M−162]$^+$, 303[M−162-162]$^+$	95.6 ± 4.2	59.0 ± 0.5
2	Cyanidin 3,5-diglucoside	11.8–11.9	276, 515	611	449[M−162]$^+$, 287[M−162-162]$^+$	8.8 ± 0.4	6.7 ± 0.2
3	Petunidin 3,5-diglucoside	12.8	274, 525	641	479[M−162]$^+$, 317[M−162-162]$^+$	68.0 ± 1.7	53.5 ± 1.1
4a	Peonidin 3,5-diglucoside	14.0–14.1	275, 525	625	463[M−162]$^+$, 301[M−162-162]$^+$	4.7 ± 0.7	7.1 ± 2.3
4b	Delphinidin 3-glucoside			465	303[M−162]$^+$		
5	Malvidin 3,5-diglucoside	14.9	274, 526	655	493[M−162]$^+$, 331[M−162-162]$^+$	32.0 ± 1.5	29.2 ± 2.1
6a	Delphinidin acetyl-diglucoside	15.7–15.9	278, 519	669	507[M−162]$^+$, 465[M−204]$^+$, 303[M−162-204]$^+$	0.4 ± 0.0	0.3 ± 0.1
6b	Cyanidin 3-glucoside			449	287[M−162]$^+$		
7	Petunidin 3-glucoside	17.0–17.3	275, 527	479	317[M−162]$^+$	1.1 ± 0.0	1.7 ± 0.4
8	Malvidin 3-glucoside	19.4–19.6	285, 528	493	331[M−162]$^+$	0.4 ± 0.1	0.8 ± 0.2

[a] Peaks numbered according to Supplementary Fig. 1.
[b] Range of retention time on C_{18} Shim-pack column.
[c] Linear gradient of water/methanol, both with 5%$_{v/v}$ formic acid.
[d] FE – functional extract.
[e] cyd 3-glu – Cyanidin 3-glucoside.
[f] Calculated using the percentage of the area and the content of monomeric anthocyanins given in Table 1.

$[M]^+$ at m/z 669 led to MS/MS fragments at m/z 507$[M-162]^+$, 465$[M-204]^+$ and 303$[M-162-204]^+$ (Table 2). In this case, losses of 162 u and 204 u corresponded, respectively, to a unit of hexose and of an acetylated hexose (162 + 42 u) (Cuyckens & Claeys, 2004), and the fragment at m/z 303 is characteristic of the aglycone delphinidin. Furthermore, the elution order in relation to dpn 3,5-diglucoside is consistent with what is expected from the reversed-phase elution, e.g., the acylated anthocyanins elute after their corresponding non-acylated anthocyanins (Wu & Prior, 2005).

The major anthocyanins found in jambolão were delphinidin 3,5-diglucoside (45%), petunidin 3,5-diglucoside (32%) and malvidin 3,5-diglucoside (15%). These results are consistent with those reported in previous studies with jambolão fruits, where the major anthocyanins were identified as 3,5-diglucosides of delphinidin (23–33%), petunidin (32–35%) and malvidin (21–38%) (Brito et al., 2007; Li et al., 2009a; Veigas et al., 2007). In addition to these anthocyanins, Brito et al. (2007) and Li et al. (2009a, 2009b) also identified 3,5-diglucosides of cyanidin and peonidin.

The phenolic compounds shown in Table 3 (chromatogram in Fig. S3 from Supplementary data) were mainly identified by the mass spectra characteristics, since ionisation in the positive and negative modes gave complementary information, such as the case where only the protonated molecule ($[M+H]^+$) with sodium adduct $[M+Na]^+$ was detected in the positive mode. The presence of the deprotonated molecule ($[M-H]^-$) allowed the confirmation of the molecular weight of the compounds.

The identification of gallic acid (peak 2) was based on the characteristics of UV–Vis and mass spectra (Table 3) compared to literature data (Cuyckens & Claeys, 2004; Nuengchamnong & Ingkaninan, 2009) and confirmed by co-chromatography. This phenolic acid showed λ_{max} at 271 nm, characteristic of phenolic acids derived from hydroxybenzoic acid. Moreover, the mass spectra obtained from both ESI^+ (fragment at m/z 153) and ESI^- ($[M-H]^-$ at m/z 169) showed the same characteristics as the ones obtained from the standard analysed under the same conditions.

Peak 1 was tentatively identified as galloyl-glucose ester based on the elution order on reversed phase relative to free gallic acid (peak 2), detection of $[M-H]^-$ at m/z 331, and loss of 162 u, equivalent to the elimination of an hexose unit, giving the fragment ion at m/z 169 corresponding to gallic acid. The $[M+Na]^+$ at m/z 355 was observed in the ESI^+ analysis. Furthermore, this compound also showed λ_{max} at 278 nm, characteristic of phenolic acids. Moreover, the galloyl-glucose ester (peak 1) showed the same MS/MS fragmentation pattern as the galloyl-glucose ester found in jambolão wine (Nuengchamnong & Ingkaninan, 2009).

With regard to flavonols, myricetin (peak 12) was identified by co-chromatography with standard, UV–Vis and mass spectra characteristics (Table 3), which are consistent with those expected for this flavonol (Fabre et al., 2001; Lin & Harnly, 2007) and for the standard analysed under the same conditions.

Peaks 8, 9, 10 and 11 were identified as myricetin glucoside, myricetin pentoside, myricetin rhamnoside and myricetin acetyl-rhamnoside, respectively. The following elution order is expected on reversed phase for the same aglycone: hexoside < pentoside < deoxyhexoside, and acylated derivatives elute after their non-acylated flavonoids (Lin & Harnly, 2007; Wu & Prior, 2005). In addition, the λ_{max} values at 349–355 nm, about 20 nm lower than the λ_{max} of myricetin (371 nm), indicate the typical hypsochromic effect of flavonol glycosides in relation to its aglycone (Lin & Harnly, 2007). The mass spectra indicated the presence of the aglycone at m/z 319 (ESI^+) and at m/z 317 (ESI^-), which corresponds to myricetin. In addition, the MS/MS fragmentation pattern obtained from these ions (m/z at 319 and m/z at 317) showed the same fragments at m/z 301, 273, 245, 165 and 153 as those found for myricetin. In the case of myricetin glucoside (peak 8), the loss of 162 u, both in positive and negative modes, indicated the presence of an hexose in the molecule, whereas the loss of 132 u indicated the presence of a pentose in peak 9 (myricetin pentoside). However, the analysis by MS itself does not allow distinguishing whether the sugar is xylose or arabinose, which are the most commonly pentoses found in fruits. For myricetin rhamnoside (peak 10), the loss of 146 u from $[M-H]^-$ (m/z at 463) is characteristic of a deoxyhexose unit, and rhamnose is the only deoxyhexose found in fruit flavonoids. Finally, the MS/MS spectrum of myricetin acetyl-rhamnoside (peak 11) showed a loss of 188 u, corresponding to an acetylated rhamnose unit (146 + 42 u) (Cuyckens & Claeys, 2004; Mahmoud, Marzouk, Moharram, El-Gindi, & Hassan, 2001). The C3 position is the most likely location for all these glycosides (Cuyckens & Claeys, 2004).

Table 3
Chromatographic and spectroscopic characteristics, obtained by HPLC-DAD-MS/MS, of non-anthocyanic phenolic compounds from jambolão.

Peak[a]	Compound (class)	t_R (min)[b]	λ_{max}[b] (nm)	$[M+H]^+$/$[M+Na]^+$ (m/z)	MS/MS in ESI^+ (m/z)	$[M-H]$ (m/z)	MS/MS in ESI^- (m/z)
1	Galloyl-glucose ester (phenolic acid)	4.2	278	n.d.[c]/355	293, 251, 263, 233, 185	331	271, 241, 211, 169$[M-H-162]^-$, 125
2	Gallic acid (phenolic acid)	5.5	271	n.d.	153$[M+H-18]^+$[d], 125	169[d]	125[d]
3	Dihydromyricetin diglucoside (flavanonol)	7.4	336	645/667	483[d]/505$[M+Na-162]^+$, 487, 343$[M+Na-162-162]^+$, 325	643	481$[M-H-162]^-$, 463, 355, 319$[M-H-162-162]^-$, 301
4	Unidentified	9.2	340	n.d.	n.d.	643	505, 481, 463, 355, 301, 283
5	Dihydroquercetin diglucoside (flavanonol)	11.1	337	n.d./651	489$[M+Na-162]^+$, 471, 327$[M+Na-162-162]^+$, 309	627	465$[M-H-162]^-$, 303$[M-H-162-162]^-$, 285
6	Methyl-dihydromyricetin diglucoside (flavanonol)	13.4	338	n.d./681	663, 519$[M+Na-162]^+$, 501, 357$[M+Na-162-162]^+$, 339	657	495$[M-H-162]^-$, 477, 333$[M-H-162-162]^-$, 315
7	Dimethyl-dihydromyricetin diglucoside (flavanonol)	18.8	338	n.d./695	677, 533$[M+Na-162]^+$, 515, 371$[M+Na-162-162]^+$,353	671	509$[M-H-162]^-$, 371, 347$[M-H-162-162]^-$, 329, 261
8	Myricetin glucoside (flavonol)	34.1	355	481/503	319$[M+H-162]^+$/341$[M+Na-162]^+$, 185	479	317$[M-H-162]^-$
9	Myricetin pentoside (flavonol)	37.5	355	n.d./n.d.	319[d], 285, 273, 245, 153	449	317$[M-H-132]^-$
10	Myricetin rhamnoside (flavonol)	38.3	349	n.d./n.d.	319[d], 301, 273, 245, 165, 153	463	317$[M-H-146]^-$
11	Myricetin acetyl-rhamnoside (flavonol)	44.7	348	n.d./n.d.	319, 273, 245, 165, 153	505	317$[M-H-188]^-$
12	Myricetin (flavonol)	45.1	371	319/n.d.	301, 273, 245, 217, 165, 153	317	271, 179, 151

[a] Peaks numbered according to Supplementary Fig. 2.
[b] Linear gradient of water/acetonitrile, both with 2%$_{v/v}$ of formic acid.
[c] n.d.: Not detected.
[d] Fragments detected only in MS (in source).

For flavanonols, considering the biosynthetic flavonoid pathway (proposed in Fig. S4 from Supplementary data), the aglycones at *m*/*z* 321 (ESI^+, peak 3) and at *m*/*z* 305 (ESI^+, peak 5) were identified as dihydromyricetin and dihydroquercetin, respectively, since these flavanonols are precursors of myricetin (peak 12). Considering that simple flavonoids with a hydroxyl in ring B may be modified during biosynthesis through hydroxylation and methylation reactions (Heller & Forkmann, 1994), the aglycones at *m*/*z* 335 (ESI^+, peak 6) and at *m*/*z* 349 (ESI^+, peak 7) were identified as methyl and dimethyl derivatives of dihydromyricetin diglucoside (peak 3) (Fig. S3 from Supplementary data). As discussed for diglycosylated anthocyanins, in the case of these four flavonoids, the occurrence of both MS/MS fragments $[M-H-162]^-$ and $[M-H-162-162]^-$ indicates the presence of two glucose molecules in different positions, probably at carbons 3 and 7, since these positions are favoured for glycosylation in flavanonols and flavonols, and the occurrence of 5-glucoside is rare in flavonoids with carbonyl at C4 (Cuyckens & Claeys, 2004).

For all flavonoids with losses of 162 u, galloyl-glucose ester (peak 1), myricetin glucoside (peak 8) and diglucosides of dihydromyricetin (peak 3), dihydroquercetin (peak 5), methyl-dihydromyricetin (peak 6), and dimethyl-dihydromyricetin (peak 7), the hexose was assigned as glucose due to the fact that this monosaccharide was the only hexose found in the anthocyanins identified in jambolão in the present and previous studies (Brito et al., 2007; Li et al., 2009a, 2009b; Veigas et al., 2007).

This is the first time that the identification of non-anthocyanic flavonoids is reported in jambolão fruits. However, gallic acid, myricetin, myricetin 3-O-α-L-rhamnopyranoside and myricetin 3-O-(4″-O-acetyl)-α-L-rhamnopyranoside, all found in the fruit, were previously identified through MS and NMR in jambolão leaves (Mahmoud et al., 2001).

3.3. Composition of carotenoids

The carotenoids found in jambolão were identified based on the combined information obtained from the elution order on C_{30} column, and characteristics of UV–Vis and mass spectra (Table 4) compared to standards and published data (Britton et al., 2004; De Rosso & Mercadante, 2007a, 2007b). The MS/MS fragments, characteristic of the polyenic chain and functional groups, allowed the confirmation of the assigned protonated molecule. The identification of all-*trans*-lutein (peak 4), all-*trans*-zeaxanthin (peak 5), all-*trans*-β-cryptoxanthin (peak 7), all-*trans*-α-carotene (peak 11), and all-*trans*-β-carotene (peak 12) was confirmed by co-chromatography with standards. A detailed description of carotenoid identification in fruits using the information above was already reported by De Rosso and Mercadante (2007a, 2007b).

The profile of carotenoids from jambolão is marked by the presence of all-*trans*-lutein, 43.7% of the total carotenoids, and all-*trans*-β-carotene (25.4%), along with their *cis* isomers (Table 4, Fig. S5 and S6 from Supplementary data).

As far as we are concerned, there are no other studies reporting the composition of carotenoids from jambolão. The profile of jambolão carotenoids is similar to that of camu–camu (*M. dubia*), other fruit also belonging to the Myrtaceae family, where the major carotenoids were all-*trans*-lutein (45.2–55.0%) and β-carotene (13.0–20.5%) (Zanatta & Mercadante, 2007).

3.4. Antioxidant capacity of the functional extract

Considering that the jambolão functional extract has high contents of phenolic compounds, mainly anthocyanins, and negligible carotenoids (Table 1), the following discussion about antioxidant activity was based on the anthocyanins behaviour.

The same dilution of FE used for the $ABTS^{\bullet+}$ test in buffer was used to measure the UV–Vis spectra (data not shown) and the CIELAB colour parameters in all pH conditions (1.0, 3.0, 5.0, 7.0 and 9.0). These results are shown in Table 5.

The UV–Vis spectra and the colour characteristics, together with the values of absorbance at 520 nm (Table 5) reveal the displacement of the characteristic equilibrium showed by anthocyanins at different pH conditions. At pH 1.0 the anthocyanins were predominantly in the flavylium cation form, whereas the proportion of this form significantly decreased at pH 3.0 and almost disappeared at pH 5.0. In fact, at pH 5.0 the absence of absorption bands in the visible spectrum indicates that the anthocyanins present in the functional extract were mostly in the colourless forms of hemiacetals and/or chalcones (Table 5).

Colour parameters are consistent with the results obtained by UV–Vis, considering that at pH 1 the hue (h_{ab}) value was in the red-purple region, and the chroma value was 2–20 times higher

Table 4
Chromatographic and spectroscopic characteristics, and contents of carotenoids from jambolão, obtained by HPLC-DAD-MS/MS.

Peak[a]	Compound	t_R (min)	λ_{max} (nm)[b]	%III/II	$\%A_B/A_{II}$	$[M+H]^+$ (*m*/*z*)	MS/MS fragment ions (*m*/*z*)	μg/100 g
1	*cis*-Neoxanthin or *cis*-violaxanthin	7.9	411, 435, 463	n.c.[c]	n.c.	601	$583[M+H-18]^+$, $565[M+H-18]^+$, $547[M+H-18-18-18]^+$, 221	0.6 ± 0.0
2	*cis*-Lutein	10.1	328, 413, 439, 465	33	n.c.	569	$551[M+H-18]^+$, $533[M+H-18-18]^+$, $495[M+H-18-56]^+$	1.3 ± 0.1
3	*cis*-Lutein	11.1	328, 413, 438, 465	33	n.c.	569	$551[M+H-18]^+$, $533[M+H-18-18]^+$, $495[M+H-18-56]^+$	0.7 ± 0.1
4	All-*trans*-lutein	12.0	420, 444, 472	66	0	569	$551[M+H-18]^+$, $533[M+H-18-18]^+$, $495[M+H-18-56]^+$, $477[M+H-92]^+$	39.0 ± 2.2
5	All-*trans*-zeaxanthin	14.1	423, 450, 476	25	0	569	$551[M+H-18]^+$, $533[M+H-18-18]^+$, $477[M+H-92]^+$	1.7 ± 0.0
6	Phytoene	18.7	272, 285, 294	0	0	545	489, 435, 395, $339[M-205]^+$	5.6 ± 0.9
7	All-*trans*-β-cryptoxanthin	22.7	424, 451, 477	25	0	553	$535[M+H-18]^+$, 495	0.3 ± 0.1
8	Phytofluene	23.9	329, 347, 366	n.c.	n.c.	543	461, $406[M+H-137]^+$, $337[M-205]^+$	2.9 ± 0.0
9	15-*cis*-β-Carotene	25.7	420, 449, 472	n.c.	n.c.	537	$444[M-92]^+$	3.1 ± 0.1
10	13-*cis*-β-Carotene	27.0	335, 420, 444, 470	17	41	537	$444[M-92]^+$	3.8 ± 0.0
11	All-*trans*-α-carotene	29.3	420, 447, 472	n.c.	0	537	$481[M+H-56]^+$, $444[M-92]^+$	2.7 ± 0.0
12	All-*trans*-β-carotene	33.5	425, 451, 478	27	0	537	$444[M-92]^+$	22.7 ± 1.6
13	9-*cis*-β-Carotene	35.7	337, 420, 447, 472	25	4	537	$444[M-92]^+$	4.9 ± 0.2

[a] Peaks numbered according to Supplementary Fig. 3.
[b] Linear gradient of methanol/MTBE.
[c] n.c.: Not calculated.

Table 5
TEAC values, percentage of protection against $^1O_2$, CIELAB colour parameters and absorbance at 520 nm obtained for the functional extract (FE) in different pH conditions.

Buffer – pH	Abs_{520nm}[a]	CIELAB colour parameters[a]			ABTS ($TEAC_{15min}$)[b]	$^1O_2$ (% protection)[c]	ORAC (TEAC)[b]
		L^*	h_{ab}	C^*			
KCl/HCl[d] – pH 1.0	0.1188	96.3	340°	9.9	4.8 ± 0.6	60.6 ± 4.1	n.d.[h]
Citrate or acetate[e,f] – pH 3.0	0.0181	100.0	354°	1.4	5.8 ± 0.2	62.3 ± 1.1	n.d.
Citrate[e] – pH 5.0	0.0025	99.6	114°	0.4	12.7 ± 1.1	n.d.	n.d.
Phosphate[g] – pH 7.0 or 7.4	0.0374	95.7	266°	5.1	9.7 ± 0.9	n.d.	16.4 ± 0.1
Borate[e] – pH 9.0	0.0406	n.a.[i]	n.a.	n.a.	11.0 ± 0.6	n.d.	n.d.

[a] Proportion of extract in buffer solutions of 0.35%$_{v/v}$.
[b] mol Trolox/g fruit.
[c] Proportion of extract in the reaction: 2.45%$_{v/v}$ (represents 2.1 g MA/mL).
[d] 0.25 M.
[e] 0.4 M.
[f] Citrate buffer for ABTS and colour measurements and acetate buffer for $^1O_2$.
[g] 0.4 M and pH 7.0 for ABTS, 0.075 M and pH 7.4 for ORAC.
[h] n.d.: Not detected.
[i] n.a.: Not determined.

than those obtained at other pH conditions (Table 5). In addition, the FE had the lowest values of C^* at pH 3 and 5 (1.4 and 0.5, respectively) due to high concentration of the colourless forms. Finally, the bathochromic shift in the UV–Vis spectra observed at pH 7.0 and 9.0 as compared to pH 1.0, along with the colour characteristics at pH 7 ($C^* = 5.1$ and h_{ab} in blue region) indicated a shift in the equilibrium towards formation of the quinonoidal bases.

The values of the decay constants (k_{DMA} and k_{DMA+FE}), used to calculate the percentage of protection against the $^1O_2$ (Table 5), were obtained from exponential fits for the first-order decay curves of DMA at 375 nm, in the presence and absence of jambolão FE at pH 1.0 and 3.0 conditions (data not shown). The proportion of functional extract used in these analyses (2.45%$_{v/v}$) was equivalent to monomeric anthocyanin concentration of 2.1 μg/ml. The results obtained (about 60% of protection at both pH conditions) corresponds to an activity higher than those reported by Wang and Jiao (2000), where percentages of protection against the $^1O_2$ between 8% (blueberry) and 15% (strawberry) were obtained when a juice proportion of 5%$_{v/v}$ was used.

Regarding the $ABTS^{\cdot+}$ scavenging capacity, the TEAC value at pH 5 was 2.2–2.7 times higher when compared to TEAC values at pH 1.0 and 3.0 (Table 5). These results indicated that the colourless forms of anthocyanins tend to have a greater free radical scavenging capacity than the flavylium cation form. Since the TEAC values under pH 7.0 and 9.0 conditions were similar to the one obtained at pH 5.0, both hemiacetals/chalcones and quinonoidal base forms show similar $ABTS^{\cdot+}$ scavenging capacities. The increase in the free radical scavenging capacity of anthocyanins with increasing pH was due to the higher reducing capacity showed by the colourless (hemiacetals/chalcones) and quinonoidal base forms of anthocyanins as compared to the flavylium cation species (Vieyra et al., 2009).

TEAC values obtained at pH 5.0–9.0 (9.7–12.7 μmol Trolox/g fruit) are in the same range as the ones reported for jambolão fruits (15 μmol Trolox/g fruit, unbuffered aqueous solution) (Luximon-Ramma et al., 2003). The results for both TEAC (9.7 μmol Trolox/g fruit, pH 7.0) and ORAC (16.4 μmol Trolox/g fruit, pH 7.4) found in this study were also close to the ones obtained for juice samples of different varieties of cherry, 20–26 μmol Trolox/g fruit for $ABTS^{\cdot+}$, and 12–19 μmol Trolox/g fruit for ORAC, both at pH 7.4 (Blando, Gerardi, & Nicoletti, 2004).

In summary, two very important classes of bioactive compounds were characterised in jambolão fruit, and for the first time the compositions of carotenoids and of non-anthocyanic phenolic compounds were reported. The free radical scavenging capacity of the jambolão functional extract varied according to the pH values, with a tendency to increased activity at higher pH values. Regarding the protection against singlet oxygen, the functional extract showed higher antioxidant features as compared to those from other fruits rich in anthocyanins.

Acknowledgments

The authors acknowledge Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2010.12.007.

References

Benherlal, P. S., & Arumughan, C. (2007). Chemical composition and in vitro antioxidant studies on *Syzygium cumini* fruit. *Journal of the Science of Food and Agriculture, 87*, 2560–2569.
Blando, F., Gerardi, C., & Nicoletti, I. (2004). Sour cherry (*Prunus cerasus* L.) anthocyanins as ingredients for functional foods. *Journal of Biomedicine and Biotechnology, 5*, 253–258.
Brito, E. S., Araújo, M. C. P., Alves, R. E., Carkeet, C., Clevidence, B. A., & Novotny, J. A. (2007). Anthocyanins present in selected tropical fruits: Acerola, jambolão, jussara, and guajiru. *Journal of Agricultural and Food Chemistry, 55*, 9389–9394.
Britton, G., Liaaen-Jensen, S., & Pfander, H. (2004). *Carotenoids handbook*. Basel: Birkhäuser.
Cuyckens, F., & Claeys, M. (2004). Mass spectrometry in the structural analysis of flavonoids. *Journal of Mass Spectrometry, 39*, 1–15.
De Rosso, V. V., Hillebrand, S., Montilla, E. C., Bobbio, F. O., Winterhalter, P., & Mercadante, A. Z. (2008). Determination of anthocyanins from acerola (*Malpighia emarginata* DC.) and açai (*Euterpe oleracea* Mart.) by HPLC-PDA-MS/MS. *Journal of Food Composition and Analysis, 21*, 291–299.
De Rosso, V. V., & Mercadante, A. Z. (2007a). Identification and quantification of carotenoids, by HPLC-PDA-MS/MS, from Amazonian fruits. *Journal of Agricultural and Food Chemistry, 55*, 5062–5072.
De Rosso, V. V., & Mercadante, A. Z. (2007b). HPLC-PDA-MS/MS of anthocyanins and carotenoids from dovyalis and tamarillo fruits. *Journal of Agricultural and Food Chemistry, 55*, 9135–9141.
Fabre, N., Rustan, I., Hoffmann, E., & Quetin-Leclercq, J. (2001). Determination of flavone, flavonol, and flavanone aglycones by negative ion liquid chromatography electrospray ion trap mass spectrometry. *Journal of the American Society for Mass Spectrometry, 12*, 707–715.
Hassimotto, N. M. A., Genovese, M. I., & Lajolo, F. M. (2005). Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps. *Journal of Agricultural and Food Chemistry, 53*, 2928–2935.
Heller, W., & Forkmann, G. (1994). Biosynthesis of flavonoids. In J. B. Harborne (Ed.), *The flavonoids – Advances in research since 1986* (pp. 499–535). New York: Chapman and Hall/CRC.
Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J. A., & Prior, R. L. (2002). High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *Journal of Agricultural and Food Chemistry, 50*, 4437–4444.
Lee, J., Durst, R. W., & Wrolstad, R. E. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and

wines by the pH differential method: Collaborative Study. *Journal of AOAC International, 88*, 1269–1278.

Li, L., Adams, L. S., Chen, S., Killian, C., Ahmed, A., & Seeram, N. P. (2009a). *Eugenia jambolana* Lam. berry extract inhibits growth and induces apoptosis of human breast cancer but not non-tumorigenic breast cells. *Journal of Agricultural and Food Chemistry, 57*, 826–831.

Li, L., Zhang, Y. J., & Seeram, N. P. (2009b). Structure of anthocyanins from *Eugenia jambolana* fruit. *Natural Product Communications, 4*, 217–219.

Lin, L.-Z., & Harnly, J. M. (2007). A screening method for the identification of glycosylated flavonoids and other phenolic compounds using a standard analytical approach for all plant materials. *Journal of Agricultural and Food Chemistry, 55*, 1084–1096.

Longo, L., Scardino, A., Vasapollo, G., & Blando, F. (2007). Anthocyanins from *Eugenia myrtifolia* Sims. *Innovative of Food Science and Emerging Technologies, 8*, 329–332.

Luximon-Ramma, A., Bahorun, T., & Crozier, A. (2003). Antioxidant actions and phenolic and vitamin C contents of common Mauritian exotic fruits. *Journal of the Science of Food and Agriculture, 83*, 496–502.

Mahmoud, I. I., Marzouk, M. S. A., Moharram, F. A., El-Gindi, M. R., & Hassan, A. M. K. (2001). Acylated flavonol glycosides from *Eugenia jambolana* leaves. *Phytochemistry, 58*, 1239–1244.

Nuengchamnong, N., & Ingkaninan, K. (2009). On-line characterization of phenolic antioxidants in fruit wines from family myrtaceae by liquid chromatography combined with electrospray ionization tandem mass spectrometry and radical scavenging detection. *LWT – Food Science and Technology, 42*, 297–302.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*, 1231–1237.

Singleton, V. L., & Rossi, J. A. Jr., (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158.

Veigas, J. M., Narayan, M. S., Laxman, P. M., & Neelwarne, B. (2007). Chemical nature, stability and bioefficacies of anthocyanins from fruit peel of *Syzygium cumini* Skeels. *Food Chemistry, 105*, 619–627.

Vieyra, F. E. M., Boggetti, H. J., Zampini, I. C., Ordoñez, R. M., Isla, M. I., Alvarez, R. M. S., et al. (2009). Singlet oxygen quenching and radical scavenging capacities of structurally-related flavonoids present in *Zuccagnia punctata* Cav. *Free Radical Research, 43*, 553–564.

Wang, S. Y., & Jiao, H. (2000). Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. *Journal of Agricultural and Food Chemistry, 48*, 5677–5684.

Waterman, P. G., & Mole, S. (1994). *Analysis of phenolic plant metabolites*. Oxford: Blackwell Scientific Publications.

Wu, X., & Prior, R. L. (2005). Systematic identification and characterization of anthocyanins by HPLC-ESI-MS/MS in common foods in the United States: Fruits and berries. *Journal of Agricultural and Food Chemistry, 53*, 2589–2599.

Zanatta, C. F., Cuevas, E., Bobbio, F. O., Winterhalter, P., & Mercadante, A. Z. (2005). Determination of anthocyanins from camu–camu (*Myrciaria dubia*) by HPLC-PDA, HPLC–MS, and NMR. *Journal of Agricultural and Food Chemistry, 53*, 9531–9535.

Zanatta, C. F., & Mercadante, A. Z. (2007). Carotenoid composition from the Brazilian tropical fruit camu–camu (*Myrciaria dubia*). *Food Chemistry, 101*, 1526–1532.

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radical. *Food Chemistry, 64*, 555–559.