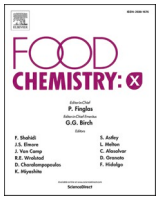
Food Chemistry: X 14 (2022) 100268

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



The bioactive constituents and antioxidant activities of ten selected Brazilian Cerrado fruits

Paulo Rogério Siriano Borges [a,*], Merete Edelenbos [b], Erik Larsen [b], Thais Hernandes [c], Elisângela Elena Nunes [a], Eduardo Valério de Barros Vilas Boas [a], Caroline Roberta Freitas Pires [d]

[a] Department of Food Science, Federal University of Lavras, 37.200-000, Lavras, MG, Brazil
[b] Department of Food Science, Aarhus University, 48 8200 Aarhus, Denmark
[c] Faculty of Nutrition, Federal University of Mato Grosso, 78.060-900, Cuiabá, MT, Brazil
[d] Federal University of Tocantins, 77006-356 Palmas, TO, Brazil

ARTICLE INFO

Keywords:
Tocopherol
Phenolic
Vitamin C
Carotenoids

ABSTRACT

This study measured the total levels of phenolic, anthocyanin, carotenoid, and tocopherol compounds, and vitamin C in ten fruits from the Brazilian Cerrado: araçá-boi, bacaba, bacupari, biribá, cajuí, curriola, marmelada-espinho, mirindiba, murici, and puçá-preto. Five extracts were prepared from each fruit using solvents with different polarities. The Trolox equivalent antioxidant activity, oxygen radical absorbance capacity, and inhibition of β-carotene bleaching were determined for each extract. Scott-Knott test and principal component analysis showed that the analyzed fruits were rich sources of different classes of bioactive compounds, with levels comparable to those in commonly consumed fruits such as guavas, and various berries and citrus fruits. To our knowledge, this is the first comprehensive study of the bioactive compounds and antioxidant activities of biribá, cajuí, marmelada-espinho, and mirindiba. Moreover, mirindiba was found to be a rich source of vitamin C and phenolics, with an average level of carotenoids and tocopherols.

1. Introduction

Fruit and vegetable consumption is associated with a healthy lifestyle and reduces the incidence of chronic and degenerative diseases (Bramley et al., 2000; Lima, Azevedo, de Souza, Nunes, & Vilas Boas, 2015; Malta et al., 2012; Muller, 1997; Perez-Gutierrez, Muniz-Ramirez, Gomez Gomez, & Bautista Ramirez, 2010; Zevallos, 2021). These benefits are related to the antioxidant, anti-inflammatory, and cytoprotective properties of the bioactive compounds found in these foods, including phenolics, carotenoids, ascorbic acid, and tocopherols (Chun, Lee, Ye, Exler, & Eitenmiller, 2006; Goncalves, Lajolo, & Genovese, 2010; Proteggente et al., 2002; Ramful, Tarnus, Aruoma, Bourdon, & Bahorun, 2011; Vasco, Ruales, & Kamal-Eldin, 2008; Zevallos, 2021). In this respect, the Brazilian Cerrado encompasses a large biodiversity, with a wide variety of plants that have been explored by local people (Goncalves et al., 2010; Oliveira, Yamada, Fagg, & Brandao, 2012; Rufino et al., 2010; Souza, Pimenta, Queiroz, Borges, & Souza Carneiro, 2012). The use of edible fruits, medicinal plants, and ornamentals from the Cerrado is common folk knowledge and some research studies regarding these edible fruits and other food sources have also been published (Abadio Finco et al., 2012; de Souza et al., 2012; Mariutti, Rodrigues, & Mercadante, 2013; de Oliveira et al., 2020). Such research has shown the great potential for Cerrado fruits to prevent chronic degenerative diseases and premature aging, due to their high levels of bioactive compounds (Goncalves et al., 2010; Lima et al., 2015; Malta et al., 2012; Perez-Gutierrez et al., 2010; Santos, Oliveira Filho, Sousa, Ribeiro, & Egea, 2021).

Among the wide variety of native Cerrado fruits, araçá-boi (*Eugenia stipitata*), bacaba (*Oenocarpus distichus*), bacupari (*Garcinia brasiliensis*), biribá (*Rollinia mucosa*), cajui (*Anacardium humile*), curriola (*Pouteria ramiflora*), marmelada-espinho (*Alibertia verrucosa*), mirindiba (*Buchenavia tomentosa*), murici (*Byrsonima crassifolia*), and puçá-preto (*Mouriri pusa*) are widely used locally owing to their unique flavors and empirically attributed medicinal properties (Abadio Finco et al., 2012; Goncalves et al., 2010; Neri-Numa, Carvalho-Silva, Morales, Malta, Muramoto, Macedo Ferreira, de Carvalho, & Ruiz, 2013; Oliveira et al., 2012; Perez-Gutierrez et al., 2010; Rufino et al., 2010). In this respect, the key approach to assessing the potential of new fruits is to examine

* Corresponding author.
E-mail address: paulosiriano@hotmail.com (P.R.S. Borges).

https://doi.org/10.1016/j.fochx.2022.100268
Received 17 August 2021; Received in revised form 20 January 2022; Accepted 22 February 2022
Available online 24 February 2022

their antioxidant activities and determine the main groups of antioxidants present (Khoo, Clausen, Pedersen, & Larsen, 2011; Neri-Numa et al., 2013; Proteggente et al., 2002; Rufino et al., 2010; Souza et al., 2012).

Phenolics constitute the largest group of bioactive compounds found in fruits, including anthocyanins, which provide the red, purple, and blue colors of fruits (Abadio Finco et al., 2012; Ferreira-Zielinski et al., 2014; Lee, Durst, & Wrolstad, 2005). The levels of both colorless and colored phenolics with varied polarities correlates with antioxidant activity *in vitro* (Ferreira-Zielinski et al., 2014; Kajdzanoska, Petreska, & Stefova, 2011; Khoo et al., 2011; Proteggente et al., 2002; Xu & Chang, 2007). Carotenoids are also responsible for fruit colors ranging from yellow to red hues; many of these nonpolar pigments show vitamin A activity and all of them have antioxidant activity in polar and nonpolar systems (Astrid Garzon et al., 2012; Burns, Fraser, & Bramley, 2003; Ferreira-Zielinski et al., 2014; Mariutti et al., 2013; Muller, 1997). Vitamin C, comprising ascorbic and dehydroascorbic acid, is also a powerful polar antioxidant and fruits are the main source of vitamin C in common diets (Hernandez, Lobo, & Gonzalez, 2006; Proteggente et al., 2002; Ramful et al., 2011). Vitamin C can provide antioxidant activity *in vitro*, as well as actively restoring vitamin E *in vivo* (Proteggente et al., 2002; Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, & Hawkins Byrne, 2006). Vitamin E or tocopherols are lipophilic antioxidants that promptly neutralize imminent membrane damage caused by chain reactions involving free radicals (Bramley et al., 2000; Huang, Ou, Hampsch-Woodill, Flanagan, & Deemer, 2002). Moreover, tocopherols can be restored by vitamin C, carotenoids, and phenolic compounds *in vitro* and *in vivo* (Bramley et al., 2000). The tocopherol content of fleshy fruits is often overlooked, since they are not considered to be rich sources of vitamin E, however, increasing fruit consumption has complemented vitamin E intake in some diets (Burns et al., 2003; Chun et al., 2006).

There is no simple universal method to measure the antioxidant activities of bioactive compounds, since there are many free radicals and many classes of antioxidants with a range of polarities (Huang, Ou, Hampsch-Woodill, Flanagan, & Prior, 2002; Kajdzanoska et al., 2011; Proteggente et al., 2002; Xu & Chang, 2007). Such complexity demands extraction of antioxidants using different solvents, resulting in a range of responses in different assays *in vitro*, which often hinders comparison of results (Hernandez et al., 2006; Kajdzanoska et al., 2011; Prior, Wu, & Schaich, 2005; Thaipong et al., 2006; Xu & Chang, 2007). To address this, standardized microplate methods for measuring the antioxidant activities of polar and nonpolar compounds, such as the determination of Trolox equivalent antioxidant activity (TEAC), oxygen radical absorbance capacity (ORAC), and β-carotene bleaching assays were revisited and standardized (Huang et al., 2002a, b; Prieto, Rodriguez-Amado, Vazquez, & Murado, 2012; Prior et al., 2005). The use of standardized procedures for sample extraction and preparation can enhance the detection of particular classes of antioxidants by these assays (Huang et al., 2002a; Jensen, Blachez, Egebo, & Meyer, 2007).

TEAC primarily detects the single electron transfer (SET) potential of antioxidants, while ORAC measures their potential with respect to hydrogen atom transfer (HAT) (Prior et al., 2005). When SET and HAT mechanisms occur simultaneously in foods, TEAC and ORAC are often performed in parallel to evaluate the antioxidant activity of the fruit samples; additionally, Trolox (a water-soluble tocopherol analog) is used as a standard in both methods, facilitating data comparison (Prior et al., 2005; Proteggente et al., 2002; Thaipong et al., 2006). The β-carotene bleaching assay measures the sample's ability to prevent discoloration of β-carotene during heat-induced oxidation; this involves HAT. This method has the advantage of being performed in an emulsion system, integrating the effects of polar and nonpolar compounds acting simultaneously (Mariutti et al., 2013; Prieto et al., 2012; Prior et al., 2005; Rufino et al., 2010). Moreover, the use of α-tocopherol as a standard in the β-carotene bleaching assay produces results that are comparable with the TEAC and ORAC assays, which use Trolox as a standard (Mariutti et al., 2013; Prieto et al., 2012).

The use of principal component analysis (PCA) provides a comprehensive overview of multiple sets of experimental data by reducing large databases to two-dimensional plots, exposing the associations between the bioactive compounds and antioxidant activities of analyzed fruits (Ferreira-Zielinski et al., 2014; Khoo, Clausen, Pedersen, & Larsen, 2012; Khoo et al., 2011).

The importance of native fruits, both for feeding local communities in Brazil and the current international market demand for new fruits with novel flavors and functional properties cannot be understated. The present study quantified the main groups of bioactive compounds (phenolics, anthocyanins, carotenoids, vitamin C, and tocopherols) in ten native fruits from the Brazilian Cerrado and assessed TEAC, ORAC, and β-carotene bleaching in the presence of five extracts of different polarities from each fruit. These Brazilian fruits were then compared with well-known sources of antioxidants found in the literature. Additionally, the association between the bioactive components of Brazilian fruits and their antioxidant activities were analyzed by PCA.

2. Materials and methods

2.1. Chemicals and reagents

2,2′-Azobis-2-methylpropanimidamide, dihydrochloride (AAPH) was purchased from Cayman Europe (Tallinn, Estonia), ethanol was purchased from Kemetyl (Haninge, Sweden), and phosphate-buffered saline-ethylenediaminetetraacetic acid (PBS-EDTA) was purchased from Lonza (Braine, Belgium). Formic acid, oxalic acid, potassium hydroxide, and sodium hydroxide were purchased from Merck (Darmstadt, Germany). (+)-α-Tocopherol, β-carotene, methyl-β-cyclodextrin, 2,2′-azino-bis (3-ethyl-benzthiazo-line-6-sulphonic acid) (ABTS), 2-propanol, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), strong basic resin (Ambersep 900 OH), acetone, acetonitrile, ethyl acetate, fluorescein, Folin-Ciocalteu reagent, gallic acid, heptane, hexane, L (+)-ascorbic acid, linoleic acid, methanol, and Tween 20® were purchased from Sigma-Aldrich (Missouri, USA). Pure water was obtained from an SG Ultra-Pure water system (SG Water, Barsbuttel, Germany).

2.2. Plant material and sample preparation

Ten native fruits from the Brazilian Cerrado were selected for this study (Table 1). Fruits were harvested manually from twenty trees of each species between November 2012 and October 2013 in Cerrado regions located in the states of Tocantins and Mato Grosso, Brazil. The common name, scientific name, family, collection site, fruit coloration, size, and dry weights are described in Table 1. Around 10 kg of each fruit was harvested, and only ripe fruits free of visual defects were selected. These were washed, dried with paper towels, measured, weighed, frozen in liquid nitrogen, packaged in polyethylene pouches to prevent dehydration, and kept at −18 °C in the dark during the 12-h transportation to the Federal University of Lavas, Brazil. The fruits were then stored at −80 °C until sample preparation.

Sample preparation was performed quickly to avoid thawing. Only the edible parts of the fruit were used. For the araçá-boi, bacaba, bacupari, cajuí, mirindiba, and murici, the peel and pulp were mashed by hand. For the biribá, curriola, and marmelada-espinho, only the flesh (endocarp) was used; the fruits were first peeled and then homogenized. Since puçá-preto fruit is consumed both with and without peel, the peel and the pulp were first separated and then homogenized, prepared, and analyzed separately throughout the study as two different samples. After homogenization, samples were immediately refrozen at −80 °C on glass plates, before freeze-drying (LIOBRAS L101 Freeze-drier; São Paulo, Brazil) at −40 °C. The dry matter content (Table 1) was calculated gravimetrically by weighing three sub-samples of each fruit before and after freeze-drying. The freeze-dried samples were subsequently milled and stored in hermetically sealed pouches at 5 °C until chemical

Table 1
Characteristics of analyzed fruits from Brazilian Cerrado.

Common name	Species	Family	Origin (City, State)	Peel color	Pulp color	Fruit sizerange (cm)[a]	Dry matter[b]
Aracá-boi	*Eugenia stipitata*	Myrtaceae	Cuiabá, Mato Grosso	Yellow	Yellow	8 ± 0,8	11.1 ± 1.2
Bacaba	*Oenocarpus distichus*	Arecaceae	Peixe, Tocantins	Greyish-purple	Greyish-purple	2.2 ± 0,7	74.4 ± 0.7
Bacupari	*Rheedia brasiliensis*	Clusiaceae	Santo Antonio do Leverger, Mato Grosso	Orange	Orange	2,3 ± 0,3	17.7 ± 0.6
Biribá	*Rollinia mucosa*	Annonaceae	Cuiabá, Mato Grosso	Yellow	White	8 ± 0,9	19.3 ± 0.4
Cajuí	*Anacardium humile*	Anacardiaceae	Dueré, Tocantins	Red	White	3.2 ± 0,5	6.2 ± 0.4
Curriola	*Pouteria ramiflora*	Sapotaceae	Santo Antonio do Leverger, Mato Grosso	Green	White	7 ± 2	11.6 ± 2.4
Marmelada-espinho	*Alibertia verrucosa*	Rubiaceae	Santo Antonio do Leverger, Mato Grosso	Yellow	White-Grey	3 ± 1,4	22.6 ± 1.1
Mirindiba	*Buchenavia tomentosa*	Combretaceae	Dueré, Tocantins	Yellow-green	Yellow	2,5 ± 0,5	29.4 ± 0.5
Murici	*Byrsonima crassifolia*	Malpighiaceae	Dueré, Tocantins	Yellow	Yellow	1,5 ± 0,5	28.1 ± 0.4
Puçá-preto	*Mouriri pusa*	Melastomataceae	Gurupi, Tocantins	Black	Orange	2,7 ± 0,2	63.6 ± 2.0 45.5 ± 2.0[c]

[a] Values obtained in the present study from 60 fruits measured.
[b] Average ± standard deviation ($n = 3$).
[c] peel.

analyses were conducted at the Department of Food Science, Aarhus University, Årslev, Denmark.

2.3. Bioactive compounds

Bioactive compounds were extracted and quantified from 11 samples using each of the approaches described below, in triplicate.

2.3.1. Total phenolics

Total phenolics were extracted according to Kajdzanoska et al. (2011), with slight modifications. Freeze-dried sample (0.5 g) was mixed with 10 mL of methanol solution containing acetic acid (H_2O:MeOH:HAc, 19:80:1, v/v/v) in a 15-mL argon-filled centrifuge tube (SARSTEDT, Nümbrecht, Germany) and stirred for 2 h at 8 °C in dim light on a MS2-Minishaker (IKA®, Königswinter, Germany). This mixture was centrifuged for 4 min at 13,000 rpm and 5 °C on a Sorvall RC-5B Plus centrifuge (Buch and Holm, Herlev, Denmark) and the resulting supernatant was filtered through a 0.45-μm RR Q-Max® nylon filter (Frisenette, Knebel, Denmark) into a brown 2-mL vial (VWR® INTERNATIONAL, Radnor, Pennsylvania, USA) prior to analysis. Quantification was performed using a microplate Folin-Ciocalteu method (Magalhaes, Santos, Segundo, Reis, & Lima, 2010). In brief, 50 μL of gallic acid or filtered sample was mixed with 50 μL Folin-Ciocalteu reagent (diluted 1:5 with H_2O, v/v) prior to by adding 100 μL 0.7 M sodium hydroxide. After 3 min, the absorbance was measured at 760 nm using a Synergy 2 multi-code microplate reader from BioTek (Vermont, USA). Water was used as a blank control. The total phenolic content was quantified as mg gallic acid equivalents per 100 g fresh weight (mg GAE/100 g FW).

2.3.2. Total anthocyanins

Total monomeric anthocyanin was extracted according to Jensen et al. (2007). Freeze-dried sample (0.5 g) was mixed with 20 mL ethanolic solution containing hydrochloric acid (H_2O:EtOH:HCl, 40.9:50:0.1, v/v/v) in a 50-mL argon-filled centrifuge tube (SARSTEDT) and stirred for 15 min at 8 °C in dim light. The mixture was centrifuged for 15 min at 13,000 rpm and 4 °C. The supernatant was filtered through a 0.22-μm Q-Max® acetate filter (Frisenette) directly into a 2-mL brown vial prior to analysis. Anthocyanins were quantified as the difference between sample absorption at 510 nm in pH 1.0 buffer (0.025 M KCl) (aq) and sample absorption at 700 nm in pH 4.5 buffer (0.4 M sodium acetate) (aq), as described by Lee et al. (2005). In brief, two 75-μL aliquots of the filtered sample were mixed with either 125 μL pH 1 buffer or 125 μL pH 4.5 buffer. After 15 min, the absorbance was measured using a microplate reader. The total anthocyanin content was quantified as cyanidin-3-glucoside equivalents per 100 g FW (mg/100 g FW).

2.3.4. Total carotenoids

Extraction of carotenoids was performed according to Larsen and Christensen (2005). Freeze-dried sample (1 g) was homogenized with 20 mL cold acetone (100%) in a 50-mL argon-filled centrifuge tube and left for 20 min. The mixture was then centrifuged for 4 min at 13,000 rpm and 4 °C. Ten milliliters of supernatant was stirred with 1 g Ambersep 900 OH for 30 min to saponification. The supernatant was then filtered through a 0.45-μm RR Q-Max® nylon filter directly into a 2-mL brown vial. High-performance liquid chromatography (HPLC) analysis was performed on a Dionex Ultimate 3000 Series HPLC equipped with a DAD-3000 (RS) diode array detector, a TCC-300SD column compartment, a WPS-3000SL autosampler and a 3000 Pump Series, all obtained from Dionex (Dionex Softron GmbH, Germering, Germany). Carotenoids were separated on a Hypersil Gold C_{18} analytical column (250 × 4.6 mm; 5-μm particle size) that was protected by a Hypersil Gold C_{18} guard cartridge (15 × 4 mm); both were obtained from Agilent Technologies (Santa Clara, California, USA). The mobile phase, gradient, and further chromatographic conditions were described previously (Larsen & Christensen, 2005). Identification and quantification of β-carotene was conducted using a calibration curve constructed using an authentic standard; other carotenoids were quantified according to Rodriguez-Amaya (2001), using the extinction coefficient in acetone. Results were expressed as mg total carotenoids per 100 g FW (mg/100 g FW). Data were processed with Chromeleon® version 6.8 software (Dionex Corporation).

2.3.5. Vitamin C

Extraction of vitamin C was performed according to Hernandez et al. (2006), with slight modifications. Freeze-dried sample (1 g) was homogenized with 10 mL cold (8 °C) aqueous solution (H_2O:OA, 99.5:0.5, v/w) in a 15-mL argon-filled centrifuge tube for 60 *sec* and subsequently stirred for 15 min in dim light. Homogenates were centrifuged for 5 min at 13,000 rpm and 4 °C and the resulting supernatant was filtered through a 0.22-μm Q-Max® acetate filter directly into a brown 2-mL vial prior to analysis. Separation and quantification of vitamin C were performed according to Li (2013), with slight modifications, using the HPLC apparatus previously described. The system was equipped with a Kinetex XB-C_{18} (4.6 × 150 mm; 2.6-μm particle size) column from Phenomenex (Torrance, California, USA). The mobile phase consisted of 0.1% formic acid (aq) (solvent A) and 100% acetonitrile (solvent B). The gradient was 0–100% B over 0–3.6 min, 100% B from 3.6 to 5 min, 100

0% B from 5 to 7 min. The flow rate was 0.8 mL/min, and the oven temperature was 30 °C. A 10-μL sample was injected and the peak area was recorded at 245 nm. Peaks were processed using Chromeleon® version 6.8 software and results were expressed as mg vitamin C per 100 g FW (mg/100 g FW), using an authentic ascorbic acid standard.

2.3.6. Total tocopherols

The extraction procedure was performed according to the European standard EN 12822. Freeze-dried sample (1 g) was saponified in a mixture of 5 mL ascorbic acid (EtOH:AA, 99.9:0.1, v/w), 5 mL ethanol (H_2O:EtOH, 96:4, v/v), 4.5 mL absolute methanol, and 3.5 mL saturated KOH, directly into a 25-mL argon-filled brown glass vial (VWR® INTERNATIONAL). First, samples were homogenized for 60 *sec*, saponified for 90 min at 70 °C in the dark, and then quickly cooled to −25 °C. One milliliter of this mixture was mixed with 2.5 mL heptane in an argon-filled 15-mL centrifuge tube, stirred for 30 *sec*, and centrifuged for 5 min at 13,000 rpm and 4 °C. The heptane fraction containing the tocopherols was transferred to a brown argon-filled vial and the mixture was extracted a second time with 2.5 mL heptane, as described above. The resulting supernatants were combined and filtered through a 0.45-μm RR Q-Max® nylon filter directly into a 2-mL brown vial prior to analysis. Chromatographic separation was performed according to Kamal-Eldin, Gorgen, Pettersson, and Lampi (2000) with the HPLC system described previously, equipped with an RF-2000 fluorescence detector connected to a ZORBAX RX-SIL (4.6 mm × 150 mm; 5 μm particle size) column (Agilent Technologies). The mobile phase consisted of hexane modified with 2-propanol (1.5 % v/v). The flow rate was 0.5 mL/min, and the oven temperature was 30 °C. A 50-μL sample was injected and the total run time was 20 min with isocratic elution. The excitation wavelength was 295 nm and the emission wavelength was 327 nm. Identification and quantification of α-tocopherol were achieved by comparison with authentic standard. Other tocopherol isomers, beta-, gamma-, delta-tocopherols and alpha-, beta-, gamma-, and delta-tocotrienols, were identified according to Kamal-Eldin et al. (2000) and quantified according to EN 12,822 methodology using the fluorescence response relative to α-tocopherol. Peaks were processed with Chromeleon® version 6.8 software and results were expressed as mg tocopherols per 100 g FW (mg/100 g FW).

2.4. Antioxidant activity

ORAC, TEAC, and β-carotene bleaching inhibition were assessed in triplicate in the eleven samples prepared using the five approaches described in section 2.3, as shown. These samples were stored at −80 °C in brown vials filled with argon prior to antioxidant activity assessments. For each fruit, the extracts were named according to the extraction solution used, as follows: phenolic compounds in methanol (MeOH), anthocyanins in aqueous ethanol (EtOH), carotenoids in acetone (Acet), ascorbic acid in water (H_2O), and tocopherols in heptane (Hept) (Table 3). Additionally, the respective pure extraction solution was used as a blank control for the antioxidant activity measurements. For the carotenoid (Acet) and tocopherol (Hept) extracts, pre-treatment with methyl-β-cyclodextrin was performed as described by Huang et al. (2002a) to increase the solubility of the bioactive compounds.

2.4.1. ORAc

The ORAC assay was performed as described by Huang et al. (2002b). Twenty-five microliters of sample extract or standard solution were placed in a Nunc 96-well black plate (Thermo Fisher Scientific, Leicestershire, UK), 150 μL of fluorescein solution (1.2×10^{-8} mM in 75 mM phosphate buffer, pH 7.42) was added, and the plates were incubated for 30 min at 37 °C. The assay was initiated by adding 25 μL AAPH solution (15 mM in 75 mM phosphate buffer, pH 7.42). Fluorescence was recorded every minute for one hour at an excitation wavelength of 485 nm and an emission wavelength of 515 nm using the microplate reader previously described. The net area under the curve (net-AUC) in relation to the blank control was calculated (Prior et al., 2005). Trolox was diluted in pure ethanol and results were expressed as Trolox equivalents per gram of FW (μM TEq/g FW).

2.4.2. TEAc

TEAC was measured according to Khoo et al. (2011). In brief, 50 μL filtered sample or standard solution was mixed with 200 μL ABTS radical buffer solution and the absorbance was read after 10 min at a wavelength of 414 nm, using the microplate reader previously described. Trolox was diluted in pure ethanol and results were expressed as μM TEq/g FW.

2.4.3. β-carotene bleaching

Discoloration of β-carotene was measured according to Prieto et al. (2012), with slight modifications. One milligram of beta-carotene was dissolved in 10 mL dichloromethane prior to dissolving 25 μL linoleic acid and 200 mg Tween-20 in 1 mL of this mixture. Dichloromethane was then removed under vacuum using a rotary evaporator operating at 40 °C in dim light. Oxygenated pure water (50 mL at 50 °C) was added to the mixture and vigorously shaken by hand to form an emulsion. A second emulsion that lacked β-carotene was prepared at room temperature (18 °C) following the procedure described above. The assay was started by adding a 50-μL aliquot of emulsion without β-carotene to the 96-well microplate followed by 30 μL sample extract or standard solution, gently mixing to avoid evaporation of solvents. Next, a 200 μL aliquot of the β-carotene/linoleic acid emulsion at 50 °C was applied, stirred, and immediately read at 492 nm every 5 min for 3 h at 50 °C using a microplate reader that was pre-heated to 50 °C. Data were plotted as fixed-percent inhibition in relation to the first measurement, and discoloration of β-carotene between 120 and 180 min was used for net-AUC integration (Prieto et al., 2012; Prior et al., 2005). α-Tocopherol authentic standard was diluted in heptane and results were expressed as μM TPEq/g FW.

2.5. Data analysis

Bioactive compound levels and antioxidant activity measurements were obtained in three replicates. These were initially compared by one-way analysis of variance ($p < 0.05$), followed by the Scott-Knott test, to identify significant differences between the fruits. A multivariate analysis approach comprising PCA was implemented using Statistica 7.0 software (Stat-Soft Inc., Tulsa, Okla., USA). The dependent variables were autoscaled to standardize their statistical importance, and no detected results were considered zero. PCA was applied to asses similarities between fruit samples (11 samples × 3 replications; n = 33 parcels) according to the bioactive compound levels (33 samples × 5 measurements = 165 parcels) and the antioxidant activity measurements (165 extracts × 3 measurements = 495 parcels).

3. Results and discussion

3.1. Bioactive compounds

All of the analyzed fruits had detectable total phenolic levels in the following order: mirindiba > bacaba > puçá-preto (peel) > puçá-preto (pulp) > bacupari > murici > biribá = marmelada-espinho = cajuí = araçá-boi = curriola (Table 2). Souza et al. (2012) classified native Cerrado fruits according to their total phenolic levels, following the scale proposed by Vasco et al. (2008). Following these parameters, we found mirindiba, bacaba, and puçá-preto peel and pulp to be high-level sources of phenolic compounds (>500 mg GAE/100 g), comparable to "super fruits" such as acerola, camu-camu (Rufino et al., 2010), blackberries, raspberries, and blueberries (Abadio Finco et al., 2012). In the second group of medium phenolic compound sources (100–500 mg GAE/100 g), we classified bacupari and murici alongside strawberries, red plums (Proteggente et al., 2002), guavas (Vasco et al., 2008), and

Table 2
Total phenolics, total anthocyanins, total carotenoids, Vitamin C, and total tocopherols in 10 Brazilian native fruits from cerrado (mg/100 fresh matter).

Fruit	Total phenolic[a]	Total anthocyanins[b]	Total carotenoids	Vitamin C	Total tocopherols
Araçá-boi	35,7 g	nd	0.9 d	8.3c	0,60 e
Bacaba	1244,6b	116.7 a	nd	nd	1,20c
Bacupari	208,4 e	nd	0.5 e	1.6c	1,19c
Biribá	46,4 g	nd	nd	1.9c	0,87 d
Cajui	36,3 g	110.6 a	nd	7.5c	0,13 g
Curriola	34,2 g	nd	nd	3.5c	0,43f
Marmelada-espinho	46,3 g	nd	nd	2.0c	1,12c
Mirindiba	2827,1 a	nd	1.4c	2018.4 a	4,05 a
Murici	143,9f	nd	1.4c	36.4b	0,78 d
Puçá-preto (pulp)	512,3 d	nd	1.5b	2.9c	1,15c
Puçá-preto (peel)	610,1c	36.4b	1.8 a	2.7c	1,94b
CV (%)	5.6	6.7	5.4	5.4	4.7

Values followed by the same letter in the column are equal accordingly the Scott-Knott means test ($p < 0.05$); n = 3; nd = not detected.
[a] galic acid equivalent (GAE).
[b] cyanidin-3-glucoside equivalents.

sour cherries (Khoo et al., 2011). The fruits considered to represent low sources of phenolic compounds (<100 mg GAE/100 g), namely biribá, marmelada-espinho, cajuí, araçá-boi, and curriola were comparable to highly consumed fruits, including apples, peaches, pears (Proteggente et al., 2002), passion fruit, and mangoes (Vasco et al., 2008). Since consumption of phenolic compounds is important for proper health, these findings indicated that the Brazilian Cerrado fruits analyzed here represent important food sources for local consumers and provide promising raw materials to meet the market demand for new products (Goncalves et al., 2010).

The bacaba, cajuí, and puçá-preto peel had detectable levels of total anthocyanins (Table 2), giving them greyish-purple, red, and black colors, respectively (Table 1). The anthocyanin content in bacaba detected in this study was three-fold higher than that reported by Astrid Garzon et al. (2012) for fruits that were also collected in Tocantins State, Brazil. The anthocyanin levels in bacaba, cajuí, and puçá-preto peel (36.4–116.7 mg/100 g FW) are comparable to those reported in the literature for açaí, camu-camu (Rufino et al., 2010), strawberries (Kajdzanoska et al., 2011), red grapes (Jensen et al., 2007), and sour cherries (Khoo et al., 2011). Apart from their total phenolic content, bacaba, cajuí, and puçá-preto are thus also rich sources of anthocyanins.

According to their carotenoid levels, the fruits were ranked as follows: puçá-preto peel > puçá-preto pulp > murici = mirindiba > araçá-boi > bacupari (Table 2). Bacaba, biribá, cajuí, curriola, and marmelada-espinho did not have detectable levels of carotenoids, an expected outcome, considering the colors of their edible parts (Table 1). In the context of the screening study performed by Muller (1997) to analyze carotenoid levels in 28 fruit species, araçá-boi, bacupari, mirindiba, murici, and puçá-preto were classified as average sources of carotenoids for human consumption, similar to apricots, plums, and clementines. We also observed three major carotenoids in the samples analyzed: (*all-E*)-zeaxanthin, (*all-E*)-lutein, and (*all-E*)-β-carotene were present in araçá-boi, as well as (*all-E*)-zeaxanthin and (*all-E*)-β-carotene in mirindiba, murici, and puçá-preto pulp and peel (data not shown). Astrid Garzon et al. (2012) found similar major carotenoid isomers in araçá-boi growing in the Colombian Amazon forest: (*all-E*)-zeaxanthin, (*all-E*)-lutein, and (*all-E*)-β-carotene. Moreover, these authors found similar total carotenoid levels (0.81 mg/100 g FW) as those identified in the present study (Table 2). Furthermore, a detailed description of the carotenoid composition of murici collected from Belém, Pará State, Brazil (Mariutti et al., 2013) showed similarities with the present findings, with predominantly (*all-E*)-lutein and (*all-E*) zeaxanthin detected. The total carotenoid content in murici from Tocantins (Table 2) was higher than that found in the fruit from Pará State, Brazil (0.7 mg/100 g; Mariutti et al., 2013) or Ceará State, Brazil (1.1 mg/100 g; Rufino et al., 2010). For puçá-preto, Rufino et al. (2010) found higher levels of total carotenoids in a mix of pulp and peel from fruits collected from the Ceará State (4.2 mg/100 g) than was detected in the present analysis of peel and pulp separately (Table 2). No published data were found in relation to the carotenoid profiles of puçá-preto, mirindiba, or bacupari. The detailed characterization of carotenoids in these fruits represents an important step toward the accurate and precise identification of isomers, vitamin A activity, and bioactivity potential (Astrid Garzon et al., 2012; Burns et al., 2003; Mariutti et al., 2013). However, due the complexity of this detailed analysis, the present screening study focused on the total carotenoid content and its antioxidant activity. Further studies detailing the carotenoid composition of puçá-preto, mirindiba, and bacupari are necessary.

The vitamin C content was as follows: mirindiba > murici > araçá-boi = bacupari = biribá = cajuí = curriola = marmelada-espinho = puçá-preto pulp and peel (Table 2). There was no detectable vitamin C in the bacaba samples. Fruits were grouped according to their vitamin C content, following the classification proposed by Ramful et al. (2011) for citrus fruits. Mirindiba was classified as a high source of vitamin C (>50 mg/100 g FW), comparable to oranges, papayas (Hernandez et al., 2006), guavas, and passion fruits (Vasco et al., 2008); this fruit had values similar to the two richest sources of vitamin C found in the literature, acerola and camu-camu (Goncalves et al., 2010; Rufino et al., 2010). Murici was classified as a medium source of vitamin C (30–50 mg/100 g FW), similar to mandarins, clementines (Ramful et al., 2011), strawberries (Vasco et al., 2008) and raspberries (Proteggente et al., 2002). Fruits with low vitamin C levels (<30 mg/100 g FW), including araçá-boi, bacupari, biribá, cajuí, curriola, marmelada-espinho, and pulp and peel of puçá-preto, still had similar levels to those reported in highly consumed fruits such as red plums, green grapes, pears, apples (Proteggente et al., 2002), lemons (Vasco et al., 2008), pineapples, and mangoes (Hernandez et al., 2006). These results indicated that seasonally available fruit in the Cerrado provided the same daily intake of vitamin C for locals as commercially available fruit.

The total tocopherol content was as follows: mirindiba < puçá-preto peel < puçá-preto pulp = bacaba = bacupari = marmelada-espinho < biribá = murici < araçá-boi < curriola < cajuí (Table 2). At least one tocopherol isomer was found in each sample analyzed; the most frequent were α-tocopherol and β-tocopherol, followed by other isomers (data not shown). Bacaba, bacupari, marmelada-espinho, mirindiba, and the peel and pulp of puçá-preto contained similar total tocopherol levels (1.12–4.05 mg/100 g FW) as blackberries, red raspberries, bottled green olives (Chun et al., 2006), barley, oats, and white rice (Bramley et al., 2000). Other fruits from this study contained the same level (0.60–0.87 mg/100 g FW) as apples, figs, grapes, peaches (Chun et al., 2006), coconuts, carrots, broccoli, cabbages, and asparagus (Bramley et al., 2000). These results indicate that native fruits from the Brazilian Cerrado also provide a complementary source of vitamin E. Although fresh fruits, vegetables, and grains are not considered the richest tocopherol sources, they contribute to total vitamin E intake if they are consumed more than primary sources (e.g., nuts and vegetable oils) in some diets

(Bramley et al., 2000; Burns et al., 2003; Chun et al., 2006)

3.2. Antioxidant activity

All five bacaba extracts showed higher ORAC antioxidant activity values than the other fruit extracts, and the methanol, ethanol and heptane extracts showed the highest TEAC signal (Table 3), indicating a possible predominance of HAT over SET mechanisms (Huang et al., 2002; Prior et al., 2005). The high content of total phenolics in bacaba fruits (including the second highest anthocyanin level) (Table 2) were primarily responsible for these ORAC and TEAC activities, confirming results reported by Astrid Garzon et al. (2012). Furthermore, we found up to six times more ORAC activity (Table 3) and three times more anthocyanins (Table 2) than were observed by Astrid Garzon et al. (2012). These results highlight the influence of total phenolics, including anthocyanins, on the antioxidant activity of bacaba fruits, and its potential as a rich source of bioactive compounds for consumers.

All five Mirindiba extracts showed greater inhibition of β-carotene bleaching than the other fruit extracts (Table 3). The high content of total tocopherol found in heptane extracts of mirindiba fruits (Table 2) may relate to this high activity in the β-carotene bleaching assay (Table 3). Similarly, methanol and acetone, which extract tocopherols (Burns et al., 2003; EN 21282, 2000; Prieto et al., 2012), produced high levels of inhibition of β-carotene bleaching. These results were also consistent with the high levels of phenolic compounds (extractable in methanol, ethanol, acetone, or water; Jensen et al., 2007; Kajdzanoska et al., 2011; Khoo et al., 2012; Thaipong et al., 2006), and vitamin C (primarily extractable in aqueous solutions; Hernandez et al., 2006; Thaipong et al., 2006) found in mirindiba fruits (Table 2). Similarly, acetone and water extracts of mirindiba had the highest TEAC values (Table 3). High levels of total tocopherol, total phenolics and vitamin C combined, as found in mirindiba fruits, can act either in polar or nonpolar media (Prior et al., 2005; Thaipong et al., 2006; Xu & Chang, 2007), resulting in the high antioxidant activities observed in this study. The results indicate a possible predominance of HAT over SET mechanism in mirindiba, as previously reported in bacaba samples.

Methanolic, ethanolic, and aqueous extracts of the puçá-preto pulp and peel showed high antioxidant activity in the ORAC assay, as did these and the heptane extracts in the TEAC assay (Table 3). Rufino et al. (2010) assessed TEAC in a mix of puçá-preto pulp and peel and found values similar to those observed in this study for ethanol and heptane extracts of pulp and peel analyzed separately (Table 3). These authors associated the high antioxidant activity with the total phenolic content (868 ± 51 mg GAE/100 g), which was similar to that observed in the present study (Table 2). Remarkably, the levels of bioactive compounds (Table 2) and antioxidant activity (Table 3) were higher in the puçá-preto peel than in the pulp. Moreover, anthocyanins were only detected in the peel (Table 2). In this respect, we only observed inhibition of β-carotene bleaching in the acetone and aqueous extracts of the peel (Table 3). Given that anthocyanins are extractable in acetone and aqueous solutions with or without acidification (Jensen et al., 2011; Kajdzanoska et al., 2011; Khoo et al., 2012) and are often considered to be the major contributors to high antioxidant activity *in vitro* in polyphenol-rich fruits (Abadio Finco et al., 2012; Khoo et al., 2011; Proteggente et al., 2002), we suggest that the high antioxidant activity of the peel was due to the anthocyanins present. Additionally, the puçá-preto results indicated simultaneous activities involving HAT and SET mechanisms.

The methanol murici extract showed considerable inhibition of β-carotene degradation, and its acetone and heptane extracts showed good ORAC antioxidant activity (Table 3). Murici fruits collected from the Brazilian states of Pará (Mariutti et al., 2013) and Goiás (Malta et al., 2012) have been studied for their high peroxyl radical scavenging potential *in vitro*, as well as for their anti-genotoxic and anti-mutagenic properties *in vivo*. Such properties are primarily attributed to carotenoids and phenolic constituents (Malta et al., 2012; Mariutti et al., 2013; Perez-Gutierrez et al., 2010), both of which are extractable in acetone or methanol (Jensen et al., 2007; Larsen & Christensen, 2005; Xu & Chang, 2007). Moreover, the vitamin C content of murici is also associated with antioxidant activity *in vitro* for fruits collected from Ceará (Rufino et al., 2010) and Minas Gerais State (Souza et al., 2012). In this study, tocopherol-rich extracts (Hept) of murici collected in Tocantins State showed ORAC activity (Table 3), indicating that this fruit provides a complementary source of tocopherols, in addition to carotenoids, phenolics, and vitamin C.

The other fruits studied, including araçá-boi, bacupari, biribá, cajuí,

Table 3
Oxygen radical absorbance capacity (ORAC), trolox equivalent oxidant activity (TEAC) and inhibition of β-carotene bleaching (β-car) in five extracts obtained; total phenolics in methanol (MeOH), total anthocyanins in aqueous ethanol (EtOH), total carotenoids in acetone (Acet), Vitamin C in water (H2O), and total tocopherols in heptane (Hept).

Fruit	ORAC (μM TEq/g FW)[a]					TEAC (μM TEq/g FW)[a]					β-car (μM TPEq/g FW)[b]				
	MeOH	EtOH	Acet	H_2O	Hept	MeOH	EtOH	Acet	H_2O	Hept	MeOH	EtOH	Acet	H_2O	Hept
Araçá-boi	8,7 i	54,3h	39,3 g	44,5 i	24,9 e	11,7h	33,6 g	4.5 e	8,3h	25,7h	2.0 e	nd	3,6c	12,6 e	nd
Bacaba	85,3 a	394,3 a	367,9 a	284,4 a	210,6 a	71,7 a	198,1 a	nd	55,3b	175,4 a	nd	nd	13,0c	72,7b	nd
Bacupari	nd	nd	75,3 e	53,5h	31,4 e	11,0h	34,1 g	12.2b	8,7h	27,0h	6.0 d	nd	14,0c	5,4 e	nd
Biribá	14,3 g	95,3f	99,6 d	80,2 g	40,3 d	18,7f	55,1f	10.1c	14,2f	44,9 g	nd	10.7b	1,5 d	24,4 d	21.8b
Cajuí	4,3 j	28,1 i	26,8h	24,2 j	11,4f	6,3 j	18,1 i	5.4 d	4,6 i	14,1 i	nd	6.41b	12,5c	4,6 e	0.55 d
Curriola	11,7h	56,0 g	53,0f	46,6 i	23,1 e	12,1h	35,0 g	nd	8,7h	27,6h	7.2 d	4.95b	11,8c	8,3 e	nd
Marmelada-espinho	16,3f	111,0 e	72,9 e	93,2f	46,4 d	16,1 g	26,9h	1.5 e	11,4 g	54,6f	11.2c	5.63b	38,3b	17,2 d	10.5c
Mirindiba	34,8c	195,8c	211,8b	167,0c	88,8b	33,6c	105,9 d	61.7 a	294,4 a	93,1c	104.7 a	47.5 a	136,5 a	97,1 a	93.8 a
Murici	24,4 e	nd	126,2c	113,2 e	53,4c	29,2 e	84,6 e	nd	21,2 e	67,0 e	24.6b	nd	3,6c	19,9 d	nd
Puçá-preto (pulp)	31,4 d	175,2 d	77,3 e	144,7 d	54,2c	32,3 d	109,3c	1.2 e	27,4 d	87,8 d	nd	nd	nd	nd	nd
Puçá-preto (peel)	39,5b	230,7b	38,8 g	177,9b	42,7 d	44,9b	131,6b	nd	34,1c	110,3b	nd	nd	13.9c	56.0c	nd
CV (%)	3.4	2.75	4.7	2.4	9.4	3.8	1.9	2.3	1.06	2.5	9.8	18.1	6.3	10	11.2

Values followed by the same letter in the column are equal accordingly the Scott-Knott means test ($p < 0.05$); n = 3; nd = not determined.
[a] trolox equivalent per 100 g of fresh matter.
[b] tocopherol equivalent per 100 g of fresh matter.

curriola, and marmelada-espinho, had lower antioxidant activity values (Table 3). However, even these lower TEAC values were in the same range as those observed by Khoo et al. (2011) in 34 cultivars of sour cherry, and by Ramful et al. (2011) in 21 citrus varieties. Moreover, their ORAC values were in the same range as those found by Thaipong et al. (2006) for four varieties of guava, and those reported by Khoo et al. (2012) in fifteen cultivars of blackcurrant. These results confirm that native fruits from the Brazilian Cerrado provide sources of bioactive compounds with high antioxidant potentials *in vitro* that are on a par with other well-known fruits. To our knowledge, out of the six fruits mentioned above, only araçá-boi fruit growing in the Colombian (Astrid Garzon et al., 2012) and Brazilian (Neri-Numa et al., 2013) Amazon have been studied for their high antioxidant activity *in vitro*, as well as for their anti-proliferative and anti-mutagenic properties *in vivo*; these activities were attributed to their carotenoid and phenolic constituents. The present study found similar total carotenoid levels (Astrid Garzon et al., 2012), higher TEAC activity (Astrid Garzon et al., 2012), lower total phenolic levels (Astrid Garzon et al., 2012; Neri-Numa et al., 2013), and lower ORAC (Neri-Numa et al., 2013) in araçá-boi growing in the Brazilian Cerrado, as compared with previous studies.

3.3. Principal component analysis

The PCA model was able to distinguish four groups of fruits (Fig. 1). PC 1 explained 52.30% of the data variability and PC 2 explained 29.48% of variability, totaling 81.78%. The PCA model showed a close relationship between β-carotene bleaching measurements in methanol, ethanol, aqueous, and heptane mirindiba samples, as well as between TEAC measurements in aqueous and acetone extracts (Fig. 1). This confirms the statistical analysis in Table 3, except for the β-carotene bleaching assessment in acetone. Moreover, the antioxidant activity of mirindiba was closely associated with the total phenolic, vitamin C, and total tocopherol levels (Fig. 1), corroborating the bioactive compounds measurements in Table 2. The tocopherol levels correlated with the inhibition of β-carotene oxidation *in vitro* (Mariutti et al., 2013; Prieto et al., 2012), and the antioxidant activity in oils and nuts (Burns et al., 2003; Chun et al., 2006), but are rarely found at such levels in fleshy fruits. Furthermore, TEAC measurements in acetone and aqueous solutions are often correlated with the total phenolic (Proteggente et al., 2002; Ramful et al., 2011; Thaipong et al., 2006; Vasco et al., 2008) and vitamin C content across a range of fruits (Proteggente et al., 2002; Rufino et al., 2010; Souza et al., 2012; Thaipong et al., 2006), as found for mirindiba. PCA confirmed that mirindiba fruit was rich in bioactive compounds and represented a new powerful antioxidant source.

Methanol, ethanol, and heptane bacaba extracts showed the closest relationship across all measurements in ORAC, as well as with TEAC measurements (Fig. 1), confirming the statistical analysis shown in Table 3. PCA did not show the expected relationship between antioxidant activity and the total phenolic and anthocyanin levels in bacaba (Table 2), even with autoscaling of the data. In this respect, we assessed the Pearson products for the bacaba samples. There were significant correlations between the total phenolic content and ORAC measurements in acetone (0.64; $p < 0.05$), and aqueous (0.62; $p < 0.05$) extracts, and between TEAC measurements in acetone (0.83; $p < 0.01$) and aqueous (0.96; $p < 0.01$) extracts. This indicates a predominance of total phenolic contribution to the antioxidant activity, despite the presence of anthocyanins. The correlation between total phenolic and ORAC measurements in other fruits was reported previously (Proteggente et al., 2002; Thaipong et al., 2006). Regarding the total anthocyanin content, ORAC and TEAC are highly sensitive to total anthocyanins (Khoo et al., 2011, 2012; Prior et al., 2005; Proteggente et al., 2002); however, Pearson products and PCA were occasionally unable to identify a significant correlation between fruit anthocyanin levels and antioxidant activity. Similar results were previously reported for extracts obtained from 15 blackcurrant cultivars using the ORAC assay (Khoo et al., 2012), and 18 non-traditional fruits from Brazil using the TEAC assay (Rufino et al., 2010).

For the puçá-preto peel, PCA identified close relationships between the total carotenoid, total anthocyanin, and β-carotene bleaching measurements in acetone extracts, confirming the statistical analyses in Tables 2 and 3. PCA indicated that the inhibition of β-carotene degradation by puçá-preto peel extracts was primarily attributed to carotenoids and anthocyanins. However, PCA did not identify a significant correlation between the TEAC and ORAC values in puçá-preto peel extracts (Table 3); this was similar to the situation in the bacaba samples (Khoo et al., 2012; Rufino et al., 2010).

Araçá-boi, bacupari, biribá, cajuí, curriola, marmelada-espinho, murici, and puçá-preto pulp were located within the same group in the PCA plot (Fig. 1), which showed a weaker correlation between the levels of bioactive compounds and the antioxidant activity measurements than the other samples analyzed. Araçá-boi, puçá-preto and murici were previously described in the literature as having high antioxidant potential *in vitro* (Astrid Garzon et al., 2012; Goncalves et al., 2010; Rufino et al., 2010; Souza et al., 2012) and bioactivity *in vivo* (Malta et al., 2012; Mariutti et al., 2013; Neri-Numa et al., 2013; Perez-Gutierrez et al., 2010). In view of this association, we suggest that more detailed studies are required to investigate the bioactive compounds in bacupari, biribá, cajuí, curriola, and marmelada-espinho fruits.

4. Conclusion

Brazilian native fruits investigated in this study contain a high concentration of antioxidants, comparable to commonly consumed fruits such as plums, oranges, guavas, and various berries and citrus fruits. As

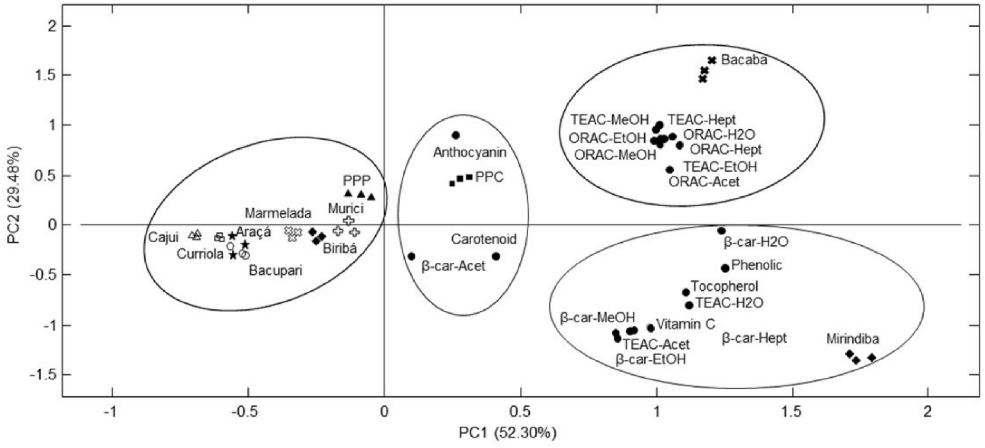


Fig. 1. PCA score plot for araçá-boi, bacaba, bacupari, biribá, cajuí, curriola, marmelada-espinho, mirindiba, murici, pulp (PPP) and peel (PPC) of puçá-preto, and loadings plot for total phenolic, total anthocyanins, total carotenoids, vitamin C, total tocopherols and antioxidant activities measured by β-carotene bleaching, ORAC and TEAC assays in five extracts obtained; phenolic compounds in methanol (MeOH), anthocyanins in aqueous ethanol (EtOH), carotenoids in acetone (Acet), Vitamin C in water (H2O), and tocopherols in heptane (Hept).

previously described in the literature, bacaba, puçá-preto, and murici were found to be rich sources of bioactive compounds. Subsequently, we report that mirindiba had significantly higher levels of carotenoids and tocopherols than camu-camu, acerola, and various berries, in addition to the high vitamin C and total phenolic content. To our knowledge, this is the first comprehensive study of the bioactive compounds and antioxidant activities of biribá, cajuí, marmelada, and mirindiba.

CRediT authorship contribution statement

Paulo Rogério Siriano Borges: Conceptualization, Methodology, Formal analysis, Data curation, Writing – original draft, Funding acquisition. **Merete Edelenbos:** Resources, Supervision, Funding acquisition. **Erik Larsen:** Methodology, Formal analysis. **Thais Hernandes:** Resources. **Elisângela Elena Nunes:** Visualization. **Eduardo Valério de Barros Vilas Boas:** Resources, Visualization, Supervision, Project administration, Funding acquisition. **Caroline Roberta Freitas Pires:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We wish to thank the Coordination for the Improvement of Higher Level - or Education - Personnel (Capes), process number 10058/13-3, and to Aarhus University, for their financial support.

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