



Evaluation of bioactive compounds potential and antioxidant activity in some Brazilian exotic fruit residues



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ARTICLE INFO

Keywords:

Achachairu
Araça-boi
Bacaba
Fruit residues
Antioxidant capacity
Phenolic compounds

ABSTRACT

The agroindustrial residues have been recognized as important sources of some prominent chemical compounds and hence a viable strategy of obtaining bioactive compounds could be applied to them. The present study was aimed to investigate the presence of bioactive compounds and the antioxidant activity of some Brazilian exotic fruits (achachairu, araçá-boi, bacaba) residues. The antioxidant capacity of fruit residues was evaluated by ORAC, FRAP and ABTS assays. The contents of total phenolic compounds, flavonoids, chlorophylls and carotenoids were determined. The identification and quantification of the phenolic compounds were performed by using the UHPLC-QqQ-MS/MS system. The compounds cinnamic acid, *p*-coumaric acid, epicatechin and quercetin were identified and quantified in all fruits residues. The residue with the highest antioxidant capacity was bacaba for ORAC ($15,285.51 \pm 20.38 \mu\text{mol TE}/100 \text{ g}$) and FRAP ($16,916.37 \pm 10.01 \mu\text{mol TE}/100 \text{ g}$) assays, as well as total phenolic compounds in its methanolic extract ($1537.45 \pm 73.35 \text{ mg GAE}/100 \text{ g}$).

1. Introduction

Brazil occupies third position in the production of fruits in the world. There is a large industry which is involved with the processing of the fruit juices whereby a large volume of residues is generated which can be better exploited for the production of highly valorized substances (Forster-Carneiro, Berni, Dorileo, & Rostagno, 2013). These by-products from different fruit processing industries are traditionally discarded as waste and these are currently being recognized as important source of valuable chemicals. In fruit processing, peel and seeds are the two main by-products and their extracts contain a considerable amount of bioactive compounds (Bataglion, Da Silva, Eberlin, & Koolen, 2015; Goot et al., 2016).

Bioactive compounds occur in small amounts in food and are considered as non-nutritional ingredients, but vital for the maintenance of human health (Patil, Jayaprakasha, Chidambaramurthy, & Vikram, 2009). In addition, polyphenols have been found to be the main constituents of fruits and in their residues (Bataglion et al., 2015). Polyphenols are compounds that have > 9000 substances identified. These can be divided into some groups according to their chemical structure: flavonoids (isoflavonoids, anthocyanidins, flavanols, flavonols, flavanones, and flavones) and nonflavonoids (hydroxycinnamic and hydroxybenzoic acids, stilbenoids, lignoids, and coumarins) (Tsao, 2010). In

general, these compounds exhibit anti-inflammatory and antioxidant effects (Kang et al., 2011), besides characterizing a reduced risk of diseases such as certain forms of cancer, inflammation, cataracts, macular and cardiovascular degeneration and degenerative diseases (Sergent, Piront, Meurice, Toussaint, & Schneider, 2010; Snyder et al., 2011).

The bioavailability and function of phenolic compounds present in foods of plant origin in the human body is dependent of many varying factors such as bioaccessibility, food matrix effect, transporters, molecular structures and metabolizing enzymes (Rein et al., 2013). In addition, a refined knowledge about these residues and its chemical characterization would contribute to its use as a source of bioactive compounds (Abdennacer et al., 2015).

The achachairu (*Garcinia humilis*), araçá-boi (*Eugenia stipitata*) and bacaba (*Oenocarpus bacaba*) are edible exotic fruits cultivated in the north and northeast region of Brazil. These fruits are used for the production of beverages and industrialized products such as pulp, ice creams etc. which are widely appreciated by the local population (Shanley & Medina, 2005). Most of the studies on these fruits have been undertaken on their pulp and practically no or very little work has been reported on their residues regarding the presence of bioactive compounds, mainly in relation to the identification and quantification of phenolic and flavonoid compounds.

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Genovese, Pinto, and De Souza (2008) determined the bioactive compounds and antioxidant activity of the araçá-boi pulp and characterized the fruit as an important source of phenolics and flavonoids. Gonçalves, Lajolo, and Genovese (2010) investigated the antioxidant and antidiabetic capacity of araçá-boi pulp and mainly identified the presence of compounds viz. quercetin and kaempferol, highlighting its potential as inhibitor of enzymes of carbohydrate metabolism. Cui et al. (2010) studied the mangosteen (*Garcinia mangostana*), fruit of the same genus as achachairu (*G. humilis*), object of this study, and identified the presence of several phytochemical compounds in peel, rind and aril parts of the fruit, indicating that phenolic acids are mainly located in the pericarp of the fruit and hydroxybenzoic acid derivatives are the major phenolic acids. Finco et al. (2012) evaluated the antioxidant activity of the bacaba pulp and tentatively identified 14 compounds suggesting that bacaba is a promising source of phenolic compounds. Thus it could be concluded from the existing publications that no work has been reported on the residues of achachairu, araçá-boi and bacaba fruits in order to tap their potential for bioactive compounds.

Recent studies on bioactive compounds in fruit extracts have demonstrated that the Ultra High Performance Liquid Chromatography coupled to tandem Mass Spectrometry (UHPLC-QqQ-MS/MS) is a powerful tool in the identification and characterization of organic compounds (Garzón, Narváez-Cuenca, Vincken, & Gruppen, 2017; Souza et al., 2016). Thus, the aim of this work was to determine the presence of bioactive (carotenoids, chlorophylls, phenols and flavonoids) compounds, and to identify and quantify the phenolic and flavonoid compounds by the UHPLC-QqQ-MS/MS system in residues of Brazilian exotic tropical fruits viz. achachairu, araçá-boi, bacaba as well as to evaluate their antioxidant capacity.

2. Materials and methods

2.1. Samples

The residues of araçá-boi and bacaba were obtained from a juice processing industry in Manaus, Amazonas, while the residues of achachairu were collected from a local ice cream shop in Aracaju, Sergipe which processes the pulp of the fruit. The residues were transported in plastic containers maintained at -18°C to the Laboratory of Flavor and Chromatographic Analysis, Federal University of Sergipe, São Cristóvão, Sergipe, Brazil.

The residues of the achachairu, araçá-boi and bacaba were composed of the remains of seeds, peel and to a very small extent of pulp left after their processing for the production of juices or pasteurized pulp. Immediately after its arrival, these residues materials were grinded until they reach a particle size about 2×2 mm.

The approximate yield of each residue corresponds to 70% of the fruit for the achachairu, 27% for the araçá-boi and 56% for the bacaba fruit. The residues were stored in different polyethylene bags at -18°C until analysis. All analyses were done in triplicate for each sample.

2.2. Chemicals

Sodium hydroxide, calcium carbonate, hydrochloric acid, sodium acetate, acetic acid, sodium carbonate, acetone, potassium persulfate were supplied from Synth (São Paulo, Brazil). Ethanol, methanol and ferric chloride were purchased from Neon (São Paulo, Brazil) and phenolphthalein from Dinâmica (São Paulo, Brazil). Folin-Ciocalteu phenol reagent, aluminum chloride, 2,20-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,4,6-tripyridyl-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2'-azobis(2-methylpropionamide) dihydrochloride (AAPH), fluorescein, formic acid (HPLC grade), acetonitrile (HPLC grade), and the phenolic standards such as caffeic acid, cinnamic acid, chlorogenic acid, ferulic acid, gallic acid, *p*-coumaric acid, vanillic acid, (+)catechin, (–)epicatechin, eriodictyol, ethyl gallate, naringenin, quercetin, rutin and

vanillin were obtained from Sigma Aldrich (St. Louis, MO, USA).

2.3. Physico-chemical composition of fruit residues

2.3.1. Moisture

The moisture content was determined on an infrared moisture meter (GEHAKA model IV 2500) by direct reading.

2.3.2. Titratable acidity

The determination was made by titrating with the standardized alkali solution, according to the methodology cited by AOAC (2000, method no. 942.15). Five grams of the sample were weighed, which was transferred to a 125 mL Erlenmeyer flask with the addition of 50 mL of water. Two to four drops of phenolphthalein solution was added and subsequently the mixture was titrated with 0.1 M sodium hydroxide solution until pink. Titratable total acidity was expressed as a percentage of citric acid.

2.3.3. Soluble solids ($^{\circ}\text{Brix}$)

The soluble solids content was determined according to the protocol determined by AOAC (2000, method no. 932.12). An aliquot of each sample was placed on the refractometer prism (The Electron Machine Corporation, model DSA E-Scan), duly calibrated with distilled water, under which direct reading was obtained.

2.3.4. pH

The determination was performed by direct reading in pH meter (HANNA, model HI 2210), according to the method proposed by the AOAC (2000, method no. 945.10). Ten grams of the sample was weighed into a beaker and it was diluted by addition of 100 mL of water. The contents were stirred until the particles were uniformly suspended. With the apparatus previously calibrated with the buffer solutions of pHs 4 and 7, the pH of respective samples was read.

2.4. Extraction of residues

The fruit residues were extracted with different solvents (water and methanol) in order to evaluate the best potential for the extraction of bioactive compounds. The methanolic extraction was followed by the methodology described by Rehman (2006), with alterations. Two grams of the residues of each fruit was weighed and diluted in 15 mL of methanol. The mixture was then maintained in shaker (SOLAB, Brazil, SL 222) at room temperature ($29 \pm 2^{\circ}\text{C}$) for 24 h. The aqueous extraction was performed by autoclaving 2 g of the fruit residues with 15 mL of distilled water at 121°C for 15 min.

All extracts, obtained as above, were centrifuged (Eppendorf Centrifuge, 5810 R) at 24°C at 12,000 rpm for 15 min and the supernatants collected in 100 mL amber colored bottles which were stored in refrigerator (8°C) until the time of use.

2.5. Bioactive compounds

2.5.1. Chlorophyll and carotenoids

For the analysis of chlorophyll and carotenoids, the method of Lichtenthaler (1987) was used. For extraction, 2 g of the samples was weighed, macerated, added to 0.2 g of calcium carbonate and 7 mL of 80% acetone. The extract was filtered through a 25 mL volumetric flask and the volume filled with the solvent itself. The absorbance was measured in a spectrophotometer (Molecular Devices, Sunnyvale, CA, USA; SpectraMax M2) at wavelengths of 470 nm for total carotenoids, 647 nm and 663 nm for chlorophyll a and b, respectively.

The concentrations of total chlorophyll (a and b) and total carotenoids contents were determined according to Eqs. (1.1), (1.2) and (1.3), respectively.

$$\text{Chlorophyll } a \text{ (Ca)} = [12.25 A_{663.2} - 2.79 A_{646.8}] \quad (1.1)$$

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