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Quantification of bioactive compounds in pulps and by-products of tropical fruits from Brazil

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ABSTRACT

This study aimed to quantify the levels of resveratrol, coumarin, and other bioactives in pulps and by-products of twelve tropical fruits from Brazil obtained during pulp production process. Pineapple, acerola, monbin, cashew apple, guava, soursop, papaya, mango, passion fruit, surinam cherry, sapodilla, and tamarind pulps were evaluated as well as their by-products (peel, pulp's leftovers, and seed). Total phenolic, anthocyanins, yellow flavonoids, β-carotene and lycopene levels were also determined. Resveratrol was identified in guava and surinam cherry by-products and coumarin in passion fruit, guava and surinam cherry by-products and mango pulp. These fruit pulp and by-products could be considered a new natural source of both compounds. Overall, fruit by-products presented higher ($P < 0.05$) bioactive content than their respective fruit pulps. This study provides novel information about tropical fruits and their by-products bioactive composition, which is essential for the understanding of their nutraceutical potential and future application in the food industry.

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1. Introduction

Fruit consumption is no longer merely a result of taste and personal preference, but has become a concern of health due to the vital fruit nutrients content. In addition to essential nutrients, most fruits feature considerable amounts of micronutrients, such as minerals, fibers, vitamins and secondary phytochemical compounds. Increasing evidence shows the importance of these micronutrients for human health (Obon, Diaz-Garcia, & Castellar, 2011; Rufino et al., 2010). Diets rich in phytochemicals, such as carotenoids and phenolic compounds, have been associated with a reduced risk of diseases such as certain types of cancer, inflammation, cardiovascular, cataracts, macular degeneration and neurodegenerative diseases (Bueno et al., 2012; Sergent, Piront, Meurice, Toussaint, & Scheinder, 2010; Snyder et al., 2011; Tanaka, Shnimizu, & Moriwaki, 2012).

Tropical fruit consumption is increasing on domestic and international markets due to growing recognition of its nutritional and therapeutic value. Brazil boasts a large number of underexploited native and exotic fruit species of potential interest to the agro-industry and a possible future source of income for the local population. These fruits represent an opportunity for local growers to gain access to special markets where consumers lay emphasis on exotic character and the presence of nutrients capable of preventing degenerative diseases (Alves, Brito, Rufino, & Sampaio, 2008). In addition, there is the potential use of these tropical fruit pulps and their by-products to isolate specific phytochemicals for application in nutraceutical supplements, dietary additives, new food and pharmaceutical products, contributing to the recovery of agro-industrial process waste, with major industrial, economic and environmental impact (Ayala-Zavala et al., 2011). Therefore, the identification and quantification of phytochemicals in pulps and by-products of tropical fruits are of utmost importance to substantiate their potential health benefits in human nutrition.

Brazil is third in production of fresh and processed fruits worldwide, followed by China and India (FAO., 2009). For tropical fruits, Brazil is considered the major producer in the world; with 47% of its production used in the fresh fruit market and 53% in processing (IBRAF., 2009). The fruits included in this study play an important economic role, either in the international market or locally in certain countries of tropical America. More specifically, these fruits

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are harvested and processed for further commercialization in the Northeast region of Brazil. The mass of by-products obtained as a result of processing tropical crops may approach or even exceed that of the corresponding valuable product affecting the economics of growing tropical crops (Miljkovic & Bignami, 2002). For instance, by-products resulting from the processing of papaya, pineapple and mango represent approximately 10–60% of fruit weight (Ayala-Zavala, Rosas-Dominguez, Vega-Vega, & Gonzalez-Aguilar, 2010). By-products of fruits are made up of peels, rinds, seeds, and unused flesh that are generated by different steps of the industrial process and normally have no further usage and are commonly wasted or discarded (Ajila, Bhat, & Rao, 2007). In the past, this costly problem has been mitigated to some extent by processing the by-products further to yield a product that presents less of a disposal problem or that has some marginal economic value (Ayala-Zavala et al., 2010). The economics of processing tropical crops could be improved by developing higher-value use for their by-products. It has now been reported that the by-products of tropical fruits contain high levels of various health enhancing substances that can be extracted to provide nutraceuticals (Gorinstein et al., 2011). In addition, the full utilization of fruits could lead the industry to a lower-waste agribusiness, increasing industrial profitability. The use of the entire plant tissue could have economic benefits to producers and a beneficial impact on the environment, leading to a greater diversity of products (Peschel et al., 2006).

A number of studies for determination of the bioactive composition of tropical fruits have been reported (Barreto, Benassi, & Mercadante, 2009; Pierson et al., 2012; Rufino et al., 2010; Sousa, Pereira, Queiroz, Borges, & Carneiro, 2012); however, a detailed comprehensive characterization including their by-products and individual phenolic compounds (resveratrol and coumarin) has not been reported so far. Furthermore, variations in sample preparation may also affect results greatly, yielding conflicting and non-comparable results, and this is a problem deserving attention from researchers.

Taking into account the potential of compounds present in pulps and by-products of tropical fruits as anti-inflammatory and antioxidant agents, and the fact that very few reports exist to date on the characterization of polyphenolic and carotene compounds in these products, this study aimed to quantify and compare the major bioactive compounds found in pulp and by-products of commercialized tropical fruits from Brazil.

2. Materials and methods

2.1. Chemical reagents

Resveratrol, coumarin, gallic acid standards and solvents used for HPLC analysis (acetonitrile and methanol) were obtained from Sigma Aldrich (Steinheim, Germany). All other reagents were analytical grade and were purchased from VWR International (Radnor, PA).

2.2. Samples and sample preparation

Samples consisted of fresh, non-pasteurized frozen pulps of pineapple (*Ananas comosus* L.), acerola (*Malpighia emarginata* D.C.), monbin (*Spondias mombin* L.), cashew apple (*Anacardium occidentale* L.), guava (*Psidium guajava L.*), sourspop (*Annona muricata* L.), papaya (*Carica papaya* L.), mango (*Mangifera indica* L.), passion fruit (*Passiflora edulis* Sims), surinam cherry (*Eugenia uniflora* L.), sapodilla (*Manikara zapota* L.) and tamarind (*Tamarindo indica* L.) were obtained from fruit processing plants in the state of Ceará, Brazil. The by-products were used from the production process of pulps, obtained after pulping of: pineapple (peel and pulp's leftovers), acerola (seed), cashew apple (peel and pulp's leftovers), guava (peel, pulp's leftovers, and seed), soursop (pulp's leftovers and seed), papaya (peel, pulp's leftovers, and seed), mango (peel and pulp's leftovers), passion fruit (seed), surinam cherry (pulp's leftovers), and sapodilla (peel, pulp's leftovers and seed). By-products samples containing different fruit sessions were evaluated together as a group. The samples were obtained in December 2011.

All samples (pulp and by-products) were freeze-dried at −50 °C under 5 mtorr (9.67×10^{-5} psi) vacuum for 48 h in a Labconco Freeze Dry-5 dryer (Labconco, MO). The freeze-dried material was stored in a desiccator protected from light until further use. Moisture content was determined for all samples following AOAC method 920.151 (data not shown) (AOAC, 1995).

2.3. Anthocyanins and yellow flavonoids determination

Analyses of anthocyanins and yellow flavonoids were carried out as described by Francis (1982). Briefly, 1 g of each freeze-dried sample was suspended in 10 ml of extraction solution (1.5 N HCl in 85% ethanol). Samples were homogenized, transferred to a 50 ml volumetric flask, and extracted for 13 h under refrigeration in the dark. After this period, the extracts were filtered (Whatman No. 1 filter paper) and absorbance at 535 nm (anthocyanins) and 374 nm (yellow flavonoids) were measured in a Shimadzu UV-1800 spectrophotometer (Columbia, MA). The content of anthocyanins and yellow flavonoids were calculated using Equation 1 and absorption coefficients of 982 and 766 $(g/100\,ml)^{-1}cm^{-1}$, respectively.

$$\text{Anthocyanins content}\,(mg/100g\,d.b.) = \frac{(\text{ABS}\times\text{dilution factors})\times 1000}{(\text{sample dried weight}\times \varepsilon^{1\%}_{1\,cm,535})} \quad (1)$$

where *ABS* is absorbance reading of sample at 535 nm, and $\varepsilon^{1\%}_{1\,cm,535}$ is the absorption coefficient for anthocyanins. Yellow flavonoids content was calculated using the same equation with absorbance reading at 374 nm and its respective absorption coefficient.

2.4. β-Carotene and lycopene determination

β-Carotene and lycopene were extracted and quantified according to the method described by Nagata and Yamashita (1992). Briefly, 1 g of each freeze-dried sample was suspended in 10 ml of extraction solution ((2:3) acetone: hexane) and mixed for 1 min. Samples were filtered (Whatman No. 1) and spectrophotometric readings were obtained at 453, 505, 645, and 663 nm and results were expressed as μg of β-carotene or lycopene/100 g dry basis (d.b.).

2.5. Total phenolic determination

Total phenolics content were determined by the Folin–Ciocalteu method (Waterhouse, 2002). First, freeze-dried samples were weighed (10–25 g) in centrifuge tubes and extracted sequentially with 40 ml of 50% (v/v) ethanol in water solution at room temperature for 1 h. Tubes were centrifuged at 2540 g for 15 min and the supernatant was recovered. Then, 40 ml of 70% (v/v) acetone in water was added to the residue, extracted for 60 min at room temperature, and centrifuged for a second time (2540g for 15 min). Ethanol and acetone extracts were combined, made up to 100 ml with distilled water and used for *Folin–Ciocalteu* analysis. Extracts (1 ml) were mixed with 1 ml of *Folin–Ciocalteu* reagent (1:3), 2 ml of 20% (w/v) sodium carbonate solution and 2 ml of distilled water. After 1 h, absorbance at 700 nm was measured using a spectrophotometer. Results were expressed as gram of gallic acid equivalents per 100 g of sample dry basis (GAE/100 g d.b.).