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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.).

2.6. Resveratrol and coumarin analysis by HPLC

For quantification of resveratrol, freeze-dried samples (5 g) were suspended in 60 ml of extraction solution (ethanol:water at 1:1 ratio) and heated for 30 min at 70 °C under constant stirring. Following, extracts were filtered (Whatman No. 1) and concentrated under vacuum at 70 °C using a rotoevaporator (Buchi R-210 Rotavapor, Buchi Co., New Castel, DE). The concentrate was resuspended in 5 ml methanol and analysed by HPLC.

For quantification of coumarin, 1 g of freeze-dried samples was suspended in 10 ml of extraction solution (ethanol:water at 1:1 ratio), macerated until completely homogeneous, and allowed to rest for 24 h at room temperature. The material was filtered (Whatman No. 1) and the extract obtained was analyzed by HPLC.

Analysis were conducted in HPLC Shimadzu LC-20A system equipped with LC-20DA pump, manual injector with a fixed volume of 100 μL, CTO-20A column oven set at 40 °C, running LC Solution software with UV–Vis detector model SPD-20A. The column was a Nova Pack C18 (CLC-ODS, 3 μm; 4.6 × 250 mm).

For resveratrol quantification, the method described by Sautter et al. (2005), with modifications, was used. Briefly, the mobile phase consisted of water acidified to pH 2.9 using phosphoric acid (H_3PO_4) (Solution A) and acetonitrile (solution B) in a ratio of 75A:25B, isocratic with a flow rate of 1.2 ml/min, with an injection volume of 100 μL, UV detection at 306 nm, and a total run time of 15 min per sample at 40 °C. For coumarin quantification, the mobile phase consisted of water (Solution A) and methanol (Solution B) in a ratio of 60A:40B, isocratic with a flow rate of 1.0 ml/min, injection volume of 100 μl, UV detection at 274 nm, and a total run time of 15 min per sample at 40 °C. The parameters obtained in the validation of the methods are shown on Table 1.

Standard curves for resveratrol and coumarin were prepared under the same conditions. Resveratrol (0.46, 0.092, 0.046, 0.0184 and 0.0092 mg/ml) and coumarin (43.6, 21.8, 10.9, 5.45 and 2.73 mg/ml) standards were diluted in methanol. Initially, sample injections were made with resveratrol and coumarin standards, using the internal standard method, in order to identify these compounds in the sample runs. The co-injection consisted of sample and standard compound in a ratio of 1:1 with standard concentrations of 0.4 mg/ml and 1 mg/ml in methanol for resveratrol and coumarin, respectively.

2.7. Statistical analysis

This experiment was based on a completely randomized design with equal replications. For all analyses, determinations were made in triplicate as independent experiments. Data analysis was performed using JMP v. 9 software (SAS Institute, Cary, NC) for anthocyanins, yellow flavonoids, β-carotene, lycopene, total phenolics, resveratrol, and coumarin. Differences between variables were tested for significance by one-way analysis of variance (ANOVA). Significantly different means ($p < 0.05$) were separated by the Tukey's test. Data are presented as mean ± SD (standard deviation).

Table 1
Method validation for the chromatographic analysis of resveratrol and coumarin in pulp and by-products of tropical fruits.

Parameter	Resveratrol	Coumarin
Limit of detection (ng/mL)	0.56×10^{-2}	0.55
Limit of quantification (ng/mL)	1.84×10^{-2}	1.09
Linearity[a]	$y = 1.94 \times 10^{8}x + 182465.10$	$y = 101{,}172x + 102{,}127$
Correlation coefficient (R^2)	0.999	0.999
Retention time (min)	5.05	6.82

[a] x is the concentration in μg/ml and y is the peak area at designated UV wavelength.

3. Results and discussion

3.1. Anthocyanins and yellow flavonoids determination

The results for anthocyanins and yellow flavonoids content are listed on Table 2. Anthocyanins are brightly-colored compounds responsible for much of the red, blue, and purple coloring of fruits. They are especially abundant in berries such as blueberries and blackcurrants (Kahkonen, Hopia, & Henonen, 2001). Acerola and surinam cherry (pulp and by-product) showed the highest ($P < 0.05$) levels of total anthocyanins. An interesting finding was that the by-products of cashew apple, papaya, mango, passion fruit, and surinam cherry showed higher ($P < 0.05$) levels of total anthocyanins than those obtained in their pulps, which provide potential applications for nutraceutical supplements, dietary additives and/or pharmaceutical products. No anthocyanins were detected on soursop and sapodilla pulps and sapodilla by-product.

Except for surinam cherry and acerola, total anthocyanins values for fruit pulps were lower in comparison to common berries, such as, red grapes (137.8 mg/100 g d.b.), strawberries (236 mg/100 g d.b.), red raspberries (647.9 mg/100 g d.b.), cherries (616.2 mg/100 g d.b.), and blackberries (2954.2 mg/100 g d.b.) (Wu et al., 2006). Comparable levels of total anthocyanins were observed on different cultivars of apple and peach, with values ranging from 8.2 to 84.8 mg/100 g d.b. and 0.8 to 3.1 mg/100 g d.b., respectively (Segantini, Leonel, Lima, Costa, & Ramos, 2012; Wu et al., 2006).

Only monbin, passion fruit, surinam cherry and tamarind pulps and acerola, cashew apple, papaya, mango, passion fruit and surinam cherry by-products presented yellow flavonoids in their content (Table 2). The values obtained for yellow flavonoids in the fruit pulps are in the same range of those reported for tropical fruits (Rufino et al., 2010); although, similar values are observed difference in the origin of fruit samples makes a comparison difficult. By-products samples showed higher levels ($P < 0.05$) of yellow flavonoids than the pulps, similar to the results obtained for total anthocyanins.

3.2. β-Carotene and lycopene determination

According to Almeida et al. (2011), foods rich in antioxidants play an essential role in the prevention of diseases. The antioxidant capacities of fruits vary depending on their contents of vitamin C, vitamin E, carotenoids, and particularly -carotene (von Lintig, 2010), and lycopene (Shami & Moreira, 2004) as well as flavonoids and other polyphenols (Saura-Calixto & Goni, 2006).

The results for β-carotene and lycopene content are listed on Table 3. Carotenoids are tetraterpenoids found throughout the flowering plant kingdom as a pigment mostly responsible for the red, orange or yellow color of fruits and are important vitamin A precursors. As they are found widely in plants, it is not surprising that a large number of carotenoids have been reported in tropical fruit species (Pierson et al., 2012). Acerola and papaya pulps showed the highest ($P < 0.05$) content of β-carotene. Surinam cherry by-product has shown to be an excellent source of β-carotene when compared to the other by-products. Similar levels of β-carotene have been previously reported for tropical fruits (Assuncao & Mercadante, 2003; Chen, Tai, & Chen, 2007; Dias, Camoes, & Oliveira, 2009; Segantini, Leonel, Lima, Costa, & Ramos, 2012). Although a general trend is followed difference in the origin of fruit samples makes a comparison difficult. Furthermore, these fruits

Table 2
Anthocyanins and yellow flavonoid levels in pulps and by-products of tropical fruits.

Fruits	Anthocyanins (mg/100 g dry basis)		Yellow Flavonoids (mg/100 g dry basis)	
	Pulp	By-product	Pulp	By-product
Pineapple	$11.62 \pm 2.10^{b}_{x}$	$10.10 \pm 0.27^{a}_{x}$	nd	nd
Acerola	$144.27 \pm 6.53^{c}_{x}$	$245.90 \pm 24.38^{b}_{y}$	nd	98.05 ± 0.19^{b}
Monbin	$7.32 \pm 0.71^{a,b}$	np	54.22 ± 1.65^{b}	np
Cashew apple	$7.62 \pm 0.76^{a,b}_{x}$	$14.74 \pm 0.29^{a}_{y}$	nd	44.91 ± 4.43^{a}
Guava	$8.79 \pm 0.88^{a,b}_{y}$	$0.90 \pm 0.03^{a}_{x}$	nd	31.41 ± 3.10^{a}
Soursop	nd	nd	nd	nd
Papaya	$1.87 \pm 1.87^{a}_{x}$	$22.43 \pm 2.23^{a}_{y}$	nd	97.30 ± 0.96^{b}
Mango	$7.85 \pm 0.80^{a,b}_{y}$	$2.29 \pm 0.23^{a}_{x}$	nd	26.47 ± 0.27^{a}
Passion fruit	$3.48 \pm 0.26^{a}_{x}$	$3.70 \pm 0.39^{a}_{y}$	$60.37 \pm 5.91^{b}_{y}$	$43.08 \pm 4.00^{a}_{x}$
Surinam cherry	$226.90 \pm 2.92^{d}_{x}$	$1021.22 \pm 42.53^{c}_{y}$	$102.45 \pm 9.04^{c}_{x}$	$207.87 \pm 19.43^{c}_{y}$
Sapodilla	nd	1.07 ± 0.11^{a}	nd	nd
Tamarind	2.92 ± 0.23^{a}	np	15.06 ± 0.49^{a}	np

Results expressed by average ± standard deviation ($n = 3$). nd: not detected, np: not performed.
x,y means within a row which are not followed by a common capital letter are significantly different ($P < 0.05$).
a,b means within a column which are not followed by a common lowercase letter are significantly different ($P < 0.05$).

Table 3
β-carotene and lycopene levels of pulps and by-products of tropical fruits.

Fruits	β-Carotene (μg/100 g dry basis)		Lycopene (μg/100 g dry basis)	
Common name	Pulp	By-product	Pulp	By-product
Pineapple	$42.86 \pm 4.26^{a}_{x}$	$156.10 \pm 15.63^{b,c}_{y}$	nd	nd
Acerola	$2623.57 \pm 262.42^{e}_{y}$	$272.83 \pm 27.24^{c}_{x}$	nd	nd
Monbin	1422.77 ± 142.30^{d}	np	nd	np
Cashew apple	$454.19 \pm 45.44^{b}_{y}$	$179.14 \pm 17.92^{c}_{x}$	nd	nd
Guava	$52.12 \pm 5.69^{a}_{y}$	$26.67 \pm 2.67^{a}_{x}$	$35.01 \pm 3.62^{a}_{y}$	$18.11 \pm 1.83^{a}_{x}$
Soursop	nd	nd	nd	nd
Papaya	2024.68 ± 199.86^{e}	490.29 ± 0.08^{d}	$2077.04 \pm 208.04^{c}_{y}$	$85.52 \pm 0.08^{a}_{x}$
Mango	$953.60 \pm 95.36^{c}_{y}$	$58.26 \pm 5.83^{a,b}_{x}$	nd	nd
Passion fruit	$1362.07 \pm 136.22^{d}_{y}$	$57.93 \pm 5.80^{a,b}_{x}$	nd	nd
Surinam cherry	$1564.06 \pm 156.45^{d}_{y}$	$1110.85 \pm 111.11^{e}_{x}$	$1445.16 \pm 1.42^{b}_{y}$	$693.26 \pm 69.29^{b}_{x}$
Sapodilla	nd	nd	$41.93 \pm 4.21^{a}_{x}$	$36.48 \pm 2.21^{a}_{x}$
Tamarind	1.20 ± 0.12^{a}	np	0.09 ± 0.01^{a}	np

Results expressed by average ± standard deviation ($n = 3$). nd: not detected, np: not performed.
x,y means within a row which are not followed by a common capital letter are significantly different ($P < 0.05$).
a,b means within a column which are not followed by a common lowercase letter are significantly different ($P < 0.05$).

and by-products should not be considered a rich source of carotenoids, where values as high as 161 mg/100 g d.b. for wine palm (*Mauritia vinifera*) one of the most important vitamin A precursors in the Brazilian flora has been reported (Godoy & Rodriguez-Amaya, 1998; Rufino et al., 2010).

Lycopene is considered the carotenoid with the greatest capacity to eliminate the singlet oxygen. Studies have demonstrated that lycopene protects lipid molecules, low-density lipoproteins, proteins, and DNA against free radical attack, playing an essential role in the protection against diseases (Agarwal & Rao, 2000; Porrini et al., 2005). From the fruits evaluated only surinam cherry, papaya, sapodilla, guava and tamarind pulps and surinam cherry, papaya, sapodilla and guava by-products showed detectable levels of lycopene in their content (Table 3). All of the pulps and byproducts analyzed had low concentrations of lycopene compared with tomatoes, a lycopene-rich fruit. Carvalho, Fonseca, Silva, Boiteux, and Giordano (2005) studied different tomato hybrids and concluded that the content of lycopene in the ripe fruit varies from 149.6 to 191.6 mg/100 g d.b.

3.3. Total phenolic determination

Following the example of previous studies (Vasco, Ruales, & Kamal-Eldin, 2008) that tested fruits from Tropical regions for their polyphenol contents, we classified our fruits into three categories: low (<500 mg GAE/100 g d.b.), medium (500–2500 mg GAE/100 g d.b.) and high (>2500 mg GAE/100 g d.b.). Acerola pulp had the highest levels of total phenolic compounds followed by cashew apple, surinam cherry, and soursop (Table 4). For by-products, surinam cherry showed the highest levels ($P < 0.05$) of total phenolic compounds followed by acerola, cashew apple, and pineapple (Table 4). These fruit pulps and by-products could therefore be categorized as having a high concentration of phenolic compounds, consequently an excellent source of phenolic compounds. All the other fruit pulps evaluated, except for sapodilla pulp could be categorized as having a medium content of phenolic compounds, and consequently be considered as a good source of phenolic compounds. Similar observation could be done for fruit by-products, with exception of mango and passion fruit, all the other by-products could be considered as a good source (medium content) of phenolic compounds.

Almeida et al. (2011) reported 445.6 mg GAE/100 g d.b. for papaya, 298.6 mg GAE/100 g d.b. for pineapple and 122.2 mg GAE/100 g d.b. for tamarind and these values are lower than the levels reported here. Similarly, Sousa, Pereira, Queiroz, Borges, and Carneiro (2012) found 1491.5 mg GAE/100 g d.b. for soursop. Bagetti et al. (2011) found 3026 mg GAE/100 g d.b. for surinam cherry which is similar to the values obtained in this study. These

Table 4
Total phenolic content of pulps and by-products of tropical fruits.

Fruits	Total phenolic (mg GAE/100 g dry basis)	
	Pulp	By-product
Pineapple	990.76 ± 81.39$^{b,c}_{x}$	2787.09 ± 225.38$^{e}_{y}$
Acerola	29093.47 ± 799.68$^{g}_{y}$	7265.29 ± 16.78$^{g}_{x}$
Monbin	925.84 ± 46.84^{a,b}	np
Cashew apple	5286.49 ± 250.34$^{f}_{x}$	6588.41 ± 370.32$^{f}_{y}$
Guava	1723.06 ± 111.58$^{c}_{x}$	1987.19 ± 8.06$^{d}_{y}$
Soursop	2886.60 ± 119.05$^{d}_{y}$	1439.63 ± 22.32$^{c}_{x}$
Papaya	1263.70 ± 126.97$^{b,c}_{y}$	783.37 ± 25.38 $^{a,b}_{x}$
Mango	652.59 ± 22.53$^{a,b}_{y}$	376.12 ± 37.62$^{a}_{x}$
Passion fruit	765.09 ± 15.95$^{a,b}_{y}$	451.06 ± 40.63$^{a}_{x}$
Surinam cherry	3957.20 ± 194.45$^{e}_{x}$	12696.03 ± 313.39$^{h}_{y}$
Sapodilla	209.45 ± 20.03$^{a}_{x}$	1053.43 ± 105.41 $^{b,c}_{y}$
Tamarind	923.34 ± 53.35^{a,b}	np

The results are expressed as mean ± standard deviation ($n = 3$). GAE = Gallic acid equivalent. np: not performed.
x,y means within a row which are not followed by a common capital letter are significantly different ($P < 0.05$).
a,b means within a column which are not followed by a common lowercase letter are significantly different ($P < 0.05$).

differences could be related to several factors that can influence the levels of bioactive compounds in fruits and their by-products, such as type of cultivation, climate, fruit variety, and time of the year (Deng, West, & Jensen, 2010).

3.4. Resveratrol and coumarin determination

Resveratrol was identified in guava and surinam cherry by-products (Table 5) with levels of resveratrol of 25.67 and 112.51 μg/g d.b., respectively, results not reported so far in the literature. Resveratrol was not detected in the other fruits and by-products samples. Resveratrol is a natural stilbene found in many vegetable species, generally in two forms: trans-(E) and cis (Z), with *trans*-Resveratrol isomer being recognized for its biological activity (Medina-Bolivar et al., 2007; Sautter et al., 2005). *trans*-Resveratrol displays antioxidant and anti-inflammatory properties (Kalantari & Das, 2010). *trans*-Resveratrol has been widely studied in grapes and red wines. Even though many factors such as the plant variety, environmental conditions, extraction procedure and solvent used during extraction can influence the quantification of this compound in plant tissues (Roldan, Palacios, Caro, & Perez, 2003), it reaches about 40.6 μg/g in the aqueous extract from Thompson seedless dried grapes (Zhao & Hall, 2008). Other edible and non-edible sources of resveratrol include dark chocolate (0.4 μg/g d.b.) (Counet, Callemien, & Collin, 2006), peanuts (0.03–0.14 μg/g d.b.) (Sanders, McMichael, & Hendrix, 2000), cranberry 5355.6 ng/g d.b. (Borowska, Mazur, Kopciuch, & Buszewski, 2009) and grape skin and pomace (1.17–5.32 mg/100 g d.b. *Muscadine* variety) (Casas et al., 2010). When compared to the content of this compound in other commercial polyphenolic extract as determined by Counet et al. (2006), and Casas et al. (2010) one can notice that the content of this compound in guava and surinam cherry by-products (2.57 mg/100 g and 11.25 mg/100 g dry weight), even if present in lower quantities than in commercial red wine (337 mg/100 g dry extract), is still higher than in white grape pomace (0.9 mg/100 g d.b.), skin (3.1 mg/100 g d.b.), stem (1.7 mg/100 g d.b.), and seed (0.2 mg/100 g d.b.). Therefore, guava and surinam cherry by-products can be considered as a rich source of resveratrol. That is particularly interesting considering that this compound has a wide range of nutraceutical and phytopharmaceutical properties.

Table 5
Levels of resveratrol and coumarin in pulp and by-products of tropical fruits.

Sample	Level (mg/100 g dry basis)
Resveratrol Content	
Guava by-product	25.67 ± 0.67[a]
Surinam cherry by-product	112.51 ± 2.01[b]
Coumarin Content	
Guava by-product	102.49 ± 4.26[b]
Passion fruit by-product	60.28 ± 6.04[a]
Surinam cherry by-product	71.18 ± 6.84[a]
Mango pulp	57.39 ± 6.13[a]

The results are expressed as mean ± standard deviation ($n = 3$).
[a,b] Within a column which are not followed by a common lowercase letter are significantly different ($P < 0.05$).

Coumarin (1,2-benzopyrone) is a natural product having a sweet-herbaceous and cherry flower-like odor (Yang et al., 2009). Coumarins are lactones derived from *o*-hydroxycinnamic acids by cyclization and ring closure between the *o*-hydroxy and carboxyl groups. These compounds are present mainly in the families *Asteraceae*, *Apiaceae*, *Fabaceae*, *Lamiaceace*, and *Poaceae*. Coumarin occurs in plant tissue in bound form as the *trans*-*o*-glucosyloxycinnamic acid (Kovacik & Repcak, 2008). Coumarin and its derivatives have been associated to antimicrobial and anti-inflammatory activities with antioxidative activities (Ikeda, Wada, Nishigaki, & Nakashima, 2009; Kovacik & Repcak, 2008). Coumarin was detected and quantified in guava, passion fruit, surinam cherry by-products and mango pulp with values ranging from 57.39 to 102.49 μg/g d.b. (Table 5), results not reported so far in the literature. Coumarin was not detected in the other fruits and by-products samples. The fruits and by-products analyzed in this study were compared to coumarin levels in *Lamiaceace* family plants and cinnamon bark, which are known to have high concentrations of coumarin and are widely used in traditional medicine and horticulture (such as lavender, salvia, rosemary, oregano, and basil). Levels of coumarin ranged from 14.3 to 276.9 μg/mg dried weight for *Lamiaceace* family plants, with the genera *Lavandula* and *Salvia* showing the highest content (Lee et al., 2011) and as high as 29.4 mg/g d.b. for ethanolic extract of cinnamon bark (Ho, Chang, & Chang, 2013). One can see that the content of coumarin in the by-products and pulp observed even if present in lower quantities than cinnamon bark is still considerably higher than other commercially available sources such as *Lamiaceace* family plants. Consequently, these fruit by-products and pulp analyzed in this study can be regarded as a rich natural source of coumarin.

4. Conclusion

Overall the fruit by-products showed higher ($P < 0.05$) levels of β-carotene and lycopene, as well as anthocyanins and yellow flavonoids when compared to the fruit pulps studied. Regarding the considerable amounts of anthocyanins, yellow flavonoids and phenolic compounds, our results indicate promising perspectives for the exploitation of these non-traditional tropical fruit species and their by-products with considerable levels of nutrients. The considerable amount of resveratrol and coumarin for some of the fruits and by-products included in this study are likely to draw attention to these species and most importantly to their by-products as potential commodities. Guava and surinam cherry by-products presented resveratrol in their constitutions and can be considered a rich source of this compound. Similarly, for coumarin, passion fruit, guava and surinam cherry by-products and mango pulp can be considered rich natural sources of coumarin. The data obtained in this study add valuable information to current knowledge of the nutritional properties of tropical fruits and their by-products,

especially if one considers the broad spectrum of properties of the compounds identified.

This study showed that agro-industrial by-products are good sources of bioactive compounds and the exploitation of these abundant and low-cost renewable resources could be anticipated for the pharmaceutical and food industries with opportunities of developing new nutraceutical and/or pharmaceutical products, reduction of industrial waste and cost, and ultimately providing a positive economic and environmental impact.

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