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Evaluation of Total Phenolic Compounds and Antioxidant Activity in Amazon Fruit

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In this work, nine fruits cultivated in the northern Amazon were studied: abiu (Pouteria caimito), acerola (Malpighia emarginata), araçá (Psidium cattleianum), bacuparí (Rheedia gardneriana), biribá (Rollinia mucosa), caçarí (Myrciaria dubia) (Annona squamosa), graviola (Annona muricata) and taperebá (Spondias mombin L.). The total phenolic compounds were evaluated in the pulp, seed and bark by means of the colorimetric reaction of Folin Ciocateau, as well as the antioxidant capacity in the different extracts. DPPH method and on the other hand by the iron reduction method. As the fruits that presented a greater quantity of phenolic compounds are in mg gallic acid. $100g^{-1}$ sample, we have: bark of camu camu (1241.1 ± 12.04), followed by the abiu bark with (1132,43 ± 8,10), araçá pulp (1080,21 ± 1,1) and Pulp of acerola (1071.4 ± 22.2). Evaluating the antioxidant capacity, the evaluated fruits that present a higher value of antioxidant capacity are the Araçá seed with EC50 value of (471,23 ± 21,23 g / g DPPH) and for iron reduction of (57,21 ± 4.11 mmol Fe_2SO_4 / g), followed by the EC50 of abiu bark (521.71 ± 1.34 g / g DPPH) and iron reduction of 411.43 ± 27.12 µmol Fe_2SO_4 / g), and for the camu-camu pulp (549.24 ± 21.13 g / g DPPH) and for reduction of iron (235.47 ± 11.44 mmol Fe_2SO_4 / g). Multivariate analysis methods were applied through Principal Component Analysis (PCA) with pulp having the highest correlation between data variability with 93.6% according to PCA.

1. Introduction

Phenolic compounds are part of secondary metabolites of plants, mainly showing the function of protecting the plant against organisms and pests, consequently, influencing the nutritional value of food, and sensorial quality, besides conferring physical-chemical attributes such as color, texture, bitterness and astringency (Evertte et al., 2010). The phenolic compounds present bioactive potential as anti-inflammatory, antioxidant and antitumor (BOGANI et al., 2007; WENG AND YEN, 2012).
According to Abe et al. (2007), the phenolic compounds can be classified between flavonoids and non-flavonoids, and the flavonoids are catechins, epicatechins, epigallocatechins, caempferol, quercertin, myricetin, anthocyanins, rutin and naringenin, and within non-flavonoids, phenolic acids, hydroxybenzoic acid, Hydroxycinnamic acid and resveratrol. The problem with the above phenolic compounds according with Fang and Bhandari (2010), the problem with instability is that insulation techniques increase the physical stability of

these compounds, thus increasing the stability, thus protecting the phenolic composition of the interaction of others Compounds in the food, in addition to having their release controlled, thus increasing their bioactivity.
A parameter used to measure the amount of total phenolic compounds in biological samples is the total antioxidant capacity, which can be used for pure compounds and for matrices of food plants (Choi et al.,2002). In this way, the total phenolic compounds and the antioxidant potential will be evaluated and for nine fruits grown in the northern Amazon (abiu, acerola, camu-camu, bacupari, fruit of the earl, graviola, araçá, biribá and taperebá). Iron reduction method, and DPPH, as well as total phenolic compounds by the Folin-Ciocateau method, and statistically correlated using multivariate analysis analysis techniques PCA with infostar program version 2016.

2. Materials and methods

2.1 Sample preparation

The samples of the different species studied in this work were collected "in the markets in several points of the State of Roraima, in the markets of the city of Boa Vista and directly with producers who owned the fruits, in order to prepare a representative composite sample for each fruit. their botanical names are shown in Table 1.

Table 1 - Names and families of cultivated fruits cultivated in the Northern Amazon.

Scientific name	Family	Common Name in Brazil
Pouteria caimito	Sapotáceas	Abiu
Malpighia emarginata	Malpighiaceae	Acerola
Psidium cattleianum	Myrtaceae	Araçá
Rheedia gardneriana Planch & Triana	Clusiaceae	Bacuparí
Rollinia mucosa	Annonaceae	Biribá o fruta da condesa
Myrciaria dubia (Krunth) Mc Vaugh, Myrtaceae	Myrtaceae	Camu-camu
Annona squamosa	Annonaceae	Fruta-do-conde
Annona muricata	Annonaceae	Graviola
Spondias mombin L.	Anacardiaceae	Taperebá

The samples were taken to the laboratory of Environmental Chemistry of the Federal University of Roraima, where they were selected those that had an excellent state of conservation, washed previously with distilled water, then with 1% sodium hypochlorite solution and again with distilled water.
The material was separated into pulp, bark and seed, they were taken to the laboratory of the Agronomic Research Center (NUPAGRI), at the Agricultural Sciences Center, Campus de Cauamé, UFRR, where they were lyophilized in LÍOTOP liquefaction model L 101 for 48 hours until drying the material. Subsequently, the material was dried, milled with LABOR model SP31, and placed in hermetically sealed bags and stored protected from light until analysis.

2.2 Determination of total phenolic compounds

The determination of the total phenolic compounds (CFT) was done according to the methodology proposed by Wolfre et al. (2013) where methanolic extracts were prepared from the extraction of 4.0 grams of lyophilized material with 35 mL of 80% (v/v) methanol acidified with 0.5% (v/v) hydrochloric acid, in falcon tubes and were subsequently placed in a bath with water at 90 °C for 30 minutes, the supernatant being separated and remaining material was added again 35 mL and treated under the same conditions as above.
The fractions were then pooled and centrifuged at 6000 rpm for 30 minutes. The samples were placed in amber glasses and stored in the refrigerator at 2 °C until the analysis.

2.3 Determination of antioxidant activity

The determination of the antioxidant activity in the different extracts was by different methods: the method of extinguishing the absorption of the radical 1,1-diphenyl-2-picrylhydrazyl (DDPH); and the iron reduction method. The DDPH method was developed using visible ultraviolet molecular absorption spectrophotometry, measured at 515 nm (MIRANDA; FRAGA, 2006) in Shimadzu UV-1800.
The methodology of iron reduction was described by Barros et al. (2010), using different concentrations of the methanolic extracts. 0.5 ml aliquots of each concentration were mixed with 0.5 ml sodium phosphate buffer (200 mmol L^{-1}, pH 6.6) and 0.5 ml potassium ferrocyanide (1% w/v, in water). The mixture was incubated for 20 minutes at 50 °C using 0.5 mL of trichloroacetic acid (10% w/v).

3. Results and discussion

In the Table 2 shows the values of total phenolic compounds for the different fruit samples studied using the Folin Ciocateau test, using gallic acid as standard, with the calibration curve of y = 0,0173 x + 0,0431 r^2 = 0.994

Table 2 :Total phenolic compounds in Amazonian fruits.

Fruit		mg gallic acid.100^{-1} g sample
Abiu	pulp	900.2 ± 7.3
	peel	1132.43 ± 8.1
	seeds	611.34 ± 6.2
Acerola	pulp	1071.4 ± 22.2
	peel	1042.1 ± 17.4
	seeds	312.3 ± 11.1
Araçá	pulp	1080.21 ± 1.1
	peel	110.14 ± 12.4
	seeds	941.3 ± 11.2
Bacuparí	pulp	31.2 ± 1.1
	peel	78.21 ± 2.1
	seeds	54.11 ± 1.3
Biribá	pulp	101.3 ± 2.2
	peel	85.4 ± 1.1
	seeds	209.4 ± 12.1
Camu camu	pulp	1741.2 ± 34.3
	peel	1241.1 ± 12.0
	seeds	241.2 ± 7.4
Fruta-do-conde	pulp	11.3 ± 0.7
	peel	92.2 ± 1.2
	seeds	58.3 ± 0.4
Graviola	pulp	89.2 ± 2.2
	peel	427.3 ± 11.2
	seeds	632.3 ± 2.4
Taperebá	pulp	524.3 ± 11.4
	peel	558.3 ± 10.12
	seeds	7.13 ± 0.21

Vasco et al. (2008) classify the polyphenolic compounds in different categories according to the content of polyphenols in the samples being from below to 100 mg $100g^{-1}$ EGA, (average of gallic acid in 100 grams of sample), average for concentrations between 100- 500 mg $100g^{-1}$ EGA and high for values having greater than 500 mg EGA.100 g^{-1}.

In the case of the samples studied in this work, they present low values according to the previous classification of the tapereba seed with values of 7.31 ± 0.21 mg $100g^{-1}$ EGA, the bacupari with low values of phenolic compounds in all parts of the fruit, presenting 31.2 ± 1.1 mg $100g^{-1}$ EGA for the pulp, 85.4 ± 1.1 mg $100g^{-1}$ EGA for the seed and 78.21 ± 2.1 mg $100g^{-1}$ EGA for the waterfall Another fruit that presents a low value of phenolic compounds is the pulp of the fruit of the count with 11,3 ± 0,7 mg $100g^{-1}$EGA.Among the fruits that present average values are the acerola seed with 312.3 ± 11.1 mg $100g^{-1}$ EGA, the araçá cascade with 110.4 ± 12.4 mg $100g^{-1}$ EGA, the biribá both the pulp with 101.3 ± 2.2 mg $100g^{-1}$ EGA as the seed with 209.43 ± 12.1 mg $100g^{-1}$ EGA, the camu-camu seed with 241.1 ± 7.4 mg $100g^{-1}$ EGA, the shell and seed of

graviola with 427.3 ± 11.2 mg $100g^{-1}$ EGA for the waterfall and 632.3 ± 2.4 mg $100g^{-1}$ EGA for the seed and finally the tapereba pulp with 524.3 ± 11.4 mg $100g^{-1}$ and the taperebá cascade with 558.3 ± 10.1 mg $100g^{-1}$.
The remaining samples showed high values of total phenolic compounds, with camu-camu with a concentration of 1241.1 ± 12.4mg $100g^{-1}$ and pulp with 1741.2 ± 34.3mg $100g^{-1}$, being the values within the ranges given by Maeda et al. (2007), where they find values between 1100-1800 mg AGE. 100 g^{-1}. Other fruits that present high values of phenolic compounds are the acerola pulp with 1071.4 ± 22.2 mg $100g^{-1}$, the acerola pulp of araçá with 1080.21 ± 1.1 mg $100g^{-1}$ and the bark of abiu with 1132.43 ± 8.1 mg $100g^{-1}$.
The high concentration of phenolic compounds in camu-camu peel and pulp is related to the high concentration of vitamin C present in the fruit, because according to Yuyama et al. (2002), it possesses up to 6000 mg.$100g^{-1}$ of ascorbic acid. In addition to vitamin C, camu-camu contains other compounds with antioxidant activity such as non-antoniic flavonoids such as rutin (1.3 mg.$100g^{-1}$/fresh weight) and quercetin (2.4 mg.$100g^{-1}$/ fresh weight, and in the presence of a high solubility in the diet.
Table 3 shows the percentage of antioxidant activity done with the DPPH method, iron reduction and EC_{50} for the different fruits studied, with the calibration curve of DPPH y = 0.0099 x + 0.0077 with r^2 = 0.997 and for the iron reduction method y = 0.00036x + 0.08172 with r^2 = 0.998.

Table 3 : Antioxidant activity EC_{50} by the DPPH method and reduction of iron.

Fruit		**Antioxidant capacity**	
		DPPH	**Reduction of iron**
		EC_{50} (g/g $DPPH^{\cdot}$)	**µmol Fe_2SO_4/g**
Abiu	pulp	912.04 ± 3.17	170.04 ± 32.04
	peel	521.71 ± 1.34	411.43 ± 27.12
	seeds	781.14 ± 3.4	217.11 ± 7.22
Acerola	pulp	647.11 ± 12.34	124.01 ± 17.04
	peel	712.23 ± 21.12	71.17 ± 0.69
	seeds	1517.18 ± 19.23	42.31 ± 4.38
Araçá	pulp	531.22 ± 12.04	31.54 ± 1.10
	peel	1321.18 ± 23.83	17.31 ± 2.34
	seeds	471.23 ± 21.23	57.21 ± 4.11
Bacuparí	pulp	1231.48 ± 12.45	11.17 ± 1.11
	peel	2121.72 ± 22.23	7.23 ± 1.1
	seeds	1611.32 ± 28.11	9.11 ± 1.1
Biribá	pulp	1411.31 ± 11.04	109.23 ± 17.22
	peel	1591.31 ± 14.28	92.41 ± 14.31
	seeds	1212.15 ± 21.17	217.31 ± 28.11
Camu camu	pulp	549.24 ± 21.13	235.47 ± 11.44
	seeds	612.34 ± 17.05	147.23 ± 8.32
	peel	1231.45 ± 43.76	98.05 ± 15.11
Fruta-do-conde	pulp	1711.04 ± 11.04	165.11 ± 21.12
	seeds	1511.08 ± 7.31	191.24 ± 17.11
	peel	1634.22 ± 21.22	180.12 ± 4.18
Graviola	pulp	1517.31 ± 12.34	104.11 ± 17.21
	peel	1211.21 ± 4.23	215.12 ± 11.21
	seeds	1187.04 ± 12.04	161.11 ± 31.04
Taperebá	pulp	1931.24 ± 23.12	18.04 ± 7.11
	peel	1811.31 ± 17.05	19.11 ± 3.23
	seeds	n.d.	n.d.

According to the above, observing the results presented in table 3 and compared with the results discussed in table 2, there is a correlation between the values of antioxidant activity with the phenolic compounds, due to that when the concentration of phenolic compounds decreases in the samples , the amount of substances that are reduced by the iron also decreases, increasing the value of the IC_{50}, being smaller the antioxidant activity.
Comparing the results obtained with other works for Amazonian fruits developed by Rufino et al. (2010), where they evaluated different fruit pulps, they determined by the FRAP technique the antioxidant capacity of acerola pulp with a value of (148 ± 16) µmol $Fe_2SO_4.g^{-1}$, a result close to the value found in this work, for the camu-camu pulp (279 ± 1.5) µmol $Fe_2SO_4.g^{-1}$, being a value close to that found in this work and for the tapereba pulp (11,08 ± 0,2) µmol $Fe_2SO_4.g^{-1}$, a slightly lower value than that found in the present study (18.04 ± 7.11) µmol $Fe_2SO_4.g^{-1}$, but presenting low antioxidant activity.
On the other hand, Canuto et al. (2010), evaluated the antioxidant activity of fruit pulps, using trolox, and found antioxidant activity for the pulp of abiu (0.8 ± 0.1) µmol.L-1 trolox, for the pulp of the arapa , 0 ± 0.1) µmol.L-1 trolox, and for graviola pulp (2,2 ± 0,1) µmol.L-1 trolox, the lowest antioxidant activity values being those determined by the reduction of iron.

3.2. Statistical analysis

The analyzes of main components were carried out jointly for the evaluated systems (abiu, bacupari, acerola, graviola, camu-camu fruit of the earl, araça, biribá and taperebá), independently for each part of the fruit, in order to (% IC50 DPPH, reduction of iron and total phenolic compounds in the different parts of the fruit), in order to find a new set of variables (main components), uncorrelated, that explain the structure of the variation, being represented the weight of each variable analyzed in each component (axes).
In the *blipot* (Figure 1), the results of the analysis of the main components (PCA) for the different fruits were explained, explaining the 93.6% of the original variability of the data retained in these components for the pulps, 90.3% for the skin and 81% in the casso of the seeds.

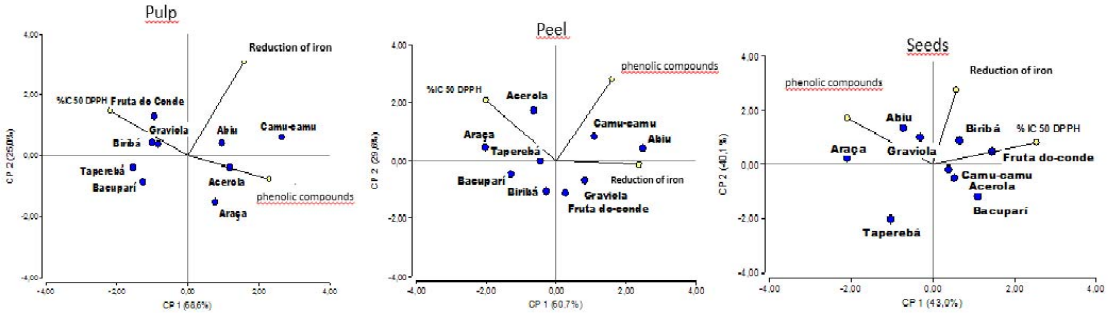

Figure 1- Distribution of the original variables between the different fruits on the first and second main component (CP1 and CP2).

For the pulps, the arrangement of the sequence shows that the systems can be grouped into two sets, the first main component (CP1), with contribution of 68.6% of the total variance explained, however most of the variables that were strongly affected contributing in a positive way to CP1 (total phenolic compounds and reduction of iron) and inversely with the percentage of IC50 inhibition of DPPH as discussed above. For the skin, the first main component (CP1) contributed with 60.7% being shaved the same variables as in the case of the pulp by the main component 1.
Finally, in the case of the seeds, the first main component (CP1) contributed with 43% of the total variance explained, however, most of the variables that were strongly affected contributed positively to CP1 (percentage of DPPH inhibition and iron reduction) and inverse with phenolic compounds.

4. Conclusions

It is observed that several fruits such as camu-camu, acerola and bark of abiu, which present a good contribution of phenolic compounds as well as high free antiradical activity, indicating the presence of bioactive compounds, being a great incentive to revalue these Amazonian fruits and elaboration of herbal products with phytotherapeutic interest or as functional foods.

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