# Nutrients and bioactive compounds in wild fruits from the Brazilian Amazon rainforest

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## Abstract

The Amazon Rainforest presents a great diversity of wild fruits that constitute an important part of the diet of many families. The present study investigated the nutritional composition of pequiá (*Caryocar villosum* (Aubl.)), camapu (*Physalis angulata* L.), tucumã (*Astrocaryum vulgare* Mart.), uxi (*Endopleura uchi* (Huber.)), and bacaba (*Oenocarpus bacaba* Mart.). Gravimetric methods were used to determine moisture and ash after drying in an oven and a muffle furnace, respectively. Total phenolics by Folin-Ciocalteu method, carotenoids by HPLC-DAD, and minerals by atomic absorption spectrophotometry were used. *C. villosum* obtained the highest concentration of carbohydrates and K; *P. angulata* showed the highest concentration of Mg; *A. vulgare* obtained the highest concentration of dietary fiber and carotenoids; *E. uchi* had the highest concentration of Ca and the highest total energy value; and *O. bacaba* showed the highest concentrations of the main nutrients analyzed; carbohydrates manifested in greater quantity in the species *C. villosum*, while phenolics were in the species *A. vulgare* and *O. bacaba*. The nutritional values found in these fruits are superior to those of other species consumed in Brazil, especially those arising from genetically improved crops.

Keywords: regional foods; Amazonian fruits; vitamins; nutritional value.

**Practical Application:** These fruits are widely consumed in their place of cultivation and are part of the families' eating habits. They are sold at fairs and markets in the region, with fixed prices that fluctuate according to their production seasonality; they are consumed in nature and also through other forms of preparation that result in by-products being consumed and sold locally.

### **1 INTRODUCTION**

The Brazilian flora, distributed along its biomes, contains a wide variety of food resources, among them wild fruits, which are used to feed families and for the commercialization of these species in fairs and markets, becoming an important source of income for these families (Polmann et al., 2021). It is worth mentioning that, from the point of view of food security, these species can be acquired at low cost since many propagate in native environments, in the forest, in agroforestry backyards, and close to homes (Dawson et al., 2014). However, although these species are part of the food habits of many families, their nutritional composition is still poorly known due to the lack of studies on them (Paula Filho et al., 2015; Souza et al., 2012).

The Amazon biome, due to the richness of its flora, has a wide variety of food species, mainly fruits, which, together with cassava flour (*Manihot esculenta* Crantz.) and fish, represent the staple food of rural populations residing in this region (Machado et al., 2021). Considering the relationship of these species with the diets of many families, it is assumed that these fruits

can be sources of various nutrients. However, this assumption is still in a hypothetical field since many species of wild fruits in the Amazon biome have not yet been properly studied (Faria et al., 2021).

This is a partially accepted hypothesis since the works that have been dedicated to investigating the nutritional concentration of some of these species have been inconclusive, which may occur mainly due to cultivation conditions and the sampling procedure of the investigated species (Gibson et al., 2010).

Although these species are part of the food base of populations in the Brazilian Amazon, studies on nutritional factors and malnutrition carried out in this region have found a prevalence of malnutrition in these populations (Khambalia et al., 2011).

Considering these aspects, the present study aimed to investigate the concentration of macronutrients and fiber, carotenoids, total phenolics, antioxidant activity, and minerals in five species of Amazonian fruits that were collected directly from the forest and, in this way, contribute to filling a gap in information that still exists on the composition and recommendations for daily intake of nutrients from these species.

Received 26 Feb., 2023.

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Funding: CNPQ (Process: 402994/2017-5), CAPES, FAPEAP (Science Network Program - Nº 06/2017), and PROPESPG/UNIFAP (Researcher Assistance Program - Nº 14/2017).

Accepted 29 Aug., 2023.

## 2 MATERIALS AND METHODS

## 2.1 Collection and preparation of the samples

The species were collected in May 2017 at the Rio Cajari Extractive Reserve (latitude S 00°90'27" and longitude W 51°85'32"), Amapá, Brazil (Figure 1), packed and transported in Styrofoam boxes with ice to avoid degradation of the samples, so that the other chemical analyses were carried out in the following hours. Five replicates were performed for each species, and each replicate was represented by a different location; around 1 kg of material was collected per replicate from at least three different plants. The samples were protected from light and transported in polystyrene boxes for 3 h to the Embrapa Amapá.

In the laboratory, the samples were washed and dried on paper towels. Three quarters of the samples were dried in an oven  $(105 \pm 1^{\circ}C \text{ for } 24 \text{ h})$  to perform the analysis of macronutrients, fibers, and minerals, and one quarter of the fresh samples were packed in aluminum foil, placed on ice inside Styrofoam boxes, and transported immediately by air for 16 h to the laboratory at the Universidade Federal de Viçosa (FUV), Brazil, for the analysis of carotenoids, total phenolics, and antioxidant activity.

The samples were dried, the inedible parts were discarded, and the edible parts were homogenized in a food processor, packed in polyethylene bags and aluminum foil, and stored at  $-4 \pm 1^{\circ}$ C until chemical analyses.

The dried samples were later transported from the Embrapa Amapá to the FUV, where the other analyses of macronutrients, fibers, and minerals were carried out.

## 2.2 Macronutrient and fiber analysis

Moisture was investigated by gravimetry after drying in an oven at 105°C, proteins by the micro-Kjeldahl method, and lipids by gravimetry after extraction in ethyl ether using a soxhlet apparatus (AOAC, 2010). Feed fiber by enzymatic-gravimetric method and ash by gravimetry after drying in a muffle furnace at 550°C (Detmann et al., 2012).

Carbohydrates were determined by the difference using the Equation 1:

[100 - % moisture - % lipids - % proteins - % dietary fibers - % ash] (1)

The total energy value was estimated considering the conversion factors of 4 kcal  $g^{-1}$  of protein or carbohydrate and 9 kcal  $g^{-1}$  of lipids (Frary & Johnson, 2005).

## 2.3 Analysis of total phenolics and antioxidant activity

It was carried out at the laboratory of the FUV, using five repetitions for each species analyzed according to the methods proposed by Bloor (2001), using an extraction solution (methanol:water = 60:40 v/v) in a fresh sample to obtain the supernatant, where the antioxidant tests were performed and the total phenolics determined according to Singleton et al. (1999). The substrate reading was performed by absorbance at 765 nm using a Shimadzu UV-VIS spectrophotometer. An analytical curve of gallic acid at concentrations ranging from 0.01 to 0.1 g L<sup>-1</sup> was prepared using the regression equation (Y = 0.1045x + 0.0052;  $R^2 = 0.984$ ) to express the results in milligrams of gallic acid equivalents.

The method for determining the antioxidant capacity was based on the decolorization of a solution composed of stable DPPH\* radicals (Duarte-Almeida et al., 2006). The free radical scavenging activity (FRSA) of the extracts was performed according to the method proposed by Blois (1958). Absorbance was measured at 517 nm.

The equation used to eliminate the DPPH\* radical was as Equation 2:



**Figure 1**. Unconventional food plants collected in the Rio Cajari Extractive Reserve, Amapá, Brazil, 2017. (A) Tucumã; (B) Pequiá; (C) camapu; (D) uxi; and (E) bacaba.

Radical-scavenging activity (%) = 
$$100\%$$
-{(Abs<sub>sample</sub>-Abs<sub>blank</sub>/Abs<sub>control</sub>) x100} (2)

Where:

Abs<sub>control</sub>: the absorbance of the control (DPPH\* solution without the sample);

Abs<sub>sample</sub>: the absorbance of the test sample (DPPH\* solution plus test sample);

 $Abs_{blank}$ : the absorbance of the extraction solution by itself, without sample or DPPH<sup>\*</sup> solution.

### 2.4 Extraction and analysis of carotenoids

They were carried out in five replications at the FUV, Brazil, using methods validated in this same laboratory (Cardoso et al., 2013; Paula Filho et al., 2015).

For the extraction of carotenoids, the following reagents were used for analysis: acetone, petroleum ether, and glacial acetic acid. For the analysis of the compounds, the following HPLC-grade reagents were used: ethyl acetate, methanol, and acetonitrile.

The vitamin A value was calculated according to the recommendations of the *Institute of Medicine* (IOM, 2011), where 1 retinol activity equivalent (RAE) corresponds to 1  $\mu$ g of retinol, 12  $\mu$ g of  $\beta$ -carotene, and 24  $\mu$ g of other provitamin carotenoids.

#### 2.5 Determination of minerals

Minerals were extracted from dry samples, according to Malavolta et al. (1997). Three replicates were used, and each replicate was analyzed in triplicate by means of nitric-perchloric digestion, in which two extracts were prepared. In extract 1, 0.5 g of dry sample of the species was weighed, 2 drops of kerosene and 4 mL of p.a. 65% nitric acid were added, and digestion was carried out for approximately 12 h. In extract 2, 5 mL of extract 1 were added to 20 mL of distilled water and stirred for 30 min; then, the solution was prepared, consisting of 0.5 mL of extract 2 + 21 mL of distilled water + 2.5 mL of solution 725 + 1 mL of 2% vitamin C, which was then shaken and measured.

The mineral measures were obtained through a solution analyzed in an atomic absorption spectrophotometer with an induced argon plasma source and with the following conditions: power of 1,300 W, refrigerant air flow of 15 L min<sup>-1</sup>, auxiliary air flow of 0.7 L min<sup>-1</sup>, carrier air flow of 0.5 L min<sup>-1</sup>, sample introduction rate of 1.5 mL min<sup>-1</sup>, and use of a Perkin Elmer nebulizer.

Only K was determined by flame photometry (Malavolta et al., 1997).

#### 2.6 Experimental design and statistical analysis of data

A completely randomized design was used with five replications for carotenoid analysis and three replications for macronutrient and fiber analyses. To assess the range of linearity of analytical standards, data obtained from peak areas were used for linear regression analysis and to calculate the correlation coefficient ( $R^2$ ). To compare the means of the treatments that presented differences, the Duncan test was used at a level of 5% probability. Statistical analysis was performed using the SAS software (Statistical Analysis System), version 9.2 (2008), licensed for FUV.

### **3 RESULTS**

All the analyzed fruits presented concentrations of the investigated nutrients, among which the species *A. vulgare* stood out, which presented the highest concentrations of the investigated nutrients (dietary fiber, DPPH,  $\beta$ -carotene, vitamin A, Cu, and Fe).

#### 3.1 Macronutrients and fiber

The physical characteristics of fruit species influence the composition of some macronutrients; for example, a species with a higher moisture content tends to have a lower fiber content, and vice versa (Dembitsky et al., 2011). Among the five species analyzed in the present study (Table 1), *P. angulata* L. is a berry-type fruit, while the others are of the drupe type. Considering these differences, it is noted that *P. angulata* L. has more water in its structure and a lower fiber concentration than the other analyzed species.

### 3.2 Total phenolics and antioxidant activity

The concentration of total phenolics can contribute to increasing the antioxidant capacity of the species (Chandrasekara & Shahidi, 2010). In the present study, *O. bacaba* presented the highest concentration of total phenolic compounds (Table 2); however, it did not present the highest antioxidant activity. This difference can be explained by the fact that the concentration of other compounds, such as flavonoids, coumarins, anthocyanins, and rutin,

Fable 1. Centesimal composition and	l total energy value in fruits co	ollected in the Rio Cajari Extracti	ve Reserve, Amapá, Brazil.
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Variables (g 100 g <sup>-1</sup> )	C. villosum	P. angulata	A. vulgare	E. uchi	O. bacaba
Moisture	$27.2\pm2.4^{\rm d}$	$79.1\pm4.7^{\text{a}}$	$56.5\pm0.9^{\mathrm{b}}$	$44.3\pm0.4^{\circ}$	$46.7\pm0.5^{\circ}$
Proteins	$1.10\pm0.05^{\circ}$	$1.16\pm0.01^{\circ}$	$1.85\pm0.01^{\rm b}$	$0.88\pm0.03^{\rm d}$	$3.27\pm0.05^{\rm a}$
Lipids	$25.81\pm0.15^\circ$	$2.60\pm0.25^{\rm e}$	$18.29\pm0.59^{\rm d}$	$50.86\pm0.17^{\text{a}}$	$30.27\pm1.47^{\rm b}$
TDF	$2.44\pm0.01^{\rm b}$	$1.90\pm0.17^{\mathrm{e}}$	$3.07\pm0.06^{\text{a}}$	$2.37\pm0.05^{\circ}$	$2.30\pm0.14^{\rm d}$
Ash	$0.94\pm0.01^{a}$	$0.54\pm0.01^{\rm d}$	$0.73\pm0.04^{\circ}$	$0.85\pm0.04^{\rm b}$	$0.73\pm0.03^{\rm c}$
Carbohydrates	$42.51\pm2.42^{\text{a}}$	$14.71\pm1.14^{\circ}$	$19.50\pm0.91^{\rm b}$	$0.77\pm0.15^{\rm d}$	$16.74\pm1.09^{\circ}$
Total energy value (kcal 100 g <sup>-1</sup> )	$406.76 \pm 8.22^{a}$	$86.87\pm4.45^{\rm e}$	$250.06\pm3.78^{\rm d}$	$464.33\pm1.94^{\text{a}}$	$352.50 \pm 8.97^{\circ}$

Values expressed in dry matter; mean of three repetitions; TDF: total dietary fiber.

Means followed by the same letter on the line do not differ by the test of Duncan at the 5% level of significance.

which were not investigated in the present study, influences the antioxidant activity of plant sources (Eberhardt et al., 2000).

The species *C. villosum* and *E. uchi* did not have these parameters analyzed because the samples had degraded between the collection and analysis sites, caused by the high geographic distance and transport time.

### 3.3 Carotenoids

Among the total carotenoids analyzed, only  $\beta$ -carotene was found, which in this case is equivalent to the proportion of  $\beta$ -carotene. If other carotenoids ( $\beta$ -cryptoxanthin and lycopene) were identified, the amount of total carotenoids would increase and the proportion of  $\beta$ -carotene would decrease, which in this case was predominant (Figure 2, Table 3).

### **3.4** Minerals

Ash concentration reflects the amount of minerals present in a food source. In the present study, all investigated minerals were identified in the analyzed species (Table 4). The elements detected in greater amounts were Ca, K, and Mn.

## **4 DISCUSSION**

### 4.1 Macronutrients and fiber

In Table 1, the highest concentration of dietary fiber was found in *A. vulgare*. This is a globose fruit, 4.4–6.5 cm in

diameter, orange in color, with a fleshy and sweet mesocarp. The Brazilian Food Composition Table (NEPA, 2011) shows dietary fiber values for this species equivalent to almost four times the values found in the present study (12.7 g 100 g<sup>-1</sup>); however, the probable reason for this difference is unknown. In addition to these results, only one study was found in which the authors (Santos et al., 2018) found dietary fiber values higher than those of the present study (35.95 g 100 g<sup>-1</sup>); however, it is noteworthy that it is the species *A. huaimi* Mart., and the samples were collected in another Brazilian biome, the Atlantic Forest.

The drupe fruit species (*C. villosum*, *A. vulgare*, *E. uchi*, and *O. bacaba*) showed the highest concentrations of lipids. There are results for the concentration of lipids in a study carried out on native fruits of the Amazon (Berto et al., 2015), in



Figure 2. Carotenoid of chromatogram in fruits of *A. vulgare* collected in the Rio Cajari Extractive Reserve, Amapá, Brazil.

Compounds	C. villosum	P. angulata	A. vulgare	E. uchi	O. bacaba
Total phenolics (mg de EAG* 100 g <sup>-1</sup> )	nd	$20.4\pm8.22^{\circ}$	$35.63\pm9.76^{\text{b}}$	nd	$60.66 \pm 11.79^{a}$
DPPH (% ARR**)	nd	$52.51 \pm 11.49^{\text{b}}$	$90.01\pm1.6^{\rm a}$	nd	$89.99 \pm 1.53^{\text{a}}$

Values expressed on fresh matter; mean of five repetitions; nd: not detected.

Means followed by the same letter on the line do not differ by the test of Duncan at the 5% level of significance; \*gallic acid equivalent; \*\*radical scavenging activities of hydro-alcohol of samples of fruits.

Table 3. Carotenoids in fruits collected in the Rio Cajari Extractive Reserve, Amapá, Brazil.

Compounds (mg 100 g <sup>-1</sup> )	C. villosum	P. angulata	A. vulgare	E. uchi	O. bacaba
Carotenoids	nd	$1.41 \pm 0.65^{\rm b} (100)$	$31.56 \pm 3.13^{a} (100)$	nd	$0.99 \pm 0.47^{ m b}  (100)$
Vitamin A value (RAE µg 100 g <sup>-1</sup> )	nd	$117.69 \pm 53.97^{\rm b}$	$2,630.28 \pm 260.95^{a}$	nd	$82.63\pm42.16^{\text{b}}$

Values expressed on fresh matter; mean of five repetitions; nd: not detected.

Means followed by the same letter on the line do not differ by the test of Duncan at the 5% level of significance.

Table 4. Minerals in fruits collected in the Rio C	Cajari Extractive Reserve, A	Amapá, Brazil
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Minerals (mg 100 g <sup>-1</sup> )	C. villosum	P. angulata	A. vulgare	E. uchi	O. bacaba
Р	$27.17\pm2.65^{\rm b}$	$21.87\pm2.87^{\circ}$	$25.97 \pm 1.98^{\text{b}}$	$38.04\pm2.52^{\text{a}}$	$39.47\pm3.44^{\text{a}}$
K	$26.25\pm1.24^{\rm a}$	$24.37\pm1.07^{\text{d}}$	$25.97 \pm 1.22^{\text{b}}$	$25.03\pm0.78^{\circ}$	$24.88 \pm 1.17^{\circ}$
Ca	$42.94\pm3.25^{\mathrm{b}}$	$37.88\pm2.45^{\text{d}}$	$43.67\pm3.62^{\text{b}}$	$57.82\pm3.26^{\text{a}}$	$38.96 \pm 3.28^{\circ}$
Mg	$10.31 \pm 2.11^{\circ}$	$12.27\pm3.75^{\text{a}}$	$11.62\pm3.28^{\rm b}$	$9.98\pm3.65^{\circ}$	$9.44\pm2.81^{\rm d}$
Cu	$0.73\pm0.21^{\rm d}$	$0.95\pm0.32^{\rm b}$	$1.38\pm0.73^{\text{a}}$	$0.69\pm0.28^{\rm d}$	$0.87\pm0.23^{\circ}$
Mn	$4.53\pm1.55^{\mathrm{b}}$	$3.93 \pm 1.38^{\circ}$	$4.68\pm1.52^{\rm b}$	$5.24\pm1.77^{\text{a}}$	$3.58\pm1.23^{\circ}$
Fe	$2.17\pm0.82^{\circ}$	$3.55\pm1.32^{\rm b}$	$4.06\pm1.43^{\text{a}}$	$3.26\pm0.99^{\rm b}$	$1.47\pm0.69^{\rm d}$
Zn	$1.06\pm0.26^{\rm b}$	$0.77\pm0.06^{\circ}$	$0.62\pm0.12^{\circ}$	$1.27\pm0.43^{\rm a}$	$0.86\pm0.07^{\circ}$

Values expressed on fresh dry; mean of three repetitions.

Means followed by the same letter on the line do not differ by the test of Duncan at the 5% level of significance.

which the authors found values for *C. villosum* (14.63 g 100 g<sup>-1</sup>) and *E. uchi* (20.48 g 100 g<sup>-1</sup>) lower than those found here. It is worth mentioning that these differences may be related to the harvesting time and fruit maturation point.

The concentration of lipids influences the result of the total energy value of the fruits, making this nutrient the main aggregating component in the calculation of the energy available in plant food sources. In the present study, this was reflected in the fact that the species that presented the highest concentration of lipids also presented the highest total energy value. However, different results were found for the same species in other available sources, such as 262.00 kcal 100 g<sup>-1</sup> for *A. vulgare* (NEPA, 2011), 296.55 kcal 100 g<sup>-1</sup> for *C. villosum* (Berto et al., 2015), and 302.56 kcal 100 g<sup>-1</sup> for *E. uchi* (Berto et al., 2015).

However, as already pointed out, this difference may be associated with a series of factors, such as sampling and harvesting of species, as well as environmental factors (soil fertility, availability of water, and light, among others), that can influence the nutritional composition of these native species.

#### 4.2 Total phenolics and antioxidant activity

The literature presents some results on the concentration of total phenolic compounds in *O. bacaba* (1,759.27 mg EAG 100 g<sup>-1</sup>) (Barreto et al., 2009) and in *P. angulata* (36.92 mg EAG 100 g<sup>-1</sup>) (Medina-Medrano et al., 2015), where concentrations higher than those found in the present study were observed. The differences may be related to a number of other parameters that were not investigated. However, the values found in the present study showed statistical significance and conveyed the antioxidant capacity of wild fruit species to fight free radicals that can promote oxidative stress and cellular aging (Almeida et al., 2011).

Regarding the FRSA, the percentages of *A. vulgare* and *O. bacaba* stood out. The literature shows some results that differ from those found in the present study for *O. bacaba* (34.25% of ARR) (Finco et al., 2012) and *P. angulata* (3.05% of ARR) (Medina-Medrano et al., 2015). However, the results found in the present study for total phenolics and antioxidant activity show that there was a relationship between these compounds, which indicates the influence and contribution of total phenolics to antioxidant activity in plant sources (Kevers et al., 2007).

Regarding the concentration of phenolic compounds and the oxidant activity in these fruit species, the identification of these parameters occurred through a methodology used in conventional fruit analysis, which contributes to these species, still little known in the conventional market, establishing themselves as potential sources of nutrients that can contribute to the food security of many families, especially those residing in rural areas of the Amazon.

### 4.3 Carotenoids

The highest concentrations of  $\beta$ -carotene were found in *A*. *vulgare*, which may be associated with the red pigmentation of the pulp of this fruit (Table 3). A study by Rodriguez-Amaya (1996) showed concentrations of 10.7 mg 100 g<sup>-1</sup> of  $\beta$ -carotene

for this fruit. These results represent one-third of the values found in the species analyzed in the present study, which reinforces the high concentration of this nutrient in *A. vulgare*.

Regarding the vitamin A content observed in the analyzed species, there are results for *A. vulgare* in the Brazilian food composition table (NEPA, 2011), which presents an RAE value equivalent to  $1,181 \mu g 100 g^{-1}$ , which represents only 45% of that found in the present study.

In general, there is a lack of studies that investigate these parameters in wild fruit species that enable comparison with others, which demonstrates the originality of the present research.

#### 4.4 Minerals

The *E. uchi* species showed high concentrations of some minerals when compared to the others. The results found, although high in comparison to the other fruits, are inferior to those of other works found in the academic literature for P (10.82 mg 100 g<sup>-1</sup>), Mg (80.77 mg 100 g<sup>-1</sup>), and other minerals (Berto et al., 2015).

Some studies on the concentration of minerals for species of the genus *Astrocaryum* present results closer to those found for *A. vulgare* (Santos et al., 2018). However, it is worth noting that the concentration of minerals alone is not a reliable indicator of their amounts absorbed by the body, as some minerals such as Ca, Fe, Zn, Cu, and Mg can form insoluble complexes with anti-nutritional factors (phytate, oxalate) and consequently decrease their bioavailability (Leal et al., 2010).

It is known that wild fruits are part of the eating habits of riverine populations in the Amazon (Machado et al., 2021); however, there are records of factors linked to malnutrition in these populations (Khambalia et al., 2011). This reality leaves some doubts, since several of the fruits consumed by this population group have high concentrations of some macronutrients, vitamins, and minerals. One of the possible causes may be related to the bioavailability of these compounds in these plant sources, making it necessary to carry out studies to investigate this situation.

In general, the concentration of macronutrients and vitamins found in the analyzed fruits is quantitatively equivalent to the most consumed fruits in Brazil (banana (*Musa* spp.), orange (*Citrus x sinensis* (L.)), apple (*Malus domestica* Borkh.), watermelon (*Citrulus lanatus* (Thunb.), among others), as observed in the Brazilian Food Composition Table (NEPA, 2011).

#### **5 CONCLUSION**

The fruit species analyzed in the present study showed a high concentration of macronutrients and vitamins when compared to the most consumed fruits in Brazil. This condition, as well as its antioxidant potential, indicates the possibility of these species being recommended in diets, as well as the development of agronomic protocols that allow their cultivation, since these species are still found in native environments with little, or none, program that allows their multiplication among farmers. It is important to highlight the positive aspects of these fruits for the promotion of food and nutritional security of the families that consume them, which are the fact that they spontaneously propagate in native environments, close to the residences of these families, without demanding financial costs for their production and management, in addition to allowing the acquisition of these nutrients without financial costs, benefiting the families that consume them, and depending on these species for daily consumption.

Moreover, in the scenario of facing the problem of hunger in Brazil, especially in some municipalities in the Amazon, this information is new for agro-food science and may motivate further studies and the implementation of food programs.

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