



**HAL**  
open science

## Mineral and bromatological assessment and determination of the antioxidant capacity and bioactive compounds in native Amazon fruits

Magda Márcia Becker, Carolina Marques Mandaji, Gaëlle Catanante,  
Jean-Louis Marty, Gilvanda Silva Nunes

### ► To cite this version:

Magda Márcia Becker, Carolina Marques Mandaji, Gaëlle Catanante, Jean-Louis Marty, Gilvanda Silva Nunes. Mineral and bromatological assessment and determination of the antioxidant capacity and bioactive compounds in native Amazon fruits. *Brazilian Journal of Food Technology*, 2018, 21, pp.e2018022. 10.1590/1981-6723.02218 . hal-03928365

**HAL Id: hal-03928365**

**<https://hal.science/hal-03928365>**

Submitted on 7 Jan 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Mineral and bromatological assessment and determination of the antioxidant capacity and bioactive compounds in native Amazon fruits

### *Avaliação mineral, bromatológica, capacidade antioxidante e compostos bioativos em frutos nativos amazônicos*

Magda Márcia Becker<sup>1,2\*</sup>, Carolina Marques Mandaji<sup>1</sup>, Gaëlle Catanante<sup>2</sup>, Jean-Louis Marty<sup>2</sup>, Gilvanda Silva Nunes<sup>1</sup>

<sup>1</sup> Universidade Federal do Maranhão (UFMA), Departamento de Tecnologia Química, Programa de Pós-graduação em Biodiversidade e Biotecnologia da Rede Bionorte (PPG-BIONORTE), São Luís/MA - Brasil

<sup>2</sup> Université de Perpignan Via Domitia (UPVD), Laboratoire BAE, Perpignan, France

#### \*Corresponding Author

Magda Márcia Becker, Universidade Federal do Maranhão (UFMA), Programa de Pós-graduação em Biodiversidade e Biotecnologia da Rede Bionorte (PPG-BIONORTE), Avenida dos Portugueses, 1966, Bacanga, CEP: 65080-805, São Luís/MA – Brasil, e-mail: magda.becker@etudiant.univ-perp.fr

**Cite as:** Mineral and bromatological assessment and determination of the antioxidant capacity and bioactive compounds in native Amazon fruits. *Braz. J. Food Technol.*, v. 21, e2018022, 2018.

Received: Feb. 08, 2018; Accepted: Apr. 30, 2018

#### Abstract

The proximate compositions, mineral contents, antioxidant capacity and bioactive compounds of 7 native Amazon fruits were chemically evaluated. The majority of the fruits showed high moisture contents (> 63.02%), and ash, total crude protein and total carbohydrate contents in the ranges of 0.22–2.07%, 0.17–2.44% and 7.17–41.71%, respectively. High levels of total lipids were found in uxi (23.25%) and monguba (18.67%). A wide range of mineral contents was detected and the highest levels were found in the samples of monguba seeds (Ca, Cu, Mg, and Zn), uxi pulp (Fe, and Mn) and pajurá pulp (Na). All the fruits showed antioxidant capacity, but the pajurá revealed the highest potential, statistically similar to that of acerola ( $p < 0.05$ ). The highest vitamin C contents were found in bacuri and cupuaçu and the highest phenolic compound contents in monguba and pajurá fruits, but flavonoids were only detected in pajurá. A statistical correlation between the Na content and antioxidant capacity was also observed. Based on the results obtained, the fruits analyzed are suitable for use in the human diet, in the food and cosmetics industries as well as in pharmaceutical compositions.

**Keywords:** Native fruits; Amazon; Chemical composition; Minerals; Antioxidant capacity; Bioactive compounds.

#### Resumo

A composição bromatológica, o conteúdo mineral e a capacidade antioxidante de 7 frutos nativos da Amazônia foram avaliados. Os frutos mostraram, em sua maioria, alto conteúdo de umidade (> 63,02%) e teores de cinzas, proteína bruta total e carboidratos totais na faixa de 0,22-2,07%, 0,17-2,44% e 7,17-41,71%, respectivamente. Os maiores teores em lipídios foram obtidos nos frutos de uxi (23,25%) e monguba (18,67%). Uma ampla variedade de minerais foi detectada, sendo as maiores concentrações obtidas nas amostras de sementes de monguba (Ca, Cu, Mg e Zn), polpas de uxi (Fe e Mn) e pajurá (Na). Todos os frutos mostraram atividade antioxidante, em que a polpa de pajurá revelou o maior potencial, semelhante estatisticamente à acerola ( $p < 0,05$ ). Maiores teores em vitamina C foram obtidos nos frutos de bacuri e cupuaçu, fenólicos totais na monguba e pajurá, enquanto flavonóides foram determinados somente nos frutos de pajurá. Uma correlação positiva entre o teor de Na e a capacidade antioxidante também foi observada. Baseado nos resultados obtidos, os frutos analisados são adequados para uso na dieta humana, nas indústrias de alimentos e cosméticos, bem como em composições farmacêuticas.

**Palavras-chave:** Frutas nativas; Amazônia; Composição química; Minerais; Capacidade antioxidante; Compostos bioativos.

#### 1 Introduction

The Brazilian Amazon Region is formed of a complex mosaic of endemic areas with a rich diversity of fruit species which are distributed in accordance with their biota specificities (SILVA et al., 2005).

The region shows great bioavailability of fruit species with approximately 220 edible fruit producing plant species, representing 44% of the native fruit diversity in Brazil (NEVES et al., 2012).



## Mineral and bromatological assessment and determination of the antioxidant capacity and bioactive compounds in native Amazon fruits

Becker, M. M. et al.

Recognized sources of nutrients, fruits comprise nutritionally important foods for the human diet and in recent years have received increased attention due to epidemiological evidence regarding the regular consumption of vegetables, which reduces the mortality and morbidity due to some chronic diseases (RUFINO et al., 2010; ALISSA; FERNS, 2012; BORGES et al., 2013). The protective effect has been attributed to the presence of constituents like minerals and high levels of bioactive compounds with antioxidant properties (NUNES et al., 2011; KAHL et al., 2012; LIU, 2013; KOZŁOWSKA; SZOSTAK-WEGIEREK, 2014; WANG et al., 2013).

Data on the composition of native fruits is essential to encourage national and international marketing; assist the food, cosmetics, bio cosmetics and other industries and support policies to protect the environment and biodiversity. In addition, knowledge of the composition aids quality control and food safety as well as evaluating the adequacy of intake of individual nutrients or populations.

Information regarding the nutritional composition of Brazilian fruits is still scarce, especially those found in the Amazon Region, but on the other hand, there is an evident need for better use of its natural resources. Considering the potential benefits that knowledge regarding the nutritional composition of fruits can offer to human health, the aim of this study was to determine the physical and chemical properties, mineral contents and antioxidant capacities of seven native Amazon fruits, some of which have been studied and parameters assessed by other authors.

## 2 Materials and methods

### 2.1 Reagents

Analytical grade chemicals were employed in the preparation of all solutions. Deionized water (Milli-Q Millipore 18.2MΩ cm<sup>-1</sup>) was used in all experiments. All the plastic articles and glassware were cleaned by soaking in dilute nitric acid (1:9). The standard analyte solutions for calibration procedures were produced by diluting stock solutions of 1000 mg.L<sup>-1</sup> of the elements under investigation (Ca, Cu, Fe, Mg, Mn, Na and Zn; from Merck Millipore Certipur®, Specsol®). The other reagents used were: nitro blue tetrazolium (NBT, N6876), hypoxanthine (HX, H9377), xanthine oxidase (XOD from bovine milk, X4376), petroleum ether, phenolphthalein, sodium hydroxide (NaOH), sulphuric acid (H<sub>2</sub>SO<sub>4</sub>), potassium iodide (KI), dry starch, potassium iodate (KIO<sub>3</sub>), nitric acid (HNO<sub>3</sub>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and oxide yttrium (Y<sub>2</sub>O<sub>3</sub>), all purchased from Sigma-Aldrich Corp (Nasdaq-Sial, Darmstadt, Germany).

### 2.2 Sample collection

Seven native Amazon fruits were included in this study: abiu (*Pouteria caimito*), bacuri (*Platonia insignis*), biribá (*Rhollinea orthopetala*), cupuaçu (*Theobroma grandiflorum*),

monguba (*Pachira aquatica*), pajurá (*Couepia bracteosa*) and uxi (*Saccoglottis uchi*).

From 1 to 5 kg of each fruit sample, in the complete physiological maturity stage, were collected during the appropriate seasonal period in the states of Amazonas, Maranhão, and Roraima. A voucher specimen of each plant was deposited in the herbarium of the Integrate Museum of Roraima. After collection, the samples were refrigerated and taken to the laboratory of the Group of Environmental Studies and Analysis (GEAA) at the Federal University of Maranhão, Brazil, where they were washed in deionized water and stored at -20 °C until analysed.

### 2.3 Bromatological analysis

The moisture content, total ash content, hydrogen potential (pH), acidity in citric acid, crude protein content and total lipids content were determined according to the AOAC methods (CUNNIFF, 1997). The total carbohydrate content was determined by difference, subtracting the sum of the crude protein, total lipids, moisture and ash contents from 100 (MERRILL; WATT, 1973). The total energy value was estimated according to the Atwater conversion values using 4 Kcal/g for protein and carbohydrates, and 9 Kcal g<sup>-1</sup> for lipids (MERRILL; WATT, 1973). All the analyses were carried out in triplicate.

### 2.4 Antioxidants

#### 2.4.1 Antioxidant capacity

The procedure used followed the method of Cortina-Puig et al. (2009) with some modifications. A reaction mixture was prepared consisting of 50 mM K-PBS containing 0.1 mM EDTA (pH 7.5), 25 μM HX, 50 μM NBT, the antioxidant fruit extract (distilled water for the blank) and 0.2 U.mL<sup>-1</sup> XOD, which was added last. The increase in absorbance at 560 nm was recorded for 15 min using a Beckman DU520 UV-Vis Spectrophotometer. Stock solutions of NBT, HX and XOD were prepared in K-PBS at pH 7.5. All the spectrometric assays were carried out in triplicate.

In the method, O<sub>2</sub><sup>•-</sup> radicals and aciduric compounds were generated in vitro by the HX/XOD system. The O<sub>2</sub><sup>•-</sup> radicals reduce the NBT reagent (yellow colour) into formazan (purple colour), which is measured spectrophotometrically at 560 nm. The presence of radical scavengers (the antioxidant sample) generates inhibition (competitive) in the formation of formazan, leading to a decrease in its production rate and consequently in absorbance.

The % superoxide Radical Scavenging Capacity (RSC) of the plant extracts was calculated using Equation 1:

$$\text{RSC}(\% \text{O}_2^{\bullet-} \text{ scavenging}) = 100 - \left[ \frac{A_{\text{AOX}} - A_0}{C_{100} - C_0} \times 100 \right] \quad [1]$$

## Mineral and bromatological assessment and determination of the antioxidant capacity and bioactive compounds in native Amazon fruits

Becker, M. M. et al.

Where:  $A_{\text{AOX}}$  is the AOX absorbance;  $A_0$  is the blank AOX absorbance;  $C_{100}$  is the control absorbance; and  $C_0$  is the blank control absorbance.

### 2.4.2 Total phenolic compounds

The total phenolic compound content was determined according to the method of Pueyo and Calvo (2009) and Berker et al. (2010). 100  $\mu\text{L}$  of ethanolic pulp extract (1:1), 630  $\mu\text{L}$  deionised water, 20  $\mu\text{L}$  of HCl (1 mol  $\text{L}^{-1}$ ), 150  $\mu\text{L}$   $\text{K}_3\text{Fe}(\text{CN})_6$  (1% m/v), 50  $\mu\text{L}$  sodium dodecyl sulphate (1% v/v) and 50  $\mu\text{L}$   $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  (0.2% m/v) were added to a cuvette. The absorbance was read at 750 nm after 30 minutes using a Shimadzu UV-probe spectrophotometer. The calibration curve was obtained using standard gallic acid solutions (1, 2, 4 and 8  $\mu\text{g mL}^{-1}$ ). The results were expressed in gram equivalents of gallic acid per 100 g of pulp (GAE.100  $\text{g}^{-1}$ ).

### 2.4.3 Determination of the flavonoid content

The flavonoid concentration was determined by adapting the spectrophotometric procedure described by Chaillou et al. (2004) and Teles (2014). Aliquots of 0.2 mL of methanolic pulp extract (1:1) and 0.2 mL methanolic  $\text{AlCl}_3$  solution (5% m/v) were added to a cuvette and the volume completed to 2 mL with concentrated methanol. After 30 minutes, the absorbance was read at a wavelength of 425 nm using a Shimadzu UV-probe spectrophotometer. The calibration curve was obtained using standard quercetin solutions. The results were expressed in milligram equivalents of quercetin per 100 g of pulp (QEE.100 $\text{g}^{-1}$ ).

### 2.4.4 Ascorbic acid

The vitamin C concentration was determined by redox titration using an iodine solution (IAL, 2008).

## 2.5 Mineral elements

### 2.5.1 Digestion procedure

The sample digestion procedure was carried out in a closed microwave oven according to the following

AOAC steps (AOAC, 2002). The resulting solution was diluted to 25.0 mL with deionized water in a volumetric flask before being analysed by ICP-OES. Blanks were prepared for each sample batch. Yttrium was used as the internal standard at a concentration of 2  $\text{mg.L}^{-1}$  and all the analyses were carried out in triplicate.

### 2.5.2 ICP-OES operational conditions

The concentrations of three macroelements (Ca, Mg and Na) and four microelements (Fe, Mn, Zn and Cu) were determined in the selected fruits. The measurements were determined simultaneously in an ICP OES (Shimadzu, model 9820) equipped with a concentric nebulizer, which allowed for the choice of the minitorch configuration between the radial or axial mode in an integrated unit. The operational conditions are summarized in Table 1.

### 2.5.3 Performance characteristics

The analytical method performance was evaluated considering the following figures of merit according to Skoog et al. (2008): practical linear range; precision, by calculating the relative standard deviation (RSD) for each analysis under repeatable conditions; and the sensitivity, estimated by the limits of detection and quantification (LOD and LOQ, respectively).

The accuracy of the complete ICP-OES analysis was estimated through addition and recovery experiments of the analytes for two samples (biribá and uxi) at two concentration levels.

## 2.7 Statistical analysis

The results were expressed as the mean value with the respective RSD (%) of three replications. The statistical differences were analyzed using one-way ANOVA followed by Tukey's test at the 95% confidence level ( $p \leq 0.05$ ). The correlation analysis was applied and expressed as Pearson's correlation coefficient ( $r$ ). The statistical analysis was carried out using Statistica, 8.0.

**Table 1.** ICP-OES operational conditions used to determine the elements in the selected Amazon fruits.

Parameter	Value
Radio frequency power	1.2 (kW)
Plasma argon flow rate	10 ( $\text{L.min}^{-1}$ )
Auxiliary argon flow rate	0.6 ( $\text{L.min}^{-1}$ )
Carrier gas	0.7 ( $\text{L.min}^{-1}$ )
Exposure time	30 (s)
Solvent rinse time	30 (s)
Peristaltic pump rotation speed	20-60 (rpm)
View direction	Radial for Mg and Na Axial for Ca, Cu, Fe, Mn and Zn
Nebulizer	Concentric
Emission lines ( $\lambda$ nm)	Ca (183.801); Cu (327.396); Fe (259.940); Mg (383.826); Mn (257.610); Na (589.592); Zn (213.856).

## Mineral and bromatological assessment and determination of the antioxidant capacity and bioactive compounds in native Amazon fruits

Becker, M. M. et al.

### 3 Results and discussion

#### 3.1 Bromatological analysis

The results of the bromatological analysis of the native Amazon fruits are shown in Table 2.

In general, the analyzed fruits presented high moisture contents (> 63.02%), except for *uxi* fruit (31.72). The moisture contents were shown to be similar to those reported for the respective pulps of *abiu* (LOVE; PAULL, 2011), *biribá*, *pajurá* (BERTO et al., 2015), *cupuaçu* (UNICAMP, 2006) and *uxi* (MARX et al., 2002; BERTO et al., 2015). The total ash contents < 2.07% were found for all the samples and the highest value was observed for *biribá* fruit.

The total lipids contents ranged from 0.06% to 23.25% for *biribá* and *uxi* pulps, respectively. Other studies have reported 10–31% total lipids for *uxi* (MARX et al., 2002; BRASIL, 2015; BEZERRA et al., 2006) and *Monguba* seeds and *uxi* pulps can be considered as rich natural sources of total lipids (18.67 to 23.25%). This fact favours the use of their oils as raw materials for the food, pharmaceutical and cosmetic industries.

Crude proteins are primary components of living things, and the main sources of protein in human consumption tend to be animal products, which normally also have high fat and saturated fat contents. Thus the presence of a high protein level in a plant points towards a possible increase in its food value. Moreover, a protein based bioactive compound could also be isolated from the original fruits (THOMSEN et al., 1991). In the present study, the highest crude protein content was found in *monguba*, followed by *uxi* fruit. The *Monguba* fruit is still very little used by Brazilians and therefore devalued economically, but the

results showed a high oil content and a significant amount of protein, showing its potential for industrial exploitation.

Carbohydrates are the main energy reserves of plant foods. In all organisms, carbohydrates make up the building blocks of cells and supply potential energy to maintain life. The total percent of carbohydrate varied greatly amongst the samples, and their values were influenced primarily by the moisture content. The highest total carbohydrate percentages were found for the *uxi* (41.71%) and *pajurá* (35.03%) pulps.

The nutritional parameter of total energy is directly related to the total lipids, crude proteins and total carbohydrate levels found in the samples. Almost all the samples evaluated presented high total energy values and only the *biribá* and *cupuaçu* pulps exhibited total energy values below 100 Kcal 100g<sup>-1</sup>. Thus these fruits could be included in energy-restricted diets whereas the others could be employed in high-caloric diets. It was observed that the Amazon fruits with higher total energy values also presented higher total lipids and lower moisture contents.

The highest pH value was 6.76 for *abiu* fruit, whilst the highest citric acid content was found in *cupuaçu* (pH 4.09 and 1.78 g of citric acid per 100g of pulp).

#### 3.2 Mineral elements

Plants are a source of minerals that are essential nutrients for the maintenance of human health. The recommended dietary allowance (RDA) is a parameter used to stipulate the nutrient levels that meet the needs of most healthy individuals (INSTITUTE OF MEDICINE, 2006). According to these parameters, the average daily requirements for adult males (19 to 30 years) of the minerals evaluated

**Table 2.** Proximate composition of the selected *in nature* Amazonian fruits with their respective RSD (%).

Amazon fruit	Moisture	Ash	Lipids	Crude protein (g.100 g <sup>-1</sup> )	Carbohydrate	Acidity* (g.100 g <sup>-1</sup> )	Total energy (kcal.100 g <sup>-1</sup> )	pH
<b>Abiu</b>	71.73 (0.21)	0.33 (9.34)	0.32 (3.57)	0.17 (0.00)	27.44	0.056 (5.04)	113.37	6.76 (0.60)
<b>Bacuri</b>	91.20 (0.15)	0.22 (11.69)	0.38 (9.94)	0.34 (0.00)	7.85	0.56 (5.46)	36.22	3.60 (1.35)
<b>Biribá</b>	89.46 (8.24)	2.07 (8.33)	0.06 (6.15)	1.04 (9.17)	7.37	0.16 (5.56)	34.16	5.80 (4.56)
<b>Cupuaçu</b>	82.32 (0.25)	1.09 (3.09)	0.57 (10.6)	0.40 (2.50)	15.63	1.78 (9.71)	69.22	4.09 (0.51)
<b>Monguba</b>	70.35 (1.20)	1.37 (1.65)	18.67 (6.98)	2.44 (5.22)	7.17	0.34 (8.69)	206.47	6.70 (3.37)
<b>Pajurá</b>	63.02 (0.53)	0.91 (3.10)	0.11 (10.83)	0.93 (0.00)	35.03	0.16 (10.63)	144.82	5.51 (3.79)
<b>Uxi</b>	31.72 (2.48)	1.15 (3.80)	23.25 (11.77)	2.17 (4.90)	41.71	0.24 (6.85)	384.74	4.44 (3.74)

\*Acidity in citric acid. All results are presented together with the respective Relative Standard Deviation (RSD). N = 3.

## Mineral and bromatological assessment and determination of the antioxidant capacity and bioactive compounds in native Amazon fruits

Becker, M. M. et al.

are as follows: Na: 1.3 to 1.5 g/day<sup>-1</sup>; Ca: 1 g/day<sup>-1</sup>; Mg: 310 to 400 mg/day<sup>-1</sup>; Cu: 0.9 mg/day<sup>-1</sup>; Fe: 8 to 18 mg/day<sup>-1</sup>; Mn: 1.8 to 2.3 mg/day<sup>-1</sup> and Zn: 8 to 11 mg/day<sup>-1</sup>.

Table 3 shows the mineral concentrations (mg 100 g<sup>-1</sup>) found in the native Amazon fruits with their respective RSD (%), LOD and LOQ (mg L<sup>-1</sup>).

The highest Ca, Cu, Mg and Zn contents were found in the *monguba* fruit, representing 5.6%, 83.0%, 21.9% and 12.4% of the RDA (INSTITUTE OF MEDICINE, 2006) for these minerals, respectively. The *Monguba* fruit can be classified (BRASIL, 1998) as a food very rich in Cu, followed by the *uxi* (35.7%), *bacuri* (17.7%), *pajurá* (15.6%) and *abiu* (22.3%) fruits. Copper functions as a component of several metalloenzymes which act as oxidases in the reduction of molecular oxygen. Symptoms associated with its deficiency include normocytic, hypochromic anemia; leucopenia; and neutropenia; and osteoporosis in copper-deficient infants and growing children. Copper toxicity is generally rare except in individuals genetically susceptible to an increased risk of the adverse effects from an excess copper intake.

Therefore these fruits can be included in the diet to improve human health (INSTITUTE OF MEDICINE, 2006). *Monguba* fruit can also be considered an excellent source of Mg.

The highest contents of Fe and Mn were found in *uxi* pulp, with 15% and 29% of the RDA for these minerals, respectively. Fe is a critical component of several proteins, including enzymes, cytochromes, myoglobin and hemoglobin, the latter of which transports oxygen throughout the body. Iron deficiency anemia is the most common nutritional deficiency in the world (INSTITUTE OF MEDICINE, 2006) and *uxi* pulp could be used to prevent and/or treat this problem. Of the world's estimated 7 billion people, 1.6 billion suffer from iron deficiency (WHO, 2008, 2009). In turn, Mn is involved in the formation of bone and in specific reactions related to the amino acid, cholesterol and carbohydrate metabolisms. Although Mn deficiency may contribute to one or more clinical symptoms, a clinical deficiency has not been clearly associated with poor dietary intakes by healthy individuals (INSTITUTE OF MEDICINE, 2006).

**Table 3.** Minerals contents (mg. 100 g<sup>-1</sup>) (wet weight basis) in the samples studied, with their respective RSD (%), LOD and LOQ (mg.L<sup>-1</sup>), and recoveries (%).

Mineral	Na	Ca	Mg	Cu	Fe	Mn	Zn
LOD	0.1044	0.065	0.0020	0.0004	0.0005	7.12 10 <sup>-6</sup>	0.0003
LOQ	3.49	2.42	0.022	0.0017	0.0029	1.2 10 <sup>-5</sup>	0.0017
<b>Abiu</b>	44.35 (8.58)	9.49 (6.16)	8.29 (8.20)	0.20 (6.94)	0.29 (10.77)	0.08 (7.14)	0.27 (9.29)
<b>Bacuri</b>	13.58 (5.22)	7.03 (4.95)	7.01 (4.97)	0.16 (9.63)	0.20 (8.14)	0.02 (6.86)	0.64 (8.90)
<b>Biribá</b>	1.12 (4.48)	34.42 (3.84)	25.83 (8.73)	0.09 (5.33)	0.22 (4.13)	0.11 (5.15)	0.18 (10.16)
<b>Cupuaçu</b>	1.24 (1.54)	17.48 (8.00)	36.27 (6.21)	0.11 (5.65)	0.32 (5.05)	0.12 (5.58)	0.34 (7.35)
<b>Monguba</b>	1.14 (2.08)	55.89 (4.11)	87.53 (4.97)	0.75 (6.18)	0.44 (9.85)	0.20 (8.45)	0.99 (8.32)
<b>Pajurá</b>	68.56 (6.72)	19.22 (4.01)	21.33 (2.24)	0.14 (9.36)	0.37 (4.79)	0.22 (2.42)	0.69 (6.55)
<b>Uxi</b>	2.79 (9.95)	22.27 (6.24)	40.65 (1.39)	0.32 (7.46)	1.20 (5.79)	0.67 (2.80)	0.51 (6.08)
<b>RECOVERIES %</b>							
<b>Biribá</b>	93.13 (2.85)	108.63 (1.63)	107.29 (0.82)	110.50 (0.64)	101.50 (6.62)	97.22 (1.21)	116.50 (4.86)
<b>Uxi</b>	103.10 (11.14)	108.1 (4.91)	109.6 (1.61)	106.60 (3.11)	102.60 (5.00)	103.20 (7.74)	109.50 (7.66)
<b>Uxi</b>	102.0 (5.79)	102.75 (5.30)	110.63 (1.60)	106.50 (9.99)	107.25 (6.92)	100.28 (2.35)	112.00 (12.94)
<b>Uxi</b>	98.10 (4.18)	96.6 (2.45)	103.6 (3.63)	103.50 (1.57)	103.20 (7.01)	95.40 (2.84)	101.50 (2.96)

All results are presented together with the respective *Relative Standard Deviation* (RSD). N = 3.

## Mineral and bromatological assessment and determination of the antioxidant capacity and bioactive compounds in native Amazon fruits

Becker, M. M. et al.

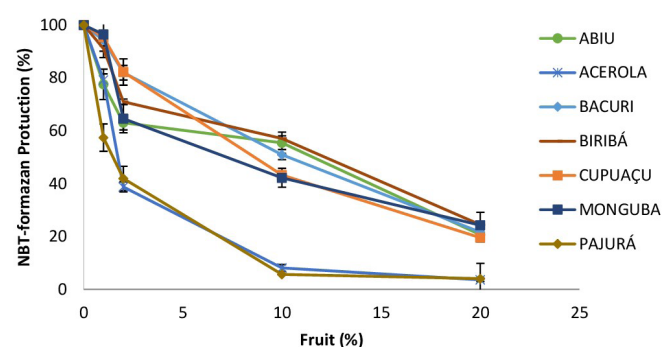
The highest Na content was found in *pajurá* fruit, although this amount only represents 4.5% of the RDA when 100 g of the fruit is ingested by an adult man. In general, most of the results for mineral contents were similar to those reported in the literature (BERTO et al., 2015; SMITH et al., 2014; LOVE; PAULL, 2011; CANUTO et al., 2010; SILVA, 2008; UNICAMP, 2006; AGUIAR, 1996).

### 3.3 Antioxidants

Figure 1 shows the results obtained for antioxidant capacity expressed as a function of the production rate of formazan for different masses (% m/v) of the fruits analyzed, and the standard deviation for each analysis, except for the *uxi* pulp, which showed a smaller antioxidant capacity than the others and was not included. Although *acerola* is not an Amazon fruit, this fruit was used for comparative purposes, due to its very high ascorbic acid content and antioxidant potential (NUNES et al., 2011; LIMA et al., 2011).

It was observed that all the fruits showed antioxidant capacity. The inhibition of  $O_2^{\bullet-}$  radicals generated by the antioxidant action of the selected fruits was revealed by the smaller amount of NBT reduced to formazan when the reaction catalyzed by XOD proceeded in the presence of the diluted pulps. As expected, the superoxide radical scavenging capacity (RSC) was highest for *acerola* (96.39%), but the *pajurá* fruit presented a very similar result (95.93%).

The RSC values for the other fruits were as follows: *cupuaçu* (80.45%), *abiu* (79.33%), *bacuri* (78.35%), *monguba* (75.74%) and *biribá* (75.55%). In order to evaluate the closeness of the results obtained, a one-way ANOVA test was applied followed by Tukey's test, so as



**Figure 1.** Antioxidant capacity expressed as a function of the production rate of formazan for different concentrations.

to identify significant differences between the average values obtained with 20% dilutions of the fruits (Table 4). The ANOVA showed significant differences ( $p < 0.05$ ) in the antioxidant capacities of the fruits studied. According to Tukey's test, the fruits could be separated into two groups: the first formed by the *acerola* and *pajurá* fruits, which presented no significant difference ( $p < 0.05$ ) in their antioxidant activities; and the second composed of the other fruits, presenting statistical similarity between them in relation to their antioxidant behaviour.

Interestingly, but not intentionally, a significant correlation ( $R^2 = 0.84$ ) between the sodium concentration and antioxidant capacity was observed (Table 5). Normally, the cultivation system (CARDEÑOSA et al., 2016), colour and the ascorbic acid/anthocyanin/polyphenol compound contents (CARDEÑOSA et al., 2016; SUMCZYNSKI et al., 2015) are the main parameters imposing a significant influence on the antioxidant capacity in vegetables and fruits, but almost no scientific publication has reported the effect of Na content on this important nutritional feature. It is known that agricultural conditions such as soil type, growing location, climate and harvesting season directly influence the content of macroelements in agricultural crops (CARDEÑOSA et al., 2016; ROP et al., 2009). Specifically, regarding the Amazon fruits here evaluated, some of them were collected in locations in which the soils have a relatively saline character and the weather has striking tropical characteristics, such as the sampling points of Maranhão state. From the biological point of view, sodium plays a key role in biochemical processes that prevent the imbalance between the production of reactive oxygen species and the antioxidant defense system (SARKADI et al., 2006).

Table 6 shows the concentrations obtained for vitamin C, phenolic compounds and flavonoids in the fruits studied, as well as the antioxidant capacity for comparative purposes.

The vitamin C contents of the fresh fruits were in the range from 5.20 to 52.59 mg 100 g<sup>-1</sup> for the *Monguba* and *Cupuaçu* fruits, respectively. The Institute of Medicine (2006) has established an RDA of 90 mg of vitamin C for a healthy adult, which allows one to classify the *bacuri* and *cupuaçu* pulps as high vitamin C content items, according to Brasil (1998), while *uxi* can be classified as a source of this nutrient. Comparing the results obtained with the literature data, it can be seen that the vitamin C contents obtained were within the ranges reported for *abiu* (BRASIL, 2015; CANUTO et al., 2010), *bacuri* (BRASIL, 2015),

**Table 4.** Average percent formazan production, F value and Tukey's test.

ABIU	ACEROLA	BACURI	BIRIBÁ	CUPUAÇU	MONGUBA	PAJURÁ	F
20.67 ± 2.75 <sup>b</sup>	3.61 ± 0.64 <sup>a</sup>	21.66 ± 0.90 <sup>b</sup>	24.45 ± 0.25 <sup>b</sup>	19.56 ± 1.38 <sup>b</sup>	24.26 ± 4.90 <sup>b</sup>	4.07 ± 5.76 <sup>a</sup>	48.99

F = value calculated (ANOVA; F critical value = 3.11 for 10 and 12 degrees of freedom and 95% confidence level). Means with the same letter are not statistically different at 5% significance in Tukey's test.

## Mineral and bromatological assessment and determination of the antioxidant capacity and bioactive compounds in native Amazon fruits

Becker, M. M. et al.

**Table 5.** Pearson's correlation - results between the antioxidant capacity, bioactive compounds and sodium content for the fruits studied.

	Vitamin C	Phenolic compounds	Flavonoids	Antioxidant capacity	Na
Vitamin C	1.00				
Phenolic compounds	-0.55	1.00			
Flavonoids	-0.25	0.68	1.00		
Antioxidant capacity	0.04	0.32	0.45	1.00	
Na	-0.42	0.40	0.81	0.84	1.00

**Table 6.** Vitamin C, phenolic compounds and flavonoids content, as well as the antioxidant capacity.

		Bioactive Compounds			
		Vitamin C	Phenolic compounds	Flavonoids	Antioxidant capacity
		(mg.100 g <sup>-1</sup> )	(GAE.100 g <sup>-1</sup> )	(QEE.100 g <sup>-1</sup> )	(RSC %)
Amazon Fruits	Abiu	5.34 ± 0.44 <sup>a</sup>	4.30 ± 0.47 <sup>a,b</sup>	ND	79.33 ± 2.75 <sup>a,b</sup>
	Bacuri	28.31 ± 3.23	5.21 ± 0.27 <sup>a</sup>	ND	78.3 ± 0.90 <sup>a,b</sup>
	Biribá	10.00 ± 0.13 <sup>a</sup>	5.45 ± 0.21 <sup>a</sup>	ND	75.55 ± 0.25 <sup>a</sup>
	Cupuaçu	52.59 ± 0.33	3.05 ± 0.08 <sup>b</sup>	ND	80.45 ± 1.38 <sup>a,b</sup>
	Monguba	5.20 ± 0.16 <sup>a</sup>	13.28 ± 1.28 <sup>c</sup>	ND	75.74 ± 4.90 <sup>a</sup>
	Pajurá	7.72 ± 0.69 <sup>a</sup>	14.46 ± 1.11 <sup>c</sup>	2.25 ± 0.10	95.93 ± 5.76
	Uxi	15.04 ± 2.03	5.56 ± 0.05 <sup>a</sup>	ND	23.25 ± 5.86 <sup>*</sup>

ND = undetectable signal. \*RSC for fruits extracts at 20% (w/v). Means followed by the same letter in the same column do not differ significantly from each other by the Tukey test at the 5% probability level.

*cupuaçu* (BRASIL, 2015; GONÇALVES, 2008) and *uxi* (GONÇALVES, 2008; MARX et al., 2002). There were no records of the vitamin C concentration for *monguba* and *pajurá* fruits, and therefore this paper is the first to present data on the ascorbic acid content of these fruits.

The highest phenolic compound concentrations were obtained for the *Monguba* and *Pajurá* fruits, which reflects on the flavour and technological characteristics of these fruits as well as on their nutritive and functional potentials (ROCHA et al., 2013).

Only the *pajurá* pulp showed quantifiable concentrations of flavonoids.

Evaluating the correlation between the bioactive compound composition and antioxidant capacity, a positive correlation ( $R^2 = 0.68$ ) was observed between the flavonoids and the phenolic compounds, and between the flavonoids and the Na content ( $R^2 = 0.84$ ) (Table 5).

In general, the antioxidant capacities were high for most of the Amazon fruits studied, but amongst them, the *pajurá* fruit was shown to have the highest antioxidant capacity against the oxidizing effects of  $O_2^{\bullet-}$  radicals of physiological importance. Thus, extracts of all the fruits, but especially *pajurá*, may be considered as promising sources of bioactive compounds with high antioxidant properties, exhibiting great potential for application in the pharmaceutical, cosmetic and food industries. Up to the completion of this study, the literature reviewed had no mentioned of any other fruit with antioxidant properties

similar to those of *acerola*, and thus the *pajurá* fruit was truly a great revelation.

## 4 Conclusions

The Amazon region remains the world location with the largest plant diversity, and it is common to find fruits with high nutritional potential. These properties were unknown even to the inhabitants of the region and to the Brazilian people as a whole. In this study, seven native fruits making up part of the diet of Amazonian inhabitants were evaluated with respect to their nutritional and antioxidant properties.

The fruits showed the expected variations for the bromatological parameters and a good mineral content, each being rich in one or more nutrients. All the fruits showed high antioxidant capacity, but the *pajurá* fruit showed the highest one, statistically equal to that of *acerola* fruit, and can therefore be explored in various application fields.

The fruits studied can be considered as valuable food supplements due to their positive influence on the nutrition status, thus increasing human productivity and longevity. The antioxidant study revealed these fruits as promising sources of bioactive compounds with high antioxidant properties, exhibiting great potential for application in the pharmaceutical, cosmetic and food industries.

These results can contribute to both composing the Brazilian Food Composition Table and to Brazilian food safety. Notably, this study is one of the first to provide a detailed evaluation of the nutritional compositions of



## Mineral and bromatological assessment and determination of the antioxidant capacity and bioactive compounds in native Amazon fruits

Becker, M. M. et al.

fruits poorly explored in the Amazon region. ICP-OES methods were used and the chemical composition of some compounds was presented for the first time for the *monguba* and *pajurá* fruits and their nutritional potential revealed. It is interesting to mention that these nutritive and antioxidant fruits are native to the Amazon region, and thus the industrial exploitation of such fruits must be supported.

### References

- AGUIAR, J. P. L. Table of nutrient composition of Amazonian foods. **Acta Amazonica**, v. 26, n. 1-2, p. 121-126, 1996. <http://dx.doi.org/10.1590/1809-43921996261126>.
- ALISSA, E. M.; FERNES, G. A. Functional foods and nutraceuticals in the primary prevention of cardiovascular diseases. **Journal of Nutrition and Metabolism**, v. 2012, p. 569486, 2012. <http://dx.doi.org/10.1155/2012/569486>. PMID:22570771.
- ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS – AOAC. **Official Methods of Analysis of the AOAC 999.10**: determination of Lead, Cadmium, Zinc, Copper and Iron in Foods. Atomic absorption spectrophotometry after microwave digestion. Rockville: AOAC, 2002.
- BERKER, K. I.; GÜÇLÜ, K.; TOR, İ.; DEMIRATA, B.; APAK, R. Total antioxidant capacity assay using optimized ferricyanide/prussian blue method. **Food Analytical Methods**, v. 3, n. 3, p. 154-168, 2010. <http://dx.doi.org/10.1007/s12161-009-9117-9>.
- BERTO, A.; SILVA, A. F.; VISENTAINER, J. V.; MATSUSHITA, M.; SOUZA, N. E. Proximate compositions, mineral contents and fatty acid compositions of native Amazonian fruits. **Food Research International**, v. 77, p. 441-449, 2015. <http://dx.doi.org/10.1016/j.foodres.2015.08.018>.
- BEZERRA, V. S.; PEREIRA, S. S. C.; FERREIRA, L. A. M. Características físicas e físico-químicas do uxi (Endopleura uchi Cuatrec.). In: **ANAI DO CONGRESSO BRASILEIRO DE PLANTAS OLEAGINOSAS, ÓLEOS, GORDURAS E BIODIESEL**, 3., 2006, Varginha. Lavras: UFLA, 2006. pp. 379-383.
- BORGES, G. D. S. C.; GONZAGA, L. V.; JARDINI, F. A.; MANCINI FILHO, J.; HELLER, M.; MICKE, G.; COSTA, A. C. O.; FETT, R. Protective effect of Euterpe edulis M. on Vero cell culture and antioxidant evaluation based on phenolic composition using HPLC ESIMS/MS. **Food Research International**, v. 51, n. 1, p. 363-369, 2013. <http://dx.doi.org/10.1016/j.foodres.2012.12.035>.
- BRASIL. Ministério da Saúde. Portaria nº 27, de 13 de janeiro de 1998. Aprova o Regulamento Técnico referente à Informação Nutricional Complementar (declarações relacionadas ao conteúdo de nutrientes), constantes do anexo desta Portaria. **Diário Oficial [da] República Federativa do Brasil**, Brasília, DF, 16 jan. 1998.
- BRASIL. Ministério da Saúde. **Alimentos regionais brasileiros**. 2. ed. Brasília: Secretaria de Atenção à Saúde, Departamento de Atenção Básica, 2015.
- CANUTO, G. A. B.; XAVIER, A. A. O.; NEVES, L. C.; BENASSI, M. T. Physical and chemical characterization of fruit pulps from amazonia and their correlation to free radical scavenger activity. **Revista Brasileira de Fruticultura**, v. 32, n. 4, p. 1196-1205, 2010. <http://dx.doi.org/10.1590/S0100-29452010005000122>.
- CARDEÑOSA, V.; GIRONES-VILAPLANA, A.; MURIEL, J. L.; MORENO, D. A.; MORENO-ROJAS, J. M. Influence of genotype, cultivation system and irrigation regime on antioxidant capacity and selected phenolics of blueberries (*Vaccinium corymbosum* L.). **Food Chemistry**, v. 202, p. 276-283, 2016. <http://dx.doi.org/10.1016/j.foodchem.2016.01.118>. PMID:26920295.
- CHAILLOU, L. L.; HERRERA, H. A.; MAIDANA, J. F. Estudio de própolis de Santiago Del Estero, Argentina. **Revista Ciencia e Tecnologia de Alimentos**, v. 24, n. 1, p. 11-15, 2004. <http://dx.doi.org/10.1590/S0101-20612004000100003>.
- CORTINA-PUIG, M.; MUÑOZ-BERBEL, X.; ROUILLON, R.; CALAS-BLANCHARD, C.; MARTY, J. L. Development of a cytochrome c-based screen-printed biosensor for the determination of the antioxidant capacity of orange juices. **Bioelectrochemistry (Amsterdam, Netherlands)**, v. 76, n. 1-2, p. 76-80, 2009. <http://dx.doi.org/10.1016/j.bioelechem.2009.04.004>. PMID:19447685.
- CUNNIFF, P. (Ed.). **Official methods of analysis of the Association of Official Analytical Chemists**. 16th ed. Gaithersburg: AOAC, 1997.
- GONÇALVES, A. E. S. S. **Avaliação da capacidade antioxidante de frutas e polpa de frutas nativas e determinação dos teores de flavonóides e vitamina C**. 2008. 133 f. Dissertação (Mestrado em Ciência de Alimentos)-Universidade de São Paulo, 2008.
- INSTITUTE OF MEDICINE. **Dietary reference intakes: the essential guide to nutrient requirements**. Washington: The National Academy Press, 2006.
- KAHL, V. F. S.; REYES, J. M.; SARMENTO, M. S.; SILVA, J. Mitigation by vitamin C of the genotoxic effects of nicotine in mice, assessed by comet assay and micronucleus induction. **Mutation Research**, v. 744, n. 2, p. 140-144, 2012. <http://dx.doi.org/10.1016/j.mrgentox.2012.01.008>. PMID:22331007.
- KOZŁOWSKA, A.; SZOSTAK-WEGIEREK, D. Flavonoids - food source and health benefits. **Roczniki Panstwowego Zakladu Higieny**, v. 65, n. 2, p. 79-85, 2014. PMID:25272572.
- LIMA, V. L. A. G.; MELO, E. A.; PINHEIRO, I. O.; GUERRA, N. B. Antioxidant capacity of anthocyanins from acerola genotypes. **Food Science and Technology**, v. 31, n. 1, p. 86-92, 2011. <http://dx.doi.org/10.1590/S0101-20612011000100011>.
- LIU, R. H. Health-promoting components of fruits and vegetables in the diet. **Advances in Nutrition**, v. 4, n. 3, p. 384S-392S, 2013. <http://dx.doi.org/10.3945/an.112.003517>. PMID:23674808.
- LOVE, K.; PAULL, R. E. **Rollinia**. Honolulu: College of Tropical Agriculture and Human Resources – CTAHR, University of Hawaii at Mānoa, 2011. (Publication F\_N-21).

## Mineral and bromatological assessment and determination of the antioxidant capacity and bioactive compounds in native Amazon fruits

Becker, M. M. et al.

- MARX, F.; ANDRADE, E.; ZOGHBI, M.G.B.; MAIA, J. Studies of edible Amazonian plants. Part 5: chemical characterisation of Amazonian Endopleura uchi fruits. **European Food Research and Technology**, v. 214, n. 4, p. 331-334, 2002. <http://dx.doi.org/10.1007/s00217-001-0477-7>.
- MERRILL, A. L.; WATT, B. K. **Energy value of foods: basis and derivation**, revised. Washington: ARS United States Department of Agriculture, 1973. 105 p. (Agriculture Handbook, no. 74).
- NEVES, L. C.; CAMPOS, A. J.; BENEDETTE, R. M.; TOSIN, J. M.; CHAGAS, E. A. Characterization of the antioxidant capacity of natives fruits from the Brazilian Amazon region. **Revista Brasileira de Fruticultura**, v. 34, n. 4, p. 1165-1173, 2012. <http://dx.doi.org/10.1590/S0100-29452012000400025>.
- NORMAS ANALÍTICAS DO INSTITUTO ADOLFO LUTZ – IAL. **Métodos químicos e físicos para análise de alimentos**. 4. ed. São Paulo: Instituto Adolfo Lutz, 2008.
- NUNES, R.S.; KAHL, V. F. S.; SARMENTO, M.S.; RICHTER, M. F.; COSTA-LOTUFO, L. V.; RODRIGUES, F. A. R.; ABIN-CARRIQUIRY, J. A.; MARTINEZ, M. M.; FERRONATTO, S.; FERRAZ, A. B. F.; SILVA, J.. Antigenotoxicity and antioxidant activity of Acerola fruit (*Malpighia glabra* L.) at two stages of ripeness. **Plant Foods for Human Nutrition**, v. 66, n. 2, p. 129-135, 2011. <http://dx.doi.org/10.1007/s11130-011-0223-7>. PMID:21503669.
- PUEYO, I. U.; CALVO, M. I. Assay conditions and validation of a new UV spectrophotometric method using microplates for the determination of polyphenol content. **Fitoterapia**, v. 80, n. 8, p. 465-467, 2009. <http://dx.doi.org/10.1016/j.fitote.2009.06.008>. PMID:19540907.
- ROCHA, M. S.; FIGUEIREDO, R. W.; ARAÚJO, M. A. M.; MOREIRA-ARAÚJO, R. S. R. Caracterização físico-química e atividade antioxidante (in vitro) de frutos do cerrado piauiense. **Revista Brasileira de Fruticultura**, v. 35, n. 4, p. 933-941, 2013. <http://dx.doi.org/10.1590/S0100-29452013000400003>.
- ROP, O.; JURIKOVA, T.; MLCEK, J.; KRAMAROVA, D.; SENGE, Z. Antioxidant activity and selected nutritional values of plums (*Prunus domestica* L.) typical of the White Carpathian Mountains. **Scientia Horticulturae**, v. 122, n. 4, p. 545-549, 2009. <http://dx.doi.org/10.1016/j.scienta.2009.06.036>.
- RUFINO, M. S. M.; ALVES, R. E.; BRITO, E. S.; PÉREZ-JIMÉNEZ, J.; SAURA-CALIXTO, F.; MANCINI-FILHO, J. Bioactive compounds and antioxidant capacities of 18 nontraditional tropical fruits from Brazil. **Food Chemistry**, v. 121, n. 4, p. 996-1002, 2010. <http://dx.doi.org/10.1016/j.foodchem.2010.01.037>.
- SARKADI, B.; HOMOLYA, L.; SZAKÁCS, G.; VÁRADI, A. Human multidrug resistance ABCB and ABCG transporters: participation in a chemoinmunity defense system. **Physiological Reviews**, v. 86, n. 4, p. 1179-1236, 2006. <http://dx.doi.org/10.1152/physrev.00037.2005>. PMID:17015488.
- SILVA, B. L. A. **Caracterização lipídica e protéica das amêndoas da munguba (*Pachira aquatica* Aubl.)**. 2008. 85 f. Dissertação (Mestrado em Ciência e Tecnologia de Alimentos)-Programa de Pós-graduação em Ciência e Tecnologia de Alimentos, Centro de Tecnologia, Universidade Federal da Paraíba, João Pessoa, 2008.
- SILVA, J. M. C.; RYLANDS, A. B.; FONSECA, G. A. B. The fate of the Amazonian areas of endemism. **Conservation Biology**, v. 19, n. 3, p. 689-694, 2005. <http://dx.doi.org/10.1111/j.1523-1739.2005.00705.x>.
- SKOOG, D. A.; WEST, D. M.; HOLLER, F. J.; CROUCH, S. R. **Fundamentals of analytical chemistry**. 8th ed. São Paulo: Thomson Learning, 2008.
- SMITH, R. E.; TRAN, K.; RICHARDS, K. M.; LUO, R. Bioactive acetogenins in Brazilian fruits. **Revista Magistra**, v. 26, p. 626, 2014.
- SUMCZYNSKI, D.; BUBELOVA, Z.; SNEYD, J.; ERB-WEBER, S.; MLCEK, J. Total phenolics, flavonoids, antioxidant activity, crude fibre and digestibility in non-traditional wheat flakes and muesli. **Food Chemistry**, v. 174, p. 319-325, 2015. <http://dx.doi.org/10.1016/j.foodchem.2014.11.065>. PMID:25529687.
- TELES, C. V., 2014. **Caracterização química e avaliação da atividade antioxidante in vitro do extrato rico em polifenóis das folhas de *Syzygium cumini* (L.) Skeels**. 2014. 130 f. Dissertação (Mestrado em Ciências da Saúde)-Universidade Federal do Maranhão, São Luís.
- THOMSEN, S.; HANSEN, H. S.; NYMAN, U. Ribosome inhibiting proteins from in vitro cultures of Phytolacea dodecandra. **Planta Medica**, v. 57, n. 3, p. 232-236, 1991. <http://dx.doi.org/10.1055/s-2006-960080>. PMID:1896521.
- UNIVERSIDADE ESTADUAL DE CAMPINAS - UNICAMP. **Tabela Brasileira de Composição de Alimentos - TACO**. versão 2. 2. ed. Campinas: UNICAMP; NEPA, 2006
- WANG, L.; CHEN, J. Y.; XIE, H. H.; JU, X. R.; LIU, R. H. Phytochemical profiles and antioxidant activity of adlay varieties. **Journal of Agricultural and Food Chemistry**, v. 61, n. 21, p. 5103-5113, 2013. <http://dx.doi.org/10.1021/jf400556s>. PMID:23647066.
- WORLD HEALTH ORGANIZATION – WHO. **Global prevalence of vitamin A deficiency in populations at risk 1995–2005**. Geneva: Vitamin and Mineral Nutrition Information System – VMNIS, 2009.
- WORLD HEALTH ORGANIZATION – WHO. **Worldwide prevalence of anaemia 1993–2005**. Geneva: Vitamin and Mineral Nutrition Information System – VMNIS, 2008.