

## ORIGINAL ARTICLE

# Nutritional characterization, antioxidant activity and bergenin content of the pulp of *Endopleura uchi*

Régis Tribuzy de OLIVEIRA<sup>1</sup>, Lorena Mota de CASTRO<sup>2\*</sup> , Whendel Mesquita do NASCIMENTO<sup>5</sup>, Maria Letícia de Sousa GOMES<sup>1</sup>, Roseane Pinto Martins de OLIVEIRA<sup>6</sup>, Ana Cecilia Nina LOBATO<sup>1</sup>, Rita de Cássia Saraiva NUNOMURA<sup>3</sup>, Carlos Victor Lamarão PEREIRA<sup>1</sup>, Sandra Patrícia ZANOTTO<sup>4</sup>

<sup>1</sup> Universidade Federal do Amazonas (UFAM), Av. Rodrigo Octávio, 6200, Coroado I, 69080-900, Laboratory of Agricultural Products Technology, Manaus - Amazonas, Brazil

<sup>2</sup> Universidade Federal do Amazonas (UFAM), Biodegradation Laboratory, Manaus - Amazonas, Brazil

<sup>3</sup> Universidade Federal do Amazonas (UFAM), Chemistry Department, Manaus - Amazonas, Brazil

<sup>4</sup> Universidade Federal do Amazonas (UFAM), Centre of Multidisciplinary Support, Centre of Biotechnology of Amazonia, Manaus - Amazonas, Brazil

<sup>5</sup> Universidade Federal do Amazonas (UFAM), Centre of Bioinformatics and Computational Biology, Biotechnology Department, Manaus - Amazonas, Brazil

<sup>6</sup> Universidade Federal do Amazonas (UFAM), Animal Anatomy and Physiology Laboratory, Manaus - Amazonas, Brazil

\* Corresponding author: [lorenamcastro.uea@gmail.com](mailto:lorenamcastro.uea@gmail.com);  <https://orcid.org/0000-0002-2122-2775>

## ABSTRACT

The yellow uxi (*Endopleura uchi*) is a tree native to the Amazon and its fruits are appreciated in the region. It is rich in total phenols and its bark is known to have high bergenin content, an isocoumarin derivative that presents several pharmacological activities. Yet the nutritional and functional properties of the fruit are still little known. We analyzed the nutritional, centesimal, and mineral properties, and the antioxidant activity and total phenol content of the alcoholic extract of yellow uxi fruit pulp from different locations in the Amazon. The bergenin content was also quantified. Average pulp yield was  $45.66 \pm 4.44\%$  (w/w), with  $60.1 - 89.2 \text{ g } 100 \text{ g}^{-1}$  of moisture and  $1.28 - 1.32 \text{ g } 100 \text{ g}^{-1}$  of ash (dry basis). Calcium ( $78.2 - 87.1 \text{ mg } 100 \text{ g}^{-1}$ ) and potassium ( $260.2 - 395 \text{ mg } 100 \text{ g}^{-1}$ ) were the most abundant minerals in the pulp. Aluminum concentration was high compared to other Amazonian fruits ( $23.7 - 28.7 \text{ mg } 100 \text{ g}^{-1}$ ). The high caloric value of the pulp ( $325.3 \pm 20.9 \text{ Kcal } 100 \text{ g}^{-1}$ ) is attributed to its lipid ( $32 - 44.9\%$ ) and carbohydrate ( $48.2 - 64.1\%$ ) content. The scavenging activity of DPPH was  $1.95 - 20.68\%$ , which was highly associated with the total phenolic content ( $16.91 - 30.07 \mu\text{g GAE mg}^{-1}$ ). Bergenin content was  $180.8 \pm 55.3 \text{ mg } 100 \text{ g}^{-1}$ . We conclude that *E. uchi* pulp has high caloric and mineral content, and it is also a source of bergenin, thus this fruit has a potential nutritional and functional value.

**KEYWORDS:** Amazonian fruit; yellow uxi; proximate analysis; functional food

## Caracterização nutricional, atividade antioxidante e teor de bergenina da polpa de *Endopleura uchi*

### RESUMO

O uxi amarelo (*Endopleura uchi*) é uma árvore nativa da região amazônica e suas frutas são apreciadas na região. Ele é rico em fenóis e sua casca tem alto teor de bergenina, um derivado da isocumarina conhecido por apresentar diversas atividades farmacológicas. No entanto, as propriedades nutricionais e funcionais do fruto ainda são pouco conhecidas. Nós analisamos as propriedades nutricionais, centesimais e minerais, e a atividade antioxidante e o teor de fenólicos do extrato alcoólico da polpa do fruto do uxi amarelo de diferentes localidades da Amazônia. O teor de bergenina também foi quantificado. O rendimento médio de polpa foi de  $45,66 \pm 4,44\%$  (m/m), com  $60,1 - 89,2 \text{ g } 100 \text{ g}^{-1}$  de umidade e  $1,28 - 1,32 \text{ g } 100 \text{ g}^{-1}$  de cinzas (base seca). Cálcio ( $78,2 - 87,1 \text{ mg } 100 \text{ g}^{-1}$ ) e potássio ( $260,2 - 395 \text{ mg } 100 \text{ g}^{-1}$ ) foram os minerais mais abundantes na polpa. A concentração de alumínio foi alta em comparação com outras frutas amazônicas ( $23,7 - 28,7 \text{ mg } 100 \text{ g}^{-1}$ ). O alto valor calórico da polpa ( $325,3 \pm 20,9 \text{ Kcal } 100 \text{ g}^{-1}$ ) é atribuído ao seu teor de lipídios ( $32 - 44,9\%$ ) e carboidratos ( $48,2 - 64,1\%$ ). A atividade sequestradora do DPPH foi de  $1,95 - 20,68\%$ , altamente associada ao teor de fenólicos totais ( $16,91 - 30,07 \mu\text{g GAE mg}^{-1}$ ). A quantidade de bergenina na polpa foi de  $180,8 \pm 55,3 \text{ mg } 100 \text{ g}^{-1}$ . Concluímos que a polpa de *E. uchi* possui alto teor calórico e mineral, além de ser fonte de bergenina, portanto, este fruto possui valor nutricional e funcional potencial.

**PALAVRAS-CHAVE:** fruto amazônico; uxi amarelo; análise centesimal, alimento funcional

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## INTRODUCTION

About 44% of all native Brazilian fruits are native to the Amazon region (Donadio 2002), and many of these present expressive concentrations of bioactive compounds, which are responsible for their functional properties. Some features of so-called functional foods and their positive effects on target functions that go beyond basic nutrition have already been investigated (Montero *et al.* 2020). Some Amazonian fruits have distinct nutritional characteristics, with high potential for incorporation into the human diet (Neves *et al.* 2015; Pinto *et al.* 2021). Several endemic species of the Amazon region are rich in antioxidants. For example, açai, *Euterpe oleracea* Mart (Arecaceae) is rich in polyphenols (Portinho *et al.* 2012), and camu-camu, *Myrciaria dubia* (H.B.K.) Mc Vough (Myrtaceae), tucumã, *Astrocaryum aculeatum* Meyer (Arecaceae), cubiu, *Solanum sessiliflorum* Dunal (Solanaceae), and yellow uxi, *Endopleura uchi* (Huber) Cuatrec (Humiriaceae) have high total phenol content (Gonçalves *et al.* 2010; Maeda *et al.* 2006).

The yellow uxi (or simply uxi) is a canopy tree (Pinto *et al.* 2021). Native Amazonian peoples use the fruits of the yellow uxi as a complement to their daily diet, and the bark of the trunk is used in the treatment of inflammations of the female urinary tract and diabetes, mainly in the form of infusions (Machado *et al.* 2010; Magalhães *et al.* 2007; Marx *et al.* 2002; Silva and Teixeira 2015). In the region of Belém, in the eastern Brazilian Amazon, yellow uxi is used for its timber, and its oil, fruits, and trunk bark are used in traditional medicines to treat arthritis, cholesterol, and sinusitis (Herrero-Jáuregui *et al.* 2009; Shanley *et al.* 2012). The bark of yellow uxi is rich in bergenin, a 4-O-methyl gallic acid C-glycoside derivate, which shows anti-inflammatory, antifungal, hepatoprotective, and neuroprotective activities (Bastos *et al.* 2020; Muniz *et al.* 2020; Machado *et al.* 2010; Nunomura *et al.* 2009; Pontes *et al.* 2009).

Dietary fibers, antioxidant compounds, vitamins, micronutrients such as calcium, iron, zinc and iodine, macronutrients such as magnesium, carbohydrates, fats and proteins, and amino acids are some of the main nutrients present in functional foods (López-Varela *et al.* 2002). However, little is known on the potential of the fruits of yellow uxi as a functional food (Magalhães *et al.* 2007; Marx *et al.* 2002; Santos Rolim *et al.* 2020). For functional foods, the relevant characteristics are the content in dietary fibers, antioxidant compounds, vitamins, micronutrients such as calcium, iron, zinc and iodine, macronutrients such as magnesium, carbohydrates, fats and proteins, and amino acids (López-Varela *et al.* 2002). Therefore, the objective of this study was to evaluate the centesimal composition, mineral content, antioxidant activity, and bergenin content of the pulp of yellow uxi from several locations in the Brazilian states of Pará and Amazonas.

## MATERIAL AND METHODS

### Chemicals

The chemical reagents diphenyl-1-picrylhydrazyl (DPPH), dimethylsulfoxide (DMSO 99.9%), gallic acid (99%), Folin & Ciocalteu's phenol reagent (10%), and bergenin used in this study were purchased from Sigma-Aldrich™ (St. Louis, MO, USA). Absolute ethanol (99.5%) was purchased from Labsynth® (Diadema, São Paulo, Brasil) and HPLC grade methanol from Tedia (Mexico City, CMX, Mexico). Sanitizer solution was purchased from AudaxCo (Vinhedo, São Paulo, Brasil) and water was purified with a Milli-Q gradient system (Millipore, Milford, MA, USA).

### Fruit selection and pulp processing

Yellow uxi (*Endopleura uchi*) fruit samples were purchased in four Brazilian Amazonian cities, directly from the fruit producers, at local markets in Belém (1°27'7.665"S, 48°30'13.310"W, state of Pará), Santarém (2°25'09"S, 54°43'46"W, Pará), Rio Preto da Eva (2°41'55.472"S, 59°42'12.724"W, state of Amazonas), and Parintins (2°37'54"S, 56°44'49"W, Amazonas). The fruits were both harvested and purchased in February 2019, at the beginning of the ripening period of *E. uchi*. Fruits that had mechanical imperfections, fungi, and/or appeared not to be ripe were discarded (Cecchi 2003; Magalhães *et al.* 2007; Sá Hyacienth *et al.* 2019).

The fruits were packed in a polyethylene thermal box and sent by air to the Laboratory of Plant Products and Bioactive Compounds (LPVCB) at Universidade Federal do Amazonas (UFAM), Manaus, Brazil. Subsequently, each fruit was washed in running water to remove residues and microorganisms adhered to the fruit peel. Subsequently, the fruits were soaked for 5 min in a sanitizer solution (Prokitchen, AudaxCo, SP, Brazil) in a ratio of 1 g of sanitizer to 5 L of water (IAL 2008; Machado *et al.* 2010). Then, the pulp of the fruit from each location was separated from the peel and the seeds using stainless steel knives. The fruit pulps were distributed in centrifuge tubes by locality, frozen at -80 °C (Cryo Cube f570, Eppendorf), and lyophilized in a freeze dryer (FreeZone Plus 2.5 L, Labconco).

### Preparation of extracts

The lyophilized pulp samples were subjected to maceration in ethanol:water (7:3) following the method of Silva and Teixeira (2015), with modifications. The samples were weighed on an analytical balance (AY220, Shimadzu, Kyoto, KYO, Japan) and placed in four 500-mL Erlenmeyer flasks, to which 200 mL of ethanol:water (7:3) were added. Then, the extracts were placed in an ultrasound bath (Q3.0 Ultronique, Eco-Sonics, Indaiatuba, SP, Brazil) and cleaned at a frequency of 40 kHz for 20 min, at 30 °C, and these steps were repeated another four times. After this, the samples were filtered for

removal of the particulate matter. This step was performed in the Mycotheque Culture Collection Laboratory (LCCM) at UFAM. At the end of this process, the ethanol was removed in a rotary evaporator under reduced pressure at 40 °C (Te-211, Tecnal, Piracicaba, SP, Brazil) and, finally, the residue was lyophilized to obtain the extract. This step was carried out at the Laboratory of Separation Processes (LPS) at UFAM. The methanolic extract was obtained by cold maceration of the pulp *in natura* in order to prevent loss of thermosensitive material. The material was extracted with methanol at the ratio of 2:1 w/v. After 48h of maceration, each sample was vacuum filtered and the filtrate solvent was removed in a rotary evaporator at 40 °C. The samples were placed in a sand bath for 24 h at 30 °C to remove residual moisture.

### Moisture and ash

The determination of each parameter was performed in triplicate for the pulp sample from each location. The moisture content was determined by drying in a sterilization oven (SL-100, Solab, Piracicaba, SP, Brazil) set at 105 °C, according to method # 925.09 of AOAC International (AOAC 2005). A 3.0-g aliquot of each sample was weighed in aluminum capsules, which were kept in an oven for 24 hours and removed when the mass remained constant. Exactly 3.0 g of dry pulp of each sample were calcined for 4-6 hours in a muffle furnace set at 550 °C, until reaching constant weight, according to method # 923.03 (AOAC 2005). Moisture content was expressed in both wet (% w/w) and dry (g 100 g<sup>-1</sup>) basis and ashes were expressed in terms of g 100 g<sup>-1</sup> of dry matter.

### Lipids, proteins and carbohydrates

The lipid content in the pulp was determined according to the methodology described by Detmann *et al.* (2012), i.e., 2.0 g of pulp subjected to continuous extraction using the Goldfish method, in which petroleum ether was used as the extraction solvent for 4 hours, with a condensation rate of 5 drops s<sup>-1</sup>. After removal of the solvent and complete drying of the sample in an oven, it was weighed, and the lipid content determined according to the difference. The protein content was determined according to method # 981.10 (AOAC 2005), with modifications proposed by Detmann *et al.* (2012) i.e., 200 mg of the dried pulp was digested in a block digester, added to 2.0 g of digester mixture (Na<sub>2</sub>SO<sub>4</sub>/CuSO<sub>4</sub>·5H<sub>2</sub>O 20:1) and 5 mL of concentrated H<sub>2</sub>SO<sub>4</sub>. The mixture was heated to 400 °C until it became translucent. After adding 10 mL of distilled water, the mixture was distilled in a Kjeldahl distilling apparatus in a NaOH solution. The steam resulting from distillation was titrated with HCl 0.05 M and the result was expressed in terms of g 100 g<sup>-1</sup> of dry matter. The nitrogen-free extract fraction (NIFEXT), which is equivalent to the carbohydrate content, was obtained by the calculation of the difference between the other fractions analyzed

(moisture, protein, lipid, and ash), according to the AOAC (2005) methods. According to the method recommended by ANVISA (2003), the caloric value was determined by the sum of the products of the multiplication of lipid values by 9 Kcal g<sup>-1</sup>, carbohydrates, and proteins by 4 Kcal g<sup>-1</sup>. All analyses were performed in triplicate per sample/location.

### Mineral analysis

The minerals analyzed in the pulp were aluminium (Al), calcium (Ca), iron (Fe), phosphorus (P), magnesium (Mg) and potassium (K). A total of 1,000 mg of each dried pulp sample in the form of a fine powder and 4,000 mg of wax were dried in a separate oven at a temperature of 105 °C for 24 hours. The wax and pulp were mixed and placed in a 10,000 kgf press. All analyses were carried out in triplicate per sample/location.

The samples were examined in an XRF (X-ray fluorescence) spectrometer (WDXRF Rigaku Supermini 200, Rigaku Co., Japan), which had been previously calibrated with the standards for geological reference quantification GBW 3125, 7105 and 7113 (Barros *et al.* 2012). The concentrations of a given element were correlated with the respective emission peaks. Each detected mineral was quantified using external standards dissolved in boric acid (H<sub>3</sub>BO<sub>3</sub>), in six predetermined concentrations, and these standards were subjected to the same conditions of the samples. The quantifications were according to intensity (cps uA<sup>-1</sup>) using the program ZSX – Spectrometer Status (Rigaku Corporation).

### Total phenolic content

The concentration of total phenols was determined according to the method described by Kim *et al.* (2003). First, 10 µL of the extract at a concentration of 1 mg mL<sup>-1</sup>, diluted in DMSO, were added to each well, plus 50 µL of Folin & Ciocalteu phenol reagent 10% and then incubated in the dark for 8 min, at room temperature. After this time, a saturated solution of 0.4% sodium carbonate was added and incubated again for 3 min. Then, the absorbance reading at 620 nm was performed on the microplate reader (Multimode Detector DTX 800, Beckman Coulter Biomek, Indianapolis, IN, USA). An analytical gallic acid curve (7.8125 - 250 µg mL<sup>-1</sup>) was constructed to express the total phenol content (TPC) in terms of mgs of gallic acid equivalent (GAE) per 100 g of dry extract mass. All analyses were run in triplicate per sample/location.

### Antioxidant activity

Antioxidant activity was assessed by DPPH radical scavenging activity assay, determined according to the methodology by Burits and Bucar (2000), with modifications, in order to allow the use of 96-well microplates. In each well, we placed 30 µL of the extract and control (DMSO) in the concentration of 1 mg mL<sup>-1</sup>, and subsequently added 270

$\mu\text{L}$  of the working solution of DPPH:ethanol  $0.05 \mu\text{g mL}^{-1}$ . The plate was incubated for 30 min at room temperature in the dark, and the reading was carried out at 492 nm on a microplate reader. The inhibition percentage (%) calculations were performed based on control absorbance. All analyses were performed in triplicate for ethanolic and methanolic extracts of the pulp samples of each locality. Antioxidant activity was expressed in terms of % DPPH radical elimination activity.

### Bergenin content

This analysis was performed for only one replicate per sample/location. The bergenin content was determined for 1.0 mg of each lyophilized extract of the pulp using the adapted method of Nunomura *et al.* (2009), and high-performance liquid chromatography (HPLC), using a chromatograph (Thermo Accela™, Thermo Fisher Scientific, Hudson, NH, USA) equipped with PDA, UV detector, and operating at the wavelength of 272 nm with a retention time of 5.27 min (Figure 1).

For injection into the HPLC column, 1 mg of extract was solubilized in ultra-pure water and HPLC grade methanol (1:1, v/v). The separation was carried out in a C-18 (2) column ( $150 \times 4.60 \text{ mm}$ ,  $5 \mu\text{m}$ , Phenomenex). Isocratic elution systems were used with ultra-pure water and HPLC grade methanol (7:3). The flow rate of the mobile phase was  $1 \text{ mL min}^{-1}$ . The calibration curve was constructed in the range of 10-150 ppm, using bergenin (Sigma, St Louis, MO, USA) as an external standard, and obtained a linear correlation coefficient ( $R^2$ ) of 0.9997. For the estimation of the limit of quantification (LOQ) and limit of detection (LOD), the method for determination was based on the signal-to-noise ratio (S/N), which presented a LOQ value of  $9.64 \text{ mg L}^{-1}$  and a LOD value of  $2.89 \text{ mg L}^{-1}$ . The analysis was carried out at

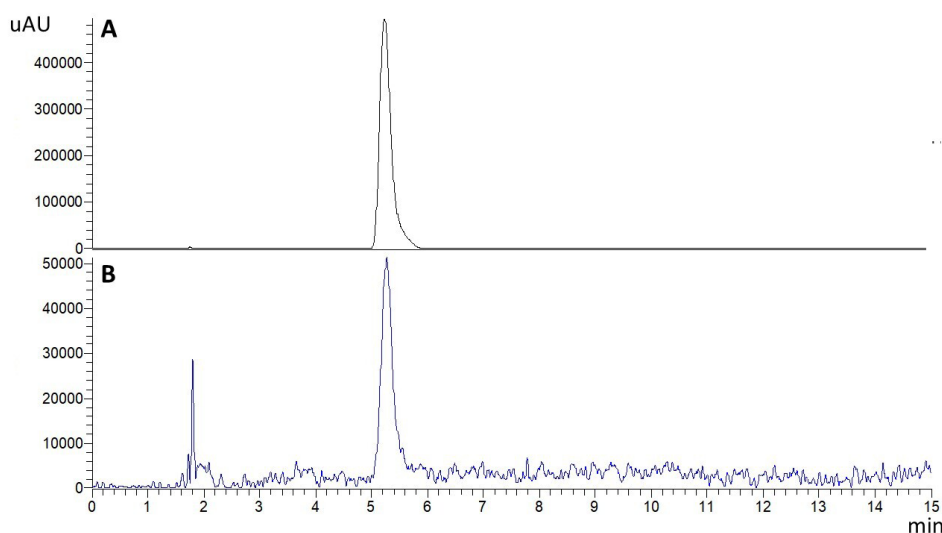
the Laboratory of Chromatography and Mass Spectrometry (LABCEM) at UFAM.

### Statistical analysis

All analyses were performed in triplicate and the results were expressed as mean  $\pm$  standard deviation. The data were submitted to the Shapiro-Wilk normality test and homogeneity of variance was evaluated using Levene's test. Each extract was evaluated separately. At the 0.05 significance level, DPPH data was not drawn from a normally distributed population, so the Kruskal-Wallis test, followed by Dunn's post hoc test, was conducted to compare mean values of fruits from the different locations. The remaining variables were significantly drawn from a normally distributed population according to the Shapiro-Wilk normality test ( $p > 0.05$ ). One-way analysis of variance (ANOVA) of the means was performed in order to compare the results of extracts obtained from fruits from the different locations. When the ANOVA was significant, pairwise differences between means were tested with a post-hoc Tukey test. A significance level of 5% ( $p \leq 0.05$ ) was used in all cases. The relation between TPC and DPPH was analyzed with the Spearman correlation. All analyses were done using Origin Pro® 2021b software.

## RESULTS

Overall, the mass of the yellow uxi fruit was composed of  $50.0 \pm 5.2\%$  seeds,  $45.7 \pm 4.4\%$  pulp, and  $1.1 \pm 0.5\%$  peel (w/w). The overall average moisture content of the pulp was  $43.7 \pm 4.5\%$  (wet basis) (Table 1). The moisture content of fruit from Belém tended to be significantly lower than in the other localities. There was no significant difference in ash content among localities. However, as for moisture, it is possible to observe higher average values in fruit pulps obtained in Parintins and Santarém.



**Figure 1.** Chromatographic profile of bergenin standard (A) and the ethanolic extract of the fruit pulp from *Endopleura uchi* (B) showing bergenin as the major component at 272 nm ( $t_R = 5.27 \text{ min}$ ). This figure is in color in the electronic version.



**Table 1.** Macro and micronutrient content of the pulp of *Endopleura uchi* fruit pulp from four localities in the Brazilian Amazon.

Parameter	Locality			
	Parintins	Rio Preto da Eva	Santarém	Belém
Moisture* (% / g 100 g <sup>-1</sup> )	45.8 ± 4.1 <sup>a</sup> / 85.1 ± 13.6 <sup>a</sup>	44.3 ± 3.4 <sup>ab</sup> / 80.0 ± 11.2 <sup>ab</sup>	47.1 ± 0.6 <sup>a</sup> / 89.2 ± 2.2 <sup>a</sup>	37.5 ± 0.9 <sup>b</sup> / 60.1 ± 2.5 <sup>b</sup>
Ash (g 100 g <sup>-1</sup> )	1.32 ± 0.10 <sup>a</sup>	1.27 ± 0.05 <sup>a</sup>	1.25 ± 0.05 <sup>a</sup>	1.28 ± 0.00 <sup>a</sup>
Proteins (g 100 g <sup>-1</sup> )	3.43 ± 0.70 <sup>ab</sup>	2.23 ± 0.60 <sup>b</sup>	5.44 ± 0.57 <sup>a</sup>	3.38 ± 0.62 <sup>ab</sup>
Lipids (g 100 g <sup>-1</sup> )	32.0 ± 3.49 <sup>b</sup>	36.4 ± 2.66 <sup>ab</sup>	44.9 ± 1.22 <sup>a</sup>	33.4 ± 2.17 <sup>b</sup>
Carbohydrates (g 100 g <sup>-1</sup> )	64.1 ± 7.37 <sup>a</sup>	59.17 ± 4.89 <sup>b</sup>	48.21 ± 1.44 <sup>c</sup>	62.26 ± 2.49 <sup>ab</sup>
<b>Minerals (mg 100 g<sup>-1</sup>)</b>				
Aluminium	24 ± 1.9 <sup>a</sup>	28.7 ± 8.0 <sup>a</sup>	23.7 ± 4.7 <sup>a</sup>	25.9 ± 3.8 <sup>a</sup>
Calcium	83.8 ± 7.6 <sup>a</sup>	82.6 ± 3.6 <sup>a</sup>	87.1 ± 5.8 <sup>a</sup>	78.2 ± 4.0 <sup>a</sup>
Iron	0.6 ± 0.5 <sup>a</sup>	2.7 ± 0.8 <sup>a</sup>	3.7 ± 1.8 <sup>a</sup>	2.5 ± 1.2 <sup>a</sup>
Phosphorus	38.0 ± 4.8 <sup>a</sup>	31.7 ± 4.4 <sup>a</sup>	35.6 ± 3.2 <sup>a</sup>	29.7 ± 2.5 <sup>a</sup>
Magnesium	19.9 ± 0.0 <sup>ab</sup>	16.4 ± 0.0 <sup>b</sup>	33.9 ± 4.4 <sup>a</sup>	19.6 ± 1.8 <sup>b</sup>
Potassium	395.0 ± 2.5 <sup>a</sup>	260.2 ± 5.5 <sup>d</sup>	308.9 ± 2.9 <sup>b</sup>	286.2 ± 4.3 <sup>c</sup>

Values are the mean ± standard deviation of three replicates.

Different superscript letters within lines indicate significant pairwise differences at  $p \leq 0.05$ , according to a post-hoc Tukey's test.

\*Moisture content on a wet and dry weight basis.

The overall average protein content of the pulp was  $3.6 \pm 1.3\%$ . There was a tendency for significantly higher values in Santarém, and significantly lower levels in Rio Preto da Eva (Table 1). The carbohydrate content of the pulp ranged between 48.2 and 64.1 %, with the lowest value found in Santarém. The lipid content varied from 32.0 and 44.9 %, with significantly higher levels in Santarém. The high lipid content, together with the carbohydrate content, resulted in a high calorific content ( $325.3 \pm 20.9$  Kcal 100 g<sup>-1</sup> of fresh pulp).

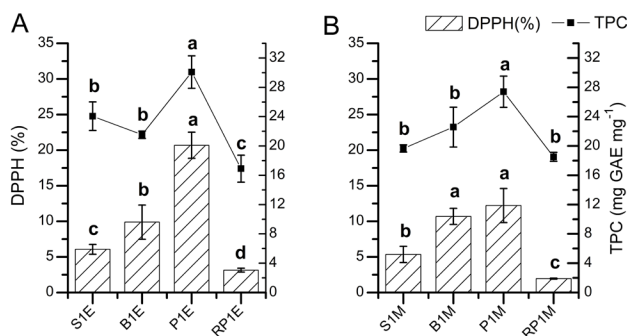
Regarding mineral quantification, potassium levels differed significantly in all locations, and magnesium levels tended to be significantly higher in Santarém (Table 1). There was no significant difference among localities for the other minerals. The aluminum content varied from 23.7 to 28.7 mg 100 g<sup>-1</sup>.

The total phenolic content of the ethanolic and methanolic extracts was significantly higher in the sample from Parintins and was significantly lower in the ethanolic extract from Rio Preto da Eva (Figure 2). A similar profile was observed in antioxidant activity of the DPPH radical, with significantly higher levels in Santarém (also in Belém for the ethanolic extract) and significantly lower levels in Rio Preto da Eva (Figure 2). There was a positive correlation between TPC and DPPH ( $r = 0.90476$ ;  $p = 0.00201$ ). The overall average bergenin content of the pulp was  $180.8 \pm 0.54$  mg 100 g<sup>-1</sup> (dry matter basis), varying from 105.2 mg 100 g<sup>-1</sup> in Rio Preto da Eva to 236.0 mg 100 g<sup>-1</sup> in Belém (Table 2).

## DISCUSSION

The moisture content of the yellow uxi fruit pulp in this study was close to that determined for uxicuruá, *Duckesia verrucosa* (Ducke) Cuatrin (Humiriaceae) (42.3%) (Aguiar 1996). Our *E. uchi* samples and *D. verrucosa* present a

similar profile to other Amazonian oily fruits such as buriti, *Mauritia flexuosa* L. f. (Arecaceae) and patawa, *Oenocarpus bataua* Mart. (Arecaceae), which have average moisture levels of  $50.5 \pm 1.1\%$  and  $33.5 \pm 0.3\%$ , respectively (Darnet *et al.* 2011). The ash content, which is directly related to the content of inorganic elements, tended to be higher in yellow uxi than in other Amazonian fruit pulps such as



**Figure 2.** Total phenolic content (TPC) and elimination of free radicals DPPH of the ethanolic (A) and methanolic (B) extracts of the fruit pulp of *Endopleura uchi* from different localities in the Brazilian Amazon. Columns/squares represent the mean and bars the standard deviation. Means followed by the same lowercase letter did not differ at the 5% level of significance by Tukey's test of multiple comparisons (TPC) and Dunn's post hoc test (DPPH).

**Table 2.** Quantification of total bergenin content in hydroethanolic (7:3) extract of fruit pulp of *Endopleura uchi* from four localities in the Brazilian Amazon.

Sample origin	Yield (%.m/m)	Bergenin (mg g <sup>-1</sup> extract)	Bergenin (mg 100 g <sup>-1</sup> pulp DM)
Santarém	5.72	30.77	180.1
Belém	4.98	41.23	236.0
Parintins	5.34	35.82	201.7
Rio Preto da Eva	5.85	19.68	105.2

DM: dry matter

abiu, *Pouteria caimito* (Ruiz & Pav.) Radlk (Sapotaceae), achachairu, *Garcinia humilis* Vahl (Clusiaceae), araçá-boi, *Eugenia stipitata* McVaugh (Myrtaceae), bilimbi, *Averrhoa bilimbi* L. (Oxalidaceae) and yellow mangosteen, *Garcinia xanthochymus* Hook.f. (Clusiaceae) (0.19 - 0.49%) (Virgolin *et al.* 2017). Moisture and ash content were also similar to those obtained in other studies of yellow uxi pulp, respectively, 48.9% and 1.04% (Berto *et al.* 2015) and 37.5% and 1.1% (Marx *et al.* 2002). In the states of Amazonas and Pará, yellow latosol soils predominate, which are highly weathered soils with high acidity and poor in exchangeable bases (Fontes *et al.* 2016; Sombroek 1984). The similarity in the mineral content of yellow uxi pulps among the sampled locations may be related to similarities in the soil composition between the municipalities where the fruits were grown.

The protein content of 1.9 to 2.9 % (fresh weight) in our uxi samples is in accordance with the generally low protein profile of Amazonian oily fruits (Andrade Júnior *et al.* 2014). For example, the palm fruits buriti, *M. flexuosa* and patawa, *O. bataua* have a protein content of  $3.7 \pm 0.02$  and  $4.9 \pm 0.05\%$ , respectively (Andrade Júnior *et al.* 2014). *Endopleura uchi* and *D. verrucosa* from the state of Amazonas had protein content of 2.20 and 2.72%, respectively (Aguiar 1996). The recommended dietary reference values for protein intake vary between 46 and 56 g per day (Meyers *et al.* 2006), therefore, 100 g of yellow uxi provides, on average, 4% of the recommended daily protein intake. The fruits from Santarém tended to have higher average lipid and protein content, therefore lower carbohydrate content. Based on these results, 100 g of the fresh pulp of yellow uxi supplies 19.6 to 29.9% of the recommended daily carbohydrate consumption, based on the recommended dietary allowance (RDA) of 130 g day<sup>-1</sup> (Meyers *et al.* 2006).

The total lipid content of the uxi pulp in our study was  $20.5 \pm 2.6\%$ , which is similar to those obtained by Berto *et al.* (2015) (20.5%) and Shanley *et al.* (2005) (20.2%) for uxi fruit pulp. Our data can be considered more representative, since we worked with a larger sample size from a wider geographical range and a larger number of analytical replicates than the cited authors. Our lipid content values were similar to those for other Amazonian fruits, such as açai (*E. oleracea*) (40.8%) and buriti (19.0%), which allows inferences about the aggregate potential of yellow uxi for oil production (Menezes *et al.* 2008; Darnet *et al.* 2011).

Uxi is highly valued in Belém, where people extract its oil for cooking and traditional medicine (Shanley *et al.* 2005). Compared to other Brazilian fruits, yellow uxi oil is described as being of excellent quality, presenting the highest phytosterol content (360.0 mg 100 g<sup>-1</sup>) and the second highest tocopherol content ( $504.7 \pm 118.3 \mu\text{g g}^{-1}$ ), only lower than that of buriti pulp ( $1,129.8 \pm 0.0 \mu\text{g g}^{-1}$ ) (Costa *et al.* 2010). The fruits of *E. uchi* from Belém had the highest energy level (351.52 Kcal

100 g<sup>-1</sup>), which is mainly due to the lipid and carbohydrate content, which makes yellow uxi a highly caloric fruit that is comparable to the pulp of açai (489.4 Kcal 100 g<sup>-1</sup>) (Menezes *et al.* 2008). Other species of the Humiriaceae family are edible, and high concentrations of fatty oils are found in the flesh and seeds of some species of *Humiria* (Schultes 1979). The fruits of Humiriaceae show potential for further investigation regarding their nutritional value.

The aluminum content of the uxi pulp obtained in our study is the highest among Amazonian fruit pulps such as araçá, *Psidium cattleianum* Sabine (Myrtaceae) (0.14 mg 100 g<sup>-1</sup>) and açai, *E. oleracea* (0.36 mg 100 g<sup>-1</sup>) (Menezes *et al.* 2008; Montero *et al.* 2020). The pulp of *Miconia albicans* (Sw.) (Melastomataceae), one of the most common species of the Cerrado (savanna) biome, has an aluminum content 10 times higher than that of yellow uxi ( $260.0 \pm 0.3 \text{ mg } 100 \text{ g}^{-1}$ ), which is likely related to the high aluminum content in the predominant latosol soil type in Cerrado (Pasta *et al.* 2019). Likewise, the predominant soil in the sampled municipalities, as mentioned previously, is the yellow latosol, which has characteristically low levels of exchangeable bases and phosphorus, low natural fertility, and a high saturation of aluminum (Maia and Marmos 2010). Aluminum is a non-essential metal, and most of the consumed aluminum is eliminated in the urine. However, it has been related to health risks in humans since it can affect cognition when aluminum, under continuous exposure conditions, enters the brain (Wisniewski and Wen 2007). Currently, the minimum oral risk level (MRL) for intermediate exposure durations established by the US health authority is of 1.0 mg Al kg<sup>-1</sup> day<sup>-1</sup> (ATSDR 2008), while the FAO has established the MRL of 2.0 mg Al kg<sup>-1</sup> day<sup>-1</sup> (JECFA 2011). Based on these reference values and considering the average adult human weight of 60 kg, the consumption of approximately 416 - 831 g day<sup>-1</sup> of yellow uxi fresh pulp would be within the safe limits and would imply only a small risk of contamination of the body.

For iron, values from 2.5 to 4.0 mg were found in 100 g of yellow uxi pulp, which is equivalent to 15.2 - 21.5% of the recommended daily iron intake of 10 mg day<sup>-1</sup> (ANVISA 2005). Iron performs numerous functions, mainly in the transport of oxygen in the blood through hemoglobin, and participating in enzymatic reactions, and its deficiency can cause chronic anemia (Franco 2008; Guyton and Hall 2011; Politi *et al.* 2010).

A high potassium content, as found for the uxi pulp in this study, is a common profile for Amazonian fruits (Montero *et al.* 2020). The consumption of 100 g of fresh uxi pulp would provide 3.7% of the recommended daily potassium intake (Meyers *et al.* 2006). Potassium is necessary for maintaining regular heart function, and also plays a role in the contraction of skeletal muscles, intracellular fluid maintenance, nerve

conduction, energy metabolism and synthesis of proteins and nucleic acids (Franco 2008; Guyton and Hall 2011).

Calcium content in native Amazonian fruits ranges from  $4.5 \pm 0.02$  mg  $100\text{ g}^{-1}$  in abiu, *P. caimito*, to  $52.2 \pm 0.13$  mg  $100\text{ g}^{-1}$  in sweetsop, *Annona squamosa*, L. (Annonaceae) (Rogez *et al.* 2004; Montero *et al.* 2020). We obtained Ca levels of around 47 mg  $100\text{ g}^{-1}$  for uxi pulp (fresh weight). As such, 100 g of yellow uxi pulp can supply 4.7% of the recommended daily Ca intake of 1,000 mg  $\text{day}^{-1}$  (ANVISA 2005). The average P content of 18.9 % was higher than that reported by Berto *et al.* (2015) ( $10.8 \pm 0.2$  mg  $100\text{ g}^{-1}$ ) and similar to that reported by Marx *et al.* (2002) (24.4 mg  $100\text{ g}^{-1}$ ) for uxi fresh pulp. The recommended daily phosphorus intake is 700 mg  $\text{day}^{-1}$  (ANVISA 2005), and the pulp of yellow uxi supplies 2.7% of this demand. Phosphorus participates with calcium in bone building, especially, during growth, besides acting in the reduction of hypercalciuria (Heaney 2004).

Santos Rolim *et al.* (2020) analyzed fruits from Parintins, Amazonas, and, for 100 g of uxi pulp, obtained 14 mg of Al, 40.7 mg of Ca, 380 mg of K, 22 mg of Mg, and 27 mg of P, which correspond to lower levels than those obtained from our samples. On the other hand, the mineral content values by Marx *et al.* (2002) varied from ours for Ca (96 mg), Fe (1.2 mg), Mg (70 mg), and P (39 mg). These differences may be due to factors such as soil, climate, and fruit-harvesting period, which are directly linked to the amount of nutrients in fruit (Shanley and Medina 2005; Gregory and Nortcliff 2013), and which are not standardized or even known among the studies compared in here. Since the chemical composition of fruits is influenced by environmental conditions, evaluating this composition locally is necessary in order to provide reliable nutritional information to local populations (Aguar 1996).

The significant correlation between the total phenolic contents and their antioxidant capacity can be attributed to the extraction method used, and corroborates the results obtained by Bastos *et al.* (2020) of a high antioxidant capacity and TPC in ethanol-based extracts of yellow uxi tree bark. The ethanol-based extracts showed the highest content of gallic acid and phenolic compounds. This association between the antioxidant capacity and the phenolic compounds present in the pulp was also described by Politi *et al.* (2011) for the bark of *E. uchi*. The TPC values found in our study (90.4 - 149.8 mg GAE  $100\text{ g}^{-1}$  dry mass) are consistent with those obtained by Neves *et al.* (2015) (121.4 - 182.3 mg GAE  $100\text{ g}^{-1}$ ) for uxi pulp. Relative to our values of DPPH free radical scavenging activity for all fruit-pulp extracts (2.0 - 22.5% at a concentration of 1,000  $\mu\text{g mL}^{-1}$ ), the values obtained by Politi *et al.* (2011) for aqueous and hydroalcoholic bark extracts of yellow uxi (79.5 - 90.6% at a concentration of 250  $\mu\text{g mL}^{-1}$ ) showed a much higher antioxidant activity. In addition to bergenin and its derivatives, which are the main components in uxi bark extracts (Silva and Teixeira 2015), other phenolic

compounds were identified, such as gallic acid, 5-galoylquinic acid, 3,5-di-O-galoylquinic acid and gallate of galocatechin (Bastos *et al.* 2020), which may jointly contribute to this higher antioxidant capacity in uxi bark.

Bergenin is the most representative compound in yellow uxi tree bark extracts, comprising 87.6 - 88.7% (w/w) of the dry extract (Silva and Teixeira 2015). This  $\alpha$ -glucoside of 4-O-methyl gallic acid was initially identified in the uxi fruit by Magalhães *et al.* (2007). This is the first report on the quantification of this substance in the fruit. Our results suggest that the fruit pulp of yellow uxi can also be a source of bergenin, which contributes to the characterization of this pulp as a functional food. From 180.1 to 236 mg of bergenin were found in 100 g of yellow uxi pulp (dry weight). In comparison with other parts of the *E. uchi* plant, the highest content of bergenin was found in the bark (4,170 - 4,750 mg  $100\text{ g}^{-1}$ ), twigs (2,660 - 3,440 mg  $100\text{ g}^{-1}$ ), and leaves (2,230 - 3,870 mg  $100\text{ g}^{-1}$ ) (Muniz *et al.* 2020). Several *in vivo* and *in vitro* studies have shown biological activities of bergenin, such as neuroprotective (Takahashi *et al.* 2003), hepatoprotective (Popov *et al.* 2005), anti-inflammatory (Nunomura *et al.* 2009), and anti-arthritis (Nazir *et al.* 2007). The anti-inflammatory action of bergenin was discovered by selectively inhibiting the enzyme that catalyzes inflammatory reactions, i.e., cyclooxygenase 2 (COX-2), which produces prostaglandins (Cossio *et al.* 2017; Nunomura *et al.* 2009) and does not present collateral gastrointestinal disorders (Murias *et al.* 2004). An *in vivo* study in diabetes mellitus-induced rats found that the administration of 10 mg  $\text{kg}^{-1}$  of bergenin provided a hepatoprotective function (Sagadevan and Ramalingam 2016). Although this substance presents positive properties, Sá Hyacienth *et al.* (2020) showed that bergenin was predicted to be carcinogenic in mice and rats based on *in silico* toxicological analysis that resulted in bergenin having an  $\text{LD}_{50}$  of 290 mg  $\text{kg}^{-1}$ , which differs significantly from the  $\text{LD}_{50}$  > 2,000 mg  $\text{kg}^{-1}$  reported by Nazir *et al.* (2007). Other authors concluded that bergenin does not present any toxicological danger for humans (Sá Hyacienth *et al.* 2020), and that, based on literature reports, no evidence was found of this substance being cytotoxic to healthy cells (Salimo *et al.* 2023). Further *in vivo* tests are required to evaluate the efficacy and dosage safety of bergenin in uxi pulp.

In summary, the fruit of yellow uxi shows potential for dietary supplementation of inorganic minerals, especially for children, women and the elderly who need more care with their diet (Cavalcante 2010). As mentioned, however, caution is encouraged in its consumption due to the levels of aluminum present in the pulp.



## CONCLUSIONS

We determined that the fruit pulp of yellow uxi (*Endopleura uchi*) has a high caloric content that is primarily attributed to its lipid and carbohydrate content. It is a valuable source of macro and micronutrients, with emphasis on the minerals calcium, iron, phosphorus, potassium, and magnesium, all of which are important in the regulation of human metabolism. The alcoholic extracts of the lyophilized pulp of yellow uxi showed DPPH scavenging activity that is highly associated with the total phenolic content, however, it is lower than the values already reported in the literature for the bark of the uxi tree. In addition, the pulp of yellow uxi contained lower bergenin content than that reported for the bark. Bergenin is associated with the prevention of inflammatory diseases and reduction of oxidative stress. The composition of the uxi fruit pulp showed nutritional and functional values that are compatible with use as a dietary supplement, although the high aluminum content implies the need for moderation in consumption, and the *in vivo* efficacy and dose-dependent safety of bergenin need to be further assessed.

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**DATA AVAILABILITY**

The data that support the findings of this study are not publicly available.

