

# Nutritional composition of the pulp of Pajurá (*Couepia bracteosa* Benth.), an underutilized fruit from the Amazon

Lais Tatiele Massing<sup>1</sup>, Rosa Helena V Mourão<sup>1,2</sup>, Leoneide Érica M Bouillet<sup>2</sup>, Regiane Sablina A Bernardes<sup>2</sup>, Eloisa Helena A Andrade<sup>3</sup>, Agnaldo Tremea<sup>4</sup>, Jesus Nazareno S Souza<sup>5</sup> and José Guilherme S Maia<sup>3\*</sup>

<sup>1</sup>Programa de Pós-Graduação em Biociências, Universidade Federal do Oeste do Pará, 68035-110 Santarém, PA, Brazil

<sup>2</sup>Laboratório de Bioprospecção e Biologia Experimental, Universidade Federal do Oeste do Pará, 68035-110 Santarém, PA, Brazil

<sup>3</sup>Programa de Pós-Graduação em Química, Universidade Federal do Pará, 66075-900 Belém, PA, Brazil

<sup>4</sup>Instituto Nacional de Colonização e Reforma Agrária, 68040-060 Santarém, PA, Brasil

<sup>5</sup>Faculdade de Engenharia de Alimentos, Universidade Federal do Pará, 66095-780 Belém, PA, Brasil

## Abstract

*Couepia bracteosa* Benth. (Chrysobalanaceae), known as pajurá, is a native fruit that occurs spontaneously in the Amazon region, with an exotic flavor and low commercial exploitation. This study describes the biochemical and nutritional composition and the antioxidant and hemagglutinating activities for the natural and lyophilized Pajurá pulps. Fruits are characterized by their high percentage of sugar and total fiber content (34.7%), significant antioxidant capacity (DPPH, 53.42%;  $\beta$ -carotene/linoleic acid, 69.85%) and the absence of haemagglutinating activity. The percentile of vitamin C (22.42 mg/100 g), total phenolic compounds (124.53 mgGAE/100 g) and total carotenoids (2898.76  $\mu$ g/100 g) were also evaluated. Primary fatty acids found in its lipid fraction were oleic (30.2%), lauric (18.1%), palmitic (16.1%), and myristic (11.1%) acids. Also, the pulp presented a pleasant aroma with a high content of (*E*)- $\beta$ -Ocimene (49.2%). It is presumed that results can contribute to a better fruit utilization, which may have potential application as a novel functional food.

## Introduction

In addition to their organoleptic characteristics, many fruits have attracted the attention of the scientific research and consumers due to the presence of minerals, vitamins, bioactive compounds, and fibers. Fruits with these properties can contribute to the risk reduction of chronic non-communicable diseases, such as cancer and cardiovascular diseases [1,2].

In Brazil, it is estimated that 44% of the native fruits are located in the Amazon region, considered a valuable natural reserve of food and medicinal plants worldwide. However, most of the native fruits from the Amazon are wild or cultivated only for the local markets, not having yet defined their economic potential. This fact is due to the absence of studies related to the conditions of crop growth, lack of data on the composition of the fruits, their nutritional value, lack of knowledge about the presence of bioactive compounds and the possibilities of commercialization [2,3].

*Couepia bracteosa* Benth. (Chrysobalanaceae), popularly known as Pajurá, is a fruitful species of the Brazilian Amazon, occurring in the Pará, Amazonas, and Roraima states. The tree is between 12 to 25 meters long, produces globular fruit type drupe of brown epicarp and yellowish pulp, thick and granular, with a pleasant aroma and sweet taste. The harvest occurs between November and May, and the fruit is consumed regionally in nature or as an ingredient in desserts [4]. Previously, the proximate composition, fatty acid and mineral content, and identification of some phenolic and carotenoid compounds were analyzed and determined in the pulp of *C. bracteosa* [5-7]. Also, there are records of bioactive compounds identified in other *Couepia* species, such as the conjugated fatty acids in the seed oils of *C. edulis*

and *C. longipendula* [8,9], phenolic compounds in *C. paraensis* and *C. ulei* [10,11], and an oleanane triterpenoid from *C. polyandra* with anticancer activity [12].

Although the consumption of Amazonian fruits has a regional predominance, these fruits present great possibilities for their use in agroindustry. Also, scientific research that seeks to identify functional properties in fruits also promotes their commercial value and, as a consequence, can benefit family farming and contribute to the sustainable development of the region. The most significant challenge for the exploration and commercialization of new fruit species, particularly in the most distant and hard-to-reach areas is the preservation of their organoleptic characteristics and their nutritional value, given the prospect of high perishability during transport [13].

Nowadays, the post-harvest loss of fruit in Brazil reaches 30%. These losses stem from the choice of inappropriate cultivars, improper postharvest techniques, poor transport conditions and inadequate storage for the products. On the other hand, there is a growing interest in processed fruits, both domestically and abroad the country, due to the demand for healthy foods that are convenient and ready for

**Correspondence to:** José Guilherme S. Maia, Programa de Pós-Graduação em Química, Universidade Federal do Pará, 66075-900 Belém, PA, Brazil, E-mail: gmaia@ufpa.br

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consumption, with a long shelf time and to providing seasonal fruits throughout the year. In this context, the dehydration process becomes a real option to maintain the quality of the fruits, both for direct consumption and in the form of ingredients for new preparations. As the fruits are rich in vitamins and antioxidants, among other heat-sensitive nutrients, when processed by conventional dehydration methods may have reduced their nutritional value and organoleptic characteristics. Thus, the lyophilization process is considered one of the most adequate to maintain the essential aspects of the fruits [14].

The present work aimed to characterize the biochemical and nutritional composition of the natural and lyophilized pulps of *C. bracteosa*, including their physicochemical and analytical aspects, the centesimal composition, antioxidant properties, total carotenoids and phenolic compounds, and the hemagglutinating activity. This study was carried out with the intention of identifying characteristics and benefits for the human consumption of Pajurá, as well as to detect other functional attributes for the fruit.

## Materials and methods

### Drugs and reagents

The reagents Folin-Ciocalteu, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), gallic acid, catechin, rutin, linoleic acid,  $\beta$ -carotene and the methylated fatty acid solution (37 Component Fame Mix) were purchased from Sigma-Aldrich (USA). The LMW GE 17044601 (97 to 14.4 KD) molecular weight calibration standard (BIO-RAD, Brazil) was used. The other reagents and solvents were all from analytical grade.

### Collection data, fruit physical characterization and sample preparation

The fruits of *Couepia bracteosa* were collected in the community of Alter do Chão (coordinates 02°30'18.7" S and 54°56'39.4" W), municipality of Santarém, the state of Pará, Brazil, February 2016. Plant samples (aerial parts) were deposited in the herbarium of Universidade Federal do Oeste do Pará (UFOPA), under the number HSTM 000094. After cleaning and sanitization, ten fruits were randomly chosen for the following biometric analyses: (1) transverse and longitudinal diameter of the fruit and seed, measured with a standard caliper; (2) individual weighing of the whole fruit, pulp, seed, and bark, with the aid of a digital scale. The fresh and lyophilized pulp were stored at -18°C, for further analysis.

### Physical-chemical characterization of the pulp

Reducing sugars of the natural and lyophilized pulps were determined by titration using a Fehling's solution, and the values were expressed as percentages of reducing sugars for 100 g of pulp. Total Soluble Solids (TSS) were quantified by refractometry and the result shown in Brix degree [15]. All the tests were performed in triplicate. The granulometric analysis, water solubility index (WSI) and water absorption index (WAI) were determined to identify possible technological properties of the material resulting from the process of pulp lyophilization. The granulometry was measured in six different sieves, with openings between 75 and 600  $\mu$ m [16]. The WSI and WAI values were obtained by diluting the sample in distilled water (1:10). The mixture was stirred and centrifuged (30 min) and the supernatant transferred to an oven (105°C, 4 h). Then, after cooling and weighing the WSI value was determined. The resulted precipitate was again considered, and the difference between the weight dry and weight wet was calculated for the determination of the WAI value.

### Centesimal composition of the pulp

Moisture, ash and protein contents (g/100 g) were determined according to the recommendations of Instituto Adolfo Lutz [15]. Total nitrogen was converted to protein multiplying its value by the conversion factor of 6.25. Molar weight of soluble protein was obtained by SDS-PAGE electrophoresis [17]. Lipid content was quantified by the method of Bligh and Dyer, according to Instituto Adolfo Lutz [15]. Total fibers (soluble and insoluble) were calculated by the method of Cunniff and Association of Official Analytical Chemists [18]. The carbohydrate content (g/100 g) was determined by exclusion in the analysis of the centesimal composition. The total energy value was estimated by multiplying the conversion factor of 16.8 kJ per g for carbohydrates and proteins, and 37.8 kJ per g for lipids. Vitamin C was quantified by Tillmans method, according to Instituto Adolfo Lutz [15].

### Fatty acid analysis

Lipid fraction from the pulp (100 g) was extracted in a Soxhlet apparatus using hexane and acetone as solvents. The lipid esterification was performed subsequently, according to Khan and Scheinmann [19]. The fatty acid methyl esters identification was performed by GC-MS, using QP-2010-Plus equipment (Shimadzu Corporation, Tokyo, Japan) and a silica capillary column (Rtx-5ms, 30 m $\times$ 0.25 mm $\times$ 0.25  $\mu$ m), with the aid of the MS Solution software and standard libraries [20,21]. The conditions of analysis were: injector temperature 250°C; oven temperature programming starting in 100°C (5 min) and then with a gradient of 4°C/min up to 260°C (20 min); carrier gas, helium (1.2 ml/min); split-type injection of 1.0  $\mu$ l of the sample; ionization by electron impact, 70 eV; temperatures of ion source and transfer line in 220°C and 250°C, respectively. The constituents were identified by comparison of their retention times and mass spectra with those of standards fatty acids existing in the libraries.

### Extraction and analysis of the volatile concentrates

The natural and lyophilized pulps (5 g, each) were subjected to extraction (2 h) with a simultaneous distillation-extraction microsystem to obtain their volatile concentrates, using a Likens & Nickerson-type apparatus [22] and pentane as the solvent (4 ml). The analysis of the volatile concentrates was carried out by GC-MS under working conditions already mentioned, except the oven temperature programming which was 60-240°C (3°C/min). Mass spectra were obtained by automatic scanning (0.3 s, each), with mass fragments varying from 35 to 400 m/z. Retention index was calculated for all volatile components using a homologous series of alkanes (C<sub>8</sub>-C<sub>20</sub>, Sigma-Aldrich), according to van den Dool & Kratz [23]. Constituents were identified by comparing their retention indices and mass spectra libraries (molecular mass and fragmentation pattern), as well as consulting the mass spectra literature.

### Detection of lectins with hemagglutinating activity

The detection of lectins with hemagglutinating activity in the Pajurá pulp was performed using microtiter plates [24]. The natural and lyophilized pulps, after serial dilution in sodium phosphate buffer (1:2, 1:4 and 1:8, pH 7.4), were incubated with a mouse erythrocyte suspension (4%, 50  $\mu$ l), at room temperature. The visual and magnifying glass readings were performed to analyze the erythrocytes hemagglutination, after 60 and 90 min. Wells containing only the sodium phosphate buffer and the erythrocyte suspension was used as the control.

### Preparation of extracts to determine antioxidant capacity, total phenolics total and carotenoids

The lipophilic extract was obtained according to Rodriguez-Amaya [25]. Briefly, the sample (6 g) was macerated with cooled acetone (50 ml). The resulting extract was filtered and transferred to a separatory flask where petroleum ether (40 ml, triplicate) and distilled water (150 ml) were added. After phase separation, water and acetone were discarded. The petroleum ether phase was filtered on anhydrous sodium sulfate and transferred to a volumetric flask, supplemented with an additional part of the solvent. The hydrophilic extract was prepared with a solution of acetone (70.0%), distilled water (29.5%) and acetic acid (0.5%) [3]. The ratio between the sample and solution was 1:1 for natural pulp and 2:8 for lyophilized pulp. The mixture was homogenized in a shaker incubator (80 rpm, 1 h), at room temperature and then filtered. The process was repeated two times. Ethanolic extracts for the natural and lyophilized pulps were prepared at the proportion of 1:2 and 1:10 (pulp:ethanol), respectively. The mixtures were stirred (1 h), centrifuged (5000 rpm) at the temperature of 15°C (10 min) and filtered.

### Determination of total carotenoids

Carotenoids were quantified according to Rodriguez-Amaya [25] based on the lipophilic extracts of the pulps and the absorbance reading at 450 nm. The results were calculated by the absorbance coefficient of petroleum ether and expressed in micrograms of  $\beta$ -carotene equivalents per 100 g of pulp.

### Determination of total phenolic compounds

The content of total phenolic compounds was determined by the Folin-Ciocalteu colorimetric method [26]. An aliquot of hydrophilic extract (500  $\mu$ l) was added to a sodium carbonate solution (1250  $\mu$ l, 75 g/l) and the Folin-Ciocalteu solution (250  $\mu$ l, 1 N). The mixture was homogenized and incubated sheltered from the light (30 min) and then the reading performed at 735 nm. A calibration curve for the gallic acid was constructed, and the results were expressed in milligrams of gallic acid equivalents per 100 g of pulp.

### Antioxidant activity by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

The DPPH antioxidant activity of ethanolic extract of the pulps was determined by the method of Miliauskas and colleagues [27]. Aliquots of the extracts in natural (75  $\mu$ l, concentration from 0.14 to 4.72 mg/ml, on dry basis) and lyophilized (75  $\mu$ l, concentration from 0.069 to 2.24 mg/ml, on dry basis) were added to the DPPH methanolic solution (2925  $\mu$ l, 60  $\mu$ M). The absorbance at 517 nm of the solutions was measured after 5, 15, 30, 45, 60, 75 and 90 min of reaction. Trolox (1 to 5  $\mu$ g/ml, in ethanol) was used as the standard, and the results were expressed in inhibition concentration ( $IC_{50}$ , mg/ml) and inhibition percent (I%).

### Antioxidant activity by $\beta$ -carotene and linoleic acid assay

A reaction mixture was prepared with linoleic acid (20  $\mu$ l), tween 40 (265  $\mu$ l),  $\beta$ -carotene solution (1.0 ml, 0.2 mg/ml in chloroform), and water (50 ml), previously saturated with oxygen (30 min). Then, the orange-yellow color mixture was vigorously stirred. The test was performed by the addition of an aliquot of the reactive solution (2.5 ml) to the ethanolic extracts (200  $\mu$ l, 6 mg/ml of the natural and lyophilized pulps, on dry basis) and incubated in a water bath (15 min, 50°C). The absorbance reading at 470 nm was monitored at the zero time, every 15 min, for 2 h. The same procedure was performed for the control

(ethanol) and the standard (Trolox in ethanol, 1 mg/ml). The results were expressed as inhibition percent (I%) [28].

### Statistical analysis

All assays were performed in triplicate, and the results expressed as the mean $\pm$ standard deviation and subjected to analysis of variance (ANOVA), using the Assistat 7.7 beta software.

## Results and discussion

### Fruit biometric characterization

Biometric features of the *C. bracteosa* fruit are described in Table 1. The fruits are oviform with a mean longitudinal length of 9.5 $\pm$ 1.1 cm and a cross-section of 6.75 $\pm$ 0.1 cm, with an average weight of 257.8 $\pm$ 46.5 g (Figure 1). In percentage terms, the pulp, bark, and seed have mean values of 51.3%, 22.6%, and 26.1%, respectively. These results are very close to that early described [4,29]. By comparison, although the fruits have been collected in distinct areas of the Amazon (Pará and Amazonas states), it is observed that there are similar physical characteristics, which presupposes a homogeneity for the species. The analyzed samples showed a medium yield profile for this fruit pulp, contributing to its probable use as a new source of food and with significant nutritional value. Other Amazon traditional fruits such as açai (*Euterpe oleracea* Mart.), bacuri (*Platonia insignis* Mart.) and pequiá (*Caryocar villosum* Pers.), classified as low yield fruit pulps, have good acceptance and are considered of significant economic value [29].

### Centesimal composition of pulp

The centesimal composition and some physicochemical characteristics of the *C. bracteosa* pulp is shown in Table 2. Composition analysis of the natural and lyophilized pulps, on a dry basis, has revealed that this fruit is a source of vitamin C, minerals (ashes), proteins, lipids and mainly carbohydrates (60.39 $\pm$ 0.08 g/100 g and 59.79 $\pm$ 0.46 g/100 g, respectively), of which 30 to 40% are classified as reducing sugars (monosaccharides). The reducing sugar content in the natural and lyophilized pulps (41.58 $\pm$ 3.52% and 34.1 $\pm$ 2.45%), as well as the total

Table 1. Biometric features for the fruit of *Couepia bracteosa*

Biometric features	Description
Fruit, longitudinal diameter (cm)	9.5 $\pm$ 1.1
Fruit, transverse diameter (cm)	6.76 $\pm$ 0.1
Seed, longitudinal diameter (cm)	6.43 $\pm$ 0.6
Seed, transverse diameter (cm)	4.21 $\pm$ 0.2
Whole fruit (g, %)	257.8 $\pm$ 46.5 (100%)
Thick bark, fruit (g, %)	58.35 $\pm$ 11.0 (22.6%)
Seed, fruit (g, %)	67.20 $\pm$ 11.6 (26.1%)
Pulp, fruit (g, %)	132.25 $\pm$ 28.5 (51.3%)

Results expressed as a mean $\pm$ standard deviation (n=10).



Figure 1. Whole fruit, pulp and seed of Pajurá (*Couepia bracteosa* Benth.).

**Table 2.** Centesimal composition and physical-chemical characteristics of the natural and lyophilized pulps of *Couepia bracteosa*, on a dry basis

Description	Natural Pulp	Lyophilized pulp
Moisture (%)	62.21±0.44 <sup>b</sup>	10.35±0.13 <sup>a</sup>
Ash (% g/100g)	1.98±0.14 <sup>a</sup>	1.98±0.14 <sup>a</sup>
Proteins (% g/100g)	2.68±0.05 <sup>a</sup>	2.74±0.25
Lipids (5, g/100g)	0.28±0.01 <sup>b</sup>	0.73±0.03 <sup>a</sup>
Carbohydrates (% g/100g)	60.39±0.08 <sup>a</sup>	59.79±0.46 <sup>a</sup>
Soluble fibers (% g/100g)	0.50±0.0 <sup>b</sup>	0.50±0 <sup>a</sup>
Insoluble fibers (% g/100g)	34.17±0 <sup>b</sup>	34.2±0 <sup>a</sup>
Total food fibers (% g/100g)	34.67±0 <sup>b</sup>	34.7±0 <sup>a</sup>
Total caloric value (kJ/100g)	1070.21±2.62 <sup>b</sup> (254.81 kcal)	1080.28±1.56 <sup>a</sup> (257.21 kcal)
Vitamin C (mg/100g)	18.42±0.0 <sup>a</sup>	22.42±1.49 <sup>a</sup>
Total soluble solids (TSS, %)	31.76±0.0 <sup>a</sup>	32.80±1.39 <sup>a</sup>
Reducing sugars (glucose %)	41.58±3.52 <sup>a</sup>	34.12±2.45 <sup>a</sup>
Water solubility index (%)		3.77±0.03
Water absorption index (g/g)		2.63±0.08

Results expressed as a mean±standard deviation (n=3). Means followed by the same letter in the columns do not differ significantly from each other by the Tukey test, at the probability level of 5%.

soluble solids (31.76±0.0% and 32.80±1.39%), are related to the degree of sweetness of the fruit and demonstrate that the Pajura has palatable attributes to consumption, both in natural and lyophilized pulps. Also, the reducing sugars and other components responsible for aroma and flavor have unique sensory qualities, which may lead to the formulation of differentiated food products and, with these characteristics, could arouse the interest of consumers and the agribusiness.

The granulometric analysis of the pulverized lyophilized pulp has determined the presence of granules with different diameters, where the particles equal to or less than 75 µm were predominant (55.31%). Particle size influences the rheological characteristics of pulverized foods, such as water absorption, solubility, and intumescence. At the same time, small particles have a more substantial contact surface, which is advantageous when used in a blend of ingredients [30]. The natural pulp presented a water content of 62.2%, which guarantees a higher pulp yield for the fruit in the dehydration process when compared to banana (68.2%), pineapple (86.3%) and acerola (*Malpighia punicifolia* L.) (90.5%) [31], for example. The evaluation of the water solubility index (3.77±0.03%) and the water absorption index (2.63±0.08 g/g) in the lyophilized pulp allowed to identify the hygroscopic characteristics of the pulp and its quality in technological terms, taking into account the incorporation and development of a new food product. Also, it suggests the choice of suitable packaging for product storage. Thus, the features of the lyophilized Pajurá pulp show great potential for its use as a food ingredient, considering that solubility and granulometry ensure that it is easily incorporated into preparations.

In the natural and lyophilized Pajurá pulps, the values obtained for ash (1.98±0.14 g/100 g) and protein (2.68±0.05 g/100 g and 2.74±0.25 g/100 g) content are within the mean previously observed for several fruits [31]. Electrophoresis analysis showed that the soluble proteins of the fresh and lyophilized pulps have the same molecular weight, which justifies that the dehydration process did not affect the protein structure of the fruit. About vitamin C, it was observed that the natural and lyophilized Pajurá pulps (100 g, on a dry basis) could provide about 30% of the daily intake of vitamin C (men, 75 mg; women, 60 mg) [32]. The results indicate that the vitamin C present in the fruit pulp was preserved during the lyophilization process. Thus, although the fruit does not offer a high concentration of vitamin C, the consumption of fresh and lyophilized pulp could complement the daily nutritional

requirement. The Pajurá pulp is distinguished by the presence of a significant amount of fibers (in natural, 34.67 g/100 g; lyophilized, 34.70 g/100 g), which can classify the fruit as a food with substantial functional property, whose value is higher than those recommended in Brazil for other fruits (2.5 g per 100 g, in average) [33]. Between ninety-five fruits described in the Brazilian Food Composition Table [34], only Tucumã (*Astrocaryum aculeatum* G. Mey.) (12.7 g/100 g), Macaúba (*Acrocomia aculeata* Jacq.) (13.4 g/100 g), and Pequi (*Caryocar brasiliense* Camb.) (19 g/100 g) have a high fiber content as Pajurá [31].

The composition of other natural Pajurá pulp, sampled in the state of Amazonas, Brazil, was previously reported [5]. In that work, moisture, ashes, proteins, and carbohydrates presented values lower than those obtained in the present study, particularly regarding the total caloric value, which was only 712.86 kJ, while the result of the current analysis showed 1070.21±2.62 kJ. This difference can be attributed to the Pajurá high fiber content, which was not previously determined by those authors. The role of fiber in the gastrointestinal system is still involved and related to its solubility. Soluble fiber is associated with the reduced blood cholesterol levels, the reduced rate of gastric emptying, and absorption of carbohydrates and lipids. Insoluble fiber increases the peristalsis, avoiding constipation. Fibers are fermented by the intestinal flora, producing short chain fatty acids. Some studies have related the consumption of fibers from fruits, vegetables, and cereals, with the risk reduction of colon cancer, for diabetes control, cardiovascular disease and obesity, among other health problems [35].

It should be noted that in the lyophilization process the content of volatile constituents is primarily preserved, which may intensify the flavor of the fruit, a fact of significant interest in establishing a new product based on the fruit pulp. Also, with the dehydration, there is an increase in the energy value of the pulp and, as a consequence, a reduction in the size of the portion of fruit to be consumed or even added to other preparations. At the same time, the lyophilization process reduces the perishability of the fruit, which would result in the fermentation of carbohydrates. This practice will allow a longer shelf life and fruit availability. Thus, the consumption of pajurá or the use of lyophilized pulp as ingredients of new preparations may be utilized by the food industry to benefit the health of future consumers. Also, due to the high concentration of fibers, the fruit can be exploited using its extraction for pharmacological purposes or as a food supplement. However, other studies are still needed to identify these fibers and their effect on the human body.

### Fatty acids composition

The fatty acids composition of the Pajurá pulp is shown in Table 3. Eighteen fatty acids were identified, with predominance of oleic acid (C18:1, 30.2%), lauric acid (C12:0, 18.1%), palmitic acid (C16:0, 16.1%), myristic acid (C14:0, 11.1%), stearic acid (C18:0, 6.4%) and capric acid (C10:0, 3.7%). Low contents were observed for the following long-chain fatty acids: arachidic (C20:0, 2.2%), behenic (C22:0, 0.7%) and lignoceric (C24:0, 0.7%). Lauric and oleic acids are essential for human nutrition, with proven benefits associated with their consumption. It has antioxidant and anti-inflammatory properties, reducing the risk of cardiovascular diseases [36]. Oleic acid (ω9), one of the most common monounsaturated fatty acids and present in food oils, is the principal constituent of olive oil and has potential action in the primary and secondary prevention of cardiovascular diseases [37]. These results stimulate new research to verify the beneficial effect of Pajurá consumption for prevention and treatment of pathologies associated with eating habits, as is the case of chronic non-communicable diseases.

**Table 3.** Composition of fatty acid methyl esters of the lipid fraction of natural pulp of *Couepia bracteosa*

RT	Symbology	Fatty acid, methyl ester	Oil%
11.408	C10:0	Capric acid, methyl ester	3.7
15.950	C11:0	Undecanoic acid, methyl ester	0.2
19.608	C12:0	Lauric acid, methyl ester	18.1
20.042	C14:1(9) $\omega$ 5	Myristoleic acid, methyl ester	0.7
22.725	12-Me-C13:0	Isomyristic acid, methyl ester	0.2
26.942	C14:0	Myristic acid, methyl ester	11.1
30.042	C15:0	Pentadecanoic acid, methyl ester	0.4
33.792	C16:1(9) $\omega$ 7	Palmitoleic acid, methyl ester	1.4
34.050	C16:0	Palmitic acid, methyl ester	16.1
36.025	14-Me-C15:0	Isopalmitic acid, methyl ester	1.2
36.842	C17:0	Heptadecanoic acid, methyl ester	1.5
38.967	C18:2(9,12) $\omega$ 6	Linoleic acid, methyl ester	0.6
39.733	C18:1(9) $\omega$ 9	Oleic acid, methyl ester	30.2
40.667	C18:0	Stearic acid, methyl ester	6.4
43.075	C18:1(11) $\omega$ 7	Vaccenic acid, methyl ester	0.3
46.008	C20:0	Arachidic acid, methyl ester	1.8
51.400	C22:0	Behenic acid, methyl ester	0.7
56.458	C24:0	Lignoceric acid, methyl ester	0.7
Total (%)			95.3

RT=retention time (on RTX-5ms capillary column)

### Volatile concentrates composition from the pulps

Constituents identified in the volatile concentrates from the natural and lyophilized pulps are listed in Table 4. Eight components were identified, whose sums correspond to 98.4% and 100.0%, respectively. The constituents were (*E*)- $\beta$ -ocimene (33.1% and 49.2%), (2*E*)-hexenal (20.8% and 5.3%), naphthalene (13.7% and 14.3%), 2-ethylhexanol (13.3% and 18.1%), hexanal (11.1% and 6.8%), eugenol (3.2% and 2.7%), 2,3-hexadienone (3.2% and 1.8%) and phenylacetaldehyde (traces and 1.8%), in decreasing order. (*E*)- $\beta$ -Ocimene, (2*E*)-hexenal and naphthalene were reported as significant constituents in the fruits of Pitanga (*Eugenia uniflora* L.) [38], Guava (*Psidium guajava* L.) [39] and Bagaçu (*Talauma ovata* A. St.-Hil.) [40]. Volatile constituents are produced during fruit growth and maturation, and their compositions depend on environmental factors, planting conditions, maturation stage, post-harvest period and storage period. Thus, controlled storage conditions may contribute to the formation of the characteristic fruit flavor [41]. The identification of aroma constituents helps to determine the appropriate procedures during the fruit processing, having in mind to guarantee its high quality and to maintain the original flavor in the final product [42].

### Detection of lectins and haemagglutinating activity

Lectins belong to a family of proteins that can bind to specific carbohydrates, reversibly and without altering their structure. Lectins are present in foods of the plant origin such as cereals, legumes, vegetables, and fruits [43]. The analysis of the hemagglutinating activity may indirectly indicate the presence or absence of lectins in Pajurá. Because of their ability to bind to erythrocytes, lectins are employed in research related to biological processes such as cell proliferation, apoptosis, and neoplastic cell metastasis. Studies have shown that the ingestion of some types of lectins may be detrimental to health due to its possible attachment to epithelial cells, with consequent damage to the intestinal epithelium. This fact makes it permeable to toxins, reducing nutrient absorption and possibly triggering inflammatory diseases related to malnutrition [43]. The extracts obtained from the natural and lyophilized pajurá pulps did not present hemagglutinating activity when tested in erythrocytes of mice, suggesting the absence

of lectins. However, analysis of the hemagglutinating activity using human erythrocytes and from other animal species becomes necessary to confirm the lack of lectins, since these can react with different blood groups. Taking into account that Pajurá is a wild fruit and little used, research proving the safety of its consumption will be necessary to avoid possible nutritional disorders that may harm the health of consumers.

### Determination of antioxidant capacity and total carotenoids and phenolic compounds

The results of the antioxidant capacity assays, which was conducted with the DPPH radical and the  $\beta$ -carotene/linoleic acid system, as well as for determination of total carotenoids and phenolic compounds, are described in Table 5.

The antioxidants interact with the DPPH through the transfer of electrons or the donation of hydrogen neutralizing its character of free radical [44]. The natural and lyophilized Pajurá pulps, on a dry basis, showed inhibition values (I%) of  $27.20 \pm 0.75\%$  and  $53.42 \pm 3.19\%$ , at the concentration of 4.72 mg/ml and 2.24 mg/ml, respectively. According the classification proposed by [1], the lyophilized pulp can be considered as having a median antioxidant capacity in comparison to the natural pulp which showed the antioxidant activity with a twice lower value. This difference may be associated with the enzymatic reactions which occur in the natural pulp because it contains a higher content of water and an adequate environment for the oxidation/degradation reactions of the bioactive compounds with antioxidant activity.

In the bleaching oxidation system of  $\beta$ -carotene, frequently used to evaluate the inhibition of free radicals generated by linoleic acid peroxidation, the Pajurá pulp showed an inhibition value (I%) of  $32.81 \pm 0.19\%$  of the natural pulp and  $69.85 \pm 3.33\%$  of the lyophilized pulp, on a dry basis and at concentrations of 6 mg/ml. According Rufino and coworkers [28], the natural pulp of Pajurá presented a median antioxidant capacity (<40%), while its lyophilized pulp showed a significant antioxidant capacity (between 40 and 70%). These results

**Table 4.** Constituents identified in the volatile concentrates of the natural and lyophilized pulps of *Couepia bracteosa*

Constituents	RI <sub>Calc</sub>	RI <sub>Lit</sub>	in natura Pulp (%)	Lyophilized Pulp (%)
2,3-Hexadienone	789	781 <sup>a</sup>	3.2	1.8
Hexanal	800	801 <sup>b</sup>	11.1	6.8
(2 <i>E</i> )-Hexenal	848	846 <sup>b</sup>	20.8	5.3
2-Ethylhexanol	1027	1030 <sup>a</sup>	13.3	18.1
Phenylacetaldehyde	1043	1036 <sup>b</sup>	tr	1.8
( <i>E</i> )- $\beta$ -Ocimene	1046	1044 <sup>b</sup>	33.1	49.2
Naphthalene	1182	1178 <sup>b</sup>	13.7	14.3
Eugenol	1358	1356 <sup>b</sup>	3.2	2.7
Total			98.4	100.0

RI<sub>Calc</sub>=Calculated retention index; RI<sub>Lit</sub>=Literature retention index; tr: traces (<0,05%); <sup>a</sup>=FFNSC library [21]; <sup>b</sup>=Adams library [20].

**Table 5.** Determination of the antioxidant capacity (DPPH radical scavenging and  $\beta$ -carotene / linoleic acid assays) and total phenolic compounds and total carotenoids in the natural and lyophilized pulps of *Couepia bracteosa*, on dry basis

Description	Natural Pulp	Lyophilized Pulp
DPPH (I%)	$27.20 \pm 0.75^b$	$53.42 \pm 3.19^a$
DPPH (IC <sub>50</sub> mg/ml)	$8.8 \pm 0.34^b$	$2.08 \pm 0.12^a$
$\beta$ -carotene/linoleic acid (I%)	$32.81 \pm 0.19^b$	$69.85 \pm 3.33^a$
Total phenolic compounds (mg GAE/100g)	$124.53 \pm 12.98^a$	$117.44 \pm 2.7^a$
Total carotenoids ( $\mu$ g $\beta$ -caroteneE/100g)	$1168.74 \pm 19.05^b$	$2898.76 \pm 41.41^a$

Results expressed as mean  $\pm$  standard deviation (n=3). Means followed by the same letter in the columns do not differ significantly from each other by the Tukey test, at the probability level of 5%.

are close to that obtained from the açai pulp (*Euterpe oleracea* Mart.): natural açai, 31.9%; lyophilized açai, 76.1% [28].

The natural and lyophilized Pajurá pulps, on a dry basis, have shown low values for the total phenolic compounds (124.53 mgGAE/100 g and 117.44 mgGAE/100 g, respectively), very close to uvaia (*Eugenia pyriformis* Camb.) and cashew (*Anacardium occidentale* L.) (127 mgGAE/100 g and 118 mgGAE/100 g, respectively), and relatively lower when compared with the value of açai (454 mgEAG/100 g) [28]. Tannins, flavonoids, and phenolic acids make up a group of molecules derived from the secondary metabolism of plants, which are identified as phenolic compounds with high antioxidant power. More than 7,000 flavonoids have been identified in recent years and, due to the discovery of numerous pharmacological activities related to these compounds, the plants have been extensively studied to determine, quantify and discover new molecules and properties of the phenolic compounds [45]. Acacetin sulfate, oleuropein, and caffeic acid were found in the Pajurá pulp [6,7]. The moderate antioxidant activity observed for the Pajurá pulp, in the present study, could be attributed to these phenolic compounds, previously identified in the plant.

Carotenoids are known for their functional properties and significant antioxidant capacity, acting in the prevention of cardiovascular diseases and macular degeneration, such as provitamin A [46]. The natural and lyophilized Pajurá pulps are primary sources of carotenoids (1168.74±19.05 mg/100 g and 2898.76±41.41 mg/100 g, respectively), with similar content to the carotenoids present in açai and acerola pulps (2800±0.4 mg/100 g and 1400±0.1 mg/100 g, respectively) [28]. It is very likely that the lyophilized pulp kept the bioactive compounds and the antioxidant activity of the in natural pulp, which confirms the lyophilization process as a useful technique in the conservation of perishable foods. The major carotenoids found in the Pajurá pulp were all-*trans*-neochrome and all-*trans*-β-carotene, with an approximate value of 43 µg per g extract [6].

## Conclusion

The Pajurá fruit stands out for its high content of total fibers, mainly insoluble fibers, that may be useful in the treatment of constipation and intestinal health. Also, it has potential as a functional food because it is a source of vitamin C, total phenolic compounds, carotenoids and showed significant antioxidant activity. Additionally, it presents other excellent characteristics, with a good pulp yield, high sugar content, and low acidity, which are considered desirable aspects for appealing new fruits to the direct consumer and the food industry. The lyophilization process was efficient in the preservation of nutritional constituents and bioactive compounds, proving to be a useful tool that may offer an increase in the shelf life of these fruits, as well as their availability during the low season, with secure distribution to other different locations from the origin. Thus, as a poorly consumed and underutilized fruit, the Pajurá can contribute to the establishment of a more healthy and varied diet for the man, besides its contribution to increasing the productive chain and the sustainable development of the Amazon region.

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