

Article

Co-Product of Pracaxi Seeds: Quantification of Epicatechin by HPLC-DAD and Microencapsulation of the Extract by Spray Drying

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Abstract: In the industrial processing of fruits, co-products are generated, which are often not used. The pracaxi co-product, obtained by cold pressing its seeds, contains phenolic compounds with antioxidant activity, which in this work were extracted and microencapsulated by spray drying. The pracaxi extract was characterized by Fourier-transform infrared spectroscopy (FTIR) and high-performance liquid chromatography (HPLC-DAD), and its antioxidant activity was quantified by the ABTS and DPPH assays. Total polyphenol and flavonoid contents in the extract and microparticles were determined by UV-Vis spectrophotometry. Microparticles were then characterized regarding their moisture content, morphology (by scanning electron microscopy), size, polydispersity index and zeta potential. The FTIR spectra revealed functional groups that may be related to phenolic compounds. The extract showed good antioxidant activity according to both selected assays, while the HPLC-DAD analysis evidenced epicatechin as the main compound, whose content was quantified and validated according to the guidelines of recognized national and international agencies. The total polyphenol contents were 20.61 ± 0.20 mg gallic acid equivalent (GAE)/g in the extract and 18.48 ± 0.10 mg GAE/g in the microparticles, while the total flavonoid contents were 28.29 ± 0.70 mg quercetin equivalent (QE)/g and 13.73 ± 0.10 mg QE/g, respectively. Microparticles had a low moisture content, spherical shape, size less than $1 \mu\text{m}$ and negative zeta potential. Furthermore, they were able to satisfactorily retain phenolic compounds, although in a smaller amount compared to the extract due to small losses resulting from degradation during the drying process. These results, taken as a whole, demonstrate that the pracaxi co-product can be a promising candidate in obtaining products of interest for the cosmetic and food sectors by aiming to exploit its antioxidant activity.

Keywords: Amazonian fruits; flavonoids; validation; antioxidant activity; spray drying



Citation: da Silva, R.L.; Ferreira, L.M.d.M.C.; Silva-Júnior, J.O.C.; Converti, A.; Ribeiro-Costa, R.M. Co-Product of Pracaxi Seeds: Quantification of Epicatechin by HPLC-DAD and Microencapsulation of the Extract by Spray Drying. *Processes* **2024**, *12*, 997. <https://doi.org/10.3390/pr12050997>

Academic Editor: Agnieszka Zgoła-Grzeskowiak

Received: 17 April 2024

Revised: 11 May 2024

Accepted: 11 May 2024

Published: 14 May 2024



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1. Introduction

Pentaclethra macroloba (Willd.) Kuntze (pracaxi) is an oilseed plant from the Amazon region that is widely used in the cosmetic industry due to the light-yellow liquid oil that results from pressing its seeds at room temperature [1,2]. However, the secondary product generated by pressing, called pracaxi co-product, is little explored, which suggests investigating its possible exploitation.

The liquid and solid co-products generated by the industrial processing of natural raw materials, despite containing biodegradable organic matter, accumulate in the environment and cause imbalances in the ecosystem. However, the increase in ecological awareness,

which began at the end of the 20th century, has led to the realization that one of the humanity's biggest challenges for the coming decades is to balance the production of goods and services, economic growth, social equality and environmental sustainability [3,4]. Given this, the Brazilian agroindustry increasingly requires the appropriate techniques for treating such co-products, whose applicability, however, is often limited by the lack of knowledge of their nutritional and economic values, for example, in the production of animal feed [5,6].

The vast majority of the co-products from vegetable raw materials are considered to have no economic value, despite their bioactive potential due to their high contents of enzymes, proteins and secondary metabolites (polyphenols, flavonoids, carotenoids, etc.). For this reason, they have become the target of research aimed at developing new products [7,8]. These bioactive compounds, such as phenolic compounds, have in fact antioxidant potential that may be exploited to treat various pathologies [9,10], thus contributing to a better quality of life for the population.

Among the phenolic compounds found in co-products, flavonoids stand out, especially epicatechin, which has high antioxidant activity and the potential to reduce the risks of cardiovascular diseases and tumors as well as the level of low-density lipoprotein (LDL) [11,12].

Antioxidants play an important role in the human body's defense system, as they are able to delay or prevent cell oxidation, which is responsible for cell aging and many degenerative processes in humans [12]. Furthermore, antioxidants have a wide application in the pharmaceutical, cosmetic and nutritional sectors, as they exhibit a wide range of biological properties, such as anti-allergic, anti-inflammatory and antimicrobial activities [13]. Despite these advantages, bioactive compounds are unstable and susceptible to oxidation, polymerization and condensation when exposed to variations in light, oxygen, temperature and pH, among others, which can reduce their biological potential [14–19].

Microencapsulation, which consists of coating small particles or droplets of the extract using one or more encapsulating agents, has therefore become an alternative to minimize these losses. Among these agents, maltodextrin, a carbohydrate formed by the partial hydrolysis of starch thanks to the action of acids and/or enzymes [20–22], has emulsifying properties and low hygroscopicity, viscosity and cost and allows good film formation, in addition to improving the taste and odor of products [20]. In this sense, the spray drying of plant extracts, despite exposure to high temperatures, is used to promote the protection and stability of their active components, in addition to increasing their application potential [23–25] thanks to spray drying's microencapsulation capacity [26].

In this context, exploring the potential of the pracaxi co-product by evaluating its antioxidant activity becomes promising, in addition to contributing to reducing waste, minimizing the environmental impact and adding value to the production chain. Therefore, in this work, the epicatechin-rich pracaxi co-product extract was microencapsulated by spray drying, with the aim of preserving its stability and bioactivity in order to prepare new products with possible applications in the pharmaceutical, cosmetic and food sectors.

2. Materials and Methods

2.1. Chemicals, Reagents and Standards

Sodium carbonate, sodium hydroxide, aluminum chloride and potassium bromide were purchased from Synth (São Paulo, Brazil), while potassium persulfate was from Impex (São Paulo, Brazil). Gallic acid, caffeic acid, 2-hydroxycinnamic acid, *trans*-cinnamic acid, catechin, epicatechin, quercetin, rutin, 2,2'-azino-bis [3-ethylbenzothiazoline-6-sulfonic acid] (ABTS) ($\geq 98\%$), 2,2-diphenyl-1-picrylhydrazyl (DPPH), (\pm)-6 hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and Folin–Ciocalteu reagent were acquired from Sigma-Aldrich (São Paulo, Brazil), while maltodextrin with dextrose equivalent (DE) 10 was from Corn Products (São Paulo, Brazil).

2.2. Obtaining Raw Material from Pracaxi Co-product

The pracaxi seed co-product obtained from the Amazon oil company underwent a cooking process at 65 °C for 45 min and was then subjected to cold pressing to extract the oil, which generated the co-product, the raw material used in this study. The co-product was collected shortly after oil extraction and transferred to the Pharmaceutical Nanotechnology Laboratory of the Faculty of Pharmacy (Nanofarm) of the Federal University of Pará (UFPA). The material was dehydrated for seven days in an oven (model SL-102 SOLAB[®], Piracicaba, Brazil) with air circulation at a temperature of 40 ± 2 °C until a constant weight was obtained. Next, the sample was crushed in a knife mill to reduce the size of the particles, placed in polyethylene bags and stored under refrigeration (−18 °C) to guarantee material stability.

2.3. Preparation of Pracaxi Co-product Extract

The hydroethanolic extract of the pracaxi co-product was obtained by percolation using 70:30 (v/v) ethanol:water as the extraction solvent. In the percolator, which contained filter paper with a diameter of 12.5 cm, 1 kg of pracaxi seed co-product was added and 10 L of solvent was dripped intensely (with solvent renewal) until exhaustion, according to the Phytotherapy Form of the *Brazilian Pharmacopoeia* (2nd Edition) with adaptations [27]. After percolation, the extract was concentrated in a rotary evaporator (model 840, Fisatom[®], São Paulo, Brazil) at a temperature of 40 °C under low pressure until complete ethanol evaporation. The obtained crude extract was stored in a freezer (−18 °C).

2.4. Determination of Total Polyphenols

To a 10 mL volumetric flask containing 100 µL of the sample, 500 µL of the Folin–Ciocalteu reagent and 6 mL of distilled water were added. After stirring for 1 min, 2 mL of 10% (w/v) sodium carbonate solution was added and the mixture was stirred for another 30 s before the volume was completed with distilled water. The solution was left to rest for 2 h, and readings were taken on a UV-Vis spectrophotometer (model UV-1800, Shimadzu[®], Kyoto, Japan) at a wavelength of 760 nm [28]. The standard curve was constructed using gallic acid solutions at concentrations of 6 to 20 mg/mL. The results were expressed in milligrams of gallic acid equivalent per gram of crude extract (mg GAE/g). The blank consisted of a solution containing all reagents except the sample.

2.5. Determination of Total Flavonoids

To a 10 mL volumetric flask containing 800 µL of the sample, 400 µL of 2.5% (v/v) aluminum chloride solution was added, and the volume was completed with distilled water. The solutions remained at rest for 30 min in the absence of light. Then, readings were taken on the same spectrophotometer mentioned above at a wavelength of 425 nm. The quercetin standard curve was constructed using concentrations of 5 to 35 mg/mL. The results were expressed as milligram of quercetin equivalent per gram of crude extract (mg QE/g) [29]. The blank consisted of a solution containing all reagents except the sample.

2.6. Identification and Quantification of Epicatechin by High-Performance Liquid Chromatography (HPLC-DAD)

2.6.1. HPLC-DAD Analyses

HPLC-DAD analyses were performed using a high-performance liquid chromatograph (model 1260 Infinity[®], Agilent, Santa Clara, CA, USA). The standards used were gallic acid, caffeic acid, 2-hydroxycinnamic acid, *trans*-cinnamic acid, catechin, epicatechin, quercetin and rutin. The standard solutions at a concentration of 0.5 mg/mL and the extract at a concentration of 1 mg/mL were filtered through membranes with a 0.22 µm pore diameter (MF Millipore[®], Bedford, MA, USA). After injecting 10 µL of the sample or standard, the analysis was performed on an Xbridge C18 column (250 × 4.6 mm, 5 µm particle size) maintained at 30 °C. The mobile phase, composed of acidified water formic acid pH 3 (solvent A) and methanol (solvent B), was fed at a flow rate of 0.8 mL/min according to the

following elution program: 10 min, 5% solvent B and 95% solvent A; 15 min, 25% solvent B and 75% solvent A; 24 min, 35% solvent B and 65% solvent A; 33 min, 80% solvent B and 20% solvent A; return to the initial condition within 36 min. Peaks were recorded in the wavelength range of 190 to 400 nm and detection at wavelengths of 254, 280, 325 and 365 nm. Peaks were confirmed by comparing the retention times and UV spectra with those of reference standards. To locate the peak corresponding to the compounds in the analyzed sample, co-elution was carried out using a 100 µL aliquot of each standard in 1 mL of the sample, and injection was performed under the same conditions as for the extract [30].

2.6.2. Validation of the Epicatechin Quantification Method

The epicatechin quantification method was validated according to the parameters linearity, precision, accuracy, robustness, limits of detection and quantification of ICH Q2 (R1) [31] and Resolution RE 166/17 of the Brazilian National Health Surveillance Agency (ANVISA) [32].

Linearity

To evaluate linearity, a standard solution of 1 mg/mL epicatechin in methanol was used to prepare diluted solutions at different concentrations (31.25, 62.50, 125, 250 and 500 µg/mL). An analytical curve was then constructed and used under the chromatographic conditions described in Section 2.6.1. The results were plotted on a graph of concentration versus area, from which the coefficient of determination (R^2) and the straight-line equation were obtained.

Precision

Precision was assessed at two levels, repeatability (intra-run) and intermediate precision (inter-run). Repeatability was evaluated based on 6 sample determinations at a concentration of 125 µg/mL, injected on the same day by the same analyst under the same chromatographic conditions. On the other hand, intermediate precision, also known as reproducibility, was evaluated by two different analysts, on different days, carrying out 6 analyses of the standard sample at the same concentration (125 µg/mL). It was possible to establish these parameters through the coefficient of variation (CV%) defined according to Equation (1):

$$CV\% = \frac{\text{Standard deviation}}{\text{Mean concentration}} \times 100 \quad (1)$$

Accuracy

Accuracy was assessed by the recovery assay, using three concentrations (31.25, 125 and 500 µg/mL), all in triplicate. The recovery value (R%) was obtained according to Equation (2):

$$R\% = \frac{\text{Mean experimental concentration}}{\text{Theoretical concentration}} \times 100 \quad (2)$$

Robustness

Using the same concentration (125 µg/mL), two variations were carried out in triplicate at an oven temperature of 30 ± 2 °C and a mobile phase flow rate of $0.8 \text{ mL/min} \pm 0.1 \text{ mL/min}$.

Limits of Detection and Quantification

The limits of detection and quantification were calculated based on the standard deviation under the slope of the calibration curve, multiplied by 3 and 10 for detection and quantification, respectively.

2.7. Determination of Total Antioxidant Activity

2.7.1. Antioxidant Activity by the ABTS Radical Cation Assay

The antioxidant activity of the crude extract of the pracaxi co-product was evaluated via the capture of the ABTS radical cation (ABTS^{•+}) using the same UV-Vis spectrophotome-

ter mentioned above (model UV-1800, Shimadzu[®], Kyoto, Japan). For analysis, a Trolox standard curve was prepared with concentrations varying from 100 to 2000 μM . To obtain the ABTS⁺ solution, 5 mL of the ABTS stock solution (7 mM) was allowed to react with 88 μL of the potassium persulfate solution (140 Mm) at room temperature (25 ± 0.5 °C) in the absence of light for 16 h. After that, 1 mL of the mixture was diluted in ethanol until reaching an absorbance of 0.7 ± 0.05 at a wavelength of 734 nm. Then, 30 μL of the crude extract of the pracaxi co-product was added to 3.0 mL of the ABTS⁺ solution and, 6 min after vortex homogenization, readings were carried out at the same wavelength [33]. Only ethanol was used in the blank. The analysis was performed in triplicate, and the results were expressed in $\mu\text{MTrolox/g}$ of sample.

2.7.2. Antioxidant Activity by the DPPH Radical Assay

The antioxidant activity of the crude extract was also assessed by the DPPH radical capture method (DPPH[·]), as described by Brand-Williams et al. [34], with adaptations from Silva et al. [35]. For this purpose, 75 μL of crude pracaxi extract and 2925 μL of methanolic DPPH[·] Solution (25 mg/mL) were used. The reaction was performed, protected from light, at room temperature, for an incubation period of 30 min, with the absorbance measured in the same spectrophotometer mentioned above at a wavelength of 515 nm. Methanolic DPPH[·] solution only was used as the blank. A calibration curve was prepared with Trolox solutions at concentrations of 25 to 1000 μM . The assay was performed in triplicate, and the results were expressed in $\mu\text{MTrolox/g}$ of sample.

2.7.3. Antioxidant Activity by the Ferric Reducing Antioxidant Power (FRAP) Assay

Antioxidant activity measurements via the iron reduction method were performed on the same spectrophotometer mentioned above at a wavelength of 595 nm. The FRAP reagent was prepared by mixing 100 mL of 0.3 M acetate buffer, 10 mL of a 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution and 10 mL of a 20 mM aqueous ferric chloride solution. In a dark environment, an aliquot of 90 μL of the pracaxi co-product crude extract was transferred into 270 μL of distilled water and 2.7 mL of FRAP reagent, then the mixture was homogenized by vortexing and was kept in a water bath at 37 °C for 30 min. Readings were then carried out at 595 nm. FRAP reagent was used as a blank to calibrate the spectrophotometer. Antioxidant activity was calculated based on a Trolox standard curve (160–1600 μM). The final concentration was expressed in $\mu\text{M Trolox/g}$ of sample [36].

2.8. Preparation of Microparticles from the Pracaxi Co-product Extract

We prepared a 5% (*w/v*) maltodextrin solution as the wall material by adding 20 g of maltodextrin into 400 mL of distilled water and a 10% (*w/v*) solution of crude pracaxi extract by homogenizing 5 g of the extract in 50 mL of distilled water. Then, 20 mL of the extract solution was added to the maltodextrin solution and the resulting mixture was stirred for 30 min and fed to a spray dryer (model LM-MSD 1.0, Labmaq Brasil[®], São Paulo, Brazil) operated at a drying air inlet temperature of 160 °C and a feed flow rate of 10.0 mL/min. Finally, the dried material was weighed in a container protected by aluminum foil and stored in a desiccator at room temperature [28].

2.8.1. Drying Yield

The drying yield (*DY*), expressed as a percentage, was calculated according to Equation (3) [37]:

$$DY (\%) = \left[\left(\frac{W-M}{S_c} \right) \times Q_e \right] + \text{Adj} \times 100 \quad (3)$$

where *W* is the mass recovered in the spray dryer collector (g), *M* is the mass of moisture in the dry extract (g), *S_c* is the solids content of the dry extract (g/100 g of DE), *Q_e* is the total mass placed for spray drying (g) and *Adj* is the total mass of maltodextrin used for drying (g).

2.8.2. Extraction of Phenolic Compounds from Microparticles

To 0.4 g of microparticles produced in the spray dryer, 2 mL of a 50:8:42 (*v/v/v*) ethanol/acetic acid/water solution was added. The suspension was homogenized by vortexing for 1 min, placed in an ultrasonic bath (model CD-4820, Cleaner Kondentech[®], São Paulo, Brazil) for 20 min and centrifuged (model 5804 R, Eppendorf[®], São Paulo, Brazil) at 7500 rpm for 15 min. The supernatant was filtered through membranes with a 0.45 µm pore diameter [11] and used to determine the polyphenol and flavonoid contents of according to Sections 2.4 and 2.5.

2.8.3. Microencapsulation Yield

The microencapsulation yield was calculated, as a percentage, according to Equation (4) [28]:

$$Y_{\text{TP}} \text{ (or } Y_{\text{TF}}) = \left(\frac{\text{TP (or TF) in the microparticle}}{\text{TP (or TF) in the crude extract before microencapsulation}} \right) \times 100 \quad (4)$$

where TP and TF are the contents of total polyphenols or total flavonoids and Y_{TP} and Y_{TF} are their respective microencapsulation yields.

2.9. Characterization of Microparticles

2.9.1. Content of Total Polyphenol (TP)

A 100 µL aliquot of the solution containing the phenolic compounds extracted from the microparticles was transferred to a 10 mL volumetric flask along with 500 µL of Folin–Ciocalteu reagent and 6 mL of distilled water. After resting for 1 min, 2 mL of 10% (*w/v*) sodium carbonate solution was added and the mixture stirred for 30 s, completing the flask volume with distilled water. After resting for 2 h, readings were taken in the same UV-Vis spectrophotometer mentioned above at 760 nm. A calibration curve with gallic acid concentrations ranging from 6 to 20 mg/mL was used to interpolate the results of the total polyphenol content expressed in mg GAE/g [38]. The analysis was carried out on both the microparticles and the crude extract before drying.

2.9.2. Content of Total Flavonoids (TF)

In a 10 mL volumetric flask, 800 µL of the solution containing the phenolic compounds extracted from microparticles and 400 µL of 2.5% (*w/v*) aluminum chloride solution were added together; the volume was completed with distilled water. After leaving the system to rest for 30 min in the absence of light, readings were taken using the same UV-Vis spectrophotometer mentioned above at a wavelength of 425 nm. A calibration curve of quercetin at concentrations between 5 and 35 mg/mL was prepared to determine the flavonoid content expressed in mg QE/g [29]. The analysis was also performed on the extract before drying.

2.9.3. Moisture Content

The moisture content was calculated based on the weight loss of particles before and after drying. To this end, the particles were subjected to a temperature of 105 °C in an oven until constant weight [14].

2.9.4. Size, Polydispersity Index and Zeta Potential of Microparticles

Particle size, polydispersity index and zeta potential were determined via dynamic light scattering using a Zetasizer (model 2000, Malvern Instruments[®], Malvern, UK). For this purpose, before reading, the microparticles were diluted in ultrapure water in a ratio of 1:100 (*v/v*) [39].

2.9.5. Particle Morphology by Scanning Electron Microscopy

Particle morphology was examined with a scanning electron microscope (VEGA[®] 3, Tescan, Brno, Czech Republic). The microparticles were placed on stubs with carbon tape, metallized with gold/palladium to allow the electrical conductivity necessary for image formation (SC7620 sputter coater, Quorum Technologies, Kent, UK) and observed in secondary electron mode with magnitudes of 250 \times , 1000 \times and 5000 \times [14].

3. Results

3.1. Identification and Quantification of Epicatechin

Identification of the epicatechin peak in the HPLC analysis of crude extract was based on the comparison of the retention time and DAD spectrum with the epicatechin standard and confirmed by co-injection. The chromatogram of the crude extract shows an intense peak at a retention time of 21.05 min (280 nm) for epicatechin at a concentration of 69 $\mu\text{g}/\text{mL}$. Figure 1 shows the chromatographic profiles of the extract, the epicatechin standard and the extract plus epicatechin (co-elution), respectively.

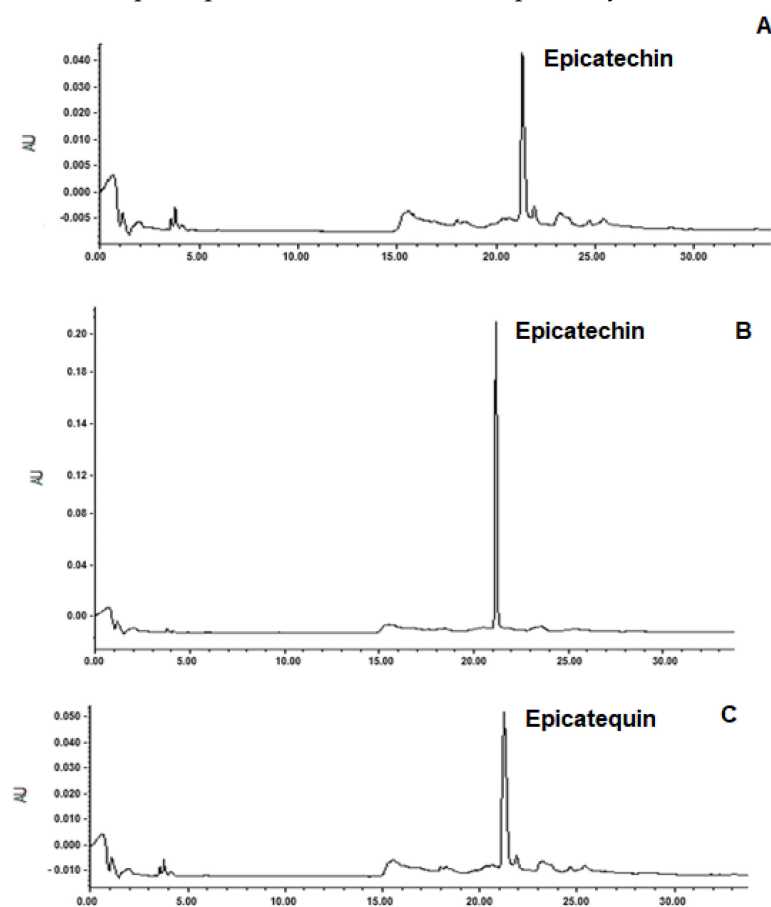


Figure 1. HPLC-DAD chromatograms of (A) pracaxi extract and (B) epicatechin standard. (C) Co-elution of the standard with the extract (280 nm). AU = arbitrary units.

3.2. Validation

The method used to quantify epicatechin was linear, as seen in Figure 2.

The value of the coefficient of variation of repeatability (intra-run) (Table 1) was lower than 5%, i.e., the limit established by ANVISA [32]. Even the coefficient of variation of reproducibility, also called intermediate precision (inter-run) (Table 1), whose measurement was made by two different analysts on distinct days, remained within the limit established by legislation and showed very little difference compared to that of repeatability. Therefore, it can be concluded that the method proposed in this work to quantify epicatechin in the pracaxi extract proved to be accurate.

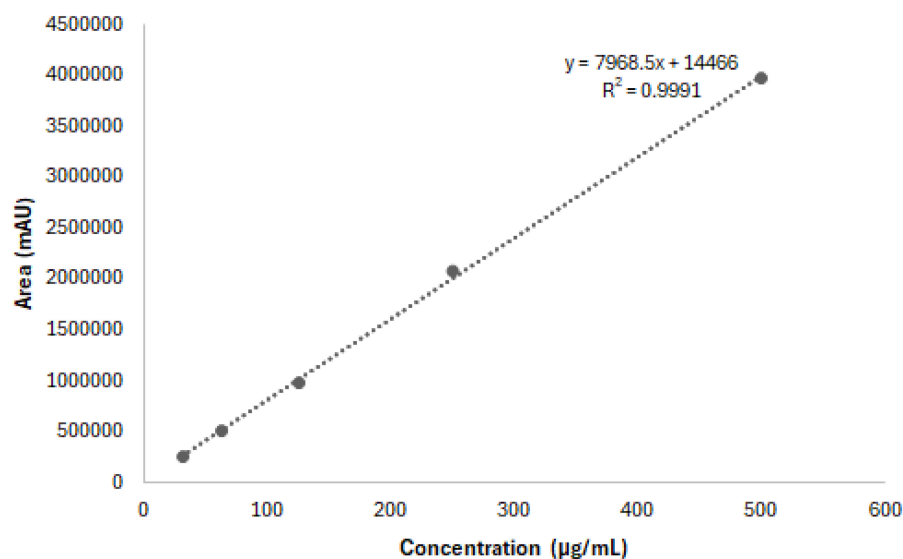


Figure 2. Epicatechin calibration curve used for its quantification in the extract for the HPLC method (31.25 a 500 µg/mL).

Table 1. Precision by repeatability (intra-run) and reproducibility (inter-run) of the epicatechin standard at a concentration of 125 µg/mL.

Precision	Average (µg/mL)	^a CV%
Repeatability	130.60 ± 3.75	2.87
Reproducibility	130.50 ± 3.60	2.76

^a CV = Coefficient of variation.

Furthermore, according to the results obtained in this work, the method proved to be accurate, as the recovery value remained in the range of 80 to 120% (Table 2), as recommended by ANVISA [32].

Table 2. Accuracy results of the chromatographic method for low, medium and high concentrations, all performed in triplicate.

Standard (µg/mL)	31.25	125	500
Average (µg/mL)	31.61	125.43	551.20
Recovery (%)	101.17	100.35	110.24

In the robustness assessment (Table 3), it was observed that even when varying the oven temperature (reduction to 28 °C or increase to 32 °C) and the mobile phase flow rate (reduction to 0.7 mL/min or increase to 0.9 mL/min) there was no significant influence on the values of peak area and retention time. Furthermore, the area values were similar to those obtained in the precision and accuracy assessments (concentration of 125 µg/mL), with the retention time (21 min) corresponding to that of the epicatechin standard obtained in the chromatographic analysis. The coefficient of variation for both parameters remained below the 5% limit established by ANVISA [32], which shows that the method was robust in relation to the changes made.

A detection limit of 3.79 µg/mL and a quantification limit of 12.64 µg/mL were obtained by mathematical extrapolation from the calibration curve in accordance with the analytical methods validation guide [32].

Table 3. Robustness results for parameters subjected to variations, i.e., the oven temperature and the mobile phase flow rate.

Parameters	Variation	Area (mAU)	Retention Time (min)	^a CV %
Oven temperature–Mobile phase flow rate	28 °C– 0.7 mL/min	1,008,325	21.68	1.15
		1,030,253	21.68	
		1,013,049	21.64	
	32 °C– 0.9 mL/min	1,053,434	21.23	1.21
		1,061,086	21.24	
		1,078,367	21.33	

^a CV = Coefficient of variation.

3.3. Antioxidant Activity

The antioxidant activities of the pracaxi co-product extract and the standard used (ascorbic acid) by the ABTS, DPPH and FRAP methods are listed in Table 4.

Table 4. Antioxidant activity of the pracaxi co-product crude extract by the ABTS, DPPH and FRAP methods.

Sample	ABTS (μMTrolox/g)	DPPH (μMTrolox/g)	FRAP (μMTrolox/g)
Standard (ascorbic acid)	2380.04 ± 0.0000	1132.68 ± 0.005	2408.57 ± 0.058
Co-product extract	910.82 ± 7.33	906.68 ± 1.20	1549.89 ± 0.020

3.4. Characterization of Microparticles

3.4.1. Contents of Total Polyphenols, Total Flavonoids and Microencapsulation Yield

As there are still no data in the literature on the presence of phenolic compounds in the co-product of pracaxi seeds; however, the determination of is of great significance due to the fact that these compounds are considered to be responsible for the majority of biological activities found in plant species. So, the contents of total polyphenols and flavonoids were assessed in both the crude extract and microparticles (Table 5), and the microencapsulation yield was also determined for the microparticles.

Table 5. Total polyphenol and flavonoid contents in the crude extract and microparticles and respective microencapsulation yields.

	Phenolic Compounds		Microencapsulation Yield	
	Total Polyphenols (mg GAE/g)	Total Flavonoids (mg QE/g)	Total Polyphenols (%)	Total Flavonoids (%)
Crude extract	20.61 ± 0.20	28.29 ± 0.70	-	-
Microparticles	18.48 ± 0.10	13.73 ± 0.10	89.66 ± 0.20	48.53 ± 0.30

3.4.2. Drying Yield and Moisture Content

The drying yield was 20.28 ± 1.20%, and the microparticles were found to have a low moisture content (3.46 ± 0.06%).

3.4.3. Size, Polydispersity Index and Zeta Potential of Microparticles

The microparticles were smaller than 1 μm in size, with a polydispersity index (PDI) value revealing polydisperse and heterogeneous characteristics, and had a relatively large negative zeta potential (Table 6).

Table 6. Physical characteristics of microparticles prepared by spray drying.

Particle Size (μm)	Polydispersity Index (PDI)	Zeta Potential (mV)
0.38 ± 0.11	0.43 ± 0.12	-42.80 ± 0.10

Results expressed as means and standard deviations ($n = 3$).

3.4.4. Scanning Electron Microscopy Examination

The photomicrographs of microparticles illustrated in Figure 3 show their spherical shape and smooth surface, in addition to confirming their heterogeneous size. Moreover, there was little agglomeration among the microparticles and no evidence of the presence of ruptures, which guarantees the protection of the active ingredients inside them.

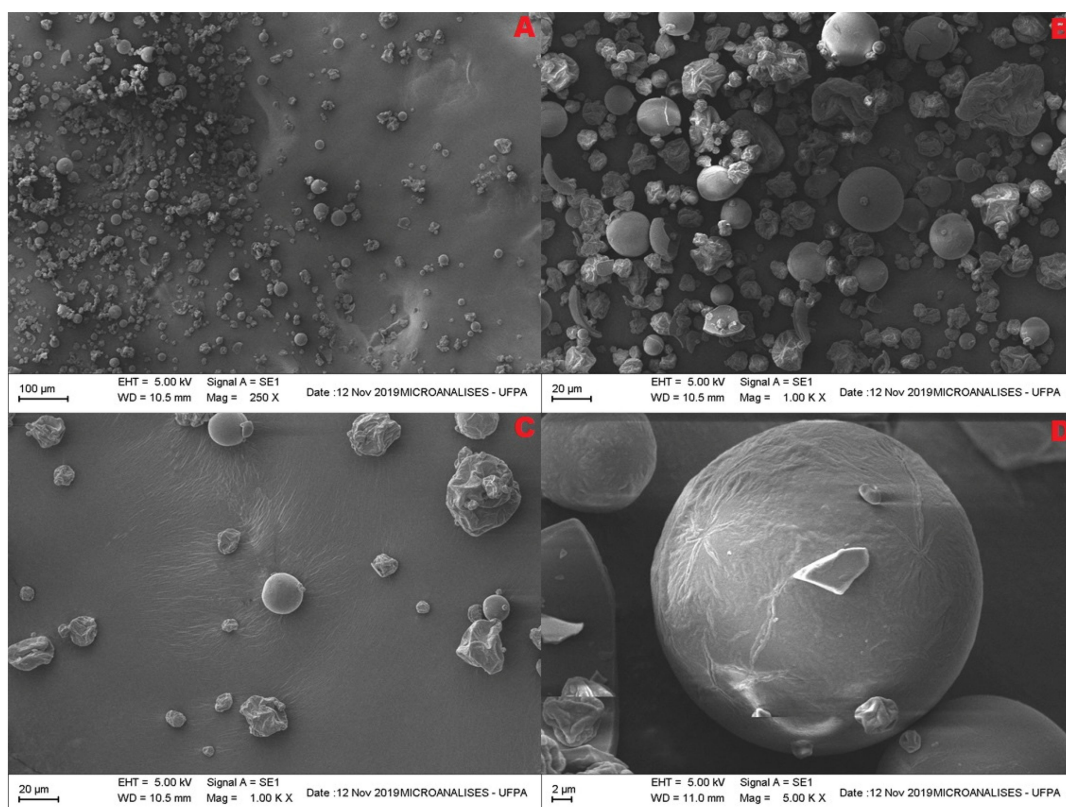


Figure 3. Photomicrographs of pracaxi co-product microparticles obtained by spray drying. Magnitude: (A) (250 \times), (B,C) (100 \times), (D) (5000 \times).

4. Discussion

The physicochemical characterization of agroindustrial co-products is an important step in the identification of their chemical constituents, which can lead to their better exploitation through valorization [40]. The extract chromatogram displays several other peaks that may be related to the presence of phenolic acids and flavonoids. However, since it is a complex mixture, a good separation of these peaks is necessary to obtain spectra that are more characteristic [28].

The presence of epicatechin in the pracaxi co-product extract is promising because of the antioxidant activity displayed by this flavonoid, which is considered capable of reducing free radicals and consequently cell damage [11]. Gabbay Alves et al. [37] identified epicatechin as one of the main phenolic compounds present in cocoa co-product extract. Da Costa et al. [41] also reported the presence of epicatechin in the crude extract of the cupuaçu seed co-product. Epicatechin acts as a vasodilator, being able to dilate blood vessels throughout the body, increase blood flow toward the extremities and brain and reduce blood

pressure [42]. Furthermore, evidence demonstrates its ability to modulate immunological and inflammatory functions as well as its use in malaria prophylaxis [43–45].

The method for identifying epicatechin by HPLC-DAD in the pracaxi co-product was validated to ensure that it provides reliable information, which is a fundamental step in determining any metabolite of interest using new analytical methods [46]. In validating the analytical method, the peak areas were directly proportional to the concentration of the epicatechin standard, therefore being considered linear. In fact, coefficient of determination values very close to 1.000, as seen in this work ($R^2 = 0.9991$), indicate a minimum dispersion for the set of experimental points, corroborating the RE 166/17 resolution of the Brazilian National Surveillance Agency (ANVISA) [32], for which R^2 , to be acceptable, must be at least 0.99.

The high levels of epicatechin, detected by the HPLC-DAD method, and of total polyphenols and flavonoids in the pracaxi co-product extract can justify its good antioxidant activity determined by both ABTS and DPPH methods. It is worth mentioning that at least two methods are needed to evaluate antioxidant activity in order to obtain acceptable reliability for the results [47,48].

Antioxidant activity is linked to the number of hydroxyls present mainly in the structure of the extracted flavonoids, which means that, depending on the type of flavonoid present in the extract, the antioxidant capacity may be greater or lesser in different samples [49]. Compounds with antioxidant activity are mainly concentrated in plant peels and seeds. As the pracaxi co-product was obtained from pressing the seeds, this justifies its good antioxidant capacity [50], which opens up the possibility of its use in the development of new products.

The pracaxi co-product extract showed good antioxidant activity via the use of the ABTS, DPPH and FRAP methods. The values found were close to that for ascorbic acid, which is used as a standard, thus suggesting that the extract, mainly because it is an agroindustrial co-product, could be used as a promising natural antioxidant.

From this perspective, spray drying stands out among the alternatives to microencapsulate bioactive compounds due to its ability to effectively remove water from the product, thus avoiding microbiological attack as well as degradation and oxidation reactions. After spray drying, parameters such as the microencapsulation yield must be evaluated to check the efficiency of the process [11].

The high microencapsulation yield of the total polyphenols found in the microparticles containing the pracaxi co-product indicates that, in general, these phenolic compounds were little degraded in relation to the crude extract. However, the microencapsulation yield of total flavonoids was less than 50%, which suggests that these compounds were particularly sensitive to the high temperature of the spray drying process, despite the short exposure in the drying chamber [23].

The high temperatures in the drying chamber of the spray dryer during microencapsulation may have led to the degradation of a portion of the epicatechin present in the pracaxi co-product extract, thus significantly reducing the total flavonoid content in the microparticles compared to the crude extract. Furthermore, the use of only maltodextrin as a coating agent may have limited the protection of epicatechin, which could be solved by combining it with other wall materials to improve process efficiency [51]. Although the value for the total flavonoid microencapsulation yield suggested that the drying process was responsible for the degradation of about one half of these compounds, it still preserved a significant fraction of the other bioactive polyphenols in the dried sample.

The lower the moisture content of the microparticles, the greater their physical, chemical and microbiological stability [52,53]. The result of this work was an indicator of the quality of the powder, which showed low moisture content, probably due to the high temperature used for spray drying (160 °C).

High spray drying inlet temperatures are known to favor water evaporation due to heat transfer from hot air to water droplets [14,54]. The low moisture content found in the microparticles of the pracaxi co-product is an important finding, as it can avoid the

chemical degradation of polyphenols and flavonoids that often occurs when their water content is high [55].

The drying yield is another important parameter in the evaluation of powder quality. Several factors may have contributed to the low value for this parameter observed in this study ($20.28 \pm 1.20\%$), such as the loss of powder due to microparticle adhesion onto the wall of the drying chamber, the transport of a portion of fine particles directly to the drying filter, thus bypassing the collector [56], and the presence of sugars and lipids in the extract [57]. Ferreira et al. [28], who microencapsulated tucumã co-product extract by spray drying, observed that the higher the concentration of maltodextrin, the higher the drying yield, probably due to the increase in the content of solids. The relatively low maltodextrin concentration used in this study (5%) may also have been responsible for the low drying yield.

Particle size is one of the most important parameters to be considered in microparticle evaluations, as it influences some important characteristics such as their optical properties and viscosity, among others. It is related to the concentration of the wall material, i.e., the lower the concentration of the wall material, the smaller the particle size [58]; consequently, the larger the surface area, the more improved the bioavailability and solubility of the encapsulated bioactive compounds [57]. From this point of view, therefore, the low concentration of maltodextrin used in this study, despite having impaired the drying yield, may have favored the formation of small microparticles ($0.38 \pm 0.11 \mu\text{m}$). Da Costa et al. [41] found an even smaller size ($0.27 \mu\text{m}$) for microparticles containing cupuaçu co-product using 5% maltodextrin as an encapsulating agent.

It is known that the lower the value of the polydispersity index (PDI), the greater the homogeneity of microparticles and vice versa [59]. PDI values < 0.2 indicate low polydispersity or monodispersity, while values above 0.4, such as the one observed in this study (0.43 ± 0.12), indicate polydispersity and heterogeneity [60].

As for the zeta potential, its relatively large and negative value ($-42.80 \pm 0.10 \text{ mV}$) indicates microparticles that are stable in colloidal dispersions, in which repulsive interactions tend to minimize their agglomeration [41,60]. The low tendency for the microparticles to agglomerate was confirmed in scanning electron microscopy photomicrographs.

Morphological examination of the microparticles revealed no cracked walls, which suggests nucleus protection, as air permeability was likely to be minimal [37,53]. The use of high drying air temperatures in spray drying, like the one used in this study, makes the particles more morphologically defined [53]. Da Costa et al. [41] reported photomicrographs where microparticles containing cupuaçu co-product had a spherical and regular structure without agglomeration. On the other hand, Gabbay Alves et al. [37] observed microparticles containing cocoa co-product that had a rounded shape, without roughness and without any rupture, like those observed in this study.

5. Conclusions

Chemical characterization by UV-Vis spectrophotometry revealed high levels of total polyphenols and total flavonoids in the pracaxi co-product extract, and high-performance liquid chromatography revealed the presence of flavonoids. Epicatechin was the main flavonoid identified, which suggests that it may be largely responsible for the good antioxidant activity of the extract. In relation to the microparticles obtained by spray drying, the process led to degradation of a portion of total polyphenols and flavonoids, which impaired the microencapsulation yield. The greatest losses were observed for total flavonoids. The microparticles showed a low moisture content and small size, in addition to good morphological characteristics, as a result of the conditions used in the drying process. The results obtained open up the possibility of their use in different biotechnological processes, as a potential natural source of antioxidants in foods and pharmaceutical and cosmetic products or as a raw material to obtain new functional ingredients for the food industry. This would lead to better exploitation of the pracaxi co-product compared to its current disposal as a

waste, which would also avoid environmental problems, in addition to leading to better environmental sustainability of natural products from the Amazon rainforest.

Author Contributions: Conceptualization, R.M.R.-C., A.C. and J.O.C.S.-J.; Methodology, R.L.d.S. and L.M.d.M.C.F.; Investigation, R.L.d.S. and L.M.d.M.C.F.; Writing—original draft, R.L.d.S. and R.M.R.-C.; Writing—review and editing, R.M.R.-C., A.C. and J.O.C.S.-J.; Supervision, R.M.R.-C. and J.O.C.S.-J.; Project administration, R.M.R.-C.; Funding acquisition, R.M.R.-C., A.C. and J.O.C.S.-J. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Coordination for the Improvement of Higher Education Personnel (CAPES, R.L.d.S. postgraduate scholarship), finance code 001. National Council for Scientific and Technological Development (CNPq), process: 401816/2022-2. Financing Agency of Studies and Projects (FINEP), process: 0058/124, for the implementation of Nanofarma/UFFPA.

Data Availability Statement: All the data are presented in this work.

Acknowledgments: The authors thank the Dean of Research and Graduate Studies (PROPESP) of Federal University of Pará (UFFPA) for providing the infrastructure for developing this research.

Conflicts of Interest: The authors declare no conflicts of interest.

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