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Chapter

# Agro-industrial By-Products from Amazonian Fruits: Use for Obtaining Bioproducts

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## Abstract

Fruit processing contributes significantly to the agricultural exportation of the Amazonian; however, it generates large amounts of solid waste, despite its high content of bioactive compounds and nutritional properties, and they are discarded in the environment. Therefore, in order to add economic value and potential reuse of agro-industrial by-products from cocoa, cupuassu, pracaxi, and tucumã, we investigated the chemical characteristics of the seed by-product resulting from the industrial extraction of these oils. The investigation of the nutritional and chemical composition of by-product was submitted to green extraction, besides other qualitative and quantitative techniques for the characterization of the main bioactive compounds. The extracts obtained from these by-products had a significant total polyphenol content and antioxidant activity. HPLC analysis identified and quantified some flavonoids present in these by-products (gallic, caffeic and protocatechuic acid, epigallocatechin-3-gallate, epicatechin, catechin, and quercetin). The oil from these species is widely used in the treatment of skin scarring and inflammation and is also used by the cosmetic industry. These results show that these by-products have a great potential for use, since they still have bioactive substances in their composition, which could alternatively be used in the pharmaceutical, cosmetic, or food industries.

**Keywords:** by-product, antioxidant activity, flavonoids, reuse, value-added applications, Amazonian fruit

## 1. Introduction

Brazil is currently one of the world's three largest fruit producers, associated with China and India. Brazil makes up 45.9% of world production a year, 2014 accounted for approximately 830.4 million tons of fruit [1]; many of them come from the Amazon region, where there is a diversity in economic fruit species, with huge agro-industrial and nutritional potential in the development of new products [2].

In addition, the Amazon region is home to a large biodiversity of plant species that produce fruit and oilseeds and stand out for their environmental conditions (climate and soil) [3]. Because it has a huge territorial extension, diverse fauna and flora, it is a source of life and income for approximately 200,000 families that collect native fruits, whose commercialization is responsible for 10% of the total income from extractivism [4]. In this sense, countless native species of fruit plants from the Amazon present economic, technological, and nutritional potential.

Given the large production of fruits, mainly for the juice industry, the processing of these fruits generates a huge amount of by-products resulting from seeds, peel, and part of the fruit pulp, resulting in an amount of about 30–40% of the production of these fruits [5]. Given the increase in fruit production, there is an increase in the generation of the so-called agro-industrial by-products, which causes an economic impact, as there is no proper reuse.

Indeed, some studies report nutrient concentrations in fruit by-products even higher than in pulp [6, 7]. The so-called tropical fruit processing by-products have been increasingly used as food additives and sources of bioactive compounds such as polyphenols [8–10]. In addition, the appropriate reuse of these by-products can reduce the environmental impact associated with their disposal, adding value to the entire production chain. Thus, the physicochemical characterization of these by-products and the quantification of their bioactive compounds are of great concern to add value and improve their commercial and industrial reuse, preserving the biome [11, 12].

In the literature, there are several studies related to agro-industrial byproducts from fruits of the amazon region with the objective of finding a sustainable destination, among which are worth mentioning those related to the cocoa (*Theobroma cacao*) [13], cupuassu (*Theobroma grandiflorum*) [10], pracaxi (*Pentaclethra macroloba*) [14], and tucumã (*Astrocaryum vulgare* Mart) [15] by-products.

These species are native to tropical forests, originating from the Brazilian Amazon. Cocoa (*Theobroma cacao* L.) belongs to the Malvaceae family, has two varieties, Criollo and Forastero, and is 15–25 cm long and 8–13 cm in diameter, and the pulp is characterized by a thick mass of about from 20 to 40 seeds [16]. In 2015–2016, cocoa production was 3.9 million tons, of which 16.96% came from America, 73.11% from Africa, and 9.93% from Asia and Oceania. In contrast, in the same period, 16 million tons of by-products were generated, with Africa being the largest producer (73.12%), followed by America (16.88%), and Asia and Oceania (10.00%) [16].

Cupuassu [*Theobroma grandiflorum* (Willd. Ex. Spreng.) K. Schum.] belongs to the genus *Theobroma*, the latter being composed of 22 species of tropical plants from the Americas, including cocoa (*Theobroma cacao* L.). Among the Amazonian fruits, it is the one that brings together the best conditions of industrial use, and its pulp has great possibilities of use in the food and cosmetics industry. Due to the various applications in cooking, cupuassu has been arousing economic interest because its pulp is widely used in home and industrial production of various specialties. From the seeds, cupulate<sup>®</sup> is produced. And also in the cosmetics industry, its fat is considered an important emollient [10].

*Pentaclethra macroloba* (Willd.) Kuntze is popularly known as pracaxi, paracaxi, or paroá-caxi, belonging to the Fabaceae family. The pracaxi is an oilseed plant from the Amazon region found in Guyana and some parts of Central America [17, 18]. The pracaxi fruit is in the form of a pod of 20–25 cm, curved and dark brown in color, when ripe and contains 4–8 seeds [19]. From the seed is extracted an oil, which is used in the treatment of ulcers and wounds, besides being healing and presenting insecticidal properties against the *Aedes aegypti* mosquito. In the cosmetics industry, it is used in the production of hair products [20, 21].

Fruit abbreviation	Amazonian fruit's species	Parts of plants used	Application	Main phenolic compounds
CA	Cocoa.	Pulp and seed	Food and cosmetics industry	Catechin, epicatechin and gallic acid [13]
СР	Cupuassu	Pulp and seed	Food and cosmetics industry	Epicatechin and glycosylated quercetin [10]
PX	Pracaxi	Tree bark and seed	Ulcers and wounds treatment, inseticidal and cosmetics industry	Catechin [14]
ТМ	Tucumã	Pulp and seed	Food and cosmetics industry	Gallic acid [15]

### Table 1.

Some by-products of Amazonian fruits: application and predominant biocompounds.

Tucumã (*Astrocaryum vulgare* Mart.) is an oleaginous fruit whose mesocarp is fibrous and nutritious, yellow-orange in color, rich in lipids and compounds such as pro-vitamin A [4]. The by-product of tucumã is also an excellent source of carotenoids [4]. The food industry uses its pulp to produce creams and ice creams. After obtaining the pulp, the tucumã seed is discarded (tons each year) [22]. The cosmetic industry uses tucumã pulp for oil extraction, which is used in skin moisturizing cosmetics, body lotions, and hair care products.

Since the waste of Amazonian fruit by-products, and consequently their antioxidant potential, due to the presence of bioactive compounds (e.g., polyphenols) (**Table 1**), it is possible to highlight some alternatives for better use of these byproducts. In this sense, its use as enriched ingredients in food formulations with nutritional and functional properties [10] stands out, for supplementation/complementation in cookies, bread, cereal bars, cakes, and pastes.

Given the high nutritional and economic (underutilized) value of by-products, many studies have been conducted with a common goal, their reuse [10, 13, 15, 23–28]. In this perspective, the valorization of agri-food by-products is presented not only as a necessity, but as an opportunity to obtain new products with added value and a great impact on the economy of industries. Thus, several authors have demonstrated that the vast diversity of fruits found in the Brazilian territory, especially the Amazon, presents nutritional richness and can be better utilized directly by the population and also by the food or cosmetic industries [10, 25, 28, 29]. To this end, further studies are needed to better understand the nutritional, functional, and economic potential of fruit by-products, especially those found in the Brazilian Amazon.

### 2. Nutritional composition

Brazilian fruit by-products, particularly those from the Amazon region, need further investigation in order to obtain more information on their nutritional composition. Relevant data on the chemical composition of those traditionally inedible parts such as peel and seeds are even rarer. In addition, large amounts of by-products from these fruits are not consumed regularly; among them are seeds that are generally wasted in the environment [8]. Therefore, this chapter aimed to gather information on the nutritional potential of cocoa (CA), cupuassu (CP), pracaxi (PX), and tucumã (TM) seed by-products, with the objective

### Bioactive Compounds in Nutraceutical and Functional Food for Good Human Health

Parameter (g/100 g)	$\mathbf{CA}^{*}$	$\mathbf{CP}^{*}$	$\mathbf{PX}^{\star}$	$\mathbf{TM}^{*}$
Lipids	33.5 ± 0.5	24.4 ± 0.8	14.9 ± 0.1	15.5 ± 0.4
Proteins	17.3 ± 0.4	14.2 ± 0.3	21.5 ± 0.6	11.1 ± 0.2
Total fibers	15.0 ± 0.4	22.3 ± 0.3	20.9 ± 0.7	41.4 ± 0.7
Carbohydrates VET <sup>a</sup>	42.9 ± 0.4 539.8 ± 2.3	26.6 ± 0.5 382.0 ± 2.0	32.9 ± 0.0 352.5 ± 3.3	63.1 ± 0.8 436.4 ± 1.8

VET means energetic value.\*Results expressed as mean of triplicates  $\pm$  standard deviation, expressed on a dry basis. <sup>a</sup>Values expressed in (kcal/100 g).

### Table 2.

Nutritional composition of Amazonian fruit by-products: Cocoa (CA) [13], cupuassu (CP) [10], pracaxi (PX) [14], and tucumã (TM) [15].

(depending on the results) of encouraging its consumption by the population, taking advantage of its use as ingredients in animal feed and even human food formulations.

The results of the nutritional composition of the CA, CP, PX, and TM by-products are presented in **Table 2**.

Amazonian fruit by-products: CA, CP, PX, and TM showed important, up-todate, and reliable nutritional values on the macronutrient composition of these by-products. As expected, because it is organic matter, carbohydrates were the most abundant macronutrients in the by-product studied. The CA, CP, PX, and TM also presented values of lipids, total fibers, and protein, and considerable energy value (**Table 2**). Thus, the contents of these macronutrients were higher than those reported for cupuassu pulp [5]. Protein content was 42% higher than fermented or roasted cupuassu seeds and 56% higher than cocoa seeds [7]. Compared to the studied by-products, pracaxi presented almost twice the protein content. And the by-product of the tucumã seed had the highest total fiber content, which may be related to its seed size (up to 22.9 mm in diameter) [30].

Compared to the cupuassu seeds, the cocoa seeds presented 17.74% higher protein content, 37.16% carbohydrate content, and 27.25% lipid content, while total fiber content was lower 33.41% (**Table 2**) [10, 13].

Given the above, the CA, CP, PX, and TM seed by-products presented significant nutritional values of macronutrients (carbohydrates, proteins, lipids, and crude fibers) (**Table 2**), suggesting the possibility of their reuse by the food industry, as a possible food supplement, as it is a great alternative for food product enrichment, by increasing its nutritional value with a low-cost raw material and its importance as a source of human and animal food.

### 3. By-product processing

The industrial processing to obtain the industrial by-products (**Figure 1**) is similar; usually this process is performed from the fruit, where the first step is the separation of the pulp (**Figure 1A**), and the seeds are then subjected to a cooking process at 65°C for 45 min (**Figure 1B,C**) and then pressed (**Figure 1D**) to remove crude oil or butter (raw material for the cosmetic industry). Therefore, the resulting by-product (residual cake) is usually discarded by the industry, but it can be used as a raw material and proceeds to the standardization stage, being added in an oven with air circulation (40 ± 2°C) until obtaining of constant weight (**Figure 1E**). After dehydration, it was pulverized and from this extraction is performed (**Figure 1F**), obtaining the biocompounds (**Figure 1G**) [31–33].

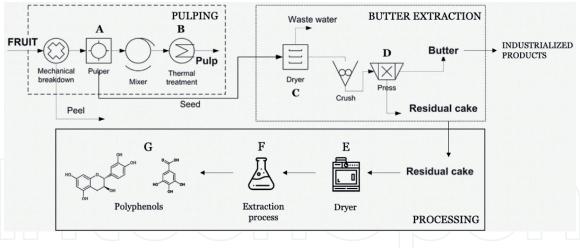


Figure 1.

Residue processing and obtaining biocompounds (adapted from González et al. [31]).

### 4. Extractive process

### 4.1 Green extraction

Green extraction is based on efficient and conscientious use of plant raw materials to ensure an extraction process with better yield conditions. In this sense, this model aims to optimize the extractive process and reduce extraction time, number of operations, energy consumption, and amount of waste generated and processing costs [34].

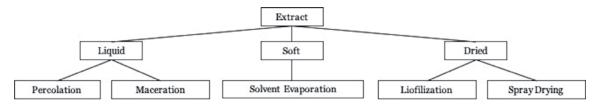
Good manufacturing practices are in this context to improve the elective parameters during the extraction process, in order to optimize the steps during the plant cultivation process, ensuring the lowest water consumption and the reduction of pesticide and fertilizer use. In addition, it is possible to develop genetic improvement protocols to obtain extracts with the highest concentration of biocompounds of industrial interest. Techniques that allow efficient production and a reduction in the generation of environmental waste should be pointed out. The use of natural, less toxic, easy to degrade solvents or with a lower risk of environmental contamination is one of the most recent bets on the production of new products [34].

### 4.2 Obtaining the extract

Extracts are preparations obtained from medicinal plant derivatives (powder), which may be in liquid (fluid extract), semi-solid (soft extract), or solid (dry extract) form. They can be obtained by various methods, as shown in **Figure 2** (Adapted from Silva Junior et al. [35]).

Maceration is an extractive process in which the proportion of vegetable drug powder and solvent volume influences the efficiency of the method; generally, the ratio 1:5 or 1:10 (plant drug/extract) is used. The plant material will be in contact with the solvent at rest in a closed container; at certain time intervals, the mixture should be agitated, and the final process time is variable [35, 36].

Percolation is an extractive methodology in which it is obtained by exhaustion. Prior to this process, maceration of the plant drug should be performed. To perform the technique, in a percolator, the vegetable drug and the solvent must be added. The volume should be 1:10 (plant drug/extract). The percolator faucet should be opened, and the liquid flow rate varies according to the velocity and can be classified as slow (1.0 mL/min), moderate (1.0-3.0 mL/min), and fast (3.0-5.0 mL/min) [36].



### Figure 2.

Types of extracts and their methods of obtaining.

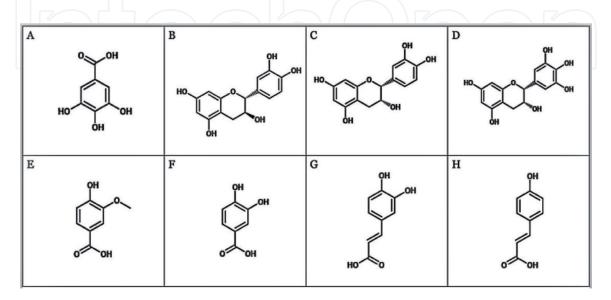
To obtain the extracts of the by-products of CA [32], CP [33], PX [14], and TM [15], the percolation methodology was performed.

The standardization of plant material for extracts and their fractions requires applying techniques that characterize it to ensure correct use according to quality parameters, among which stand out the identification and quantification of the main classes of secondary metabolites and chemical markers, as well as investigation of pharmacological activities of industrial interest [35].

### 5. Chemical composition

Polyphenols (**Figure 3**) are the secondary metabolites found mostly in both fruit and by-products. Many factors may alter the contents of polyphenols, as well as other biocompounds in a plant species, such as the area under cultivation, the maturation time, climatic conditions during the cultivation stage, the season of the harvest year, and the storage of the crop raw material. In addition, the seeds of CA, CP, PX, and TM during their processing go through a heating step, and the temperature used can contribute negatively in reducing the levels of the assets [37]. They are derived from phenylpropanoids, where their main representatives are phenols, lignins, and flavonoids [6].

Flavonoids are low molecular weight compounds and are derived from benzog-pyrone, are responsible for the pigmentation of flowers, and protect against damage caused by light, fungi, or parasites. They have a chemical structure consisting of 15 carbon atoms, characterized by the presence of a diphenylpropan ( $C_6-C_3-C_6$ ) attached to two benzene rings (A and B), and depending on the oxidation of the pyran ring, the ring A is derived from the acetate/malonate route, and ring B is derived from phenylalanine, and its name is variable according to ring C [6].



### Figure 3.

Polyphenol structural formula: A) gallic acid; B) (+)-catechin; C) (–)-epicatechin; D) (–)-epigallocatechin; E) vanillic acid; F) protocatechuic acid; G) caffeic acid; H) p-coumaric acid (adapted from Alves [32]).

## 5.1 Qualitative analysis

## 5.1.1 Determination of biocompound contents by ultraviolet spectrophotometry (UV: Vis)

Pre-formulation studies are of fundamental importance in relation to quality control tests, which are based on analyzes that allow the characterization of the raw material for the evaluation of its potential to be used as a finished product asset [27]. The characterization of an extract implies the definition of the contents of its main chemical constituents, as well as moisture, color, particle size distribution, viscosity, and technological properties, ensuring the safety, efficacy and quality of a product. When formulating products containing natural actives, the standard identification of markers or the development of methods that allow the quantification of purified chemical groups before, during, and after the obtaining process is essential [38].

There are many qualitative analytical methods used to identify total polyphenol levels (**Table 3**) such as UV–vis spectrophotometry, where one of the main colorimetric methods used is the Folin–Ciocalteu reagent. For the analysis, in a 25 mL volumetric flask, 4.8 ml of deionized water, 0.2 ml of sample, and 0.5 ml of Folin–Ciocalteu reagent are added. To this mixture is added 1.0 mL of 20% sodium carbonate solution, and then deionized water should be added to complete the volume of 10 mL and homogenize. The reaction system should be kept for 1 hour at room temperature and protected from light. After time, aliquots were collected and analyzed by UV–vis spectrophotometer (Perkin Elmer, Wellesley, MA, USA) at a wavelength of 725 nm. TP was standardized against gallic acid and expressed as micrograms of gallic acid equivalents per gram of dried extract (mgGAE/g) [39].

The total flavonoid contents (**Table 3**) can be determined by spectrophotometric analysis using aluminum chloride and for the extracts as described by [39, 40] for the analysis of CA, CP, PX, and TM, the UV–vis spectrophotometer was used (Perkin Elmer, Wellesley, MA, USA) to a wavelength of 510 nm.

### 5.1.2 Fourier transform infrared spectroscopic profile identification (FT-IR)

Infrared absorption spectroscopy is used in the literature to identify possible characteristic functional groups in organic compounds, providing important information on the chemical structure of the sample [38].

Samples	TP(mg/g)	TF (mg/g)	Source
CA	229.6 ± 3.24 <sup>a</sup>	$0.68 \pm 0.02^{b}$	[13]
СР	$50.1 \pm 5.30^{a}$	$5.92 \pm 3.40^{\circ}$	[10]
PX	$2.66 \pm 0.01^{a}$	$0.11 \pm 0.25^{d}$	[14]
TM	$1.35 \pm 0.08^{a}$	$0.33 \pm 0.01^{\rm b}$	[15]

Results are expressed as mean triplicate ± standard deviation.

<sup>*a</sup>GAE = gallic acid equivalent.*</sup>

<sup>b</sup>EQE = quercetin equivalent.

<sup>c</sup>ECA = catechin equivalent.

<sup>d</sup>ERT = rutin equivalent.

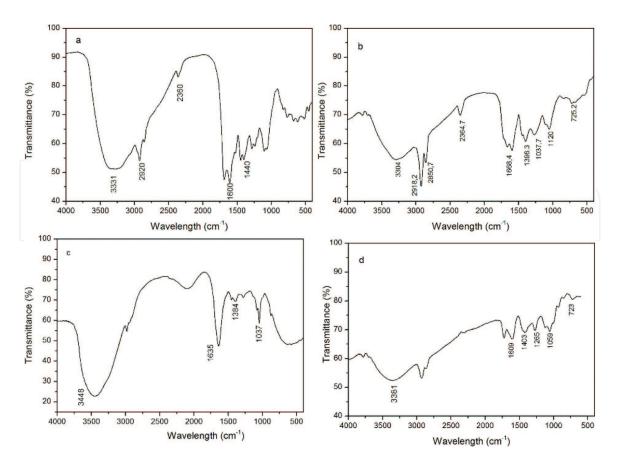
### Table 3.

Total polyphenol (TP) and total flavonoid (TF) content of crude extracts of CA, CP, PX, and TM by-products.

The identification of the functional bands using the Fourier transform infrared absorption spectroscopy (Figure 4) was performed in the CA by-product extract, where it was possible to observe bands at 1600, 2920, and 3331 cm<sup>-1</sup>, which correspond to C-C stretching of aromatic ring by phenol group, C-H stretching of aromatic ring by phenol group, and O-H stretching of phenol group, respectively [27, 41]. In the CP by-product extract, the bands  $1037 \text{ cm}^{-1}$  corresponding to acid vibrations, 1120 cm<sup>-1</sup> alcohol vibrations, 1668 cm<sup>-1</sup> esters and sulfonic vibrations, and 2931 cm<sup>-1</sup> O-H axial deformation of alcohol groups were displayed [33]. In PX, bands were shown at 1037, 1384, 1635, and 3448 cm<sup>-1</sup> for C-O axial deformation of alcohol and phenols, C-H axial deformation vibration of methyl, C=C axial deformation of carbonyl ring, and -OH deformation vibration of carbohydrates and carboxylic acids, respectively [38]. And the TM by-product extract exhibited bands at 1403 cm<sup>-1</sup> aromatic ring stretching vibration, 1609 cm<sup>-1</sup> ketone C=O stretching vibration, and 3381  $\text{cm}^{-1}$  free –OH stretching vibration [42]. All bands observed in CA, CP, PX, and TM were correlated to chemical structures present in polyphenols [27, 33, 38, 42].

### 5.1.3 Thermal analysis

Thermogravimetric analysis (TGA) is a technique that assesses the loss in mass of a substance as a function of temperature, it allows a variability of results to be observed, such as the temperature range at which the sample is degraded, until the temperature of the sample remains stable, at which temperature a change in physical state such as melting, among others, occurs. In addition, it is possible to plot a derivative on top of the TGA curve; this analysis can show which temperature range the greatest loss of



### Figure 4.

Identification of the functional bands using FT-IR absorption spectroscopy. The extracts of the by-products were compressed into Kbr and scanned in the 4000-400 cm<sup>-1</sup> wavelength absorption range, with a resolution of 2.0 cm<sup>-1</sup> and a scan number of 20 scans. a) CA [27], b) CP [33], c) PX [14], and d) TM [15].

mass occurs in [27]. This technique has been used for the evaluation of by-products and their extracts, such as CA [27], CP [10], PX [14], and TM [15].

The analysis of behavior and thermal stability can be employed in the quality control of raw materials and evaluation in the development of herbal medicines [43]. The thermogravimetric analysis (**Figure 5**) of the CA, CP, PX, and TM extracts presented on average three events of mass loss. The first corresponds to the evaporation of solvents, such as water. The second event represents successive reactions and may be related to loss of sugars. And the latter confers with the degradation and carbonization of organic matter from biocompounds [14, 15, 27, 28].

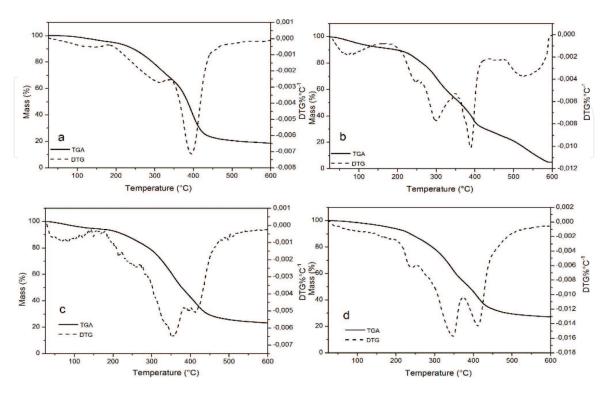
Such analysis is important to evaluate the thermal stability of the extracts, bearing in mind that prior knowledge has the purpose of guaranteeing the physical-chemical stability of the thermal constituents present in the extracts [38, 44]. The thermogravimetric study makes it possible to obtain information on the relationship between humidity and the maximum temperature of stability of the extract [44].

### 5.2 Quantitative analysis

### 5.2.1 High-performance liquid chromatography (HPLC)

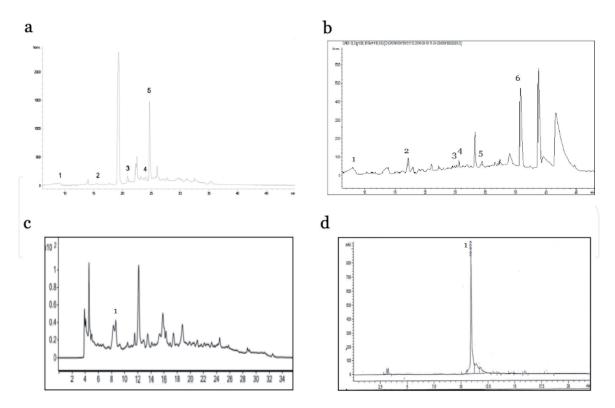
High-performance liquid chromatography is a separation technique that is among the main techniques used to determine polyphenol and flavonoid levels. Natsume et al. [45] point out that it is possible to carry out the identification and quantification of those elements in plant extracts and their derivatives; the analysis applied may be: reverse-phase HPLC (RP-LC) and reverse-phase HPLC-mass spectrometry (RP-LC/MS), and in particular, when it comes to the genus *Theobroma*, the majority of flavonoids observed in the species were catechin and epicatechin (**Figure 6**).

The literature points to the activity of certain flavonoids (**Table 4**), such as those identified and quantified in CA and CP a possible correlation with interesting



#### Figure 5.

TGA and DTG curves of the by-product extracts obtained at 25 to 600° C at 10° C/ min under N2 atmosphere and flow of 50 mL/min: a) CA [28], b) CP [10], c) PX [14], and d) TM [15].



### Figure 6.

Chromatograms about the phenolic compounds (280 nm) identified in the extracts of the by-products of a) CA [28], where 1—gallic acid, 2—protocatechuic acid, 3—catechin, 4—epigallocatechin-3-gallate, and 5—epicatechin; b) CP [33], where 1—gallic acid, 2—protocatechuic acid, 3—epigallocatechin-3-gallate, 4—epicatechin, 5—-p-coumaric acid, and 6—glycosylated quercetin; c) PX [14], where 1—catechin; d) TM [15], where 1—gallic acid.

Compounds	CA <sup>a</sup>	CA <sup>b</sup>	CP <sup>a</sup>	$\mathbf{CP}^{\mathbf{b}}$	PX <sup>a</sup>	$\mathbf{P}\mathbf{X}^{\mathbf{b}}$	$\mathbf{TM}^{\mathrm{a}}$	$\mathbf{T}\mathbf{M}^{b}$
Gallic acid	9.05	1.10	8.80	0.06	_		10.03	20.0
Protocatechuic acid	17.08	1.12	17.32	0.33	_		_	
<i>p</i> -coumaric acid	_	_	29.5	0.01	_		_	
Catechin	23.20 <sup>a</sup>	11.00	_	_	9.0	0.21	_	
Epigallocatechin- 3-gallate	24.80	0.66	25.45	0.07	_		_	
Epicatechin	24.80 <sup>a</sup>	24.00	25.9	0.21	F			
Glycosylated quercetin	$\exists$	GI	35.6	0.28	况			
Quercetin <sup>c</sup>			40.78	0.58				

<sup>*a*</sup>Retention time (min).

<sup>b</sup>Concentration (mg/g).

<sup>c</sup>Viewed at 369 nm.

### Table 4.

Polyphenolic compounds detected at 280 nm by HPLC in the extract of CA, CP, and PX by-products and UHPLC in the extract of TM by-product [10, 13–15].

pharmacological activities, such as cardiac protector [46] reducing oxidative stress [47]. The phenolic acids, such as gallic acid, found in CA, CP, and TM, confers antioxidant properties both to food and to the body, so they are indicated for the treatment and prevention of cancer, cardiovascular diseases, and other illnesses [48]. According to Natsume et al. [45], some flavonoids may be related to the reduction of the probability of developing atherosclerosis, since the ingestion of these biocomposites contribute to the inhibition of oxidation of low-density lipoprotein (LDL).

## 6. Antioxidant activity

The compounds containing antioxidant activity inhibit and/or diminish the effects caused by free radicals, protecting the cells against the harmful effects of oxygenated and nitrogenous free radicals, formed during the oxidative process of the cells [49]. The evaluation of the antioxidant activity of plant material shall be determined by at least two methods, in order to obtain a slightly more complete context of this activity [50]. The use of ABTS colorimetric methods, together with DPPH, bring better reproducibility and sensitivity [51]. Given this, the antioxidant capacity of the raw extracts of the cocoa, cupuassu, pracaxi, and tucumã by-products was evaluated by the ABTS<sup>+</sup> and DPPH methods, being performed in triplicate.

**Table 5** shows the antioxidant activities of CA, CP, PX, and TM extracts. According to the results, CP extract was the one that presented the best antioxidant activity, both by the ABTS<sup>+</sup> and by the DPPH methods, while the CA presented the lowest values, determined by both methods. The four by-product extracts showed good results for the ABTS<sup>+</sup> method.

These results were higher than those reported in the literature by Leong and Shui [52], who achieved values of the radical ABTS<sup>+</sup> for some fruits, such as mango, passion fruit, pineapple, and guava that were 38.0; 5.5; 7.7; and 20.9  $\mu$ M Trolox/g, respectively.

Studies have demonstrated that the ABTS<sup>+</sup> assay can be used to evaluate the antioxidant activity of a wide variety of substances [53], being commonly applied to determine the antioxidant activity in plants and based on the antioxidant capacity to neutralize the ABTS cation radical [54].

The antioxidant capacity values presented by different by-products CA, CP, PX, and TM, in addition to the vegetable sample difference, may also be related to different types of plant samples, which lead to different levels of phenolic compounds in extracts [55].

The results presented by the different by-products bring perspectives on the use of their antioxidant capabilities and may be better used in the development of new products.

Extract	ABTS⁺ <sup>*</sup> μM Trolox/g	DPPH <sup>*</sup> µM Trolox/g	
CA	225.0 ± 3.46	6.74 ± 0.20	
СР	1497.82 ± 5.78	1717.73 ± 5.54	
РХ	597.23 ± 0.37	599.54 ± 0.01	
ТМ	1247.88 ± 3.60	326.0 ± 1.21	

*Results expressed as a mean of triplicate* ± *standard deviation.* 

### Table 5.

Values of antioxidant activity, by ABTS and DPPH methods, of raw extracts of CA [13], CP [10], PX [14], and TM [15].

## 7. Drying by spray drying

Microencapsulation is a technique that aims to protect the assets, from possible causes that produce their instability, such as oxidation, humidity, and photolysis, among others. For this purpose, the sample is surrounded by a polymeric layer that was denominated an encapsulating agent [13]. Therefore, there are several works in this area, which aim to guarantee and/or increase the stability of phenolic

compounds present in tropical fruit by-products, including cocoa, cupuassu, pracaxi, and tucumã [13, 28, 56, 57].

Thus, the spray drying technique was chosen for drying and obtaining microencapsulated extract of CA, CP, PX, and TM by-products, in order to protect the phenolic compounds against oxidation and environmental factors. For the drying of the materials, different conditions of inlet temperature (IT) and feed flow (FF) were used, in addition to the encapsulating agents.

To confirm the encapsulation, from the microencapsulated dry extract, the total polyphenol (TP) and total flavonoid (TF) contents were determined, besides their polyphenol ( $Y_{TP}$ ) and flavonoid ( $Y_{TF}$ ) microencapsulation yields, as well as the antioxidant activity by the ABTS<sup>+</sup> method. Analysis of scanning electron microscopy (SEM) of microparticles from microencapsulated extracts of Amazonian fruit by-products was performed.

**Table 6** shows the results obtained in the drying of cocoa, cupuassu, pracaxi, and tucumã extracts. Maltodextrin was the encapsulating agent for drying all extracts of fruit by-products, and a percentage of 5.0% was used. For CA extract, chitosan was also used in a percentage of 0.5%. Chitosan was used as a polymer for encapsulation as the microparticles formed with the cocoa extract by-product because it was used in pisciculture, since maltodextrin is readily soluble in water [32]. For the CA and CP extracts, FF = 2.5 and 5.0 mL/min were used, respectively, while for both, IT was 170°C. For PX and TM extracts, the FF value was 10 and 7.5 mL/min, and the IT was 160 and 100°C, respectively.

The TM extract showed the highest values for all methods used in relation to the other extracts. It was observed that the higher TP content influenced a higher antioxidant activity for all extracts, where the TM extract had the highest content of polyphenols and, consequently, higher antioxidant activity by the ABTS<sup>+</sup> method. Despite the difference in the TP contents for the extracts of CA (80.44 ± 2.84 mgGAE/g) and CP (38.93 ± 1.24 mgGAE/g), the two presented values of  $Y_{PT}$  close to 64.87 ± 0.16% and 67.20 ± 1.90%, respectively.

The CP and TM extracts showed better microencapsulation yields ( $Y_{PT}$  and  $Y_{FT}$ ), being above 50%. The  $Y_{PT}$  of the CP and TM extracts were close, where 96.50 ± 0.10 and 93.95 ± 2.62% of the polyphenols were present in the microparticles of the extracts, respectively, suggesting that they were not affected by the high temperatures used.

All extracts presented higher antioxidant activities in relation to the studies performed by Rezende et al. (129.16–155.24  $\mu$ M Trolox/g) in the drying of acerola by-product extract; this may be due to use of gum arabic, along with maltodextrin, as an encapsulation agent, besides the plant material used and drying parameters [28, 59].

ME	TP <sup>*</sup> (mgGAE/g)	<b>TF</b> <sup>*</sup> ( <sup>**</sup> )	Y <sub>PT</sub> <sup>*</sup> (%)	Y <sub>FT</sub> * (%)	ABTS⁺ <sup>*</sup> (µMTrolox/g)
CA	80.44 ± 2.84	22.52 ± 2.34	31.98 ± 2.08	64.87 ± 0.16	623.76 ± 20.06
СР	38.93 ± 1.24	11.28 ± 0.37	93.95 ± 2.62	67.20 ± 1.90	435.13 ± 4.1
PX	19.06 ± 0.32	12.46 ± 0.21	31.88 ± 0.02	21.90 ± 0.13	163.28 ± 0.32
TM	130.00 ± 0.024	27.17 ± 0.002	96.50 ± 0.10	83.02 ± 0.01	956.01 ± 7.63

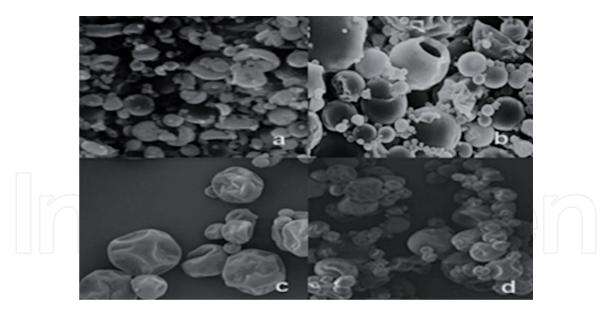
Results expressed as a mean of triplicate ± standard deviation.

\*\*mgQE/g for microencapsulated CA, PX, and TM extracts; mgCE/g for microencapsulated CP extracts.

ME = microencapsulated extracts; TP = total polyphenols; TF = total flavonoids; YPT and YFT = microencapsulation yields of polyphenols and flavonoids, respectively.

### Table 6.

Values of microencapsulated extracts of CA [13], CP [10], PX [14], and TM [15] obtained by spray drying.



#### Figure 7.

*Photomicrograph (SEM) of the microencapsulated extract of Amazonian fruit by-products. a) CA [13]; b) CP [10]; c) PX [14], magnification in 1.000x; d) TM, magnification in 5.000x [15].* 

With the drying results of the raw extracts of the Amazonian fruit by-products, it was observed that all of them maintained the presence of bioactive compounds and also showed antioxidant activity, using maltodextrin as an encapsulating agent.

There are a variety of polymers that can be used in microencapsulation; maltodextrin is of natural origin and is used as a wall material for encapsulation of various plant extracts because it has advantages such as biocompatibility, biodegradability, low toxicity, and reduced moisture in the wall of the microparticle [60].

The SEM analysis was performed for all four by-products in order to verify the formation of microparticles after spray drying. In the microencapsulated extract of the cocoa by-product (**Figure 7a**), the microparticles did not present any type of cracks and were not very grouped, besides not being rough. For the microencapsulated extract of the cupuassu by-product, the microparticles exhibited a very regular spherical structure, with low agglomeration, few ruptures, and heterogeneous size (**Figure 7b**), indicating poor structure deformation.

**Figure 7c** shows the photomicrographs of the microparticles present in the extract of the pracaxi by-product, where it is possible to verify that in general, the particles presented a spherical shape and rough surface characteristic of particles obtained by the spray drying method, which occurred probably during the drying and cooling process [56]. The presence of heterogeneous sizes and aggregate formation was also observed.

The microparticles of the microencapsulated extract of the tucumã by-product (**Figure 7d**) showed morphologies with low deformation in their structures and heterogeneity. Its external surfaces were without cracks and thus does not lead to rupture, which is essential to ensure greater protection of the asset. In general, they exhibit regular spherical shapes, although some are rounded, without strong agglomeration that may be due to the repulsion of loads, with varied size and presence of roughness.

### 8. Industrial application

The reuse of vegetable waste (by-products) is a viable alternative to contribute to the production chain segment, reduce costs, and contribute to the reduction of environmental contamination [32]. In this context, they have a wide spectrum of use in the food, pharmaceutical, cosmetic and veterinary industries. The use of residues of certain fruits as raw material in the food industry in place of synthetic antioxidants and in the production of food that can be included in human food, such as biscuits, breads, cereal bars, cakes, and pastes among other products is of great economic interest and has represented an important segment in industries [26]. By-products have been used in innovative biotechnological processes to obtain enzymes with proteolytic and keratinolytic properties [57].

The exploration of by-products of fruit and vegetable processing, as a source of functional compounds and their application in cosmetics, is a promising field, used in personal, perfumery, and cosmetics hygiene products [25]. Animal feed supplementation is one of the most frequent applications for plant by-products. Its indications on the market are pisciculture, poultry, cattle, and pigs [23, 58]. The use of nutraceuticals in diets is adopted by improving the development, performance, and immunity of the animal. In this segment, enzymes, nucleotides, chitin, chitosan, vitamins, antioxidants, and plant extracts stand out in this segment [59].

The application is possible, thanks to the levels of nutrients that they possess, because they have an expressive amount of protein, nutrients, minerals, and bioactive compounds and ensure good digestibility [33, 60] contributing to generate a low-cost product with promising characteristics for its use.

The use of elements with a low-cost and easy access enables the use of byproducts from industrial processing as a strategy to optimize the entire course of the productive stage [61]. Several studies use by-products of vegetable origin as a raw material for industrial reuse [39, 62]. These matrices present nutrient contents significantly interesting for total or partial use in fish feed supplementation [63]. Within this perspective, the cocoa and pracaxi extracts from the by-product present all the prerequisites to be applied in this market [13, 14].

In this perspective, the elaboration and characterization of flours, from fruit byproducts, have been the object of numerous studies, which point to good nutritional characteristics and potential for their application as ingredients in food [33]. Due to their nutritional characteristics, cupuassu and tucumã flours emerge as a highly desirable food ingredient to enrich other foods [33, 64].

The market is made up of niches of consumers of natural foods (energy products), such as athletes, sportsmen, children, and workers who need to eat caloric foods [65]. As a result of the growing interest of consumers for more nutritious natural foods, with good intake of carbohydrates, proteins, vitamins, minerals, fibers, and an adequate balance of calories, the market for cereal bars has been increasing [64].

In view of the above, the extracts obtained from the by-products of cupuassu and tucumã can be used in this segment. The by-product of cupuassu can be used in the enrichment of multimixture flour, which is incorporated in the feeding of children in a state of infant malnutrition, a project already applied by the Sociedade Bíblica do Brasil in partnership with the Pastoral da Criança [33] and the by-product of tucumã in the preparation of bakery products (in the form of bread and cookies) and pasta in the production of cereal bars explored as functional food [15, 64].

### 9. Conclusions

The cocoa, cupuassu, pracaxi, and tucumã seed by-products presented concentrations of macronutrients such as proteins, fibers, total fats, and carbohydrates as ingredients potentially to be used as food or animal feed. In addition, these extracts showed significant antioxidant activity, and phenolic compounds (including protocatechuic acid, gallic acid, caffeic acid, and *p*-coumaric acid) and flavonoids (quercetin, glucosylated quercetin, epicatechin, catechin, and epigallocatechin-3-gallate)

were the most abundant compounds in these extracts. By means of the response surface methodology, it was determined that the optimal conditions for the microencapsulation of cocoa, cupuassu, and pracaxi seeds by-product extract are: IT = 170°C, FF = 2.5 mL/min, and MD = 5.0%; IT = 170°C, FF = 5.0 mL/min, and MD = 5.0%; IT = 160°C, FF = 10.0 mL/min and MD = 5.0%, respectively, and optimal conditions for microencapsulation of tucumã seeds IT = 100°C, FF = 7.5 mL/min, and MD = 5.0%. Under these conditions, the microparticles were obtained with good stability and some heterogeneity, with spherical structure, confirming the efficiency of the microencapsulation process with the use of maltodextrin as a drying adjuvant.

Therefore, it is suggested that the microencapsulated extracts of cocoa, cupuassu, pracaxi, and tucumã seed by-products can be used in the food, cosmetic, pharmaceutical, and veterinary industries And used as a potential source of nutrients to be deployed as an integrator of alternative human food or animal feed, an opportunity in which they acquire economic value and at the same time reducing the environmental impact related to their disposal in the environment.

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## **Conflict of interest**

The authors declare that there is no conflict of interest.

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