

# The Wasted Fractions of Pequi Fruit are Rich Sources of Dietary Fibers and Phenolic Compounds

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## Abstract

Considering the scarcity of studies on the nutrients and phenolic compounds in the wasted fractions of the pequi (*Caryocar brasiliense* Camb.) fruit processing, this study investigated the proximate composition, identified the phenolic compounds, and quantified the gallic and ellagic acids in the shell (peel and external mesocarp), and in the external mesocarp of pequi. The shell and the external mesocarp of the pequi fruit presented high concentrations of total dietary fibers, soluble fibers and phenolic compounds, mostly the freeze-dried pequi shell, which showed approximately 50% total dietary fibers, 20% soluble dietary fibers and 10% polyphenols, with remarkable antioxidant capacity. The phenolics identified in the pequi shell and external mesocarp were gallic acid, ellagic acid, and quercetin. In addition, protocatechuic acid, catechin, *p*-coumaric acid, and luteolin were identified for the first time in the pequi by-products. The freeze-dried pequi shell showed twice the gallic and ellagic acids concentrations compared to those of the external mesocarp. The wasted by-products of pequi, especially the pequi shell, are rich in healthy phytochemicals with the potential to be used by the food and pharmaceutical industries as ingredients in functional plant-based products or nutraceuticals.

**Keywords:** bioactive compounds, Brazilian Savannah fruit, food residue, functional food by-products, phenolics profile, prebiotic compounds

## 1. Introduction

*Caryocar brasiliense* Camb. is the most prevalent species of the genus *Caryocar* spp. in the Brazilian Savannah (Cerrado) (Oliveira-Filho & Ratter, 2002), and its fruit is called pequi. The pequi fruit encompasses a shell, composed of an exocarp (peel) and an external mesocarp (thick and whitish mass), and a core (pyrene) composed of an internal yellow-orange mesocarp, which is the edible pulp of the fruit (Nascimento-Silva & Naves, 2019). The pequi pulp is the most consumed and studied fraction of the fruit, with economic and social importance for the producing regions (Silva, Pinto, Teixeira, & Drumond, 2020). However, the pequi shell, which represents approximately 80% of the fruit, is poorly studied and usually discarded in the environment, as solid waste, during the agro-industrial processing of the pequi fruit (Nascimento-Silva & Naves, 2019).

Regarding the nutritional and bioactive properties of the pequi shell, few reports have shown that it is a source of dietary fiber and polyphenols (Leão, Franca, Oliveira, Bastos, & Coimbra, 2017), with high antioxidant capacity (Cangussu, Leão, Oliveira, & Franca, 2021; Rocha, Melo, Paula, Nobre, & Abreu, 2015). Despite being a poorly explored by-product, an *in vitro* study demonstrated that the extract of pequi shell inhibit the  $\alpha$ -glucosidase and  $\alpha$ -amylase enzymes and reduce blood glucose (Caldeira et al., 2021). In another study, the pequi shell was effective in minimizing effects of chronic cardiotoxicity induced by chemotherapy in the myocardium of rats (Moura et al., 2017). These effects are possibly associated with the bioactive compounds of the pequi shell.

Considering the potential of the wasted fractions of the pequi fruit as functional by-products, and the importance of phenolic compounds for human health, this study analyzed the proximate composition, identified the phenolic

compounds, and quantified the gallic and ellagic acids in two fractions of the pequi fruit: the shell and the external mesocarp.

## 2. Material and Methods

### 2.1 Plant Material

The fruits were collected during the harvest period (November), in the State of Tocantins (Presidente Kennedy municipality - S 8° 32' 20" W 48° 30' 19"), a Brazilian state with representative occurrence of the *C. brasiliense* species of the Cerrado biome. After harvesting, fruits were selected, cleaned and shelled. The samples were divided into shell (peel and external mesocarp) and external mesocarp (peeled shell), which were freeze-dried, vacuum-packed in low-density polyethylene packages and stored at -20 °C until analysis.

### 2.2 Proximate Composition

The proximate composition was evaluated by the following analyses AOAC (2019): moisture (method 925.45b), total nitrogen by micro-Kjeldahl and conversion to protein using the factor 6.25 (method 960.52), ashes (method 923.03), and dietary fiber (insoluble and soluble fractions) by enzymatic–gravimetric method (method 985.29). The lipids content was quantified according to Bligh and Dyer (1959). The carbohydrates content was estimated by difference, subtracting the moisture, ashes, lipids, proteins and total dietary fiber values from 100. The total energy value was calculated considering the Atwater conversion factors: 9 kcal/g of lipids and 4 kcal/g of proteins and carbohydrates.

### 2.3 Total Phenolic Compounds

The total phenolic compounds were determined as described by Genovese, Pinto, Gonçalves and Lajolo (2008). The lyophilized shell or external mesocarp (0.5 g) was homogenized with 25 mL of 50% methanol in the ultrasonic bath for 60 min at 25 °C. The mixture was centrifuged at 4000 rpm for 10 min at 25 °C to obtain the extracts. The extracts (0.25 mL) were mixed with 2.75 mL of Folin-Ciocalteu solution (3%), and 0.25 mL of sodium carbonate (10%), and the solution was kept at room temperature for 60 min, in a dim light environment. The total phenolic compounds were quantified using a standard curve for gallic acid (Concentration = [Absorbance + 0.0448] / 0.0069;  $r = 0.9961$ ) at 725 nm by a UV/Visible spectrophotometer (V-630, Jasco, Easton, MD, USA). The result was expressed in mg of gallic acid equivalents (GAE) per 100 g of sample.

### 2.4 Phenolic Compounds Identification and Quantification by HPLC-ESI-HRMS/MS

The samples were extracted (100 mg) in 3 mL of methanol for 30 min using an ultrasonic bath (AltSonic Clean 3IA). Each sample and a blank (methanol) were filtered through a cellulose acetate filter (0.22 µm) and injected in the liquid chromatography coupled to high-resolution tandem mass spectrometry method (HPLC-ESI-HRMS/MS). The chemicals for the HPLC analyses were purchased from Sigma-Aldrich (St. Louis, MO, USA).

The HPLC-ESI-HRMS/MS system consisted of a HPLC Ultimate 3000 (Thermo Scientific, Waltham, MA, USA) coupled to a high-resolution mass spectrometer Q-Exactive (Thermo Scientific). The H-ESI in negative mode source was used to monitor the compounds by HRMS and in the Parallel Reaction Monitoring (PRM) experiments. The chromatographic separation was carried out using ACE C18 column (4.6 mm × 100 mm, 3.0 µm) at 20 °C column temperature with mobile phases of deionized water (A) and acetonitrile (B), both acidified with 0.1% formic acid. The applied gradients were 93-70 (A%) from 0 to 10 min, 70-50 (A%) from 10 to 15 min, 50-30 (A%) from 15 to 18 min, 30-20 (A%) from 18 to 20 min, 20-0 (A%) from 20 to 23 min, 100 (B%) from 23 to 26 min, 0-93 (A%) from 26 to 28 min, and 93 (A%) from 28 to 33 min; with 0.3 mL/min flow rate and 10 µL injection volume. The MS parameters were used in the following conditions: spray voltage 4 kV, sheath gas flow rate 30, auxiliary gas flow rate 10, capillary temperature 350 °C, auxiliary gas heater temperature 300 °C, S-lens 55 and mass range  $m/z$  150-700.

Polyphenols were identified by injecting a stock solution with 14 standards (gallic acid, protocatechuic acid, gentisic acid, caffeic acid, p-coumaric acid, vanillic acid, ellagic acid, catechin, epicatechin, rutin, quercetin, naringenin, luteolin and kaempferol). The analyses were carried out in the negative ESI ionization mode and phenolic compounds were identified by the retention times and fragments generated in the PRM experiment, compared to commercial standards. Considering the abundance of the gallic and ellagic acids observed in the qualitative analysis and their biological effects, the quantification of these two derivatives was performed. For building the gallic acid quantification curve ( $y = 209427 + 10939.4x$ ;  $r = 0.9967$ ), the SRM (Selected Reaction Monitoring) experiment with collision energy equal to 10 (EC) was used, monitoring the charge masses 169.01 and 300.99 relative to the deprotonated molecules of gallic acid and acid ellagic, respectively. Gallic acid solutions were prepared at the following concentrations in ppm: 10, 25, 50 and 100. Data was processed in

Xcalibur™ software.

### 2.5 Antioxidant Capacity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method was carried out according to Brand-Williams, Cuvelier and Berset (1995) at 517 nm. An aliquot (0.1 mL) of the extract was mixed with 3.9 mL of DPPH solution (25 mg/L). The mixture was reacted for 120 min in a dim light environment at 25 °C. Trolox was used as the standard for the calibration curve (Concentration = [0.5992 – Absorbance]/0.0006; r = -0.9969). The antioxidant capacity was expressed as μmol of trolox equivalents (TE) per g of sample.

### 2.6 Statistical Analysis

The results were reported as mean ± standard deviation of three replicates and subjected to Student's *t*-test for mean comparisons. Pearson's correlation test was carried out between the total phenolic content and antioxidant capacity (DPPH). A 5% probability was considered statistically significant. The statistical analysis was performed using R software (R Foundation for Statistical Computing, Vienna, Austria), version 4.0.3.

## 3. Results and Discussion

### 3.1 Nutritional Composition

The pequi shell and external mesocarp showed a high concentration of moisture and dietary fiber, and low concentrations of ashes, lipids, proteins and energy (approximately 50 kcal/100 g, fresh weight) (Table 1). These results are compatible with the chemical composition of the exocarp with external mesocarp of *C. brasiliense* from Minas Gerais, Brazil (Monteiro, Silva, Martins, Barin, & Rosa, 2015). It is worth highlighting that the freeze-dried pequi shell (9.2% moisture) has a remarkable high concentration of total dietary fibers (49.1 g/100 g), with almost 40% soluble fibers (19.4 g/100g). These values are higher than those reported in pequi shell flour (6.4 to 8.2% moisture), of 42.1 to 43.3 g/100 g for total dietary fibers, and 9.4 to 10.4 g/100 g for soluble fibers (Leão et al., 2017; Bemfeito et al., 2020). Thus, the pequi shell and external mesocarp can be used to improve the nutritional value of healthy and low-calorie food products, for instance, for replacing wheat flour in the formulation of cookies (Soares Júnior et al., 2009) and brownies (Reis Filho et al., 2018). Considering the high content of soluble fibers, some studies investigated the pectin yield of the pequi shell (dried) and found values from 12 to 20 g/100 g (Leão, Botelho, Oliveira, & Franca, 2018), and from 9 to 56 g/100 g for the external mesocarp (dried) (Leão et al., 2018; Siqueira, Alves, Vasconcelos, Damiani, & Soares Júnior, 2012). These results confirm the potential of the pequi shell as a source of pectin, a dietary fiber considered a candidate to prebiotic, which can be used by gut microbiota providing health benefits (Rezende, Lima, & Naves, 2021). Therefore, studies on the prebiotic effects of the pequi shell are warranted.

Table 1. Proximate composition, total phenolic and antioxidant capacity of the shell and external mesocarp of *C. brasiliense*

Parameter	Samples	
	Shell	External mesocarp
Proximate composition (g/100 g)		
Moisture	76.70 ± 0.16 <sup>b</sup>	82.38 ± 0.49 <sup>a</sup>
Ashes	0.68 ± 0.01 <sup>a</sup>	0.54 ± 0.02 <sup>b</sup>
Lipids	0.35 ± 0.00 <sup>a</sup>	0.27 ± 0.00 <sup>b</sup>
Proteins	1.12 ± 0.02 <sup>a</sup>	0.79 ± 0.03 <sup>b</sup>
Total dietary fiber	12.59 ± 0.09 <sup>a</sup>	8.27 ± 0.02 <sup>b</sup>
Insoluble fiber	7.61 ± 0.23 <sup>a</sup>	5.86 ± 0.05 <sup>b</sup>
Soluble fiber	4.98 ± 0.13 <sup>a</sup>	2.42 ± 0.02 <sup>b</sup>
Carbohydrates	8.56 ± 0.09 <sup>a</sup>	7.75 ± 0.47 <sup>b</sup>
Energy value (kcal/100 g)	42.66 ± 0.27 <sup>a</sup>	36.60 ± 1.96 <sup>b</sup>
Total phenolics (mg GAE/100 g)	2283.64 ± 38.52 <sup>a</sup>	2135.70 ± 75.28 <sup>b</sup>
Antioxidant capacity (μmol TE/g)	194.49 ± 3.85 <sup>a</sup>	187.70 ± 4.37 <sup>b</sup>

Values (fresh weight) are mean ± standard deviation (n=3). In the same row, means with different letters differ significantly by Student's *t*-test (p < 0.05). GAE: Gallic acid equivalent. TE: trolox equivalents. External mesocarp: shell without exocarp.

### 3.2 Phenolic Compounds and Antioxidant Capacity

A high concentration of total phenolic compounds was found in the shell and external mesocarp, even though the

high moisture (approximately 80%) of these by-products (Table 1). In a previous study performed with the shell dry extract of *C. villosum* from the Amazon region, the total phenolic content varied from 4,153 to 4,523 mg GAE/100 g (Yamaguchi et al., 2017). In our study, the content of phenolic compounds in the freeze-dried shell approaches 10% of the product (8,820 mg GAE/100 g), value higher than those reported by Monteiro et al. (2015) in the exocarp with external mesocarp of *C. brasiliense*, from 1,136 to 7,858 mg GAE/100 g (dry extract). These data reinforce the potential of pequi shell as a functional by-product.



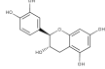
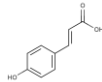
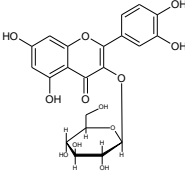
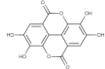
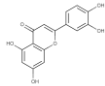
The phenolic compounds identified in the shell and external mesocarp of the pequi fruit were gallic acid, protocatechuic acid, catechin, *p*-coumaric acid, ellagic acid, glycosylated compounds quercetin and luteolin (Table 2, Figure 1). In addition, compounds derived from ellagic acid and glycosylated quercetin generated in the PRM experiment were observed. Thus, in the pequi shell, ellagic acid derivatives ( $C_{20}H_{15}O_{13}$ ,  $\delta=3.829$ ) were identified in 12.08 min and 23.76 min; and glycosylated quercetin derivative ( $C_{21}H_{19}O_{12}$ ,  $\delta=3.342$ ), in 25.93 min. In the external mesocarp, only ellagic acid derivatives ( $C_{20}H_{15}O_{13}$ ,  $\delta=3.829$ ) were found in 12.08 min and 23.76 min. Roesler, Catharino, Malta, Eberlin and Pastore (2008) reported the presence of *p*-hydro benzoic acid, gallic acid, quinic acid, quercetin, and quercetin 3-O-arabinose in the *C. Brasiliense* peel (exocarp) from the state of Goiás, Brazil, and ethyl gallate and ellagic acid were also reported in pequi peel flour (Cangussu et al., 2021).

The presence of protocatechuic acid, catechin, *p*-coumaric acid, and luteolin in the shell and external mesocarp of the pequi fruit are reported for the first time in our study. Protocatechuic acid is a phenolic acid that is found in potato and onion peels. Several health benefits have been attributed to its consumption, such as anti-inflammatory, antioxidant, antimicrobial, anti-carcinogenic, and anti-atherosclerotic activity (Rashmi & Negi, 2020). Catechin and luteolin, flavonoids found in medicinal plants, have strong anti-inflammatory activity (Aziz, Kim, & Cho, 2018; Matsui, 2015). Moreover, recent evidence suggests that catechin and its microbiota-derived metabolites improve gut barrier function and endotoxemia (Hodges, Sasaki, & Bruno, 2020). Thus, the use of pequi shell is recommended for the development of new food products, and its inclusion in humans' diet is important for health promotion and disease prevention.

The freeze-dried shell and external mesocarp of the pequi fruit showed a high concentration of gallic acid. The pequi shell showed twice the gallic acid concentration (320 mg/100 g) compared to that of the external mesocarp (160 mg/100 g). These concentrations represent 3.6% and 1.4% of the total phenolic content (dry weight), respectively. The values observed in pequi shell are compatible with those reported in the pequi peel flour, from 117 to 431 mg/100 g (Cangussu et al., 2021). The gallic acid and its derivative compounds are found in green and black teas, avocado, blackcurrant, grapes, guava, mango, mulberry and pomegranate, which has been increasingly investigated for their protective effects on obesity (Dludla et al., 2019). A study with obese rats observed that oral supplementation of gallic acid reduced liver steatosis, body weight and plasma insulin levels (Sousa et al., 2020). Thus, the freeze-dried pequi shell may be used as a supplement or nutraceutical in the management of obesity, as its low energy value and high gallic acid concentration. Moreover, gallic acid and its derivatives also have great potential in the management of gastrointestinal diseases through interaction with the gut microbiota and modulation of the immune response (Yang et al., 2020).

A high concentration of ellagic acid also was found in the freeze-dried pequi shell (1,320 mg/100 g) and external mesocarp (680 mg/100 g), which represent 14% and 6% of the total phenolic content (dry weight), respectively. The ellagic acid content observed in pequi shell is compatible with those reported by Cangussu et al. (2021), from 306 to 1,630 mg/100 g, according to different tested extraction times (30 min to 12 h). The main dietary sources of ellagic acid are berries, especially, raspberries, with the concentrations varying from 265 to 1,795 mg/100 g of fresh weight (Landete, 2011). Ellagic acid is present in the foods of several forms, including free, glycosylated, or as complex polymers called ellagitannins, which can be metabolized by hydrolysis in the gut microbiota, producing metabolites like the urolithins (Rós, Giner, Marín, & Recio, 2018). Studies report that ellagic acid acts as antioxidant, anti-inflammatory, neuroprotective, and hepatoprotective, as well as the protection against diabetes, cardiovascular disease, and cancer (Kang, Buckner, Shay, Gu, & Chung, 2016; Rós et al., 2018). A clinical trial with oral administration of urolithin A to healthy, sedentary elderly individuals, for 4 weeks, showed that this ellagic acid metabolite improves mitochondrial and cellular health following its regular consumption (Andreux et al., 2019). As the freeze-dried pequi shell has notable content of ellagic acid, it may be considered an excellent source of ellagic acid with great potential health benefits.

Table 2. Phenolic compounds identified through the PRM experiment in the shell and external mesocarp of *C. brasiliense* by HPLC-HRMS

RT (min)	Standard RT	Detected mass [M - H]	Calculated mass	Molecular formula	Error (ppm)	Identified compound	Fragments (m/z)
14.25	14.27	169.01318	169.01370	C <sub>7</sub> H <sub>6</sub> O <sub>5</sub>	0.001	gallic acid 	169.01318 125.02316
19.21	19.22	153.01814	153.01879	C <sub>7</sub> H <sub>6</sub> O <sub>4</sub>	0.622	protocatechuic acid 	153.01814 109.02817
20.08	20.08	289.07159	289.07122	C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>	3.201	catechin 	289.07159 245.08145 205.04997 179.03389 125.02304
25.11	25.08	163.03891	163.03952	C <sub>9</sub> H <sub>8</sub> O <sub>3</sub>	- 0.065	p-coumaric acid 	163.03891 119.04893
25.55	25.59	463.08844	463.09548	C <sub>21</sub> H <sub>20</sub> O <sub>12</sub>	2.888	quercetin 3-O-β-glucoside and/or quercetin 3-O-β-galactoside 	300.02747 271.02481 255.02481 255.02972 243.02954 178.99771 163.00272 151.00261
26.36	26.38	300.99881	300.99845	C <sub>14</sub> H <sub>6</sub> O <sub>8</sub>	2.546	ellagic acid 	300.99881 283.99646 229.01391 185.02354
28.23	28.31	285.04044	285.03992	C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>	3.773	luteolin 	257.04535 241.05034 217.05003 199.03934 175.03917 151.00264 133.02837 107.01260

RT - retention time. Source of phenolic compounds' chemical structures: Phenol-Explorer database (www.phenol-explorer.eu).

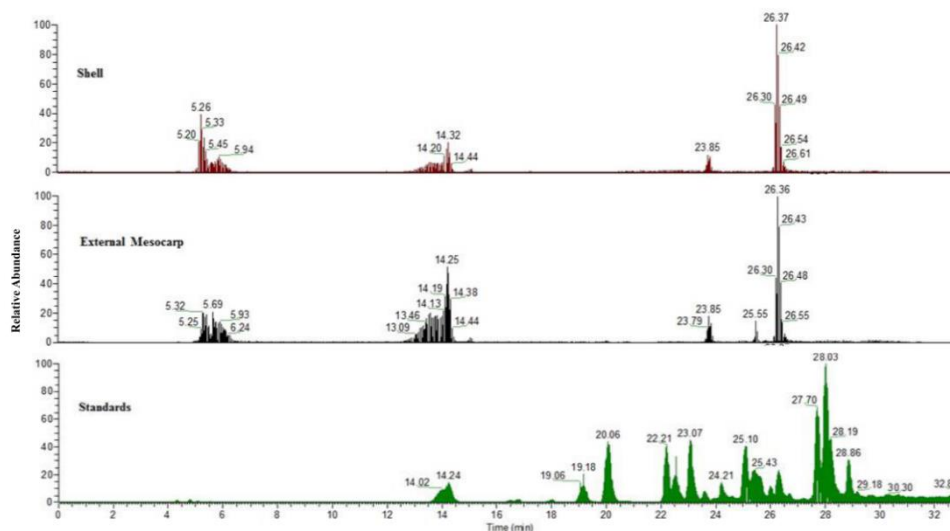


Figure 1. Total ion chromatograms of the shell and external mesocarp of *C. brasiliense* and phenolic compounds standards, negative ESI ionization mode

The high polyphenol content was associated with the high antioxidant capacity of the shell and external mesocarp of the pequi fruit, by the high correlation found between total phenolic compounds and DPPH, with an R-value of 0.9524. The antioxidant capacity of the shell is higher than that of the external mesocarp (Table 1), and this attribute may be explained in part by the high concentrations of gallic and ellagic acids in the pequi by-products, as these compounds have the ability to scavenge free radicals (Rós et al., 2018). Exocarp and external mesocarp of pequi also presented high antioxidant capacity by three different methods (ABTS, FRAP and  $\beta$ -caroten/linoleic acid system) (Bemfeito et al., 2020), as well as the pequi peel, with values similar to those of tested commercial antioxidants (Rocha et al., 2015).

The concentrations of dietary fibers, the total phenolic compounds and the gallic and ellagic acids, and the antioxidant capacity of the pequi shell were higher than those of the external mesocarp. This finding is probably explained by the high concentrations of dietary fibers and polyphenols in the pequi peel. Phenolic compounds are concentrated in the peels of the fruits, as they protect the plant against ultraviolet light, pathogens, parasites, and predators (Mojzer, Hrnčić, Škerget, Knez, & Bren, 2016). On the other hand, concerning the technological properties and functionalities in food applications, no significant differences were found between the pequi shell flours, with or without exocarp (Leão et al., 2017).

The nutritional and functional attributes evidence the pequi by-products as suitable ingredients to the development of plant-based foods, such as meat analogs, beverages, bakery, and pasta products (Tan, Nawaz, & Buckow, 2021). Nevertheless, it is worth highlighting that *in vivo* studies on antioxidant and prebiotic properties of the pequi by-products are desirable.

#### 4. Conclusion

The shell and the external mesocarp of the pequi fruit contain high concentrations of total dietary fibers, soluble dietary fibers and phenolic compounds, mostly the freeze-dried pequi shell, which contains approximately 50% total fibers, 20% soluble fibers and 10% polyphenols. Gallic acid, ellagic acid, and quercetin were identified in the shell and external mesocarp of pequi, and the ellagic acid is present in remarkably high concentrations. Moreover, for the first time, protocatechuic acid, catechin, *p*-coumaric acid, and luteolin were identified in the pequi by-products. The peel and external mesocarp of the pequi fruit, usually discarded in the environment as solid wastes, have a great potential to be used in different healthy food systems, dietary supplements, and nutraceutical products. The shell is especially indicated as biomass rich in phytochemicals in the formulation of functional plant-based foods.

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### Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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