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Physical and chemical parameters, total phenols and the antioxidant activity of Pequi (*Caryocar brasiliense* Camb)

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Pequi is a Brazilian cerrado fruit with nutritive and sensory characteristics and good taste and is used in numerous typical dishes. The purpose of this study was to analyze the physical and chemical characteristics, total phenols and the antioxidant activity and the scanning electron microscope (SEM) of pequi pulp, mesocarp and peel. Physical and chemical analyses such as antioxidants and phenolic compounds compositions were performed. Physical analyses showed high variation in length, equatorial diameter, weight and volume characteristics. Green peel, dark gray mesocarp and bright yellow pulp were observed. Significant differences were observed in the physical and chemical analyses of the fruit, with the pulp showing the most relevant characteristics such as high presence of lipids and proteins. The SEM of the peel and mesocarp showed a multiform, irregular and fibrous structure while the pulp showed irregular aspect, with the presence of granules and fibers. The highest antioxidant activity (EC_{50}=280.1) was seen in pulp and the lowest (EC_{50}=4.89) in peel. However, the highest concentration (204.37 mg EAG/g) of phenols is in the peel and the lowest (26.55 mg EAG/g) in the pulp. This study concluded that the fruit pulp presents potential for body maintenance and human consumption.

Key words: Ether extract, color, pulp, reuse.

INTRODUCTION

Cerrado is a region with many fruit tree species, many of which are unexplored fruits with pleasant and exotic sensory peculiarities such as color, taste and smell. Werneck (2011) reports that pequi (*Caryocar brasiliense* Camb.) is a fruit from the Brazilian *cerrado* with commercial and nutritional importance. Its typical exotic smell and taste are attractive characteristics. The fruit has high nutritional value, as it is a source of protein,

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Figure 1. Morphological structure of pequi (*Caryocar brasiliense* Camb.).

lipid, vitamin A, minerals and β-carotene (Alves et al., 2009). Pequi is comprised of pericarp and seed. Its pericarp is green, greenish brown epicarp, dark gray external mesocarp and yellow internal mesocarp which is the edible part of the fruit, and brown endocarp (Figure 1).

Maia et al. (2007) report that pequi is economically unexplored for oil production, as its mesocarp may be used to replace butter and its pit may be cooked and used in several typical dishes of Brazilian cerrado. The consumption of *Caryocar brasiliense* fruits is not only associated with taste and nutritional value, clinical and epidemiological studies have shown evidence indicating these fruits have immunological, therapeutic and anti-inflammatory characteristics mainly due to their antioxidant activity and presence of vitamins A and E (Saraiva et al., 2010). The antioxidant characteristics are related to phenolic compounds which present enzyme activity in the body by blocking oxidation of compounds in foods like fats, and reducing the action of reactive oxygen species (Moreira and Mancini Filho, 2004). Phenolic compounds are chemical structures that present hydroxyls and aromatic rings in simple and polymer forms which enables their antioxidant activity. There are several known sources of natural antioxidants and some are commonly found in the plant kingdom (Angelo and Jorge 2007).

Considering the above facts, the objective of this study was to evaluate the physical and chemical characteristics such as weight, diameter, proteins, lipids, total phenolics, antioxidant activity and scanning electron microscopy (SEM) of the various layers (epicarp, mesocarp and endocarp) of pequi fruits of the region of Belo Horizonte, MG.

MATERIALS AND METHODS

General experimental procedures

The reagents used in this study were analytically pure (AP). The Folin-Ciocalteu reagent was acquired from Sigma-Aldrich, DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical from Aldrich Co. and gallic acid from Vetec. Absorption was measured using a UV-V spectrophotometer (Biospectro SP 220).

Vegetal material

Pequi fruits were manually harvested in February 2013 from native trees located in a farm in the municipality of Lagoa Santa in the metropolitan region of Belo Horizonte, MG (19° 37′ 45″ S and 43° 53′ 23″ W) and taken to the Laboratório de Frutas e Hortaliças of the Instituto Federal Goiano – Rio Verde Campus. The fruits were selected in terms of size, color, absence of mechanical injuries and spots. They were then sanitized in chlorinated water (100 $mg.L^{-1}$), for 15 min and dried. For the analyses of fiber and scanning electron microscope, the fruit pulp, mesocarp and peel were dried and degreased using the Soxhlet method [with petroleum ether reagent (IAL, 2008)] and ground to meal consistency at 6 mm granulometry.

Preparation of crude extracts for the antioxidant activity and total phenols

The vegetal material (1 g) for the antioxidant activity was ground to

small particles, placed in 40 mL methanol solution, 50% (V/V) in agitation for 1 h, centrifuged at 600 rpm for 15 min and the suspended particles collected. With the residue, a new extraction was conducted with 40 mL acetone solution, 70% (V/V) in agitation for 1 h, centrifuged again, and the suspended particles collected. The two extracts were placed in a 100 mL amber volumetric flask whose meniscus was checked with water.

Determination of total phenols

1 g of the vegetal material for the analysis of total phenols was ground and solubilized in 100 mL ethanol in agitation for 1 h. Then, it was filtered and stored in an amber flask until the analysis was conducted. For the determination of total phenols present in the ethanolic extract samples, the Folin-Ciocalteu method with modifications (Sousa et al., 2007) was employed. The amount of 100 µL was taken from the ethanolic extract and agitated with 500 µL Folin-Ciocalteu reagent; 7.4 mL distilled water for 1 min; after whihc 2 ml Na_2CO_3 at 15% was added to the mix and agitated for 30 s, making up 10 mL solution. The solution was placed at rest for 2 h until reading was conducted in triplicates with absorbance at 750 nm using glass cuvettes, ethanol and all reagents as "blank", but not the sample. Total phenols (TP) were determined using the calibration curve built with gallic acid patterns (10 to 350 µg/mL) and expressed as mg of GAE (gallic acid equivalents) per g of the sample. The gallic acid calibration equation showed a correlation coefficient R^2 of 0.996.

Quantitative analysis of antioxidant activity

The antioxidant activity evaluation of pequi (peel, mesocarp and pulp) was determined through its ability to sequestrate DPPH free radical according to the methodology described by Sharma and Bhat (2009) with adaptations. When the DPPH solution is in contact with a substance that can donate a hydrogen atom, the reduced form of the resulting radical, presents a reduced color (Ali et al., 2009).

To determine DPPH calibration curve, a portion with different concentrations of DPPH (10, 20, 30, 40, 50 and 60 µM) was transferred to a glass cuvette and the reading conducted using a spectrophotometer at 515 nm. Methanol was used as blank for the appliance calibration. To calculate the straight-line equation, the DPPH concentrations were plotted (µM) on X axis and the respective concentrations on Y axis. The equation from the calibration curve had a correlation coefficient R^2 of 0.999.

Using the crude extract previously diluted in methanol at three concentrations (25, 50 and 100% V/V), the amount of 0.1 mL from each dilution was transferred to a test tube with 3.9 mL DPPH reagent. Readings were conducted half an hour later for sample stabilization, with the equipment previously calibrated. With the calibration curve equation and absorbance values after 30 min for every tested concentration, the concentrations expressed as EC_{50} were tested.

Centesimal composition of pequi and aspect

The physical evaluation of pequi was based on length (mm) and equatorial diameter (mm) measured with digital calipers. Volume was determined with fruit immersion in a graduated polypropylene jar with distilled water by recording the amount (mL) of fluid displaced. Fruit weight was determined using an analytical scale of three decimal places with the results expressed in grams (g).

Physicochemical composition of pequi *in natura* was determined as follows: fruit moisture according to methodology no. 925.09 of AOAC (2000) until a constant weight was obtained; ethereal extract according to methodology no 925.38 of AOAC (2000); crude protein content according to themicro-Kjeldahl method no 920.87 of AOAC (2000); ash according to gravimetric method no. 923.03 of AOAC (2000), with calcination at 550ºC and sample resting in FORNITEC muffle, model 1926, Brazil.

Color

The fruit aspect was evaluated in relation to color parameters according to the system CIELab; L*, a*, b* in a colorimeter (Colorquest II, Hunter Associates LaboratoryInc., Virginia). The evaluation used 10º observation angle and D65 as standard illuminant, which corresponds to natural daylight. The results were expressed as L*, a* and b* values, where L* (luminosity or brightness) values ranging from black (0) to white (100), a* values ranging from green (-60) to red (+60) and b* values ranging from blue (-60) to yellow (+60).

Scanning electron microscope

For the structural analysis of peel, mesocarp and pulp meal, scanning electron microscope (SEM) was used with samples which were placed on stabs, coated with a film of gold and analyzed microscopically. The evaluation was conducted at the Multiuser Laboratory of High-Resolution Microscopy at the Physics Universidade Federal de Goiás. The analysis used Jeol scanning electron microscope, JSM – 6610, equipped with EDS, Thermo scientific NSS Spectral Imaging.

Statistical analysis

Statistical analysis of the physical and chemical parameters and Pequi color was performed using statistical software in a completely randomized design. Means were compared using the Tukey test at 5% probability.

RESULTS AND DISCUSSION

Physical analyses of pequi fruits

Pequi fruits presented length values of 63.17 and 37.64 mm for fruit and internal mesocarp (Table 1); greater than those reported by Vera et al. (2005) (58 mm) and Oliveira et al. (2009) (55.83 mm). The equatorial diameter of pequi fruits found in this study was lower than the value (64.8 mm) reported by Vera et al. (2005). Its $L.ED^{-1}$ ratio indicates pequi has a circular shape and its internal mesocarp is also circular, but with more accentuated length in relation to the fruit width.

The fruits presented mean weight of 132.04 g, close to the value (140 ± 64.91 g) reported by Cordeiro et al. (2013) in pequi fruits from Cuiabá region. However, Oliveira et al. (2009) reported lower weight (90.48 g) in a similar study. The reported difference in values confirms the high variability presented by pequi fruits. The volume of the fruit and internal ranged from 560 to 30.3, respectively. Pequi fruits presented considerable genetic variability, highlighting the differences between physical values for fruits from the same region and confirming the importance of studies on such characteristics.

Table 1. Mean and standard deviation values of length (L), equatorial diameter (ED), length/equatorial diameter ($L.ED^{-1}$) ratio, weight and volume of the fruit and internal mesocarp of pequi (*C. brasiliense* Camb.).

Parameter	Fruit	Internal mesocarp
Length (mm)	63.17 ± 5.36	37.64 ± 5.01
Equatorial diameter (mm)	61.42 ± 5.76	26.14 ± 5.70
$L.ED^{-1}$ ratio	1.03 ± 0.08	1.44 ± 0.05
Weight (g)	132.04 ± 26.22	31.04 ± 7.92
Volume (mL)	560 ± 162.88	30.3 ± 9.00

Table 2. Mean and standard deviation values of L, a*, b* for pulp, mesocarp and peel of pequi fruits (*C. brasiliense* Camb.).

Parameter	Pulp	Mesocarp	Peel
L*	76.40 a ± 4.34	56.38 b ± 6.60	48.30^{c} ± 4.97
a*	25.65 a ± 5.53	4.71 b ± 2.85	-0.05^{c} ± 2.50
b*	45.66 a ± 8.29	41.12 b ± 5.90	10.74^{c} ± 3.57

Distinct small letters on the line present significant difference between each other through Tukey's test at 5% probability.

Siqueira et al. (2012) reported that the physical properties of fruits contribute to designing new equipment for post-harvest processes (drying, cleaning, classification and peeling) and storage, stressing that it may help adaptations to existing processes for better processing results.

Pequi fruit color analyses

Color is one of the attributes used in evaluating food quality; since visual analysis is one of the first felt to be used in choosing a food and also being a critical feature in choosing and accepting a product. Pequi fruit color coordinates presented significant differences for pulp, mesocarp and peel (Table 2). Coordinate L* represents how light or dark the fruit is, confirming pequi pulp is the lightest portion of the fruit, while the peel is the darkest. Pequi pulp presented 76.40 for coordinate L*, but Cordeiro et al. (2013) reported values between 48.65 and 60.08 in pequi pulp from the state of Mato Grosso, MT. Parameter a* ranges from green (-60) to red (+60); a* values for pequi pulp in this study were 25.65, lower than for pequi pulps from the state of Mato Grosso (37.27) and Goiás (85.5) (Cordeiro et al., 2013; Vera et al., 2005). Pequi pulp showed small incidence of red or green color due to the fact that the value found in this study is between the limit values of this parameter. Pequi mesocarp and peel present greenish color and low incidence of red color; thus, the values analyzed for pequi peel confirm the greenish or greenish brown color for the fruit and yellowish color for the internal mesocarp (Lima et al., 2007).

Parameter b*, ranging from blue (-60) to yellow (+60) shows that pequi pulp, mesocarp and peel do not present blue color; pequi pulp has strong yellow color, which contributes to its commercial attractiveness *in natura*.

Physicochemical characteristics of pequi

Table 3 shows the results of pequi physicochemical characterization, presented with significant differences between the values. Pequi pulp presented 54.40 g/100 g mean values of moisture. Vera et al. (2005) reported similar mean values of moisture while studying fruits from two regions of Goiás; 48.13g/100 g for fruits from the region of Mambaí, and 54.34 g/100 g for fruits from the region of Araguapaz. In this study, pequi pulp presented lower contents of moisture and higher contents of protein, ethereal extract and ash when compared to mesocarp and peel. However, the data confirm high contents of soluble solids in the mesocarp where the moisture content is greater.

In pulp, a high protein value of (3.02 g/100 g) was observed when compared to the peel (1.06 g/100 g). Machado et al. (2013) reported 2.83 g/100 g and 2.93 g/100 g protein content in pulp and endocarp of pequi fruits. However, Sousa et al. (2011) reported 9.64 g/100 g protein content in pequi pulp.

The ethereal extract values of 56.74 g/100 g for pulp, 3.57 g/100 g for mesocarp and 0.73 g/100 g for peel were found in this study. Machado et al. (2013) reported lower values for pequi pulp and endocarp (26.30 g/100 g and 19.13 g/100 g, respectively).The food chemical composition table shows that the value of ethereal extract from pequi compares to that of fruits like babassu and avocado (Franco, 1982). However, Oliveira et al. (2006)

Table 3. Mean and standard deviation values of moisture, protein, ethereal extract, ash, soluble solids, pH, titratable acidity and pulp reducing sugars, mesocarp and peel of pequi fruits (*C. brasiliense* Camb.).

Parameter	Pulp	Mesocarp	Peel
Moisture (g/100 g)	54.40 c ± 0.65	83.41 a ± 0.77	73.98[b] ± 0.28
Protein (g/100 g)	3.02 a ± 0.02	1.06 b ± 0.03	0.60[b] ± 0.35
Ethereal Extract (g/100 g)	56.74 a ± 8.05	3.57 b ± 0.49	0.73[b] ± 0,02
Ash (g/100 g)	0.67 a ± 0.02	0.41 a ± 0.25	0.63[a] ± 0.01
Soluble Solids (Brix)	7.33 b ± 1.53	10.0 a ± 0.00	8.90[ab] ± 0.17
pH	6.90 a ± 0.04	4.80 b ± 0.03	4.56[b] ± 0.26
Titratable Acidity (g/100 g)	1.52 a ± 0.22	5.37 b ± 0.57	6.60[b] ± 0.78
Reducing Sugars (g/100 g)	1.2 b ± 0.01	2.2 a ± 0.01	2.3[a] ± 0.01

Distinct small letters on the line present significant difference between each other through Tukey's test at 5% probability.

reported that pequi oil has predominantly unsaturated fatty-acids, which makes it an oil of excellent quality. An increasing proportion of ash (0.41 g/100 g) was found in pequi mesocarp peel (0.63 g/100 g) and pulp (0.67 g/100 g); unlike the values reported by Gondim et al. (2005) who said that pequi peels present important amounts of ash when compared to pulps. This fact may be related to the greater availability of minerals in pequi pulp, which is the most tasteful portion of the fruit. Sousa et al. (2012) and Oliveira et al. (2010) found lower values than those observed in this study for pequi pulp, 0.39 and 0.6%, respectively.

The contents of soluble solids for pulp, mesocarp and peel were 7.33, 10.0 and 8.90 °Brix, respectively. Lower values (15 °Brix) were found for ripe cerrado fruits, (Silva et al., 2009). For minimally processed and stored pequi, 12 °Brix was reported after a 15-day period (Damiani et al., 2008).

Significant differences were observed when pH of pequi pulp, mesocarp and peel were compared. Pequi pulp pH (6.90) was lower than the value reported by Pinedo et al. (2010) who found 7.36 pH for pequi pulps, higher than the values of 6.58 to 6.97 reported by Vera et al. (2005). According to Chitarra and Chitarra (2005), pH values increased with reduced titratable acidity as reported in this study when comparing pequi pulp, mesocarp and peel. Souza et al. (2013) reported that unlike other tropical fruits, pequi shows low acidity.

The values of titratable acidity were 1.52, 5.37 and 6.70 g titratable acidity/100 g for pulp, mesocarp and peel, respectively. These data showed significant differences. Titratable acidity of fruits may be influenced by sample preparation, harvesting time and ripening. Pequi fruits stored in modified atmosphere (Souza et al., 2007) presented lower values than those reported in this study (0.049g titratable acidity/100g).

This study found that the amount of carbohydrate for pequi pulp, mesocarp and peel were 1.2, 2.2 and 2.3 g/100 g, respectively. Sousa et al. (2011) reported 0.40 g/100 g carbohydrate values. Santos et al. (2010) reported 3.75 g/100 g of pulp reducing sugars in pequi fruits from the region of Barra do Bugres.

Analyses of total phenols and antioxidant activity of pequi fruits

Pequi peel showed considerable amount of total phenols, followed by pequi mesocarp and pulp with lower amounts (Table 4). Pequi peel presented 204.37 mg EAG/g for total phenols. In cerrado fruits analyzed by Roesler et al. (2007), pequi peel presented the greatest amount (209.37 ± 3.57 g GAE.kg $^{-1}$) of total phenols,

Pequi pulp presented a value of 26.55 mg EAG/g for phenolic compounds. However, Lima (2008) found greater values, 209 mg EAG/100 g, as well as Oliveira (2009) who found 104.12 mg EAG/100 g for *Caryocar coriaceum* species. According to Asami et al. (2003), the variation in values of phenolic compounds in the fruits is related to the use of solvents and extraction method. It is also associated with fruit species, cultivar, ripening and climate conditions. Roesler et al. (2008) reported that phenolic compounds are directly related to the antioxidant properties of the material and according to Vieira et al. (2011), a greater antioxidant activity reduces the EC_{50} value that is, a smaller amount of extract will be required to reduce the radical by 50%. Pequi pulp is rich in lipids and pequi trees are cultivated in places with solar ray incidence, factors that help generate free radicals and consequently, antioxidant properties (Lima et al., 2007).

Considering the results, pequi fruits present potential antioxidant effect, mostly concentrated on pequi peel, followed by its mesocarp and pulp. The antioxidant activity is influenced by sample storage, plantation conditions and preparation of extract and solvents employed. Machado et al. (2013) reported that the antioxidant activity of pequi fruits is related to the presence of polyphenols, carotenoids and vitamin C in fruits.

Table 4. Mean values of total phenols and antioxidant activity for pulp, mesocarp and peel of pequi (*C. brasiliense* Camb.).

Parameter	Pulp	Mesocarp	Peel
Total phenols (mg EAG/g)	26.55 ± 5.87	139.85 ± 7.71	204.37 ± 3.71
Antioxidant activity (EC_{50})	280.14 ± 5.77	197.69 ± 5.11	4.89 ± 2.82

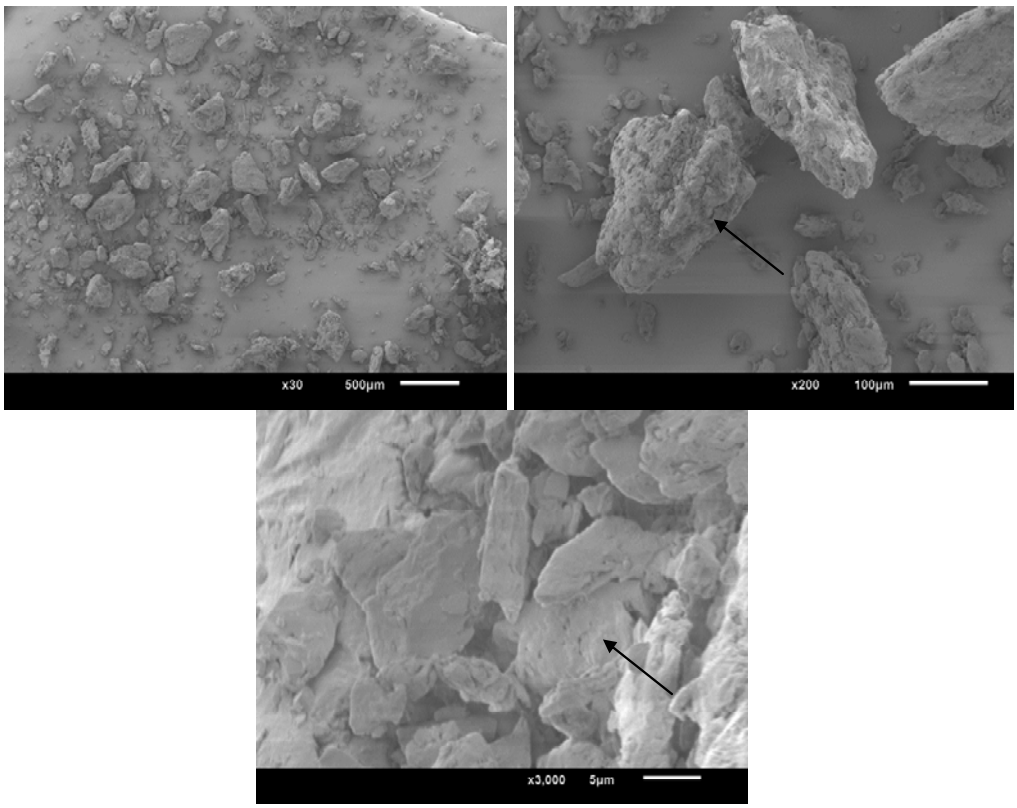

Figure 2. SEM images of pequi (*C. brasiliense* Camb.) mesocarp meal.

Scanning electron microscope (SEM)

The structural analyses of pequi mesocarp meal are illustrated in Figure 2. Degreased pequi mesocarp meal has a multiform, irregular and fibrous structure. However, the sample presented defined structures which were not observed for lyophilized pequi with sucrose treatment in which crystalline cores were created (Alves, 2007). Thestructural characteristics of the mesocarp suggest that its meal can have a high fiber content which may be used for human consumption. The presence of amorphous starch granules and fibrous structures was observed, as reported by Borba et al. (2005) in sweet potato meal.

The structural analyses of pequi peel are illustrated in Figure 3. Degreased pequi peel meal presented similar aspect to degreased pequi mesocarp meal analyzed in this study, therefore showing its fibrous characteristics. Fiorda et al. (2013) reports that starch granules have characteristic circular and concave-convex structure; however, fiber structures have geometric aspect with gaps and incidence of permeable pores that contribute to water absorption. The spherical and uniform structure and geometric surfaces confirm the values of reducing sugars found in this study, with pequi peel presenting the highest values when compared to the mesocarp and pulp; results that may be interesting for human nutrition.

The structural analyses of pequi pulp meal are illustrated in Figure 4. The analyses show that degreased pequi pulp meal also presented irregular aspect with granules, and surface with the presence of fibers, unlike the mesocarp meal which shows porous surface.

Essential oil in vegetal cells is retained in the cytoplasmic vacuoles. When the solvent flows during the degreasing process, these structures rupture due to mechanical or physicochemical damage and the essential

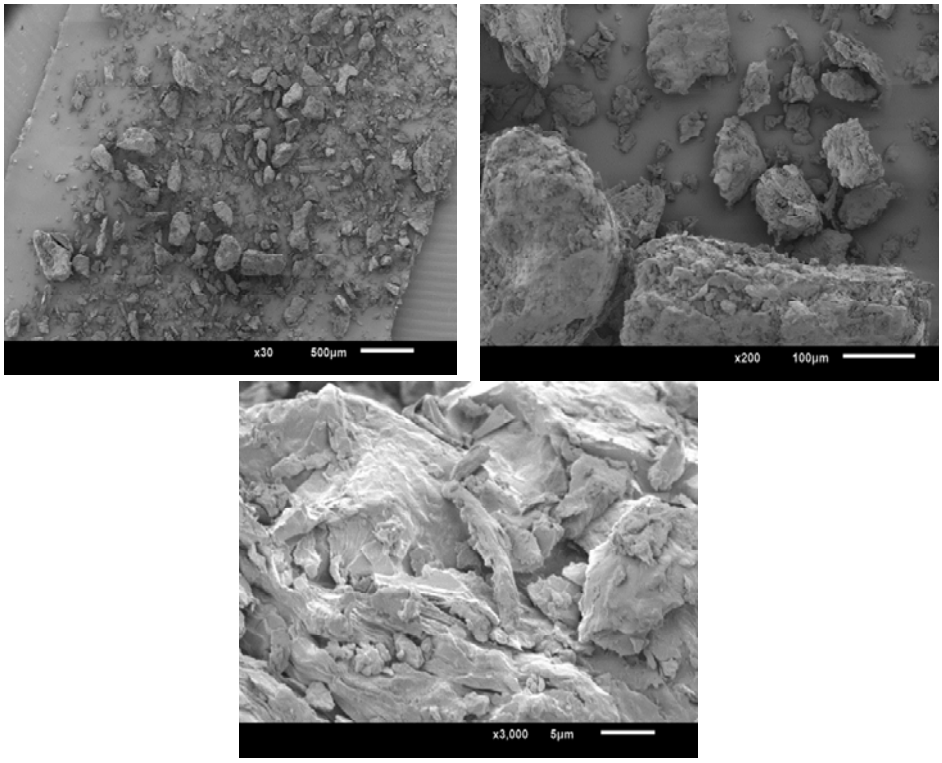

Figure 3. SEM images of pequi (*C. brasiliense* Camb.) peel meal.

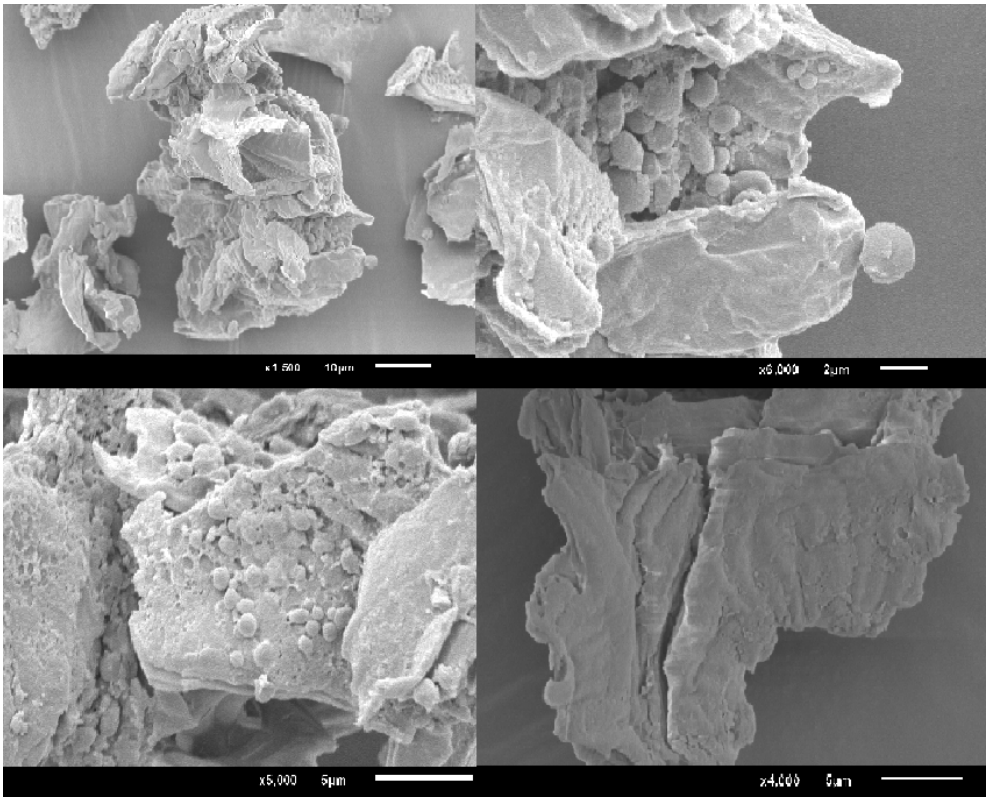

Figure 4. SEM images of pequi (*C. brasiliense* Camb.) pulp meal.

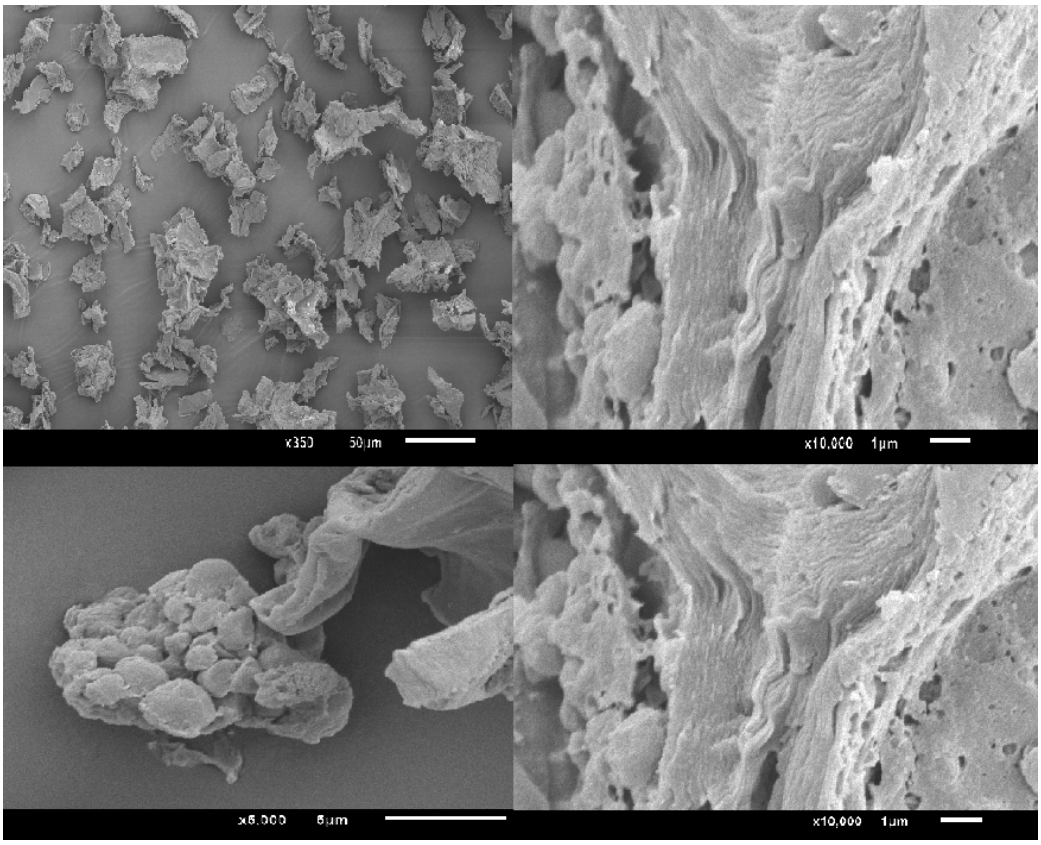

Figure 5. SEM images of pequi (*C. brasiliense* Camb.) pulp meal.

oil is released. Thus, the action of solvents used in meal degreasing may be analyzed (Figure 5) due to spaces among fibrous and starchy structures (HESS, 1975). This fact can be confirmed by analyzing the fat quantification in this study, which showed that the highest amounts of lipids are in pequi pulp and this would rupture the vacuoles of vegetal cells. Pequi pulp, with its pleasant sensory characteristics is excellent for human nutrition by adding fibers to human diet.

Conclusion

Pequi pulp, which can be used in innumerous dishes, has considerable amounts of minerals, lipids and proteins which are important nutrients for human nutrition. The high antioxidant effect of pequi pulp in relation to pequi mesocarp and skin shows the importance of this fruit to help maintain body health. However, pequi peel presented a high amount of phenols, which requires additional studies on peel utilization.

Conflict of Interest

The authors have not declared any conflict of interest.

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