

ORIGINAL ARTICLE

Nutritional potential of *Vasconcellea quercifolia* A. St.-Hil. green fruit flour

Potencial nutricional da farinha de frutos verdes de Vasconcellea quercifolia A. St.-Hil.

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Abstract

Non-conventional food plants have a variety of bioactive compounds with nutritional value. *Vasconcellea quercifolia* A. St.-Hil., belonging to the Caricaceae family, is a dietary alternative with excellent nutritional composition. This study aimed at characterizing the nutritional composition of mountain papaya (*V. quercifolia*) green fruit flour, in order to incorporate it in a functional food. For that purpose, the flour was characterized regarding its macro and micronutrients, anti-nutritional factors, pH, water activity, and color. This flour showed contents of carbohydrate of 22.31%; protein of 9.65%; dietary fiber of 32.80%; lipids of 14.95%, 63.56% of which are unsaturated fatty acids, especially oleic acid; and ash of 9.10%, with higher concentrations for potassium, calcium and magnesium. Therefore, *V. quercifolia* flour had good nutritional characteristics and might be used as supplementary food.

Keywords: Native fruits; NCFP's; Functional food; Dietary fiber; Nutritional; Nutrients.

Resumo

As plantas alimentícias não convencionais têm uma variedade de compostos bioativos com valor nutricional. O jaracatiá (*Vasconcellea quercifolia*) pertence à família Caricaceae e é uma alternativa dietética com uma excelente composição nutricional. Este estudo teve como objetivo caracterizar a composição nutricional da farinha de frutos verdes do jaracatiá (*V. quercifolia*), a fim de aplicá-la em um alimento funcional. Para isso, a farinha foi caracterizada em relação a macro e micronutrientes, fatores antinutricionais, pH, atividade da água e cor. Esta farinha apresentou teores de carboidratos de 22,31%; proteínas de 9,65%; fibra alimentar dietética de 32,80%; lipídios de 14,95%, dos quais 63,56% são ácidos graxos insaturados, especialmente ácido oleico; e cinzas de 9,10%, com maiores concentrações para potássio, cálcio e magnésio. Portanto, a farinha de *V. quercifolia* tem características nutritivas e pode ser utilizada como suplemento alimentar.

Palavras-chave: Frutas nativas; PANCS; Alimento funcional; Fibra dietética; Nutricional; Nutrientes.



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1 Introduction

Brazil houses approximately 20% of all biodiversity worldwide and has the richest flora in the world. However, due to cultural reasons, most agricultural activities in the country are based on exotic species, leading to the underuse of native plants, which, in turn, leads to low diversity of foods derived from this category (Kinupp & Lorenzi, 2014). However, native species have high phytochemical variety and nutritional value, which allows them to be economically exploited.

Most of them are part of a category that contemplates Non-Conventional Food Plants (NCFP's). Within this category, emphasis is placed on underused native fruits with peculiar characteristics and high contents of proteins, minerals, vitamins, dietary fibers, and bioactive compounds. Underutilized fruits refer to those species that have potential to help to enhance diets but are not yet relevant in global agriculture due to their limited competitiveness with other crops (Pacheco et al., 2018). Because of a long natural selection process, native species might have resistant genes to climatic changes and to certain phytopathogens, compared to exotic crops (Kinupp & Lorenzi, 2014).

Vasconcellea quercifolia A. St.-Hil. is a native arboreal species of Brazil, belonging to the Caricaceae family which, in turn, is comprised of six genera (*Carica*, *Cylicomorpha*, *Jacaratia*, *Jarilla*, *Horovitzia*, and *Vasconcellea*) and approximately 50 species. Popularly known as “mamãozinho-do-mato” or “jacaratiá” (mountain papaya), it has a wide occurrence in Brazil, from southern Bahia to Rio Grande do Sul, and might also be found in tropical and subtropical areas of Central America, South America, and Africa. Its flowering occurs from September to October and its fruiting occurs from January to March (Kinupp & Lorenzi, 2014).

This species is included in the list of NCFP, which integrates species with high potential for use in diets, although it might be currently underexploited, and even sometimes considered as weeds (spontaneous plants), and also, there is no large-scale cultivation in Brazil, however, it is a native species which is easily adapted to climatic conditions, with fast growth, easy cultivation and early fruit production. According to Kinupp & Barros (2008), its fruits had significant starch, protein, lipid, and dietary fiber contents, as well as potassium and nitrogen, which are the most abundant mineral composition.

Additionally, Folharini et al. (2019) evaluated green and ripe fruits of the species in physical-chemical terms and nutritional composition, concluding that green fruits had higher levels of protein and fiber, whereas ripe fruits had higher levels of ash and carbohydrates. However, more studies are required in order to discover their dietary importance, thus boosting production, marketing, and consumption beyond the regional scope.

In view of the Brazilian flora diversity and the possibility of using plants and their phytochemicals properties in the development of new biotechnological products, native species have become a potential concern for both health and agri-food industry. Thus, native plants are alternatives as new types of food that are not only nutritional, but also economically feasible. Therefore, in order to prize biodiversity, this study aimed to evaluate the food potential of flour obtained from the green fruits of *V. quercifolia*, as well as determining its chemical composition, techno-functional properties and nutritional characteristics.

2 Materials and methods

2.1 Flour preparation

Green fruits of mountain papaya (*V. quercifolia*) were collected from specimens of existing species in the municipalities of Arroio do Meio, Rio Grande do Sul (RS), Brazil (coordinates 29°24'0.7" S and 51°57'16.7" W) and Cruzeiro do Sul (RS), Brazil (coordinates 29°32'39.01" S and 52°3'15.31" W; 29°29'7.8" S and 52°0'51.8" W). Samplings were conducted in the periods ranging from January to March 2017, and from January to March 2018, which correspond to the species' fruiting period. After sampling, fruits were taken to the laboratory where they were mixed prior to preparing the flour.

The methodology proposed by Santos et al. (2015) was used to prepare the flour. Green fruits were selected first, and those with damaged parts or injuries were discarded. After that, they were weighed and then rinsed in three steps as following: the first step, with running water to remove dirt that was more conspicuous; the second step, submerging fruits in a 1% sodium hypochlorite solution (v/v); and the third step, with running water to remove residual chlorine. After that, they were longitudinally sliced and oven-dried with air circulation and renovation (Solab, SLA 102, Brazil) at 65 °C, until they reached constant weight. Dried fruits were grounded using an industrial blender and the flour obtained was weighed for calculating yield.

2.2 Proximate composition and energy value

V. quercifolia flour was analyzed regarding its centesimal composition according to standard protocols for analysis of food and all assays were performed. The flour sample was analyzed in triplicate on all tests, with the mean and standard deviation of the results being performed.

In accordance to Horwitz & Latimer (2012), fibers were analyzed according to methodology n° 991.43, the insoluble and soluble fractions of the dietary fiber were determined, as well as the total dietary fiber, which was obtained by adding the insoluble and soluble fractions, as recommended by the same method. Lipids were analyzed using the Mojonnier method (AOAC n° 2000.18), where 2.0 g of flour was extracted in 70 mL of hexane for 2 hours in total, after the lipid extract was dried, cooled and weighed. Ash was determined based on the incineration of 1.0 g of the sample in a muffle furnace at 550 °C (Quimis, Q318M), until the complete elimination of organic matter. At last the ashes were weighed (AOAC no 900.02).

Proteins were determined using Kjeldahl method (no 991.20) (Horwitz & Latimer, 2012), where 1.0 g of the sample was inserted in digester tubes, in which 20 mL of sulfuric acid and 5.0 g of catalytic mixture was added, after that, the sample was distilled by drag. Afterwards, the titration was performed to determine the protein content. Moisture was determined using a drying oven (De Leo, DL-SED) by evaluating water loss (105 °C) until complete drying and constant weight, both according to the Analytical Standards of Instituto Adolfo Lutz (2008). Carbohydrate content per 100 g of sample and energetic values were defined according to the equations described in Equations 1 and 2 (Brasil, 2003), respectively:

Equation 1 Carbohydrates:

$$\text{Carbohydrate \%} = 100 - (\% \text{moisture} + \% \text{ash} + \% \text{protein} + \% \text{lipids} + \% \text{fibers}) \quad (1)$$

Equation 2 Energy value:

$$\text{kcal / 100 g} = (\% \text{lipids} \times 9) + (\% \text{carbohydrates} \times 4) + (\% \text{proteins} \times 4) \quad (2)$$

2.3 Determination of lipid profile

In the methylation step, the extracted lipid residues were dissolved in 3.0 mL of chloroform and 3.0 mL of diethyl ether. After dryness, 2.0 mL of 7% (w / v) boron trifluoride in methanol and 1.0 mL of toluene were added. The mixture remained in an oven for 45 minutes at 100 °C with gentle stirring every 10 minutes. After cooling, 5.0 mL of water, 1.0 mL of hexane and about 1.0 g of sodium sulfate were added. The upper layer, containing the fatty acid methyl esters, was dried and filtered.

Fatty acids present in the lipid sample extracted were analyzed using Gas Chromatography coupled to Mass Spectrometry (GC-MS) (Shimadzu, QP-2010 Plus, Japan) with an Rtx-5MS (5% of diphenyl, 95% of dimethylpolysiloxane) column (30 m; 0.25 mm; 0.25 µm), oven temperature of 60 °C, injection volume 1.0 µL, and hexane as sample solvent. The integration of the methyl ester peaks obtained was compared with the Mass Spectral Database library (NIST/EPA/NIH, NIST11) and used to calculate the fatty acid concentration

present in the sample. The analysis was performed at least in triplicate ($n = 3$) and was expressed as the average \pm standard deviation.

2.4 Determination of minerals

For the determination of minerals, the methodology adapted from Altundag & Tuzen (2011) was used, in which the flour sample was digested in a wet basis, assisted by heating in a microwave oven (Anton Paar Multiwave PRO), and weighing 0.5 g of sample and 5 mL of nitric acid (ultrapure reagents using *sub-boiling* distillation). Heating ramp up to 180 ° C was made. After digestion, contents of phosphorus (P), selenium (Se), zinc (Zn), calcium (Ca), magnesium (Mg), nickel (Ni), iron (Fe), manganese (Mn), sodium (Na), and potassium (K) were determined using an Agilent 4200 microwave-induced plasma optical emission spectrometer (MIP OES) (Agilent Technologies, Melbourne, Australia), equipped with a OneNeb nebulizer and a mist chamber. Nitrogen used to maintain plasma was extracted from atmospheric air using a 4107 Nitrogen Generator (Agilent Technologies, Melbourne, Australia). Measurements were performed in triplicate at a pump speed of 15 rpm, inlet and settling time of 15 seconds each, reading time of 3 seconds, and using automatic correction of background signal by subtracting spectra from control and from samples. The analysis was performed at least in triplicate ($n = 3$) and was expressed as the average \pm standard deviation.

2.5 Determination of amino acids

The samples were hydrolyzed with 6 N hydrochloric acid for 24 hours. Thus, the released amino acids were reacted with Phenylisothiocyanate (PITC), separated by High-Performance Liquid Chromatography (HPLC) in reverse phase, and detected by ultraviolet (UV) at 254 nm. Quantification was performed by multilevel internal calibration, with the aid of Alpha-Aminobutyric Acid (AAAB) as an internal standard. The analysis was performed at least in triplicate ($n = 3$) and was expressed as the average \pm standard deviation.

2.6 Analysis of water activity, color, pH and anti-nutritional factors

Water activity was determined using an AquaLab equipment (Aqualab Lite - Decagon, Pullman, WA 99163, USA), where the sample was inserted in the equipment reader. Color parameters (L^* , a^* , b^*) were determined using a Konica Minolta CM-5 colorimeter (Chiyoda, Tokyo, Japan), in which the L^* -axis, with oscillation of 0 to 100, indicates the variation in color from black to white; the a^* -axis indicates variation from red (+) to green (-); and the b^* -axis indicates variation from yellow (+) to blue (-). For the determination of pH, 10.0 g of sample was diluted in 100 mL of water and analyzed using an electronic pH meter (Mettler Toledo, Seven Compact S220, Switzerland).

Anti-nutritional factors were also evaluated in the flour sample. Tannins were determined according to method n° 952.03 of AOAC (Horwitz & Latimer, 2012) and phytates were determined using the MA-CQ.179 method, based on the methodology by Latta & Eskin (1980). All analysis was performed at least in triplicate ($n = 3$) and was expressed as the average \pm standard deviation.

2.7 Analysis of the techno-functional and physicochemical properties

For the Water Absorption Index (WAI) and the Oil Absorption Index (OAI), 1 g of sample was weighed to form a suspension in 10 mL of water and oil, respectively, and these were subjected to mixing in a horizontal agitator for 3 minutes. After resting for 30 minutes, the suspensions were centrifuged at 700 g for 10 minutes; the supernatant was discarded and the wet precipitate was weighed. The indexes were obtained as the ratio between the weight of the supernatant and the weight of dry matter and expressed in g of water or oil absorbed per g of dry matter (Drakos et al., 2017).

The Water Solubility Index (WSI) was determined according to the methodology adapted from Wani et al. (2016), where 2.5 g of sample were dispersed in 25 g of distilled water, followed by mixing for 30 minutes. The mixture was centrifuged and more distilled water was added up to 32.5 g. This procedure was followed by centrifugation at 4,000 rpm for 15 minutes. Solubility index was determined as the ratio between the weight of solids dissolved in the supernatant and the dry weight of sample, multiplied by 100.

Bulk density was determined through the total mass of the sample that occupied a volume of 5 mL. True density was determined through the liquid displacement method, utilizing soybean oil as the immersion fluid (Pragati et al., 2014). Porosity was calculated utilizing the relation between bulk and true density, subtracted from 1, as given by Equation 3:

Equation 3 Porosity:

$$\text{Porosity \%} = (1 - \text{bulk density} / \text{true density}) \times 100 \quad (3)$$

Antioxidant activity was determined through the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, where 100 μL of previously prepared sample extract were added to 3.9 mL of a 0.06 mM DPPH methanolic solution. Absorbances were read at 515 nm after 60 minutes in a UV-Vis spectrophotometer (model SP-22, Biospectro, Brazil). Antioxidant activity was expressed as the percent free-radical inhibition efficiency (Rebey et al., 2019), given by $[(1 - \text{ABS sample}) / \text{ABS blank}] \times 100$.

All analyses were performed at least in triplicate ($n = 3$) and were expressed as the average \pm standard deviation.

3 Results and discussion

3.1 Flour yield

The *V. quercifolia* green fruit flour yield was 16.87%. Considering other flours with nutritional characteristics, this content was high when compared to the 9% yield of yacon potato flour (*Smallanthus sonchifolius*) reported by Rodrigues et al. (2011). Although yacon has considerable pulp, its yield flour was lower than the flour derived from *V. quercifolia* fruits found in the present study. On the other hand, green banana flour, known for its potential as functional food, had a yield of approximately 30% depending on the cultivar. This high value of yield is associated with the lower amount of water in the fruits, as they have lower moisture than others (Silva et al., 2015).

3.2 Chemical composition

A total dietary fiber of 32.80% was recorded in *V. quercifolia* green fruit flour (Table 1), indicating that the flour has promising nutritional values regarding human consumption. ANVISA's Resolution n° 54 (november 12, 2012) defines that food with functional properties must contain at least 3.0 g of fibers per 100 g of analyzed sample; it must also present metabolic or physiological activity that favors growth, development, and maintenance of the organism (Wendling & Weschenfelder, 2013; Brasil, 2012).

Moreover, the results found in the present study were similar to those found in papaya skin flour, which had 33.5% of fibers (Bokaria & Ray, 2016), thus emphasizing that this property is typical of the Caricaceae family. This content was even higher when compared to green banana flour, which might have from 6 to 15% of fiber in its composition (Santos et al., 2018). However, fiber concentration in mountain papaya flour was lower than on coconut flour, which presented approximately 60% of fiber (Trinidad et al., 2006). The fact that total dietary fiber content is higher than gross fiber content might be primarily due to cellulosic glucose, e.g. xylose. Additionally, all species compared above are exotic in Brazil, and therefore, the use of the native species *V. quercifolia* encourages the appreciation and preservation of local biodiversity. This result is also interesting as total fibers might have prebiotic effects in the growth of some bacteria beneficial to gut microbiota (Liutkevičius et al., 2016).

Furthermore, *V. quercifolia* flour had much higher lipid content (14.95%) (Table 1) than green banana flour (1.3%) (Silva et al., 2015) and papaya skin flour (0.1%) (Bokaria & Ray, 2016). However, other flours

with functional properties common in the daily human diet also had high lipid content, such as chia flour and soy flour, of 21.22% and 22.61%, respectively (Huerta et al., 2018). *V. quercifolia* could be compared to other plant-based flours with functional characteristics and might help if ingested as part of a lipid-rich diet.

Table 1. Chemical composition of *Vasconcellea quercifolia* flour.

Components	Composition (g/100 g of flour)
Total Dietary Fiber	32.80 ± 0.01
Lipids	14.95 ± 0.01
Ash	9.10 ± 0.14
Proteins	9.65 ± 0.41
Moisture	11.19 ± 1.03
Carbohydrate	22.31 ± 1.37
Energetic value (kcal/100 g)	262.39 ± 7.21

Values are mean ± standard deviation of three determinations (n=3). The results were determined on a wet basis.

Most of the lipid content in *V. quercifolia* flour is comprised of unsaturated fatty acids, due to the presence of oleic acid (C18:1n-9) as its majority element (58.22%) (Table 2). It is followed by a saturated acid, palmitic acid (C16:0), at 18.87%. Ijarotimi et al. (2013) described similar values in *Moringa oleifera* Lam. seed flour, in which the major compounds were the fatty acids: oleic acid (13.18%); palmitic acid (13.48%); and stearic acid (12.62%), which are also the most abundant fatty acids found in this study. It is important to emphasize the difference between saturated and unsaturated fatty acids in *V. quercifolia* flour, since saturated fatty acids inhibit the removal of Low Density Lipoproteins (LDL) from the plasma, whereas unsaturated fatty acids play a protective role, and might reduce LDL levels and triglycerides in the blood. Additionally, oleic acid might act in the prevention of rheumatoid arthritis due to changes in production of inflammatory response mediators (Ijarotimi et al., 2013).

Saturated fatty acids have different effects on the plasma concentration of lipoprotein cholesterol fractions, e.g. lauric (C12:0) and myristic acids (C14:0), which increase LDL cholesterol, whereas stearic acid (C18:0) has no effect (Food and Agriculture Organization of the United Nations, 2010). Although *V. quercifolia* flour has myristic acid, it occurs in low concentrations, and most of the lipid composition consists of unsaturated fatty acids (65%). Therefore, this product might help in the daily uptake of fatty acids that, in turn, help to reduce cholesterol and triglycerides in the organism. The ingestion of foods rich in essential fatty acids provide suitable nutritional components for several functions throughout the body, including development, brain activity, and basic physiological needs.

Table 2. Fatty acids identified in *Vasconcellea quercifolia* flour.

Fatty acids	Composition (g/100 g of lipids)
Myristic acid (C14:0) [§]	1.04 ± 0.04
Palmitoleic acid (C16:1)	3.05 ± 0.23
Palmitic acid (C16:0) [§]	18.87 ± 1.35
Oleic acid (C18:1n-9)	58.22 ± 3.56
Stearic acid (C18:0) [§]	8.55 ± 1.60
Gondoic acid (C20:1n-9)	2.29 ± 0.03
Behenic acid (C22:0) [§]	2.36 ± 0.37

Values are mean ± standard deviation of three determinations (n=3). [§]Saturated fatty acids. The results were determined on a wet basis.

Ash corresponds to 9.10% of flour chemical composition (Table 1). This value is similar to the content observed for other *in natura* fruit flour of same botany family (Santos et al., 2014). The major minerals found in this work were potassium and calcium (Table 3). An analysis of *Passiflora edulis* f. *flavicarpa* albedo flour showed that calcium, potassium, and magnesium were also majority minerals in passion fruit flour, with 1.36%, 3.14%, and 0.87%, respectively (Silva et al., 2019). It is worth noting that calcium, magnesium, and potassium contents obtained in *V. quercifolia* flour were lower than in flours of other native plants, and despite that, this food item does contribute with the daily uptake of minerals.

Table 3. Mineral composition of *Vasconcellea quercifolia* flour.

Minerals	Composition (ppm)
Selenium (Se)	0.04 ± 0.00
Calcium (Ca)	5.36 ± 0.12
Magnesium (Mg)	3.52 ± 0.25
Iron (Fe)	0.04 ± 0.00
Potassium (K)	26.25 ± 1.37

Values are mean ± standard deviation of three determinations (n=3). The results were determined on a wet basis.

The protein concentration found in *V. quercifolia* (Table 1) was higher than the one found by Olawuni et al. (2018) in green banana flour (3 g/100 g), which also has functional properties, and both flours are sources of plant proteins. Since some population groups still have a diet with limited access to animal proteins due to scarcity, consumption of protein-rich plant sources, such as *V. quercifolia* flour, could help to prevent or treat depletions related to this nutrient. The high content and quality of the protein from certain cereals promote antioxidant action, even if moderately, and can inhibit lipid peroxidation and act as a free-radical scavenging and metal chelator (Ijarotimi et al., 2013). Therefore, *V. quercifolia* flour can be a dietary source of proteins, as it provides approximately 9 g of proteins in each 100 g of its mass.

Among the amino acids that comprise these proteins (Table 4), the most abundant Essential Amino Acids (EAA) were lysine (1.52 g/100 g of protein), followed by leucine (0.93 g/100 g of protein), threonine (0.90 g/100 g of protein), and valine (0.89 g/100 g of protein). Leucine, isoleucine, and valine help the recovery of multiple trauma and burns, and they promote the reestablishment of normal metabolic processes when the liver is debilitated. On the other hand, lysine and leucine help in bone development, stimulate the immune system, and reduce triglycerides in the blood. Plant proteins are typically classified as having low biological value because they lack some essential amino acids. Cereals, for instance, are low in lysine, while legumes are low in methionine (Tinoco et al., 2015). However, this does not occur in *V. quercifolia* flour, as it is rich in both lysine and methionine, which helps to increase the biological value of this product.

Furthermore, the flour studied has a balanced concentration of non-essential amino acids. Among these non-essential amino acids, glutamic acid (3.03 g/100 g of protein), proline (1.96 g/100 g of protein), aspartic acid (1.42 g/100 g of protein), and arginine (1.36 g/100 g of protein) occurred in higher concentrations (Table 4). The values of glutamic acid in *M. oleifera* flour found by Ijarotimi et al. (2013) are similar to the ones found in the present study and it is also the most abundant non-essential amino acid, with 17.87 g/100 g. Additionally, Tinoco et al. (2015), studying pumpkin seed flour, reported that glutamic acid, arginine, and aspartic acid were majority amino acids, with 5.63 g, 4.91 g, and 2.94 g per 100 g, respectively. Thus, according to the obtained results, *V. quercifolia* flour is an important source of proteins for human beings, with important essential amino acids that can contribute with the daily uptake of these compounds and for the nutritional enrichment of foods.

Table 4. Essential and non-essential amino acids in *Vasconcellea quercifolia* flour.

Essential amino acids	Composition (g/100 g of protein)	Food and Agriculture Organization of the United Nations (2010) Composition (g/100 g of protein)
Lysine	1.52 ± 0.06	4.5 ± 0.05
Leucine	0.93 ± 0.12	5.9 ± 0.44
Threonine	0.90 ± 0.02	2.3 ± 0.01
Valine	0.89 ± 0.05	3.9 ± 0.37
Histidine	0.71 ± 0.02	1.5 ± 0.28
Isoleucine	0.69 ± 0.03	3.0 ± 0.06
Phenylalanine	0.62 ± 0.01	-
Methionine	0.03 ± 0.01	-
Non-essential amino acids	Composition (g/100 g of protein)	
Glutamic acid	3.03 ± 0.25	
Proline	1.96 ± 0.03	
Aspartic acid	1.42 ± 0.31	
Tyrosine	1.42 ± 0.06	
Arginine	1.36 ± 0.20	
Serine	1.00 ± 0.05	
Glycine	0.87 ± 0.14	
Alanine	0.87 ± 0.04	
Total AA	14.46	

Values are expressed as mean ± standard deviation of three determinations (n=3). The results were determined on a wet basis.

With an 11.19% content of moisture, the flour evaluated had higher value than coconut flour, with 3.6% (Trinidad et al., 2006). However, this result still lies within the range considered as safe to prevent microbiological growth in flours. Increased moisture and water activity in flours are related to the drying time of raw materials and to relative air humidity conditions, as well as to the temperature of the environment where it was stored (Alves et al., 2014).

V. quercifolia flour had 22.31% of carbohydrates and an energetic value of 262.39 kcal/100 g (Table 1). This carbohydrate value might be considered low compared to the value found in pequi (*Caryocar brasiliense* Camb.) flour (49%), which is also a native Brazilian species (Leão et al., 2017). Even so, *V. quercifolia* flour might help in the daily uptake of carbohydrates in a balanced diet, as this macronutrient acts as a primary source of energy for living organisms (glucose and starch), as plant structure and support (cellulose), or even as responsible for the sweet taste of fruits (sucrose and fructose). Most of these carbohydrates tend to be starch that resists degradation by α -amilase and are thus not digested in the small intestine. Indeed, it works as a substrate for microorganisms present in the colon of healthy individuals and might be considered a functional compound (Silva et al., 2015). Additionally, the lower carbohydrate content causes this flour to have lower energetic value (262.39 kcal /100 g) (Table 1) when compared to calories found in green banana flour (Santos et al., 2018). Fruits rich in fibers, carbohydrates, and lipids might be used in a functional diet as prebiotic products and to supplement foods with high nutritional value.

The flour of green fruit *V. quercifolia* had higher concentrations of chemical constituents when compared to the unripe fruit *in nature* evaluated by Folharini et al. (2019), except for the humidity, which the flour has a reduction of 78% in relation to fresh fruits. This difference in composition is associated with the drying process of the fruits for the preparation of the flour, which leads to a higher concentration of its constituents due to the loss of water. The maturation period of the green fruit, location and seasonality of the collection can also influence the phytochemical composition of the fruits.

3.3 Physicochemical analyses

V. quercifolia flour has an acidic character (pH 5.21 ± 0.02). This corroborates reports by Olawuni et al. (2018), who found values that might vary from 4.6 to 7.63 using green banana flour, depending on fruit ripening level. Additionally, both products derive from green fruits. There is no difference between the pH recorded for green fruits *in nature* (Folharini et al., 2019). The evaluation of acidity in post-harvest fruits is an important way to evaluate flour flavor, and pH is directly affected by ripening level.

The water activity value of $0.470 (\pm 0.002)$ in *V. quercifolia* flour is similar to that reported by Alves et al. (2014) studying jaboticaba (*Plinia jaboticaba* (Vell.) Berg) skin flour (approximately 0.42). The results of the present study lie below water activity values for the development of bacteria (0.90), yeasts (0.8), filamentous fungi (0.6), halophilic bacteria (0.65), and osmophilic yeasts (0.62).

In color parameters of *V. quercifolia* flour, its luminosity value $L^* = 40.53 (\pm 0.05)$ lies between the values found for pequi flour (Leão et al., 2017), with $L^* = 55$, and jaboticaba skin flour, with $L^* = 37$ (Alves et al., 2014). Luminosity variation is an important characteristic in product appearance; moreover, the darkening of food products might be an indication of organoleptic changes. *V. quercifolia* flour had a color that tends to yellow, because b^* value (24.33 ± 0.62) was higher than a^* (7.72 ± 0.08), which indicated the predominance of this coloration. Leão et al. (2017) also reported yellow coloration as being a characteristic of pequi flour. Flour color is evaluated, particularly, for measures of luminosity (L) and yellow intensity (+b). Luminosity is affected by bran content or foreign material, while yellow intensity is related to the amount of pigments in the raw material. This confirms that *V. quercifolia* flour had a yellow appearance and low luminosity, which is a characteristic of the green fruits of this species.

3.4 Anti-nutrients (tannins and phytates)

Tannins form complexes with proteins, thus rendering them insoluble and inactivating enzymes. Additionally, they can bind to macromolecules such as starch, causing a reduction of nutritional values in foods. Other negative effects are also attributed to tannins, such as undesirable color due to enzyme darkening reactions, astringency in taste, damages to intestinal mucosa, and interference in the uptake of iron, glucose, and vitamin B12 (Benevides et al., 2015).

The total tannin content found in the flour elaborated in this study was $1.97 \text{ g}/100 \text{ g} (\pm 0.035)$, similar to the result observed in canola bran ($1.9 \text{ g}/100 \text{ g}$) (Pena et al., 2010). One of the limitations of flours with high tannin concentrations as an alternative dietary ingredient can be their unpleasant astringent taste, which is caused by soluble tannin forming insoluble complexes with salivary proteins. In non-oxidized form, tannins interact with proteins through hydrogen bonds or hydrophobic interactions. The oxidation of tannins results in the formation of quinones, which can covalently bond to some functional groups of proteins. Tannin complexation with proteins turns them insoluble and promotes enzymatic inactivation. This reaction is the basis of this biological effect and is directly related to pH (Benevides et al., 2015). On the other hand, Ferreira & Arêas (2010) claimed that a tannin content of $1.3 \text{ g}/100 \text{ g}$ in amaranth flour did not impair the absorption of minerals such as calcium, meaning it should not disturb the bioavailability of minerals in this concentration, which also suggested that the tannin concentration in *V. quercifolia* flour would not affect

mineral absorption by the organism either, and may only result in a slightly astringent taste in food, but not enough to influence the main sensory characteristics.

Phytic acid, on the other hand, was reported with a lower concentration, 0.57 g/100 g (± 0.02), than the tannin content, and also lower than those reported by Hager et al. (2012) in quinoa, whole wheat, and buckwheat flours, which were, respectively, 1.34, 0.77, and 0.64 g/100 g; thus showing that the latter had higher phytate levels than *V. quercifolia* flour. The low concentration of this compound is a positive factor, as phytic acid content can still be significantly reduced by water soaking, germination and/or fermentation processes, which favor phytase activation. Phytate is considered an anti-nutritional factor because it has high chelating activity, which might decrease the bioavailability of certain elements, such as calcium, magnesium, iron, and zinc. It also negatively affects the absorption of other nutrients, such as amino acids, proteins, and starch (Hager et al., 2012).

3.5 Techno-functional and physicochemical properties

The WAI is an usual indicator of how flours may be incorporated in food formulations. The flour of green fruit of *V. quercifolia* showed a WAI of 4.46 g of water/g of sample (Table 5), this composition was similar to other vegetable flours, such as what was reported by Santana et al. (2017) for passion fruit flour (4.85 g/g). The water absorption of vegetable-based flours is usually attributed to the high dietary fiber content normally found in these flours (Santana et al., 2017).

Regarding the OAI, the flour of the present work showed a composition of 1.13 g of oil/g of sample (Table 5), inferior to the OAI found in two distinct brands of oatmeal flour (2.75 and 1.70 g/g) studied by Santana et al. (2017). This difference may be related to a larger presence of hydrophobic groups in the proteins present in oatmeal flour. In addition, high fiber content may prevent some oil absorption, which can explain the lower contents found in this work. High OAI determine whether or not the flour can be used in meat products or in emulsified products, such as cake batter, mayonnaise or salad dressings, soups, processed cheese, and meat extenders (Rodríguez-Ambríz et al., 2005).

The flour of *V. quercifolia* showed 12.33% of solubility in water, values that are close to the ones found for passion fruit (10%) and grape (13%) flours. However, this solubility was lower if compared with soybean, wheat, and oatmeal flours (Santana et al., 2017). Flours with a higher solubility index may be used in foods that require low temperatures during preparation or as ingredients in formulations that require more soluble ingredients (Santana et al., 2017).

Table 5. Techno-functional and physical-chemical properties in *Vasconcellea quercifolia* flour.

Parameters	Results
Water Absorption Index (WAI)	4.46 g/ g \pm 0.13
Oil Absorption Index (OAI)	1.13 g/ g \pm 0.03
Solubility index	12.33% \pm 0.23
Bulk density	0.35 g/ cm ³ \pm 0.02
True density	0.92 g/ cm ³ \pm 0.08
Porosity	63.09% \pm 1.26
Antioxidant activity	91.00% \pm 0.06

Values are expressed as mean \pm standard deviation of three determinations (n=3). The results were determined on a wet basis.

The density of a flour is directly related to its ability to absorb water. The flour evaluated in this work showed a bulk density of 0.35 g/cm³, and the presence of many empty spaces explains the low density.

According to Khan & Saini (2016), flours that display a higher bulk density, finer grain size, and a tendency to compact easily have a greater difficulty in absorbing water, which can be observed in flaxseed flour (0.47 g/cm³). The value found for the true density of the *V. quercifolia* flour was 0.92 g/cm³, lower than the one found by Khan & Saini (2016) for the flaxseed flour (1.77 g/cm³). This difference may be due to the flour processing steps or the origin of the matrix, which may vary and result in more or less spherical particles, thus reducing the empty space between them.

Porosity is a measure of the voids between the solid particles of a material. Pores can be filled by fluids, including gas or water. Air-filled porosity allows gases to move within the material. The flour of *V. quercifolia* showed a porosity of 63.09%, a result similar to that described by Khan & Saini (2016) for wheat flour (64.44%), but lower than flaxseed flour (73.01%).

Antioxidants can act at different stages of the oxidative process in food and in cell membranes. In biological systems, antioxidants protect against oxidation, preventing diseases associated to oxidative stress (Sardarodiyani & Sani, 2016). The flour analyzed in this study showed an antioxidant activity of 91%. The DPPH scavenging activity in *V. quercifolia* flour, expressed as IC₅₀, was 150 µg/mL for 50% anti-radical action. The IC₅₀ found was lower when compared to amaranth flour (294-317 µg/mL) (Karamać et al., 2019), thus highlighting a larger antioxidant capacity of the flour of green fruits of *V. quercifolia* compared to other flours and at lower contents.

4 Conclusion

Green mountain papaya (*V. quercifolia*) fruit flour had high protein, carbohydrate, and fiber contents, as well as the presence of some amino acids and minerals, thus presenting nutritional characteristics suitable for human consumption. The nutrient profile, especially the fiber content, points to the potential of using the flour in food formulations and biotechnological products as a substrate for probiotics, for example, serving as a carbon source for cell maintenance.

Furthermore, *V. quercifolia* is an underutilized native species in Brazil, with potential use in human diet, and is favored by its easy propagation. Therefore, this study aimed to promote an insertion of a native plant species in the regional economy, valuing native flora and encouraging the use of NCFP's. More research is required in order to discover its food importance, boosting production and consumption, in addition to regional uses and fresh consumption of NCFP's. The present study indicated the applicability of this native fruit in the development of new products with significant nutritional value.

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