Nutritional profile, bioactive compounds and antioxidant capacity of jatobá-da-mata (Hymenaea courbaril, var. stilbocarpa) by product

Perfil nutricional, compuestos bioactivos y capacidad antioxidante de subproductos del jatobá-da-mata (Hymenaea courbaril, var. stilbocarpa)

ABSTRACT
Jatobá (Hymenaea sp.) is an arboreal legume native to the Brazilian Savannah (Cerrado) and its fruit has a high functional potential, but the jatobá-da-mata specie has been poorly explored. This study evaluated the nutritional profile, bioactive compounds and antioxidant capacity of jatobá-da-mata byproducts: pulp flour, fibrous pulp residue and sap. Jatobá fruits were pulped to obtain pulp flour and fibrous pulp residue, and the jatobá tree’s sap was obtained in a typical extraction location in the Cerrado (Brazilian Savannah). Fibrous pulp residue and pulp flour had high protein (11 and 12 g/100 g) and dietary fiber (49 and 44 g/100 g) content, respectively, and the fibrous residue showed the highest total and insoluble fibers, ash and vitamin C content. Regarding polyphenols, the fibrous residue showed a high concentration (775 mg GAE/100 g); an intermediate value in pulp flour (462 mg GAE/100 g); and considerable content in sap (181 mg GAE/100 g). Jatobá residue and pulp flour are suitable ingredients for the formulation of functional foods, and the sap is a promising non-caloric product with potential health benefits.

Keywords: Antioxidants; Ascorbic acid; Bioactive compounds; Fibers; Phenolics.

RESUMEN
Jatobá (Hymenaea sp.) es una leguminosa arbórea originaria de la sabana brasileña y su fruto tiene un alto potencial funcional, pero la especie jatobá-da-mata ha sido poco explorada. El objetivo del estudio fue evaluar el perfil nutricional, compuestos bioactivos y capacidad antioxidante de subproductos del jatobá-da-mata: harina de la pulpa, residuo fibroso de la pulpa y la savia. Los frutos fueron despulpados para obtener la harina juntamente con el residuo fibroso de la pulpa, y la savia de la planta fue obtenida en un lugar exclusivo de extracción en el Cerrado (Sábana brasileña). El residuo fibroso y la harina de la pulpa contienen un alto contenido de proteína (11 y 12 g/100 g) y de fibra dietética (49 y 44 g/100 g), respectivamente, y el residuo fibroso mostró el mayor contenido de fibras totales y insolubles, cenizas y vitamina C. En cuanto a los polifenoles, el residuo fibroso presentó alta concentración (775 mg AGE/100 g); la harina de la pulpa, un valor intermedio (462 mg AGE/100 g); y la savia, un contenido considerable (181 mg AGE/100 g). El residuo fibroso y la harina de la pulpa son ingredientes adecuados en la formulación de alimentos funcionales, y la savia es un producto no calórico prometedor con potenciales beneficios a la salud.

Palabras clave: Ácido ascórbico; Antioxidantes; Bioactivos; Compuestos Fenólicos; Fibras.

INTRODUCTION
The Cerrado (Brazilian Savannah) is the second largest Brazilian biome and contains a diverse flora, with a high...
potential for healthy functional foods and nutraceuticals. Although many native species have been identified, there is little information about their nutritional and functional potential. One of the plants widely distributed all over the Cerrado area is the jatobá (Hymenaea sp.), an arboreal legume belonging to the Fabaceae or Leguminosae family and to the Caesalpiniaeeae subfamily. Jatobá can be found in the Brazilian states of Piauí, Bahia, Goiás, Minas Gerais, Mato Grosso do Sul and São Paulo.

The fruit of the species *H. stigonocarpa* Mart., popularly known as jatobá-do-cerrado, has been the most researched. Likely the sweeter and smoother flavor of the pulp compared to other species is associated with its wide distribution in the Cerrado. In addition, the smaller size of the tree (up to 10 m) has likely influenced the research with this species. By contrast, jatobá-da-mata (*H. courbaril* L., var. stilbocarpa) reaches 15 to 20 m height and can be found in the most fertile lands of the biome, the forests of the Cerrado, which justify its name.

The pulp is the jatobá byproduct used most by the Cerrado population. It is farinaceous and slightly sweet with a peculiar flavor, usually consumed in nature and as an ingredient of several dishes. The fibrous pulp residue is the residue retained in the sieve after sifting the pulp flour, and jatobá sap is obtained by bleeding directly from the trunk of the plant. Neither byproduct is usually consumed, but the sap is popularly used in folk medicine because of its alleged medicinal properties, such as anti-anemic, anti-inflammatory and as a remedy for stomach ulcers. Although research on its therapeutic effects is scarce, it was reported that the sap of *H. courbaril* is an efficient inhibitor of genotoxic action.

There is little knowledge about the nutritive and bioactive profiles of jatobá pulp. Silva et al. reported significant amounts of dietary fibers and minerals, such as calcium and magnesium, and carotenoids, mainly lutein, in jatobá-do-cerrado (*Hymenaea stigonocarpa* Mart.) pulp. As far as we know, there is no similar information in the literature about the fibrous pulp residue and the sap of any species of the genus *Hymenaea*.

The consumption of antioxidant-rich foods can enhance enzyme activity, prevent the oxidation of low-density lipoproteins (LDL), act on oxidation-related initiation, and promotion and tumor progression processes, thus reducing the risk of chronic diseases. The antioxidant potential of the pulp of jatobá-do-cerrado (*Hymenaea stigonocarpa* Mart.) was investigated by Arakaki et al., who reported high phenolic acid and tannin content, and antioxidant activity estimated by DPPH assay. However, jatobá-da-mata (*Hymenaea courbaril*, var. stilbocarpa) has still been little explored in the scientific literature concerning the nutritive and bioactive properties of its pulp. Thus, the objective of this work was to investigate the nutritional profile, bioactive compounds and the antioxidant capacity of byproducts of jatobá-da-mata, a fruit native to Goiás State, Brazil.

MATERIALS AND METHODS

Plant material and sample preparation

Jatobá-da-mata (*Hymenaea courbaril* L., var. stilbocarpa) fruits were collected in the harvest period (August-September), in Senador Canedo City, Goiás State, Brazil. Fruits with the shell intact were selected, packed in cotton bags and transported for later sanitization and storage. The fruits were peeled and the pulp was extracted in a domestic multiprocessor for 30 s, and the contents were sieved to obtain the flour. The fibrous residue retained in the sieve was roasted using a conventional electric oven at 70°C for 60 min and then ground in an analytical mill (IKA, model A-11). Jatobá-da-mata sap was purchased from a typical extraction location in the Cerrado area, situated in the rural region of the city of Pirenópolis, Goiás State. Pulp flour, fibrous pulp residue and sap samples (Figure 1) were stored at -18°C until analysis. A density of 1.14 g/mL was considered for weighing the sap.

Figure 1: Jatobá-da-mata and its byproducts: A - jatobá-da-mata fruit; B - pulp flour; C - untreated and D - roasted and ground fibrous pulp residue; E - sap.

Proximate composition and energy value

Moisture content was determined by drying at 105°C, and total nitrogen content was analyzed according to the micro-Kjeldahl method and then converted to crude protein using the 6.25 factor. Total fat content was analyzed by the Bligh and Dyer method. The total soluble and insoluble dietary fibers were determined by an enzymatic, gravimetric, standard method. Ash analysis was conducted by incineration in a muffle
The standard used for all three methods was a methanolic ABTS+ free radical capture assay, as described by Re et al.\textsuperscript{15}; the Fe\textsuperscript{3+} reduction (FRAP) by Benzie and Strain\textsuperscript{16}, and ABTS+ free radical capture assay, as described by Re et al.\textsuperscript{17}. The standard used for all three methods was a methanolic solution of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) at different concentrations. The results were expressed in μmol of Trolox equivalent per gram of fresh sample.

**Vitamin C**

Vitamin C was analyzed according to Tillman’s method described by the Instituto Adolfo Lutz\textsuperscript{19}. Aliquots of pulp flour and fibrous pulp residue were homogenized with 60 mL of acid solution (15 g of metaphosphoric acid, 40 mL of acetic acid and 450 mL of distilled water) in a magnetic stirrer (LS Logen Scientific, Stirring hot plate model) for 2 min, and the mixture was filtered. A 30 mL aliquot of the sap was homogenized with the same volume of acidic solution and filtered. From the filtrate of each sample, a new aliquot of 10 mL was taken and 10 mL of the same acid solution was added in triplicate and titrated with Tillman’s solution.

**Extraction procedure**

The extraction was carried out according to the method described by Silva et al.\textsuperscript{2}, with modifications. To obtain the extract, 300 mg of pulp flour and fibrous residue were homogenized with 25 mL of 60% acetone in distilled water (v/v), using a magnetic stirrer (LS Logen Scientific, Stirring hot plate model), for 30 min at room temperature. These mixtures were then centrifuged at 15,000 x g at 4 °C for 15 min. The supernatant was collected and the volume completed to 25 mL with 60% acetone. Jatobá sap (900 mg) was dissolved in 30 mL of the same solvent. The extracts were used for the total phenolics and antioxidant capacity analyses.

**Total phenolic compounds**

The total phenolic compounds analysis was carried out using the Folin-Ciocalteau reagent, according to the procedure described by Singleton and Rossi\textsuperscript{21}. Sample extracts (0.25 mL), in triplicate, were added to 0.25 mL of Folin-Ciocalteau reagent and 2.5 mL of distilled water, shaken for 10 s in a vortex and rested for 10 min. Subsequently, 0.25 mL of 10% sodium carbonate solution was added, and the solution was shaken for 10 s and rested for 60 min. The absorbance was measured at 765 nm using a UV/Vis spectrophotometer (Jasco, model V-630, Tokyo, Japan). The results were expressed as mg of gallic acid per 100 g of fresh sample.

**Antioxidant capacity**

The antioxidant capacity was performed in vitro using three methods: DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical sequestration assay, according to Brand-Williams et al.\textsuperscript{3}; the Fe\textsuperscript{3+} reduction (FRAP) by Benzie and Strain\textsuperscript{16}, and ABTS+ free radical capture assay, as described by Re et al.\textsuperscript{17}. The standard used for all three methods was a methanolic solution of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) at different concentrations. The results were expressed in μmol of Trolox equivalent per gram of fresh sample.

**Statistical analysis**

The results were expressed as mean ± standard deviation of three replicates. The data analyzed using Analysis of Variance and Tukey test for mean comparisons. Pearson’s correlation coefficient was used to evaluate the influence of vitamin C and phenolic contents on antioxidant capacity, and the correlation between the results of DPPH, FRAP and ABTS assays. For all analyses, a 5% probability was considered statistically significant. Statistical calculations were performed using Action software (Estatcamp Consultoria Estatística, São Carlos, Brazil).

**RESULTS**

Fibrous pulp residue showed higher energy, lipid, total and insoluble fiber and ash compared to pulp flour. Fibrous residue and pulp flour had high dietary fiber (49 and 44 g/100 g), mainly insoluble fiber, and protein (11 and 12 g/100 g) content, respectively. Sap presented insignificant amounts of these nutrients because it is a fluid containing 99% moisture, thus, its energy production is inexpressive and it can be considered a non-caloric product (Table 1).

Fibrous pulp residue also had the highest vitamin C concentration, total phenolic content and antioxidant capacity (by DPPH, FRAP and ABTS assays) compared to the other jatobá byproducts analyzed. The sap had the lowest results, except for the antioxidant capacity by ABTS test, having a value similar to that of pulp flour (Table 2). There was a high and positive correlation between antioxidant compounds (vitamin C and phenolics) and antioxidant capacity of the samples. In addition, a strong and positive correlation was observed between the antioxidant capacity assays (Table 3).

**DISCUSSION**

Pulp flour and fibrous pulp residue of jatobá-da-mata are rich in dietary fibers, including soluble fiber, and polyphenols, and have a relatively low calorie count. The fibrous residue is also a good source of vitamin C and has a high antioxidant capacity. Jatobá tree’s sap is a non-caloric fluid, with important amounts of polyphenols and considerable antioxidant capacity. No study on the nutritional and bioactive properties of the jatobá fibrous pulp residue and sap were found in literature, thus, these data are being reported for the first time in the present study.

The high dietary fiber content of pulp flour and fibrous pulp residue (44 and 49 g/100 g), and their considerable amounts of soluble fibers (around 20% of the total fiber), are comparable to those reported for jatobá-do-cerrado pulp flour of 44.3 g/100 g\textsuperscript{2} and 60.6 g/100 g\textsuperscript{2}. These fiber concentrations are higher than those of other fiber-rich foods, such as whole cereals (approximately 10 g/100 g)\textsuperscript{2}.

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Table 1. Proximate composition and total energy value of jatobá-da-mata byproducts: pulp flour, fibrous pulp residue and sap.

<table>
<thead>
<tr>
<th>Component (g/100g)</th>
<th>Pulp flour</th>
<th>Fibrous pulp residue</th>
<th>Sap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>16.5b ± 0.10</td>
<td>8.64c ± 0.04</td>
<td>99.0a ± 0.10</td>
</tr>
<tr>
<td>Protein</td>
<td>11.5a ± 0.19</td>
<td>10.5b ± 0.38</td>
<td>Trace</td>
</tr>
<tr>
<td>Lipids</td>
<td>4.53b ± 0.06</td>
<td>7.66a ± 0.13</td>
<td>Trace</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>20.9a ± 0.23</td>
<td>20.6a ± 0.25</td>
<td>Trace</td>
</tr>
<tr>
<td>Total dietary fiber</td>
<td>44.1b</td>
<td>48.7a</td>
<td>&lt; 0.1c</td>
</tr>
<tr>
<td>Insoluble fiber</td>
<td>34.8b</td>
<td>39.5a</td>
<td>&lt; 0.1c</td>
</tr>
<tr>
<td>Soluble fiber</td>
<td>9.3a</td>
<td>9.2a</td>
<td>Not detected</td>
</tr>
<tr>
<td>Ash</td>
<td>2.45b ± 0.03</td>
<td>3.87a ± 0.03</td>
<td>0.08c ± 0.001</td>
</tr>
<tr>
<td>Energy value (kcal/100g)</td>
<td>170b ± 0.61</td>
<td>193a ± 0.65</td>
<td>Trace</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation (n= 3). Results are expressed on wet basis. In the same row, different letters indicate difference in means by the Tukey test (p ≤ 0.05). The term “trace” indicates values lower than 0.06, according to Greenfield and Southgate.

Table 2. Antioxidant compounds and antioxidant capacity determined by different methods in jatobá-da-mata byproducts: pulp flour, fibrous pulp residue and sap.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Antioxidant compounds</th>
<th>Antioxidant capacity (µmol TE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vitamin C (mg/100g)</td>
<td>Total phenolics (mg GAE/100g)</td>
</tr>
<tr>
<td>Pulp flour</td>
<td>6.68b ± 0.32</td>
<td>462b ± 12.5</td>
</tr>
<tr>
<td>Fibrous pulp residue</td>
<td>53.5a ± 2.55</td>
<td>775a ± 6.08</td>
</tr>
<tr>
<td>Sap</td>
<td>0.10c ± 0.01</td>
<td>181c ± 3.09</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation (n= 3). Results are expressed on wet basis. GAE: Gallic acid equivalent; TE: Trolox equivalent. In the same column, different letters indicate difference in means by the Tukey test (p ≤ 0.05).

Table 3. Pearson correlation coefficients between antioxidant compounds and antioxidant capacity, and between three antioxidant capacity assays of jatobá-da-mata byproducts: pulp flour, fibrous pulp residue and sap.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Antioxidant compounds</th>
<th>Antioxidant capacity assays</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vitamin C</td>
<td>Phenolics</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Phenolics</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>DPPH</td>
<td>0.932*</td>
<td>0.999*</td>
</tr>
<tr>
<td>FRAP</td>
<td>0.899*</td>
<td>0.984*</td>
</tr>
<tr>
<td>ABTS</td>
<td>0.993*</td>
<td>0.886*</td>
</tr>
</tbody>
</table>

* Significant Pearson correlation (p < 0.05).
edible seeds and nuts (8 to 14 g/100 g); and baru pulp (18 g/100 g), another arboreal legume native to the Cerrado (Dipteryx alata Vog.)

Therefore, the pulp flour and the pulp fibrous residue of jatobá-da-mata are byproducts with high potential for the formulation of healthy foods. They are potential ingredients to be used in different food systems, aiming to increase fiber intake, for instance, to regulate bowel functioning. Insoluble fiber assists in intestinal function, as it increases fecal volume through water retention and decreases intestinal transit time. The soluble fibers are also known as functional compounds because of their physiological effects, such as decreasing the glycemic response and plasma cholesterol, as well as its prebiotic action22.

In addition to the health benefits of fiber-rich food consumption, the foods with high concentration of fiber usually have low calories, as observed in jatobá-da-mata byproducts (Table 1). Jatobá-da-mata pulp flour and fibrous residue presented energy values equivalent to those reported for jatobá-do-cerrado pulp flour: 153 kcal/100 g2 and 193 kcal/100 g4. The calorie content of flours usually used in the formulation of bakery products19 are higher than that of the jatobá pulp flour. Considering the high prevalence of overweight and obesity worldwide, and obesity-related diseases, such as cardiovascular diseases, diabetes and cancer23, the use of natural and alternative low calorie byproducts is an important strategy for public health. In addition to low caloric concentration, jatobá-da-mata pulp and fibrous residue presented higher protein contents than those of jatobá-do-cerrado pulp flour (1.7 to 8.1 g/100 g)1,2,4,24, and baru pulp flour (3.2 g/100 g)21.

Lipid content of pulp fibrous residue is higher than that reported for jatobá-do-cerrado pulp flour (3.8 g/100 g)3, but the pulp flour lipid concentration is similar (Table 1). Ash concentrations of jatobá-da-mata byproducts are compatible with the values reported in the literature for jatobá-do-cerrado pulp flour1,2,4,24, mainly in fibrous pulp residue (around 4 g/100 g). This ash content indicates high mineral concentration; therefore, the analysis of minerals in jatobá-da-mata pulp byproducts is recommended. Calcium and magnesium content stood out from other minerals in jatobá-do-cerrado pulp flour – 249 mg/100 g and 135 mg/100 g, respectively1.

Apart from the high fiber and ash content, and relatively low calories, the fibrous pulp residue showed a high concentration of vitamin C (Table 2), having a value that is close to those of citruseous fruits, such as oranges (45 to 59 mg/100 g)19. The relatively low vitamin C value of the pulp flour is compatible with the value reported for jatobá-do-cerrado pulp flour (8.9 mg/100 g)4.

The fibrous residue of jatobá-da-mata flour had a high polyphenol concentration, which is close to jatobá-do-cerrado flour content (786 mg GAE/100 g)1. Jatobá-da-mata flour is a good source of polyphenols, having a concentration which is close to that reported for hydrocetic extract of jatobá-do-cerrado flour, of 536 mg GAE/100 g, and higher than that of baru pulp flour (292 mg GAE/100 g)21. Furthermore, the sap phenolic content can be comparable to the values reported for other Cerrado fruits, such as cashew (Anacardium occidentale) (184 mg GAE/100 g) and mamá-cadela (Brosimum guauchae) (177 mg GAE/100 g)23, and higher than those of cagaita, chichá, cajuí and macaúba24. Therefore, jatobá-da-mata byproducts analyzed in the present study represent good options for healthy foods and diets, especially the sap, considering it is a non-caloric product.

Jatobá-da-mata byproducts showed higher antioxidant capacity by DPPH assay than jatobá-do-cerrado flour (1.44 to 14.66 μmol TE/g), which was analyzed by different extracts2. Considering FRAP results, the pulp flour and fibrous residue presented higher values than those reported for jatobá-do-cerrado flour through methanol 60% (15.32 μmol TE/g) and water (28.65 μmol TE/g) extracts2, and the buriti (Mauritia flexuosa L.f.), a fruit native to the Amazon biome (26.95 μmol TE/g)26. However, in a study by Silva et al.2, the antioxidant capacity of 60% acetone extract of jatobá-do-cerrado pulp was higher (106.40 μmol TE/g) than those obtained in the present study for jatobá-da-mata pulp byproducts. Regarding ABTS results, there was no difference between the antioxidant capacities of the pulp flour and sap (Table 2), which values are higher than those of other Cerrado fruits, such as jenipapo (7.31 μmol TE/g) and sweet passion fruit (10.84 μmol TE/g)27.

The fibrous pulp residue had the highest antioxidant capacity among the jatobá-da-mata byproducts analyzed, which is higher than those of the Cerrado fruits previously mentioned, including murici (57.25 μmol TE/g)27.

Pearson’s correlation coefficients were statistically significant (Table 3), which indicated that vitamin C and total phenolic compounds were associated with the antioxidant capacity of jatobá-da-mata byproducts. The DPPH, FRAP and ABTS methods can be considered equivalent to estimate the antioxidant capacity of the samples. Moreover, the correlation between total phenolic compounds and DPPH, and between vitamin C and ABTS were the strongest among the three methods tested, which suggests that these assays were more sensitive to evaluate the antioxidant capacity of these bioactive compounds in jatobá byproducts.

Epidemiological studies have associated the consumption of polyphenol-rich foods with reduced risk of developing several chronic diseases, including cardiovascular diseases, type 2 diabetes, cancer and neurodegenerative disorders. Moreover, prebiotic properties of phenolic compounds have been recognized and the relationship of these properties with the prevention and management of obesity28. Nevertheless, studies on phenolic compounds, bioaccessibility and bioavailability of the functional foods, such as the byproducts of jatobá-da-mata, are necessary to confirm the action of these compounds in the human organism.

Jatobá pulp is crushed and usually sifted to produce the pulp flour used in the formulation of bakery products by the Cerrado population. This procedure wastes the fibrous residue of the pulp, decreasing the nutritional and functional values of jatobá pulp flour. Thus, we suggest investigating the
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antioxidant compounds profiles of the pulp flour with the fibrous residue, and in vivo studies on the potential health benefits of jatobá-da-mata byproducts. Research in this field will certainly add value to this species and contribute to the preservation of the Cerrado flora.

CONCLUSION
Fibrous pulp residue is a healthy ingredient with high fiber concentration, polyphenols content and antioxidant capacity, and has relatively low calories. Considering these attributes, we recommend the use of jatobá pulp flour with the fibrous pulp residue in the formulation of bakery products. The sap is a non-caloric fluid with considerable amounts of phenolic compounds and antioxidant capacity, and its popular use as a medicinal product should be investigated. The nutritive and bioactive profiles of jatobá-da-mata byproducts indicate that they are promising ingredients for the formulation of functional foods and nutraceuticals.

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