

# 'Jatobá do cerrado' (*Hymenaea stigonocarpa*): chemical composition, carotenoids and vitamins in an exotic fruit from the Brazilian Savannah

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## 'Jatobá do cerrado' (*Hymenaea stigonocarpa*): chemical composition, carotenoids and vitamins in an exotic fruit from the Brazilian Savannah.

**Abstract – Introduction.** The fruits of the Brazilian Savannah have potential to improve the human diet, income generation and, consequently, the quality of life for socially vulnerable families. Among the native fruits of the Savannah, one that stands out is the 'jatobá do cerrado' (*Hymenaea stigonocarpa*). Therefore, the physical characteristics, chemical composition (titratable acidity, soluble solids, pH, moisture, ash, proteins, lipids and total dietary fiber), occurrence and content of vitamin C (ascorbic and dehydroascorbic acids), carotenoids ( $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin and lycopene), vitamin E ( $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ -tocopherols and tocotrienols) and folates (tetrahydrofolate, 5-methyltetrahydrofolate and 5-formyltetrahydrofolate) were evaluated in pulp of 'jatobá do cerrado' from the Brazilian Savannah. **Materials and methods.** The length, diameter, mass and fruit yield were evaluated. The titratable acidity was determined by volumetric neutralization; pH by potentiometry; soluble solids by refractometry; moisture using an oven; ash using a muffle furnace; proteins by the micro-Kjeldhal method; total dietary fiber by the gravimetric non-enzymatic method; and lipids with a Soxhlet extractor. Vitamin C and carotenoids were analyzed by HPLC-DAD, and vitamin E and folates by HPLC with fluorescence detection. **Results and discussion.** The 'jatobá do cerrado' presented low pulp yield (17.1%) and moisture (8.8 g·100 g<sup>-1</sup>), and high contents of total dietary fiber (44.3 g·100 g<sup>-1</sup>), protein (5.6 mg·100 g<sup>-1</sup>) and energy (193.0 kcal·100 g<sup>-1</sup>). The fruit presented reduced contents of carotenoids and vitamin C [(0.4 and 8.9) mg·100 g<sup>-1</sup>, respectively]. The contents of vitamin E and folates [(53.5 and 495.5)  $\mu$ g·100 g<sup>-1</sup>, respectively] were higher than in other widely consumed fruits. **Conclusion.** The 'jatobá do cerrado' is a source of vitamin C, good source of folates, and excellent source of dietary fiber. Due to its nutritional value, 'jatobá do cerrado' is an important dietary alternative; thus, its consumption should be encouraged.

## Brazil / Minas Gerais / *Hymenaea stigonocarpa* / fruits / physicochemical properties / carotenoids / vitamin content / energy value

### « Jatobá do Cerrado » (*Hymenaea stigonocarpa*) : composition chimique, caroténoïdes et vitamines dans un fruit exotique de la savane brésilienne.

**Résumé – Introduction.** Les fruits de la savane brésilienne pourraient permettre d'améliorer l'alimentation humaine et de générer des revenus ; par conséquent, ils pourraient permettre d'améliorer la qualité de vie de familles socialement vulnérables. Parmi eux, « jatobá do Cerrado » (*Hymenaea stigonocarpa*) est un fruit indigène qui se démarque. De ce fait, les caractéristiques physiques, la composition chimique (acidité titrable, solides solubles, pH, humidité, cendres, protéines, lipides et fibres alimentaires totales), la présence et le contenu en vitamine C (acide ascorbique et déhydroascorbique), caroténoïdes ( $\alpha$ -carotène,  $\beta$ -carotène,  $\beta$ -cryptoxanthine et lycopène), vitamine E ( $\alpha$ ,  $\beta$ ,  $\gamma$ , et  $\delta$ -tocophérols et tocotriénols) et folates (tétrahydrofolate, 5-méthyltétrahydrofolate et 5-formyltétrahydrofolate) ont été évalués dans la pulpe de « jatobá do Cerrado » de la savane brésilienne. **Matériel et méthodes.** La longueur, le diamètre, le poids et le rendement en fruits ont été évalués. L'acidité titrable été déterminée par neutralisation volumétrique ; le pH par potentiométrie ; les solides solubles par réfractométrie ; l'humidité à l'aide d'un four ; les cendres à l'aide d'un four à moufle ; les protéines par le procédé micro-Kjeldhal ; les fibres alimentaires totales par un procédé de gravimétrie non enzymatique ; les lipides avec un extracteur Soxhlet. La vitamine C et les caroténoïdes ont été analysés par HPLC-DAD, et la vitamine E et les folates par HPLC avec détection par fluorescence. **Résultats et discussion.** Le « jatobá do Cerrado » a présenté de faibles rendements en pulpe (17,1 %) et teneurs en humidité (8,8 g·100 g<sup>-1</sup>), et des teneurs élevées en fibres alimentaires totales (44,3 g·100 g<sup>-1</sup>), protéines (5,6 mg·100 g<sup>-1</sup>) et énergie (193,0 kcal·100 g<sup>-1</sup>). Le fruit a présenté des teneurs réduites en caroténoïdes et vitamine C [(0,4 et 8,9) mg·100 g<sup>-1</sup>, respectivement] et des teneurs en vitamine E et en folates [(53,5 et 495,5)  $\mu$ g·100 g<sup>-1</sup>, respectivement] supérieures à d'autres fruits largement consommés. **Conclusion.** Le « jatobá do Cerrado » est une source de vitamine C, une bonne source de folates, et une excellente source de fibres alimentaires. En raison de sa valeur nutritive, le « jatobá do Cerrado » est une ressource importante pour l'alimentation ; sa consommation devrait donc être encouragée.

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## Brésil / Minas Gerais / *Hymenaea stigonocarpa* / fruits / propriété physicochimique / caroténoïde / teneur en vitamines / valeur énergétique

## 1. Introduction

The Savannah is the second largest biome in South America and Brazil. It covers approximately 25% of the Brazilian territory [1]. Due to its geographical location and territorial extension, the Brazilian Savannah has a wide vegetable diversity that can be used for various purposes, including the use of this fruit in the human diet. Fruits from this region have potential to improve the human diet, income generation and, consequently, the quality of life for socially vulnerable families.

Among the native fruits of the Savannah, one that stands out is the 'jatobá do cerrado' (*Hymenaea stigonocarpa*). This bean-shaped fruit has a rough dark brown bark. Inside the fruit it is observed from 1 to 6 reddish-brown seeds surrounded by a sweet edible pulp [2]. The jatobá pulp may be consumed *in natura* or as an ingredient in several food preparations.

There is little data available in the literature about the nutritional value of 'jatobá do cerrado' pulp. Furthermore, these data do not relate to fruit grown in the state of Minas Gerais, Brazil. Some studies reported that 'jatobá do cerrado' pulp from other states of Brazil was an excellent source of dietary fiber [3, 4] and presented minerals such as potassium ( $1121.0 \text{ mg}\cdot 100 \text{ g}^{-1}$ ), magnesium ( $125.0 \text{ mg}\cdot 100 \text{ g}^{-1}$ ), calcium ( $134.0 \text{ mg}\cdot 100 \text{ g}^{-1}$ ), phosphorus ( $96.0 \text{ mg}\cdot 100 \text{ g}^{-1}$ ), zinc ( $1.4 \text{ mg}\cdot 100 \text{ g}^{-1}$ ) and sodium ( $7.0 \text{ mg}\cdot 100 \text{ g}^{-1}$ ) [5]. There is no information on the proximate composition and content of bioactive compounds such as folates and vitamins A, C, and E of 'jatobá do cerrado'. These vitamins play important roles in reducing the risk of non-communicable diseases [6–9].

Knowledge of the chemical composition of foods consumed in Brazil, including the 'jatobá do cerrado', is essential for conducting research on their impacts on human nutrition and health (individual and population), adaptation and application of improved technologies to assess the content and/or biological availability of healthful components of a particular food, effects of post-harvest handling, processing and storage, stability and biological activity of

bioactive components, analysis of the functional effect of this food, and others [10].

Thus, our study evaluated physical characteristics, chemical composition, and occurrence and content of carotenoids and vitamins in 'jatobá do cerrado' pulp (*H. stigonocarpa*) from the Brazilian Savannah.

## 2. Materials and methods

### 2.1. Raw material, sample collection and preparation

'Jatobá do cerrado' fruits were collected in rural areas of the municipality of Curvelo (South latitude  $18^{\circ}45'$  and West longitude  $44^{\circ}25'$ ), Minas Gerais, Brazil.

Fruits were collected during the harvest season (June–July 2010) directly from the tree. After, the fruits were transported to the laboratory in polystyrene boxes, in a period of up to 48 h after collection. The collection area was divided into five sub-areas (repetitions), and approximately 3.0 kg of fruit were collected in each sub-area.

Morphologically perfect and completely mature fruits were selected and washed in tap water and dried on paper towels. Those fruits that presented a thin and dark brown bark, and pulp color varying from white to yellowish were considered ripe. The 'jatobá do cerrado' bark was removed using a hammer, and the pulp was separated from the seeds with a knife. Later, the fresh pulp was homogenized in a domestic food processor (Faet Multipratic, MC5 model, Brazil), packaged in polyethylene bags, and stored for up to 4 d at  $(-18 \pm 1) ^{\circ}\text{C}$  prior to analysis.

### 2.2. Physical characterization

Individual measurements of height and diameter (longitudinal and transverse) were carried out in 30 'jatobá do cerrado' fruits using a digital caliper rule (Mitutoyo, model M1, Brazil). Whole fruit (MF), bark (MB) pulp (MP) and seed (MS) masses were obtained by individual direct weighing on

a semi-analytical balance (Gehaka, BG 2000 model, Brazil). The yields of pulp, bark and seed in the fruit were calculated, respectively, by the equations  $(MP / MF) \times 100$ ;  $(MB / MF) \times 100$  and  $(MS / MF) \times 100$ .

### 2.3. Chemical analysis

The chemical analyses were performed, in three repetitions, at the Laboratory of Food Analysis of the Department of Nutrition and Health, Federal University of Viçosa, Brazil. The physicochemical characteristics of the pulp were determined according to the methods proposed by the Adolfo Lutz Institute [11]. The titratable acidity was determined by volumetric neutralization using a standard solution of sodium hydroxide  $0.1 \text{ mol}\cdot\text{L}^{-1}$ . The pH was determined by direct potentiometry and the soluble solids determined by refractometry.

The proximate composition was determined in the 'jatobá do cerrado' pulp according to the methods of the Association of Official Analytical Chemists [12]. Moisture was determined at  $105 \text{ }^\circ\text{C}$  using an oven (Nova Ética, 400 model, Brazil) and ash was determined at  $550 \text{ }^\circ\text{C}$  using a muffle furnace (Quimis, Q344 model, Brazil). Protein content was determined by the micro-Kjeldhal method and total dietary fiber by the gravimetric non-enzymatic method. Lipid concentration was determined with a Soxhlet extractor (Eletrothermo, 500WX model, Brazil), while carbohydrates were estimated by the equation:  $[100 - (\% \text{ moisture} + \% \text{ lipids} + \% \text{ proteins} + \% \text{ total dietary fiber} + \% \text{ ash})]$ . Total energy was estimated considering the conversion factors of  $4 \text{ kcal}\cdot\text{g}^{-1}$  for proteins and carbohydrates and  $9 \text{ kcal}\cdot\text{g}^{-1}$  for lipids [13].

### 2.4. Extraction and analysis of carotenoids and vitamins

Preparation and analysis of carotenoids and vitamins were performed, in five repetitions, at the Laboratory of Vitamin Analysis of the Department of Nutrition and Health, Federal University of Viçosa, Brazil. During the extraction and analysis, the samples and extracts were protected from both sunlight

and artificial light with the use of amber glass bottles, aluminum foil and blackout curtains; they were also protected from oxygen by using lids and environments with nitrogen gas in glass bottles.

#### 2.4.1. Carotenoids

Carotenoid extraction ( $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin and lycopene) was performed according to Rodriguez-Amaya *et al.* [14], with modifications. About 5 g of pulp were added to 20.0 mL of cooled acetone, homogenized in a micro-crusher (Marconi, MA 102 model, Brazil) for approximately 3 min and vacuum-filtered in a Büchner funnel, using filter paper, and was the residue maintained in the extraction tube. The extraction and filtration procedures were performed twice more on the waste until complete discoloration of the sample.

Subsequently, the filtrate was transferred in three fractions to a separatory funnel containing 50.0 mL of petroleum ether. After the transfer of each fraction, distilled water was added for phase separation (carotenoids in petroleum ether and acetone-water), and was the bottom phase (water-acetone) discarded. Anhydrous sodium sulfate was added to the ether extract for removal of residual water that could impair evaporation of the material. The ether extract was then concentrated using a rotary evaporator (Tecnal, TE-211 model, Brazil) at  $(35 \pm 1) \text{ }^\circ\text{C}$ , transferred to a 25.0-mL volumetric flask, and the volume was completed with petroleum ether. Later, the extract was then stored in a hermetically sealed amber glass bottle and stored at  $(-18 \pm 1) \text{ }^\circ\text{C}$ .

For analysis, eight milliliters of the extract were evaporated under nitrogen gas flow and the dry residue redissolved in 2.0 mL of HPLC-grade acetone (Tedia, Brazil). The extracts were filtered through HV Millex filter units, in polyethylene, with  $0.45 \text{ } \mu\text{m}$  of porosity (Millipore, Brazil), and 50  $\mu\text{L}$  were injected into the chromatographic column for analysis.

Carotenoids were analyzed using a high-performance liquid chromatography system (HPLC) (Shimadzu, SCL 10AT VP model,

Japan) comprised of a high-pressure pump (Shimadzu, LC-10AT VP), an autosampler with a loop of 50  $\mu\text{L}$  (Shimadzu, SIL-10AF) and a diode array detector (DAD) (Shimadzu, SPD-M10A). The chromatographic conditions used were developed by Pinheiro-Sant'Ana *et al.* [15], and included: HPLC system, DAD; chromatographic column Phenomenex Gemini RP-18 (250 mm  $\times$  4.6 mm, 5  $\mu\text{m}$ ) fitted with the guard column Phenomenex ODS (C18), (4 mm  $\times$  3 mm); mobile phase composed of methanol: ethyl acetate: acetonitrile (70:20:10, v/v/v); and flow rate of 1.7 mL $\cdot\text{min}^{-1}$ . The chromatograms were obtained at 450 nm.

Vitamin A concentration was calculated according to the recommendations of the Institute of Medicine [16], in which 1 Retinol Activity Equivalent (RAE) corresponds to 1  $\mu\text{g}$  of retinol, 12  $\mu\text{g}$  of  $\beta$ -carotene or 24  $\mu\text{g}$  of other provitamin A carotenoids.

#### 2.4.2. Vitamin C

Extraction of ascorbic and dehydroascorbic acids, conversion of dehydroascorbic acid into ascorbic acid and analysis of ascorbic acid were carried out according to methods reported by Campos *et al.* [17] with modifications. Roughly 3.0 g of the sample were crushed for approximately 5 min, in 15.0 mL of the extraction solution (3% metaphosphoric acid, 8% acetic acid,  $\text{H}_2\text{SO}_4$  0.3 N and 1 mM EDTA). The extract was centrifuged (Fanem, Excelsa Baby II - 206R model, Brazil) at 4000 rpm (1789 g) for 15 min, vacuum-filtered in a Büchner funnel and diluted to 25.0 mL in a volumetric flask with ultrapure water. Next, the extract was centrifuged again at 4000 rpm (1789 g) for 15 min and the supernatant frozen [ $-5 \pm 1$   $^\circ\text{C}$ ] until the time of analysis.

For conversion of dehydroascorbic acid into ascorbic acid, an aliquot of 1.0 mL of the extract obtained in the previous stage was pipetted into an amber glass and 1.0 mL of a 0.5 M Trizma buffer solution (pH 9.0) containing 40 mM dithiothreitol (DTT, Sigma-Aldrich, Germany) was added to bring the pH closer to neutral. The conversion reaction was performed at room temperature in the dark, for 10 min. Later,

0.5 mL of  $\text{H}_2\text{SO}_4$  0.4 M was added to reduce the pH prior to chromatographic injection.

Analyses were carried out by injection of 50  $\mu\text{L}$  of the extracts previously filtered in filter units with porosity of 0.45  $\mu\text{m}$ . The vitamin C analyses were performed on the same HPLC system used for analysis of carotenoids. The following chromatographic conditions were used: RP-18 Lichrospher 100 chromatographic column (250 mm  $\times$  4 mm, 5  $\mu\text{m}$ ); HPLC system, DAD, mobile phase composed of ultrapure water containing 1 mM of  $\text{NaH}_2\text{PO}_4$ , 1 mM of EDTA and pH adjusted to 3.0 with  $\text{H}_3\text{PO}_4$ ; mobile phase flow of 1.0 mL $\cdot\text{min}^{-1}$ . Chromatograms were obtained at 245 nm. The dehydroascorbic acid content was calculated by the equation: dehydroascorbic acid content = ascorbic acid content after conversion – ascorbic acid content before conversion.

#### 2.4.3. Vitamin E

Occurrence and content of the eight components of vitamin E ( $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ -tocopherols and tocotrienols) were assessed in the 'jatobá do cerrado' pulp. The extraction was carried out according to Pinheiro-Sant'Ana *et al.* [18] with some modifications. Approximately 5.0 g of the sample were weighed and added to 4.0 mL of heated ultrapure water [about ( $80 \pm 1$ )  $^\circ\text{C}$ ], 10.0 mL of isopropyl alcohol, 1.0 mL of hexane containing 0.05% of butylhydroxytoluene, 5 g of anhydrous sodium sulfate and 25.0 mL of the extraction solvent mixture (hexane:ethyl acetate, 85:15, v/v). After these procedures, the suspension was homogenized in a micro-crusher at average speed for 1 min. The suspension was then vacuum-filtered through filter paper in a Büchner funnel, and the residue was maintained in the extraction tube. The extraction was repeated with the addition of 5.0 mL of isopropyl alcohol and 30.0 mL of the solvent mixture, followed by homogenization and vacuum filtration. Next, the extract was concentrated in a rotary evaporator at ( $70 \pm 1$ )  $^\circ\text{C}$  for about 2 min and transferred to a volumetric flask. The volume was completed to 25.0 mL with the solvent mixture.

After extraction, aliquots of 5.0 mL of the extract were dried in nitrogen gas, redissolved

in 2.0 mL of HPLC-grade hexane (Tedia, Brazil) and filtered through filter units with porosity of 0.45  $\mu\text{m}$ . Analyses of vitamin E were performed by a HPLC system (Shimadzu, SCL 10AD VP model, Japan) composed of a high-pressure pump with a valve for a low-pressure quaternary gradient (Shimadzu, LC-10AD VP model, Japan), an autosampler with a loop of 50  $\mu\text{L}$  (Shimadzu, SIL-10AF model, Japan), a helium degassing system of the mobile phase (Shimadzu, DGU-2 A model, Japan) and a fluorescence detector (Shimadzu, RF10AXL model, Japan), with injection of 50  $\mu\text{L}$  of the extract.

The chromatographic conditions used for analysis were developed by Pinheiro-Sant'Ana *et al.* [18] and included a HPLC system, fluorescence detector (290 nm of excitation and 330 nm of emission), LiChrosorb chromatographic column (Si60 Phenomenex 250 mm  $\times$  4 mm, 5  $\mu\text{m}$ ), mobile phase composed of hexane:isopropanol:glacial acetic (Tedia, Brazil), in the proportions 98.9:0.6:0.5 (v/v/v), and mobile phase flow of 1.0 mL $\cdot\text{min}^{-1}$ . The total vitamin E content in 'jatobá do cerrado' was calculated by the sum of vitamin E components identified.

#### 2.4.4. Folates

Occurrence and content of folates [tetrahydrofolate (THF), 5-methyltetrahydrofolate (5-MTHF) and 5-formyltetrahydrofolate (5-FTHF)] in the 'jatobá do cerrado' pulp were assessed. The processes of extraction, deconjugation, purification and analysis of the folates were carried out according to Della Lucia *et al.* [19] with some modifications. For extraction, approximately 5 g of the sample were ground in 20.0 mL of phosphate buffer solution 0.1 M, pH 6.0, containing ascorbic acid 1% and 2-mercaptoethanol 0.1%. The obtained extract was centrifuged at 4000 rpm (1789 g) for 15 min, vacuum-filtered in the Büchner funnel and diluted to 25.0 mL in a volumetric flask with ultrapure water. Next, the extract was heated for about 12 min in a water bath at (100  $\pm$  1)  $^{\circ}\text{C}$  and cooled in an ice bath until the temperature was below (37  $\pm$  1)  $^{\circ}\text{C}$ . This cooled extract was re-centrifuged at 4000 rpm (1789 g) for

15 min and submitted to deconjugation of polyglutamates into monoglutamates.

For deconjugation, hundred  $\mu\text{L}$  of rat plasma containing the enzyme conjugase ( $\gamma$ -glutamyl carboxypeptidase) were added to 3.0 mL of the previously obtained supernatant. The extract was incubated in a water bath at (37  $\pm$  1)  $^{\circ}\text{C}$  for 3 h. This extract was then heated in boiling water for 5 min for enzyme inactivation.

Extract purification was carried out using an ion exchange column with a stationary phase composed of Q-Sepharose Fast Flow (Pharmacia, USA). The column was pre-conditioned with methanol and water (1:1) at a flow rate of 2 drops per second. The extract was then applied to the column at a flow rate of 2 drops per second. Elution of the retained folates was carried out using 1.5 mL of a sodium acetate solution (0.1 M) containing NaCl 10%, ascorbic acid 1% and 2-mercaptoethanol 0.1%. Analyses were carried out by injection of 50  $\mu\text{L}$  of the extracts, previously filtered in filter units with porosity of 0.45  $\mu\text{m}$ , in the same system used for analysis of vitamin E.

The chromatographic conditions used were: Shim Pack 100 RP18 chromatographic column (150 mm  $\times$  4.6 mm, 4.6  $\mu\text{m}$ ) (Merck, Germany), mobile phase composed of a binary gradient containing phosphate buffer solution ( $\text{NaH}_2\text{PO}_4$  30 mM, pH adjusted to 2.3 with  $\text{H}_3\text{PO}_4$ ) as eluent A, and acetonitrile as eluent B. The gradient utilized was as follows: from 0 to 5 min, 94% of eluent A + 6% of eluent B; from 5 to 25 min, a linear gradient for 75% of eluent A + 25% of eluent B; from 25 to 33 min, 75% of eluent A + 25% of eluent B; from 33 to 35 min, return to initial conditions followed by stabilization up to 50 min. The mobile phase flow rate was 0.7 mL $\cdot\text{min}^{-1}$  and fluorescence detection occurred with excitation at 290 nm and emission at 360 nm. The mobile phase was degassed with helium gas for 15 min at 100 kPa before the start of the analyses and at 50 kPa during the runs.

#### 2.4.5. Identification and quantification of carotenoids and vitamins

The identification and quantification of compounds were performed using the following

standards: vitamin E standards ( $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ -tocopherols and tocotrienols) purchased from Calbiochem<sup>®</sup>, EMD Biosciences, Inc. (USA); L-ascorbic acid purchased from Sigma-Aldrich<sup>®</sup> (Germany); folate standards - (6S)-5,6,7,8-sodium tetrahydrofolate, (6S)-5-methyl-5,6,7,8-tetrahydrofolate and (6S)-5-formyl-5,6,7,8-tetrahydrofolate - provided by Merck-Eprova<sup>®</sup> (Switzerland);  $\alpha$ -carotene and  $\beta$ -carotene isolated from concentrated carrot extract, and  $\beta$ -cryptoxanthin and lycopene isolated from extracts of papaya and tomato, respectively, by open column chromatography [20].

Identification of the compounds was performed by comparing the retention times obtained for standards and samples analyzed under the same conditions. Folates and vitamin E isomers were identified by co-chromatography, and carotenoids and ascorbic acid by comparing the absorption spectra of the standards with the peaks of interest in the samples, using the DAD.

Compounds observed in the 'jatobá do cerrado' pulp ( $\beta$ -carotene, ascorbic acid,  $\alpha$ -tocopherol,  $\gamma$ -tocotrienol, THF and 5-FTHF) were quantified by external analytical curves. Construction of standard curves was performed by injection, in duplicate, of six increasing concentrations of standard solutions in the range from (0.004 to 1.433)  $\mu\text{g}$  for  $\beta$ -carotene, (0.0589 to 5.8800)  $\mu\text{g}$  for ascorbic acid, (1.02 to 104.21) ng for  $\alpha$ -tocopherol, (3.21 to 157.6) ng for  $\gamma$ -tocotrienol, (0.04 to 462.28) ng for THF and (0.03 to 33.12) ng for 5-FTHF. Thus, a linear correlation was constructed between the peak areas and the injected concentrations of each compound.

Compounds present in the 'jatobá do cerrado' pulp were quantified based on the analytical curves and regression equations achieved for  $\beta$ -carotene ( $y = 1389460.4 x + 24320.87$ ;  $R^2 = 0.996$ ), ascorbic acid ( $y = 3277607.19 x - 66204.16$ ;  $R^2 = 0.998$ ),  $\alpha$ -tocopherol ( $y = 76030901.90 x - 66102.66$ ;  $R^2 = 0.999$ ),  $\alpha$ -tocotrienol ( $y = 28452328.82 x - 05303.68$ ;  $R^2 = 0.997$ ),  $\gamma$ -tocotrienol ( $y = 1243329487.57 x - 300446.44$ ;  $R^2 = 0.999$ ), THF ( $y = 942240050.58 x - 162371.44$ ;  $R^2 = 0.996$ ) and 5-FTHF ( $y = 710036264.81 x - 1088694.36$ ;  $R^2 = 0.996$ ). The real

concentration was achieved via calculations based on the dilutions performed.

#### 2.4.6. Quality control of analytical methods

Since the methods used to determine the content of bioactive compounds were developed and validated for analysis of other food matrices (ascorbic acid and folates in vegetables and vitamin E in vegetable oils and egg), the recovery, linearity, repeatability, limit of detection (LOD) and limit of quantification (LOQ) were assessed. The recovery tests were carried out by the addition of standards ( $\beta$ -carotene, ascorbic acid,  $\alpha$ -tocopherol,  $\gamma$ -tocotrienol, THF and 5-FTHF) to the samples. The quantity of standards added varied between 50% to 100% of the initial content observed in 'jatobá do cerrado' pulp. The recovery percentage was calculated by the equation: % recovery = (final concentration of the isomer – concentration added to the isomer) / (initial concentration of the isomer)  $\times$  100. All procedures were carried out in triplicate.

The linearity range of the compounds was carried out by injection, in duplicate, of six increasing concentrations of the standard solutions using the same chromatographic conditions employed for analysis of the extracts. Data obtained from the peak areas were used for linear regression analysis. The correlation coefficients ( $R^2$ ) acquired for each case were used to assess linearity [21].

Repeatability was evaluated by extraction in quintuplicate and analysis in duplicate of the same fruit repetition containing the carotenoids and vitamins evaluated. The repeatability evaluation was carried out by calculation of the relative standard deviation (RSD) of the peak areas and retention times of the components analyzed [21].

Assessment of the LOD was carried out by successive dilutions of the carotenoid and vitamin standards identified in the fruits, followed by determination of the lowest detectable amount as three times the value of the amplitude of the baseline noise. The LOQ was established as 10 times the LOD [22].

## 2.5. Experimental design and statistical analysis

A completely randomized design was used, with three repetitions for chemical analysis and five repetitions for analysis of carotenoids and vitamins. Data was stored in spreadsheets using the Microsoft Office Excel software system, version 2007. Averages, standard deviations and amplitude of the parameters were calculated using the SAS package (Statistical Analysis System), version 9.2 (2008), licensed for the UFV.

## 3. Results and discussion

### 3.1. Physical characteristics

The 'jatobá do cerrado' is an elongated pod with tough dark brown bark. Internally the fruit has elliptical and flat brown seeds, which are surrounded by a pale-yellow farinaceous pulp (*figure 1*).

The fruits presented a longitudinal diameter ranging from (2.5 to 3.8) cm, transverse diameter ranging from (2.7 to 4.8) cm and length from (7.4 to 13.4) cm. The average mass of fruits was 49.7 g, ranging from (20.8 to 84.2) g. This fruit presented reduced pulp yield (17.1%) and high yields of bark (56.0%) and seed (26.9%), which makes its technological exploitation difficult; however, it does not preclude its fresh use (*table I*).

### 3.2. Chemical characteristics

Information on the content of soluble solids, tritatable acidity and pH of 'jatobá do cerrado' pulp is scarce in the specialized literature. Thus, it was not possible to make



**Figure 1.** Fruits of 'jatobá do cerrado' (*Hymenaea stigonocarpa*).

comparisons with the results obtained in the present study. The 'jatobá do cerrado' pulp showed low soluble solids and moisture (*table II*). Silva *et al.* observed in fruits of the same variety a higher moisture [(11.97 to 12.94)  $\text{g}\cdot 100^{-1}$ ] than in the present study [3]. However, the fruits analyzed by Silva *et al.* were collected in the state of Goiás [3], which is located approximately 460 miles from the area where the fruits of our study were collected (Minas Gerais state, Brazil).

'Jatobá do cerrado' presented an elevated dietary fiber content which was lower than that verified by Silva *et al.*, in fruits of the same variety (47.16  $\text{g}\cdot 100\text{ g}^{-1}$ ) [3]. This content was higher than that observed in other legumes such as bean, soybean, lentil and pea [from (5.1 to 30.3)  $\text{g}\cdot 100\text{ g}^{-1}$ ] [23] and from 7 to 14 times higher than those observed in fruits considered excellent sources of dietary fiber, such as guava (6.2  $\text{g}\cdot 100\text{ g}^{-1}$ ), orange (4.0  $\text{g}\cdot 100\text{ g}^{-1}$ ) and

**Table I.** Physical characteristics of 'jatobá do cerrado' fruits (*Hymenaea stigonocarpa*) from the Brazilian Savannah.

Measure	Diameter (cm)		Length (cm)	Mass (g)			Pulp yield (%)	
	Longitudinal	Transversal		Fruit	Bark	Seed		
Mean of 30 fruits $\pm$ standard deviation	3.1 $\pm$ 0.4	3.5 $\pm$ 0.6	10.3 $\pm$ 1.6	49.7 $\pm$ 20.6	27.3 $\pm$ 10.3	13.9 $\pm$ 8.7	8.4 $\pm$ 3.9	17.1 $\pm$ 4.9
Minimum	2.5	2.7	7.4	20.8	12.2	4.9	2.6	12.6
Maximum	3.8	4.8	13.4	84.2	42.9	31.8	16.8	32.6

**Table II.**

Chemical characteristics and total energy value of the 'jatobá do cerrado' pulp (*Hymenaea stigonocarpa*) from the Brazilian Savannah. Values expressed in fresh matter. Mean of three repetitions  $\pm$  standard deviation.

Soluble solids (°Brix)	Titratable acidity (g citric acid·100 g <sup>-1</sup> )	pH	Moisture	Proteins	Lipids	Ash	Total dietary fiber	Carbohydrates	Total energy value (kcal·100 g <sup>-1</sup> )
4.8 $\pm$ 0.1	1.5 $\pm$ 0.1	5.5 $\pm$ 0.2	8.8 $\pm$ 1.0	5.6 $\pm$ 0.4	3.8 $\pm$ 1.0	3.4 $\pm$ 0.1	44.3 $\pm$ 2.3	34.1 $\pm$ 3.3	193.0 $\pm$ 11.9

**Table III.**

Repeatability, limits of detection and quantification, linearity range, and recovery of carotenoids and vitamins in the 'jatobá do cerrado' pulp (*Hymenaea stigonocarpa*) from the Brazilian Savannah.

Compound	Repeatability		Detection limit (µg·mL <sup>-1</sup> )	Quantification limit (µg·mL <sup>-1</sup> )	Range of linearity (µg)	Recovery (%)
	Peak area	Retention time				
	(Relative standard deviation)					
β-carotene	3.3	0.9	6.422	64.221	0.0045 – 1.4333	93.2
Ascorbic acid	2.3	1.1	12.321	123.214	0.0589 – 5.8800	93.7
α-tocopherol	4.8	0.9	0.025	0.251	0.0010 – 0.1042	92.5
γ-tocotrienol	5.5	1.2	0.042	0.740	0.0033 – 0.1576	92.2
THF	2.4	0.9	0.003	0.031	0.00004 – 0.04622	91.2
5-FTHF	2.9	0.9	0.002	0.021	0.00003 – 0.03319	87.4

THF: tetrahydrofolate; 5-FTHF: 5-formyltetrahydrofolate.

tangerine (3.1 g·100 g<sup>-1</sup>) [10]. Since jatobá presented farinaceous pulp and with a high content of dietary fiber, the fruit has the potential to be an ingredient in products including porridge, cakes, breads, crackers, high-fiber crackers and cookies.

Due to its low moisture, 'jatobá do cerrado' showed a high lipid content and energy value when compared with most traditional fruits (*table II*) and contents similar to those observed by Silva *et al.* [3] (2.05 g·100 g<sup>-1</sup> and 158.7 kcal·100 g<sup>-1</sup>, respectively). These authors also verified contents of carbohydrate (27.1 g·100 g<sup>-1</sup>), ash (4.0 g·100 g<sup>-1</sup>) and protein (6.7 g·100 g<sup>-1</sup>) similar to those observed in our study.

The proteic content in jatoba was lower than that of other legumes. According to Deshpande and Damodaran, legumes are generally characterized by a relatively high protein content (from 17% to 50% dry basis) [24]. The differences between the chemical composition of jatoba and other legumes may be due to the fact that, in the jatoba,

the edible portion refers to the fruit pulp, whereas in the other legumes the edible portion refers to the seed [4].

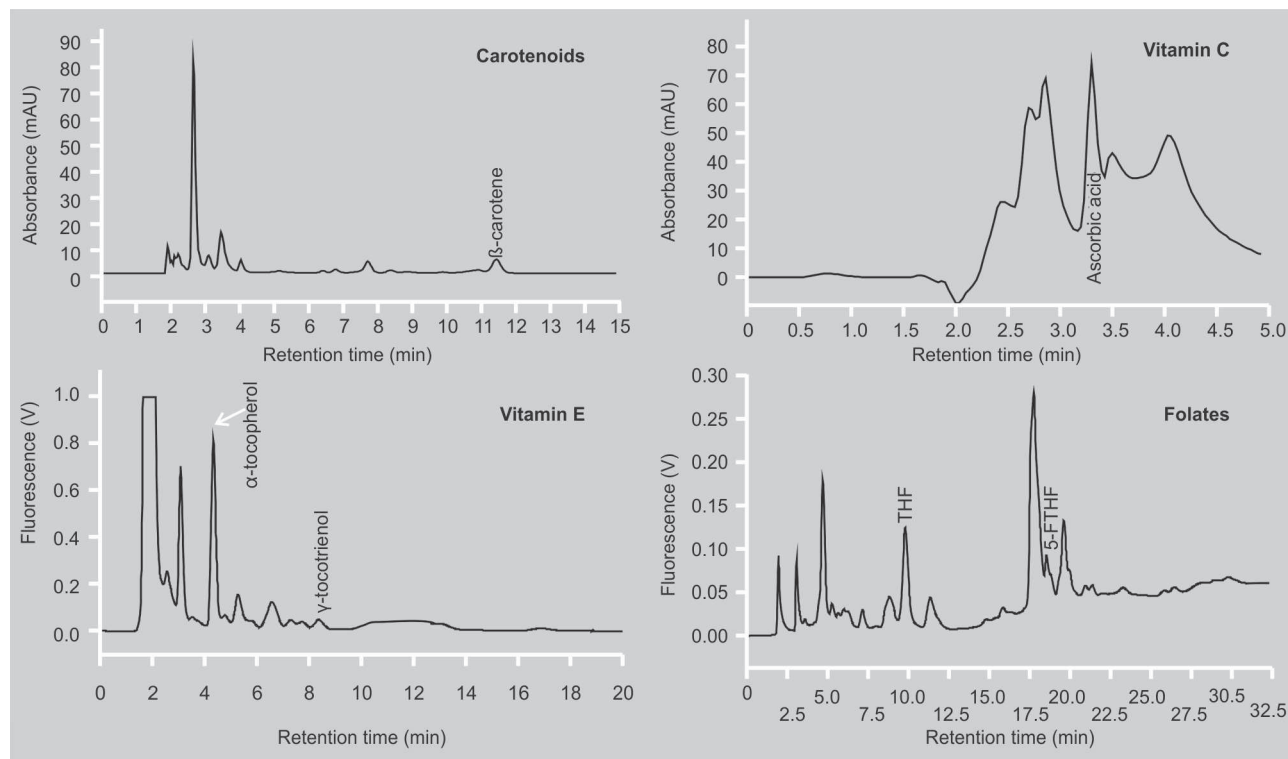
### 3.3. Carotenoids and vitamins

#### 3.3.1. Quality of analytical methods

Quality control tests of the analysis methods used indicated the reliability of the analysis conditions. The methods utilized reduced the chances of carotenoid and vitamin loss during the extraction and analyses, and allowed the detection of reduced concentrations of the analyzed compounds (*table III*).

Recovery of the standards added to the samples ranged from 87.4% to 97.3%, with an average of 91.7%. Repeatability of the β-carotene and vitamin isomer analyses presented a RSD in relation to the peak areas and retention time (RT) below 5.5% and 1.3%, respectively (*table III*).





The LOD for  $\beta$ -carotene and vitamins ranged between  $0.002 \mu\text{g}\cdot\text{mL}^{-1}$  and  $12.321 \mu\text{g}\cdot\text{mL}^{-1}$ . The LOQ, considered as 10 times the value of LOD, ranged from  $(0.02 \text{ to } 123.21) \mu\text{g}\cdot\text{mL}^{-1}$ . The linearity range of the compounds analyzed presented ratios between the maximum and minimum injected concentrations greater than 60 times and the correlation coefficients ( $R^2$ ) were greater than 0.996 (table III).

### 3.3.2. Qualitative composition

The analysis methods allowed a good resolution of the peaks, which assured adequate quantification of the compounds (figure 2). The presence of  $\beta$ -carotene (RT = 11.4 min), ascorbic acid (RT = 3.3 min),  $\alpha$ -tocopherol (RT = 4.5 min),  $\gamma$ -tocotrienol (RT = 8.6 min), THF (RT = 10.2 min) and 5-FTHF (RT = 19.3 min) was observed in 'jatobá do cerrado' pulp.

### 3.3.3. Carotenoid and vitamin content

Information on the content of carotenoids, vitamin C, vitamin E and folates in fruits from the Savannah are scarce in the specialized

literature. Furthermore, there is no data on the presence and content of these compounds in 'jatobá do cerrado'. The absence of nutritional data on 'jatobá do cerrado' indicates the significance of this study, as well as the need for new studies on the presence and content of carotenoids and vitamins in fruits, mainly those from the Savannah.

'Jatobá do cerrado' pulp presented a reduced carotenoid content ( $\beta$ -carotene), similar to that of other legumes (pea, soybean, lentils and bean) [23]. This content was lower than that observed in other fruit from the Savannah such as araticum ( $4.98 \text{ mg}\cdot 100 \text{ g}^{-1}$ ) [25] and cagaita ( $0.77 \text{ mg}\cdot 100 \text{ g}^{-1}$ ) [26], and in fruits considered sources of these compounds, such as mango ( $1.63 \text{ mg}\cdot 100 \text{ g}^{-1}$ ), papaya ( $7.48 \text{ mg}\cdot 100 \text{ g}^{-1}$ ) and guava ( $7.33 \text{ mg}\cdot 100 \text{ g}^{-1}$ ) [27]. Despite a reduced vitamin A value in jatoba, this value was higher than those observed in traditional fruits including strawberry ( $4.14 \text{ RAE}\cdot 100 \text{ g}^{-1}$ ), carambola ( $18.2 \text{ RAE}\cdot 100 \text{ g}^{-1}$ ) and nectarine ( $25.7 \text{ RAE}\cdot 100 \text{ g}^{-1}$ ) [28] (table IV).

The vitamin C content in 'jatobá do cerrado' was higher than that in soybean

**Figure 2.** HPLC analysis of carotenoids, vitamin C, vitamin E and folates in the 'jatobá do cerrado' pulp (*Hymenaea stigonocarpa*) from the Brazilian Savannah. THF: tetrahydrofolate; 5-FTHF: 5-formyltetrahydrofolate.

**Table IV.**

Content of carotenoids and vitamins in the 'jatobá do cerrado' pulp (*Hymenaea stigonocarpa*) from the Brazilian Savannah. Values are expressed in fresh matter. Mean of five repetitions  $\pm$  standard deviation.

Total carotenoids ( $\beta$ -carotene) (mg·100 g <sup>-1</sup> )	Vitamin A value (RAE·100 g <sup>-1</sup> )	Total vitamin C (ascorbic acid) (mg·100 g <sup>-1</sup> )	Total vitamin E	$\alpha$ -tocopherol	$\gamma$ -tocotrienol ( $\mu$ g·100 g <sup>-1</sup> )	Total folates	THF	5-FTHF
0.4 $\pm$ 0.1	32.4 $\pm$ 9.7	8.9 $\pm$ 1.9	495.5 $\pm$ 37.5	439.9 $\pm$ 41.1	55.5 $\pm$ 18.3	53.5 $\pm$ 4.2	26.9 $\pm$ 0.9	26.5 $\pm$ 4.4

RAE: Retinol Activity Equivalent; THF: tetrahydrofolate; 5-FTHF: 5-formyltetrahydrofolate.

**Table V.**

Contribution of 100 g of 'jatobá do cerrado' pulp (*Hymenaea stigonocarpa*) to supplying the daily recommendations of nutrients for children, adult men and pregnant women.

Age group	Percentage of intake adequacy					
	Protein	Dietary fiber	Vitamin A	Vitamin C	Folates	Vitamin E ( $\alpha$ -tocopherol)
Children aged between 4 and 8 years	29.5	177.1	8.1	35.6	26.7	6.3
Adult men aged between 19 and 30 years	10.0	116.5	3.6	9.9	13.4	2.9
Pregnant women	7.9	158.1	4.2	11.9	8.9	2.9

(6.0 mg·100 g<sup>-1</sup>), bean (1.2 mg·100 g<sup>-1</sup>) and pea (4.4 mg·100 g<sup>-1</sup>) [23]. However, this content was lower than that observed in fruits widely consumed by the Brazilian population and sources of this vitamin, such as papaya (80.2 mg·100 g<sup>-1</sup>), mango (17.5 mg·100 g<sup>-1</sup>), guava (85.9 mg·100 g<sup>-1</sup>) [27], orange (57.0 mg·100 g<sup>-1</sup>), lemon (31.0 mg·100 g<sup>-1</sup>) and passion fruit (20.0 mg·100 g<sup>-1</sup>) [10].

The pulp of jatoba showed an  $\alpha$ -tocopherol content higher than that observed in the bean (280  $\mu$ g·100 g<sup>-1</sup>), similar to that of lentil (490  $\mu$ g·100 g<sup>-1</sup>) and lower than that of soybean (850  $\mu$ g·100 g<sup>-1</sup>) [23].  $\alpha$ -Tocopherol, the biologically active compound of vitamin E, was the major component observed in 'jatobá do cerrado' pulp, corresponding to 88% of the total vitamin E. The concentration of vitamin E in 'jatobá do cerrado' was higher than in other widely consumed fruits such as pear (420  $\mu$ g·100 g<sup>-1</sup>), strawberry (410  $\mu$ g·100 g<sup>-1</sup>) and banana (150.00  $\mu$ g·100 g<sup>-1</sup>), and lower than peach (790  $\mu$ g·100 g<sup>-1</sup>) and grape (540  $\mu$ g·100 g<sup>-1</sup>) [29].

Among the folates investigated in 'jatobá do cerrado' pulp, THF and 5-FTHF were encountered, which corresponded to 50.4% and

49.6% of the total content respectively. Folate content was approximately 2 times greater than that observed in fruits that present a high folate content, such as strawberry (24.09  $\mu$ g·100 g<sup>-1</sup>), blackberry (25.00  $\mu$ g·100 g<sup>-1</sup>), orange (30.00  $\mu$ g·100 g<sup>-1</sup>) and papaya (38.15  $\mu$ g·100 g<sup>-1</sup>) [23], and higher than that of other fruits of the Savannah, such as cagaita (25.74  $\mu$ g·100 g<sup>-1</sup>) [26], araticum (27.36  $\mu$ g·100 g<sup>-1</sup>) [25] and pequi (5.16  $\mu$ g·100 g<sup>-1</sup>) [30].

### 3.3.4. Nutritional value of 'jatobá do cerrado' pulp as a source of nutrients

Philippi classified foods as "sources" of a nutrient if they meet from 5% to 10% of the Dietary Reference Intake (DRI), as "good sources" if they meet from 10% to 20% of the DRI, and as "excellent sources" if they meet more than 20% of the DRI [31]. Considering the recommendations for proteins, dietary fibers, folates, vitamin A, vitamin C and vitamin E for children aged between 4 and 8 years, adult men between 19 and 30 years and pregnant women [32–34], the consumption of 100 g of 'jatobá do cerrado' pulp is an excellent source of dietary fiber for the three groups. It is at least a source of protein

for pregnant women, adult men and children (table V).

Additionally, it is a source of vitamin A for children, a source of vitamin C for adult men and a good source for pregnant women and an excellent source for children. 'Jatobá do cerrado' pulp is a source of vitamin E for children, a source of folates for pregnant women, a good source for adult men and an excellent source for children.

#### 4. Conclusion

'Jatobá do cerrado' from the Brazilian Savannah has a low pulp yield which may impair its technological use. Its pulp was farinaceous, with reduced moisture, which makes it very important for technological processing of porridge, cakes, breads, crackers, high-fiber crackers and cookies.

The fruit pulp presented a high total dietary fiber content and energy value and was classified as a source of vitamin C, good source of folates, and excellent source of dietary fiber for adults.

Due to its nutritional value, 'jatobá do cerrado' is an important dietary alternative, thus its consumption should be encouraged. It can contribute to improved diet quality, especially for families lacking a balanced diet and those living in socially vulnerable conditions.

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**« Jatobá do Cerrado » (*Hymenaea stigonocarpa*): composición química, carotenoides y vitaminas en un fruto exótico de la sabana brasileña.**

**Resumen – Introducción.** Los frutos de la sabana brasileña podrían permitir mejorar la alimentación humana y generar ingresos. En consecuencia, podrían permitir mejorar la calidad de vida de familias socialmente vulnerables. Entre estos frutos, « jatobá do Cerrado » (*Hymenaea stigonocarpa*) es un fruto indígena que resalta. Por consiguiente, se evaluaron las características físicas, la composición química (acidez valorable, sólidos solubles, pH, humedad, cenizas, proteínas, lípidos y fibras alimentarias totales), la presencia y el contenido de vitamina C (ácido ascórbico y dehidroascórbico), carotenoides ( $\alpha$ -caroteno,  $\beta$ -caroteno,  $\beta$ -criptoxantina y licopeno), vitamina E ( $\alpha$ ,  $\beta$ ,  $\gamma$ , y  $\delta$ -tocoferoles y tocotrienoles) y folatos (tetrahidrofolato, 5-metil-tetrahidrofolato y 5-formil tetrahidrofolato) en la pulpa de « jatobá do Cerrado » de la sabana brasileña. **Material y métodos.** Se evaluaron la longitud, el diámetro, el peso y el rendimiento en frutos. La acidez valorable fue determinada por neutralización volumétrica, el pH por potenciometría, los sólidos solubles por refractometría, la humedad con la ayuda de un horno, las cenizas con la ayuda de un horno de mufla, las proteínas por el método micro Kjeldahl, las fibras alimentarias totales por un procedimiento de gravimetría no-enzimática, los lípidos con un extractor Soxhlet. La vitamina C y los carotenoides se analizaron por HPLC-DAD, y la vitamina E y los folatos por HPLC con detección por fluorescencia. **Resultados y discusión.** El « jatobá do Cerrado » presentó escasos rendimientos en pulpa (un 17,1%) y contenidos de humedad ( $8,8 \text{ g}\cdot 100 \text{ g}^{-1}$ ), y altos contenidos en fibras alimentarias totales ( $44,3 \text{ g}\cdot 100 \text{ g}^{-1}$ ), proteínas ( $5,6 \text{ mg}\cdot 100 \text{ g}^{-1}$ ) y energía ( $193,0 \text{ kcal}\cdot 100 \text{ g}^{-1}$ ). El fruto presentó bajos contenidos en carotenoides y vitamina C [(0,4 et 8,9)  $\text{mg}\cdot 100 \text{ g}^{-1}$ , respectivamente] y contenidos en vitamina E y en folatos [(53,5 y 495,5)  $\mu\text{g}\cdot 100 \text{ g}^{-1}$ , respectivamente] superiores a otros frutos muy consumidos. **Conclusión.** El « jatobá do Cerrado » es una fuente de vitamina C, una buena fuente de folatos, y una excelente fuente de fibras alimentarias. Dado su valor nutritivo, el « jatobá do Cerrado » es un recurso importante para la alimentación. Por lo tanto, su consumo debería potenciarse.

**Brasil / Minas Gerais / *Hymenaea stigonocarpa* / frutas / propiedades fisicoquímicas / carotinoides / contenido vitamínico / valor energético**