

# 'Araçá of Cerrado' from the Brazilian Savannah: physical characteristics, chemical composition, and content of carotenoids and vitamins

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## 'Araçá of Cerrado' from the Brazilian Savannah: physical characteristics, chemical composition, and content of carotenoids and vitamins.

**Abstract – Introduction.** The Brazilian Savannah has one of the richest biodiversities in the world and contains native fruits presenting high nutritional value. These fruits are the basis for sustaining wildlife and also serve as a food source for the population. Among these, the 'araçá of Cerrado' (*Psidium firmum* O. Berg), a fruit found naturally in the Brazilian Savannah, stands out. Therefore, we evaluated the physical characteristics, chemical composition, and occurrence and content of vitamin C, carotenoids, vitamin E and folates in 'araçá of Cerrado' from the Savannah of Minas Gerais, Brazil. **Materials and methods.** Titratable acidity was determined by volumetric neutralization, pH by potentiometry, soluble solids by refractometry, moisture using an oven, ash using a muffle furnace, protein by the micro-Kjeldhal method, total dietary fiber by the gravimetric non-enzymatic method, and lipids with a Soxhlet extractor. Vitamin C (ascorbic and dehydroascorbic acids) and carotenoids ( $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin and lycopene) were analyzed by HPLC-DAD. Vitamin E ( $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ -tocopherols and  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ -tocotrienols) and folates (tetrahydrofolate, 5-methyltetrahydrofolate and 5-formyltetrahydrofolate) were analyzed by HPLC with fluorescence detection. **Results and discussion.** The araçá presented a high pulp yield (81.43%), soluble solids (11.93 °Brix), moisture (77.46%), dietary fiber (12.32 g·100 g<sup>-1</sup>) and folates (47.25 mg·100 g<sup>-1</sup>). It presented a low total energy value of 43.19 kcal·100 g<sup>-1</sup>, and low contents of carotenoids (0.32 mg·100 g<sup>-1</sup>) and vitamin E (336.43 mg·100 g<sup>-1</sup>). Vitamin C was not found in the fruit. **Conclusion.** 'araçá of Cerrado' fruit stands out due to its high yield and showed good nutritional value, being classified as an excellent source of dietary fiber and at least a source of folates. Thus, its consumption should be encouraged.

**Brazil / Minas Gerais / *Psidium firmum* / fruits / physicochemical properties / carotenoids / vitamin content / energy value**

## « Araçá du Cerrado », fruit de la savane brésilienne : caractéristiques physiques, composition chimique et teneur en caroténoïdes et vitamines.

**Résumé – Introduction.** La savane brésilienne est l'une des biodiversités les plus riches au monde ; elle contient des fruits indigènes présentant une haute valeur nutritionnelle. Ces fruits permettent à la faune de se nourrir et ils servent également de source de nourriture pour les populations. Parmi ceux-ci, la présence d' « araçá du Cerrado » (*Psidium firmum* O. Berg), un fruit naturellement présent dans la savane brésilienne, est à souligner. De ce fait, nous avons évalué les caractéristiques physiques, la composition chimique, la fréquence et la teneur en vitamine C, caroténoïdes, vitamine E et folates dans le fruit d' « araçá du Cerrado » de la savane de Minas Gerais, au Brésil. **Matériel et méthodes.** 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 (acides ascorbique et déhydroascorbique) et les caroténoïdes ( $\alpha$ -carotène,  $\beta$ -carotène,  $\beta$ -cryptoxanthine et lycopène) ont été analysés par HPLC-DAD ; la vitamine E ( $\alpha$ ,  $\beta$ ,  $\gamma$  et  $\delta$ -tocophérols et  $\alpha$ ,  $\beta$ ,  $\gamma$  et  $\delta$ -tocotriénols) et les folates (tetrahydrofolate, 5-méthyltetrahydrofolate et 5-formyltetrahydrofolate) par HPLC avec détection par fluorescence. **Résultats et discussion.** Le fruit de l'araçá a présenté un fort rendement en pulpe (81,43 %) et des teneurs élevées en solides solubles (11,93 °Brix), humidité (77,46 %), fibres alimentaires (12,32 g·100 g<sup>-1</sup>) et folates (47,25 mg·100 g<sup>-1</sup>). Il a montré une faible valeur énergétique totale de 43,19 kcal·100 g<sup>-1</sup>, et de faibles teneurs en caroténoïdes (0,32 mg·100 g<sup>-1</sup>) et en vitamine E (336,43 mg·100 g<sup>-1</sup>). La vitamine C n'a pas été trouvée dans ce fruit. **Conclusion.** Le fruit « araçá du Cerrado » se distingue par son rendement élevé ; étant considéré comme une excellente source de fibres alimentaires et une source de folates, il se révèle avoir une bonne valeur nutritive. Sa consommation doit donc être encouragée.

**Brésil / Minas Gerais / *Psidium firmum* / fruits / propriété physicochimique / caroténoïde / teneur en vitamines / valeur énergétique**

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Received 22 October 2012  
Accepted 20 December 2012

Fruits, 2013, vol. 68, p. 467–481  
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DOI: 10.1051/fruits/2013095  
[www.fruits-journal.org](http://www.fruits-journal.org)

RESUMEN ESPAÑOL, p. 481

## 1. Introduction

The Savannah is the second largest biome in South America and Brazil, and covers approximately 25% of the Brazilian territory [1]. Due to its extensive area and privileged geographical position, it is the most characteristic Brazilian biome. The Brazilian Savannah presents a wide variety of micro-environments and numerous springs and streams that feed the major river basins of the country. Furthermore, it stands out due to its plant heterogeneity and its numerous native species, which are still little studied [2].

Fruit species native to the Savannah may have high nutritional value and contribute to a healthy diet. These fruits are potential sources of macro- and micronutrients such as dietary fiber, carbohydrates, lipids, vitamins and carotenoids. They also have particular characteristics such as various shapes, bright colors, *sui generis* flavors and intense sensory aspects that make their use attractive [2, 3].

The 'araçá of Cerrado' or 'araçá rasteiro' (*Psidium firmum* O. Berg), a species belonging to the family Myrtaceae, is widely distributed in Brazil and in other parts of the world. This plant provides tasty and edible fruits which are utilized in animal feeding, in agribusiness and in human nutrition [3]. In human nutrition the fruit is consumed fresh or in the form of regional delicacies such as juices, frozen fruit pulps, ice creams, jams, liqueurs, sweets and other products [3]. Furthermore, tea of the leaves is used in folk medicine to combat diarrhea and the tree is used in landscaping and restoration of degraded areas [4].

Thus, the present study evaluated the physical and physicochemical characteristics, occurrence and content of carotenoids, vitamin C, vitamin E and folates, and the nutritional value of 'araçá of Cerrado' from the Savannah of Minas Gerais, Brazil.

## 2. Materials and methods

### 2.1. Raw material collection and sample preparation

'Araçá of Cerrado' fruits (*Psidium firmum* O. Berg) were collected during the harvest

season (December 2010 to March 2011), in an area of native vegetation typical of the Savannah, in Diamantina (south latitude 18°14' and west longitude 43°36'), Minas Gerais, Brazil. The fruits were collected directly from the tree to avoid injuries due to its fragile shell. To obtain five repetitions, the collection area was divided into sub-areas, and approximately 0.4 kg of fruits were collected in each sub-area ( $\pm 35$  fruits). In the laboratory, the fruits were selected according to the degree of maturation, based on the color and texture features. Ripe fruits were considered those with a predominantly yellow shell and soft texture. Subsequently, the fruits were washed with tap water to eliminate surface dirt from the collection site and dried on paper towels.

After this process, the shell was removed and discarded, and the edible portion (seed + pulp) was homogenized in a food processor (Faet Multipratic, MC5, Brazil), packaged in plastic bags, and stored for up to 4 days in the freezer at  $(-18 \pm 1)$  °C.

### 2.2. Physical characterization

For physical characterization, individual measurements of diameter and height were carried out in 30 fruits using a digital caliper (Mitutoyo, Brazil). The mass of the fruit (MF), edible portion (MP) and shell (MS) were obtained by individual direct weighing on a semi-analytical scale (Gehaka, BG 2000, Brazil). The pulp yield was calculated using the equation  $[(MP / MF) \times 100]$ .

### 2.3. Physicochemical analysis

Titrateable acidity (TA), soluble solids (SS), the [soluble solids / titrateable acidity] ratio, pH, moisture, ash, protein, lipids and total dietary fiber were determined according to the methodologies proposed by the Institute Adolfo Lutz and Association of official Analytical Chemistry, respectively [5, 6]. The titrateable 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 were determined by refractometry, using a portable refractometer. The

[SS / TA] ratio was obtained by dividing the content of soluble solids by titratable acidity. Moisture content was determined using an oven at 105 °C (New Ethics, 400, Brazil) and ash using a muffle furnace at 550 °C (Quimis, 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, Brazil), while available carbohydrates were calculated as the difference, using 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 kcal·g<sup>-1</sup> for proteins and available carbohydrates, and 9 kcal·g<sup>-1</sup> for lipids [5]. Analyses were performed on three repetitions at the Laboratories of Food Analysis and Vitamin Analysis in the Department of Nutrition and Health, Federal University of Viçosa (DNS-UFV), Brazil.

## 2.4. Extraction and analysis of carotenoids and vitamins

Analyses of carotenoids and vitamins were performed in the Laboratory of Vitamin Analysis (LAV) of DNS-UFV. During 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, and were also protected from oxygen by using lids and nitrogen gas environments in the glass bottles. The analyses were performed in five repetitions.

### 2.4.1. Extraction and analysis of carotenoids

The occurrence and content of the main carotenoids in fruits ( $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin and lycopene) were investigated in 'araçá of Cerrado' pulp. Extraction was performed according to Rodriguez-Amaya *et al.* [7], with modifications. About 5 g of pulp were weighed, supplemented with 60 mL of cooled acetone (divided into three volumes of 20 mL), homogenized in

a micro-crusher (Marconi, MA 102, Brazil) for approximately 3 min, and vacuum-filtered in a Büchner funnel using filter paper. Then, the filtrate was transferred in three fractions to a separatory funnel containing 50 mL of cooled petroleum ether. Each fraction was washed with distilled water for complete removal of acetone. Anhydrous sodium sulfate was added to the ether extract to remove any residual water. Subsequently, the ether extract was concentrated using a rotary evaporator (Tecnal, TE-211, Brazil) at  $(35 \pm 1)$  °C and transferred to a 25-mL volumetric flask, where the volume was completed with petroleum ether. This extract was then transferred to a hermetically sealed amber glass bottle and stored at  $(-18 \pm 1)$  °C.

For the chromatographic analysis, a 10-mL aliquot of the extract was evaporated under nitrogen gas flow, and the dry residue redissolved in 2 mL of HPLC-grade acetone (Tedia, Brazil). The extracts were filtered through HV Millex filter units, in polyethylene, with 0.45  $\mu\text{m}$  of porosity (Millipore, Brazil), and 50  $\mu\text{L}$  was injected into the column for analysis. Carotenoid analyses were performed by high-performance liquid chromatography (HPLC) and the chromatographic conditions developed by Pinheiro-Sant'Ana *et al.* [8], with modifications: a HPLC-DAD system (Shimadzu, SCL 10ATVP, Japan) comprising a high-pressure pump (Shimadzu, LC-10AT VP, Japan), autosampler with 50- $\mu\text{L}$  loop (Shimadzu SIL-10AF, Japan) and diode array detector (DAD) (Shimadzu SPD-M10A, Japan); a chromatography column (Phenomenex Gemini RP-18; 250 mm  $\times$  4.6 mm, 5  $\mu\text{m}$ ) fitted with a guard column (Phenomenex ODS; 4 mm  $\times$  3 mm), mobile phase consisting of methanol: ethyl acetate:acetonitrile (70:20:10, v/v/v) at a flow rate of 2 mL·min<sup>-1</sup> and run time of 15 min. Chromatograms were obtained at 450 nm.

Vitamin A concentrations were calculated according to the recommendations of the Institute of Medicine [9], 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. Extraction and analysis of vitamin C

The occurrence and content of ascorbic acid (AA) and dehydroascorbic acid (DHA) were investigated in 'araçá of Cerrado' pulp.

Extraction and analysis of ascorbic acid were carried out according to the conditions proposed by Campos *et al.* [10], with modifications. For the extraction, about 5 g of pulp were ground for about 3 min in 15 mL of extraction solution composed of ultrapure water supplemented with metaphosphoric acid 3%, acetic acid 8%, sulfuric acid 0.8% and EDTA 0.0294%. The extract was centrifuged (Fanem, Excelsa Baby II - 206R, Brazil) at 4,000 rpm (1,789 g) for 15 min, vacuum-filtered on filter paper in a Buchner funnel, and diluted to 25 mL in a volumetric flask with ultrapure water. Subsequently, the extract was again centrifuged at 14,000 rpm (21.913 g) for 5 min and the supernatant stored under refrigeration [(5 ± 1) °C] until analysis. The conversion of dehydroascorbic acid into ascorbic acid was performed according to Campos *et al.* [10]. A 2-mL aliquot of the extract obtained in the previous stage was pipetted into an amber glass, and supplemented with 0.8 mL of 1.2 M Trizma buffer solution (pH 9.0) containing 40 mM dithiothreitol (Sigma-Aldrich, Germany) to bring the pH closer to neutral (pH 6.0). For the conversion reaction, the extract was maintained at rest for 10 min at room temperature and away from light. Subsequently, a H<sub>2</sub>SO<sub>4</sub> 0.4 mM content of 0.9 mL was added to the extract to lower the pH (pH 2.0). The HPLC analyses were performed from the injection of 50 µL of extract previously filtered in filter units with a porosity of 0.45 µm. Analysis of vitamin C was performed using the same HPLC system used for analysis of carotenoids and the chromatographic conditions used were: a chromatographic column (RP-18 Lichrospher 100: 250 mm × 4 mm, 5 µm), HPLC-DAD system, mobile phase composed of ultrapure water with 1 mM of NaH<sub>2</sub>PO<sub>4</sub>, 1 mM of EDTA and pH adjusted to 3.0 with H<sub>3</sub>PO<sub>4</sub>, mobile phase flow of 1.0 mL min<sup>-1</sup>, and run time of 5 min. Chromatograms were obtained at 245 nm [10]. The dehydroascorbic acid content was calculated using the

equation: [dehydroascorbic acid content = ascorbic acid content after conversion – ascorbic acid content before conversion] [9].

#### 2.4.3. Extraction and analysis of vitamin E

The occurrence and content of the eight components of vitamin E ( $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherols, and  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocotrienols) in 'araçá of Cerrado' pulp were investigated. The extraction and analysis of the compounds were performed according to Pinheiro-Sant'Ana *et al.* [11]. Approximately 10 g of pulp were supplemented with 4 mL of heated ultrapure water [(about (80 ± 1) °C)], 10 mL of isopropyl alcohol, 1 mL of hexane containing BHT 0.05% and 5 g of anhydrous sodium sulfate. Gradually, 25 mL of the extraction solvent mixture (hexane:ethyl acetate, 85:15, v/v) were added to the suspension. After these procedures, the suspension was homogenized in a micro-crusher at average speed for 1 min. Once ground, the sample was vacuum-filtered through filter paper in a Büchner funnel, and the residue was maintained in an extraction tube. The extraction was repeated with the addition of 5 mL of isopropyl alcohol and 3 mL of the solvent mixture, with subsequent homogenization and vacuum filtration. Then the extract was concentrated in a rotary evaporator at (70 ± 1) °C for about 2 min, transferred to a volumetric flask and the volume completed to 25 mL with the solvent mixture. After extraction, aliquots of the extract (5 mL) were dried under nitrogen gas, redissolved in 2 mL of HPLC-grade hexane (Tedia, Brazil) and filtered through filter units with porosity of 0.45 µm. Analyses were performed by HPLC with injection of 50 µL of the extract in a HPLC system (Shimadzu SCL 10AD VP, Japan), comprising a high-pressure pump (Shimadzu LC-10AD VP, Japan), autosampler with 50-µL loop (Shimadzu SIL - 10AF, Japan) and fluorescence detector (Shimadzu, RF10AXL, Japan). The chromatographic conditions used for the analysis were developed by Pinheiro-Sant'Ana *et al.* [11], which included: HPLC system; fluorescence detection (290 nm excitation and 330 nm emission); Luna chromatography column (Si60 Phenomenex, 250 mm × 4 mm, 5 µm), fitted with a Phenomenex guard column (Si60,

4 mm × 3 mm), mobile phase composed of [hexane:isopropanol:acetic acid] (Tedia, Brazil) (98.9:0.6:0.5, v/v/v), mobile phase flow of 1 mL·min<sup>-1</sup>, and run time of 20 min. The total content of vitamin E was calculated by summing the identified vitamin E components.

#### 2.4.4. Extraction and analysis of folates

The occurrence and content of tetrahydrofolate (THF), 5-methyltetrahydrofolate (5-MTHF) and 5-formyltetrahydrofolate (FTHF-5) in the pulp of ‘araçá of Cerrado’ were also investigated. Extraction and analysis were performed according to Della Lucia *et al.* [12], with some modifications. Approximately 5 g of the pulp were ground in 20 mL of phosphate buffer solution 0.1 M, pH 6.0, containing ascorbic acid 1% and 2-mercaptoethanol 0.1%. The suspension was centrifuged at 4000 rpm (1789 g) for 15 min, vacuum-filtered in a Buchner funnel using filter paper, and diluted to 25 mL in a volumetric flask with ultrapure water. Then, the extract was heated for about 12 min in a water bath at (100 ± 1) °C and cooled in an ice bath until the temperature fell below (37 ± 1) °C. The cooled extract was centrifuged again at 4000 rpm (1789 g) for 15 min and submitted to deconjugation of the polyglutamates into monoglutamates. For deconjugation, rat plasma (100 µL) containing the enzyme conjugase ( $\gamma$ -glutamyl carboxypeptidase) was added to 3 mL of the previously obtained supernatant, and the extract was incubated in a water bath at (37 ± 1) °C for 3 h. Then, the extract was heated in boiling water for 5 min to inactivate the enzyme. Purification of the extract was performed 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 two drops per second, and the extract was applied to the column at a flow rate of two drops per second. Then, the retained folates were eluted in 1.5 mL of sodium acetate solution (0.1 M) containing NaCl 10%, ascorbic acid 1% and 2-mercaptoethanol 0.1%. Analyses were carried out by injecting 50 µL of the extracts, previously filtered in filter units with porosity of 0.45 µm, into the same system used for analysis of vitamin E, which in this case

was also equipped with a helium-degassing system for the mobile phase (Shimadzu DGU-2, Japan). The chromatographic conditions used for analyses included: a Shim Pack 100 RP18 chromatographic column (150 mm × 4.6 mm, 4.6 µm) (Merck, Germany), and a mobile phase composed of a binary gradient containing phosphate buffer solution (NaH<sub>2</sub>PO<sub>4</sub> 30 mM, pH adjusted to 2.3 with H<sub>3</sub>PO<sub>4</sub>) as eluent A, and acetonitrile as eluent B. The gradient utilized was as follows: from 0 to 5 min, 94% of the eluent A + 6% of the eluent B; from 5 to 25 min, a linear gradient to 75% of A + 25% of B; from 25 to 33 min, 75% of A + 25% of B; from 33 to 35 min, return to initial conditions followed by stabilization until 50 min. The mobile phase flow was 0.7 mL·min<sup>-1</sup> and fluorescence detection occurred with excitation at 290 nm and emission at 360 nm. The mobile phase was degassed with helium for 15 min at 100 kPa before initiating the analyses, and at 50 kPa during the runs [12].

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

Identification and quantification of compounds were performed using the following standards:  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ -tocopherols and  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ -tocotrienols from Calbiochem®, EMD Biosciences, Inc. (USA); L-ascorbic acid from Sigma-Aldrich® (Germany); (6S)-tetrahydrofolate-5,6,7,8 sodium, (6S)-5-methyl-5,6,7,8-tetrahydrofolate and (6S)-5-formyl-5,6,7,8-tetrahydrofolate were supplied by Merck-Eprova® (Switzerland);  $\alpha$ -carotene and  $\beta$ -carotene were isolated from concentrated extract of carrots, and  $\beta$ -cryptoxanthin and lycopene were isolated from extracts of papaya and tomato, respectively, by open-column chromatography [13].

Identification of compounds was performed by comparison of the retention times obtained for the standards and samples analyzed under the same conditions. In addition, carotenoids and ascorbic acid were identified by comparing the absorption spectra of the standards and samples using the DAD, and the folates and vitamin E by co-chromatography.

For quantification of the compounds, external standard curves were used. Appropriate dilutions were made from the standard solutions in order to achieve concentrations comparable with those observed in the pulp of 'araçá of Cerrado'. Solutions of each compound present in the pulp of the fruit were prepared for this purpose ( $\beta$ -carotene,  $\beta$ -cryptoxanthin,  $\alpha$ -tocopherol,  $\gamma$ -tocopherol, THF, 5-MTHF and 5-FTHF).

The construction of standard curves was performed by injection, in duplicate, of six increasing concentrations of the standard solutions in the range from (0.2060 to 6.0321)  $\mu\text{g}$  for  $\beta$ -carotene; (0.0045 to 1.4333  $\mu\text{g}$  for  $\beta$ -cryptoxanthin; (0.0010 to 0.1042)  $\mu\text{g}$  for  $\alpha$ -tocopherol; (0.0035 to 0.1040)  $\mu\text{g}$  for  $\gamma$ -tocopherol, (0.00004 to 0.04622)  $\mu\text{g}$  for THF, (0.00001 to 0.01077)  $\mu\text{g}$  for 5-MTHF, and (0.00003 to 0.03312)  $\mu\text{g}$  for 5-FTHF. Thus, a linear correlation was constructed between the peak areas and the injected concentrations of each compound.

Quantification of compounds in 'araçá of Cerrado' was based on the analytical curves and regression equations achieved for  $\beta$ -carotene ( $y = 1421302.23x + 3563.819$ ,  $R^2 = 0.999$ ),  $\beta$ -cryptoxanthin ( $y = 1730130.1624x - 8057.5853$ ,  $R^2 = 0.999$ ),  $\alpha$ -tocopherol ( $y = 93284137.0896x + 47566.8009$ ,  $R^2 = 0.999$ ),  $\gamma$ -tocopherol ( $y = 234829959.333x + 731230.429$ ,  $R^2 = 0.995$ ), THF ( $y = 942240050.58x - 162371.44$ ;  $R^2 = 0.996$ ), 5-MTHF ( $y = 1237294689.6711x - 59476.9661$ ,  $R^2 = 0.998$ ) and 5-FTHF ( $y = 710036264.81x - 1088694.36$ ,  $R^2 = 0.996$ ). The real concentration was achieved via calculations based on the dilution factors.

## 2.6. Quality control of the analytical methods

Tests of recovery, linearity, repeatability, limit of detection (LOD) and limit of quantification (LOQ) were performed for quality control of analytical methods for vitamins and carotenoids. The recovery tests were conducted by addition of standards ( $\beta$ -carotene,  $\beta$ -cryptoxanthin,  $\alpha$ -tocopherol,  $\gamma$ -tocopherol, THF, 5-MTHF and 5-FTHF) to the samples. The quantity of the standard

added varied between 50% and 100% of the initial average concentration observed in the pulp. The percent recovery was calculated using the equation:  $\{\% \text{ recovery} = [(\text{final concentration of compound} - \text{concentration of compound added}) / (\text{initial concentration of compound})] \times 100\}$ . All procedures were performed in triplicate.

The linearity range of compounds was performed by injection, in duplicate, of six standard solutions of increasing concentrations using the same chromatographic conditions used for analysis of the extracts. Data obtained for the peak areas were used for linear regression analysis. The coefficient of determination ( $R^2$ ) obtained in each case was used to evaluate the linearity [14].

Repeatability tests were performed by extraction and analysis in quintuplicate of the same repetition of a pulp sample containing vitamins and carotenoids. Repeatability was assessed by calculating the relative standard deviation (RSD) of the peak areas and retention times of the analyzed components [14].

The limits of detection were determined by successive dilution of the vitamin standards identified in the fruit, followed by determination of the smallest detectable amount. The limit of quantification was established as three times the amplitude of the baseline noise and as ten times the limit of detection [15].

## 2.7. Categorization of 'araçá of Cerrado' as a source of vitamins

The categorization of 100 g of 'araçá of Cerrado' as a source of vitamins for children (aged 4 to 8 years), pregnant women and adult men (aged 19 to 30 years) was performed using the criteria proposed by Philippi [16], which classifies food as a "source" when it supplies 5% to 10% of the Dietary Reference Intake (DRI), "good source" when it supplies 10% to 20% of the DRI and "excellent source" when supplying more than 20% of the DRI.

## 2.8. Experimental design and statistical analysis

The experimental design was completely randomized with three repetitions for physicochemical analyses and five repetitions for analysis of carotenoids and vitamins. Data was stored in spreadsheets using Microsoft Office Excel 2007. Means, standard deviations and ranges of the parameters were calculated using the SAS package (Statistical Analysis System), version 9.2 (2008), licensed to UFV.

## 3. Results and discussion

### 3.1. Physical characterization

The 'araçá of Cerrado' is a round and slightly flattened berry with a yellowish shell, and juicy and fleshy pulp containing cream-colored seeds (*figure 1*).

Fruits of the 'araçá of Cerrado' presented an average diameter and height of 2.79 cm and 3.00 cm, respectively. The fruit mass varied between (7.28 and 18.71) g (average of 12.73 g). The shell and edible portion masses were 2.34 g and 10.39 g, respectively. The fruit presented a high yield of the edible portion (81.43%), which is an important feature for its economic and technological exploitation for product development. This yield was greater than that observed in 'araçá pêra' (*Psidium acutangulum* D.C.) (75.67%) growing wild in the Amazon region, Brazil [17].

The physical characteristics of 'araçá of Cerrado' were smaller than those found in different varieties of guava (*Psidium guajava* L.) [18, 19]. In guava, the fruit mass, diameter and height ranged from (90.8 to 244.5) g, (5.84 to 7.60) cm and (5.3 to 7.79) cm, respectively [18, 19].

Cerrado fruits are not domesticated and therefore their chemical content and physical characteristics may vary significantly. These characteristics may be affected by the edaphoclimatic characteristics of the fruit collection sites. Furthermore, differences between the physical characteristics observed in the 'araçá of Cerrado' and in other



**Figure 1.** Fruits of the 'araçá of Cerrado' (*Psidium firmum* O. Berg). fruits such as guava and other varieties of araçá may reflect the intrinsic characteristics of each fruit, which are of different species.

### 3.2. Physicochemical characterization

The main indices used by the food industry related to quality of fruit pulps for elaboration of products are the soluble solids (SS), titratable acidity (TA), the [SS / TA] ratio and the pH. Knowledge of SS content in the

fruit is important since the greater the amount of SS, the lower the quantity of sugar required to be added to the pulp during processing, thus reducing the production cost and increasing product quality [20].

Pulp of 'araçá of Cerrado' showed a high SS content, which was higher than that found in 'araçá pêra' from the Amazon (8.56 °Brix), 'araçá of campo' (*Psidium guineensis* Sw) from the state of Minas Gerais (8.8 °Brix) and guava (5.50 °Brix) [17, 21, 22]. Therefore, the development of products using pulp of the 'araçá of Cerrado' may be profitable due to its SS content since less sugar is required.

Another criterion for the classification of fruit flavor, odor, stability and quality is the determination of TA [20]. The 'araçá of Cerrado' analyzed in our study presented TA content lower than that of 'araçá pêra' from the Amazon (2.67 g citric acid·100 g<sup>-1</sup>) [17] and higher than observed in different varieties of guava [(0.4 and 0.8) g citric acid·100 g<sup>-1</sup>] [18, 22]. A high [SS / TA] ratio was observed in the 'araçá of Cerrado' pulp. This parameter is related to fruit quality in terms of maturity and flavor, showing the balance between sugars and acidity which makes it more palatable, highlighting its sweet and pleasant taste [20].

The pH of 'araçá of Cerrado' (3.49) was higher than that observed in 'araçá pêra' (2.77) [17] and lower than that reported in 'araçá of campo' (3.99) [21] and in different varieties of guava (from 3.75 to 4.22) [18, 22]. This difference may be due to the comparison of different fruit species and edaphoclimatic differences among the sampling sites.

### 3.3. Chemical characterization

Data regarding the chemical characteristics, and carotenoid and vitamin contents of araçá pulp are scarce, especially for the species 'araçá of Cerrado'. This makes it difficult to perform comparisons with the results obtained in our study. Thus, the 'araçá of Cerrado' was compared with fruits considered as sources of the nutrient analyzed, fruits belonging to the genre *Psidium* (guava and other varieties of araçá) and native fruits from the Savannah. The 'araçá of Cerrado' presented high

moisture content (77.46 g·100 g<sup>-1</sup>) (table D), but it was lower than that observed in 'araçá pêra' (82.49 g·100 g<sup>-1</sup>), 'araçá of campo' (80.41 g·100 g<sup>-1</sup>) [17, 21] and guava (85.7 g·100 g<sup>-1</sup>) [23]. As observed in the 'araçá of Cerrado', fruits generally have high moisture content. This characteristic, combined with the fragility of its shell, can lead to a rapid degradation of the 'araçá of Cerrado'. Therefore, it is recommended that it be consumed or utilized in product development immediately, or cooled for storage.

Pulp from 'araçá of Cerrado' presented a high dietary fiber content (12.32 g·100 g<sup>-1</sup>) (table D), which was higher than that found in 'araçá pêra' collected in the Amazon, Brazil (9.66 g·100 g<sup>-1</sup>) [17], and up to five times higher than that observed in fiber sources such as guava (6.2 g·100 g<sup>-1</sup>), jambo (5.1 g·100 g<sup>-1</sup>), orange (4.0 g·100 g<sup>-1</sup>), tangerine (3.1 g·100 g<sup>-1</sup>), cherry (3.2 g·100 g<sup>-1</sup>) and apple (2.4 g·100 g<sup>-1</sup>) [25]. It can be considered an excellent source of fiber since it may provide 41% of the recommended intake for adults (30 g per day) [26]. Due to the high content of dietary fiber, this fruit can help control intestinal transit and reduce both serum glucose and cholesterol [27].

The 'araçá of Cerrado' presented low lipid content (table D) and thus its consumption should be encouraged, especially by overweight individuals and those with lipid disorders. However, this content (1.07 g·100 g<sup>-1</sup>) was higher than that found in 'araçá of campo' collected in the Brazilian state of Minas Gerais (0.33 g·100 g<sup>-1</sup>) [21] and guava (0.5 g·100 g<sup>-1</sup>) [23]. The protein content (1.45 g·100 g<sup>-1</sup>) was lower than that of 'araçá of campo' (1.87 g·100 g<sup>-1</sup>) [21] and higher than that of guava (0.9 g·100 g<sup>-1</sup>) [23].

The energy value of 'araçá of Cerrado' pulp was low (43.19 kcal·100 g<sup>-1</sup>, table D), and thus its consumption should be encouraged, especially by overweight individuals. This value was similar to that observed in 'araçá of campo' from the state of Mato Grosso do Sul, Brazil (44.5 kcal·100 g<sup>-1</sup>) [24], and lower than that in 'araçá of campo' from the state of Minas Gerais (78.2 kcal·100 g<sup>-1</sup>) [21].



**Table I.** Physicochemical characteristics and total energy of 'araçá of Cerrado' pulp (*Psidium firmum* O. Berg) from the Savannah (Diamantina, Minas Gerais, Brazil). Values are expressed in fresh matter; data present the mean  $\pm$  standard deviation of three repetitions.

Soluble solids (SS) ( $^{\circ}$ Brix)	Titratable acidity (TA) (g citric acid $\cdot$ 100 g $^{-1}$ )	[SS / TA] ratio	pH	Moisture	Ash	Proteins (g $\cdot$ 100 g $^{-1}$ )	Total dietary fiber	Lipids	Available carbohydrates	Total energy value (kcal $\cdot$ 100 g $^{-1}$ )
11.93 $\pm$ 0.66	1.14 $\pm$ 0.12	10.52 $\pm$ 1.80	3.49 $\pm$ 0.07	77.46 $\pm$ 0.38	0.75 $\pm$ 0.08	1.45 $\pm$ 0.13	12.32 $\pm$ 0.53	1.07 $\pm$ 0.36	6.91 $\pm$ 0.61	43.19 $\pm$ 5.08

**Table II.**

Repeatability, limit of detection and quantification, linearity range and recovery of vitamins found in 'araçá of Cerrado' pulp (*Psidium firmum* O. Berg) from the Savannah (Diamantina, Minas Gerais, Brasil).

Compounds	Repeatability (%)		Limit of detection	Limit of quantification	Range of linearity ( $\mu\text{g}$ )	Recovery (%)
	Peak area	Retention time				
	Relative standard deviation					
$\beta$ -carotene	2.24	0.34	6.422	64.221	0.2060 – 6.0321	93.1
$\beta$ -cryptoxanthin	1.04	0.60	6.961	69.613	0.0045 – 1.4333	97.4
$\alpha$ -tocopherol	1.06	1.72	0.025	0.251	0.0104 – 0.1040	94.3
$\gamma$ -tocopherol	0.82	1.28	0.025	0.252	0.0022 – 0.1076	98.7
THF	2.55	0.70	0.003	0.031	0.00004 – 0.04622	98.8
5-MTHF	4.37	0.72	0.002	0.024	0.00001 – 0.01077	97.2
5-FTHF	1.99	0.59	0.002	0.021	0.00003 – 0.03319	85.7

THF: tetrahydrofolate.

5-MTHF: 5-methyltetrahydrofolate.

5-FTHF: 5-formyltetrahydrofolate.

### 3.4. Carotenoids and vitamins

#### 3.4.1. Quality of analytical methods

The tests for quality control of the analytical methods demonstrated that the analysis conditions were reliable, with little likelihood of loss of vitamins during extraction and analysis, allowing the detection of low concentrations of the compounds examined (table II).

In the repeatability test, the relative standard deviation in relation to the peak areas and retention times was lower than 4.37% and 1.72%, respectively. The limit of detection for the compounds analyzed varied between 6.422 mg·mL<sup>-1</sup> and 6.961 mg·mL<sup>-1</sup> and the limit of quantification ranged from 64.22 mg·mL<sup>-1</sup> to 69.61 mg·mL<sup>-1</sup>. The linearity range for each compound analyzed was large and the coefficients of determination ( $R^2$ ) were greater than 0.995. Percent recovery of the vitamin standards added to the samples ranged from 85.7% to 98.8% with a mean of 94.9% (table II).

#### 3.4.2. Qualitative composition

Typical chromatograms of the carotenoid and vitamin analyses of 'araçá of Cerrado' from the Savannah of Minas Gerais showed that in the pulp the following compounds

were found:  $\beta$ -cryptoxanthin (retention time - RT: 4.14 min),  $\beta$ -carotene (RT: 9.20 min),  $\alpha$ -tocopherol (RT: 6.93 min),  $\gamma$ -tocopherol (RT: 12.04 min), THF (RT: 8.50 min), 5-MTHF (RT: 10.3 min) and 5-FTHF (RT: 18.04 min) (figure 2).  $\alpha$ -Carotene, lycopene, ascorbic acid, dehydroascorbic acid,  $\beta$ , and  $\delta$ -tocopherols, and  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ -tocotrienols were not identified in the fruit.

#### 3.4.3. Carotenoid and vitamin content

Information regarding the occurrence and content of carotenoids and vitamins in 'araçá of Cerrado' pulp are scarce in the scientific literature. In contrast to other fruits of the *Psidium* family, the 'araçá of Cerrado' did not present vitamin C. However, it is herein emphasized that the presence of this vitamin is reported in other fruit species, including fruits also popularly called araçá-cagão (*Psidium rufum*) [28].

The carotenoid content in 'araçá of Cerrado' (table III) was lower than that observed in sources of this nutrient, such as papaya (0.38 mg·100 g<sup>-1</sup>), guava (1.05 mg·100 g<sup>-1</sup>) and mango (1.47 mg·100 g<sup>-1</sup>) [29], and in fruits from the Brazilian Savannah such as 'jatobá of Cerrado' (0.39 mg·100 g<sup>-1</sup>), cagaita (0.77 mg·100 g<sup>-1</sup>), araticum (4.98 mg·100 g<sup>-1</sup>) and pequi (8.10 mg·100 g<sup>-1</sup>) [30–33]. This content was superior to that found

in mangaba from the Brazilian Savannah ( $0.12 \text{ mg}\cdot 100 \text{ g}^{-1}$ ) [34].

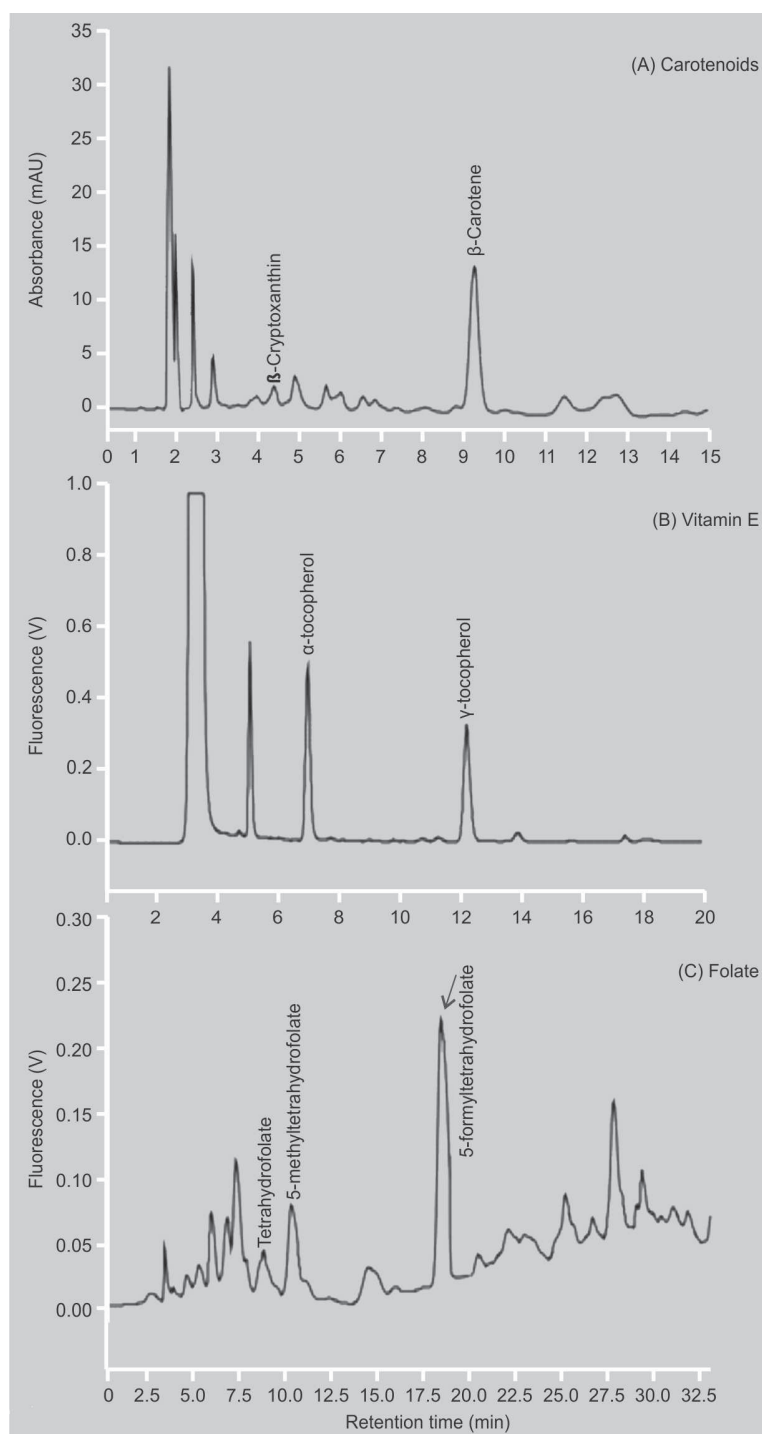
The 'araçá of Cerrado' presented low vitamin E, which was composed mainly of  $\alpha$ -tocopherol (table III). This content was higher than cooked pequi from the Savannah ( $170.81 \text{ }\mu\text{g}\cdot 100 \text{ g}^{-1}$ ) [33] and lower than that found in araticum ( $494.04 \text{ }\mu\text{g}\cdot 100 \text{ g}^{-1}$ ) [32], jatobá ( $495.54 \text{ }\mu\text{g}\cdot 100 \text{ g}^{-1}$ ) [30] and mangaba ( $2732.47 \text{ }\mu\text{g}\cdot 100 \text{ g}^{-1}$ ) [34] from the Brazilian Savannah. Although low, the presence of vitamin E in this fruit is very important since, as well as other Savannah fruits with low vitamin E contents, it is present in areas where food sources of this vitamin may not be available. Furthermore, this nutrient is an important antioxidant and thus helps in the prevention of several chronic non-communicable diseases [35].

The folate concentration found in 'araçá of Cerrado' pulp was high (table III). This content was similar to or higher than that observed in fruits that have the highest folate content such as orange ( $30 \text{ }\mu\text{g}\cdot 100 \text{ g}^{-1}$ ) and guava ( $49 \text{ }\mu\text{g}\cdot 100 \text{ g}^{-1}$ ) [25], and in fruits of the Brazilian Savannah such as pequi ( $5.16 \text{ }\mu\text{g}\cdot 100 \text{ g}^{-1}$ ) [33], cagaita ( $25.74 \text{ }\mu\text{g}\cdot 100 \text{ g}^{-1}$ ) [31] and araticum ( $27.36 \text{ }\mu\text{g}\cdot 100 \text{ g}^{-1}$ ) [32]. This nutrient is very important for the prevention of anemia and for appropriate development of the nervous system of the fetus during pregnancy [36].

#### 3.4.4. Nutritional value of 'araçá of Cerrado' pulp as a source of vitamins

Pulp from 'araçá of Cerrado' stood out not only because of the concentrations of vitamin A and vitamin E, but also because it can be considered a source of vitamin A for children by supplying more than 6% of the daily recommendation (table IV). Thus, the inclusion of 'araçá of Cerrado' fruit in the daily diet of children can contribute to the reduction in cases of vitamin A deficiency, mainly diagnosed in this age group.

Araçá pulp was shown to be an excellent source of folates for children, relevant for preventing cases of folate deficiency anemia [36]. For adults it may be considered a good source and for pregnant women it can be classified as a source (table IV).



**Figure 2.** HPLC analysis of carotenoids (A), vitamin E (B) and folate (C) in 'araçá of Cerrado' pulp (*Psidium firmum* O. Berg) from the Cerrado Savannah of Minas Gerais (Diamantina, Minas Gerais, Brazil). Chromatographic conditions can be found in the *Materials and methods* section.

**Table III.** Carotenoid and vitamin contents in 100 g of araçá pulp (*Psidium firmum* O. Berg) from the Savannah (Diamantina, Minas Gerais, Brasil). Values are expressed as fresh weight.

Compounds	(mg·100 g <sup>-1</sup> )	Mean of 5 replicates ± standard deviation		%
		(mg·100 g <sup>-1</sup> )	RAE·100 g <sup>-1</sup> (µg·100 g <sup>-1</sup> )	
Vitamin C	Not detected	-	-	-
Carotenoids	0.32 ± 0.05	-	-	100.0
β-carotene	0.30 ± 0.05	-	-	94.3
β- cryptoxanthin	0.02 ± 0.01	-	-	5.7
Vitamin A	-	26.20 ± 4.03	-	100.0
Vitamin E	-	-	336.33 ± 104.94	100.0
α-tocopherol	-	-	231.65 ± 65.43	68.9
γ- tocopherol	-	-	104.68 ± 40.57	31.1
Folates	-	-	47.25 ± 12.15	100.0
THF	-	-	7.96 ± 1.94	16.8
5-MTHF	-	-	5.58 ± 1.33	11.8
5-FTHF	-	-	33.70 ± 10.26	71.4

RAE: Retinol Activity Equivalent.

THF: tetrahydrofolate.

5-MTHF: 5-methyl-tetrahydrofolate.

5-FTHF: 5-formyl-tetrahydrofolate.

**Table IV.** Contribution of 100 g of 'araçá of Cerrado' pulp (*Psidium firmum* O. Berg) to supplying the daily recommendations for vitamins for children, pregnant women and adult men. Calculation is based on the Recommended Dietary Allowance (RDA) of the Dietary Reference Intakes (DRIs) for the respective age groups and nutrients [37, 38].

Compounds	Content per portion (100 g)	Daily intake adequacy (%)		
		Children	Adult men	Pregnant women
Vitamin A	26.20 Retinol Activity Equivalent	6.55	3.26	3.40
Vitamin E	231.65 µg α-tocopherol	3.30	1.54	1.54
Folates	47.25 µg	23.62	11.81	7.87

## 4. Conclusion

The 'araçá of Cerrado' fruit showed high yield, an important characteristic for its economic and technological exploitation for product development. Contents of soluble sugars and titratable acidity were high, indicating the balance between sugars and acidity, making the fruit more palatable. 'Araçá of Cerrado' pulp showed high dietary fiber content and was considered an excellent source of this nutrient.

Despite the low contents of carotenoids and vitamin E, 'araçá of Cerrado' proved to be an excellent source of folates for children, a good source for adults, and a source for pregnant women.

Overall, 'araçá of Cerrado' pulp showed good nutritional value and its consumption should be encouraged, especially by socially vulnerable families and groups located in areas of typical Savannah vegetation.

## Acknowledgments

The authors thank the FAPEMIG (*Fundação de Amparo à Pesquisa de Minas Gerais*) and CNPq (*Conselho Nacional de Desenvolvimento Científico e Tecnológico*) for their financial support.

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**« Araçá de Cerrado », fruta de la sabana brasileña: características físicas, composición química y contenido en carotenoides y vitaminas.**

**Resumen – Introducción.** La sabana brasileña constituye una de las biodiversidades más ricas del mundo; contiene frutas indígenas que presentan un alto valor nutricional. Dichas frutas permiten la alimentación no solo de la fauna local, sino de las comunidades de personas. Entre ellas, cabe destacar la presencia de « araçá de Cerrado » (*Psidium firmum* O. Berg), una fruta existente de forma natural en la sabana brasileña. Por ello, hemos evaluado las características físicas, la composición química, la frecuencia y el contenido en vitamina C, carotenoides, vitamina E y folatos de la fruta de « araçá de Cerrado » de la sabana de Minas Gerais, en Brasil. **Material y métodos.** La acidez valorable se determinó por neutralización volumétrica; el pH por potenciometría; los sólidos solubles por refractrometría; la humedad con un horno; las cenizas con un horno de mufla; las proteínas mediante el método micro-Kjeldhal; las fibras alimenticias totales mediante un procedimiento de gravimetría no enzimática; los lípidos con un extractor Soxhlet. La vitamina C (ácido ascórbico y dehidroascórbido) y los carotenoides ( $\alpha$ -caroteno,  $\beta$ -caroteno,  $\gamma$ -critoxantina y licopeno) se analizaron por HPLC-DAD; la vitamina E ( $\alpha$ ,  $\beta$ ,  $\gamma$  y  $\delta$ -tocoferoles y  $\alpha$ ,  $\beta$ ,  $\gamma$  y  $\delta$ -tocotrienoles) y los folatos (tetrahidrofolato, 5-metiltetrahidrofolato y 5-formiltetrahidrofolato) mediante HPLC con detección por fluorescencia. **Resultados y discusión.** La fruta de araçá presenta una alta producción de pulpa (81,43 %) y contenidos elevados en sólidos solubles (11,93 °Brix), humedad (77,46 %), fibras alimentarias (12,32 g·100 g<sup>-1</sup>) y folatos (47,25 mg·100 g<sup>-1</sup>). Mostró un débil valor energético total, de 43,19 kcal·100 g<sup>-1</sup>, y bajo contenido en carotenoides (0,32 mg·100 g<sup>-1</sup>) y vitamina E (336,43 mg·100 g<sup>-1</sup>). No se encontró vitamina C en esta fruta. **Conclusión.** La fruta « araçá de Cerrado » destaca por su alto rendimiento. Considerada como una excelente fuente de fibras alimentarias y folatos, demuestra presentar un valor nutricional óptimo. Por tanto, debe fomentarse su consumo.

**Brasil / Minas Gerais / *Psidium firmum* / frutas / propiedades fisicoquímicas / carotinoides / contenido vitamínico / valor energético**

