

# Araticum (*Annona crassiflora* Mart.) from the Brazilian Cerrado: chemical composition and bioactive compounds

Leandro de Moraes CARDOSO<sup>1\*</sup>, Daniela da Silva OLIVEIRA<sup>1</sup>, Sabrina de Freitas BEDETTI<sup>1</sup>, Hércia Stampini Duarte MARTINO<sup>2</sup>, Helena Maria PINHEIRO-SANT'ANA<sup>1</sup>

<sup>1</sup> Lab. Vitam. Anal., Dep. Nutr. Health, Fed. Univ. Viçosa, PH Rolfs Avenue, s/n, Viçosa, Minas Gerais, 36571-000, Brazil, lcardoso.nutricao@hotmail.com

<sup>2</sup> Dep. Nutr. Health, Fed. Univ. Viçosa, PH Rolfs Avenue, s/n, Viçosa, Minas Gerais, 36571-000, Brazil

## Araticum (*Annona crassiflora* Mart.) from the Brazilian Cerrado: chemical composition and bioactive compounds.

**Abstract – Introduction.** The Brazilian Cerrado presents a wide vegetable diversity, which is used for different purposes, especially in human feeding. Among the various fruits of the Cerrado, the araticum (*Annona crassiflora* Mart.) stands out, due to its high nutritional value and technological potential. The physical characteristics, chemical composition (titratable acidity, pH, moisture, ash, total dietary fiber, lipids and proteins), 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 araticum from the Cerrado of Minas Gerais, Brazil. **Materials and methods.** Vitamin C and carotenoids were analyzed by HPLC-DAD, and vitamin E and folates by HPLC with fluorescence detection. **Results and discussion.** Araticum pulp presented a high energy value (95.12 kcal·100 g<sup>-1</sup>), as well as elevated contents of dietary fiber (6.80 g·100 g<sup>-1</sup>), carotenoids (4.98 mg·100 g<sup>-1</sup>) and vitamin A value (288.79 RAE·100 g<sup>-1</sup>). The contents of vitamin C, folates and vitamin E were 5.23 mg·100 g<sup>-1</sup>, 27.36  $\mu$ g·100 g<sup>-1</sup> and 494.04  $\mu$ g·100 g<sup>-1</sup>, respectively. **Conclusion.** Araticum presented high energy value and high dietary fiber content. It is a source of vitamin C and folates, and an excellent source of vitamin A.

**Brazil / Minas Gerais / *Annona crassiflora* / fruits / physicochemical properties / carotenoids / vitamin content / energy value**

## Araticum (*Annona crassiflora* Mart.) du Cerrado brésilien : composition chimique et composés bioactifs.

**Résumé – Introduction.** Le Cerrado brésilien héberge une large diversité végétale qui est utilisée à des fins diverses, notamment pour l'alimentation humaine. L'araticum (*Annona crassiflora* Mart.), à fort potentiel nutritionnel et technologique, se distingue parmi les différents fruits du Cerrado. Les caractéristiques physiques, la composition chimique (acidité tritable, pH, humidité, cendres, fibres alimentaires totales, lipides et protéines), la présence de vitamine C (acide ascorbique et déhydroascorbique) et sa teneur, ainsi que les quantités de 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 évaluées dans des fruits d'araticum du Cerrado de l'État du Minas Gerais, au Brésil. **Matériel et méthodes.** La composition en vitamine C et caroténoïdes a été analysée par HPLC-DAD, et celle en vitamine E et folates par HPLC avec détection par fluorescence. **Résultats et discussion.** La pulpe d'araticum a présenté une forte valeur énergétique (95,12 kcal·100 g<sup>-1</sup>), ainsi que des teneurs élevées en fibres alimentaires (6,80 g·100 g<sup>-1</sup>), caroténoïdes (4,98 mg·100 g<sup>-1</sup>) et vitamine A (288,79 RAE·100 g<sup>-1</sup>). Les teneurs en vitamine C, folates et vitamine E ont été de 5,23 mg·100 g<sup>-1</sup>, 27,36  $\mu$ g·100 g<sup>-1</sup> et 494,04  $\mu$ g·100 g<sup>-1</sup>, respectivement. **Conclusion.** Les fruits d'araticum ont présenté une forte valeur énergétique ainsi que des teneurs élevées en fibres alimentaires. Ils se sont révélés être une source de vitamine C et de folates, et une excellente source de vitamine A.

**Brésil / Minas Gerais / *Annona crassiflora* / fruits / propriété physicochimique / caroténoïde / teneur en vitamines / valeur énergétique**

\* Correspondence and reprints

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## 1. Introduction

The Cerrado is the second largest biome of Brazil and covers an area of approximately 204 Mha, corresponding to 22% of the Brazilian territory [1]. This biome stands out due to its vegetable diversity, which is used for different purposes, especially in human feeding.

The Annonaceae family presents a wide variety of species including atemoya (*Annona cherimola* Mill), sweetsop (*Annona squamosa*), soursop (*Annona muricata*) and araticum (*Annona crassiflora* Mart.). Araticum, also known as *panã* or *marolo*, is an exotic fruit of the Brazilian Cerrado that presents high nutritional and technological potential. The fruit has a slightly sweet pulp with a pleasant smell and strong flavor, which makes it well accepted by consumers [2]. Studies demonstrate that the use of araticum pulp in the formulation of products, including yogurt, is an interesting option for the industry, since these products have a good acceptance and purchase intent by the consumer [3, 4].

Araticum plays an important social role since it is used as a food complement and contributes to income generation, especially for socially vulnerable families. Besides its social significance, the consumption of this fruit, as well as fruits in general, may be related to numerous health benefits, such as lower incidence and mortality by cancer, and cardiovascular and cerebrovascular diseases [5]. The protection offered by fruits to the human organism is mainly associated to the presence of bioactive compounds with antioxidant properties, including carotenoids, vitamin C, vitamin E and folates. These compounds act as reducing agents, protecting the organism against oxidative stress [6–9].

Data related to the chemical composition and content of bioactive compounds of araticum are rare in the specialized literature. Knowledge on the nutritional composition of foods consumed in Brazil, including this fruit, is essential to assess the availability of nutrients and consumption by populations and individuals, verify the nutritional adequacy of diets, evaluate nutritional status,

conduct research on the relationship between diet and disease, and develop new products, among others [10].

Therefore, this study aimed to evaluate the physical characteristics, chemical composition and nutritional value of araticum from the Cerrado of Minas Gerais state, Brazil.

## 2. Materials and methods

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

Araticum fruits (*Annona crassiflora* Mart.) were collected in the rural area of the municipality of Curvelo (18°45' south latitude and 44°25' west longitude), Minas Gerais state, Brazil.

Fruits with full physiological maturity were collected during harvest season (from January to March 2010), after naturally falling from the trees. The collection area was divided into sub-areas for the acquisition of five repetitions. Three kilograms of fruits were collected in each sub-area. Samples were transported overland from the collection site to the laboratory in polystyrene boxes, up to 36 h after collection. The morphologically perfect and completely mature fruits (with soluble solids more than 19 °Brix) were washed with tap water to remove surface dirt and dried at room temperature. Later, the fruit pulp was manually separated from the seeds and homogenized in a domestic food processor (Faet Multipratic, MC5 model, Brazil). This procedure was carried out for each of the five repetitions.

### 2.2. Physical characterization

Individual measurements of height and diameter were carried out on 30 araticum fruits with the use of a digital caliper rule (Mitutoyo, model M1, Brazil). The mass of whole fruits (MF), skin (MS), pulp (MP) and seeds (MS) was obtained by direct individual weighing on a semi-analytical balance (Gehaka, BG 2000 model, Brazil). The pulp

yield was calculated by the equation  $(MP / MF) \times 100$ .

### 2.3. Chemical analysis

The chemical analyses were performed at the Laboratory of Food Analysis of the Department of Nutrition and Health, Federal University of Viçosa, Brazil. Titratable acidity, soluble solids, pH [11]; moisture, ash, proteins, lipids and total dietary fiber [12] were determined in the araticum pulp with three repetitions. Moisture was determined using an oven at 105 °C and ash quantified using a muffle furnace (Quimis, Q320M was model, Brazil) at 550 °C. 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 calculated as the difference, using the equation:  $[100 - (\% \text{ moisture} + \% \text{ lipids} + \% \text{ proteins} + \% \text{ total dietary fiber} + \% \text{ ash})]$ . The total energy value of the araticum pulp was estimated considering the conversion factors of 4 kcal·g<sup>-1</sup> of protein or carbohydrate and 9 kcal·g<sup>-1</sup> of lipid [13].

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

The preparation and analysis of carotenoids, vitamin C, vitamin E and folates in araticum fruits were performed at the Laboratory of Vitamin Analysis of the Department of Nutrition and Health, Federal University of Viçosa, Brazil, with five repetitions. During the stages of 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

The occurrence and content of the major carotenoids ( $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin and lycopene) in araticum pulp were investigated. Carotenoids were

extracted according to the method proposed by Rodriguez-Amaya *et al.* [14] with modifications. Approximately 5.0 g of pulp were weighed, added to 60.0 mL of cooled acetone (divided into three volumes of 20.0 mL), homogenized in a micro-crusher (Marconi, MA 102 model, Brazil) for approximately 5 min, and vacuum-filtered in a Büchner funnel on filter paper. Next, the filtrate was transferred in three fractions to a separation funnel containing 50.0 mL of cooled petroleum ether; then, each fraction was washed with distilled water for complete removal of acetone. Anhydrous sodium sulfate was added to the ether extract for removal of residual water that could impair evaporation of the material. The extract in ether was then concentrated using a rotary evaporator (Tecnal, TE-211 model, Brazil) at  $(35 \pm 1)$  °C, transferred to a 25.0-mL volumetric flask, and the volume was completed with petroleum ether. This extract was stored in a hermetically sealed amber glass bottle and stored at a temperature of  $(-18 \pm 1)$  °C.

For analysis, eight milliliters of the ether extract were evaporated under nitrogen gas flow and the dry residue was 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  $\mu\text{m}$  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 model, Japan), an autosampler with a loop of 50  $\mu\text{L}$  (Shimadzu, SIL-10AF model, Japan) and a diode array detector (DAD) (Shimadzu, SPD-M10A model, Japan). The following chromatographic conditions were used: HPLC system, DAD; RP-18 Phenomenex Gemini chromatographic column, 250 mm  $\times$  4.6 mm, 5  $\mu\text{m}$ , fitted with a Phenomenex ODS (C18) guard column, 4 mm  $\times$  3 mm; and mobile phase composed of methanol:ethyl acetate:acetonitrile (Tedia, Brazil), in the proportions 80:10:10, with flow rate of 2.0 mL·min<sup>-1</sup> [15]. The chromatograms were obtained at 450 nm.

Vitamin A concentration was calculated according to recommendations of the Institute of Medicine [16], in which 1 Retinol Activity Equivalent (RAE) corresponds to 1 µg of retinol, 12 µg of β-carotene, and 24 µg of other provitamin A carotenoids.

#### 2.4.2. Vitamin C

The contents of ascorbic acid and dehydroascorbic acid in the araticum pulp were assessed. Extraction and analysis of ascorbic acid were carried out according to methods reported by Campos *et al.* [17] with modifications. Roughly 5.0 g of the sample were crushed for approximately 5 min, in 15.0 mL of the extraction solution (3% metaphosphoric acid, 8% acetic acid, H<sub>2</sub>SO<sub>4</sub> 0.3 N and 1 mM EDTA). The obtained 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 was frozen [(−5 ± 1) °C] until the time of analysis.

Conversion of dehydroascorbic acid into ascorbic acid was carried out according to Campos *et al.* [17]. An aliquot of 1.2 mL of the extract obtained in the previous stage was pipetted into an amber glass. Next, 1.0 mL of a 0.5 M Trizma buffer solution (pH 9.0) containing 40 mM dithiothreitol (DTT, Sigma-Aldrich, Germany) was added, aiming to increase the pH to approximately neutral. The conversion reaction lasted 10 min and was performed at room temperature in the dark. Later, 0.6 mL H<sub>2</sub>SO<sub>4</sub> 0.4 M was added to reduce the pH prior to chromatographic injection.

Analyses were carried out via injection of 50 µL of the extracts previously filtered in filter units with porosity of 0.45 µm. The vitamin C analyses were performed on the same HPLC system used for analysis of carotenoids and the following chromatographic conditions were used: RP-18 Lichrospher 100 chromatographic column (250 mm × 4 mm, 5 µm); HPLC system, DAD, mobile phase composed of ultrapure water containing 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>. 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 vitamin E components (α-, β-, γ- and δ-tocopherols and tocotrienols) in the araticum pulp were assessed. The extraction process was carried out according to Guinazi *et al.* [18] with some modifications. Approximately 5.0 g of the sample were weighed and 4.0 mL of heated ultrapure water were added [about (80 ± 1) °C]. Then, 10.0 mL of isopropyl alcohol, 1.0 mL of hexane containing 0.05% of butylhydroxytoluene and 5 g of anhydrous sodium sulfate were added. Twenty-five milliliters of the extraction solvent mixture (hexane:ethyl acetate, 85:15, v/v) were slowly added. After these procedures, the sample was triturated in a micro-crusher at average speed for 1 min. The suspension was then vacuum-filtered in a Büchner funnel through filter paper, 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 ± 1) °C for about 2 min and transferred to a volumetric flask where 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 µm. 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 µL (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 Guinazi *et al.* [18] and included the 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:isopropyl alcohol:glacial acetic acid (Tedia, Brazil), in the proportions 98.9:0.6:0.5, and mobile phase flow rate of 1.0  $\text{mL}\cdot\text{min}^{-1}$ . The total vitamin E content in araticum was calculated by the sum of vitamin E components identified.

#### 2.4.4. Folates

The occurrence and content of folates (THF, 5-MTHF and 5-FTHF) in the araticum pulp were investigated. 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 the extraction, approximately 5 g of the sample were ground in 20.0 mL of a phosphate buffer solution 0.1 M, pH 6.0, containing 1% ascorbic acid and 0.1% 2-mercaptoethanol. The obtained suspension was centrifuged 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 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 fell below  $(37 \pm 1)^\circ\text{C}$ . The cooled extract was re-centrifuged at 4000 rpm (1789 g) for 15 min and submitted to deconjugation of polyglutamates into monoglutamates.

For deconjugation, 100.0  $\mu\text{L}$  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. The extracts were then heated in boiling water for 5 min for enzyme inactivation.

Extract purification was carried out using an ion exchange column, with the stationary phase composed of Q-Sepharose Fast Flow (Pharmacia, USA), connected to a peristaltic pump (Pharmacia Biotech, P1 model, USA).

The column was pre-conditioned with methanol and water (1:1) at a flow of two drops per second. The extract was then applied to the column at a flow of two drops per second. Elution of the retained folates was carried out with 1.5 mL of a sodium acetate solution (0.1 M) containing 10% NaCl, 1% ascorbic acid and 0.1% 2-mercaptoethanol. Analyses of folates were carried out from the 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 following chromatographic conditions were used: 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 a 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 following gradient was utilized: from 0 to 5 min 94% of the eluent A + 6% of the eluent B; from 5 to 25 min, 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 until 50 min. The mobile phase flow rate was 0.7  $\text{mL}\cdot\text{min}^{-1}$  and fluorescence detection occurred with excitation at 290 nm and emission at 360 nm. The mobile phase was degassed with helium 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  $\delta$ -tocopherol and tocotrienol) 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 (THF), (6S)-5-methyl-5,6,7,8-tetrahydrofolate (5-MTHF) and (6S)-5-formyl-5,6,7,8-tetrahydrofolate (5-FTHF) 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

**Table I.**

Minimum and maximum concentrations of the standards used for the construction of analytical curves and the regression equations used for quantification of the compounds.

Compounds	Concentration of the standards (µg)		Regression equation	R <sup>2</sup>
	Minimum	Maximum		
α-carotene	0.0330	2.0600	4,383,677.42 x + 2,996,199.63	0.999
β-carotene	0.0045	1.4333	1,730,130.16 x – 8,057.58	0.999
Lycopene	0.0003	0.0546	1,389,460.94 x + 24,320.87	0.996
Ascorbic acid	0.0589	5.8800	3,277,607.19 x – 66,204.16	0.998
α-tocopherol	0.0010	0.1042	76,030,901.90 x – 66,102.66	0.999
α-tocotrienol	0.0020	0.2041	28,452,328.82 x – 105,303	0.997
THF	0.00004	0.04622	942,240,050.58 x – 162,371.44	0.996
5-MTHF	0.00001	0.01077	1,237,294,689.67 x – 259,476.97	0.994

THF: tetrahydrofolate; 5-MTHF: 5-methyltetrahydrofolate.

of papaya and tomato, respectively, by open column chromatography [14].

For the identification of the compounds, injections of carotenoid and vitamin standard mixtures were carried out and the retention times (RT) achieved for the standards were compared with those of the samples. The carotenoids and AA were also identified by comparison of the absorption spectra of the standard and the peaks of interest in the samples, using a DAD, and the folate and vitamin E components, by co-chromatography.

The compounds observed in araticum pulp (α-carotene, β-carotene, lycopene, ascorbic acid, α-tocopherol, α-tocotrienol, THF and 5-MTHF) were quantified by external standard curves constructed from injection, in duplicate, of six increasing concentrations of standard solutions (table D). A linear correlation was calculated between the peak areas and the concentrations of each compound injected.

#### 2.4.6. Quality control of analytical methods

Linearity, recovery, repeatability, the limit of detection (LOD) and the limit of quantification (LOQ) were assessed. The linearity range of the compounds was determined by the injection of six increasing concentrations of the standard solutions under

the same chromatographic conditions employed for analysis of the extracts. Data obtained for the peak areas were used for linear regression analysis. The correlation coefficient (R<sup>2</sup>) determined for each case was used to assess linearity [20].

The recovery tests were carried out by the addition of standards (α-carotene, β-carotene, lycopene, ascorbic acid, α-tocopherol, α-tocotrienol, THF and 5-MTHF) to the samples. The quantity of standard added varied between 50 to 100% of the initial content observed in araticum pulp. The recovery percentage was calculated using the equation: % recovery = [(final concentration of the isomer – concentration added to the isomer) / (initial concentration of the isomer)] × 100. All procedures were carried out in quintuplicate.

Repeatability was evaluated by extraction in quintuplicate and analysis in duplicate of the same repetition containing the carotenoids and vitamins evaluated. Repeatability was assessed by calculation of the variation coefficient for both the retention time and peak area of the components analyzed [20].

The LOD was assessed by successive dilutions of the vitamin and carotenoid standards identified in the fruits, followed by determination of the smallest detectable quantity, established as three times the value of the magnitude of the baseline

**Table II.**

Chemical characteristics and total energy value of the araticum pulp (*Annona crassiflora* Mart.) of the Cerrado (Curvelo, Minas Gerais, Brazil). Values are expressed in fresh matter. Means of 3 repetitions  $\pm$  standard deviation.

Soluble solids (°Brix)	Titrateable 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> )
(g·100 g <sup>-1</sup> )									
22.54 $\pm$ 1.91	0.54 $\pm$ 0.11	4.89 $\pm$ 0.01	73.32 $\pm$ 0.87	1.52 $\pm$ 0.11	3.50 $\pm$ 0.05	0.47 $\pm$ 0.01	6.80 $\pm$ 0.49	14.39 $\pm$ 0.61	95.12 $\pm$ 2.60

noise. The LOQ was established as 10 times the LOD [21].

### 2.5. Experimental design and data 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. Average 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 characterization

Araticum is a rounded berry, with resistant brown bark. Its pulp is composed of cone-shaped buds, with color ranging from light yellow to pinkish. Inside it, each bud contains a dark brown seed (*figure 1*).

Diameter of the araticum fruit ranged from (9.10 to 11.90) cm and the height from (11.50 to 15.10) cm. The araticum presented high mass (1.13 kg) and pulp yield (52.9%), with significant variation for these parameters, from (0.63 to 1.65) kg and from 51.5% to 57.7%, respectively. The pulp yield of fruits from the Minas Gerais state was lower than that of fruits from the Goiás state (55.7%) [22]. The wide variation in physical characteristics of fruits may be attributed to



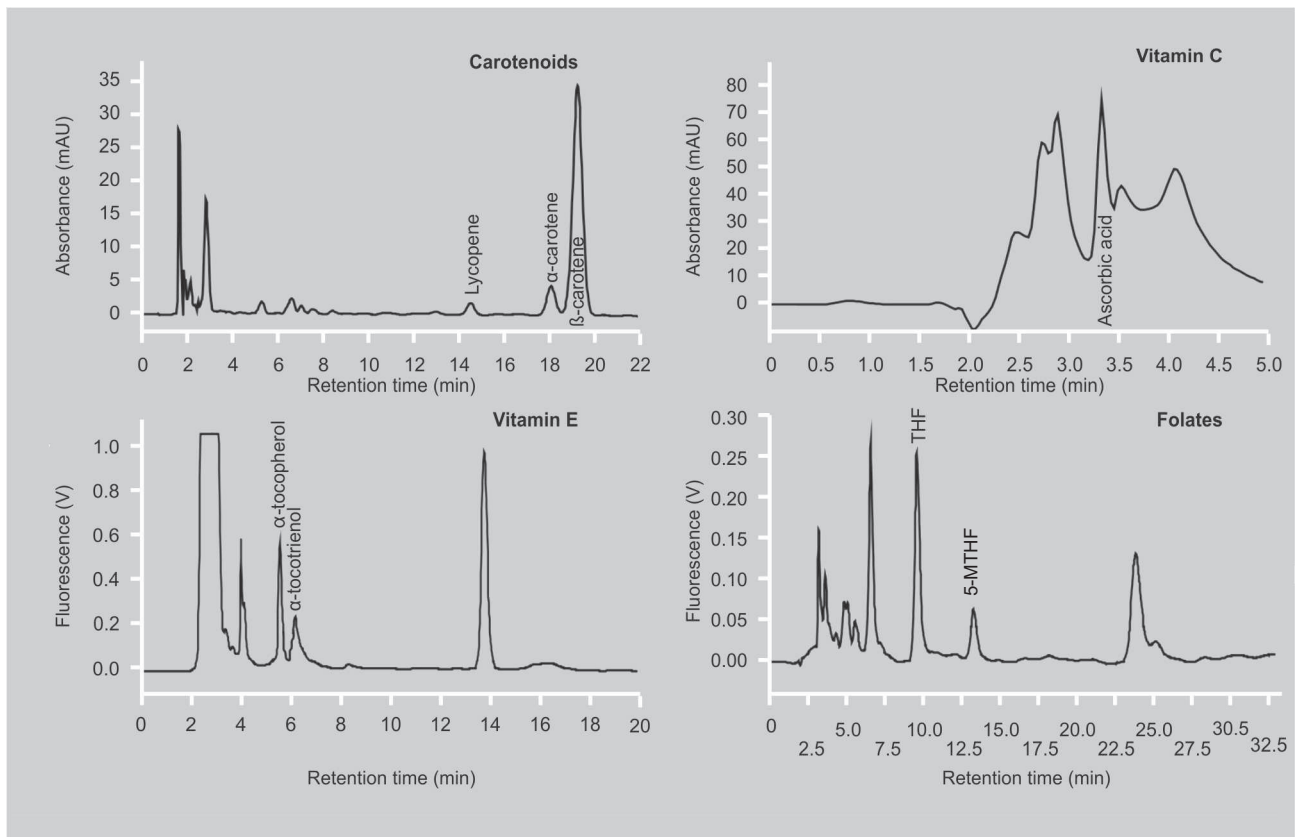
**Figure 1.** Araticum fruits (*Annona crassiflora* Mart.).

edaphoclimatic differences between the collection sites of the fruits since the state of Goiás is located approximately 460 miles from the area where the fruits of this study were collected (Minas Gerais state).

The physical characteristics of araticum proved to be different from those observed in other species of Annonaceae. The fruit mass and pulp yield were greater than those observed by Neves and Yuhara [23] in different varieties of atemoya: (275 to 357) g for fruit mass and 45.2% to 51.8% for pulp yield. However, these physical characteristics of araticum were lower than those observed by Sacramento *et al.* [24] in different varieties of soursop: (2.39 to 3.2) kg for fruit mass and 83.12% to 85.85% for pulp yield.

### 3.2. Chemical characterization

Araticum pulp presented reduced titrateable acidity and pH (*table II*). High soluble solids and moisture contents were observed, which enable the pulp to be used in sweets, jams and yogurts. Roesler *et al.* [22] and Silva *et al.* [25] observed, in fruits of



**Figure 2.** Analysis by HPLC of carotenoids, vitamin C, vitamin E and folates in araticum pulp (*Annona crassiflora* Mart.) of the Cerrado (Curvelo, Minas Gerais, Brazil). THF: tetrahydrofolate; 5-MTHF: 5-methyltetrahydrofolate.

Goiás, moisture content similar to that found in the present study ( $70.56 \text{ g}\cdot 100 \text{ g}^{-1}$  and  $76.05 \text{ g}\cdot 100 \text{ g}^{-1}$ , respectively).

Regarding macronutrients, araticum pulp presented a high total dietary fiber content, which was 44% greater than that found by Silva *et al.* in fruits of the Cerrado in Goiás, Brazil ( $4.72 \text{ g}\cdot 100 \text{ g}^{-1}$ ) [25]. The dietary fiber content in the pulp was on average 2.7 times greater than that observed in fruits of the *Annonaceae* family such as soursop ( $1.9 \text{ g}\cdot 100 \text{ g}^{-1}$ ), sweetsop ( $3.4 \text{ g}\cdot 100 \text{ g}^{-1}$ ) and atemoya ( $2.1 \text{ g}\cdot 100 \text{ g}^{-1}$ ) [10].

Araticum pulp presented a high lipid content ( $3.50 \text{ g}\cdot 100 \text{ g}^{-1}$ ), which was greater than that observed by Roesler *et al.* ( $2.36 \text{ g}\cdot 100 \text{ g}^{-1}$ ) in fruits of the same species collected in the state of Goiás [22], as well as in soursop, sweetsop and atemoya (on average,  $0.3 \text{ g}\cdot 100 \text{ g}^{-1}$ ) [10]. Due to the high amount of macronutrients in its composition, araticum presented high energy density. The contents of proteins, carbohydrates

and the total energy observed in the araticum pulp from the Cerrado of Minas Gerais were similar to those found in fruits of the Cerrado of Goiás, Brazil. In 100 g of araticum pulp, Silva *et al.* observed 1.22 g of proteins, 12.78 g of carbohydrates and total energy of 90.47 kcal [25].

### 3.3. Carotenoids and vitamins

#### 3.3.1. Qualitative composition

The analysis methods allowed good peak resolution, which assured adequate quantification of the compounds (*figure 2*). The run times of the carotenoids, ascorbic acid, vitamin E and folate analyses were (22, 5, 20 and 33) min, respectively. Araticum pulp presented lycopene (retention time: RT = 14.6 min),  $\alpha$ -carotene (RT = 18.2 min),  $\beta$ -carotene (RT = 19.5 min), ascorbic acid (RT = 3.3 min),  $\alpha$ -tocopherol (RT = 5.7 min),  $\alpha$ -tocotrienol (RT = 6.2 min), THF



**Table III.**

Repeatability, limits of detection and quantification, range of linearity, and recovery of carotenoids and vitamins in the araticum pulp (*Annona crassiflora* Mart.) of the Cerrado (Curvelo, Minas Gerais, Brazil).

Compounds	Repeatability (relative standard deviation)		Detection limit	Quantification limit	Range of linearity ( $\mu\text{g}$ )	Recovery (%)
	Peak area	Retention time				
$\alpha$ -carotene	2.28	1.30	7.861	78.612	0.0330 – 2.0600	96.3
$\beta$ -carotene	2.62	0.61	6.422	64.221	0.0045 – 1.4333	91.2
Lycopene	2.43	0.52	5.312	53.119	0.0003 – 0.0546	90.8
Ascorbic acid	1.40	0.40	12.321	123.214	0.0589 – 5.8800	98.4
$\alpha$ -tocopherol	7.38	0.34	0.025	0.251	0.0010 – 0.1042	98.3
$\alpha$ -tocotrienol	2.90	1.10	0.074	0.741	0.0020 – 0.2041	95.2
THF	4.98	0.91	0.003	0.031	0.00004 – 0.04622	89.3
5-MTHF	5.42	1.27	0.002	0.024	0.00001 – 0.01077	91.0

THF: tetrahydrofolate; 5-MTHF: 5-methyltetrahydrofolate.

(RT = 9.9 min) and 5-MTHF (R = 13.3 min). In the samples,  $\beta$ -cryptoxanthin,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherols and tocotrienols, and 5-MTHF were not detected. Information on the chromatographic profiles of the carotenoid and vitamin analyses in the araticum pulp, obtained by HPLC, is not available in the literature, which prevented comparisons related to the chromatographic profiles achieved in this study.

### 3.3.2. Quality of analytical methods

The linearity range of the compounds analyzed with a DAD ( $\alpha$ -carotene,  $\beta$ -carotene, lycopene and ascorbic acid) presented ratios between the maximum and minimum concentrations injected of up to 230 times. In the compounds evaluated by fluorescence detection, the ratio between these concentrations was 100 times for the vitamin E components and 1000 times for the folates. It was observed that there was a wide linearity range for each compound and the correlation coefficients ( $R^2$ ) were greater than 0.994, which assured the acquisition of reliable data (table I).

Repeatability of the carotenoid and vitamin isomers present in the araticum presented a relative standard deviation in relation to the peak areas and retention times below 7.4% and 1.3%, respectively

(table III). The results suggest that the analysis conditions are reliable.

The limit of detection (LOD) achieved for the carotenoids and vitamins identified in the araticum ranged from (0.002 to 12.321)  $\mu\text{g}\cdot\text{mL}^{-1}$ . The limit of quantification (LOQ), considered to be 10 times the value of LOD, ranged from (0.02 to 123.21)  $\mu\text{g}\cdot\text{mL}^{-1}$ . It can be observed that the limits of detection and quantification were low, which allowed the detection of reduced concentrations of these compounds (table III).

Recovery of the standards added to the samples varied from 89.3% to 98.4%, with a mean of 93.8%. These results indicate good recovery percentages of the components analyzed, which decreases the chances of losses during the extraction process and analysis.

### 3.3.3. Content of carotenoids and vitamins

There is little data available in the literature related to the content of carotenoids and vitamin C in araticum pulp, especially obtained using reliable analysis methods, such as HPLC. Information on vitamin E and folate contents in fruits is also scarce in the literature and there is no information regarding the presence of these vitamins in exotic

fruits, such as araticum. The lack of nutritional data on araticum 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 Cerrado.

The araticum pulp presented a carotenoid content greater than those found in traditional fruits, such as banana, orange, peach and passion fruit [26] (*table IV*). However, this content was approximately 35% lower than that observed in excellent sources of carotenoids, including papaya ( $7.48 \text{ mg}\cdot 100 \text{ g}^{-1}$ ), guava ( $7.34 \text{ mg}\cdot 100 \text{ g}^{-1}$ ) [27] and cooked pequi pulp from the Brazilian Cerrado ( $8.10 \text{ mg}\cdot 100 \text{ g}^{-1}$ ) [28].

The  $\alpha$ -carotene and  $\beta$ -carotene were the major carotenoids in the araticum pulp, corresponding to 59.9% (*table IV*). These carotenoids present provitamin A activity, thus play an important nutritional role, especially in developing countries, where hypovitaminosis A is one of the most serious problems in public health. The vitamin A value in the araticum pulp was greater than that observed in fruits marketed in the state of Minas Gerais, Brazil, such as acerola ( $175.00 \text{ RAE}\cdot 100 \text{ g}^{-1}$ ), khaki ( $58.40 \text{ RAE}\cdot 100 \text{ g}^{-1}$ ), carambole ( $18.20 \text{ RAE}\cdot 100 \text{ g}^{-1}$ ), sweet passion fruit ( $89.50 \text{ RAE}\cdot 100 \text{ g}^{-1}$ ), nectarine ( $25.70 \text{ RAE}\cdot 100 \text{ g}^{-1}$ ) and peach ( $48.50 \text{ RAE}\cdot 100 \text{ g}^{-1}$ ) [29].

The vitamin C content observed in araticum was lower than that observed in atemoya ( $10.10 \text{ mg}\cdot 100 \text{ g}^{-1}$ ), soursop ( $19.10 \text{ mg}\cdot 100 \text{ g}^{-1}$ ), sweetsop ( $35.90 \text{ mg}\cdot 100 \text{ g}^{-1}$ ) [10] and in native Cerrado fruits, such as mangaba ( $190.00 \text{ mg}\cdot 100 \text{ g}^{-1}$ ) [30], cagaita ( $34.11 \text{ mg}\cdot 100 \text{ g}^{-1}$ ) [31] and 'jatobá do cerrado' ( $8.90 \text{ mg}\cdot 100 \text{ g}^{-1}$ ) [32].

The  $\alpha$ -tocopherol content in araticum pulp was greater than that observed in soursop pulp ( $80 \mu\text{g}\cdot 100 \text{ g}^{-1}$ ) [26] (*table IV*). The total content of vitamin E was higher than that in traditional fruits, as observed by Chun *et al.* in strawberry ( $410.00 \mu\text{g}\cdot 100 \text{ g}^{-1}$ ) and pear ( $420.00 \mu\text{g}\cdot 100 \text{ g}^{-1}$ ) [33].

Araticum presented a folate content approximately twice that observed in soursop [(14.00 to  $19.00 \mu\text{g}\cdot 100 \text{ g}^{-1}$ )] [26, 34] and sweetsop ( $14.00 \mu\text{g}\cdot 100 \text{ g}^{-1}$ ) [26]; similar to that found in berry ( $25.00 \mu\text{g}\cdot 100 \text{ g}^{-1}$ ) and strawberry ( $24.09 \mu\text{g}\cdot 100 \text{ g}^{-1}$ ); and lower than that encountered in papaya ( $38.15 \mu\text{g}\cdot 100 \text{ g}^{-1}$ ) and orange ( $30.00 \mu\text{g}\cdot 100 \text{ g}^{-1}$ ) [26] (*table IV*).

### 3.3.4. Nutritional value of araticum pulp as a source of vitamins

Philippi classifies 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 [35].

Considering the recommendations of vitamins (folates, vitamin A, vitamin C and vitamin E) for children between 4 and 8 years old, adult men between 19 and 30 years old and pregnant women [16, 36, 37], it was observed that 100 g of the araticum pulp was an excellent source of vitamin A for the three groups (*table V*). The fruit was shown to be a source of vitamin C for adults and pregnant women and an excellent source of this vitamin for children. Araticum was also a source of folates for adults and a good source of folates for children.

Despite the small contribution of araticum pulp to supply the daily recommendations for vitamin E, it should be noted that this contribution has a significant biological value, since araticum is consumed mainly by individuals from socially vulnerable families residing in rural areas. Therefore, araticum is present in locations where food sources of this vitamin may not be available over the entire year or may be insufficient to meet the needs of certain groups. Moreover, during the harvest season, araticum can be freely obtained by the families that take part in extractivism activities in the Cerrado, or purchased at low cost (US\$ 0.90 per fruit on average). Thus, this fruit could be an important tool to reduce the risk of food and nutritional insecurity.

**Table IV.** Content of carotenoids and vitamins in the araticum pulp (*Annona crassiflora* Mart.) of the Cerrado (Curvelo, Minas Gerais, Brazil). Values are expressed in fresh matter. Means of 5 repetitions  $\pm$  standard deviation.

Total carotenoids	$\alpha$ -carotene	$\beta$ -carotene	Lycopene	Vitamin A value	Vitamin C (ascorbic acid)	Total vitamin E	$\alpha$ -tocopherol	$\alpha$ -tocotrienol	Total folates	THF	5-MTHF
( $\mu\text{g}\cdot 100\text{ g}^{-1}$ )	( $\text{mg}\cdot 100\text{ g}^{-1}$ )	( $\text{mg}\cdot 100\text{ g}^{-1}$ )	( $\text{mg}\cdot 100\text{ g}^{-1}$ )	( $\text{RAE}\cdot 100\text{ g}^{-1}$ )	( $\text{mg}\cdot 100\text{ g}^{-1}$ )	( $\mu\text{g}\cdot 100\text{ g}^{-1}$ )	( $\mu\text{g}\cdot 100\text{ g}^{-1}$ )	( $\mu\text{g}\cdot 100\text{ g}^{-1}$ )	( $\mu\text{g}\cdot 100\text{ g}^{-1}$ )	( $\mu\text{g}\cdot 100\text{ g}^{-1}$ )	( $\mu\text{g}\cdot 100\text{ g}^{-1}$ )
$4.98 \pm 1.12$	$2.98 \pm 0.78$	$1.97 \pm 0.33$	$0.02 \pm 0.01$	$288.79 \pm 36.91$	$5.23 \pm 7.19$	$494.04 \pm 16.8$	$163.11 \pm 7.76$	$332.94 \pm 9.61$	$27.36 \pm 2.31$	$21.97 \pm 2.11$	$5.39 \pm 0.51$

RAE: Retinol Activity Equivalent; THF: tetrahydrofolate; 5-MTHF: 5-methyltetrahydrofolate.

**Table V.** Contribution of 100 g of araticum pulp (*Annona crassiflora* Mart.) to supply the daily recommendations of vitamins for children, adult men and pregnant women.

Age group	Percentage of intake adequacy <sup>1</sup>			
	Vitamin A value	Vitamin C	Folates	Vitamin E
Children aged between 4 and 8 years	72.2	20.9	13.7	2.3
Adult men aged between 19 and 30 years	32.1	5.8	6.8	1.1
Pregnant women	37.5	7.0	4.6	1.1

<sup>1</sup> Calculation based on the Recommended Dietary Allowance (RDA) of the Dietary Reference Intakes (DRIs) for the respective age groups and nutrients [16, 36, 37].

#### 4. Conclusion

In conclusion, araticum fruit of the Cerrado of Minas Gerais, Brazil, presented high mass, pulp yield, soluble solids and moisture, which make it very important for technological processing, especially for the manufacture of sweets, jams and yoghurts.

The fruit pulp presented a high energy value as well as elevated contents of dietary fiber. The fruit showed numerous bioactive compounds (lycopene,  $\alpha$ -carotene,  $\beta$ -carotene, ascorbic acid,  $\alpha$ -tocopherol,  $\alpha$ -tocotrienol, THF and 5-MTHF) and proved to be a source of vitamin C and folates, and an excellent source of vitamin A.

Araticum is an important food alternative that may contribute to the access to appropriate diets, especially in regions with high levels of food insecurity and in places where there may be scarcity or absence of traditional foods considered as sources of these nutrients in the Brazilian diet.

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### **Araticum (*Annona crassiflora* Mart.) del Cerrado brasileño: composición química y compuestos bioactivos.**

**Resumen – Introducción.** El Cerrado brasileño alberga una amplia diversidad vegetal que se emplea para fines diversos, sobre todo, para la alimentación humana. El araticum (*Annona crassiflora* Mart.), con un fuerte potencial nutricional y tecnológico, se distingue entre los diferentes frutos del Cerrado. Se evaluaron las características físicas, la composición química (acidez valorable, pH, humedad, cenizas, fibras alimentarias totales, lípidos y proteínas), la presencia de vitamina C (ácido ascórbico y dehidroascórbico) y su contenido, así como sus cantidades de carotenoides ( $\alpha$ -caroteno,  $\beta$ -caroteno,  $\beta$ -criptoxantina y licopeno), vitamina E ( $\alpha$ -,  $\beta$ -,  $\gamma$ -, y  $\delta$ -tocoferoles y tocotrienoles) y folatos (tetrahidrofolato, 5-metiltetrahidrofolato y 5-formil tetrahidrofolato) en los frutos de araticum del Cerrado del estado de Minas Gerais, en Brasil.

**Material y métodos.** La composición de vitamina C y carotenoides se analizó por HPLC-DAD, y la de vitamina E y folatos por HPLC con detección por fluorescencia. **Resultados y discusión.** La pulpa de araticum presentó un fuerte valor energético ( $95,12 \text{ kcal}\cdot 100 \text{ g}^{-1}$ ), así como altos contenidos en fibras alimentarias ( $6,80 \text{ g}\cdot 100 \text{ g}^{-1}$ ), carotenoides ( $4,98 \text{ mg}\cdot 100 \text{ g}^{-1}$ ) y vitamina A ( $288,79 \text{ RAE}\cdot 100 \text{ g}^{-1}$ ). Los contenidos en vitamina C, folatos y vitamina E fueron de  $5,23 \text{ mg}\cdot 100 \text{ g}^{-1}$ ,  $27,36 \text{ }\mu\text{g}\cdot 100 \text{ g}^{-1}$  et  $494,04 \text{ }\mu\text{g}\cdot 100 \text{ g}^{-1}$ , respectivamente. **Conclusión.** Los frutos de araticum presentaron un fuerte valor energético, así como altos contenidos en fibras alimentarias. Resultaron ser una fuente de vitamina C y de folatos, y una excelente fuente de vitamina A.

**Brasil / Minas Gerais / *Annona crassiflora* / frutas / propiedades fisicoquímicas / carotinoides / contenido vitamínico / valor energético**

