

# Chemical characteristics and bioactive compounds of cooked pequi fruits (*Caryocar brasiliense* Camb.) from the Brazilian Savannah

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## Chemical characteristics and bioactive compounds of cooked pequi fruits (*Caryocar brasiliense* Camb.) from the Brazilian Savannah.

**Abstract – Introduction.** The Brazilian Savannah presents a wide vegetable diversity that produces fruit that have unique characteristics and should be better characterized and exploited. The agricultural-economic exploration of the fruits from the Savannah contributes to income generation and may play an important role in improving human nutrition. The aim of our research was to evaluate the physical characteristics (diameter, height and mass), chemical composition (moisture, ash, proteins, lipids, dietary fiber, titratable acidity, soluble solids and pH), occurrence and content of vitamin C (ascorbic acid and dehydroascorbic acid), 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) in pequi from the Savannah of Minas Gerais, Brazil. **Materials and methods.** Analyses of carotenoids and vitamin C were carried out by HPLC-DAD, and vitamin E and folates by HPLC with fluorescence detection. **Results.** The pulp of cooked pequi presented high contents of dietary fiber ( $9.9 \text{ g}\cdot 100 \text{ g}^{-1}$ ), lipids ( $33 \text{ g}\cdot 100 \text{ g}^{-1}$ ), carotenoids ( $8.11 \text{ mg}\cdot 100 \text{ g}^{-1}$ ), vitamin C ( $14.33 \text{ mg}\cdot 100 \text{ g}^{-1}$ ), vitamin A ( $514.38 \text{ RAE}\cdot 100 \text{ g}^{-1}$ ) and total energy ( $317.19 \text{ kcal}\cdot 100 \text{ g}^{-1}$ ). Contents of folates and vitamin E were  $5.1 \mu\text{g}\cdot 100 \text{ g}^{-1}$  and  $110.52 \mu\text{g}\cdot 100 \text{ g}^{-1}$ , respectively. **Conclusion.** The pulp of cooked pequi presents a high energy value, as well as elevated contents of dietary fiber and lipids. It is a good source of vitamin C and an excellent source of vitamin A. The nutritional value and the wide availability of pequi make it an important tool for the reduction of food and nutritional insecurity.

**Brazil / Minas Gerais / *Caryocar brasiliense* / fruits / cooking / chemical composition / carotenoids / vitamin content / energy value**

## Caractéristiques chimiques et composés bioactifs des fruits cuits de *Caryocar brasiliense* Camb. (péqui) de la savane brésilienne.

**Résumé – Introduction.** La savane brésilienne présente une large diversité d'espèces végétales qui produisent des fruits à caractéristiques uniques, qui gagneraient à être mieux caractérisés et exploités. L'exploitation agricole-économique des fruits de savane contribue à la génération de revenus et peut jouer un rôle important pour l'amélioration de la nutrition humaine. Le but de nos recherches a été d'évaluer les caractéristiques physiques (diamètre, hauteur et poids), la composition chimique (eau, cendres, protéines, lipides, fibres alimentaires, acidité titrable, solides solubles et pH), la présence et la teneur en vitamine C (acide ascorbique et déhydroascorbique), ainsi que celle en 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) dans les fruits de péquis de la savane du Minas Gerais, au Brésil. **Matériel et méthodes.** L'analyse des caroténoïdes et de la vitamine C a été effectuée par HPLC-DAD, et celle de la vitamine E et des folates par HPLC avec détection par fluorescence. **Résultats.** La pulpe cuite de péqui a présenté des teneurs élevées en fibres alimentaires ( $9,9 \text{ g}\cdot 100 \text{ g}^{-1}$ ), lipides ( $33 \text{ g}\cdot 100 \text{ g}^{-1}$ ), caroténoïdes ( $8,1 \text{ mg}\cdot 100 \text{ g}^{-1}$ ), vitamine C ( $14,33 \text{ mg}\cdot 100 \text{ g}^{-1}$ ), vitamine A ( $514,38 \text{ RAE}\cdot 100 \text{ g}^{-1}$ ) et valeur énergétique totale ( $317,19 \text{ kcal}\cdot 100 \text{ g}^{-1}$ ). Les teneurs en folates et vitamine E ont été de  $5,1 \mu\text{g}\cdot 100 \text{ g}^{-1}$  et  $110,52 \mu\text{g}\cdot 100 \text{ g}^{-1}$ , respectivement. **Conclusion.** La pulpe cuite de péqui présente une forte valeur énergétique, ainsi que des teneurs élevées en fibres alimentaires et en lipides. Elle est une bonne source de vitamine C et une excellente source de vitamine A. La valeur nutritionnelle du péqui et sa grande disponibilité dans la savane du Minas Gerais, au Brésil, en font un atout important pour la lutte contre l'insécurité alimentaire et nutritionnelle.

**Brésil / Minas Gerais / *Caryocar brasiliense* / fruits / cuisson / composition chimique / caroténoïde / teneur en vitamines / valeur énergétique**

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## 1. Introduction

The Savannah covers a considerable area of the Brazilian territory and thus presents a wide variation in climate, soil, fauna and flora. Native plant species of the Savannah have been explored for many purposes, including human consumption. Fruits from this region are consumed fresh or in the form of juices, liqueurs, ice creams, jellies and jams.

Pequi (*Caryocar brasiliense* Camb.) from the Brazilian Savannah plays an important economic role, due to commercialization of the fruits and derived products, as well as nutritional role. This fruit is a drupe consisting of a green epicarp that contains one to four pyrenes. The mesocarp is divided into outer and inner sections (pulp), which surround the pyrenes [1]. Pyrenes are widely marketed for acquisition of the pulp, which is commonly cooked.

Fresh pulp of pequi presents excellent nutritional value and high content of fibers, lipids and total energy [2]. The fatty acid profile of fresh pequi is mainly composed of oleic and palmitic acids (55.9% and 35.4%, respectively) [2]. The unsaturated fatty acids, including oleic acid, are involved in the modulation of serum levels of triglyceride and cholesterol [3]. However, there is no information about the content of macronutrients in cooked pequi pulp cultivated in the state of Minas Gerais, Brazil.

Although there are many publications about the chemical composition of pequi fruit from Minas Gerais, Brazil, there is no information about vitamin E and folate even for fresh pequi. So, our work reports the first study regarding these micronutrients that protect the body against oxidative stress [4, 5].

Fresh pequi pulp also presents bioactive compounds such as vitamin C and carotenoids [6–8]. These compounds are effective antioxidants that interrupt the chain of low partial pressure of oxygen and may reduce the risk of developing cancer, and cardiovascular and cerebrovascular diseases [9, 10]. However, information is scarce regarding the presence of these compounds in cooked pequi pulp.

The objective of our study was to evaluate the chemical characteristics and occurrence and content of bioactive compounds (carotenoids, vitamin C, vitamin E and folates) in cooked pequi pulp obtained from the Savannah of Minas Gerais, Brazil.

## 2. Materials and methods

### 2.1. Raw material

The pequi fruits (*Caryocar brasiliense* Camb.) were collected in the rural area of the municipality of Curvelo (south latitude 18°45' and west longitude 44°25'), Minas Gerais, Brazil.

### 2.2. Standards

The vitamin E standards ( $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherol and tocotrienol) were purchased from Calbiochem<sup>®</sup>, EMD Biosciences, Inc. (USA). L-ascorbic acid was purchased from Sigma-Aldrich<sup>®</sup> (Germany). The following folate standards were used (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). The standards of  $\alpha$ -carotene and  $\beta$ -carotene were isolated from concentrated extract of carrot, while  $\beta$ -cryptoxanthin and lycopene were isolated from extracts of papaya and tomato, respectively, by open column chromatography [11].

### 2.3. Collection and preparation of the samples

The fruits were collected during the harvest season (from January to March 2010) after naturally falling from the trees. To achieve five repetitions, the collection area was split into sub-areas, and, in each sub-area, approximately 5.0 kg of fruits were collected. The samples were transported from the collection site to the laboratory in Styrofoam boxes up to 36 h after collection.

The morphologically perfect fruits were washed with tap water for removal of surface dirt and dried with paper towels. The pequi skin (epicarp + external mesocarp)

was removed with a kitchen knife in order to obtain the pyrenes (internal mesocarp + endocarp + almond). Next, the pyrenes were cooked in boiling water, at the proportion of 1:1 (m/v), for 15 min. After cooking and natural cooling, the pulp (internal mesocarp) was removed from the pyrenes by scraping with a spoon and homogenized in a food processor (Faet Multipratic, model MC5). This procedure was carried out for each of the five repetitions.

## 2.4. Physical characterization

Individual measurements of height, longitudinal diameter and cross-sectional diameter were obtained from 30 fruits using a digital caliper rule (Mitutoyo). The mass of fruit (MF), skin (Msk), pyrenes (MPy), pulp (MPu) and seeds (MSe) was obtained by direct weighing on a semi-analytical balance (Gehaka, BG 2000). Yields of pulp, skin and seeds were calculated, respectively, by the equations  $(MPu / MF) \times 100$ ,  $(Msk / MF) \times 100$  and  $(MSe / MF) \times 100$ .

## 2.5. Chemical analyses

The chemical analyses were performed, with three repetitions, at the Laboratory of Food Analysis of the Department of Nutrition and Health, Federal University of Viçosa, Brazil. Titratable acidity, soluble solids and pH [12] were determined in the fresh pequi pulp; moisture, ash, proteins, lipids and total dietary fiber [13] were determined in the cooked pequi pulp. Carbohydrates were calculated as the difference, by the formula:  $[100 - \% \text{ moisture} - \% \text{ lipids} - \% \text{ proteins} - \% \text{ total dietary fiber} - \% \text{ ash}]$ . The total energy value of the pequi pulp was estimated considering the conversion factors of  $4 \text{ kcal} \cdot \text{g}^{-1}$  of protein or carbohydrate and  $9 \text{ kcal} \cdot \text{g}^{-1}$  of lipid [14].

## 2.6. Extraction and analysis of carotenoids and vitamins

For extraction and analysis of carotenoids and vitamins, the following HPLC-grade reagents were used: acetone, hexane, isopropanol, ethyl acetate, methanol and acetonitrile (Tedia, Brazil) and glacial acetic acid (Vetec, Brazil). Analyses of carotenoids and

vitamin C were carried out by high performance liquid chromatography (HPLC) with a diode array detector (DAD), and vitamin E and folates by HPLC with fluorescence detection.

### 2.6.1. Carotenoids

Carotenoids ( $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin and lycopene) were extracted in acetone and transferred to petroleum ether [15]. Later, the extract in petroleum ether was saponified with a methanol solution containing 10% KOH [16].

The chromatographic conditions used were developed by Pinheiro-Sant'Ana *et al.* [17], and included: HPLC system; a DAD with detection at 450 nm; a chromatographic column Phenomenex Gemini RP-18, 250 mm  $\times$  4.6 mm, 5  $\mu\text{m}$ , fitted with a 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  $1.7 \text{ mL} \cdot \text{min}^{-1}$ .

Vitamin A concentration was calculated according to recommendations of the Institute of Medicine [18], in which 1 Retinol Activity Equivalent (RAE) is equivalent to 1  $\mu\text{g}$  of retinol, 12  $\mu\text{g}$  of  $\beta$ -carotene, or 24  $\mu\text{g}$  of other provitamin A carotenoids.

### 2.6.2. Vitamin C

Ascorbic acid (AA) and dehydroascorbic acid (DHA) were extracted in buffer (3% metaphosphoric acid, 8% acetic acid, sulfuric acid 0.3 N and 1 mM EDTA) [19]. Subsequently, the DHA was converted into AA using dithiothreitol [19]. For analysis of AA, the chromatographic conditions proposed by Campos *et al.* were used [19]. DHA content in the pulp was calculated using the formula:  $\text{DHA content} = \text{AA content after conversion} - \text{AA content before conversion}$ .

### 2.6.3. Vitamin E

The vitamin E isomers ( $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherols and tocotrienols) in cooked pequi pulp were extracted using an extraction solution composed of hexane: ethyl acetate (85:15 v/v), isopropanol, ultrapure water, hexane containing 0.05% BHT (Butylated HydroxyToluene) and sodium sulfate [20]. For analysis, the chromatographic conditions proposed by Guinazi *et al.* [20] were

**Table I.**

Regression equations and correlation coefficients for compounds identified in cooked pequi pulp.

Compound	Regression equation	R <sup>2</sup>
β-cryptoxanthin	1661804.24 x + 18345.98	0.999
β-carotene	1389460.94 x + 24320.87	0.996
Ascorbic acid	3277607.19 x - 66204.16	0.998
α-tocopherol	76030901.90 x - 66102.66	0.999
α-tocotrienol	28452328.82 x - 105303.68	0.997
γ-tocopherol	93182765.60 x + 170331.40	0.997
γ-tocotrienol	124332948.76 x - 300446.44	0.997
(6S)-5,6,7,8-sodium tetrahydrofolate (THF)	942240050.58 x - 162371.44	0.996
(6S)-5-methyl-5,6,7,8-tetrahydrofolate (5-MTHF)	1237294689.67 x - 259476.97	0.994
(6S)-5-formyl-5,6,7,8-tetrahydrofolate (5-FTHF)	710036264.81 x - 1088694.36	0.996

used with a mobile phase composed of hexane:isopropanol:glacial acetic acid (98.9:0.6:0.5, v/v/v).

#### 2.6.4. Folates

The processes of extraction and deconjugation of the folate (THF, 5-MTHF and 5-FTHF) were carried out according to Della Lucia *et al.* [21]. After these steps, the extract was purified according to methods proposed by the same authors with the following modifications. The extract was purified using an ion exchange column, with the stationary phase of Q-Sepharose Fast Flow. The column was pre-conditioned with methanol and water (1:1) at a flow of two drops per second. The extract was applied to the column at a flow rate of two drops per second. Then, elution of the retained folates was carried out with 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 injecting 50 µL of the extracts, previously filtered in filter units with porosity of 0.45 µm. The chromatographic conditions used were developed by Della Lucia *et al.* [21].

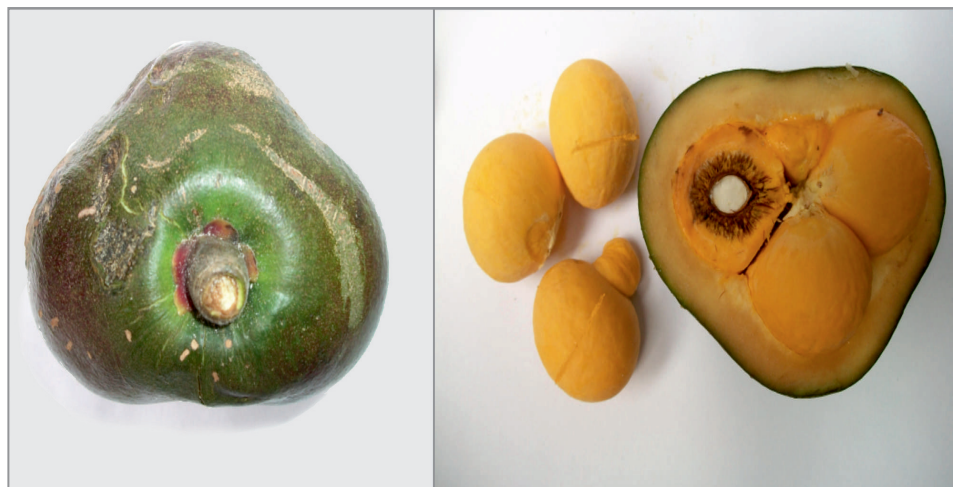
#### 2.6.5. Identification and quantification of carotenoids and vitamins

For identification of the compounds, injections of carotenoid and vitamin standard mixtures were carried out and the retention

times (RT) obtained for the standards were compared with those of the samples. Carotenoids and ascorbic acid were identified by comparison of absorption spectra of the standard and the peaks of interest in the samples, using a DAD, and the folate and vitamin E isomers by co-chromatography.

The isomers found in the cooked pequi pulp (β-cryptoxanthin, β-carotene, ascorbic acid, α-tocopherol, α-tocotrienol, γ-tocopherol, γ-tocotrienol, THF, 5-MTHF and 5-FTHF) were quantified by external standard curves. Construction of standard curves was performed by injection, in duplicate, of six increasing concentrations of standard solutions in the range from (0.033 to 2.060) µg·mL<sup>-1</sup> for β-carotene; (0.004 to 1.433) µg·mL<sup>-1</sup> for β-cryptoxanthin; (0.0589 to 5.8800) µg·mL<sup>-1</sup> for ascorbic acid; (1.0 to 104.2) ng·mL<sup>-1</sup> for α-tocopherol; (2.0 to 204.1) ng·mL<sup>-1</sup> for α-tocotrienol; (2.2 to 107.6) ng·mL<sup>-1</sup> for γ-tocopherol; (3.3 to 157.6) ng for γ-tocotrienol; (0.04 to 46.22) ng·mL<sup>-1</sup> for THF; (0.01 to 10.77) ng·mL<sup>-1</sup> for 5-MTHF; and (0.03 to 33.12) ng·mL<sup>-1</sup> for 5-FTHF. Thus, a linear correlation was determined between the peak areas and the injected concentrations of each compound.

The compounds present in the cooked pequi pulp were quantified based on the analytical curves and regression equations achieved for them (table I). Real concentrations were obtained by calculations based on the dilutions utilized.



**Figure 1.**  
Fruits of pequi (*Caryocar brasiliense* Camb.).

### 2.6.6. Quality control of analytical methods

Recovery, linearity, repeatability, the limit of detection (LOD) and the limit of quantification (LOQ) were assessed. Recovery tests of the standards were carried out by the addition of  $\beta$ -cryptoxanthin,  $\beta$ -carotene, ascorbic acid,  $\alpha$ -tocopherol,  $\alpha$ -tocotrienol,  $\gamma$ -tocopherol,  $\gamma$ -tocotrienol, THF, 5-MTHF and 5-FTHF to the samples at the proportion of 50% to 100% of the original average concentration observed for the species. The recovery percentage was calculated by the formula:

$$\% \text{ recovery} = \frac{\{(\text{final concentration of the isomer}) - (\text{concentration added to the isomer})\}}{(\text{initial concentration of the isomer})} \times 100.$$

All the procedures were carried out in triplicate.

Determination of 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 for the peak areas was used for linear regression analysis; and the correlation coefficient ( $R^2$ ) acquired in each case was used to assess linearity [22].

Repeatability was determined by extraction in quintuplicate and analysis in duplicate of the same fruit sample containing the

carotenoids and vitamins evaluated. Calculation of the relative standard deviation (RSD) of the peak areas and retention times of the components analyzed was then performed to evaluate repeatability [22].

Assessment of the limit of detection was carried out by successive dilutions of the carotenoid and vitamin standards identified in the fruits until they were the lowest detectable amount as was classified as three times the value of the amplitude of the baseline noise. The limit of quantification was established as 10 times the limit of detection [23].

## 3. Results and discussion

### 3.1. Physical characterization

Pequi fruits are drupes containing one to six pyrenes. The fruit peel consists of a green pericarp and grayish-brown outer mesocarp. Pyrenes are composed of the internal yellow mesocarp, which is the edible portion of the fruit, of the thorny endocarp and of the almond (*figure 1*).

The fruits presented a longitudinal diameter ranging from (5.9 to 11.1) cm, transverse diameter ranging from (5.7 to 8.9) cm and height from (5.1 to 7.8) cm. The average mass was 240.12 g, ranging from (120.98 to 412.54) g. Fruits from the state of Minas Gerais presented masses nearly two times greater than fruits from the state of Goiás (125.06 g), and, consequently, greater



**Table II.**

Chemical characteristics and total energy value of cooked pulp of pequi (*Caryocar brasiliense* Camb.) from the Savannah (Curvelo, Minas Gerais, Brazil). Values are expressed in fresh matter (mean of three repetitions  $\pm$  standard deviations).

Soluble solids (°Brix)	Titratable acidity (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> )
7.4 $\pm$ 0.5	0.6 $\pm$ 0.1	6.5 $\pm$ 0.1	51.7 $\pm$ 0.3	2.2 $\pm$ 0.1	33.1 $\pm$ 0.3	0.5 $\pm$ 0.1	9.9 $\pm$ 0.2	2.7 $\pm$ 0.4	317.2 $\pm$ 3.2

height, longitudinal diameter and transverse diameter [24]. The pequi of Minas Gerais state presented a low pulp mass (18.90 g) and low pulp yield (7.9%); however, these values were higher than observed in fruits harvested in other states of Brazil [24]. The wide variation in physical characteristics of fruits may be attributed to 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 fruit present elevated yields of the peel and seeds (78.3% and 13.8%, respectively) which may hinder transportation and technological exploitation of the fruit. Because pequi has a high peel yield, it is necessary to carry out studies that seek better technological utilization of the peel. Soares Júnior *et al.* observed that, in cookies formulated with flour from the peel of pequi, the replacement of up to 25% of wheat flour by flour of the peel of pequi is a viable alternative, with economic and ecological benefits [25].

### 3.2. Chemical characterization

Pulp of fresh pequi presented reduced titratable acidity and soluble solids (*table II*). The pH of the pulp varied within the range reported by Vera *et al.* [24] (6.6 to 6.7).

There is no data in the literature on the content of macronutrients in cooked pequi pulp. The cooked pequi pulp presented moisture content within the range of (48.1 to 54.3) g·100 g<sup>-1</sup> observed by Vera *et al.* [24] in fresh pequi pulp. Lipids corresponded to approximately 33.0% of the macronutrients present in pulp and contributed to 95.0% of the total energy of the pequi (*table II*). Lima

*et al.* observed a total energy value and lipid content in the fresh pequi pulp similar to those found in the present study (358.4 kcal·100 g<sup>-1</sup> and 33.40 g·100 g<sup>-1</sup>, respectively) [2].

The content of dietary fiber in the pulp was, on average, 4.5 times greater than that encountered in fruits commonly recognized as good sources of dietary fiber, such as plum (2.40 g·100 g<sup>-1</sup>) and Tommy mango (2.10 g·100 g<sup>-1</sup>) [7]. The contents of proteins, carbohydrates and ash observed in cooked pequi from the Savannah of Minas Gerais were lower than those of fresh fruits from the Savannah of Goiás, where 3.00 g of proteins, 11.45 g of carbohydrates and 0.63 g of ash were encountered in 100 g of pulp [2].

### 3.3. Carotenoids and vitamins

#### 3.3.1. Recovery, repeatability, limits of detection and quantification, and linearity

Recovery of the standards added to the analyzed samples ranged from 89.0% to 97.4%, with an average of 93.1% (*table III*). These results indicated good recovery percentages of the components analyzed, which decreases the chances of losses during the extraction and analysis processes.

Repeatability of the carotenoid and vitamin isomers present in the cooked pequi pulp presented a relative standard deviation in relation to the peak areas and retention times lower than 7.2% and 2.3%, respectively (*table III*). The results obtained indicate the reliability of the analysis conditions used in our study.

Due to the low limits of detection and quantification achieved for the carotenoid and vitamin isomers present in the cooked

**Table III.**

 Repeatability, limits of detection and quantification, range of linearity and recovery of carotenoids and vitamins in the cooked pulp of pequi (*Caryocar brasiliense* Camb.) of the Savannah (Curvelo, Minas Gerais, Brazil).

Compound	Repeatability		Detection limit	Quantification limit	Range of linearity (µg)	Recovery (%)
	Peak area	Retention time				
	Relative standard deviation		(µg·mL <sup>-1</sup> )			
β-cryptoxanthin	7.20	0.97	6.961	69.61	0.0045 – 1.4333	91.3
β-carotene	4.15	0.72	6.422	64.21	0.0330 – 2.0600	94.2
Ascorbic acid	1.37	0.57	12.321	123.21	0.0589 – 5.8800	97.1
α-tocopherol	3.57	0.89	0.025	0.25	0.0010 – 0.1042	95.3
α-tocotrienol	0.89	1.10	0.042	0.74	0.0020 – 0.2041	97.4
γ-tocopherol	4.84	2.34	0.025	0.25	0.0022 – 0.1076	94.3
γ-tocotrienol	6.73	1.12	0.042	0.74	0.0033 – 0.1576	91.9
THF	1.89	0.91	0.003	0.03	0.00004 – 0.04622	89.3
5-MTHF	1.80	1.57	0.002	0.02	0.00001 – 0.01077	91.0
5-FTHF	2.43	0.83	0.002	0.02	0.00003 – 0.03312	89.0

THF: (6S)-5,6,7,8-sodium tetrahydrofolate.

5-MTHF: (6S)-5-methyl-5,6,7,8-tetrahydrofolate.

5-FTHF: (6S)-5-formyl-5,6,7,8-tetrahydrofolate.

pequi pulp, the methods of analysis allowed identification and quantification of reduced concentrations of these compounds. The limit of detection for the carotenoids and vitamins was between (0.002 and 12.321) µg·mL<sup>-1</sup> (table III). The limit of quantification, considered as 10 times the value of the limit of detection, thus ranged from (0.02 to 123.21) µg·mL<sup>-1</sup>.

The linearity range of the compounds analyzed with UV-visible detection (β-carotene, β-cryptoxanthin and ascorbic acid) presented ratios greater than 60 times between the maximum and minimum injected concentrations (table III). In the compounds evaluated with fluorescence detection, the ratio between these concentrations was 100 times for the vitamin E isomers and 1000 times for the folate isomers. It was observed that the linearity range for each compound was wide and the correlation coefficients ( $R^2$ ) were higher than 0.994, which ensured the acquisition of reliable data.

### 3.3.2. Qualitative composition

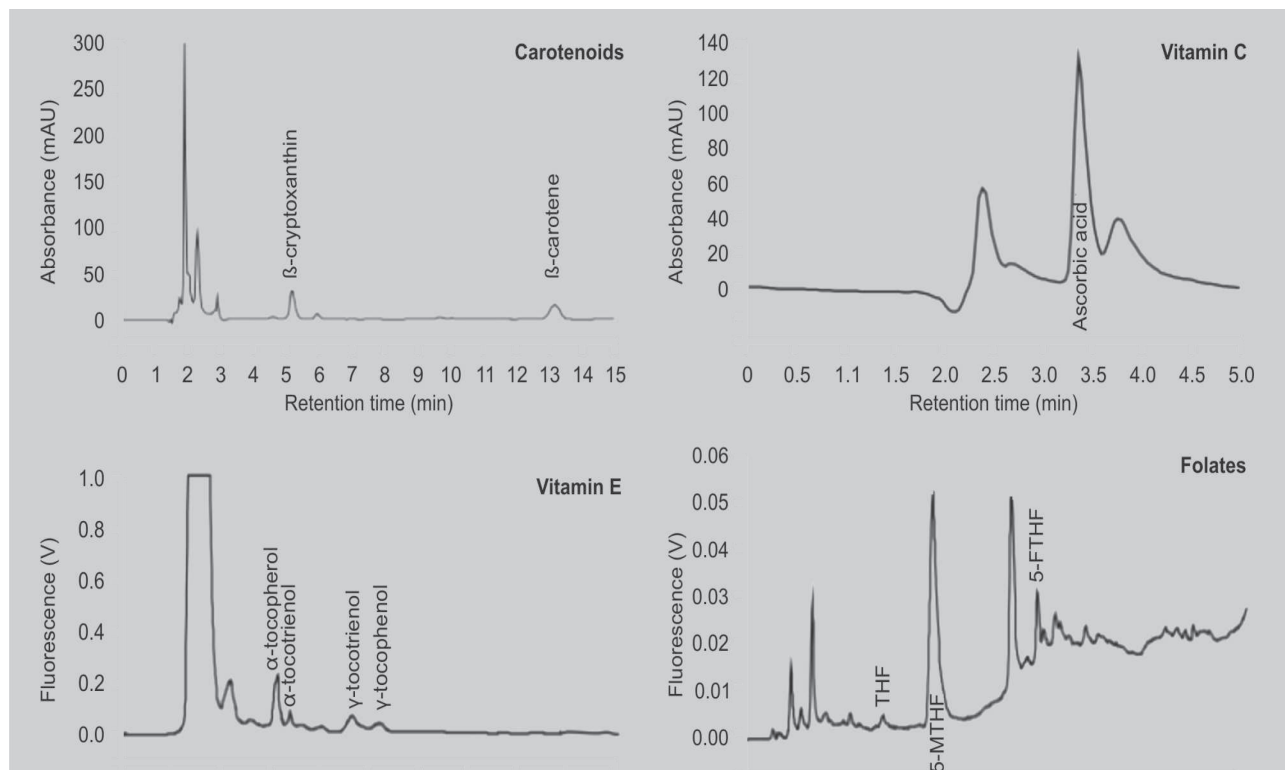
The analysis methods allowed adequate identification of all compounds investigated and quantification of β-cryptoxanthin [Retention Time (RT): 5.2 min], β-carotene

(RT: 13.2 min); ascorbic acid (RT: 3.5 min); α-tocopherol (RT: 6.3 min), α-tocotrienol (RT: 6.9 min), γ-tocopherol (RT: 9.3 min), γ-tocotrienol (RT: 10.6 min); THF (RT: 9.5 min), 5-MTHF (RT: 12.2 min) and 5-FTHF (RT: 19.6 min) (figure 2).

### 3.3.3. Content of carotenoids and vitamins

There are reports in the literature on the presence of carotenoids and vitamin C in the fresh pulp of pequi [6–8]. However, data is limited on the content of these compounds in pequi pulp submitted to thermal treatment, especially data obtained by reliable methods of analysis such as HPLC. There is no information on the presence and content of vitamin E and folates in the pulp of fresh or cooked pequi.

The cooked pequi pulp presented a carotenoid content similar to that found in fruits which are considered sources of carotenoids such as papaya (7.48 mg·100 g<sup>-1</sup>) and guava (7.34 mg·100 g<sup>-1</sup>) [26] (table IV) and higher than in other fruits of the Cerrado, such as araticum and cagaita [(4.98 and 0.77) mg·100 g<sup>-1</sup>, respectively] [27, 28]. This content was approximately 50% lower than that found by Ramos *et al.* [8] in the cooked pequi pulp from the Savannah of Goiás



**Figure 2.** Analysis by HPLC of carotenoids, vitamin C, vitamin E and folates in cooked pulp of pequi (*Caryocar brasiliense* Camb.) of the Savannah

(15.40 mg·100 g<sup>-1</sup>). It should be highlighted that, different from the present study in which the presence and content of four carotenoid isomers were investigated, Ramos *et al.* identified and quantified seven isomers (β-carotene, β-cryptoxanthin, ζ-carotene, cryptoflavin, anteraxanthin, zeaxanthin and mutaxanthin) [8].

Among the carotenoids identified in the cooked pequi, a high concentration of β-carotene and β-cryptoxanthin was observed. These carotenoids play an important role in human health due to their provitamin A activity. The content of β-carotene and β-cryptoxanthin observed in this study was, on average, four times higher than that found by Ramos *et al.* [8] [(0.79 and 1.21) mg·100 g<sup>-1</sup>, respectively]. This variation can be attributed to soil and climatic influences of the collection site of the fruits, state of ripeness at the time of collection, or difference in the cooking procedure of the pyrene (method of cooking, water quantity, time) [6, 29] (table IV).

The vitamin A value in the cooked pequi was greater than that observed by Ramos *et al.*

in cooked pequi pulp (374.8 RAE·100 g<sup>-1</sup>) and fresh pequi pulp (494.0 RAE·100 g<sup>-1</sup>) [8]. When compared with the fruits marketed in the state of Minas Gerais, the vitamin A value was greater than that of acerola (175.0 RAE·100 g<sup>-1</sup>), khaki (58.4 RAE·100 g<sup>-1</sup>), sweet passion fruit (89.5 RAE·100 g<sup>-1</sup>) and papaya (76.7 RAE·100 g<sup>-1</sup>) [30].

The cooked pequi presented a vitamin C content greater than that found in the fresh pulp of pequi by the Brazilian Center for studies and research on food (8.30 mg·100 g<sup>-1</sup>) [7]. Considering that the cooking process leads to reduction in vitamin C content in the pequi pulp, our results suggest that the initial vitamin C content in fruits collected in Minas Gerais was also higher than that of fruits analyzed by that Brazilian center. The content of vitamin C in cooked pequi was, on average, 25% lower than that observed in fruits widely consumed by the Brazilian population and considered sources of vitamin C such as mango (17.5 mg·100 g<sup>-1</sup>) [26] and passion fruit (20.0 mg·100 g<sup>-1</sup>) [7].

The vitamin E content that we found in the cooked pulp of pequi was higher than that



**Table IV.**

 Content of carotenoids and vitamins in cooked pulp of pequi (*Caryocar brasiliense* Camb.) of the Savannah (Curvelo, Minas Gerais, Brazil). Values are expressed in fresh matter.

Compounds	Mean of 5 repetitions $\pm$ standard deviation			%
	RAE $\cdot$ 100 g <sup>-1</sup>	mg $\cdot$ 100 g <sup>-1</sup>	$\mu$ g $\cdot$ 100 g <sup>-1</sup>	
Vitamin A	514.38 $\pm$ 118.12	–	–	100.0
Carotenoids	–	8.10 $\pm$ 2.23	–	100.0
$\beta$ -cryptoxanthin	–	3.86 $\pm$ 0.97	–	47.6
$\beta$ -carotene	–	4.24 $\pm$ 1.26	–	52.4
Vitamin C	–	14.33 $\pm$ 0.32	–	100
Ascorbic acid	–	13.06 $\pm$ 0.53	–	91.2
Dehydroascorbic acid	–	1.27 $\pm$ 0.45	–	8.8
Vitamin E	–	–	170.81 $\pm$ 11.39	100.0
$\alpha$ -tocopherol	–	–	58.77 $\pm$ 12.70	34.4
$\alpha$ -tocotrienol	–	–	50.07 $\pm$ 6.45	29.3
$\gamma$ -tocopherol	–	–	38.32 $\pm$ 4.31	22.4
$\gamma$ -tocotrienol	–	–	23.65 $\pm$ 0.52	13.8
Folates	–	–	5.16 $\pm$ 0.01	100.0
THF	–	–	0.84 $\pm$ 0.02	16.3
5-MTHF	–	–	4.32 $\pm$ 0.01	83.6
5-FTHF	–	–	0.01 $\pm$ 0.00	0.2

RAE: Retinol Activity Equivalent.  
 THF: (6S)-5,6,7,8-sodium tetrahydrofolate.  
 5-MTHF: (6S)-5-methyl-5,6,7,8-tetrahydrofolate.  
 5-FTHF: (6S)-5-formyl-5,6,7,8-tetrahydrofolate.

found by Chun *et al.* in banana (150.00  $\mu$ g $\cdot$ 100 g<sup>-1</sup>) and lower than that of kiwi (1450.00  $\mu$ g $\cdot$ 100 g<sup>-1</sup>), avocado (2750.00  $\mu$ g $\cdot$ 100 g<sup>-1</sup>), peach (790.00  $\mu$ g $\cdot$ 100 g<sup>-1</sup>), grape (540.00  $\mu$ g $\cdot$ 100 g<sup>-1</sup>), pear (420.00  $\mu$ g $\cdot$ 100 g<sup>-1</sup>) and strawberry (410.00  $\mu$ g $\cdot$ 100 g<sup>-1</sup>) [31].

The cooked pequi presented a low folate content (5.16  $\mu$ g $\cdot$ 100 g<sup>-1</sup>), which was lower than that encountered in banana (8.00  $\mu$ g $\cdot$ 100 g<sup>-1</sup>) [32], mango (14.09  $\mu$ g $\cdot$ 100 g<sup>-1</sup>), mulberry (25.00  $\mu$ g $\cdot$ 100 g<sup>-1</sup>) and strawberry (24.09  $\mu$ g $\cdot$ 100 g<sup>-1</sup>) [33].

### 3.3.4. Nutritional value of the pulp of cooked pequi as a source of vitamins

Foods may be classified as “sources” of a nutrient if they provide 5% to 10% of the Dietary Reference Intake (DRI), as “good sources” if they provide 10% to 20% of the DRI and as “excellent sources” if supplying more than 20% of the DRI [34]. Considering the recommendations of folates, and vitamins A, C and E for adult men between

19 and 30 years old [35–37], it was observed that the pulp of cooked pequi was a source of vitamin C and an excellent source of vitamin A.

It was observed that the consumption of 100 g of pulp supplied 57.3% and 66.9% of the recommended dietary allowance (RDA) of vitamin A for adult men and pregnant women, respectively, and exceeded the RDA for children between 4 and 8 years old (128.8%). The vitamin C content observed in 100 g of cooked pequi pulp provides 57.3%, 15.9% and 19.1% of the recommendations for children, adult men and pregnant women, respectively.

Consumption of cooked pequi pulp offers a modest contribution of vitamin E (less than 1.0% of RDA) and folates (less than 2.6% of RDA) to the three groups. Despite the small contribution of pequi for supplying the recommended amounts of folates and vitamin E, this contribution is very important, since pequi is present in

places where other sources of these vitamins may not be available during the year, or may not be sufficient to meet the needs of certain groups. Similar to several fruits of the savannah, pequi is widely available during the harvest season and can be obtained freely from the families that take part in extractivism activities in the savannah or purchased at a very low cost (on average, US\$ 1.10 per kg).

#### 4. Conclusions

The pequi fruit from the state of Minas Gerais presents a high mass content, but reduced pulp yield, which may hamper its technological utilization. Cooked pequi pulp presented excellent nutritional value and total energy higher than most Brazilian fruits. It presented high contents of dietary fiber and lipids, and is a source of vitamin C and excellent source of vitamin A. The nutritional value and the wide availability of pequi make it an important tool for the reduction of food and nutritional insecurity.

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### **Características químicas y compuestos bioactivos de los frutos cocidos de *Caryocar brasiliense* Camb. (pequi) de la sabana brasileña.**

**Resumen – Introducción.** La sabana brasileña presenta una gran diversidad de especies vegetales que producen frutos de características singulares, que ganarían si fueran mejor caracterizados y explotados. La explotación agrícola-económica de los frutos de la sabana contribuye a generar ingresos y puede desempeñar un papel importante para la mejora de la nutrición humana. El objetivo de nuestras investigaciones fue la evaluación de las características físicas (diámetro, altura y peso), la composición química (agua, cenizas, proteínas, lípidos, fibras alimentarias, acidez valorable, sólidos solubles y pH), la presencia y el contenido de vitamina C (ácido ascórbico y dehidroascórbico), así como el 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 pequi de la sabana de Minas Gerais, en Brasil. **Material y métodos.** El análisis de los carotenoides y de la vitamina C se efectuó por HPLC-DAD, y el de la vitamina E y de los folatos por HPLC con detección por fluorescencia. **Resultados.** La pulpa cocida de pequi presentó altos contenidos de fibras alimentarias ( $9,89 \text{ g}\cdot 100 \text{ g}^{-1}$ ), lípidos ( $33,07 \text{ g}\cdot 100 \text{ g}^{-1}$ ), carotenoides ( $8,10 \text{ mg}\cdot 100 \text{ g}^{-1}$ ), vitamina C ( $14,33 \text{ mg}\cdot 100 \text{ g}^{-1}$ ), vitamina A ( $514,38 \text{ RAE}\cdot 100 \text{ g}^{-1}$ ) y valor energético total ( $317,19 \text{ kcal}\cdot 100 \text{ g}^{-1}$ ). Los contenidos de folatos y vitamina E fueron de  $5,1 \text{ }\mu\text{g}\cdot 100 \text{ g}^{-1}$  y  $110,52 \text{ }\mu\text{g}\cdot 100 \text{ g}^{-1}$ , respectivamente. **Conclusión.** La pulpa cocida de pequi presenta un fuerte valor energético, así como altos contenidos de fibras alimentarias y lípidos. Es una buena fuente de vitamina C y una excelente fuente de vitamina A. El valor nutricional del pequi y su gran disponibilidad en la sabana de Minas Gerais, en Brasil, hacen que este fruto sea una baza importante para la lucha contra la inseguridad alimentaria y nutricional.

**Brasil / Minas Gerais / *Caryocar brasiliense* / frutas / cocción / composición química / carotenoides / contenido vitamínico / valor energético**