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Original Research Article

Caryocar brasiliense Camb. fruits from the Brazilian Cerrado as a rich source of carotenoids with pro-vitamin A activity

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ABSTRACT

Caryocar brasiliense Camb. is the best-known fruit of the Brazilian Cerrado. This fruit has yellow pulp containing several carotenoids and has a strong and exotic flavor; however, its composition may vary due to factors such as genotypic differences, seasonality, cultivation forms, climatic and soil characteristics, maturation stage, type of storage and processing. Therefore, the present study aimed to determine the chemical composition of carotenoids with pro-vitamin A activity of *C. brasiliense* fruits from 18 Brazilian municipalities. The vitamin A value expressed as μg Retinol Activity Equivalent (RAE) in the fresh fruit and carotenoid profile were determined by high-performance liquid chromatography coupled to a diode array detector (HPLC- DAD). The main carotenoids found were β-cryptoxanthin and β-carotene. High variation in vitamin A values were observed, especially among the fruits from Gato-Preto-MA with (1.4 μg RAE/100 g fresh fruit) and Januária-MG (719 μg RAE/100 g fresh fruit). PC1 and PC2 explored about 98.80% of the data variance in the multivariate analysis, Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) and organized the *C. brasiliense* fruits in three clusters by decreasing vitamin A values. The fruits from the municipalities of Januária, Japonvar, Arinos, Salinas and Montes Claros showed higher carotenoid content.

1. Introduction

The Brazilian Cerrado is the largest neotropical savanna formation in South America and the second-largest ecosystem in Brazil, which spans 200 million hectares. It covers about 24 % of the Brazilian territory and displays a great diversity of flora and fauna (Batlle-Bayer et al., 2010; IBGE, 2019). The *Caryocar brasiliense* Camb. plant, commonly called *pequizeiro*, is one of the most representative species of this ecosystem in this important biome. This plant produces a fruit with a yellow pulp and a strong and exotic flavor. It is considered an important source of carotenoids and lipids, so it has been widely used by the Cerrado population due to its nutritional, medicinal, ornamental, oleaginous and taniferous qualities (Cordeiro et al., 2013; Ribeiro et al., 2014; Nascimento-Silva and Naves, 2019; Nascimento-Silva et al., 2020).

Several studies have been conducted due to the commercial, gastronomic and agro-industrial potential of *C. brasiliense* species (Ascari et al., 2010), highlighting the nutritional and nutraceutical values of its fruits (Oliveira et al., 2006; Lima et al., 2007; Cordeiro et al., 2013; Geőcze et al., 2013; Pinto et al., 2018; Nascimento-Silva et al., 2020). In these studies, the proximate composition of the *C. brasiliense* fruits have shown contents above 40 % in lipids, in addition to presenting proteins, carbohydrates, fiber, ash, and vitamin C. Its yellow-orange pulp is indicative of the presence of carotenoids such as β-carotene and β-cryptoxanthin, which are carotenoids with pro-vitamin A activity (IOM, 2000, 2001; Yeum and Russell, 2002; Vargas-Murga et al., 2016).

However, previous studies have reported variations in the chemical

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composition of fruits associated with factors such as genotypic differences (Golubkina et al., 2018; Léchaudel et al., 2018; Kyriacou et al., 2018); seasonality (Mirdehghan and Rahemi, 2007; Golubkina et al., 2018); cultivation forms (Rouphael et al., 2010); climatic and soil characteristics (Assunção and Mercadante, 2003; Moretti et al., 2010; Léchaudel et al., 2018; Kyriacou et al., 2018); maturation stage (Msaada et al., 2009; Kyriacou et al., 2018); and the type of storage and processing (Rico et al., 2007; Kyriacou et al., 2018).

While some data exist, detailed studies comparing the carotenoid composition of the most important Brazilian Cerrado fruit genotypes remain scarce.

In this sense, the present study aimed to determine the chemical composition of carotenoids with pro-vitamin A activity of *C. brasiliense* fruit pulps from 18 municipalities of the Brazilian Cerrado and to verify the classifying patterns of these fruits according to vitamin A values through multivariate analysis.

2. Materials and methods

2.1. Fruit sampling

C. brasiliense fruits were collected during the harvesting period between December and January. Traditionally, this fruit is considered ripe and suitable for consumption when it falls off the plant. Therefore, to best emulate traditional harvesting and quality at consumption, in this study we used fruits that were on the ground and were not damaged or malformed (Figs. S1a and S1b (Supplementary material)). The main sampling site was Minas Gerais state in which the Cerrado biome covers 57 % of the total vegetation area, and corresponds to 16 % of all Brazilian Cerrado. Although the percentage of the Cerrado biome in the states of Maranhão and Tocantins is smaller (11 % and 12 %, respectively), the geographical interplay between the growing areas and their neighboring flora must be considered because the vegetation in these states is located in regions bordering the Amazon Forest (IBGE, 2019). Samples from a total of 18 locations were collected.

We collected fruits from several plants (3–5 different plants growing in the same area) in each municipality studied, totaling approximately 25 kg of fruit. These fruits were packed in isothermal boxes with ice and immediately transported to the laboratory after harvesting.

The intact (unpeeled) fruit units were washed and stored in a freezer in the laboratory at −18 °C until extraction and chromatographic analysis.

Fruits from each municipality were registered and deposited into the collection of the VIC Herbarium in the Department of Plant Biology (DBV), UFV. The details of each registered fruit are shown in Table S1 (Supplementary material).

2.2. Reagents

Petroleum ether, acetone, ethyl acetate, acetonitrile and methanol PA-grade solvents were purchased from Vetec Quimica Fina Ltda (São Paulo, Brazil). All solvents were filtered through 0.45 μm polyethylene membrane purchased from Supelco Inc. (Bellefonte, USA). β-Carotene, lutein, neoxanthin, violaxanthin standards were purchased from Sigma-Aldrich (St. Louis, Missouri, USA); zeaxanthin and β-cryptoxanthin were purchased from Indofine Chemical Company (Hillsborough, NJ, USA). BHT (2,6-bis(1,1-dimethylethyl)-4-methylphenol) was purchased from Merck (Burlington, Massachusetts, USA). Anhydrous sodium sulfate, sodium hydroxide, and potassium hydroxide were purchased from Vetec Quimica Fina Ltda (São Paulo, Brazil) with purity greater than 99 %.

2.3. Equipment

The equipment used in this study included a Kindly centrifuge (São Paulo, Brazil), a Scilogex vortex (USA), a Black & Decker KPMHC3IT domestic food microprocessor (São Paulo, Brazil), a Marconi MA-102 stainless steel crusher (Piracicaba, São Paulo, Brazil), a Marconi MA 120/TH rotary evaporator (Piracicaba, São Paulo, Brazil), a Heto VR-Maxi CS3 vacuum concentrator (Waltham, Massachusetts, USA) and a Shimadzu analytical scale (Barueri, Sao Paulo, Brazil).

2.4. Extraction methods

The analysis of individual carotenoids was carried out at Purdue University, West Lafayette, Indiana, USA, and the analysis of the carotenoids pro-vitamin A were performed at the Universidade Federal de Viçosa, Minas Gerais, Brazil.

2.4.1. Determination of individual carotenoids

Individual carotenoids were analyzed at Purdue University, so it was necessary to dehydrate the fruit pulp for shipment to the United States.

For this part of the study, we send 500 g of fruit pulp, which corresponds to approximately 10–12 fruits. Samples that could represent a specific geographic region was sent. Thus, five samples from Minas Gerais state (Arinos, Curvelo, Januária, Montes Claros and Salinas), one sample from Tocantins state (Paranã) and one from Maranhão state (Barra do Corda) were sent for analysis.

The *C. brasiliense* pulps were dehydrated by the lyophilization method in a vacuum concentrator until reaching moisture of around 7%. All sample handling and dehydration processes were carried out in the dark to minimize the degradation of the carotenoids by light. The samples were placed in opaque plastic containers, labeled and sent to Purdue University (USA).

The methodology used was in accordance with that described in Ferruzzi et al. (2006). All sample preparation and pigment extraction steps were performed under yellow light to minimize carotenoid photo-oxidation and photo-isomerization reactions. The analyses were performed in duplicate.

Approximately 3.00 g of lyophilized fruit pulp were dispersed in 4 mL of distilled water. This mixture was saponified with NaOH solution in methanol (30 % m/v) for 30 min in the absence of light at 37 °C. Next, the carotenoids were extracted with 4 mL petroleum ether/acetone (3:1 v/v) containing 0.1 % (m/v) of butylated hydroxytoluene solution (BHT). The extracts were then vortexed for 30 s and centrifuged at 3500 rpm (5000 × g) for 5 min until phase separation. The organic phase was removed and the remaining residue was extracted with petroleum ether (3 × 10 mL). The combined petroleum ether fractions were dried under nitrogen leaving a solid residue that was dissolved in methanol/ethyl acetate (50:50 v/v), filtered through 0.45 μm polyethylene membrane and stored in amber vials at -5 °C until the time of analysis (on the same day).

2.4.2. Determination of pro-vitamin A carotenoids

Pro-vitamin A carotenoids were determined at Universidade Federal de Viçosa with all of the 18 fruit samples being submitted to this analysis. For this stage of the study, the fruits were thawed and immediately subjected to the extraction stage, so it was not necessary to dehydrate the pulps. The moisture content for each sample was determined according to the analytical norms of the Adolfo Lutz Institute (IAL, 1985).

Carotenoid extraction was performed according to the methodology proposed by Rodriguez et al. (1976). The fruit peels were initially removed and the fruit pulp was crushed and homogenized in a domestic food microprocessor. Next, 20 mL of cooled acetone at 10 °C were added to 5.00 g of fruit pulp and extraction was carried out with a stainless steel crusher. This process was repeated two more times totaling 60 mL of cold acetone (or until the extract was colorless). The total extract obtained was filtered through a filter paper; the filtrate was further transferred to a separating funnel containing 50 mL of cooled petroleum ether at 10 °C. Each fraction was washed with distilled water (3 × 20 mL) to remove all acetone and the pigments were consequently transferred to the petroleum ether phase. These petroleum ether phases were stored in amber vials at −5 °C until the time of analysis (on the same

day).

All of the obtained extracts were submitted to the saponification stage. This procedure was performed according to Mercadante (1999). First, 50 mL of the petroleum ether extract containing the carotenoids and 50 mL of potassium hydroxide (KOH) methanolic solution (10 % m/v of KOH and 0.5 % m/v of BHT) were added into a 250 mL amber Erlenmeyer flask. The mixture was stirred under a nitrogen atmosphere for 1 min, and then the Erlenmeyer cap was sealed with aluminum foil and parafilm. The mixture was left in the dark for 16 h at room temperature. After saponification, the mixture was washed three times with distilled water in a separating funnel to remove all of the saponification solution. The petroleum ether carotenoid solution was dried over anhydrous sodium sulfate, filtered and the solvent was removed in a rotary evaporator at 37 °C under reduced pressure. The obtained pigments were dissolved in petroleum ether (25 mL) and stored in amber vials at −5 °C until analysis (on the same day). This procedure was performed with all of the 18 fruit sample extracts.

2.5. Chromatographic analysis

2.5.1. Determination of individual carotenoids

The individual carotenoid content analyses were carried out according to the methods described by Kean et al. (2008). The analyses were performed using a Hewlett-Packard 1090A high-performance liquid chromatography (HPLC) system coupled to a 79880A diode array detector (DAD). A Waters Corp. polymeric C30 reversed-phase chromatographic column with dimensions of 250 mm × 2.0 mm (3 μm particle size) was employed (Milford, MA).

The elution was carried out using a mobile phase binary system composed of: Phase A - methanol/ammonium acetate (1 mol L^{-1}) (98: 2 v/v); and Phase B - ethyl acetate. The mobile phase flow was 0.37 mL min^{-1}. The elution started with 100 % of phase A and linear gradient to 80:20 v/v ratio to be reached within 6 min. This proportion was maintained for 2 min before it was returned to initial conditions at 3 min, remaining under such conditions for 3 min before the next analysis. The total analysis time was 14 min. Detection and preliminary identification of each carotenoid were performed using a diode array detector between 250 and 600 nm and comparison to previous reports from our group (Lipkie et al., 2013; Aragón et al., 2018).

Quantification of individual carotenoids was performed by following the validated protocol described by Kean et al. (2008). For this, multilevel response curves were constructed at 450 nm with authentic carotenoid standards for lutein, zeaxanthin, β-cryptoxanthin, α-carotene, and β-carotene. Due to a lack of authentic standards, (*Z*)-isomers of lutein, zeaxanthin, and β-carotene were tentatively identified based on comparison of electronic absorption spectra and elution profiles using similar chromatography conditions, elution order as well as diode array spectra to previous studies (Kurilich and Juvik, 1999; Ferruzzi et al., 2006; Goltz et al., 2012). Zeinoxanthin was similarly tentatively identified by comparison to previous studies (De Oliveira and Rodriquez-Amaya, 2007; Scott and Eldridge, 2005; Kean et al., 2007). Levels of these carotenoids in test foods and grains were estimated based on response curves for corresponding all-E-isomer (for (*Z*)-isomers) and lutein (for zeinoxanthin). Intraday coefficients of variation (CV) for extraction and analysis were 3.1, 2.7, 5.1, 3.9, and 5.7 % for (all-*E*)--lutein, (all-E)-zeaxanthin, (*Z*)-lutein + (*Z*)-zeaxanthin, zeinoxanthin, and β-carotene, respectively.

2.5.2. Determination of pro-vitamin A carotenoid content

For the chromatographic analysis, 4 mL of the total petroleum ether extract (25 mL) was dried under nitrogen gas flow and the residue was then dissolved in 2 mL of acetone (HPLC-grade). Next, the extract was filtered on HV-Millex polyethylene membrane of 0.45 μm porosity and stored in amber vials until the time of analysis (on the same day).

Pro-vitamin A carotenoid content was determined according to the methodology proposed by Pinheiro-Sant'ana et al. (1998). A Shimadzu SCL-10A VP high-performance liquid chromatography (HPLC) system with a Shimadzu SOD-M10 AVP diode array detector (DAD), along with a Phenomenex FR C18 chromatographic column (250 mm × 4.6 mm) and mobile phase composed of methanol:ethyl acetate:acetonitrile (80:10:10 v/v/v) were used for the analyses. The mobile phase flow was 2.0 mL min^{-1} in isocratic elution; the running time was 20 min and the wavelength was 450 nm. Each carotenoid was identified by comparing their UV/visible spectra and retention times of each standard of carotenoid with the peaks present in the chromatograms of the analyzed extracts.

Quantitative analyses were performed according to the methodology proposed by Rodriguez-Amaya (1999). To do so, an external standardization using β-carotene and β-cryptoxanthin as standards was applied. The β-cryptoxanthin was isolated from *Carica papaya* fruits, according to Kimura and Rodriguez-Amaya (2002). The injection was performed in duplicate using six increasing concentrations of standard solutions between 0.0103 and 2.0600 μg mL^{-1} (β-carotene) and 0.0057 and 1.4333 μg mL^{-1} (β- cryptoxanthin). The actual concentration of the carotenoid standards was determined by spectrophotometry and adequately corrected. The equation and coefficients used to calculate the concentrations were: equation 1:

$$C\ (\mu g\ mL^{-1}) = Abs \times 10^4/\mathcal{E}$$

In which: C = actual concentration; ABS = maximum absorbance; Ɛ = molar absorptivity coefficient of the carotenoid in the solvent used.

Carotenoid concentrations with pro-vitamin A activity were obtained from the analytical curves.

2.6. Calculation of vitamin A activity

The vitamin A value of the pro-vitamin A carotenoids were calculated according to the recommendations of the Institute of Medicine (IOM, 2001; Yeum and Russel, 2002).

The vitamin A value was calculated as μg Retinol Activity Equivalent (RAE) per 100 g fresh weight according to Eq. 2:

$$\mu g\ RAE = (\mu g\ \beta\text{-carotene mass}/12) + (\mu g\ \beta\text{-cryptoxanthin mass}/24). \quad (2)$$

2.7. Analysis of variance

We used a completely randomized experimental design with three replications to study the averages of the 18 fruit samples, where each sample represents the fruits of a Brazilian municipality. The ANOVA was performed using the ASSISTAT Version 7.6 beta statistical program (2011).

2.8. Multivariate analysis

The obtained data were arranged in a matrix consisting of variables (columns) such as moisture, β-cryptoxanthin and β-carotene contents. The objects of this matrix were the fruits from 18 Brazilian municipalities (lines). The matrix was auto-scaled and then the principal component analysis (PCA) and hierarchical cluster analysis (HCA) were performed. It should be noted that HCA (cluster) was performed using the averaging algorithm. Data were processed using MATLAB software, version 5.3, employing the PLS_Toolbox chemometric package, version 2.0 (Wise and Gallagher, 1999).

3. Results and discussion

In this study, *C. brasiliense* fruits were collected from three Brazilian states, totaling 18 municipalities in the Cerrado biome (Fig. 1). The sampling sites were the state of Minas Gerais, where the Cerrado biome corresponds to 16 % of the Brazilian Cerrado, and the states of Maranhão

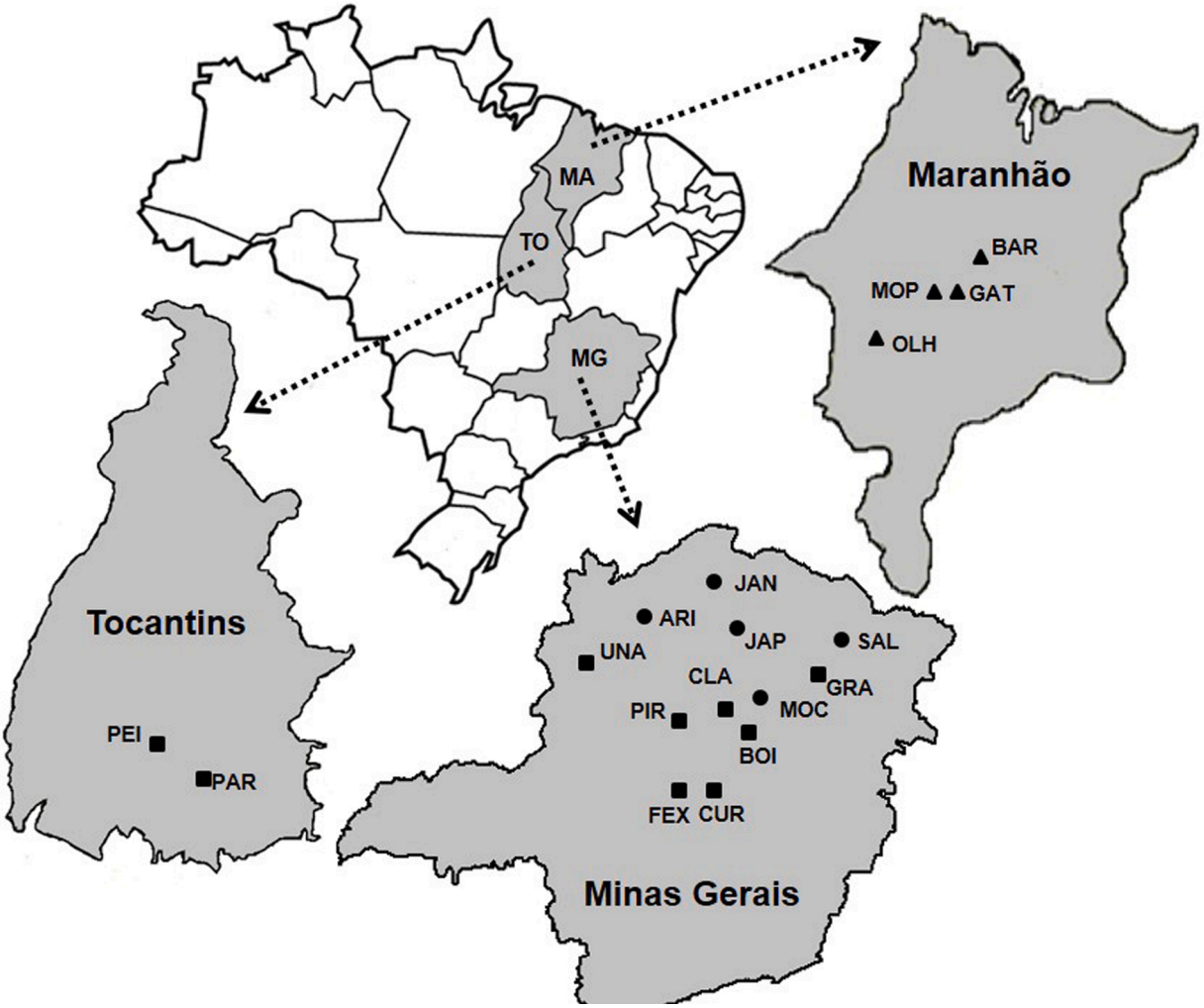

Fig. 1. Map of Brazil showing the states of Minas Gerais, Maranhão and Tocantins. The sample codes are related to the municipalities of origin. BAR - Barra do Corda, GAT - Grajaú I (Gato preto), OLH - Grajaú II (Olhos d'Agua), MOP – Monte Pascoal, ARI - Arinos, OX - Bocaiúva, CLA - Claro dos Porções, CUR - Curvelo, FEX - Felixlândia, GRA - Grão Mogol, JAN - Januária, JAP - Japonvar, MOC - Montes Claros, PIR - Pirapora, SAL - Salinas, UNA - Unaí, PAR - Paranã and PEI - Peixe.

and Tocantins with 11 % and 12 % of this biome, respectively.

Therefore, the first part of this study involved preparing *C. brasiliense* pulp extract representatives from samples obtained in seven locations, representing the three states. The extracts were analyzed by high-performance liquid chromatography (HPLC) and a typical chromatogram is presented in Fig. 2.

The identification and quantification of each carotenoid present in the pulp extracts of *C. brasiliense* are presented in Table 1.

The main carotenoids found in the fruit pulp of *C. brasiliense* are neoxanthin, violaxanthin, lutein, zeaxanthin, β-cryptoxanthin and β-carotene. These compounds have previously been described in studies of *C. brasiliense* pulp (Azevedo-Meleiro and Rodriguez-Amaya, 2004; Biazotto et al., 2019; Nascimento-Silva and Naves, 2019) and other Brazilian fruits (Vargas-Murga et al., 2016). The (15*Z*)-β-carotene, (13*Z*)-β-carotene, α-carotene and (9*Z*)-β-carotene were detected but not quantified due to low levels.

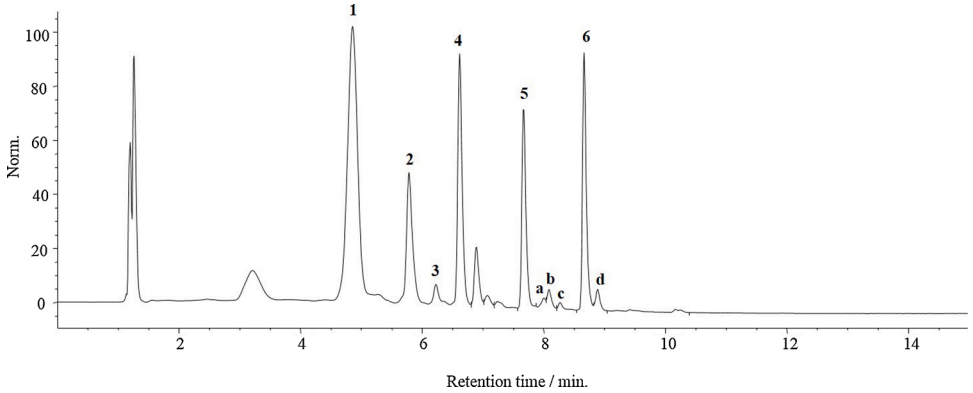

Fig. 2. Representative chromatogram of *C. brasiliense* pulp extract by HPLC-DAD. Compounds identified: 1 – Neoxanthin, 2 – Violaxanthin. 3 – Lutein, 4 – Zeaxanthin, 5 - β-Cryptoxanthin, 6 - β-Carotene, a – (15*Z*)-β-carotene, b – (13*Z*)-β-carotene, c - α-carotene and d – (9*Z*)-β-carotene. Chromatographic conditions were: Waters Corp polymeric C30 reverse phase chromatography column of 250 mm × 2.0 mm (3 μm particle size) (Milford, MA). The mobile phase was Phase A - methanol/ammonium acetate (1 mol L^{-1}) (98: 2 v/v) and Phase B - ethyl acetate, mobile phase flow was 0.37 mL min^{-1} and 450 nm.

Table 1
Carotenoid content (mg/100 g dry weight) in fruit pulp of *C. brasiliense*.

Carotenoids	Samples						
	MA	MG					TO
	BAR	ARI	CUR	JAN	MOC	SAL	PAR
Neoxanthin	0.00**Aa**	2.78 ± 0.01**Ba**	5.66 ± 0.01**Ca**	10.2 ± 0.03**Da**	0.851 ± 0.01**Ea**	2.81 ± 0.01**Ba**	1.54 ± 0.01**Fa**
Violaxanthin	0.00**Aa**	0.558 ± 0.00**Bb**	0.743 ± 0.00**Cb**	2.94 ± 0.01**Db**	0.215 ± 0.00**Eb**	0.788 ± 0.00**Fb**	0.316 ± 0.00**Gb**
Lutein	0.00**Aa**	0.284 ± 0.00**Bc**	0.136 ± 0.00**Cc**	0.404 ± 0.00**Dc**	0.0550 ± 0.00**Ec**	0.0670 ± 0.00**Fc**	0.0845 ± 0.00**Gc**
Zeaxanthin	0.190 ± 0.00**Ab**	1.35 ± 0.02**Bd**	1.77 ± 0.00**Cd**	4.13 ± 0.01**Dd**	0.958 ± 0.01**Ed**	1.58 ± 0.01**Fd**	1.33 ± 0.02**Bd**
β-Cryptoxanthin	0.00**Aa**	0.390 ± 0.00**Be**	0.406 ± 0.00**Be**	2.69 ± 0.01**Ce**	0.408 ± 0.00**Be**	0.942 ± 0.01**De**	0.193 ± 0.00**Ee**
β-Carotene	0.0845 ± 0.00**Ac**	3.68 ± 0.02**Bf**	6.03 ± 0.00**Cf**	13.3 ± 0.01**Df**	1.35 ± 0.00**Ef**	4.09 ± 0.01**Ff**	1.85 ± 0.03**Gf**

Values are mean of 2 replicates.
Means followed by the same uppercase letters in the rows, or lowercase letters in the columns do not differ by the Tukey test at the 5%.
States: MA – Maranhão; MG – Minas Gerais; TO – Tocantins; Municipalities: BAR - Barra do Corda, ARI – Arinos, CUR – Curvelo, JAN – Januária, MOC - Montes Claros, SAL – Salinas and PAR – Paranã.

The results reported herein show the lowest content of carotenoids for *C. brasiliense* fruits from Barra do Corda-MA, where only two carotenoids were quantified (zeaxanthin and β-carotene). On the other hand, the fruits from municipalities of Minas Gerais showed the highest content of carotenoids, mainly the fruits from Januária-MG.

The xanthophylls, zeaxanthin and lutein were detected in almost all *C. brasiliense* samples (except the BAR-MA sample), in which the fruits from Januária-MG again stood out for their high content. These compounds are of interest since they are associated with preventing eye diseases such as cataract and macular degeneration (Krinsky et al., 2003; Sabour-Pickett et al., 2012). Thus, this fruit may be considered a source of these bioactive pigments and as such these fruits could contribute to healthier diets (Biazotto et al., 2019; Nascimento-Silva and Naves, 2019). It is important to highlight that fruit pulp of *C. brasiliense* from Januária-MG showed the highest content of these specific carotenoids around 4.53 mg/100 g dry weight (total sum of lutein + zeaxanthin).

β-Carotene was the main carotenoid found in five of these seven studied samples of *C. brasiliense* (ARI, JAN, MOC, SAL and PAR). This compound has been widely studied and reported in the scientific literature (Burri, 1997; Aruna and Baskaran, 2010; Van Loo-Bouwman et al., 2014; Vargas-Murga et al., 2016), and it is a major food carotenoid; it is a pro-vitamin A with 100 % vitamin A activity. Therefore, the second part of this study evaluated the carotenoids content with pro-vitamin A activity in fruit pulps of *C. brasiliense* from 18 Brazilian municipalities within the Cerrado biome.

3.1. Pro-vitamin A carotenoids

The β-carotene and β-cryptoxanthin contents in the fruit pulp of *C. brasiliense* of the present study are in agreement with previous reports (Azevedo-Meleiro and Rodriguez-Amaya, 2004; Biazotto et al., 2019; Nascimento-Silva and Naves, 2019). These two carotenoids were detected in six *C. brasiliense* samples (ARI, CUR, JAN, MOC, SAL and PAR), while β-cryptoxanthin was only not detected in the BAR-MA sample. The results of quantitative analyses of pro-vitamin A carotenoids in the *C. brasiliense* pulp extracts of 18 municipalities are shown in Fig. 3.

The results revealed that all samples showed higher β-carotene content compared to β-cryptoxanthin. A high variation in the vitamin A value (μg RAE/100 g fresh fruit pulp) was similarly found (Table 2), highlighting the great difference between the fruits from Grajaú-MA (Gato Preto), which almost had a zero index (1.42 μg/100 g), while fruits from Januária-MG stood out for their high vitamin A value in comparison with the other samples. *C. brasiliense* fruits from Januária-MG, Japonvar-MG, Salinas-MG, Montes Claros-MG and Arinos-MG had higher vitamin A value than those from the other municipalities.

In this context, the municipalities from Minas Gerais such as Arinos, Januária, Japonvar, Montes Claros and Salinas are located in transition regions between Cerrado and Caatinga, so they have climatic conditions with lower rainfall and higher solar incidence when compared to the municipalities of Tocantins and Maranhão states which, despite having a climate with high temperatures, are in areas bordering those typical of

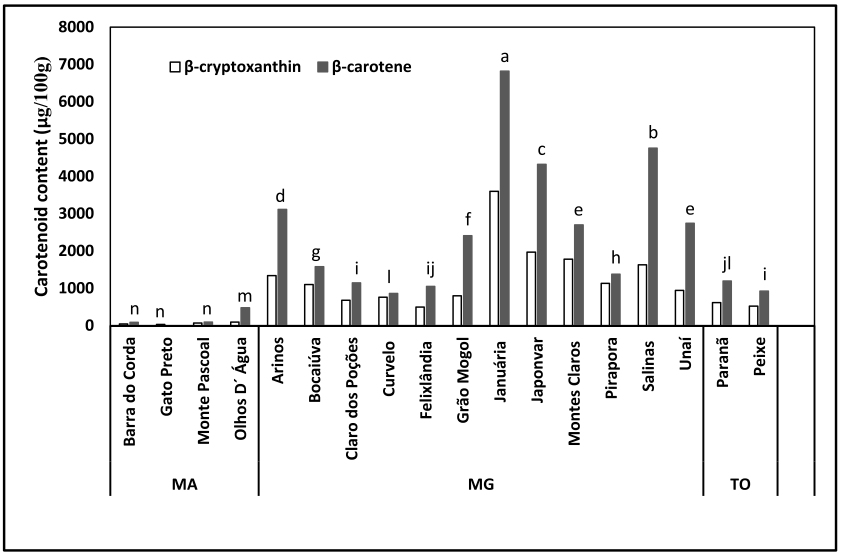

Fig. 3. Pro-vitamin A carotenoid content in fresh fruit pulp of *C. brasiliense* *Means followed by the same letter do not differ statistically from each other by the Duncan test at 5% significance.

Table 2
Percentage dietary contribution to requirements of vitamin A from fruit pulp of *C. brasiliense* from each municipality, for women and men.

State*	Municipalities	Code	µg RAE**	% Daily Contribution ***	
				Women	Men
MA	Barra do Corda	BAR	9n	1	1
	Grajaú I (Gato Preto)	GAT	1n	0	0
	Grajaú II (Olhos D'Água)	OLH	44m	6	5
MG	Monte Pascoal	MOP	11n	2	1
	Arinos	ARI	315d	45	35
	Bocaiúva	BOI	177h	25	20
	Claro dos Poções	CLA	124j	18	14
	Curvelo	CUR	103 L	15	11
	Felixlândia	FEX	108I	15	12
	Grão Mogol	GRA	234g	33	26
	Januária	JAN	718a	103	80
	Japonvar	JAP	442c	63	49
	Montes Claros	MOC	299e	43	33
	Pirapora	PIR	162i	23	18
	Salinas	SAL	464b	66	52
	Unaí	UNA	268f	38	30
TO	Paranã	PAR	125j	18	14
	Peixe	PEI	99 L	14	11

Means followed by the same letter do not differ statistically from each other by the Duncan test at 5% significance.
* State of MA – Maranhão state; MG – Minas Gerais state and TO – Tocantins.
** 1 µg Retinol Activity Equivalent (RAE) equals 1 µg of retinol; µg RAE values are for a 100 g portion of fresh fruit pulp of *C. brasiliense*.
*** Based on the IOM recommendations (2001), in which recommended dietary allowance is 900 µg RAE/day for men 14 years and older and 700 µg RAE/day for women 19 years and older, non-pregnant and non-lactating.

equatorial climate with high rainfall incidence.

Another possibility to explain such differences is that some selective preference for genotypes with higher potential for carotenoid production could be taking place under these stress conditions, resulting in genetic variability. A previous study showed genetic diversity indices among plants from the same municipality; however, greater similarities were observed between plants from nearby municipalities due to the greater possibility of gene flow (Melo-Júnior et al., 2004).

In this study, vitamin A content from the 18 *C. brasiliense* fruit samples are presented as µg RAE (retinol activity equivalent) per 100 g fresh weight, as shown in Table 2.

The contribution to the vitamin A supply from *C. brasiliense* fruits of these municipalities is presented in Table 2. The contribution is based on the IOM recommendations (2001), in which the recommended dietary allowance is 900 µg RAE/day for men 14 years and older, and 700 µg RAE/day for women 19 years and older, non-pregnant and non-lactating.

The vitamin A value found for fruits from Januária-MG was also higher in comparison with other typical Cerrado fruits such as cagaita (*Eugenia dysenterica*) and coquinho azedo (*Butia capitata*), which showed RAE values of 45 and 146 µg RAE/100 g fresh weight, respectively (Faria et al., 2008; Cardoso et al., 2011). Tropical fruits such as mango (*Mangifera indica* L.) similarly revealed 96−112 µg RAE/100 g fresh weight (Mercadant and Rodriguez-Amaya, 1998), guava (*Psidium guajava* L.) had 185 µg RAE/100 g fresh weight (Padula and Rodriguez-Amaya, 1986) and papaya (*Carica papaya* L.) presented 22.7–55.2 µg RAE/100 g fresh weight (Wall et al., 2010).

3.2. Multivariate analysis

The PCA interpreted the behavior of the carotenoid samples from *C. brasiliense* fruits from different municipalities, showing that 98.40 % of the explained variance for the behavior information related to the β-carotene content, β-cryptoxanthin content, as well as moisture content are explained by PC1 which accumulated 77.01 %, while PC2 accumulated 21.09 % of the information. Thus, it is possible to notice the separation of groups from the municipalities originating from the samples in the graph of the scores, Figs. 4 and 6, as well as the components responsible for the grouping, as shown in Fig. 5.

The samples with moisture content varied between 45.5 and 58.37 % are in group 1, showing lower β-cryptoxanthin content than the other samples ranging from 34 to 91 µg/100 g dry weight, and β-carotene ranging from not detected to 480 µg/100 g dry weight, arranged in PC2 positive and PC1 negative (Barra do Corda, Olhos D'Água, Monte Pascoal) and negative PC2 and negative PC1 (Gato Preto).

Only one sample (Claro das Porções) in group 2 has a moisture content below 50 % (with 45.62 %), while the other samples (Bocaiúva, Paranã, Peixe, Curvelo, Pirapora and Felixlândia) vary between 50.5 and 58.1 %, in which PC1 is negative and PC2 is positive and negative; only the Bocaiúva sample is located in PC1 and PC2 positive, which is mainly due to the 58.1 % moisture content.

In addition, the β-cryptoxanthin content in this group varied between 49.9 and 113 µg/100 g dry weight and β-carotene ranged from 86 to 138 µg/100 g dry weight. The samples in group 3 (which is found in PC1 positive) have high moisture and β-carotene content, ranging from 53.4 %–59.8 % and 2410–4760 µg/100 g dry weight respectively, whereas the β-cryptoxanthin content were between 800 and 1970 µg/100 g dry weight.

The samples with the highest moisture content (Montes Claros and Japonvá) are found in PC1 and PC2 positive. This group was distinguished from the others by the high moisture, β-cryptoxanthin and β-carotene contents. Only the Januária sample was not grouped, as it presented higher content of all components. The separation made between PC1 and PC2 of the samples shows that the municipalities with the highest moisture, β-cryptoxanthin and β-carotene contents are in the positive PC2 and the others are in the negative PC2, whereas the samples with the highest β-cryptoxanthin and β-carotene contents are arranged in the positive PC1.

It was observed that PC1 and PC2 components together accumulated 98.10 % of the total data variance. Furthermore, the results obtained in the score and dendrogram graphs (Fig. 6) revealed that fruit samples were grouped into three clusters according to the decreasing order of carotenoid content.

The fruits from Minas Gerais state showed the highest amounts of µg RAE/100 g fresh weight, as can be seen in Table 2, thus justifying the

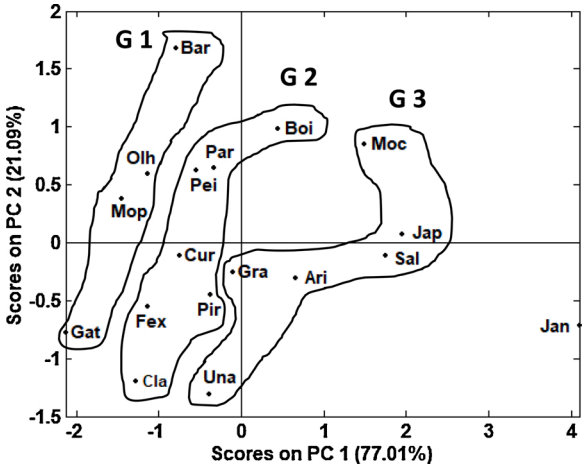

Fig. 4. PCA score plot for *C. brasiliense* fruit samples, where PC1 and PC2 explained 98.28 % of the total variance. Considering the concentrations of carotenoids with pro-vitamin A activity, vitamin A value and pulp moisture content. BAR - Barra do Corda, GAT - Grajaú I (Gato Preto), OLH - Grajaú II (Olhos d'Agua), MOP – Monte Pascoal, ARI - Arinos, BOI - Bocaiúva, CLA - Claro dos Porções, CUR - Curvelo, FEX - Felixlândia, GRA - Grão Mogol, JAN - Januária, JAP - Japonvar, MOC - Montes Claros, PIR - Pirapora, SAL - Salinas, UNA - Unaí, PAR - Paranã and PEI - Peixe.

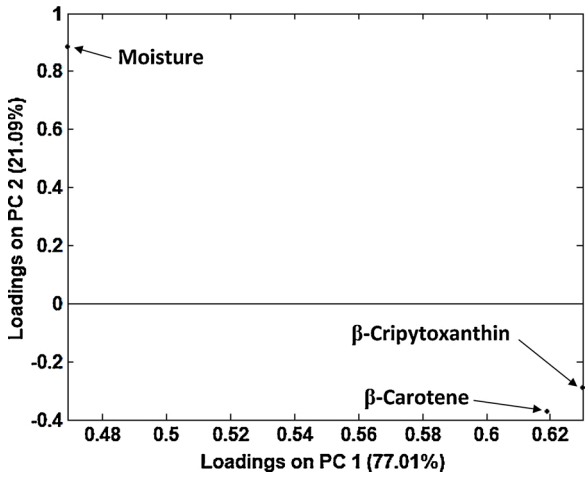

Fig. 5. PCA loading plot of the variables for the pulp carotenoids from 18 *C. brasiliense* fruit samples as moisture, β-cryptoxanthin and β-carotene content values.

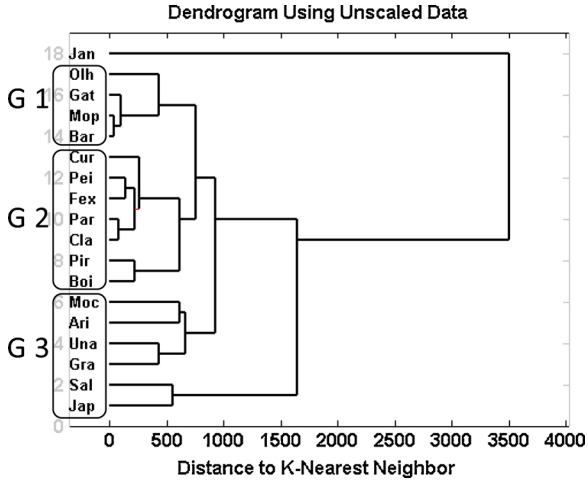

Fig. 6. Dendrograms for *C. brasiliense* fruit samples obtained by Multivariate Analysis, considering the concentrations of carotenoids with pro-vitamin A activity. BAR - Barra do Corda, GAT - Grajaú I (Gato Preto), OLH - Grajaú II (Olhos D'Água), MOP – Monte Pascoal, ARI - Arinos, BOI - Bocaiúva, CLA - Potion Clear, CUR - Curvelo, FEX - Felixlandia, GRA - Grão Mogol, JAN - Januária, JAP - Japonvar, MOC - Montes Claros, PIR - Pirapora, SAL - Salinas, UNA - Unaí, PAR - Paranã and PEI - Peixe.

formation of Group 1 consisting of the municipalities of Gato Preto, Barra do Corda, Olhos D'Água and Monte Pascoal. *C. brasiliense* fruits from Maranhão state showed the lowest carotenoid content, as can be seen in Fig. 3 and Table 2, justifying the formation of cluster 1.

The other fruits, including those from municipalities of Tocantins and Minas Gerais, presented intermediate β-cryptoxanthin and β-carotene contents, thus justifying the formation of cluster 2. The other fruits, including those from municipalities Minas Gerais, presented high moisture, β-cryptoxanthin and β-carotene contents, thereby justifying the formation of the cluster 3. Therefore, it was demonstrated that the multivariate analysis enabled classifying *C. brasiliense* fruits according to their decreasing concentrations of carotenoids with pro-vitamin A activity, and grouped the fruits according to their geographical location.

It is important to note while we attempted to maintain traditional harvesting parameters (i.e. collect fruit undamaged after it had fallen from the tree), this method may not have fully controlled for ripeness level. As specific parameters such as ^{o} Brix and other ripeness indicators were not collected it is possible that some variation could be due to variable level of ripeness. While our efforts represent the content of carotenoids and pro-vitamin A activity in a culturally appropriate way, future studies that assess content relative to fruit maturity are needed to clarify the potential impacts of the traditional harvest practice on nutritional quality.

4. Conclusions

The 18 *C. brasiliense* fruit samples showed significant differences regarding vitamin A content. However, these differences were smaller between samples which were geographically closer to each other. This is probably due to the edaphoclimatic similarities, as well as the possibility of greater gene flow among these populations.

The *C. brasiliense* fruits from Januária-MG showed the highest vitamin A value (μg RAE/100 g fresh fruit pulp) and had a high content of other carotenoids as neoxanthin, violaxanthin, lutein and zeaxanthin. Therefore, such specimens should be recommended as components of germplasm bank formation studies interested in selecting species with superior genotypes/chemotypes in terms of their nutrient content. The neoxanthin and β-carotene were the major carotenoids in all fruits studied.

Author statement

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Luiz C. A. Barbosa: Resources; Funding acquisition; Supervision; Project administration; Supervision; Roles/Writing - original draft; Writing - review & editing.

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Cláudio F. Lima: Supervision; Writing - review & editing.

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Declaration of Competing Interest

All the authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jfca.2021.103943.

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