# FRUITS OF *Butia capitata* (Mart.) Becc AS GOOD SOURCES OF β -CAROTENE AND PROVITAMINA[1]

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**ABSTRACT -** *Butia capitata* is a palm tree, widely found in the Brazilian savanna. Their fruits are largely used by local communities to prepare juices, jellies and ice-creams. The main objective of this work was to determine the carotenoids profile of *Butia capitata* fruits and their provitamin A values. Total carotenoids content ranged from 11.1 to 43.9 μg.g$^{-1}$; β-carotene was the predominant carotenoid (5.2-22.8 μg.g$^{-1}$), followed by γ-carotene, phytoene, phytofluene, ζ-carotene, α-cryptoxanthin (or zeinoxanthin) and α-carotene. Provitamin A values varied from 50 to 200 RAE.100g$^{-1}$. This result suggests that *B. capitata* pulp may be a good source of β-carotene and provitamin A.
**Index terms**: Brazilian savanna, palm tree, carotenoid.

## *Butia Capitata* (Mart.) Becc COMO FONTE DE β – CAROTENO E PROVITAMINA

**RESUMO -** *Butia capitata* é uma palmeira largamente distribuída no cerrado brasileiro. Seus frutos são utilizados pelas comunidades locais para preparar sucos, geleias e sorvetes. O objetivo foi determinar o perfil de carotenoides e o valor pró-vitamina A dos frutos de *Butia capitata*. Os teores de carotenoides totais variaram entre 11,1-43,9 μg.g$^{-1}$; o β-caroteno foi o carotenoide predominante (5,2-22,8 μg.g$^{-1}$), seguido pelo γ-caroteno, fitoeno, fitoflueno ζ-caroteno, α-criptoxantina (ou zeinoxantina) e α-caroteno. Os valores de pró-vitamina A variaram entre 50-200 RAE.100g$^{-1}$, sugerindo que a polpa de *B. capitata* pode ser uma boa fonte de pró-vitamina A.
**Termos para indexação**: Cerrado, palmeira, carotenoides.

## INTRODUCTION

*Butia capitata* (Mart.) Becc. is a nearly 2.6 m palm tree, also known as coquinho-azedo or butiá, native of the Brazilian savanna biome, growing under sandy soils such as dunes and restingas (MARCATO; PIRANI, 2006). Fruits of *B. capitata* are harvested from the wild between november and february. However, frozen pulp can be storage for commercialization, promoting income to the local farms during all year.

*Butia capitata* seeds present a rich oil nut, with a similar composition to coconut oil (*Cocos nucifera*) (FARIA et al., 2008a). The fruit presents an orange, strongly aromatic pulp, which is widely appreciated, especially for preparing juices, jams and liquor. Previous studies of the pulp indicate a high content of lipids (2.5 %), phenolic compounds (163-259 mg.100g$^{-1}$ of pulp), potassium (462 mg.100g$^{-1}$), neutral detergent fiber (6.2 %) and vitamin C (53 mg.100g$^{-1}$ of pulp) (FARIA et al., 2008b).

Carotenoids are substances with special properties that no other groups of substances possess and that form the basis of their many varied functions and actions in all kinds of living organisms (BRITTON, 1995). Many flowers and fruit present yellow, orange and red colors due carotenoid pigments. Other less obvious roles make carotenoids essential components in oxygenic photosynthetic organisms. Other carotenoids show an antioxidant activity, which may prevent diseases associated with oxidative damages such as aging, cancer, liver alcoholic damages, heart and neurodegenerative diseases. Some carotenoids, such as beta-carotene, are provitamin A, developing several vitamin A role in the body. Carotenoids are abundant in fruits and dark green leafages (BRITTON, 1995; OLSON, 1999; OLIVER; PALOU, 2000; TAPIERO et al., 2004).

The purpose of this study was to valuate by the first time the composition of the carotenoids and the provitamin A value in the pulp of *Butia capitata.*

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## MATERIAL AND METHODS

Samples of fresh mature fruits of *B. capitata* were randomly collected from eleven different places at North of Minas Gerais state, Brazil. The fruits were transported under refrigeration, in styrofoam boxes and kept frozen (-20 ºC) until analysis. The ripe stage of the fruits was determined by the fruit firmness and surface color orange (some fruit samples were red due anthocyanin pigments present in some fruit samples but not in others; anthocyanin was detected by color change in acidic and basic pH). Fruits free of defects were selected and the pulp, along with a thin peel, was manually separated from the seeds, using a stainless knife and then homogenized in a multiprocessor.

Butylated hydroxytoluene (BHT) was purchased from Sigma, petroleum ether (40-60 ºC) was from Riedel and *n*-hexane was from Merck. Other analytical grade chemicals were obtained from local marked (Vetec).

Carotenoids were analyzed according to Rodriguez-Amaya (1999). **The pigments from 20 g of** *B. capitata* pulp were extracted in a mortar, using 1 g of hyflosupercel and 4 to 5 share of 50-70 mL of cold acetone (4 ºC), until complete residue lack of color. The extract was vacuum filtered and the pigments were transferred from the acetone to the petroleum ether; acetone was washed with distillated water. Butylated hydroxytoluene (0.1 %) was added to minimize the oxidation of the carotenoids and water residue was eliminated with anhydrous sodium sulfate**.** The extract was saponified overnight using equal volume of 10 % potassium hydroxide in methanol. Saponified extract was washed with distillated water, until a pH near to neutrality.

The petroleum ether extract was vacuum concentrated until 10-20 ml and applied into a magnesium oxide - hyflosupercel (1:2) (w/w) open-column (20 x 120 mm). The carotenoids were separated using petroleum ether or hexane, containing increasing amounts of acetone (4-100 %). Acetone was removed from each fraction through washing with water and the spectrum was recorded using a Hitachi U-2000 recording spectrophotometer (Tokyo).

The carotenoids eluted from open-column chromatography (OCC) were identified according to: a) its column elution order, using the profile of carotenoids standards as the references, obtained in the same chromatographic conditions; b) its visible and ultraviolet absorption spectra: wavelength maximum absorbed ($\lambda_{max}$) and its fine structure (% III/II, which is expressed by the ratio between the peak height of the longest-wavelength absorption band (III) and the middle absorption peak, generally $\lambda_{max}$ (II), making up the minimum between the two peaks as the baseline, multiplied by 100); c) its chromatographic properties ($R_F$) on silica thin layer and open-column; and d) by specific chemical reactions (DAVIES, 1976; BRITTON et al., 1995; RODRIGUEZ-AMAYA, 1999).

Calculation of the concentration of each identified carotenoid was made according RODRIGUEZ-AMAYA (1999), using the respective absorbance at a specified wavelength ($\lambda_{max}$**)** obtained on UV-visible recording spectrophotometer and the absorption coefficients $A^{1\%}_{1cm}$ for each carotenoid (DAVIES, 1976; BRITTON et al., 1995). The provitamin A was calculated according to the new conversion factor (IOM, 2001), in wich 12 µg of β-carotene and 24 µg of γ-carotene correspond to 1 RAE (retinol activity equivalent). The average computer calculated (Excel) were determined for carotenoid concentration and provitamin A value.

## RESULTS AND DISCUSSION

Table 1 shows identifying parameters for the carotenoids from *B. capitata* pulp. It was observed that open-column of Vetec magnesium oxide with high sulfur content developed with acetone petroleum ether (or hexane) gradient show the best resolution for *B. capitata* carotenoids, without reduction of carotene content.

Using thin-layer chromatography (TLC), all carotenoid fractions were developed with the solvent front and showed 0.99 $R_F$, meaning that all the carotenoids found in the *B. capitata* pulp were carotenes (the absence of functional group epoxide or hydroxyl is shown by their behavior on TLC), except for the last fraction ($R_F$=0.5), which was a monohydroxilated carotenoid.

The first colorless fraction (phytoene and phytofluene) were identified as by the elution order in the open-column and by the typical fine structure of these carotenoids in a minor wavelength band ($\lambda_{max}$ 285 for phytoene and $\lambda_{max}$ 347 for phytofluene), consistent with acyclic carotenoids with three and five conjugated double bonds, respectively.

Fraction two (α-carotene) has been detected only in three samples of *B. capitata* and showed a typical pattern of elution very close to β-carotene. It differs from that one by the elution order and the clearer yellow color in the open-column. Also, they differ based on the maximum wavelength band at 445 nm, associated to the *trans*-configuration (lower than the *trans*-β-carotene - 449 nm), and the fine structure

more defined in the same solvent.

Fraction three (β-carotene) was predominant in all samples of *B. capitata*. It presented an orange color band eluted after α-carotene by open-column. Fraction profile of fine structure and maximum wavelength absorption band (449 nm) is in agreement with literature data (BRITTON et al., 1995; RODRIGUEZ-AMAYA, 1999) and with the *trans*-β-carotene standard extracted of leafy vegetable.

Fraction four (ζ-carotene) showed a very clear yellow band by open-column and elution profile by TLC consistent with non-hydroxilated carotenoid. The spectrum presented well defined peaks (fine structure) at 399 and 242 nm, typical of ζ-carotene when compared with literature data (DAVIES, 1976; BRITTON et al., 1995; RODRIGUEZ-AMAYA, 1999) and with ζ-carotene standard extracted of passion fruit.

Fraction five (poly-*cis*-γ-carotene) was negative for reduction of keto- and apocarotenoids. It presented a maximum absorption band at 433 nm and 456 nm before and after iodine isomerization, respectively, with batachromic shift of 24 nm and with a fine structure profile similar to γ-carotene after iodine isomerization, consistent with poly-*cis*-γ-carotene, according to Davies (1976).

Fraction six (γ-carotene) presented a pink band by open-column and an elution profile consistent with non-hydroxilated carotenoid by TLC, being found in *trans*- and *cis*-configuration. Its maximum absorption band, spectral fine structure and elution pattern, matched with data described for the γ-carotene (CAVALCANTE, 1991).

Fraction seven (not identified carotene) was negative for reduction for keto- or apo-carotenoids. It showed an elution pattern in the thin-layer (TLC) similar to a non-hydroxilated carotenoid and a profile of spectral fine structure low defined.

Fraction eight presented an elution pattern in the open-column and in TLC consistent to a monohydroxy carotenoid with same chromophore of α-criptoxanthin and the zeinoxanthin (III/II = 60) (RODRIGUEZ-AMAYA, 1999). Due to its reduced amount, the methylation test to confirm the hydroxyl position was not performed.

The average concentration of total carotenoids in the *B. capitata* pulp was 36.1 $\mu g.g^{-1}$. The β-carotene was the predominant carotenoid (16.1 $\mu g.g^{-1}$), followed by phytoene, poly-*cis*-γ-carotene, phytofluene, γ-carotene and small levels of not identified carotene, α-cryptoxanthin (or zeinoxanthin) and ζ-carotene (Table 2). Some of these carotenes were not found in all samples, which is usual, as long as the analyzed samples proceeded from different places. The average values were calculated with the respective number of samples containing carotenoids (Table 2). Considering that *B. capitata* is wildly distributed in the savanna biome, it is expected a large range of fruit shapes and carotenoids content. Variation in the carotene composition of fruits and vegetables has been widely reported in the literature, as a result of the effects of several factors such as the genetic, soil type, climatic conditions and the exposure to sun light (RODRIGUEZ-AMAYA, 1999).

If compared with other fruits usually consumed in Brazil (RODRIGUEZ-AMAYA et al., 2008a) and in the USA (HOLDEN et al., 1999), the *B. capitata* fruits can be considered a very good source of β-carotene and total carotenoids. It also showed to be richer in β-carotene then the available fruit information from Austrian (MURKOVIC et al., 2000) and Indonesian (SETIAWAN et al., 2001) carotenoids database.

The β-carotene contributed with 92 % of the provitamin A value of the *B. capitata* pulp (50-200 RAE.$100g^{-1}$). This value is quite similar to the ones found in conventionally consumed fruits rich in provitamin A carotene, such as mango (35-215 RAE.$100g^{-1}$) and acerola (35-325 RAE.$100g^{-1}$) (RODRIGUEZ-AMAYA et al., 2008b). The occurrence of high content of provitamin A carotene and lipids (2.6 %) (FARIA et al., 2008b) in the *B. capitata* pulp suggests that this fruit may represent a source of a bioavailable provitamin A for the local people from the Brazilian savannas. As verified in fruits of *Acrocomia aculeate* (RAMOS et al., 2007), a native palm tree of Brazilian savanna, the high lipid content of palm fruits seems to facilitate the solubility of carotenoids in the intestinal lumen improving the absorption of the carotenoids presented in the diet.

This is the first report on *B. capitata* carotenoids. Considering the social, economic and cultural importance of the *B. capitata* to the Brazilian savanna biome, this palm tree can became a potential product to the economy of rural populations. According to the daily needs of vitamins recommended by The American Medicine Institute, a glass of juice containing 100 g of *B. capitata* pulp can provide 40 % of the daily needs of vitamin A for children under eight years-old. In the North of Minas Gerais State, the *B. capitata* juice has been added to the public school lunch, and can became an important food supply to dietary complement.

**TABLE 1** – Identifying parameters for the carotenoids from *B. capitata* pulp.

| Carotenoid[a] | Elution solvent | $\lambda_{max}$ (nm)[b] | | | Rf[c] | Isomers[d] (nm) | % III/II[e] |
|---|---|---|---|---|---|---|---|
| Phytoene | Petroleum ether | 274 | 285 | 297 | nd | nd | 6 |
| Phytofluene | petroleum ether | 331 | 347 | 367 | nd | nd | 89 |
| Trans-α-carotene | petroleum ether (4 % acetone) | | 445 | 474 | 0.99 | -2 | 38 |
| Trans-β-carotene | petroleum ether (4 % acetone ) | (426) | 449 | 477 | 0.99 | -3 | 25 |
| Trans-ζ-carotene | petroleum ether (4-6 % acetone) | 378 | 399 | 424 | 0.99 | -3 | 113 |
| Poly-*cis* γ-carotene | petroleum ether (10 % acetone) | (407) | 433 | (457) | 0.99 | +24 | 6 |
| γ-carotene | petroleum ether (15 % acetone) | 433 | 457 | 483 | 0.99 | 0 | 31 |
| Not identified *trans*-carotene | petroleum ether (20 % acetone) | 415 | 438 | 464 | 0.99 | -3 | 57 |
| *Trans*-α-criptoxanthin or zeinoxanthin | 30 % acetone | 420 | 442 | 471 | 0.5 | -2 | 64 |

[a]Carotenoids developed on magnesium oxide / hyphlosupercel open-column, listed in order of their elution; [b]maximum wavelength on petroleum ether; [c]the thin layer values ($R_F$); [d]shift of $\lambda_{max}$ (nm) after iodine reaction; [e]ratio between the absorption height peaks of the upper wavelength (III) and the middle wavelength (II), taking as baseline the minimum among both, multiplied by 100; nd: not determined; parenteses indicate a sholder.

**TABLE 2 -** Diversity of carotenoid from *B. capitata* pulp.

| Carotenoid | Average (variation) (μg.g$^{-1}$)[a] | Composition (% of total carotenoid) |
|---|---|---|
| Phytoene | 5.7 (1.8-8.6) | 16.3 |
| Phytofluene | 4.4 (1.5-7.4) | 11.5 |
| α-carotene | 0.1 (0-0.1) | - |
| β-carotene | 16.1 (5.2-22.8) | 45.8 |
| ζ-carotene | 0.8 (0.2-1.3) | 2.4 |
| Poly-*cis*-γ-carotene | 4.7 (1.8-10.1) | 13.5 |
| γ-carotene | 2.9 (1.3-4.7) | 8.3 |
| Not identified-*trans*- carotene | 0.7 (0.3-1.1) | 2.2 |
| α-criptoxanthin or zeinoxanthin | 0.8 (0.2-1.4) | - |
| Total carotenoids (μg.g$^{-1}$) | 36.1 (11.1- 43.9) | 100 |
| Provitamin A (RAE.100g$^{-1}$) [b] | 146.2 (50- 200.0) | |

[A](N=11, B-carotene; N=9, Γ-carotene, poli-cis-γ-carotene and α-cryptoxantin; n=8, γ-γ-carotene; n=7, ζ-carotene; n=6, phytoene and phytofluene; n=3, α-carotene); [b]conversion factor [12 μg of β-carotene e 24 μg de γ-carotene = 1 RAE].

## CONCLUSION

This result suggests that *B. capitata* pulp may be a good source of β-carotene and provitamin A and also reinforce the cultural and nutritional relevance of the *B. capitata* species and the importance of its conservation in the Brazilian savanna.

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