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Carotenoids and ascorbic acid from cashew apple (*Anacardium occidentale* L.): variety and geographic effects

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Abstract

Cashew apple is the pseudofruit of the cashew tree, native to Brazil. Different varieties of cashew apple were collected in Brazil, three being from Piauí State (Northeast) and two from São Paulo State (Southeast). In all the fruits, β-carotene (16.6–67.9 μg/100 g), β-cryptoxanthin (7.7–64.4 μg/100 g), α-carotene (5.9–51.9 μg/100 g) and 9-*cis*- + 13-*cis*-β-carotene (3.3–15.6 μg/100 g) were found. In general, the levels of carotenoids were higher in the red than in the yellow cashew apples, from both regions; for example, the levels of α- and β-carotene were about 1.8 and 1.3 times higher in the red than in the yellow fruits from the Southeast and Northeast, respectively. In contrast, ascorbic acid (AA) values were slightly higher in the yellow variety. Elongated red and yellow fruits also presented slightly higher AA contents than the rounded red ones. The total carotenoid levels of the rounded red fruits were 1.5 and 1.7 times lower than those found in the yellow and red varieties, respectively, all being from the Northeast region. Yellow fruits from the Northeast presented 1.7 times higher provitamin A levels than those from the Southeast whereas, for the red variety, the values were similar. The yellow and red varieties from the Northeast showed non statistically higher AA levels than those from the Southeast.

Keywords: Cashew apple; Carotenoids; Ascorbic acid; Varieties; Geographic locations; *Anacardium occidentale*

1. Introduction

One of the tropical fruits most highly consumed in the Northeast of Brazil is the cashew apple. The pseudofruit, known as cashew apple, is the part of the tree that connects it to the cashew nut, the real fruit, and a well-known product around the world. The cashew apple is a hard, pear-shaped, small and non climacteric fruit, and is found in three colours: yellow, orange and red. The most commonly commercialized ones are the yellow and red fruits.

The origins of the cashew apple are the North and Northeast regions of Brazil. The name comes from the Tupi indians, and means the nut that produces itself. Today, it is also cultivated in India and Africa because of the colonization process. The tropical climate and dry soil are ideal for its cultivation. The ripening process takes place from September to January, and a 4-year-old tree can produce from 100 to 150 kg of cashew apple per year (Tassara & Silva, 2002). Although Brazil produced 1,640,156 tons of cashew apple in 1996, the pseudofruit was only exploited as fresh fruit or as industrial products such as juice, pulp, jam, alcoholic beverages, sweets and honey, in about 10% of the total amount produced (EMBRAPA, 1999).

Studies of nutrient contents of different cultivars/varieties have been conducted on various fruits and vegetables because important nutrients have been found in significantly different amounts. In Brazil, studies with mango (Godoy & Rodriguez-Amaya, 1989; Mercadante & Rodriguez-Amaya, 1998) and papaya (Kimura, Rodriguez-Amaya, & Yokoyama, 1991) showed quantitative and qualitative differences in the carotenoid contents which account for different vitamin A values. Vitamin C levels were also verified in other studies, showing differences between cultivars, as for example, in kiwi fruit (Selman, 1983), melon (Wills, 1987) and guava (Padula & Rodriguez-Amaya, 1986).

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Environmental differences, such as temperature, soil and solar intensity are not always mentioned in the studies, but they can affect the total nutrient values. In Brazil, studies usually compare fruits from the Northeast (hot climate) and the Southeast (moderate climate) regions. Papaya (Kimura et al., 1991), guava (Padula & Rodriguez-Amaya, 1986) and mango (Mercadante & Rodriguez-Amaya, 1998), from the Northeast region, were investigated and presented, respectively 4.4, 1.8–3.5 and 2.3 times higher β-carotene contents than those from the Southeast region. The higher temperature of the Northeast Brazilian region should be the major reason for the significantly higher content of β-carotene in fruits from this region because carotenoid biosynthesis is slow at low temperatures (Britton, 1998). Studies have also compared seasons, such as summer and winter. Tomatoes cultivated in the summer presented almost twice the ascorbic acid content as those cultivated during the winter (Marchesini & Brugnatelli, 1992), when the solar incidence is lower than during the summer season.

With respect to the carotenoid composition and ascorbic acid (AA) content, the present study had two objectives: (a) to verify the compositional differences amongst the three varieties of cashew apple commercialized in Brazil, and (b) to investigate environmental effects on these fruits. This seems to be the first planned study of the changes of both carotenoids and AA in a non-climacteric tropical fruit.

2. Materials and methods

2.1. Cashew apple characterization

Three different varieties of ripe cashew apple (elongated yellow, elongated red and rounded red) were analysed. Botanically, they are not considered established cultivars (Silva, 1998), but cashew apple can be classified according to form, rounded or elongated, and colour, red or yellow.

For all varieties of cashew apple, the samples were weighed and measured, after removing the cashew nuts. For all varieties of cashew apple, the samples were weighed and measured, after removing the cashew nuts. Both varieties from the Southeast region were smaller (5.6–6.1 cm) and lighter (59.0±21.8 g for red to 63.7±26.3 g for yellow) than the same varieties from the Northeast region (6.8–7.2 cm; 74.3±4.9 g for red to 96.9±7.8 g for yellow). Rounded red cashew apples from the Northeast were the heaviest fruits (102.6±24.1 g). At the same time, pH and Brix were verified. The contents of soluble solids (°Brix) ranged from 10.2 to 12.6, and pH from 3.8 to 4.5, for all varieties of cashew apple. According to Silva (1998), ripe cashew apple should present a pH range from 3.5 to 4.6, and Brix values from 9.8 to 14.0. All the fruits studied were in agreement with these values.

2.2. Samples

For each variety, five lots, consisting of 5–8 fruits each, were collected in the harvesting season during five consecutive weeks in the year 2000, this being March for those from the Southeast and September for those coming from the Northeast. The lots of elongated red and yellow fruits from Valinhos, São Paulo State (Southeast region) came from the same farm, as also did the lots of elongated red and yellow cashew apples from Piauí State (Northeast region), both States located in Brazil. At the same time, rounded red cashew apple came from a different farm, located in the same area of Piauí.

The fruits (pulp and peel) were homogenized in a blender. Samples, from 30 to 75 g, were taken for carotenoid analysis and samples of 10–20 g for ascorbic acid, both in duplicate.

2.3. Standards

Standards of lutein, β-cryptoxanthin, 9-*cis*-, 13-*cis*- and 15-*cis*-β-carotene were provided by Hoffmann-La Roche (Basel, Switzerland), showing purity of 98%, 98%, 96%, 95% and 92%, respectively, by high-performance liquid chromatography (HPLC) analysis.

α-Carotene and β-carotene were extracted from carrot according to the extraction procedure described below, followed by thin-layer chromatography (TLC) separation on MgO (Mallinckrodt)/kieselguhr (Merck) (1:1) with 3% acetone in petroleum ether as mobile phase. Two major bands were separated. The yellow one ($R_f = 0.5$), corresponding to α-carotene, and the orange one ($R_f = 0.3$), corresponding to β-carotene, were scraped off and eluted with diethyl ether. The purity was 97% for α-carotene and 95% for β-carotene, demonstrated by HPLC.

For the vitamin C analysis, an L-ascorbic acid standard (Merck) was used.

2.4. Carotenoid determination

The carotenoids were exhaustively extracted with acetone and saponified with 10% methanolic KOH for 12 h at room temperature, according to the method of Mercadante, Rodriguez-Amaya, and Britton (1997). All the extractions were conducted in duplicate and each duplicate was injected twice into the HPLC. Separation was achieved using a Waters HPLC equipped with a photodiode array detector (Waters, model 996). The equipment also included an on line degasser, a Rheodyne injection valve with a 20 μl loop and an external oven. The data acquisition and processing were performed by the Millenium Waters software. For all the samples, carotenoid separation was carried out on a C_{18} Vydac 218TP54 column, 250×4.6 mm i.d. (5 μm particle

size), with 100% MeOH as mobile phase at a flow rate of 1 ml/min and column temperature set at 29 °C. The chromatograms were processed at the maximum absorption wavelengths (λ_{max}). The spectra were obtained between 250 and 600 nm.

The carotenoids were identified according to the following parameters: chromatographic behaviour on the C_{18} HPLC column and TLC on silica (Mercadante & Rodriguez-Amaya, 1991), UV–visible spectrum (λ_{max} and shape) compared with data available in the literature (Britton, 1995; Davies, 1976) and co-chromatography with authentic standards. The methylation test with acidified methanol (Davies, 1976; Eugster, 1995), monitored by HPLC and by TLC on silica with petroleum ether/diethyl ether (1:1) as mobile phase, was performed for zeinoxanthin. The epoxide–furanoxide rearrangement was monitored spectrophotometrically (Davies, 1976).

For the external standardization, calibration curves were constructed with a minimum of five concentration levels, each one in triplicate, and the concentration levels were chosen to include those of the samples. Carotenoid quantification was performed by comparison of the peak area of the sample with that of the standard, injected daily. Since the following carotenoids were present at low concentrations in the samples and standards were not available, violaxanthin was quantified using the lutein area, zeinoxanthin and *cis*-β-cryptoxanthin using the β-cryptoxanthin area and *cis* isomers of β-carotene by the all-*trans*-β-carotene area.

The NAS-NRC (1989) conversion factor was used to calculate vitamin A value. Since in the present work, 9-*cis* and 13-*cis*-β-carotene eluted together, they were both quantified as 50% of β-carotene activity.

2.5. Ascorbic acid determination

The method used for the AA determination was the official method of the AOAC (1984, 1995), as modified by Benassi and Antunes (1988), where the extractor solvent, metaphosphoric acid, was replaced by 1% oxalic acid, since the soft yellow colour of the products did not interfere in the colour change turning point.

To the samples, 50 ml of oxalic acid (Merck) were added, keeping them in a dark room for 15 min. Aliquots of 5–10 ml were diluted in 50 ml of oxalic acid for titration with 0.2% dichlorophenol–indophenol (Merck). The AA concentration was calculated by comparison with that known L-ascorbic acid standard, prepared and titrated daily.

2.6. Statistical analysis

To evaluate the differences amongst the varieties and locations of the cashew apples, analyses of variance were conducted using the "General Linear Models" (SAS/STAT, 1987). Mean comparisons were performed through contrasts. Contrasts were carried out for varieties: elongated yellow and red from Piauí (columns 1 and 2, Table 2), the same varieties from São Paulo (columns 4 and 5, Table 2), elongated and rounded red from Piauí (columns 2 and 3, Table 2) and elongated yellow and rounded red from Piauí (columns 1 and 3, Table 2).

For location, the contrasts were applied to the yellow variety from São Paulo and Piauí (columns 1 and 4, Table 2) and to the elongated red fruits from both regions (columns 2 and 5, Table 2).

The same analyses were conducted considering the variability within the lots of each fruit variety.

3. Results and discussion

3.1. General

All the varieties presented the same major carotenoids: lutein, zeinoxanthin, *cis*- and *trans*-β-cryptoxanthin, α-carotene and β-carotene (*cis* and *trans*), whose structures are shown in Fig. 1, in different concentrations, according to their variety and geographic

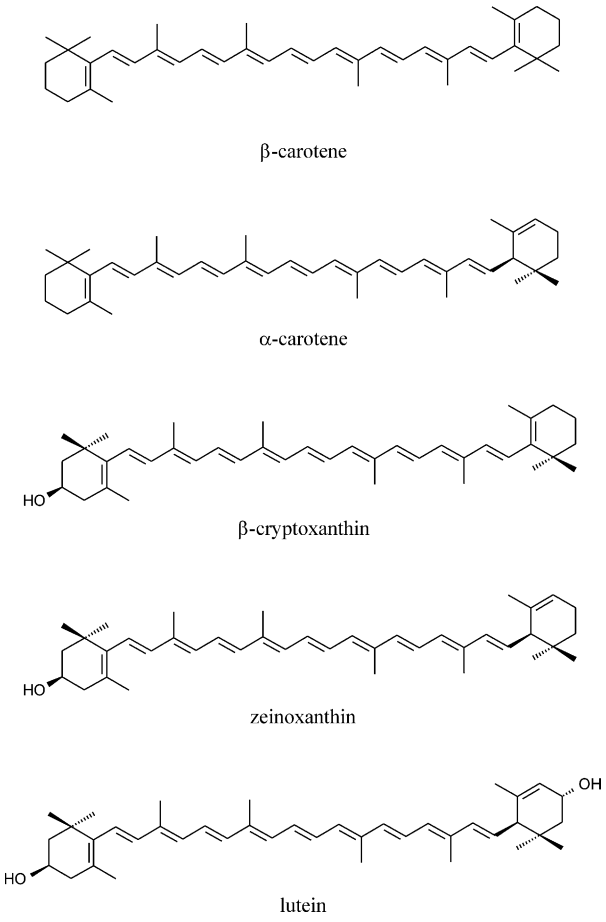

Fig. 1. Main carotenoids found in the cashew apple samples.

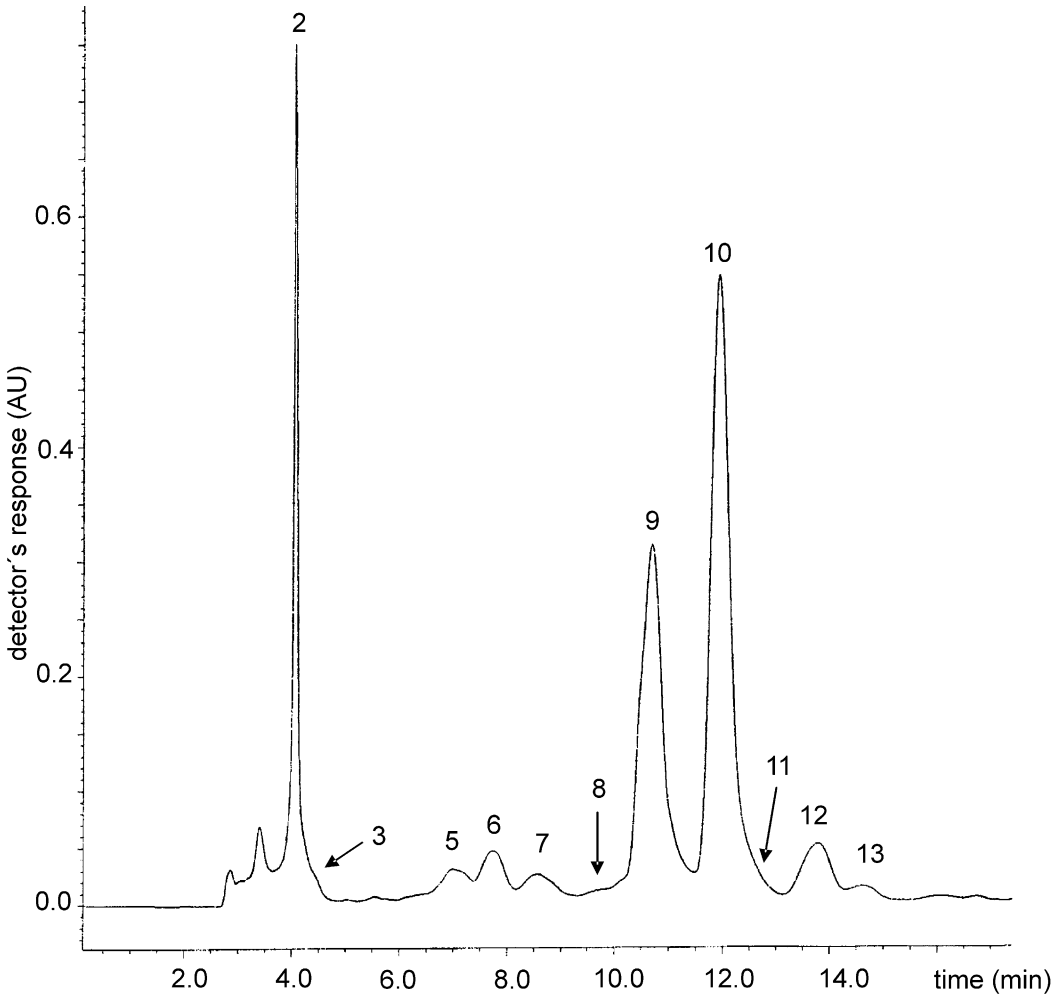

Fig. 2. Chromatogram, obtained by HPLC, of carotenoids from red Southeast cashew apple. Chromatographic conditions: C_{18} Vydac column, T of 29 °C, mobile phase: 100% MeOH at 1 ml/min. Detection at λ_{max}. Peak identification is given in Table 1.

Table 1
Main properties, obtained by HPLC, of carotenoids of all cashew apple samples

Peak[a]	Carotenoid	t_R (min)[b]	λ_{max}[c] (nm)	%III/II
1	Violaxanthin	2.8–3.5	416, 437, 466	98
2	Lutein	3.9–4.5	420, 443, 471	53
3	Not identified	4.4–4.5	420	0
4	Zeaxanthin	4.5–5.3	422, 448, 475	25
5	Zeinoxanthin	5.8–6.1	423, 443, 471	50
6	β-Cryptoxanthin	6.5–7.0	(420), 449, 476	29
7	*cis*-β-Cryptoxanthin	7.3–7.6	335, (420), 445, 470	22
8	ζ-Carotene	8.2–9.0	380, 401, 425	112
9	α-Carotene	10.2–11.0	423, 443, 472	49
10	β-Carotene	11.3–12.0	(425), 450, 477	23
11	Phytofluene	11.9–12.6	330, 347, 365	96
12	9-*cis*-β-Carotene	13.0–14.0	335, (422), 446, 471	12 13
12	13-*cis*-β-carotene	13.0–14.0	338, (425), 443, 468	13
13	Not identified	13.5–15.2	380, 400, 424	134

[a] Numbered according to the chromatograms shown in Figs. 1 and 2.
[b] Range from 100 runs.

location. Fig. 2 shows the most common carotenoid pattern in red cashew apple from the Southeast region and Fig. 3, the most common one in yellow cashew apple from the Northeast. Peak identification and characterization are presented in Table 1.

As expected for reversed-phase columns, polar carotenoids with two hydroxy groups, such as violaxanthin and lutein, eluted before the monohydroxy carotenoids (zeinoxanthin and β-cryptoxanthin). The carotenes, ζ-carotene, α-carotene, β-carotene and *cis*-β-carotene, were the last to elute under these conditions. Separation of 9-*cis* and 13-*cis*-β-carotene was not achieved, but they were identified separately through co-elution with standards. Lutein, β-cryptoxanthin, α-carotene and β-carotene co-eluted with their specific standards, showing similar UV–visible spectra characteristics to those presented by Britton (1995) and Davies (1976).

The identity of zeinoxanthin was confirmed by the negative response to methylation after 3 and 6 h of reaction, observed by no change in the R_f and t_R in the TLC and HPLC systems, respectively. This identification is important because α-cryptoxanthin and zeinoxanthin have identical UV–visible spectra and similar chromatographic behaviour. Furthermore, α-cryptoxanthin presents provitamin A activity, whereas zeinoxanthin does not.

Table 2 shows the carotenoid composition of all the cashew apples analyzed. β-Carotene and β-cryptoxanthin were the major carotenoids in all the varieties of cashew apple from the Northeast region, whereas in all fruits from the Southeast, β-carotene and α-carotene were the major carotenoids.

Violaxanthin, lutein, zeinoxanthin and *cis*-β-cryptoxanthin were found for the first time in fresh cashew apple. Cecchi and Rodriguez-Amaya (1981) identified α-carotene, β-carotene, ζ-carotene, *cis*-β-carotene, cryptoxanthin and auroxanthin in red and yellow cashew apples from two regions in Brazil.

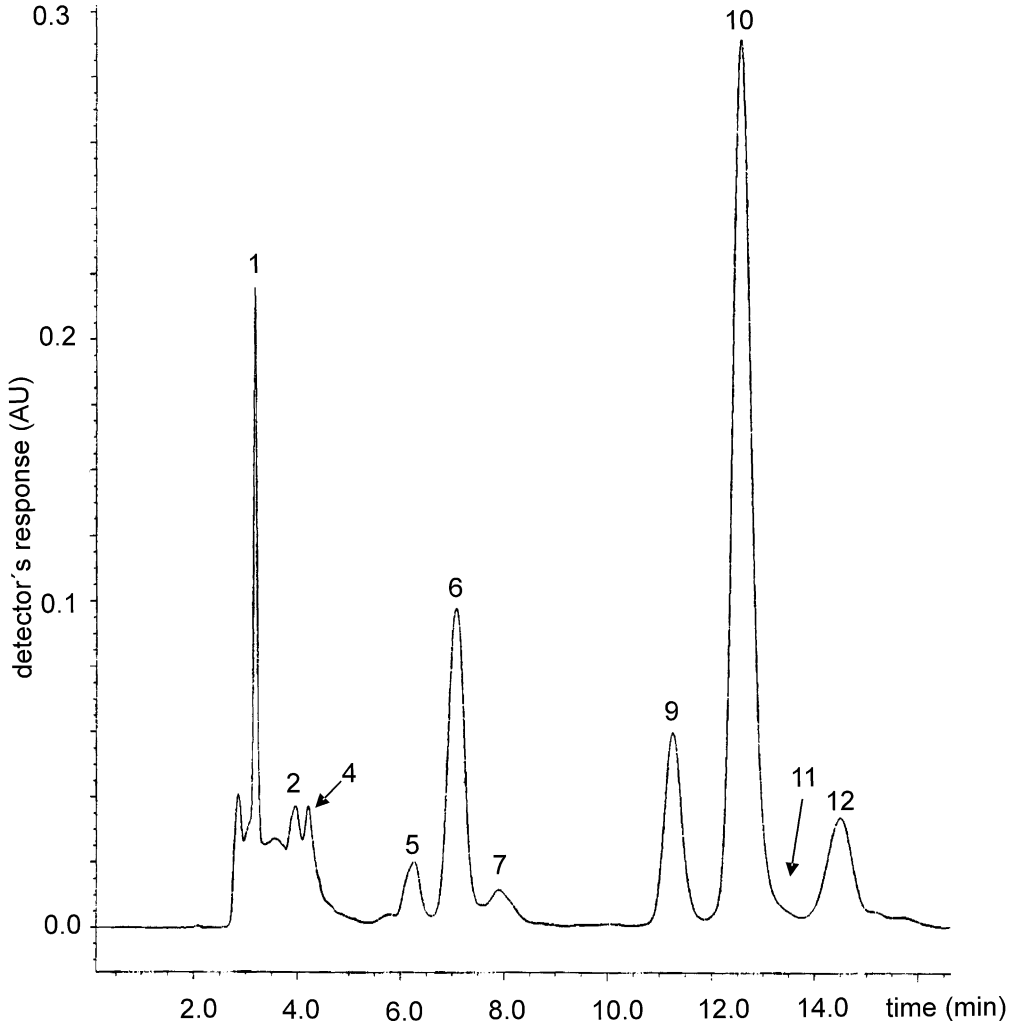

Fig. 3. Chromatogram, obtained by HPLC, of carotenoids from yellow Northeast cashew apple. Chromatographic conditions: C_{18} Vydac column, *T* of 29 °C, mobile phase: 100% MeOH at 1 ml/min. Detection at λ_{max}. Peak identification is given in Table 1.

Table 2
Carotenoid composition (μg/100 g), vitamin A value (RE/100 g) and AA content (mg/100 g) of cashew apple varieties

Carotenoids	Piauí State			São Paulo State	
	Yellow	Red	Red rounded	Yellow	Red
Violaxanthin	8.2±3.5	6.9±0.7	9.6±2.5	ND	ND
Lutein	4.6±1.2	4.6±1.5	4.7±1.3	12.9±3.8	14.2±1.6
Zeaxanthin	1.3±1.3	ND	2.8±1.5	ND	ND
Zeinoxanthin	9.1±0.7	9.8±2.0	4.8±1.0	7.4±2.7	5.1±0.0
β-Cryptoxanthin	53.0±11.6	59.6±26.7	64.4±12.9	9.7±1.5	7.65±1.0
cis-β-Cryptoxanthin	8.3±2.5	8.6±4.4	7.6±1.5	1.1±2.4	5.9±2.2
α-Carotene	25.3±10.5	35.2±17.9	5.9±1.5	26.9±5.1	51.9±8.5
β-Carotene	51.4±25.2	67.9±22.9	16.6±2.8	38.1±10.8	66.8±11.0
9- +13-*cis*-β-Carotene	10.5±4.8	11.0±3.8	3.3±0.6	8.2±1.1	15.6±4.4
Not identified	ND	ND	ND	0.1±0.2	0.7±1.5
Total	174±39.7	204±41.5	119±16.7	98.8±16.5	155±23.6
Vitamin A value	17.0±5.7	15.8±5.3	9.5±1.1	9.9±2.4	17.3±2.4
Ascorbic acid	121±18.2	118±28.7	104±22.3	109±21.2	106±20.0

Mean and standard deviation of five sample lots. ND: not detected. RE: retinol equivalent

3.2. Variety differences

In general, highly significant differences were observed between the elongated red and yellow varieties of cashew apple cultivated in the Southeast region. α-Carotene, β-carotene *cis*-β-carotene, and consequently the vitamin A value, and also the total carotenoid contents were significantly higher ($P<0.0001$) in the red variety, being almost twice that of the yellow variety. The level of *cis*-β-cryptoxanthin was 5.4 times higher in the red than in the yellow cashew apples, but as a result of the high coefficient of variation value among the five lots, this difference was not statistically different ($P=0.1332$) Although the lutein concentration was slightly higher in the red variety, this difference was not significant ($P=0.5588$). On the other hand, the β-cryptoxanthin level was 1.3 times higher in the yellow variety ($P=0.5655$).

The same tendency was found between elongated red and yellow cashew apples from the Northeast region, the contents of zeinoxanthin ($P=0.3237$), β-cryptoxanthin ($P=0.1231$), *cis*-β-cryptoxanthin ($P=0.7362$) and *cis*-β-carotene ($P=0.5919$) being slightly higher in the red variety than in the yellow one. In fact this tendency was only significant for α-carotene ($P=0.0126$), β-carotene ($P=0.0063$) and total carotenoid contents ($P=0.0030$), which were about 1.3 times higher in the red variety. Violaxanthin ($P=0.0301$) and zeaxanthin ($P=0.0001$) levels were 1.3 times higher in the yellow variety.

In contrast to carotenoids, AA values were higher in the yellow than in the red varieties from both regions (Table 2), but these differences were not significant ($P=0.6408$ for the Southeast and $P=0.2626$ for the Northeast). When the variability within lots was considered, P values became statistically significant ($P=0.0116$ and $P=0.0001$, respectively).

The comparison among the three varieties from the Northeast region showed that elongated red and yellow fruits presented significantly higher levels of zeinoxanthin ($P=0.0001$), α-carotene ($P=0.0001$), β-carotene ($P=0.0001$), *cis*-β-carotene ($P=0.0001$), total carotenoid levels ($P=0.0001$) and vitamin A values ($P=0.0001$) than rounded red fruits from the same region (Table 2). *cis*-β-Cryptoxanthin followed the same tendency as observed above, whereas violaxanthin, lutein, zeaxanthin and β-cryptoxanthin presented greater values in red rounded cashew apples. As can be seen in Table 2, rounded red cashew apples (104 mg/100 g) presented significantly lower levels of AA than the elongated red (118 mg/100 g, $P=0.0018$) and yellow fruits (125 mg/100 g, $P=0.0406$).

Cecchi and Rodriguez-Amaya (1981) also reported higher carotenoid concentration in red cashew apple than in the yellow variety, from São Paulo State. Statistical analyses were not performed because just one lot was analyzed. These authors did not evaluate different forms of cashew apple and no other study comparing elongated red and rounded red was found in the literature.

Falade (1981) studied different varieties of cashew apples from Nigeria and found a higher AA content in elongated red and rounded red fruits than in elongated yellow ones, but no statistical analysis was reported.

3.3. Geographic effects

Two regions in Brazil were compared, the Northeast and the Southeast. They were specially chosen because the solar intensity and the temperatures are higher in the Northeast than in the Southeast. Qualitative differences between the regions were observed in the carotenoid profile. Northeastern fruits showed the presence of violaxanthin and as major carotenoids, β-carotene and β-cryptoxanthin (Fig. 3), whereas in the South-

eastern fruits, violaxanthin was absent and β-carotene and α-carotene were the major carotenoids (Fig. 2).

When comparing yellow cashew apples from both regions, the fruits from the Northeast region presented 1.7 times higher concentrations of total carotenoid ($P = 0.0001$) and provitamin A ($P = 0.0001$) than those from the Southeast. The carotenoids that contributed to this higher content in the Northeast region were especially *cis*- and *trans*-β-cryptoxanthin, whose levels were, respectively, 7.5 and 5.5 times higher in the fruits from the Northeast. Although the contents of β-carotene and *cis*-β-carotene were 1.3 times higher in the fruits from the Northeast, the differences were not significant ($P = 0.0057$ and 0.4301, respectively).

As for the yellow variety, the elongated red cashew apple from the Northeast also presented significantly higher *cis*- and *trans*-β-cryptoxanthin ($P = 0.0001$), their levels being, respectively, 7.8 and 1.5 times higher than those from the Southeast. The β-carotene levels ($P = 0.2525$) were similar for both regions. In contrast, the α-carotene (1.5 times, $P = 0.0158$), lutein (2.9 times, $P = 0.0001$) and *cis*-β-carotene (1.4 times, $P = 0.0001$) contents were significantly higher in the Southeastern fruits.

Yellow and red cashew apples from the Northeast region presented higher AA contents than those from the Southeast (Table 2). These differences were significant between yellow cashew apples from both regions ($P = 0.0148$), but not as significant for the red variety ($P = 0.0713$).

In general, for variety difference, as well as for geographic effects, statistical analysis (considering the variability within the lots) showed higher significant differences, i.e. lower P values.

Unlike our results, Cecchi and Rodriguez-Amaya (1981) showed no difference between the yellow and red varieties cultivated in different regions. In fact just one lot of each region was analysed by these authors.

Cecchi and Rodriguez-Amaya (1981) reported higher carotenoid content and vitamin A value in red cashew apples from the Northeast and Southeast regions than those found in this study. The differences in the vitamin A values reached 1.9 times between the studies. On the other hand, for yellow cashew apples from both regions the values reported by these authors were similar to those shown in the present study (Table 2).

Falade (1981) reported higher AA levels than in this study, but it is important to mention that differences among countries are more expected since soils and climates are different between the two countries.

It is noteworthy that, in the present study, the elongated yellow and red cashew apples from the Northeast region presented a standard deviation (S.D.) amongst the lots of 47 and 33%, respectively. Rounded red fruits, also from the Northeast, showed lower S.D. (16%). For the elongated southeastern fruits, the S.D. values were lower, being 29% for yellow cashew apple and 14% for red ones.

4. Conclusions

Highly significant differences in the carotenoid and AA contents were observed between the different varieties of cashew apple from the two regions. In general, elongated red presented higher carotenoid levels than the yellow variety. In contrast, AA values were higher in the yellow variety from both regions.

When comparing the different regions, both fruits from the hottest region (Northeast) presented significant higher total carotenoid concentration and vitamin A value than those from the Southeast due to the much higher *cis*- and *trans*-β-cryptoxanthin levels in the fruits from the Northeast. The same tendency was observed for AA.

Cashew apple fruits can be considered a good source of vitamin C but not so good for provitamin A.

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