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Carotenoid composition from the Brazilian tropical fruit camu–camu (Myrciaria dubia)

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Abstract

Camu–camu (Myrciaria dubia) is a small berry, native to the Amazon, known as a rich source of ascorbic acid. The carotenoid composition of this fruit was determined using high performance liquid chromatography-diode array detection on C_{18} and C_{30} columns. Fruits produced in two different regions of São Paulo State, Iguape and Mirandópolis, were analysed. All-trans-lutein was the major carotenoid in camu–camu fruits from both regions, ranging from 45% to 55% of the total carotenoid content (160.5 \pm 93.1 µg/100 g for Iguape and $601.9 \pm 75.6 \,\mu g/100 \,g$ for Mirandopolis fruits), followed by β -carotene, violaxanthin and luteoxanthin. The levels of lutein, b-carotene, violaxanthin, luteoxanthin and other minor carotenoids were significantly higher in the camu–camu produced in Mirandópolis region, most probably due to the higher temperature and light exposure found in this region, in comparison to those from Iguape. Maturation was also an important feature affecting batches from the same region. $© 2006 Elsevier Ltd. All rights reserved.$

Keywords: Carotenoids; Camu–camu; Myrciaria dubia; Tropical fruit; HPLC–PDA; Climatic effects

1. Introduction

Camu–camu (Myrciaria dubia (HBK) McVaugh) is a small Amazonian bush that occurs naturally in areas of periodic flooding, such as lowlands around river courses and lakes [\(Rodrigues et al., 2004\)](#page-6-0). Its fruit is a round-shaped berry averaging 2.5 cm in diameter. During the ripening process, the colour changes from green to hues, varying from red to purple. Because of its particularly high ascorbic acid content (1380–1490 mg/100 g pulp and 2050 mg/100 g peel) [\(Justi, Visentainer, Souza, & Matsushita, 2000](#page-5-0); unpublished results), and potassium levels, as well as the presence of carotenoids and anthocyanins, camu–camu is considered to have high nutritional value according to the Amazon Research National Institute (INPA). This fruit is considered to be one of the richest sources of vitamin C in Brazil, presenting higher content than in acerola (1125–1790 mg/

100 g of fresh pulp) ([Maatta, Kamal-Eldin, & Torronen,](#page-5-0) [2003; Visentainer, Matsushita, Souza, & Vieira, 1997](#page-5-0)) and cashew apples from Northern and Southern Brazilian States $(106-121 \text{ mg}/100 \text{ g})$ (Assunção & Mercadante, 2003).

As well as their colorant properties, the carotenoids are known to have several others biological functions, such as vitamin A activity, cancer-preventing effects, cardiovascular disease protective effects and can also reduce the risk of cataract and age-related macular degeneration [\(Van](#page-6-0) [den Berg et al., 2000](#page-6-0)).

Despite the great commercial potential of camu–camu, few reports have been published about its phytochemical contents. The identification of the major carotenoids from camu–camu was first studied by [Azevedo-Meleiro and](#page-5-0) [Rodriguez-Amaya \(2004\),](#page-5-0) but the indication of the major carotenoids was based on HPLC peak area of only one analysis. The composition of anthocyanins from camu–camu, was determined by [Zanatta, Cuevas, Bobbio, Winterhalter,](#page-6-0) [and Mercadante \(2005\).](#page-6-0) Cyanidin-3-glucoside was the major anthocyanin followed by delphinidin-3-glucoside.

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Considering São Paulo State as the main Brazilian consumer centre and the high potential of camu–camu fruits, the purpose of this study was to evaluate the carotenoid composition of camu–camu cultivated in two different regions of São Paulo State.

2. Materials and methods

2.1. Samples

Fruits of camu–camu produced in two different regions of São Paulo State (Brazil), Iguape and Mirandópolis, were analysed. From each region, three batches, consisting of about 2 kg each, were harvested, from May to July 2003, for quantitative analysis. Previous qualitative analysis was performed using samples harvested in July 2002, in Mirandópolis. The ripe camu–camu fruits were manually peeled, and the peel obtained was homogenized in a blender.

2.2. Camu–camu characterization

Fruits were weighed and measured. At the same time, the pH and Brix values were measured in the pulp. Within the batches no statistical difference was observed; however, the camu–camu from Mirandópolis was significantly smaller $(22.1 \pm 0.3 \text{ mm}, P = 0.013)$ and lighter $(6.9 \pm 1.3 \text{ mm})$ 0.6 g, $P = 0.0019$ than the fruits produced in Iguape $(24.0 \pm 0.1 \text{ mm}; 8.6 \pm 0.1 \text{ g})$. The soluble solids (°Brix) varied from 6.5 to 8.5 for the fruits from Mirandópolis and from 6.0 to 6.5 for the fruits from Iguape. The pH ranged from 2.6 to 2.9 and from 2.4 to 2.8 for the fruits from Mirandópolis and Iguape, respectively. These values are in the range of those found for fruits of camu–camu produced in Peru [\(Zapata & Dufour, 1993\)](#page-6-0).

2.3. Standards

DSM Nutritional Products (Switzerland) provided standards of lutein, purity of 96%, zeaxanthin (99%), β -cryptoxanthin (99%), β -apo-8'-carotenal (98%), β -apo-10'carotenal (95%) , β -apo-12'-carotenal (80%) , rubixanthin (97%), γ -carotene (99%) and lycopene (98%), all determined by high performance liquid chromatography-photodiode array detector (HPLC–PDA) analysis.

Standards of α -carotene and β -carotene were prepared from carrot, according to the extraction procedure described below, followed by separation by column chromatography (CC) on MgO/Hyflosupercel and crystallisation with petroleum ether and methanol. The crystallization was repeated to obtain purity of 99% for α -carotene and 98% for β -carotene, both demonstrated by HPLC–PDA.

2.4. Carotenoid determination

The carotenoids were exhaustively extracted from the crushed peel with acetone, transferred to petroleum ether/diethyl ether and saponified with 10% methanolic KOH, according to [Mercadante and Rodriguez-Amaya](#page-6-0) [\(1998\)](#page-6-0). All extractions were conducted in duplicate, and each duplicate was injected twice into the HPLC.

Separation was achieved using a Waters HPLC coupled to a PDA detector (Waters, model 996), equipped with a quaternary solvent delivery system (Waters, model 600), an on-line degasser, a Rheodyne injection valve with a $20 \mu L$ loop and an external oven. The data acquisition and processing were performed by the Millennium Waters software. For quantitative analysis, carotenoids separations were carried out on a C₁₈ Nova-Pak ODS, $300 \text{ mm} \times 3.9 \text{ mm}$ (4 µm particle size) column, using as mobile phase a linear gradient of acetonitrile/ $H₂O$ /ethyl acetate starting at $88:10:2$ (v/v) reaching $85:0:15$ in 15 min, then, for the fruits from Iguape one more step from 85:0:15 to 70:0:30 in 30 min, at a flow rate of 1 mL/min and column temperature set at $29 \degree C$. For qualitative analysis, the carotenoid separation was also performed on a C_{30} YMC 250 mm \times 4.6 mm (3 µm particle size) column, using as mobile phase a linear gradient of methanol/methyl tertbutyl ether (MTBE)/H₂O starting at 90:5:5 (v/v/v) reaching 95:5:0 in 12 min, 89:11:0 in 25 min, 75:25:0 in 40 min, 50:50:0 in 60 min and returning to the initial condition in 2 min, at a flow rate of 1 mL/min and column temperature set at 33 °C [\(Mouly, Gaydou, & Corsetti, 1999](#page-6-0)). Chromatograms were processed at 450 nm and the spectra were obtained between 250 and 600 nm. Immediately before HPLC–PDA analysis, the crude extract was dissolved in acetonitrile/ethyl acetate (85:15) for C_{18} column separation while for analysis on the C_{30} column the extract was dissolved in methanol/MTBE (30:70) and further filtered in a Millipore filter $(0.22 \text{ }\mu\text{m})$.

The carotenoids were identified according to the following parameters: chromatographic behaviour on C_{18} and C_{30} HPLC columns, TLC on silica gel and on MgO/kieselguhr [\(Mercadante, Britton, & Rodriguez-Amaya, 1998\)](#page-5-0), UV–vis spectra (λ_{max} and shape) compared to literature data ([Britton, 1995; Davies, 1976; Mercadante & Egeland,](#page-5-0) [2004](#page-5-0)) and co-elution with authentic standards. Pigments were also isolated according to [Mercadante et al. \(1998\)](#page-5-0) and submitted to chemical tests. A methylation test with acidified methanol ([Davies, 1976; Eugester, 1995\)](#page-5-0), monitored by thin layer chromatography (TLC) on silica gel with petroleum ether/diethyl ether (1:1) as mobile phase, was performed for mutatoxanthin $(5,8$ -epoxy-zeaxanthin). The epoxide-furanoid rearrangement and isomerization catalyzed by iodine were monitored spectrophotometrically ([Davies, 1976\)](#page-5-0).

For quantification, calibration curves were constructed for lutein, zeaxanthin, β -cryptoxanthin and β -carotene with a minimum of seven concentration levels, each one in duplicate, and the concentration levels were chosen to include those of the samples. Carotenoid quantification was performed by comparison of peak area of the sample with that of the standards peak area, injected daily. Since the following carotenoids were present in the samples at low concentrations or the respective standards were not

available, neoxanthin, violaxanthin, luteoxanthin, 5,8 epoxy-lutein (flavoxanthin), 5,6-epoxy-lutein (taraxanthin) and 9-cis-lutein were quantified using the all-trans-lutein peak area; 5,8-epoxy-zeaxanthin (mutatoxanthin) and 5,6 epoxy-zeaxanthin (antheraxanthin) using the zeaxanthin area; sintaxanthin using β -cryptoxanthin area and 5,8epoxy-b-carotene and not identified-427 using the alltrans-β-carotene area.

The [NAS-NRC \(1989\)](#page-6-0) conversion factor was used to calculate vitamin A value.

2.5. Statistical analysis

To evaluate the differences in the carotenoid concentrations between the different regions and within the batches, analysis of variance were conducted using one-way ANOVA (Software Origin 5.0).

3. Results and discussion

3.1. Carotenoid identification

The carotenoid profiles from camu–camu fruits produced in Mirandópolis and Iguape regions harvested in the year of 2003 were very similar, as shown by the HPLC–PDA chromatograms in Fig. 1.

HPLC analysis showed the separation of 16 carotenoids on the C_{18} column. Peak identification and characterization are presented in [Table 1.](#page-3-0) The first xanthophylls to elute from the reversed-phase column were neoxanthin, cis-neoxanthin, violaxanthin, cis-violaxanthin and luteoxanthin, identified from their UV/vis spectra, chromatographic behaviour and the cis-isomers also by the lower λ_{max} and spectral fine structure (%III/II) values in comparison to the all-*trans* compounds. Neoxanthin and violaxanthin, also isolated by CC and TLC, showed a hypsochromic shift of, respectively, 20 and 40 nm, after epoxide-furanoid rearrangement.

The monoepoxides, 5,8-epoxy-lutein, 5,6-epoxy-lutein, 5,8-epoxy-zeaxanthin and 5,6-epoxy-zeaxanthin, were identified from their UV/vis spectra, chromatographic behaviour and by their lower λ_{max} of, respectively, 22, 6, 24 and 6 nm, in comparison to the equivalent non-epoxide carotenoids. The identity of 5,8-epoxy-zeaxanthin was finally confirmed by the negative response to methylation after 3 and 6 h of reaction, observed by no change in the R_f on silica gel TLC developed with petroleum ether/acetone (65:35) as mobile phase.

Lutein, zeaxanthin, sintaxanthin, b-cryptoxanthin and $5,8$ -epoxy- β -carotene were the last xanthophylls to elute. Their identification was based on chromatographic behaviour and UV/vis spectra features. Since sintaxanthin has no fine structure (%III/II = 0), which is typical of apocarotenals, and possessed a λ_{max} in the range of those verified for β -apo-8'-carotenal (461 nm) and β -apo-10'-carotenal (448), a co-elution with standards of β -apo-8'-carotenal, β -apo-10'-carotenal and β -apo-12'-carotenal was performed. However, sintaxanthin did not co-elute with those standards, confirming the presence of a ketone group in the molecule and a longer carbon chain when compared to apo-10'-carotenal. Small amounts of cis - β -cryptoxanthin, which eluted together with β -cryptoxanthin, were verified

Fig. 1. Chromatograms, obtained by HPLC–PDA, of the carotenoids extract from camu–camu produced in 2003, in Iguape and Mirandópolis regions. Chromatographic conditions: see text. Peak identification and characterization is given in [Table 1](#page-3-0). Processed at 450 nm.

Table 1

Chromatographic and spectroscopic characteristics, carotenoid composition and vitamin A value of camu–camu harvested in Iguape and Mirando´polis, São Paulo State, Brazil

Peak No. ^a	Carotenoid	$t_{\rm R}$ (min)		λ_{max} (nm) ^d	$\frac{9}{6}$ III/II	$\%A_{\text{B}}/A_{\text{II}}$	Concentration $(\mu g/100 g)^e$	
		$MP1^b$	MP2 ^c				Iguape	Mirandópolis
	Neoxanthin	$5.5 - 6.2$	6.2	415, 440, 467	84	Ω	3.9 ± 1.7 ^{f,g}	20.8 ± 13.3^g
\overline{c}	cis-Neoxanthin	$5.8 - 6.3$	$6.3 - 6.5$	329, 410, 438, 466	88	21	2.1 ± 1.8 ^r	16.0 ± 10.2^g
3	Violaxanthin (all- <i>trans</i> [*] + <i>cis</i>)	$8.8 - 9.1$	$8.9 - 9.2$	328, 417, 441, 471	88	θ	$12.0 \pm 19.3^{\text{f}}$	115.6 ± 62.4^g
4	Luteoxanthin	$8.4 - 9.4$	$9.5 - 9.6$	398, 423, 449	115	θ	21.5 ± 13.4^f	60.0 ± 20.7 ^g
5	Not identified-436	9.9	$9.9 - 10.1$	411, 436, 466	77	nc	$2.3 \pm 2.7^{\text{t}}$	$26.1 \pm 6.1^{\rm f}$
6	$Flavoxanthin* + mixture$	$9.9 - 10.3$	10.2	402, 425, 451	10	nc	$9.4 \pm 4.9^{\rm f}$	$6.7 \pm 0.6^{\rm f}$
	5,6-Epoxy-lutein	12.4	12.4	416, 441, 471	54	nc	2.1 ± 3.7^f	$8.6 \pm 8.6^{\rm t}$
8	5,8-Epoxy-zeaxanthin	$12.1 - 13.6$	nd	337, (403), 430, 454	57	nc	$9.2 + 9.8$	nd
9	5,6-Epoxy-zeaxanthin	13.2	13.2	406, 426, 447, 472	32	θ	12.9 ± 22.3 ^r	47.7 ± 22.7^g
10	All-trans-lutein	$14.9 - 16.2$	$15.1 - 16.4$	(423), 447, 476	60	θ	$160.5 \pm 114.0^{\text{f}}$	601.9 ± 92.5^8
11	Zeaxanthin	$15.7 - 17.1$	$15.9 - 17.3$	(428) , 454, 481	33	θ	$22.9 \pm 13.0^{\rm f}$	38.0 ± 12.7 ^f
12	Sintaxanthin	$23.6 - 27.4$	$25.0 - 25.2$	(420), 453, 484	θ	$\mathbf{0}$	$1.11 \pm 0.4^{\text{f}}$	1.0 ± 0.3^f
13	β -Cryptoxanthin (all- <i>trans</i> [*] + <i>cis</i>)	$24.5 - 26.1$	$26.7 - 26.9$	(428) , 454, 481	18	θ	9.9 ± 3.7 ^f	6.9 ± 0.2^f
14	$5,8$ -Epoxy- β -carotene	$27.8 - 29.2$	$30.8 - 30.9$	(404) , 428, 453	42	θ	$3.6 \pm 2.4^{\text{t}}$	1.2 ± 2.0^f
15	Not identified-427	$29.8 - 31.1$	$30.7 - 31.9$	(405) , 427, 453	55	θ	$8.6 \pm 9.4^{\circ}$	$2.8 \pm 3.7^{\text{t}}$
16	β-Carotene	$33.4 - 35.3$	$40.4 - 41.1$	(428), 454, 481	20	θ	$72.8 \pm 74.6^{\rm f}$	142.3 ± 23.7^g
	Total carotenoids						$354.8 \pm 260.5^{\text{f}}$	1095.3 ± 237.0^8
	Vitamin A value $(RE/100 g)$						$14.2 \pm 13.4^{\mathrm{f}}$	$24.5 \pm 4.1^{\rm f}$

nd, not detected; nc, not calculated; and *, major.

^a Numbered according to [Fig. 1](#page-2-0). b Mobile phase used for camu–camu from Iguape.

 c Mobile phase used for camu–camu from Mirandópolis.

^d Linear gradient of acetonitrile/water/ethyl acetate.

^e Mean and standard deviation of three batches.

 f,g Different superscripts in the same row indicate mean difference at significant level of 5% .

by its lower values of λ_{max} (337, (425), 451, 474), %III/II (18) and $\%A_B/A_H$ (38) in comparison to the all-*trans* isomer. Lutein, zeaxanthin and β -cryptoxanthin also had their identities confirmed by co-elution with standards.

Only one carotene, all-*trans*- β -carotene, was found in the samples harvested in 2003, identified by its UV/vis spectra and chromatographic behaviour. The identity of all-trans-b-carotene was also confirmed by co-chromatography with standard.

The fruits harvested in 2002 showed some differences in relation to the camu–camu collected in 2003, both from Mirandópolis. The analysis performed by HPLC showed the separation of 27 carotenoids on the C_{30} column ([Fig. 2\)](#page-4-0) and 19 on the C_{18} column (data not shown). Using large amounts of sample (200 g), the separation by CC followed by TLC on silica and MgO/kieselguhr allowed the isolation of 26 carotenoids. It is worthwhile to highlight that the C_{30} column presents a special characteristic, when compared to C_{18} columns, which allows molecules to be separated according to their size and space conformation despite polarity interactions. These features permitted more efficient separations of geometric isomers [\(Sander,](#page-6-0) [Sharpless, Craft, & Wise, 1994](#page-6-0)).

In these samples the separation of 9-cis-lutein and 13 *cis*-lutein was completely achieved on the C_{30} column. The lutein *cis*-isomers showed different UV/vis spectra λ_{max} , fine structure and *cis* peak intensity (% A_B/A_{II}), *cis*lutein exhibited λ_{max} values at 329, 416, 441, 468 nm, %III/II of 41 and % A_B/A_H of 29, whereas 13-*cis*- or 13[']-

cis-lutein showed values of λ_{max} at 329, 415, 437, 464 nm, %III/II of 51 and % A_B/A_H of 46. These values and elution order were similar to literature data, in which identification was confirmed by NMR [\(Brunner, 1997](#page-5-0)).

Other xanthophylls not found in the samples used for quantification, such as 5,6,5',6'-diepoxy-ß-cryptoxanthin, rubixanthin, cis-rubixanthin and citranaxanthin were also observed. Rubixanthin had its identity also confirmed by co-elution with standard and its isomer by showing lower λ_{max} (3 nm).

Several carotenes were found in the separations of the extract of the camu–camu harvested in 2002. Prolycopene, ε -carotene and α -carotene were identified from their UV/ vis spectra and chromatographic behaviour, as were δ -carotene, γ -carotene, *cis-* γ -carotene, lycopene and 9-*cis*-lycopene which were observed only during separations on the C_{30} column and by TLC on MgO/kieselguhr. The presence of a *cis* isomer was observed eluting with all-*trans*- β -carotene on the C_{18} column analysis. However, the complete separation of 9- cis - β -carotene was only resolved on the C_{30} column, the identification of which was based on its UV/vis spectrum, λ_{max} 3 nm lower in comparison to the all-trans compound, % III/II of 18 and % A_B/A_H of 16, similar to literature data [\(Mercadante, Steck, & Pfander,](#page-6-0) [1999](#page-6-0)). Phytofluene identification was based on its UV/vis spectrum, chromatographic behaviour and its fluorescence under UV light at 254 nm on TLC, although it eluted together with all-*trans*- β -carotene on the C₁₈ column or with 5,8-epoxy- β -carotene on the C₃₀ column.

Fig. 2. Chromatogram, obtained by HPLC–PDA on C₃₀ column, of the carotenoids extract from camu–camu produced in 2002 in Mirandópolis region. Chromatographic conditions: see text. Processed at 450 nm. Peak identification: (1) neoxanthin, (2) violaxanthin, (3) cis-violaxanthin, (4) luteoxanthin, (5) cis-lutein, (6) 13-cis-lutein, (7) all-trans-lutein, (8) zeaxanthin, (9) 5,6,5',6'-diepoxy-β-cryptoxanthin, (10) not identified, (11) β-cryptoxanthin, (12) 5,8epoxy- β -carotene + phytofluene, (13) mixture, (14) cis- ζ -carotene, (15) cis-rubixanthin, (16) α -carotene, (17) rubixanthin, (18) ζ -carotene, (19) all-trans- β carotene, (20) prolycopene, (21) 9-cis- β -carotene, (22) cis- γ -carotene, (23) all-trans- γ -carotene, (24) sintaxanthin, (25) citranaxanthin, (26) . 9-cis-lycopene, (27) lycopene.

Sintaxanthin and citranaxanthin, the latter only detected on the C_{30} column, may be considered as an analysis artefact, since these carotenoids were only found in extracts in which acetone was used as extraction solvent. These carotenoids are, respectively, the product of an aldol condensation reaction of β -apo-10'-carotenal and β -apo-8'-carotenal with acetone. Experiments, where only ethyl acetate was used as extraction solvent, showed the presence of β -apo-10'-carotenal using TLC with MgO/kieselguhr separation. The presence of β -apo-8'-carotenal was not observed using TLC separations, owing probably to its very low concentration.

This was the first time that the carotenoids from camu– camu were fully quantified: [Azevedo-Meleiro and Rodri](#page-5-0)[guez-Amaya \(2004\)](#page-5-0), using one batch of camu–camu, only reported the identification of lutein as the major carotenoid, along with β -carotene and zeaxanthin, and similar peak areas for neoxanthin, β -cryptoxanthin, 5,6-epoxy- β carotene and cis-b-carotene.

3.2. Climatic conditions effect on the carotenoid composition

Lutein was the major carotenoid in camu–camu fruits from both regions, representing 45% and 55% of total carotenoid content for the fruits produced in Iguape and

Mirandópolis, respectively. β -Carotene represented 20% and 13%, while violaxanthin represented 5% and 9%, and luteoxanthin 6% and 5% of the total carotenoid contents, respectively, for camu–camu from Iguape and Mirandópolis. The quantitative composition of carotenoids from camu–camu is presented in [Table 1](#page-3-0).

Although the carotenoid profile was very similar for the samples from both regions, the fruits from Mirandópolis contained significantly higher contents of total carotenoids $(P = 0.0002)$ than those from Iguape. The levels of lutein were 3.7 times higher $(P \le 0.0001)$ in the fruits from Mirandópolis than in those from Iguape; the concentration of β -carotene was almost two times higher ($P = 0.0044$) in the samples from Mirandópolis. As a result of the higher β carotene concentration, the vitamin A value was 1.7 times higher in the fruits from Mirandópolis. However, the difference was not significant ($P = 0.26$), due to the variability within the batches produced in Iguape.

Other minor peaks were significantly higher in the camu–camu produced in Mirandópolis, such as neoxanthin $(P = 0.0064)$, cis-neoxanthin $(P = 0.0047)$, violaxanthin $(P = 0.0001)$, luteoxanthin $(P = 0.0017)$ and antheraxanthin ($P = 0.0137$). Although the difference was not statistically significant, the levels of zeaxanthin were also higher in the fruits from Mirandópolis. In contrast, the contents of

 β -cryptoxanthin, 5,8-epoxy- β -carotene and not identified-427 were higher in the camu–camu from Iguape. In addition, mutatoxanthin was not detected during quantitative analysis of camu–camu fruits from Mirandópolis.

Considering that carotenoids accumulation is influenced by many external environmental factors, weather characteristics from both regions were verified. During the month of May that preceded the harvesting season, the average of the minimum and maximum temperatures registered in Mirandópolis were higher (17.2 \degree C and 31.5 \degree C) than those observed in Iguape (14.5 °C and 25.9 °C). During the same period rainfall was higher in Iguape (22.5 mm) than in Mirandópolis (12.5 mm), suggesting longer periods of light exposure in the Mirandópolis region. These weather conditions indicate that higher temperature and light exposure were the main factors responsible for the significantly higher carotenoid contents found in the camu–camu from Mirandópolis. However, since the fruits were collected in different regions of the same state, the influence of soil conditions and use of herbicides cannot be excluded. Direct correlations between high temperatures, light exposure and increased carotenoid contents were previously reported for acerola collected during two consecutive years from the same plantation (De Rosso & Mercadante, 2005). Despite the different origins of the fruits, previous surveys on acerola (Cavalcante & Rodriguez-Amaya, 1992) and mango ([Mercadante & Rodriguez-Amaya, 1998\)](#page-6-0) also reported higher carotenoid contents in fruits harvested in hotter regions

There were also significant differences within batches in the levels of neoxanthin ($P = 0.0001$), cis-neoxanthin ($P =$ 0.0061), violaxanthin $(P = 0.0003)$, luteoxanthin $(P = 0.0061)$ 0.0005), antheraxanthin ($P = 0.0008$), lutein ($P = 0.0041$), zeaxanthin ($P = 0.0084$) and β -carotene ($P = 0.025$) in the samples produced in Mirandópolis. Whereas for the batches produced in Iguape, significant differences were observed in the contents of *cis*-neoxanthin ($P = 0.039$), violaxanthin ($P = 0.0056$), luteoxanthin ($P = 0.011$), not identified-436 ($P = 0.0003$), mutatoxanthin ($P = 0.0036$), antheraxanthin ($P = 0.0014$), lutein ($P = 0.0026$), zeaxanthin $(P = 0.031)$ and 5,8-epoxy- β -carotene $(P = 0.028)$. Considering that the batches from the same region were harvested in an interval less than 2 weeks, this variation is probably due to maturity differences between the batches, since camu–camu, like other climacteric fruits, undergoes a variety of chemical and physical changes during ripening that influence carotenoids biosynthesis. The same tendency was observed for mango ([Mercadante &](#page-6-0) [Rodriguez-Amaya, 1998](#page-6-0)) and acerola (Lima et al., 2005).

In comparison to some tropical fruits such as acerola (De Rosso & Mercadante, 2005), caja (Hamano & Mercadante, 2001) and passion fruit ([Silva & Mercadante, 2002\)](#page-6-0), camu–camu can not be considered a good source of carotenoids and provitamin A. However, camu–camu fruit contains higher carotenoid and provitamin A contents, when compared to cashew-apple (Assunção & Mercadante, 2003), and its lutein concentration is proportionally higher than all of these fruits and comparable to some leafy vegetables, which are good lutein sources ([Ramos & Rodriguez-](#page-6-0)[Amaya, 1987](#page-6-0)).

In summary, all-trans-lutein was found to be the major carotenoid in camu–camu fruits from both regions, followed by β -carotene, violaxanthin and luteoxanthin. A positive correlation was observed between high temperature and light exposure and an increase in carotenoid content.

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