Carotenoids from Yellow Passion Fruit (*Passiflora edulis*)

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The following 13 carotenoids from yellow passion fruit (*Passiflora edulis*) were conclusively identified: phytoene, phytofluene, ζ-carotene (principal carotenoid), neurosporene, β-carotene, lycopene, prolycopene, monoepoxy-β-carotene, β-cryptoxanthin, β-citraurin, antheraxanthin, violaxanthin, and neoxanthin. Electron impact mass spectrometry, complemented by UV−visible spectrophotometry and co-chromatography, besides ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy for prolycopene, was used for this purpose. Seven of the carotenoids identified are reported as passion fruit carotenoids for the first time.

Keywords: *Carotenoids; passion fruit; identification*

INTRODUCTION

Considering the many functions or actions attributed to carotenoids, there is worldwide effort to obtain reliable analytical data on these compounds. Since there is a large number of natural carotenoids, one of the problems is conclusive identification. Incomplete or conflicting information about the carotenoid composition of fruits is found in the literature.

Native to Brazil, passion fruit is now widely produced throughout the tropics. Brazilian production of this fruit surpasses that of mango, guava, and papaya, and this country is the leading exporter of passion fruit juice (Chan, 1993). The purple passion fruit is preferentially consumed fresh, and the yellow fruit is used as raw material for juice processing.

The first investigation on passion fruit carotenoids was carried out by Pruth and Lal in 1958. Phytofluene, ζ-carotene, β-carotene, α-carotene and three unidentified pigments with absorption maxima at 400 and 425 nm were found in purple passion fruit. Leuenberger and Thommen (1972) detected phytofluene, ζ-carotene, β-carotene, and, additionally, cryptoxanthin, auroxanthin, mutatoxanthin, β-apo-12′-carotenal, and β-apo-8′-carotenal. In comercial-processed passion fruit juice, presumably from yellow passion fruit, Cecchi and Rodriguez-Amaya (1981) detected α-carotene, β-carotene, ζ-carotene, neurosporene, lycopene, γ-carotene, aurochrome, cryptocrome, and auroxanthin. ζ-Carotene was always found as principal carotenoid, leading Gross (1987) to comment that the carotenoid pattern was uncommon for fruits. Identification in these three papers was based on UV−visible absorption spectra and co-chromatography; the latter work included some chemical reactions.

Passion fruit was one of four fruits subjected to screening by TLC (Mercadante and Rodriguez-Amaya, 1991), demonstrating the presence of 5 carotenes, 1 monohydroxy-, 3 dihydroxy-, 1 trihydroxy-, and 2 other carotenoids. This indicated the occurrence of a greater number of carotenoids than previously reported.

Mass spectrometry (MS) can be used to confirm the identity of carotenoids because the molecular ion is almost always obtained, and the characteristic fragments produced under electron impact can indicate the type of end groups. Nuclear magnetic resonance (NMR) analysis may prove a carotenoid structure beyond doubt, including the geometry of the double bonds. MS and NMR have been used extensively to elucidate the structures of carotenoids in algae, fungi, and bacteria, but both have been underutilized in relation to food carotenoids.

MATERIALS AND METHODS

Three batches, consisting of seven fruits each, of yellow passion fruits (*Passiflora edulis* var. *flavicarpa*), at the fully ripe stage, were bought in Campinas, São Paulo State (Brazil). Only the edible portion (mesocarp - about 400 g per batch) was analyzed.

To prevent contamination during extraction and chromatography, plastic materials, filter paper, and blender were avoided. All solvents (reagent grade) were redistilled, and peroxides were removed from diethyl ether by the addition of reduced iron powder prior to distillation. Solvents used in the final stage of purification were also filtered through an alumina (grade I) column. Silica G (Merck) and MgO/(BDH)/kieselguhr (Merck) (1:1) thin layer plates (20 × 20 cm, 0.25 mm thickness) were prepared in the laboratory and activated at 110 °C for 1 h. The silica gel plates were previously run with diethyl ether, and those that would be used to separate epoxycarotenoids were prepared with silica gel slurry to which a pellet of NaOH was added to avoid epoxide-furanoxide rearrangement, one of the principal causes of artifact formation during carotenoid analysis (Schiedt and Liaaen-Jensen, 1995).

The carotenoids were extracted with acetone, using a mortar and pestle, transferred to diethyl ether in a separatory funnel with the addition of water, saponified overnight with 10% KOH in methanol, washed free of alkali, and concentrated in a rotary evaporator (T < 35 °C). The extract (ca. 2 g) was applied on an open column packed with 60 g of neutral alumina (grade III) (2.5 i.d. × 8 cm height), and three fractions were separated. The first fraction (fraction 1 − carotenes and

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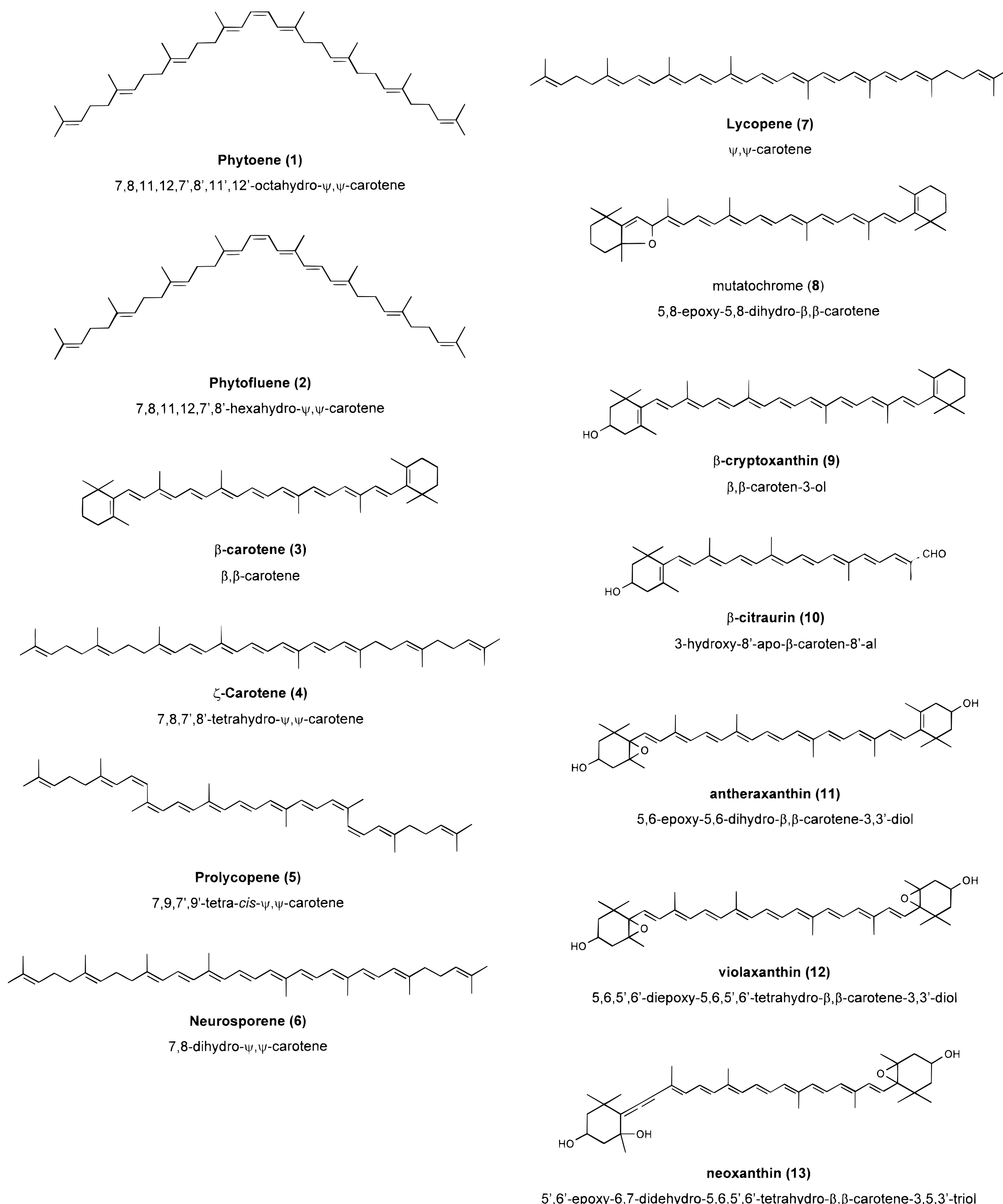
Chart 1



epoxycarotenoid) was eluted with petroleum ether, the second fraction (fraction 2 − monohydroxy- and keto-carotenoids) with 60−90% diethyl ether in petroleum ether, and the last fraction (fraction 3 − polyhydroxycarotenoids) with 0−30% ethanol in ether. Each fraction was concentrated to dryness under N_2.

Fraction 1 was submitted to thin-layer chromatography (TLC) on silica gel, which was developed with petroleum ether. Six bands (1.1−1.6) were scraped off and desorbed with diethyl ether. Band 1.1 ($R_f = 0.8$, silica gel TL) was further separated on MgO/kieselguhr TLC plates with petroleum ether as mobile phase. Two bands were obtained, the upper one ($R_f = 0.9$, MgO TL), located by its green fluorescence under UV light, consisted of phytoene **(1)** and phytofluene **(2)**, and the lower band ($R_f = 0.8$, MgO TL) *β*-carotene **(3)**. Further attempt to separate **1** and **2** by TLC were unsuccessful. Bands 1.2 ($R_f = 0.7$, silica gel TL) and 1.3 ($R_f = 0.6$, silica gel TL) were purified by TLC on MgO/kieselguhr, with petroleum ether and 10% acetone in petroleum ether as mobile phases, respectively. The major bands were scraped off, yielding ζ-caroteno **(4)** ($R_f = 0.4$, MgO TL) and prolycopene **(5)** ($R_f = 0.3$, MgO TL), respectively. Band 1.4 ($R_f = 0.55$, silica TL) was shown on MgO TLC to be composed of at least eight trace compounds and was discarded. Further purification of band 1.5 ($R_f = 0.4$, silica TL) by TLC on MgO, with 40% acetone in petroleum ether as mobile phase, gave neurosporene **(6)** ($R_f = 0.4$, MgO TL) and lycopene **(7)** ($R_f = 0.1$, MgO TL) as major pigments. Epoxy-*β*-carotene **(8)** was purified from band 1.6 ($R_f = 0.1$, MgO TL) by TLC on MgO with 10% acetone in petroleum ether.

Fraction 2 was separated into three bands (2.1−2.3) by TLC on silica gel with petroleum ether/diethyl ether (2:3) as mobile phase. Band 2.1 ($R_f = 0.9$, silica gel TL) was discarded because it was shown to be composed of at least seven trace pigments on MgO TLC with 10% acetone in petroleum ether as mobile phase. Bands 2.2 ($R_f = 0.8$, silica gel TL) and 2.3 ($R_f = 0.5$, silica gel TL) were further purified by TLC on MgO with 10

Table 1. Molecular Ion and Characteristic Fragments of Passion Fruit Carotenoids

carotenoid	m/z (% relative abundance) molecular ion	characteristic fragments
phytoene	544 (20)	69 (98), 137 (100), 339 (38)
phytofluene	542 (21)	69 (98), 137 (87), 337 (40), 405 (7.1)
ζ-carotene	540 (55)	69 (100), 137 (56), 403 (18), 446 (2.5)
neurosporene	538 (17)	69 (100)
β-carotene	536 (61)	105 (95), 137 (42), 399 (1.6), 430 (1.5), 444 (22)
lycopene	536 (29)	69 (100), 430 (4.5), 444 (4.1), 467 (2.7)
prolycopene	536 (22)	69 (100), 430 (3.6), 444 (4.9), 467 (2.3)
mutatochrome	552 (3.6)	69 (100), 205 (45), 446 (1.1), 472 (5.5)
β-cryptoxanthin	552 (14)	105 (95), 442 (1.7), 460 (5.9), 534 (5.9)
β-citraurin	432 (17)	105 (100), 239 (8.6), 279 (4.3), 414 (12)
antheraxanthin	584 (0.2)	105 (25), 221 (6.4), 352 (0.8), 492 (0.1), 504 (0.3), 566 (0.1)
violaxanthin	600 (0.9)	105 (62), 181 (27), 221 (46), 352 (5.4), 440 (2.2), 520 (1.2)
neoxanthin	600 (0.7)	105 (95), 181 (37), 221 (66), 352 (4.9), 502 (2.6), 520 (0.5), 564 (1.5), 582 (0.8)

and 15% of acetone in petroleum ether to give β-cryptoxanthin **(9)** (R_f = 0.4, MgO TL) and β-citraurin **(10)** (R_f = 0.3, MgO TL), respectively.

Fraction 3 was submitted to TLC on silica which was developed with ether. Three bands (3.1−3.3) were scrapped off. Band 3.1 (R_f = 0.7, silica gel TL) had at least three pigments when separated by TLC on MgO with 40% acetone in petroelum ether as mobile phase and was discarded. Further purification of band 3.2 (R_f = 0.5, silica gel TL) by TLC on MgO developed with 25% acetone in petroleum ether gave antheraxanthin **(11)** (R_f = 0.8, MgO TL) and violaxanthin **(12)** (R_f = 0.2, MgO TL). From band 3.3 (R_f = 0.2, silica gel TL), neoxanthin **(13)** was purified by TLC on MgO with 30% acetone in petroleum ether as mobile phase (R_f = 0.3, MgO TL).

Immediately before mass spectrometry, all carotenoids were repurified through a neutral alumina minicolumn (grade III for carotenes, III or IV for the other carotenoids).

Co-chromatography was performed in a high-performance liquid chromatograph equipped with Kontron pumps and Waters PDA 990 photodiode array detector. The authentic samples (β-carotene, ζ-carotene, lycopene, β-cryptoxanthin and violaxanthin) were kindly provided by Hoffmann-La Roche, Basel, and β-citraurin was a gift from Prof. Molnár (Pécs, Hungary). The following stationary and mobile phase systems (at 1 mL/min) were employed: for carotenes, C_{18} Vydac 218 TP54 with methanol (MeOH) as mobile phase and for xanthophylls, a nitrile Nucleosil column with 20% of ethyl acetate (EtOAc) in hexane as mobile phase, both columns with 5 μm particle size, 250 × 4.6 i.d. mm.

For the NMR measurements, fraction 1 (from alumina column) was purified by semipreparative HPLC, with Nucleosil C_{18} column (5 μm, 250 × 10 i.d. mm) and 5% of EtOAc in MeOH at 4 mL/min, to give prolycopene (t_R = 18 min).

For the UV−visible spectra, petroleum ether was used as solvent, spectral fine structure is given as %III/II and relative intensity of cis peak as $\%A_B/A_{II}$ (Britton, 1995). The electron impact mass spectra were obtained with a VG model Quattro instrument. Direct insertion probe was used at an ionizing voltage of 70 eV and ion source temperature of 240 °C. The spectra obtained were compared with those presented in the literature (Enzell et al. 1969; Vetter et al., 1971; Enzell and Back, 1995). The nuclear magnetic resonance spectrum was recorded on a Bruker DRX-400 equipment, at 23 °C under argon in $CDCl_3$ (99.95%) that had been passed twice through an alumina minicolumn. Chemical shifts of ^1H and ^{13}C resonances (δ) were related to residual solvent signals. Complete proton and carbon line assignments were achieved by ^1H, ^{13}C, DEPT and H,H−COSY (correlated spectroscopy), and HMQC (heteronuclear multiquantum coherence) experiments.

Prolycopene **(5)** was submitted to isomerization catalyzed by I_2 and β-citraurin **(10)** to reduction reaction according to procedures described by Davies (1976) and Eugster (1995).

RESULTS AND DISCUSSION

Thirteen carotenoids from passion fruit were tentatively identified by their chromatographic behavior and UV−visible spectra and the identification confirmed by mass spectrometry and co-chromatography. The structures are shown in Chart 1, the characteristic MS fragments and molecular ions in Table 1.

Phytoene **(1)** and phytofluene **(2)** were collected together, located by the fluorescence of phytofluene under UV light. The UV−visible absorption spectrum of **1** showed λ_{max} at 277, 287, and 299 nm and no significant spectral fine structure (%III/II 10), whereas **2** displayed UV−visible λ_{max} at 332, 348, and 367 nm, with high spectral fine structure (%III/II 87). These values are consistent with acyclic carotenoids with three and five conjugated double bonds, respectively. The mass spectrum showed two peaks of high mass at m/z 544 ($C_{40}H_{64}$) and 542 ($C_{40}H_{62}$), corresponding to the molecular ion of **1** and **2**, respectively. Characteristic fragments were also observed at m/z 405 $[M - 137]^+$, due to the loss of the 7,8-dihydro-ψ end group in **2**, and at m/z 337 $[M - 205]^+$ for **2** and 339 $[M - 205]^+$ for **1**, due to elimination of 7,8,11,12-tetrahydro-ψ end group, and at m/z 69, 137, and 205 for **1** and **2**. These fragments are due to the cleavage of the activated single bonds between nonconjugated double bonds, leading to the production of abundant ions at m/z 69, 137, and 205 separated by 68 mass units (mu) (Enzell et al., 1969). As expected, both carotenes did not show fragments corresponding to in-chain elimination of toluene and xylene.

The mass spectrum of β-carotene (**3**) showed the same molecular ion as those of lycopene and prolycopene, which is consistent with the formula $C_{40}H_{56}$. The assignment was confirmed by the absence of distinct terminal group fragmentation in the mass spectrum since the loss of the β-ring $[M - 137]^+$ results in a very low intensity peak. The ratio $[M - 92]^+/[M - 106]^+$ was 14.6, similar to the 12.9 ratio obtained by Schwieter et al. (1965). This value is a good indication of a bicyclic carotenoid (Britton and Young, 1993). The UV−visible absorption spectrum with λ_{max} at (425), 450, and 476 nm was typical of β-carotene, with low spectral fine structure (%III/II 25), consistent with nine conjugated double bonds in the polyene chain and two in β-rings. β-Carotene eluted together with an authentic sample in the reversed-phase HPLC system, with t_R of 16.0 min.

The mass spectrum of symmetrical ζ-carotene **(4)** showed the molecular ion at m/z 540, which is consistent with $C_{40}H_{60}$, and mass characteristic fragments at m/z

Table 2. NMR Data of Prolycopene from Passion Fruit

C−n°	^{1}H δ (ppm), signal multiplicity, coupling constant (Hz)	^{13}C δ (ppm)
1, 1′		131.73[a]
2, 2′	5.07, m	123.95
3, 3′	2.06, m	26.69
4, 4′	2.07, m	40.34
5, 5′		140.93[a]
6, 6′	6.11, d ($J = 11.7$)	122.47
7, 7′	6.30, dd ($J = 11.7$; $J = 11.6$)	126.20[b]
8, 8′	6.02, d ($J = 11.6$)	125.89
9, 9′		135.52
10, 10′	6.03, d ($J = 11.1$)	129.84
11, 11′	6.47, dd ($J = 11.1$; $J = 15.0$)	126.16[b]
12, 12′	6.25, d ($J = 15.0$)	136.10
13, 13′		136.36
14, 14′	6.20 (B,B′ part of AA′BB′ system)	131.97
15, 15′	6.57 (A,A′ part of AA′BB′ system)	129.73
16, 16′	1.651, s	25.66
17, 17′	1.561, s	17.62
18, 18′	1.797, s	16.60
19, 19′	1.998, s	24.74
20, 20′	1.871, s	12.69

[a,b] Assignment may be interchanged.

403 $[M - 137]^+$ and 137 due to the loss of the 7,8-dihydro-ψ end group. The higher abundance of the peak at *m/z* 446 (M − 94, 2.5%) than that of toluene elimination (M − 92, 0.5%) confirmed the presence of seven conjugated double bonds in the molecule (Enzell and Back, 1995), which was also indicated by the absorption maxima in the UV−visible spectrum at 378, 400, and 425 nm and high spectral fine structure (%III/II 103). ζ-Carotene eluted together with a synthetic sample in the reversed-phase HPLC system, with t_R of 17.5 min.

Prolycopene (**5**) and lycopene (**7**) show very similar mass spectra because the high ionization temperature to which the sample is exposed promotes isomerization, therefore it is not possible to distinguish these two carotenoids by MS. The mass spectra of **5** and **7** showed the molecular ion at *m/z* 536 ($C_{40}H_{56}$) and fragments at *m/z* 467 $[M - 69]^+$ and 69 (base peak), both characteristic of the ψ end group. Peaks corresponding to in-chain elimination at *m/z* 444 $[M - 92]^+$ and 430 $[M - 106]^+$ were also present. The UV−visible absorption spectrum of **7** displayed λ_{max} at 444, 470, and 500 nm and spectral fine structure (%III/II 65), whereas the spectrum of **5** showed a single broad absorption band (λ_{max} at 436 with shoulders at 415 and 461 nm) with little spectral fine structure. After iodine-catalyzed isomerization, the spectrum of **5** assumed the λ_{max} of **7** and increased spectral fine structure (%III/II 55). Lycopene eluted together with an authentic sample in the reversed-phase HPLC system, with t_R of 20.6 min.

The ^{1}H and ^{13}C NMR data (Table 2) of prolycopene (**5**) were identical with those found in the literature (Hengartner et al., 1992; Englert, 1995). The isomerization shift values ($\Delta\delta = \delta_{(Z)} - \delta_{(all-E)}$) found by comparing corresponding chemical shifts in the prolycopene and (*all-trans*)-lycopene were in agreement with those cited by Hengartner et al. (1992) and Englert (1995), proving unequivocally the configuration assigned to **5**.

The UV−visible spectrum of neurosporene **(6)** showed λ_{lmax} at 415, 439, and 468 nm with high spectral fine structure (%III/II 100), indicating an acyclic carotenoid with nine conjugated double bonds. The mass spectrum of **4** presented the molecular ion at *m/z* 538 and principal characteristic fragments at *m/z* 401 $[M - 137]^+$ and 69 (base peak), corresponding to elimination of 7,8-dihydro-ψ and ψ end groups, respectively.

As previously reported (Britton, 1995) for the UV−visible spectra, the degree of fine structure increased from phytoene (**1**) (with three conjugated double bonds) to carotenoid **4** (seven conjugated double bonds) and then decreased from **6** to **7** as the chromophore is extended. The ratio $[M - 92]^+/[M - 106]^+$ obtained from the mass spectra for ζ-carotene (2.25), neurosporene (1.25), and lycopene (0.94) decreased as the number of conjugated double bonds increased (from seven to nine and eleven, respectively). This behavior has already been pointed out by Enzell et al. (1968), Enzell (1969), Francis (1972), and Enzell and Back (1995). The ratio for prolycopene (1.36) was higher than that for lycopene, possibly because of steric influence or variations in conditions between runs; however, the relative importance of steric and eletronic effects remains uncertain (Enzell and Back, 1995).

Despite the presence of a peak due to contamination from silica gel (515 mu) in the mass spectrum, the presence of monoepoxy-β-carotene (**8**) was confirmed by the expected molecular ion at *m/z* 552, which is consistent with $C_{40}H_{56}O$. The loss of 80 mu (this peak was more intense than the molecular ion) due to the presence of an epoxide group at the possible positions 5,6 or 5,8 and the peaks at *m/z* 336, 205, and 165 indicated that this epoxy group was located on a nonhydroxylated β-ring. Since the mass spectrum cannot differentiate between 5,6- and 5,8-epoxide groups, the type of epoxide was assigned by the UV−visible λ_{max}, which were 20 nm lower (at 430 and 455 nm) than those of β-carotene, demonstrating the presence of one 5,8-epoxy group.

As expected, β-cryptoxanthin (**9**) presented the same UV−visible absorption maxima of β-carotene. The mass spectrum showed molecular ion at *m/z* 552, which is consistent with $C_{40}H_{56}O$, and fragments at *m/z* 534 $[M - 18]^{\bullet+}$ corresponding to the loss of a hydroxy group and 460 $[M - 92]^{\bullet+}$ due to toluene elimination from the isoprenic chain. The ratio $[M]^{\bullet+}/[M - 18]^+$ obtained was higher than 1.0, i.e., the intensity of the molecular ion peak exceeded that of loss of H_2O, indicating that the hydroxy group was not allylic. The ratio obtained in this work was 2.4, whereas Britton and Young (1993) reported a ratio of 4.0. β-Cryptoxanthin eluted together with an authentic sample in the reversed-phase HPLC system, with t_R of 12.2 min.

β-Citraurin (**10**) was found in only one of three different batches of fruits. The UV−visible absorption spectrum showed a single broad absorption band at 446 nm, becoming three-peaked (at 390, 421, and 446 nm) with λ_{max} at shorter wavelengths after reduction by $NaBH_4$. The mass spectrum showed the molecular ion at *m/z* 432 ($C_{30}H_{40}O_2$) and fragments at *m/z* 414 $[M - 18]^{\bullet+}$ due to the elimination of a hydroxyl group and at 279 $[M - 153]^+$ and 239 $[M - 193]^+$, corresponding to the loss of a β-ring with a hydroxyl substituent. After reduction, with the transformation of the aldehyde to a primary alcohol, the pigment gave the molecular ion at *m/z* 434 and fragments at *m/z* 416 and 398, corresponding to consecutive losses of one and two molecules of water. Authentic β-citraurin showed t_R of 12.1 min on HPLC with a nitrile column.

The UV−visible absorption spectrum of antheraxanhin (**11**) showed λ_{max} at 422, 447, and 474 nm, 6 nm lower than those of β-carotene, reflecting the presence of one epoxy group at the 5,6-position. The molecular

ion appeared at *m/z* 584, corresponding to the formula $C_{40}H_{56}O_3$, and characteristic mass fragments were observed at *m/z* 566 $[M - 18]^{\bullet+}$, corresponding to loss of a hydroxy group, and at 504 $[M - 80]^{\bullet+}$, 352 and 221, which indicate a ring with a hydroxyl and epoxide substituents. The ratio $[M]^+/[M - 18]^{\bullet+}$ obtained was 2.0, indicating that the hydroxy groups were not in allylic positions.

The absorption maxima at 416, 440, and 464 nm in the UV–visible spectrum of violaxanthin (**12**) were consistent with the presence of two epoxy substituents at the 5,6- and 5′,6′-positions. The mass spectrum showed the molecular ion at *m/z* 600 ($C_{40}H_{56}O_4$) and fragments at *m/z* 564 $[M - 18-18]^{\bullet+}$, due to the elimination of two hydroxyls, at 520 $[M - 80]^{\bullet+}$ and 440 $[M - 80-80]^{\bullet+}$, representing losses of one and two epoxide groups, respectively. The peaks at *m/z* 352, 221, and 181 indicated that the epoxy substituents were in rings with hydroxy groups. Violaxanthin eluted together with an authentic sample in the nitrile HPLC system, with t_R of 28.0 min.

Neoxanthin (**13**) displayed λ_{max} at 416, 438, and 466 nm. Like violaxanthin (**12**), **13** presented the molecular ion at *m/z* 600. The characteristic fragments at *m/z* 582 $[M - 18]^{\bullet+}$, 564 $[M - 18-18]^{\bullet+}$, 520 $[M - 80]^{\bullet+}$, and 502 $[M - 18-80]^{\bullet+}$, the latter being more intense than the molecular ion, allowed an easy distinction from **12**.

Aside from ζ-carotene (principal carotenoid), prolycopene and violaxanthin, the other carotenoids were present in very low or trace amounts, making their isolation very difficult.

Of the 13 carotenoids identified in the present study, phytoene, prolycopene, mutatochrome, β-citraurin, antheraxanthin, violaxanthin, and neoxanthin are being reported for the first time as passion fruit carotenoids. α-Carotene, reported by Pruth and Lal (1958) and Cecchi and Rodriguez-Amaya (1981), and γ-carotene detected by the latter authors were not found in the present study. Likewise, we did not detect β-apo-12′-carotenal, β-apo-8′-carotenal, mutatoxanthin, and auroxanthin which were reported by Leuenberger and Thommen (1972); auroxanthin together with aurochrome and cryptochrome were also found by Cecchi and Rodriguez-Amaya. Since the samples analyzed by the latter authors were processed, the 5,8,5′,8′-diepoxy carotenoids aurochrome, cryptochrome, and auroxanthin could have been formed from β-carotene, β-cryptoxanthin, and violaxanthin, respectively, during the processing. These carotenoids can also be produced during extraction and analysis, so reports on their natural occurrence should be taken with caution. This also applies to the β-carotene epoxide, mutatochrome, identified in the present investigation.

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