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Identification and Quantification of Carotenoids, By HPLC-PDA-MS/MS, from Amazonian Fruits

VERIDIANA V. DE ROSSO AND ADRIANA Z. MERCADANTE*

Department of Food Science, Faculty of Food Engineering, State University of Campinas (UNICAMP), P.O. Box 6121, CEP 13083-862 Campinas, SP, Brazil

The major and minor carotenoids from six fruits, buriti (*Mauritia vinifera*), mamey (*Mammea americana*), marimari (*Geoffrola striata*), peach palm (*Bactrys gasipaes*), physalis (*Physalis angulata*), and tucuma (*Astrocaryum aculeatum*), all native to the Amazonia region, were determined by high-performance liquid chromatography−photodiode array detector−mass spectrometry detector (HPLC-PDA-MS/MS), fulfilling the recommended criteria for identification. A total of 60 different carotenoids were separated on a C_{30} column, *all-trans*-β-carotene being the major carotenoid found in all fruits. The presence of apo-10′-β-carotenol, found in mamey, was not previously reported in foods. In addition, this is the first time that the identification of β-zeacarotene in natural sources is supported by MS data. The total carotenoid content ranged from 38 μg/g in marimari to 514 μg/g in buriti. All fruits analyzed can be considered good sources of provitamin A, especially buriti, with 7280 RE/100 g.

KEYWORDS: Carotenoids; HPLC-MS/MS; Amazonian fruits; buriti; mamey; marimari; palm oil; peach palm; physalis; tucuma

INTRODUCTION

Carotenoids are a class of natural pigments widely distributed in vegetables and fruits and also added as additives, being responsible for the yellow-reddish color of many foods. Apart from their colorant properties, the carotenoids are related to important functions and physiological actions, provitamin A activity being the most known one. In addition, a positive correlation has been observed between ingestion of vegetables and fruits containing carotenoids and prevention of several chronic−degenerative diseases, such as cancer, inflammation, cardiovascular disease, cataract, and age-related macular degeneration, among others (*1*−*5*).

Due to all of these beneficial actions, the composition of carotenoids in fruits and vegetables has been extensively reported. Nevertheless, because inconclusive or incorrect identifications of carotenoids are found in the literature, it is strongly recommended that the following minimum criteria for identification be fulfilled: UV−visible spectrum (maximum absorption wavelengths (λ_{max}), spectral fine structure, peak *cis* intensity) should be in agreement with the chromophore suggested; chromatographic properties verified in two systems, including cochromatography with an authentic standard, and a mass spectrum should be obtained, allowing at least the confirmation of the molecular mass (*6*−*8*). However, the mass spectrometry (MS) technique does not distinguish stereoisomers (*9*), and for this purpose nuclear magnetic resonance (NMR) spectroscopy must be employed (*10*−*12*).

Unfortunately, in most of the studies concerning the carotenoid composition in fruits and vegetables, including some tropical ones, the carotenoid identification was solely based on elution order on the chromatographic column and the UV−visible spectra characteristics; sometimes coelution with standard and chemical derivatizations were included (*13*−*20*).

The Amazon basin is rich in genetic resources of fruits and oleaginous plants, and their economic exploitation is potentially of great importance for the region. Among the fruits and oleaginous plants extracted in the Amazon, many are exceptionally rich in micronutrients, particularly in antioxidants, such as carotenoids, anthocyanins, and other polyphenols. In addition, some of the Amazonian fruits can be considered ideal carotenoid natural sources for consumption or supplementation because the concomitant presence of high amounts of oil and carotenoids in these fruits considerably improves the oral bioavailability of these compounds (*21*, *22*).

In the present paper, the major and minor carotenoids from seven fruits native to the Amazonian region were identified by high-performance liquid chromatography−photodiode array detector−mass spectrometry (HPLC-PDA-MS/MS), fulfilling the recommended above criteria.

MATERIALS AND METHODS

Materials. Methanol (MeOH), methyl *tert*-butyl ether (MTBE), ethyl acetate (EtOAc), and acetonitrile for HPLC were obtained from Merck (Darmstadt, Germany) or from Mallinckrodt Baker (Philipsburg, NJ). The other reagents were all of analytical grade from Labsynth (Diadema, Brazil). The samples and solvents were filtered through Millipore (Billerica, MA) membranes (0.22 and 0.45 μm) prior to HPLC analysis.

* Corresponding author (e-mail azm@fea.unicamp.br; fax 55-19-3521-2153).

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Standards of *all-trans*-lutein, *all-trans*-zeaxanthin, *all-trans*-β-cryptoxanthin, *all-trans*-α-carotene, *all-trans*-β-carotene, 9-*cis*-β-carotene, 13-*cis*-β-carotene, 15-*cis*-β-carotene, *all-trans*-γ-carotene, *all-trans*-lycopene, 9-*cis*-lycopene, *all-trans*-β-zeacarotene, *cis*-β-zeacarotene, β-apo-8′-carotenal, β-apo-10′-carotenal, and β-apo-12′-carotenal were provided by Dr. Werner Simon from DSM Nutritional Products (Basel, Switzerland), showing purity between 95 and 99% by HPLC.

Samples. The fruits from the Amazonian region, buriti (*Mauritia vinifera*), mamey (*Mammea americana*), marimari (*Geoffrola striata*), peach palm (*Bactrys gasipaes*), physalis (*Physalis angulata*), and tucuma (*Astrocaryum aculeatum*), were obtained in Manaus City (Amazonas State, Brazil) and kept frozen at −18 °C until analysis. The commercial palm oil (*Elaeis guineensis*) was acquired in a supermarket in Campinas City, São Paulo State, Brazil. The peel and seeds of the fruits, about 2 kg each, were manually removed, and the pulp was homogenized.

Carotenoid Extraction. The carotenoids were exhaustively extracted from the pulps with acetone, transferred to petroleum ether (30−70 °C)/diethyl ether, and saponified overnight at room temperature with 10% methanolic KOH (*13*−*15*). Due to the high oil content in tucuma, palm oil, buriti, and peach palm, it was necessary to physically remove the oil, as follows: prior to ether transference, the carotenoid extract was kept in the freezer (temperature < −18 °C) for 2 h, followed by filtration using cold glassware and washing with cold acetone.

HPLC-PDA-MS/MS. The analysis was carried out in a Shimadzu HPLC (Kyoto, Japan) equipped with quaternary pumps (model LC-20AD), on-line degasser, and a Rheodyne injection valve (Rheodyne LCC, Rohnert Park, CA) with a 20 μL loop. The equipment included, connected in series, a PDA detector (Shimadzu, model SPD-M20A) and a mass spectrometer with an ion-trap analyzer and APCI ionization source from Bruker Daltonics, model Esquire 4000 (Bremem, Germany). The UV−visible spectra were obtained between 250 and 600 nm, and the chromatograms were processed at 450 nm. The MS parameters were as follows: positive mode; current corona, 4000 nA; source temperature, 450 °C; dry gas, N_2, temperature, 350 °C; flow, 60 L/h; nebulizer, 5 psi; MS/MS fragmentation energy, 1.4 V. The mass spectra were acquired with scan range of *m*/*z* from 100 to 700.

For all of the samples, carotenoid separation was carried out on a C_{30} YMC column (3 μm, 250 × 4.6 mm i.d.) (Waters, Wilmington, MA) using as mobile phase a linear gradient of MeOH with 0.1% triethylamine (TEA)/MTBE from 95:5 to 70:30 in 30 min, to 50:50 in 20 min, and maintaining this proportion for 35 min. The flow rate was 0.9 mL/min, and the column temperature was set at 22 °C. The same samples were also separated on a C_{18} Novapak (Waters) column (4 μm, 4.0 × 300 mm) using as mobile phase a linear gradient of acetonitrile (0.1% TEA)/water/EtOAc from 88:10:2 to 85:0:15 in 25 min, maintaining this proportion until the end of the run, at 1 mL/min, and the column temperature was set at 29 °C (data not discussed). Although addition of TEA to the mobile phase increased the carotenoid recovery from the column (*23*, *24*) and improved the peak separation, this base was excluded from the mobile phase when the samples were analyzed by the MS detector because TEA shows high proton affinity, being more easily ionized in the APCI source, and as a result the carotenoid ion signals decreased.

The carotenoids were identified according to the following combined information: elution order on C_{30} and C_{18} HPLC columns, cochromatography with authentic standards, UV−visible spectrum (λ_{max}, spectral fine structure, peak *cis* intensity), and mass spectrum compared with data available in the literature (*9*, *10*, *24*−*31*). In addition, reduction with $NaBH_4$ was also carried out (*32*).

The carotenoids were quantified by HPLC, using external calibration curves for lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene, γ-carotene, and lycopene with a minimum of five concentration levels. Neoxanthin, violaxanthin, and luteoxanthin were quantified using the curve of lutein; the β-cryptoxanthin epoxides, α-cryptoxanthin, and zeinoxanthin using the curve of β-cryptoxanthin; β-carotene epoxides by the curve of β-carotene; and the *cis* isomers of lycopene, α-carotene, β-carotene, δ-carotene, and γ-carotene using the curve of the corresponding *all-trans* isomer. The NAS−NRC conversion factor was used to calculate the vitamin A value (*33*).

RESULTS AND DISCUSSION

Carotenoid Identification. The characteristics of the carotenoids separated in all of the fruits analyzed are shown in **Table 1**, and the identification is discussed according to the elution order on the C_{30} column. The MS and MS/MS spectra figures are presented in supplemental Figure 1 (Supporting Information).

10′-Apo-β-caroten-10′-ol (peak 1a). The UV−visible spectrum, λ_{max} at 380, 400, and 421 nm, defined spectral fine structure (III/II = 33%), and the protonated molecule at *m*/*z* 379 was similar to the data from the literature for apo-10′-β-carotenol isolated from flowers (*34*). In addition, the identity was confirmed by coelution with the product obtained by reduction with $NaBH_4$ of the apo-10′-β-carotenal standard. Among the fruits analyzed, apo-10′-β-carotenol was found in only mamey. This is the first report of the occurrence of apo-10′-β-carotenol in foods, because it has been previously found in only petals of white and yellow roses (*34*).

Neoxanthin. all-trans-Neoxanthin (peak 2) and 9-*cis*-neoxanthin (peak 3) showed characteristic UV−visible spectra, with a hypsochromic shift of 4 nm, decreased spectral fine structure, and high intensity of the *cis* peak for the *cis* isomer compared to the corresponding *all-trans* isomer. The identification as 9-*cis* was supported by cochromatography with neoxanthin extracted from kale (*29*), which coeluted with peak 3. The molecular mass of neoxanthin was confirmed by the protonated molecule at *m*/*z* 601 and by consecutive losses of three hydroxy groups, at *m*/*z* 583 $[M + H - 18]^+$, 565 $[M + H - 18 - 18]^+$, and 547 $[M + H - 18 - 18 - 18]^+$ from the protonated molecule, verified in the MS/MS. In addition, other fragments were detected, both from the MS/MS and in-source fragmentations, at *m*/*z* 393, resulting from the cleavage of the double bond allylic to the allenic carbon, as well as the fragment at *m*/*z* 221 corresponding to an epoxy substituent in a β-ring with a hydroxyl group (*9*, *30*, *31*). It is worth highlighting that the fragments resultant from the loss of 80 mass units (u), which are characteristic of epoxy carotenoids ionized by electron impact (*9*, *30*, *31*), were not found using APCI in the present study. Both isomers of neoxanthin were found in only tucuma.

Neochrome. The presence of the 5,8-furanoid group in peak 4a was indicated by its UV−visible spectrum with λ_{max} 20 nm lower than that of neoxanthin. However, the mass spectra cannot differentiate between the 5,6-epoxy and 5,8-furanoid groups because the high ionization temperature itself promotes this rearrangement. The MS showed the presence of another compound, and thus only the fragment at *m*/*z* 583 $[M + H - 18]^+$ was assigned. Peak 4, which contains neochrome, was found in only mamey.

Apo-12′-violaxanthal. Peak 5 was identified as apo-12′-violaxanthal, considering the lack of fine structure in the UV−visible spectrum, elution order, and the molecular mass compared to the literature (*29*, *35*). The MS/MS showed an intense fragment at *m*/*z* 365, due to the loss of water from the protonated molecule. In fact, the loss of water was previously verified in the APCI mass spectra of steroids, which present in the structure a carbonyl alone or along with an alcohol function (*36*). The apo-12′-violaxanthal was identified in only mamey, which contained also considerable amounts of *all-trans*- and *cis*-violaxanthin, suggesting that this apocarotenoid may be derived from oxidative degradation of violaxanthin.

Violaxanthin. Peak 7 was identified as *all-trans*-violaxanthin considering the UV−visibled spectrum characteristics, the protonated molecule at *m*/*z* 601, and the fragments at *m*/*z* 583 $[M + H - 18]^+$ and 565 $[M + H - 18 - 18]^+$, due to losses of hydroxyl groups, and at *m*/*z* 221, all formed from 601 u at

Table 1. Chromatographic, UV–Vis, and Mass Spectroscopy Characteristics of Carotenoids from Amazonian Fruits, Obtained by HPLC-PDA-MS

peak[a]	carotenoid	t_R[b] (min)	λ_{max}[c] (nm)	% III/II	% A_B/II	$[M + H]^+$ (*m*/*z*)	fragment ions (*m*/*z*)
1a	10′-apo-β-caroten-10′-ol	8.0	380, 400, 421	33	0	379	361 [M + H – 18], 305 [M + H – 56], 255, 223
1b	not identified 1	8.0	nd[e]	nd	nd	601	583 [M + H – 18], 565 [M + H – 18 – 18], 547 [M + H – 18 – 18 – 18], 509 [M + H – 92], 491 [M + H – 18 – 92], 393, 221
2	*all-trans*-neoxanthin	8.0–8.2	416, 442, 468	78	0	601	583 [M + H – 18], 565 [M + H – 18 – 18], 547[M + H – 18 – 18 – 18], 509 [M + H – 92], 491 [M + H – 18 – 92], 393, 221
3	9-*cis*-neoxanthin	8.5	327, 416, 438, 468	75	31	601	583 [M + H – 18], 565 [M + H – 18 – 18], 547 [M + H – 18 – 18 – 18], 509 [M + H – 92], 393, 221
4a	neochrome	9.4	399, 424, 447	nc[f]	nc	601	583 [M + H – 18]
4b	not identified 2	9.4	nd	nd	nd	569	551 [M + H – 18], 387, 218
5	apo-12′-violaxanthal[d]	10.0	437	0	0	383	365 [M + H – 18]
6	not identified 3	10.9	443	0	0	411	391, 355, 329, 259
7	*all-trans*-violaxanthin	11.1	422, 444, 471	82	0	601	583 [M + H – 18], 565 [M + H – 18 – 18], 509 [M + H – 92], 491 [M + H – 18 – 92], 221
8	*cis*-violaxanthin	11.8–12.5	326, 410, 434, 463	78	40	601	583 [M + H – 18], 565 [M + H – 18 – 18], 491 [M + H – 18 – 92], 221
9	luteoxanthin	14.1	400, 421, 444	100	0	601	583 [M + H – 18]
10	*all-trans*-lutein	14.1–15.2	420, 444, 472	60	0	569	551 [M + H – 18], 533 [M + H – 18 – 18], 477 [M + H – 92], 463 [M + H – 106], 459 [M + H – 18 – 92]
11	*cis*-8′-apo-caroten-8′-al 1	14.5	265, 455	0	12	417	399 [M + H – 18], 359, 333, 317
12	*cis*-lutein	14.7	353, 418, 439, 469	27	20	569	551 [M + H – 18]
13	*cis*-8′-apo-caroten-8′-al 2	14.9	265, 452	0	10	417	399 [M + H – 18], 359, 333, 317
14	*all-trans*-zeaxanthin	15.7–16.1	425, 450, 476	20	0	569	551 [M + H – 18], 533 [M + H – 18 – 18], 463 [M + H – 106]
15	phytoene	17.0–18.3	276, 286, 300	0	0	545	489, 435, 395, 339 [M – 205]
16	5,6-epoxy-β-cryptoxanthin	18.3	420, 445, 471	52	0	569	551 [M + H – 18], 459 [M + H – 18 – 92], 221
17	5,8-epoxy-β-cryptoxanthin	19.6–20.4	420, 427, 456	50	0	569	551 [M + H – 18], 459 [M + H – 18 – 92], 221
18	zeinoxanthin	20.4–20.9	423, 445, 472	60	0	553	535 [M + H – 18], 495, 443, 361
19	*cis*-phytofluene	20.4–21.0	330, 347, 366	66	0	543	461, 406 [M + H – 137], 338 [M + H – 205]
20	5,6-epoxy-α-carotene[d]	21.5	418, 441, 469	10	0	553	535 [M + H – 18], 495, 205
21	not identified 4	23.2–23.4	420, 449, 477	27	0	553	535 [M + H – 18], 443
22	15-*cis*-α-carotene or 13-*cis*-α-carotene	23.3	330, 418, 441, 467	11	45	537	481 [M + H – 56], 444 [M – 92]
23	5,6-epoxy-β-carotene	23.5	418, 445, 471	50	0	553	535 [M + H – 18], 461 [M + H – 92], 205
24	13-*cis*-α-carotene or 13′-*cis*-α-carotene	23.7	330, 417, 438, 466	31	43	537	481 [M + H – 56], 444 [M – 92]
25	*all-trans*-α-cryptoxanthin	23.8–24.0	420, 445, 473	61	0	553	535 [M + H – 18], 479, 461 [M + H – 92], 439
26	*all-trans*-phytofluene	24.4	330, 347, 366	94	0	543	461, 406 [M + H – 137], 338 [M + H – 205]
27	*all-trans*-β-cryptoxanthin	25.0	421, 450, 476	20	0	553	535 [M + H – 18], 495, 461 [M + H – 92]
28	di-*cis*-α-carotene[d]	25.2	331, 416, 437, 464	25	32	537	481 [M + H – 56], 444 [M – 92], 413
29	15-*cis*-β-carotene	26.1–26.3	337, 420, 449, 472	10	61	537	444 [M – 92]
30	*cis* isomers mixture	26.8	334, 345, 367, 439	nc	52	537	444 [M – 92]
31	5,8-epoxy-β-carotene	26.8	405, 430, 452	nc	nc	553	535 [M + H – 18], 461 [M + H – 92], 205
32	13-*cis*-β-carotene	26.6–27.9	338, 420, 444, 470	12	47	537	444 [M – 92]
33	not identified 5	28.3	470	0	0	nd	439, 421, 397, 369
34	not identified 6	29.2	470	0	0	575	501, 463, 436, 339, 311, 265
35	not identified 7	29.4	401, 424, 449	20	0	537	nd
36	di-*cis*-β-carotene 1[d]	29.7	337, 420, 444, 470	8	40	537	444 [M – 92]
37	*all-trans*-ζ-carotene	29.7	379, 399, 423	108	0	541	472 [M + H – 69], 404 [M + H – 137], 364, 337
38	*all-trans*-α-carotene	29.9	420, 445, 473	66	0	537	481 [M + H – 56], 444 [M – 92]
39	di-*cis*-β-carotene 2[d]	31.1–31.8	338, 415, 440, 469	14	13	537	444 [M – 92]
40	not identified 8	32.1	313, 402, 427, 452	50	11	537	nd
41	9-*cis*-α-carotene	33.6	330, 420, 444, 472	60	09	537	481 [M + H – 56], 444 [M – 92]
42	*all-trans*-β-carotene	35.0–35.8	421, 452, 478	25	0	537	444 [M – 92]
43	*all-trans*-β-zeacarotene	35.9–36.8	402, 428, 453	62	0	539	455, 402 [M + H – 137], 310 [M + H – 137 – 92]
44	9-*cis*-β-carotene	37.1–38.0	338, 420, 447, 472	20	18	537	444 [M – 92]
45	mixture	38.5	433, 454	nc	nc	537	nd

Table 1. (Continued)

peak[a]	carotenoid	t_R[b] (min)	λ_{max}[c] (nm)	% III/II	% A_B/II	$[M + H]^+$ (*m*/*z*)	fragment ions (*m*/*z*)
46	*cis*-δ-carotene 1	39.8–40.0	348, 430, 453, 481	20	42	537	444 [M – 92]
47	*cis*-β-zeacarotene 1	42.1–42.6	403, 428, 453	56	0	539	nd
48	not identified 9	42.5	312, 420, 447, 473	nc	nc	537	444 [M – 92]
49	*cis*-δ-carotene 2	43.6	349, 430, 454, 484	41	35	537	nd
50	*cis*-β-zeacarotene 2	43.9	403, 428, 453	60	0	539	455, 402 [M + H – 137], 310 [M + H – 137 – 92]
51	*cis*-δ-carotene 3	44.2	349, 430, 453, 482	41	42	537	444 [M – 92]
52	*all-trans*-δ-carotene	46.8	430, 455, 485	50	0	537	481 [M + H – 56], 444 [M – 92]
53	*cis*-δ-carotene 4	48.2	350, 428, 454, 482	45	13	537	nd
54	*cis*-γ-carotene 1	52.1	300, 360, 429, 453, 482	45	18	537	nd
55	*cis*-γ-carotene 2	52.4	300, 360, 435, 459, 491	44	30	537	nd
56	*cis*-γ-carotene 3	54.4–55.4	431, 461, 492	50	0	537	467 [M – 69], 444 [M – 92]
57	*all-trans*-γ-carotene	54.6–56.5	430, 461, 492	68	0	537	467 [M – 69], 444 [M – 92]
58	*cis*-γ-carotene 4	58.3	300, 360, 435, 460, 491	65	16	537	467 [M – 69], 444 [M – 92]
59	*cis*-γ-carotene 5	59.3	300, 360, 434, 459, 491	66	22	537	nd
60	9-*cis*-lycopene	72.3	290, 360,440, 466, 497	75	12	537	467 [M – 69], 444 [M – 92]

[a] Numbered according to **Figures 2**–**8**. [b] Retention time on the C_{30} column. [c] Linear gradient of methanol/MTBE. [d] Tentative identification. [e] Not detected. [f] Not calculated.

both MS/MS and in-source fragmentations. Peak 8, identified as *cis*-violaxanthin, had a similar mass spectrum, lower λ_{max} and spectral fine structure values, and high *cis* peak intensity compared to peak 7. Violaxanthin was detected in only mamey, and its *cis* isomer was also found in tucuma.

Luteoxanthin. Peak 9 was tentatively identified as luteoxanthin considering the UV−visible spectrum characteristics, the protonated molecule at *m*/*z* 601, and the fragment at *m*/*z* 583 $[M + H - 18]^+$ formed in-source. However, the ion at *m*/*z* 221, characteristic of an epoxy substituent in a ring with a hydroxyl group, was not detected. Luteoxanthin was detected in only mamey, being most probably formed by an epoxy-furanoid rearrangement from violaxanthin.

8′-Apo-caroten-8′-al. Two *cis* isomers of 8′-apo-caroten-8′-al (peaks 11 and 13) were identified by their UV−visible spectra, both with no spectral fine structure. The mass spectra of both isomers showed the protonated molecule at *m*/*z* 417, along with the fragment at *m*/*z* 399 $[M + H - 18]^+$, as observed in the *all-trans*-8′-apo-caroten-8′-al standard MS spectrum. The *cis* isomers also eluted at retention times similar to those of the isomers formed by thermal isomerization of *all-trans*-8′-apo-caroten-8′-al. Although not found in the samples analyzed, the *all-trans*-8′-apo-caroten-8′-al standard eluted in 19 min and showed λ_{max} at 462 nm in the same chromatographic conditions. The 8′-apo-caroten-8′-al isomers were detected in only mamey.

Lutein. *all-trans*-Lutein (peak 10) and *cis*-lutein (peak 12) showed characteristic UV−visible spectra, with a hypsochromic shift of 5 nm for the *cis* isomer. The identifications of both lutein isomers were indicated by their mass spectra with the protonated molecule at *m*/*z* 569 and fragments at *m*/*z* 551 $[M + H - 18]^+$ and 533 $[M + H - 18 - 18]^+$. In addition, the MS/MS showed the presence of fragments at *m*/*z* 477 and 463, resulting from the respective losses of toluene ($[M + H - 92]^+$) and xylene ($[M + H - 106]^+$) from the polyene chain, and at *m*/*z* 459 due to consecutive losses of hydroxyl and toluene. Another characteristic feature of the APCI-MS, previously observed with electron impact ionization (*25*, *31*), was the fragment with 551 u corresponding to the loss of the hydroxyl group in the ϵ-ring, with higher intensity than the protonated molecule (569 u). Coelution with the *all-trans*-lutein standard confirmed the identity. Lutein was found in physalis, tucuma, buriti, marimari, and palm oil.

Zeaxanthin. Peak 14 was identified as *all-trans*-zeaxanthin considering the UV−visible and mass spectra characteristics and confirmed by coelution with the *all-trans*-zeaxanthin standard. As expected, the mass spectrum showed the protonated molecule at *m*/*z* 569 and fragments at *m*/*z* 551 $[M + H - 18]^+$ and 533 $[M + H - 18 - 18]^+$. The higher intensity of the protonated molecule peak (569 u) compared to the fragment at *m*/*z* 551 indicated that the hydroxyl group was not allylic to the double bond, in contrast to that observed for lutein. Zeaxanthin was detected in mamey, marimari, physalis, and tucuma.

Phytoene. Peak 15 was identified as phytoene by comparing the UV−visible spectrum (λ_{max} and fine structure) with that given in the literature (*29*). The mass spectrum showed the molecular ion at *m*/*z* 545 and the most abundant fragment ion in the MS−MS spectrum at *m*/*z* 339, corresponding to the cleavage of the closest single bond allylic to the conjugated triene. The geometrical isomer form was not assigned because the UV−visible and mass spectra of *cis* and *all-trans* isomers would be identical, although the 15-*cis*-phytoene is more abundant in plants and in most microorganisms (*12*, *29*). Phytoene was detected in buriti, mamey, marimari, and physalis.

β-Cryptoxanthin Epoxides. 5,6-Epoxy-β-cryptoxanthin (peak 16) and 5,8-epoxy-β-cryptoxanthin (peak 17) were identified considering the UV−visible spectrum characteristics, as λ_{max} was 18 nm lower for the 5,8-furanoid group than that of the 5,6-epoxide. However, the mass spectra of both epoxides were very similar, with the protonated molecule at *m*/*z* 569 and fragment ions at *m*/*z* 551 due to loss of a hydroxyl group and at *m*/*z* 221, indicating that the epoxide was in a ring with a hydroxyl group. Both epoxides were found in marimari and physalis, whereas 5,6-epoxy-β-cryptoxanthin was detected in buriti.

Phytofluene. The *cis* (peak 19) and *all-trans* isomers (peak 26) showed the same absorption wavelengths; nevertheless, the spectral fine structure of the *cis* isomer (III/II = 66%) was much lower than that of the *all-trans*-phytofluene (III/II = 94%). The protonated molecule was detected at *m*/*z* 543 in the mass spectra; in addition, the MS/MS showed the presence of fragments at *m*/*z* 406 and 338, formed by bis-allylic cleavage of the single bonds between the C-7 and C-8 $[M + H - 137]^+$ and the C-11 and C-12 $[M + H - 205]^+$. The presence of both isomers was

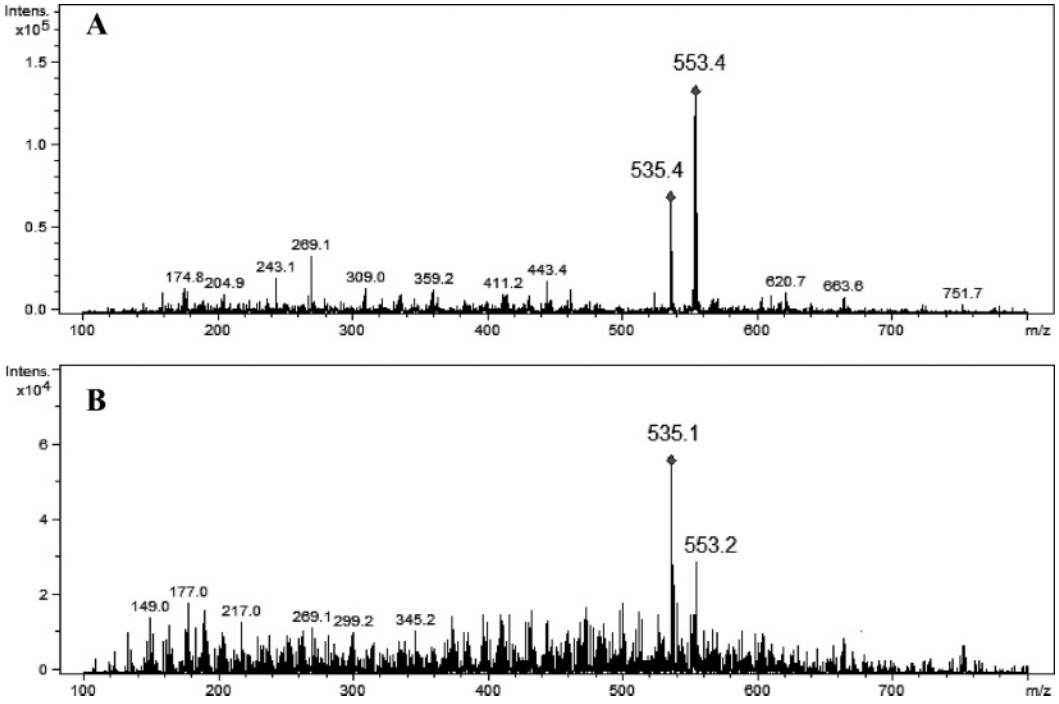

Figure 1. Mass spectra with APCI of (**A**) zeinoxanthin and (**B**) α-cryptoxanthin.

observed only in physalis; *cis*-phytofluene was found in marimari and *all-trans*-phytofluene in mamey.

5,6-Epoxy-α-carotene. Peak 20 was tentatively identified as 5,6-epoxy-α-carotene considering the UV−visible and mass spectra similar to the data from the literature (*29*). The mass spectrum showed the protonated molecule at *m*/*z* 553 and fragments at *m*/*z* 535 and 205, resulting, respectively, from the loss of a hydroxyl group and an epoxide group. Although the λ_{max} was 4 nm lower than that observed for the 5,6-epoxy-*β*-carotene, the highest spectral fine structure excluded the assignment of peak 20 as a *cis* isomer of 5,6-epoxy-*β*-carotene. In addition, this carotenoid was found in only palm oil, which is rich in α-carotene.

Zeinoxanthin and α-Cryptoxanthin. Zeinoxanthin (peak 18) and α-cryptoxanthin (peak 25) have the same chemical formula ($C_{40}H_{56}O$) and, therefore, protonated molecule (*m*/*z* 553) and also the same chromophore. The only structural difference between them is the position of the hydroxyl group, which is located either in the *ϵ*-ring allylic to the double bond as in the case of α-cryptoxanthin or in the *β*-ring as in zeinoxanthin. The differentiation between zeinoxanthin and α-cryptoxanthin was carried through comparison of the intensity of the protonated molecule peak (*m*/*z* 553) with that of the fragment of 535 u $[M + H - 18]^+$. Zeinoxanthin showed a more intense protonated molecule peak compared to the fragment with 535 u (**Figure 1A**), whereas for α-cryptoxanthin the contrary was observed (**Figure 1B**), confirming the facility of the loss of the hydroxyl group allylic to the double bond, as reported in the literature (*26*). Physalis and tucuma contained both xanthophylls, whereas zeinoxanthin was found in palm oil and α-cryptoxanthin in buriti, peach palm, and marimari.

β-Cryptoxanthin. Peak 27 was identified as *all-trans-β*-cryptoxanthin, with the UV−visible spectrum similar to those from zeaxanthin and *β*-carotene, all possessing the same chromophore. As expected, the protonated molecule was detected at *m*/*z* 553, along with less intense fragments at *m*/*z* 535 $[M + H - 18]^+$ and 461 $[M + H - 92]^+$, resulting from the loss of a hydroxyl group and toluene, respectively. The identification was confirmed through coelution with the *all-trans-β*-cryptoxanthin standard. *β*-Cryptoxanthin was detected in mamey, marimari, physalis, and tucuma.

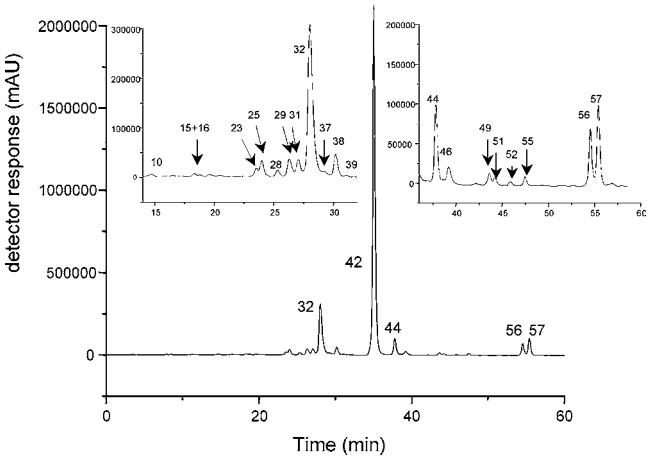

Figure 2. Chromatogram (processed at 450 nm), obtained by HPLC-PDA-MS, of the carotenoids from buriti. See text for chromatographic conditions. Peak identification and characterization are given in **Table 1**.

β-Carotene Epoxides. 5,6-Epoxy-*β*-carotene (peak 23) and 5,8-epoxy-*β*-carotene (peak 31) were identified considering the UV−visible spectra characteristics, with the presence of a 5,8-furanoid group indicated by its UV−visible spectrum with λ_{max} 15 nm lower than that of 5,6-epoxy-*β*-carotene. Because MS cannot differentiate between the 5,6-epoxy and 5,8-furanoid groups, both mass spectra showed the protonated molecule at *m*/*z* 553, and the MS/MS spectra showed the presence of fragment ions at *m*/*z* 551 and 461 corresponding to the losses of the hydroxyl group and toluene, respectively, and at *m*/*z* 205, which indicated the presence of a *β*-ring with an epoxy group. The 5,6-epoxy-*β*-carotene was found in buriti and the respective furanoid in buriti, mamey, marimari, physalis, and tucuma.

ζ-Carotene. The *all-trans-ζ*-carotene (peak 37) was identified considering the λ_{max} and high fine structure in UV−visible spectrum. The protonated molecule was detected at *m*/*z* 541, and the MS/MS showed the presence of fragments at *m*/*z* 472

Table 2. Carotenoid Composition and Vitamin A Value of Amazonian Fruits

fruit	carotenoid concentration (μg/g)	total carotenoid (μg/g)	vitamin A value (RE/100 g)
buriti	*all-trans*-β-carotene (372.32), 13-*cis*-β-carotene (59.23), 9-*cis*-β-carotene (18.57), *all-trans*-γ-carotene (14.76), *cis*-γ-carotene 3 (9.88), 15-*cis*-β-carotene (8.87), 5,8-epoxy-β-carotene (7.44), *cis*-δ-carotene 1 (5.46), *cis*-δ-carotene 2 (3.67), *all-trans*-α-carotene (3.23), *cis*-δ-carotene 3 (2.42), *cis*-γ-carotene 2 (2.33), *all-trans*-δ-carotene (2.09), *all-trans*-α-cryptoxanthin (1.28), di-*cis*-α-carotene (1.25), 5,6-epoxy-β-carotene (0.41), phytoene (0.34), di-*cis*-β-carotene 2 (0.11), 5,6-epoxy-β-cryptoxanthin (0.10), *all-trans*-ζ-carotene (0.08), *all-trans*-lutein (0.03)	513.87	7280
mamey	*all-trans*-β-carotene (20.37), 10′-apo-β-caroten-10′-ol + not identified 1 (15.19), *cis*-8′-apo-caroten-8′-al 2 (4.76), *cis*-8′-apo-caroten-8′-al 1 (4.28), 9-*cis*-β-carotene (3.62), *all-trans*-phytofluene (2.32), *all-trans*-β-cryptoxanthin (1.71), not identified 5 (1.35), *all-trans*-violaxanthin (1.12), 13-*cis*-β-carotene (1.10), *cis*-violaxanthin (1.02), neochrome + not identified 2 (0.86), not identified 6 (0.72), *all-trans*-α-carotene (0.65), apo-12′-violaxanthal (0.65), *all-trans*-zeaxanthin (0.61), *all-trans*-β-zeacarotene (0.52), luteoxanthin (0.41), *all-trans*-γ-carotene (0.30), *cis*-β-zeacarotene 1 (0.28), *cis*-γ-carotene 4 (0.27), phytoene (0.21), *cis*-β-zeacarotene 2 (0.18), not identified 3 (0.03), *cis*-phytofluene + not identified 4 (0.03)	62.53	688
marimari	*all-trans*-β-carotene (23.21), 13-*cis*-β-carotene (4.87), 9-*cis*-β-carotene (3.75), 5,8-epoxy-β-carotene (1.56), 15-*cis*-β-carotene (1.07), *all-trans*-α-cryptoxanthin (0.67), *cis*-phytofluene (0.58), phytoene (0.38), not identified 7 (0.37), 5,8-epoxy-β-cryptoxanthin (0.36), 5,6-epoxy-β-cryptoxanthin (0.24), *all-trans*-β-cryptoxanthin (0.23), *all-trans*-lutein (0.21), *all-trans*-zeaxanthin (0.17), di-*cis*-β-carotene 2 (0.13), *all-trans*-α-carotene (0.11), not identified 4 (0.08)	37.98	605
palm oil	*all-trans*-β-carotene (65.77), *all-trans*-α-carotene (22.34), 9-*cis*-β-carotene (17.46), 9-*cis*-α-carotene (8.23), 13-*cis*-β-carotene (6.21), *cis*-γ-carotene 3 (2.89), *cis*-δ-carotene 1 (2.32), *all-trans*-γ-carotene (1.76), di-*cis*-α-carotene (0.44), 13-*cis*-α-carotene or 13′-*cis*-α-carotene (0.34), *cis* isomers mixture (0.31), 5,8-epoxy-β-carotene (0.24), 15-*cis*-α-carotene or 13-*cis*-α-carotene (0.21), di-*cis*-β-carotene 2 (0.13), 5,6-epoxy-α-carotene (0.13), *all-trans*-lutein (0.11), 5,8-epoxy-β-cryptoxanthin (0.10), di-*cis*-β-carotene 1 (0.04)	129.03	1535
peach palm	*all-trans*-β-carotene (55.51), *all-trans*-δ-carotene (45.77), *all-trans*-γ-carotene (35.43), *cis*-γ-carotene 4 (28.35), 9-*cis*-lycopene (8.44), *cis*-δ-carotene 1 (5.22), 13-*cis*-β-carotene (4.02), *cis*-γ-carotene 1 (3.25), *cis*-γ-carotene 2 (2.26), 9-*cis*-β-carotene (2.21), *cis*-γ-carotene 3 (2.11), *cis*-δ-carotene 2 (2.09), *all-trans*-α-carotene (1.78), *cis*-δ-carotene 3 (0.86), *cis*-γ-carotene 5 (0.13), *all-trans*-α-cryptoxanthin (0.12), 15-*cis*-β-carotene (0.08), 5,8-epoxy-β-carotene (0.03)	197.66	1491
physalis	*all-trans*-β-carotene (62.23), 9-*cis*-β-carotene (2.91), *all-trans*-α-cryptoxanthin (2.65), *all-trans*-β-cryptoxanthin (1.87), *all-trans*-lutein (1.44), zeinoxanthin (1.42), 13-*cis*-β-carotene (1.39), *all-trans*-α-carotene (1.25), 5,8-epoxy-β-carotene (1.03), 5,6-epoxy-β-cryptoxanthin (0.61), *cis*-phytofluene (0.55), 15-*cis*-β-carotene (0.49), *all-trans*-zeaxanthin (0.40), *cis*-lutein (0.35), *all-trans*-δ-carotene (0.35), not identified 4 (0.33), phytoene (0.30), 5,8-epoxy-β-cryptoxanthin (0.25), *all-trans*-phytofluene (0.26), *all-trans*-γ-carotene (0.23), *cis*-δ-carotene 4 (0.21), not identified 9 (0.20), *cis*-γ-carotene 3 (0.12), *cis*-γ-carotene 4 (0.05)	80.89	1108
tucuma	*all-trans*-β-carotene (47.36), *all-trans*-α-carotene (1.68), *all-trans*-β-cryptoxanthin (1.64), 13-*cis*-β-carotene (1.60), *all-trans*-α-cryptoxanthin (1.30), zeinoxanthin (1.02), *all-trans*-lutein (0.79), *cis*-γ-carotene 3 (0.89), not identified 8 (0.82), 15-*cis*-β-carotene (0.80), 5,8-epoxy-β-carotene (0.76), *cis*-β-zeacarotene 2 (0.65), *cis*-β-zeacarotene 1 (0.60), *all-trans*-δ-carotene (0.52), *all-trans*-β-zeacarotene (0.44), mixture (0.36), *all-trans*-γ-carotene (0.35), *all-trans*-neoxanthin (0.26), *cis*-violaxanthin (0.24), *cis*-neoxanthin (0.18), *all-trans*-zeaxanthin (0.16), *all-trans*-ζ-carotene (0.14), *cis*-lutein (0.04)	62.65	850

and 404, formed by bis-allylic cleavage between the C-3 and C-4 $[M + H - 69]^+$ and between the C-7 and C-8 $[M + H - 137]^+$ single bonds. The identification was confirmed through cochromatography with an authentic standard. This carotene (peak 37) was observed in buriti and tucuma.

α-Carotene Isomers. *all-trans*-α-Carotene (peak 38) shows the same chromophore as lutein, and therefore the UV−visible absorption spectrum resembled that of this xanthophyll. The identification was confirmed through chromatographic behavior, coelution with the *all-trans*-α-carotene standard, and mass spectrum. The *cis* isomers of α-carotene (peaks 22, 24, and 41) were tentatively identified beyond comparison of data from the literature (*24*, *28*). However, the information did not allow the differentiation between 15-*cis*- and 13-*cis*-α-carotene as peak 22, 13-*cis*- and 13′-*cis*-α-carotene as peak 24, and 9-*cis*- and 9′-*cis*-α-carotene as peak 41. Taking into consideration that a *cis* peak at 330 nm was observed in the UV absorption spectra of all of the mono-*cis* isomers of α-carotene (peaks 22, 24, and 41), a di-*cis*-α-carotene was also tentatively identified as peak 28 due to the presence of a peak at 331 nm. In addition, the mass spectra of all isomers (peaks 22, 24, 28, 38, and 41) showed the protonated molecule at *m*/*z* 537, with the most abundant fragment ions in the MS/MS spectra at *m*/*z* 481 and 444, corresponding to the losses of the ϵ-ring and toluene, respectively. *all-trans*-α-Carotene was detected in all samples, whereas the isomers were found in commercial palm oil.

β-Carotene Isomers. Peaks 29, 32, 42, and 44 were identified as 15-*cis*-, 13-*cis*-, *all-trans*-, and 9-*cis*-β-carotene, respectively, considering the UV−visible spectra characteristics, chromatographic behavior, coelution with standards, and mass spectra. Two di-*cis*-β-carotene (peaks 36 and 39) were tentatively identified considering the presence of the *cis* peak in the range of 337−338 nm, as found in all of the mono-*cis* isomers of β-carotene (peaks 29, 32, and 44). The mass spectra of all isomers of β-carotene showed the protonated molecule at *m*/*z* 537 and a fragment ion in the MS/MS at *m*/*z* 444 $[M - 92]^+$, corresponding to the loss of the toluene from the polyene chain.

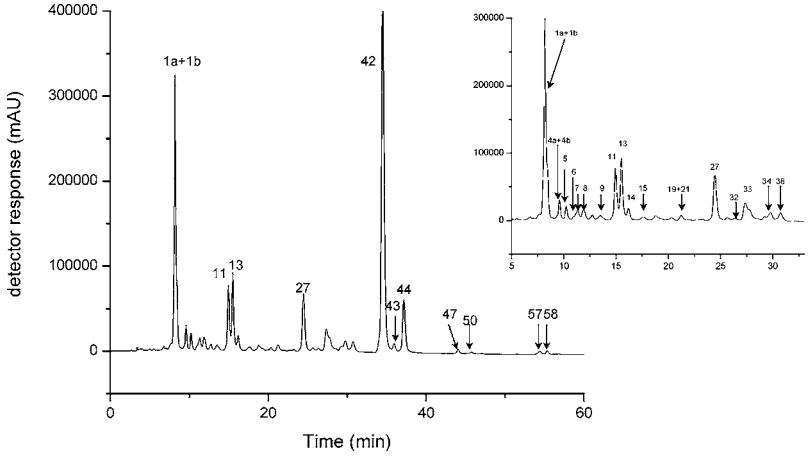

Figure 3. Chromatogram (processed at 450 nm), obtained by HPLC-PDA-MS, of the carotenoids from mamey. See text for chromatographic conditions. Peak identification and characterization are given in **Table 1**.

all-trans- and 13-*cis*-β-carotene were found in all fruits analyzed, whereas the other isomers were less widespread.

β-Zeacarotene Isomers. The *all-trans*-β-zeacarotene (peak 43) and its two *cis* isomers (peaks 47 and 50) were identified by comparison of UV−visible spectra, chromatographic behavior, mass spectra, and coelution with the standards. The mass spectrum of *all-trans*-β-zeacarotene showed the protonated molecule at *m*/*z* 539 and the fragment ions in the MS/MS spectrum at *m*/*z* 402 and 310, corresponding to the losses of the β-ring and of β-ring plus toluene, respectively. The differentiation between the *all-trans* and *cis* isomers of β-zeacarotene was done through the spectral fine structure values, with the *cis* isomers showing lower %III/II than the *all-trans*-β-zeacarotene, even though the λ_{max} values were similar for the *all-trans* and *cis* isomers. These three isomers of β-zeacarotene were found in tucuma and mamey. This is the first time that the identification of this carotenoid in natural sources is supported by MS data; in previous papers the identification was based solely on UV−visible spectrum information (*16*, *18*, *37*, *38*).

δ-Carotene Isomers. Peak 52 was identified as *all-trans*-δ-carotene by comparison of UV−visible and mass spectra with those given in the literature (*29*). Peaks 46, 49, 51, and 53 were tentatively assigned as *cis* isomers of δ-carotene considering the lower %III/II and λ_{max} values compared to *all-trans*-δ-carotene and the presence of the *cis* peak at 348−350 nm. As expected, the mass spectra of all isomers showed the protonated molecule at *m*/*z* 537, besides fragment ions in the MS/MS spectra at *m*/*z* 481 and 444, corresponding to losses of the ϵ-ring and toluene, respectively. In the MS/MS spectrum of peak 46 the fragment at *m*/*z* 481 was not detected. δ-Carotene was not found in mamey and marimari.

γ-Carotene Isomers. The identification of *all-trans*-γ-carotene (peak 57) was confirmed by comparison of UV−visible and mass spectra with data from the literature (*29*) and coelution with the standard of *all-trans*-γ-carotene. Peaks 54, 55, 58, and 59 were tentatively identified as *cis* isomers of γ-carotene due to the presence of *cis* peaks at 300 and 360 nm, whereas peak 56 was tentatively assigned as 5′-*cis*-γ-carotene due to the absence of the *cis* peak, identical λ_{max}, and lower spectral fine structure compared to those values of the corresponding *all-trans* isomer. The protonated molecule of all isomers of γ-carotene (peaks 54−59) was detected at *m*/*z* 537, and the MS/MS spectra showed fragments at *m*/*z* 467 and 444, due to elimination of the ψ-end group and toluene, respectively. γ-Carotene was found in all fruits but marimari.

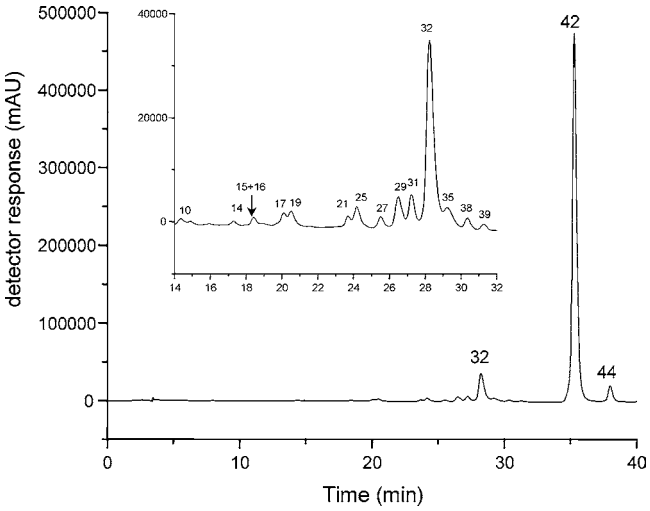

Figure 4. Chromatogram (processed at 450 nm), obtained by HPLC-PDA-MS, of the carotenoids from marimari. See text for chromatographic conditions. Peak identification and characterization are given in **Table 1**.

9-cis-Lycopene. Peak 60 was identified as 9-*cis*-lycopene, comparing the elution order on the C_{30} column, UV−visible and mass spectra characteristics with those given in the literature (*10*, *39*), and coelution with the authentic standard. The mass spectrum of 9-*cis*-lycopene showed the protonated molecule at *m*/*z* 537 and the fragment ions in the MS/MS spectrum at *m*/*z* 467 and 444, corresponding to losses of the ψ-end group and toluene, respectively.

Regarding the APCI ionization of carotenoids, it is interesting to highlight that the MS/MS spectra of xanthophylls showed that losses of toluene and xylene were derived from the protonated molecule $[M + H]^+$, whereas for carotenes the ions were formed by free radical fragmentation from the radical cation $[M]^+$. In addition, the fragment due to loss of the ϵ-ring, that when detected is of low intensity in the electron impact ionization (*29*), was observed at high intensity in the MS/MS of α-and δ-carotene using APCI, allowing its differentiation also by MS from other carotenes with the same molecular weight.

Quantitative Composition. According to **Table 2**, the total carotenoid content was much higher in buriti, followed by peach palm, palm oil, physalis, similar values for tucuma and mamey, and finally marimari.

The HPLC chromatogram of the carotenoids from buriti is shown in **Figure 2**, with the separation of 21 carotenoids. The

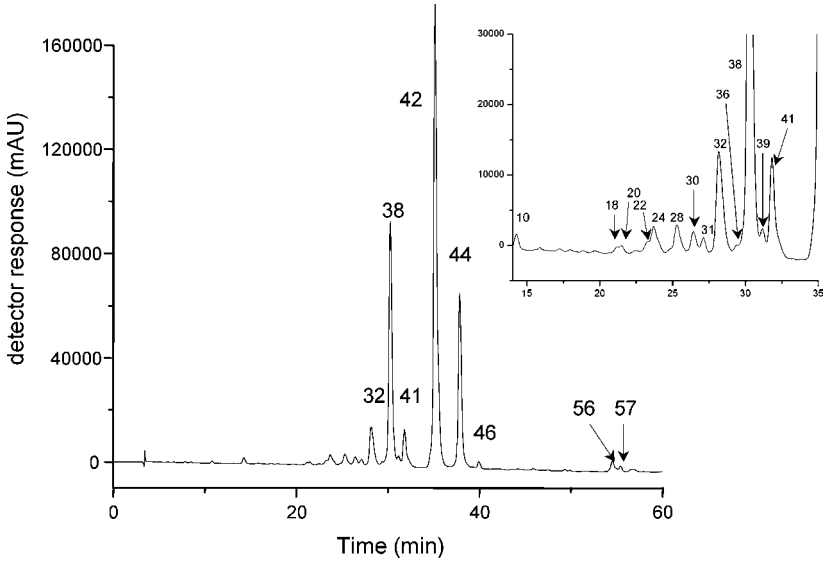

Figure 5. Chromatogram (processed at 450 nm), obtained by HPLC-PDA-MS, of the carotenoids from palm oil. See text for chromatographic conditions. Peak identification and characterization are given in **Table 1**.

all-trans-β-carotene was the major carotenoid, representing 72.4% of total content in this fruit, followed by 13-*cis-β*-carotene and 9-*cis-β*-carotene, which contributed 11.5 and 3.6%, respectively. The 18 minor carotenoids represented 12.5% of the total content. The level of *all-trans-β*-carotene found in this study was similar to those previously reported of 360 μg/g (*16*).

Figure 3 shows the chromatogram of the carotenoids from mamey, with the separation of 24 peaks, the principal carotenoids being *all-trans-β*-carotene, representing 32.6% of the total content, followed by 10′-apo-*β*-caroten-10′-ol (24.3%) and two *cis* isomers of 8′-apo-caroten-8′-al (7.6 and 6.8%). The level of *all-trans-β*-carotene (14.1 ± 4.1 μg/g) determined in a previous study (*16*) was lower than that found in the present study (20.37 μg/g). In addition, the identification of *all-trans-β*-apo-8′-carotenol and *all-trans-β*-apo-10′-carotenal is most probably incorrect in this other study, because it was based only on the visible absorption spectrum, chromatographic behavior, and reduction reaction (*16*).

Although 17 carotenoids from marimari were separated on the C_{30} column (**Figure 4**) and 15 identified, this fruit contained mainly *β*-carotene, with the *all-trans* isomer representing 61.1%, the 13-*cis* isomer 12.8%, and the 9-*cis* isomer 9.9% of the total carotenoid content in this fruit. The other minor carotenoids, each <4.1%, summed to 16.2% of the total content. As far as we know, this is the first report on the carotenoid composition of marimari.

The chromatogram, presented in **Figure 5**, of the carotenoids from palm oil shows a complex carotene mixture, with 13 carotenes among the 18 carotenoids separated. The *all-trans-β*-carotene was the major carotenoid, representing 51% of the total content followed by *all-trans*-α-carotene (17.3%), 9-*cis-β*-carotene (13.5%), and 9-*cis*-α-carotene (6.4%). Most probably due to the heat treatment used to achieve enzyme inactivation in the fruit, a high percentage of *cis* isomers, 30%, was found in the commercial palm oil. In another study using a C_{30} column, 13 carotenoids were separated, with *all-trans-β*-carotene and *all-trans*-α-carotene also as major carotenoids (*17*). However, the carotenoid levels were about 3 times higher than those found in the present study, and echinenone (1.5%), a carotenoid so far only found in algae, bacteria, and marine animals (*29*), was detected in palm oil (*17*).

Figure 6 presents the chromatogram of the carotenoids of peach palm, showing the preponderance of *all-trans-β*-carotene

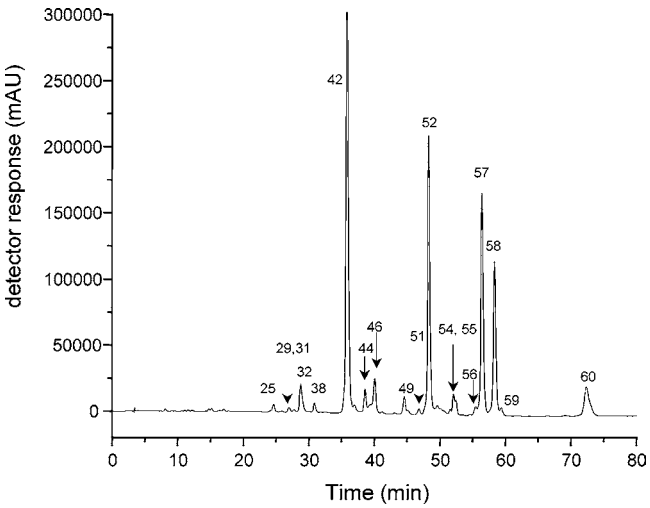

Figure 6. Chromatogram (processsed at 450 nm), obtained by HPLC-PDA-MS, of the carotenoids from peach palm. See text for chromatographic conditions. Peak identification and characterization are given in **Table 1**.

(28.1%), followed by *all-trans-δ*-carotene (23.1%), *all-trans-γ*-carotene (17.9%), and *cis-γ*-carotene (14.3%). The other 14 minor carotenoids identified corresponded to 16.6% of the total content. The provitamin A activity reported for peach palm, also harvested in Amazonia, was higher, 1718 RE/100 g (*40*), than the value found in the present study.

A typical HPLC-PDA chromatogram of the carotenoids from physalis is presented in **Figure 7**, showing the separation of 24 carotenoids on the C_{30} column, among them 22 identified. *all-trans-β*-Carotene was the major carotenoid, contributing 76.8% to the total carotenoid content, followed by 9-*cis-β*-carotene and *all-trans*-α-cryptoxanthin, contributing around 3.6 and 3.4%. All of the other minor carotenoids represented only 16.2% of the total content. Studies regarding the carotenoid composition in physalis were not found in the literature.

As can be seen in **Figure 8**, 24 carotenoids from tucuma were separated, and among them 21 were identified. The *all-trans-β*-carotene was found to be the major carotenoid, representing 75% of total carotenoid content in this fruit, followed by 13-*cis-β*-carotene, *all-trans*-α-carotene, *all-trans-β*-cryptoxanthin,

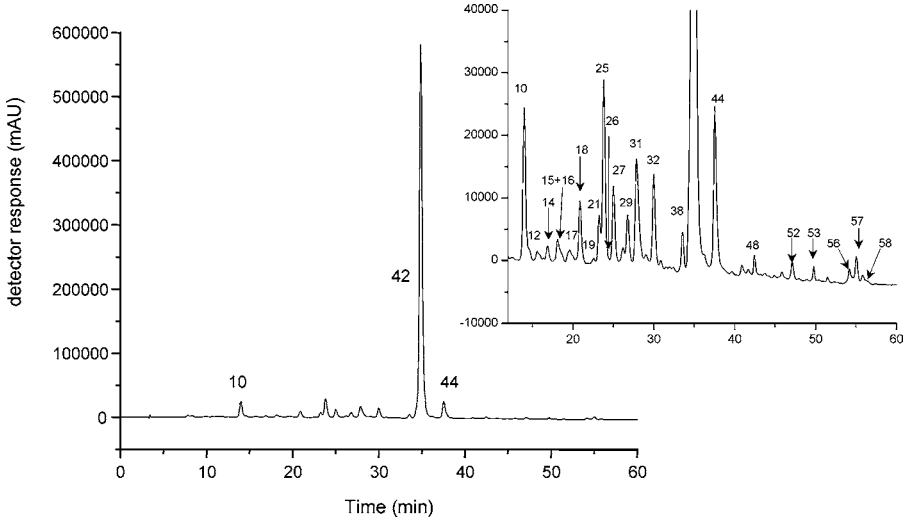

Figure 7. Chromatogram (processed at 450 nm), obtained by HPLC-PDA-MS, of the carotenoids from physalis. See text for chromatographic conditions. Peak identification and characterization are given in **Table 1**.

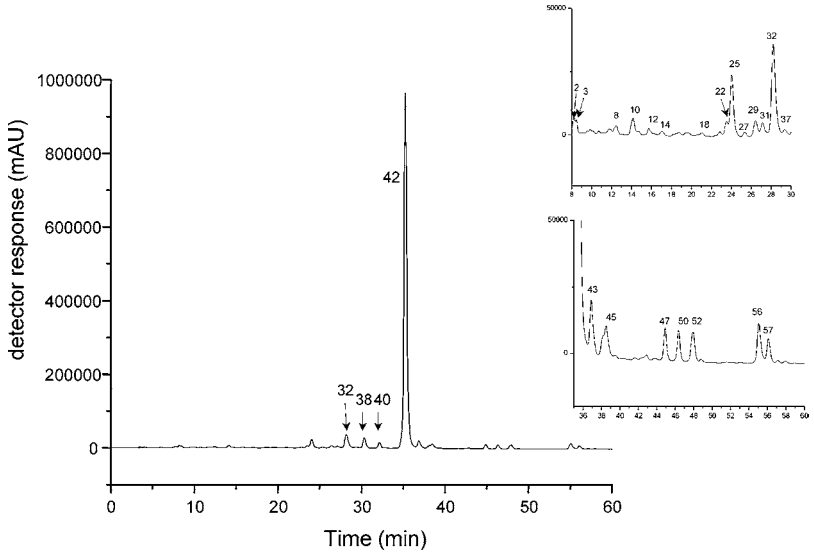

Figure 8. Chromatogram (processed at 450 nm), obtained by HPLC-PDA-MS, of the carotenoids from tucuma. See text for chromatographic conditions. Peak identification and characterization are given in **Table 1**.

and *all-trans*-α-cryptoxanthin, each representing from 2.0 to 2.8% of the total carotenoid content. The other 19 minor carotenoids, summing to 15% of the total content, were found at <2.0% each. As far as we are know, the carotenoid composition of tucuma has not been previously reported.

Provitamin A Activity. Among the Amazonian fruits evaluated in this study, the provitamin A activity decreased in the following order: buriti, palm oil, peach palm, physalis, tucuma, mamey, and marimari (**Table 2**).

All fruits analyzed are good sources of provitamin A, considering the vitamin A values of some tropical fruits, such as caja (120 RE/100 g) (*41*), mango cultivars Tommy Atkins and Keitt (96−251 RE/100 g) (*42*), acerola cultivar Olivier (148−283 RE/100 g) (*14*), camu-camu (14−24 RE/100 g) (*15*), and papaya (19−74 RE/100 g) (*43*), and even of green leafy vegetables (429−640 RE/100 g) (*44*). It should be remembered that not all Amazonian fruits are rich in carotenoids, as, for example, acai (*45*) and camu-camu (*46*), which possess anthocyanins as major pigments.

In the present study, HPLC-PDA-MS/MS with APCI was successfully applied for the determination of major and minor carotenoids in some fruits from the Amazonian region for the first time. In addition, the carotenoid composition of physalis, marimari, and tucuma was not reported before in the literature. Although peach palm and palm oil are important sources of provitamin A, buriti is the richest source of provitamin A among all foods listed in the USDA-NCC database (*47*). To prevent carotene deficiency, the use of these natural resources from Amazonia should be commercially exploited.

Supporting Information Available: MS and MS/MS carotenoid spectra, obtained by APCI ionization, of all peaks separated by HPLC on a C_{30} column, from the Amazonian fruits. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Krinsky, N. I.; Landrum, J. T.; Bone, R. A. Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. *Annu. Rev. Nutr.* **2003**, *23*, 171−201.

(2) Coyne, T.; Ibiebele, T. I.; Baadr, P. D.; Dobson, A.; McClintock, C.; Dunn, S.; Leonard, D.; Shaw, J. Diabetes mellitus and serum carotenoids: findings of a population-based study in Queensland, Australia. *Am. J. Clin. Nutr.* **2005**, *82*, 685−693.

(3) Van den Berg, H.; Faulks, R.; Granado, H. F.; Hirschberg, J.; Olmedilla, B.; Sandmann, G.; Southon, S.; Stahl, W. The potential for the improvement of carotenoids levels in foods and the likely systemic effects. *J. Sci. Food Agric*. **2000**, *80*, 880−912.

(4) Fraser, M. L.; Lee, A. H.; Binns, C. W. Lycopene and prostate cancer: emerging evidence. *Expert. Rev. Anticancer Ther*. **2005**, *5*, 847−854.

(5) Stahl, W.; Sies, H. Bioactivity and protective effects of natural carotenoids. *Biochim. Biophys. Acta, Mol. Basis Dis.* **2005**, *1740*, 101−107.

(6) Schiedt, K.; Liaaen-Jensen, S. Isolation and analysis. In *Carotenoids Vol. 1A: Isolation and Analysis*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser Verlag: Basel, Switzerland, 1995; pp 81−108.

(7) Liaaen-Jensen, S. Combined approach: identification and structure elucidation of carotenoids. In *Carotenoids Vol. 1B: Spectroscopy*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser Verlag: Basel, Switzerland, 1995; pp 343−354.

(8) Pfander, H.; Riesen, R.; Niggli, U. HPLC and SFC of carotenoids—scope and limitations. *Pure Appl. Chem.* **1994**, *66*, 947−954.

(9) Mercadante, A. Z.; Rodriguez-Amaya, D.; Britton, G. HPLC and mass spectrometric analysis of carotenoids from mango. *J. Agric. Food Chem*. **1997**, *45*, 120−123.

(10) Mercadante, A. Z.; Steck, A.; Pfander, H. Carotenoids from guava (*Psidium guajava* L.): isolation and structure elucidation. *J. Agric. Food Chem*. **1999**, *47*, 145−151.

(11) Aman, R.; Biehl, J.; Carle, R.; Conrad, J.; Beifuss, U.; Schieber, A. Application of HPLC coupled with DAD, APCI-MS and NMR to the analysis of lutein and zeaxanthin stereoisomers in thermally processed vegetables. *Food Chem.* **2005**, *92*, 753−763.

(12) Tiziani, S.; Schwartz, S. J.; Vodovotz, Y. Profiling of carotenoids in tomato juice by one- and two-dimensional NMR. *J. Agric. Food Chem*. **2006**, *54*, 6094−6100.

(13) Assunção, R. B.; Mercadante, A. Z. Carotenoids and ascorbic acid from cashew apple (*Anacardium occidentale* L.): variety and geographic effects. *Food Chem.* **2003**, *81*, 495−502.

(14) De Rosso, V. V.; Mercadante, A. Z. Carotenoid composition of two Brazilian genotypes of acerola (*Malpighia punicifolia* L.) from two harvests. *Food Res. Int.* **2005**, *38*, 1073−1077.

(15) Zanatta, C. F.; Mercadante, A. Z. Carotenoid composition from the Brazilian tropical fruit camu-camu (*Myrciaria dubia*). *Food Chem*. **2007**, *101*, 1526−1532.

(16) Godoy, H. T.; Rodriguez-Amaya, D. B. Occurrence of *cis*-isomers of provitamin A in Brazilian fruits. *J. Agric. Food Chem*. **1994**, *42*, 1306−1313.

(17) Darnoko, D.; Cheryan, M.; Moros, E.; Jerrel, J.; Perkins, E. G. Simultaneous HPLC analysis of palm carotenoids and tocopherols using a C-30 column and photodiode array detector. *J. Liq. Chromatogr. Relat. Technol*. **2000**, *23*, 1873−1885.

(18) Mortesen, A. Analysis of a complex mixture of carotenes from oil palm (*Elaeis guineensis*) fruit extract. *Food Res. Int.* **2005**, *38*, 847−853.

(19) Dhuique-Mayer, C.; Caris-Veyrat, C.; Ollitrault, P.; Curk, F.; Amiot, M-.J. Varietal and interspecific influence on micronutrient contents in citrus from the Mediterranean area. *J. Agric. Food Chem.* **2005**, *53*, 2140−2145.

(20) Xu, C.-J.; Fraser, P. D.; Wang, W.-J.; Bramley, P. M. Differences in carotenoid content of ordinary citrus and lycopene-accumulating mutants. *J. Agric. Food Chem*. **2006**, *54*, 5474−5481.

(21) Olson, J. A. Absorption, transport, and metabolism of carotenoids in humans. *Pure Appl. Chem.* **1994**, *66*, 1011−1016.

(22) Parker, R. S. Carotenoids, 4. Absorption, metabolism and transport of carotenoids. *FASEB J.* **1996**, *10*, 542−551.

(23) Hart, D. J.; Scott, K. J. Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurement of the carotenoid content of vegetables and fruits commonly consumed in the UK. *Food Chem.* **1995**, *54*, 101−111.

(24) Emenhiser, C.; Simunovic, N.; Sander, L. C.; Schwartz, S. J. Separation of geometrical carotenoid isomers in biological extracts using a polymeric C_{30} column in reversed-phase liquid chromatography. *J. Agric. Food Chem*. **1996**, *44*, 3887−3893.

(25) Meléndez-Martínez, A. J.; Britton, G.; Vicario, I. M.; Heredia, F. J. Identification of isolutein (lutein epoxide) as *cis*-antheraxanthin in orange juice. *J. Agric. Food Chem*. **2005**, *53*, 9369−9373.

(26) Schlatterer, J.; Breithaupt, D. E. Cryptoxanthin structural isomers in oranges, orange juice and other fruits. *J. Agric. Food Chem*. **2005**, *53*, 6355−6361.

(27) Meléndez-Martínez, A. J.; Britton, G.; Vicario, I. M.; Heredia, F. J. Identification of zeinoxanthin in orange juices. *J. Agric. Food Chem*. **2005**, *53*, 6362−6367.

(28) Emenhiser, C.; Englert, G.; Sander, L. C.; Ludwig, B.; Schwartz, S. J. Isolation and structural elucidation of the predominant geometrical isomers of α-carotene. *J. Chromatogr., A* **1996**, *719*, 333−343.

(29) Britton, G.; Liaaen-Jensen, S.; Pfander, H. *Carotenoids Handbook*; Birkhäuser: Basel, Switzerland, 2004.

(30) Enzell, C. R.; Back, S. Mass Spectrometry. In *Carotenoids. Vol. 1B: Spectroscopy*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser: Basel, Switzerland, 1995; pp 261−320.

(31) Mercadante, A. Z.; Britton, G.; Rodriguez-Amaya, D. Carotenoids from yellow fruit (*Passiflora edulis*). *J. Agric. Food Chem*. **1998**, *46*, 4102−4106.

(32) Eugster, C. H. Chemical derivatization: microscale tests for the presence of commom fuctional groups in carotenoids. In *Carotenoids Vol. 1A: Isolation and Analysis*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser Verlag: Basel, Switzerland, 1995; pp 71−80.

(33) NRC. *Recommended Dietary Allowances*, 10th ed.; National Academy of Sciences: Washington, DC, 1989.

(34) Märki-Fischer, E.; Eugster, C. H. Isolation of 10′-apo-β-carotene-10′-ol and (3*R*)-10′-apo-β-carotene-3,10′-diol (galloxanthin) from rose flowers. *Helv. Chim. Acta* **1987**, *70*, 1988−1993.

(35) Molnár, P.; Szabolcs, J. β-citraurin epoxide, a new carotenoid from Valencia orange peel. *Phytochemistry* **1980**, *19*, 633−637.

(36) Ma, Y.-C.; Kim, H.-Y. Determination of steroids by liquid chromatography/mass spectrometry. *J. Am. Soc. Mass Spectrom*. **1997**, *8*, 1010−1020.

(37) Almeida-Muradian, L. B.; Penteado, M. V. C. Carotenoids with provitamin A value of some Brazilian sweet potato (*Ipomea batatas* Lam.) cultivars. *Rev. Farm. Bioquim. Univ. S. Paulo* **1992**, *28*, 145−154.

(38) Lee, C. Y. Changes in carotenoid content of carrots during growth and post-harvest storage. *Food Chem.* **1986**, *20*, 285−293.

(39) Nunes, I.; Mercadante, A. Z. Production of lycopene crystals from tomato waste. *Cienc. Tecnol. Aliment*. **2004**, *24*, 440−447.

(40) Yuyama, L. K. O.; Cozzolino, S. M. F. Effect of supplementation with peach palm as source of vitamin A: study with rats. *Rev. Saude Publica* **1996**, *30*, 61−66.

(41) Hamano, P. S.; Mercadante, A. Z. Composition of carotenoids from commercial products of caja (*Spondias lutea*). *J. Food Compos. Anal*. **2001**, *14*, 335−343.

(42) Mercadante, A. Z.; Rodriguez-Amaya, D. Effects of ripening, cultivar differences, and processing on the carotenoid composition of mango. *J. Agric. Food Chem*. **1998**, *46*, 128−130.

(43) Wall, M. M. Ascorbic acid, vitamin A, and mineral composition of banana (*Musa* sp.) and papaya (*Carica papaya*) cultivars grown in Hawaii. *J. Food Compos. Anal*. **2006**, *19*, 434−445.

(44) Hulshof, P. J. M.; Xu, C.; Van den Bovenkamp, P.; West, C. E. Application of a validated method for the determination of provitamin A carotenoids in Indonesian foods of different maturity and origin. *J. Agric. Food Chem*. **1997**, *45*, 1174−1179.

(45) Schauss, A. G.; Wu, X.; Prior, R. L.; Ou, B.; Patel, D.; Huang, D.; Kababick, J. P. Phytochemical and nutrient composition of the freeze-dried amazonian palm berry, *Euterpe olerace* Mart. (acai). *J. Agric. Food Chem*. **2006**, *54*, 8598−8603.

(46) Zanatta, C. F.; Cuevas, E.; Bobbio, F. O.; Winterhalter, P.; Mercadante, A. Z. Determination of anthocyanins from camu-camu (*Myrciaria dubia*) by HPLC-PDA, HPLC-MS and NMR. *J. Agric. Food Chem*. **2005**, *53*, 9531−9535.

(47) U.S. Departament of Agriculture (USDA). USDA-NCC Carotenoid Database for U.S. Foods, 1998; http://www.nal.usda.gov/fnic/foodcomp/Data/car98/car98.htm (accessed April 10, 2007).

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