JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Identification and Quantification of Xanthophyll Esters, Carotenes, and Tocopherols in the Fruit of Seven Mexican Mango Cultivars by Liquid Chromatography–Atmospheric Pressure Chemical Ionization–Time-of-Flight Mass Spectrometry [LC-(APcI$^+$)-MS]

J. de Jesus Ornelas-Paz,† Elhadi M. Yahia,*,‡ and Alfonso Gardea-Bejar†

Centro de Investigación en Alimentación y Desarrollo, A.C., Hermosillo, Sonora, Mexico, and Universidad Autónoma de Querétaro, Avenida de las Ciencias s/n, Juriquilla, 76230 Querétaro, Mexico

A liquid chromatography–mass spectrometry (LC-MS) method was developed to simultaneously identify and quantify carotenoids and tocopherols in the fruit of seven mango cultivars grown in Mexico. Fruit maturity was characterized objectively, and carotenoids and tocopherols were isolated by solvent extraction and analyzed by HPLC coupled to a C_{30} stationary phase and diode array, fluorescence, and mass (time-of-flight) detectors. All cultivars had a similar carotenoid pattern, in which *all-trans*-β-carotene and dibutyrates of *all-trans*-violaxanthin and 9-*cis*-violaxanthin were the most abundant. The content of *all-trans*-β-carotene ranged between 0.4 and 2.8 mg/100 g, and 'Haden' and 'Ataulfo' mangoes had the highest amount. The amounts of *all-trans*-violaxanthin and 9-*cis*-violaxanthin (as dibutyrates) ranged between 0.5 and 2.8 mg/100g and between 0.4 and 2.0 mg/100 g, respectively. The content of α-tocopherol was low (200–500 μg/100 g). The results of this study indicate that *all-trans*-β-carotene, *all-trans*-violaxanthin, and 9-*cis*-violaxanthin are the most abundant carotenoids in mango grown in Mexico.

KEYWORDS: ***Mangifera indica*** **L.; carotenoids; xanthophyll esters; vitamin E; vitamin A; carotenoid stereoisomers**

INTRODUCTION

Mango (*Mangifera indica* L.) is a popular fruit in many countries due to its exotic flavor, attractive appearance, and nutritional value. Mexico is the fourth largest producer and the biggest exporter of mango; more than 58 different cultivars of this fruit are cultivated in Mexico (*1*). The per capita consumption of mango in Mexico is high (*1*), and therefore this fruit is an important source of several important nutritional and health components such as dietary fiber (*2*), polyphenols (*3*), ascorbic acid (*4*), and carotenoids (*5*). The yellow-orange color of this fruit is caused by its high content of carotenoids, which are lipid-soluble compounds, correlated with protective health effects, such as against some types of cancer (*6*), age-related macular degeneration (*7*), and heart diseases (*8*). In addition, some carotenoids, such as *all-trans*-β-carotene, are precursors of vitamin A. Vitamin A has many positive effects on human health (*9*), and its deficiency is still causing problems, especially for children, in many countries around the world (*10*).

The most abundant carotene of mango is *all-trans*-β-carotene, whereas the most important xanthophylls seem to be violaxanthin and its isomers (*11*, *12*). Mercadante et al. (*13*) quantified several carotenoids in saponified extracts of 'Keitt' mangoes and concluded that the most predominant xanthophylls were *all-trans*-violaxanthin and 9-*cis*-violaxanthin, accounting for 38 and 18% of total carotenoid content, respectively, although other xanthophylls can be more important depending on the type of cultivar (*14*–*16*).

For carotenoid analysis, a cool or hot saponification step is usually used to remove unwanted compounds and for hydrolyzing xanthophyll esters. However, this step may cause transformations and losses of carotenoids (*17*), so further analysis of crude extracts is needed to study their natural forms. Several esters of violaxanthin and neolutein were tentatively identified in crude extracts of 'Alphonso' mango (*16*). Burns et al. (*18*) found three violaxanthin esters in mango from Costa Rica, but no identification was provided. The predominant xanthophyll esters in crude extracts of 'Kent' mangoes were identified as dibutyrates of *all-trans*- and 9-*cis*-violaxanthin by LC-(APcI$^+$)-MS analyses (*19*). However, the amounts of *all-trans*-violaxanthin and 9-*cis*-violaxanthin as butyric acid diesters were not

* Author to whom correspondence should be addressed (telephone/fax 52-442-2281416; e-mail yahia@uaq.mx).
† Centro de Investigación en Alimentación y Desarrollo.
‡ Universidad Autónoma de Querétaro.

10.1021/jf0706981 CCC: $37.00
Published on Web 07/11/2007

given. Information on the tocopherol content in mango is very scarce, but it seems to be very low (*18*).

The objective of this study was to develop a LC-MS method to simultaneously identify and quantify tocopherols and the most important carotenoids in crude extracts of the fruit of seven mango cultivars grown in Mexico.

MATERIALS AND METHODS

Chemicals and Solvents. High-performance liquid chromatography (HPLC) grade methanol, acetone, *n*-hexane, 2-propanol, reactive grade benzene, anhydrous granular sodium sulfate, calcium carbonate, and $Na_2S_2O_3$ were purchased from J. T. Baker (Baker Mallinckrodt, Mexico). 2,6-Di-*tert*-butyl-4-methylphenol (BHT) was obtained from Merck KGaA (Darmstadt, Germany). Diethyl ether, *tert*-butyl methyl ether (MTBE) HPLC grade, a mixture of *all-trans*-α/*β*-carotene (purity = 95%) from carrots, and α- and *δ*-tocopherol (purity = 95 and 90%, respectively) were from Sigma-Aldrich (St. Louis, MO), and *all-trans*-violaxanthin (purity = 95%) was from CaroteNature GmbH (Lupsingen, Switzerland). HPLC grade water was prepared by a Milli-Qplus purification system (Millipore Corp., Bedford, MA).

Plant Material. Fresh mango fruit of the cultivars 'Ataulfo', 'Manila', 'Criollo', 'Paraíso', 'Haden', 'Kent', and 'Tommy Atkins' at ripe stage were obtained from a local market in Querétaro, Mexico. Fruits were selected for uniform size and color and freedom from blemishes and defects and were maintained at 28 °C and 50% relative humidity until analysis.

Indicators of Fruit Physiological Maturity. The physiological maturity stage of the fruit was determined by total soluble solids content (°Brix) and objective color measurements. °Brix was measured in the juice obtained from a representative portion of each fruit, using a hand refractometer (Atago Co. Ltd., Osaka, Japan). Color was measured with a Minolta spectrophotometer (Minolta Co. Ltd., Osaka, Japan), which was calibrated with the white pattern during each sampling time. External color was longitudinally determined on three points of each flat side of the fruit (six points for each fruit). For flesh (internal) color a big slice from a flat side of each fruit was obtained, and color was determined longitudinally on three equidistant points. *L**, *a**, *b**, *C**, and *h*° values were recorded.

Preparation of Samples. The extraction procedure of carotenoids and tocopherols was carried out as described by Pott et al. (*20*) with slight modifications (*21*). Fresh mango pulp from each fruit (6 g) was ground by a homogenizer (Ika Works Inc., Wilmington, NC) in the presence of calcium carbonate (0.2 g) and methanol (15 mL). The homogenate was filtered through a filter paper, adding methanol until retained solids became colorless. The methanolic extract was mixed with 50 mL of a mixture of hexane/acetone (1:1, v/v) containing 0.1% of BHT. After vigorous stirring, 40 mL of 10% sodium sulfate was added for phase separation. The upper layer was separated, washed several times with water, and evaporated in a rotavapor at 35 °C. For saponification, the residue was dissolved in 30 mL of diethyl ether, 0.2 mL of 40% methanolic KOH were added, and the mixture was kept for 16 h in the dark at room temperature. After completion of the saponification step, the extract was washed with water and evaporated as described above. Saponified and unsaponified residues were dissolved in 2 mL of 2-propanol, filtered through a polyethylene membrane of 0.45 μm pore size (Millipore Corp., Bedford, MA), and 25 μL was injected onto the HPLC system.

I_2-Catalyzed Photoisomerization of Carotenoids. Cis isomers of violaxanthin were generated for identification purposes according to the method of Molnár et al. (*22*). A quantity of 0.11 mg of *all-trans*-violaxanthin was dissolved in 1 mL of benzene containing 0.002 mg of I_2. The solution was exposed to daylight until equilibrium was reached within 40 min. The reaction mixture was then washed with 5% Na_2SO_3 (50 mL), evaporated at reduced pressure (35 °C), and redissolved in 2-propanol (2 mL) prior to HPLC analysis.

HPLC-MS Analytical Conditions. Samples containing carotenoids and tocopherols were automatically injected into an HP 1100 series HPLC system (Hewlett-Packard GmbH, Waldbronn, Germany) equipped with an online degasser, a diode array detector (DAD), and a fluorescence detector (FLD). Spectra for all peaks were recorded between 200 and 500 nm (each 2 nm). Peak purity, which indicates the mean purity value of all spectra, was determined automatically by DAD. Individual signals for 9-*cis*-violaxanthin, *all-trans*-violaxanthin, and *all-trans*-*β*-carotene were monitored at 436, 439, and 452 nm, respectively. The FLD was set at excitation and emission wavelengths of 294 and 326 nm, respectively, for tocopherol detection. The HPLC system was equipped with a 150 × 4.6 mm i.d., 3 μm C_{30} reversed-phase column (YMC Inc., Milford, MA), which was kept at 15 °C. After pilot studies, the most appropriate mobile phase was found to be composed of water (A), methanol (B), and MTBE (C) with the following gradient program: 4% A, 95.2% B, and 0.8% C at 0 min, decreasing to 4% A, 55.3% B, and 40.7% C within 78 min at a flow rate of 0.75 mL/min.

Mass spectra of mango carotenoids were obtained using the chromatographic system described above (without FLD), fitted to an 6210 time-of-flight (TOF) mass spectrometer (Agilent, Palo Alto, CA) equipped with an atmospheric pressure chemical ionization ($APcI^+$) interface and Mass Hunter manager software (A.02.01). The $APcI^+$-MS system was operated in positive ion mode. High-purity nitrogen (99.999%) was used as nebulizing (20 psi) and drying gas (5 L/min). Other $APcI^+$-MS parameters were as follows: gas and vaporizer temperatures, 325 and 350 °C, respectively; corona, capillary, fragmentor, and skimmer voltages, 4 μA, 3000, 200, and 60 V, respectively.

Carotenoids were identified by comparing their retention time and UV−vis data with those obtained with reference standards as well as cochromatography with added standards and using their mass spectra (*m*/*z* 100−1200). Quantitative data for α-tocopherol and *trans* carotenoids were obtained by calibration curves constructed with pure compounds. Quantification of cis isomers of carotenoids was based on calibration curves of their parent *trans* carotenoids.

Statistical Analysis. The Tukey−Kramer honestly significant difference test was used as a comparison of statistical significance. All data analyses were performed using JMP statistical software (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Fruit Ripening Characteristics. Color and other fruit quality parameters are shown in **Table 1**. Fruits of all cultivars had excellent external appearance and were ripe according to their taste (°Brix) (*23*, *24*). 'Manila' and 'Ataulfo' fruit exhibited a uniform yellow-orange peel color, whereas 'Criollo', 'Haden', 'Paraíso', and 'Kent' had yellow-orange with some pink, purple, or reddish regions. 'Tommy Atkins' mangoes had slight greenish regions on the peel. In contrast to the peel, the flesh of all mango cultivars presented a uniform orange-yellow color. 'Ataulfo' and 'Haden' mangoes had the deepest pigmentation and the lowest hue (*h*°) values in flesh, whereas 'Paraíso' mangoes exhibited the highest *h*° values. The *h*° values of the peel of 'Manila', 'Ataulfo', and 'Haden' mangoes were similar to those of the pulp, and therefore this measurement of external color might be used as a reliable nondestructive indicator of maturity of these cultivars. On the other hand, 'Ataulfo' and 'Haden' mangoes had the highest *a** values in flesh. High *a** values and low *h*° values in flesh have been correlated with high *β*-carotene content (*5*, *25*).

Separation and Identification of Carotenoids from Mango Tissue Using Diode Array Detection. Several solvent systems were tried in pilot studies. The best results were obtained when the ternary system (described above) composed of methanol/MTBE/water was employed. These solvents along with a C_{30} column have been successfully applied for carotenoid analysis from foods and human serum and tissues (*26*), allowing for separation of polar and nonpolar carotenoids, as well as geometric and positional isomers. Existing methods for carotenoid analysis in fruit by HPLC commonly use room (uncontrolled or controlled) temperatures in the range of 25−35 °C (*27*, *28*). In our study, good separation of α-tocopherol,

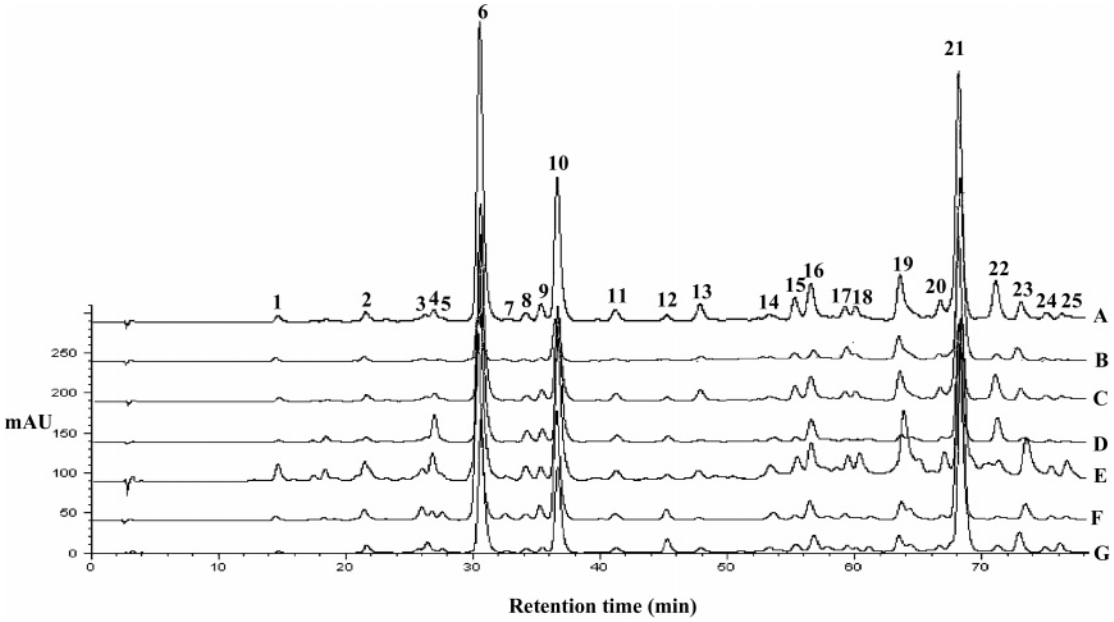

Figure 1. Typical chromatographic patterns at 452 nm of crude extracts of fruit of 'Manila' (**A**), 'Ataulfo' (**B**), 'Haden' (**C**), 'Tommy Atkins' (**D**), 'Paraíso' (**E**), 'Criollo' (**F**), and 'Kent' (**G**) mangoes. For precise peak assignment see **Table 2**.

Table 1. Fruit Quality Parameters of Mango Cultivars[a]

	cultivar						
variable	Manila	Ataulfo	Criollo	Haden	Kent	Paraíso	Tommy Atkins
weight (g) ($n = 4$)	243.3 ± 5.8	269.3 ± 24.4	139.4 ± 4.0	320.4 ± 9.7	350.3 ± 26.0	569.5 +11.4	407.4 ± 20.0
°Brix ($n = 8$)	21.3 ± 0.3	16.4 ± 0.6	17.7 ± 0.4	18.3 ± 0.5	16.4 ± 0.6	12.8 ± 0.3	13.6 ± 0.2
peel							
L^* ($n = 24$)	71.4 ± 0.6	68.6 ± 0.4	60.7 ± 0.7	55.7 ± 1.3	47.3 ± 1.4	60.2 ± 1.2	54.5 ± 0.7
a^* ($n = 24$)	10.9 ± 0.2	18.1 ± 0.5	21.4 ± 0.8	19.3 ± 1.1	23.6 ± 1.3	19.87 ± 1.0	9.8 ± 1.5
b^* ($n = 24$)	38.2 ± 0.5	43.8 ± 0.4	32.5 ± 0.9	26.6 ± 2.0	21.0 ± 2.4	29.6 ± 1.6	26.3 ± 1.1
C^* ($n = 24$)	39.8 ± 0.5	47.4 ± 0.3	39.3 ± 0.3	34.3 ± 1	33.2 ± 1.7	36.6 ± 0.8	29.14 ± 1.0
h° ($n = 24$)	74.0 ± 0.3	67.5 ± 0.6	56.5 ± 1.7	51.9 ± 3.7	39.6 ± 4.1	55.1 ± 2.7	70.5 ± 3.3
pulp							
L^* ($n = 12$)	59.6 ± 1.2	66.0 ± 0.6	58.4 ± 1.4	59.1 ± 1.3	59.3 ± 1.3	60.2 ± 2.1	60.1 ± 0.7
a^* ($n = 12$)	17.4 ± 0.8	23.7 ± 0.5	18.1 ± 0.9	24.6 ± 0.5	20.3 ± 1	15.9 ± 0.6	21.7 ± 0.7
b^* ($n = 12$)	50.8 ± 2.3	50.3 ± 1.1	50.0 ± 1.3	39.2 ± 1.9	45.4 ± 2.1	51.1 ± 1.6	58.8 ± 0.9
C^* ($n = 12$)	53.8 ± 2.2	55.6 ± 1	53.3 ± 1.3	46.4 ± 1.9	49.8 ± 2.2	53.6 ± 1.6	62.7 ± 1.0
h° ($n = 12$)	70.9 ± 0.7	64.7 ± 0.7	70.1 ± 1	57.5 ± 0.9	65.8 ± 0.8	72.6 ± 0.6	69.8 ± 0.5

[a] Data represent the mean of several measurements of the peel or pulp of mangoes ± standard error.

carotenes, and free and esterified xanthophylls from mango was achieved at 15 °C, probably due to the fact that at low temperature an increased selectivity is presented as a consequence of the population of the ordered trans conformations of C_{30} alkyl chains exceeds the population of disordered gauche conformations, as has been described by Albert (*29*). The chromatographic carotenoid pattern in all seven cultivars showed 25 common carotenoids (**Figure 1** and **Table 2**). The initial identification of such peaks was carried out by comparing their spectral characteristics with those previously reported using a similar mobile phase (water/methanol/MTBE). Several peaks were tentatively identified as *all-trans*-violaxanthin and 9-*cis*-violaxanthin containing compounds (**Figure 1** and **Table 2**), according to the UV−vis data reported by Pott et al. (*19*) for such xanthophylls. If maximum absorption wavelengths for *all-trans*- (439 nm) and 9-*cis*-violaxanthin (436 nm) are compared, a difference of 3 nm can be noted, which is a typical characteristic that distinguishes these isomers (*13*). The spectral maximum for peak 13 (**Figure 1** and **Table 2**) was similar to that reported for *cis*-β-cryptoxanthin (*27*). Peak 18 (**Figure 1** and **Table 2**) was assigned to *all-trans*-neoxanthin on the basis of the UV−vis data (415, 438, and 467 nm) reported for this carotenoid (*30*). Peaks 16, 21, and 22 (**Figure 1** and **Table 2**) were identified as isomers of β-carotene on the basis of their elution order and spectral maxima: 443, 452, and 446 nm for 13-*cis*-β-carotene, *all-trans*-β-carotene, and 9-*cis*-β-carotene (*31*). Peaks 3, 4, and 8 (**Figure 1** and **Table 2**) were not clearly identified.

When crude extracts were subjected to saponification, peaks 6 and 10 (**Figure 1** and **Table 2**) along with other small peaks disappeared, but two intense peaks (peaks 1 and 27 in **Figure 2**) with spectroscopic characteristics typical for *all-trans*- and 9-*cis*-violaxanthin were visible in the polar region of the chromatogram. This demonstrates that these xanthophylls are the most important in mangoes. Additionally, as a consequence of the saponification step, another unidentified peak (peak 26 in **Figure 2**), having maxima at 417, 441, and 469 nm, was observed at 12.7 min. Peaks 4, 13, 16, 21, and 22 (**Figures 1** and **2** and **Table 2**) were unaffected by saponification, and therefore these compounds were considered to be unesterified.

To further investigate the identity of the important carotenoids, reference compounds were employed. Due to the unavailability of reference material for 9-*cis*-violaxanthin it had to be prepared starting from its trans isomer. Thermal and iodine-catalyzed photoisomerization methods are commonly used for

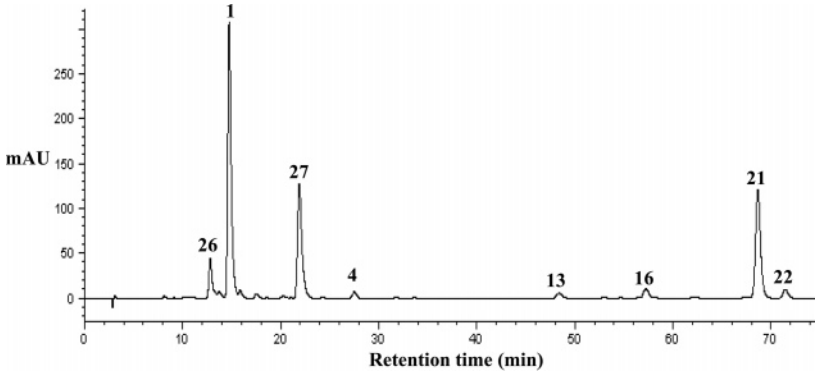

Figure 2. Typical chromatographic pattern at 452 nm of saponified extract of ripe 'Manila' mango. Peak 26 represents an unknown compound; peaks 1 and 27 correspond to free *all-trans*-violaxanthin and 9-*cis*-violaxanthin, respectively. Tentative identification data for peaks are shown in **Table 2**.

Table 2. Tentative Identification Data of Carotenoids in Mango

peak[a]	t_R (min)	cis peak, λ_{maxI}, λ_{maxII}, λ_{maxIII}[b] (nm)	III/II[c] (%)	purity (%)	APcI$^+$-MS data (m/z)[d]	compound
1	14.6	328 (3.0), 416, 439, 469	92	94.5	601 (100)*,[e] 583 (31), 534 (38), 429 (35), 39760 (22)	free *all-trans*-violaxanthin
2	21.7	328 (1.5), 417, 439, 469	94	95.8	671 (100)*, 653 (44), 583 (4), 565 (7)	*all-trans*-violaxanthin butyrate
3	26.3	329 (5.1), 411, 434, 462	52	100	601 (26), 427 (100), 409 (58), 339 (18)	not identified
4	27.5	328 (1.9), 417, 439, 469	97	100	427 (9), 409 (34), 395 (100)	*all-trans*-violaxanthin derivative
5	27.9	328 (1.0), 414, 436, 465	91	90.3	671 (100)*, 653 (40), 583 (2), 565 (7)	9-*cis*-violaxanthin butyrate
6	30.8	328 (22.5), 416, 439, 469	93	99.9	741 (100)*, 723 (69), 653 (7), 635 (11), 565 (2), 547 (3)	*all-trans*-violaxanthin dibutyrate
7	32.8	328 (0.7),413, 436, 465	87	100	741 (89)*, 723 (66), 613 (23), 583 (4), 534 (2), 443 (32), 149 (100)	9-*cis*-violaxanthin ester
8	34.3	328 (2.36), 407, 432, 460	20	100	545 (100)*, 529 (5), 411 (16), 397 (16)	not identified
9	35.8	329 (2.36), 415, 439, 469	91	99.6	769 (100)*, 751 (76), 663 (8), 635 (6), 411 (16), 397 (17)	*all-trans*-violaxanthin butyrate-caproate
10	36.8	328 (18.2), 413, 436, 465	89	100	741 (100)*, 723 (57), 653 (5), 635 (9), 565 (1), 547 (2)	9-*cis*-violaxanthin dibutyrate
11	41.7	328 (1.9), 413, 436, 465	89	100	705 (10), 661 (12), 617 (13), 567 (35), 545 (100), 411 (14), 397 (17)	9-*cis*-violaxanthin ester
12	45.8	328 (1.3), 413, 436, 465	87	86.2	797 (12)*, 661 (12), 650 (15), 632 (64), 617 (13), 411 (11), 397 (16)	9-*cis*-violaxanthin ester
13	48.3	331 (1.2), 419, 445, 474	58	99.8	553 (100)*, 535 (18), 395 (14)	free *cis*-β-cryptoxanthin
14	54.2	328 (0.8), 416, 439, 469	93	100	664 (100)*, 646 (78), 409 (21)	*all-trans*-violaxanthin ester
15	56.2	328 (1.3), 416, 439, 469	96	99.7	853 (100)*, 835 (87), 621 (18), 409 (14)	*all-trans*-violaxanthin ester
16	57.2	340 (18.9), NA,[f] 443, 468	16	100	537 (100)*, 529 (4), 457 (5), 409 (11), 397 (3), 391 (7)	13-*cis*-β-carotene
17	59.8	328 (1.3), 418, 439, 469	94	99.4	907 (100)*, 889 (91), 877 (51), 853 (14), 749 (76)	*all-trans*-violaxanthin ester
18	60.6	328 (1.4), 416, 438, 468	73	100	863 (100)*, 781 (1), 660 (2)	*all-trans*-neoxanthin ester
19	64.2	328 (2.2), 418, 439, 469	90	99.6	881 (100)*, 863 (89), 793 (4), 771 (5), 653 (4), 635 (5)	*all-trans*-violaxanthin ester
20	67.7	328 (1.2), 414, 436, 465	92	100	881 (88)*, 863 (64), 775 (4), 674 (7)	9-*cis*-violaxanthin ester
21	69	340 (18.6), 427, 452, 478	21	100	537 (100)*, 457 (8), 397 (4), 391 (9)	*all-trans*-β-carotene
22	72.1	340 (3.6), 426, 446, 472	33	99.8	537 (100)*, 457 (6), 409 (41), 397 (47), 391 (7), 383 (19)	9-*cis*-β-carotene
23	74.1	328 (1.5), 418, 439, 469	93	96.8	909 (100)*, 891 (87), 803 (6), 635 (5)	*all-trans*-violaxanthin ester
24	76.2	NA, 415, 436, 465	91	100	855 (100)*	9-*cis*-violaxanthin ester
25	77.2	NA, 414, 436, 465	88	89.9	987 (100)*	9-*cis*-violaxanthin ester

[a] Numbered according to chromatogram in **Figure 1**. [b] Intensity (mAU) of cis peak given in parentheses. [c] Spectral fine structure obtained according to the method of Lee et al. (*32*). [d] Fragment ion, corresponding percentage of relative intensity (in parentheses). [e] *, quasimolecular ion ([M ± H]$^+$). [f] NA, not available.

the generation of cis isomers of carotenoids. Violaxanthin thermal isomerization induces mainly the 13-cis isomer, whereas iodine-catalyzed photoisomerization favors the generation of the 9-cis isomer (*22*). When the *all-trans*-violaxanthin standard compound was injected, an intense peak with maximum absorption wavelengths at 416, 439, and 469 nm and a peak purity of 100 was observed at 14.6 min (**Figure 3A**). In samples subjected to the isomerization process one additional peak (peak 27) with maximum absorption wavelengths at 413, 436, and 466 nm appeared at 21.7 min (**Figure 3B**). The retention times and UV−vis data of both compounds coincided with those observed for peaks 1 and 27 in saponified extracts of mango (**Figure 2**).

A mixture of *all-trans*-α/β-carotene was used for the identification of peak 21. Retention time (69 min) and spectroscopic characteristics (427, 452, and 478 nm) of reference material for *all-trans*-β-carotene were identical to those observed for peak 21 in crude and saponified extracts of mango (**Figures 1** and **2** and **Table 2**).

The spectral fine structures (% III/II) of peaks from standard compounds of *all-trans*-violaxanthin (95), 9-*cis*-violaxanthin (92), and *all-trans*-β-carotene (24) were similar to those observed in mango samples, although some slight variations were observed, probably due to the fact that the purity of some peaks was less than 100% (**Table 2**). In general, % III/II values found in this study are in agreement with the values found in the literature (*32*, *33*).

Separation and Identification of Tocopherols from Mango Flesh Using Fluorescence Detection. Suitable HPLC methods for the separation of tocopherols commonly use normal phases, because with reversed phases it is hardly possible to separate β- and γ-tocopherols (*34*), although some other reports have indicated the opposite (*35*, *36*). Strohschein et al. (*37*) have

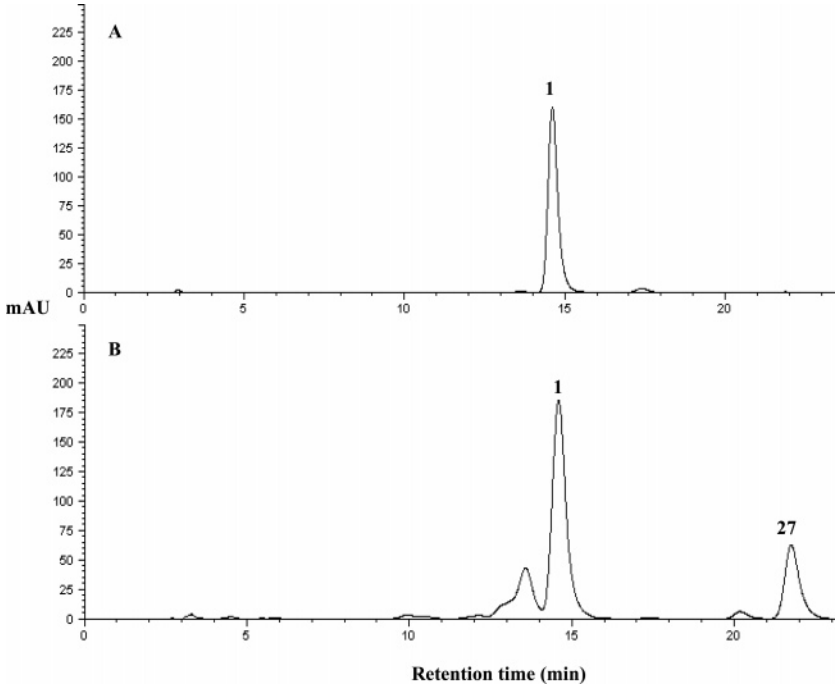

Figure 3. HPLC chromatograms obtained with standard compounds of carotenoids. Samples of *all-trans*-violaxanthin before (**A**) and after (**B**) iodine-catalyzed photoisomerization were recorded at 439 nm. Peaks 1 and 27 correspond to free *all-trans*-violaxanthin and 9-*cis*-violaxanthin. Unnumbered peaks were not studied.

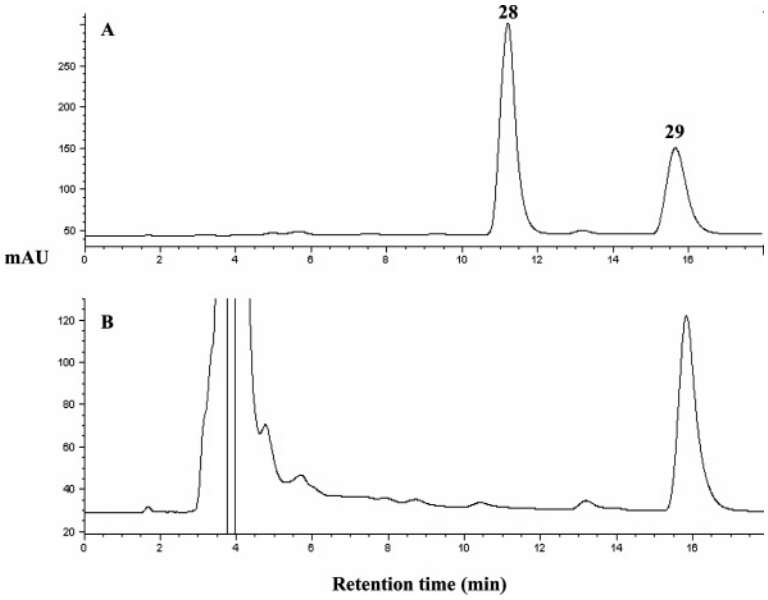

Figure 4. HPLC separation of tocopherol isomers using (**A**) authentic standard compounds or (**B**) crude extract of 'Tommy Atkins' mango at λ (excitation) = 294 nm and λ (emission) = 326 nm. Peaks 28 and 29 correspond to δ- and α-tocopherol, respectively.

demonstrated that the separation of β- and γ-tocopherols is possible with a polymeric C_{30} column. With the method developed in this study standards of α-/δ-tocopherol were separated (peaks 28 and 29 in **Figure 4A**). The retention times for δ-tocopherol and α-tocopherol were 11.2 and 15.7 min, respectively. However, in mango samples only α-tocopherol was detected (**Figure 4B**).

Mass Spectrometry of Mango Carotenoids. Mass spectrometric analysis was performed for crude extracts of mango (**Table 2**). The $APcI^+$-MS conditions used allowed a good fragmentation pattern (protonated ions) of unesterified carotenoids and carotenoids esters of low molecular weight (m/z < 741). Peaks 1 and 3 presented different fragmentation patterns but the same quasimolecular ion (601, $[M + H]^+$), which corresponds to the molecular mass of protoned violaxanthin. Although the UV−vis data of peak 3 were similar to those reported for 13-*cis*-violaxanthin (*38*), its mass spectra and elution order did not allow its adequate identification. The protoned molecule of peak 4 was not identified; however, because its UV−vis data corresponded to the *all-trans*-violaxanthin (*19*) and because only ions of m/z below 601 were observed in its mass spectra, we believe that peak 4 was an *all-trans*-violaxanthin derivative. The absorption spectra of carotenoids depend on the number and configuration (cis/trans) of their conjugated double bounds (*39*), so it can be assumed that peak 4 resulted from mass losses from the rings of violaxanthin but that the conjugated double bounds of the skeleton are intact. Undoubtedly, the loss of oxygen from

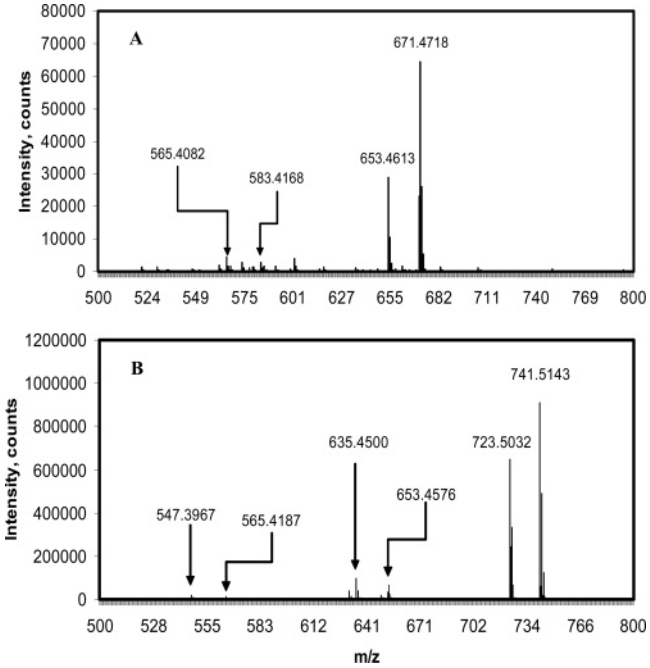

Figure 5. Mass spectra (APcI$^+$ mode) of *all-trans*-violaxanthin butyrate (**A**) and *all-trans*-violaxanthin dibutyrate (**B**) (corresponding to peaks 2 and 6, respectively, in **Figure 1** and **Table 2**), as examples of fragmentation pattern of carotenoid monoesters and diesters from crude mango extracts. Fragments belonging to the main fragmentation pathway are labeled.

the rings of violaxanthin could lead to a less polar compound. This could explain the higher retention time for peak 4 than that observed for *all-trans*-violaxanthin. The former ion of *m*/*z* 553 in the mass spectra of peak 16 confirmed the presence of protonated β-cryptoxanthin, whereas the quasimolecular ion for peaks 16, 21, and 22 (537, $[M + H]^+$) led to the identification of β-carotene isomers. The presence of cis/trans isomers of violaxanthin, β-cryptoxanthin, and β-carotene has been reported for some mango cultivars (*12*, *13*, *25*).

Some monoesters and diesters of violaxanthin were identified. Mass spectra for peaks 2 (**Figure 5A**) and 5 showed the same quasimolecular ion (*m*/*z* 671, $[M + H]^+$), which successively lost water (*m*/*z* 653) and one molecule of butyric acid of 88 Da (*m*/*z* 565). The ion resulting from the loss of butyric acid from the quasimolecular ion without the loss of water (*m*/*z* 583) was observed for both peaks. Thus, peaks 2 and 5 were tentatively identified as butyrates of *all-trans*/9-*cis*-violaxanthin. Cano and de Ancos (*16*) identified some violaxanthin monoesters in 'Alphonso' mango fruit according to their HPLC behavior and UV−vis spectra, but the fatty acid involved in such esters was not identified. The mass spectra for peaks 6 (**Figure 5B**) and 10 exhibited the same quasimolecular ion (*m*/*z* 741 $[M + H]^+$), which showed the successive loss of water (*m*/*z* 723) from the epoxy or hydroxyl groups, one neutral molecule of butyric acid (*m*/*z* 635), and the loss of another molecule of butyric acid (*m*/*z* 547). In addition, the mass spectra of both peaks revealed the sequential loss of the two molecules of butyric acid from the quasimolecular ion (*m*/*z* 653 and 565, respectively) without loss of water. This fragmentation pattern for diesters of violaxanthin has been previously reported in potatoes and mango (*19*, *40*). Thus, peaks 6 and 10 were identified as dibutyrates of *all-trans*-violaxanthin and 9-*cis*-violaxanthin. Our findings are in agreement with those generated by Pott et al. (*19*), who concluded that dibutyrates of *all-trans*-violaxanthin and 9-*cis*-violaxanthin were the main esters of violaxanthin in crude extracts of 'Kent' mangoes.

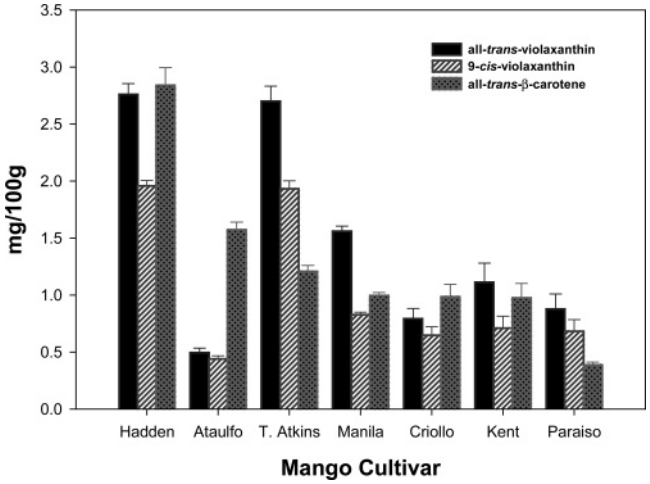

Figure 6. Content of selected carotenoids in the pulp of several mango cultivars. Error bars indicate the standard error of the mean of eight individual observations for each cultivar.

The mass spectrum for peak 9 showed that the quasimolecular ion (*m*/*z* 769, $[M + H]^+$) lost water (*m*/*z* 751), leading after that to two daughter ions, indicating the loss of butyric acid (*m*/*z* 663) and caproic acid of 116 Da (*m*/*z* 635). However, the ion corresponding to the loss of the two fatty acids without loss of water was not found in the mass spectrum. Peak 9 was identified as butyrate−caproate of *all-trans*-violaxanthin.

The mass spectra for other peaks were not clear, and they were identified as esters of *all-trans*/9-*cis*-violaxanthin or neoxanthin on the basis of their UV−vis data and the fact that they disappeared after the saponification step.

Quantitative Analysis of Carotenoids and Tocopherols. Three carotenoids (*all-trans*-β-carotene and *all-trans*/9-*cis*-violaxanthin dibutyrates) were predominant in the carotenoid pattern of all seven mango cultivars studied. Because two of these carotenoids were esters, native extracts were used for quantification, to avoid errors possibly caused by the saponification process. The most abundant carotenoid in the fruits of 'Ataulfo', 'Crillo', and 'Haden' was *all-trans*-β-carotene, whereas in the other cultivars *all-trans*-violaxanthin (as dibutyrate) was predominant. The content of *all-trans*-violaxanthin was higher than that of 9-*cis*-violaxanthin (as dibutyrates) in all cultivars (**Figure 6**). 'Haden' mangoes had high contents of the three carotenoids, and its *all-trans*-β-carotene concentration was highest compared to those of all other cultivars. 'Ataulfo' mango had a high amount of *all-trans*-β-carotene but the lowest amount of xanthophylls compared to the other cultivars. Godoy and Rodriguez-Amaya (*41*) have demonstrated that generally the most important carotenoid of mango is *all-trans*-β-carotene, representing 48−84% of the total carotenoid content, depending on cultivar and fruit maturity stage (*42*). The contents of *all-trans*-β-carotene in 'Tommy Atkins' and 'Kent' mangoes were similar to those reported by Pott et al. (*20*). The amounts of *all-trans*- and 9-*cis*-violaxanthin in all of the studied cultivars were similar to those reported in 'Keitt' mangoes by Mercadante et al. (*13*), but higher than those found in Taiwanese mangoes (*12*). These findings are in accordance with those reported by Mercadante et al. (*13*), who demonstrated that in general the most abundant carotenoids in mango are *all-trans*-β-carotene and *all-trans*- and 9-*cis*-violaxanthin, although the exact content and composition depend on the cultivar and the physiological stage of the fruit (*41*, *42*).

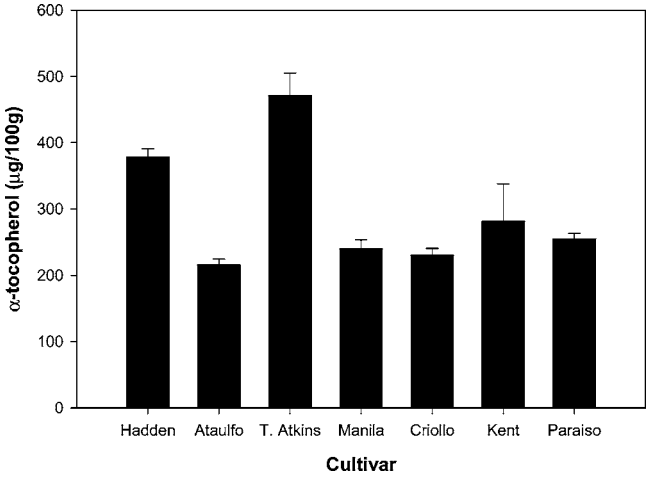

Figure 7. Content of α-tocopherol in the pulp of several mango cultivars. Error bars indicate the standard error of the mean of eight individual observations for each cultivar.

'Haden' and 'Tommy Atkins' mangoes had the highest α-tocopherol contents (**Figure 7**); however, the content of this vitamer in all mango cultivars was considerably higher than those reported for Costa Rican mangoes (*18*).

The results shown in this study confirm that *all-trans*-β-carotene, *all-trans*-violaxanthin, and 9-*cis*-violaxanthin are the most abundant carotenoids in mango, and the levels of these compounds and of α-tocopherol in Mexican mangoes are high in comparison with those of mangoes of other geographical origins.

LITERATURE CITED

(1) Yahia, E. M.; Ornelas, P. J. De J.; Ariza, F. R. *El Mango*; Editorial Trillas: México, DF, Mexico, 2006.

(2) Ramulu, P.; Rao, P. U. Total, insoluble and soluble dietary fiber contents of Indian fruits. *J. Food Compos. Anal.* **2003**, *16*, 677−685.

(3) Gorinstein, S.; Zemser, M.; Haruenkit, R.; Chuthakorn, R.; Grauer, F.; Martin-Belloso, O.; Trakhtenberg, S. Comparative content of total polyphenols and dietary fiber in tropical fruits and persimmon. *J. Nutr. Biochem.* **1999**, *10*, 367−371.

(4) Vinci, G.; Botré, F.; Mele, G.; Ruggieri, G. Ascorbic acid in exotic fruits: a liquid chromatographic investigation. *Food Chem.* **1995**, *53*, 211−214.

(5) Vázquez-Caicedo, A. L.; Neidhart, S.; Carle, R. Postharvest ripening behavior of nine Thai mango cultivars and their suitability for industrial applications. *Acta Hortic.* **2004**, *645*, 617−625.

(6) Bertram, J. S.; Vine, A. L. Cancer prevention by retinoids and carotenoids: independent action on a common target. *Biochim. Biophys. Acta*, **2005**, *1740*, 170−178.

(7) Bruno, R. S.; Medeiros, D. M. Lutein, zeaxanthin, and age-related macular degeneration. *J. Nutraceuticals Funct. Med. Foods* **2000**, *3*, 79−85.

(8) Palace, V. P.; Khaper, N.; Qin, Q.; Singal, P. K. Antioxidant potentials of vitamin A and carotenoids and their relevance to heart disease. *Free Radical Biol. Med.* **1999**, *26*, 746−761.

(9) Wolf, G. Multiple functions of vitamin A. *Physiol. Rev.* **1984**, *64*, 873−937.

(10) Graebner, I. T.; Siquiera, E. M. A.; Arruda, S. F.; de Souza, E. M. T. Carotenoids from native Brazilian dark-green vegetables are bioavailable: a study in rats. *Nutr. Res.* **2004**, *24*, 671−679.

(11) Wilberg, V. C.; Rodriguez-Amaya, D. B. HPLC quantitation of major carotenoids of fresh and processed guava, mango and papaya. *Lebens.-Wiss. -Technol.* **1995**, *28*, 474−480.

(12) Chen, J. P.; Tai, C. Y.; Chen, B. H. Improved liquid chromatographic method for determination of carotenoids in Taiwanese mango (*Mangifera indica* L.). *J. Chromatogr., A* **2004**, *1054*, 261−268.

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

(14) Ben-Amotz, A.; Fishler, R. Analysis of carotenoids with emphasis on 9-*cis*-β-carotene in vegetables and fruits commonly consumed in Israel. *Food Chem.* **1998**, *62*, 515−520.

(15) Setiawan, B.; Sulaeman, A.; Giraud, D. W.; Driskell, J. A. Carotenoid content of selected Indonesian fruits. *J. Food Compos. Anal.* **2001**, *14*, 169−176.

(16) Cano, M. P.; de Ancos, B. Carotenoid and carotenoid ester composition in mango fruit as influenced by processing method. *J. Agric. Food Chem.* **1994**, *42*, 2737−2742.

(17) Kimura, M.; Rodriguez-Amaya, D. B.; Godoy, H. T. Assessment of the saponification step in the quantitative determination of carotenoids and provitamins A. *Food Chem.* **1990**, *35*, 187−195.

(18) Burns, J.; Fraser, P. D.; Bramley, P. M. Identification and quantification of carotenoids, tocopherols and chlorophylls in commonly consumed fruits and vegetables. *Phytochemistry* **2003**, *62*, 939−947.

(19) Pott, I.; Breithaupt, D. E.; Carle, R. Detection of unusual carotenoid esters in fresh mango (*Mangifera indica* L. Cv. Kent). *Phytochemistry* **2003**, *64*, 825−829.

(20) Pott, I.; Marx, M.; Neidhart, S.; Mühlbauer, W.; Carle, R. Quantitative determination of β-carotene stereoisomers in fresh, dried, and solar-dried mango (*Mangifera indica* L.). *J. Agric. Food Chem.* **2003**, *51*, 4527−4531.

(21) Yahia, E. M.; Ornelas-Paz, J. de J.; Gardea, A. Extraction, separation and partial identification of 'Ataulfo' mango fruit carotenoids. *Acta Hortic.* **2006**, *712*, 333−338.

(22) Molnár, P.; Deli, J.; Zsila, F.; Steck, A.; Pfander, H.; Tóth, G. Preparation and (E/Z)-isomerization of the diastereoisomers of violaxanthin. *Helv. Chim. Acta* **2004**, *87*, 11−27.

(23) Acosta, R. M.; Neito, A. D.; Mena, N. G.; Teliz, O. D.; Vaquera, H. H.; Nieto, A. R. Effect of post-harvest temperatures on the development of internal darkening in mango fruits (*Mangifera indica* L) cv. Haden and their quality. *Acta Hortic.* **2000**, *509*, 401−412.

(24) Durigan, J. F.; de Almeida Teixeira, G. H.; Castanharo, N. M.; Domarco, R. E. Postharvest conservation of 'Tommy Atkins' mango fruit influenced by gamma radiation, wax, hot water, and refrigeration. *Acta Hortic.* **2004**, *645*, 601−604.

(25) Vázquez-Caicedo, A. L.; Sruamsiri, P.; Carle, R.; Neidhart, S. Accumulation of *all-trans*-β-carotene and its 9-*cis* and 13-*cis* stereoisomers during postharvest ripening of nine Thai mango cultivars. *J. Agric. Food Chem.* **2005**, *53*, 4827−4835.

(26) Sander, L. C.; Sharpless, K. E.; Pursch, M. C_{30} stationary phases for the analysis of food by liquid charomatography. *J. Chromatogr., A* **2000**, *880*, 189−202.

(27) Emenhiser, C.; Sander, L. C.; Schwartz, S. J. Capability of a polymeric C_{30} stationary phase to resolve *cis*−*trans* carotenoid isomers in reversed-phase liquid chromatography. *J. Chromatogr., A* **1995**, *707*, 205−206.

(28) Breithaupt, D. E.; Wirt, U.; Bamedi, A. Differentiation between lutein monoester regioisomers and detection of lutein diesters from marigold flowers (*Tagetes erecta* L.) and several fruits by liquid chromatography−mass spectrometry. *J. Agric. Food Chem.* **2002**, *50*, 66−70.

(29) Albert, K. Correlation between chromatographic and physicochemical properties of stationary phases in HPLC: C_{30} bonded reversed-phase silica. *Trends Anal. Chem.* **1998**, *17*, 648−658.

(30) Giuffrida, D.; Salvo, F.; Salvo, A.; La Pera, L.; Dugo, G. Pigments composition in monovarietal virgen olive oils from various sicilian olive varieties. *Food Chem.* **2007**, *101*, 833−837.

(31) Breitenbach, J.; Braun, G.; Steiger, S.; Sandmann, G. Chromatographic performance on a C_{30}-bonded phase of monohydroxycarotenoids with variable chain length or degree or desaturation and of lycopene isomers synthesized by various carotene desaturases. *J. Chromatogr., A* **2001**, *936*, 59−69.

(32) Lee, H. S.; Castle, W. S.; Coates, G. A. High-performance liquid chromatography for the characterization of carotenoids in the new sweet orange (Earlygold) grown in Florida, USA. *J. Chromatogr., A* **2001**, *913*, 371−377.
(33) Taylor, K. L.; Brackenridge, A. E.; Vivier, M. A.; Oberholster, A. High-performance liquid chromatography profiling of the major carotenoids in *Arabidopsis thaliana* leaf tissue. *J. Chromatogr., A* **2006**, *1121*, 83−91.
(34) Kamal-Eldin, A.; Görgen, S.; Pettersson, J.; Lampi, A.-M. Normal-phase high-performance liquid chromatography of tocopherols and tocotrienols. Comparison of different chromatographic columns. *J. Chromatogr., A* **2000**, *881*, 217−227.
(35) Richheimer, L. S.; Kent, C. M.; Bernard, W. M. Reversed-phase high-performance liquid chromatographic method using a pentafluorophenyl bonded phase for analysis of tocopherols. *J. Chromatogr., A* **1994**, *677*, 75−80.
(36) Abidi, L. S.; Mounts, L. T. Reversed-phase high-performance liquid chromatographic separations of tocopherols. *J. Chromatogr., A* **1997**, *782*, 25−32.
(37) Strohschein, S.; Pursch, M.; Lubda, D.; Albert, K. Shape selectivity of C_{30} phases for RP-HPLC separation of tocopherol isomers and correlation with MAS NMR data from suspended stationary phases. *Anal. Chem.* **1998**, *70*, 13−18.
(38) Meléndez-Martínez, A. J.; Vicario, M. I.; Heredia, F. J. Geometrical isomers of violaxanthin in orange juice. *Food Chem.* **2007**, *104*, 169−175.
(39) Meléndez-Martínez, J. A.; Britton, G.; Vicario, I. M.; Heredia, F. J. Relationship between the colour and the chemical structure of carotenoid pigments. *Food Chem.* **2007**, *101*, 1145−1150.
(40) Breithaupt, D.; Bamedi, A. Carotenoids and carotenoid esters in potatoes (*Solanum tuberosum* L.): new insights into an ancient vegetable. *J. Chromatogr., A* **2002**, *50*, 7175−7181.
(41) Godoy, H. T.; Rodriguez-Amaya, D. B. Carotenoid composition of commercial mangoes from Brazil. *Lebens.-Wiss. -Technol.* **1989**, *22*, 100−103.
(42) Mercadante, A. Z.; Rodriguez-Amaya, D. B. Effects of ripening, cultivar differences and processing on the carotenoid composition of mango. *J. Agric. Food Chem.* **1998**, *46*, 128−130.

Received for review March 9, 2007. Revised manuscript received May 25, 2007. Accepted May 29, 2007.

JF0706981