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Improved liquid chromatographic method for determination of carotenoids in Taiwanese mango (*Mangifera indica* L.)

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Abstract

An HPLC method was developed to determine the various carotenoids in Taiwanese mango (*Mangifera indica* L.). Initially, the peel and seed of mangoes were removed, the pulps were cut into pieces, freeze-dried, ground into powder, extracted and subjected to HPLC analysis. A mobile phase of methanol–isopropanol (99:1, v/v) (A) and methylene chloride (100%) (B) with the following gradient elution was developed: 100% A and 0% B in the beginning, maintained for 15 min, decreased to 70% A in 45 min, maintained for 15 min and returned to 100% A in 65 min. A total of 25 carotenoids were resolved within 53 min by using a C-30 column with flow rate at 1 mL/min and detection at 450 nm. α-Carotene was used as an internal standard to quantify all the carotenoids. All-*trans*-β-carotene was present in largest amount (29.34 μg/g), followed by *cis* isomers of β-carotene (9.86 μg/g), violaxanthin and its *cis* isomers (6.40 μg/g), neochrome (5.03 μg/g), luteoxanthin (3.6 μg/g), neoxanthin and its *cis* isomers (1.88 μg/g), zeaxanthin (1.16 μg/g) and 9- or 9′-*cis*-lutein (0.78 μg/g).

Keywords: Taiwanese mango; Carotenoid; Food analysis

1. Introduction

Mango, a popular fruit commodity produced in the summer of Taiwan and other countries in the world, has been shown to be a rich source of carotenoids [1,2]. Numerous studies have demonstrated that carotenoids, such as lutein and β-carotene possess antioxidant activity and thus may enhance LDL degradation and prevent cardiovascular disease [3,4]. Of the various carotenoids in plants, lycopene has been reported to exhibit the highest antioxidant activity, followed by β-cryptoxanthin, β-carotene, lutein and zeaxanthin [5]. Therefore, it is important to learn about the variety and amount of carotenoids in mango. The content and variety of carotenoids in mango can be affected by many factors, i.e., growth condition, maturity and cultivar [1,6]. For instance, the major carotenoids in Bahiaand Keift varieties of Brazil are all-*trans*-violaxanthin, all-*trans*-β-carotene and 9-*cis*-violaxanthin, while in some other cultivars, such as Bourbon and Haden, the dominant carotenoids are β-carotene, luteoxanthin and violaxanthin [6,7]. John et al. [8] further pointed out that phytofluene is the main carotenoid in ripen mango, whereas β-carotene is the major carotenoid in unripen mango. Obviously, the maturity and environmental conditions have a great impact on the carotenoid profile in mango.

The separation of carotenoids in mango has been previously achieved by using reversed-phase HPLC with a C-18 column [9] or normal-phase HPLC with a nitrile column [6]. However, the resolution of geometrical isomers of carotenoids by employing a C-18 column has been shown to be inadequate [10]. Emenhiser et al. [10] further proved that a C-30 column could provide better resolution of geometrical isomers of carotenoids than a C-18 column. As no information is available as to the level and variety of carotenoids in Taiwanese mango, this study was undertaken to use a C-30 column and develop an HPLC method for determination.

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2. Experimental

2.1. Materials

A total of 150 mangoes with an average weight of 750 g were purchased from a farm located in the Tainan county, Taiwan and transported to the laboratory on the same day immediately. Mangoes were washed with tap water, peel and seed removed and the pulps were cut into pieces, freeze-dried and ground into fine powder prior to extraction. Spinach, used for preparation of neoxanthin and violaxanthin standards, was obtained from a local supermarket and also cut into pieces, freeze-dried and ground into fine powder. Yellow corn, used for preparation of zeaxanthin standard, were also freeze-dried and ground into fine material. All-*trans*-lutein, all-*trans*-β-carotene and all-*trans*-α-carotene standards were from Sigma (St. Louis, MO, USA). Chemicals, including potassium hydroxide, anhydrous sodium sulfate and magnesium oxide were from Riedel-de Häen Co. (Barcelona, Spain). Diatomaceous earth was from J.T. Baker Co. (Phillipsburg, NJ, USA). A YMC C30 column (250 mm × 4.6 mm i.d., 5 μm) was from Waters Co. (Milford, MA, USA). The TLC plates (Silica gel 60 F254) were from Merck (Germany).

2.2. Instrumentation

The HPLC instrument consists of two Jasco PU-980 pumps (Jasco Co., Tokyo, Japan), a Rheodyne model 7161 injector (Rheodyne Co., Rohnert Park, CA), a model DP-4010 degasser (Sanwa Tsusho Co., Tokyo, Japan), a Jasco MD-915 photodiode-array detector and a Borwin computer software. The rotary evaporator (model N-1) was from Eyela Co. (Tokyo, Japan). The freeze-dryer (model FD-24) was from Chin-Ming Co. (Taipei, Taiwan). The sonicator (model 2210R-DTH) was from Branson Co. (Danbury, CT, USA). The low-temperature incubator (model TL520R) was from Sheng-Long Co. (Taipei, Taiwan). The spectrophotometer (model CE3021) was from Cecil Co. (Cambridge, UK).

2.3. Extraction of carotenoids from dried mango

One-gram dried mango was mixed with 30 mL hexane–ethanol–acetone–toluene (10:6:7:7, v/v/v/v) in a 100 mL volumetric flask. After shaking for 1 h, 2 mL 40% methanolic KOH was added and the solution was saponified at 25 °C in the dark for 16 h. Then, 30 mL of hexane was added for partition of carotenoids, shaken for 1 min and 10% sodium sulfate solution was added and diluted to volume. After shaking for 1 min, the upper layer was collected. The lower layer was repeatedly extracted twice and the supernatant was also collected. The upper extracts were pooled, evaporated to dryness, dissolved in 1 mL methanol–methylene chloride–isopropanol (89:1:10, v/v/v) and filtered through a 0.2-μm membrane filter for HPLC analysis.

2.4. Preparation of neoxanthin and violaxanthin standards

Because of absence of commercial neoxanthin and violaxanthin standards, both were prepared from spinach, using a method described by Chen et al. [11]. Prior to development, the TLC plates (20 cm × 20 cm) were activated at 110 °C for 1 h, placed in glass tanks lined with filter paper and 150 mL methanol–acetone–hexane (1:29:70, v/v/v) was poured into the tank for saturation for 30 min. Ten microliters of carotenoid extract was applied to the TLC plate, using a syringe. The chromatograms were developed for a distance of 16 cm, after which both neoxanthin and violaxanthin bands were scraped individually and each was poured into a glass funnel attached to a small side-arm filtration flask. A total of 30 spots were collected separately for violaxanthin and neoxanthin bands and both were eluted with acetone with the absorbance measured at 443 nm for violaxanthin and 439 nm for neoxanthin, using a spectrophotometer. The concentrations were calculated, using the following formula [12]:

$$\text{Concentration (g/mL)} = \frac{E}{E^{1\%}_{1\text{cm}} \times 100}$$

where E is the absorbance and $E^{1\%}_{1\text{cm}}$ is the extinction coefficient (violaxanthin 2550 and neoxanthin 2243). The concentrations of violaxanthin and neoxanthin were calculated to be 6.32 and 2.58 μg/mL, respectively.

2.5. Preparation of zeaxanthin standard

Zeaxanthin standard was prepared from yellow corn, using a method described by Quackenbush et al. [13]. Initially, 25 gm yellow corn powder was mixed with 150 mL hexane in a flask and the mixture was shaken in a shaker for 1 h. Then, 30 mL of 40% methanolic KOH was added and the solution was saponified at 25 °C in the dark for 16 h, after which 150 mL hexane was added for extraction of carotenoids. The mixture was shaken for 1 min and stood in the dark for 1 h until two layers were formed. The upper layer was collected and concentrated to 10 mL, of which 5 mL was collected and poured into a column (30 cm × 20 mm i.d.) containing a mixture of 15 g magnesium oxide and 15 g diatomaceous earth. Anhydrous sodium sulfate was poured into the column to form a layer of 1 cm above the adsorbent. A solvent system of hexane-acetone with a proportion of 95:5 and 90:10 (v/v) was used to elute carotenes and zeinoxanthin, respectively. Both β-cryptoxanthin and lutein were eluted separately with hexane–acetone–ethanol (89:10:1, v/v/v), while zeaxanthin eluted with the same solvent system with a ratio of 88:10:2 (v/v/v). The zeaxanthin band was evaporated to dryness and dissolved in acetone with the absorbance measured at 452 nm. The concentration was calculated to be 52.45 μg/mL based on a formula shown above and an extinction coefficient of 2340 ($E^{1\%}_{1\text{cm}}$) as reported by Davies [12].

2.6. Isomerization of both lutein and β-carotene standards

One-gram lutein standard was dissolved in 10 mL methylene chloride and poured into 10–20 mL vials so that each vial contains lutein at a concentration of 100 mg/mL. The vials were placed in an incubator and illuminated at 25 °C for 24 h. Similarly, 1 gm β-carotene standard was dissolved in 25 ml methylene chloride and poured into 25–20 mL vials so that each vial contains β-carotene at a concentration of 40 mg/mL. All the vials were exposed to four fluorescent tubes (20 W each) with a distance of 30 cm and light intensity of 2000–3000 lux. Both illuminated standards were evaporated to dryness separately, dissolved in methanol–methylene chloride–isopropanol (89:1:10, v/v/v) and filtered through a 0.2-μm membrane filter for HPLC analysis.

2.7. Determination of recovery

Two concentrations each of carotenoid standards, including all-*trans*-lutein (5 and 10 μg/mL), all-*trans*-β-carotene (5 and 10 μg/mL), zeaxanthin (5.3 and 10.5 μg/mL), neoxanthin (2.6 and 1.3 μg/mL) and violaxanthin (6.3 and 3.2 μg/mL) were added to dried mangoes (1 g) for extraction. After HPLC analysis, the recovery of each carotenoid was calculated based on the ratio of the concentration obtained after HPLC to the concentration added in the beginning. The recoveries of neoxanthin, violaxanthin, zeaxanthin, all-*trans*-lutein and all-*trans*-β-carotene were found to be 86.6, 94.3, 92.6, 81.0 and 79.2%, respectively. Because of absence of commercial standards and similarity in extinction coefficient, the quantification of *cis* isomers of carotenoids was based on the recoveries of their parent *trans*-form carotenoids.

2.8. HPLC analysis of carotenoids in dried mango

Various binary and ternary solvent systems in isocratic or gradient mode were used to compare the separation efficiency of carotenoids in mango. In addition, the different sample solvents were also compared. The separation efficiency was evaluated by retention factor (κ') and separation factor (α). After various studies, the most appropriate mobile phase was found to be composed of methanol–isopropanol (99:1, v/v) (A) and methylene chloride (100%) (B) with the following gradient elution: 100% A and 0% B in the beginning, maintained for 15 min, decreased to 70% A in 45 min, maintained for 15 min and returned to 100% A in 65 min. A C-30 column was used with flow rate at 1.0 mL/min and detection at 450 nm. The most suitable sample solvent was found to be methanol–methylene chloride–isopropanol (89:1:10, v/v/v) and the injection volume was 20 μL. A total of 25 carotenoids were resolved within 53 min. The various carotenoids were identified by comparing retention time and absorption spectra of unknown peaks with reference standards as well as cochromatograghy with added standards. In addition, the *cis* isomers were tentatively identified based on the spectrum characteristics and *Q* ratios as reported in the literature [14,15]. Quantification was carried out using an internal standard all-*trans*-α-carotene. A fixed concentration of all-*trans*-α-carotene (10 μg/mL) was mixed with various concentrations of all-*trans*-luetin (1, 5, 8, 10 and 20 μg/mL), all-*trans*-β-carotene (1, 5, 10, 20 and 30 μg/mL) and zeaxanthin (1.1, 5.3, 10.5, 26.2 and 52.5 μg/mL) separately. The standard curves of all-*trans*-lutein, all-*trans*-β-carotene and all-*trans*-zeaxanthin were each prepared by plotting concentration ratio against area ratio. The correlation coefficient (r^2) and regression equations were obtained, using Microsoft Excel XP software. A high correlation coefficient ($r^2 > 0.99$) was found for all the standard curves and the regression equations for all-*trans*-zeaxanthin, all-*trans*-lutein and all-*trans*-β-carotene were $y = 0.8206x + 0.0691$, $y = 0.9316x + 0.0602$ and $y = 2.1624x + 0.015$, respectively. The standard curves for neoxanthin and violaxanthin were not prepared because of purity problem, as evidenced by the presence of several peaks on the HPLC chromatogram when both collecting from TLC were injected separately into HPLC. Thus, both neoxanthin and violaxanthin were quantified by calculating the area ratio of each to all-*trans*-α-carotene and multiplying the concentration of all-*trans*-α-carotene. The data were subjected to analysis of variance and Duncan's multiple range test, using SAS [16]. The purity of each peak was automatically determined with a Jasco photodiode-array detector.

2.9. Determination of limits of detection (LOD) and quantification (LOQ)

Both LOD and LOQ were measured based on a method described by the International Conference on Harmonization [17]. Three concentrations were prepared for zeaxanthin (1.1, 5.3 and 10.5 μg/mL), lutein (1.0, 5.0 and 8.0 μg/mL) and β-carotene (1.0, 2.0 and 10.0 μg/mL). Each was analyzed three times and the calibration curves were obtained by plotting concentration against area. The LODs for zeaxanthin, lutein and β-carotene were 0.2, 0.2 and 0.2 μg/g, respectively, while the LOQs were 0.7, 0.5 and 0.5 μg/g, based on a formula described in a previous study [18].

3. Results and discussion

3.1. HPLC analysis of carotenoids in dried mango

In view of the complexity of carotenoids in mango [6,9], a gradient solvent system of methanol–isopropanol and methylene chloride described in the method section was developed to resolve 25 carotenoids in dried mango. Fig. 1 shows the HPLC chromatogram of carotenoids in Taiwanese mango. A total of 26 carotenoids, including internal standard all-*trans*-α-carotene were resolved. With the exception of several peaks, most carotenoids were adequately resolved. The κ' values for all peaks ranged from 1.44 to 19.85, indicating that a proper solvent strength was controlled. Dolan [19]

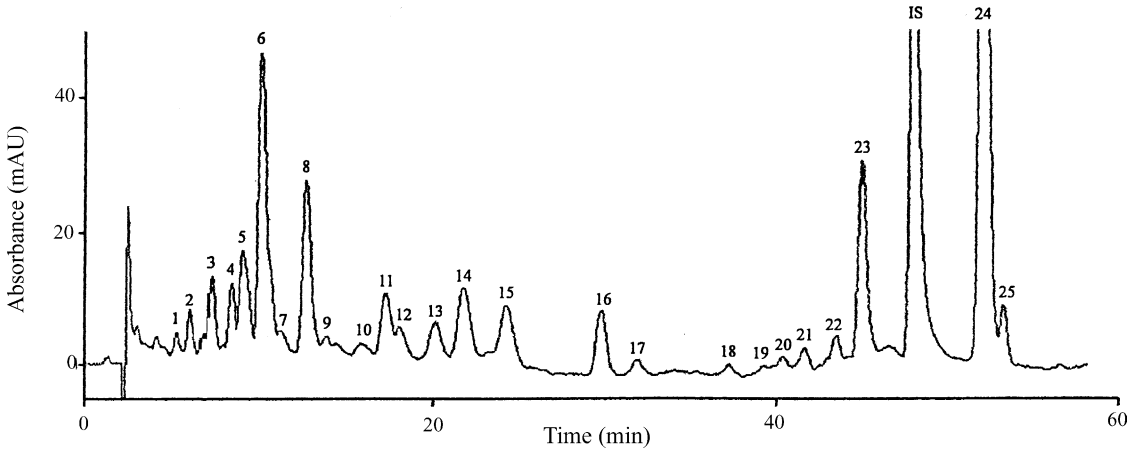

Fig. 1. HPLC chromatogram of carotenoids in dried mango.

reported that for separation of complicated components, the κ' values can be ranged between 0.5 and 20. The α values for all peaks were greater than 1, implying that a good selectivity of mobile phase to sample components was achieved. With the exception of peaks 2 and 9, the purities of all the other peaks were higher than 90% (Table 2).

Fig. 2 shows the HPLC chromatogram of all-*trans*-lutein standard after illumination at 25 °C for 48 h. A total of 11 peaks were resolved. Peak 9 was positively identified as all-*trans*-lutein and the other peaks were tentatively identified as *cis*-isomers of lutein based on spectra characteristics and *Q*-ratios as reported in the literature (Table 3). Peaks 5 and 7 were thus identified as 13- or 13′-*cis*-lutein, while peaks 10 and 11 were identified as 9- or 9′-*cis*-lutein. A large hypsochromic shift may indicate the presence of *cis*-lutein for peaks 1–4 [15]. Fig. 3 shows the HPLC chromatogram of all-*trans*-β-carotene standard after illumination at 25 °C for 24 h. Likewise, a total of 11 peaks were resolved and peak 7 was positively identified as all-*trans*-β-carotene whereas the other peaks were tentatively identified as *cis*-isomers of β-carotene (Table 4). Similarly, peaks 4 and 5 were tentatively identified as 15- or 15′-*cis*-β-carotene, whereas peaks 6 and 8 were identified as 13- or 13′-*cis*-β-carotene. No *cis* position was assigned to the other peaks because of inconsistent wavelength shift and no *Q*-ratio is available. Peaks 9–11 were excluded as *cis* isomers of β-carotene because of presence of abnormal absorption spectra. The retention behavior and spectra characteristics of all the peaks in Figs. 2 and 3 were compared with those unknown peaks in Fig. 1 for further identification of the various carotenoids in dried mango. Both illuminated lutein and β-carotene standard solutions were also added to the mango extract separately and injected into HPLC for co-chromatography.

Peaks 16 and 24 were positively identified as all-*trans*-zeaxanthin and all-*trans*-β-carotene based on the criteria shown in the method section. Peaks 1–15 were tentatively identified as neoxanthin, violaxanthin and their *cis* isomers or derivatives, on the basis of a hypsochromic shift of about 20 nm for neoxanthin and 40 nm for violaxanthin after addition of a few drops of 0.1 N methanolic HCl to the sample extract (Table 1). The collection of both neoxanthin and violaxanthin spots from TLC as well as injection into HPLC also

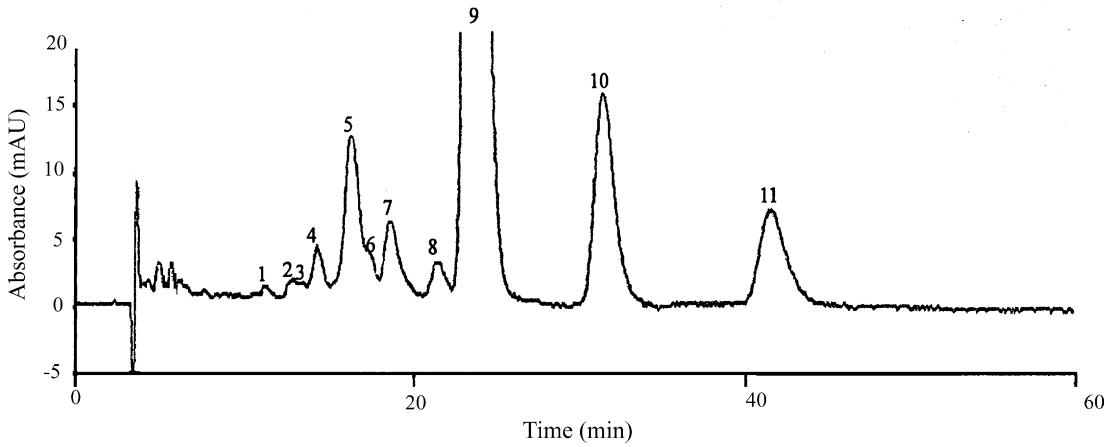

Fig. 2. HPLC chromatogram of all-*trans*-lutein standard after illumination at 25 °C for 48 h. Chromatographic conditions described in text. Peaks: (1) *cis*-lutein; (2) *cis*-lutein; (3) *cis*-lutein; (4) *cis*-lutein; (5) 13- or 13′-*cis*-lutein; (6) *cis*-lutein; (7) 13- or 13′-*cis*-lutein; (8) *cis*-lutein; (9) all-*trans*-lutein; (10) 9- or 9′-*cis*-lutein; (11) 9- or 9′-*cis*-lutein.

Table 1
Tentative identification data for all-*trans* plus *cis* forms of carotenoids in dried mango

Peak no.	Compound	Retention time (min)	λ (nm, in-line)[a]				λ (nm, reported)				Epoxide test hypsochromic shift			*Q*-ratio found	*Q*-ratio reported
1	Violaxanthin	6.19		435	465		415	441	471[b]		399	435		0.22	–
2	Neoxanthin	6.85	411	435	465		415	438	468[b]		417	441		0.35	–
3	Neochrome	8.08		417	441		400	422	449[b]					0.25	–
4	Neoxanthin	9.24	412	435	465		415	438	468[b]	393	417	441		0.18	–
5	Neochrome	9.90		417	441		401	423	449[c]					0.22	–
6	Violaxanthin	10.64	411	435	465		415	438	468[b]	393	417	441		0.1	–
7	*cis*-Neoxanthin	11.88	411	429	459						410	440		0.28	–
8	Neochrome	13.12		417	441		401	423	449[c]					0.10	–
9	Luteoxanthin	14.52		423	441	465	409	431	460[d]		404	422		0.22	0.52[d]
10	*cis*-Violaxanthin	15.92	393	423	453		409	431	460[d]	375	393	417		0.19	0.52[d]
11	*cis*-Violaxanthin	17.57		429	459		409	431	460[d]		416	434		0.13	–
12	*cis*-Violaxanthin	18.32	411	435	459		334 419	442	466[e]		416	440		0.40	0.33[e]
13	Luteoxanthin	20.30	393	417	441		–				398	421		0.14	–
14	Luteoxanthin	22.28	339	423	447		–				402	427		0.16	–
15	Luteoxanthin	24.59	399	423	447						401	426		0.16	–
16	Zeaxanthin	30.45		447	471		424	454	478[e]					–	0.06[e]
17	*cis*-Zeaxanthin	31.98	417	440	470		–							–	–
18	*cis*-Zeaxanthin	37.37	416	441	470		–							–	–
19	*cis*-Zeaxanthin	40.11		435	471		–							–	–
20	9- or 9′-*cis*-Lutein	41.18	411	435	453	465	334 419	442	466[e]					0.11	0.19[e]
21	*cis*-β-Carotene	41.84	429	447	465		–							0.44	–
22	*cis*-β-Carotene	43.16	429	441	465		413	437	458[f]					0.68	–
23	15- or 15′-*cis*-β-Carotene	44.64	411	441	465		421	443	470[g]					0.43	0.43[g]
	All-*trans*-α-carotene (IS)	48.08		447	476									–	
24	All-*trans*-β-carotene	51.33	423	447	477		426	454	478[e]					–	0.08[e]
25	13- or 13′-*cis*-β-carotene	52.82	423	441	465		419	442	465[h]					0.31	0.35[h]

[a] A gradient mobile phase of methanol–2-proponol (99:1, v/v) and methylene chloride (from 100:0 to 70:30, v/v) was used.
[b] A mobile phase of acetone and water (from 70:30 to 100:0, v/v) was used by Razungles et al. [21].
[c] A mobile phase of MeCN + MeOH (75:25, v/v) (solvent A), MTBE (solvent B) and water (solvent C) (from 95:5:0 to 26:74:0, v/v/v) was used by Lee et al. [22].
[d] A mobile phase of hexane + 0.1% (v/v) diisopropylamide (solvent A) and dichloromethane + 2% (v/v) methanol (solvent B) in ratio 62A:38B was used by Phillip et al. [23].
[e] A mobile phase of methanol–methylene chloride–isopropanol (89:1:10, v/v/v) was used by Tai and Chen [14].
[f] A mobile phase of methanol–methylene chloride (99:1, v/v) was used by Chen et al. [24].
[g] A gradient mobile phase of 1-butanol–acetonitrile (30:70, v/v) and methylene chloride (from 99:1 to 90:10, v/v) was used by Lin and Chen [15].
[h] A mobile phase of 1-butanol–acetonitrile–methylene chloride (30:70:10, v/v/v) was used by Lee and Chen [18].

Table 2
Retention time, retention factor (κ'), separation factor (α), purity and content (μg/g) of carotenoids in dried mango

Peak no.	Compound	Retention time (min)	κ'[a]	α[b]	Peak purity (%)	Content (μg/g)
1	Violaxanthin	6.19	1.44	1.18 (1, 2)[c]	95.9	0.27
2	Neoxanthin	6.85	1.70	1.18 (1, 2)[c]	82	0.56
3	Neochrome	8.08	2.19	1.29 (2, 3)[c]	96.5	0.86
4	Neoxanthin	9.24	2.65	1.21 (3, 4)[c]	96.1	0.86
5	Neochrome	9.90	2.91	1.10 (4, 5)[c]	95	1.66
6	Violaxanthin	10.64	3.2	1.10 (5, 6)[c]	97.3	4.33
7	*cis*-Neoxanthin	11.88	3.69	1.08 (7, 8)[c]	95	0.46
8	Neochrome	13.12	4.18	1.13 (8, 9)[c]	87	2.51
9	Luteoxanthin	14.52	4.73	1.13 (9, 10)[c]	97.2	0.30
10	*cis*-Violaxanthin	15.92	5.29	1.12 (10, 11)[c]	96.5	0.23
11	*cis*-Violaxanthin	17.57	5.94	1.12 (11, 12)[c]	97.1	1.05
12	*cis*-Violaxanthin	18.32	6.23	1.05 (12, 13)[c]	97.4	0.52
13	Luteoxanthin	20.30	7.02	1.13 (13, 14)[c]	92	0.69
14	Luteoxanthin	22.28	7.80	1.11 (14, 15)[c]	96.7	1.36
15	Luteoxanthin	24.59	8.71	1.12 (15, 16)[c]	98	1.25
16	Zeaxanthin	30.45	11.02	1.27 (16, 17)[c]	99.4	1.16
17	*cis*-Zeaxanthin	31.98	11.63	1.05 (17, 18)	99.4	ND
18	*cis*-Zeaxanthin	37.37	13.75	1.18 (18, 19)	99.9	ND
19	*cis*-Zeaxanthin	40.11	14.83	1.08 (19, 20)[c]	99.9	ND
20	9- or 9′-*cis*-Lutein	41.18	15.26	1.03 (20, 21)[c]	99.9	0.78
21	*cis*-β-Carotene	41.84	15.52	1.02 (21, 22)[c]	99.9	0.56
22	*cis*-β-Carotene	43.16	16.04	1.03 (22, 23)[c]	99.9	0.62
23	15- or 15′-*cis*-β-Carotene	44.65	16.63	1.02 (24, 25)[c]	97.1	7.20
	All-*trans*-α-carotene (IS)	48.08	18.0	1.04 (26, 27) [c]	96.6	
24	All-*trans*-β-carotene	51.33	19.27	1.07 (27, 28)[c]	92.4	29.34
25	13- or 13′-*cis*-β-Carotene	52.82	19.85	1.03 (28, 29)[c]	99.9	1.48

[a] κ': Retention factor.
[b] α: Selectivity (separation factor).
[c] Numbers in parentheses represent values between two peaks.

confirm this result. Peaks 9 and 13–15 were tentatively identified as luteoxanthin because a hypsochromic shift of 20 nm occurred when compared to violaxanthin before epoxide test and a further shift of 20 nm was shown after epoxide test, indicating the conversion from luteoxanthin to auroxanthin under acidic condition [12]. Peaks 1, 6 and 10–12 were tentatively identified as violaxanthin or its *cis* isomers, since a large hypschromic shift of about 40 nm was found after epoxide test, implying the formation of auroxanthin from violaxanthin. Peaks 2, 4 and 7 were tentatively identified as neoxanthin or its *cis* isomers as a hypsochromic shift of about 20 nm was shown after epoxide test, revealing the formation of neochrome from neoxanthin in the presence of HCl. Peaks 3, 5 and 8 were tentatively identified as neochrome or its *cis*

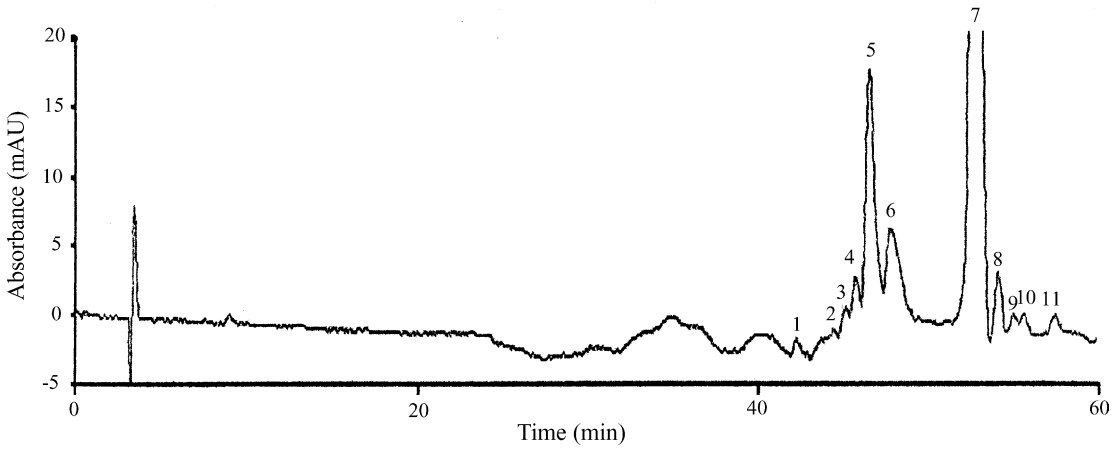

Fig. 3. HPLC chromatogram of all-*trans*-β-carotene standard after illumination at 25 °C for 24 h. Chromatographic conditions described in text. Peaks: (1) *cis*-β-carotene; (2) *cis*-β-carotene; (3) *cis*-β-carotene; (4) 15- or 15′-cis-β-carotene; (5) 15- or 15′-cis-β-carotene; (6) 13- or 13′-cis-β-carotene; (7) all-*trans*-β-carotene; (8) 13- or 13′-cis-β-carotene.

Table 3
Identification data for all-*trans* and *cis* forms of lutein after illumination of all-*trans*-lutein standard at 25 °C for 48 h

Peak no.	Compound	Retention time (min)	κ'[a]	Peak purity (%)	λ (nm, in-line)[b]				λ (nm, reported)				*Q*-ratio found	*Q*-ratio reported
1	*cis*-Lutein	11.14	2.14	92.8	327	405	423	447	–[c]				0.69	–
2	*cis*-Lutein	12.70	2.61	90.1	333	405	423	441	–				0.28	–
3	*cis*-Lutein	13.14	2.80	86.6	339	405	429	447	–				0.35	–
4	*cis*-Lutein	14.26	3.05	91.6	339	405	423	453	–				0.53	–
5	13- or 13′-*cis*-Lutein	16.08	3.62	93.6	327	406	435	459	334	419	442	466[d]	0.33	0.33[d]
6	*cis*-Lutein	17.33	3.92	91.5	339	417	441	464	–				0.22	–
7	13- or 13′-*cis*-Lutein	18.57	4.27	94.6	333	405	435	459	334	419	442	466[d]	0.38	0.33[d]
8	*cis*-Lutein	21.48	5.09	91.6	333	429	441	465	–				0.22	–
9	All-*trans*-lutein	23.56	5.68	99.5			441	465		426	448	472[d]	–	0.06[d]
10	9- or 9′-*cis*-Lutein	31.38	7.90	96.4	321		435	459	334	419	442	466[d]	0.09	0.19[d]
11	9- or 9′-*cis*-Lutein	41.56	10.76	92.5	345		435	459	334	419	442	466[d]	0.10	0.19[d]

[a] κ': Retention factor.
[b] A gradient mobile phase of methanol–isopropanol (99:1, v/v) and methylene chloride (from 100:0 to 70:30, v/v) was used.
[c] (–): Data not available.
[d] A mobile phase of methanol–methylene chloride–isopropanol (89:1:10, v/v/v) was used by Tai and Chen [14].

Table 4
Identification data for all-*trans* and *cis* forms of β-carotenen after illumination of all-*trans*-β-carotenen standard at 25 °C for 24 h

Peak no.	Compound	Retention time (min)	κ'[a]	Peak purity (%)	λ (nm) (in-line)[b]				λ (nm) (reported)			*Q*-ratio found	*Q*-ratio reported
1	*cis*-β-Carotene	42.33	10.37	99.9			442	471	413	437	458[d]	–[c]	–
2	*cis*-β-Carotene	44.50	11.06	99.9	327	417	441	464	413	437	458[d]	–	–
3	*cis*-β-Carotene	45.17	11.22	99.9	327	417	447	471	413	437	458[d]	–	–
4	15- or 15′-*cis*-β-Carotene	45.80	11.74	99.9	327	417	441	464	421	443	470[e]	0.41	0.43[e]
5	15- or 15′-*cis*-β-Carotene	46.56	11.91	99.7	327		441	465	421	443	470[e]	0.43	0.43[e]
6	13- or 13′-*cis*-β-Carotene	47.85	12.31	99.7	327		447	477	419	442	465[g]	0.32	0.35[g]
7	All-*trans*-β-carotene	52.69	13.84	93.5			453	477	426	454	478[f]	0.04	0.08[f]
8	13- or 13′-*cis*-β-Carotene	54.09	14.29	99.9	333		447	471	419	442	465[g]	0.35	0.35[g]

[a] κ': Retention factor.
[b] A gradient mobile phase of methanol–isopropanol (99:1, v/v) and methylene chloride (from 100:0 to 70:30, v/v) was used.
[c] (–): Data not available.
[d] A mobile phase of methanol–methylene chloride (99:1, v/v) was used by Chen et al. [24].
[e] A gradient mobile phase of 1-butanol–acetonitrile (30:70, v/v) and methylene chloride (from 99:1 to 90:10, v/v) was used by Lin and Chen [15].
[f] A mobile phase of methanol–methylene chloride–isopropanol (89:1:10, v/v/v) was used by Tai and Chen [14].
[g] A mobile phase of acetone–hexane (3:97, v/v) was used by Tsukida et al. [25].

isomers on the basis of a hypsochromic shift of 20 nm when compared to neoxanthin before epoxide test and no wavelength change was observed after epoxide test. Peaks 17–19 were identified as *cis*-zeaxanthin, since a hypsochromic shift occurred when compared to all-*trans*-zeaxanthin. Similarly, peak 20 was tentatively identified as 9- or 9′-*cis*-lutein. Peak 23 was tentatively identified as 15- or 15′-*cis*-β-carotene, while peak 25 was identified as 13- or 13′-*cis*-β-carotene [15]. Both peaks 21 and 22 were identified as *cis* isomers of β-carotene, however, no *cis* position is assigned because there are no *Q*-ratio values in the literature available.

The contents of various carotenoids in Taiwanese mango is shown in Table 2. Obviously the epoxy-containing carotenoids, such as neoxanthin and violaxanthin constitute a large portion. Nevertheless, the amounts of all-*trans*-β-carotene plus its *cis* isomers were found to be the largest, followed by violaxanthin plus its *cis* isomers, neochrome, luteoxanthin, neoxanthin plus its *cis* isomers, zeaxanthin and 9- or 9′-*cis*-lutein. This result is different from a report by Mercadante et al. [6], who showed that violaxanthin was present in the highest level, followed by β-carotene. As described before, this difference may be attributed to cultivar and geographic effects [1]. In addition, the analytical procedure used in study may account for this difference. In our study, a total of 25 carotenoids were separated, while in some other studies, a total of 14 carotenoids were resolved by Mercadante et al. [6] and 6 carotenoids by Pott et al. [20]. Apparently, the separation method developed in this study provided a better resolution power than the other methods. Further research is necessary to study the stability of carotenoids in Taiwanese mango as affected by various processing methods.

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