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Determination of *cis*- and *trans*- α- and β-carotenoids in Taiwanese sweet potatoes (*Ipomoea batatas* (L.) Lam.) harvested at various times

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ABSTRACT

A HPLC method was improved to determine sweet potato carotenoids rapidly with good separation efficiency. A C30 column and a gradient solvent system consisting of methanol–acetonitrile–water (84/14/2, v/v/v, solvent A) and dichloromethane (solvent B) (a mixture of 80% A and 20% B was used initially, and then the mixing was programmed to 55% B within 15 min and kept to the end) were used for analysis. The flow rate was 1 ml/min and detection was at 450 nm. A total of 11 all-*trans* and *cis* forms of α- and β-carotene in Taiwanese sweet potato (*Ipomoea batatas* (L.) Lam.) could be resolved within 16 min. The orange-fleshed sweet potato (Tainung 66) had higher total carotenoid content than the yellow-fleshed one (Tainung 57) at the same harvest time. The total carotenoid levels in both crops harvested at various times were in the order: October > July > April > January.

1. Introduction

Sweet potato (*Ipomoea batatas* (L.) Lam.) (belongs to *convoluvlaceae* family) is an important and valuable staple crop worldwide (Food and Agriculture Organization, 1987). It is a nutritious and generous food source for humans and animals as well as a raw material for manufacturing starch, sugar, alcohol and so on (Kozai et al., 1997; Saiful Islam, Kubota, Takagaki, & Kozai, 2002). The crop grows throughout the tropics and subtropics (Scott, 1992), especially in Asian and African countries where account for about 95% of the world's sweet potato production (Mok, Zhang, & Carey, 1997). Sweet potato has high yield even though it was cultivated in adverse situations (Simonne, Kays, Koehler, & Eilenmiller, 1993). In Taiwan, the annual production of this crop is about 0.2 million metric tons (Yearly Report of Taiwan's Agriculture, 2005). It can be harvested throughout the year.

Some reports (K'osambo, Carey, Misra, Wilkes, & Hagenimana, 1998; Van Jaarsveld, Marais, Harmse, Nestel, & Rodriguez-Amaya, 2006) indicated that sweet potato contains abundant β-carotene particularly for that with orange flesh. Carotenoids are antioxidants that could suppress singlet oxygen ($^1O_2$) forming and lipid peroxidation (Burton & Ingold, 1984; Foote & Denny, 1968); moreover, they could reduce incidence of certain cancers and coronary heart disease in humans (Gester, 1993; Ziegler, 1989). However, high dose β-carotene supplements could not show protective effects against cancer and cardiovascular disease (Paiva & Russell, 1999). The agricultural research organizations of Taiwan have remarkable results in cultivation, harvesting technologies and post-harvest treatments of sweet potato. Huang, Chang, and Shao (2006) have evaluated the antioxidant activity of Taiwanese sweet potato by assessing contents of total phenols, flavonoids, anthocyanins and vitamin C. However, there are no thorough reports on the content of carotenoids in sweet potato cultivated in Taiwan.

The reversed-phase high performance liquid chromatography (RP-HPLC) has been used routinely to determine carotenoids because of its satisfactory separation efficiency (Chen, Tai, & Chen, 2004; Inbaraj, Chien, & Chen, 2006). Furthermore, some investigations (Chen et al., 2004; Lin & Chen, 2003; Sander, Sharpless, & Pursch, 2000) manifested that a C30 column had better resolution than a C18 column for separation of carotenoids and their geometric isomers.

In the present study, we improved a HPLC method with a C30 column for rapid and efficient analysis of carotenoids and their isomers in sweet potato. The contents and compositions of these compounds in Tainung 66 (with orange flesh) and Tainung 57 (with yellow flesh), the major and popular sweet potatoes in Taiwan, harvested in four different seasons of the year (2007) were also surveyed.

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2. Materials and methods

2.1. Materials

Tubers of sweet potatoes (*Ipomoea batatas* (L.) Lam.) with orange flesh (Tainung 66) (ca ~4 cm in diameter and ~12 cm long) or yellow flesh (Tainung 57) (ca ~6 cm in diameter and ~18 cm long) were randomly harvested from the same fields in Taichung County, Taiwan in the middle January (winter), the middle April (spring), the middle July (summer), and the middle October (autumn) in 2007, respectively. Each sample was collected about 10 kg. After harvesting, these tubers were treated immediately as following: tubers were cleaned, peeled and cut into 4 mm thick slices with a Cucina™ slicer (model: HR7633) (Koninklijke Philips Electronics Co., Suzhou, Jiangsu, China) followed by lyophilization in the freeze-drying system (Vestech Scientific Co. Ltd., Taipei, Taiwan). The freeze-dried slices were then ground by the RT08 grinder (Rong-Tsong Co. Taipei, Taiwan) and were used to extract carotenoids at once. The moisture contents in the fresh tubers of the two sweet potatoes were measured as well.

2.2. Chemicals and standards

All-*trans* forms of α-carotene and β-carotene standards were obtained from Sigma Co. (St. Louis, MO, USA). Solvents used for the extraction and determination of carotenoids, e.g. acetonitrile (ACN), methanol (MeOH), dichloromethane (CH_2Cl_2), ethanol (EtOH), acetone and *n*-hexane were purchased from Merck Co. (Darmstadt, Germany). Deionized water (dd H_2O) was prepared by Ultrapure™ water purification system (Lotun Co., Ltd. Taipei, Taiwan). Chemicals such as magnesium carbonate ($MgCO_3$), potassium hydroxide (KOH) and butylated hydroxy toluene (BHT) were purchased from Merck Co. (Darmstadt, Germany).

2.3. Extraction of carotenoids from sweet potato

The method was based on that reported by Subagio, Morita, and Sawada (1996) and Chen et al. (2004). One gram of sweet potato powder was mixed with 30 mL of hexane/acetone/EtOH (2/1/1, v/v/v) containing 0.1 g of $MgCO_3$ and 0.05 g BHT. After shaking for 0.5 h, 8 mL of 40% methanolic KOH was added and the solution was saponified for 3 h under nitrogen gas at 25 °C. After filtering through a 0.45 μm Teflon membrane (Millipore Co., Bedford, MA), the extract was quickly transferred to a separatory funnel, washed with 50 mL of distilled water and the solvent was removed with nitrogen gas. The carotenoid extract was dissolved in 1 mL of MeOH/CH_2Cl_2 (1/1, v/v) containing 0.1% BHT immediately for HPLC analysis. All preparative and extractive procedures were performed in dim lighting.

2.4. Isomerization of all-trans forms of α- and β-carotene in carotenoid extraction procedure

All-*trans* form of α- or β-carotene standard (1 mg) was dissolved in 30 mL of hexane/acetone/EtOH (2/1/1, v/v/v) containing 0.1 g of $MgCO_3$ and 0.05 g BHT, and done as the procedure described in Section 2.3.

2.5. HPLC separation of carotenoids in sweet potato

A *PrimeLine*™ Gradient Model 500 G HPLC pump system (Analytical Scientific Instruments, Inc., El Sobrante, CA, USA) with an S-3210 photodiode-array detector (PDA) (Schambeck SFD GmbH, Bad Honnef, Germany) was used for carotenoid determination. The analytical condition was improved from that reported by Inbaraj et al. (2006). The column was a YMC C30 reverse-phase analytical column (250 × 4.6 mm, 5 μm) (Waters Co., Milford, MA, USA) kept at 25 °C. A gradient solvent system consisting of MeOH/ACN/dd H_2O (84/14/2, v/v/v) (solvent A) and CH_2Cl_2 (solvent B) was employed: a mixture of 80% A and 20% B was used initially, and then the mixing was programmed linearly to 55% B within 15 min and retained from 15 to 18 min. The flow rate was 1 ml/min and detection was at 450 nm. HPLC separation efficiency was estimated through the separation factor (α) and resolution (*Rs*). The limits of detection (LODs) and quantification (LOQs) for all-*trans* α-carotene and all-*trans* β-carotene standards were determined by the signal-to-noise ratio (S/N) of 3 and 10, respectively. The reproducibility for each carotenoid was measured by run-to-run and day-to-day for six times and the standard deviation was calculated. The peak purities were obtained through the S-3210 PDA automatically.

2.6. Identification of carotenoids in sweet potato

Comparing of retention time and absorption spectra of unknown peaks with reference standards and addition of carotenoid standards to samples for co-chromatography executed the identification of varied carotenoids in extracts. Furthermore, the *cis* isomers of carotenoids were tentatively assigned based on the spectral characteristics and *Q*-ratio values reported in the literatures (Inbaraj et al., 2006; Lee & Chen, 2003).

2.7. Preparation of standard curve

Five concentrations of all-*trans* forms of α-carotene and β-carotene were injected at 20 μl into HPLC (the range for 450 nm detection is 0.01–10 μg), and the linear regression equation for each standard curve was acquired by plotting the quantity of standard compound injected against the peak area. The regression equation and the correlation coefficient (r^2) were calculated using Chrom-Manager Multisystem (Analab Co., Taipei, Taiwan). The commercial *cis*-form of carotenoids were not available, therefore the *cis* isomers were quantified based on the calibration curves of their corresponding all-*trans* form of carotenoid standards because of similarity in extinction coefficient (Lee & Chen, 2001).

2.8. Determination of recovery

The recoveries were determined by adding a mixture of all-*trans* α-carotene and all-*trans* β-carotene standards (each weighing 0.1, 0.5, 1 and 2 mg) in MeOH/CH_2Cl_2 (1/1, v/v) to 1 g of freeze-dried sweet potato powder and extracted as described above. The extracts were then subjected to HPLC determination. The recovery of each all-*trans* form of standards was calculated from the analytical result and the original amount of carotenoid used. The recoveries of *cis* isomers were also gauged to be equivalent to their corresponding all-*trans* form of carotenoid standards.

2.9. Statistical analysis

The standard calibration equations of carotenoids, recoveries and quantitative analyses were determined in triplicate and the mean values were calculated. The data subjected to analysis of variance (ANOVA) and Duncan's multiple range tests were administered to resolve significance between means, at a level of $p < 0.05$.

3. Results and discussion

3.1. HPLC analysis of carotenoids in sweet potato

Inbaraj et al. (2006) has developed a HPLC method that could resolve 33 carotenoids including their isomers in the microalga *Chlorella pyrenoidosa* simultaneously and reveal high resolution. Initially, we used the same method to determine carotenoids in the major Taiwanese orange-fleshed (Tainung 66) and yellow-fleshed (Tainung 57) sweet potatoes and found that they only contained all-*trans* α-carotene, all-*trans*-β-carotene and their *cis* isomers. However, the analytical condition did not show good efficiency to separate these sweet potato carotenoids (some peaks overlapped obviously above the baseline) and should take longer determination time (around 42 min, data not showed). We, therefore, improved the analytical condition further to cope with these difficulties. Fig. 1 presents the chromatograms of carotenoids in Tainung 57 and 66 sweet potatoes. It indicates that the improved condition had good separation efficiency and appropriate separation time (around 16 min) for sweet potato carotenoids. Table 1 shows the separation factor (α), resolution (*Rs*) and purity of carotenoids in Taiwanese sweet potato after analysis. The α and *Rs* values for all peaks were greater than 1 and 0.9, respectively; moreover, the good reproducibility, RSD < 2.1% for retention times and RSD < 3.7% for integrated areas, of these carotenoids is also presented in Table 2. Therefore, the mobile phase was feasible to analyze the carotenoid composition in Taiwanese sweet potato. The purities of all peaks were higher than 97% (Table 1).

For the assignments of the sweet potato carotenoids, peaks 9 and 10 were explicitly assigned as all-*trans*-α-carotene and all-*trans*-β-carotene, respectively, based on the criteria described in Section 2.5. Peaks 1, 2 and 3 were tentatively assigned as *cis*-, di-*cis*- and *cis*-β-carotene, respectively, because a hypsochromic shift of 6, 35 and 6 nm occurred and the *Q*-ratio values were similar to those reported in the papers (Lin & Chen, 2003; Inbaraj et al., 2006); nevertheless, *cis* position could not be assigned further from the information of related researches. Peaks 4 and 5 showed hypsochromic shifts of 4 and 9 nm, respectively, and the *Q*-ratio values conformed to those reported by Inbaraj et al. (2006). Both of them were tentatively assigned as 13- or 13′-*cis*-α-carotene; however, we could not confirm which peak was the real 13-*cis*-α-carotene from the available data. Peaks 6 and 7 were tentatively assigned as 15- or 15′-*cis*-β-carotene and 13- or 13′-*cis*-β-carotene, respectively, based on a hypsochromic shift of 12 and 12 nm and the *Q*-ratio values in the literatures (Lin & Chen, 2003; Inbaraj et al., 2006). Both of peaks 8 and 11 were tentatively assigned as 9- or 9′-*cis*-β-carotene, according to a hypsochromic shift of 6 and 6 nm and the *Q*-ratio values presented in the article of Inbaraj et al. (2006). β-Carotene is a symmetrical molecule (half of the molecule being a mirror image of the other half), thus 9-*cis*- and 9′-*cis*-β-carotene are the same molecule such that there cannot be two peaks for this molecule. However, both of the spectral characteristics and *Q*-ratio values of these two peaks fitted the requirement of 9- or 9′-*cis*-β-carotene assignment, and therefore we tentatively assigned them as this compound. Table 3 shows the spectral data and *Q*-ratio values on comparison with those reported for the thorough array of all-*trans* and *cis* forms of carotenoids in Taiwanese sweet potato.

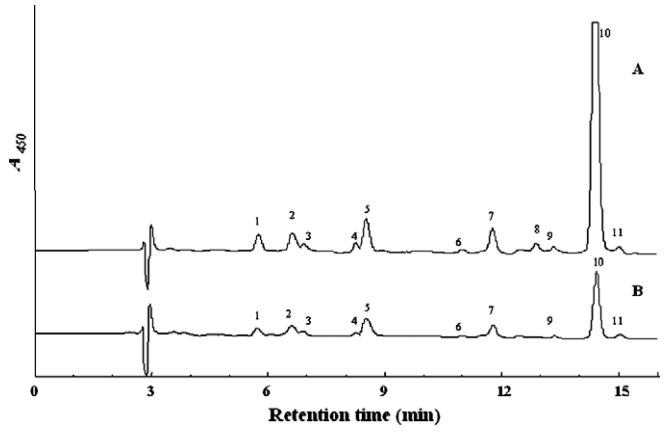

Fig. 1. HPLC chromatograms of carotenoid extracts of orange-fleshed (A) and yellow-fleshed (B) sweet potatoes harvested in October The analytical conditions are described in Section 2.5. See Table 3 for the assignment of peaks.

Table 1
Separation factor (α), resolution (*Rs*), and purity of carotenoids after analysis of Taiwanese sweet potato extract[a]

Peak no.	Compound	Retention time (min)	α[b]	*Rs*[c]	Peak purity (%)
1	*cis*-β-Carotene	5.7	–	–	99.2
2	di-*cis*-β-Carotene	6.6	1.3 (1/2) [d]	2.6 (1/2)	98.2
3	*cis*-β-Carotene	6.9	1.1 (2/3)	1.0 (2/3)	99.8
4	13- or 13′-*cis*-α-Carotene	8.3	1.3 (3/4)	4.0 (3/4)	98.5
5	13- or 13′-*cis*-α-Carotene	8.6	1.1 (4/5)	0.9 (4/5)	97.5
6	15- or 15′-*cis*-β-Carotene	11.0	1.4 (5/6)	7.3 (5/6)	99.7
7.	13- or 13′-*cis*-β-Carotene	11.8	1.1 (6/7)	2.5 (6/7)	99.2
8	9- or 9′-*cis*-β-Carotene	12.9	1.1 (7/8)	3.3 (7/8)	99.9
9	All-*trans*-α-Carotene	13.4	1.1 (8/9)	1.5 (8/9)	99.9
10	All-*trans*-β-Carotene	14.3	1.1 (9/10)	2.6 (9/10)	98.2
11	9- or 9′-*cis*-β-Carotene	15.0	1.1 (10/11)	2.2 (10/11)	99.9

[a] The analytical conditions are as in Section 2.5.
[b] $\alpha = t_{R2} - t_0/t_{R1} - t_0$, where t_{Rn} = retention time of an analyte, t_0 = retention time of an unretained peak.
[c] $Rs = 2\,(t_{R2} - t_{R1})/(w_1 + w_2)$, where w_n = band width of an analyte at the baseline.
[d] Values in parentheses represent two neighboring peaks.

Lessin, Catigani, and Schwartz (1997) developed a method with a C30 column and a mobile phase of methanol/methyl *tert*-butyl ether (89/11, v/v) at a flow rate of 1 ml/min to determine carotenoids in sweet potato. This method could separate β-carotene isomers (*all-tans*, 9-*cis*, 13-*cis* and 15-*cis*) and α-carotene isomers (*all-tans*, 9-*cis*, 13-*cis* and 13′-*cis*) simultaneously within 65 min. Van Jaarsveld et al. (2006) used a C30 column with 85/15 (v/v) methanol/methyl *tert*-butyl ether as mobile phase at a flow rate of 0.5 ml/min to resolve only two carotenoids, *trans*- and *cis*-β-carotene in sweet potato. Nevertheless, the method should take about 60 min. They also employed a C18 column and a mobile phase with acetonitrile containing 0.05% triethylamine, methanol and ethyl acetate (from 95/5/0, v/v/v to 60/20/20, v/v/v) at a flow rate of 0.5 ml/min to determine the two compounds; it should take about 35 min as well. Our method could determine 11 α- and β-carotene isomers in Taiwanese sweet potato within 16 min simultaneously.

3.2. Carotenoid content in sweet potato

The LODs and LOQs were 1.8 and 6.0 ng for all-*trans* α-carotene, and 2.6 and 8.7 ng for all-*trans*-β-carotene, respectively. Solutions containing 0.01–20 μg of these carotenoid standards were used to obtain the standard calibration curves at 450 nm detection, they were linear and reproducible. The linear regression equations for all-*trans* α-carotene and all-*trans* β-carotene were $Y = 26902\ X - 750.2$ ($r^2 = 0.9999$) and $Y = 31826\ X - 1824.1$ ($r^2 = 0.9998$) (Y is the value of the peak area and X is the value of sample quantity (ng)), respectively.

Table 2
Reproducibility of the carotenoids in Taiwanese sweet potato extract

Peak no.	Compound	RSD[a] (%)			
		Retention time		Integrated area	
		Run-to-run	Day-to-day	Run-to-run	Day-to-day
1	*cis*-β-Carotene	0.42	0.77	1.45	2.09
2	di-*cis*-β-Carotene	0.51	1.15	2.81	2.89
3	*cis*-β-Carotene	0.64	1.24	3.31	2.96
4	13- or 13′-*cis*-α-Carotene	0.62	1.20	3.02	3.46
5	13- or 13′-*cis*-α-Carotene	0.49	2.06	2.64	2.52
6	15- or 15′-*cis*-β-Carotene	0.38	1.51	1.94	2.21
7	13- or 13′-*cis*-β-Carotene	0.84	1.08	2.62	3.43
8	9- or 9′-*cis*-β-Carotene	0.73	1.65	2.82	3.62
9	All-*trans*-α-carotene	0.55	0.82	1.36	2.57
10	All-*trans*-β-carotene	0.41	0.86	2.77	4.52
11	9- or 9′-*cis*-β-Carotene	0.67	1.32	1.92	2.51

[a] The result was obtained from 10 mg (carotenoids extract)/ml with six measurements. The analytical conditions are as in Section 2.5.

Table 3
Identification data for all-*trans* and *cis* forms of carotenoids in Taiwanese sweet potato

Peak no.	Compound	Retention time (min)	λ (nm) (in-line)[a]	λ (nm) (reported)	Q-ratio[a,d] found	Q-ratio reported
1	*cis*-β-Carotene	5.7	426, 452, 474	427, 452, 476[b]	0.44	0.45[b]
2	di-*cis*-β-Carotene	6.6	403, 423, 458	404, 422, 458[c]	0.66	0.68[c]
3	*cis*-β-Carotene	6.9	425, 452, 476	427, 452, 476[b]	0.43	0.45[b]
4	13- or 13′-*cis*-α-Carotene	8.3	416, 445, 469	416, 446, 470[b]	0.51	0.52[b]
5	13- or 13′-*cis*-α-Carotene	8.6	416, 440, 465	416, 440, 464[b]	0.40	0.40[b]
6	15- or 15′-*cis*-β-Carotene	11.0	421, 445, 474	422, 446, 476[c]	0.38	0.37[c]
7	13- or 13′-*cis*-β-Carotene	11.8	422, 446, 476	422, 446, 476[b]	0.46	0.46[b]
8	9- or 9′-*cis*-β-Carotene	12.9	421, 452, 476	422, 452, 476[b]	0.25	0.26[b]
9	All-*trans*-α-Carotene	13.4	426, 449, 476	426, 449, 476[b]	0.10	0.10[b]
10	All-*trans*-β-Carotene	14.3	430, 458, 482	430, 458, 482[b]	0.12	0.12[b]
11	9- or 9′-*cis*-β-Carotene	15.0	427, 452, 476	428, 452, 476[b]	0.20	0.20[b]

[a] A mobile phase of methanol–acetonitrile–water (84/14/2, v/v/v) and methylene chloride (from 80/20, v/v to 45/55, v/v) was used in the study (the analytical conditions are as in Section 2.5).
[b] A mobile phase of methanol–acetonitrile–water (84/14/2, v/v/v) and methylene chloride (from 100/0, v/v to 45/55, v/v) used by Inbaraj et al. (2006).
[c] A mobile phase of acetonitrile-1-butanol (70/30, v/v) and methylene chloride (from 99/1, v/v to 90/10, v/v) used by Lin and Chen (2003).
[d] Q-ratio: the height ratio of the *cis* peak to the main absorption peak.

The recoveries of added all-*trans* α-carotene and all-*trans* β-carotene (each weighing 0.1–2 mg) in sweet potato powder were between 98.85% and 99.21% (Table 4); they did not show significant difference. The moisture contents in Tainung 57 and Tainung 66 were 69.43% and 68.12%, respectively. The isomerization of all-*trans* forms of α- and β-carotene in the procedure of carotenoid extraction was assessed as well. Fig. 2 shows that the two compounds would be isomerised in the procedure; however, the transformation from all-*tarns* to *cis* isomers was only around 1% (w/w) (Table 5). Carotenoid isomerization is mainly driven by light and temperature (Lee & Chen, 2001; Lin & Chen, 2003; Inbaraj et al., 2006). The carotenoid extraction procedure was carried out at 25 °C in dim lighting; the slight carotenoid isomerization was observed in the investigation.

Table 4
The recoveries of added carotenoids in Taiwanese sweet potato after extraction

Added amount (mg)	% Recovery (%CV)[A,B]	
	Compound	
	All-*trans*-α-carotene	All-*trans*-β-carotene
0.10	98.85 (3.19) a	99.02 (4.12) a
0.50	99.13 (2.57) a	99.21 (3.51) a
1.00	99.02 (4.07) a	99.19 (2.76) a
2.00	99.20 (3.06) a	99.14 (2.53) a

Values bearing different letters in the same column are significantly different. ($p < 0.05$).
The analytical conditions are as in Section 2.5.
[A] All values are the means of triplicate analyses.
[B] Values in parentheses are the coefficient of variation (%).

Table 6 shows that the orange-fleshed sweet potato (Tainung 66) had significantly higher total carotenoid content than the yellow-fleshed one (Tainung 57) at the same harvest time. Both Tainung 57 and Tainung 66 harvested in October contained the highest total carotenoid amount and the second and third were those harvested in July and April, respectively. Those harvested in Jan. had the lowest total carotenoid level. Some literatures (Amaducci & Pritoni, 1998; Baert, 1997; Saengthongpinit & Sajjaanantakul, 2005) demonstrated that harvest time would affect the content and composition of bioactive compounds. Pęksa, Golubowska, Rytel, Lisińska, and Aniolowski (2002) indicated that greater influence of harvest time on bioactive compounds was due to various environmental and weather conditions such as growing temperature. In spite of different harvest times, all-*trans*-β-carotene was always the major carotenoid compound in the two crops. Our results could respond to the report of Takahata, Noda, and Nagata (1993); they indicated that many sweet potato cultivars contained β-carotene mostly. Tainung 57 harvested in the four months could not be observed to contain 9- or 9′-*cis*-β-carotene and even that

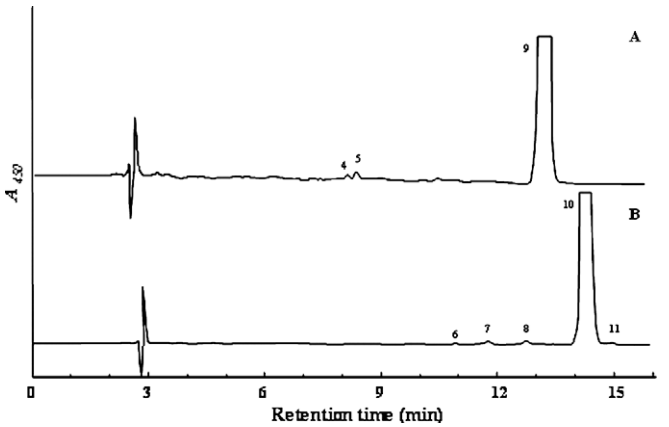

Fig. 2. HPLC chromatograms of all-*trans* forms of α- (A) and β-carotene (B) standards going through carotenoid extraction procedure as Section 2.3. The analytical conditions are described in Section 2.5. See Table 3 for the assignment of peaks.

Table 5
Changes of all-*trans* forms of α- and β-carotene standards going through carotenoid extraction procedure[a]

Peak no.	Compound	Estimated standard			
		All-*trans* α-carotene		All-*trans* β-carotene	
		Content (μg)	% Of original carotene amount[b]	Content (μg)	% Of original carotene amount[b]
1	*cis*-β-Carotene	–	–	ND[c]	0
2	di-*cis*-β-Carotene	–	–	ND	0
3	*cis*-β-Carotene	–	–	ND	0
4	13- or 13′-*cis*-α-Carotene	3.82 ± 0.27	0.38	–	–
5	13- or 13′-*cis*-α-Carotene	4.51 ± 0.30	0.45	–	–
6	15- or 15′-*cis*-β-Carotene	–	–	1.48 ± 0.01	0.15
7	13- or 13′-*cis*-β-Carotene	–	–	2.76 ± 0.01	0.28
8	9- or 9′-*cis*-β-Carotene	–	–	3.45 ± 0.02	0.35
9	All-*trans*-α-Carotene	990.46 ± 10.22	99.05	–	–
10	All-*trans*-β-Carotene	–	–	989.03 ± 11.30	98.90
11	9- or 9′-*cis*-β-Carotene	–	–	2.83 ± 0.01	0.28

All values are mean ± SD obtained by triplicate analyses. The analytical conditions are as in Section 2.5.
[a] Carotenoid extraction procedure is as in Section 2.3.
[b] One microgram of all-*trans* forms of α- or β-carotene standard was used for estimation
[c] ND = not detected.

harvested in January could not be found having *cis* isomers of α-carotene.

Huang, Tanudjaja, and Lum (1999) determined the contents of α-carotene and β-carotene in 18 sweet potato varieties grown in Hawaii. They observed that β-carotene content varied from 6.7 to 13.1 mg/100 g fresh wt in seven orange-fleshed, <0.1–0.6 mg/100 g fresh wt in seven yellow/white-fleshed, and <0.1–0.5 mg/100 g fresh wt in four purple-fleshed sweet potato varieties. The contents of α-carotene were <0.1 mg/100 g fresh wt in most sweet potato varieties except two orange-fleshed (contained 0.3 and 1.5 mg/100 g fresh wt) and one purple-fleshed sweet potato varieties (contained 0.2 mg/100 g fresh wt). Nonetheless, they did not identify each α- and β-carotene isomers in these sweet potato varieties and investigate the influence of harvest time on carotenoids. The contents of α- (between 0.11 and 0.80 mg/100 g fresh wt) and β-carotene (between 0.98 and 2.67 mg/100 g fresh wt) in these Taiwanese yellow-fleshed sweet potatoes harvested at various times were higher than those in Hawaiian ones. Lessin et al. (1997) found that fresh sweet potatoes obtained from NJ, USA contained all-*trans*-β-carotene only, whereas Van Jaarsveld et al. (2006) indicated that there were *trans*- and *cis*-β-carotenes in raw sweet potatoes grown in South Africa. We found that all-*trans* and *cis* isomers of α- and β-carotenes existed in raw Taiwanese sweet potatoes. Dinan, Harmatha, and Lafont (2001) expressed that the cultivar, the geographic locality and so on would also influence the content and composition of bioactive compounds in plants. K'osambo et al. (1998) described that epoxy-derivatives of *β*-carotene existed in sweet potato; however, we could not find them in Taiwanese sweet potatoes. Rodriguez-Amaya (1999) reminded that the occurrence of these epoxides was due to the oxidative degradation of β-carotene in the initial products; moreover, they also could form during analytical period.

4. Conclusion

The improved HPLC method in this study could be employed to determine α-carotene and β-carotene including their all-*trans* and *cis* forms in sweet potato efficiently and rapidly. The orange-fleshed sweet potato had significantly higher total carotenoid content than the yellow-fleshed one at the identical harvest time. Regardless of flesh color, all-*trans* β-carotene was the major carotenoid in sweet potatoes; the compound and its *cis* isomers were much higher than all-*trans* α-carotene and its *cis* isomers. Orange-fleshed sweet potatoes contained higher *trans* carotene than *cis* one, whereas yellow-fleshed sweet potatoes presented opposite results. Form the viewpoint of carotenoid amount, the optimum harvest time of sweet potatoes is autumn. Weather may play the crucial factor to influence carotenoid content in the crops. The

Table 6
Carotenoid contents in Taiwanese sweet potato harvested at various times

Peak no.	Compound	Content (mg/ 100 g fresh wt)							
		Harvest time of Tainung 66 (with orange flesh)				Harvest time of Tainung 57 (with yellow flesh)			
		January	April	July	October	January	April	July	October
1	*cis*-β-Carotene	0.31 ± 0.02 e	0.37 ± 0.03 c	0.42 ± 0.03 b	0.53 ± 0.05 a	0.15 ± 0.01 g	0.27 ± 0.02 f	0.35 ± 0.02 cd	0.33 ± 0.01 de
2	di-*cis*-β-Carotene	0.15 ± 0.01 g	0.29 ± 0.01 c	0.27 ± 0.01 d	0.57 ± 0.03 a	0.17 ± 0.01 f	0.21 ± 0.02 e	0.28 ± 0.01 cd	0.38 ± 0.03 b
3	*cis*-β-Carotene	0.12 ± 0.01 g	0.21 ± 0.02 e	0.30 ± 0.02 b	0.40 ± 0.02 a	ND	0.22 ± 0.02 d	0.17 ± 0.01 f	0.29 ± 0.01 c
4	13- or 13′-*cis*-α-Carotene	0.14 ± 0.01 f	0.31 ± 0.02 a	0.20 ± 0.02 d	0.25 ± 0.02 b	ND	0.12 ± 0.01 g	0.24 ± 0.02 c	0.15 ± 0.01 e
5	13- or 13′-*cis*-α-Carotene	0.24 ± 0.01 e	0.32 ± 0.03 d	0.52 ± 0.04 b	0.65 ± 0.05 a	ND	0.20 ± 0.01 f	0.26 ± 0.02 e	0.47 ± 0.03 c
6	15- or 15′-*cis*-β-Carotene	0.19 ± 0.02 d	0.17 ± 0.01 e	0.41 ± 0.04 a	0.35 ± 0.02 b	ND	0.13 ± 0.01 f	0.23 ± 0.01 c	0.22 ± 0.01 c
7	13- or 13′-*cis*-β-Carotene	0.30 ± 0.02 e	0.35 ± 0.02 c	0.42 ± 0.02 b	0.47 ± 0.03 a	0.11 ± 0.01 h	0.15 ± 0.01 g	0.24 ± 0.01 f	0.32 ± 0.02 d
8	9- or 9′-*cis*-β-Carotene	0.22 ± 0.01 d	0.45 ± 0.03 c	0.57 ± 0.03 b	0.61 ± 0.04 a	ND	ND	ND	ND
9	All-*trans*-α-Carotene	0.12 ± 0.01 e	0.19 ± 0.01 c	0.28 ± 0.02 a	0.27 ± 0.01 a	0.11 ± 0.01 f	0.11 ± 0.01 f	0.12 ± 0.01 e	0.18 ± 0.01 d
10	All-*trans*-β-Carotene	2.13 ± 0.14 d	3.05 ± 0.25 c	3.35 ± 0.27 b	3.76 ± 0.23 a	0.41 ± 0.03 g	0.63 ± 0.05 f	0.75 ± 0.05 ef	0.83 ± 0.06 e
11	9- or 9′-*cis*-β-Carotene	0.15 ± 0.01 f	0.26 ± 0.02 c	0.23 ± 0.02 de	0.38 ± 0.03 a	0.14 ± 0.01 f	0.24 ± 0.02 d	0.22 ± 0.02 e	0.30 ± 0.02 b
Total *cis* isomers of α-carotene		0.38 ± 0.02 e	0.63 ± 0.05c	0.72 ± 0.06 b	0.90 ± 0.07 a	ND	0.32 ± 0.02 f	0.50 ± 0.04 d	0.62 ± 0.04 c
Total *cis* isomers of β-carotene		1.44 ± 0.10 e	2.10 ± 0.19 c	2.62 ± 0.23 b	3.31 ± 0.22 a	0.57 ± 0.04 g	1.22 ± 0.12 f	1.49 ± 0.08 e	1.84 ± 0.10 d
Total amount		4.07 ± 0.29 d	5.97 ± 0.50 c	6.97 ± 0.58 b	8.24 ± 0.53 a	1.09 ± 0.06 h	2.28 ± 0.20 g	2.86 ± 0.18 f	3.47 ± 0.21 e

All values are mean ± SD obtained by triplicate analyses.
ND = not detected.
Values bearing different letters in the same row are significantly different ($p < 0.05$).
The analytical conditions are as in Section 2.5.

results could provide the referable information for sweet potato exploitation.

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