Analysis and Stability of Carotenoids in the Flowers of Daylily (*Hemerocallis disticha*) as Affected by Various Treatments

C.-Y. Tai and B. H. Chen*

Department of Nutrition and Food Science, Fu Jen University, Taipei, Taiwan 242

The analysis and stability of carotenoids in the flowers of daylily (*Hemerocallis disticha*) as affected by soaking and drying treatments were studied. The various carotenoids in the flowers of daylily were analyzed using a reversed-phase C_{30} HPLC column and a mobile phase of methanol/methylene chloride/2-propanol (89:1:10, v/v/v) with methanol/methylene chloride (45:55, v/v) as sample solvent. Twenty-one pigments were resolved, of which 14 carotenoids were identified, including neoxanthin, violaxanthin, violeoxanthin, lutein-5,6-epoxide, lutein, zeaxanthin, β-cryptoxanthin, all-*trans*-β-carotene, and their cis isomers, based on spectral characteristics and Q ratios. Prior to hot-air-drying (50 °C) or freeze-drying, some of the daylily flowers were subjected to soaking in a sodium sulfite solution (1%) for 4 h. Under either the hot-air- or the freeze-drying treatment, the amounts of most carotenoids were higher in the soaked daylily flowers than in those that were not soaked. With hot-air-drying, the amount of cis carotenoids showed a higher yield in soaked samples than in nonsoaked samples. However, with freeze-drying, only a minor change of each carotenoid was observed for both soaked and nonsoaked samples. Also, air-drying resulted in a higher loss of carotenoids than freeze-drying.

Keywords: *Daylily; HPLC; carotenoid; freeze-drying; air-drying*

INTRODUCTION

Carotenoids are one of the major phytochemicals in nature, and they are widely distributed in plants as well as in the animal kingdom. In the past decade the biological functions of carotenoids have drawn considerable interest from scientists. For instance, carotenoids have been shown to exert protective effects against cardiovascular and eye diseases, as well as skin and stomach cancers (Canfield et al., 1993; Clinton, 1998; Nguyen and Schwartz, 1999). In addition, several carotenoids, such as α- and β-carotene, possess vitamin A activity. Numerous studies have also shown that carotenoids may act as antioxidants through a mechanism of quenching singlet oxygen (Palozza and Krinsky, 1992) or free radicals (Jørjensen and Skibsted, 1993). Obviously, the significance of carotenoids to mankind as neutraceuticals is indisputable.

The flowers of daylily (*Hemerocallis disticha*) have been used commonly in Chinese cuisine for a long time. Daylily flowers can be cooked either when fresh or dehydrated, and thus they can still be used after preservation for a long period of time by dehydrating. The dehydrated daylily flowers possess flavor and texture that are different from those of the fresh ones. However, the dehydration process often results in a reduction of color due to pigment degradation and browning reaction (Food Industry, 1987). To maintain a better color, the manufacturer usually treats daylily flowers with sodium hydrogen sulfite solution prior to dehydration (Food Industry, 1987). In addition, the application of sodium hydrogen sulfite prevents the growth of microorganisms such as fungi (Lindsay, 1996). Nevertheless, this additive has been found to cause allergic reactions in some people, especially asthma patients (Lindsay, 1996). Both carotenoids and flavonoids contribute to the yellow color in dried daylily flowers (Food Industry, 1987). However, the various carotenoids present in daylily flowers remain unknown. As sodium hydrogen sulfite possesses the ability to preserve the color of dried daylily flowers, the effect of this chemical on the retention of carotenoids in the flowers during soaking and dehydration also needs to be elucidated.

The effect of various heating treatments such as boiling (Nguyen and Schwartz, 1999), steaming (Khachik et al., 1992), stewing (Khachik et al., 1992), frying (Speek et al., 1988), canning (Chen et al., 1995; Cano et al.,1996; Lessin et al.,1997), microwave heating (Khachik et al., 1992, Chen, 1992; Chen and Chen, 1993), and baking (Chandler and Schwartz, 1988) on the carotenoid losses in foods have been reported. However, the effect of various drying methods on the carotenoid stability in the daylily flowers has not yet been studied. The objectives of this study were to analyze and determine, by liquid chromatography, the changes of various carotenoids in the daylily flowers under soaking and drying treatments.

MATERIALS AND METHODS

Instrumentation. The HPLC instrument consisted of a Jasco PU-980 pump (Jasco Co., Tokyo, Japan), a Rheodyne model 7161 injector (Rheodyne Co., Rohnert Park, CA), a Phenomenex DG-440 degassing system (Phenomenex Co., Torrance, CA), and a Jasco MD-915 photodiode-array detector; a Borwin computer software (Jasco Co.) was used to process data. A YMC Carotenoid S5μm C_{30} column (25 cm × 4.6 mm i.d.)(Waters Co., Milford, MA) was used for separation of carotenoids in the dried daylily flowers. The spectrophotometric determinations were made with a Beckman DU-70 double-beam spectrophotometer (Irvine, CA). The homogenizer (model

* To whom correspondence should be addressed. Telephone: 886-22-902-1219. Fax: 886-22-902-1215. E-mail: nutr1007@fujens.fju.edu.tw.

10.1021/jf000956t CCC: $19.00
Published on Web 11/03/2000

HG-2800) was from Hsiang-Tai Co. (Taipei, Taiwan). The rotary evaporator was from Rikakikai Co. (Tokyo, Japan). The freeze-dryer was from Kingmech Co., Ltd. (Taipei, Taiwan). The hot air-dryer was manufactured by Autopack Co. (TYPE-KVEO-1, Taipei, Taiwan).

Materials. Fresh daylily flowers were harvested in August from a farm located in the eastern part of Taiwan. The flowers were picked in the early morning, then transported to our lab, and were stored at 4 °C prior to drying. All-*trans*-β-carotene standard, which was found to contain trace amounts of cis isomers by HPLC analysis, was purchased from Sigma Co. (St. Louis, MO). All-*trans*-lutein with a purity of 85% was purchased from Fluka (Bushs, Swizerland). All solvents used in this study were HPLC grade. Acetonitrile and toluene were purchased from Mallinckrodt Co. (Paris, KY); methanol was from Merck (Darmstadt, Germany); *n*-hexane was from Labscan Co. (Bangkok, Thailand); ethanol, anhydrous sodium sulfate, and potassium hydroxide were from Riedel-de Haën Co. (Seelze, Germany); sodium hydrogen sulfite was from Hayashi Pure Chemical Co. (Osaka, Japan).

Dehydration of Daylily Flowers. Fresh daylily flowers were subjected to four treatments. Treatment A consisted of one batch of flowers (2 kg) being dried directly under hot air flow (48 °C) for at least 60 h until the final moisture content reached 10%, and a total of 3 batches was used. In treatment B, daylily flowers (2 kg) from one batch were soaked in 1% of sodium hydrogen sulfite solution for 4 h and were drained for 10 min prior to dehydration in the hot air-dryer (48 °C); the final moisture content was about 10%, and a total of 3 batches was used. Treatment C used the flowers (400 g) from one batch, which were dried directly in the freeze-dryer (−53 °C, 0.06 Torr) for 48 h until the final moisture content reached 7%, and a total of 3 batches was used. In treatment D the flowers (400 g) were soaked in 1% of sodium sulfite solution for 4 h and were drained for 10 min prior to drying in the freeze-dryer (−53 °C, 0.06 Torr); the final moisture content was about 7%, and a total of 3 batches was used. A total of 6 kg of fresh daylily flowers was used for each treatment of A and B, and 1.2 kg for each treatment of C and D.

Extraction of Carotenoids. A modified AOAC method (Chen and Yang, 1992) used to determine the contents of carotenes and xanthophylls in dried plant materials and mixed feeds was used to extract carotenoids in the daylily flowers.

Three grams of dried daylily flowers were ground into powder with a grinder. The powder (0.5 g) was mixed with 30 mL of extractant (hexane/ethanol/acetone/toluene, 10:6:7:7, v/v/v/v) in a flask and swirled gently for 1 h. Two mL of 40% methanolic KOH was added to the flask, and the contents were stirred for 1 min. The mixture was then left in the dark for 16 h for saponification. Thirty mL of hexane was added to the flask and swirled for 1 min; the mixture was brought to volume (100 mL) with 10% Na_2SO_4. The mixture was placed in the dark for 1 h until two phases were separated. The upper phase (5 mL), containing carotenoids, was placed in a tube and evaporated to dryness under nitrogen. The carotenoid extracts were dissolved in 1 mL of methanol/methylene chloride (45:55, v/v). The solution was filtered through a 0.2-μm membrane filter and stored at −70 °C for subsequent analysis by HPLC.

Preparation of β-Cryptoxanthin, Zeaxanthin, and α-Carotene Standards. Fresh yellow corn was freeze-dried and ground into powder. Twenty-five grams of the powder was mixed with 150 mL of hexane in a flask and stirred for 1 h for extraction. Thirty mL of 40% methanolic KOH was added to the flask. After the mixture was stirred for 1 min, it was left in the dark for 16 h for saponification. Hexane (150 mL) was added to the flask and swirled for 1 min, and then 50 mL of 10% Na_2SO_4 was added. The flask was left standing in the dark for 1 h until two phases were separated. The upper phase was collected and concentrated to 10 mL using a rotary evaporator. Carotenoid extract (5 mL) was pipetted onto a glass column (30 cm × 24 mm i.d.), which was packed with a mixture of MgO (15 g) and diatomaceous earth (15 g). A layer of anhydrous sodium sulfate (1 cm) was placed on the top, and a suction flask was connected to the bottom of the column. A hexane/acetone mixture (90:10, v/v) was used to elute the carotene band (first fraction). Five bands were observed when eluted with hexane/acetone/methanol (84:15:1, v/v/v) and the top band was identified as β-cryptoxanthin. The seventh band eluted by hexane/acetone/methanol (84:15:2, v/v/v) was identified as zeaxanthin (Quackenbush et al., 1961). The zeaxanthin and β-cryptoxanthin fractions were further purified by an HPLC Vydac 201TP54 column (25 cm × 4.6 mm i.d.) packed with 5-μm particles (Hesperia, CA) and a mobile phase of methanol/methylene chloride (99:1, v/v). The eluates of zeaxanthin (15 runs) and β-cryptoxanthin (25 runs) peaks were collected individually and dried under a flow of nitrogen, and then redissolved in 1 mL of methanol/methylene chloride (45:55, v/v). Their purities were 98.7 and 97.4%, respectively, as determined by a Jasco MD-915 photodiode-array detector.

All-*trans*-α-carotene, which was used as internal standard in this study, was extracted from carrot juice (Chen et al., 1995). Carrot juice (100 mL) was mixed with 50 mL of hexane in a flask. The mixture was gently stirred for 1 min in the dark and then left standing for 1 h until two phases were separated. Ten mL of the upper phase (hexane) was dried under a stream of nitrogen and then redissolved in 1 mL of methanol/methylene chloride (45:55, v/v). Purification of all-*trans*-α-carotene was also carried out using an HPLC Vydac 201TP54 column and a mobile phase of methanol/methylene chloride (99:1, v/v) (Chen et al., 1995). The eluates of all-*trans*-α-carotene peak from 20 runs were pooled and dried under a stream of nitrogen, and then redissolved in 1 mL of methanol/methylene chloride (45:55, v/v). The concentration of all-*trans*-α-carotene was approximately 250 μg/mL with a purity 99.8%.

HPLC Analysis of Carotenoids. A mobile phase of methanol/methylene chloride/2-propanol (89:1:10, v/v/v), with methanol/methylene chloride (45:55, v/v) as sample solvent, was used to separate the various carotenoids in the dried daylily flowers. The initial flow rate was 1.0 mL/min for 15 min, increased to 2 mL/min at 25 min, and then held for 30 min. The sensitivity was 0.005 AUFS with detection wavelength of 450 nm and injection volume of 20 μL. The purity of each peak was automatically determined by a Jasco photodiode-array detector. Most carotenoids were identified by comparing absorption spectra of unknown peaks in various solvents with those of purified carotenoid standards or with those reported in the literature (Tsukida et al., 1982; Quackenbush, 1987; Chandler and Schwartz, 1987; Chen et al., 1991; Yen and Chen, 1995; Chen et al., 1995), or by cochromatography with added standards. The epoxy-containing carotenoids were identified using an epoxide test, which was conducted by adding 10 μL of 0.1 N HCl to 20 μL of sample. The mixture was injected into HPLC for analysis of absorption spectrum of each peak. The cis carotenoids were identified based on spectral characteristics and Q ratios as described in some previous studies (Chen and Chen, 1993; Chen at al., 1995).

Quantitation. For quantitation, the daylily extract was spiked with 50 μg/mL of internal standard all-*trans*-α-carotene. The calibration curves of all-*trans*-β-carotene, all-*trans*-lutein, zeaxanthin, and β-cryptoxanthin were prepared at 6 concentrations (0 to 270 μg/mL). The coefficient of linearity of the standard curve was obtained by plotting the peak area ratios (*Y* axis) of each carotenoid standard to the internal standard versus the concentration ratios (*X* axis) of each carotenoid standard to the internal standard. All the calibration curves gave a good linearity ($R^2 > 0.99$). Because several standards such as neoxanthin, violaxanthin, violeoxanthin, and lutein peroxide are not commercially available, the quantitation of these carotenoids was based on the calibration curve of lutein. Likewise, the cis isomers of all-*trans*-β-carotene, all-*trans*-lutein, zeaxanthin, and β-cryptoxanthin were each quantified based on the standard curves of the parent trans compound. Duplicate injections were performed and the mean values were determined.

Because of the moisture difference in the dried samples, the data from all treatments were calculated on a dry weight basis for comparison.

Color Determination of Dried Daylily Flowers. A color difference meter was used to measure *L*, *a*, and *b* of dried daylily flowers of various treatments, of which *L*, *a*, and *b*

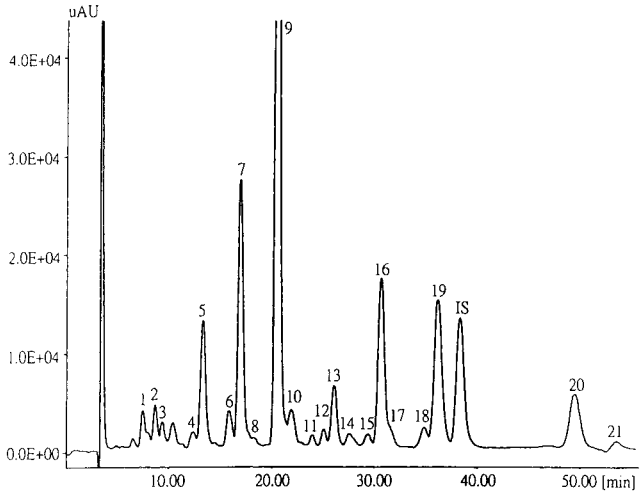

Figure 1. HPLC chromatagram of carotenoids extracted from dried daylily flowers. Chromatagraphic conditions described in text. Peaks: 1, neoxanthin; 2, violaxanthin; 3, violeoxanthin; 4, 13-*cis*-lutein 5,6-epoxide; 5, lutein 5,6-epoxide; 6, 13-*cis*-lutein; 7, all-*trans*-lutein; 8, 9-*cis*-lutein; 9, zeaxanthin; 10, 13-*cis*-zeaxanthin; 11, unknown; 12, unknown; 13, unknown; 14, unknown; 15, unknown; 16, β-cryptoxanthin; 17, 13-*cis*-β-cryptoxanthin; 18, *cis*-β-cryptoxanthin; 19, *cis*-β-cryptoxanthin; 20, all-*trans*-β-carotene; 21, 9-*cis*-β-carotene; IS, all-*trans*-α-carotene.

indicate lightness, red, and yellow, respectively. Hue can be expressed as a/b, whereas chroma is expressed as $(a^2 + b^2)^{1/2}$. Six dried daylily flowers from each treatment of 3 batches were randomly selected for color measurement. Each flower was measured at 3 locations including top, middle, and bottom. The *L*, *a*, and *b* values were an average of these 3 spots of the dried daylily flowers of 3 batches.

Statistical Analysis. The data were subjected to analysis of variance using a SAS program (PROC ANOVA) and Duncan's multiple range test procedures of the statistical analysis (*SAS/STAT Guide for Personal Computers*, 1985).

RESULTS AND DISCUSSION

Separation of Carotenoids in Dried Daylily Flowers. Figure 1 shows the HPLC chromatogram of the various carotenoids in the dried daylily flowers. Twenty-one pigments were separated using an HPLC reversed-phase C_{30} column. Initially, we employed a reversed-phase C_{18} column and various solvent systems for separation of carotenoids, however, no good resolution was achieved. Instead, the application of a reversed-phase C_{30} column and a mobile phase of methanol/methylene chloride/2-propanol (89:1:10, v/v/v) with sample solvent methanol/methylene chloride (45:55, v/v) was found to show a better resolution when compared to that of a C_{18} column. However, the retention times of all the peaks for the former were much longer than the latter. Apparently, this can be attributed to a greater hydrophobic interaction between carotenoids and the stationary phase of a C_{30} column. It has been demonstrated that the polymeric C_{30} stationary phase provides excellent resolution of all-trans carotenoids and possesses outstanding shape selectivity toward the geometric isomers of β-carotene (Emenhiser et al., 1995). Delgado-Vargas and Paredes-López (1997) also used a reversed-phase C_{30} column to resolve four lutein isomers and zeaxanthin in marigold flowers. Likewise, Rentel et al. (1998) also used a C_{30} column to separate various isomers of lycopene and β-carotene. Although the C_{30} column prolongs the retention time of each peak, it enhances the resolution power of both geometrical and positional isomers of carotenoids.

Identification of Carotenoids. Table 1 shows the identification data of the various carotenoids in the dried daylily flowers. All-*trans*-lutein and all-*trans*-β-carotene were identified by comparing absorption spectra and retention times of the unknown peaks with reference standards. The visible absorption spectrum (in line) of peak 7 (purity 96.0%) was 430, 448, and 472 nm, which was close to that in a report by Chen et al. (1995) and was identical to the all-*trans*-lutein standard used in this study. In addition, the retention times of peak 7 and all-*trans*-lutein standard were similar. For confirmation, all-*trans*-lutein standard was added to the extract for cochromatography and peak 7 was found indeed to be all-*trans*-lutein. Following the same approach, peak 20 with maximum absorption wavelength at 426 (shoulder), 454, and 478 nm was identified as all-*trans*-β-carotene with a purity of approximately 91%.

Because of the lack of standards, zeaxanthin and β-cryptoxanthin were extracted from corn grains and purified using a modified procedure of Quackenbush et al. (1961). The isolated zeaxanthin and β-cryptoxanthin bands were further purified by HPLC and reached a purity of 98.7 and 97.4%, respectively, with maximum absorption wavelengths 425 (shoulder), 454, and 478 nm for both. By comparison of retention times and maximum absorption wavelengths of unknown peaks with reference standards and cochromatography, peaks 9 and 16 were identified as zeaxanthin and β-cryptoxanthin, respectively.

For further confirmation, peak 7 (all-*trans*-lutein), peak 9 (zeaxanthin), peak 16 (β-cryptoxanthin), and peak 20 (all-*trans*-β-carotene) were purified. Purification of these four pigments was conducted by employing a C_{30} column and the same mobile phase used to resolve the carotenoids in the daylily flowers extract. The eluate from each peak (15 runs) was collected and pooled, and was evaporated under a nitrogen flow to dryness. Table 2 shows the identification data of both purified compounds and the standards in hexane and acetone. Comparison of spectra of the isolated peaks with the standards and the reported data indicated these four peaks were all-*trans*-lutein, zeaxanthin, β-cryptoxanthin, and all-*trans*-β-carotene.

The identification of cis isomers of carotenoids was based on a hypsochromic shift (approximately 4 nm for mono-cis and longer for di-cis isomers) as compared to all-*trans*-carotenoids (Zechmeister, 1944; Davis, 1976; Goodwin, 1981). It has been reported that the presence of central cis isomers, such as 13- or 15-*cis*-carotenoids, would result in a significant absorption in the ultraviolet region (320−380 nm) (Zechmeister, 1944; Davis, 1976; Goodwin, 1981). The ratio of absorption at maximum to the absorption at cis peak is defined as the Q ratio (Tsukida et al., 1982; Quackenbush, 1987; Saleh and Tan, 1991), which may also be used to identify the cis isomers. In addition, all-*trans*-lutein, zeaxanthin, β-cryptoxanthin, and all-*trans*-β-carotene standards were each heated at 50 °C for 120 min separately to facilitate formation of cis isomers and minimize degradation of carotenoids at the same time. Collection of the eluates of each cis isomer peak and addition to the sample extract for cochromatography would facilitate the identification of cis carotenoids (Chen et al., 1995). Peaks 6 and 8 were identified as 13-*cis*-lutein and 9-*cis*-lutein, respectively, which were based on a hypsochromic shift

Table 1. Identification Data for Carotenoids in Dried Daylily Flowers

peak no.	compound	λ (nm) (in-line)[a]	λ (nm) reported[b]	solvent	epoxide test hypsochromic shift	Q ratio found	Q ratio reported[c]
1	neoxanthin	418, 442, 472	415, 438, 467	ethanol	398, 424, 454	-[e]	-
2	violaxanthin	418, 442, 472	417, 440, 469	ethanol	382, 406, 436	-	-
3	violeoxanthin	(412)[d],436, 466	(415), 436, 464	ethanol	(382), 400, 430	-	-
4	13-*cis*-lutein 5,6-epoxide	334, (412), 436, 466	-	-	(400), 424, 448	2.5	-
5	lutein 5,6-epoxide	(420), 442, 472	420, 442, 471	ethanol	(407), 430, 454	-	-
6	13-*cis*-lutein	334, (419), 442, 466	(419), 439, 465	methanol−methylene chloride (99:1)	-	3.0	2.6
7	all-*trans*-lutein	(426), 448, 472	422, 445, 474	ethanol	-	15.6	11.5
8	9-*cis*-lutein	334, (419), 442, 466	440, 467	MeOH	-	5.2	8.6
9	zeaxanthin	(425), 454, 478	424, 449, 476	acetone	-	15.6	-
10	13-*cis*-zeaxanthin	340, (419), 448, 472	-	-	-	2.5	-
11	unknown-1	(418), 442, 466	-	-	-	-	-
12	unknown-2	(418), 448, 472	-	-	-	-	-
13	unknown-3	(419), 448, 472	-	-	-	-	-
14	unknown-4	(412), 442, 466	-	-	-	-	-
15	unknown-5	334, (412), 442, 466	-	-	-	-	-
16	β-cryptoxanthin	(425), 454, 478	(428), 449, 473	ethanol	-	15.6	-
17	13-*cis*-β-cryptoxanthin	334, (419), 448, 472	-	-	-	4.3	-
18	*cis*-β-cryptoxanthin	340, 448, 472	-	-	-	11.1	-
19	*cis*-β-cryptoxanthin	340, 448, 472	-	-	-	9.3	-
20	all-t*rans*-β-carotene	(426), 454, 478	(424), 452, 476	acetone	-	12.6	11.2
21	9-*cis*-β-carotene	352, 448, 472	(425), 446, 473	acetone-hexane (3:97)	-	6.5	8.3

[a] Eluent used as solvent (methanol/methylene chloride/2-propanol, 89:1:10, v/v/v. [b] Reported values of visible spectra were from Quackenbush (1987), Saleh and Tan (1991), Chen et al., (1991), Chen and Chen (1993), Yen and Chen (1995), Khachik et al., (1999), Hornero-Mendez and Minguez-Mosquera (2000). [c] Q ratios were reported by Chen et al. (1995). [d] Values in parentheses represent shoulder. [e] Data not available.

Table 2. Identification Data for Several Carotenoid Standards and HPLC Peaks from Daylily Flower Extract

compound	λ (nm) in hexane	λ (nm) reported[a]	λ (nm) in acetone	λ (nm) reported[a]
all-*trans*-lutein standard (from Fluka)	424, 444, 472	422, 444, 473	426, 446, 474	-[b]
peak 7 (all-*trans*-lutein)	424, 442, 470	-	426, 446, 472	-
zeaxanthin (from corn)	424, 446, 475	-	426, 450, 476	424, 449, 476
peak 9 (zeaxanthin)	424, 446, 475	-	426, 450, 476	-
β-cryptoxanthin (from corn)	422, 445, 475	-	422, 448, 476	425, 449, 476
peak 16 (β-cryptoxanthin)	422, 446, 475	-	424, 448, 476	-
all-*trans*-β-carotene standard (from Sigma)	426, 448, 476	423, 446, 475	426, 452, 476	(429), 452, 478
		424, 448, 476		424, 452, 476
peak 20 (all-*trans*-β-carotene)	424, 448, 476	-	426, 452, 478	-
peak 5 (lutein 5,6-epoxide)	422, 444, 470	420, 444, 470	426, 450, 476	-

[a] Reported values of visible spectra were from Bauernfeind (1981), Chen et al. (1991), Khachik et al. (1999), and Hornero-Mendez and Minguez-Mosquera (2000). [b] Data not available.

of 6 nm when compared to all-*trans*-lutein. The Q ratios were 3.0 and 5.2, respectively. Cochromatography also indicated that they were indeed the cis isomers of all-*trans*-lutein. Peaks 10 and 17 were tentatively identified as 13-*cis*-zeaxanthin and 13-*cis*-β-cryptoxanthin, respectively, which were based on a hypsochromic shift of 6 nm when compared to all-trans reference standards. Cochromatography also indicated that they were probably the cis isomers of zeaxanthin and β-cryptoxanthin. The Q ratios for peaks 10 and 17 were 2.5 and 4.3, respectively. Peak 21 was identified as 9-*cis*-β-carotene based on hypsochromic shift of 6 nm when compared to the all-*trans*-β-carotene, and a cis peak at 352 nm. Cochromatography also indicated that it was 9-*cis*-β-carotene. Peaks 18 and 19 were tentatively identified as cis isomers of β-cryptoxanthin because a hypsochromic shift of 6 nm and a cis peak at 340 nm occurred. Surprisingly, the retention times between these two peaks and β-cryptoxanthin were more than 5 min apart. This result is expected because a greater hydrophobic interaction between cis carotenoids and their trans compounds could occur on the C_{30} stationary phase.

Peak 1 exhibited a hypsochromic shift of 18 nm using an epoxide test, and the visible absorption spectra were similar to those reported by Chen et al. (1991) and Yen and Chen (1995). Therefore, it was identified as neoxanthin, and the purity was 82%. Both violaxanthin and violeoxanthin were identified by a hypsochromic shift of 36 nm and by comparison of the absorption spectra to the reported data (Chen et al., 1991; Yen and Chen, 1995). The purities of the peaks were 98 and 76%, respectively. Lutein 5,6-epoxide was identified according to a hypsochromic shift of 12 nm and the similarity of the reported absorption spectra (Chen et al., 1991; Yen and Chen, 1995). 13-*cis*-Lutein 5,6-epoxide was also tentatively identified base on its spectrum characteristics and Q ratio. A hypsochromic shift of 6 nm when compared to lutein 5,6-epoxide, and a Q ratio of 2.5, suggested that this peak was probably a 13-cis isomer of lutein 5,6-epoxide (Yen and Chen, 1995).

Peaks 11 to 15 did not exhibit a hypsochromic shift using an epoxide test, suggesting that they were not carotenoid epoxides. Because of the lack of standards and literature support, these pigments were not identified. The application of some other techniques such as LC−MS and LC−NMR would be necessary for identification of these unknown carotenoids.

With regard to quantification, zeaxanthin was present in the largest amount in dried daylily flowers, followed by β-cryptoxanthin, all-*trans*-β-carotene, and all-*trans*-

Table 3. Retention Times (RT) and Contents (μg/mL) of Carotenoids in Dried Daylily Flowers under Four Treatments by HPLC Analysis

			treatment[a]			
peak no.	compound	RT (min)	A	B	C	D
1	neoxanthin	6.45	1.03[b]**	0.89[b]	0.97[b]	3.26[a]
2	violaxanthin	7.61	1.10[c]	0.65[d]	2.72[b]	3.41[a]
3	violeoxanthin	8.59	0.74[c]	0.42[d]	3.34[a]	1.61[b]
4	13-*cis*-lutein 5,6-epoxide	12.21	1.62[b]	2.17[a]	1.67[b]	1.76[b]
5	lutein 5,6-epoxide	13.13	0.89[d]	2.52[c]	16.66[b]	20.80[a]
6	13-*cis*-lutein	15.65	7.81[b]	12.69[a]	4.49[c]	4.37[c]
7	all-*trans*-lutein	16.72	19.90[c]	27.46[b]	34.53[a]	34.08[a]
8	9-*cis*-lutein	18.07	0.84[a]	0.48[b]	0.47[b]	0.42[b]
9	zeaxanthin	20.25	103.26[b]	222.77[a]	225.94[a]	226.25[a]
10	13-*cis*-zeaxanthin	21.65	0.67[c]	1.95[b]	3.29[a]	3.56[a]
11	unknown	23.72	1.52[a]	1.32[ab]	1.29[b]	1.19[b]
12	unknown	24.81	1.24[d]	1.52[c]	1.80[b]	2.16[a]
13	unknown	25.79	6.24[b]	9.21[a]	8.82[a]	10.21[a]
14	unknown	27.24	0.35[b]	0.63[b]	2.25[a]	2.27[a]
15	unknown	29.03	1.24[c]	1.14[c]	1.53[b]	1.78[a]
16	β-cryptoxanthin	30.24	40.09[c]	71.63[ab]	67.30[b]	76.33[a]
17	13-*cis*-β-cryptoxanthin	34.43	trace	trace	trace	trace
18	*cis*-β-cryptoxanthin	35.73	3.33[c]	4.46[b]	5.26[ab]	6.18[a]
19	*cis*-β-cryptoxanthin	37.87	49.21[c]	75.79[b]	78.28[b]	99.10[a]
20	all-*trans*-β-carotene	48.32	21.17[b]	38.10[a]	40.88[a]	37.68[a]
21	9-*cis*-β-carotene	52.85	0.10[b]	2.87[b]	4.46[a]	3.02[b]
	total		262.35	478.67	505.95	539.44

[a] Treatments: A, daylily flowers were dried under hot air; B, daylily flowers were soaked in 1% $NaHSO_3$ prior to hot-air-drying; C, daylily flowers were freeze-dried; D, daylily flowers were soaked in 1% $NaHSO_3$ prior to freeze-drying. [b] Each value of means bearing different letters within the same raw is significantly different ($p<0.05$).

lutein. The cis isomers are relatively minor in quantity, and the dehydration process possibly enhanced the formation of cis isomers. However, by comparison of the yield of cis isomers between treatments A and C as well as treatments B and D (hot-air-drying vs freeze-drying), treatments A and B (hot-air-drying) did not result in a significant increase of the amount of cis isomers with the exception of 13-*cis*-lutein. This outcome may be explained by the faster degradation rate of the pigments under the hot-air treatment. In addition to the cis forms, the carotenoid epoxides were also present in small amounts.

Effects of Various Treatments on Stability of Carotenoids. Table 3 shows the concentrations of carotenoids in dried daylily flowers as affected by four treatments. The concentrations of most carotenoids for treatment B were higher than those for treatment A. This is probably because in the former treatment (B) the daylily flowers were soaked in 1% sodium hydrogen sulfite solution prior to dehydration. The addition of sodium hydrogen sulfite has been reported to possess antioxidant activity (Lindsay, 1996), and thus contributed to the higher retention of carotenoids of samples from treatment B. Obviously, the application of sodium hydrogen sulfite solution could protect carotenoids from undergoing oxidative degradation during hot-air-drying. However, the use of sulfite salts in foods has been severely regulated because of possible adverse reactions to these compounds by asthmatic patients (Lindsay, 1996). The total amount of carotenoids in daylily flowers of treatment A was 262.35 μg/mL, which was 216.32 μg/mL less than that of treatment B. For each carotenoid, the loss of zeaxanthin was reduced by 54%, β-cryptoxanthin 44%, all-*trans*-lutein 28%, 13-*cis*-lutein 39%, and all-*trans*-β-carotene 44%.

The total amount of carotenoids of daylily flowers from treatments C and D were 505.95 and 539.44 μg/mL, respectively. Again, this phenomenon showed that soaking in 1% sodium hydrogen sulfite solution results in a higher retention of carotenoids. However, the difference of carotenoids between treatments C and D was not so pronounced as compared to that between treatments A and B. This outcome indicated that the protective effect of sodium hydrogen sulfite toward carotenoids by freeze-drying is minor because it was conducted under an extremely low temperature and high vacuum condition. Thus, the oxidative degradation of carotenoids during freeze-drying was minimized. The major carotenoids such as zeaxanthin, all-*trans*-lutein, all-*trans*-β-carotene, and most other carotenoids only showed minor change for treatments C and D. However, the epoxy-containing carotenoids such as lutein 5,6-epoxide, neoxanthin, and violeoxanthin showed greater change. This result revealed that the carotenoid epoxides may be more vulnerable to the soaking process than the other carotenoids.

By comparing treatments A and C, a 48% loss of total carotenoids was observed for hot-air-dried samples. For each carotenoid,the loss of zeaxanthin was 54%, β-cryptoxanthin 40%, all-*trans*-β-carotene 48%, and all-*trans*-lutein 42%. Most significantly, about 95% of lutein 5,6-epoxide was degraded during hot-air-drying. Also, the contents of violeoxanthin and violaxanthin were reduced by 78 and 60%. respectively, while both neoxanthin and 13-*cis*-lutein 5,6-epoxide did not show significant change. Interestingly, for samples from treatments B and D, only about 11% loss of total carotenoids was found, demonstrating that soaking is a necessary step before hot-air-drying.

Although freeze-drying is capable of retaining a higher amount of carotenoids, it may not be appropriate for commercial production of dried daylily flowers because of high capital cost. The advantage of using sodium hydrogen sulfite was undoubtful; however, the safety of this compound is a major concern. Finding a proper substitute may be a solution to this problem in the future.

Effects of Various Treatments on the Change of Color. Table 4 summarizes the *L*, *a*, *b*, hue, and chroma values of dried daylily flowers under various treatments.

Table 4. Changes of Color of Dried Daylily Flowers under Various Treatments

	color[a]				
treatment	L	a	b	hue	chroma
A	38.2[c]	3.6[c]	20.3[b]	0.18	20.6
B	40.5[b]	4.7[a]	22.0[a]	0.21	22.5
C	43.1[a]	4.4[b]	22.3[a]	0.20	22.7
D	43.5[a]	4.3[b]	23.1[a]	0.19	23.5

[a] Each value of means bearing different letters within the same column is significantly different ($p<0.05$).

Treatment D was found to possess the highest *L* value, followed by treatments C, B, and A. However, no significant difference ($p>0.05$) was found between C and D. The *b* value change exhibited a similar trend, implying that treatments D, C, and B would result in the highest intensity of yellow color. In contrast, the *a* value was highest in treatment B, followed by C, D, and A. This result indicated that samples under soaking and subsequent hot-air-drying would produce a product of deep orange color. The chroma change also displayed the same trend as *L* and *b* values, whereas the hue change was the same as that of the *a* value. The changes of *L*, *b*, and chroma values correlated well to the amount of carotenoids in dried daylily flowers under various treatments. That is, samples containing the largest amount of carotenoids also possessed the highest *L*, *b* and chroma values. As the brightness, redness, and yellowness are the most important criteria to evaluate the color quality of dried daylily flowers, the consumers often prefer bright yellow to orange color product. Based on such a preference, the flowers from treatments D and C should be the first choice of all samples. As for samples from treatment A, which was directly dehydrated under hot-air, they displayed a dull color and were considered a poor product.

In conclusion, the application of a C_{30} column shows a better resolution than a C_{18} column for separation of carotenoids in dried daylily flowers. However, the retention times of carotenoids for the former are much longer. Fresh daylily flowers treated with 1% sodium hydrogen sulfite solution were found to effectively inhibit the loss of carotenoids during hot-air-drying. Freeze-drying is a better method than hot-air-drying for prevention of degradation of carotenoids. This study is a preliminary work for studying the carotenoid change in daylily flowers during processing. Further research is necessary to identify the unknown pigments and prevent degradation of carotenoids during processing.

LITERATURE CITED

Canfield, L. M.; Krinski, N. I.; James, A. O., Eds. *Carotenoids in Human Health*; New York Academy of Science: New York, 1993.

Cano, M. P.; Ancos, B.; Lobo, G.; Monreal, M. Effect of freezing and canning of papaya slices on their carotenoid composition. *Z. Lebensm.-Unters.-Forsch.* **1996**, *202*, 279−284.

Chandler, L. A.; Schwartz, S. J. HPLC separation of *cis*−*trans* carotene isomers in fresh and processed fruits and vegetables. *J. Food Sci.* **1987**, *52*, 669−672.

Chandler, L. A.; Schwartz, S. J. Isomerization and losses of *trans*-β-carotene in sweet potatoes as affected by processing treatments. *J. Agric. Food Chem.* **1988**, *36*, 129−133.

Chen, B. H. Studies on the stability of carotenoids in garland chrysanthemum (*Ipomoea* spp.) as affected by microwave cooking and conventional heating. *J. Food Prot.* **1992**, *5*, 296−300.

Chen, B. H.; Chen, Y. Y. Stability of chlorophylls and carotenoids in sweet potato leaves during microwave cooking. *J. Agric. Food Chem.* **1993**, *41*, 1315−1320.

Chen, B. H.; Yang, S. H. An improved analytical method for determination of carotenes and xanthophylls in dried plant materials and mixed feeds. *Food Chem.* **1992**, *44*, 61−66.

Chen. B. H.; Yang, S. H.; Han, L. H. Characterization of major carotenoids in water convolvulus (*Ipomoea aquatica*) by open-column, thin-layer and high-performance liquid chromatography. *J. Chromatogr.* **1991**, *543*, 147−155.

Chen, B. H.; Chen, T. M.; Chien, J. T. Kinetic model studying the isomerization of α- and β-carotene during heating and illumination. *J. Agric. Food Chem.* **1994**, *42*, 2391−1397.

Chen, B. H.; Peng, H. Y.; Chen, H. E. Changes of carotenoids, color, and vitamin A contents during processing of carrot juice. *J. Agric. Food Chem.* **1995**, *43*, 1912−1918.

Clinton, S. K. Lycopene: Chemistry, biology, and implications for human health and disease. *Nutr. Rev.* **1998**, *56*, 35−51.

Cutler, R. G. Antioxidant and aging. *Am. J. Clin. Nutr.* **1991**, *53*, 373−379.

Davis, B. H. Carotenoids. In *Chemistry and Biochemistry of Plant Pigments*; Goodwin, T. W., Ed.; Academic Press: New York, 1976; Vol. II, pp 38−165.

Delgado-Vargas, F.; Paredes-López, O. Effects of enzymatic treatments of marigold flowers on lutein isomeric profiles. *J. Agric. Food Chem.* **1997**, *45*, 1097−1102.

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.* **1995**, *707*, 205−216.

Food Industry Research and Development Institute of Taiwan. The post-harvested treatment of daylily flowers. *Food Industry* **1987**, *19*(8), 58−59 (in Chinese).

Goodwin, T. W. *The Biochemistry of the Carotenoids*; Chapman and Hall: New York, 1981; Vol. I, pp 1−29.

Hornero-Méndez, D.; Mínguez-Mosquera, M. I. Carotenoid Pigments in *Rosa mosqueta* hips, an alternative carotenoid source for foods. *J. Agric. Food Chem.* **2000**, *48*, 825−828.

Jørjensen K.; Skibsted, L. H. Carotenoid scavenging of radicals. Effect of carotenoid structure and oxygen partial pressure on antioxidative activity. *Z. Lebensm.-Unters.-Forsch* **1993**, *196*, 423−429.

Khachik, F.; Beecher, G. R. Synthesis of C_{45}-β-carotene, a potentially useful internal standard for quantification of hydrocarbon carotenoids by High-Performance Liquid Chromatography. *Ind. Eng. Chem. Prod. Res. Dev.* **1986**, *25*, 671−675.

Khachik, F.; Goli, M. B.; Beecher, G. R.; Holden, J.; Lusby, W. R.; Tenorio, M. D.; Barrera, M. R. Effect of food preparation on qualitative and quantitative distribution of major carotenoids of tomatoes and several green vegetables. *J. Agric. Food Chem.* **1992**, *40*, 390−398.

Khachik, F.; Steck, A.; Pfander, H. Isolation and structural elucidation of (13Z, 13′Z, 3R, 3′R, 6′R)-lutein from marigold flowers, kale, and human plasma. *J. Agric. Food Chem.* **1999**, *48*, 455−461.

Lessin, W. J.; Catigani, G. L.; Schwartz, S. J. Quantification of *cis*−*trans* isomers of provitamin A carotenoids in fresh and processed fruits and vegetables. *J. Agric. Food Chem.* **1997**, *45*, 3728−3732.

Lindsay, R. C. Food additives. In *Food Chemistry*; Fennema, O. R., Ed.; Marcel Dekker: New York, 1996; pp 768−823.

Nguyen, M. L.; Schwartz, S. J. Lycopene: chemical and biological properties. *Food Technol.* **1999**, *53*, 38−45.

Palozza, P.; Krinsky, N. I. Antioxidant effects of carotenoids in vivo and in vitro: An overview. *Methods Enzymol.* **1992**, *213*, 403−420.

Quackenbush, F. W. Reverse phase HPLC separation of *cis* and *trans*-carotenoids and its application to β-carotenes in food materials. *J. Liq. Chromatogr.* **1987**, *10*, 643−653.

Quackenbush, F. W.; Firch, J. G.; Rabourn, W. J.; McQuistan, M.; Petzold, E. N.; Kargl, T. E. Analysis of carotenoids in corn grain. *J. Agric. Food Chem.* **1961**, *9*, 132−135.

Rentel, C.; Strohschein, S.; Albert, K.; Bayer, E. Silver-plated vitamins: a method of detecting tocopherols and carotenoids in LC/ESI-MS. *Anal. Chem.* **1998**, *70*, 4394−4400.

Saleh, M. H.; Tan, B. Separation and identification of *cis*−*trans* carotenoid isomers. *J. Agric. Food Chem.* **1991**, *39*, 1438−1443.

SAS/STAT Guide for Personal Computers, version 6; SAS Instruments: Cary, NC, 1985.

Speek, A. J.; Speek-Saichua, S.; Schreurs, W. H. P. Total carotenoid and *β*-carotene contents of Thai vegetables and the effect of processing. *Food Chem.* **1988**, *27*, 245−257.

Tsukida, K.; Saiki, K.; Takii, T.; Koyama, Y. Separation and determination of *cis*/*trans*-*β*-carotene by high performance liquid chromatography. *J. Chromatogr.* **1982**, *345*, 359−364.

Yen, W. J.; Chen, B. H. Isolation of xanthophylls from Taiwanese orange peels and their effects on the oxidation stability of soybean oil. *Food Chem.* **1995**, *53*, 417−425.

Zechmeister, L. *Cis*−*trans* isomerization and stereochemistry of carotenoids and diphenyl polyenes. *Chem. Rev.* **1944**, *34*, 267−344.

Received for review July 31, 2000. Revised manuscript received September 12, 2000. Accepted September 12, 2000. This study was supported by a grant (NSC 89-2313-B-030-003) from the National Science Council, Taiwan, ROC.

JF000956T