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Pigment change of freeze-dried carotenoid powder during storage

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Abstract

The stability of pigment in freeze-dried carotenoid powder during dark storage at 4, 25, or 45°C, or under light at 25°C was studied. Carrot pulp waste was used as raw material for processing carotenoid powder by freeze-drying. The various carotenoids were analyzed by HPLC with photodiode-array detection. Results showed that the amounts of all-*trans* forms of all three compounds, α-carotene, β-carotene and lutein, decreased with increasing storage temperature or illumination time. The major isomers formed during dark storage were 13-*cis*-α-carotene, 13-*cis*-β-carotene and 13-*cis*-lutein, while 9-*cis*-α-carotene, 9-*cis*-β-carotene and 9-*cis*-lutein were favored during illumination. The degradation rate of the total amount of all-*trans* plus *cis* forms of each pigment fits a first order model, and the highest rate constant (day^{-1}) was found for β-carotene, followed by α-carotene and lutein. Both the Hunter *L* and *b* values of the powder decreased with increasing storage time and temperature, while the Hunter *a* value showed insignificant change ($p > 0.05$).

1. Introduction

Carotenoids such as β-carotene have long been demonstrated to be capable of providing some medical or health benefits, including the possible prevention and treatment of skin cancer and cardiovascular disease (Base, van den Berg, van der Plas & Haenen, 1996; Van Poppel, 1996). Although some controversial results have been reported regarding the potential treatment of lung cancer by β-carotene (Blumberg & Block, 1994; Nicol, Maudet & Savoure, 1994), β-carotene is still an important biological compound because of its provitamin A activity (Olsen, 1989).

In recent years, carrot juice has become an important beverage commodity because it contains high amount of both α- and β-carotene (Chen, Peng & Chen, 1995, 1996). One of the major by-products during processing of carrot juice is carrot pulp waste, which was also reported to contain significant amounts of α- and β-carotene (Chen & Tang, 1998). Thus it would be an advantage to the food industry if the carrot pulp waste could be reprocessed and utilized. In a recent study, Chen and Tang (1998) reprocessed carrot pulp waste into carotenoid powder by spray-drying, and found that the total amount of carotenoids decreased with both increasing time and temperature. Although spray drying is a promising technique for possible commercial production of carotenoid powder in the future, the stability of powder may be diminished because of disruption of powder granules under high temperature treatment during processing (Bhandari, Dumoulin, Richard, Noleau & Lebert, 1992). To remedy this problem, the application of some other processing methods such as freeze-drying can also be employed. The objectives of this study were to process carrot pulp waste into carotenoid powder by freeze-drying and to determine the pigment stability during storage.

2. Materials and methods

2.1. Materials

All-*trans*-α-carotene and all-*trans*-β-carotene standards were purchased from Sigma Co. (St. Louis, MO, USA). Six working solutions of all-*trans*-α-carotene, 1, 5, 10, 25, 50 and 70 μg/ml, were each prepared by dissolving an appropriate amount of all-*trans*-α-carotene in 100 ml of methanol/methylene chloride (99:1, v/v) in a brown volumetric flask. Likewise, six working solutions of all-*trans*-β-carotene, 10, 25, 50, 75, 100 and 130

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μg/ml, were each prepared by dissolving an appropriate amount of all-trans-β-carotene in 100 ml of methanol/methylene chloride (99:1, v/v) in a brown volumetric flask. Lutein standard was prepared from spinach leaves using open-column chromatography as described by Chen and Tang (1998), and six working solutions of lutein, 1, 2, 5, 10, 15 and 20 μg/ml, were each prepared in a 100-ml volumetric flask. All of these standards were prepared under dimmed light and nitrogen gas was flushed into the flask before sealing. Solvents used for extraction of carotenoids, such as petroleum ether, methanol, acetone and hexane were analytical grade. The HPLC grade solvents, such as methanol and methylene chloride, were filtered through a 0.2-μm membrane filter and degassed by sonication prior to use. All of the organic solvents were purchased from Mallinckrodt Co. (Paris, KY, USA). Anhydrous sodium sulfate, potassium hydroxide, and sodium chloride were from RDH Co. (Seelze, Germany). A stainless-steel 5-μm Vydac 201TP54 C_{18} column (25 cm×4.6 mm I.D.) (Hesperia, CA, USA) was used for separation of carotenoids by HPLC.

2.2. Instrumentation

The HPLC instrument consists of an SSI 222D pump (Scientific System Inc., State Collage, PA, USA) and a Shimadzu SPD-M6A photodiode-array detector (Tokyo, Japan). An Axxiom 727 integration software system (Axxiom Chromatography, Inc., Calabasas, CA, USA) was used to process data. The colour difference meter (ND1001 DP) was from Japan Electric Chemical Co. (Tokyo, Japan). The freeze-dryer (FD-24) was from Chin-Ming Co. (Taipei, Taiwan). Microcentrifuge tubes were from Millipore Co. (Bedford, MA, USA) and the Polytron homogenizer (PT10-35) was from Kinematica Ag Littau Co. (Switzerland).

2.3. Extraction of carotenoids from carrot pulp waste

A method similar to that described by Chen and Tang (1998) was used. Ninety grams of carrot pulp was mixed with 90 ml acetone and 135 ml hexane, after which the mixture was blended for 1 min and filtered through a Whatman No. 2 paper. The filtrate was poured into a separating funnel, and the residue was washed twice, with 45 ml acetone and once with 45 ml hexane. The filtrates were pooled and poured into the same separating funnel. The combined filtrate was washed with 100 ml water four times, and the lower layer was discarded. The upper layer, containing carotenoid pigment, was collected and placed in a separating funnel, after which 90 ml methanolic potassium hydroxide (40%) was added, and the mixture was shaken vigorously at room temperature for 2 h for saponification. The solution was then washed with water several times, and the lower layer was discarded. The upper phase was collected again and filtered through anhydrous sodium sulfate. The filtrate was then collected for processing into powder by freeze-drying. All of the extraction procedures were performed under dimmed light, and, whenever possible, nitrogen gas was flushed into the flask to assure adequate inertness.

2.4. Processing of carotenoid powder by freeze-drying

A substrate of 35 g sucrose and 25 g gelatin was placed in a flask, and 80 ml distilled water was added. The solution was thoroughly mixed in a water bath at 45°C, which was used as an aqueous phase. The carotenoid extract (250 ml), prepared from the carrot pulp waste, was concentrated to about 25 ml using a rotary evaporator at 40°C. The aqueous phase was homogenized (5000 rpm) in a Polytron homogenizer (PT10-35), and the carotenoid extract was added gradually so that the carotenoid extract could be dissolved into the substrate. After 35 min, the carotenoid pigment was completely coated with the mixture. The emulsion was then subjected to freeze drying with a vacuum 0.4 mm Hg and drying time 24 h, after which the dried product was ground into powder using a grinder. Approximately 55 g of powder was obtained. The same procedure was repeated 10 times and a total of about 550 g was obtained for the stability study of carotenoid powder during storage.

2.5. Stability of carotenoid powder during storage

Five-gram aliquots of powder were placed in 72 25-ml brown bottles filled with nitrogen gas; 24 were stored at 4°C, 24 at 25°C and 24 at 45°C for 12 weeks. A separate incubator was used for each temperature treatment. Two bottles were randomly removed from each incubator every 2 weeks, and 4-g powder from each bottle was collected for HPLC analysis of carotenoids. For illumination, 5-g aliquots of powder were placed in 24 25-ml translucent bottles filled with nitrogen gas and stored at 25°C for 12 weeks in an incubator. A fluorescent tube was suspended about 30 cm above the bottles, where the light intensity measured about 1500 lux. Two bottles were randomly removed from the incubator every 2 weeks, and 4 g powder from each bottle was collected for HPLC analysis of carotenoids.

2.6. Extraction of carotenoids from powder

A method similar to that used by Chen and Tang (1998) was used. Four grams of powder was mixed with 40 ml water and 80 ml petroleum ether/acetone (1:1, v/v) in a blender, and the mixture was blended for 30 s prior to centrifuging at 10 000 *g* for 15 min. The upper phase containing carotenoid pigment was collected in a

separating funnel and washed with 40 ml saturated saline three times. The upper layer was again collected, and solvent was evaporated using a rotary evaporator. The residue was dissolved in 400 (μl methanol/methylene chloride (45:55, v/v) and transferred to a microcentrifuge tube for centrifugation at 4000 *g* for 30 min. Then the supernatant was filtered through a 0.2-μm membrane filter for analysis of carotenoids by HPLC. Likewise, all of the extraction procedures were conducted under dimmed light, and, whenever possible, nitrogen gas was flushed into the flask to assure adequate inertness.

2.7. HPLC analysis of carotenoids in freeze-dried powder

A method similar to that developed by Chen et al. (1995) was used. A mobile phase of methanol/methylene chloride (99:1, v/v) with a sample solvent of methanol/methylene chloride (45:55, v/v) and a Vydac 201TP54 C_{18} column were used to separated all-*trans*-lutein, all-*trans*-α-carotene, all-*trans*-β-carotene and their *cis* isomers. The flow rate was 0.9 ml/min with a detector sensitivity 0.08 AUFS and wavelength 450 nm. The identification of all-*trans*-lutein, all-*trans*-α-carotene and all-*trans*-β-carotene was carried out by comparing retention times and absorption spectra of unknown peaks with reference standards. In addition, the identification of various cis forms of carotenoids was based on spectral characteristics and Q ratios as described in some previous studies (Chen, Chen & Chien, 1994; Chen et al., 1995). Quantitation of each pigment was made using a method described by Chen and Tang (1998). Duplicate samples were analyzed for each treatment and the mean value was determined. The data were subjected to analysis of variance and Duncan's multiple range test (SAS, 1985).

2.8. Color change of carotenoid powder during storage

The color change of carotenoid powder during storage was studied by using a Color Difference Meter, which was used to determine the Hunter *L*, *a* and *b* values. "*L*" was used to denote brightness of powder, while "*a*" denotes red and "*b*" yellow.

2.9. Kinetic analyses

The degradation rate constants of the total amount of all-*trans* plus *cis* forms of lutein, α-carotene and β-carotene were determined based on a method described by Chen et al. (1994) and Chen and Huang (1998). The correlation coefficient (r^2) was measured from the plot of the logarithm of the total amounts of lutein, α-carotene or β-carotene versus time. The degradation rate constant (day^{-1}) was calculated using the following formula:

$$K = -\ln(CA/CAo)/t$$

where CA: the total concentration of lutein, α-carotene or β-carotene after storage; CA_0: the initial con-centration of lutein, α-carotene or β-carotene; t: storage time.

3. Results and discussion

3.1. Changes of all-trans-lutein and its cis isomers

Table 1 shows the concentration changes of all-*trans*-lutein and its *cis* isomers in the carotenoid powder during storage under light and in the dark at 4, 25 and 45°C. Freeze-dried carotenoid powder was found to contain 9-*cis*-lutein (0.7 μg/g), 13-cis-lutein (1.06 μg/g) and all-*trans*-lutein (7.23μg/g). At 4°C, the concentration of 13-*cis*-lutein increased by 0.18 μg/g, while no significant change ($p > 0.05$) was observed for 9-*cis*-lutein after 12 weeks storage. In contrast, a decrease of 0.64 μg/g was found for all-*trans*-lutein. A similar trend

Table 1
Concentration changes of lutein and its *cis* isomers in the freeze-dried carotenoid powder during storage at various temperatures[a,b]

Lutein (μg/g)				
Temperature	Time (weeks)	9-*cis*	13-*cis*	All-*trans*
4°C	0	0.70a	1.06a	7.23a
(dark)	2	0.69a	1.07a	7.17a
	4	0.71a	1.11b	7.08b
	6	0.71a	1.13b	6.96b
	8	0.70a	1.17bc	6.81c
	10	0.71a	1.21c	6.71c
	12	0.71a	1.24c	6.59c
25°C	0	0.70a	1.06a	7.23a
(dark)	2	0.70a	1.08a	7.14a
	4	0.71a	1.11b	7.02b
	6	0.71a	1.17c	6.87b
	8	0.72a	1.24c	6.65bc
	10	0.71a	1.28d	6.46c
	12	0.72a	1.32d	6.31d
45°C	0	0.70a	1.06a	7.23a
(dark)	2	0.70a	1.09a	7.11b
	4	0.71a	1.16b	6.88b
	6	0.72ab	1.24b	6.64bc
	8	0.72ab	1.34c	6.38c
	10	0.73b	1.38cd	6.18cd
	12	0.75b	1.43d	5.92d
25°C	0	0.70a	1.06a	7.23a
(light)	2	0.70a	1.08a	7.08b
	4	0.71a	1.11b	6.85c
	6	0.70a	1.15bc	6.57c
	8	0.73b	1.21c	6.25cd
	10	0.73b	1.25cd	5.96d
	12	0.73b	1.30d	5.71e

[a] Mean of duplicate analyses.
[b] Values in the same column bearing different letters are significantly different ($p < 0.05$).

was observed at 25°C, i.e. an increase of 0.26 μg/g for 13-*cis*-lutein and a loss of 0.92 μg/g for all-*trans*-lutein occurred. Likewise, the amount of 9-*cis*-lutein showed no significant change ($p > 0.05$). At 45°C, the amount of 13-*cis*-lutein increased by 0.37 μg/g while all-*trans*-lutein decreased by 1.31μg/g over a 12-week period. Interestingly, the level of 9-*cis*-lutein showed a minor increase (0.05 μg/g). Similarly, the amount of all-*trans*-lutein showed a declined trend under light at 25°C. Following a 12-week storage period, a loss of 1.52 μg/g was found, which was higher than dark storage. This result implied that light energy can be more destructive to all-*trans*-lutein. However, the level of 13-*cis*-lutein only increased by 0.24 μg/g. This is probably because 13-*cis*-lutein may further undergo degradation as soon as it is formed from all-*trans*-lutein. It has been reported that both isomerization and degradation of carotenoids may proceed simultaneously under light storage (Chen et al., 1994; Pesek & Warthesen, 1990). For 9-*cis*-lutein, this was only a minor change.

From the results shown above it may be concluded that the higher the storage temperature, the greater the destruction of all-*trans*-lutein. Also, 13-*cis*-lutein was more readily formed than 9-*cis*-lutein during storage in the dark, and the amounts of both formed were greater at high temperature than at low temperature. It has been reported that the central *cis* isomers of carotenoids, such as 13-*cis*-lutein are more suseptible to formation during heating or dark storage, because of the low activation energy required for isomerization (Chen et al., 1995, 1996; Zechmeister, 1944). In additon, the amount of 13-*cis*-β-carotene formed in the dark was higher than that under light.

Table 2
Concentration changes of α-carotene and its *cis* isomers in the freeze-dried carotenoid powder during storage at various temperatures[a,b]

α-carotene (μg/g)					
Temperature	Time (weeks)	9-*cis*	13-*cis*	15-*cis*	All-*trans*
4°C	0	1.07a	1.88a	0.74a	58.72a
(dark)	2	1.08a	1.89a	0.73a	57.37a
	4	1.10a	1.91a	0.74a	55.77b
	6	1.12ab	1.95b	0.75a	54.14b
	8	1.14b	2.00	0.75a	52.57bc
	10	1.16b	2.04bc	0.76a	51.01c
	12	1.18b	2.08c	0.77a	49.44c
25°C	0	1.07a	1.88a	0.74a	58.72a
(dark)	2	1.09a	1.92a	0.75a	57.12a
	4	1.12b	1.98ab	0.76a	55.14b
	6	1.17b	2.06b	0.78b	52.59bc
	8	1.21c	2.13c	0.79b	49.75c
	10	1.24cd	2.19c	0.81bc	47.28c
	12	1.27d	2.25d	0.84c	44.98d
45°C	0	1.07a	1.88a	0.74a	58.72a
(dark)	2	1.10a	1.95b	0.76a	56.71b
	4	1.15b	2.03b	0.79ab	53.59b
	6	1.25c	2.15c	0.83b	49.48c
	8	1.33c	2.28cd	0.86c	45.08c
	10	1.38cd	2.40d	0.89c	41.39d
	12	1.42d	2.52e	0.91c	38.14d
25°C	0	1.07a	1.88a	0.74a	58.72a
(light)	2	1.14b	1.90b	0.76a	56.59b
	4	1.25b	1.95b	0.79ab	53.47b
	6	1.43c	2.02bc	0.84b	48.58c
	8	1.56d	2.08c	0.89c	42.98d
	10	1.69d	2.14d	0.94c	38.75c
	12	1.79e	2.20d	0.99d	34.99e

[a] Mean of duplicate analyses.
[b] Values in the same column bearing different letters are significantly different ($p < 0.05$).

3.2. *Changes of all*-trans-α-*carotene and its* cis *isomers*

Table 2 shows the concentration change of all-*trans*-α-carotene and its *cis* isomers during storage under light and in the dark at 4, 25 and 45°C. All-*trans*-α-carotene was present at a level of 58.72 μg/g in the powder, 13-*cis*-α-carotene 1.88 μg/g, 9-*cis*-α-carotene 1.07 μg/g and 15-*cis*-α-carotene 0.74 μg/g. After 12 week storage at 4°C, the levels of 9-*cis*- and 13-*cis*-α-carotene increased by 0.11 and 0.20 μg/g, respectively, while no significant change ($p > 0.05$) was found for 15-*cis*-α-carotene, and a loss (9.28 μg/g) was observed for all-*trans*-α-carotene. At 25°C, a further increase of 0.20, 0.37 and 0.10 μg/g occurred for 9-*cis*-, 13-*cis*- and 15-*cis*-α-carotene, respectively, while a decline (13.74 μg/g) was found for all-*trans*-α-carotene. Similar trend was observed at 45°C; the concentrations of 9-*cis*-, 13-*cis*- and 15-*cis*-α-carotene increased by 0.35, 0.64 and 0.17 μg/g, respectively, and a loss (20.58 μg/g) was found for all-*trans*-α-carotene over a 12-week storage period. After light storage at 25°C for 12 weeks, the amounts of 9-*cis*-, 13-*cis*- and 15-*cis*-α-carotene increased by 0.72, 0.32 and 0.25 μg/g, respectively, while a decline (23.7 μg/g) was found for all-*trans*-α-carotene. In contrast to the result shown above, 9-*cis*-α-carotene was more susceptible to formation than 13-*cis*-α-carotene during illumination.

By comparing the results shown above, 13-*cis*-α-carotene was more abundant than the other *cis* isomers of α-carotene during storage in the dark. This phenomenon was also shown for 13-*cis*-lutein as previously described. However, during light storage, 9-*cis*-α-carotene was more abundant. In addition, the amounts of *cis* isomers of α-carotene formed were greater at high temperature than at low temperature.

3.3. *Changes of all*-trans-β-*carotene and its* cis *isomers*

Table 3 shows the concentration change of all-*trans*-β-carotene and its *cis* isomers during storage under light conditions and in the dark at 4, 25 and 45°C. All-*trans*-β-carotene was present in the powder in largest amount (103.14 μg/g), followed by 13-*cis*-β-carotene (5.47 μg/g), 15-*cis*-β-carotene (2.57 μg/g) and 9-*cis*-β-carotene (1.93 μg/g). The loss of all-*trans*-β-carotene reached 18.67

Table 3
Concentration changes of β-carotene and its *cis* isomers in the freeze-dried carotenoid powder during storage at various temperatures[a,b]

β-carotene (μg/g)						
Temp.	Time (weeks)	9-*cis*	13-*cis*	15-*cis*	13,15-di-*cis*	All-*trans*
4°C	0	1.93a	5.47a	2.57a	n.d.[c]	103.14a
(dark)	2	1.94a	5.50a	2.57a	n.d.	101.04b
	4	1.96a	5.55ab	2.56a	n.d.	98.79c
	6	1.99b	5.61b	2.57a	n.d.	95.04c
	8	2.03bc	5.69b	2.55a	n.d.	90.77cd
	10	2.06c	5.75b	2.55a	n.d.	87.62d
	12	2.08c	5.81c	2.55a	n.d.	84.47d
25°C	0	1.93a	5.47a	2.57a	n.d.	103.14a
(dark)	2	1.95a	5.52b	2.58a	n.d.	99.01b
	4	2.00b	5.64bc	2.60ab	n.d.	93.87c
	6	2.07c	5.77c	2.63b	n.d.	86.64cd
	8	2.12c	5.89c	2.66bc	0.98a	81.34e
	10	2.18d	5.98d	2.68c	1.00a	76.76f
	12	2.23d	6.08d	2.69c	1.04a	73.54f
45°C	0	1.93a	5.47a	2.57a	n.d.	103.14a
(dark)	2	1.96a	5.57b	2.59a	n.d.	98.61b
	4	2.06b	5.72b	2.62ab	0.99a	92.91cd
	6	2.14c	5.91bc	2.66b	0.99a	84.69d
	8	2.25c	6.10c	2.70b	1.02a	75.37d
	10	2.34d	6.28c	2.72bc	1.05ab	67.36f
	12	2.43e	6.45d	2.74c	1.10b	61.46f
25°C	0	1.93a	5.47a	2.57a	n.d.	103.14a
(light)	2	2.04b	5.52b	2.61ab	n.d.	96.41b
	4	2.27bc	5.60bc	2.65b	1.02a	89.29bc
	6	2.35c	5.70c	2.70bc	1.03a	80.06c
	8	2.58c	5.83d	2.75c	1.05a	70.76d
	10	2.75d	5.93de	2.78d	1.11ab	63.64e
	12	2.88d	6.02e	2.80d	1.18b	54.96f

[a] Mean of duplicate analyses.
[b] Values in the same column bearing different letters are significately different ($p < 0.05$).
[c] n.d.: not detected.

μg/g after 12-week storage at 4°C, while no significant change ($p > 0.05$) occurred for 15-*cis*-β-carotene. In contrast, both 9-*cis*- and 13-*cis*-β-carotene showed an increase by 0.15 and 0.34 μg/g, respectively. At 25°C, a further loss (29.6 μg/g) was found for all-*trans*-β-carotene, while an increase was found for 9-*cis*-β-carotene (0.30 μg/g), 13-*cis*-β-carotene (0.61 μg/g) and 15-*cis*-β-carotene (0.12 μg/g). Interestingly, one more *cis* isomer, 13,15-di-*cis*-β-carotene, was formed after 6 weeks. The formation of 13,15-di-*cis*-β-carotene may be due to conversion of 13-*cis*- or 15-*cis*-β-carotene (Chen et al., 1994, 1995). In addition, both 9-*cis*- and 13-*cis*-β-carotene were reported to be interconvertible through formation of all-*trans*-β-carotene (Chen et al., 1994; Pesek & Warthesen, 1990; Pesek, Warthesen & Taoukis, 1990). A similar phenomenon was found at 45°C, i.e. the loss of all-*trans*-β-carotene was 41.68 μg/g, while an increase was found for 9-*cis*-β-carotene (0.50 μg/g), 13-*cis*-β-carotene (0.98 μg/g), 15-*cis*-β-carotene (0.17 μg/g) and 13,15-di-*cis*-β-carotene (1.10 μg/g), over a 12-week storage period. This result further demonstrated that the amounts of mono-*cis* or di-*cis* isomer would be substantially increased under high temperature storage. Of the various isomers, 13-*cis*-β-carotene was formed in largest amount during storage, followed by 9-*cis*-β-carotene, 15-*cis*-β-carotene and 13,15-di-*cis*-β-carotene. However, the amounts of both 9-*cis*-and 13-*cis*-β-carotene formed were lower than that reported by Pesek et al. (1990), who studied the isomerization of all-*trans*-β-carotene dissolved in hexane, during dark storage at 45°C. This is probably because that sample solvent hexane may promote isomerization of all-*trans*-β-carotene (Pesek et al.). Also, the β-carotene solution was in a homogeneous state, which should be more susceptible to isomerization than the powder, which is in a solid state used in this study.

After light storage at 25°C for 12 weeks, the amount of all-*trans*-β-carotene declined by 48.18 μg/g, while an increase of 0.95, 0.55, 0.23 and 1.18 μg/g was found for 9-*cis*-, 13-*cis*-, 15-*cis*- and 13,15-di-*cis*-β-carotene, respectively. On the contrary, 9-*cis*-β-carotene occurred at a higher level than 13-*cis*-β-carotene. This phenomenon was also showed for 9-*cis*-β-carotene formation during light storage. This is probably because the activation energy required for isomerization of 9-*cis*-β-carotene was greater than that of 13-*cis*-β-carotene, which would favor formation of the former because of sufficient energy dissipated by light during storage (Pesek & Warthesen, 1990; Chen et al., 1994). It was also found that light storage can be more destructive to all-*trans*-β-carotene than dark storage. It has been established that both degradation and isomerization of all-*trans*-β-carotene can proceed simultaneously during illumination, and the dominant reaction depends upon several factors such as temperature, light intensity, and the presence of catalyst (Pesek & Warthesen, 1990; Pesek et al., 1990). The amounts of 9-*cis*-, 13-*cis*-, 15-*cis*- or 13,15-di-*cis*-β-carotene, formed under light storage, were higher than dark storage. Several researchers reported that some drastic treatments, such as illumination or canning, may facilitate formation of di-*cis* isomers suh as 13,15-di-*cis*-β-carotene (Chen et al., 1994, 1995).

Table 4 shows the degradation rate constants of the total amount of all-*trans* plus *cis* forms of lutein, α-carotene and β-carotene during storage. The degradation rate of each pigment was assessed to fit a first-order model because a linear correlation ($r^2 > 0.95$) was observed for the plot of the logarithm of the total concentration of each pigment versus time. The degradation rate constants (day^{-1}) of α-carotene under light storage at 25°C or in the dark at 4, 25 and 45°C were 0.039, 0.013, 0.020 and 0.032, respectively, while that of β-carotene was found to be 0.043, 0.015, 0.024 and 0.037. This result implies that β-carotene is more susceptible to degradation than α-carotene, probably because the former possesses more conjugated carbon–carbon double bonds, which can be more reactive than the latter. For lutein, the degradation rate constant

Table 4
Rate constants of lutein, α-carotene and β-carotene in freeze-dried carotenoid powder during storage at various temperatures

	Rate constant (day^{-1})		
Temperature	Lutein[a]	α-Carotene[b]	β-Carotene[c]
4°C (dark)	0.004	0.013	0.015
25°C (dark)	0.006	0.020	0.024
45°C (dark)	0.009	0.032	0.037
25°C (light)	0.013	0.039	0.043

[a] Lutein includes all-*trans*-lutein and its *cis* isomers.
[b] α-Carotene includes all-*trans*-α-carotene and its *cis* isomers.
[c] β-Carotene includes all-*trans*-β-carotene and its *cis* isomers.

(day^{-1}) was less than α-carotene or β-carotene, which may be attributed to the formation of lutein–gelatin complex in the powder, and the stability of powder was thus greatly enhanced (Bryant, McCord, Unlu & Erdman, 1992).

From the results shown above, it may be concluded that the stability of freeze-dried carotenoid powder can be substantially improved by storage at low temperature such as 4°C. In a similar study, Chen and Tang (1998) studied the stability of spray-dried carotenoid powder and found that the degradation rate constants (day^{-1}) of the total amount of all-trans plus cis forms of α-carotene, β-carotene and lutein were 0.049, 0.058 and 0.015, respectively, during light storage at 25°C. These data were higher than those shown in this study, indicating that spray-dried powder is more susceptible to degradation loss than freeze-dried powder during storage. This may be explained as follows: during spray drying, the powder granule may undergo shrinkage because of hot air penetration, which in turn results in formation of numerous tiny pores on the surface of powder. In contrast, during freeze-drying, no shrinkage or deformation of powder granule occurred because of sublimation of water from ice crystals at the freezing temperature (Geji-Hansen & Flink, 1977; Zilberboim, Kopelman & Talmon, 1986). In addition, the amount of *cis* isomers of each pigment formed by freeze drying was less than that by spray-drying. This should have some impact on the quality of powder because it has been well documented that the formation of *cis*-carotenoid isomers would decrease colour intensity and provitamin A activity (Chen et al., 1995; Chen & Tang, 1998).

Table 5 shows the color change of freeze-dried carotenoid powder during light storage at 25°C or in the dark at 4, 25 and 45°C. Results showed that the Hunter *L* value (brightness) decreased with increasing storage time and temperature. However, only a minor change was observed for the Hunter *a* value, implying that red is not a major contributing factor to the color of carotenoid powder. For the Hunter *b* value (yellow), it decreased significantly ($p > 0.05$) after 12 week storage under light or in the dark. Also, the Hunter *b* value

Table 5
Changes in Hunter *L*, *a* and *b* values of freeze-dried carotenoid powder during storage[a,b,c]

Storage time (weeks)	Storage temperature			
	4°C (dark)	25°C (dark)	45°C (dark)	25°C (light)
Hunter L *value*				
0	92.8a	92.8a	92.8a	92.8a
2	92.6a,A	92.5ab,A	92.4b,A	92.4ab,A
4	92.5a,A	92.1bc,B	92.0b,B	92.2b,AB
6	92.1b,A	91.7cd,AB	91.5c,B	91.1c,C
8	91.9b,A	91.5d,AB	91.1c,B	90.7cd,C
10	91.6bc,A	91.3de,A	90.5d,B	90.4de,B
12	91.5c,A	91.1e,A	90.5d,B	90.1e,B
Hunter a *value*				
0	0.3a	0.3a	0.3a	0.3a
2	0a,A	0.2a,A	0a,A	0.3a,A
4	0.1a,A	0a,A	0.2a,A	0.2a,A
6	0.2a,A	0.1a,A	0.1a,A	0.2a,A
8	0a,A	0.1a,A	0a,A	0a,A
10	0.3a,A	0.2a,A	0.2a,A	0.2a,A
12	0.3a,A	0.1a,A	0.2a,A	0a,A
Hunter b *value*				
0	32.5a	32.5a	32.5a	32.5a
2	32.3a,A	32.3a,A	32.2a,A	31.8b,B
4	32.0a,A	31.2b,B	30.7b,C	30.9c,BC
6	31.0b,A	30.4c,B	29.6c,C	29.2d,C
8	30.7bc,A	29.9d,B	28.7d,C	28.5e,C
10	30.4cd,A	29.3e,B	28.1de,C	27.5f,C
12	30.1d,A	29.1e,B	27.9e,C	27.3f,D

[a] Average of duplicate analysis.
[b] Values in the same column bearing different letters (a–f) are significantly different ($p < 0.05$).
[c] Values in the same row bearing different letters (A–D) are significantly different ($p < 0.05$).

decreased to a greater extent at high temperature than at low temperature. Both the Hunter *L* and *b* values decreased by 2.7 and 5.2, respectively, after 12 weeks light storage. However, the Hunter a value only showed a slight change. This result revealed that with increasing storage time and temperature, both the brightness and yellow colour of powder decreased, mainly because of degradation of carotenoids or formation of *cis* isomers.

In conclusion, the amount of all-*trans*-carotenoids in the freeze-dried powder decreased with increasing storage temperature or illumination time. The 13-*cis* type isomers of carotenoids dominated under dark storage, while the 9-*cis* type was favored under light storage. Di-*cis* isomers, such as 13,15-di-*cis*-β-carotene, could be formed under high temperature storage or illumination. The degradation rate of the total amount of all-*trans* plus *cis* forms of each pigment fits a first-order model, and the highest rate constant (day^{-1}) was found for β-carotene, followed by α-carotene and lutein. Both the Hunter *L* and *b* values of freeze-dried powder decreased with increasing storage temperature and illumination time, while no significant change ($p > 0.05$) of the Hunter *a* value was observed. The freeze-dried powder

showed a higher pigment stability than the spray-dried powder during storage.

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