Processing and Stability of Carotenoid Powder from Carrot Pulp Waste

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Carrot pulp waste was used as raw material for processing carotenoid powder by spray-drying, and the stability of carotenoid in the powder was studied under light and dark storage at various temperatures. The various carotenoids were analyzed by HPLC with photodiode array detection. Results showed that the most appropriate condition for processing carotenoid powder by spray-drying consists of 15% solid content of feed, with inlet air temperature of 135−145 °C and outlet air temperature of 90−100 °C. The total amount of all-trans plus cis forms of lutein, α-carotene, or β-carotene in the carotenoid powder decreased with increasing storage time and temperature, and the degradation rate of each fits the first-order model. The major cis isomers formed in the dark were 13-*cis*-α-carotene and 13-*cis*-β-carotene, whereas 9-cis isomers of both α- and β-carotene predominated under light. A high correlation was also observed between color changes and carotenoid content.

Keywords: *Carotenoid powder; carrot pulp waste; spray-drying; carotenoid stability*

INTRODUCTION

In the past decade carotenoids such as β-carotene have created considerable attention because of their possible protective effect against some types of cancers (Bast et al., 1996; Santos et al., 1996; Van Poppel, 1996). However, the results of some recent studies indicated that the consumption of β-carotene in excess in the diet may exert harmful effects to male smokers with lung cancer (Blumberg and Block, 1994; Nicol et al., 1994). Nevertheless, β-carotene is still an important biological compound because of its provitamin A activity (Olsen, 1989). Theoretically, β-carotene possesses 100% vitamin A activity, whereas α-carotene possesses 50% vitamin A activity.

In Taiwan the consumption of carrot juice has increased steadily in recent years because of consumers' demand for healthy drinks. Carrot juice has been reported to contain high amounts of α- and β-carotene (Munsch and Simard, 1983; Heinonen, 1990; Chen et al., 1995, 1996). According to a report by the Commission of Fruits and Vegetables in Taiwan (1995), the consumption of carrot juice reached 2.5 million tons in 1994. Since only ∼60−70% juice is extracted from carrot, a large amount of carrot pulp waste is produced, which creates a major disposal problem for the food industry. However, it has been reported that the carrot pulp waste still contains a significant amount of carotenoids (Krishna, 1985; Bao and Chang, 1994). Thus, it would be a great advantage to the food industry if the carotenoids in the carrot pulp could be extracted and processed into powder, which could be used as a color additive in various foods.

The objectives of this study were (1) to process carotenoid powder from carrot pulp waste by spray-drying and (2) to study the stability of carotenoids in the powder under light and dark storage at various temperatures.

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MATERIALS AND METHODS

Materials. *all-trans*-α-Carotene and *all-trans*-β-carotene standards were purchased form Sigma Chemical Co. (St. Louis, MO). Six *all-trans*-α-carotene concentrations, 1, 5, 10, 25, 50, and 70 μg/mL, were prepared by dissolving an appropriate amount of *all-trans*-α-carotene in 100 mL of methanol/methylene chloride (99:1 v/v). Likewise, six *all-trans*-β-carotene concentrations, 10, 25, 50, 75, 100, and 130 μg/mL, were prepared by dissolving an appropriate amount of *all-trans*-β-carotene in 100 mL of methanol/methylene chloride (99:1 v/v). Gelatin was obtained from Hsen-Yuan Chemical Co. (Taipei, Taiwan). Sucrose was from Aldrich Chemical Co. (Milwaukee, WI). Anhydrous sodium sulfate, potassium hydroxide, and sodium chloride were from RDH Co. (Seelze, Germany). Solvents used for extraction including absolute alcohol, acetone, and hexane were of 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). Approximately 5 kg of carrot pulp was obtained from a local plant.

Preparation of Lutein Standard. Lutein standard was prepared from spinach by column chromatography using a method similar to that described by Chen et al. (1991). A 25-g sample of fresh spinach leaves was mixed with 2.5 g of magnesium carbonate, and the mixture was extracted with 75 mL of hexane/acetone/methanol/toluene (10:7:6:7 v/v/v/v) in a 250-mL volumetric flask. The solution was blended for 1 min and passed through a Whatman No. 2 filter paper, and the filtrate was poured into another 250-mL volumetric flask. The solution was saponified by adding 25 mL of 40% methanolic potassium hydroxide and then allowed to stand at ambient temperature for 16 h. Hexane (75 mL) was added to the flask, and the mixture was diluted to volume with 10% sodium sulfate solution. After shaking vigorously for 1 min and standing in the dark for 1 h, 5 mL of the upper phase was pipetted onto a column (30 cm × 12.5 mm i.d.) containing a mixture of activated magnesium oxide and diatomaceous earth (1:1) for open-column chromatography. Hexane/acetone (95:5 v/v) was used to elute carotenes. Three colored bands were observed when eluted with hexane/acetone/methanol (85:15:1 v/v/v). The first band collected was identified as lutein on the basis of spectral characteristics and *Q* ratios as

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described in some previous studies (Chen et al., 1991, 1995). For quantification, the eluate of lutein was evaporated to dryness and dissolved in an appropriate volume of methanol/methylene chloride (99:1 v/v). The absorbance was measured at 450 nm. Six lutein concentrations, 1, 2, 5, 10, 15, and 20 μg/mL, were obtained by using the formula

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

where E is absorbance and $E^{1\%}_{1\text{cm}}$ is the extinction coefficient (2500).

Instrumentation. The HPLC instrument consists of an SSI 222D pump (Scientific System Inc., State College, PA) and a Shimadzu SPD-M6A photodiode-array detector (Tokyo, Japan). Data were processed with Axxiom 727 integration software (Axxiom Chromatography Inc., Calabasas, CA). A stainless steel 5-μm Vydac 201TP54 C_{18} column (25 cm × 4.6 mm i.d.) (Hesperia, CA) was used. A Beckman DU-70 double-beam spectrophotometer (Fullerton, CA) was used to record the spectra. The color difference meter, ND1001DP, was from Japan Electric Chemical Co. (Tokyo, Japan). The mobile minor spray-dryer was from Niro Atomizer Co. (Denmark). The FD-24 freeze-dryer was from Chin-Ming Co. (Taipei, Taiwan). The vs-6 funnel shaker was from Hsiang-Tai Co. (Taipei, Taiwan). Microcentrifuge tubes were from Millipore Co. (Bedford, MA). The homogenizer (Polytron PT10-35) was from Kinematica Ag Littau Co. (Switzerland).

Extraction of Carotenoids from Carrot Pulp Waste. Thirty grams of carrot pulp was placed in a blender, to which a mixture of 30 mL of acetone and 45 mL of hexane was added. The solution was thoroughly blended for 1 min and passed through a Whatman No. 2 filter paper, and the filtrate was poured into a separating funnel. The residue was washed twice with 15 mL of acetone and once with 15 mL of hexane. The filtrates were pooled and poured into the same separating funnel. The combined filtrate was washed four times with 100 mL of water, and the lower layer was discarded. The upper phase containing the carotenoids was collected and mixed with 30 mL of methanolic potassium hydroxide (40%) in a separating funnel, which 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 containing the carotenoids was collected and filtered through anhydrous sodium sulfate. The solution was evaporated to dryness in a rotary evaporator at 40 °C, dissolved in methanol/methylene chloride (45:55 v/v), and filtered through a 0.2-μm membrane filter for HPLC analysis. For processing of carotenoid powder, a 90-g carrot pulp sample was used for preparation of carotenoid extract. The extraction procedure was the same as that described above with the exception that the solvents employed were 3 times greater in volume than those originally used for extraction of carotenoids.

Processing of Carotenoid Powder by Spray-Drying. A substrate of 35 g of sucrose and 25 g of gelatin was mixed and placed in a flask, and 80 mL of distilled water was added. After thorough mixing, the substrate was heated in a water bath (45 °C) until it was dissolved. This solution was used as an aqueous phase. The carotenoid extract (250 mL) prepared from a 90-g carrot pulp sample was concentrated to 25 mL using a rotary evaporator at 40 °C. The aqueous phase was homogenized (5000 rpm) in a Polytron PT10-35 homogenizer, and the carotenoid extract was added gradually so that the carotenoid pigment could be dissolved into the substrate. After 35 min, the carotenoid pigment was completely coated with the substrate. The solid content of the emulsion was controlled at 15% by adding 260 mL of water. The emulsion was then subjected to spray-drying with the following conditions: solid content of feed (the emulsion), 15%; inlet air temperature, 135−145 °C; outlet air temperature, 90−100 °C; and drying time, 45 min. Approximately 40 g of carotenoid powder was obtained. The same procedure was repeated 15 times, and a total of ∼600 g of carotenoid powder was collected.

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 of powder from each bottle was taken for HPLC analysis of carotenoids. To evaluate light effects, 5-g aliquots of powder were placed in 24 25-mL translucent bottles filled with nitrogen and stored at 25 °C for 12 weeks in an incubator. A fluorescent tube was suspended ∼30 cm above the bottles, where the light intensity measured 1500 lx. Two bottles were randomly removed from the incubator every 2 weeks, and 4 g of powder from each bottle was taken for HPLC analysis of carotenoids.

Extraction of Carotenoids from Powder. Four grams of powder was mixed with 40 mL of water in a blender, and 80 mL of petroleum ether/acetone (1:1 v/v) was added. The solution was blended for 25 s and then centrifuged at 10000*g* for 15 min. The upper layer was collected and placed in a separating funnel and washed three times with 40 mL of saturated saline. The upper phase containing the carotenoids was then collected, and solvent was evaporated to dryness. The residue was dissolved in 400 μL of methanol/methylene chloride (45:55 v/v) and placed in a microcentrifuge tube for centrifugation (4000*g*, 30 min). The supernatent was filtered through a 0.2-μm membrane filter for carotenoid analysis by HPLC.

HPLC Analysis of Carotenoids in Carrot Pulp Waste and Powder. This method was similar to that developed by Chen et al. (1995). 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 separate *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 of 0.08 AUFS and wavelength of 450 nm; injection volume was 20 μL. The purity of each peak was automatically calculated on the basis of an equation described in the operation manual of a Shimadzu SPD-M6A photodiode array detector. The recovery was obtained by adding a 3-mL mixture of *all-trans*-lutein, *all-trans*-α-carotene, and *all-trans*-*β*-carotene to a carrot pulp waste or a carotenoid powder sample, and extraction was performed as described previously. After quantification by HPLC, the recovery data for both samples were obtained by dividing the amount of carotenoids following extraction and HPLC analysis by the amount of caroteniods added to the sample. The identification of *all-trans*-lutein, *all-trans*-α-carotene, and *all-trans*-*β*-carotene was conducted by comparison of retention times and absorption spectra of unknown peaks with reference standards. In addition, the identification of cis carotenoids was based on spectral characteristics and *Q* ratios as described in some previous studies (Chen and Chen, 1994; Chen et al., 1994, 1995). Quantitation of each carotenoid was carried out using an external calibration method. Six concentrations of *all-trans*-lutein, *all-trans*-α-carotene, and *all-trans*-*β*-carotene ranging from 1 to 130 μg/mL were injected onto the HPLC, and the calibration curve for each pigment was obtained by plotting concentration against area. The calibration curves of each pigment gave good linearity ($r^2 = 0.99$). Due to the absence of cis carotenoid standards, the quantitation of cis isomers of *all-trans*-lutein, *all-trans*-α-carotene, and *all-trans*-*β*-carotene was calculated as *all-trans*-lutein, *all-trans*-α-carotene, and *all-trans*-*β*-carotene equivalents, respectively. 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). The degradation rate constants of the total amount of all-trans and cis forms of α-carotene, *β*-carotene, and lutein were determined using a method described by Chen et al. (1994).

Color Stability of Carotenoid Powder during Storage. The color difference meter was used to measure *L*, *a*, and *b* values, of which *L* "+" stands for brightness, *a* "+" stands for redness, and *b* "+" stands for yellowness. The overall color change of carotenoid powder was monitored using ΔE values

with the formula

$$\Delta E = \sqrt{(L_1 - L_0)^2 + (a_1 - a_0)^2 + (b_1 - b_0)^2}$$

where L_0, a_0, and b_0 are the Hunter L, a, and b values of carotenoid powder before storage and L_1, a_1, and b_1 are the Hunter L, a, and b values of carotenoid powder after storage for n weeks.

RESULTS AND DISCUSSION

Processing of Carotenoid Powder. It has been well established that several parameters such as solid content of feed, inlet air temperature, and outlet air temperature have to be carefully controlled for processing spray-dried powder (Bhandari et al., 1992). Although high inlet air temperature can reduce drying time, it may result in degradation of carotenoids. Likewise, the high outlet air temperature may also result in breakdown of powder granule. The high solid content may increase the viscosity of the feed, while the low solid content may increase the moisture content of the feed. These conditions would make it difficult for spray-drying. After various studies, the most appropriate condition for processing carotenoid powder by spray-drying consists of 15% solid content of feed, with inlet air temperature of 135−145 °C and outlet air temperature of 90−100 °C.

Analysis of Carotenoids in Carrot Pulp Waste and Powder. The chromatographic conditions used for the separation of caroteniods and their cis isomers were based on a study by Chen et al. (1995), who employed a mobile phase of methanol/methylene chloride (99:1 v/v) with methanol/methylene chloride (45:55 v/v) as sample solvent and a polymeric Vydac 201TP54 column to resolve 12 pigments in carrot juice. However, with the same condition the carotenoids and their cis isomers in the carrot pulp waste and carotenoid powder were not adequately resolved. This is probably because many impurities in the carrot pulp waste and carotenoid powder may interfere with the separation of carotenoids and their cis isomers. Nevertheless, the HPLC method was found to be very reproducible, with coefficients of variation <3% in five sample analyses. Also, the retention time variation was <5% over a 12-week period. The recoveries of *all-trans*-α-carotene, *all-trans*-*β*-carotene, and *all-trans*-lutein in the presence of carrot pulp waste through the cold saponification and extraction procedures ranged from 92 to 98%. However, in the presence of carotenoid powder the recoveries of *all-trans*-α-carotene, *all-trans*-*β*-carotene, and *all-trans*-lutein ranged from 85 to 90%. This difference can be attributed to the fact that the presence of a large amount of gelatin in the powder may greatly reduce the extraction efficiency of carotenoids, and thus a lower recovery was observed. In addition, differences in extraction procedures may also account for the recovery variation. A total of 10 carotenoids, 9-*cis*-lutein (0.40 μg/g), 13-*cis*-lutein (0.51 μg/g), *all-trans*-lutein (3.75 μg/g), 9-*cis*-α-carotene (0.55 μg/g), 13-*cis*-α-carotene (0.78 μg/g), *all-trans*-α-carotene (27.44 μg/g), 9-*cis*-*β*-carotene (0.87 μg/g), 13-*cis*-*β*-carotene (2.55 μg/g), 15-*cis*-*β*-carotene (1.02 μg/g), and *all-trans*-*β*-carotene (54.23 μg/g), were found in carrot pulp waste. For carotenoid powder, a total of 12 carotenoids, 9-*cis*-lutein (0.76 μg/g), 13-*cis*-lutein (1.26 μg/g), *all-trans*-lutein (5.98 μg/g), 9-*cis*-α-carotene (1.40 μg/g), 13-*cis*-α-carotene (2.01 μg/g), 15-*cis*-α-carotene (1.03 μg/g), *all-trans*-α-carotene (36.48 μg/g), 9-*cis*-*β*-carotene (2.11 μg/g), 13-*cis*-*β*-carotene (6.35 μg/g), 15-*cis*-*β*-carotene (2.42 μg/g), 13,15-di-*cis*-*β*-carotene (1.10 μg/g), and *all-trans*-*β*-carotene (50.83 μg/g), were present. Figure 1 shows the HPLC chromatogram of 12 carotenoids in spray-dried carotenoid powder. The purity of each carotenoid ranged from 98.4 to 99.9% as determined by the photodiode array detector. With the exception of *all-trans*-*β*-carotene, the amount of each carotenoid in spray-dried carotenoid powder was found to be substantially higher than that in carrot pulp waste. This result implied that during spray-drying *all-trans*-*β*-carotene may be degraded or converted to other cis forms of isomers. In addition, two more carotenoids, 15-*cis*-α-carotene and 13,15-di-*cis*-*β*-carotene, were formed during spray-drying.

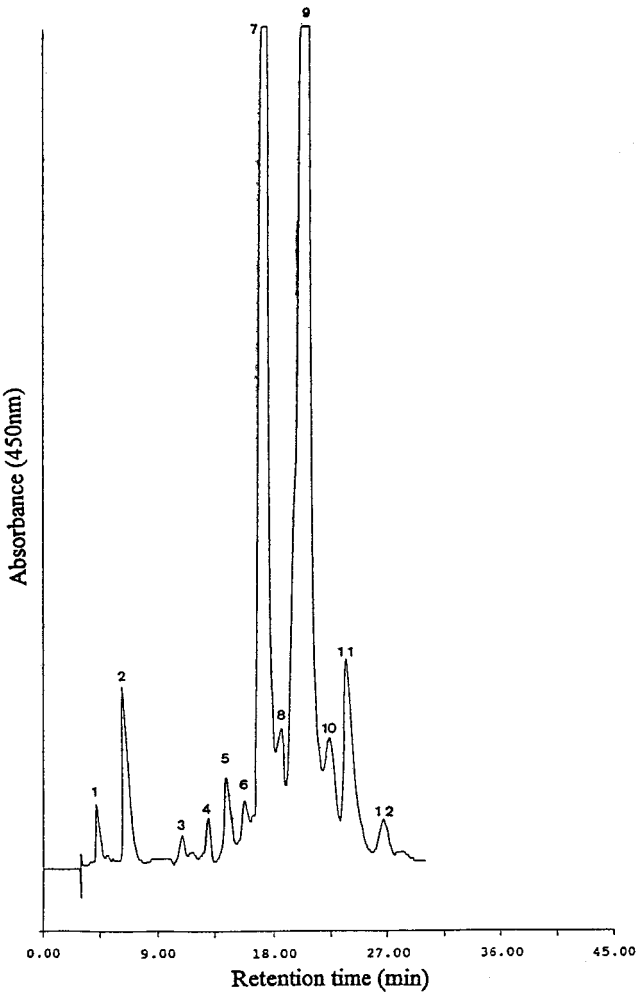

Figure 1. HPLC chromatogram of spray-dried carotenoid powder. Chromatographic conditions are described in the text. Peaks: 1, 13-*cis*-lutein; 2, lutein; 3, 9-*cis*-lutein; 4, 9-*cis*-α-carotene; 5, 13-*cis*-α-carotene; 6, 13,15-di-*cis*-*β*-carotene; 7, α-carotene; 8, 15-*cis*-α-carotene; 9, *β*-carotene; 10, 9-*cis*-*β*-carotene; 11, 13-*cis*-*β*-carotene; 12, 15-*cis*-*β*-carotene.

Stability of *all-trans*-Lutein and Its Cis Isomers in Carotenoid Powder during Storage. Table 1 shows the changes in the concentration of *all-trans*-lutein and its cis isomers in powder during storage. In the samples stored in the dark the loss of *all-trans*-lutein increased with both increasing storage temperature and time. The losses after 12 weeks at 4, 25, and 45 °C were 0.73, 1.04, and 1.45 μg/g, respectively. In contrast, the concentration of 13-*cis*-lutein increased with increasing storage temperature and time: 0.27,

Table 1. Concentration Changes of *all-trans*-Lutein and Its Cis Isomers during Storage

temp (°C)	time (weeks)	lutein[a] (μg/g) 9-*cis*	13-*cis*	*all-trans*
4 (dark)	0	0.76[a]	1.26[a]	5.98[a]
	2	0.76[a]	1.30[a]	5.88[b]
	4	0.75[a]	1.36[b]	5.80[b]
	6	0.77[a]	1.41[c]	5.62[c]
	8	0.77[a]	1.46[d]	5.48[d]
	10	0.77[a]	1.50[de]	5.35[e]
	12	0.77[a]	1.53[e]	5.25[f]
25 (dark)	0	0.76[a]	1.26[a]	5.98[a]
	2	0.77[a]	1.32[b]	5.86[b]
	4	0.77[a]	1.41[c]	5.68[c]
	6	0.78[a]	1.48[d]	5.47[d]
	8	0.77[a]	1.52[de]	5.27[e]
	10	0.78[a]	1.56[ef]	5.12[f]
	12	0.79[a]	1.59[f]	4.94[g]
45 (dark)	0	0.76[a]	1.26[a]	5.98[a]
	2	0.77[a]	1.32[b]	5.77[b]
	4	0.78[ab]	1.42[c]	5.55[c]
	6	0.80[ab]	1.54[d]	5.25[d]
	8	0.81[ab]	1.59[de]	5.00[e]
	10	0.82[b]	1.62[ef]	4.76[f]
	12	0.82[b]	1.65[f]	4.53[g]
25 (light)	0	0.76[a]	1.26[a]	5.98[a]
	2	0.76[ab]	1.29[a]	5.76[b]
	4	0.80[abc]	1.34[b]	5.31[c]
	6	0.77[ab]	1.40[c]	5.00[d]
	8	0.79[abc]	1.47[d]	4.72[e]
	10	0.82[bc]	1.52[e]	4.58[f]
	12	0.83[c]	1.58[f]	4.29[g]

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

0.33, and 0.39 μg/g, respectively. There were no significant changes ($p > 0.05$) in the concentrations of 9-*cis*-lutein at 4 and 25 °C, and there was only a minor increase at 45 °C. By comparison of the results described above, it was observed that the higher storage temperature, the faster the degradation of *all-trans*-β-carotene. Also, 13-*cis*-lutein was more abundant than 9-*cis*-lutein during storage of carotenoid powder in the dark. This phenomenon may be explained as below: the low activation energy of the 13-cis type isomer could be favored during dark storage because of the low energy provided (Zechmeister, 1944). In a similar study Pesek et al. (1990) also reported that the rate of 13-*cis*-β-carotene formation was faster than that of 9-*cis*-β-carotene when *all-trans*-β-carotene was held in the dark. During illumination at 25 °C the concentration of *all-trans*-lutein declined by 1.69 μg/g during 12 weeks of storage and 13-*cis*-lutein increased by 0.32 μg/g, but there was only a minor change in 9-*cis*-lutein. This result implied that light storage can be more destructive to *all-trans*-lutein than dark storage. The degradation rate constants (day^{-1}) of the total amount of all-trans and cis forms of lutein during storage under light at 25 °C and in the dark at 4, 25, and 45 °C for 12 weeks were 0.015, 0.005, 0.008, and 0.011, respectively (Table 2). Also, all of the degradations at various temperatures fit the first-order model since a linear correlation was observed for the plot of the logarithm of the total lutein (all-trans and cis forms) concentration versus time.

Stability of *all-trans*-α-Carotene and Its Cis Isomers in Carotenoid Powder during Storage. Table 3 shows the changes in the concentration of *all-trans*-α-carotene and its cis isomers in powder during storage. In the samples stored in the dark the loss of *all-trans*-α-carotene increased with both increasing

Table 2. Rate Constants of Lutein, α-Carotene, and β-Carotene in Spray-Dried Carotenoid Powder during Storage at Various Temperatures

temp (°C)	rate constant (day^{-1}) lutein[a]	α-carotene[b]	β-carotene[c]
4 (dark)	0.005	0.014	0.018
25 (dark)	0.008	0.026	0.031
45 (dark)	0.011	0.043	0.050
25 (light)	0.015	0.049	0.058

[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.

Table 3. Concentration Changes of *all-trans*-α-Carotene and Its Cis Isomers during Storage

temp (°C)	time (weeks)	α-carotene[a] (μg/g) 9-*cis*	13-*cis*	15-*cis*	*all-trans*
4 (dark)	0	1.40[a]	2.01[a]	1.03[a]	36.48[a]
	2	1.41[a]	2.04[ab]	1.03[a]	35.43[b]
	4	1.43[ab]	2.09[b]	1.04[a]	34.38[c]
	6	1.47[bc]	2.17[c]	1.05[a]	33.18[d]
	8	1.50[cd]	2.24[d]	1.05[a]	31.88[e]
	10	1.52[d]	2.28[d]	1.05[a]	30.76[f]
	12	1.54[d]	2.33[e]	1.07[a]	29.62[g]
25 (dark)	0	1.40[a]	2.01[a]	1.03[a]	36.48[a]
	2	1.43[a]	2.09[b]	1.03[a]	35.01[b]
	4	1.48[b]	2.19[c]	1.05[ab]	32.98[c]
	6	1.58[c]	2.33[d]	1.08[bc]	30.88[d]
	8	1.68[d]	2.45[e]	1.10[cd]	28.83[e]
	10	1.69[de]	2.60[f]	1.10[cd]	26.75[f]
	12	1.72[e]	2.68[g]	1.13[d]	24.62[g]
45 (dark)	0	1.40[a]	2.01[a]	1.03[a]	36.48[a]
	2	1.49[b]	2.13[b]	1.05[ab]	33.77[b]
	4	1.59[c]	2.28[c]	1.08[bc]	30.73[c]
	6	1.73[d]	2.50[d]	1.12[cd]	27.55[d]
	8	1.88[e]	2.78[e]	1.16[de]	24.19[e]
	10	1.95[f]	2.98[f]	1.19[ef]	21.05[f]
	12	1.99[f]	3.12[g]	1.21[f]	18.11[g]
25 (light)	0	1.40[a]	2.01[a]	1.03[a]	36.48[a]
	2	1.52[b]	2.07[a]	1.06[a]	34.02[b]
	4	1.67[c]	2.17[b]	1.11[b]	31.08[c]
	6	1.85[b]	2.29[c]	1.17[c]	27.83[d]
	8	2.08[e]	2.41[d]	1.25[d]	23.90[e]
	10	2.25[f]	2.51[e]	1.30[e]	20.03[f]
	12	2.45[g]	2.61[f]	1.34[e]	16.43[g]

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

storage temperature and time. The losses after 12 weeks at 4, 25, and 45 °C were 6.86, 11.86, and 18.37 μg/g, respectively. In contrast, the concentration of 13-*cis*-α-carotene increased with increasing storage temperature and time: 0.32, 0.67, and 1.11 μg/g, respectively. The concentration change of 9-*cis*-α-carotene showed the same trend, with increases of 0.14, 0.32, and 0.59 μg/g. There were no significant changes ($p > 0.05$) in the concentration of 15-*cis*-α-carotene at 4 °C, and there was only a minor increase at 25 and 45 °C. From the above results it can be found that both the isomerization and degradation of *all-trans*-α-carotene was greater at 45 °C than at 4 or 25 °C. This result indicated that the higher storage temperature, the faster the isomerization and degradation of *all-trans*-α-carotene. It was also observed that 13-*cis*-α-carotene was more readily formed than 9-*cis*-α-carotene in the dark. Apparently this phenomenon can be attributed to activation energy difference between 9-*cis*-α-carotene and 13-*cis*-α-carotene as explained before. During illumination at 25 °C the concentration of *all-trans*-α-carotene declined by 20.05 μg/g during 12 weeks of

Table 4. Concentration Changes of *all-trans*-β-Carotene and Its Cis Isomers during Storage

temp (°C)	time (weeks)	β-carotene[a] (μg/g) 9-*cis*	13-*cis*	15-*cis*	13,15-di-*cis*	*all-trans*
4 (dark)	0	2.11[a]	6.35[a]	2.42[a]	1.10[a]	50.83[a]
	2	2.12[a]	6.41[a]	2.43[a]	1.10[a]	49.43[b]
	4	2.15[ab]	6.49[a]	2.42[a]	1.09[a]	47.82[c]
	6	2.20[bc]	6.62[b]	2.41[a]	1.08[a]	44.87[d]
	8	2.23[cd]	6.72[bc]	2.41[a]	1.08[a]	41.80[e]
	10	2.27[de]	6.80[cd]	2.41[a]	1.08[a]	39.98[f]
	12	2.31[e]	6.90[d]	2.40[a]	1.08[a]	38.76[g]
25 (dark)	0	2.11[a]	6.35[a]	2.42[a]	1.10[a]	50.83[a]
	2	2.14[a]	6.45[a]	2.44[a]	1.10[a]	48.05[b]
	4	2.21[b]	6.60[b]	2.46[ab]	1.12[a]	44.70[c]
	6	2.33[c]	6.79[c]	2.49[bc]	1.14[ab]	40.67[d]
	8	2.39[d]	6.99[d]	2.52[cd]	1.17[bc]	36.46[e]
	10	2.47[e]	7.16[e]	2.55[de]	1.18[bc]	32.65[f]
	12	2.55[f]	7.32[f]	2.57[e]	1.20[c]	30.16[g]
45 (dark)	0	2.11[a]	6.35[a]	2.42[a]	1.10[a]	50.83[a]
	2	2.14[a]	6.54[b]	2.45[ab]	1.13[ab]	47.41[b]
	4	2.28[b]	6.80[c]	2.48[bc]	1.16[bc]	43.58[c]
	6	2.43[c]	7.04[d]	2.52[c]	1.18[cd]	37.35[d]
	8	2.57[c]	7.36[e]	2.59[de]	1.21[de]	30.51[e]
	10	2.69[e]	7.63[f]	2.63[ef]	1.24[ef]	25.44[f]
	12	2.81[f]	7.85[g]	2.67[f]	1.28[f]	20.50[g]
25 (light)	0	2.11[a]	6.35[a]	2.42[a]	1.10[a]	50.83[a]
	2	2.28[b]	6.43[a]	2.46[ab]	1.13[ab]	47.69[b]
	4	2.50[c]	6.55[b]	2.51[bc]	1.16[bc]	42.59[c]
	6	2.77[d]	6.73[c]	2.56[cd]	1.21[c]	36.41[d]
	8	3.01[e]	6.95[d]	2.61[de]	1.27[de]	28.87[e]
	10	3.21[f]	7.13[e]	2.65[e]	1.32[ef]	22.66[f]
	12	3.37[g]	7.26[f]	2.77[f]	1.36[f]	17.49[g]

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

storage, but 9-*cis*-α-carotene, 13-*cis*-α-carotene, and 15-*cis*-α-carotene increased by 1.05, 1.60, and 0.31 μg/g, respectively. With the exception of 13-*cis*-α-carotene, the amounts of both 9-*cis*-α-carotene and 15-*cis*-α-carotene formed under light were higher than those in the dark. This result implied that the 9-cis type isomer of α-carotene was favored during illumination. This is probably because during illumination both heat and light energies can be provided, and the high activation energy of 9-*cis*-α-carotene can thus be more susceptible to formation than the low activation energy of 13-*cis*-α-carotene. In some other studies Chandler and Schwartz (1987) observed that the 9-cis type isomer of β-carotene was favored during illumination, and Pesek and Warthesen (1990) also found that the 9-cis isomer of β-carotene was formed in larger amount under light storage. The degradation rate constants (day^{-1}) of the total amount of all-trans and cis forms of α-carotene during storage under light at 25 °C and in the dark at 4, 25, and 45 °C for 12 weeks were 0.049, 0.014, 0.026, and 0.043, respectively, and all of the degradations fit the first-order model because a linear correlation was observed for the plot of the logarithm of the total α-carotene (all-trans and cis forms) concentration versus time (Table 2).

Stability of *all-trans*-β-Carotene and Its Cis Isomers in Caroteniod Powder during Storage. Table 4 shows the changes in the concentration of *all-trans*-β-carotene and its cis isomers in carotenoid powder during storage. In the samples stored in the dark the loss of *all-trans*-β-carotene increased with both increasing storage temperature and time. The losses after 12 weeks at 4, 25, and 45 °C were 12.07, 20.67, and 30.33 μg/g, respectively. In contrast, the concentration of 13-*cis*-β-carotene increased with increasing storage temperature and time: 0.55, 0.97, and 1.50 μg/g, respectively. The concentration change of 9-*cis*-β-carotene showed the same trend, with increases of 0.20, 0.44, and 0.70 μg/g. There were no significant changes ($p > 0.05$) in the concentrations of both 15-*cis*-β-carotene and 13,15-di-*cis*-β-carotene at 4 °C, and there was only a minor increase at 25 and 45 °C. The formation of 13,-15-di-*cis*-β-carotene may be due to conversion of 13-*cis*-β-carotene or 15-*cis*-β-carotene (Chen et al., 1994, 1995). It has been reported that the di-cis isomer of *all-trans*-β-carotene can be formed only under drastic treatments such as canning, illumination, or storage under high temperature (Chen et al., 1994, 1995). The formation of 9-*cis*-β-carotene may be due to conversion of 13-*cis*-β-carotene or 15-*cis*-β-carotene through *all-trans*-β-carotene (Pesek and Warthesen, 1990; Pesek et al., 1990). Likewise, the formation of 13-*cis*-β-carotene may be due to conversion of 9-*cis*-β-carotene or 15-*cis*-β-carotene through *all-trans*-β-carotene. It has been well established that each mono-cis isomer can be converted to other forms of cis isomers only after it changes to the all-trans form (Chandler and Schwartz, 1987; Pesek and Warthesen, 1990; Pesek et al., 1990). From the above results it can be concluded that the higher temperature, the more susceptible to formation of mono-cis and di-cis isomers of β-carotene. Also, 13-*cis*-β-carotene was formed at a higher amount than 9-*cis*-β-carotene in the dark. This phenomenon was also observed for dark storage of *all-trans*-lutein and *all-trans*-α-carotene. During illumination at 25 °C the concentration of *all-trans*-β-carotene declined by 33.34 μg/g during 12 weeks of storage, and 9-*cis*-β-carotene, 13-*cis*-β-carotene, 15-*cis*-β-carotene, and 13,15-di-*cis*-β-carotene increased by 1.26, 0.91, 0.35, and 0.26 μg/g, respectively. It was also found that 9-*cis*-β-carotene was more plentiful than 13-*cis*-β-carotene, which can be attributed to activation energy difference as explained before. Interestingly, the amount of 13-*cis*-β-carotene formed under light storage was found to be lower than that in the dark. This result implied that illumination can facilitate the degradation of 13-*cis*-β-carotene. The degradation rate constants (day^{-1}) of the total amount of all-trans and cis forms of β-carotene during storage under light at 25 °C and in the dark at 4, 25, and 45 °C for 12 weeks were found to be 0.058, 0.018, 0.031, and 0.050, respectively, and all of the degradations fit a first-order model because a linear correlation was observed for the plot of the logarithm of the total β-carotene (all-trans and cis forms) concentration versus time. It has been well established that both degradation and isomerization of *all-trans*-β-carotene can proceed simultaneously during illumination, and the dominant reaction depends upon temperature, light intensity, and the presence of catalyst (Pesek and Warthesen, 1990; Pesek et al., 1990). Pesek and Warthesen (1990) reported that higher amounts of both 9-*cis*-β-carotene and 13-*cis*-β-carotene were formed during illumination of *all-trans*-β-carotene solution. However, in this study less 9-*cis*-β-carotene and 13-*cis*-β-carotene was formed, mainly because dry powder was used as illumination sample and the stability of *all-trans*-β-carotene can thus be greatly enhanced.

By comparison of the results shown above, it can be concluded that the degradation of *all-trans*-β-carotene was greater than that of *all-trans*-α-carotene and *all-trans*-lutein, probably because the former possesses a longer conjugated carbon−carbon double bond, which is more susceptible to temperature and illumination

Table 5. Changes in Hunter *L*, *a*, and *b* Values of Spray-Dried Carotenoid Powder during Storage[a]

storage time (weeks)	storage temp 4 °C (dark)	25 °C (dark)	45 °C (dark)	25 °C (light)
	Hunter *L* Value			
0	90.1^{a}	90.1^{a}	90.1^{a}	90.1^{a}
2	89.8^{ab,A}	89.7^{a,A}	89.7^{a,A}	89.4^{b,A}
4	89.5^{ab,A}	89.3^{b,A}	89.3^{b,A}	89.3^{b,A}
6	89.3^{c,A}	89.1^{b,AB}	88.5^{c,C}	88.8^{c,BC}
8	89.1^{cd,A}	88.9^{b,A}	88.4^{c,B}	88.2^{d,C}
10	88.8^{de,A}	88.4^{cd,B}	87.8^{d,C}	86.8^{e,B}
12	88.6^{e,A}	88.1^{d,B}	87.4^{e,C}	86.5^{e,B}
	Hunter *a* Value			
0	0.2^{a,A}	0.2^{a,A}	0.2^{a,A}	0.2^{a,A}
2	0.2 ^{a,A}	0.2^{a,A}	0.2^{a,A}	0.1^{a,A}
4	0.1 ^{a,A}	0.1^{a,A}	0.0^{a,A}	0.1^{a,A}
6	0.2 ^{a,A}	0.2^{a,A}	0.1^{a,A}	0.0^{a,A}
8	0.0 ^{a,A}	0.1^{a,A}	0.1^{a,A}	0.1^{a,A}
10	0.1 ^{a,A}	0.1^{a,A}	0.1^{a,A}	0.0^{a,A}
12	0.1 ^{a,A}	0.1^{a,A}	0.1^{a,A}	0.1^{a,A}
	Hunter *b* Value			
0	27.3^{a}	27.3^{a}	27.3^{a}	27.3^{a}
2	27.2^{a,A}	26.9^{b,AB}	26.7^{b,B}	26.6^{b,B}
4	26.9^{a,A}	26.2^{c,B}	26.4^{b,B}	25.5^{c,C}
6	26.3^{b,A}	25.8^{c,B}	24.9^{c,C}	24.4^{d,D}
8	25.5^{c,A}	24.9^{d,B}	24.1^{cd,C}	23.9^{e,C}
10	25.3^{c,A}	23.5^{e,B}	23.8^{d,B}	23.0^{f,C}
12	24.6^{d,A}	23.1^{f,B}	22.0^{e,C}	20.5^{g,D}

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

loss. The lesser loss of *all-trans*-lutein is probably due to the formation of a lutein−gelatin complex during spray-drying (Bryant et al., 1992).

Color Stability of Carotenoid Powder during Storage. Table 5 shows the Hunter *L*, *a*, and *b* value changes in carotenoid powder during storage at various temperatures. The Hunter *L* value was found to decrease with increasing storage time. After storage under light at 25 °C and in the dark at 4, 25, and 45 °C for 12 weeks, the *L* value decreased by 3.5, 1.5, 2.0, and 2.7, respectively. This result implied that the brightness of carotenoid powder decreased with increasing storage time. The Hunter *b* value showed the same trend with decreases of 6.8, 2.7, 4.2, and 5.3, indicating that the yellow color of powder also decreased with increasing storage time. In contrast, insignificant ($p > 0.05$) change was observed for the Hunter *a* value, implying that red is not a major contributing factor to the color of carotenoid powder. From the above results it can be found that light storage can be more destructive to the color of carotenoid powder than dark storage. The decrease of yellow color of carotenoid powder during storage is probably due to degradation of *all-trans*-β-carotene and formation of its cis isomers. It has been well established that the formation of cis carotenoids can decrease color intensity (Khachik et al., 1986; Chen et al., 1995). A high correlation (r^2) was observed between concentration change of carotenoids (all-trans and cis forms of α-carotene and β-carotene) and Hunter *b* value, which amounted to 0.97, 0.97, 0.96, and 0.95 for dark storage at 4, 25, and 45 °C and light storage at 25 °C, respectively. To better understand the overall color change of carotenoid powder during storage, the ΔE value must be investigated. Figure 2 shows the ΔE value changes of carotenoid powder during storage. With illumination at 25 °C and increasing storage temperature in the dark, the ΔE value shows greater

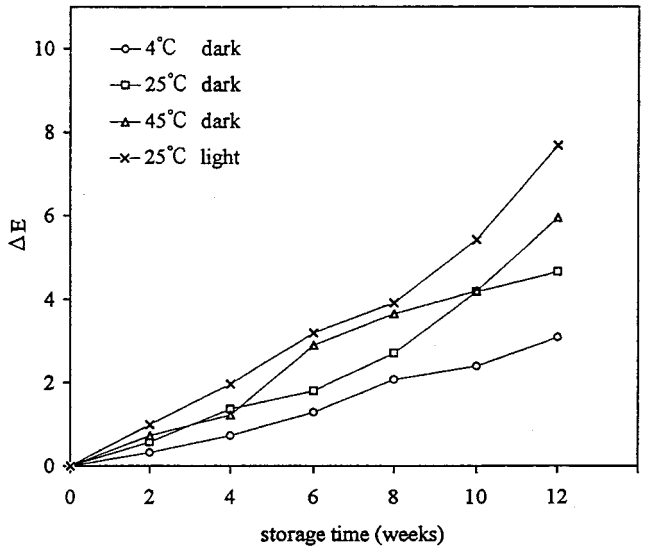

Figure 2. Changes in ΔE values of spray-dried carotenoid powder during storage.

change, implying that the color of carotenoid powder faded to a greater extent. This result also confirmed the previous finding that light storage can be more destructive to the overall color of carotenoid powder.

In conclusion, the most appropriate condition for processing carotenoid powder by spray-drying consists of 15% solid content of feed material, with inlet air temperature of 135−145 °C and outlet air temperature of 90−100 °C. The degradations of all-trans and cis forms of lutein, α-carotene, and β-carotene fit the first-order model. The 13-cis type isomers of carotenoids dominate during dark storage, while the 9-cis type is favored during light storage. For color change of carotenoid powder during storage, both the Hunter *L* and *b* values show decreases, whereas only insignificant change of the Hunter *a* value is observed.

Finally, we have to point out here that the conditions used for processing carotenoid powder in this study are only a preliminary step for possible commercial production in the future. Also, the problems of carotenoid losses during storage need to be overcome before the commercial viability can be properly assessed. It is quite possible that the stability of caroteniod powder can be greatly enhanced by employing appropriate packaging methods and storage conditions. For instance, the shelf life of carotenoid powder may be substantially increased by vacuum packaging in foil laminated sachets and storage below 0 °C. Thus, further research is needed to evaluate the effects of various processing conditions on the commercial production of carotenoid powder. In addition, the possibility of increasing the stability of carotenoid powder by using various packaging techniques has to be studied.

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