Changes of Carotenoids, Color, and Vitamin A Contents during Processing of Carrot Juice

B. H. Chen,* H. Y. Peng, and H. E. Chen

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

The effects of various processing methods on carotenoid, color, and vitamin A content changes in carrot juice were studied. Results showed that canning (121 °C, 30 min) resulted in the highest destruction of carotenoids, followed by HTST heating at 120 °C for 30 s, 110 °C for 30 s, acidification plus 105 °C heating for 25 s, and acidification. 13-*cis*-β-Carotene was formed in largest amount during heating, followed by 13-*cis*-lutein and 15-*cis*-α-carotene. The formation of 13,15-di-*cis*-β-carotene during canning was due mainly to conversion of 13-*cis*-β-carotene. Carrot juice color turned from orange to yellow with intensive treatment. The vitamin A content decreased along with increasing temperature and heating time.

Keywords: *Carotenoid; color; vitamin A; carrot juice; processing*

INTRODUCTION

In recent years demand for β-carotene has been increasing steadily because of its possible roles in the treatment of human diseases such as skin cancer (Mathews-Roth, 1985; Krinsky, 1989; Ziegler, 1989). However, the result of a recent study conducted in Finland showed that supplementation of β-carotene did not reduce the incidence of lung cancer in male smokers (Blumberg and Block, 1994; Nicol et al., 1994). Despite this controversy, β-carotene is still an important biological compound because it theoretically possesses 100% vitamin A activity. Likewise, α-carotene is also important because it theoretically possesses 50% vitamin A activity. In addition, both α- and β-carotene are present as positional isomers in foods such as carrots (Bushway, 1986; Chandler and Schwartz, 1987; Heinonen, 1990; Chen et al., 1993).

Carrots are one of the principal vegetable crops grown in Taiwan. A significant portion of carrots is discarded every year due to quality defects. However, these carrots are good sources of carotenoids and can be used in carrot beverage products such as carrot juice (Saldana et al., 1976; Sims et al., 1993). It has been reported that β-carotene constitutes a large portion (60–80%) of carotenoids in carrots, followed by α-carotene (10–40%), lutein (1–5%), and the other minor carotenoids (0.1–1.0%) (Baloch et al., 1977; Seifert and Buttery, 1978; Bushway and Wilson, 1982; Munsch and Simard, 1983; Heinonen, 1990).

Although most carotenoids are naturally present in *trans* forms, there are still significant amounts of *cis* forms of carotenoids present in vegetables (Khachik et al., 1986; Chen and Chen, 1993). The presence of these *cis* carotenoids may be due to extraction or chromatography (Khachik et al., 1986). In addition, it has been reported that processing methods such as blanching, dehydration, and canning can result in degradation or formation of *cis* isomers of carotenoids (Borchgrevink and Charley, 1966; Lee and Ammerman, 1974; Teixeira Neto et al., 1981; Bushway and Wilson, 1982; Saguy et al., 1985; Chen and Chen, 1994). Borchgrevink and Charley (1966) reported that cooking carrots in a saucepan under pressure for 50 s can result in higher loss of α- and β-carotene than that in saucepan without pressure for 19 min. This result indicated that cooking carrots under pressure can have a drastically destructive effect on carotenes. Dietz and Gould (1986) studied the effect of processing on β-carotene content of tomato juice and found that canning resulted in a higher loss of β-carotene than pasteurization. Quackenbush (1987) studied the effect of canning on β-carotene stability in carrots and found that 13-*cis*-β-carotene was formed in greater amount than 9-*cis*-β-carotene. Pesek and Warthesen (1987) demonstrated that the degradation of β-carotene in vegetable juice during illumination fits the first-order reaction.

Color is an important quality attribute of foods. As carrots are low-acid (pH 5.5–6.5) foods, the sterilization of carrot juice under high temperature is often required. However, this treatment can result in great loss of color (Stephens et al., 1971). Munsch and Simard (1983) demonstrated that the color change of carrot juice during processing correlated well to carotenoid content. To minimize loss of color and carotenoid content, the raw carrot juice is often acidified before processing so that the sterilization temperature can be lowered. Very few reports dealt with the effects of various processing methods on color and carotenoid stability in carrot juice. The purposes of this study were (1) to use appropriate mobile phase and sample solvent to separate carotenoid and its *cis* isomers in carrot juice and (2) to determine the effect of various processing methods on changes of color, carotenoids, and vitamin A contents in carrot juice.

MATERIALS AND METHODS

Materials. Fresh carrots (*Daucus carota* L. var. Sativa DC) were purchased from a local market, and a total of approximately 30 kg of carrots was obtained.

all-trans-α-Carotene, *all-trans*-β-carotene, and *all-trans*-lutein (75% purity) standards were purchased from Sigma (St. Louis, MO). Each standard was found to contain a trace amount of *cis* isomers by HPLC analysis and was used without further purification. All HPLC grade solvents such as methanol and methylene chloride were from Merck (Darmstadt, Germany). Solvents used for extraction of pigments such as hexane, acetone, toluene, and absolute alcohol were of analytical grade and were also from Merck. The HPLC grade solvents

* Author to whom correspondence should be addressed.

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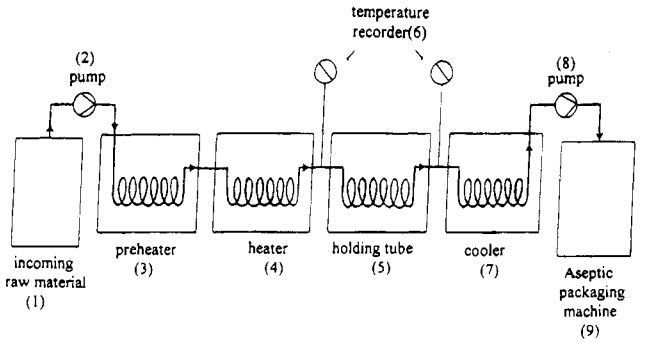

Figure 1. Carrot juice laboratory pasteurization system.

were degassed under vacuum and filtered through a 0.2-μm membrane filter prior to use.

Instrumentation. The HPLC instrument consisted of a Jasco PU-980 pump (Tokyo, Japan) with a Shimadzu SPD-M6A photodiode array detector (Tokyo, Japan) and a SIC Chromatocoder 12 integrator (Tokyo, Japan). Data were analyzed by an Axxiom 727 and dual-channel chromatography data system (Axxiom Chromatography Inc., Calabasas, CA). A Vydac 201TP54 column (250 × 4.6 mm i.d.) packed with 5-μm particle (Hesperia, CA) was used.

Speedy autoclave HL-340 was from HLMC Co. (Taipei, Taiwan). A pH meter SP-T01 was from Suntex Co. (Urdorf, Switzerland), and a color difference meter ND 1001 DP was from Japan Electric Co. (Tokyo, Japan).

Processing of Carrots. Carrots obtained from a local market were washed with 20 L of water, containing 5 ppm chlorine to reduce total bacteria count, and then rinsed with running water before peeling. The peeled carrots were cut into small pieces and ground into juice with a grinder. A total of approximately 15 L of carrot juice was obtained and divided into five portions of 3 L each. Four treatments were conducted as follows: (I) Three liters of juice was acidified to pH 4.0 with citric acid and heated at 105 °C for 30 s using a laboratory pasteurization system shown in Figure 1, which consisted of nine sections. The main function of each section is briefly described: (1) a stainless steel tank was used to receive carrot juice; (2) raw carrot juice was pumped to a preheater to start heating; (3) juice was preheated to a temperature of 70 °C; (4) preheated juice was transported to a heater to continue heating until the temperature reached 105 °C; (5) heated juice was held in the holding tube with the temperature maintained at 105 °C for 30 s; (6) a thermocouple-connected temperature recorder was installed on the exit of heater and holding tube to monitor juice temperature; (7) heated juice was cooled to 95 °C with water; (8) cooled juice was pumped and collected on an aseptic operation stand for packaging; (9) cooled juice was filled into aluminum foil bags and cooled to room temperature with ice water. (II) Three liters of juice (pH 6.1) was heated at 110 °C for 30 s using a UHT/HTST pasteurization system shown in Figure 2, which consisted of 12 sections. The main function of each section is described: (1) a stainless steel tank that can accommodate up to 8 L of juice was used to receive raw carrot juices (the temperature of the carrot juice was maintained below 30 °C); (2) carrot juice flow rate was maintained at 160 mL/min by a metering pump; (3) the flow rate of the carrot juice, which was between 0 and 500 mL/min, was shown on a flow meter; (4) the flow meter can be connected to a computer so that the average flow in a certain time interval can be shown; (5) juice was preheated to 70 °C; (6) juice was continuously heated until the temperature reached 110 °C; (7) juice was held in the holding tube with the temperature maintained at 110 °C for 30 s; (8) a thermocouple was placed on the exit of each section of the preheater, heater, and holding tube to monitor temperature change; (9) the cooling tank was divided into three sections, with the first two sections using running water and the last section using cyclable ice water, and product temperature was controlled between 0 and 30 °C; (10) a pressure-controlled needle valve was used to control pressure in the tube so that sample can be maintained in a liquid state; (11) an aseptic surge tank was used to create an aseptic environment so that the pasteurized product can be stored properly for packaging; (12) an aseptic packaging machine was used to flush nitrogen gas into the container, which was then sealed. (III) Three liters of juice was also heated in a UHT/HTST pasteurization system shown in Figure 2. The processing procedure was the same as in treatment II with the exception that the temperature used was 120 °C. (IV) Three liters of juice was preheated to 71 °C and then filled into eight cans (15.2 × 5.8 cm) and processed in a still retort with temperature at 121 °C for 30 min.

Extraction of Carotenoids. A modified AOAC method (Chen and Chen, 1992) used for determination of carotenes and xanthophylls in dried plant materials and mixed feeds was used to extract carotenoids from carrot juice.

Four milliliters each of fresh and processed carrot juice was mixed with 30 mL of extractant (hexane–acetone–absolute alcohol–toluene = 10:7:6:7 v/v/v/v) and 6 mL of 40% methanolic KOH in a 100-mL volumetric flask. After shaking for 1 min, the mixture was left standing in the dark for 16 h for saponification. Thirty milliliters of hexane was added to the flask and swirled gently for 1 min and then diluted to volume with 10% Na_2SO_4. The mixture was left standing in the dark for 1 h until two phases were separated. The upper phase containing carotenoids was evaporated to dryness and dis-

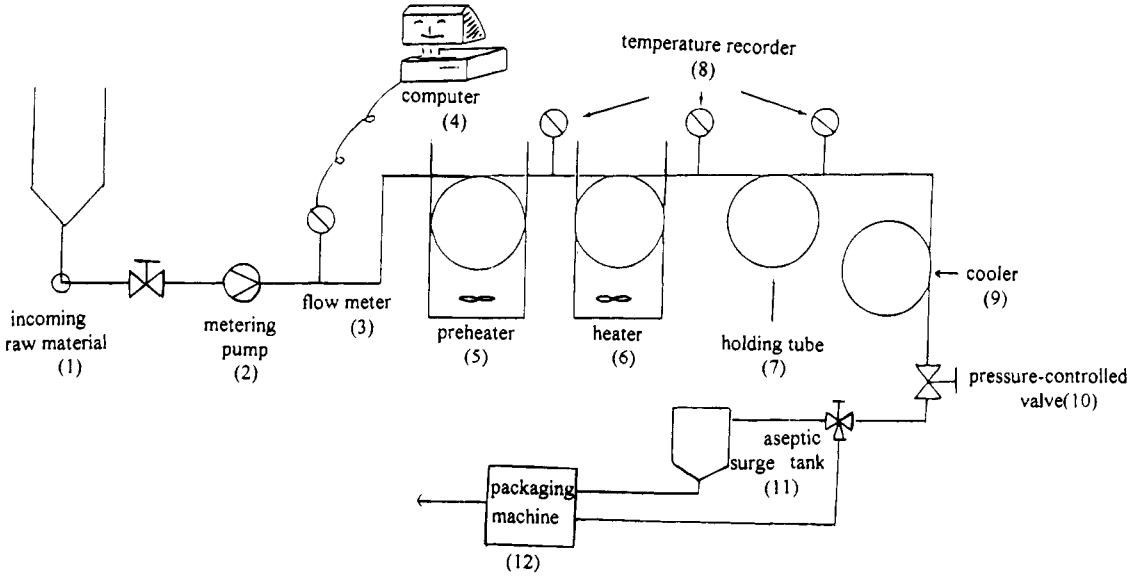

Figure 2. UHT/HTST pasteurization system.

Table 1. Identification Data for α-Carotene and Its *cis* Isomers by HPLC

pigment	visible spectra[b] in-line[a]	visible spectra[b] max reported[b]	visible spectra[b] solvent	probable purity[f] (%)	Q ratio found	Q ratio reported
15-*cis*-α-carotene	(417),[e] 439, 465	418, 437, 466	methanol	0.9827–0.9830	2.0	2.5[d]
α-carotene	(421), 444, 472	420, 445, 474	petroleum ether	0.9999–0.9999	11.7	<12[c]
13-*cis*-α-carotene	(418), 439, 464	441, 466	methanol–chloroform (94:6)	0.9977–0.9989	3.8	3.7[c]
9-*cis*-α-carotene	421, 442, 468	442, 468	methanol–chloroform (94:6)	0.9709–0.9728	8.3	8.6[c]

[a] Eluant used as solvent (methanol/methylene chloride = 99:1 v/v). [b] Reported values of visible spectra are from three references: Quackenbush (1987), Chen and Chen (1993), and Chen and Chen (1994). [c] Reported values of *Q* ratio are from a reference by Quackenbush (1987). [d] Reported values of *Q* ratio are from a reference by Chen and Chen (1994). [e] Values in parentheses represent shoulder on absorption curves. [f] Determined by photodiode array detector.

Table 2. Identification Data for β-Carotene and Its *cis* Isomers by HPLC

pigment	visible spectra in-line[a]	visible spectra max reported[b]	visible spectra solvent	probable purity[g] (%)	Q ratio found	Q ratio reported
13,15-di-*cis*-β-carotene	(413),[h] 437, (458)	436	hexane	0.9992–0.9993	6.2	7.0[f]
15-*cis*-β-carotene	(425), 446, 470	425, 446, 474	acetone–hexane (3:97)	0.9998–0.9998	1.5	1.9[c]
β-carotene	(425), 450, 475	(423), 444, 470	petroleum ether	0.9998–0.9999	11.2	12.7[d]
9-*cis*-β-carotene	(423), 444, 470	425, 446, 473	acetone–hexane (3:97)	0.9934–0.9963	8.3	8.5[e]
13-*cis*-β-carotene	425, 446, 470	423, 444, 469	acetone–hexane (3:97)	0.9998–0.9998	2.0	2.3[e]

[a] Reported values of visible spectra are from three references: Tsukida et al. (1982), Chandler and Schwartz (1988), and Chen and Chen (1993). [b] Eluant used as solvent (methanol–methylene chloride = 99:1 v/v). [c] Reported values of *Q* ratio are from a reference by Tsukida et al. (1982). [d] Reported values of *Q* ratio are from a reference by Saleh and Tan (1991). [e] Reported values of *Q* ratio are from a reference by Quackenbush (1987). [f] Reported values of *Q* ratio are from a reference by Tsukida et al. (1982). [g] Determined by photodiode array detector. [h] Values in parentheses represent shoulder on absorption curves.

Table 3. Identification Data for Lutein and Its *cis* Isomers by HPLC

pigment	visible spectra[b] in-line[a]	visible spectra[b] max reported	visible spectra[b] solvent	probable purity[f] (%)	Q ratio found	Q ratio reported
9-*cis*-lutein	(420),[e] 442, 467	440, 467	methanol (94:6)	0.9435–0.9824	8.6	8.6[c]
lutein	(424), 444, 470	424, 444, 472	acetonitrile–methanol–methylene chloride (80:18:2)	0.9999–0.9998	11.5	<12[c]
13-*cis*-lutein	(419), 439, 465	437, 463	acetonitrile–methanol–methylene chloride (80:18:2)	0.9653–0.9997	2.6	2.5[d]

[a] Eluant used as solvent (methanol–methylene chloride = 99:1 v/v). [b] Reported values of visible spectra are from two references: Quackenbush (1987) and Saleh and Tan (1991). [c] Reported values of *Q* ratio are from a reference by Quackenbush (1987). [d] Reported values of *Q* ratio are from a reference by Saleh and Tan (1991). [e] Values in parentheses represent shoulder on absorption curves. [f] Determined by photodiode array detector.

solved in 10 mL of methanol–methylene chloride (45:55 v/v). The solution was filtered through a 0.2-μm membrane filter and stored at −30 °C for HPLC analysis.

HPLC Analysis of Carotenoids. 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 ODS column (Vydac 201TP54) were used to separate carotenoids and their *cis* isomers in carrot juice. The flow rate was 1.0 mL/min with sensitivity at 0.16 AUFS and detection wavelength at 450 nm. Injection volume was 20 μL. Due to the low concentration of *cis* isomers of carotenoids present in carrot juice, a mixture of α-carotene, β-carotene, and lutein standards was heated at 121 °C for 30 min to obtain a higher concentration of *cis* isomers. Also, each peak of these isomers was scanned, and 10 injections of eluates from *cis* peaks were collected and injected into HPLC for cochromatography so that a positive identification can be confirmed. After cochromatography, the purity of each peak was checked by collecting spectra from the upslope, apex, and downslope portions of the peak, and then the spectra were normalized and overlaid to see if there was any difference in curve shape. As no difference in curve shape was observed for most peaks, the purity of these peaks was assessed to be close to 100% (Tables 1–3). The purity number of each peak was determined automatically based on an equation described in the appendix of the operation manual of a Shimadzu SPD-M6A photodiode array detector. As peak purity was determined after cochromatography, "probable purity" is a more appropriate term for each peak in this study. Identification was also made by comparing absorption spectra and *Q* ratios with reference values reported in the literature (Tsukida et al., 1982; Quackenbush, 1987; Saleh and Tan, 1991; Chen and Chen, 1993). The spectra characteristics of *cis* carotenoids were described in a previous study (Chen and Chen, 1994; Chen et al., 1994).

Quantification of Carotenoids and Vitamin A. Each peak was quantified using absolute calibration curves. The calibration curves for α-carotene, β-carotene, and lutein were prepared by area measurement of reference compounds at eight concentrations ranging from 5 to 120 μg/mL. The calibration curves of each compound gave good linearity (r^2 = 0.99). As no *cis* standards are commercially available, *cis* isomers of α-carotene, β-carotene, and lutein were calculated as α-carotene, β-carotene, and lutein equivalents, respectively. Duplicate analyses were conducted and mean values determined. Vitamin A was quantified using the following formula:

1 retinol equivalent (RE) = 1 mg of retinol
= 6 mg of β-carotene
= 12 mg of other provitamin A carotenoids

Color Determination of Carrot Juice. A color difference meter was used to measure *L*, *a*, and *b* of carrot juice, of which *a* indicates red, *b* indicates yellow, and *L* indicates lightness. Hue can be expressed as *a*/*b*, while chroma is expressed as $(a^2 + b^2)^{1/2}$.

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

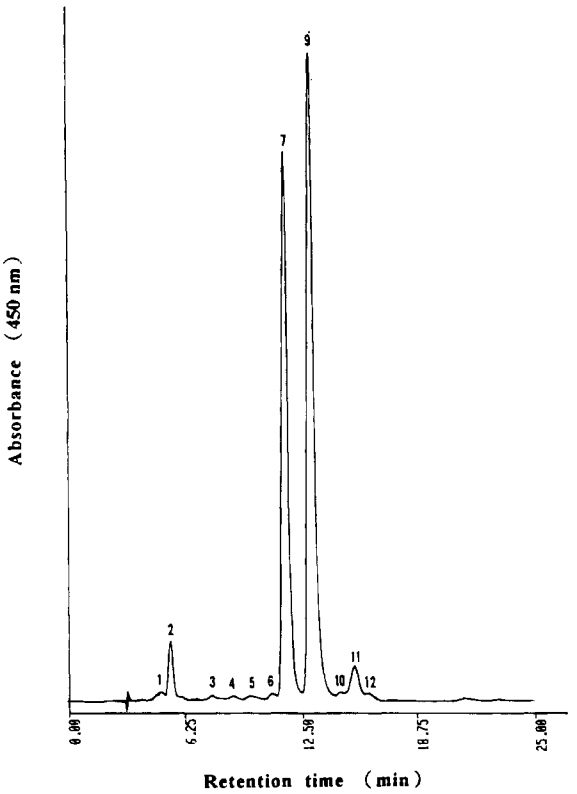

Figure 3. HPLC chromatogram of carotenoids from fresh carrot juice by employing a mobile phase of methanol–methylene chloride (99:1 v/v). Sample solvent: methanol–methylene chloride (45:55 v/v). Chromatographic conditions are described in the text. Peaks: 1, 13-*cis*-lutein; 2, lutein; 3, 9-*cis*-lutein; 4, 9-*cis*-α-carotene; 5, 13-*cis*-α-carotene; 6, 13,15-di-*cis*-β-carotene; 7, α-carotene; 9, β-carotene; 10, 9-*cis*-β-carotene; 11, 13-*cis*-β-carotene; 12, 15-*cis*-β-carotene.

RESULTS AND DISCUSSION

Separation of Carotenoids and Their *cis* Isomers in Carrot Juice. The chromatographic conditions used for the separation of carotenoids and their *cis* isomers in carrot juice were based on a study by Chen and Chen (1994), who employed a mobile phase of methanol–methylene chloride (99:1 v/v) with 100% hexane as sample solvent and a polymeric Vydac 201TP54 column to resolve four *cis* isomers of β-carotene and three *cis* isomers of α-carotene. However, with the same conditions the carotenoids and their *cis* isomers in carrot juice were not adequately resolved. This may be explained as follows: (1) It has been reported that column to column variability for polymeric columns is greater than for monomeric columns (Epler et al., 1992); thus, the separation efficiency of carotenoid isomers can be affected even for polymeric columns from the same batch. (2) Carotenoids present in carrot juice are more complicated. In addition to *cis* isomers of α- and β-carotene, carrot juice also contains lutein and its *cis* isomers. (3) Sample solvent hexane has a lower solubility for lutein and its *cis* isomers, which may be prevented from eluting the column. According to a paper by Chen and Chen (1994), the selection of an appropriate sample solvent is very important because it can change mobile phase polarity and thus effect separation efficiency of carotenoid isomers. By using the same mobile phase and changing sample solvent as methanol–methylene chloride (45:55 v/v), it was found that all *cis* isomers of carotenoids in carrot juice were resolved. Figure 3 shows the HPLC chromatogram of carotenoids and their *cis* isomers in raw carrot juice. Twelve peaks were resolved and identified as 13-*cis*-lutein, lutein, 9-*cis*-lutein, 9-*cis*-α-carotene, 13-*cis*-α-carotene, 15-*cis*-α-carotene, 13,15-di-*cis*-β-carotene, α-carotene, β-carotene, 9-*cis*-β-carotene, 13-*cis*-β-carotene, and 15-*cis*-β-carotene on the basis of spectra characteristics and *Q* ratios shown in Tables 1–3. The simultaneous separation of *cis* isomers of α-carotene, β-carotene, and lutein has been difficult. Compared to other work, the separation in this study is good, as shown by the capacity factor (k') of each peak, which was ideally controlled between 1 and 10. However, the resolution for some *cis* isomers is not adequate.

Table 4. Concentration Changes (Micrograms per Milliliter) of α-Carotene and Its *cis* Isomers under Various Processing Treatments

	treatment[a]					
compd	control	acidified	I[b]	II[c]	III[d]	IV[e]
α-carotene	27.6[a]	26.5[a]	25.4[a]	15.0[b]	12.7[c]	10.9[d]
9-*cis*-α-carotene	0.2[a]	0.2[a]	0.2[a]	0.4[b]	0.5[c]	0.5[c]
13-*cis*-α-carotene	0.2[a]	0.3[a]	0.4[b]	0.6[c]	0.7[e]	0.5[d]
15-*cis*-α-carotene	0.0[a]	0.0[a]	0.0[a]	1.5[b]	2.1[d]	1.3[c]

[a] Each value of means bearing different letters within the same row is significantly different ($p < 0.05$). [b] Carrot juice acidified to pH 4.0 and heated at 105 °C for 30 s. [c] Carrot juice (pH 6.1) heated at 110 °C for 30 s. [d] Carrot juice (pH 6.1) heated at 120 °C for 30 s. [e] Carrot juice (pH 6.1) heated at 121 °C for 30 min for canning.

As most carotenoids are naturally present in *trans* forms, the presence of *cis* carotenoids in raw carrot juice is probably due to extraction. It has been reported that selection of an appropriate extracting solvent is very important because of the possibility of inducing isomer formation (Khachik et al., 1986; Pesek et al., 1990). For instance, chlorinated solvent was found to promote isomerization of β-carotene (Pesek et al., 1990). Also, the isomerization of β-carotene was found to be greater in nonpolar solvents than in polar solvents (Zechmeister, 1944). In contrast, Khachik et al. (1986) used a combination of light petroleum ether–acetone and diethyl ether–methanol as extracting solvent and found that no significant change in either qualitative or quantitative distribution of carotenoids was observed. On the basis of the results of these studies, the extracting solvents used in this study may also cause isomer formation. However, this needs to be further investigated.

β-Carotene was present in largest amount (62.5 μg/mL) in carrot juice, followed by α-carotene (27.6 μg/mL) and lutein (6.0 μg/mL). This result is similar to that reported by Kim and Gerber (1988), who found that β-carotene was present in carrot juice at a concentration of 67.9 μg/mL and α-carotene at 43.5 μg/mL. The small difference is probably due to variability, pressing methods, and maturity of carrots (Stephens et al., 1971; Heinonen, 1990; Sims et al., 1993). For *cis* isomers of carotenoids, 13-*cis*-β-carotene was present in largest amount (3.4 μg/mL), followed by 13,15-di-*cis*-β-carotene (1.3 μg/mL), 15-*cis*-β-carotene (1.1 μg/mL), 9-*cis*-β-carotene (1.1 μg/mL), 13-*cis*-lutein (0.6 μg/mL), 9-*cis*-lutein (0.4 μg/mL), 13-*cis*-α-carotene (0.2 μg/mL), and 9-*cis*-α-carotene (0.2 μg/mL) (Tables 4–6).

Carotenoid Stability during Processing of Carrot Juice. All of the carotenoids and their *cis* isomers only showed minor changes after acidification and heating at 105 °C for 25 s using the laboratory pasteurization system shown in Figure 1, indicating neither treatment resulted in significant isomerization of carotenoids. It has been reported that a high concentration of acid can result in isomerization of β-carotene (Zech-

Table 5. Concentration Changes (Micrograms per Milliliter) of β-Carotene and Its *cis* Isomers under Various Processing Treatments

	treatment[a]					
compd	control	acidified	I[b]	II[c]	III[d]	IV[e]
β-carotene	62.5[a]	61.1[a]	59.7[a]	34.4[b]	32.8[b]	28.3[c]
9-*cis*-β-carotene	1.1[a]	1.1[a]	1.2[b]	2.5[c]	3.1[d]	4.8[e]
13-*cis*-β-carotene	3.4[a]	3.5[a]	4.5[b]	8.0[c]	10.8[d]	7.7[c]
15-*cis*-β-carotene	1.1[a]	1.2[a]	1.5[b]	2.6[c]	3.3[e]	3.0[d]
13,15-di-*cis*-β-carotene	1.3[a]	1.4[a,b]	1.4[b]	1.7[c]	1.9[d]	2.8[e]

[a] Each value of means bearing different letters within the same row is significantly different ($p < 0.05$). [b] Carrot juice acidified to pH 4.0 and heated at 105 °C for 30 s. [c] Carrot juice (pH 6.1) heated at 110 °C for 30 s. [d] Carrot juice (pH 6.1) heated at 120 °C for 30 s. [e] Carrot juice (pH 6.1) heated at 121 °C for 30 min for canning.

Table 6. Concentration Changes (Micrograms per Milliliter) of Lutein and Its *cis* Isomers under Various Processing Treatments

	treatment					
compd	control	acidified	I[b]	II[c]	III[d]	IV[e]
lutein	6.0[a]	5.2[b]	4.6[c]	4.2[c]	3.2[d]	3.0[e]
9-*cis*-lutein	0.4[a]	0.4[a]	0.5[b]	0.4[a]	0.6[c]	0.6[c]
13-*cis*-lutein	0.6[a]	0.7[a]	0.8[b]	0.9[c]	1.5[d]	1.5[d]

[a] Each value of means bearing different letters within the same row is significantly different ($p < 0.05$). [b] Carrot juice acidified to pH 4.0 and heated at 105 °C for 30 s. [c] Carrot juice (pH 6.1) heated at 110 °C for 30 s. [d] Carrot juice (pH 6.1) heated at 120 °C for 30 s. [e] Carrot juice (pH 6.1) heated at 121 °C for 30 min for canning.

meister, 1944). However, in some other papers pH has been reported to have only a minor effect on β-carotene isomerization in solvents or in food systems (Schwartz and Patroni-Killam, 1985; Sian and Ishak, 1991). Apparently the effect of pH on β-carotene stability can be attributed to time of exposure to acid, concentration of acid, and the system in which β-carotene exists. In this study carrot juice was heated immediately following acidification, indicating that the short exposure time of β-carotene to acid did not result in isomerization. Also, the HTST (105 °C, 25 s) treatment has only a minor effect on β-carotene isomerization (Table 5). Similar results were observed for α-carotene and lutein under acidification of carrot juice to pH 4.0 and heating at 105 °C for 25 s (Tables 4 and 6).

Figure 4 shows the HPLC chromatogram of carrot juice (pH 6.1) heated at 110 °C for 30 s using the UHT/HTST pasteurization system shown in Figure 2. A loss of 45% was found for β-carotene. In contrast, all *cis* isomers of carotenoids increased significantly ($p < 0.05$) during heating. 13-*cis*-β-Carotene was formed in largest amount (4.6 μg/mL), followed by 15-*cis*-β-carotene (1.5 μg/mL), 9-*cis*-β-carotene (1.4 μg/mL), and 13,15-di-*cis*-β-carotene (0.4 μg/mL). This result implied that 13-*cis*-β-carotene can be more easily formed than the other *cis* isomers during heating. A similar trend was found by Pesek et al. (1990), who reported that the formation rate of 13-*cis* was greater than that of 9-*cis* during heating of β-carotene. Similar to β-carotene, a loss of 45% was found for α-carotene, and all *cis* isomers of α-carotene increased significantly ($p < 0.05$) during heating. The only difference is that 15-*cis*-α-carotene was formed in largest amount (1.5 μg/mL), followed by 13-*cis*-α-carotene (0.4 μg/mL) and 9-*cis*-α-carotene (0.2 μg/mL). Compared to 13-*cis*-β-carotene, 13-*cis*-α-carotene was formed in a lesser amount, probably because this isomer was more susceptible to heat loss. For lutein and its *cis* isomers, a loss of 30% was found for lutein, and 13-*cis*-lutein was found in greater amount than 9-*cis*-lutein.

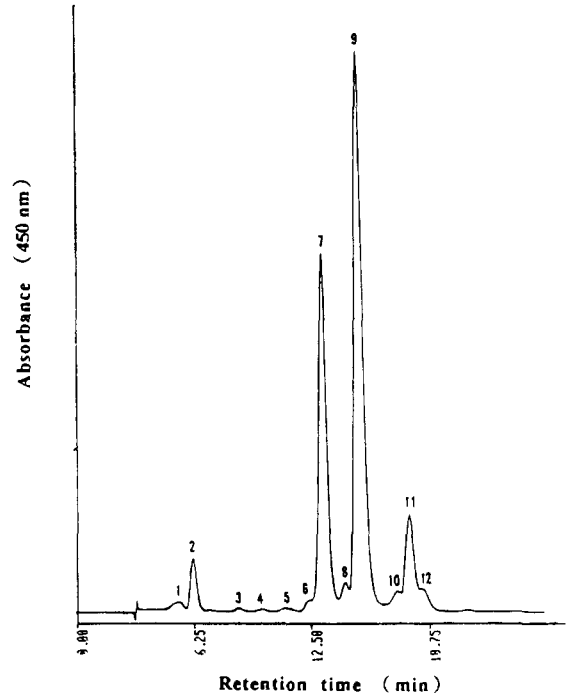

Figure 4. HPLC chromatogram of carotenoids from carrot juice after heating at 110 °C for 30 s. A mobile phase of methanol–methylene chloride (99:1 v/v) and a sample solvent of methanol–methylene chloride (45:55 v/v) were used. Chromatographic conditions are described in the text. Peaks: 1, 13-*cis*-lutein; 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.

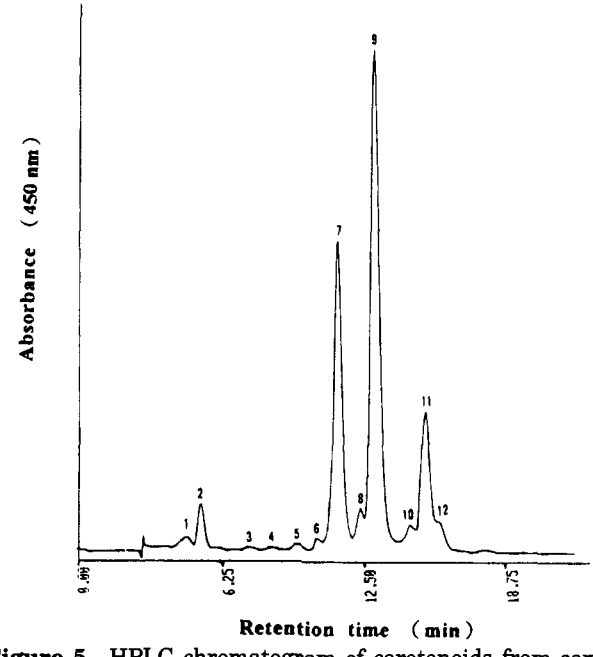

Figure 5. HPLC chromatogram of carotenoids from carrot juice after canning at 121 °C for 30 min. A mobile phase of methanol–methylene chloride (99:1 v/v) and a sample solvent of methanol–methylene chloride (45:55 v/v) were used. Chromatographic conditions are described in the text. Peaks: 1, 13-*cis*-lutein; 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.

The HPLC chromatogram of carrot juice (pH 6.1) heated at 120 °C for 30 s using the UHT/HTST pasteurization system (Figure 2) was similar to that in Figure 4 and is not shown in the text.

Table 7. Changes in Color of Carrot Juice under Various Processing Treatments

treatment	color[a,f]			chroma[f]	hue[f]
	L	a	b		
control	38.4[a]	+32.5[a]	+24.3[b]	40.6	1.3
acidified	39.9[b]	+32.1[b]	+24.7[b]	40.5	1.3
I[b]	38.0[c]	+30.1[c]	+25.1[a]	40.9	1.1
II[c]	34.4[d]	+22.0[d]	+21.4[c]	30.7	1.0
III[d]	32.5[e]	+18.5[e]	+20.9[d]	27.9	0.9
IV[e]	27.3[f]	+15.8[f]	+18.9[d]	26.6	0.7

[a] Each value of means bearing different letter within the same column is significantly different ($p < 0.05$). [b] Carrot juice acidified to pH 4.0 and heated at 105 °C for 30 s. [c] Carrot juice (pH 6.1) heated at 110 °C for 30 s. [d] Carrot juice (pH 6.1) heated at 120 °C for 30 s. [e] Carrot juice (pH 6.1) heated at 121 °C for 30 min for canning. [f] Each value is an average of duplicate sample mean.

Compared to control treatment, β-carotene concentration decreased further by 48%. 13-*cis*-β-Carotene was also formed in largest amount (7.4 μg/mL), followed by 15-*cis* (2.2 μg/mL), 9-*cis* (2.0 μg/mL), and 13,15-di-*cis* (0.6 μg/mL). The degradations of α-carotene and lutein showed the same trend as in treatment II; i.e., each decreased further by 54 and 47%, respectively. For *cis* isomers of α-carotene and lutein, 9-*cis*-, 13-*cis*-, and 15-*cis*-α-carotene and 9-*cis*- and 13-*cis*-lutein increased by 0.3, 0.5, 2.1, 0.2, and 0.9 μg/mL, respectively.

Figure 5 shows the HPLC chromatogram of carrot juice (pH 6.1) heated at 121 °C for 30 min for canning using a speedy autoclave. Compared to the other treatments, β-carotene increased steadily with the exception that both 13-*cis* and 15-*cis* decreased. As the amount of 15-*cis*-β-carotene decreased only slightly, it is reasonable to assume that the formation of 13,15-di-*cis*-β-carotene was due mainly to conversion of 13-*cis*-β-carotene. In a previous study Chen et al. (1994) demonstrated that 13-*cis*-β-carotene can be further converted to 13,15-di-*cis*-β-carotene during iodine-catalyzed photoisomerization. Obviously, 13,15-di-*cis*-β-carotene can only be formed under drastic treatments such as canning or illumination. However, no 13,15-di-*cis*-β-carotene was observed when β-carotene crystal was heated at 150 °C for 30 min (Chen et al., 1994). This is probably because the slightly acidic nature of carrot juice (pH 6.1) can catalyze the formation of 13,15-di-*cis*-β-carotene during canning. Compared to control treatment, α-carotene decreased further by 60% after canning. In contrast to treatment III, both 13-*cis*- and 15-*cis*-α-carotene concentrations decreased slightly while 9-*cis*-α-carotene remain unchanged. This result indicated that both 13-*cis*- and 15-*cis*-α-carotene formed during HTST heating can be further degraded during canning. For lutein and its *cis* isomers, lutein decreased by 50%, and both 9-*cis*- and 13-*cis*-lutein only showed insignificant change during canning.

Color Stability during Processing of Carrot Juice. The formation of *cis* isomers of carotenoids during heating can not only lower vitamin A activity but also reduce its color intensity (Bauernfeind, 1981). Table 7 shows the color change of carrot juice during processing. The lightness (L) of carrot juice increased from 38.4 to 39.9 after acidification and then decreased to 38.0 after 105 °C heating. It has been reported that blanching carrots with acid can increase the brightness of carrot juice and decrease the precipitation of carrot juice during processing (Stephens et al., 1971). Sims et al. (1993) also demonstrated that blanching carrots with acid can improve the color and turbidity of heated or canned juice. However, the lightness decreased from 38.4 to 34.4, 32.5, and 27.3 after 110 and 120 °C heating and canning, respectively. This result indicated that the brightness of carrot juice can be decreased greatly under drastic treatments such as canning. The yellowness (b) and redness (a) of carrot juice also decreased with increasing temperature and heating time. Both chroma and hue showed the same trend. The drastic decrease of hue during canning of carrot juice was accompanied by a color change from orange to yellow. Munsch and Simard (1983) reported that carrot juice color change during processing can be correlated to carotenoid content and formation of *cis* carotenoid isomers. From Table 7 it can be clearly seen that the carotenoid content decreased along with intensive heat treatments. The formation of *cis* carotenoid isomers showed the reverse trend.

Table 8. Vitamin A Contents of α- and β-Carotene of Carrot Juice

compd	treatment					
	control	acidified	I[a]	II[b]	III[c]	IV[d]
α-carotene						
μg/mL	27.6	26.5	25.4	15.0	12.7	10.9
RE/mL[e]	2.3	2.3	2.1	1.2	1.1	0.9
β-carotene						
μg/mL	62.5	61.1	59.7	34.4	32.8	28.3
RE/mL[f]	10.4	10.2	10.0	5.7	5.5	4.7
vitamin A contents from α- and β-carotene						
RE/mL[g]	12.7	12.4	12.1	7.0	6.5	5.6

[a] Carrot juice acidified to pH 4.0 and heated at 105 °C for 30 s. [b] Carrot juice (pH 6.1) heated at 110 °C for 30 s. [c] Carrot juice (pH 6.1) heated at 120 °C for 30 s. [d] Carrot juice (pH 6.1) heated at 121 °C for 30 min for canning. [e] RE represents retinol equivalent, RE/mL = 0.083 × (μg of α-carotene). [f] RE/mL = 0.167 × (μg of β-carotene). [g] RE/mL = 0.167 × (μg of β-carotene) + 0.083 × (μg of α-carotene).

Vitamin A Content Change during Processing of Carrot Juice. Table 8 shows the change of vitamin A activity from α- and β-carotene during processing of carrot juice. A total of vitamin A content (12.7 RE/mL) was found in raw carrot juice, of which β-carotene constitutes about 80%. Similar to carotenoid change, the vitamin A content also decreased along with intensive heat treatments. Canning resulted in the highest destruction of vitamin A (55.7%), followed by 120 °C heating (48.8%), 110 °C heating (45.2%), acidification plus 105 °C heating (5.1%), and acidification (2.6%).

From the above discussions it can be concluded that canning resulted in the highest destruction of carotenoids, followed by HTST heating and acidification. 13-*cis*-β-Carotene is formed in largest amount during heating, followed by 13-*cis*-lutein and 15-*cis*-α-carotene. Canning can result in the formation of 13,15-di-*cis*-β-carotene. Both color and vitamin A contents decreased along with increasing temperature and heating time. Further research is necessary to determine carotenoid, color, and vitamin A content changes during storage of carrot juice.

LITERATURE CITED

Baloch, A. K.; Buckle, K. A.; Edwards, R. A. Separation of carrot carotenoids on hyflo super-celmagnesium oxide-calcium sulfate thin layers. *J. Chromatogr.* **1977**, *139*, 149–155.

Bauernfeind, J. C. Natural food colors. In *Carotenoids as Colorants and Vitamin A Precursors*; Bauernfeind, J. C., Ed.; Academic Press: New York, 1981; pp 1–37.

Blumberg, J.; Block, G. The α-tocopherol, β-carotene cancer prevention study in Finland. *Nutr. Rev.* **1994**, *52*, 242–245.

Borchgrevink, N. C.; Charley, H. Color of cooked carrots related to carotene content. *J. Am. Diet. Assoc.* **1966**, *49*, 116–121.

Bushway, R. J. Determination of α- and β-carotene in some raw fruits and vegetables by high-performance liquid chromatography. *J. Agric. Food Chem.* **1986**, *34*, 409–412.

Bushway, R. J.; Wilson, A. M. Determination of α- and β-carotene in fruits and vegetables by high performance liquid chromatography. *Can. Inst. Food Sci. Technol. J.* **1982**, *15*, 165–169.

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.

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.; Chuang, J. R.; Lin, J. H.; Chiu, C. P. Quantification of provitamin A compounds in Chinese vegetables by high-performance liquid chromatography. *J. Food Prot.* **1993**, *56*, 51–54.

Chen, B. H.; Chen, T. M.; Chien, J. T. Kinetic model for studying the isomerization of α- and β-carotene during heating and illumination. *J. Agric. Food Chem.* **1994**, *42*, 2391–2397.

Chen, T. M.; Chen, B. H. Optimization of mobile phases for the HPLC of cis-trans carotene isomers. *Chromatographia* **1994**, *39*, 346–354.

Dietz, J. M.; Gould, W. A. Effects of process stage and storage on retention of beta-carotene in tomato juice. *J. Food Sci.* **1986**, *51*, 837–848.

Epler, R. G.; Sander, L. C.; Ziegler, R. G.; Wise, S. A.; Craft, N. E. Evaluation of reversed-phase liquid chromatographic column for recovery and selectivity of selected carotenoids. *J. Chromatogr.* **1992**, *595*, 89–101.

Heinonen, M. I. Carotenoids and provitamin A activity of carrot (Daucus carota L.) cultivars. *J. Agric. Food Chem.* **1990**, *38*, 609–612.

Khachik, F.; Beecher, G. R.; Whittaker, N. F. Separation, identification and quantification of the major carotenoid and chlorophyll constituents in extracts of several green vegetables by liquid chromatography. *J. Agric. Food Chem.* **1986**, *34*, 603–616.

Kim, H.; Gerber, L. E. Influence of processing on quality of carrot juice. *Korean J. Food Sci. Technol.* **1988**, *20*, 683–689.

Krinsky, N. I. Carotenoids and cancer in animal model. *J. Nutr.* **1989**, *119*, 123–126.

Lee, W. G.; Ammerman, G. A. Carotene stereoisomerization in sweet potatoes as affected by rotating and still retort canning processes. *J. Food Sci.* **1974**, *39*, 1188–1190.

Mathews-Roth, M. M. Carotenoids and cancer prevention experimental and epidemiological studies. *Pure Appl. Chem.* **1985**, *51*, 649.

Munsch, M. H.; Simard, R. E. Relationships in color and carotene content of carrot juices. *Can. Inst. Food Sci. Technol. J.* **1983**, *16*, 173–178.

Nicol, M.; Maudet, M.; Savoure, N. Commented publication: about the A.T.B.C. Finland study. *Med. Nutr.* **1994**, *30*, 212–217.

Olsen, J. A. Provitamin A function of carotenoids: the conversion of β-carotene to vitamin A. *J. Nutr.* **1989**, *119*, 105–108.

Pesek, C. A.; Warthesen, J. J. Photodegradation of carotenoids in a vegetable juice system. *J. Food Sci.* **1987**, *52*, 744–746.

Pesek, C. A.; Warthesen, J. J.; Taoukis, P. S. A kinetic model for equilibration of isomeric β-carotenes. *J. Agric. Food Chem.* **1990**, *38*, 41–45.

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.

Saguy, I.; Goldman, M.; Karel, M. Prediction of β-carotene decoloration in model system under static and dynamic conditions of reduced oxygen environment. *J. Food Sci.* **1985**, *50*, 526–530.

Saldana, G.; Stephens, T. S.; Lime, B. J. Carrot beverages. *J. Food Sci.* **1976**, *41*, 1243–1244.

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 ed.; SAS Instruments: Cary, NC, 1985.

Schwartz, S. J.; Patroni-Killam, M. Detection of cis-trans carotene isomers by two-dimensional thin-layer and high-performance liquid chromatography. *J. Agric. Food Chem.* **1985**, *33*, 1160–1163.

Seifert, R. M.; Buttery, R. G. Characterization of some previously unidentified sesquiterpenes in carrot roots. *J. Agric. Food Chem.* **1978**, *26*, 161–163.

Sian, N. K.; Ishak, S. Carotenoid and anthocyanin contents of papays and pineapple: influence of blanching and predrying treatments. *Food Chem.* **1991**, *39*, 175–185.

Sims, C. A.; Balaban, M. O.; Matthews, R. F. Optimization of carrot juice color and cloud stability. *J. Food Sci.* **1993**, *58*, 1129–1131.

Stephens, T. S.; Saldana, G.; Brown, H. E.; Griffiths, F. P. Stabilization of carrot juice by dilute treatment. *J. Food Sci.* **1971**, *36*, 36.

Tee, E. S. Carotenoids and retinoids in human nutrition. *Crit. Rev. Food Sci. Nutr.* **1992**, *31*, 103–103.

Teixeira Neto, R. O.; Karel, M.; Saguy, I.; Mizrahi, S. Oxygen uptake and β-carotene decoloration in a dehydrated food. *J. Food Sci.* **1981**, *46*, 665–676.

Tsukida, K.; Saiki, K.; Takii, T.; Koyama, Y. Separation and determination of cis/trans-β-carotene by high-performance liquid chromatography. *J. Chromatogr.* **1982**, *245*, 359–364.

Zechemeister, L. Cis-trans isomerization and stereochemistry of carotenoids and diphenylpolyenes. *Chem. Rev.* **1944**, *34*, 267–322.

Ziegler, R. G. A review of epidemiologic evidence that carotenoids reduce the risk of cancer. *J. Nutr.* **1989**, *119*, 116–122.

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