

# Kinetic Model for Studying the Isomerization of $\alpha$ - and $\beta$ -Carotene during Heating and Illumination

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The thermoisomerization and iodine-catalyzed photoisomerization of *all-trans*- $\alpha$ - and *all-trans*- $\beta$ -carotene were kinetically studied using regression models. Carotene samples were heated at varied temperatures or exposed to a 20 W light for varied lengths of time. Isomerization and degradation reactions were monitored using HPLC with diode array detection. Four *cis* isomers of  $\beta$ -carotene and three *cis* isomers of  $\alpha$ -carotene were separated and detected. The degradations of both carotenes under heating at 150 °C or iodine/light treatment may fit the reversible first-order model. 9-*cis* and 13-*cis* were the major  $\beta$ -carotene isomers formed during heating, while 13,15-di-*cis* was favored during iodine-catalyzed photoisomerization. The formation of 9-*cis* and 13-*cis* from *all-trans*- $\alpha$ -carotene was dependent upon the extent of heat or iodine/light treatment, and the latter was formed in greater amount under either treatment.

**Keywords:**  $\alpha$ -Carotene;  $\beta$ -carotene; thermoisomerization; photoisomerization; kinetic study

## INTRODUCTION

In the past decade carotenoids have drawn considerable attention because of their possible roles in the treatment of human disease such as skin cancer (Krinsky, 1989; Moon, 1989; Ziegler, 1989). In addition, some carotenoids possess vitamin A activity. Of the various vitamin A precursors, *all-trans*- $\beta$ -carotene is the most important because of its high (theoretically 100%) vitamin A activity (Olsen, 1989; Rodriguez-Amaya, 1989; Tee, 1992). Some other carotenoids, such as *all-trans*- $\alpha$ -carotene, have only half the biological value of *all-trans*- $\beta$ -carotene. Both *all-trans*- $\alpha$ - and *all-trans*- $\beta$ -carotenes are also present as positional isomers in foods such as carrots (Chandler and Schwartz, 1987; Heinonen, 1990; Chen et al., 1993).

Due to the presence of a long chain of conjugated carbon-carbon double bonds, carotenoids are susceptible to light, oxygen, heat, and acid degradations. Sweeney and Marsh (1971) reported that processing of vegetables could result in a 15–35% loss of vitamin A because of the conversion of *trans*- $\beta$ -carotene to its *cis* isomers. Chen (1992) also found that the relative amount of *cis*- $\beta$ -carotene in vegetables increased with heating time when the output power of the microwave was 180 W. To accurately determine the provitamin A activity in foods, the National Research Council of the United States (NAS) (Food and Nutrition Board, 1980) suggested that the *cis* isomers of  $\beta$ -carotene formed during the processing of foods have to be separated and quantified.

Jensen et al. (1982) studied the *trans*-*cis* photoisomerization of *all-trans*- $\beta$ -carotene in the presence of chlorophyll *a* and found that the amount of three isomers, 9-, 13-, and 15-*cis*- $\beta$ -carotene were all initially linearly dependent upon the illumination dose. This result implied that the three isomers were primary products originating from *all-trans* and not formed from each other by secondary isomerization. The authors also demonstrated that *all-trans* is the primary product after isomerization of 15-*cis* and that the other isomers were formed only after a considerable amount of *all-trans* had accumulated. Chandler and Schwartz (1987)

studied the iodine-catalyzed photoisomerization of *all-trans*- $\beta$ -carotene and found that the 9-*cis* isomer was formed in larger amounts than 13-*cis* in the initial stage of isomerization. However, the concentration of 13-*cis* reached a plateau and a lower quantity of 9-*cis* was found during equilibrium. In another study dealing with the photodegradation of *all-trans*- $\beta$ -carotene in model dispersions, Pesek and Warthesen (1988) found that the relative amounts of *cis* isomers increased with time of exposure. In a later study, Pesek and Warthesen (1990) studied the photoisomerization and photodegradation of *all-trans*- $\beta$ -carotene and found that the 13-*cis* isomer was formed in greater amounts under dark storage conditions, while the accumulation of the 9-*cis* isomer was favored under lighted storage. Apparently, the instability of the 13-*cis* isomer during illumination is due to the fact that the activation energy for *trans*-*cis* isomerization about the central double bond is less than that about the other double bonds (Zechmeister, 1944).

Theoretically there are numerous *cis*- $\beta$ -carotene isomers possible during illumination and heating; however, only two *cis* forms have been kinetically studied so far. Also, the formation of *cis*- $\alpha$ -carotene isomers during heating and illumination was not known. Therefore, it is necessary to separate more *cis* forms of  $\alpha$ - and  $\beta$ -carotene so that the exact mechanism of isomer formation during heating and illumination can be established.

## MATERIALS AND METHODS

**Instrumentation.** The HPLC instrument consisted of an SSI 222D pump (Scientific System Inc., State College, PA) with a Linear 206 photodiode array detector (Linear Instrument, Reno, NV) and a Vydac 201TP54 column (25 cm  $\times$  4.6 mm i.d.) packed with 5- $\mu$ m particle size (Hesperia, CA). An Advantec SF-2120 fraction collector (Tokyo, Japan) was used to collect eluates. The data were stored and processed with an Axxiom 727 dual-channel chromatography data system (Axxiom Chromatography Inc., Calabasas, CA). Spectrophotometric determinations were made with a Beckman DU-70 double-beam spectrophotometer (Irvine, CA).

**Materials.** *trans*- $\alpha$ -Carotene and *trans*- $\beta$ -carotene standards were purchased from Sigma (St. Louis, MO). Both

standards were found to contain trace amounts of cis isomers by HPLC analysis and were used without further purification. All HPLC grade solvents such as acetonitrile, methanol, methylene chloride, chloroform, and hexane were purchased from Merck (Darmstadt, Germany) and were degassed under vacuum and filtered through a 0.2- $\mu$ m membrane filter prior to use.

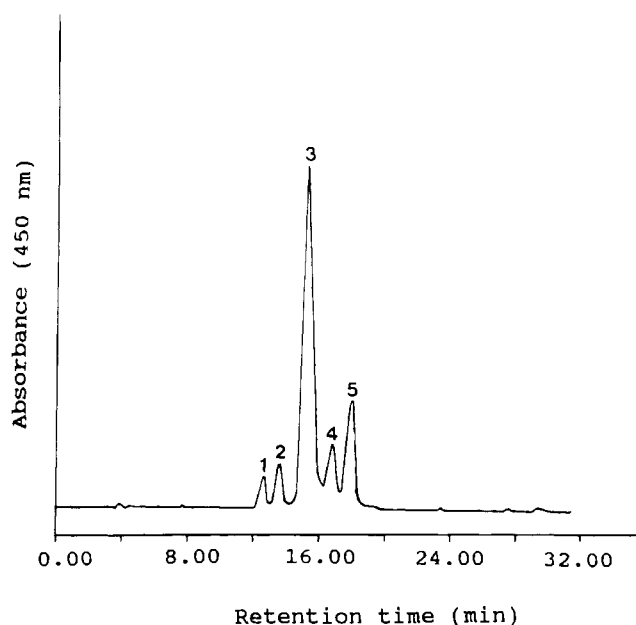
**Heating and Illumination of *all-trans*- $\alpha$ - and *all-trans*- $\beta$ -Carotene.** Approximately 5 mg of crystallized *all-trans*- $\alpha$ - and *all-trans*- $\beta$ -carotene each was placed in a 2-mL glass vial with a Teflon-coated screw cap. Each vial was heated in an oven for 10, 20, and 30 min at 50, 100, or 150 °C. Each heated sample was dissolved in 100 mL of hexane for *all-trans*- $\alpha$ -carotene or 100 mL of methanol-chloroform (45:55) for *all-trans*- $\beta$ -carotene, and then the solution was filtered through a 0.2- $\mu$ m membrane filter under vacuum prior to HPLC analysis. Twenty microliters of heated sample was injected into the HPLC. For iodine-catalyzed photoisomerization, approximately 5 mg of sample was dissolved in 100 mL of hexane and 0.1 mg of iodine was added. The solution was then illuminated in a chamber for 5, 10, 15, and 20 min at 25 °C under fluorescent light. The fluorescent tube (General Electric 20 W) was suspended approximately 20 cm above the vial, where the light intensity measured approximately 2000 lx. After illumination, the solvent (hexane) was removed under vacuum for *all-trans*- $\beta$ -carotene, which was then dissolved in the same solvent as described for heating. The solutions of *all-trans*- $\alpha$ - and *all-trans*- $\beta$ -carotene each were filtered through a 0.2- $\mu$ m membrane filter, and 20  $\mu$ L of sample was collected and injected into the HPLC. All injections were conducted in duplicate.

**Chromatographic Separation of Cis Isomers of  $\alpha$ - and  $\beta$ -Carotene.** A binary solvent system of acetonitrile-methanol (90:10) and a polymeric column (Vydac 201TP54) were used to separate *all-trans*- $\beta$ -carotene and its cis isomers with methanol-chloroform (45:55) as sample solvent. For the separation of *all-trans*- $\alpha$ -carotene and its cis isomers, a mobile phase of methanol-methylene chloride (99:1) and the same type of column with hexane as sample solvent were used (Chen and Chen, 1994). The flow rates for both systems were maintained at 1.0 mL/min with sensitivity at 0.2 AUFS and detection wavelength at 450 nm. The eluates of cis isomers from 10 HPLC runs were pooled, and the absorption spectra were determined. Both *all-trans*- $\alpha$ - and *all-trans*- $\beta$ -carotene were identified by cochromatography with added standards. In addition, cis isomers were identified on the basis of the following rules:

1. The absorption wavelength of cis isomers will result in a hypsochromic shift of about 4 nm (Davis, 1976; Goodwin, 1981).
2. Cis isomers are accompanied by a reduction in fine structure and a hypsochromic effect on absorbance (Goodwin, 1981).
3. The central cis isomers such as 13-,13'- or 15-,15'-cis have a strong peak in the UV region at about 340 nm (Goodwin, 1981).
4. Di-cis isomers are shifted to shorter wavelength than mono-cis isomers (Tsukida et al., 1982).
5. Mono-cis or di-cis isomers can also be identified by their *Q* ratio. *Q* ratio can be defined as the absorbance ratio of the cis peak to the middle main absorption peak (Tsukida et al., 1982; Quackenbush, 1987; Saleh and Tan, 1991).

**Quantification of Cis Isomers of  $\alpha$ - and  $\beta$ -Carotene.** Each peak was quantified using absolute calibration curves. The calibration curves for *all-trans*- $\alpha$ - and *all-trans*- $\beta$ -carotene were prepared by area measurement of reference compounds at six concentrations ranging from 20 to 120  $\mu$ g/mL. The calibration curves gave good linearity for both ( $r^2 = 0.99$ ), and the relative standard deviations were less than 3%. In the absence of reliable cis standards, cis isomers of  $\alpha$ -carotene were calculated as *all-trans*- $\alpha$ -carotene and cis isomers of  $\beta$ -carotene as *all-trans*- $\beta$ -carotene equivalents. Duplicate injections were conducted, and values of mean were determined.

**Kinetic Analyses of Data.** Only data obtained for *all-trans*- $\alpha$ - and *all-trans*- $\beta$ -carotene under iodine/light treatment or heating at 150 °C were analyzed kinetically using regression



**Figure 1.** HPLC chromatogram of *all-trans*- $\beta$ -carotene standard under heating at 150 °C for 30 min. A binary solvent system of acetonitrile-methanol (90:10) was employed. Peaks: (1) 13,15-di-cis- $\beta$ -carotene; (2) 15-cis- $\beta$ -carotene; (3) *all-trans*- $\beta$ -carotene; (4) 9-cis- $\beta$ -carotene; (5) 13-cis- $\beta$ -carotene.

models. The reaction rate constants for the reversible and irreversible reactions were determined by solving a series of differential equations that represented the kinetics of the proposed models. The coefficient of each differential equation was determined by fitting the regression models to the experimental data with the use of a statistical analysis system (SAS, 1985). All of the coefficients in the best-fitted models were significantly different ( $p < 0.05$ ) using regression analysis.

## RESULTS AND DISCUSSION

Both *all-trans*- $\alpha$ - and *all-trans*- $\beta$ -carotene standards were found to contain trace amounts of cis isomers. It has been reported that chlorinated solvents can promote isomerization of trans conjugated polyenes such as retinyl palmitate and  $\beta$ -carotene (Pesek et al., 1990; Saleh and Tan, 1991). Also, isomerization of  $\beta$ -carotene was found to be higher in nonpolar solvents than in polar solvents (Pesek et al., 1990). Hence, this may be responsible for some of the cis forms found in the  $\alpha$ - and  $\beta$ -carotene standards because sample solvent hexane (100%) was used for the former and methanol-chloroform (45:55) for the latter. In a previous study the authors demonstrated that selection of an appropriate sample solvent is very important because it can affect the HPLC separation of cis-trans carotene isomers (Chen and Chen, 1994). The purity of *all-trans*- $\alpha$ - and *all-trans*- $\beta$ -carotene standards as determined by diode array detector was assessed to be 93 and 87%, respectively. Figure 1 shows the HPLC chromatogram of *all-trans*- $\beta$ -carotene standard under heating at 150 °C for 30 min. Four cis forms were resolved and identified as 13,15-di-cis-, 15-cis-, 9-cis-, and 13-cis- $\beta$ -carotene on the basis of spectral characteristics and *Q* ratio (Table 1). The HPLC chromatogram of *all-trans*- $\alpha$ -carotene under heating at 150 °C for 30 min is shown in Figure 2. Three cis forms were resolved and identified as 15-cis-, 13-cis-, and 9-cis- $\alpha$ -carotene (Table 2).

In general, the relative amounts of *all-trans*- $\beta$ -carotene and its four cis isomers are only slightly affected when heated at 50 or 100 °C over a 30-min

**Table 1. Identification Data for  $\beta$ -Carotene and Its Cis Isomers by HPLC**

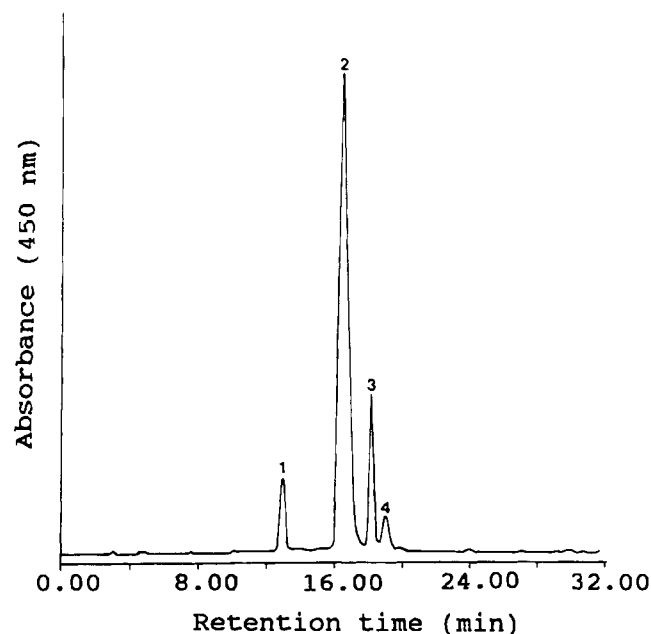
peak no.	pigment	visible spectra <sup>a</sup>			Q ratio		
		in-line <sup>b</sup>	off-line <sup>b</sup>	max reported	solvent	found	reported
1	13,15-di-cis- $\beta$ -carotene	(411), <sup>e</sup> 434, 456	(410), 433, 456	436	hexane	7.5	7.0 <sup>c</sup>
2	15-cis- $\beta$ -carotene	(421), 443, 470	(420), 442, 469	425, 446, 474	acetone-hexane (3:97)	2.3	1.9 <sup>c</sup>
3	$\beta$ -carotene	(424), 447, 475	(424), 446, 473	(424), 448, 476	hexane	11.8	12.7 <sup>d</sup>
4	9-cis- $\beta$ -carotene	(421), 444, 471	(420), 442, 469	425, 446, 473	acetone-hexane (3:97)	8.7	8.2 <sup>d</sup>
5	13-cis- $\beta$ -carotene	(420), 442, 467	(419), 442, 465	423, 444, 469	acetone-hexane (3:97)	2.9	2.8 <sup>c</sup>

<sup>a</sup> Reported values of visible spectra are from Quackenbush (1987), Chandler and Schwartz (1987), and Chen and Chen (1993). <sup>b</sup> Measured in solvent (acetonitrile-methanol = 80:20). <sup>c</sup> Reported values of Q ratio are from Tsukida et al. (1982). <sup>d</sup> Reported values of Q ratio are from Saleh and Tan (1991). <sup>e</sup> Values in parentheses represent shoulder on absorption curves.

**Table 2. Identification Data for  $\alpha$ -Carotene and Its Cis Isomers by HPLC**

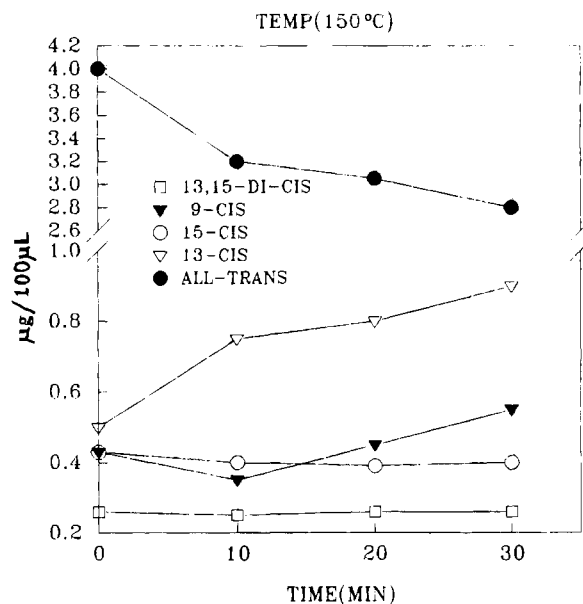
peak no.	pigment	visible spectra <sup>a</sup>			Q ratio		
		in-line <sup>b</sup>	off-line <sup>b</sup>	max reported	solvent	found	reported
1	15-cis- $\alpha$ -carotene	418, 437, 466	416, 437, 466	— <sup>c</sup>	— <sup>c</sup>	2.5	— <sup>c</sup>
2	$\alpha$ -carotene	(421), <sup>d</sup> 440, 468	(420), 440, 468	420, 445, 474	petroleum ether	13.1	<12 <sup>a</sup>
3	13-cis- $\alpha$ -carotene	(417), 436, 463	(417), 436, 463	441, 466	methanol-chloroform (94:6)	4.1	3.7 <sup>a</sup>
4	9-cis- $\alpha$ -carotene	(417), 437, 464	(415), 436, 462	442, 468	methanol-chloroform (94:6)	8.9	8.6 <sup>a</sup>

<sup>a</sup> Reported values of visible spectra and Q ratio are from Quackenbush (1987). <sup>b</sup> Measured in solvent (100% methanol). <sup>c</sup> Data not available. <sup>d</sup> Values in parentheses represent shoulder on absorption curves.



**Figure 2.** HPLC chromatogram of *all-trans*- $\alpha$ -carotene standard under heating at 150 °C for 30 min. A binary solvent system of methanol-methylene chloride (99:1) was employed. Peaks: (1) 15-cis- $\alpha$ -carotene; (2) *all-trans*- $\alpha$ -carotene; (3) 13-cis- $\alpha$ -carotene; (4) 9-cis- $\alpha$ -carotene.

period. However, with temperature at 150 °C the losses of *all-trans*- $\beta$ -carotene were greater and amounted to 0.80, 0.95, and 1.20  $\mu\text{g}/100 \mu\text{L}$  for heating times of 10, 20, and 30 min, respectively (Figure 3). The degradation of *all-trans*- $\beta$ -carotene during heating at 150 °C may fit the reversible first-order model. The selection of the reversible first-order model rather than the zero-order model was based on the following reasons: (1) It is easier to explain the kinetics with a concentration-dependent model, which was used by many authors (Pesek and Warthesen, 1988, 1990; Pesek et al., 1990). (2) The reversible first-order model proposed in this study fits well the experimental data. (3) One of the requirements to evaluate the rate constants of the forward and backward reactions individually for the zero-order model is that the concentration of the reactant has to be much higher than that of the product. However, in our study neither reaction can be evaluated individually because the initial concentrations of 9-cis-



**Figure 3.** Concentration changes of *all-trans*- $\beta$ -carotene and its cis isomers under heating at 150 °C for 10, 20, and 30 min.

**Table 3. Rate Constants of  $\alpha$ - and  $\beta$ -Carotene Isomerization during Heating at 150 °C**

reaction	rate constants <sup>a</sup>		
	$k_1^b$	$k_{-1}^b$	$r^2^c$
$\beta$ -carotene			
all-trans $\rightleftharpoons$ 13-cis	0.0125	0.0339	0.996
all-trans $\rightleftharpoons$ 9-cis	0.0043	0.0184	0.990
$\alpha$ -carotene			
all-trans $\rightleftharpoons$ 13-cis	0.0323	0.0998	0.994
all-trans $\rightleftharpoons$ 9-cis	0.0038	0.0449	0.980
all-trans $\rightleftharpoons$ 15-cis	0.0067	0.0407	0.990

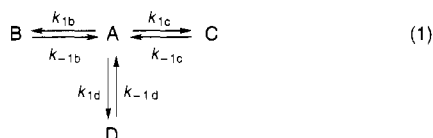
<sup>a</sup> All rate constants are significantly different ( $p < 0.05$ ) by regression analysis. <sup>b</sup>  $k_1$  and  $k_{-1}$  are the rate constants of forward and backward reactions of monomolecular conversion, respectively. <sup>c</sup>  $r^2$  represents the correlation of determination.

13-cis-, and 15-cis- $\beta$ -carotene are too high; hence, in the beginning of the reaction the backward reaction rate cannot be neglected as compared to the forward reaction. Data of correlation determination based on the reversible first-order model are shown in Table 3.

The amount of 9-cis- $\beta$ -carotene did not increase pronouncedly until the temperature reached 150 °C. The

formation of 9-*cis*- $\beta$ -carotene should be due to conversion of *all-trans*- $\beta$ -carotene. It has been reported that both 9-*cis*- and 13-*cis*- $\beta$ -carotenes are the two major *cis* forms which can be found during heating and illumination of *all-trans*- $\beta$ -carotene (Chandler and Schwartz, 1987; Pesek et al., 1990; Chen and Chen, 1993). Also, it was found that 13-*cis* was formed at a faster rate than was 9-*cis* when heated at 150 °C, and the increased amounts of the former were 0.25, 0.30, and 0.41  $\mu\text{g}/100 \mu\text{L}$  for heating times of 10, 20, and 30 min, respectively. It has been established that *all-trans* can be converted to either 9-*cis* or 13-*cis* during spontaneous isomerization and lighted storage (Chandler and Schwartz, 1987; Pesek and Warthesen, 1990; Pesek et al., 1990). In this study the conversion of *all-trans* to 15-*cis* or 13,15-*di-cis* during heating was not observed, indicating that 9-*cis* and 13-*cis* are the two major  $\beta$ -carotene *cis* isomers formed during heating.

The concentration changes of *all-trans*- $\beta$ -carotene and its *cis* isomers during heating at 50 and 100 °C over a period of 30 min were too small to fit a regression model. However, with the heating temperature increased to 150 °C the kinetic model for the reversible equilibrium of *all-trans*- $\beta$ -carotene isomerization can be easily established. This is because a high temperature dramatically increased both the forward and backward rates of isomeric compounds toward the equilibrium concentration within a short period of heating time. This result also showed that the isomerization of *all-trans*- $\beta$ -carotene during heating at 150 °C may fit the reversible first-order reaction. The proposed model was equilibrated in monomolecular conversion of *all-trans*- $\beta$ -carotene to its corresponding *cis* isomers



where A is *all-trans*- $\beta$ -carotene, B is 13-*cis*- $\beta$ -carotene, C is 9-*cis*- $\beta$ -carotene, and D is 15-*cis*- $\beta$ -carotene.  $k_1$  is the rate constant of the monomolecular conversion reaction of *all-trans*- $\beta$ -carotene to its *cis* isomers, and  $k_{-1}$  is the rate constant of the monomolecular conversion reaction of *cis* isomers to *all-trans*.

When the regression model fits the experimental data, the best-fit rate equations were solved by a differential method and were written as

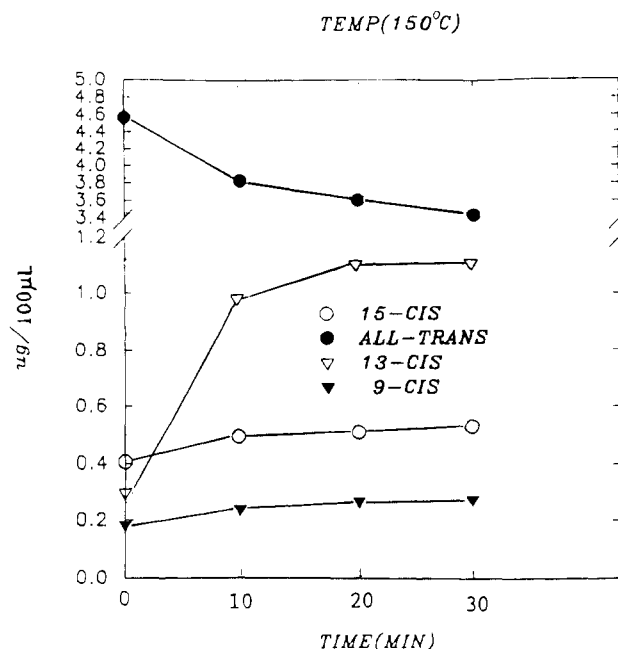
$$\frac{d[A]}{dt} = -(k_{1b} + k_{1c} + k_{1d})[A] + k_{-1b}[B] + k_{-1c}[C] + k_{-1d}[D] \quad (2)$$

$$\frac{d[B]}{dt} = k_{1b}[A] - k_{-1b}[B] \quad (3)$$

$$\frac{d[C]}{dt} = k_{1c}[A] - k_{-1c}[C] \quad (4)$$

$$\frac{d[D]}{dt} = k_{1d}[A] - k_{-1d}[D] \quad (5)$$

The corresponding rate constants of *all-trans*- $\beta$ -carotene isomerization with heating temperature of 150 °C are shown in Table 3. It was observed that the reversible conversion rate between *all-trans*- and 13-*cis*- $\beta$ -carotene was about 2–3-fold faster than that between *all-trans*- and 9-*cis*- $\beta$ -carotene. As for the conversion between *all-trans*- $\beta$ -carotene and its *cis* isomers, the backward reactions were faster than the forward reactions by 2–3-fold. This finding is similar

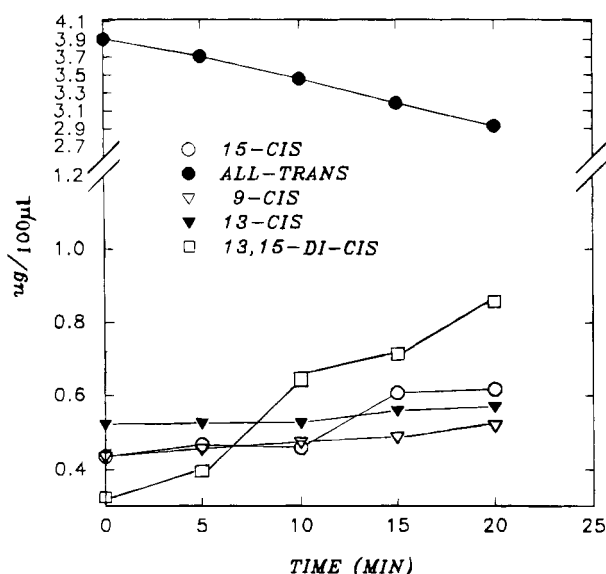


**Figure 4.** Concentration changes of *all-trans*- $\alpha$ -carotene and its *cis* isomers under heating at 150 °C for 10, 20, and 30 min.

to that in the study of Pesek et al. (1990), who reported that the rate of formation of 13-*cis*- $\beta$ -carotene was faster than that of 9-*cis*- $\beta$ -carotene when *all-trans*- $\beta$ -carotene was held in the dark at 45 °C over 28 days. The authors also found that the formation of 9-*cis* from *all-trans*- $\beta$ -carotene was the slowest reaction.

The concentration changes of *all-trans*- $\alpha$ -carotene and its *cis* isomers during heating were somewhat different from those of *all-trans*- $\beta$ -carotene and its *cis* isomers. The amounts of *all-trans*- $\alpha$ -carotene are only slightly affected with temperature at 50 or 100 °C over a 30-min period. However, with temperature increased to 150 °C the losses of *all-trans*- $\alpha$ -carotene reached 0.75, 0.98, and 1.20  $\mu\text{g}/100 \mu\text{L}$  for heating times of 10, 20, and 30 min, respectively (Figure 4). The degradation of *all-trans*- $\alpha$ -carotene during heating at 150 °C may also fit a reversible first-order model. Both 9-*cis*- and 15-*cis*- $\alpha$ -carotene showed only minor changes under heating treatment. For 13-*cis*- $\alpha$ -carotene, it did not increase pronouncedly until temperature reached 150 °C, and the increased amounts were 0.95, 1.10, and 1.12  $\mu\text{g}/100 \mu\text{L}$  for the same heating time. Also, it was found that 13-*cis*- $\alpha$ -carotene was formed at a higher concentration than 9-*cis*- or 15-*cis*- $\alpha$ -carotene. This result implied that *all-trans*- $\alpha$ -carotene can be easily converted to 13-*cis*- $\alpha$ -carotene during heating. By comparison of data in Figures 3 and 4, it can be found that 13-*cis*- $\alpha$ -carotene was formed in greater amount than 13-*cis*- $\beta$ -carotene during heating, indicating that the thermo-isomerization of *all-trans*- $\alpha$ -carotene may be greater than that of *all-trans*- $\beta$ -carotene.

Similar to the kinetic model of *all-trans*- $\beta$ -carotene isomerization shown in Table 3, *all-trans*- $\alpha$ -carotene was in a higher rate of equilibration with 13-*cis* than with 9-*cis* or 15-*cis*. The rate constant of the reversible conversion between *all-trans*- and 13-*cis*- $\alpha$ -carotene was higher than that between *all-trans*- and 9-*cis* or 15-*cis*. It was also found that the backward reactions from the *cis* isomers to form *all-trans*- $\alpha$ -carotene were all faster than the forward reactions, and the ratio of the rate constant of the former to the latter varied from 3.1 (*all-trans*  $\rightleftharpoons$  13-*cis*) to 6.0 (*all-trans*  $\rightleftharpoons$  15-*cis*) to 11.8 (*all-trans*  $\rightleftharpoons$  9-*cis*).

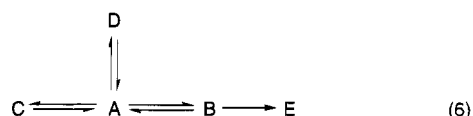


**Figure 5.** Concentration changes of *all-trans*- $\beta$ -carotene and its cis isomers under iodine/light treatment for 5, 10, 15, and 20 min.

Figure 5 shows the concentration changes of *all-trans*- $\beta$ -carotene and its cis isomers after iodine-catalyzed photoisomerization for varied lengths of time. The losses of *all-trans*- $\beta$ -carotene after exposure under fluorescent light for 5, 10, 15, and 20 min were 0.21, 0.44, 0.82, and 1.10  $\mu\text{g}/100\ \mu\text{L}$ , respectively, indicating that the amount of *all-trans*- $\beta$ -carotene decreased with increasing exposure time. The degradation of *all-trans*- $\beta$ -carotene may account for two reactions proceeding simultaneously under iodine/light treatment, i.e., a reaction catalyzed by the formation of free radicals and a reversible first-order reaction proposed for heating at 150 °C. In contrast to the result described above, the amount of 13,15-di-*cis*- $\beta$ -carotene increased greatly during iodine-catalyzed photoisomerization, indicating that this isomer can be formed more easily than the other cis forms of isomers. It has been reported that if a mono-*cis* carotenoid forms a certain di-*cis* carotenoid, it is reasonable to assume that the two *cis* carotenoids have one *cis*-position in common (Zechmeister, 1944). Therefore, it can be postulated that the large increase of 13,15-di-*cis*- $\beta$ -carotene was due mainly to conversion of either 13-*cis*- or 15-*cis*- $\beta$ -carotene. The amount of 13,15-di-*cis*- $\beta$ -carotene increased substantially after exposure time reached 10 min and above; meanwhile, 13-*cis* remained almost the same. As the amount of 15-*cis*- $\beta$ -carotene increased pronouncedly at the same time, it can be inferred that the formation of 13,15-di-*cis*- $\beta$ -carotene was due in large part to conversion of 13-*cis*- $\beta$ -carotene. It was also observed that the concentration changes of 9-*cis*- and 13-*cis*- $\beta$ -carotene during iodine-catalyzed photoisomerization are almost the same. This result seems to be contradictory to that described above, which showed that 13-*cis* was favored during heating of *all-trans*- $\beta$ -carotene. This is probably because no 13,15-di-*cis*- $\beta$ -carotene was observed as a result of heating. It is also possible that the activation energies for heating and illumination are different. It has been reported that the activation energy for heating and illumination for *trans*-*cis* isomerization about the central double bond is less than that about the other double bonds (Zechmeister, 1944), and hence the activation energy required for isomerization will be lower for 13-*cis* than for 9-*cis*. Chandler and Schwartz (1987) reported that 9-*cis*- $\beta$ -carotene was kinetically favored during illumination.

Pesek and Warthesen (1990) also found that formation of the 9-*cis* isomer was favored under lighted condition. However, in another study Pesek et al. (1990) reported that 13-*cis* was formed at a faster rate than 9-*cis* during spontaneous isomerization of *all-trans*- $\beta$ -carotene in the dark. Apparently the difference can be attributed to intensity of light and modes of illumination.

To further examine the mechanism of iodine-catalyzed photoisomerization of *all-trans*- $\beta$ -carotene, the following model



was proposed, where A is *all-trans*- $\beta$ -carotene, B is 13-*cis*- $\beta$ -carotene, C is 9-*cis*- $\beta$ -carotene, D is 15-*cis*- $\beta$ -carotene, and E is 13,15-di-*cis*- $\beta$ -carotene. This model likely consists of two types of reactions: (1) one is the irreversible bimolecular conversion involving the propagation of free radical; (2) the other is the reversible reaction of monomolecular conversion as described for the isomerization of *all-trans*- $\beta$ -carotene at 150 °C.

On the basis of the regression analysis of the experimental data, the best-fit rate equations with coefficients being significantly different ( $p < 0.05$ ) were as follows:

$$10^4 \times d[\text{B}]/dt = 6.8[\text{E}]^2 + 5.8[\text{A}][\text{D}] - (4.4[\text{A}] + 14.4[\text{E}])[\text{B}] \quad (r^2 = 0.996) \quad (7)$$

$$10^3 \times d[\text{E}]/dt = 1.96[\text{B}]^2 - 9.65[\text{E}]^2 \quad (r^2 = 0.999) \quad (8)$$

$$10^2 \times d[\text{C}]/dt = 5.57[\text{A}] - 161.5[\text{C}] + 2.57[\text{C}]^2 \quad (r^2 = 0.643) \quad (9)$$

$$10^3 \times d[\text{D}]/dt = 2.74[\text{A}][\text{D}] - 660.5[\text{D}] \quad (r^2 = 0.968) \quad (10)$$

Of these rate equations, the formation of 13-*cis*- and 13,15-di-*cis*- $\beta$ -carotene fits the first type of reaction, while that of 9-*cis*- and 15-*cis*- $\beta$ -carotene fits the second type of reaction. The possibility of an isomerization reaction occurring in a bimolecular collision is low in the diluted solution used in our experiment unless the isomer was in an activated state. Thus, it is reasonable to assume that all of the bimolecular conversion may involve propagation of free radicals between the same or different isomers in the reaction. Although further studies are needed to understand the actual mechanism of this complex reaction, the data from the kinetic study clearly showed that all mono-*cis* isomers approached an equilibrium concentration, and each of them could be converted to other forms of *cis* isomers only after it changed to *all-trans* forms. As described above, 13,15-di-*cis*- $\beta$ -carotene was likely formed from the bimolecular conversion of 13-*cis*- $\beta$ -carotene. This is clearly shown in the kinetic study: the di-*cis* isomer formation is solely dependent upon the concentration of the 13-*cis* isomer and not the concentration of either the 9-*cis* or 15-*cis* isomer. The concentration of 13-*cis*- $\beta$ -carotene during the reaction is low and is quickly converted to the 13,15-di-*cis* isomer after its formation from the *all-trans* isomer. Nevertheless, the conversion rates of 9-*cis* and 15-*cis* isomers to approach an equilibrium were higher than those of 13-*cis* and 13,15-di-*cis* isomers.

Figure 6 shows the concentration changes of *all-trans*- $\alpha$ -carotene and its *cis* isomers during iodine-catalyzed photoisomerization. After illumination for 5, 10, 15, and 20 min, the losses of *all-trans*- $\alpha$ -carotene were 1.50, 1.62, 1.85, and 2.08  $\mu\text{g}/100 \mu\text{L}$ , respectively. As the losses of *all-trans*- $\alpha$ -carotene were greater than those of *all-trans*- $\beta$ -carotene, it may be postulated that the former was degraded faster than the latter under iodine/light treatment. Like *all-trans*- $\beta$ -carotene, the degradation of *all-trans*- $\alpha$ -carotene during iodine-catalyzed photoisomerization also fits the proposed model as shown above. This model consisted of two types of reactions with the exception that the formation of the 13,15-di-*cis* isomer was not observed in the reaction. The amount of 13-*cis*- $\alpha$ -carotene increased substantially after exposure time reached 5 min, indicating that this isomer was more easily formed than the other *cis* isomers. For 9-*cis*- and 15-*cis*- $\alpha$ -carotene, both showed only minor changes over a 20-min illumination period. It was also observed that 13-*cis*- $\alpha$ -carotene can be formed at a higher concentration than 13-*cis*- $\beta$ -carotene. This result does not necessarily imply that the iodine-catalyzed photoisomerization of *all-trans*- $\alpha$ -carotene is greater than that of *all-trans*- $\beta$ -carotene, mainly because most 13-*cis*- $\beta$ -carotene can be further converted to 13,15-di-*cis*- $\beta$ -carotene easily under iodine/light treatment. No formation of 13,15-di-*cis*- $\alpha$ -carotene during illumination was observed probably because this isomer was not resolved from the column by the solvent system employed in this study.

According to the proposed model for photoisomerization of *all-trans*- $\beta$ -carotene, the experimental data obtained during illumination of *all-trans*- $\alpha$ -carotene were analyzed and the best-fit rate equations with coefficients being significant at the 5% level were

$$d[B]/dt = 0.202[A] + 6.5 \times 10^{-4}[B]^2 - 0.445[B] \quad (r^2 = 0.998) \quad (11)$$

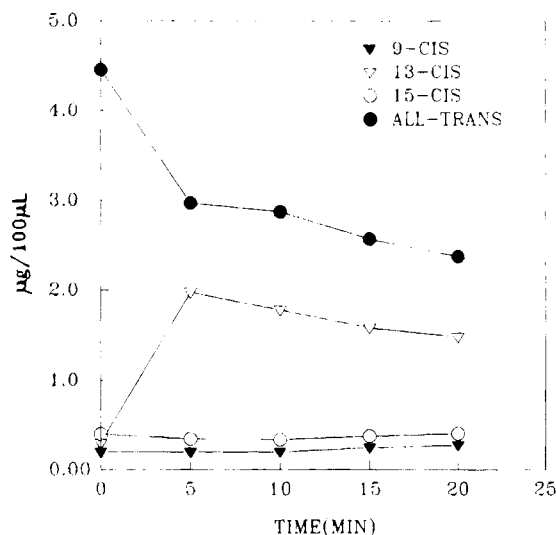
$$d[C]/dt = 1.64 \times 10^{-2}[A] - 0.268[C] - 9.5 \times 10^{-4}[A][C] + 1.58 \times 10^{-2}[C]^2 \quad (r^2 = 0.990) \quad (12)$$

$$d[D]/dt = 0.0031[D]^2 - 0.119[D] \quad (r^2 = 0.925) \quad (13)$$

where A is *all-trans*- $\alpha$ -carotene, B is 13-*cis*- $\alpha$ -carotene, C is 9-*cis*- $\alpha$ -carotene, and D is 15-*cis*- $\alpha$ -carotene.

Unlike iodine-catalyzed photoisomerization of *all-trans*- $\beta$ -carotene, both formation and degradation of 13-*cis*- $\alpha$ -carotene are much faster than that of either 9-*cis*- or 15-*cis*- $\alpha$ -carotene, and the former could be at a faster rate to approach equilibrium than the latter (9-*cis* or 15-*cis*). Nevertheless, all  $\alpha$ -carotene *cis* isomers also involved propagation of free radicals in the reaction just as the iodine-catalyzed photoisomerization of *all-trans*- $\beta$ -carotene.

From the above discussions, it may be concluded that the thermoisomerization and iodine-catalyzed photoisomerization of *all-trans*- $\alpha$ -carotene are greater than those of *all-trans*- $\beta$ -carotene, that 13-*cis*- $\alpha$ -carotene can be more easily formed than 13-*cis*- $\beta$ -carotene during heating, and that the formation of 13,15-di-*cis*- $\beta$ -carotene during iodine-catalyzed photoisomerization can be due in large part to conversion of 13-*cis*- $\beta$ -carotene. However, we have to point out here that the results observed in this experiment may not be identical to those in real food systems because of numerous factors



**Figure 6.** Concentration changes of *all-trans*- $\alpha$ -carotene and its *cis* isomers under iodine/light treatment for 5, 10, 15, and 20 min.

such as food contains water and other compounds. Thus, further research is necessary to determine if the trends shown in this study can work the same as in food systems during processing and storage.

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