Kinetic Model for Photoisomerization and Concomitant Photodegradation of β-Carotenes†

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The photoisomerization and concomitant photodegradation of β-carotene were studied. β-Carotene samples were stored at 28 °C in the dark or under 250 ft-c of light for varied lengths of time. Isomerization and photodegradation reactions were monitored by using HPLC with diode-array detection. Under lighted storage conditions, the photodegradation reactions predominated over the isomerization reactions. Degradation of total β-carotenes took place at a rate of 0.040 ± 0.005 day^{-1}. The 13-cis isomer was formed in greater amounts under dark storage conditions, while under lighted storage, accumulation of the 9-cis isomer was favored. Similar trends were observed for β-carotenes in a carrot juice sample.

INTRODUCTION

Carotenoids are important food constituents because they provide color and potential health benefits (Klaui and Bauernfeind, 1981). Processing and storage conditions can cause isomerization or degradation of carotenoids in foods (Panalaks and Murray, 1970; Sweeney and Marsh, 1971; Ogunlesi and Lee, 1979; Chandler and Schwartz, 1987). These reactions may bring about undesirable changes in color and nutritive value of a product. The color intensity of cis isomers is less than that of the all-trans molecule. The vitamin A potential of cis β-carotenes in humans is unknown, but rat studies indicate that cis isomers are no more than 50% the biopotency of *all-trans*-β-carotene (Zechmeister, 1944). A basic understanding of these reactions is important to the study of carotenoid chemistry in foods.

Advances in chromatography have made it possible to separate and characterize isomeric cartenoids. Several HPLC procedures dealing with separation of isomeric β-carotenes are reported in the literature (Bushway, 1985, 1986; Khachik et al., 1986, 1989; Chandler and Schwartz, 1987; Landen et al., 1987; Quackenbush, 1987; Tan, 1988).

The effect of light on carotenoid stability has been reported briefly in the literature (Cole and Kapur, 1957; Lovric et al., 1970; Tsukida et al., 1981; Pesek and Warthesen, 1987, 1988). Due to light-permeable packaging or additional light-exposure situations, carotenoids may isomerize or be degraded. No kinetic studies dealing with the influence of light on carotenoid isomerization and concomitant degradation have been published. The objective of this study was to determine a kinetic model for the photoisomerization and concomitant photodegradation of *all-trans*-β-carotene.

EXPERIMENTAL METHODS

Evaluation of Photoisomerization and Photodegradation in a Solvent System. To ensure that our initial sample was pure, the *all-trans*-β-carotene used in this study was isolated from a 1% cold water soluble (CWS) β-carotene powder (Hoffman-La Roche, Inc., Nutley, NJ). A 4.0-mL aliquot of a working dispersion of 10 μg/mL β-carotenes in distilled water was extracted by using the basic procedures of Hsieh and Karel (1983). The sample was placed in a 50-mL, screw-capped glass test tube fitted with an aluminum foil sleeve to minimize light exposure. Ten milliliters of petroleum ether/acetone (50:50 v/v) was added to the tube. The contents were mixed, and the liquids were allowed to separate. The ether phase was transferred to another foil-covered test tube. The extraction procedure was repeated until the ether phase was clear. The sample then was evaporated to dryness under nitrogen. β-Carotenes were redissolved in 2.0 mL of HPLC mobile phase (acetonitrile/methanol/THF, 42:58:1 v/v/v) and analyzed by using the reverse-phase HPLC procedure described under Chromatographic Procedures. The *all-trans*-β-carotene peak was collected as it eluted from the exit of the HPLC detector.

The *all-trans*-β-carotene elutions from 12 HPLC runs were pooled, concentrated to a volume of approximately 0.8 mL, and then divided into two fractions which were transferred to 1.0-mL reaction vials with Teflon-coated screw caps. One vial was stored under 250 ft-c of light, and the other was covered with aluminum foil to prevent light exposure. Both vials were stored at 28 °C. Constant light intensity was provided by standard fluorescent lights (General Electric cool white No. F15T8-CW). Both samples were analyzed for β-carotenes at selected times over a 30-day period. Data were evaluated and rate constants were determined through solution of differential rate equations.

Evaluation of Photoisomerization and Photodegradation in a Carrot Juice System. A carrot juice sample was prepared by mixing 1.00 g of freeze-dried carrot crystals (Natural Carotene Products Cooperative, Inc., Eustis, FL) with 40 mL of distilled water. The juice was filtered through Whatman No. 4 filter paper to remove large particulates. One-gram samples were placed in glass tubes, and the tubes were capped and laid horizontally in the previously described light chamber under 250 ft-c of light at 28 °C. A second set of samples was covered with aluminum foil to prevent light exposure and stored at the same temperature. Samples were extracted by using the extraction procedure of Pesek and Warthesen (1988). Basically, juice samples were extracted by using a mixture of petroleum ether/acetone (50:50 v/v). Extracts then were dried under nitrogen, and the carotenoids were resuspended in 2.0 mL of HPLC mobile phase (acetonitrile/methanol/THF, 42:58:1 v/v/v) prior to injection into the HPLC.

Chromatographic Procedures. HPLC with absorbance detection was used to separate and quantify carotenoid isomers. The HPLC system consisted of a Model 6000A pump

† Published as Paper No. 17 189 of the contribution series of the Minnesota Agriculture Experiment Station based on research conducted under Project 18-87.

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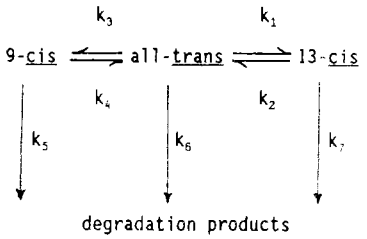

Figure 1. Schematic of photoisomerization and concomitant photodegradation reactions for β-carotene, where *k*'s represent reaction rates for designated reactions.

(Waters Associates, Inc., Milford, MA), a Model 7125 Rheodyne injector with a 10-μL loop, a Spectroflow 757 variable-wavelength detector (Kratos, Inc., Ramsey, NJ) (for fraction collection work), or a Hewlett-Packard 1040 diode-array detector and workstation computer (Palo Alto, CA). Absorbance at 436 nm was monitored, and scans from 600 to 300 nm were collected throughout the chromatographic runs. The absorptivity values of the isomers are similar at 436 nm, which permits the estimation of the percentage of each isomer present and quantitation based on absorption of an *all-trans*-β-carotene standard at this wavelength (Chandler and Schwartz, 1987). In the absence of reliable quantitative cis standards, the cis isomers were quantified by using the peak areas of *all-trans*-β-carotene.

Carotene separation was accomplished by using a Vydac 218TP54 column (Hesperia, CA) with an acetonitrile/methanol/THF (42:58:1 v/v/v) mobile phase at a flow rate of 1.0 mL/min (Pesek et al., 1990). This mobile phase and column are similar to those reported by Bushway (1986). Tentative peak identification was accomplished by using a β-carotene standard for all-trans and comparison of absorption spectra and *Q* ratios with published data (Stitt et al., 1951; Quackenbush, 1987).

Data Analysis. Linear regression was used to determine overall and all-trans photodegradation rates for β-carotenes. First-order rate constants and 95% confidence intervals were determined.

RESULTS AND DISCUSSION

A group of competing reactions exists when carotenoids are exposed to light. Photoisomerization reactions compete with photodegradation reactions. The predominant reaction will depend upon temperature, light intensity, and the presence of additional catalysts. A schematic of possible reactions is presented in Figure 1.

Our tentative identification of the two cis isomers, 9-cis and 13-cis, is based on a spectral analysis of the 9-cis peak which agrees well with published spectra and other spectral characteristics (Stitt et al., 1951; Quackenbush, 1987). Assignment of 13-cis to the second eluting cis isomer is less certain but is based on spectral characteristics and the work of Tsukida et al. (1982), who reported 9- and 13-cis isomers as the major isomers formed from all-trans. Although numerous cis isomers theoretically are possible, these are the two major cis isomers expected in food systems (Sweeney and Marsh, 1971; Chandler and Schwartz, 1987). Both cis isomers were formed in a control sample stored in the dark at 28 °C, but no overall degradation of β-carotene occurred in the control sample. The dark equilibration reactions at 28 °C appeared to follow the trends described by Pesek et al. (1990) at 45 °C except at a slower rate. This slower rate was expected because of the lower storage temperature. The 13-cis-β-carotene isomer formed at a rate approximately 3 times faster than that of the 9-cis isomer and, therefore, was present in greater amounts as equilibrium was approached.

Although the same two cis isomers were detected under lighted storage conditions, the 9-*cis*- and 13-*cis*-β-carotene isomers were present in different amounts than when the sample was not exposed to light (Figure 2). Under lighted conditions, formation of the 9-cis isomer was favored over formation of the 13-cis isomer. The light appeared to catalyze the formation of 9-cis since more 9-*cis*-β-carotene was present in samples stored under lighted conditions than in the dark. The 13-*cis*-β-carotene isomer was present in much smaller quantities with lighted than with dark conditions; therefore, it appeared that either 13-cis was less stable to light or less was formed under lighted conditions. According to Zechmeister (1944) the activation energy for trans–cis isomerization about the central double bond is less than that about the other double bonds. Also, each double bond loses some of its double-bond character to adjoining single bonds, and the amount lost increases from the ends toward the center. Therefore, the activation energy required for isomerization will be lower for 13-*cis*- than for 9-*cis*-β-carotene. This may help explain the greater instability of the 13-cis isomer.

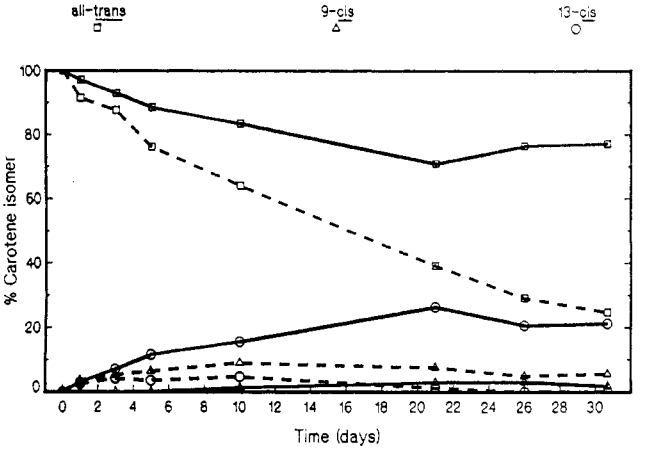

Figure 2. Photoisomerization/degradation plots for *all-trans*-β-carotene at 28 °C unexposed (—) and exposed (- - -) to 250 ft-c of light.

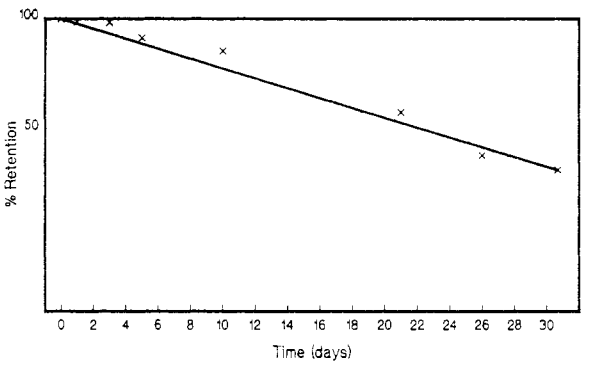

Figure 3. First-order plot of total β-carotene photodegradation at 28 °C and 250 ft-c light (r^2 = 0.985).

Under lighted conditions degradation was observed throughout the 30-day study. A first-order plot of overall photodegradation of all isomeric forms of β-carotene is presented in Figure 3. The rate of photodegradation of all isometric forms of β-carotene was 0.040 ± 0.005 day^{-1}. The rate of change in percent *all-trans*-β-carotene was 0.044 ± 0.003 day^{-1}. This indicated that although trans–cis isomerization is taking place, the change in *all-trans*-β-carotene, namely photodegradation, is the predominant reaction taking place at 28 °C under 250 ft-c of light.

To see if these same trends hold for a food system, the photostability of β-carotenes in a carrot juice sample was investigated. The carrot juice sample contained about

96% *all-trans*- and 4% 13-*cis*-β-carotene at the beginning of the experiment. Carrot juice samples stored in the dark showed no development of 9-*cis*-β-carotene after 12 days at 28 °C, and the amount of 13-cis remained fairly constant. When exposed to light (250 ft-c), the 9-cis isomer appeared in carrot juice samples after 1 day. The 9-cis isomer constituted 3–5% of the total β-carotene peak area even after 35 days. *all-trans*-β-Carotene constituted greater than 90% of the chromatogram peak areas for β-carotenes throughout the study. The level of 13-*cis*-β-carotene ranged from 2% to 4% throughout the study. This implied that the photoisomerization reactions were relatively slow in the carrot juice system as compared to the solvent system. The reaction rate probably was influenced by the presence of protective factors such as particulates in the carrot juice. Although isomerization occurred, in general, degradation of total β-carotenes in carrot juice was not a significant factor even after 35 days of storage.

From the results of this study, it can be concluded that although photoisomerization is taking place, the predominant reaction occurring is the photodegradation of *all-trans*-β-carotene. The formation of the 9-cis isomer was enhanced by light exposure with samples exposed to light containing up to 6 times more 9-*cis*-β-carotene than those stored in the dark. The 13-*cis*-β-carotene isomer was the most sensitive to light. These trends held for β-carotene in a solvent system and a carrot juice sample.

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Received for review September 12, 1989. Revised manuscript received February 12, 1990. Accepted March 1, 1990.

Registry No. *all-trans*-β-Carotene, 7235-40-7; 9-*cis*-β-carotene, 13312-52-2; 13-*cis*-β-carotene, 6811-73-0.