Oxidative Degradation Kinetics of Lycopene, Lutein, and 9-*cis* and All-*trans* β-Carotene

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ABSTRACT: The thermal and oxidative degradation of carotenoids was studied in an oil model system to determine their relative stabilities and the major β-carotene isomers formed during the reaction. All-*trans* β-carotene, 9-*cis* β-carotene, lycopene, and lutein were heated in safflower seed oil at 75, 85, and 95°C for 24, 12, and 5 h, respectively. The major isomers formed during heating of β-carotene were 13-*cis*, 9-*cis*, and an unidentified *cis* isomer. The degradation kinetics for the carotenoids followed a first-order kinetic model. The rates of degradation were as follows: lycopene > all-*trans* β-carotene ≈ 9-*cis* β-carotene > lutein. The values for the thermodynamic parameters indicate that a kinetic compensation effect exists between all of the carotenoids. These data suggest that lycopene was most susceptible to degradation and lutein had the greatest stability in the model system of the carotenoids tested. Furthermore, there was no significant difference in the rates of degradation for 9-*cis* and all-*trans* β-carotene under the experimental conditions.

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KEY WORDS: β-carotene, carotenoids, degradation, kinetics, lutein, lycopene, thermal stability.

Carotenoids are multifunctional, naturally occurring red, yellow, and orange pigments that provide provitamin A activity and peroxidation protection to cells and living organisms. The pigments are used extensively in the food industry as natural colorants and as provitamin A sources. Independent of their vitamin A activity, epidemiological studies indicate that carotenoids may function in a protective role against degenerative diseases that are influenced by oxidative stress (1–3).

Because carotenoids are highly unsaturated molecules, comprising many conjugated double bonds, they are very susceptible to oxidation. In the process of protecting compounds from harmful oxidative reactions *via* trapping free radicals (4) or quenching singlet oxygen (5–7), carotenoids may become the primary oxidizable substrate. Baloch *et al.* (8), utilizing a model system, demonstrated that β-carotene was consumed faster than linoleic acid and, consequently, that carotenoid oxidation may be a more favorable reaction than autooxidation of linoleic acid. Therefore, as antioxidants the pigments may selectively become oxidized and in turn lose their characteristic color and provitamin A function, especially in cases of severe oxidation.

The kinetics of carotenoid degradation have not been extensively studied. Degradation reactions are influenced by factors such as reaction medium, temperature, physical state, type of pigment, and environmental conditions (9,10). First-order kinetic models were reported for the photodegradation of β-carotene adsorbed onto a matrix (9), β-carotene dispersed on microcrystalline cellulose (11), and in other low-moisture model systems (12–14). However, zero-order reaction kinetics were reported for the oxidation of β-carotene dissolved in toluene and exposed to molecular oxygen (15).

It is postulated that β-carotene degradation is an autocatalytic reaction that proceeds only after an induction period, during which radicals accumulate and antioxidants are depleted (8,11,16). Philip and Francis (17) reported the absence of an induction period in the oxidation of crystalline capsanthin; therefore, carotenoid degradation is not always characterized by a distinct lag phase.

The majority of studies to date have focused on β-carotene in low-moisture and solvent-based model systems (9,15,18). In addition, limited information is available on carotenoids other than β-carotene. Previous studies with other carotenoids were also solvent- or water-based (10,17). Information regarding the kinetics and thermodynamic parameters of carotenoid pigment degradation in an oil model system may better relate to lipid-based food systems. Therefore, the objective of this research was to measure the degradation kinetics of 9-*cis* and all-*trans* β-carotene, lycopene, and lutein in an oil model system under thermal and oxidative conditions.

EXPERIMENTAL PROCEDURES

Materials. Authentic standards of all-*trans*, 9-*cis*, and 13-*cis* β-carotene were provided by Hoffmann La Roche, Inc. (Nutley, NJ). Lycopene, lutein, and safflower seed oil (without endogenous tocopherols) were obtained from Sigma Chemical Co. (St. Louis, MO). A *Dunaliella* algae-derived β-carotene preparation, Betatene™ (4% β-carotene in soy oil), which is used as a natural colorant, was provided by Betatene, Ltd., Melbourne, Australia. Structures of the carotenoid compounds are shown in Figure 1.

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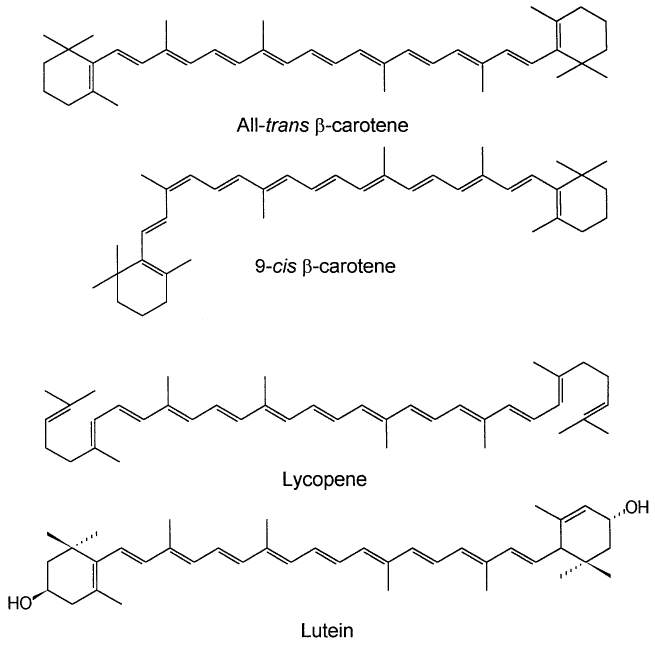

FIG. 1. Chemical structures of the carotenoids evaluated.

The solvents methanol, hexane, and petroleum ether were of certified A.C.S. grade, whereas methyl-*tert*-butyl ether (MTBE) was of high-performance liquid chromatography (HPLC) grade (Fisher Scientific Co., Fairlawn, NJ). Solvents used in the mobile phase for liquid chromatography were filtered through a 1.0-µm pore (47-mm) PTFE filter (Fisher Scientific Co.).

Dunaliella algae produce high levels of *cis* isomers of β-carotene. The 9-*cis* isomer was purified from the algae *via* chromatographic separation. The 9-*cis* isomer was identified based on its electronic absorption spectrum. The absorption spectra of carotenoid isomers are commonly used as a tool for identification (19,20).

The 9-*cis* β-carotene isomer was obtained by dissolving a small amount of the *Dunaliella* algae-derived β-carotene preparation in hexane. The mixture was filtered through a 0.45-µm (3-mm) nylon filter (MSI, Westboro, MA), and the solvent was evaporated under nitrogen. The extract was redissolved in the injection solvent, 50:50 MTBE/methanol. The 9-*cis* peak was collected *via* chromatographic separation of the sample by using a semipreparatory 5-µm C_{30} reversed-phase column (10 mm i.d. × 250 mm). The chromatographic instrumentation and procedures are outlined below.

Chromatographic instrumentation. The carotenoids and their reaction products were separated on an analytical-scale (4.6 mm i.d. × 250 mm) 3-µm C_{30} reversed-phase column, which was prepared at the National Institute of Standards and Technology (NIST; Gaithersburg, MD) as specified in its development report (21). The column is produced commercially by YMC, Inc. (Wilmington, NC). Purified standards and isolated 9-*cis* isomer from Betatene were obtained by using a semipreparatory (10 mm i.d. × 250 mm) 5-µm C_{30} reversed-phase column. The HPLC system consisted of a Waters Model U6K injector, two Model 510 pumps, a gradient controller Model 680, and a photodiode array Model 990 detector. The photodiode array detector was coupled to an NEC Powermate SX/20 computer (Boxborough, MA), and Waters 990 PDA chromatography software was used to obtain and store absorption spectra and chromatograms.

Carotenoid and oil preparations. Each carotenoid was dissolved in hexane and diluted as necessary to obtain initial concentrations of 150, 54, 35, and 66 µM for all-*trans* β-carotene, 9-*cis* β-carotene, lycopene, and lutein, respectively. The carotenoids in hexane were added directly to disposable borosilicate glass reaction tubes that contained 5 g of safflower oil. The solvent was evaporated under a stream of nitrogen. A blank sample was prepared with 5 g of safflower oil and hexane, which was evaporated under nitrogen. Triplicate tubes were prepared for the blank and each mixture.

Thermal and oxidative conditions. All-*trans* β-carotene, 9-*cis* β-carotene, lycopene, and lutein were individually heated in safflower seed oil at 75, 85, and 95°C for 24, 12, and 5 h, respectively. Samples were removed from the heating block every 2 h (75°C), 1 h (85°C), or 0.5 h (95°C). The Omnion Oxidative Stability Instrument (Archer Daniels Midland Co., Decatur, IL), which comprises a heating compartment, air inlet valves and electrical conductivity probes, was used for heating, and the samples were aerated with a continuous flow of air at 2.5 psi.

Pigment extraction and chromatographic procedures. β-Carotenes (all-*trans* and 9-*cis*) and lutein were extracted by adding 1 g of the oil mixture and 10 mL of hexane to a 100-mL volumetric flask. One mL of 1% butylated hydroxytoluene (BHT) and 22 mL of 17% potassium hydroxide were added to the mixture. Methanol was the solvent for both the BHT and potassium hydroxide mixtures. Each sample was saponified at room temperature with constant stirring for 4 h. After saponification, 15 mL petroleum ether was added, and the epilayer was removed and placed into a separatory funnel. The petroleum ether extraction was repeated five times. The combined epilayers were washed four times with 100 mL water. Saturated sodium chloride was added as needed to disrupt any emulsion. The epilayer was obtained, and the solvent was evaporated to dryness under a stream of nitrogen. The extracts were stored at −20°C under a headspace of nitrogen gas and analyzed within 48 h after storage. All extracts were redissolved in the injection solvent of 50:50 MTBE/methanol and filtered through a 0.45-µm (3-mm) nylon filter (MSI) prior to analysis. Extractions were carried out under subdued yellow light to prevent isomerization and degradation reactions.

The oil that contained dissolved lycopene was injected directly onto the analytical column; therefore, the carotenoid was not extracted from the oil. The purity and homogeneity of the oil, in conjunction with the difference in polarity between the oil and lycopene, allowed excellent chromatographic separation of the two constituents.

Binary mobile phases of MTBE/methanol were used to separate the carotenoids and monitor degradation. β-Carotenes

(all-*trans* and 9-*cis*) and lutein were separated under isocratic conditions, whereas the lycopene separation employed gradient conditions. Chromatographic separations were monitored at 452, 472, and 442 nm for β-carotene, lycopene, and lutein, respectively. The specific separation conditions for each carotenoid are outlined in Table 1.

Kinetic data analysis. A plot of natural-log carotenoid concentration vs. time (Fig. 2) was used to best fit a kinetic model and to obtain reaction rate constants.

The enthalpy of activation (ΔH) was obtained by plotting ln (k/T) versus $1/T$, and the entropy of activation (ΔS) was obtained from the expression of rate constants based on the transition state theory:

$$\ln (k/T) = \ln (K_b/h) + \Delta S/R - \Delta H/RT \quad [1]$$

where K_b is Boltzmann's constant and h is Planck's constant.

A kinetic compensation effect (isokinetic relationship) exists when there is a linear relationship between the thermodynamic parameters of enthalpy and entropy. The parallel changes occur because the parameters compensate for each other to produce minor changes in the free energy of the process (22). The expression of rate constant based on the transition state theory is represented by Equation 1.

The Gibbs free energy, ΔG, is expressed as:

$$\Delta G = \Delta H - T\Delta S = -RT \ln k \quad [2]$$

TABLE 1
Solvent Conditions for Separation of β-Carotene, Lycopene, and Lutein

Carotenoid		Flow (mL/min)	Methyl-*tert*-butyl ether (%)	Methanol (%)
β-Carotene (all-*trans* and 9-*cis*)	Isocratic	1.0	11	89
Lycopene	Gradient			
Time (min)	Initial	1.5	38	62
	7.0	1.5	38	62
	20.0	1.5	60	40
	22.0	1.5	60	40
	25.0	1.5	38	62
Lutein	Isocratic	1.0	5	95

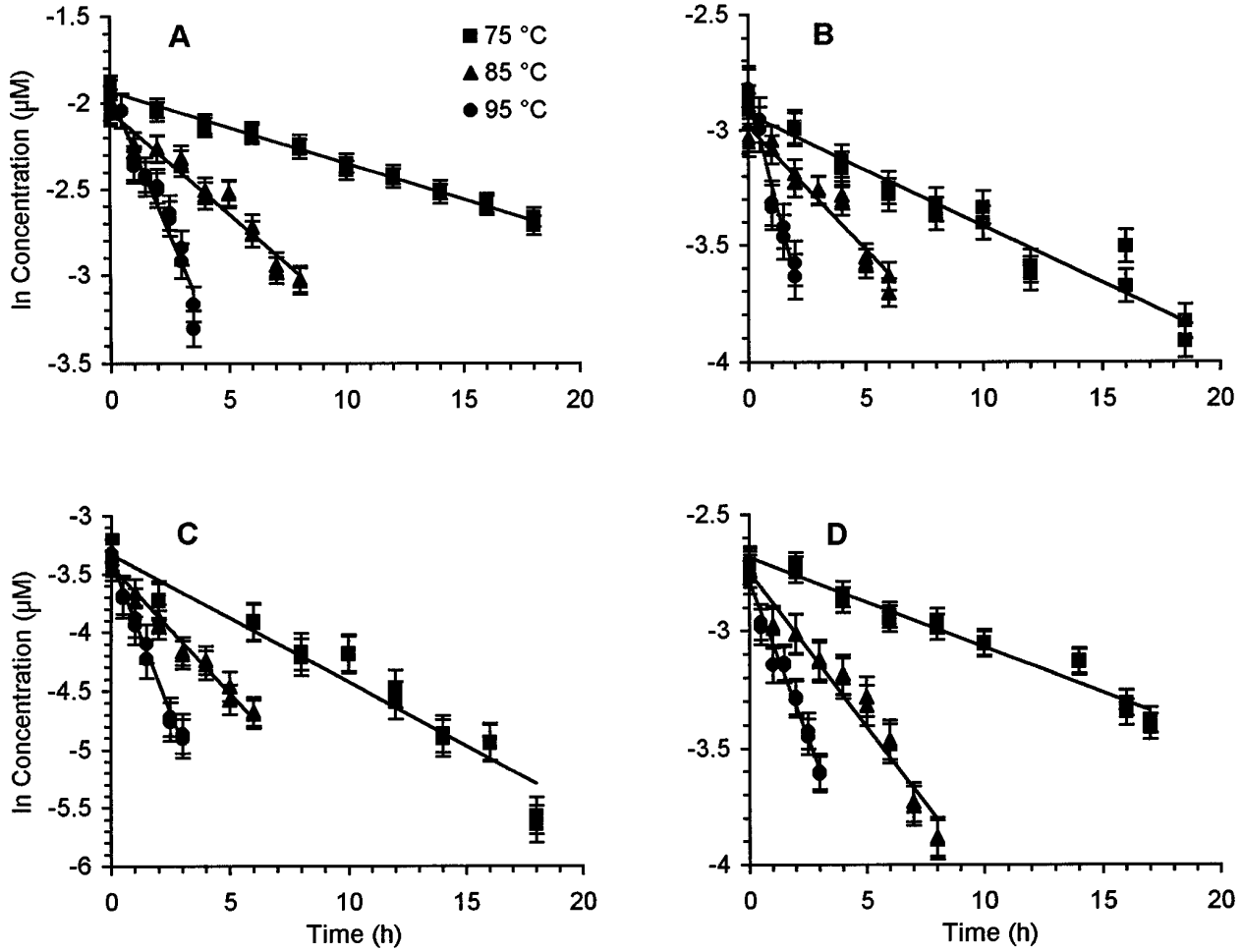

FIG. 2. Stability plots: (A) all-*trans* β-carotene, (B) 9-*cis* β-carotene, (C) lycopene, and (D) lutein heated in safflower seed oil.

Therefore, the following expression can be derived from Equations 1 and 2:

$$\Delta S = a\Delta H + b \quad [3]$$

where $a = 1/T_c$, $b = -\Delta G/T_c$. The term T_c is the isokinetic temperature, which is the temperature at which all of the reaction rate constants are equal. A linear relationship between enthalpy and entropy suggests that an isokinetic temperature exists (23).

Statistical analysis. The experiment was run with experimental units arranged as a complete randomized design. The treatments were arranged as a 3 × 4 factorial with factors given by compound (4 types) and temperature (3 levels). Two replicates for each treatment combination yielded a total of 24 experimental units (2 replicates × 3 temperatures × 4 compounds) for statistical analysis. The rate constants for the degradation of pigments were analyzed by the general linear models (GLM) procedure (24). The overall rate constants are the means of the three temperature-derived rate constants within each pigment. Error bars in the figures refer to standard errors.

TABLE 2
Reaction Rate Constants and Correlation Coefficients for β-Carotene, Lycopene, and Lutein Degradation[a,b]

Carotenoid[c]	Temperature (°C)	Rate constant (1/h)	R^2
β-Carotene[a] (all-*trans*)	75	0.042 ± 0.005	0.991
	85	0.119 ± 0.004	0.959
	95	0.326 ± 0.001	0.944
β-Carotene[a] (9-*cis*)	75	0.049 ± 0.005	0.945
	85	0.107 ± 0.002	0.924
	95	0.353 ± 0.019	0.973
Lycopene[b]	75	0.109 ± 0.000	0.945
	85	0.217 ± 0.000	0.973
	95	0.518 ± 0.003	0.990
Lutein[c]	75	0.038 ± 0.000	0.964
	85	0.132 ± 0.003	0.957
	95	0.268 ± 0.004	0.960

[a]Means ± standard error of means.
[b]The degradation kinetics for all of the carotenoids at each temperature followed a first-order kinetic model.
[c]Carotenoids followed by the same superscript letter are not significantly different ($P < 0.01$). The letters reflect the means of the three temperatures within each pigment.

RESULTS AND DISCUSSION

Degradation rate of carotenoids. Figure 2 illustrates the concentration of all-*trans* β-carotene, 9-*cis* β-carotene, lycopene, and lutein present (during heat treatment at 75, 85, and 95°C) over time. Although the samples were heated for 24, 12, and 5 h, data are reported on Figure 2 only up to 19, 8, and 3 h, because, beyond those times at each temperature, there were only trace amounts of the carotenoids remaining. The concentration values were zero and therefore not shown on the plot.

The results indicate that the oxidative degradation reaction of the carotenoids follows a first-order kinetic model, i.e., $\ln C = \ln C_0 - (k)(t)$. A first-order kinetic model has been previously reported for β-carotene degradation (9,12,13). The correlation coefficients, reaction rate constants, and their respective standard deviations are listed in Table 2.

Comparison of the overall degradation rates indicates that the rate constant for lycopene is approximately double that of the other pigments in this study. Lutein, a dihydroxy carotenoid, had the greatest stability of the carotenoids. There was no statistically significant difference in the rate of degradation between 9-*cis* β-carotene and all-*trans* β-carotene; therefore, geometric configuration does not appear to have an effect on the decomposition reaction rate. Although the relative stabilities of the β-carotene isomers did not differ under the experimental conditions, several authors have reported that *cis* and *trans* isomers may have distinct biological functions (25) and different antioxidant capacities (26,27).

Lycopene degraded more rapidly than the other carotenoids at all temperatures. This significant difference in degradation rates may relate to differences in lycopene's antioxidant activity when compared to other carotenoids. DiMascio *et al.* (28) reported that lycopene has twice the singlet oxygen quenching ability of β-carotene. This enhanced quenching ability may be related to a more efficient antioxidant capacity.

Temperature and compensation effect. The Arrhenius model and transition state theory were used to determine the influence of temperature on the reaction rates. The activation energy (E_a) and frequency factor ($\ln A$) were determined from the Arrhenius model, and enthalpy (ΔH) and entropy (ΔS) were obtained from transition state theory (Table 3).

TABLE 3
Activation Energies and Thermodynamic Parameters for β-Carotene, Lycopene, and Lutein Degradation[a]

Carotenoid	Activation energy (kcal/mol)[b]	Intercept $\ln A$	R^2	Enthalpy of activation (kcal/mol)[c]	Entropy of activation (cal/mol · K)[c]	R^2
β-Carotene (all-*trans*)	26.2 ± 0.41	3.6 ± 0.00	1.00	25.5 ± 0.41	8.05	1.00
β-Carotene (9-*cis*)	25.1 ± 4.87	3.5 ± 1.59	0.982	24.4 ± 4.87	5.0	0.981
Lycopene	19.8 ± 2.35	3.3 ± 0.87	0.993	19.1 ± 2.35	–8.5	0.993
Lutein	24.9 ± 4.90	3.5 ± 1.59	0.980	24.2 ± 4.90	4.4	0.979

[a]Means ± standard error of means.
[b]Values obtained from slopes of Arrhenius plot.
[c]Values obtained from slopes of transition state theory equations.

Figure 3 illustrates a plot of enthalpy of activation (ΔH) vs. entropy of activation (ΔS) which shows a linear compensation between the two thermodynamic parameters. The plot includes values for all of the carotenoids tested. The compensation effect suggests that each carotenoid is degraded by a similar mechanism (10,22). A compensation effect has also been reported for the degradation of chlorophylls, chlorophyllides (29), and numerous carotenoid pigments (10); acid hydrolysis of disaccharides (22); and the denaturation of whey proteins (30).

β-Carotene isomer formation. Figure 4 shows the loss of all-*trans* β-carotene and the predominant isomers formed during heating at 95°C. At the three temperatures evaluated (75°C and 85°C not shown), the 13-*cis* isomer was present in the highest concentration over the heating periods. The 9-*cis* and an unidentified *cis* isomer were present at a significantly lower level than 13-*cis* β-carotene. The geometric configuration of the isomer was identified by retention time on a C_{30} analytical column, from ultraviolet-visible (UV-vis) spectra, and by comparison to authentic standards (21,31,32). The UV-vis spectra of the unidentified β-carotene isomer closely resembled the spectra of β-carotene *cis* isomers but did not match those of authentic standards (33,34). Figure 5 is a chromatogram that shows the presence of the predominant isomers during the degradation reaction. Table 4 lists the UV-vis absorbance maxima for peaks 2–4 represented in Figure 5. An initial significant decrease in all-*trans* β-carotene was accompanied by a concurrent increase in the 13-*cis* isomer. Comparison of the unidentified and 9-*cis* isomers at the three temperatures indicates that the unidentified isomer was observed prior to an increase in the 9-*cis* isomer. Therefore, there are differences in the formation and degradation kinetics of the β-carotene isomers. Chandler and Schwartz (35) found that during heat processing, both 13-*cis* and 9-*cis* β-carotene isomers formed but 13-*cis* isomer predominated. Chen *et al.* (36) also studied the isomerization of β-carotene while heating and found that 13-*cis* was a major isomer and that the 15-*cis* and 9-*cis* isomers were present at low levels.

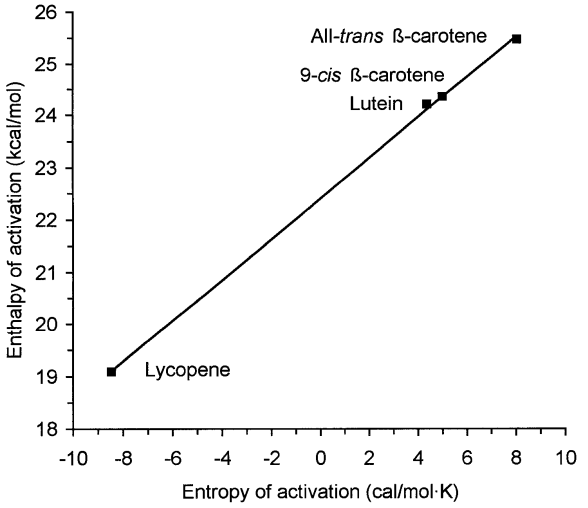

FIG. 3. Kinetic compensation effect of all-*trans* β-carotene, 9-*cis* β-carotene, lycopene, and lutein.

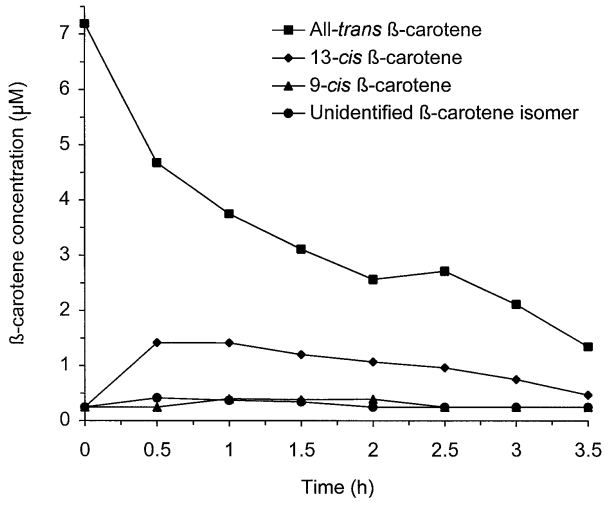

FIG. 4. Loss of all-*trans* β-carotene and predominant isomers formed during heating at 95°C.

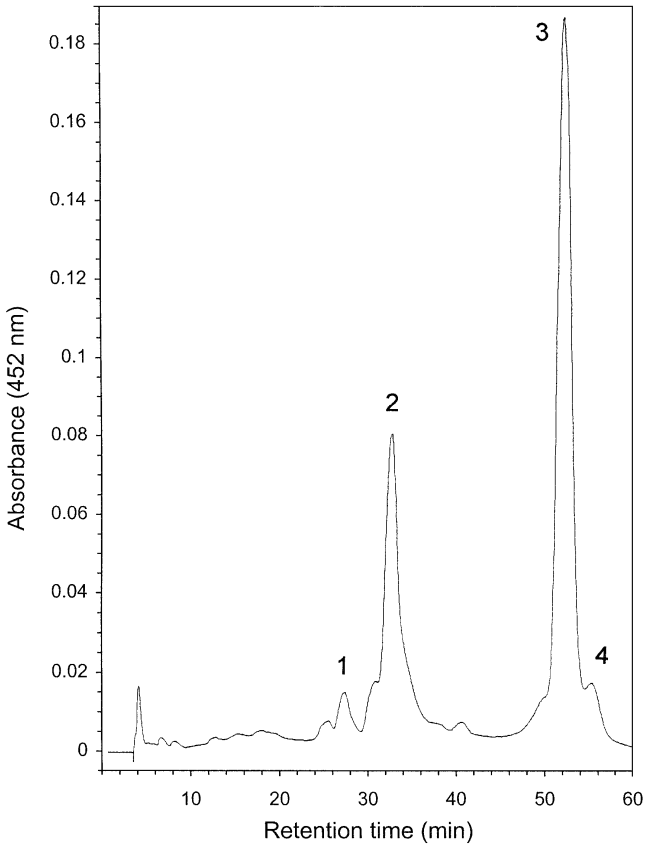

FIG. 5. Chromatographic separation of predominant isomers formed during heating of β-carotene in safflower seed oil (60 min 95°C): **1**, an unidentified *cis* β-carotene isomer; **2**, 13-*cis* β-carotene; **3**, all-*trans* β-carotene; and **4**, 9-*cis* β-carotene.

TABLE 4
Ultraviolet-Visible (UV-vis) Absorbance Maxima for β-Carotene and Selected Isomers

Peak[a]	Compound	UV-vis absorbance maxima (nm)[b]
2	13-*cis* β-carotene	339, 421, **446,** 473
3	All-*trans* β-carotene	425, **450,** 479
4	9-*cis* β-carotene	342, 423, **447**, 474

[a]The peak numbers are from Figure 5. See Experimental Procedures section for high-performance liquid chromatography methods.
[b]Absolute maxima are listed in bold.

Although numerous degradation products of lycopene and lutein were formed during exposure to the experimental conditions (data not shown), they were not monitored in this study. The degradation products of these compounds are presumably a combination of epoxides, apo-carotenals, and isomers.

In conclusion, thermal and oxidative stabilities in an oil model system vary among carotenoids. Lycopene was the most labile, indicative of reaction rate constants that were greater than those for the other carotenoids at all temperatures. Geometric configuration did not affect stability, because there was no significant difference between 9-*cis* and all-*trans* β-carotene. Lutein had the greatest stability of the carotenoids tested in the model system. Food systems are complex because of the numerous other compounds present. Compounds such as proteins, carbohydrates, and lipids may act as co-oxidants and promote oxidation or may have a protective effect. Therefore, results obtained in the pure model oil system may not be directly transposed to those observed in a more complex food matrix.

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