Degradation of Lycopene, α-Carotene, and β-Carotene During Lipid Peroxidation

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ABSTRACT: The stability and antioxidant effectiveness of lycopene, a-carotene, and b-carotene were compared during oxidation of methyl linoleate at 37 and 60 °C. Two carotene concentrations, 80 or 160 mg/g of methyl linoleate, were used to determine a concentration effect. At 37 °C, the degradation rates were: lycopene > β-carotene > α-carotene. Lycopene and α-carotene inhibited hydroperoxide formation, lycopene being the more effective antioxidant. β-Carotene inhibited hydroperoxide formation at the lower concentration but did not show an antioxidant effect at the higher concentration. At 60 °C, the carotenes degraded 6 to 8 times faster than at 37 °C and did not show an antioxidant effect. BHT or α-tocopherol effectively suppressed the hydroperoxide formation and degradation of the carotenes.

Key Words: lycopene, oxidation, carotenes, antioxidants

Introduction

EPIDEMIOLOGICAL STUDIES HAVE SHOWN THAT CONSUMPTION of vegetables and fruits high in α-carotene, β-carotene, and lycopene — such as carrots, spinach, and tomatoes — is associated with lower incidence of certain types of cancer, including lung, skin, and digestive tract cancers (Murakoshi and others 1992; Mayne and others 1994; Franceschi and others 1994; Steinmetz and Potter 1991). Carotenoids are considered to be antioxidants in biological tissue as well as in food systems.

β-Carotene was found to inhibit free-radical-mediated oxidation of methyl linoleate in solution (Burton and Ingold 1984; Terao 1989), in phosphatidylcholine liposomes (Kennedy and Liebler 1992), and in microsomal lipids (Palozza and Krinsky 1991). The antioxidant effect of β-carotene against lipid peroxidation was reported to be accompanied by degradation of the pigment and loss of color (Tsuchihashi and others 1995; Kennedy and Liebler 1992). BHT and α-tocopherol were used to prevent β-carotene degradation during lipid peroxidation in benzene solution and liposomal membranes (Tsuchihashi and others 1995). α-Tocopherol was also found to enhance the antioxidant effect of astaxanthin during peroxidation of liposomal phospholipids (Terao and others 1992). γ-Tocopherol was reported to protect the degradation of lutein and lycopene during oxidation of triglycerides (Haila and others 1996). The combination of γ-tocopherol with lutein or lycopene had an antioxidant effect, while lycopene (20 μg/g oil) alone acted as a prooxidant. The chemical and biological properties of lycopene have recently been reviewed (Nguyen and Schwartz 1999).

Carotenoids act as antioxidants against lipid peroxidation by quenching singlet oxygen and trapping free peroxyl radicals (Palozza and Krinsky 1992). Investigations have shown that singlet oxygen quenching ability of the carotenoids depends on their structural differences, such as number of conjugated double bonds, end groups (acyclic or cyclic), and substituent functional groups in the rings (Stahl and Sies 1996; Hirayama and others 1994). Di Mascio and others (1989) reported that the singlet oxygen quenching capacity of the carotenes was as follows: lycopene > α-carotene > β-carotene. In a study using 2,2'-azobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt as a free radical source, it was found that radical scavenging capacity of the carotenoids increased with the number of double bonds and the maximum overlap of the carbon-carbon double bond molecular orbitals (Miller and others 1996). Lycopene, α-carotene, and β-carotene have all 11 double bonds that in the straight molecule of lycopene fully overlap. Steric hindrance reduces the orbital overlap between the chain double bonds and the β-ring double bonds in the molecules of α- and β-carotene, leaving β-carotene with 9 fully overlapping double bonds plus 2 β-ring double bonds with reduced overlap. α-Carotene has 9 fully overlapping double bonds plus only one β-ring conjugated double bond, as the double bond of the ε-ring is not part of the conjugated double bond system. As a result, the order for their free radical scavenging abilities was: lycopene > β-carotene > α-carotene. Substituent functional groups on the rings were also found to affect radical scavenging activity. In the case of lycopene, the 2 terminal nonconjugated double bonds may also contribute to its radical scavenging capacity.

The purpose of this study was to compare the stability of lycopene, α-carotene, and β-carotene as related to their ability to inhibit lipid peroxidation. Such a study will contribute to better understanding of the role and stability of carotenoids during lipid oxidation and the loss of color of foods susceptible to lipid peroxidation. Further, the use of butylated hydroxy toluene (BHT) and α-tocopherol as potential agents against lipid peroxidation and carotenoid loss was studied.

Results and Discussion

Inhibition of Oxidation of Methyl Linoleate

To compare the effect of carotene concentration, hydroperoxide formation during oxidation of methyl linoleate at 37 and 60 °C was studied in the presence of 2 levels of lycopene, α-carotene, and β-carotene. These 2 temperatures were chosen for a comparison of antioxidant effectiveness with different rates of oxidation. The effects of 80 μg/g or 160 μg/g lycopene, α-carotene, and β-carotene on the hydroperoxide formation of 0.1 g methyl linoleate at 37 °C are shown in Fig. 1. The initial amount of hydroperoxides in the methyl linoleate was 0.02% to 0.04%. Hydroperoxides were formed in all samples from the beginning of the reaction without an observed induction period. In all cases, hydroperoxide formation was inhibited in the presence of carotenes.

Hydroperoxide formation at 37 °C followed a first order kinetic model ($\ln A = \ln A_0 - kt$). This is consistent with the first order reaction model reported for the oxidation of methyl linoleate at 37 °C (Labuza 1971). The use of apparent first order rate constants, k, allows for the comparison of reaction rates and can be used to

judge the relative effectiveness of various antioxidants. The rate constants of hydroperoxide formation and their respective standard errors are shown in Table 1. The carotenes inhibited the hydroperoxide formation as follows: lycopene > α-carotene > β-carotene. The rate constants of hydroperoxide formation in the presence of each individual carotene at the lower and higher concentrations did not differ significantly. Nevertheless, data in Table 1 show that there was a tendency of increased rate constants with the increase of α- and β-carotene concentration. This tendency did not affect the antioxidant efficiency of lycopene and α-carotene as they inhibited hydroperoxide formation at both concentrations. It suggests that higher carotene concentrations did not provide additional antioxidant activity and α- and β-carotene could potentially become pro-oxidants at higher concentrations. In the case of β-carotene, the increased rate constant of hydroperoxide formation at the higher concentration of β-carotene overlapped with that of the control sample without carotenes. Therefore, β-carotene inhibited hydroperoxide formation at concentration 80 μg/g but did not show an antioxidant effect at the higher concentration of 160 μg/g.

Monitoring β-carotene degradation during hydroperoxide formation by HPLC showed that the higher concentration of β-carotene produced increased amount of epoxy derivatives. Epoxide formation is accompanied by release of an alcoxyl radical that may continue the chain oxidation (Kennedy and Liebler 1992, Tsuchihashi and others 1995). Also, more β-carotene peroxyl radicals are formed at higher concentrations of β-carotene. Both epoxide formation and β-carotene peroxyl radical formation enhance the chain oxidation, which may explain the observed decrease of β-carotene antioxidant effectiveness at the higher concentration.

Table 1—First order rate constants for hydroperoxide formation during oxidation of methyl linoleate in hexane at 37 °C in the dark in the absence or presence of carotenes (R^2 = 0.904 for the fit of the multiple regression model to the data set)

Carotene	Rate Constant, k, × 10^{-2} hr^{-1}
Lycopene, 80 μg/g	0.508 a
Lycopene, 160 μg/g	0.511 a
α-Carotene, 80 μg/g	0.686 ab
α-Carotene, 160 μg/g	0.740 b
β-Carotene, 80 μg/g	0.922 bc
β-Carotene, 160 μg/g	1.150 cd
Methyl linoleate without carotenes (control)	1.180 d

Rates with different letters are significantly different ($p < 0.05$).
Standard error for the rate constant determination was 0.061 to 0.062.

Our results showing increased antioxidant effectiveness of lycopene as compared to α- and β-carotene are consistent with the reported increased reactivity of lycopene towards singlet oxygen quenching and free radical scavenging (Di Mascio and others 1989; Miller and others 1996). In this study, α-carotene inhibited lipid peroxidation more effectively than β-carotene. The higher singlet oxygen quenching ability of α-carotene (Miller and others 1996) and the effect of the nonconjugated double bond in the α-ionone ring of α-carotene may have enhanced its antioxidant effectiveness. Using 2,2'-azobis(2,4-dimethylvaleronitrile) as radical initiator, β-carotene was reported to increase its antioxidant effectiveness at higher initial concentrations in benzene and in

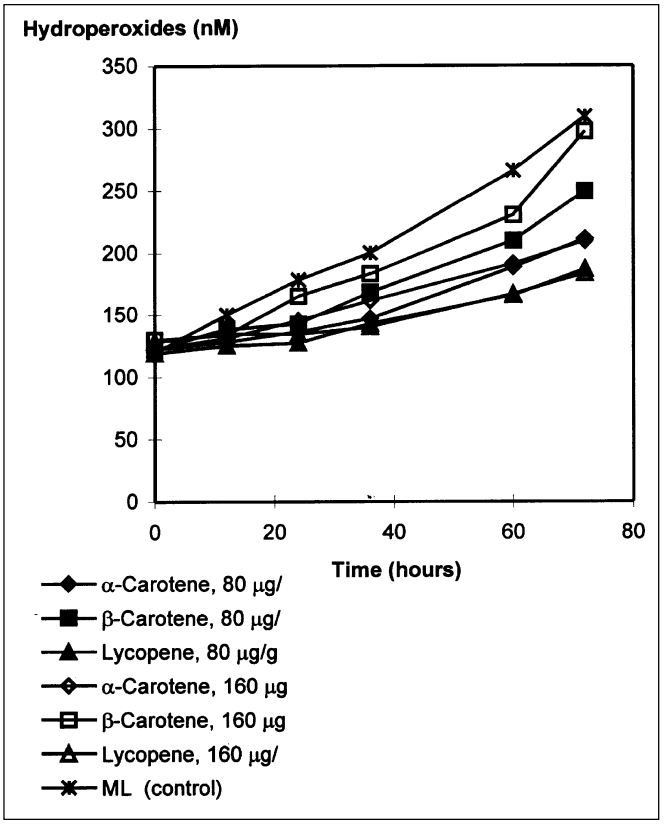

Fig. 1—Hydroperoxide formation during the oxidation of methyl linoleate (ML) in hexane at 37 °C under air in the dark in the absence and presence of carotenes. Each point represents the mean of 2 experiments.

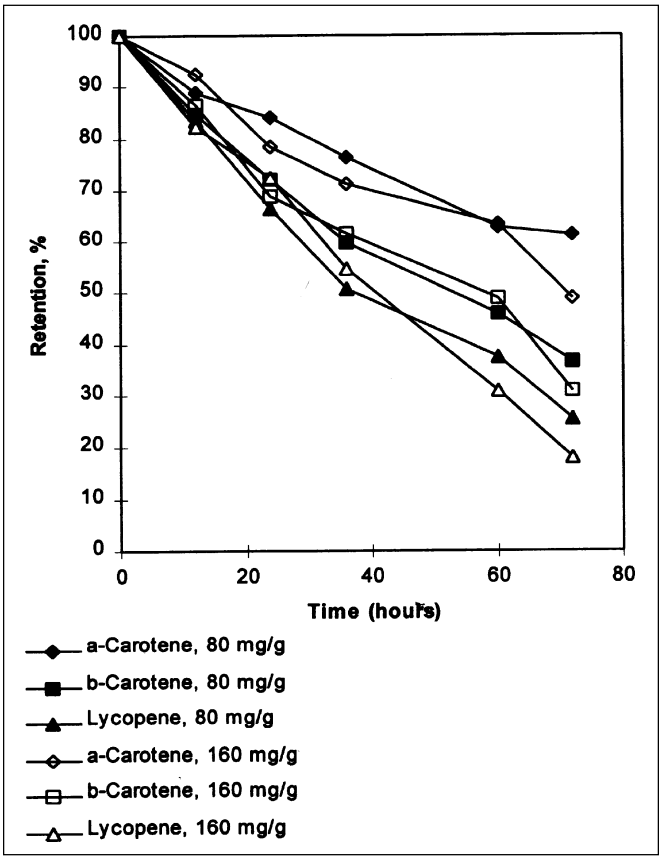

Fig. 2—Carotene degradation during the oxidation of methyl linoleate in hexane at 37 °C under air in the dark. Each point represents the mean of 2 experiments.

Table 2—First order rate constants for carotene degradation during oxidation of methyl linoleate in hexane at 37 °C in the dark. (R^2 = 0.998 for the fit of the multiple regression model to the data set)

Carotene	Rate Constant, k, × 10^{-2} hr^{-1}
Lycopene, 160 μg/g	2.116 a
Lycopene, 80 μg/g	1.823 ab
β-Carotene, 160 μg/g	1.487 b
β-Carotene, 80 μg/g	1.447 b
α-Carotene, 160 μg/g	0.923 c
α-Carotene, 80 μg/g	0.751 c

Rates with different letters are significantly different ($p < 0.05$).
Standard error for the rate constant determination was 0.098.

Table 3—First order rate constants for carotene degradation during oxidation of methyl linoleate in hexane at 60 °C in the dark. (R^2 = 0.908 for the fit of the multiple regression model to the data set)

Carotene	Rate Constant, k, × hr^{-1}
Lycopene, 160 μg/g	0.167 a
Lycopene, 80 μg/g	0.131 a
β-Carotene, 160 μg/g	0.089 b
β-Carotene, 80 μg/g	0.084 b
α-Carotene, 160 μg/g	0.075 b
α-Carotene, 80 μg/g	0.063 b

Rates with different letters are significantly different ($p < 0.05$).
Standard error for the rate constant determination was 0.009 except of 80 ug/g of α-carotene which was 0.012.

microsomal lipids (Tsuchihashi and others 1995; Palozza and Krinsky 1991). These findings are opposite to those observed in this study showing a decrease of the antioxidant effectiveness of β-carotene with increase of concentration. These differences may be due to differences in experimental conditions, such as solvents, concentration of the lipids, and β-carotene, and the presence of a radical initiator.

At 60 °C, hydroperoxide formation proceeded from 4 to 10 times faster than at 37 °C and followed a first order reaction model. The rate constants of hydroperoxide formation of the samples containing α-, β-carotene, and lycopene were from 4.3 to 5.2 to × 10^{-2} h^{-1} and did not differ significantly from the rate constant of the control sample without carotenes (5.2 × 10^{-2} h^{-1}). Therefore, at 60 °C, none of the carotenes had an inhibitory effect on the hydroperoxide formation. These results suggest that the carotenes could not effectively inhibit hydroperoxide formation at higher temperatures where the oxidation reaction was much more extensive. The increased temperature may have also decreased the carotene antioxidant efficiency by increasing the reactivity of the carotene resonance-stabilized radical adducts.

Carotene degradation

Figure 2 shows the percent of each carotene remaining over time after oxidation of methyl linoleate at 37 °C. After 72 h, lycopene had degraded by 74% to 84 %, b-carotene had degraded by 64% to 68%, and α-carotene had degraded by 36% to 50%. Degradation of α- and β-carotene was accompanied by the formation of oxygenated derivatives eluting in the HPLC analysis as single peaks before the corresponding carotenes (450 nm). Similarly, hydroxy- and epoxycarotenes were reported to elute before the corresponding carotenes on a C-18 reverse phase column (Khachik and others 1992). The degradation product of β-carotene was tentatively identified as 5,8-epoxy-5,8-dihydro-β,β-carotene by comparing the UV-vis spectrum with data from previous publications ($C_{40}H_{56}O$: UV-vis maxima at λ 405, 426, and 452) (Kennedy and Liebler 1992).

The degradation reaction of the carotenes at 37 °C followed a first order kinetic model, and this is consistent with the first order degradation of α- and β-carotene reported in previous studies (Pesek and Warthesen 1987; Wagner and Warthesen 1995). Table 2 shows the degradation rate constants of the carotenes at

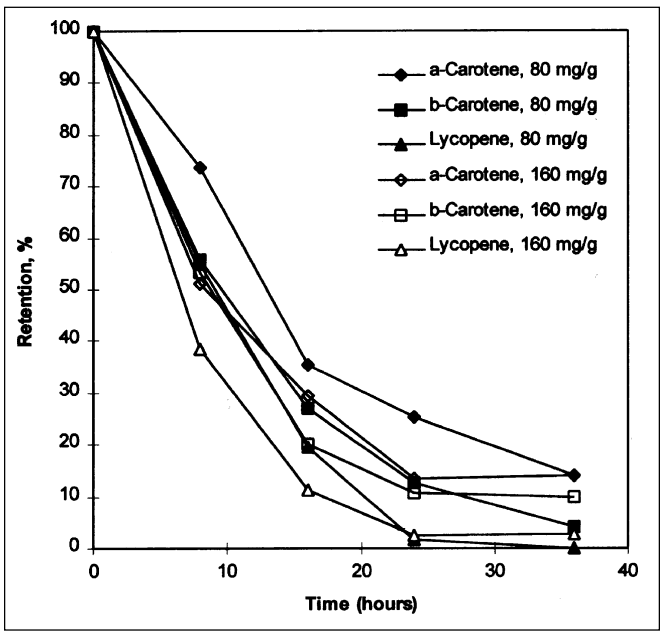

Fig. 3—Carotene degradation during the oxidation of methyl linoleate in hexane at 60 °C under air in the dark. Each point represents the mean of 2 experiments.

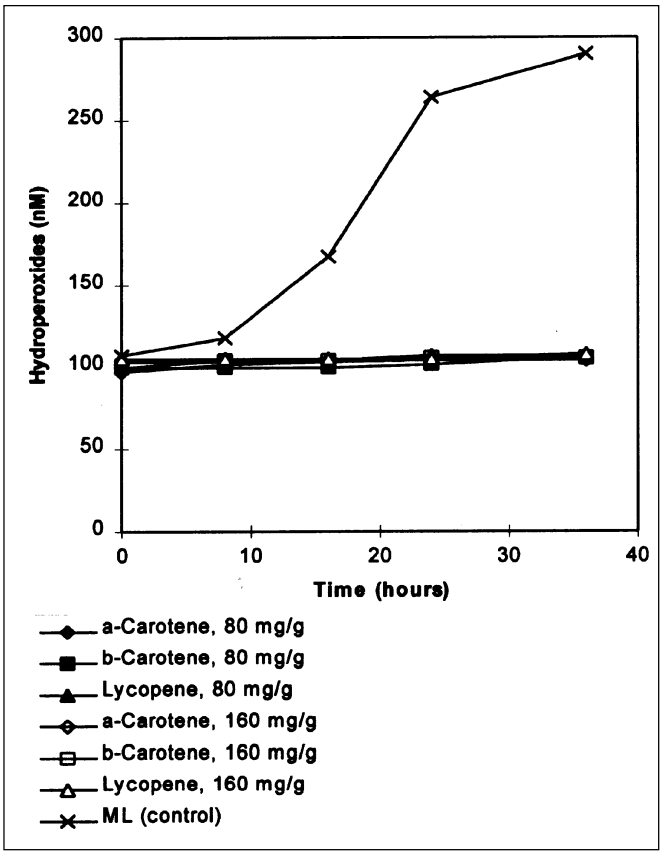

Fig. 4—Effect of 80 mg/g BHT on the oxidation of methyl linoleate (ML) in hexane at 60 °C under air in the dark in the absence and presence of carotenes. Each point represents the mean of 2 experiments.

37 °C. Lycopene degraded with the fastest rate, which may be related to its increased reactivity towards singlet oxygen and free peroxyl radicals as compared to α- and β-carotene. β-Carotene degraded with a lower rate than lycopene but faster than α-carotene. The faster degradation of β-carotene may be related to the higher reactivity of β-carotene radical adduct.

The higher concentration of β-carotene did not degrade faster than the low concentration. At the same time, the higher concentration of β-carotene did not inhibit hydroperoxide formation. This is consistent with the reported finding that when methyl linoleate is present in excess of β-carotene, the peroxyl radicals react more readily with methyl linoleate than with β-carotene and the degradation of β-carotene slows down (Tsuchihachi and others 1995). Therefore, at the higher concentration, β-carotene was acting as a prooxidant, but its autoxidation (loss of color) did not proceed faster.

At 60 °C, the carotenes degraded 6 to 8 times as fast as at 37 °C (Fig. 3). After 36 h, lycopene had almost completely degraded, β-carotene had degraded by 90% to 95%, and α-carotene had degraded by 85% to 90%. The first order degradation rate constants are shown in Table 3. The rates of degradation of the carotenes were as follows: lycopene > β-carotene ≃ α-carotene. Similarly to the degradation at 37 °C, lycopene degraded faster than α- and β-carotene, and this is consistent with its increased reactivity towards singlet oxygen and free peroxyl radicals. At 60 °C the degradation rates of α- and β-carotene did not differ significantly. This suggests that at the higher temperature, the reactivities of the radical adducts of α- and β-carotene were very close, and the carotenes degraded with almost the same rates.

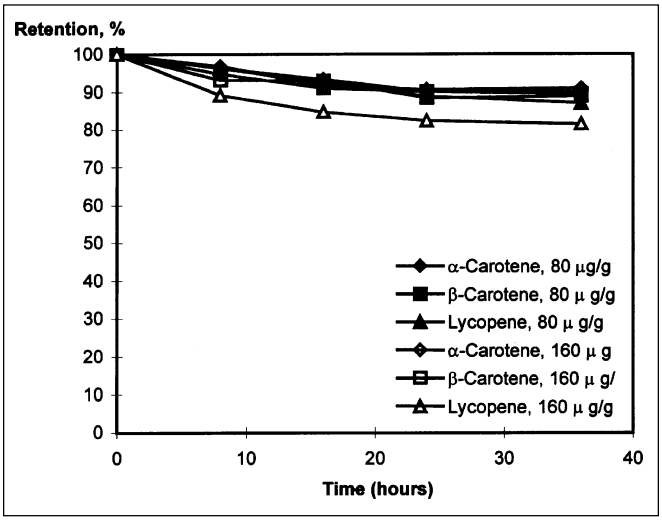

Fig. 5—Effect of 80 mg/g BHT on carotene degradation during the oxidation of methyl linoleate in hexane at 60 °C under air in the dark. Each point represents the mean of 2 experiments.

Effect of BHT or α-tocopherol on hydroperoxide formation and carotenoid degradation

The combined action of the carotenes and α-tocopherol or BHT on the oxidation of methyl linoleate was studied at 60 °C. Lycopene, α-, and β-carotene degraded very fast and did not inhibit hydroperoxide formation when added individually at the lower or higher concentration. Figure 4 shows that in the presence of carotenes (80 μg/g or 160 μg/g), 80 μg/g BHT suppressed efficiently hydroperoxide formation. The same effect was observed when using 40 μg/g α-tocopherol instead of BHT. Furthermore, in the presence of BHT or α-tocopherol, the carotene degradation was significantly slowed down. Lycopene, α-, and β-carotene degraded by only 10% to 18% as compared to more than 90% loss in the absence of BHT or α-tocopherol (Fig. 5).

Our results are in agreement with other studies reporting that tocopherols prolonged the inhibitory effect of β-carotene on singlet oxygen initiated photooxidation by preventing the decomposition of β-carotene (Terao and others 1980). α-Tocopherol and BHT were also reported to be effective antioxidants against oxidation of methyl linoleate at 50 °C in the dark (Terao and Matsushita 1986). BHT and α-tocopherol were used to protect β-carotene degradation during extrusion cooking. At the high temperature of extrusion (170 to 185 °C) 500 ppm α-tocopherol were needed to exhibit the protective effect of 50 ppm BHT (Berset and Marty 1992). On the other hand, it was reported that very low levels of carotenoids prolonged the lifetime of α-tocopherol during photooxidation of membrane phospholipids (Ojima and others 1993). A possible mechanism for the protection of the carotenes by tocopherols (and vice versa) could be a competition between the tocopherols and the carotenes for the peroxyl radicals and reactive oxygen species (Palozza and Krinsky 1991).

Our results suggest that BHT and α-tocopherol may be used as protective agents against carotene loss in lipid-containing foods and during processing at elevated temperatures.

Materials and Methods

LYCOPENE, α-CAROTENE, β-CAROTENE, AND α-TOCOPHEROL were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Methyl linoleate (99%) was also obtained from Sigma Chemical Co. For preparing stock solutions of the carotenes, approximately 1.00 mg (all 3 have the same molecular weight) of the *all-trans* standard was dissolved in 3 drops of chloroform and brought to volume (100 ml) with hexane. To prevent isomerization of the carotenoids during storage, the stock solutions were divided into smaller portions and evaporated under nitrogen for storage in the dark at −18 °C (Scott 1992). Before every experiment, an appropriate amount of the carotenoid was redissolved in hexane. Stock solutions of α-tocopherol were prepared in hexane immediately prior to sample preparation.

All solvents used were high-performance liquid chromatography (HPLC) grade. All procedures with carotenoids were performed under reduced light.

Oxidation of methyl linoleate

The oxidation of methyl linoleate (0.52 m molar in hexane) was carried out in 2.4 ml hexane in the presence or absence of 80 μg or 160 μg carotenes per gram of methyl linoleate with or without an additional antioxidant (BHT at 80 μg/g methyl linoleate or α-tocopherol at 40 μg/g methyl linoleate). The molar ratios of the antioxidants per mole of methyl linoleate were 4.4×10^{-5} and 8.8×10^{-5} for the 2 levels of carotenes, 1.06×10^{-4} for BHT, and 2.73×10^{-5} for α-tocopherol. Samples were held at 37 or 60 °C in the dark in crimped cap glass vials. Hexane was used as a solvent for the reaction because it provided solubility for the carotenes and methyl linoleate without promoting oxidation. Without total solubility of the carotenes, comparisons of effectiveness would not be as valid. Oxidation was followed by reverse-phase HPLC by monitoring of methyl linoleate hydroperoxides (Chan and Levett 1977) measuring their absorbance at 234 nm. A C18 (201TP540) analytical column (5

μm, 25 cm × 4.6 mm; VYDAC, Hesperia, Calif., U.S.A.) was used with a 20-μl injection loop. Solvent delivery of acetonitrile/methanol/2-propanol (44/54/2 by vol) was achieved with Spectra Physics SP8800 System at a flow rate 1 ml/min. Elution was monitored at 234 nm with Diode Array 1040A Hewlett Packard absorbance detector. The *cis-trans-* and *trans-trans* methyl linoleate hydroperoxides eluted at 2.8 and 3.1 min, respectively. Identification was based on their spectra (Chan and Levett 1977).

Carotene Degradation

Carotene degradation was followed spectrophotometrically (UV-Visible Spectrophotometer, Beckman Instruments, Inc., Irvine, Calif., U.S.A.) by the absorbance at 450 nm for α- and β-carotene and 470 nm for lycopene.

Data Analysis

The reaction order for the hydroperoxide formation and degradation of lycopene, α-, and β-carotene were determined using *Water Analyzer Series-Reaction Kinetics Program* (Labuza and others 1991). To calculate the rate constants of hydroperoxide formation and carotenoid degradation, multiple regression models were fitted to the data sets at each studied temperature (Weisberg 1985). Statistical differences among the rate constants were determined by multiple comparison ($p < 0.05$). All experiments were performed 2 times.

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