Effects of β-Carotene and Lycopene Thermal Degradation Products on the Oxidative Stability of Soybean Oil

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ABSTRACT: The relative oxidative stability of soybean oil samples containing either thermally degraded β-carotene or lycopene was determined by measuring peroxide value (PV) and headspace oxygen depletion (HOD) every 4 h for 24 h. Soybean oil samples containing 50 ppm degraded β-carotene that were stored in the dark at 60°C displayed significantly ($P < 0.01$) higher HOD values compared with controls. Lycopene degradation products (50 ppm) in soybean oil significantly ($P < 0.05$) decreased HOD of samples when stored in the dark. PV and HOD values for samples containing 50 ppm of either β-carotene or lycopene degradation products stored under lighted conditions did not differ significantly from controls ($P > 0.05$). However, soybean oil samples containing 50 ppm of unheated, all-*trans* β-carotene or lycopene stored under light showed significantly lower PV and HOD values than controls ($P < 0.01$). These results indicated that during autoxidation of soybean oil held in the dark, β-carotene thermal degradation products acted as a prooxidant, while thermally degraded lycopene displayed antioxidant activity in similar soybean oil systems. In addition, β-carotene and lycopene degradation products exposed to singlet oxygen oxidation under light did not increase or decrease the oxidative stability of their respective soybean oil samples.

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KEY WORDS: Beta-carotene, lycopene, oxidative stability, soybean oil.

In recent decades, soybean oil production has seen dramatic growth as a result of its widespread availability and relatively low cost. Today, soybean oil accounts for over 75% of total vegetable oil consumption in the United States, with total production exceeding its nearest competitor, cottonseed oil, by nearly 10-fold (1). A common drawback to the use of soybean oil in processed foods, however, is its degree of unsaturation and resulting susceptibility to lipid oxidation.

Carotenoids are a group of lipid-soluble pigments containing multiple conjugated double bonds that have been shown to be effective antioxidants owing to their ability to reduce excited singlet oxygen back down to its less reactive triplet state. Lee and Min (2) determined that the total singlet oxygen quenching rate of the five carotenoids lutein, zeaxanthin, lycopene, isozeaxanthin, and astaxanthin increased as their conjugated double bond count increased. The carotenoids β-carotene and lycopene, which both possess polyene systems consisting of 11 conjugated double bonds, have been reported to be particularly effective at quenching singlet oxygen (2–6). Foote and Denny (7) first reported that singlet oxygen could be effectively quenched by low concentrations of β-carotene. Di Mascio *et al.* (5) found that lycopene was the most efficient biological carotenoid singlet oxygen quencher, with a quenching rate constant of $31 \times 10^9\ M^{-1}\ s^{-1}$, over twice that of β-carotene ($14 \times 10^9\ M^{-1}\ s^{-1}$). Matsushita and Terao (3), however, reported that β-carotene's singlet oxygen quenching rate was higher than that reported for lycopene. Lee and Min (8) examined the effects of 0, 5, 10, and 20 ppm β-Carotene on the oxidation of a soybean oil/methylene chloride model system containing 4 ppm chlorophyll. β-Carotene reduced the oxidation of soybean oil at every concentration, and most effectively at the highest concentration of 20 ppm. Warner and Frankel (9), however, found that at levels ≥20 ppm, β-carotene contributed to poor flavor and color in soybean oil, whereas 5 to 10 ppm β-carotene reduced the photosensitized oxidation of the oil without decreasing oil quality.

Although carotenoids such as β-carotene and lycopene can help minimize singlet oxygen-catalyzed lipid oxidation in food products, their ability also to act as effective antioxidants through free-radical trapping mechanisms has not been as thoroughly evaluated. Though β-carotene does not have the structural features commonly associated with chain-breaking antioxidants, Burton (10) claimed that, given low oxygen partial pressures, β-carotene had the potential to act as a lipid-soluble chain-breaking antioxidant. Burton and Ingold (11) found, however, that at oxygen pressures of 150 torr or higher, β-carotene and related compounds could actually act as prooxidants in a methyl linoleate model system.

While the extended polyene structure of carotenoid pigments provides them with valuable antioxidant properties, it also causes the compounds to be easily degraded in the presence of light and/or heat (12–14). Because carotenoids such as β-carotene are widely used in industry as food colorants and/or sources of vitamin A, light or thermally induced degradation (and resulting loss of color and vitamin destruction) during or postprocessing is of great concern to food manufac-

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turers and nutritionists. Although β-carotene and lycopene can be easily degraded in this manner, very little information has been reported regarding the effects of the degraded carotenoids themselves on the oxidative stability of lipid systems. Therefore, in the present study, the oxidative stability of soybean oil samples stored either in lighted or dark conditions containing different concentrations of thermally degraded β-carotene or lycopene has been investigated.

EXPERIMENTAL PROCEDURES

Materials. β-Carotene, lycopene, and chlorophyll standards were obtained from Sigma Chemical Company (St. Louis, MO). Lycopene (>98% pure) was donated by LycoRed Natural Products Industries (Beer-Sheva, Israel). High-performance liquid chromatography (HPLC) grade methanol, methyl-*tert*-butyl ether, acetonitrile, and chloroform were purchased from Fisher Scientific (Pittsburgh, PA). Refined, bleached, and deodorized soybean oil was obtained from Abitec Corp. (Columbus, OH).

Thermal treatment of β-carotene and lycopene. A stock solution of 500 ppm β-carotene or lycopene solubilized in acetone was added to a glass vial that was then sealed airtight with a Teflon-faced rubber septum and aluminum cap (Supelco, Inc., Bellefonte, PA). A portion of the unheated 500 ppm stock solution was saved. The remaining portion in the glass vial was wrapped in aluminum foil to minimize light exposure, then placed in a water bath set at 90°C to allow thermal degradation of the carotene to occur. After the β-carotene or lycopene solution was thermally degraded, the sealed glass vial was removed from the water bath and allowed to cool to room temperature. Out of the cooled glass vial, the stock solution containing 500 ppm degraded products was collected and stored alongside the 500 ppm unheated stock solution in a freezer at –4°C for further use.

Verification of β-carotene, lycopene, and their thermal degradation by HPLC. Unheated 500 ppm stock solutions of either all-*trans* β-carotene or lycopene, along with 500 ppm solutions of either β-carotene or lycopene degradation products, were separated, verified, and quantified using a Hewlett-Packard Series 1050 HPLC system and Hewlett-Packard 3396A integrator (Palo Alto, CA). The retention time and peak size of Sigma β-carotene and lycopene standards were used for verification and quantification, respectively, of observed chromatogram peaks. An ultraviolet detector was set at 460 nm for quantitation of β-carotene, 472 nm for quantitation of lycopene, and 350 nm for quantitation of major β-carotene or lycopene thermal degradation products. Isocratic reversed-phase liquid chromatography was conducted utilizing a Hewlett-Packard ODS Hypersil analytical column (5 µm, 200 × 4.6 mm) and a mobile phase solvent system of methanol/ methyl-*tert*-butyl-ether (90:10). The flow rate for each run was 1 mL/min with a total run time of 20 min.

Sample preparation and storage for soybean oil containing β-carotene or lycopene thermal degradation products stored under the light. Aliquots from the two stock solutions of 500 ppm unheated β-carotene or lycopene, and 500 ppm degraded β-carotene or lycopene, respectively, were mixed to obtain the following three solution concentrations: (i) 250 ppm carotene + 250 ppm degraded products; (ii) 50 ppm carotene + 450 ppm degraded products; and (iii) 0 ppm carotene + 500 ppm degraded products. Exactly 2.5 mL of each solution was added in triplicate to 30 mL glass serum vials containing 22.5 mL of purified soybean oil and 3 ppm chlorophyll sensitizer. The resulting 25 mL soybean oil solutions then contained one of three final concentrations: (i) 25 ppm carotene + 25 ppm degraded products; (ii) 5 ppm carotene + 45 ppm degraded products; or (iii) 0 ppm carotene + 50 ppm degraded products. In addition, 2.5, 1.25, or 0.25 mL of 500 ppm unheated β-carotene or lycopene were added to vials containing soybean oil and chlorophyll to create 25-mL control solutions containing either 50, 25, or 5 ppm of either lycopene or β-carotene. Other controls included samples containing only oil, or oil and chlorophyll. Each vial was then sealed airtight (for headspace oxygen content determination only) with Teflon-faced rubber septa and aluminum caps and stored at 25°C in a mirrored light box (70 × 50 × 60 cm) for 24 h. The light source itself consisted of a 100-W fluorescent lamp (Lights of America, Inc., Walnut, CA) with an intensity of 1,650 lumens. The oxidative stability of the sample was determined by measuring both peroxide value (PV) (15) and the headspace oxygen content of the sample vial by thermal conductivity gas chromatography every 4 h during the aforementioned 24-h period.

Sample preparation and storage for soybean oil containing β-carotene or lycopene thermal degradation products stored in the dark. To study the effects of degraded carotenes on the oxidative stability of thermally treated soybean oil, 2.5-mL acetone solutions containing either 500 ppm β-carotene or lycopene, or 0 ppm carotene + 500 ppm degraded β-carotene or lycopene were added in triplicate to 30-mL glass serum vials containing 22.5 mL soybean oil, respectively. Each vial (including controls containing 22.5 mL soybean oil and 2.5 mL acetone) was then sealed airtight with Teflon-faced rubber septa and aluminum caps and stored at 60°C in the dark for 8 d. The oxidative stability of the sample was determined by measuring the headspace oxygen content of the sample vial by thermal conductivity gas chromatography after 8 d of storage at 60°C.

Headspace oxygen analysis. A Hewlett-Packard 5890 gas chromatograph (GC) equipped with a thermal conductivity detector and a stainless steel molecular sieve packed column (13×, 80/100; Alltech, Deerfield, IL) was utilized to determine the extent of headspace oxygen depletion (HOD) (and corresponding soybean oil oxidation). High purity (99.995%) helium gas was used as both the carrier and auxiliary gas at a rate of 30 mL/min. The injector, detector, and oven temperatures were set to 120, 150, and 40°C, respectively. Every 4 h for 24 h, 100 µL of headspace air from each sealed vial was manually injected into the GC using an airtight syringe. Ambient air was used as a reference of O_2 content to correct for day-to-day chromatographic variability. A Hewlett-Packard

HP 3396A integrator (Avondale, PA) was used to record the oxygen peak in electronic counts (16).

Statistical analysis. Two-sample *t*-test and one-way analysis of variance were used to determine the presence of differences ($\alpha = 0.05$) between two samples and groups of samples, respectively. Tukey's Studentized Range Test determined which particular sample groups were different at $\alpha = 0.05$. All the aforementioned statistical analyses were calculated by using the Minitab Version 10.1 for Windows statistical software package (6).

RESULTS AND DISCUSSION

Degraded β-carotene in the light. The control sample containing 50 ppm unheated all-*trans* β-carotene (B-car 50 ppm) had significantly lower ($P < 0.05$) HOD (Fig. 1) and PV (Fig. 2) after 24 h when compared with all other samples, which indicated that there was less oxygen uptake and subsequent oxidation of the oil. This result was not surprising considering the numerous studies citing β-carotene as an effective quencher of singlet oxygen (7,17), particularly in soybean oil model systems exposed to light (6,9). Control samples containing 5 or 25 ppm unheated all-*trans* β-carotene (B-car 5 ppm or B-car 25 ppm, respectively) were not significantly different in HOD or PV after 24 h when compared to the samples containing 5 or 25 ppm all-*trans* β-carotene + 45 or 25 ppm degraded products (5 + 45 or 25 + 25, respectively). The only dissimilarity between the 5 and 25 ppm all-*trans* β-carotene controls and their corresponding 5 + 45 or 25 + 25 degraded mixtures was the presence of degraded β-carotene in the non-control samples. Therefore, the degraded β-carotene compounds in this case did not act as either prooxidants or antioxidants in the soybean oil model system, but rather had no overall effect on the soybean oil oxidative stability under the light.

Of particular interest was the relationship in HOD and PV values between the sample containing 50 ppm degraded β-carotene (0 + 50), and the control samples containing 50 ppm all-*trans* β-carotene (B-car 50 ppm) and oil and 3 ppm chlorophyll [oil + chloro (light)], respectively. The 50 ppm degraded β-carotene sample showed a significantly higher ($P < 0.05$) level of HOD after 24 h than all the other samples (Fig. 1) except for the oil and chlorophyll only control. Similar results were seen when measuring PV (Fig. 2). Obviously, the degradation of the β-carotene polyene system from 50 ppm all-*trans* β-carotene to 50 ppm degraded products caused a dramatic change in the effects of the molecule on soybean oil stability. With the polyene system intact in the 50 ppm all-*trans* sample (B-car 50 ppm), HOD was significantly reduced. After the thermal destruction of the polyene system in the 50 ppm degraded sample (0 + 50), however, HOD and PV were much higher. This indicated that the loss of the extensive conjugated double bonds present in all-*trans* β-carotene by heating, eliminated its ability to quench singlet oxygen and prevent photosensitized oxidation of the soybean oil. Similarly, Lee and Min (2) reported a decrease in the singlet oxygen quenching rate of five carotenoids as their respective conjugated double bond counts decreased. The 50 ppm degraded β-carotene sample not only provided no antioxidant activity in the form of singlet oxygen quenching, but in fact caused a slight, though not significant, prooxidant effect when compared with the control containing only oil and chlorophyll (Fig. 1).

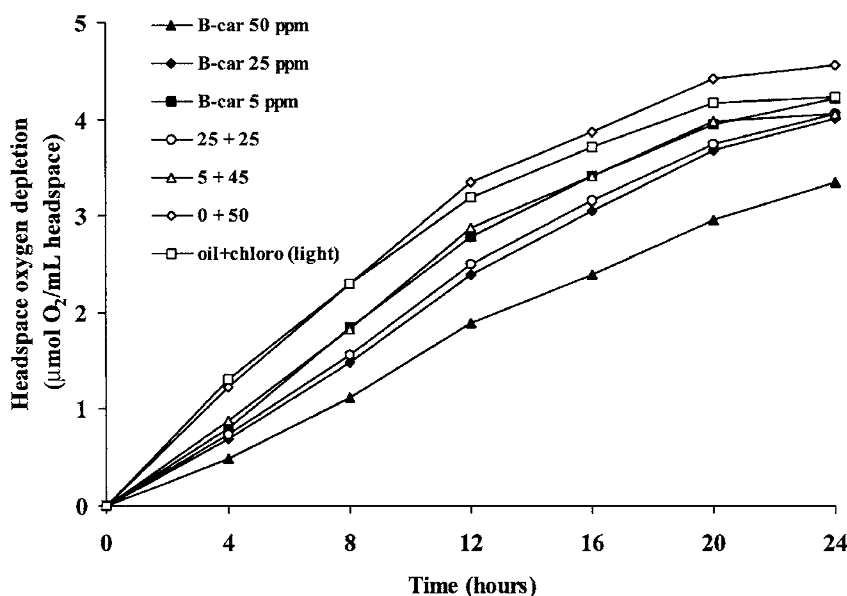

FIG. 1. Effects of β-carotene and thermally degraded β-carotene on the headspace oxygen depletion of soybean oil containing 3 ppm chlorophyll under light storage at 25°C. 25 + 25 indicates a solution containing 25 ppm all-*trans* β-carotene and 25 ppm degraded β-carotene; 5 + 45 contains 5 ppm all-*trans* β-carotene and 45 ppm degraded β-carotene; and 0 + 50 indicates a solution of only 50 ppm degraded β-carotene.

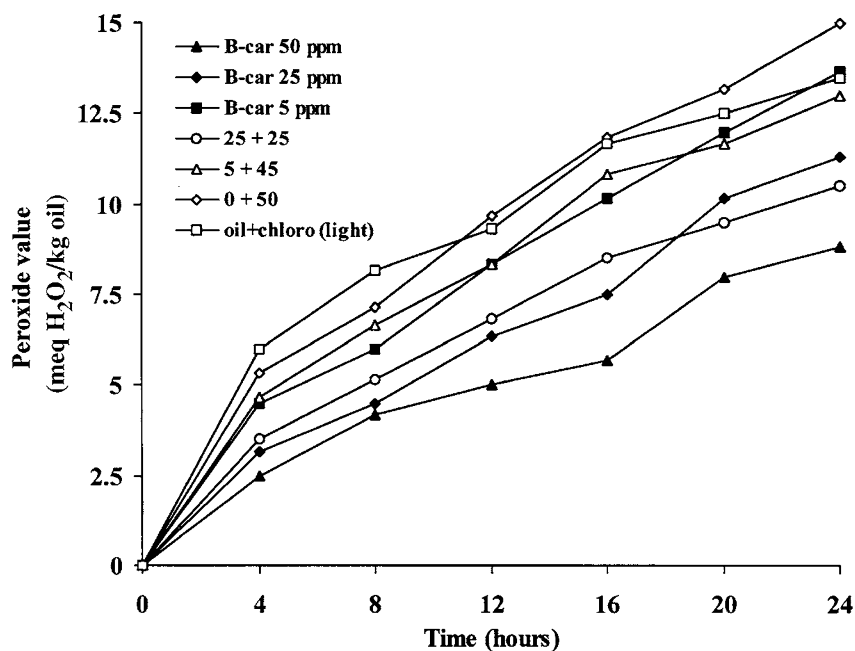

FIG. 2. Effects of β-carotene and thermally degraded β-carotene on the peroxide value of soybean oil containing 3 ppm chlorophyll under light storage at 25°C. See Figure 1 for explanation of legend.

Degraded lycopene in the light. The HOD and PV values that were recorded every 4 h during 24 h of storage in the light for the lycopene/soybean oil samples can be seen in Figures 3 and 4, respectively. In a manner similar to the 50 ppm all-*trans* β-carotene sample control, the 50 ppm all-*trans* lycopene sample control possessed HOD and PV values that were lower than all other samples throughout the 24-h time course. After 24 h of light storage the 50 ppm all-*trans* lycopene sample had significantly lower ($P < 0.05$) HOD than all other samples except for the 25 ppm all-*trans* lycopene

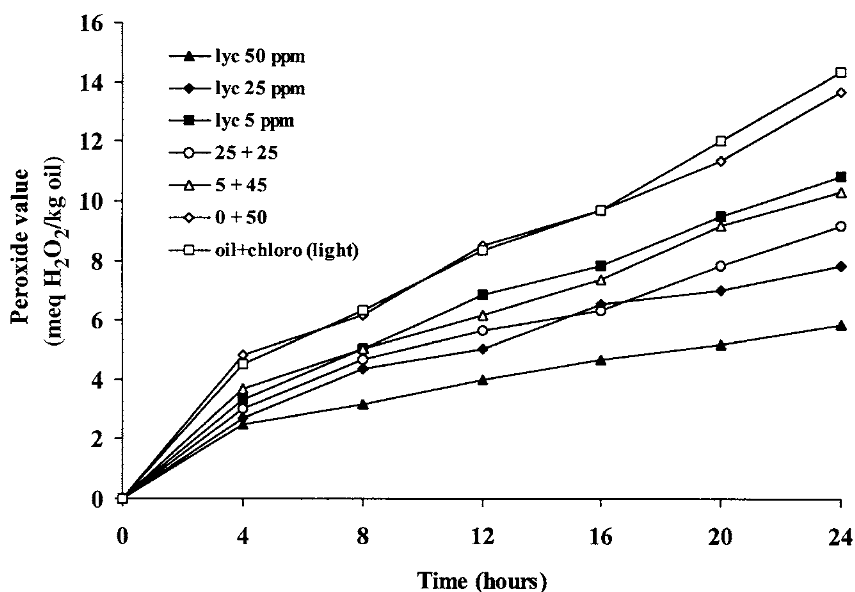

FIG. 3. Effects of lycopene and thermally degraded lycopene on the headspace oxygen depletion of soybean oil containing 3 ppm chlorophyll under light storage at 25°C. 25 + 25 indicates a solution containing 25 ppm lycopene and 25 ppm degraded lycopene; 5 + 45 contains 5 ppm lycopene and 45 ppm degraded lycopene; and 0 + 50 indicates a solution of only 50 ppm degraded lycopene.

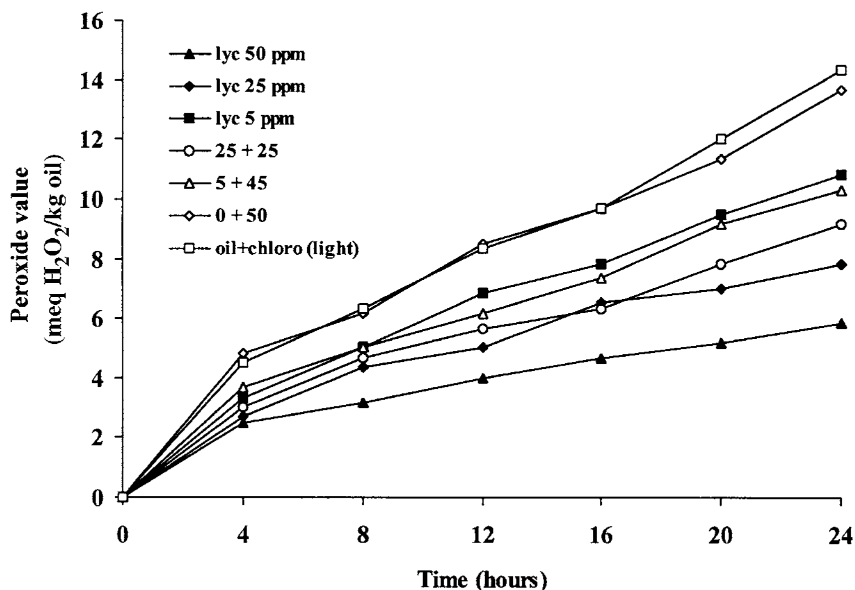

FIG. 4. Effects of lycopene and thermally degraded lycopene on the peroxide value of soybean oil containing 3 ppm chlorophyll under light storage at 25°C. See Figure 3 for explanation of legend.

sample control (Fig. 3). PV after 24 h for the 50 ppm all-*trans* lycopene sample were significantly ($P < 0.05$) lower than all other samples (Fig. 4). These results agreed with several other studies, which reported all-*trans* lycopene to be very effective in the reduction of photosensitized oxidation because of its ability to quench singlet oxygen back to its less reactive triplet state (5,17). As the all-*trans* lycopene concentration in the soybean oil control samples decreased from 50 to 5 ppm, HOD and PV increased significantly ($P < 0.05$). Higher concentrations of up to 50 ppm all-*trans* lycopene were therefore increasingly effective in preventing peroxide formation in the soybean oil. This indicated that even at concentrations as high as 50 ppm, lycopene was still the limiting factor in quenching singlet oxygen to prevent photosensitized oxidation of the soybean oil. The control samples containing 25 and 5 ppm, respectively, of all-*trans* lycopene had similar HOD and PV when compared with corresponding samples containing 25 ppm all-*trans* lycopene + 25 ppm degraded products (25 + 25) and 5 ppm all-*trans* lycopene + 45 ppm degraded products (5 + 45), respectively. A slight though again insignificant ($P > 0.05$) prooxidant effect was noted for the degraded lycopene products in the sample containing 0 ppm all-*trans* lycopene + 50 ppm degraded products (0 + 50) when compared with the control containing only oil and 3 ppm chlorophyll [oil + chloro (light)].

As was the case with β-carotene, thermal degradation of the polyene system found in all-*trans* lycopene led to a very significant increase in both HOD and PV due to a loss of antioxidant capacity. Apparently, the degraded lycopene products did not possess enough conjugated double bonds to effectively absorb energy from singlet oxygen and lower it to its ground-state triplet form. As a result, in samples containing only degraded lycopene products, singlet oxygen was able to more easily abstract electrons from the unsaturated fatty acyl chains typically found in soybean oil such as linoleic and linolenic. The result was increased peroxy-radical and peroxide formation (and higher HOD) in the soybean oil sample. When compared with controls containing only oil, however, both β-carotene and lycopene degradation products, as measured by HOD and PV, did not act as either prooxidants or antioxidants in the soybean oil model systems exposed to light. Rather, under light storage, the degradation products themselves seemed to be fairly inert.

Singlet oxygen oxidation. In order to prove that oxidation of the soybean oil stored under lighted conditions was indeed coming from singlet oxygen oxidation, two control samples were prepared, stored for 24 h, and examined for HOD (data not shown). The first control contained only soybean oil and was stored in the light at 25°C, whereas the second control contained soybean oil as well as 3 ppm chlorophyll and was stored in the dark at 25°C. After 24 h of storage in the light, HOD for the sample containing only soybean oil and no added chlorophyll was only 0.74 μmol O_2/mL, significantly less than the control in light storage containing soybean oil and 3 ppm chlorophyll (4.24 μmol O_2/mL). After 24 h of storage in the dark, the second control sample containing soybean oil as well as 3 ppm chlorophyll experienced almost no HOD (0.004 μmol O_2/mL). These results revealed that in the presence of light (9,18,19) and a photosensitizer (18,20,21), oxidation of soybean oil occurred. Interestingly, samples lacking either light exposure or chlorophyll were oxidized to a much lesser extent. Because light exposure along with the presence

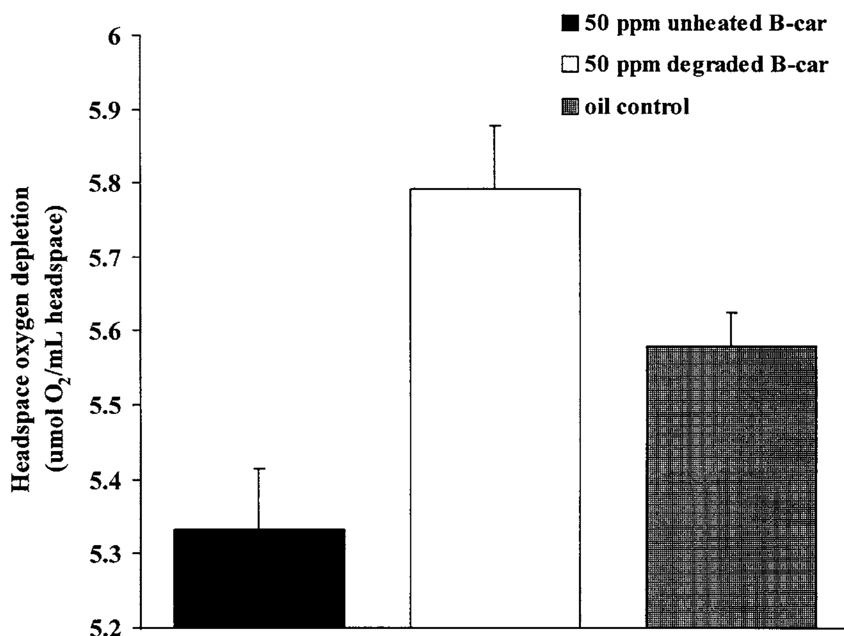

FIG. 5. Effects of thermally degraded β-carotene on the headspace oxygen depletion of soybean oil stored in the dark at 60°C.

of a sensitizer, such as chlorophyll, is necessary for singlet oxygen oxidation to occur, the data strongly supported singlet oxygen as the main source of soybean oil oxidation under light storage.

Degraded β-carotene in the dark. Unsaturated lipid systems are also prone to autoxidation initiated by free radicals, especially at elevated temperatures (11,22). The effects of β-carotene thermal degradation products on the oxidative stability of soybean oil stored in the dark at 60°C can be seen in Figure 5. After 8 d of storage, HOD occurred in each of the three samples, indicating the presence of free radical autoxidation and peroxide formation within the soybean oil. The sample containing 50 ppm degraded β-carotene displayed significantly more HOD (5.79 μmol O_2/mL) than either the oil-only control (5.58 μmol O_2/mL) or the 50 ppm all-*trans* β-carotene control (5.33 μmol O_2/mL) ($P < 0.05$). All-*trans* β-carotene has been reported by Burton (10) to act as a free radical inhibitor in the absence of light. In addition, because there was no visible loss of color in the samples containing 50 ppm all-*trans* β-carotene during the 8 d of storage at 60°C, it could be assumed that most of the β-carotene did not undergo significant polyene degradation. It was therefore not surprising that the samples containing all-*trans* β-carotene experienced the least headspace depletion. The obvious prooxidant effect of the 50 ppm degraded β-carotene compounds in the soybean oil was not expected, however, based on HOD values obtained from photosensitized oxidation data that clearly showed degraded β-carotene as having no significant effect vs. controls. The high level of significance obtained between the samples containing 50 ppm degraded β-carotene and the oil-only control ($P < 0.05$) strengthened the argument that in dark conditions at elevated temperatures β-carotene degradation products acted as prooxidants by increasing the rate of autoxidation in soybean oil.

Degraded lycopene in the dark. Lycopene degradation products were also examined for their effects on the oxidative stability of heated soybean oil in the dark. Results were very different from those observed with β-carotene degradation products (Fig. 6). Samples containing 50 ppm degraded lycopene products had the lowest HOD values of the three samples (5.34 μmol O_2/mL), which also included a 50 ppm all-*trans* lycopene control (5.36 μmol O_2/mL) and an oil-only control (5.54 μmol O_2/mL). Though the 50 ppm degraded products samples were not significantly different in HOD values from the samples containing 50 ppm all-*trans* lycopene, both were significantly lower ($P < 0.05$) than those of the oil-only controls (Fig. 6). At levels of 50 ppm, the lycopene thermal degradation products exhibited a definite antioxidant effect on the soybean oil samples when stored in the dark at elevated temperatures. Miller *et al.* (23) similarly reported lycopene as the most effective scavenger of free radical cations in a nonpolar solvent system when compared with β-carotene and various xanthophylls. Lycopene degradation products in soybean oil exposed to light were found to be largely inert, so again the results seen here were very different from previous observations with photosensitized oxidation. As verified by HPLC analysis, the polyene system in all-*trans* lycopene had been substantially degraded by thermal treatments during the formation of the thermal degradation products. The compounds created by the thermal degradation

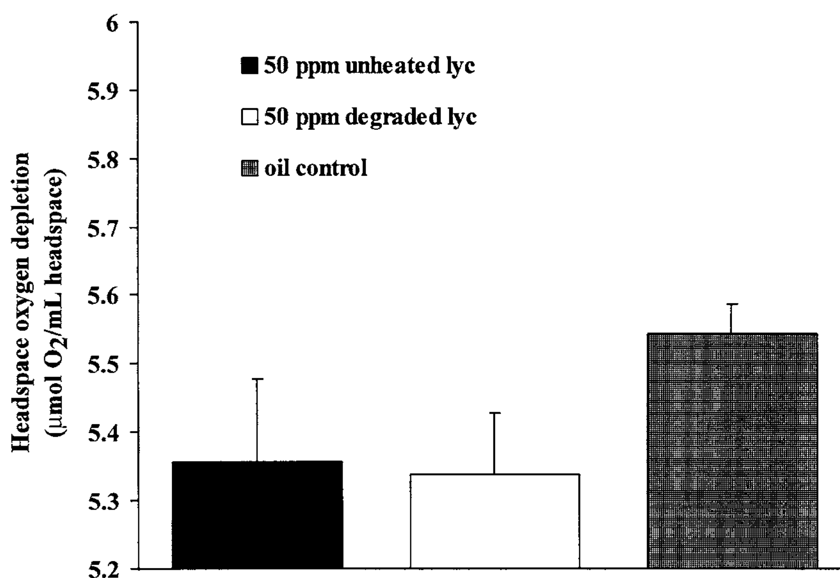

FIG. 6. Effects of thermally degraded lycopene on the headspace oxygen depletion of soybean oil stored in the dark at 60°C.

must have had some type of free radical scavenging ability that was approximately equal to that of the all-*trans* compound.

These results seemed to indicate that compounds not capable of quenching singlet oxygen and preventing photosensitized oxidation might still be very effective as peroxy radical scavengers during autoxidative processes. The type of carotenoid, as well as the conditions surrounding the oil sample, played a large role in the determination of how the carotenoid degradation products, when formed, affected the oxidative stability of the lipid system. Food processors producing products that contain nutraceutical ingredients such as β-carotene should therefore be careful to minimize degradation of the carotenoids to prevent the possible formation of prooxidant compounds that may decrease product stability.

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