Effects of Quenching Mechanisms of Carotenoids on the Photosensitized Oxidation of Soybean Oil

Mun Y. Jung and **David B. Min***
Department of Food Science and Technology, The Ohio State University, Columbus, Ohio 43210

The effects of 0, 1.0×10^{-5}, 2.5×10^{-5}, and 5.0×10^{-5} M β-apo-8′-carotenal, β-carotene, and canthaxanthin on the photooxidation of soybean oil in methylene chloride containing 3.3×10^{-9} M chlorophyll b were studied by measuring peroxide values and conjugated diene content. β-Apo-8′-carotenal, β-carotene, and canthaxanthin contain 10, 11, and 13 conjugated double bonds, respectively. The peroxide values and conjugated diene contents of oils containing the carotenoids were significantly lower ($P<0.05$) than those of control oil containing no carotenoid. As the number of conjugated double bonds of the carotenoids increased, the peroxide values of soybean oils decreased significantly ($P<0.05$). The quenching mechanisms and kinetics of the carotenoids in the photosensitized oxidation of soybean oil were studied by measuring peroxide values. The steady-state kinetics study showed that carotenoids quenched singlet oxygen to reduce chlorophyll-sensitized photooxidation of soybean oil. The singlet-oxygen quenching rate constants of β-apo-8′-carotenal, β-carotene, and canthaxanthin were 3.06×10^9, 4.60×10^9, and 1.12×10^{10} $M^{-1}sec^{-1}$, respectively.

KEY WORDS: Carotenoids, chlorophyll, photosensitized oxidation, quenching mechanisms and kinetics, singlet oxygen.

Soybean oil is susceptible to oxidation because it contains a large amount of unsaturated fatty acids. Lipid oxidation not only produces an undesirable off-flavor but also decreases the nutritional quality of lipid foods due to the loss of essential fatty acids. For the past 50 years, triplet-oxygen free-radical lipid oxidation has been extensively studied to improve the oxidative stability of fats and oils. However, it does not satisfactorily explain the initiation step of oil oxidation (1-3).

Rawls and Van Santen (4) reported that singlet oxygen participated in the initiation step of oil oxidation, and the reaction rate of singlet oxygen with linoleic acid is about 1,450 times greater than that of triplet oxygen. Singlet oxygen can be formed by photochemical, chemical, and enzymatic reactions (1). Chlorophylls have been reported as efficient photochemical sensitizers for the formation of singlet oxygen (4-7). Photochemical production of singlet oxygen is of great importance in vegetable oils that contain natural sensitizers such as chlorophylls. β-Carotene improved the flavor stability of soybean oil containing chlorophyll during light storage (6,8,9).

The detailed effects, quenching mechanisms, and kinetics of several synthetic and non-food-approved carotenoids and nickel chelates on the singlet oxygen oxidation of soybean oil have been reported by Lee and Min (10, 11). The effects, quenching mechanisms and kinetics of α- γ- and δ-tocopherols on the singlet-oxygen oxidation of soybean oil indicated that these tocopherols reduced the singlet-oxygen oxidation (12). Even though synthetic and non-food-approved carotenoids, such as lutein, zeaxanthin, lycopene, isozeaxanthin, and astaxanthin effectively reduced the singlet-oxygen oxidation of oil (10), the effects of naturally occurring and food-approved carotenoids on the singlet oxygen oxidation of vegetable oils have not been reported. The only food-approved carotenoids are β-apo-8′-carotenal, β-carotene, and canthaxanthin (13).

The objectives of this research were: i) to determine the effects of β-apo-8′-carotenal, β-carotene, and canthaxanthin, containing 10, 11, and 13 conjugated double bonds, respectively, on the photosensitized oxidation of soybean oil; and ii) to determine the quenching mechanisms and kinetics of these carotenoids in photooxidation of soybean oil containing chlorophyll.

EXPERIMENTAL PROCEDURES

Materials. Refined, bleached and deodorized soybean oil was obtained from Capital City Products Co. (Columbus, OH). Chlorophyll b was purchased from Sigma Chemical Co. (St. Louis, MO). β-apo-8′-Carotenal, β-carotene and canthaxanthin were obtained from Hoffman LaRoche Inc. (Nutley, NJ). Methylene chloride was purchased from Fisher Scientific (Pittsburgh, PA).

Preparation of purified soybean oil. Soybean oil was passed through a chromatographic column (60 cm × 4 cm) packed with a series of 100 g activated silicic acid (100 mesh, Mallinkrodt Co., Paris, KY), 30 g of a 2:1 mixture of activated charcoal (J.T. Baker Chemical Co., Phillipsburg, NJ) and celite (Sargent Welch Co., Cleveland, OH) 120 g of a 2:1 mixture of powdered sugar and celite, and 100 g activated silicic acid (6,10,12). The soybean oil passed through the chromatographic column is referred to as purified soybean oil.

Chemical analysis of purified soybean oil. Tocopherols were determined by high-pressure liquid column chromatography (14), and carotenoids were determined by a spectrometric method (15). Phospholipids, peroxides, and free acids were determined by the Ca 12-55, Cd 8-53, and Ca 5a-40 of AOCS (16) methods, respectively.

Effects of carotenoids on the photosensitized oxidation of soybean oil. To study the effects of naturally-occurring and food-approved carotenoids on the photosensitized oxidation of soybean oil, samples of 0, 1.0×10^{-5}, 2.5×10^{-5}, and 5.0×10^{-5} M β-apo-8′-carotenal, β-carotene, and canthaxanthin in 0.16 M purified soybean oil in methylene chloride containing 3.3×10^{-9} M chlorophyll b were prepared according to the method of Lee and Min (6,10). The fatty acid composition of purified soybean oil determined by the gas chromatographic method of Lee (17) was 3.8% stearic acid, 23.4% oleic acid, 53.7% linoleic acid, 7.2% linolenic acid, and 11.8% palmitic acid. The average molecular weight of the soybean oil was 870, which was derived from (890×3.8% + 884×23.4% + 878×53.7% + 872×7.2% + 806×11.8%)/100. The 0.16 molar concentration of purified soybean oil in methylene

*To whom correspondence should be addressed at Dept. of Food Science and Technology, the Ohio State University, 2121 Fyffe Rd., Vivian Hall 122, Columbus, OH 43210.

chloride was obtained from the average molecular weight of soybean oil triglycerides (10).

Ten mL of the oil sample was transferred into a 30-mL serum bottle; sample bottles were prepared in duplicate. The bottles were sealed air-tight with Teflon-coated rubber septa and aluminum caps, and placed in a light storage box described in detail by Lee and Min (6). The light source was made up of four 15-watt cool white, fluorescent lamps. The light intensity at the sample level was 4,000-lux and the temperature was 25°C. The oxidation of soybean oil was determined by measuring peroxide values and conjugated dienes every 2 hr for 8 hr by AOCS (16) methods.

Determination of the quenching mechanisms and rate constants. The quenching mechanisms and kinetics of carotenoids in chlorophyll-sensitized photooxidation of purified oil were studied by the steady-state kinetic methods of Foote and Denny (18) and Yamauchi and Matsushita (19). To study quenching mechanisms and singlet-oxygen quenching rates of the carotenoids, samples of 0.03, 0.06, 0.10, or 0.16 M purified soybean oil in methylene chloride containing 3.3×10^{-9} M chlorophyll b and 0, 0.25×10^{-5}, 0.5×10^{-5}, or 1.0×10^{-5} M β-apo-8′-carotenal, β-carotene or canthaxanthin were prepared according to the method of Lee and Min (6). Oil (15 mL) was transferred into a 30-mL serum bottle. The sample bottles were prepared in duplicate and sealed with Teflon-coated rubber septa and aluminum caps. The bottles were placed in the light storage box for 2 hr (6). Oxidation of the soybean oil was determined by measuring peroxide formation, and quenching mechanisms and quenching rate constants of the carotenoids were studied by using steady-state kinetic equations (18–20).

Statistical analysis. The peroxide values and conjugated diene contents reported in this paper are the mean values of duplicate samples. Tukey's range test (10,21) was used to ascertain the quantitative and qualitative effects of carotenoids on the peroxide values and conjugated diene contents of soybean oils during storage.

RESULTS AND DISCUSSION

Purified soybean oil. The purified soybean oil obtained by column chromatography was colorless, and did not contain detectable concentrations of peroxides, free fatty acids, phospholipids, conjugated dienes, tocopherols, or carotenoids.

Effect of carotenoids on photosensitized oxidation of soybean oil. The effect of 0, 1.0×10^{-5}, 2.5×10^{-5}, 5.0×10^{-5} M β-apo-8′-carotenal, β-carotene, or canthaxanthin on the peroxide values of purified soybean oil containing 3.3×10^{-9} M chlorophyll b during 4,000-lux light storage is shown in Table 1. The coefficient of variation of peroxide value analysis of oil was 2% (10). Preliminary studies (data not shown) showed that the peroxide values of purified soybean oil in methylene chloride containing no chlorophyll after 8 hr of storage under light was 0.5, and the peroxide values of the oils with and without chlorophyll after 8 hr of storage in the dark were 0. The peroxide values of the same sample containing chlorophyll after 8 hr under light storage was 75.9 (Table 1). Therefore, more than 99% of the peroxide value of soybean oil containing chlorophyll under light storage (Table 1) was due to the chlorophyll-photosensitized singlet-oxygen oxidation, and only less than 1% was due to triplet-oxygen autoxidation. The carotenoids reduced the peroxide value of photosensitized soybean oil, as expected (Table 1). As the concentration of the carotenoids increased from 0 to 5×10^{-5} M, the peroxide value decreased. The peroxide values of soybean oils containing the carotenoids were significantly lower ($P<0.05$) than that of the control sample containing no carotenoid (Table 1). The carotenoids acted as antioxidants in soybean oil containing chlorophyll under light storage, but not in dark storage (6). Tukey's range test for the effect of the number of conjugated double bonds of the carotenoids on the peroxide value of soybean oil is shown in Table 2. As the number of conjugated double bonds increased from 10 to 13, the peroxide value decreased significantly ($P<0.05$) at con-

TABLE 1

Tukey's Test for the Effect of Carotenoids on the Peroxide Value of Soybean Oil Containing 3.3×10^{-9}M Chlorophyll b During Light Storage at 25°C

Carotenoid ($\times 10^{-5}$M)	Peroxide value* (meq/kg oil)					
	0 hr	2 hr	4 hr	6 hr	8 hr	Mean**
Control	0	43.1	57.5	60.2	75.9	47.3^a
α-Apo-8′-carotenal (1.0)	0	22.5	28.2	35.0	39.0	24.9^b
β-Apo-8′-carotenal (2.5)	0	11.0	15.3	18.4	20.2	13.8^c
β-Apo-8′-carotenal (5.0)	0	5.8	7.3	8.1	10.3	6.3^d
β-Carotene (1.0)	0	15.0	25.2	29.6	32.3	20.4^b
β-Carotene (2.5)	0	9.4	13.5	15.7	15.7	13.3^c
β-Carotene (5.0)	0	5.0	6.7	8.1	8.1	5.8^d
Canthaxanthin (1.0)	0	10.8	21.6	23.8	25.1	14.1^b
Canthaxanthin (2.5)	0	7.7	11.7	12.3	12.6	8.8^c
Canthaxanthin (5.0)	0	4.5	6.3	7.8	8.5	5.4^c

*Peroxide value is the mean of dulicate samplings.

**Mean of peroxide values of samples after 0-, 2-, 4-, 6-, and 8-hr storage and means in a column of same carotenoid with different superscript letters are significantly different at $P<0.05$.

TABLE 2

Tukey's Test for the Effect of the Number of Conjugated Double Bonds of Carotenoids on the Peroxide Value and Conjugated Diene of Soybean Oil Containing 3.3×10^{-9} M Chlorophyll b During Light Storage at 25°C

Carotenoid in soybean oil	Number of conjugated double bonds	Peroxide value (meq/kg oil) (mean*)	Conjugated diene (%) (mean*)
(1.0×10^{-5} M)			
β-Apo-8′-carotene	10	$24.9^{a,**}$	$0.10^{a,**}$
β-Carotene	11	20.4^{b}	0.09^{ab}
Canthaxanthin	13	14.1^{c}	0.07^{b}
(2.5×10^{-5} M)			
β-Apo-8′-carotene	10	13.8^{a}	0.05^{a}
β-Carotene	11	13.3^{a}	0.05^{a}
Canthaxanthin	13	8.8^{b}	0.04^{a}
(5.5×10^{-5} M)			
β-Apo-8′-carotene	10	6.3^{a}	0.04^{a}
β-Carotene	11	5.8^{b}	0.04^{a}
Canthaxanthin	13	5.4^{c}	0.03^{a}

*Mean of peroxide values and conjugated dienes of soybean oils containing different levels of carotenoid after 0-, 2-, 4-, 6-, and 8-hr of storage (see peroxide values in Table 1 and conjugated dienes in Table 3).
**Means in a column with different superscripts are significantly different at $P<0.05$.

TABLE 3

Tukey's Test for the Effect of Carotenoids on the Conjugated Diene Content of Soybean Oil Containing 3.3×10^{-9}M Chlorophyll b During Light Storage at 25°C

Carotenoid ($\times 10^{-5}$M)	Conjugated diene* (%)					
	0 hr	2 hr	4 hr	6 hr	8 hr	Mean**
Control	0	0.17	0.23	0.26	0.34	0.20^{a}
β-Apo-8′-carotenal (1.0)	0	0.08	0.12	0.13	0.15	0.10^{b}
β-Apo-8′-carotenal (2.5)	0	0.03	0.05	0.07	0.08	0.05^{c}
β-Apo-8′-carotenal (5.0)	0	0.02	0.05	0.05	0.06	0.04^{c}
β-Carotene (1.0)	0	0.07	0.10	0.13	0.15	0.09^{b}
β-Carotene (2.5)	0	0.04	0.05	0.06	0.08	0.05^{c}
β-Carotene (5.0)	0	0.03	0.04	0.04	0.05	0.04^{c}
Canthaxanthin (1.0)	0	0.06	0.09	0.10	0.12	0.07^{b}
Canthaxanthin (2.5)	0	0.03	0.04	0.06	0.06	0.04^{c}
Canthaxanthin (5.0)	0	0.02	0.03	0.04	0.04	0.03^{c}

*Conjugated diene is the mean of duplicate samplings.
**Mean of conjugate dienes of samples after 0-, 2-, 4-, 6-, and 8-hr storage and means of a column of same carotenoid with different letters are significantly different at $P<0.05$.

centrations of 1.0×10^{-5} M, 2.5×10^{-5} M, or 5.0×10^{-5} M carotenoid.

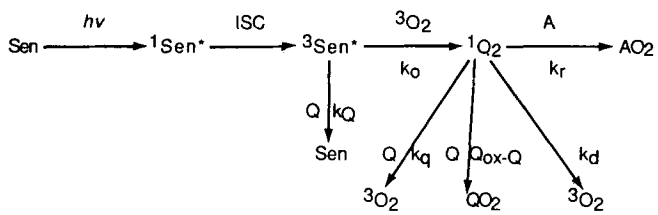
The effect of 0, 1.0×10^{-5}, 2.5×10^{-5}, and 5.0×10^{-5} M β-apo-8′-carotenal, β-carotene, or canthaxanthin on the conjugated diene content of soybean oil during light storage is shown in Table 3. Preliminary analysis showed that the coefficient of variation for conjugated diene analyses of five replicate oil samples was 2.9%. The conjugated diene content of the control sample containing 3.3×10^{-9} M chlorophyll b increased from 0 to 0.34% after 8 hr under light storage. However, the conjugated dienes of the control in the dark and the purified soybean oil containing no chlorophyll under light after 8 hr were 0 (data not shown). Table 3 showed that as the concentration of the carotenoid increased from 0 to 5×10^{-5} M, the conjugated diene content decreased. The conjugated diene contents of soybean oils containing the carotenoids were significantly lower ($P<0.05$) than that of control oil containing no carotenoid. Tukey's range test for the effect of the number of conjugated double bonds of the carotenoids on the conjugated diene contents of soybean oil is shown in Table 2. As the number of conjugated double bonds of carotenoids increased from 10 to 11, and from 11 to 13, the conjugated diene content of oil decreased, even though they were not statistically different between 10 and 11 and between 11 and 13 at $P > 0.05$. Table 2 also showed that as the double bonds of the carotenoids increased from 10 to 13, the conjugated diene content of soybean oil did not decrease significantly ($P>0.05$), but the peroxide values (Table 2) did ($P<0.05$).

The conjugated diene determination method at 233 nm may not be as sensitive as the peroxide value determination in the evaluation of singlet oxygen oxidation. Literature review generally showed that peroxide value

determination (10,22-26) is more commonly used to evaluate singlet-oxygen oxidation of oil than the conjugated diene determination (7,27), which may also indirectly suggest that the peroxide value method may be more sensitive than the conjugated diene method in the evaluation of singlet-oxygen oxidation of lipid. Lee and Min (10) reported that as the number of conjugated double bonds of carotenoids, such as lutein, zeaxanthin, lycopene, isozeaxanthin, and astaxanthin increased form 10 to 13, the protective effect of the carotenoids on the singlet-oxygen oxidation of oil increased.

The results of peroxide and conjugated diene formation suggest that canthaxanthin with 13 conjugated double bonds has the greatest antioxidant activity, followed by β-carotene with 11 conjugated double bonds, and then β-apo-8′-carotenal with 10 conjugated double bonds. However, it should be noted that as the conjugated double bonds of the carotenoids increased from 10 to 11, and from 11 to 13, the conjugated diene of the soybean oil decreased, but not significantly between 10 and 11 or between 11 and 13 at $P > 0.05$.

Quenching mechanism and rate constants of carotenoids. The schematic diagram for the formation of oxidized product (AO_2) *via* singlet-oxygen oxidation is as follows (20):



When a sensitizer (Sen), such as chlorophyll, in soybean oil absorbs light energy it becomes an excited singlet sensitizer (^{1}Sen*) and then becomes an excited triplet sensitizer (^{3}Sen*) by an intersystem crossing (ISC) mechanism. The energy of ^{3}Sen* is transferred to ordinary triplet-state oxygen ($^3O_2$) to produce singlet oxygen ($^1O_2$) by triplet-triplet annihilation. Singlet-oxygen reacts with substrate (A) to produce oxidized substrate (AO_2). The formation of oxidized products (AO_2) could be reduced by quenching of singlet-oxygen and/or excited triplet sensitizers. The antioxidant effects of the carotenoids in soybean oil containing chlorophyll under light storage must be due to the quenching of singlet-oxygen and/or chlorophyll by the carotenoids. If carotenoids reduced the chlorophyll-sensitized photooxidation of soybean by singlet-oxygen quenching, the following steady-state kinetic equation is established (20):

$$\{d[AO_2]/dt\}^{-1} = K^{-1}\{1+(k_q[Q]+k_{ox-Q}[Q] + k_d) / k_r[A]$$

where K denotes the rate of singlet-oxygen formation; AO_2, oxidized soybean oil; K_r, reaction rate constant of soybean oil with singlet oxygen; A, soybean oil; k_q, reaction rate constant of physical singlet-oxygen quenching by carotenoid; k_{ox-Q}, reaction rate constant of chemical singlet-oxygen quenching by carotenoid; Q, carotenoid, and k_d, decaying rate of singlet-oxygen. The intercepts and slopes of the plots of $[AO_2]^{-1}$ *vs.* $[A]^{-1}$ at various concentrations of quencher (Q) are K^{-1} and $K^{-1}\{(k_d + k_q [Q] + K_{ox-Q}[Q])/k_r\}$, respectively. The intercepts of the plots are independent of the concentration of quencher and the slopes are dependent on the concentration of quencher (20).

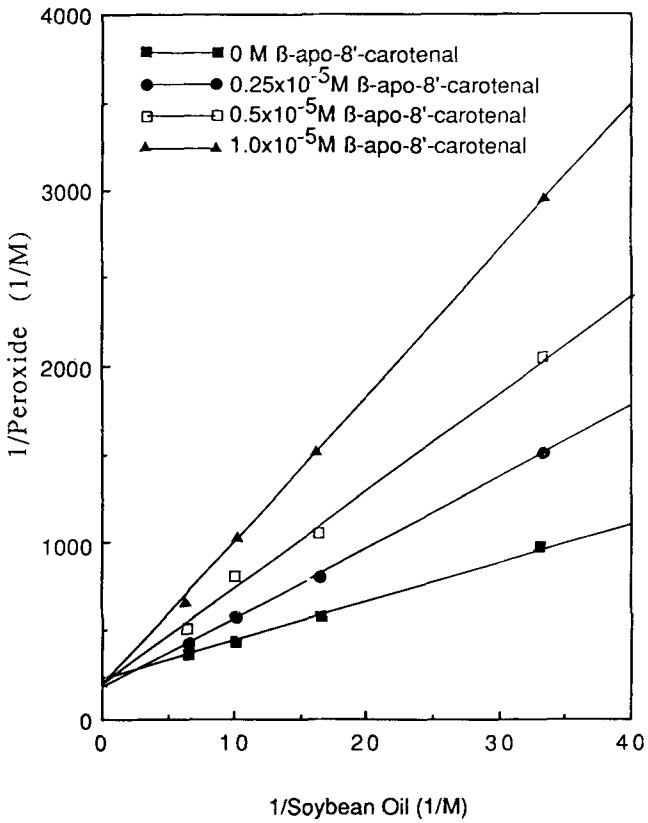


FIG. 1. Effect of β-apo-8′-carotenal on the peroxide values of purified soybean oil containing 3.3×10^{-9} M chlorophyll b during 2-hr light storage at 25°C.

The ratio of slope to intercept of the plot is $\{k_d + (k_q + k_{ox-Q})[Q]\}/k_r$. The plot of slope/intercept from the plots of $[AO_2]^{-1}$ *vs.* $[A]^{-1}$) *vs.* [Q] allows the determination of k_d/k_r from the intercept and $(k_q + k_{ox-Q})/k_r$ from the slope. If the decay rate of singlet-oxygen (k_d) is known, the reaction rate of soybean oil with singlet-oxygen (k_r) and the total singlet-oxygen quenching rate of carotenoid ($k_q + k_{ox-Q}$) can be determined (20). The k_d values are known for many solvents (28). The plots of $[AO_2]^{-1}$ *vs.* $[A]^{-1}$ for different levels of β-apo-8′-carotenal are shown in Figure 1. The intercepts were the same for different levels of the carotenoid, but the slopes of the plots increased as the concentration of β-apo-8′-carotenal increased from 0 to 1.0×10^{-5} M. Therefore, β-apo-8′-carotenal quenched singlet-oxygen to reduce the photosensitized oxidation of soybean oil. This result confirms that carotenoids quenched singlet-oxygen to reduce the singlet-oxygen oxidation of oils containing sensitizers (6,10,20).

The linear regression line for the plot of $[AO_2]^{-1}$ *vs.* $[A]^{-1}$ containing no β-apo-8′-carotenal (Fig. 1) is $y = 21.75 X + 203.6$, where $y = [AO_2]^{-1}$ and $X = [A]^{-1}$. The slope/intercept of the regression line is 0.106. Foote (20) showed that the slope/intercept of the regression line for the soybean oil containing no carotenoid is k_d/k_r. The k_d

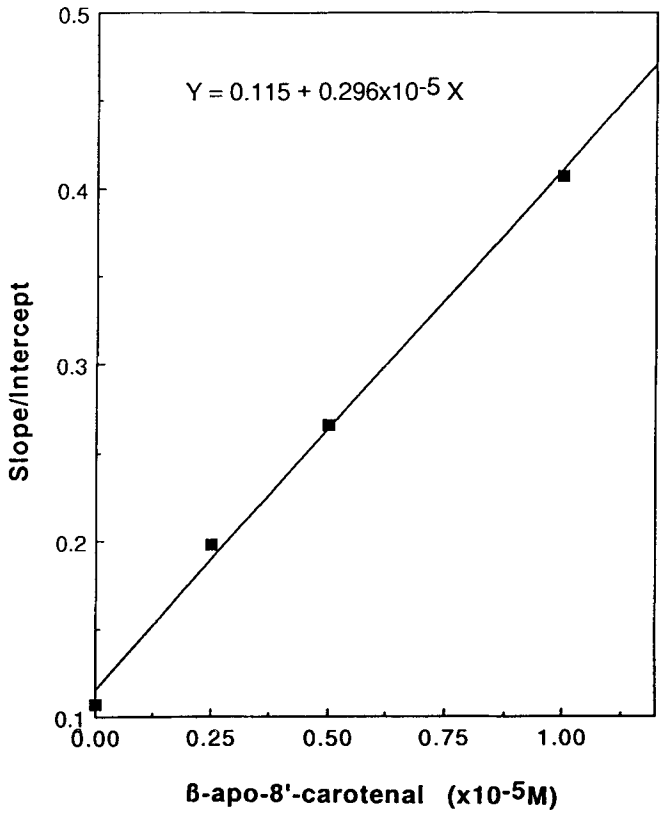


FIG. 2. The plot of slope/intercept of the plots (of 1/peroxide *vs.* 1/soybean oil shown in Fig. 1) *vs.* the concentration of β-apo-8′-carotenal.

value in methylene chloride is 1.1×10^4 sec^{-1} (29). The singlet-oxygen oxidation rate (k_r) of soybean oil is k_q/slope. Therefore, $k_r = 1.1 \times 10^4/0.106 = 1.04 \times 10^5$ M^{-1} sec^{-1} in methylene chloride. Doleiden *et al.* (30) reported that the singlet-oxygen oxidation rates of methyl oleate, methyl linoleate, and methyl linolenate were 0.67×10^5, 1.3×10^5, and 1.9×10^5 M^{-1} sec^{-1} in pyridine, respectively.

The ratios of slope/intercept of the plots containing 0, 0.25×10^{-5}, 0.5×10^{-5}, and 1.0×10^{-5} M β-apo-8′-carotenal were calculated from Figure 1 and were 0.106, 0.197, 0.265, and 0.401, respectively. To determine the singlet-oxygen quenching rate ($k_q + k_{ox-Q}$) of β-apo-8′-carotenal, the slope/intercept *vs.* [β-apo-8′-carotenal] was plotted and the plot is shown in Figure 2. The linear regression equation of the plot/intercept *vs.* [β-apo-8′-carotenal] of Figure 2 is $Y = 0.115 + 0.296 \times 10^5$ X, where Y is slope/intercept and X is [β-apo-8′-carotenal] in moles. Foote (20) reported that the slope of the plot of slope/intercept *vs.* [β-apo-8′-carotenal] is $(k_q + k_{ox-Q})/k_r$. The value of $(k_q + k_{ox-Q})$ of β-apo-8′-carotenal is slope × k_r. Since the slope of the plot for β-apo-8′-carotenal (Fig. 2) is 0.296×10^5 M^{-1} and k_r is 1.04×10^5 M^{-1} sec^{-1}, the $(k_q + k_{ox-Q})$ of β-apo-carotenal is $0.296 \times 10^5 \times 1.04 \times 10^5$ M^{-1} sec^{-1} = 3.07×10^9 M^{-1} sec^{-1}.

$[AO_2^{-1}]$ *vs.* $[A]^{-1}$ for different levels of β-carotene and canthaxanthine were plotted to determine the quenching mechanisms of the carotenoids (plots not shown). The intercepts of the plots were the same, and the slopes were different for different levels of β-carotene and canthaxanthin. Therefore, these carotenoids reduced the photosensitized oxidation of soybean oil by quenching singlet oxygen. The slope/intercept *vs.* [β-carotene] and [canthaxanthin] were also plotted to determine the singlet-oxygen quenching rates of the carotenoids (plots not shown). The singlet-oxygen quenching rates of β-carotene and canthaxanthin were calculated from the slopes of the plots, and were 4.60×10^9 and 1.12×10^{10} M^{-1} sec^{-1}, respectively. The singlet-oxygen quenching rate of β-carotene in benzene-methanol (4:1) was 5×10^9 M^{-1} sec^{-1} according to Foote and Denny (18), 6.5×10^9 M^{-1} sec^{-1} in pyridine according to Fahrenholtz *et al.* (31), and 1.3×10^{10} M^{-1} sec^{-1} in benzene according to Farmilo and Wilkinson (32). The singlet-oxygen quenching rate of β-carotene varies in different solvents (28). The quenching rates of β-apo-8′-carotenal and canthaxanthin have not been reported. Since the singlet-oxygen quenching rates of β-apo-8′-carotenal, β-carotene, and canthaxanthin are 3.06×10^9, 4.60×10^9, and 1.12×10^{10} M^{-1} sec^{-1}, respectively, the singlet-oxygen quenching rates of the food-approved carotenoids increased as the number of conjugated double bonds of the carotenoids increased. The singlet-oxygen quenching rates of synthetic and non-food-approved lutein, zeaxanthin, lycopene, isozeaxanthin, and astaxanthin increased as the number of conjugated double bonds of the carotenoids increased (10).

ACKNOWLEDGMENTS

Salaries and research support provided by state and federal funds appropriated to The Ohio Agricultural Research and Development Center, The Ohio State University, Journal Article No. 89-286.

REFERENCES

1. Korycka-Dahl, M.B., and T. Richardson, in *CRC Critical Reviews in Food Science and Nutrition*, CRC Press, Inc., Boca Raton, 1978.
2. Frankel, E.N., *Progress of Lipid Research 23*:197 (1985).
3. Whang, K., and I. Peng, *J. Food Sci. 53*:1596 (1988).
4. Rawls, H.R., and P.J. Van Santen, *J. Am. Oil Chem. Soc. 47*:121 (1970).
5. Endo, Y., R. Usuki and T. Kaneda, *Ibid. 61*:781 (1984).
6. Lee, E.C., and D.B. Min, *J. Food Sci. 53*:1894 (1988).
7. Clements, A.H., R.H. Van Den Engh, D.J. Frost, K. Hoogenhout and J.R. Nooi, *J. Am. Oil Chem. Soc. 51*:325 (1973).
8. Warner, K., and E.N. Frankel, *Ibid. 64*:213 (1987).
9. Fakourelis, N., E.C. Lee and D.B. Min, *J. Food Sci. 52*:234 (1987).
10. Lee, S.W., and D.B. Min, *J. Agr. and Food Chem. 38*:1630 (1990).
11. Lee, S.W., and D.B. Min, *Ibid. 39*:642 (1991).
12. Jung, M.Y., E.C. Lee and D.B. Min, *Ibid.*, in press (1991).
13. Code of Federal Regulation 21, U.S. Government Printing Office, Washington, D.C., 1990.
14. Carpenter, A.P., *J. Am. Oil Chem. Soc. 56*:668 (1979).
15. Proctor, A., and H.E. Snyder, *Ibid. 64*:1163 (1987).
16. Walker, R. (ed.) *Official and Tentative Methods of the American Oil Chemists' Society*, American Oil Chemists' Society, Champaign, IL, 1980.
17. Lee, E.C., *Effects of Chlorophyll, β-carotene and Tocopherol on the Singlet Oxygen Oxidation of Soybean Oil*, Ph.D. Dissertation, The Ohio State University, 1987.
18. Foote, C.S., and R.W. Denny, *J. Am. Chem. Soc. 90*:6233 (1968).
19. Yamauchi, R., and S. Matsushita, *Agri. Biol. Chem. 41*:1425 (1977).
20. Foote, C.S., in *Singlet Oxygen*, edited by H.H. Wasserman and R.W. Murray, Academic Press, New York, 1979.
21. SAS, *SAS User's Guide*, Cary, N.C., 1982.

22. Carlsson, D.J., T. Suprunchuk and D.M. Wiles, *J. Am. Oil Chem. Soc. 53*:656 (1976).
23. Carlsson, D.J., T. Suprunchuk and D.M. Wiles, *Can. J. of Chem. 52*:3728 (1974).
24. Ding, A.H., and P.C. Chan, *Lipids 19*:278 (1984).
25. Chainneaux, J., and C. Tanielian, in *Singlet Oxygen*, edited by B. Ranby and J.F. Rabek, John Wiley & Sons, New York, 1978.
26. Scott, G., in *Singlet Oxygen*, edited by B. Ranby and J.F. Rabek, John Wiley & Sons, New York, 1978.
27. Terao, A., and S. Matsushita, *J. Am. Oil Chem. Soc. 54*:234 (1977).
28. Hurst, J.R., J.D. McDonald and G.B. Schuster, *J. Am. Chem. Soc. 104*:2065 (1982).
29. Salokhiddinov, K.I., I.M. Byteva and G.P. Gurinovich, *Zh. Prikl. Spektrosk. 34*:892 (1981).
30. Doleiden, F.H., S.R. Fahrenholtz, A.A. Lamola and A.M. Trozzolo, *Photochem. Photobiol. 20*:519 (1974).
31. Fahrenholtz, S.R., F.H. Doleiden, A.M. Trozzolo and A.A. Lamola, *Ibid. 20*:505 (1974).
32. Farmilo, A., and F. Wilkinson, *Ibid. 18*:447 (1973).

[Received March 22, 1991; accepted July 22, 1991]