Quenching Mechanism and Kinetics of Ascorbyl Palmitate for the Reduction of the Photosensitized Oxidation of Oils

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ABSTRACT: Effects of 0, 500, 1000, and 1500 ppm (wt/vol) ascorbyl palmitate (AP) on the methylene-blue- and the chlorophyll-sensitized photooxidations of linoleic acid or soybean oil, either in methanol or in a solvent mixture (benzene/methanol, 4:1, vol/vol), were studied during storage under 3300 lux fluorescent light for 5 h. Steady-state kinetic approximation was used to determine a quenching mechanism and quenching rate constant of AP in the chlorophyll-sensitized photooxidation of methyl linoleate in a solvent mixture (benzene/methanol, 4:1, vol/vol). Both methylene blue and chlorophyll greatly increased the photooxidation of linoleic acid and soybean oil, as was expected. AP was extremely effective at minimizing both methylene-blue- and chlorophyll-sensitized photooxidations of linoleic acid and soybean oil, and its effectiveness was concentration-dependent. The addition of 500, 1000, and 1500 ppm AP resulted in 69.3, 83.6, and 94.6% inhibition of methylene-blue-sensitized photooxidation of linoleic acid, respectively, after 5-h storage under fluorescent light. AP showed significantly greater antiphotooxidative activity than α-tocopherol for the reduction of methylene blue-sensitized photooxidation of linoleic acid ($P < 0.05$). The steady-state kinetic studies indicated that AP quenched singlet oxygen only to minimize the chlorophyll-sensitized photooxidation of oils. The calculated total quenching rate of AP was 1.0×10^8 $M^{-1}s^{-1}$. The present results clearly showed, for the first time, the effective singlet oxygen quenching ability of AP for the reduction of photosensitized oxidation of oils.

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KEY WORDS: Ascorbyl palmitate, kinetics, linoleic acid, mechanism, photooxidation, singlet oxygen quenching, soybean oil.

Various types of oils, oil-soluble vitamins (retinyl palmitate, carotenoids, and tocopherols), cholesterol, limonene, and conjugated terpenes in citrus oils are susceptible to photooxidation during storage under light, especially when photosensitizers, such as chlorophylls, are present in the systems (1). Singlet oxygen can be formed by photochemical, chemical, and enzymatic reactions. Chlorophylls, myoglobin derivatives, riboflavin, and methylene blue are reportedly efficient photochemical sensitizers for the formation of singlet oxygen (1–3). Photochemical production of singlet oxygen is of great importance in vegetable oils that contain natural sensitizers, such as chlorophylls at 0.065–1.33 μg/g oil (4). Rawls and Van Santen (5) 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.

The effects, quenching mechanisms, and kinetics of tocopherols, carotenoids, and nickel chelates on the singlet oxygen oxidation of soybean oil have been reported previously (6–11). Tocopherols and carotenoids can be used for the practical reduction of singlet oxygen oxidation of oils and other oil-soluble components. Application of carotenoids, as effective singlet oxygen quenchers, to some oils or oil-containing foods is, however, limited because they provide yellow to red color to the products. Tocopherols do not provide color to oils, but their singlet oxygen-quenching abilities are not as effective as the carotenoids. Thus, the need for novel fat-soluble antioxidants for effective reduction of photooxidation of oils and other photolabile oil-soluble compounds is obvious, and academia and industry continue to look for novel natural antioxidants.

Ascorbic acid reportedly is an effective singlet oxygen quencher (12–17) and can be used to minimize the photooxidation of water-soluble compounds in aqueous solutions (17). Jung *et al.* (16) reported that singlet oxygen reaction rates of ascorbic acid in aqueous solutions at pH 7.5, 6.0, and 4.5 were 6.63×10^8, 5.77×10^8, and 5.27×10^8 $M^{-1}s^{-1}$, respectively. Jung *et al.* (17) also reported that addition of ascorbic acid to skim milk greatly reduced formation of light-activated off-flavor during storage under fluorescence light. Ascorbic acid cannot be used in oils because of its insolubility in oils. However, ascorbyl palmitate (AP), a fat-soluble ester of palmitic acid and ascorbic acid, could be used in oils or oil foods. AP is a substance that is generally recognized as safe (GRAS) with no specific limitation or restriction. Consumer ingestion of this antioxidant would pose no health hazard because metabolic breakdown yields ascorbic acid and palmitic acid—both normal metabolites.

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Even though it has been reported that ascorbic acid is an effective singlet oxygen quencher in aqueous solutions, the effect of AP on the photosensitized oxidations of oils has not been studied. The objectives of this research were to study the effects of AP on the methylene-blue- and chlorophyll-sensitized photooxidation of linoleic acid or of soybean oils and to determine the quenching mechanism and quenching rate constant of AP for the reduction of chlorophyll-sensitized photooxidation of oil.

MATERIALS AND METHODS

Materials. Linoleic acid, methyl linoleate, AP, α-tocopherol, chlorophyll *b*, and methylene blue were purchased from Sigma Chemical Co. (St. Louis, MO). Soybean oil without any additives was obtained from Dongbang Oil Co. (Seoul, Korea).

*Effects of AP on methylene-blue or chlorophyll-*b*-sensitized photooxidation of linoleic acid and soybean oil.* To study the effects of AP on the photosensitized oxidation of linoleic acid, samples of 0, 500, 1000, and 1500 ppm (wt/vol) AP in 1.0% (wt/vol) linoleic acid were prepared in methanol that also contained 3 ppm (wt/vol) methylene blue or 3 ppm (wt/vol) chlorophyll *b* as a photosensitizer. Samples containing 1000 ppm (wt/vol) α-tocopherol were used as a positive control in the system. The antioxidant concentration was based on the entire volume of sample.

To study the effects of AP on the photosensitized oxidation of soybean oil, samples of 0, 500, 1000, and 1500 ppm (wt/vol) AP in 10.0% (wt/vol) soybean oil were prepared in a solvent mixture (benzene/methanol, 4:1, vol/vol) that also contained 3 ppm (wt/vol) methylene blue or 3 ppm (wt/vol) chlorophyll *b* as a photosensitizer. Ten milliliters of the prepared sample was transferred, in duplicate, into 30- mL serum bottles. The surface/volume ratio of the sample was 32.15 cm^2/10 mL. The bottles were sealed airtight with poly(tetrafluoroethylene)-coated rubber septa and aluminum caps and placed in a light storage box described in detail by Jung and Min (9). The light intensity at the sample level was 3000 lux, and the temperature was 25°C. The degree of oxidation of linoleic acid and soybean oil was determined by measuring peroxide values every hour for 5 h by using the AOCS method (18).

Determination of quenching mechanism and rate constant. The quenching mechanism and kinetics of AP in chlorophyll-sensitized photooxidation of oils were studied by the steady-state kinetic method of Foote (19). To study the quenching mechanism and singlet oxygen quenching rates of AP, samples of 0.03, 0.06, 0.09, and 0.15 M methyl linoleate in a solvent mixture (benzene/methanol, 4:1, vol/vol) that also contained 3.3×10^{-6} M chlorophyll *b* and 0, 0.75×10^{-3}, 1.50×10^{-3}, and 2.50×10^{-3} M AP were prepared according to Jung and Min (9). The prepared samples (5 mL) were transferred into 30-mL serum bottles. The sample bottles were prepared in duplicate and sealed with poly(tetrafluoroethylene)-coated rubber septa and aluminum caps. The bottles were placed in the light storage box for 1 h. Oxidation of the soybean oil was determined by peroxide formation, and quenching mechanism and quenching rate constants of the AP were studied by means of steady-state kinetic equations (8,9,16,19).

Statistical analysis. All experiments were done in duplicate, and statistical analysis was accomplished by using the Statistical Analysis System (20). Duncan's multiple range test was used to ascertain the effects of AP on the photooxidation of linoleic acid and soybean oil.

RESULTS AND DISCUSSION

Effects of AP on the photosensitized oxidation of linoleic acid. Effects of 0, 500, 1000, and 1500 ppm (wt/vol) AP on methylene-blue-sensitized photooxidation of linoleic acid in methanol during 5-h storage under 3300 lux fluorescent light are shown in Figure 1. Methylene blue greatly increased the photooxidation of linoleic acid in methanol, as was expected. However, in the dark, no oxidation occurred during a 5-h storage. The peroxide value of linoleic acid in the presence of 3 ppm methylene blue after 5-h storage under light illumination was 140 meq/kg oil. Addition of either AP or α-tocopherol greatly decreased the methylene-blue-sensitized photooxidation of AP. As the concentration of AP increased, the reduction of peroxide formation in linoleic acid increased. The peroxide values of linoleic acid in the presence of 0, 500, 1000, and 1500 ppm AP after 5-h storage under light were 140, 42.0, 22, and 8 meq/kg oil, respectively. Duncan's multiple range tests showed that the peroxide values of samples treated with AP were significantly lower than the control (no ascorbyl palmitate added) after 5-h storage under fluorescent light ($P < 0.05$). AP was much more effective than α-tocopherol (Fig. 1). The peroxide value of linoleic acid in the presence of 1000 ppm α-tocopherol was 82 meq/kg oil after 5-h storage under fluorescent light, showing

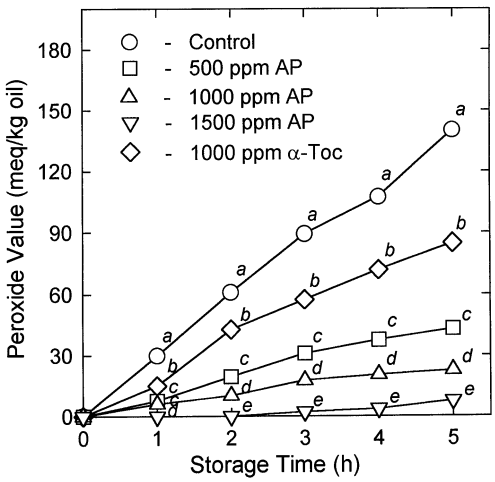


FIG. 1. Effects of 0, 500, 1000, 1500 ppm (wt/vol) ascorbyl palmitate (AP), and 1000 ppm (wt/vol) α-tocopherol (α-Toc) on methylene-blue-sensitized photooxidation of linoleic acid in methanol during storage under fluorescent light. Peroxide values with different italicized letters at the same storage time were significantly different ($P < 0.05$).

the significantly lower activity of 1000 ppm tocopherol than even 500 ppm AP ($P < 0.05$).

As expected, chlorophyll also greatly increased the photooxidation of linoleic acid (Fig. 2). The peroxide value of linoleic acid without AP was 87 meq/kg oil after 5-h storage under fluorescent light. In the dark, no oxidation (0 meq/kg oil) occurred during 5-h storage (data not shown). The photosensitizing activity of methylene blue for the oxidation of linoleic acid was greater than that of chlorophyll *b* on the basis of the same gram weight. The addition of AP also reduced the chlorophyll-sensitized photooxidation of linoleic acid. The peroxide values of linoleic acid with 0, 500, 1000, and 1500 ppm AP after 5-h storage under light were 87, 69, 65, and 53 meq/kg oil, respectively. This result showed that antioxidant activity of AP was lower in the chlorophyll-sensitized photooxidation of linoleic acid than in the methylene-blue-sensitized photooxidation of linoleic acid. The results in Figures 1 and 2 show that 1500 ppm AP resulted in 94 and 39% inhibition of methylene-blue- and chlorophyll-sensitized photooxidations of linoleic acid, respectively.

Effects of AP on the photosensitized oxidation of soybean oil. AP was extremely effective at minimizing methylene-blue-sensitized photooxidation of soybean oil (Fig. 3). As AP was increased from 500 to 1500 ppm, its effectiveness increased significantly ($P < 0.05$). The peroxide values of methylene-blue-sensitized photooxidation of soybean oil with 0, 500, 1000, or 1500 ppm after 5-h storage under fluorescent light were 89, 69, 31, and 13 meq/kg oil, respectively. AP also reduced the chlorophyll-sensitized photooxidation of soybean oil in a solvent mixture (benzene/methanol; 4:1, vol/vol) (Fig. 4). The peroxide values of chlorophyll-sensitized photooxidation of soybean oil with 0, 500, 1000, and 1500 ppm after 5-h storage under fluorescent light were 75.0, 64.5, 42.1, and 17.7 meq/kg oil, respectively. The peroxide measurement coefficients of variation shown in Figures 1–4 were 3.82, 1.91, 2.76, and 3.95, respectively. The coefficients of variation of the peroxide measurements shown in Figures 1–4 were 3.82, 1.91, 2.76, and 3.95, respectively. The results in Figures 1–4 clearly show, for the first time, that AP had a strong antiphotooxidative activity on both methylene blue- and chlorophyll-sensitized photooxidation of oils.

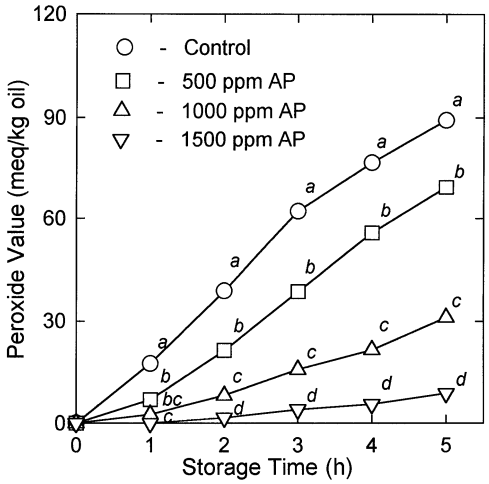


FIG. 3. Effects of 0, 500, 1000, and 1500 ppm (wt/vol) AP on methylene-blue-sensitized photooxidation of soybean oil in a solvent mixture (benzene/methanol, 4:1, vol/vol) during storage under fluorescent light. Peroxide values with different italicized letters at the same storage time were significantly different ($P < 0.05$). See Figure 1 for abbreviation.

Quenching mechanism and rate constant of AP. Because AP decreased photosensitized oxidation of oils, the authors decided to study the mechanism and the kinetics of AP for the reduction of photosensitized oxidation of oils by using a steady-state kinetic approximation. The schematic diagram

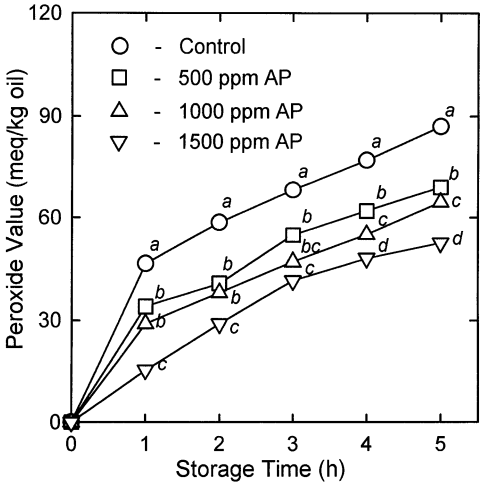


FIG. 2. Effects of 0, 500, 1000, and 1500 ppm (wt/vol) AP on chlorophyll-sensitized photooxidation of linoleic acid in methanol during storage under fluorescent light. Peroxide values with different italicized letters at the same storage time were significantly different ($P < 0.05$). See Figure 1 for abbreviation.

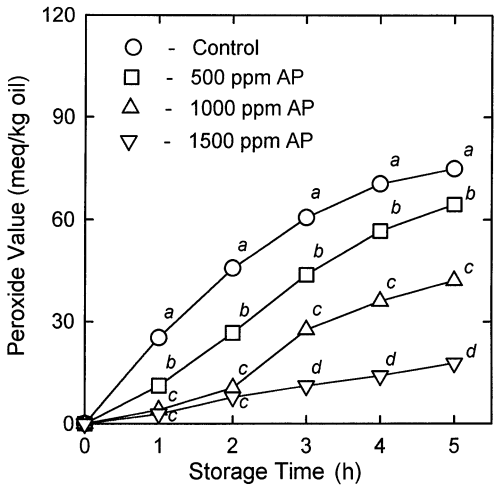
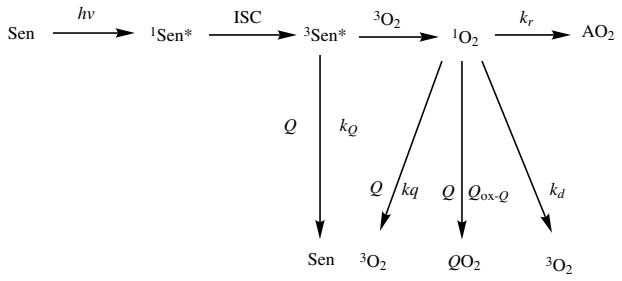


FIG. 4. Effects of 0, 500, 1000, and 1500 ppm (wt/vol) AP on chlorophyll-sensitized photooxidation of soybean oil in a solvent mixture (benzene/methanol, 4:1, vol/vol) during storage under fluorescent light. Peroxide values with different italicized letters at the same storage time were significantly different ($P < 0.05$). See Figure 1 for abbreviation.

for the formation of oxidized products (AO_2) *via* singlet-oxygen oxidation is as follows (19):



When a sensitizer (Sen), such as chlorophyll, in 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 products (AO_2). The formation of oxidized products (AO_2) could be reduced by the quenching of the singlet oxygen and/or the excited triplet sensitizer. If AP reduces sensitized photooxidation of oils by singlet oxygen quenching, the following steady-state kinetic equation is established:

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

where K denotes the rate of singlet oxygen formation; AO_2, oxidized soybean oil; k_r, reaction rate constant of methyl linoleate with singlet oxygen; A, methyl linoleate; k_q, reaction rate constant of physical singlet oxygen quenching; $k_{ox\text{-}Q}$, reaction rate constant of chemical singlet oxygen quenching by AP; Q, AP; and k_d, decaying rate of singlet oxygen.

The intercept and slope of the plots of $[AO_2]^{-1}$ vs. $[A]^{-1}$ at various concentrations of quencher (Q) are K^{-1} and $K^{-1}\{k_d + kq[Q] + k_{ox\text{-}Q}[Q]/k_r\}$, respectively. The intercepts of the plots are independent of the concentration of quencher (AP), and the slopes are dependent on the concentration of quencher (19).

The plot of $[AO_2]^{-1}$ vs. $[A]^{-1}$ for different levels of AP is shown in Figure 5. The intercepts were the same for different levels of AP, but the slopes of the plots increased as the concentration of AP increased from 0 to 2.5×10^{-3} M, indicating that AP quenched singlet oxygen only to reduce the photosensitized oxidation of oils. That is, AP reduced photosensitized oxidation of oils by the singlet oxygen quenching mechanism but not by the excited triplet sensitizer quenching mechanism (19).

The linear regression line for the plot of $[AO_2]^{-1}$ vs. $[A]^{-1}$ without AP (Fig. 5) was $Y = 51.17\ X + 38.88$, where $Y = [AO_2]^{-1}$ and $X = [A]^{-1}$. The slope/intercept ratio of the regression line was 0.7598. Foote (19) showed that the slope/intercept ratio of the regression line for the oil without quencher is k_d/k_r. The k_d value in a solvent mixture (benzene/methanol, 4:1, vol/vol) is 12×10^4 s^{-1} (21). Because the singlet oxygen

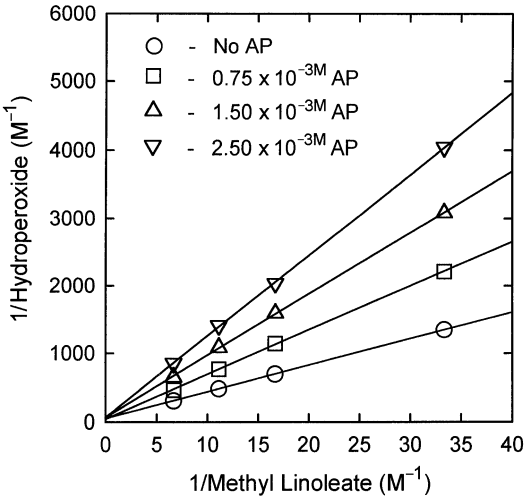


FIG. 5. Effects of AP on peroxide formation of methyl linoleate in a solvent mixture (benzene/methanol, 4:1) containing 3.3×10^{-6} M chlorophyll *b* during 1-h storage at 25°C under 3300 lux fluorescent light. See Figure 1 for abbreviation.

oxidation rate (k_r) of methyl linoleate is k_d/slope, then $k_r = 12 \times 10^4/0.7598 = 1.6 \times 10^5$ M^{-1} s^{-1} in a solvent mixture (methanol/benzene, 1:4, vol/vol). This present value (k_r) for methyl linoleate was close to the one reported previously in pyridine. Doleiden *et al.* (22) reported that the singlet oxygen oxidation rate of methyl linoleate (k_r) was 1.3×10^5 M^{-1} s^{-1} in pyridine.

For the calculation of the ratios of slope/intercept of the plots in Figure 5, the average intercept value (56.57) of the four plots was used. The ratios of slope/intercept of the plots for 0, 0.75×10^{-3}, 1.5×10^{-3}, and 2.5×10^{-3} M AP were calculated from Figure 5 and were 0.687, 1.154 1.590, and 2.330, respectively. The r^2 values for the regression lines in Figure 4 were greater than 0.996. To determine the singlet oxygen quenching rate ($k_q + k_{ox\text{-}Q}$) of AP, the slope/intercept ratio vs. [AP] was plotted (Fig. 6). The linear regression equation of the plot/intercept ratio vs. [AP] of Figure 6 was $Y = 0.646 \times 10^3 X + 0.653$, and the correlation coefficient (r^2) was 0.986. Foote (19) reported that the slope of the plot of slope/intercept ratio vs. [Q] is $k_q + k_{ox\text{-}Q}/k_r$. The value of total singlet oxygen quenching rate constant ($k_q + k_{ox\text{-}Q}$) of AP is slope $\times k_r$. Because the slope of the plot for AP (Fig. 6) was 0.646×10^3 and k_r was 1.58×10^5 s^{-1}, the total quenching rate constant ($k_q + k_{ox\text{-}Q}$) was $1.58 \times 10^5 \times 0.646 \times 10^3$ M^{-1} s^{-1} = 1.0×10^8 M^{-1} s^{-1}. The total singlet oxygen quenching rate constant of AP in a solvent mixture (benzene/ methanol, 4:1, vol/vol) is lower than previously reported for ascorbic acid in aqueous systems (16). Jung *et al.* (16) reported that the rate constants for ascorbic acid with singlet oxygen at pH 7.5, 6.0 and 4.5 were 6.63×10^8, 5.77×10^8, and 5.27×10^8 M^{-1}s^{-1}, respectively. The total quenching rate constant of AP was one order of magnitude greater than the one previously reported for α-tocopherol (8) and one order of magnitude lower than that of

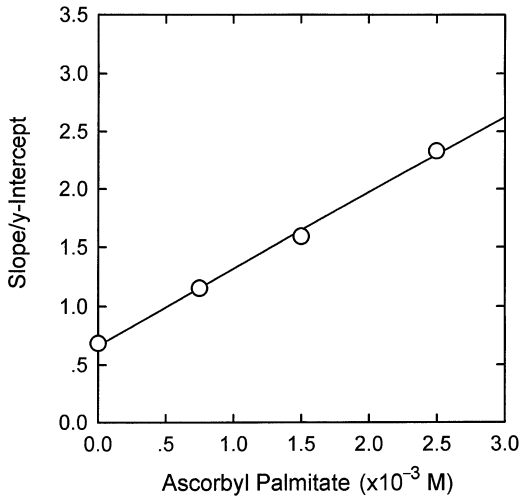


FIG. 6. The plot of slope/intercept of the plots (1/peroxide vs. 1/methyl linoleate shown in Fig. 5) vs. the concentration of ascorbyl palmitate.

β-carotene (6,9). That is, AP was about 10 times more effective than α-tocopherol in quenching singlet oxygen.

This present kinetic value for singlet-oxgyen-quenching ability of AP is consistent with its antiphotooxidative activity, shown in Figure 1. That is, AP, which had a stronger singlet oxygen quenching ability, also had a stronger antioxidative activity in photosensitized oxidation of oil than did α-tocopherol. Because the carotenoids provide yellow–red colors to oils and foods, the application of carotenoids to oils and foods is limited. It is expected that AP, which is almost colorless, might be applied to oils and oil-containing foods prevent photooxidation in various oils, oil-soluble vitamins (retinyl palmitate, tocopherols, and carotenoids), and other oil-soluble components (cholesterol, limonene, conjugated terpenes, etc.).

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