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


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 photoxidation of methyl linolate 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 photoxidation of linoleic acid (P < 0.05). The steady-state kinetic studies indicated that AP quenched singlet oxygen only to minimize the chlorophyll-sensitized photoxidation of oils. The calculated total quenching rate of AP was 1.0 x 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.


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 photoxidation 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 photoxidation 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 photoxidation 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 x 10^8, 5.77 x 10^8, and 5.27 x 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.
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 cm²/10 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...