Quenching Mechanism and Kinetics of Ascorbyl Palmitate for the Reduction of the Gamma Irradiation-Induced Oxidation of Oils

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ABSTRACT: The effects of 0, 250, 500, and 1000 ppm (wt/vol) ascorbyl palmitate (AP) on the gamma irradiation-induced oxidation of soybean oil, cottonseed oil, corn oil, tallow, lard, or linoleic acid either in a solvent mixture (benzene/methanol, 4:1 vol/vol) or in methanol, was studied immediately after gamma irradiation with a dose of 1–5 kGy. Steady-state kinetic approximation was used to determine a quenching mechanism and quenching rate constant of AP on the gamma irradiation-induced oxidation of purified soybean oil in a solvent mixture (benzene/methanol, 4:1 vol/vol). Irradiation greatly increased oxidation of all oils, as was expected. AP was extremely effective at minimizing oxidation in all oils, and its effectiveness was concentration dependent. AP showed significantly greater antioxidative activity than α-tocopherol for the reduction of oxidation in all oils ($P < 0.05$). The steady-state kinetic studies indicated that AP quenched oxygen only to minimize the oxidation of oils. The calculated total quenching rate of AP was 7.51×10^7 $M^{-1}s^{-1}$. The present results clearly show the effective oxygen quenching ability of AP for the reduction of gamma irradiation-induced oxidation of oils.

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KEY WORDS: Ascorbyl palmitate, edible oil, gamma irradiation, kinetics, mechanism, oxidation, oxygen quenching.

Gamma irradiation effectively killed food-borne pathogens on food, thereby prolonging shelf life. Numerous researchers have obtained positive results on the spoilage inhibition of food by ionizing radiation and indicated wholesomeness and applications of irradiated food (1–4). Furthermore, the U.S. Food and Drug Administration has permitted ionizing radiation of 4.5 kGy and below 7.0 kGy for fresh beef and frozen beef, respectively (5). This in turn extends the possibility of the application of gamma irradiation to high-fat foods. But, various types of oil and oil-soluble vitamins in foods are susceptible to oxidation when ionizing radiation is absorbed by matter (6), especially gamma irradiation in the initial step of oil oxidation and the reaction rate of gamma irradiation-induced oxidation with oils (7,8). These are both faster than that of autooxidation.

Many synthetic antioxidants have been prepared, the best known of which are 2-*t*-butyl-4-hydroxyanisole (BHA) and 2,6-di-*t*-4-butyl-*p*-cresol (BHT). Because of growing concerns about the potential health side effects of synthetic antioxidants, in most countries the allowed concentration of each antioxidant in any food stuff is restricted to about 0.01% (9).

Natural antioxidants, such as tocopherols and carotenoids, can be used for the practical reduction of oxidation of oils and other oil-soluble components. The application of carotenoids, as effective oxygen quenchers, to some oils or other oil-containing foods is, however, limited because they add a yellow to red color to the products (10,11). Tocopherols do not add color to oils, but their oxygen-quenching abilities are not as effective as carotenoids (12). Thus, researchers in academia and industry continue to look for novel fat-soluble natural antioxidants for the effective reduction of oxidation in oils.

Ascorbic acid is reportedly an effective oxygen quencher (13–15) and can be used to minimize the oxidation of water-soluble compounds in aqueous solution (13). Ascorbic acid cannot be used in oils because it is oil-insoluble. 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 with no specific limitations or restrictions. Ingestion of this antioxidant would pose no health hazards because metabolic breakdown yields ascorbic acid and palmitic acid, both normal metabolites (15).

Even though it has been reported that AP is an effective singlet oxygen quencher on the photosensitized oxidation of oils, the quenching rate of AP on the gamma irradiation-induced oxidation of oils has not been studied. The objectives of this research were to study the effects of AP on the gamma irradiation-induced oxidation of soybean oil, cottonseed oil, corn oil, tallow, lard, and linoleic acid, and to determine the quenching mechanism and quenching-rate constant of AP in purified soybean oil.

MATERIALS AND METHODS

Materials. Linoleic acid, AP, and α-tocopherol were purchased from Sigma Chemical Co. (St. Louis, MO). Soy-

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bean oil, cottonseed oil, corn oil, tallow, and lard without any additives were obtained from Seoul Heinz Co. (Seoul, Korea).

Effects of AP on the gamma irradiation-induced oxidation of various types of oils. To study the effects of AP on the gamma irradiation-induced oxidation of soybean oil, cottonseed oil, corn oil, tallow, and lard, samples of 0, 250, 500, and 1000 ppm (wt/vol) AP in 10.0% wt/vol of each oil were prepared in a solvent mixture of benzene/methanol (4:1 vol/vol). The solvent mixture was used for solubilizing all the necessary reagents [methanol for linoleic acid and ascorbyl palmitate, and benzene/methanol (4:1 vol/vol) for oils]. The solvent mixture of benzene and methanol was chosen for the calculation of quenching rate since the decay rate of oxygen in this solvent mixture was known according to data from Lee *et al.* (15) and Young *et al.* (16).

To study the effects of AP on the gamma irradiation-induced oxidation of linoleic acid, samples of 0, 250, 500, and 1000 ppm (wt/vol) AP in 1.0% wt/vol linoleic acid were prepared in methanol. 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 the sample.

Prepared samples (10 mL each) were transferred, in duplicate, into 30-mL serum bottles. The surface volume 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 irradiated in a cobalt-60 irradiator.

Gamma irradiation was carried out in a cobalt-60 irradiator equipped with 100 kCi activity at 15 ± 0.5°C and operated at a dose rate of 1 kGy h^{-1}. The applied dose levels were 1, 2, 3, and 5 kGy. The absorbed dose was monitored with both free radical and ceric/cerous dosimeters. The degree of oxidation on various types of oils was determined by measuring peroxide values immediately after gamma irradiation using the AOCS method Cd-53(17).

Determination of quenching mechanism and rate constant. The quenching mechanism and kinetics of the effect of AP on the gamma irradiation-induced oxidation of oils were studied by the steady-state kinetic method of Foote (18). To prepare purified soybean oil, bleached and deodorized soybean oil was prepared according to Lee and Min (19). To study the quenching mechanism and oxygen-quenching rates of AP, samples of 0.03, 0.06, 0.09, and 0.15 M purified soybean oil in a solvent mixture (benzene/methanol, 4:1 vol/vol) and 0, 0.75×10^{-3}, 1.50×10^{-3}, and 2.50×10^{-3} M AP were prepared according to Jung and Min (11). 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 irradiated with a dose of 3 kGy. Oxidation of the purified soybean oil was determined by peroxide formation, and quenching mechanism and the quenching rate constants of AP were studied by using steady-state kinetic equations (11,12,18,20).

Statistical analysis. All the experiments were done in duplicate, and statistical analysis was done with the Statistical Analysis System (Version 5, Cary, NC) (21). Duncan's multiple range test was used to ascertain the effects of AP on the gamma irradiation-induced oxidation of various types of oils.

RESULTS AND DISCUSSION

Effects of AP on the gamma irradiation-induced oxidation of various types of oils. The effects of 0, 250, 500, and 1000 ppm (wt/vol) AP on the gamma irradiation-induced oxidation of soybean oil, cottonseed oil, corn oil, tallow, lard, or linoleic acid either in a solvent mixture (benzene/methanol, 4:1 vol/vol) or in methanol after 1–5 kGy gamma irradiation are shown in Table 1.

The difference in oxidation rates for oils such as soybean oil, cottonseed oil, corn oil, tallow, lard, and linoleic acid was correlated with the degree of unsaturation.

Gamma rays greatly increased the oxidation of soybean oil in a solvent mixture, as was expected. However, the addition of either AP or α-tocopherol greatly decreased the gamma irradiation-induced oxidation of soybean oil. As the concentration of AP increased, the reduction of peroxide formation in soybean oil increased. The addition of 250, 500, and 1000 ppm AP resulted in 58.2, 69.0, and 74.1% inhibition of soybean oil, respectively, after 5 kGy gamma irradiation. Duncan's multiple range tests showed that the peroxide values of samples treated with AP were significantly lower than the control (no AP added) after gamma irradiation ($P < 0.05$). AP was much more effective than α-tocopherol. The peroxide value of soybean oil in the presence of 1000 ppm α-tocopherol after 5 kGy gamma irradiation showed significantly lower activity than even 500 ppm AP ($P < 0.05$).

The addition of AP also reduced the gamma irradiation-induced oxidation of cottonseed oil. The peroxide value of cottonseed oil with 1000 ppm α-tocopherol was not significantly different from 1000 ppm AP treatment after 5 kGy gamma irradiation ($P > 0.05$).

AP was very effective in minimizing gamma irradiation-induced oxidation of corn oil in a solvent mixture (benzene/methanol, 4:1 vol/vol). As the AP was increased from 250 to 1000 ppm, the effectiveness of AP increased significantly ($P < 0.05$).

AP also retarded the gamma irradiation-induced oxidation of tallow in a solvent mixture (benzene/methanol; 4:1 vol/vol). In the case of tallow containg 1000 ppm α-tocopherol, the peroxide values were not significantly different from 500 ppm AP treatment after 5 kGy gamma irradiation ($P > 0.05$).

Gamma irradiation-induced oxidation of lard or linoleic acid either in a solvent mixture (benzene/methanol, 4:1 vol/vol) or in methanol was inhibited by AP. As the AP was increased from 250 to 1000 ppm, the effectiveness of AP increased significantly ($P < 0.05$).

These results showed that the antioxidant activity of AP was higher than that of α-tocopherol in all oils. And, the results in Table 1 clearly show, for the first time, that AP had a

TABLE 1
Effects of 0, 250, 5000, 1000 ppm (wt/vol) Ascorbyl Palmitate (AP) and 1000 ppm (wt/vol) α-Tocopherol (Toc) on 0–5 kGy Gamma Irradiation-Induced Oxidation of Oils (meq/kg)[a]

		Treatment	Gamma irradiation dose (kGy)				
Fat and oils		(ppm)	0	1	2	3	5
Soybean oil		Control	0.00	11.25^{a}	33.45^{a}	64.20^{a}	125.75^{a}
	AP	250	0.00	9.00^{a}	16.74^{b}	25.00^{b}	52.60^{b}
		500	0.00	7.34^{a}	11.43^{c}	$20.20b^{c}$	39.00^{d}
		1000	0.00	5.40^{a}	6.76^{d}	12.40^{c}	32.60^{e}
	Toc	1000	0.00	10.40^{a}	16.37^{b}	$22.20^{b,c}$	45.00^{c}
Cotton seed oil		Control	0.00	11.40^{a}	70.48^{a}	134.00^{a}	207.50^{a}
	AP	250	0.00	8.20^{a}	17.43^{b}	28.40^{b}	103.00^{b}
		500	0.00	6.00^{a}	$11.52^{b,c}$	$20.60^{b,c}$	46.60^{c}
		1000	0.00	4.80^{a}	7.48^{c}	16.60^{c}	29.50^{d}
	Toc	1000	0.00	8.80^{a}	$12.76^{b,c}$	$21.60^{b,c}$	44.85^{c}
Corn oil		Control	0.00	12.40^{a}	60.42^{a}	115.20^{a}	203.20^{a}
	AP	250	0.00	11.43^{a}	21.64^{b}	44.38^{b}	82.36^{b}
		500	0.00	9.86^{a}	$18.75^{b,c}$	32.00^{c}	60.40^{c}
		1000	0.00	7.42^{a}	15.00^{c}	20.60^{d}	34.00^{e}
	Toc	1000	0.00	10.60^{a}	15.54^{c}	24.00^{d}	41.20^{d}
Tallow		Control	0.00	13.70^{a}	18.43^{a}	25.75^{a}	42.42^{a}
	AP	250	0.00	7.70^{b}	13.43^{b}	21.00^{b}	39.20^{b}
		500	0.00	6.60^{b}	10.51^{c}	16.00^{c}	33.00^{c}
		1000	0.00	4.60^{c}	7.06^{d}	12.00^{d}	28.00^{d}
	Toc	1000	0.00	7.00^{b}	11.43^{c}	16.60^{c}	31.84^{c}
Lard		Control	0.00	27.00^{a}	42.64^{a}	51.00^{a}	77.10^{a}
	AP	250	0.00	11.00^{b}	21.46^{b}	32.90^{b}	61.60b
		500	0.00	8.42^{b}	16.59^{c}	23.80^{c}	50.60^{c}
		1000	0.00	4.00^{e}	6.74^{e}	9.80^{e}	20.00^{e}
	Toc	1000	0.00	4.76^{c}	10.23^{d}	18.64^{d}	32.67^{d}
Linoleic acid		Control	0.00	8.60^{a}	18.43^{a}	31.40^{a}	50.40^{a}
	AP	250	0.00	8.14^{a}	14.43^{b}	23.20^{b}	37.60^{b}
		500	0.00	7.16^{a}	13.42^{b}	19.23^{c}	30.70^{c}
		1000	0.00	4.20^{b}	7.64^{d}	13.20^{e}	23.75^{e}
	Toc	1000	0.00	$5.80^{a,b}$	11.05^{c}	17.20^{d}	28.26^{d}

[a] Means within each column with the different superscript roman letters are significantly different at $P < 0.05$.

strong antioxidative effect on gamma irradiation-induced oxidation of oils.

Quenching mechanism and rate constant of AP. Since it was found that AP decreased the gamma irradiation-induced oxidation of oils, we decided to study the mechanism and the kinetics of AP for the reduction of oxidation of oils by using a steady-state kinetic approximation.

If AP reduced gamma irradiation-induced oxidation of oils by oxygen quenching, the following steady-state kinetic equation is established:

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

where $\mathrm{AO_2}$ is oxidized soybean oil; k_r, reaction rate constant of oxygen oxidation of soybean oil; A, purified soybean oil; k_q, reaction rate constant of physical oxygen quenching; $k_{\mathrm{ox\text{-}Q}}$, reaction rate constant of chemical oxygen quenching by AP; Q, AP; and k_d, decay rate of oxygen.

The intercept and slope of the plots of $[\mathrm{AO_2}]^{-1}$ vs. $[A]^{-1}$ at various concentrations of quencher (Q) are K^{-1} and $K^{-1}\{k_d + k_q[Q] + k_{\mathrm{ox\text{-}Q}}[Q]/k_r\}$, respectively. The intercepts of the plots are independent of the concentration of quencher (AP), and the slopes are dependent on the concentration of quencher (18).

The plot of $[\mathrm{AO_2}]^{-1}$ vs. $[A]^{-1}$ for different levels of AP is shown in Figure 1. 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, which meant that AP quenched oxygen only to reduce the gamma irradiation-induced oxidation of oils. That is, AP reduced gamma irradiation-induced oxidation of oils by the oxygen-quenching mechanism (18).

The linear regression line for the plot of $[\mathrm{AO_2}]^{-1}$ vs. $[A]^{-1}$ containing no AP (Fig. 1) was $y = 21.13x + 119.20$, where $y = [\mathrm{AO_2}]^{-1}$ and $x = [A]^{-1}$. The slope/intercept of the regression

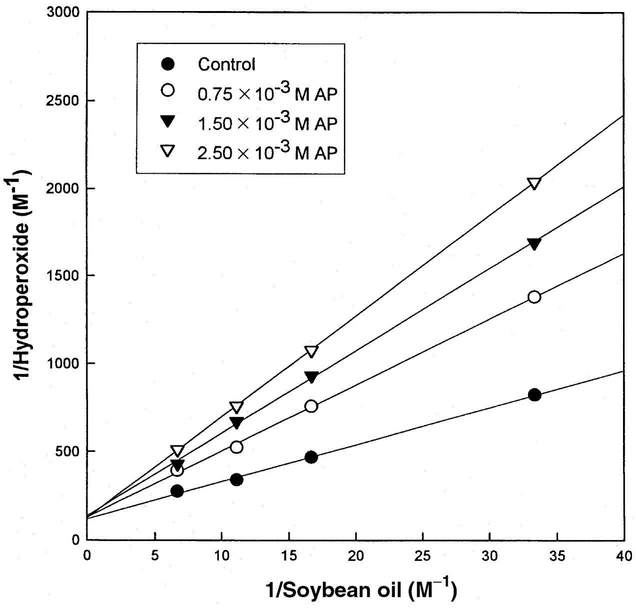

FIG. 1. Effects of ascorbyl palmitate (AP) on the peroxide formation of purified soybean oil in a solvent mixture (benzene/metanol; 4:1 vol/vol) dafter 1 h storage under 3 kGy gamma ray.

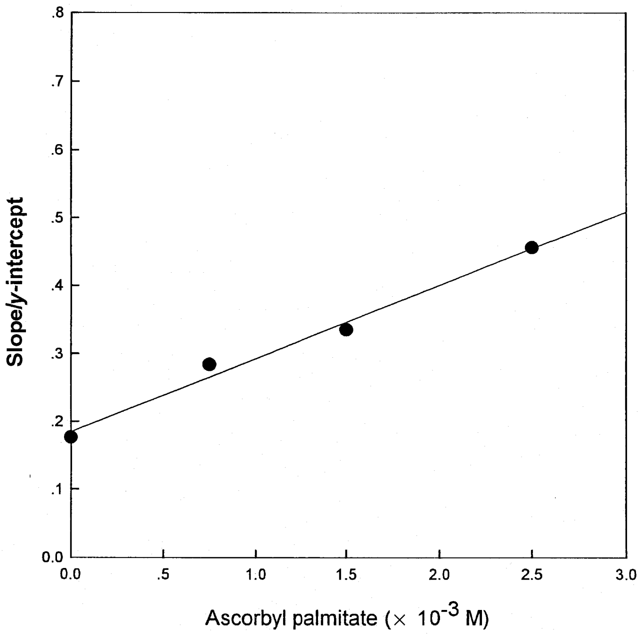

FIG. 2. The plot of the slope/intercept of 1/peroxide vs. 1/soybean oil (shown in Fig. 1) vs. the concentration of ascorbyl palmitate.

line was 0.1773. Foote (18) showed that the slope/intercept of the regression line for the oil containing no quencher is k_d/k_r. The k_d value in a solvent mixture (benzene/methanol, 4:1 vol/vol) is 1.2×10^5 s^{-1} (16). Because the oxidation rate (k_r) of soybean oil is k_d/slope, then $k_r = 1.2 \times 10^5/0.1773 = 6.77 \times 10^5$ M^{-1} s^{-1} in a solvent mixture (methanol/benzene, 1:4 vol/vol).

For the calculation of the ratios of the slope/intercept of the plots in Figure 1, the average intercept value (127.87) of the four plots was used. The ratios of the slope/intercept of the plots containing 0, 0.75×10^{-3}, 1.5×10^{-3}, and 2.5×10^{-3} M AP were calculated from Figure 1 and were 0.165, 0.293, 0.367, and 0.449, respectively. The r^2 values for the regression lines in Figure 1 were greater than 0.990. To determine the oxygen-quenching rate ($k_q + k_{\text{ox-Q}}$) of AP, the slope/intercept vs. AP was plotted (Fig. 2). The linear regression equation of the plot/intercept vs. AP of Figure 2 was $y = 1.11 \times 10^2\,x + 0.187$, and the correlation coefficient (r^2) was 0.983. Foote (18) reported that the slope of the plot of slope/intercept vs. Q is $k_q + k_{\text{ox-Q}}/k_r$. The value of oxygen quenching rate constant ($k_q + k_{\text{ox-Q}}$) of AP is slope $\times$ k_r. Because the slope of the plot for AP (Fig. 1) was 1.11×10^2 and k_r was 6.77×10^5 M^{-1} s^{-1}, the total quenching rate constant ($k_q + k_{\text{ox-Q}}$) was $1.11 \times 10^2 \times 6.77 \times 10^5$ M^{-1} s^{-1} = 7.51×10^7 M^{-1} s^{-1}.

The oxygen quenching rate constant of AP on the gamma irradiation oxidation is lower than previously reported for AP on the photosensitized oxidation of oils (15). This present kinetic value for the oxygen-quenching ability of AP is consistent with its antigamma irradiation-induced oxidative activity shown in Table 1. That is, AP, which had a stronger oxygen-quenching ability, also had a stronger antioxidative activity in gamma irradiation-induced oxidation of oil than did α-tocopherol.

It is expected that AP, which is almost colorless, might be applied to oils and oil-containing foods for the prevention of gamma irradiation-induced oxidation in various oils, oil-soluble vitamins (retinyl palmitate, tocopherols and carotenoids), other oil-soluble components (cholesterol, limonene, conjugated terpenes, etc.), and high-fat food.

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