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Factors influencing ultraviolet and electron beam irradiation-induced free radical damage of ascorbic acid

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Abstract

The oxidation of an unsaturated fatty acid and antioxidant vitamin by ultraviolet (UV) and electron beam (e^- beam) irradiation was studied. Hydroxyl radical (•OH), generated by Fe-EDTA and hemoglobin, under heat incubation or UV illumination, was compared. L-ascorbic acid (LAA; 10, 100 and 500 mM) oxidation was measured after irradiation at 0–10 kGy, in the presence and absence of myoglobin. Moreover, both UV and e^- beam irradiation at 0–20 kGy were used to oxidize a linoleic acid emulsion, both with and without LAA. The presence of hemoglobin generated less •OH, than Fe-EDTA under similar heating conditions. Generation of •OH increased with illumination by UV. LAA was oxidized by e^- beam irradiation, with an increasing rate of oxidation occurring at lower concentrations of LAA and higher dosages of irradiation. LAA (10 mM) exhibited pro-oxidant activity in the linoleic acid emulsion when exposed to both forms of irradiation, while 500 mM LAA exhibited antioxidant activity. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: UV light; Electron beam irradiation; L-ascorbic acid; Oxidation; Antioxidant activity

1. Introduction

Lipid autoxidation is the leading cause of non-microbial spoilage in foods. Polyunsaturated fatty acids (PUFA) are especially prone to autoxidation, which can lead to adverse effects on flavour, nutritive value and shelf-life of the food (Vercellotti, St. Angelo & Spanier 1992). Several factors have been linked to the initiation of lipid autoxidation. These include oxygen, transition metals, light, radiation, peroxides, free radicals, heat and certain enzymes such as lipooxygenases (Namiki, 1990).

Ultraviolet (UV) light is commonly used in the food industry to reduce microbial growth in beef (Volz, Gortner, Pitz & Miller, 1949), water (Potter & Hotchkiss, 1995) and juices (Flickinger, 1999). However, UV light can adversely affect food quality by inducing photooxidation of PUFA (Jay, 1996). Yamshoji, Yoshida and Kajimoto (1979) observed the oxidation of linoleic acid in ethanol after 15 min exposure to UV light. Oxidation of PUFA, induced by UV light was also detected in beef muscle and adipose tissues (Korhonen, Regan, Carpenter & Campion, 1981) and in rat liver microsomes (Dumont, Petit, Tarrade,, & Nouvelot, 1992). The initiation of lipid oxidation by UV light has been attributed to the nucleophilic attack of PUFA, by UV light-generated reactive oxygen species (ROS), in particular, superoxide anion, singlet oxygen and peroxyl radicals (Melo & Mahmoud, 1988; Yamashoji et al., 1979). Furthermore, UV light photooxidation of hydrogen peroxide has been shown to produce hydroxyl radical, a reactive ROS (Yamashoji et al., 1979). All ROS generated from UV light radiation can induce oxidation of other non-lipid food components, such as protein or carbohydrates (Gennadios, Rhim, Handa, Weller & Hanna, 1998; Kato, Uchida & Kawakishu, 1992).

In recent years, there has been a growing concern about irradiation-induced lipid oxidation in foods, especially in beef products, since the process of irradiation of ground beef has been permitted in the United States to reduce contamination by *E. Coli* O157:H7. Irradiation of PUFA is known to produce oxidation products, such as short-chain carbonyls and peroxides (Doty, 1965). Oxidation of the phospholipid fraction of ground beef PUFA content is the major contributor to

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the development of warm-over flavour (WOF) and rancidity in meat muscle (Giroux & Lacroix, 1998; Lefebvre, Thibault, Charbonneau & Piette, 1994). Earlier studies have also demonstrated the acceleration of lipid oxidation by irradiation in other muscle products, such as turkey meat (Hampson, Fox, Lakritz & Thayer, 1996), fish (Ghadi & Venugopal, 1991), pork (Luchsinger et al., 1996; Chen, Jo, Lee & Ahn, 1999; Lambert, Smith & Dodds, 1992), chicken (Heath, Owens & Tesch, 1990) and sausages (Ahn, Olsen, Jo, Love & Jin, 1999).

Antioxidants can be added to PUFA-rich foods to minimize lipid autoxidation. Antioxidants function by scavenging free radicals, interacting with headspace or dissolved oxygen, or chelating transition metals (Gordon, 1990). Commercially, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene, propyl gallate, and natural antioxidants, such as α-tocopherol, ascorbyl palmitate and L-ascorbic acid (LAA) are widely used due to their potent antioxidant activity (i.e. scavenging free radicals) and stability in various food systems.

The objectives of this study were to investigate the extent of oxidative damage induced by UV and electron beam irradiation and to assess the degree of antioxidant activity provided by LAA under both irradiation conditions. Several simple aqueous and emulsion model systems were used to study the underlying interactions between irradiation, LAA and oxidative reactions.

2. Materials and methods

2.1. Materials

All chemicals used were of reagent grade. Sodium dihydrogenorthophosphate, ferrous chloride, ferric chloride, metaphosphoric acid and LAA were obtained from BDH Chemicals Co. (Toronto, ON). Tween 20 was obtained from Difco Laboratories (Detroit, MI). Linoleic acid, BHA, Na_2-EDTA, thiobarbituric acid, 2,6-dichloroindophenol, Tris-HCl, Tris-NaOH, hemoglobin (Hb) and myoglobin (Mb) were purchased from Sigma Chem. Co (St. Louis, MO). Deoxyribose was acquired from Applied Science Lab Inc (Pennsylvania). Sodium phosphate, sodium hydroxide, sodium bicarbonate, ethanol, hydrogen peroxide, hydrochloric acid, trichloroacetic acid, acetic acid and ammonium thiocyanate were purchased from Fisher Scientific Co. (Fair Lawn, NJ). Only distilled deionized water was used for experimentation.

2.2. Hydroxyl radical generation

Hydroxyl radical generation was measured according to a modified method of Halliwell, Gutteridge and Aruoma (1987). The reaction mixture consisted of Na_2-EDTA (1 mM), $FeCl_3$ (10 mM) and/or Hb (0.01 g/ml), H_2O_2 (10 mM), deoxyribose (10 mM), in a Tris-buffer (5 mM, pH 7.4) containing LAA (1 mM). Samples were incubated at 37°C in a water bath for 1 h in the dark, or expose to UV light ($\lambda = 254$ nm) for 15 min at a distance of 4 cm. Following incubation, a 1 ml aliquot of the incubated mixture was mixed with 1 ml of 10% TCA and 1 ml of 0.5% TBA (in 0.025M NaOH containing 0.02% BHA) and heated in a boiling water bath for 15 min to develop colour. The pink chromogen was measured at 532 nm, against a heated reagent blank, using UV-visible recording spectrophotometer (Shimadzu UV-160).

2.3. Ascorbic acid oxidation

Two simplified model systems were used to assess the oxidation of LAA after irradiation. An aqueous model system was prepared to contain 10, 100 or 500 mM LAA (in 0.2M sodium phosphate buffer at pH 7.0) and Mb (0.01 g/ml). An emulsion model system was also prepared by adding 10, 100 or 500 mM LAA (in sodium phosphate buffer) and Mb to linoleic acid containing Tween 20. All samples from both model systems were kept at 4°C during transportation to and from the electron beam irradiation facility. Samples were irradiated at 0 (control), 2.5, 5 and 10 kGy using an electron beam accelerator (Iotron Technologies Inc., Port Coquitlam) at room temperature. After irradiation, all samples were stored at 4°C under normal lighting.

LAA in both model systems was measured by a modification of the vitamin C:2,6-dichloroindophenol method (AOAC, 1980). Prior to analysis, a metaphosphoric-acetic acid solution (15 g HPO_3 dissolved in 200 ml HOAc and diluted to 500 ml) was prepared and stored in an amber bottle at 4°C. The colour dye 2,6-dichloroindophenol (50 mg dissolved in 50 ml distilled deionized water containing 42 mg sodium bicarbonate and diluted to 200 ml with distilled deionized water) was prepared immediately before use. Ascorbic acid was determined by titrating a 7 ml mixture containing an aliquot of sample in metaphosphoric-acetic acid, against an indophenol solution until end-point was reached. Ascorbic acid content was calculated from standard curves relating specific ascorbic acid concentration to the volume of indophenol required for titration.

2.4. Ultraviolet light-induced lipid oxidation

The effects of UV light and ascorbic acid on lipid oxidation were determined using a linoleic acid emulsion system (Wijewickreme & Kitts, 1997). This model system was prepared by mixing linoleic acid, Tween 20, sodium phosphate buffer (0.2M, pH 7.0), and LAA (either 10, 100 or 500 mM) together, and adjusting the total volume with distilled deionized water. The mixture

was vortexed until an emulsion was formed. Then, the emulsion was exposed to 15 min of UV light ($\lambda = 254$ nm) at a distance of 4 cm at 4°C. After UV exposure, the emulsion was stored at ambient temperature under normal lighting.

Samples were periodically removed to measure the degree of lipid oxidation by the ferric-thiocyanate method reported by Wong and Kitts (2001). Peroxide content was determined by the oxidation of ferrous to ferric ion, as detected by colour development with thiocyanate ion in the reaction mixture, producing a characteristic absorbance at 500 nm.

2.5. Electron beam irradiation-induced lipid oxidation

The linoleic acid emulsion system mentioned in the above experiment was also exposed to 0 (control), 5, 10 and 20 kGy dosages of electron beam irradiation (Iotron Technologies, Port Coquitlam) at room temperature. After irradiation, all samples were stored at ambient temperature under normal lighting. An aliquot of the emulsion was periodically removed to measure the degree of lipid oxidation, using the ferric-thiocyanate method mentioned above.

2.6. Statistical analysis

All treatments in all experiments were carried out in triplicate and analyzed in duplicate. Each experiment was conducted twice to assess precision. Data were analyzed using an one-way ANOVA (for one variable effect) and a two-way ANOVA (for two variable effects), with Tukey's Test for mean values with significance at a probability of $P < 0.05$ (MiniTab ststistical program; MiniTab Inc., PA). Linear trend analyses, linear trend equations and correlation coefficients were determined by the Microsoft Excel program (Microsoft Corp., USA)

3. Results

3.1. Hydroxyl radical generation

The degree of hydroxyl radical (•OH) generation, represented by the quantity of oxidized deoxyribose-TBA adduct detectable at 532 nm, is presented in Fig. 1. Regardless of the effects of heating and exposure to UV, the catalyst ferrous ion (Fe^{2+}) (generated from the reduction of ferric ion by LAA) generated twice the quantity of •OH in comparison to that of (Hb). The combination of Fe^{2+} and Hb increased the total concentration of iron in the sample mixtures; however, •OH production was not found to be statistically different from that of Fe^{2+} treatment alone.

Incubating the sample mixture with Fe^{2+} and Fe^{2+} with Hb at 37°C (i.e. heat treatment) produced a significantly greater ($P < 0.05$) •OH response than with the UV exposure. An opposite trend was noted when Hb was used in place of Fe^{2+}. The combination of Hb with

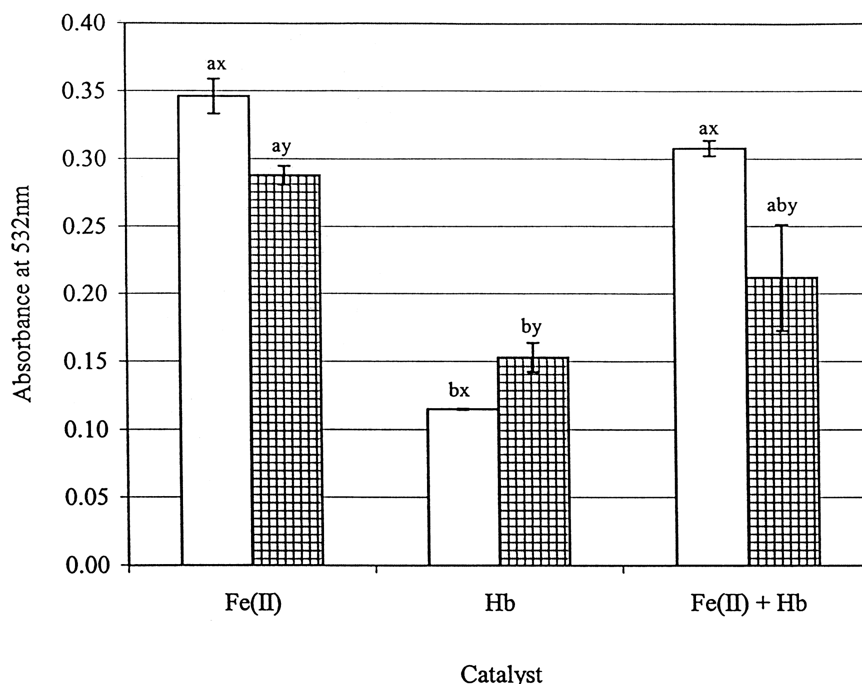

Fig. 1. Deoxyribose degradation by hydroxyl radical generated from Fenton reaction initiated by Fe(II) and/or hemoglobin under the influence of heat incubation (open columns) or UV light illumination (solid columns). [ab]Denotes the statistically significantly ($P < 0.05$) effects of various catalysts. [xy]Denotes the statistically significantly ($P < 0.05$) effects of heat and UV light.

UV exposure generated a significantly greater ($P<0.05$) •OH response than the Hb with heat incubation.

3.2. Ascorbic acid oxidation

LAA (10 mM) oxidation, induced by electron beam irradiation in the presence of Mb and linoleic acid, during 4°C storage is illustrated in Fig. 2. In general, the LAA content of all treatments decreased over time. The rate of LAA oxidation was affected by irradiation dose, in the order of 10 kGy > 5 kGy = 2.5 kGy > 0 kGy. At 10 kGy, complete oxidation of LAA in the linoleic acid emulsion occurred by day 3. In general, greater oxidation of LAA occurred in samples containing linoleic acid (approximately 15 to 20% more oxidation) throughout the storage period. No significant difference between LAA concentration values measured from day 1 to day 14 were found for all treatments. Rather a rapid loss of 10 mM LAA (i.e. between 31.8 and 96.5±0.5% of the original amount) occurred within a

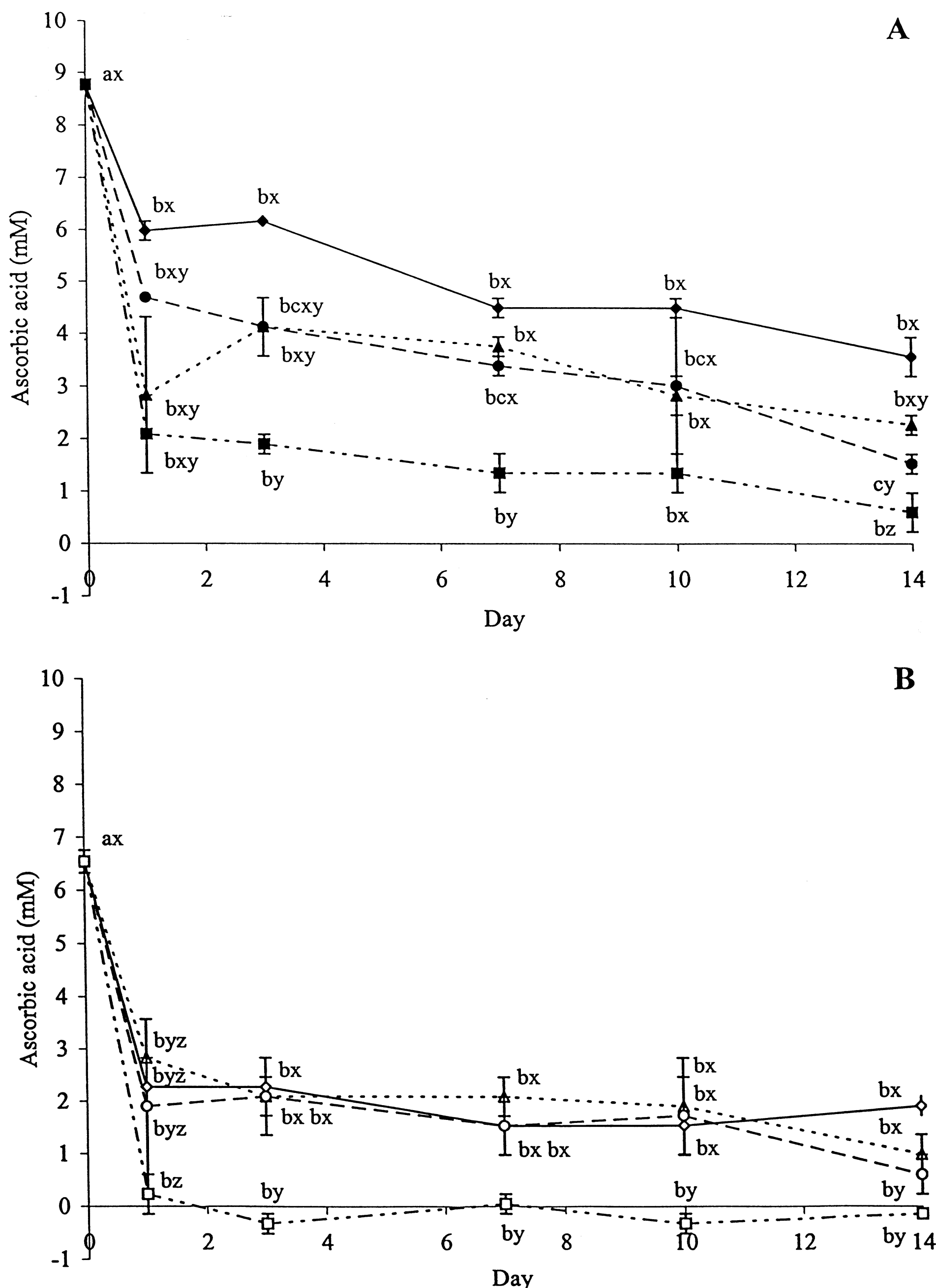

Fig. 2. Quantitative analysis of 10 mM ascorbic acid during storage as influenced by (A) the absence of linoleic acid and (B) the presence of linoleic acid with an initial exposure to irradiation at 0 (diamond), 2.5 (triangle), 5 (circle) and 10 kGy (square). [abc]Denotes the significantly different ($P<0.05$) levels of ascorbic acid within a single irradiation dose over time. [xyz]Denotes the significantly different ($P<0.05$) levels of ascorbic acid between the irradiation dosages at a particular time.

period of 24 h post-irradiation. By day 14, the final concentrations of the 10 mM LAA treatments fell within a range of 0.8–3.8 mM.

The oxidation of 100 mM LAA by electron beam irradiation is shown in Table 1. No statistical difference was observed between losses of LAA at dosages of 0, 2.5 and 5 kGy. Only the 10 kGy irradiated samples produced a significant ($p<0.05$) loss of LAA over time. A significant ($P<0.05$) decrease in LAA concentration (i.e. between 18.6 and 31.2±0.5% of initial concentration) was observed for all treatments on day 3. By day 14, however, only an average of 17.4+0.5% of the original LAA concentration was found to be oxidized in all irradiated samples. The oxidation of LAA was not significant in the presence of a linoleic acid emulsion medium.

A minimal oxidation of 500 mM LAA was observed during storage (Table 1), with general decrease occurring in the concentrations of 7.0–26.7±0.5% between day 0 and day 3. At the end of the storage period, the LAA content of all samples ranged from 80.0 to 96.2±0.5% of the original concentration. Surprisingly, fat and irradiation were found to have no significant individual or interactive effect on LAA oxidation at this concentration.

3.3. Ultraviolet light-induced lipid oxidation

Antioxidant activity of LAA against UV light-induced linoleic acid oxidation was evaluated, based on the ability of LAA to suppress the formation of lipid peroxides. The presence of peroxide was detected by the extent of Fe^{2+} oxidation to Fe^{3+}, which when coupled to thiocyanate, produces a red pigment with a maximum absorbance at 500 nm.

Fig. 3 illustrates the UV light-induced oxidation of linoleic acid. Immediately after the UV light exposure (i.e. Time 0), both the control and the 10 mM LAA sample exhibited a significantly greater ($P<0.05$) degree of lipid oxidation compared to both the 100 and the 500 mM LAA treatments. After the 46th hour post-UV light exposure, only the 10 mM LAA treatment was found to have a greater degree of lipid oxidation than the control. This observation demonstrated a possible prooxidant activity at 10 mM. At the same time, the peroxide levels present in 100 mM LAA treatment samples were not significantly different from that of control, indicating no appreciable antioxidant activity of LAA at this concentration. Maximum levels of peroxide were achieved by the 70th hour in the 10 mM LAA samples. It was not until the end of the storage period that the

Table 1
Electron beam irradiation-induced depletion of L-ascorbic acid in emulsions with and without linoleic acid during 4°C storage[a]

Ascorbic acid sample (mM)[b]	Irradiation dose (KGy)	Day of storage					
		0	1	3	7	10	14
10	0	8.77±0.01ax	5.98±0.19bx	6.17±0.01bx	4.50±0.19bx	4.50±0.19bx	3.57±0.34bx
10+LA[c]	0	6.54±0.21ay	2.27±0.19bxy	2.27±0.19by	1.53±0.56by	1.53±0.20bxy	1.90±0.19by
10	2.5	8.77±0.01ax	2.83±1.48bxy	4.13±0.56bxy	3.76±0.19bx	2.83±0.37bxy	2.27±0.22bxy
10+LA	2.5	6.54±0.21ay	2.83±0.01bxy	2.09±0.74by	2.09±0.37by	1.9±0.93bxy	0.98±0.36by
10	5	8.77±0.01ax	4.69±0.01bxy	4.13±0.56bcxy	3.39±0.21bcx	3.02±1.30bcxy	1.53±0.17cy
10+LA	5	6.54±0.21ay	1.90±1.67bxy	2.09±0.37by	1.53±0.56by	1.72±0.74bxy	0.62±0.37bz
10	10	8.77±0.01ax	2.09±0.74bxy	1.90±0.19by	1.35±0.37byz	1.35±0.30bxy	0.60±0.05bz
10+LA	10	6.54±0.21ay	0.23±0.37by	0.32±0.32bz	0.05±0.19bz	0.33±0.32by	0.15±0.14bz
100	0	102±3.62ax	87.4±1.47ax	83.0±0.01ax	90.3±4.40ax	100.1±5.87ax	91.8±8.80ax
100+LA	0	104±0.98ax	99.7±2.93abx	83.6±1.47bx	97.4±1.47bx	106±0.01abx	98.2±1.49abx
100	2.5	102±3.62ax	84.4±1.47abx	78.6±1.47bx	84.4±4.38abxy	88.8±5.87abx	90.3±1.55abx
100+LA	2.5	104±0.98ax	87.4±4.40ax	80.0±2.93ax	85.9±0.03axy	84.4±4.56ax	74.2±17.60ay
100	5	102±3.62ax	90.3±1.47bx	83.0±2.93ax	80.0±0.01axy	83.0±11.73ax	84.4±1.40ax
100+LA	5	104±0.98ax	85.9±5.87bx	80.0±0.02bx	84.4±4.41bxy	83.0±2.93bx	80.0±0.01bx
100	10	102±3.62ax	91.8±5.87ax	77.1±0.01by	81.5±1.48by	84.4±1.47bx	80.0±1.05bx
100+LA	10	104±0.98ax	81.5±1.47ax	71.3±2.94by	78.6±4.41by	83.0±5.87bx	80.0±5.87bx
500	0	546±27.56ax	478±109.38ax	400±156.25ax	525±31.25ax	572±78.13ax	650±62.50ax
500+LA	0	598±75.12ax	603±46.88ax	6031±234.38ax	588±93.75ax	744±125.00ax	619±31.25ax
500	2.5	546±27.56ax	525±31.25ax	635±78.13ax	478±46.88ax	510±15.63ax	572±15.63ax
500+LA	2.5	598±75.12ax	588±10.01ax	557±93.75ax	541±15.63ax	510±15.64ax	650±126.00ax
500	5	5461±27.56ax	432±125.00ax	494±125.00ax	682±93.70ax	588±31.25ax	525±0.05ax
500+LA	5	5980±75.12ax	510±78.13ax	400±31.25ax	525±0.05ax	494±281.25ax	728±140.63ax
500	10	546±27.56ax	385±46.88ax	494±125.00ax	588±31.25ax	525±31.99ax	478±78.13ax
500+LA	10	598±75.12ax	478±15.63ax	400±62.50ax	588±30.75ax	494±0.02ax	476±15.63ax

[a] All data are means±S.E.M.; reported in ascorbic acid concentration (mM) from a sample size of $n=6$ Columns with similar letters (abc) are not significantly ($P<0.05$) different. Rows, within a single ascorbic acid concentration, with similar letters (xyz) are not significantly ($P<0.05$) different.

[b] Ascorbic acid concentration tested (10, 100 and 500 mM).

[c] LA represents linoleic acid.

control treatment reached the highest concentrations of lipid peroxides. In contrast, the 500 mM LAA treatment was effective at preventing the UV-induced production of lipid peroxides, throughout the entire storage period.

3.4. Electron beam irradiation-induced lipid oxidation

The same linoleic acid system mentioned in the above experiment was also used to assess the antioxidant activity of 10, 100 and 500 mM LAA following electron beam (e^- beam) irradiation at 0, 5, 10 and 20 kGy.

The effectiveness of LAA treatments in retarding irradiation-induced lipid oxidation is presented in Fig. 4. All irradiated samples attained the highest production of lipid peroxides after 72 h of storage, which in general was 24 h earlier than with samples exposed to UV light. Notwithstanding this, the presence of 10 mM LAA once again demonstrated a prooxidant activity in all samples, regardless of the irradiation dosage. The 100 mM LAA sample significantly ($P < 0.05$) inhibited irradiation-induced peroxide production, in comparison to the control, with an increase in antioxidant activity occurring as the irradiation dosages increased. The 500 mM LAA treatment prevented irradiation-induced production of lipid peroxide throughout the entire storage period. A significant interaction ($P < 0.05$) was noted between LAA and the presence of the irradiation dosage for the peroxide content in the emulsion.

To investigate the relationship between electron beam irradiation dosage and lipid peroxide production, the maximum absorbance values, obtained from control samples (free of the influences of LAA on lipid oxidation) during storage, was plotted against the respective electron beam irradiation dosage (Fig. 5). A trend analysis was conducted on the plotted data and a significant ($P < 0.01$) correlation coefficient ($r^2 = 0.9780$) was determined, thus demonstrating a linear relationship between the increase in electron beam dosage (up to 20 kGy) and the increase in lipid oxidation products.

4. Discussion

In biological systems, •OH is commonly produced from the oxidation of H_2O_2 and soluble Fe^{2+} (Aruoma, Grootveld, & Halliwell 1987; Puppo & Halliwell, 1988). As expected, the addition of Fe^{2+} (from the reduction of Fe^{3+} in $FeCl_3$), solubilized by EDTA, generated the highest concentration of •OH (Fig. 1). When Fe^{2+} was replaced by Hb under the same reaction conditions, a markedly lowered generation of •OH occurred. Puppo and Halliwell (1988) have reported similar findings and concluded that intact Hb does not generate •OH by direct interaction of H_2O_2 with the heme iron, but rather by the released Fe^{2+} from the unreacted H_2O_2 oxidized metHb.

In this study, the exposure of the Hb samples to UV light generated more •OH compared to conventional heat incubation. Andley and Clark (1989) have reported the oxidation of protein and the formation of H_2O_2 and

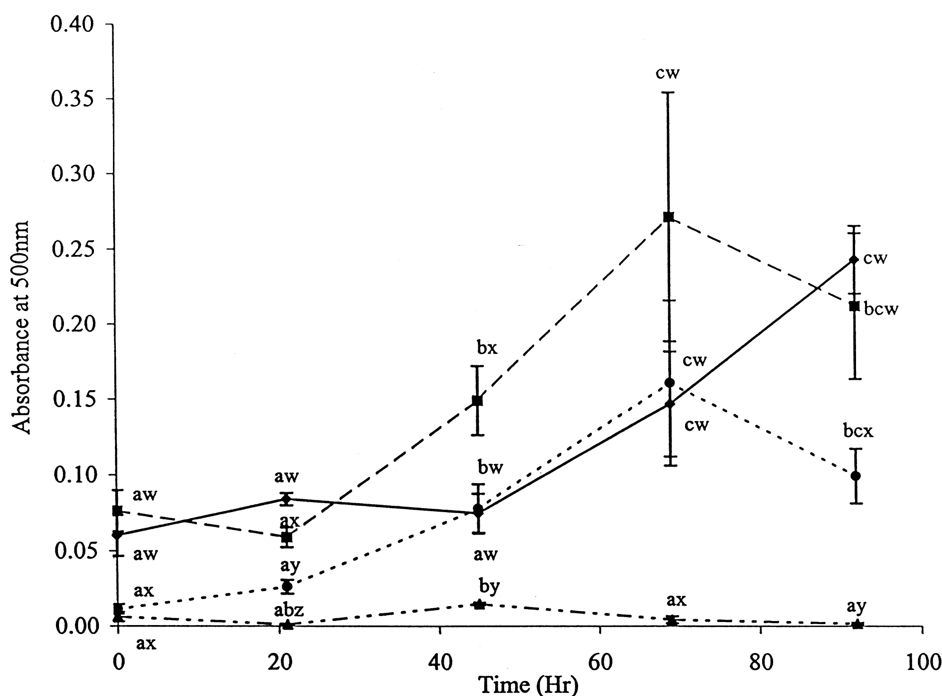

Fig. 3. Effects of ascorbic acid on UV-induced phootoxidation of linoleic acid emulsion system; 0 (diamond), 10 (square), 100 (circle), and 500 mM (triangle) ascorbic acid. [abc]Denotes the statistically significant ($P < 0.05$) levels of peroxide within a particular treatment over time. [wxyz]Denotes the statistically significantly ($P < 0.05$) effect of ascorbic acid at a particular time interval.

O_2^- by exposure to UV light under aerobic conditions. Specifically, Whitburn, Shieh, Sellers, Hoffman and Taub (1982) observed the generation of H_2O_2 by γ-radiolysis of an aqueous ferrimyoglobin solution that resulted from the union of two •OH produced from oxidized proteins. It is possible, therefore, that the observed effect of UV light on Hb catalyzed •OH generation in the present study was indeed due to the oxidation of Hb, resulting from the direct generation of •OH.

The rapid oxidation of 10 mM LAA samples after 24 h of post-irradiation storage was more pronounced when the dosage of irradiation was increased. Moderate losses of LAA in both 100 and 500 mM samples were also observed by day 3 of storage. These findings agree with the previous results of Tobback (1977), who also showed that the sensitivity of ascorbic acid increases as the dilution of ascorbic acid increases. Since ascorbic acid oxidation can also be induced by soluble iron, Mb (a chelated form of iron) was added to the ascorbic acid to enhance the electron beam induced LAA oxidation. However, the iron in Mb was probably unavailable for LAA oxidation since Hb generated less •OH than EDTA-solubilized iron (Fig. 1). Therefore, oxidation of LAA in the present study is likely due to the presence of •OH generated from radiolysis of water or the oxidation of Hb or Mb during irradiation (Fig. 1). Furthermore, our results showed that the lipid peroxyl radical scavenging activity of LAA could also lead to the oxidation of LAA in the lipid emulsion model. This suggestion was confirmed by the greater depletion of LAA

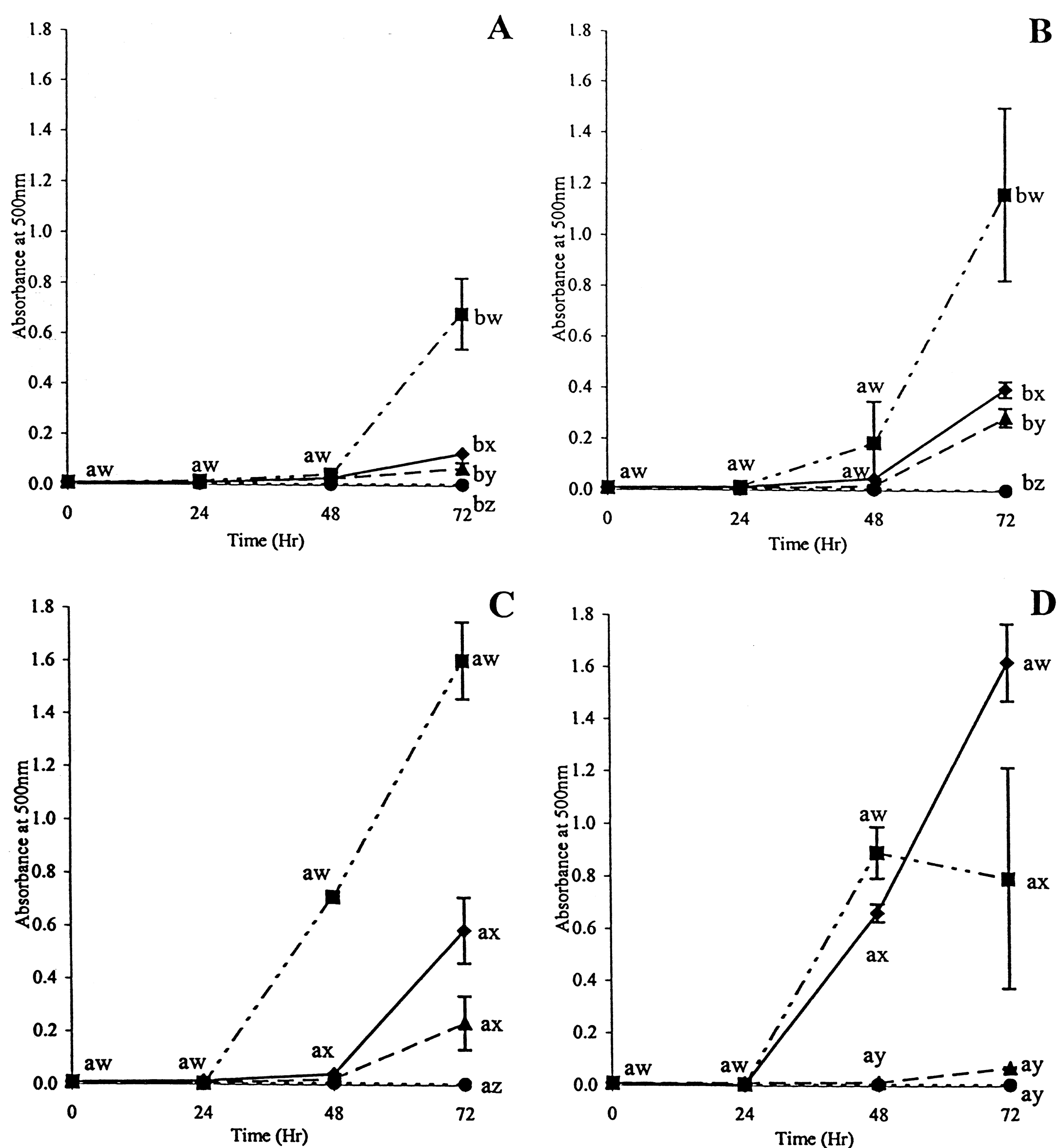

Fig. 4. Effects of 0 (diamond), 10 (square), 100 (triangle), and 500 mM (circle) ascorbic acid and electron beam irradiation at (A) 0 kGy, (B) 5 kGy, (C) 10 kGy, and (D) 20 kGy on oxidation of linoleic acid emulsion system. [abc]Denotes the statistically significant ($P<0.05$) levels of peroxide within a particular treatment over time. [wxyz]Denotes the statistically significantly ($P<0.05$) effect of ascorbic acid at a particular time interval.

observed in samples containing linoleic acid. Thus, the combination between irradiation-induced oxidation and free radical-scavenging activity of ascorbic acid collectively contributed to the rapid loss of LAA in our study. Surprisingly, the destruction of LAA detected after 24 h of storage was much longer in our study than that observed by other workers. Lee, Hendricks, and Cornforth. (1999) observed a complete oxidation of LAA in buffer, catalyzed by Cu^{2+} within 25 min of reaction at room temperature. Perhaps the storage temperature used in our study (4°C) delayed the oxidation of LAA relative to that of Lee et al. (1999). This observation has also been observed by Allan (1950), where the time for LAA oxidation in butter stored at−13°C was about 12 h, compared to 2 h when the temperature was raised to 20°C.

After an initial reduction in LAA concentration, a subsequent increase in LAA concentration, in both the 100 and 500 mM concentration samples, occurred after 3 days of storage. This temporal change of LAA content suggests an accumulation of the reversible dehydroascorbic acid (DAA), which can be regenerated back to LAA in the presence of a reducing agent. This observation did not occur in the 10 mM LAA samples, indicating a likely rapid oxidation of DAA to diketogulonic acid at low concentrations, before DAA was able to be reduced back to LAA. Tobback (1977) has also observed similar findings and proposed that a disproportionation mechanism, occurring between partly oxidized and partly reduced LAA, was the explanation. Taken together, these results suggest a potential self-regeneration process between two LAA molecules which could occur only in an oxygen-free aqueous solution. Therefore, our findings may be explained by the possibility that the dissolved oxygen in the buffer, or in the headspace of the flask (approximately 15 ml in volume), was consumed by day 3 of storage due to oxidation consumption during the aerobic oxidation pathway. This would provide a limited oxygen environment in the aqueous solution for the self-regeneration of LAA.

High intensity UV light ($\lambda = 254$ nm) induces photo-oxidation of PUFA. Melo and Mahmoud (1988) have shown, by electron pair resonance, that UV light ($\lambda < 310$ nm) produced both superoxide radical ($O_2^{\cdot-}$) and peroxyl radical (LOO•) by homolytic cleavage on PUFA under aerobic conditions. In the presence of 100 and 500 mM LAA, the scavenging of $O_2^{\cdot-}$ and/or LOO• by excess LAA could have prevented lipid peroxide formation immediately after UV light exposure. We suggest that 10 mM LAA was unable to scavenge all the generated $O_2^{\cdot-}$ and/or LOO•, which in turn resulted in a lipid peroxide content not statistically different from that of the control. Once the LAA has donated two hydrogen (H^+) atoms to free radicals, an ascorbate radical anion will be formed, before further conversion to DAA (Tannenbaum, Young & Archer, 1985). Moreover, accumulation of an ascorbate radical anion initiates lipid oxidation (Mahoney & Graf, 1986), therefore, eliciting a prooxidant effect. This observation occurred for the 10 mM LAA concentration starting at approximately 25 h of incubation, since 40% of the initial LAA content degraded at this time was coupled to a marked increase in peroxide level. By the 40th h of incubation,

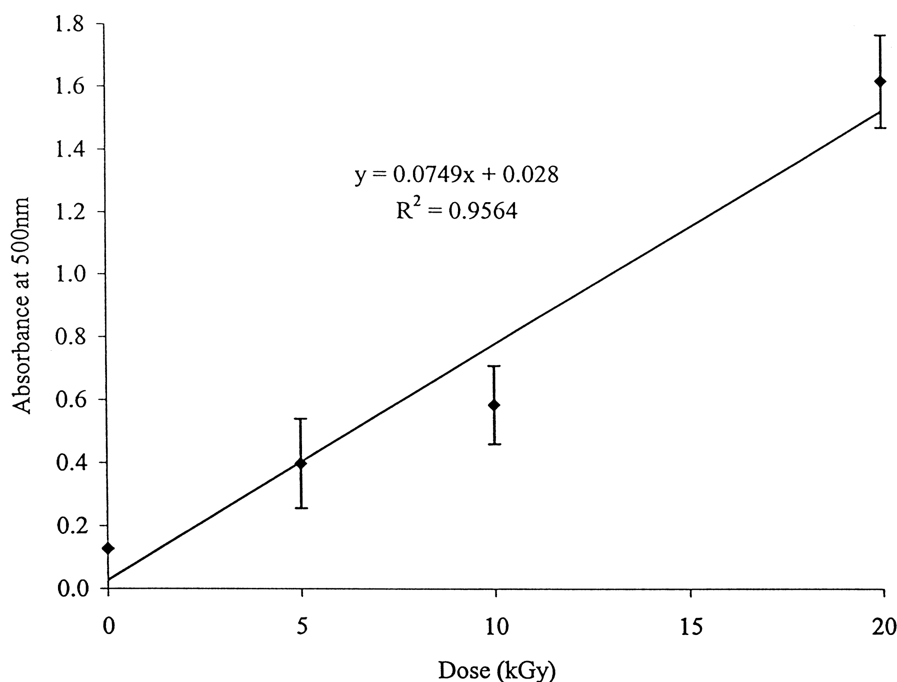

Fig. 5. A regression analysis of the relationship between irradiation dosages and oxidation of linoleic acid emulsion system without the influence of ascorbic acid.

the peroxide content present in the 100 mM LAA treatment was equal to that measured in the control. This result paralleled the finding that 25–30% of the initial LAA content had been oxidized after 40 h. Our result suggests that as the amount of peroxide formed exceeded the capacity of the remaining 100 mM LAA to scavenge free radical, an ineffective concentration for antioxidant activity against UV light photooxidation occurred. This theory is supported by the observation that 500 mM LAA treatment retained a significant amount of LAA to retard the formation of lipid peroxides during the entire storage period.

The same linoleic acid emulsion model was also subjected to e^- beam irradiation. Similar to UV exposure, irradiating the model system by e^- beam produces free radicals that can initiate lipid oxidation reactions. Since water was the main component of our model, radiolysis of water by e^- beam irradiation likely occurred to produce e^-_{aq}, $\cdot OH$ and H_2O_2, with all three by-products having the potential to initiate oxidative damage of macromolecules (Thakur & Singh, 1994). In general, oxidation reactions in the control and the 10 mM LAA treatment, at all irradiation dosages, began to increase after 48 h of storage. This change was expected, due to the loss of 40% LAA by the 24th hour of storage. Similar to the UV light-induced oxidation experiment, all 10 mM LAA treatments accumulated lipid peroxides to an extent greater than the control. In contrast, both the 100 and 500 mM LAA concentrations exhibited antioxidant activity, with 100 mM LAA retarding the degree of oxidation during propagation, while 500 mM LAA prevented propagation from occurring during 72 h storage.

Former studies have shown that a low dose of irradiation (as low as 5 Gy) of unsaturated fatty acid (e.g. methyl oleate or linoleic acid) produces lipid peroxides and that the rate of the autoxidation reaction varies markedly with the irradiation dose (Nawar, 1977). The peroxide content observed in the irradiated model system used herein was also dose-dependent, depicted by the peroxide content of irradiated control samples seen after 72 h of storage. This was best observed by the fact that an increase in the irradiation dose exhibited a linear increase in the peroxide content. However, a similar trend was not observed when LAA was added to the mixture.

5. Conclusion

Under the current experimental conditions, Hb generated very little $\cdot OH$ via the Fenton reaction in comparison to the EDTA-solubilized Fe^{2+}. However, exposure of Hb to UV light increased the production of $\cdot OH$. Therefore, the heme iron in Hb was concluded to be inactivated in the Fenton reaction when in its native form, but with possible denaturation or degradation of Hb by UV light, the release of iron from heme can participate in the Fenton reaction, the oxidation of ascorbic acid and the peroxidation of unsaturated fatty acids.

Ascorbic acid, at 10 mM, was rapidly degraded to a baseline level between 20 and 40% of the initial LAA concentration after an electron beam irradiation dose of less than 5 kGy, following 1 day of storage at 4°C. Increasing the dose of irradiation to 10 kGy completely degraded all of the added LAA after 1 day of storage. Both 100 and 500 mM LAA also exhibited a moderate (20%) loss of LAA content by day 3 of storage. However, the regeneration of LAA was thought to have occurred when an increase in LAA concentration, comparable to the original amount added, was observed after 3 days of storage.

UV ($\lambda = 254$ nm) photooxidation produced maximum peroxide after 96 h of storage while electron beam irradiation produced even higher levels of peroxide after merely 72 h of storage. Regardless of the forms of irradiation, a 10 mM LAA solution exhibited potential prooxidant activity while a 100 mM LAA solution demonstrated marginal antioxidant activity. Only 500 mM LAA solution was observed to exert superior antioxidant activity in both situations. These results indicated a biphasic, concentration dependent prooxidant/antioxidant activity of LAA in the linoleic acid emulsion under the influence of both UV and electron beam irradiation.

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