Ascorbic Acid and Ascorbic Acid 6-Palmitate Induced Oxidation in Egg Yolk Dispersions

Jacob Holm Nielsen,* Gitte Hald Kristiansen, and Henrik Jørgen Andersen

Department of Animal Product Quality, Institute of Agricultural Sciences, P.O. Box 50, DK-8830 Tjele, Denmark

The oxidation in aqueous dispersions of egg yolk powder and the influence of addition of the proposed antioxidants ascorbic acid and ascorbic acid 6-palmitate indicate that both ascorbic acid and ascorbic acid 6-palmitate propagated the oxidation of egg yolk powder dispersions. Ascorbic acid 6-palmitate was found to be more prooxidative than ascorbic acid. Moreover, it was found that addition of ascorbic acid or ascorbic acid 6-palmitate gave rise to an increase in the amount of free iron Fe(II) in the egg yolk dispersions. It is proposed that ascorbic acid and ascorbic acid 6-palmitate react with the phosvitin−Fe(III) complex found in egg yolk and release Fe(II), which subsequently propagates lipid oxidation. It appears that less oxidation occurs in egg yolk dispersions exposed to high concentrations of peroxy radicals with added ascorbic acid than egg yolk dispersions with added ascorbic acid without exposure to peroxy radicals.

Keywords: *Ascorbic acid; ascorbic acid 6-palmitate; oxidation; egg yolk; phosvitin*

INTRODUCTION

Pasteurized egg yolk or dispersions of egg yolk powder are most often used by the food industry in combination with vegetable oils and additives in the production of dressings, mayonnaises, and sauces. The high content of polyunsaturated phospholipids in egg yolk leads to processed products, which include oxidative egg yolk, susceptible to oxidation. However, only a few studies regarding oxidation in egg powder and processed egg products have been published, and they have concentrated on studies of cholesterol oxidation (van de Bovenkamp et al., 1988; Sander et al., 1989). To minimize oxidation of egg yolk powder and processed egg products, miscellaneous antioxidants are often added to these products. The use of antioxidants by the industry is often based on experience and tradition, which might give rise to unexpected problems regarding the oxidative stability of the products. Two antioxidants often used are ascorbic acid and ascorbic acid 6-palmitate, both of which are known to scavenge radicals (Doba et al., 1985) and be able to regenerate antioxidants, such as tocopherols (Niki, 1987). Ascorbic acid 6-palmitate is used as an antioxidant in the production of egg yolk powder in order to prolong its shelf life. Both ascorbic acid and ascorbic acid 6-palmitate are used as antioxidants in processed foods that contain egg yolk powder as an ingredient. Even though ascorbic acid is generally accepted as an antioxidant, studies have shown that low concentrations of ascorbic acid might indirectly become prooxidative due to its ability to reduce Fe(III) to prooxidative Fe(II), which can catalyze lipid peroxidation (Decker and Hultin, 1992). This work stresses the necessity to carefully consider which antioxidant should be used in a specific food system. The present study focuses on the influence of ascorbic acid and ascorbic acid 6-palmitate on the oxidative stability of aqueous dispersions of egg powder and illustrates how the oxidative stability is affected by ascorbic acid.

* To whom correspondence should be addressed. Phone: +45 89 99 11 63. Fax: +45 89 99 15 64. E-mail: Jacobh.nielsen @ agrsci.dk.

MATERIALS AND METHODS

Analytical Equipment. In the analysis of total oxygen scavenger capacity assay (TOSCA), a Perkin-Elmer HS40XL headspace sampler (The Perkin-Elmer Corp. Norwalk, CT) coupled with a HP 6890 gas chromatograph (Hewlett-Packard Co., Palo Alto, CA) was used. Separation of ethylene was conducted on an HP Pora Plot Q column (25 m × 0.53 mm; 40 μm film thickness) (Hewlett-Packard Co.). Oxygen consumption was measured by a Clark electrode (Rank Brothers Ltd.,-UK), and the absorbance of the Fe(II) ferrozine conjugate was detected by a HP-8453 spectrophotometer (Hewlett-Packard Co.).

Chemicals. Freshly produced egg yolk powder was a gift from Sanovo Foods A/S, Denmark. l-Ascorbic acid and ascorbic acid 6-palmitate both of analytical grade, ferrozine (3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine) for determination of free Fe(II), and α-keto-γ-methiolbutyric acid (KMBA) were purchased from Sigma (St. Louis, MO). 2,2′-Azobis(2-amidinopropane) hydrochloride (AAPH) was obtained from Polysciences, Inc. (Warrington, PA).

Oxygen Consumption. Measurement of oxygen consumption during oxidation was measured by a Clark electrode. Egg yolk powder (5 mg/mL) was dispersed in air-saturated phosphate buffer (80 mM, pH 7.40) and thermostated at 37 °C. At time $t = 0$, ascorbic acid (1 mM) was added to the sample. The consumption of oxygen was followed as a function of time.

Total Oxygen Scavenger Capacity Assay. The antioxidative capacity was evaluated in a dispersion with added KMBA where oxygen radicals will react with KMBA resulting in the formation of ethylene as described by Pryor and Tang (1978). The concentration of ethylene was used as an indicator of oxidation. Egg powder (0.3 g) was dissolved in 15 mL of phosphate buffer pH 7.40 after gentle dissolution of the egg powder using a mixer. KMBA was subsequently added to the dissolved egg powder and ascorbic acid or ascorbic acid 6-palmitate to a total volume of 8 mL in a headspace vial resulting in a final concentration of 0.010 g/mL egg powder, 250 μM KMBA, and 0, 62.5, 125, 250, or 500 μM ascorbic acid/

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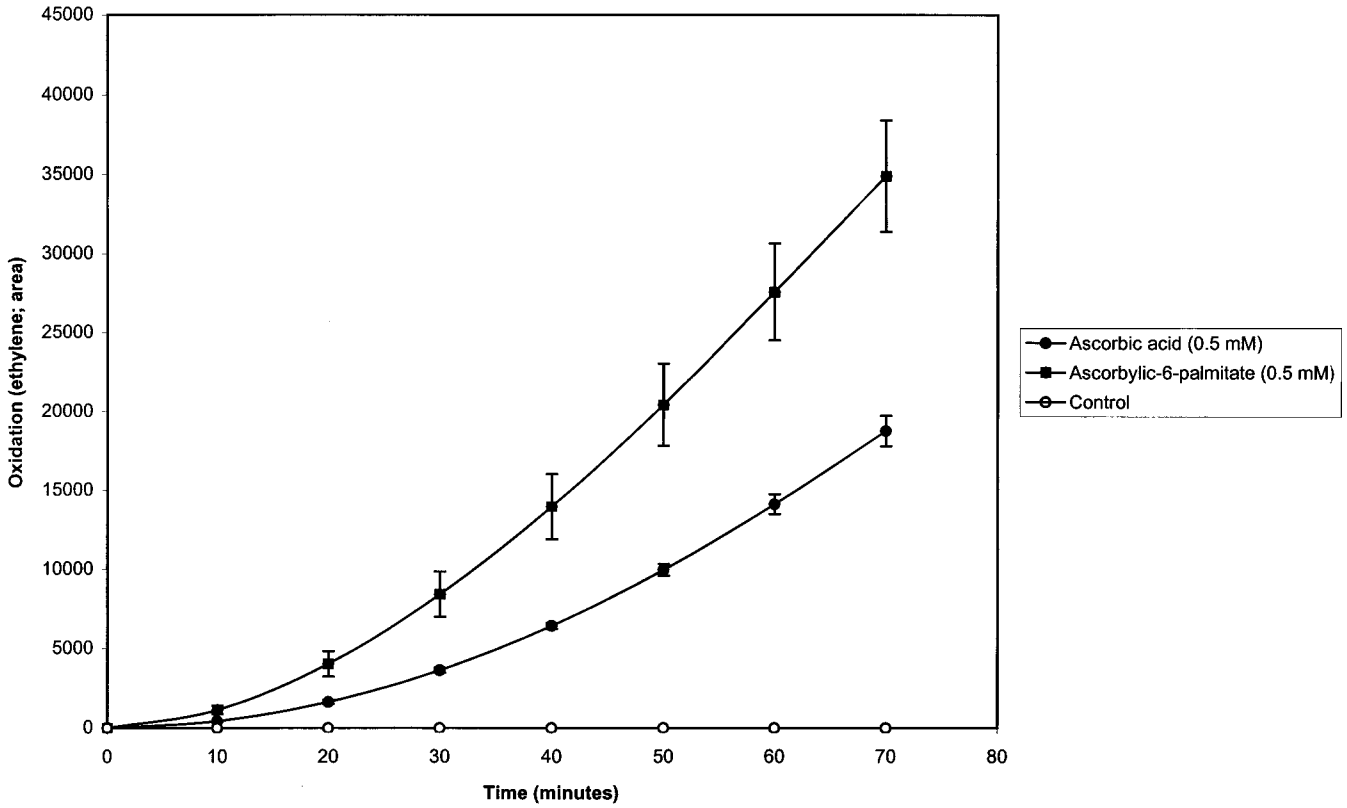

Figure 1. Oxidation of egg yolk powder expressed as the accumulation of ethylene (expressed as peak area) in an aqueous dispersion of egg yolk powder (○) and an aqueous dispersion of egg yolk powder exposed to 0.5 mM ascorbic acid (●) or 0.5 mM ascorbic acid 6-palmitate (■) (values represent mean ± SE).

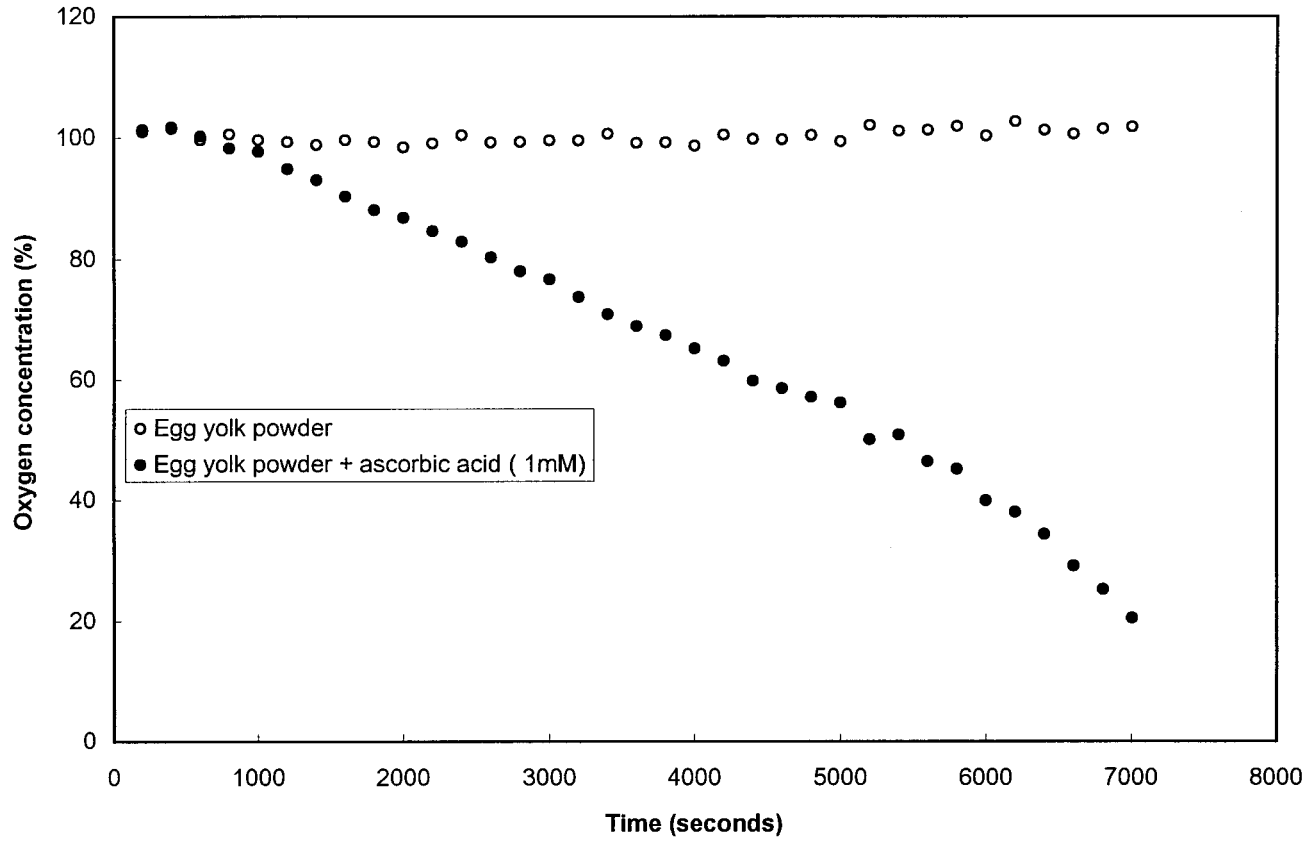

Figure 2. Oxygen consumption expressed as percentage of oxygen dissolved in an aqueous dispersion of egg yolk powder (○) and exposed to ascorbic acid (1 mM) (●).

ascorbic acid 6-palmitate. Additional experiments were performed where the azo compound AAPH was added in combination with ascorbic acid or ascorbylic-6-palmitate in order to simulate high radical flux. The sample vials were placed in the oven of an HS40XL headspace sampler at 50 °C for a period of 105 min, and sampling from the headspace was performed

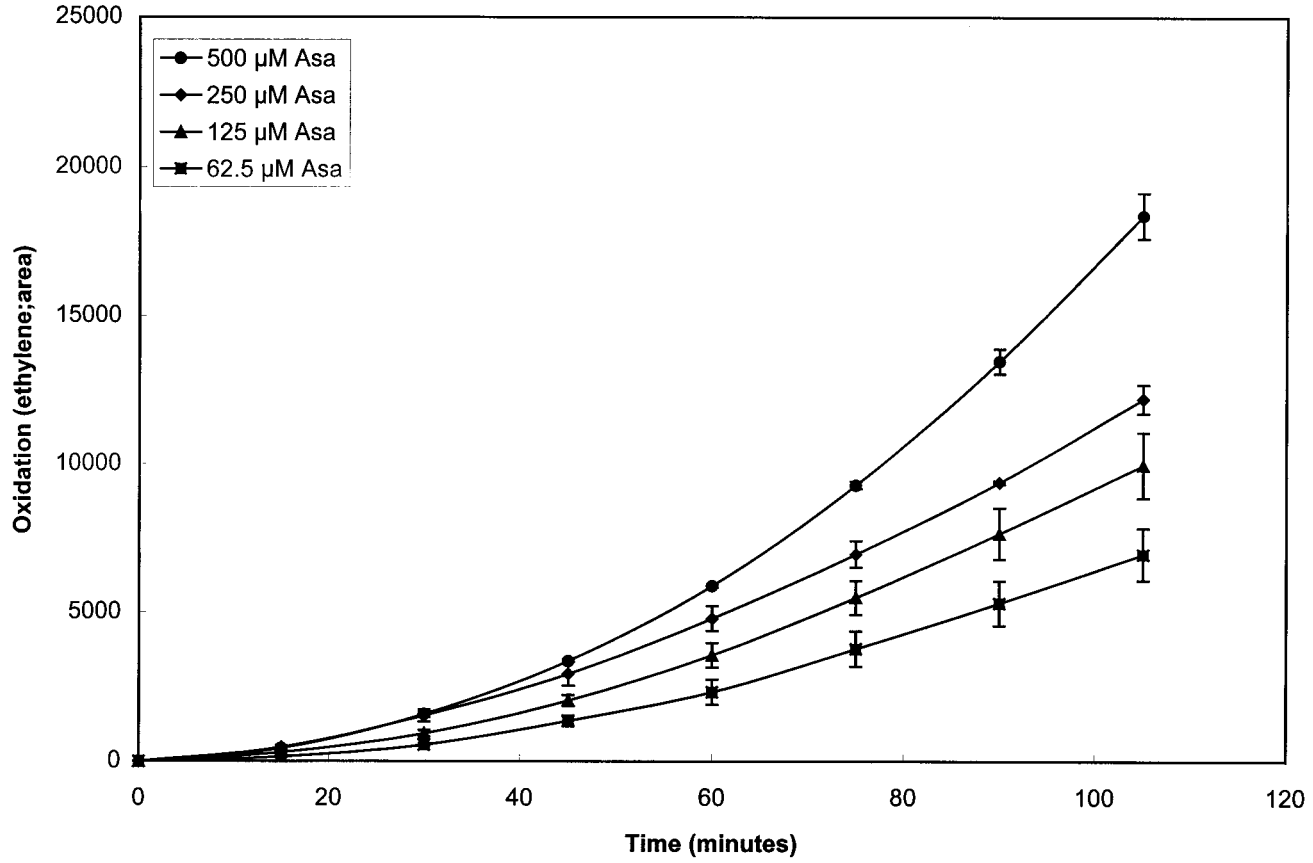

Figure 3. Oxidation of an aqueous dispersion of egg yolk powder as function of concentration of ascorbic acid) (values represent mean ± SE).

at 15 min intervals. Each sampling period had a length of 0.08 min and the sample was introduced on-column on a poro-plot column with a pressure of 10 psi and 80 °C for 5 min. The detector temperature was held at 275 °C.

Quantification of Free Fe(II). Free Fe(II) was quantified by the procedure described by Stookey (1970), where ferrozine reacts with Fe(II) to produce a colored complex. Egg yolk powder was mixed with 0.05 M phosphate buffer (pH 7.4), ferrozine, and ascorbic acid or ascorbic acid 6-palmitate to a final concentration of 0.016 mg/mL, 1 mM, and 0.5 mM, respectively. The mixtures were incubated for 30 min and subsequently centrifuged in Eppendorf tubes for 15 min at 15000*g*. The supernatant was withdrawn and measured spectrophotometrically at 562 nm. The concentration of Fe(II) was calculated using the molar absorption coefficient of the ferrozine−Fe(II) complex, $\epsilon_{562} = 27\ 100\ M^{-1}\ cm^{-1}$.

RESULTS AND DISCUSSION

Figure 1 shows that no significant formation of ethylene was observed in the TOSCA assay when pure egg yolk powder dispersions was incubated together with KMBA. In contrast, when egg yolk powder dispersions were incubated with 0.5 mM proposed antioxidant (ascorbic acid), a continuous accumulation of ethylene was observed during the incubation period. These results clearly show that addition of ascorbic acid to egg yolk dispersions induces radical formation, which then reacts with KMBA and results in the observed ethylene formation. The pro-oxidative activity of ascorbic acid in this system was moreover confirmed in a model system of dispersed egg yolk powder, where oxidation was followed by oxygen consumption during incubation (Figure 2). Additional experiments showed that the rate of oxidation of egg yolk powder was directly related to the concentration of ascorbic acid (Figure 3). This is in contrast to earlier observations of the pro-oxidative activity of ascorbic acid in other food systems, where ascorbic acid only has been found to be pro-oxidative at low concentrations.

As ascorbic acid often is replaced by the more nonpolar ascorbic acid 6-palmitate in hydrophobic food systems in order to incorporate an antioxidative activity in the hydrophobic lipid environment, this proposed antioxidant was also tested in the simple TOSCA assay used in the present study. Figure 1 shows that ascorbyl-6-palmitate, as was observed with ascorbic acid, is able to propagate oxidation of egg powder in a simple system. The accumulation of ethylene during the oxidation of egg yolk powder in the presence of ascorbic acid 6-palmitate was significantly higher than in egg powder exposed to adequate concentrations of ascorbic acid. This shows that ascorbyl-6-palmitate is more prooxidative than ascorbic acid in the tested system.

A possible explanation for the pro-oxidative behavior of both ascorbic acid and ascorbic acid 6-palmitate in the present food model system could be that they both are able to reduce Fe(III) current in egg yolk to free Fe(II). Free Fe(II) is known to catalyze heterolytic cleavage of lipid hydroperoxides resulting in free-radical formation.

To investigate whether ascorbic acid and ascorbic acid 6-palmitate are able to reduce Fe(III) to free Fe(II) in the present food model system, ferrozine, which is known to aggregate with free Fe(II) and form a colored complex, was added to egg yolk powder dispersions in combination with either ascorbic acid or ascorbic acid 6-palmitate. Figure 4 shows that Fe(II) was released in

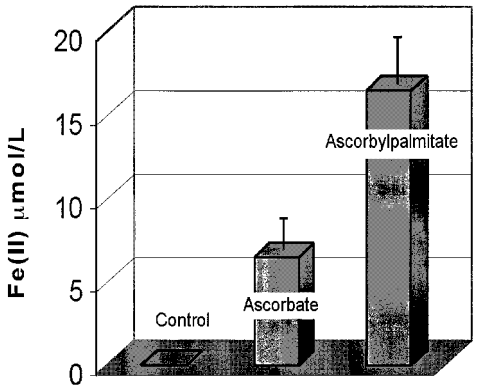

Figure 4. Release of Fe(II) from dispersed egg yolk powder analyzed as the Fe(II)−ferrozine aggregate in the presence of ascorbic acid (0.5 mM) or ascorbic acid 6-palmitate (0.5 mM) (values represent mean ± SE).

the presence of ascorbic acid and that the release of free Fe(II) was even greater in the presence of ascorbic acid 6-palmitate. These data support the above-mentioned hypothesis for the pro-oxidative activity of ascorbic acid and ascorbic acid 6-palmitate in the present system.

Moreover, recent studies by Nielsen et al. (unpublished data) have shown that ascorbic acid is able to reduce complex bound Fe(III) from the iron-binding protein phosvitin present in egg yolk to free Fe(II) (eq 1) and hereby support possible Fe(II)-induced oxidation through decomposition of lipid hydroperoxides (eq 2). The pro-oxidative effect of both the hydrophilic ascorbic acid and the lipophilic derivative is supported by the findings that phosvitin has emulsifying capabilities (Aluko and Mine, 1997; Chung and Ferrier, 1991), and it is suggested that this amphiphilic ability is responsible for the reaction of both ascorbic acid and ascorbic acid 6-palmitate. A prooxidant effect of ascorbic acid 6-palmitate has previously been reported also in combination with tocopherols (Guardiola et al., 1997).

$$\text{Fe(III)}-\text{phosvitin (complex)} + \text{Asa} \rightarrow \text{Fe(II)} + \text{Asa(ox.)} + \text{phosvitin} \quad (1)$$

$$\text{LOOH} + \text{Fe (II)} \rightarrow \text{LO}^{\cdot} + \text{OH}^{-} \quad (2)$$

To investigate how ascorbate influences the rate of oxidation in an egg yolk powder system where oxidation proceeds directly through radical-induced oxidation, rather than through Fe(II)-induced peroxidation, the azo radical initiator AAPH was added to the egg yolk dispersion. Such a system reflects, e.g., light-induced oxidation or addition of a labile oil.

AAPH is known to decompose to two carbon-centered radicals upon heating (eq 3). The carbon-centered radicals react instantly with oxygen to yield peroxy radicals capable of abstracting hydrogen from unsaturated lipids (eq 4). Figure 5 shows that AAPH can induce oxidation in an egg yolk powder dispersion. However, the test system cannot distinguish between direct reaction of the peroxy radicals generated by AAPH or radicals generated through reaction of the egg yolk powder. Nevertheless, addition of 500 μM ascorbic acid in combination with AAPH showed that ascorbic acid can reduce the rate of oxidation further than addition of the 500 μM ascorbic acid without the added AAPH. This clearly shows that ascorbic acid is an effective antioxidant in a system with a high radical flux that is in agreement with the findings by Doba et al. (1985). The underlying mechanism for this behavior might be explained through the AAPH-derived peroxy radical abstraction of a hydrogen directly from ascorbic acid (eq

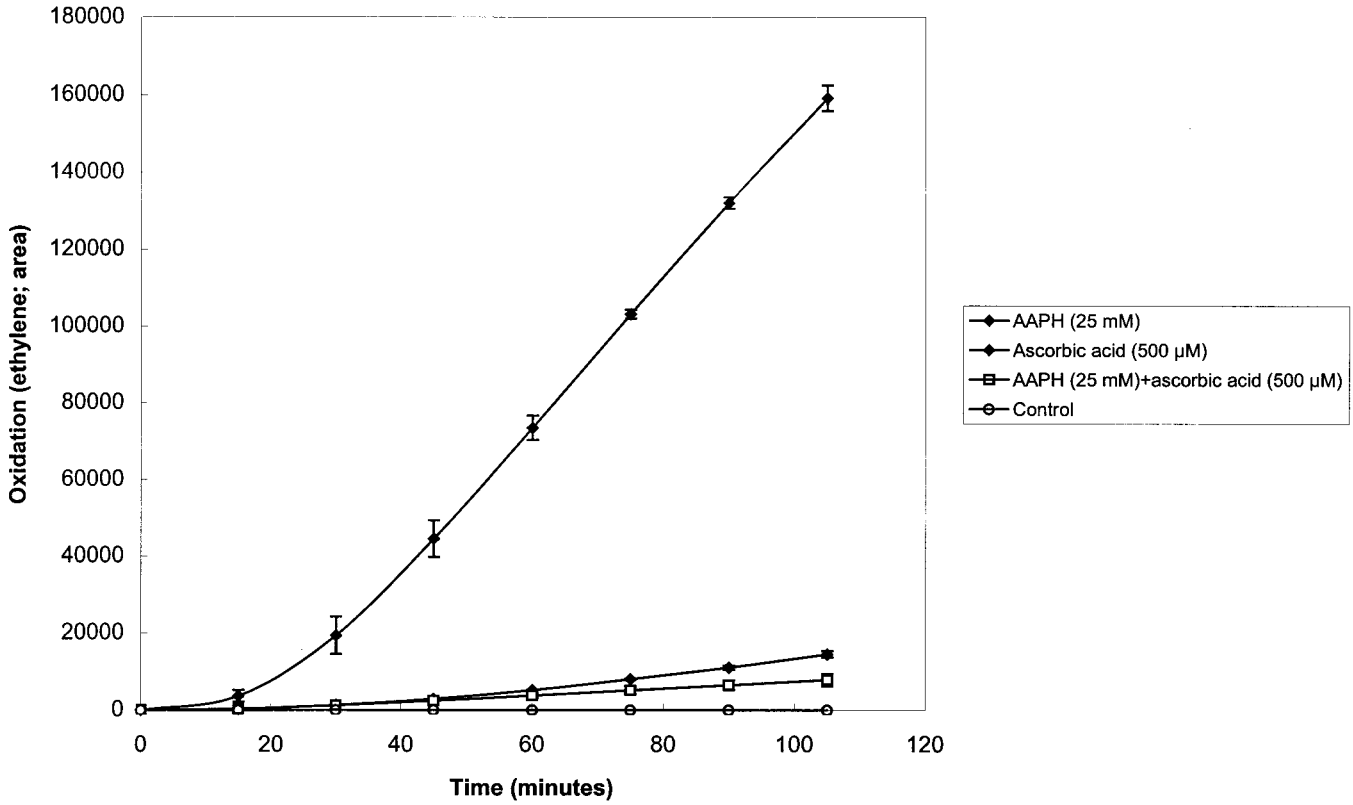

Figure 5. Oxidation of egg yolk powder expressed as the accumulation of ethylene (expressed as peak area) in the presence of AAPH (25 mM) (●), AAPH (25 mM) + ascorbic acid (500 μM) (□), or ascorbic acid (◆) (values represent mean ± SE).

5). Such a mechanism will prevent reduction of phosvitin-bound Fe(III) by ascorbic acid, so the reaction proceeds with a much lower rate and thereby minimizes Fe(II)-induced peroxidation.

$$AAPH \rightarrow A^{\cdot} + A^{\cdot} + N_2 \quad (3)$$

$$A^{\cdot} + O_2 \rightarrow ROO^{\cdot} \quad (4)$$

$$AOO^{\cdot} + Asa \rightarrow AOOH + Asa^{\cdot} \quad (5)$$

This result indicates that ascorbic acid can act as an antioxidant in egg yolk food systems with a high flux of free radicals (illustrated by AAPH) since the ascorbic acid is oxidized and thereby cannot release phosvitin-bound Fe(III). The data clearly show that, prior to addition of antioxidants to egg yolk powder or to complex foods where egg yolk is an ingredient, one should consider the oxidative balance within the products.

In conclusion, the present study shows that the proposed antioxidant ascorbic acid and its hydrophobic derivative ascorbic acid 6-palmitate induce oxidation in egg yolk dispersions most probably indirectly through release of pro-oxidative free Fe(II) from the Fe(III)-binding egg yolk protein phosvitin. In contrast, ascorbic acid delays lipid oxidation in an egg yolk dispersion with a high flux of free radicals, such as light-induced oxidation or in systems added unsaturated lipid fractions where lipid oxidation is already in progress. The explanation is most probably due to the direct reaction of ascorbic acid with the free radicals resulting in formation of the less reactive ascorbyl radical and simultaneous hindrance of the above-mentioned reduction and release of protein-bound Fe(III).

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