Antioxidative Properties of Stearoyl Ascorbate in a Food Matrix System

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Abstract: Stearoyl ascorbate or 6-O-stearoyl l-ascorbate is a lipophilic derivative of l-ascorbic acid and is commercially used in foods as a fat-soluble antioxidant and surfactant to overcome the disadvantages of using l-ascorbic acid. The objective of this research is to evaluate the antioxidative ability of stearoyl ascorbate, in the presence of wheat starch or gluten as a matrix, by measuring the unoxidized methyl linoleate available in the mixture of them after oxidation under accelerated conditions compared to that when using ascorbic acid. We observed that stearoyl ascorbate and ascorbic acid exhibited mutually adjacent antioxidative ability against oxidation of the methyl linoleate at a molar ratio of 0.0001 in presence of either wheat starch or gluten. In addition, the oxidation process in the mixture containing either stearoyl ascorbate or ascorbic acid was significantly slower than that in the mixture without stearoyl ascorbate or ascorbic acid. Moreover, by altering the initiation and propagation periods of the oxidation process, the mixture containing the stearoyl ascorbate and gluten as the matrix exhibited conspicuously slower oxidation than the mixture containing either the wheat starch or stearoyl ascorbate alone. However, increase in the ratio of stearoyl ascorbate to methyl linoleate to 0.001 or higher resulted in adverse effects due to acceleration of the oxidation process.

Key words: stearoyl ascorbate, antioxidant, methyl linoleate, gluten, wheat starch

1 INTRODUCTION

Lipid oxidation proceeds due to a free radical chain reaction between unsaturated fatty acyl groups in lipid and active oxygen species and can occur in an autocatalytic manner\textsuperscript{1}. The overall process of lipid oxidation occurs in three stages defined as initiation, propagation, and termination stages. The initiation stage involves formation of free radicals resulting from abstraction of hydrogen atoms from a fatty acid in the presence of an initiator such as lipid or other organic peroxyl radicals. At the propagation stage, lipid peroxyl radicals are produced, which can then produce lipid hydroperoxide and new alkyl radicals from abstraction of the hydrogen from other unsaturated fatty acid molecules. Finally, the termination stage is reached when two free radicals interact with each other and form non-radical species\textsuperscript{1,2}.

In many food systems, rate of lipid oxidation is dependent on reactive oxygen species, antioxidants, and contaminants, in particular transition metal ions. In addition, other prooxidants such as photosensitizers, some types of enzymes, and high amount of some types of antioxidants can accelerate the rate of lipid oxidation. Prooxidants are compounds that initiate, facilitate or accelerate lipid oxidation. Hydroperoxides are major substrates for lipid oxidation due to their composition that results in the scission of the fatty acid to produce the low molecular weight compounds. The prooxidants can also accelerate the rate of lipid oxidation by directly interacting with unsaturated fatty acids to form lipid hydroperoxides by or promoting formation of free radicals. The decomposition of hydroperoxides produces additional radicals that could be responsible for the exponential increase in oxidation rates. Elevated temperatures, light, and many prooxidants can promote the decomposition of hydroperoxides\textsuperscript{1,3,12}.

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Ascorbic acid is well known as a water-soluble antioxidant. However, due to its hydrophobicity and chemical instability, ascorbic acid can easily undergo oxidation in food systems. Stearoyl ascorbate or 6-O-stearoyl L-ascorbate is a lipophilic derivative of L-ascorbic acid, which is synthesized and used as a fat-soluble antioxidant and surfactant in foods, in particular in bread-making for strengthening and for softening bread crumbs. It has an amphiphilic structure and does not have the disadvantages of using ascorbic acid, because stearoyl ascorbate has increased solubility and stability in comparison with the ascorbic acid in fat containing foods. Moreover, ascorbic acid can undergo spontaneous oxidation as well. The autoxidation occurs via an ascorbate dianion in the absence of metal ions, and subsequently generates considerable amount of hydrogen peroxide, which is a strong oxidizing agent. It can also be oxidized by catalytic metals, and this oxidation can be accelerated by the catalytic metals themselves. The effects of ascorbate on lipid peroxidation can be due to it acting as a prooxidant or an antioxidant depending on its concentration and the form of transition metal ions in the matrix components. There are some reports showing that ascorbate can reduce metal ions to form compounds that react with oxygen and subsequently form lipid peroxidation initiators.

There are extensive studies on antioxidative abilities of ascorbic acid but only a few studies have been conducted on those of its lipophilic derivatives, in particular stearoyl ascorbate in the presence of other food matrix. Therefore, the objective of this study is to evaluate the antioxidative ability of stearoyl ascorbate against lipid oxidation, wherein the lipid oxidation occurs under an accelerated condition in the presence of a food matrix system including wheat starch or gluten.

2 EXPERIMENTAL PROCEDURES

2.1 Materials

Wheat starch, wheat gluten, and stearoyl ascorbate were purchased from Wako Pure Chemical Industries (Osaka, Japan). Ascorbic acid was obtained from Nacalai Tesque (Kyoto, Japan). Methyl linoleate was purchased from Tokyo Chemical Industry (Tokyo, Japan).

2.2 Preparation of a powdery mixture for evaluation of antioxidative ability

Various powdery mixtures were prepared by mixing thoroughly wheat starch or gluten powder with methyl linoleate in the ratio of 9:1 (w/w). After dissolving various contents of stearoyl ascorbate or ascorbic acid as the antioxidative additive into methanol, each one was added to the powdery mixture of gluten or wheat starch (5 g) at a molar ratio of the additive to methyl linoleate of 0.0001, 0.01, 0.05, and 0.1. Ascorbic acid was used to compare its antioxidative ability with stearoyl ascorbate at the molar ratio of the additive to methyl linoleate of 0.0001. Methanol was then removed by evaporation under vacuum condition in a desiccator. Each powderized mixture (20 mg) was transferred to a glass cup (1.5 cm × 3.0 cm). The oxidation of methyl linoleate in the powder system was accelerated by storing all cups in a sealed container containing potassium carbonate for controlling the relative humidity at 44% and was placed in an incubator at 65°C.

2.3 Determination of unoxidized methyl linoleate

One randomly selected cup of sample was periodically taken and the lipid fraction was extracted for determination of the unoxidized residue of methyl linoleate. One milliliter of 0.1 mol/L NaOH was added to the cup to solubilize the powder and the mixture was transferred to a centrifuge tube. Methanol (1 mL), and methyl palmitate, which was used as an internal standard in chloroform (500 μL/100 mL), were added and well mixed. After adding with 0.1 mol/L HCl (1 mL), the mixture was centrifuged at 3000 rpm for 10 min. A sample (250 μL) from the chloroform layer was transferred to a tube and evaporated under vacuum to remove the solvent. Hexane (150 μL) was added to dissolve the sample for GC analysis.

GC analysis was performed using G-3900 (Hitachi High-Tech Science Corp., Tokyo, Japan) equipped with a FID detector and a Rt-x-2330 capillary column (0.32 mm × 15 m, GL Science, Tokyo, Japan). Injector, column, and detector temperatures were 200, 180, and 200°C, respectively. Helium was used as a carrier gas at a flow rate of 1.8 mL/min.

3 RESULTS AND DISCUSSION

3.1 Antioxidative ability of stearoyl ascorbate in the matrix of wheat starch

Methyl linoleate was mixed with wheat starch as the matrix component at the weight ratio of 1:9 in the presence of an additive that was either ascorbic acid or stearoyl ascorbate. The additive was added to the mixture at the molar ratio of 0.0001. During an accelerated test, each mixture was subjected to autoxidation process. Differences in oxidation rates of methyl linoleate in the absence or presence of either stearoyl ascorbate or ascorbic acid was evaluated by measuring the unoxidized methyl linoleate content as shown in Fig. 1. We observed that ascorbic acid and stearoyl ascorbate could reduce the rate of methyl linoleate oxidation by increasing the duration of the induction period in the oxidation process. The entire oxidation process of methyl linoleate could be expressed by the autocatalytic type Eq. (1) as reported by us previously.
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and the presence of either ascorbic acid or stearoyl ascorbate is a parameter reflecting the induction period of the oxidation process. The parameters of the propagation stage as shown by higher content of the oxidized linoleic acid were not much different from each other and varied from 0.451 to 0.478. The $Y_0$ values were not much different because their $k$ and $Y_0$ values were close to each other. The result was in accordance with previous reports about the antioxidative ability of the stearoyl ascorbate in prevention of the oxidation of linoleic acid.

Thus, in the matrix of wheat starch, both ascorbic acid and stearoyl ascorbate exhibited the close antioxidative ability although the ability was not high in comparison with the mixture without any additive. The molar ratio of the additive at 0.0001 may be too low to significantly suppress auto-oxidation of methyl linoleate. Consequently, they exhibit antioxidative ability only to a minor extent.

3.2 Antioxidative properties of stearoyl ascorbate in the matrix of wheat gluten

Methyl linoleate was mixed with wheat gluten as the matrix component in the presence of an additive that was either ascorbic acid or stearoyl ascorbate. The molar ratio of the additive to the mixture of wheat gluten and methyl linoleate was 0.0001. The unoxidized methyl linoleate of all powdery mixtures was evaluated periodically as shown in Fig. 2. The mixtures containing the additive exhibited shortening of the induction period of the initiation stage, but slowed down the progress of the oxidation process at the propagation stage as shown by higher content of the unoxidized methyl linoleate at the same reaction time than that of the mixture containing no additive. The $k$ and $Y_0$ values of the oxidation process of the mixtures in the presence or absence of the additive were derived by the same way as shown in Fig. 1 and Table 1. We observed that the $k$ and $Y_0$ values of the oxidation processes of the mixtures containing the additive were lower than those of the mixture without the additive. Thus, the presence of wheat

$$dY/dt = -kY(1-Y)$$

where $Y$ is the fraction of unoxidized methyl linoleate, $t$ is the oxidation time, and $k$ is a rate constant. After integration of Eq. (1) under initial condition of $Y = Y_0$ at $t = 0$, Eq. (2) is obtained.

$$\ln[(1-Y)/Y] = kt + \ln[(1-Y_0)/Y_0]$$

$Y_0$ was introduced to conveniently solve the equation and is a parameter reflecting the induction period of the oxidation process. The parameters of $k$ and $Y_0$ for each oxidation process were estimated using regression analysis between $\ln[(1-Y)/Y]$ and $t$ as shown in the inset in Fig. 1. The $k$ and $Y_0$ values were obtained from the slopes and the ordinate intercepts, respectively, of the inset in Fig. 1. The $k$ values of the oxidation processes of the mixtures in the absence or presence of the additive were not much different from each other and varied from 0.451 to 0.478. The $Y_0$ values of the oxidation processes in the presence of either ascorbic acid or stearoyl ascorbate slightly increased in comparison with the process in the absence of the additive. According to our previous reports, the lower the $k$ values, the higher the antioxidative property of the additive added to a fatty acid, and the higher the $Y_0$ value, the longer the induction period of the oxidation process was.

3.2 Antioxidative properties of stearoyl ascorbate in the matrix of wheat gluten

Methyl linoleate was mixed with wheat gluten as the matrix component in the presence of an additive that was either ascorbic acid or stearoyl ascorbate. The molar ratio of the additive to the mixture of wheat gluten and methyl linoleate was 0.0001. The unoxidized methyl linoleate of all powdery mixtures was evaluated periodically as shown in Fig. 2. The mixtures containing the additive exhibited shortening of the induction period of the initiation stage, but slowed down the progress of the oxidation process at the propagation stage as shown by higher content of the unoxidized methyl linoleate at the same reaction time than that of the mixture containing no additive. The $k$ and $Y_0$ values of the oxidation process of the mixtures in the presence or absence of the additive were derived by the same way as shown in Fig. 1 and Table 1. We observed that the $k$ and $Y_0$ values of the oxidation processes of the mixtures containing the additive were lower than those of the mixture without the additive. Thus, the presence of wheat

![Fig. 1](image-url)

**Fig. 1** Oxidation process at 65°C of methyl linoleate in wheat starch powder in absence of an antioxidative additive (○) and presence of ascorbic acid (△) or stearoyl ascorbate (□) when the ratio of the antioxidative additive to methyl linoleate is 0.0001.

| Parameters | Wheat starch | Gluten |
|------------|--------------|--------|
|            | No additive | Ascorbic acid | Stearoyl ascorbate | No additive | Ascorbic acid | Stearoyl ascorbate |
| $k$ [%]    | 0.454        | 0.451    | 0.478   | 0.298   | 0.138   | 0.113   |
| $Y_0$      | 0.973        | 0.995    | 0.998   | 0.988   | 0.931   | 0.911   |

Table 1 Kinetic parameters of the autocatalytic type equation for the oxidation process of methyl linoleate in wheat starch or gluten in the presence or absence of an antioxidative additive. The additive is either ascorbic acid or stearoyl ascorbate and the molar ratio of the additive to methyl linoleate is 0.0001.
gluten in the mixture could reduce the rate of the oxidation process of methyl linoleate by extending the period of the propagation stage as visibly shown in the Fig. 2 and by lowering the $k$ values. However, the induction period may be slightly accelerated as shown by lowering the $Y_0$ values.

According to Table 1, it should be noted that when gluten was used as the matrix component instead of wheat starch, the $k$ value of the oxidation process of the mixtures containing either ascorbic acid or stearoyl ascorbate was obviously decreased, but the $Y_0$ value of the mixture without any additive was only slightly increased from 0.973 to 0.988. Thus, the retardation of the oxidation process of the mixture containing the gluten as the matrix may be due to not only either ascorbic acid or stearoyl ascorbate but also due to gluten. This result is in agreement with the previous reports showing that wheat gluten hydrolysates obtained from hydrolysis of gluten with various enzymes exhibit antioxidative properties according to TBA, ABTS, DPPH, and hydroxyl radical scavenging methods as well as Trolox equivalent antioxidant capacity. As shown in Figs. 1 and 2, and Table 1, the matrix of wheat starch and a higher molar ratio of the stearoyl ascorbate and methyl linoleate than 0.0001 would be examined to evaluate the antioxidative activity of the stearoyl ascorbate.

3.3 Antioxidative property of stearoyl ascorbate at its various molar ratios to methyl linoleate

According to Fig. 2, the gluten interfered with the oxidation process of methyl linoleate, therefore only wheat starch was used as the matrix to evaluate the antioxidative activity of the stearoyl ascorbate at various molar ratios of the additive to methyl linoleate, in the range of 0 to 0.1. The unoxidized methyl linoleate of each mixture was periodically measured, as shown in Fig. 3. It is obvious that the addition of the stearoyl ascorbate to the mixture at a higher molar ratio than 0.0001 could accelerate the oxidation process of the methyl linoleate by shortening the duration of the initiation and propagation stages.

Figure 4 shows the relationship between $k$ or $Y_0$ values and the molar ratios of the stearoyl ascorbate to methyl linoleate.
noleate. The $k$ values of the oxidation processes of all mixtures were close to each other and tended to slightly increase at molar ratios higher than 0.0001, which indicated a slightly faster oxidation process. In addition, the $Y_0$ values at molar ratios higher than 0.0001 tended to decrease with the increasing molar ratio, which indicated shortened induction periods. Thus, increase in the molar ratio of the stearoyl ascorbate to methyl linoleate to higher than 0.0001 made the oxidation process of the methyl linoleate faster, and not slower. The reason for this unexpected change may be that the higher amount of stearoyl ascorbate, in particular at a molar ratio higher than 0.0001 in the mixture might act as a prooxidant and directly interact with methyl linoleate to form lipid hydroperoxides or promote formation of free radicals in the mixture during acceleration of storage condition. The decomposition of lipid hydroperoxides generates more radicals, which could be responsible for the significant increase in rate of oxidation.

4 CONCLUSION

Stearoyl ascorbate in the presence of wheat starch or gluten exhibited antioxidative ability against the oxidation of methyl linoleate at the molar ratio of 0.0001 under accelerated conditions and its ability to act as an antioxidant was quite similar that of ascorbic acid. The effect of gluten when compared to that of wheat starch as the matrix of the mixture was contributed to the antioxidation effect against the methyl linoleate by altering durations of the initiation and propagation periods of the oxidation process. The appropriate molar ratio of the stearoyl ascorbate to methyl linoleate in order to be an antioxidant is 0.0001. The increase in the molar ratio to values higher than 0.0001 led to the acceleration of the oxidation process of the methyl linoleate.

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