Discoloration Kinetics of L-Ascorbyl 6-Palmitate Powders with Various Water Contents

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Received June 16, 2006; Accepted October 30, 2006

The discoloration of L-ascorbyl 6-palmitate, with water contents ranging from 2 to 10% (w/w) during storage at temperatures of 60 to 90° C, was investigated by monitoring the absorbance at 500 nm of its solutions dissolved in methanol. The discoloration process of the substrate was expressed by the modified Weibull equation under all conditions, and the kinetic parameters were evaluated. The activation energy and frequency factor were estimated based on the Arrhenius equation for the substrates of various water contents, and the enthalpy-entropy compensation held for the discoloration process. Comparison of the discoloration kinetic parameters for L-ascorbyl 6-palmitate with those for L-ascorbic acid revealed that the discoloration of L-ascorbyl 6-palmitate was weaker than that of L-ascorbic acid.

Keywords: L-ascorbyl 6-palmitate, discoloration kinetics, water content, Weibull equation

Introduction

L-Ascorbic acid (vitamin C) is used widely as an additive in foods because it is an essential nutrient for human beings and also a natural antioxidant. However, L-ascorbic acid undergoes degradation during storage and the processing of foods in which it is present. Many factors, such as the oxygen partial pressure, pH, temperature, and the presence of heavy metal ions, affect the degradation of L-ascorbic acid (Belitz and Grosch, 1999).

The discoloration of L-ascorbic acid in the solid state was also investigated (He *et al.*, 1990; Shephard *et al.*, 1999; Hung *et al.*, 2005). We reported the discoloration kinetics of L-ascorbic acid powders with water contents of 1 to 10% (w/w) during storage at temperatures of 60 to 90° C by dissolving them in water and monitoring the absorbance of the solution at 360, 450 and 500 nm (Hung *et al.*, 2005).

In recent years, L-ascorbic acid derivatives, which are acylated with a long- or medium-chain fatty acid, have been synthesized (Enomoto et al., 1991; Uragaki et al., 1993; Watanabe et al., 1999). Although L-ascorbic acid is highly hydrophilic, the acylation alters its property to improve the solubility and miscibility in a hydrophobic environment (Watanabe et al., 2003). Therefore, the lipophilic derivatives of L-ascorbic acid are used in foods rich in lipids. For example, L-ascorbyl 6-palmitate is increasingly used as a preservative in edible oil or as an antioxidant in other foods. Austria et al. (1997) reported that an L-ascorbyl 6-palmitate solution was more stable than an L-ascorbic acid solution. Špiclin et al. (2001) also examined the stability of L-ascorbyl 6-palmitate in an oil-inwater emulsion system. These examinations showed the acyl chain added a favorable characteristic to the Lascorbic acid. There is a possibility that the acylation affected the property of the L-ascorbic acid moiety. However, whether or not the acylation inhibits or suppresses the discoloration of the L-ascorbic acid moiety remains to be examined. The objective of this study is to investigate the discoloration kinetics of L-ascorbyl 6-palmitate powders.

Materials and Methods

Materials L-Ascorbyl 6-palmitate (purity,>95.0%) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan).

Sample preparation The effect of the water content on the stability of the L-ascorbyl 6-palmitate was investigated by using water contents ranging from 0 to 10% (w/w). The L-ascorbyl 6-palmitate powders (500 mg) were placed in a vial. Distilled water was pipetted into the vials to produce water contents of 2, 4, 6, 8, and 10% (w/w). In order to mix the powders with the distilled water as evenly as possible, the samples in the vials were stirred by a needle. Following that, the vials were sealed with a screw-cap. The samples were then stored at 60, 70, 80, and 90°C. The vials were taken out of the oven periodically and, after they had cooled down to room temperature to ensure evaporation of the water inside the vials was kept to a minimum, 33 mg samples were removed from each of them.

Analysis The color changes were monitored by dissolving 33 mg of the sample in 3 mL of methanol. The samples were then centrifuged at 3000 rpm for 10 min, and the absorbance of the solution was measured using a Shimadzu UV-1600 UV-visible spectrophotometer (Kyoto, Japan) from 300 to 600 nm at a sampling pitch of 2.0 nm. The optical path length was 1 cm.

The sample was also analyzed for determining the fraction of unchanged L-ascorbyl 6-palmitate by an HPLC-UV method. The HPLC-UV system was a Shimadzu LC-10 AT liquid chromatograph (Kyoto, Japan) equipped with a Shimadzu SPD-6A UV spectrophotometer and Hydrosphere C18, HS-303 column (250×4.6 mm I.D.; YMC, Kyoto,

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Fig. 1. Absorption spectra of the methanol solutions for L-ascorbyl 6-palmitate samples with 2% (w/w) water content after storage at 70°C for 1, 2, 4, 6 and 10 days. The numerical labels indicate the days of storage.

Japan). The eluent was a mixture of methanol, water, and phosphoric acid (95: 5: 0.1 by vol.) at a flow rate of 1.0 mL/min. The absorbance of the effluent was measured at 255 nm and then recorded.

Results and Discussion

Discoloration of L-ascorbyl 6-palmitate powder Figure 1 shows the typical absorption spectra of the L-ascorbyl 6palmitate sample solutions with a 2% (w/w) water content after storage at 70°C for 1 to 10 days. The absorbance at wavelengths from 300 to 600 nm increased with the storage time, although the extent of which was dependent on the wavelength. However, there was no significant difference among the changing ratios of the absorbance values at any wavelength. In our previous study (Hung *et al.*, 2005), L-ascorbic acid also showed no significant difference in the ratio at 360, 450 and 500 nm. Therefore, only the experimental data obtained at 500 nm, which reflect the yellowness of the samples, were analyzed and discussed in the present study.

Figure 2 shows the changes in the absorbance at 500 nm of the solutions containing the L-ascorbyl 6-palmitate samples consisting of various water contents and stored at different temperatures. The absorbance at 500 nm increased with the storage time at a higher rate for the sample stored at a higher temperature. The absorbance at 500 nm reached a maximum, which depended on both the water content and the storage temperature. The discoloration process of L-ascorbic acid could be well expressed by the modified Weibull equation (Hung *et al.*, 2005), which is flexible and has a potential for describing many deterioration kinetics (Cunha *et al.*, 1998):

$$(A_{\max} - A) / (A_{\max} - A_0) = \exp[-(k_d t)^{n_d}]$$
(1)

where *A* is the absorbance at time *t*, A_{max} is the maximum absorbance, A_0 is the initial absorbance (which is equal to 0 in this study), k_d is the rate constant for the discoloration, and n_d is the shape constant. The equation was also applied to the discoloration of L-ascorbyl 6-palmitate and



Fig. 2. Changes in absorbance at 500 nm for the solutions of L-ascorbyl 6-palmitate samples which had the water contents of (\bigtriangledown) 2%, (\Box) 4%, (\bigtriangleup) 6%, (\bigcirc) 8% and (\diamondsuit) 10% (w/w) and at (a) 90°C, (b) 80°C, (c) 70°C, and (d) 60°C.

the kinetic parameters, A_{max} , k_{d} and n_{d} were evaluated by best-fitting the experimental data using the Solver of Microsoft Excel[®]. The solid curves in Fig. 2 were drawn based on Eq. (1) using the estimated parameters. Agglomeration occurred for the L-ascorbyl 6-palmitate samples with water contents of 4, 6, 8 and 10% (w/w) during the storage at 70, 80 and 90°C. Although the effect of the agglomeration on the discoloration rate of the L-ascorbyl 6-palmitate powders was not clear, the kinetic parameters were estimated, including all the effects caused by any changes during the storage.

Figure 3 shows the temperature dependencies of parameters A_{max} , k_d and n_d for the L-ascorbyl 6-palmitate powders with different water contents. The maximum absorbance, A_{max} increased with both the water content and the storage temperature. The rate constant, k_d also increased with the storage temperature and generally decreased with the storage temperature and generally decreased with the water content. The shape constant, n_d was greater than unity for every water content and storage temperature, reflecting the fact that the discoloration slowly progressed during the early stage of storage with the exception of samples with a 2% (w/w) water content and stored at 60°C.

The temperature dependency of the rate constant, k_{d} , was analyzed based on the Arrhenius equation:

$$k_{\rm d} = k_{\rm d0} \exp(-E_{\rm d}/RT) \tag{2}$$

where k_{d0} is the frequency factor, E_d is the activation energy, R is the gas constant, and T is the absolute temperature. As shown in Fig. 3 (b), the plots for each sample lay roughly on a straight line, from which the E_d and k_{d0} values were evaluated.

The E_d values were plotted against the natural logarithms of the k_{d0} values for the discoloration of the L-ascorbyl 6-palmitate. For comparison, the E_d and k_{d0} values



Fig. 3. Temperature dependencies of (a) A_{max} , (b) k_{d} and (c) n values of Eq. (1) for discoloration of the L-ascorbyl 6-palmitate with various water contents. The symbols are the same as in Fig. 2.

for the discoloration of L-ascorbic acid (Hung *et al.*, 2005) were also plotted in Fig. 4. The plots for both the L-ascorbyl 6-palmitate and L-ascorbic acid lie on a straight line. Equation (3) is one of the expressions describing the enthalpy-entropy compensation (Leffer, 1955; Exner, 1964):

$$E_{\rm d} = R T_{\beta} \ln k_{\rm d0} + \gamma \tag{3}$$

where T_{β} is a parameter called the isokinetic temperature and γ is a constant. Therefore, the compensation held for the discoloration of both the L-ascorbyl 6-palmitate and L-ascorbic acid, indicates that the discoloration proceeds through the same mechanism for both substrates. The values of T_{β} and γ were estimated to be 361 K and 1.34 kJ/ mol, respectively, from the slope and intercept of the line.

Insert of Fig. 4 shows the dependencies of the E_d and k_{d0} (ln k_{d0}) values on the water contents of the L-ascorbyl 6-palmitate. The E_d and ln k_{d0} values proportionally increased with the water content, although the reason for the linear relationship is unclear.

Disappearance of L-ascorbyl 6-palmitate The fraction of the remaining L-ascorbyl 6-palmitate, which was determined by the HPLC-UV method, changed in a different manner from the discoloration. Figure 5(a) shows the relationship between the fraction of unchanged L-ascorbyl 6-palmitate, C/C_0 , and the storage time *t*, in the storage temperature of 90°C, where *C* and *C*₀ represent the concentration at time *t* and the initial concentration of L-ascorbyl 6-palmitate. The Weibull model of Eq. (2) was adopted again to describe the decomposition of L-ascorbyl 6palmitate over the storage period:



Fig. 4. Enthalpy-entropy compensation for the discoloration of (\bigcirc) L-ascorbyl 6-palmitate and (\triangle) L-ascorbic acid (Hung *et al.*, 2005). Insert: the dependencies of the activation energy E_d (\bigcirc) and the frequency factor k_{d0} ($\ln k_{d0}$) (\triangle) of the rate constant for discoloration at 500 nm of the L-ascorbyl 6-palmitate on the water content.



Fig. 5. Disappearance with time of the L-ascorbyl 6-palmitate powders (a) with the water contents of 6, 8 and 10% (w/w) at 90°C and (b) with 10% (w/w) water content at (\bigcirc) 60°C, (\square) 70°C, (\triangle) 80°C, and (\diamond) 90°C. The symbols in (a) are the same as in Fig. 2.

$$C/C_0 = \exp\left[-(k_{\rm h}t)^{n_{\rm h}}\right] \tag{2}$$

where k_h and n_h are the rate and shape constants, respectively. The kinetic parameters, k_h and n_h , were evaluated using the Solver of Microsoft Excel[®] to best-fit the experimental data. The solid curves in Fig. 5 were drawn based on Eq. (2) using the estimated parameters. The k_h value was higher for the sample with the higher water content.

Figure 5(b) shows the changes in the fraction against time for the L-ascorbyl 6-palmitate samples with the 10% (w/w) water content at different temperatures. The disappearance of the substrate could also be expressed by the the Weibull model at any temperature. For the samples with water contents less than 10% (w/w) and stored at 60°C, it was difficult to express the disappearance processes by the Weibull model because the fraction of the decreased substrate, during the experimental period, was less than 7% for the samples.

The rate constants, $k_{\rm h}$ and $n_{\rm h}$ for L-ascorbyl 6-palmitate



Fig. 6. The temperature dependencies of (a) the rate constant, k_h and (b) the shape constant, n_h for disappearance of L-ascorbyl 6-palmitate with various water contents. The symbols are the same as in Fig. 2.

samples with the water contents of 6, 8 and 10% (w/w) were estimated at 70, 80 and 90°C. The reliability of the parameters estimated for the samples with the water contents of 2 and 4% (w/w) seemed low due to the decrease in the fraction being insignificant. Therefore, the parameters were not used in the estimation of the activation energy. The temperature dependence of the $k_{\rm h}$ value could also be expressed by the Arrhenius equation (Fig. 6 (a)). The activation energy, $E_{\rm h}$ for the disappearance of the substrate was greater for the sample with the lower water content, and the $E_{\rm h}$ values for the substrates with the water contents of 6, 8 and 10% (w/w) were 230, 129 and 82 kJ/mol, respectively. The $E_{\rm h}$ values for the disappearance were higher than those for the discoloration (Fig. 4). The disappearance of the substrate includes two reactions: one is the discoloration of the substrate itself, and another is the hydrolysis of the substrate to its constituent components. As shown later, the reactions seem to proceed in a parallel fashion. Therefore, the activation energy for the hydrolysis would be higher than that for the discoloration. The lower $E_{\rm h}$ value at the higher water content would also be reasonable because the hydrolysis proceeded with relative ease at the high water concentration. The $n_{\rm h}$ values' dependence on both the temperature and the water content of the sample were insignificant and were approximately 0.8.

Comparison of discoloration of L-ascorbyl 6-palmitate and L-ascorbic acid powders Figure 7 shows the relationship between the absorbance at 500 nm and the amount of consumed L-ascorbyl 6-palmitate for the samples with water contents of 8 and 10% (w/w) stored at 70, 80 and 90 °C. The amount of consumed substrate was expressed by C_0-C where C_0 and C are the initial substrate concentra-



Fig. 7. Relationship between the absorbance at 500 nm and the amount of consumed L-ascorbyl 6-palmitate at (\Box, \blacksquare) 70°C, $(\triangle, \blacktriangle)$ 80°C and $(\diamondsuit, \diamondsuit)$ 90°C. The water contents of the L-ascorbyl 6-palmitate were (open symbols) 8% (w/w) and (closed ones) 10% (w/w). For comparison, the relationship for (\bigcirc, \bullet) the L-ascorbic acid was cited from our previous study (Hung *et al.*, 2005). C_0 and C represent the initial concentration of the L-ascorbyl 6-palmitate (or L-ascorbic acid) and that of the remaining amount at any time, respectively.

tion and the concentration of the remaining one at any time, respectively. For comparison, the relationship for L-ascorbic acid is also shown in Fig. 7. There was a linear correlation with the determination coefficient of 0.93 between the difference in the concentration, $C_0 - C_2$, and the logarithm of the absorbance at 500 nm for the L-ascorbyl 6 -palmitate. This linear correlation on the semi-logarithmic scale indicates that the absorbance exponentially increases with the disappearance of the substrate and that the substrate produces more colored substances during the late stage of being consumed. L-Ascorbic acid exhibited a rough linear correlation with the determination coefficient of 0.65 between C_0-C and the absorbance (Hung et al., 2005). The line for the L-ascorbyl 6-palmitate was under the curve for L-ascorbic acid, indicating that the discoloration per mole of consumed L-ascorbic acid is stronger than that per mole of consumed L-ascorbyl 6palmitate.

In order to examine the effect of the acylation of Lascorbic acid on the discoloration, the discoloration process was calculated based on the kinetic parameters for the L-ascorbic acid. If there is no effect, the discoloration rate should be equal to that of the L-ascorbic acid. If the acylation completely suppresses the discoloration, only L-ascorbic acid liberated from the L-ascorbyl 6-palmitate by hydrolysis discolors. The discoloration processes for the L-ascorbyl 6-palmitate powders with water contents of 2 and 4% (w/w) at 90°C were calculated using the A_{max} , n_{h} and k_{h} values for the L-ascorbic acid (Hung *et al.*, 2005) for two reaction schemes. In one scheme, the L-ascorbyl 6palmitate is first hydrolyzed to L-ascorbic acid and palmitic acid, and only the L-ascorbic acid is discolored. This scheme assumes a consecutive reaction. In the other



Fig. 8. The absorbance values at 500 nm of the solutions of the L-ascorbyl 6-palmitate with water contents of (a) 2% (w/w) and (b) 4% at 90°C. The broken and solid curves were drawn based on the assumptions that only the L-ascorbic acid produced by the hydrolysis of the L-ascorbyl 6-palmitate discolors, and that the L-ascorbyl 6-palmitate discolors in the same manner as the L-ascorbic acid, respectively. For the solid curve, the kinetic parameters for the L-ascorbic acid with the water content on a molar basis as the L-ascorbyl 6-palmitate were used (Hung *et al.*, 2005). The k_h , n_h , A_{max} , k_d and n_d values for the samples with the water contents of 2% (w/w) and 4% (w/w) were 0.0015 d⁻¹, 0.236, 1.70, 0.61 d⁻¹ and 2.75, and 0.0195 d⁻¹, 0.22, 4.06, 0.49 d⁻¹ and 2.75 respectively.

scheme, both the L-ascorbyl 6-palmitate and L-ascorbic acid discolored in the same manner. The latter scheme assumes parallel and consecutive discoloration reactions of the L-ascorbyl 6-palmitate itself and the L-ascorbic acid produced by the hydrolysis of the L-ascorbyl 6-palmitate.

The broken and solid curves in Figs. 8(a) and (b) were calculated for the former and latter schemes, respectively. The experimental results were located between the broken and solid curves for both samples with water contents of 2 and 4% (w/w). This indicates that both the L-ascorbyl 6-palmitate and L-ascorbic acid discolor, but that the extent of L-ascorbyl 6-palmitate discoloration is weaker than that of the L-ascorbic acid. This estimate coincides with the results shown in Fig. 7.

Figure 9 shows the relationships between the A_{max} value and the molar ratio of water to the L-ascorbyl 6-palmitate or L-ascorbic acid at the various temperatures. The A_{max} values for the L-ascorbyl 6-palmitate were one-third those for L-ascorbic acid or less at the same water content on a molar basis.

As mentioned above, the acylation of L-ascorbic acid with palmitic acid suppressed the discoloration of the L-ascorbyl moiety.

Conclusion

Discoloration of the L-ascorbyl 6-palmitate in the solid state was significantly affected by both the water content and storage temperature. The discoloration process could be expressed by the modified Weibull equation for all conditions, and the kinetic parameters of the equation



Fig. 9. The relationships between the A_{\max} value and the molar ratio of water to the (open symbols) L-ascorbyl 6-palmitate or (closed ones) L-ascorbic acid (Hung *et al.*, 2005) at (\bigcirc) 60°C, (\square , \blacksquare) 70°C, (\triangle , \blacktriangle) 80°C and (\diamondsuit , \blacklozenge) 90°C.

were evaluated. The temperature dependence of the rate constant was expressed by the Arrhenius equation, and the activation energy and frequency factor were evaluated. The maximum absorbance of the L-ascorbyl 6-palmitate was lower than that of L-ascorbic acid for the same water content. Thus, it was indicated that the acylation suppressed the discoloration of the L-ascorbyl moiety.

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