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Influence of Acetic Acid Solution on Heat Stability of L-Ascorbic Acid

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Abstract This study investigated the heat stability of L-ascorbic acid (AA) in acetic acid solution. To analyze the degradation of AA using high performance liquid chromatography (HPLC), AA was measured at a wavelength of 244 nm in acetic acid and 265 nm in distilled water. During the storage of AA in acetic acid or distilled water at 37°C, degradation of AA was slower in acetic acid than in distilled water. On examining various ratios of AA to acetic acid, the stability of AA at 100°C for 30 min was the highest when the concentration of acetic acid was 10 times higher than the concentration of AA. After acetic acid was added into AA degraded by heating, the AA is stabilized by reheating. Ultimately, these results indicate that degraded AA is reduced by hydrogen ions dissociated from acetic acid, and the rate of reduction of degraded AA in acetic acid solution is improved with heat processing.

Keywords: ascorbic acid, degradation, acetic acid, heat stability, reduction

Introduction

L-Ascorbic acid (AA), also known as vitamin C, is a representative water-soluble vitamin possessing a variety of biological, pharmaceutical, and dermatological functions; it promotes collagen biosynthesis, provides photoprotection, causes melanin reduction, scavenges free radicals, and enhances immunity. These functions are closely related to the established antioxidant properties of this compound in foodstuffs, cosmetics, and pharmaceutical preparations (1-7).

Vitamin C constitutes compounds exhibiting some or all of the biological activity of AA. These include esters of AA such as ascorbyl palmitate, which has 100% relative activity, synthetic forms such as 6-deoxy-L-ascorbic acid with 33% relative activity, and the primary oxidized form of AA, dehydroascorbic acid (DHA), with 80% relative activity (8). Crystalline AA is very stable in the presence of oxygen when little water is present. In aqueous solution, the AA can lead to rapid and excessive oxidative changes with conversion to DHA via monodehydroascorbic acid (MDHA). Generally, the vitamin C content is evaluated by determining the AA, MDHA, and DHA dosage because MDHA and DHA possess antiscorbutic activity equivalent to that of AA (8-11).

The stability of AA in various environments has been studied extensively (12,13). It is known to be unstable when exposed to air, moisture, light, heat, metal ions, oxygen, and food processing, and is easily decomposed into biologically inactive compounds, such as 2,3-diketo-L-gulonic acid, oxalic acid, L-threonic acid, L-xylonic acid, and L-lyxonic acid (8,14,15).

The pH of food greatly influences the oxidative stability of AA. At low pH, the fully protonated form is quite stable. As the pH approaches pK1 (4.04), the stability decreases. The maximal stability usually occurs between pH 4 and 6. However, the degradation rate is dependent on oxygen availability, the presence of antioxidants, heat processing conditions, transition metal catalysis, and a multitude of possible interactions (8). Reducing agents can convert the dehydro form back into AA. The enzymatic conversion of DHA into AA by glutathione is an important biological defense against oxidative stress (8,16).

Since AA is oxidized rapidly when in aqueous solution or during food processing, the degradation of AA is a major drawback in the design of various dosage forms for its use (16,17). To solve these drawbacks, studies have examined the influence of amino acids on the stability of AA (18), AA destruction in aqueous systems (14), the reduction of AA by strong acid, such as HCl (16), AA microencapsulation (19) and inorganic nanocapsules (5), and the stability of AA in orange juice (20). However, no experimental data are available on the influence of mild organic acids on the reduction of AA and the stability of AA during heat processing.

A mild organic acid such as acetic acid can serve as a reducing agent and supply MDHA and DHA with free hydrogen ions. The aim of this study was to investigate the influence of acetic acid on AA degradation during heating in aqueous solution.

Materials and Methods

Materials L-Ascorbic acid (AA), acetic acid, and potassium dihydrogen phosphate were purchased from Shinyo Pure Chemistry (Osaka, Japan). Sodium L-ascorbate (MDHA) was obtained from Kanto Chemical Company (Tokyo, Japan), and acetonitrile from J.T. Baker (Solusorb®; Phillipsburg, NJ, USA).

Spectrum of AA and MDHA in distilled water and an acetic acid solution To analyze changes in the AA and MDHA contents by high performance liquid chromatography (HPLC), each spectrum of AA and MDHA was examined

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in distilled water and a 0.25% acetic acid solution using a spectrophotometer (UV-1650PC; Shimadzu, Kyoto, Japan). First, 10 mg of AA and MDHA were dissolved in 100 mL samples of distilled water and a 0.25% acetic acid solution, respectively, and ultra violet (UV)-Vis spectra from 200 to 600 nm were obtained using the spectrophotometer on 1 mL samples.

Analysis of AA by HPLC The analyses of AA and MDHA were carried out by HPLC (Thermo Separation Products, Waltham, MA, USA). A 250×4.6 mm column (C_{18} column; Thermo Separation Products) was used as the solid phase. The mobile phase consisted of 50 mM potassium dihydrogen phosphate and acetonitrile at a ratio of 6:4 (18,21,22). The flow rate was 1 mL/min, and the detection wavelength of the UV detector (Spectra System UV1000; Thermo Separation Products) was set at 265 nm for the distilled water and 244 nm for the 0.25% acetic acid solution. The injection volume of 20 μL was delivered by an autosampler (Spectra System AS1000; Thermo Separation Products), and quantitation was made via electronic integration of the peak area using MultiChro™ Version 5.0 (Yullin Technology, Seoul, Korea). All samples were analyzed in triplicate.

Changes in the AA content in distilled water and an acetic acid solution A total of 10 mg AA was dissolved in 100 mL distilled water and a 0.25% acetic acid solution, and each of the AA samples was kept in the dark at 37°C for 3 days in a shaking incubator at 100 rpm (SI-300R; Jeio Tech, Seoul, Korea). On each day, the remaining AA contents in the distilled water and 0.25% acetic acid solution were measured by HPLC. All samples were analyzed in triplicate.

Heat stability of ascorbic acid in acetic acid solutions The heat stability of AA in acetic acid solutions was evaluated by 2 methods. In the first method, the heat stability of AA at various ratios of AA to acetic acid was compared with the heat stability of AA in distilled water. The AA and acetic acid solution ratios were 10:1, 1:1, and 1:10; 10 mg samples of AA were dissolved in 100 mL of 0.001, 0.01, and 0.1%(v/v) acetic acid solutions, and in 100 mL of distilled water, respectively. Each of the AA samples was heated for 10, 20, or 30 min at 100°C, and changes in the AA content for each ratio were measured by HPLC. In the second method, to analyze the heat stability of total AA after the addition of an acetic acid solution to a heated AA solution with time, 10 mg AA was dissolved in 100 mL distilled water; 9 mL AA solutions were then transferred into 3 test tubes, and the test tubes were heated for 10, 20, or 30 min at 100°C. Then, 1 mL of 1%(v/v) acetic acid solution was added to the test tubes, and each test tube was heated again for 10 min at 100°C. The AA contents before and after the addition of the acetic acid solution were measured by HPLC. All samples were analyzed in triplicate.

Results and Discussion

Comparison of the AA and MDHA spectra in distilled water vs. an acetic acid solution Figure 1 shows the spectra of AA and MDHA in both the distilled water and acetic acid solution. In the case of AA, the maximum peak was seen at 254 nm in distilled water and at 244 nm in the acetic acid solution. For MDHA, the maximum peak was seen at 265 nm in distilled water and at 244 nm in the acetic acid solution. These detection wavelengths, 254 nm for AA and 265 nm for MDHA, were similar to the majority of those previously reported for AA and MDHA using HPLC (8). However, the maximum peaks for AA and MDHA in acetic acid corresponded with the maximum peak of 244 nm reported by Eitenmiller and Landen (8) for AA in 0.1 M phosphate buffer at pH 2. Consequently, to analyze the total AA content under acidic conditions, a detection wavelength of 244 nm was used instead of 254 nm under neutral conditions.

Degradation of AA in distilled water and an acetic acid solution Figure 2 shows the change in the degradation of AA in distilled water and an acetic acid solution. The AA contents in both solutions degraded with time, and the degradation of AA in the acetic acid solution was slower than in the distilled water. Touitou *et al.* (16) reported that

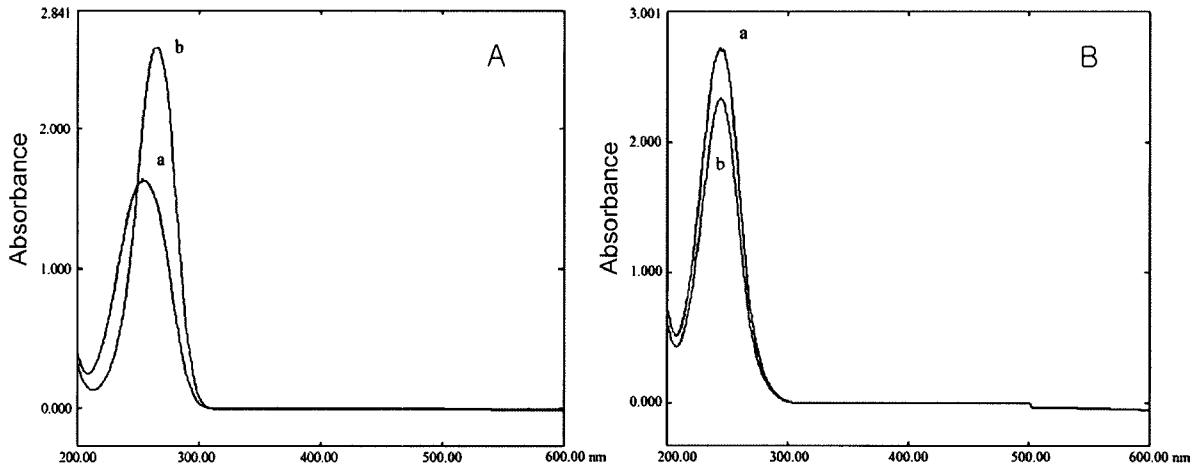

Fig. 1. UV-Vis spectra of L-ascorbic acid (a) and sodium-ascorbate (b) dissolved in distilled water (A) and 0.25% acetic acid (B) solution. L-Ascorbic acid in DW, 254 nm; sodium ascorbate in DW, 265 nm; L-ascorbic acid in 0.25% acetic acid, 244 nm; sodium ascorbate in 0.25% acetic acid, 244 nm.

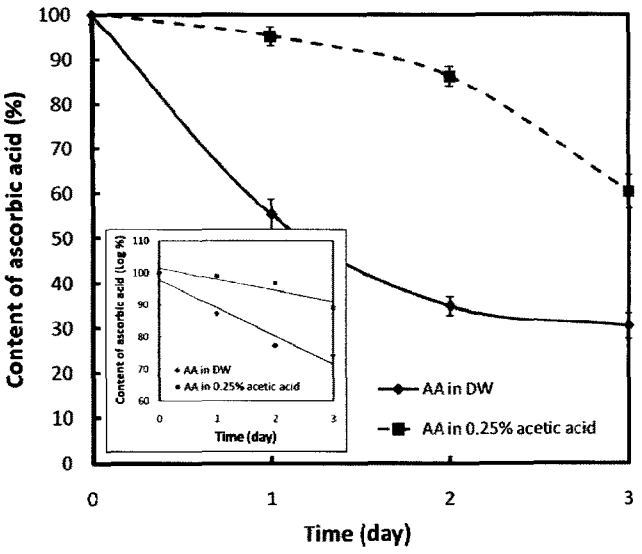

Fig. 2. Degradation with time of ascorbic acid (AA) in distilled water (DW) and 0.25% acetic acid at 37°C. Insert: semilogarithmic plot of AA degradation in DW and 0.25% acetic acid at 37°C.

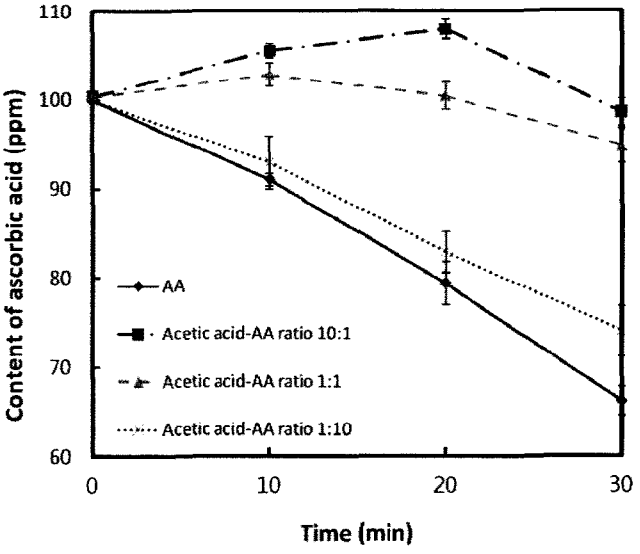

Fig. 3. The effect of acetic acid for the stability of ascorbic acid (AA) heated at 100±1°C under various ratios of acetic acid to ascorbic acid.

acidification by the addition of HCl led to a remarkable stabilization of vitamin C in solution and that the addition of a free sulfhydryl group as a reducing agent, which dissociated from glutathione completely, suppressed AA degradation and prevented the degradation of an AA solution. Therefore, we suggest that the slow degradation of AA in the acetic acid solution was likely due to the reduction of degraded AA to AA by hydrogen ions dissociated from acetic acid.

Maintenance of the stability of ascorbic acid at heat processing by acetic acid The effects of hydrogen ions dissociated from acetic acid on the degradation of AA were investigated using two methods. In the first method, the degradation of AA in distilled water and an acetic acid solution (at various ratios of AA:acetic acid) at 100°C was measured (Fig. 3). At a 1:10 ratio of AA:acetic acid, the content of AA in the acetic acid solution increased for 20 min compared to the initial content of AA, but decreased

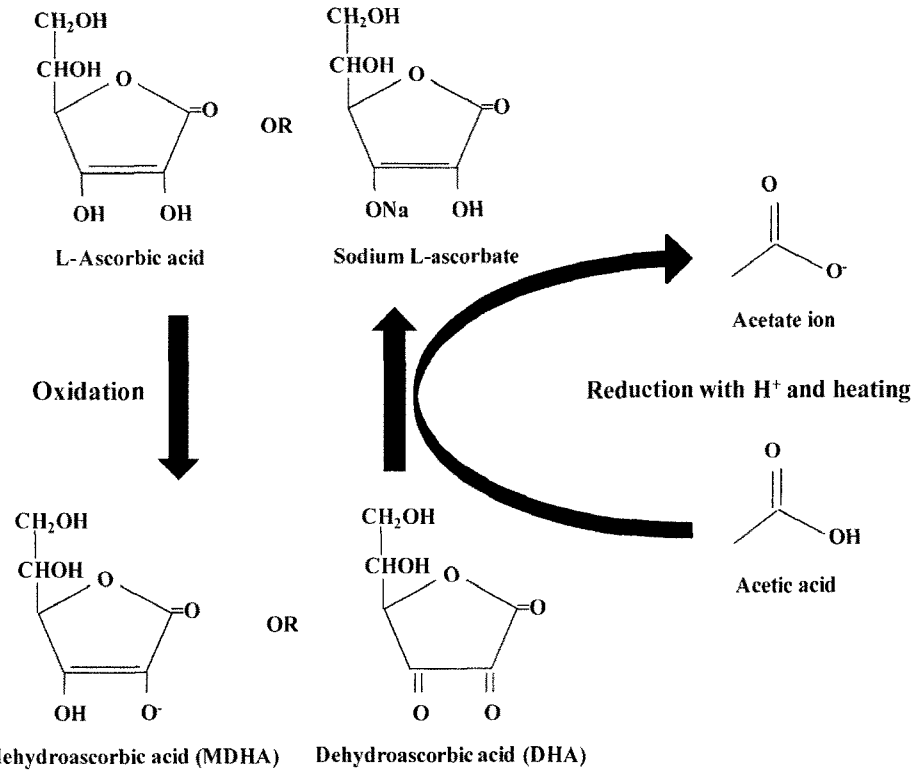

Fig. 4. Schematic for the reduction of degraded ascorbic acid by acetic acid solution and heating.

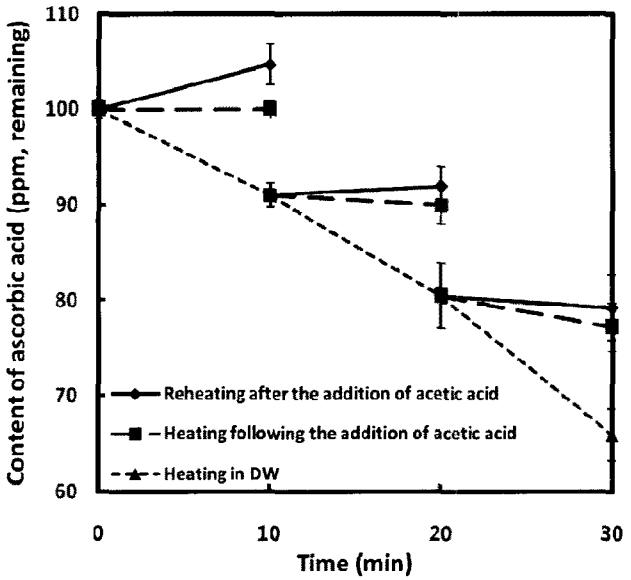

Fig. 5. The heat stability of ascorbic acid (AA) after addition of acetic acid solution to AA heated at 100±1°C.

after 10 min at a 1:1 ratio of AA:acetic acid. In contrast, the AA content in distilled water and the acetic acid solution at a ratio of 10:1 rapidly decreased. Touitou *et al.* (16) reported that the AA content improved after heating at 121°C with the addition of glutathione. These results suggest that AA is easily oxidized to the degraded AA through heating processing and that acetic acid, a mild acid, and pro-reducing agent like glutathione, through heating, may rapidly degrade into an ionized form. Thus, the concentration of free hydrogen ions dissociated from acetic acid increased and served as a reducing agent for the degraded AA. Moreover, the reduction rate of AA increased, and the heat stability of AA maintained (Fig. 4). Acetic acid, when used as a reducing agent, was more effective when the acetic acid content was higher than the AA concentration.

In the second method, the degradation of total AA at 100°C was compared after 1) heating, 2) heating following the addition of acetic acid solutions, and 3) heating after the addition of an acetic acid solution and reheating (Fig. 5) to analyze the influence of acetic acid on the heat stability of AA. The reheated AA content increased for 10 min after adding the acetic acid solution into the heated AA solution and reached a higher level than the heated AA content and the heated AA content in the acetic acid solutions. The heated AA content in distilled water declined faster than the heated AA content in the acetic acid solutions. These phenomena suggest that the degraded AA was reduced by hydrogen ions dissociated from acetic acid and that the reduction rate of the degraded AA was improved by heating. Kabasakalis *et al.* (23) reported that storage of commercial fruit juices in closed containers resulted in ascorbic acid losses ranging from 29 to 41% for 4 months, while commercial orange juice when stored in open containers lost 60 to 67% of its AA for 31 days. In open system as rapidly degraded condition of AA, this results show the possibility for the maintenance of the stability of AA during heat processing of food such as beverage.

In conclusion, AA is easily oxidized into the degraded AA as MDHA and DHA by, for example, heat and oxygen, in food processing and decomposes into biologically inactive compounds such as 2,3-diketo-L-gulonic acid, oxalic acid, L-threonic acid, L-xylonic acid, and L-lyxonic acid (1,5,8). *In vivo* MDHA and DHA are readily taken up by erythrocytes and other cells and reduced to AA, the active form of vitamin C, but if AA, MDHA, and DHA as active form of vitamin C are completely degraded to inactive forms, then it is never reduced to AA (20,24,25). To resolve these problems, the stability of total AA must be maintained or improved in food processing. The natural mild organic acids contained in food and the addition of mild organic acids such as acetic acid should maintain the stability of AA in food, and because the dissociation of hydrogen ions from acetic acid is stimulated by heating, the stability of AA in food processing can be further stabilized. Ultimately, we hope that our findings will increase the availability of the biologically active form of vitamin C in food industry.

Acknowledgments

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