Degradation of Ascorbic Acid in Aqueous Solution

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An HPLC method, for the simultaneous determination of the degradation products of ascorbic acid, was employed to investigate the degradation of ascorbic acid in aqueous solution at different pH values. After ascorbic acid aqueous solutions were heated at 100 °C for 2 h, four main degradation products, furfural, 2-furoic acid, 3-hydroxy-2-pyrone, and an unidentified compound, were separated and determined. In an acid aqueous solution, ascorbic acid was converted to 2-furoic acid and 3-hydroxy-2-pyrone via dehydroascorbic acid under aerobic conditions, whereas under anaerobic conditions, ascorbic acid degraded to furfural. Low pH conditions favored the formation of furfural, 2-furoic acid, and 3-hydroxy-2-pyrone; at extremely low pH (i.e., pH 1), the formation of furfural dominated. In an alkaline aqueous solution, the unknown compound became the main degradation product of ascorbic acid; at pH 10, only very small amounts of furfural and 3-hydroxy-2-pyrone with no 2-furoic acid were detected. Our results suggest that, in a hydrogen-ion-catalyzed environment, the anaerobic degradation of ascorbic acid to furfural is the main degradation pathway in an aqueous solution.

Keywords: *Ascorbic acid; dehydroascorbic acid; degradation; furfural; 2-furoic acid; 3-hydroxy-2-pyrone; HPLC*

INTRODUCTION

The degradation of ascorbic acid has been considered one of the major causes of quality and color changes during processing and storage of food products. The degradation processes of ascorbic acid are very complex and contain a number of oxidation/reduction and intermolecular rearrangement reactions (Kimoto et al., 1993). Dehydroascorbic acid, the oxidation form of ascorbic acid, is highly unstable in an aqueous solution, which may be converted to a variety of degradation products, such as 2-furoic acid, 3-hydroxy-2-pyrone, 5-methyl-3,4-dihydroxytetrone, furfural, etc. (Kimoto et al., 1993), depending upon the conditions of the degradation reaction.

Solomon et al. (1995) studied the effect of oxygen on the degradation of ascorbic acid and found that there was a significant effect of dissolved oxygen concentrations on the formation of dehydroascorbic acid. The oxidation of ascorbic acid led to the formation of the intermediates dehydroascorbic acid and diketogulonic acid (Kimoto et al., 1993; Ortwerth et al., 1994; Sawamura et al., 1994; Deutsch, 1998) and subsequently to L-xylosone, the further degradation to the various five-carbon compounds (Kimoto et al., 1993). 2-Furoic acid has been proved to be one of the degradation products of dehydroascorbic acid in an aqueous solution (Sawamura et al., 1994; Kimoto et al., 1993).

Under anaerobic conditions, ascorbic acid degraded via several steps to furfural instead of dehydroascorbic acid (Smoot and Nagy, 1980; Robertson and Samaniego, 1986; Rodriguez et al., 1991). In a hydrogen-ion-catalyzed anaerobic environment, the accumulation of furfural was concurrent with the degradation of ascorbic acid (Smoot and Nagy, 1980; Rodriguez et al., 1991). Robertson and Samaniego (1986) suggested that since furfural production was not significantly affected by initial dissolved oxygen levels, the anaerobic rather than the aerobic pathway is the major one for furfural formation from ascorbic acid in lemon juice. Even if under drastic thermal degradation conditions, e.g., heated at 300 °C in the absence of a solvent or at 180 °C in propylene glycol, furfural was one of the degradation products of ascorbic acid (Vernin et al., 1998).

The aim of the present study is to further investigate the degradation of ascorbic acid and dehydroascorbic acid and the effect of pH on the degradation of ascorbic acid, using an HPLC method developed previously by Yuan and Chen (1998) for the simultaneous determination of these degradation products.

EXPERIMENTAL PROCEDURES

Chemicals and Reagents. L-Ascorbic acid was purchased from Sigma Chemical Co. (St. Louis, MO). Dehydro-L-ascorbic acid, furfural, 2-furoic acid, 2,5-dimethyl-4-hydroxy-3-(2*H*)-furanone (DMHF), and 5-(hydroxymethyl)furfural (5-HMF) were purchased from Aldrich Chemicals Co. (Milwaukee, WI). HPLC-grade acetonitrile was purchased from BDH Laboratory Supplies (Poole, England).

Degradation Reaction of Ascorbic Acid and Dehydroascorbic Acid. For comparing the degradation products, ascorbic acid (25 mg) and dehydroascorbic acid (10 mg) were dissolved in 10 mL of 0.008 N hydrogen chloride solution, respectively, and heated in a water bath at 100 °C for 2 h. For identifying 3-hydroxy-2-pyrone, the degradation product of dehydroascorbic acid (Kimoto et al., 1993), ascorbic acid (25 mg), and dehydroascorbic acid (15 mg) were dissolved in 10 mL of 1 N sulfuric acid solution, respectively, and heated in a water bath at 100 °C for 1 h. For investigating the effect of pH on the degradation of ascorbic acid, an aqueous ascorbic acid solution at 10 g/L was prepared on the day of use; ascorbic acid (1 g) was dissolved in 100 mL of distilled water, and the pH of the solution was adjusted, respectively, to 1, 2, 3, 4, 5,

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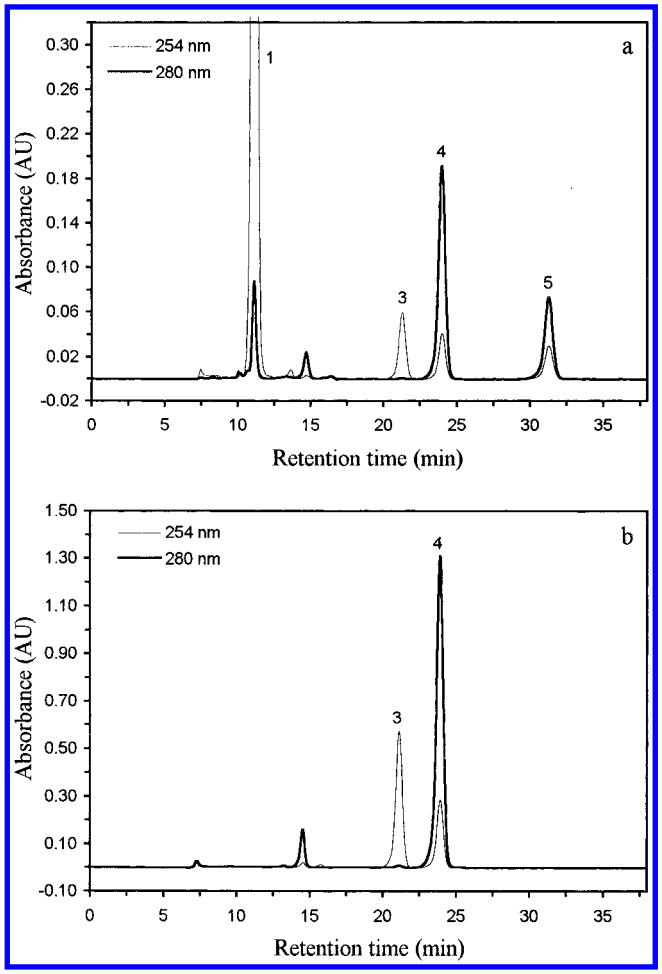

Figure 1. HPLC chromatograms of ascorbic acid (a) and dehydroascorbic acid (b) heated in 0.008 N hydrogen chloride solution at 100 °C for 2 h.

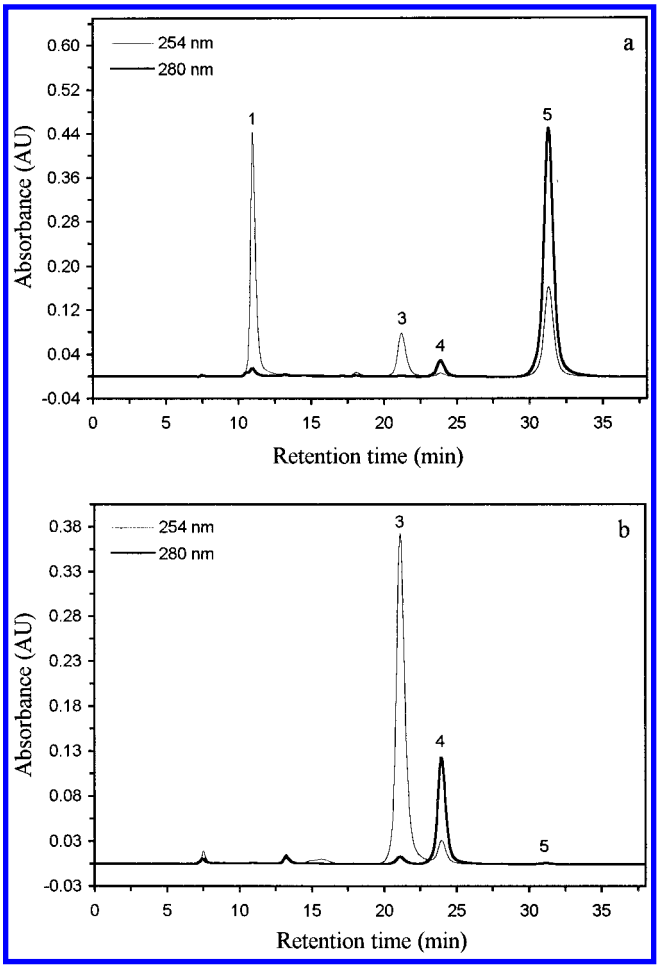

Figure 2. HPLC chromatograms of ascorbic acid (a) and dehydroascorbic acid (b) heated in 1 N sulfuric acid solution at 100 °C for 1 h.

7, 9, and 10 by the addition of either hydrogen chloride solution or sodium hydroxide solution. The ascorbic acid solutions were subsequently heated in a water bath at 100 °C for 2 h.

After heating, the reaction mixtures were quickly cooled to room temperature, and the resulting products were sampled and analyzed using HPLC.

HPLC Method. The degradation products of ascorbic acid and dehydroascorbic acid were determined by employing an HPLC method developed previously by Yuan and Chen (1998). High-performance liquid chromatography was conducted on a Waters liquid chromatograph equipped with two 510 pumps. The samples were separated by using a Bio-Rad Aminex HPX-87H column (300 × 7.8 mm). The column temperature was maintained at 25 °C. The mobile phase consisted of acetonitrile (19%) and 0.005 M sulfuric acid aqueous solution (81%). The flow rate was set at 0.5 mL/min. The samples were injected with a Rheodyne 7725i valve with a 20 μL loop. A 996 photodiode array detector (Waters) was used for the simultaneous detection of these degradation products of ascorbic acid. The tridimensional chromatogram was recorded from 190 to 400 nm. Peaks were measured at wavelengths of 254 and 280 nm, respectively, to facilitate the detection of these compounds.

RESULTS AND DISCUSSION

To compare the degradation reactions of ascorbic acid and dehydroascorbic acid, ascorbic acid (2.5 mg/mL) and dehydroascorbic acid (1.0 mg/mL) dissolved in 0.008 N hydrogen chloride solution were heated at 100 °C for 2 h, respectively, and then cooled to room temperature for subsequent HPLC analysis. The reaction mixtures were directly injected without any pretreatment, but centrifugation at 10000*g*. The chromatograms of the reaction mixtures of ascorbic acid and dehydroascorbic acid are shown in Figure 1, panels a and b, respectively. No furfural (peak 5), which was one of the degradation products of ascorbic acid (Figure 1a), was detected from the degradation products of dehydroascorbic acid (Figure 1b), indicating that furfural was not the degradation product of dehydroascorbic acid under the present degradation conditions. Peak 3, another degradation product of ascorbic acid and dehydroascorbic acid, was identified as 2-furoic acid. Peak 4, the main degradation product of ascorbic acid and dehydroascorbic acid under the present conditions, was identified using the method of Kimoto et al. (1993). When dehydroascorbic acid was heated in 1 N sulfuric acid, three compounds, 2-furoic acid, 3-hydroxy-2-pyrone possessing an absorption maximum at 295 nm, and 5-methyl-3,4-dihydroxytetrone possessing an absorption maximum at 265 nm, were produced from the degradation of dehydroascorbic acid (Kimoto et al., 1993). In the present experiment, as reported by Kimoto et al. (1993), ascorbic acid and dehydroascorbic acid were heated in 1 N sulfuric acid at 100 °C for 1 h, respectively. The chromatograms of the degradation mixtures of ascorbic acid and dehydroascorbic acid in 1 N sulfuric acid are shown in Figure 2, panels a and b, respectively. Figure 2b shows that two main degradation products (peaks 3 and 4) were formed by the degradation of dehydroascorbic acid. Peak 3 was identified as 2-furoic acid, and peak 4 was identified as 3-hydroxy-2-pyrone according to the result

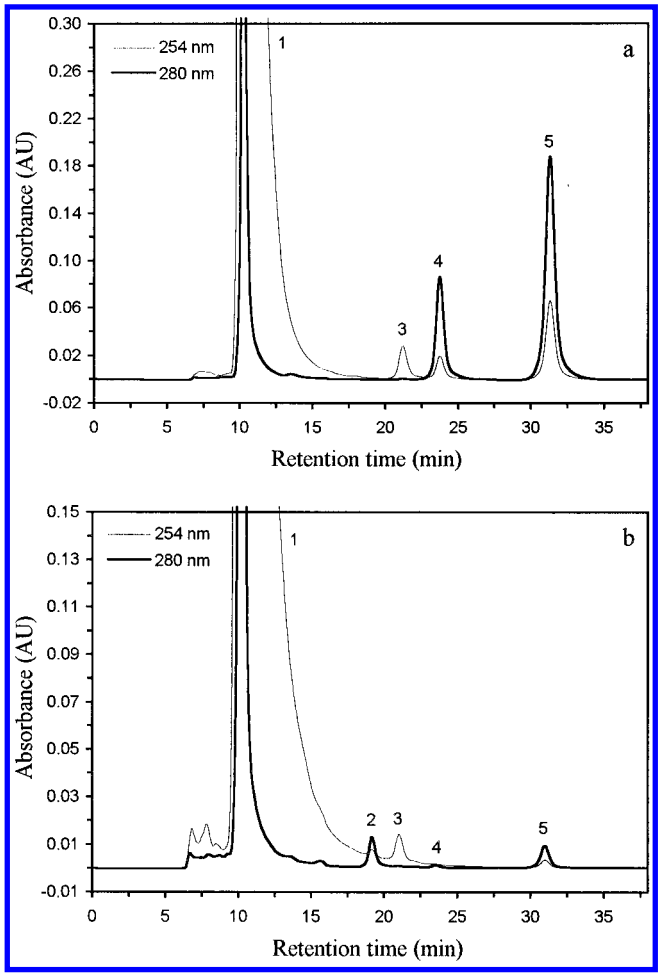

Figure 3. HPLC chromatograms of ascorbic acid heated in the solution of pH 2 (a) and pH 7 (b) at 100 °C for 2 h.

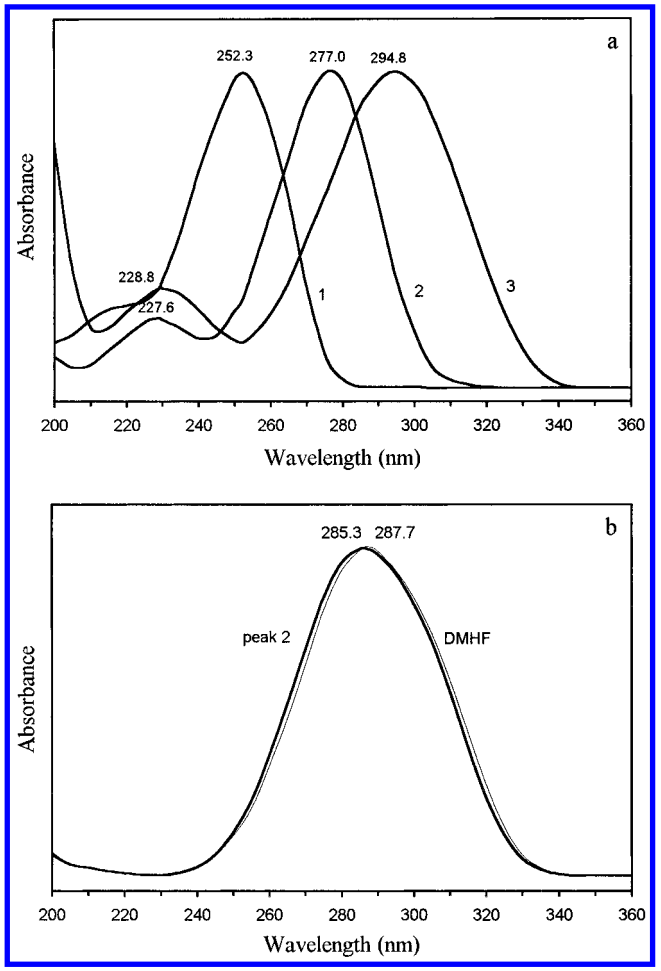

Figure 4. Absorbance spectra of (a) 2-furoic acid (1), furfural (2), and 3-hydroxy-2-pyrone (3) and (b) 2,5-dimethyl-4-hydroxy-3-(2*H*)-furanone (DMHF) and an unknown compound (peak 2).

of Kimoto et al. (1993). As shown in Figure 2a, in 1 N sulfuric acid aqueous solution, furfural (peak 5) was the main degradation product of ascorbic acid, and only a small amount of 2-furoic acid and 3-hydroxy-2-pyrone were detected. For the degradation of dehydroascorbic acid, as shown in Figure 2b, a minute amount of furfural (peak 5) was detected, indicating that in a strong sulfuric acid (1 N) aqueous solution, a very small amount of furfural could still be formed in the degradation process.

The comparison in Figures 1 and 2 shows that the relative content of 2-furoic acid and 3-hydroxy-2-pyrone in panel a is approximately proportional to that in panel b, indicating that 2-furoic acid and 3-hydroxy-2-pyrone are the degradation products of ascorbic acid via dehydroascorbic acid. As shown in Figures 1 and 2, while the concentration of acidic solution was changed, the relative contents of 2-furoic acid, 3-hydroxy-2-pyrone, and furfural were different. Therefore, the effect of pH on the formation of different degradation products of ascorbic acid was studied. The ascorbic acid solutions at different pH values were heated at 100 °C for the investigation of degradation. After heated at 100 °C for 2 h and subsequently cooled to room temperature, the mixtures of the degradation reaction of ascorbic acid were analyzed by HPLC. The chromatograms of the reaction mixtures at pH 2 and pH 7 are shown in Figure 3, panels a and b, respectively. As shown in the figures, four peaks can be seen after the peak of ascorbic acid (peak 1). The results indicated that four products were formed by the degradation of ascorbic acid on heating

Table 1. Retention Times and Absorption Maxima of Ascorbic Acid and Its Degradation Products

peak no.	retention time (min)	absorption maxima (nm)	compounds identified
1	10.2	244.1	ascorbic acid
2	19.2	285.3	unidentified compound
3	21.2	252.3	2-furoic acid
4	23.8	(228.8) 294.8	3-hydroxy-2-pyrone
5	31.3	(227.6) 277.0	furfural

at different pH values. The retention times and absorption maxima of these four compounds are summarized in Table 1. The absorbance spectra of these four compounds are shown in Figure 4, panels a and b, and the absorbance spectrum of DMHF is also shown in Figure 4b for the comparison with peak 2. As shown in Figure 4b, peak 2 gave a similar spectrum to that of DMHF, which eluted before peak 2, and the retention time was 18.3 min, indicating that peak 2 and DMHF had a similar structure.

The contents of four degradation products of ascorbic acid at different pH values were determined, and the results are shown in Figure 5, panels a and b. The contents of 3-hydroxy-2-pyrone and the unknown compound (peak 2) were measured by area comparison with 5-HMF and DMHF, respectively. The results indicated that low pH conditions favored the formation of furfural and, also, 2-furoic acid and 3-hydroxy-2-pyrone. As shown in Figure 5a, while the pH of ascorbic acid solution was lower than 2, furfural was the prime

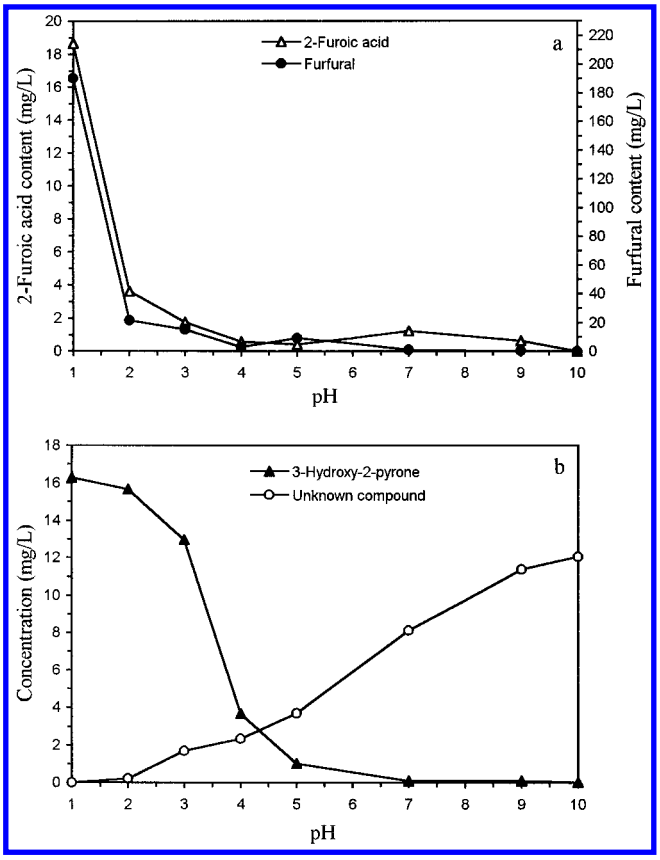

Figure 5. Effect of pH on the contents of four degradation products of ascorbic acid after heating at 100 °C for 2 h: (a) 2-furoic acid and furfural; (b) 3-hydroxy-2-pyrone and an unknown compound (peak 2).

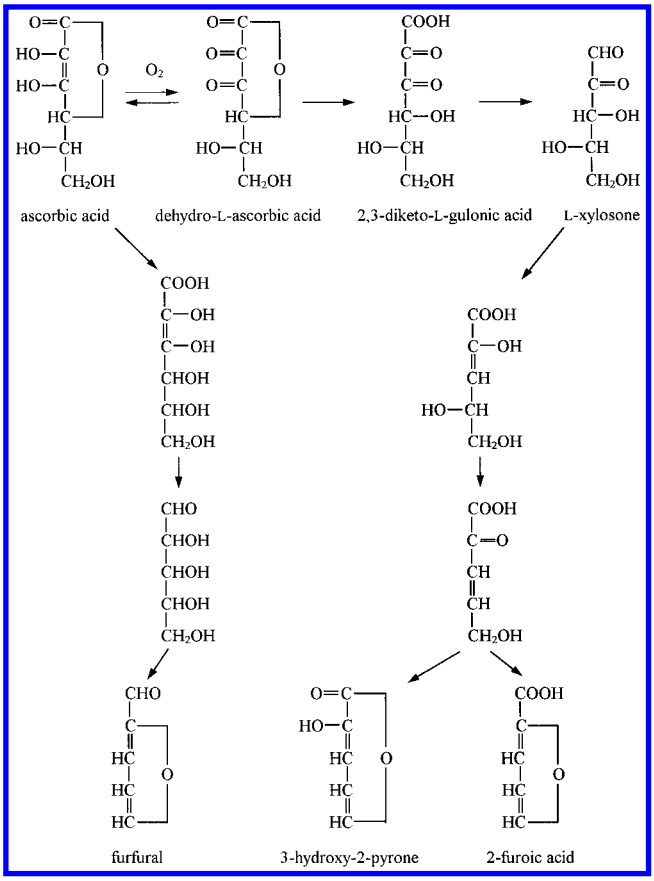

Figure 6. Possible reaction pathways for the degradation of ascorbic acid.

degradation product of ascorbic acid, and its concentration in ascorbic acid solution increased markedly from 21.6 at pH 2 to 190.2 mg/L at pH 1. Like furfural, the formation of 2-furoic acid also increased obviously while the pH of ascorbic acid solution was lower than 2. For 3-hydroxy-2-pyrone, while the pH of ascorbic acid solution was lower than 4, its content in the ascorbic acid solution increased significantly. High pH values did not favor the formation of the three degradation products (i.e., furfural, 2-furoic acid, and 3-hydroxy-2-pyrone). In contrast, high pH values could promote the production of the unknown compound (peak 2). While the pH of ascorbic acid solution was higher than 7, the unknown compound became the main degradation product of ascorbic acid. While the pH of ascorbic acid solution was 10, only a very small amount of furfural (0.1 mg/L) and 3-hydroxy-2-pyrone (0.02 mg/L) with no 2-furoic acid was detected.

Under different degradation conditions, ascorbic acid produced different transformation products possessing absorption maxima between 230 and 300 nm. The reaction types in the degradation reaction process to produce these compounds could be classified into the oxidation/reduction and the intermolecular rearrangements (Kimoto et al., 1993). According to the present result, and the schemes of Smoot and Nagy (1980), Kimoto et al. (1993), and Sawamura et al. (1994), two parallel reaction pathways for ascorbic acid degradation, under an acid condition, to furfural, 2-furoic acid, and 3-hydroxy-2-pyrone are shown in Figure 6. Under aerobic and acid conditions, ascorbic acid is oxidized to dehydroascorbic acid and then form 2,3-diketo-L-gulonic acid, i.e., hydrolyzed dehydroascorbic acid (Deutsch, 1998), the delactonized form of dehydroascorbic acid, by the cleavage of ring and the addition of water molecule. The decarboxylation of 2,3-diketo-L-gulonic acid forms L-xylosone, followed by the intermolecular redox reaction and the dehydrations to finally produce 2-furoic acid and 3-hydroxy-2-pyrone. Under anaerobic conditions, no dehydroascorbic acid is formed significantly; instead, ascorbic acid degrades by the cleavage of ring and the addition of water molecule, the decarboxylation and the intermolecular rearrangement, and dehydrations to form furfural.

Our results showed that, under an acid environment, the degradation of ascorbic acid was carried out simultaneously by two reaction pathways. That is, under hydrogen-ion-catalyzed aerobic conditions, ascorbic acid was converted to 2-furoic acid and 3-hydroxy-2-pyrone via dehydroascorbic acid, whereas under anaerobic conditions, ascorbic acid directly degraded to furfural. The results indicated that an insufficient dissolved oxygen concentration in the degradation reaction system might result in incomplete transformation of ascorbic acid to dehydroascorbic acid. Since furfural production was not significantly affected by initial dissolved oxygen levels, the anaerobic rather than the aerobic pathway was the major one for furfural formation from ascorbic acid in the present system (Robertson and Samaniego, 1986).

Temperature is an important factor affecting the degradation rate of ascorbic acid. Our results showed that, while heated at 100 and 60 °C for 2 h, the content of furfural in ascorbic acid solution (pH 4) was 2.88 and 0.01 mg/L, respectively, while the content of 3-hydroxy-2-pyrone was 3.68 and 0.4 mg/L, respectively. The content of 2-furoic acid was 0.56 mg/L at 100 °C, but

was not detected at 60 °C. The results indicated that lower temperatures might inhibit the degradation of ascorbic acid and thus limit the accumulation of various degradation products (Rodriguez et al., 1991).

As mentioned previously (Yuan and Chen, 1998), in commercial fruit juice concentrate samples with added vitamin C, the contents of furfural and 2-furoic acid were significantly higher than in other fruit juice samples without added vitamin C. The result also showed that the content of "compound 4" (Yuan and Chen, 1998), which was subsequently identified as 3-hydroxy-2-pyrone, was higher in fruit juice samples, especially in these samples with added vitamin C. The degradation of ascorbic acid is not only important in nutrition, but is also related to flavor and color changes of juices. The browning in processed fruit juices is mainly caused by the degradation of ascorbic acid. Furfural, one of the main degradation products of ascorbic acid, may undergo polymerization or combine with amino acids to form brown melanoidan pigments (Rodriguez et al., 1991; Solomon et al., 1995). Thus, it is very important to prevent the degradation of ascorbic acid in juice processing (Sawamura et al., 1994).

The results of the study indicated that there were significant effects of pH on the degradation processes and products of ascorbic acid and high temperature might promote the degradation of ascorbic acid.

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