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Browning in Ethanolic Solutions of Ascorbic Acid and Catechin

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ABSTRACT: Nonenzymatic browning occurs readily in alcoholic beverages and degrades their color quality. Ascorbic acid degradation in the presence of phenolic compounds is a major browning pathway in alcoholic beverages with fruit or fruit juice as the raw material or an ingredient. In the present study ethanolic solutions of ascorbic acid and catechin were prepared to simulate the alcoholic beverages. Ascorbic acid degradation and browning in these model solutions were investigated. Glycerol solutions with the same water activity (A_w) values as those of the ethanolic model solutions were used as controls in the evaluation of browning rate. Results showed that the aerobic degradation of ascorbic acid dominates over the anaerobic one in ethanolic solutions, that the browning rate decreases as the ethanol concentration increases, that the compound 3-hydroxy-2-pyrone may not be a good indicator of browning in ethanolic ascorbic acid−catechin solutions, and that A_w is a major factor responsible for the difference in the browning rate among ascorbic acid−catechin solutions with different ethanol concentrations.

KEYWORDS: ethanol, browning, ascorbic acid, catechin, water activity, alcoholic beverage

INTRODUCTION

Color is generally recognized as an essential quality attribute of food. Nonenzymatic browning is a major course of discoloration.[1] Ascorbic acid occurs naturally in many food materials, especially fruits. The degradation of ascorbic acid is among the common mechanisms of nonenzymatic browning in processed foods, including many alcoholic beverages that used fruit or fruit product as the raw material or in the formulation.[2,3] For example, wine coolers and alcopops are virtually ethanolic solutions of fruit juice with ethanol contents around 4 and 6% (v/v), respectively.[4,5] Fruit liqueur, which is usually made by immersing fruit or fruit product in spirit, normally contains ethanol in a concentration somewhere between 15 and 50%. Ascorbic acid is also widely used as an oxygen scavenger in winemaking, especially in the production of white wine, which normally contains about 12% ethanol.[6]

Numerous papers pertaining to ascorbic acid degradation and browning in aqueous food fluids have been published, whereas only a few deal with the behavior of ascorbic acid in alcoholic beverages. Among all alcoholic beverages, wine has been the only investigated object of browning. Model systems with an ethanol concentration within the range of 10−14%, a pH value between 3.0 and 3.7, and the presence of at least one phenolic compound were commonly used in the study of browning reactions in wine.[7−11] In a model system, Barril et al. concluded that ascorbic acid is converted to dehydroascorbic acid and hydrogen peroxide at the beginning of white wine aging. The dehydroascorbic acid then undergoes rapid degradation into a variety of intermediate compounds of browning, including carboxylic acids, ketones, and aldehydes.[7] Ascorbic acid can delay oxidative browning only briefly. After some period of storage, it may even accelerate browning.[12] Catechin was found to be a good representive of phenolic compounds as it is an oxidizable substrate that correlates well with ascorbic acid browning in wine.[6] The addition of ascorbic acid to a catechin-containing ethanolic model system resulted in an increased rate of browning with the increased production of phenolic pigments. A degradation product emanating from ascorbic acid was found to react with catechin and form colored xanthylium cations.[6,13,14]

Ethanol concentration has been recognized as a major factor in affecting the rate and mechanism of color changes in ethanolic model systems containing no ascrobic acid. Shen et al. concluded that the mechanisms of the Maillard reaction in the aqueous and ethanol systems are not the same.[15] The rate of color loss in chlorophyll *a* solution varies with ethanol concentration. At 50 °C, the rate of loss in 40% ethanol solution was 230 times that in 10% ethanol solution. Different ethanol/water ratios may constitute different chemical environments and lead to the difference in chlorophyll stability.[16] The above-described findings justify further investigations into the influence of ethanol concentration on browning in ascorbic acid−catechin solutions.

The present study was aimed to investigate the rate and mechanism of browning involving ascorbic acid and catechin in model solutions that simulate beverages with various ethanol concentrations.

MATERIALS AND METHODS

Chemicals. L-Ascorbic acid, (+)-catechin, dehydroascorbic acid, glycerol, and potassium dihydrogen phosphate were purchased from Sigma Chemical Co. (St. Louis, MO). Furfural and 2-furoic acid were obtained from Chem Service Inc. (West Chester, PA), and 3-hydroxy-2-pyrone was from Tyger Scientific Inc. (Ewing, NJ). Glacial acetic acid was obtained from J. T. Baker Co. (Phillipsburg, NJ). Acetonitrile (gradient grade), methanol (gradient grade), and orthophosphoric acid (85%) were obtained from Merck Co. (Darmstadt, Germany).

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Preparation of Model Solutions. Aqueous and ethanolic (10, 20, 30, 40, or 50%) solutions containing ascorbic acid (0.5 mg mL^{-1}) alone, catechin (0.5 mg mL^{-1}) alone, and ascorbic acid plus catechin (0.5 mg mL^{-1} each) were prepared for the evaluation of browning index and contents of ascorbic acid decomposed products. Solutions containing catechin (0.5 mg mL^{-1}) and a major ascorbic acid degradation product (dehydroascorbic acid, 2-furoic acid, or 3-hydroxy-2-pyrone at 0.1 mg mL^{-1}) were also prepared. The pH of the model solutions was adjusted to 3.0 with 1.0 and 0.1 N hydrochloric acids using a pH-Stat Controller (PHM 290, Radiometer Analytical SAS, Lyon, France). Each 4.0 mL aliquot of the solution was filled into a 4.5 mL brown glass vial. The vial was sealed with a Teflon gasket and cap. Browning index, taken as the absorbance at 420 nm,[17] and the contents of catechin, ascorbic acid, and its degradation products in the model solutions in an accelerated storage test at 45 °C were evaluated periodically up to 26 days.

Analysis of Catechin, Ascorbic Acid, and Degradation Products. The analysis was performed referring to Shinoda et al. and Nour et al. with modifications.[18,19] Catechin, ascorbic acid, and three major degradation products (furfural, 2-furoic acid, and 3-hydroxy-2-pyrone) were analyzed using a 250 mm × 4.6 mm inside diameter, 5 μm particle size, Hypersil Gold C_{18} reversed-phase high-pressure liquid chromatography (HPLC) column (Thermo Scientific, Waltham, MA). The HPLC analysis was run with a Prominence liquid chromatography system (Shimadzu, Kyoto, Japan) composed of a vacuum degasser, LC-20AT pump, SIL-20A autosampler, and SPD-M20A diode array detector. For the determination of ascorbic acid, 50 mM phosphate solution (potassium dihydrogen phosphate, of which the pH value had been adjusted to 2.8 with phosphoric acid) was chosen the mobile phase. For the simultaneous detection of catechin and the degradation products, 0.05 M acetic acid/acetonitrile (98:2 v/v) was chosen the mobile phase instead. The mobile phase was vacuum-degassed and then set at 0.7 mL/min flow rate for all of the chromatographic separations. The detection wavelength was set at the maximum optical absorbance: 244 nm for ascorbic acid, 283 nm for catechin and furfural, 254 nm for 2-furoic acid, and 300 nm for 3-hydroxy-2-pyrone. Standard curves were established with ascorbic acid, catechin, furfural, 2-furoic acid, and 3-hydroxy-2-pyrone. The injection volume was 20 μL for all samples. The analysis was supplemented with internal calibration by using the standard compounds. The degradation ratios of ascorbic acid and catechin in storage for up to 26 days were calculated periodically using the equation

$$\text{degradation ratio } (\%) = \frac{A - B}{A} \times 100$$

where A = initial concentration of ascorbic acid or catechin and B = detected concentration of ascorbic acid or catechin in storage.

Evaluation of A_w in Ethanolic Solutions. The A_w values of the ethanolic solutions were evaluated following the reported method[20−22] with modifications. The freezing points of ethanolic solutions were measured using a differential scanning calorimeter (DSC) (model DSC 822^e, Mettler-Toledo GmbH, MatChar, Switzerland). Samples of 14−18 mg were weighed into aluminum pans, and then covers were hermetically sealed into place. An empty, hermetically sealed aluminum pan was used as the reference. Before the analysis of samples, the baseline was obtained with the reference pan. After both the sample pan and the reference pan had been placed in the chamber at 25 °C, the cell block of the DSC was cooled to −70 °C at 5 °C/min and held there for 5 min, followed by heating at a rate of 5 °C/min to 25 °C. The heating thermograms of samples showed a single melting peak. The peak temperature and the enthalpy were found by analyzing the thermogram with Mettler-Toledo Star^e software V6, 1.0. The freezing point is defined as the intersection of the baseline and the tangent line of the melting peak. The A_w of a sample was calculated using the equation[23]

$$-ln\, A_w = 9.6934 \times 10^{-3} \times \theta_F + 4.761 \times 10^{-6} \times \theta_F^2$$

where θ_F (freezing point depression) = $T_0 - T_F$, T_0 = freezing point of pure water, and T_F = freezing point of sample solution.

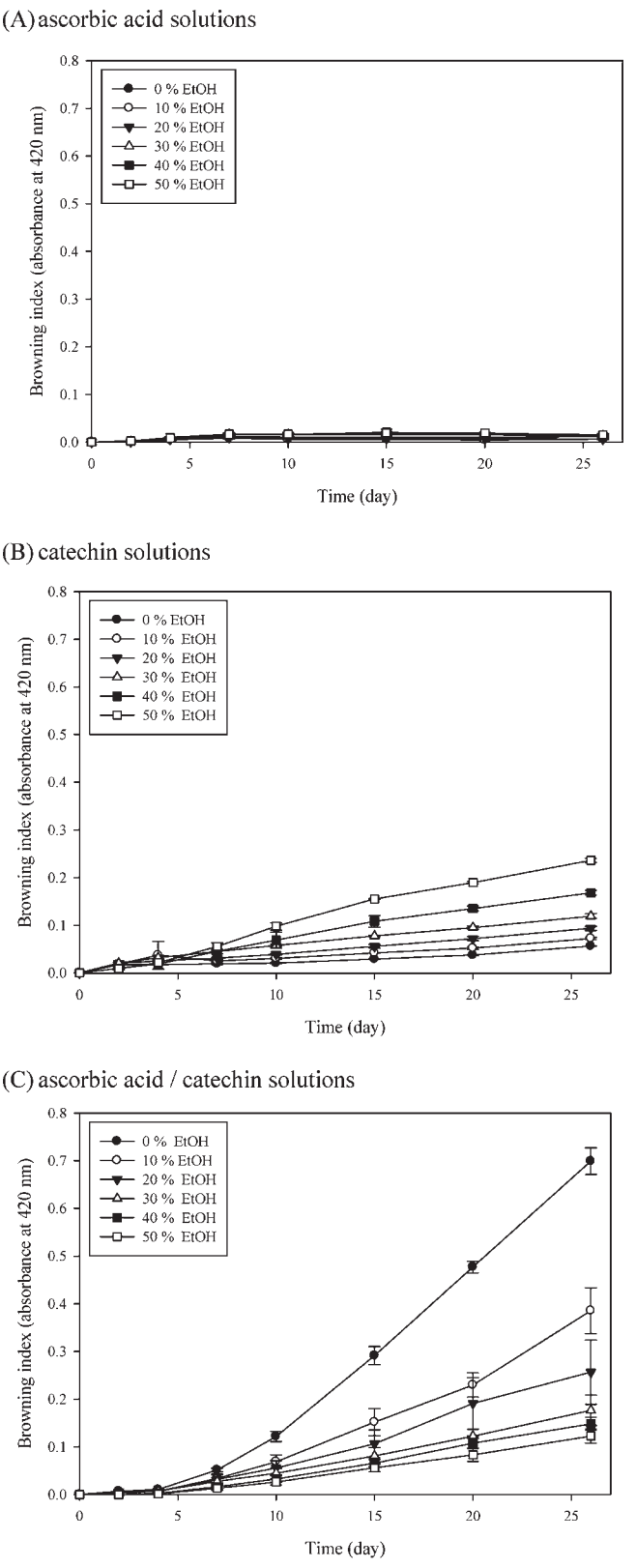

Figure 1. Browning in aqueous and ethanolic model solutions in storage at 45 °C: (A) ascorbic acid solutions (0.5 mg mL^{-1}); (B) catechin solutions (0.5 mg mL^{-1}); (C) ascorbic acid (0.5 mg mL^{-1})/ catechin (0.5 mg mL^{-1}) solutions.

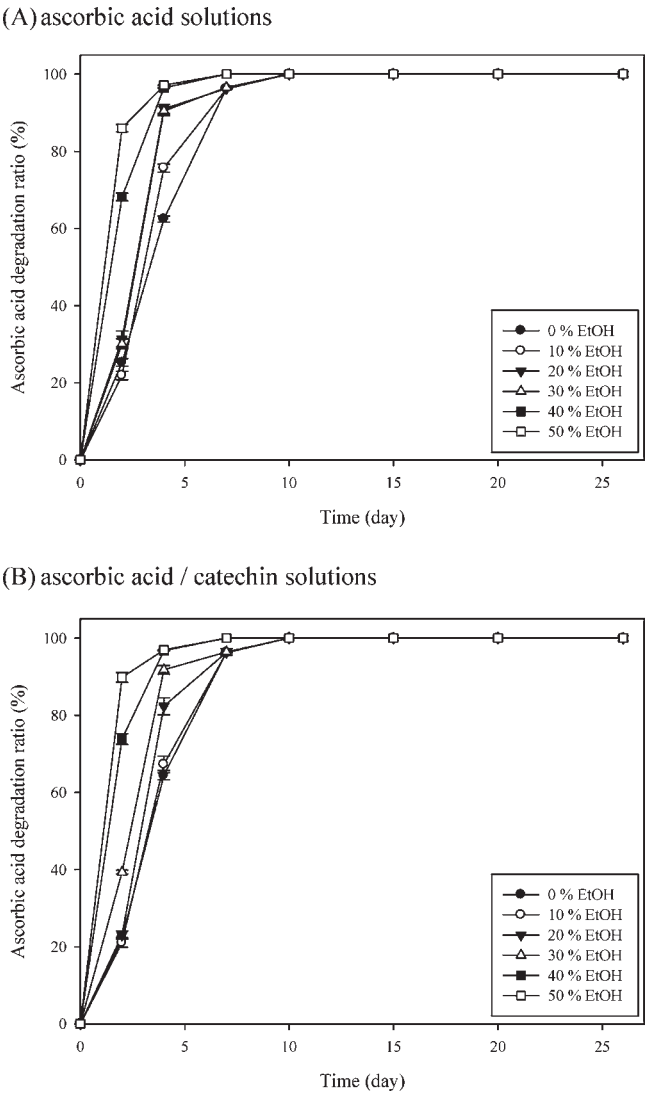

Figure 2. Ascorbic acid degradation ratios in aqueous and ethanolic model solutions in storage at 45 °C: (A) ascorbic acid solutions (0.5 mg mL^{-1}); (B) ascorbic acid (0.5 mg mL^{-1})/catechin (0.5 mg mL^{-1}) solutions.

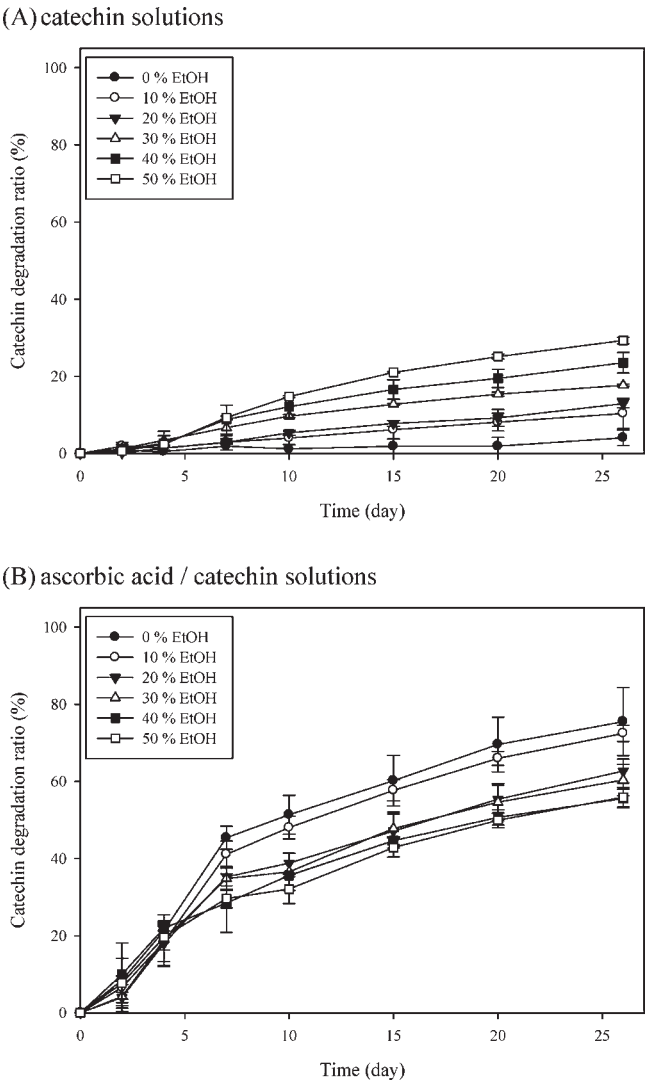

Figure 3. Catechin degradation ratios in aqueous and ethanolic model solutions during storage at 45 °C: (A) catechin solutions (0.5 mg mL^{-1}); (B) ascorbic acid (0.5 mg mL^{-1})/catechin (0.5 mg mL^{-1}) solutions.

Glycerol Model Solutions as Controls. Glycerol model solutions were used to investigate the effect of A_w on browning.[24] The glycerol model solutions were prepared by mixing glycerol with distilled water. The pH was adjusted to 3.0, the same as the ethanolic solutions, right before A_w evaluation with a portable water activity meter (Pawkit Water Activity Meter, Decagon Devices Inc., Pullman, WA). The browning index during storage of the glycerol model solutions was recorded following the same procedures as previously described for the ethanolic solutions.

Statistical Analysis. Data were presented as the mean ± standard deviation. One-way analysis of variance was used to analyze the variance among the samples. Duncan's multiple-range test, at $p < 0.05$, was used to determine significant differences among sample means.

RESULTS AND DISCUSSION

Browning in Ethanolic Model Solutions. All of the ethanolic model solutions browned gradually (Figure 1). In the solutions containing ascorbic acid (0.5 mg mL^{-1}) alone (Figure 1A), browning proceeded very slowly. The browning rates in the solutions containing catechin (0.5 mg mL^{-1}) alone and the solutions containing both ascorbic acid (0.5 mg mL^{-1}) and catechin (0.5 mg mL^{-1}) were higher than those in the solutions containing ascorbic acid (0.5 mg mL^{-1}) alone (Figure 1B,C). The concurrent presence of ascorbic acid and catechin (Figure 1C) resulted in faster browning than in solutions with either ascorbic acid or catechin alone at ethanol concentrations between 0 and 30% (Figure 1A,B). The browning rate decreased with the increase in ethanol concentration from 0% until reaching a plateau at 30%. There is no significant difference ($p > 0.05$) among the browning rates at 30, 40, and 50% ethanol concentrations (Figure 1C). Our results reconfirmed and extended the previous findings in ethanolic solutions modeled after wine at the single ethanol concentration of 12%.[6,14]

As shown in Figure 2, a higher ethanol concentration corresponds to a higher degradation rate of ascorbic acid. Residual ascorbic acid became nondetectable in all model solutions in storage at 45 °C for 10 days.

In model solutions containing catechin alone (Figures 1B and 3A), the browning index correlates well with the catechin

Table 1. Furfural, 2-Furoic Acid, and 3-Hydroxy-2-pyrone Contents in Model Solutions after 26 Days of Storage at 45 °C[a]

solution	furfural (ppm)	2-furoic acid (ppm)	3-hydroxy-2-pyrone (ppm)	total final products (2-furoic acid + 3-hydroxy-2-pyrone)
containing ascorbic acid (0.5 mg mL^{-1}) alone				
0% EtOH	ND[b]	10.55 ± 0.20c	0.90 ± 0.79 g	11.45 ± 0.99 g
10% EtOH	ND	6.74 ± 0.73e	11.04 ± 4.20f	17.78 ± 3.93ef
20% EtOH	ND	4.75 ± 0.42f	20.64 ± 6.26e	25.39 ± 6.47e
30% EtOH	ND	3.58 ± 0.26fg	45.67 ± 8.55d	49.25 ± 8.80d
40% EtOH	ND	0.60 ± 0.58 h	44.93 ± 10.54d	45.53 ± 11.12d
50% EtOH	ND	0.00 ± 0.00 h	46.43 ± 11.49d	46.43 ± 11.49d
containing ascorbic acid (0.5 mg mL^{-1}) and catechin (0.5 mg mL^{-1})				
0% EtOH	ND	18.83 ± 1.82a	50.61 ± 5.67d	69.44 ± 4.61c
10% EtOH	ND	13.00 ± 0.94b	59.59 ± 1.68c	72.59 ± 1.14c
20% EtOH	ND	8.38 ± 1.31d	74.64 ± 1.45b	83.02 ± 1.76b
30% EtOH	ND	4.87 ± 0.25f	83.95 ± 0.78a	88.83 ± 1.03ab
40% EtOH	ND	2.24 ± 0.29 g	91.73 ± 2.94a	93.97 ± 2.65a
50% EtOH	ND	0.05 ± 0.09 h	91.87 ± 2.95a	91.93 ± 3.02a

[a] Values are presented as the mean ± standard deviation. Values in each column followed by a different letter are significantly different ($p < 0.05$). [b] ND, not detected.

degradation ratio ($R^2 = 0.9536$) and faster rates of browning and catechin degradation occur at higher ethanol concentrations. The first step in the catechin degradation pathway is dehydration.[25] Ethanol may bond with water in an ethanol−water mixture.[26] The bonding between ethanol and water molecules can be expected to promote dehydration and catechin degradation. However, higher ethanol concentrations reduced the rates of browning and catechin degradation in model solutions containing both ascorbic acid and catechin (Figures 1C and 3B). Catechin degraded more rapidly in the presence of ascorbic acid (Figure 3A,B). The concurrent presence of ascorbic acid and catechin caused stronger browning and faster catechin degradation in the aqueous solution (0% ethanol concentration) than that in any ethanolic model solution (Figures 1 and 3). The breakdown products of ascorbic acid may induce catechin oxidation and form browning pigments.[14,27] Therefore, the profile of ascorbic acid degradation products was investigated in the following for elucidating the effect of ethanol concentration on browning in model solutions.

The contents of the ascorbic acid degradation products, including furfural, 2-furoic acid, and 3-hydroxy-2-pyrone, in the model solutions were evaluated at the end of the storage test, the 26th day, to elucidate the pathway of ascorbic acid degradation (Table 1). Furfural is formed in the anaerobic degradation of ascorbic acid, whereas 2-furoic acid and 3-hydroxy-2-pyrone are formed in the aerobic degradation.[28−30] Besides, furfural and 3-hydroxy-2-pyrone are recognized as browning contributors and indicators in ascorbic acid degradation.[18,31−33] In the present study, 2-furoic acid and 3-hydroxy-2-pyrone were found in all model solutions in storage at 45 °C for 26 days, whereas furfural was not, indicating the predominance of aerobic degradation of ascorbic acid over anaerobic degradation in ethanolic solutions. The increase in ethanol concentration resulted in an increase in 3-hydroxy-2-pyrone formation in the model solutions containing ascorbic acid alone and in those containing both ascorbic acid and catechin as well. However, in the ethanolic model solutions containing both ascorbic acid and catechin, the increase in ethanol concentration corresponds to a decrease in browning instead (Figure 1C). It appears that 3-hydroxy-2-pyrone is not a good indicator of browning in the degradation of ascorbic acid in ethanolic solutions.

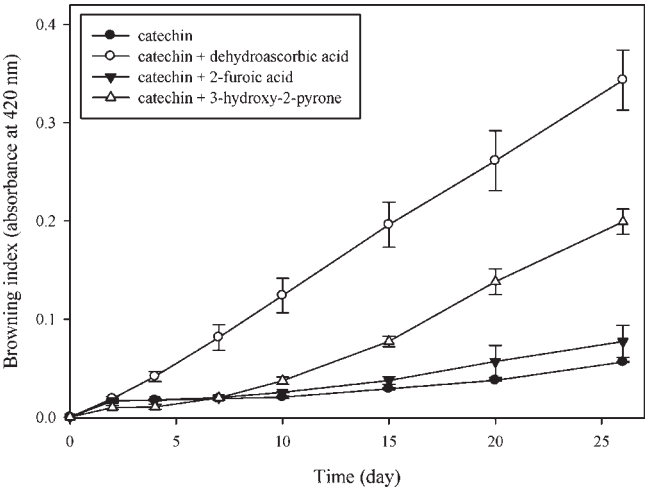

Figure 4. Browning in catechin-containing aqueous model solutions with and without ascorbic acid degradation products in storage at 45 °C.

The first step in the oxidative degradation of ascorbic acid is its oxidation into dehydroascorbic acid.[28−30] Dehydroascorbic acid is highly unstable in an aqueous solution and may be degraded into a variety of intermediate browning products, such as 2-furoic acid, 3-hydroxy-2-pyrone, and furfural,[28,34] depending upon the reaction conditions. In the present study, dehydroascorbic acid and the two degradation products, 2-furoic acid and 3-hydroxy-2-pyrone, were added to catechin-containing aqueous model solutions. As shown in Figure 4, the addition of dehydroascorbic acid and 3-hydroxy-2-pyrone resulted in an increased rate of browning in all of the catechin-containing aqueous model solutions. However, a higher extent of 3-hydroxy-2-pyrone formation corresponds to a lower browning index in ethanolic solutions instead (Figure 1C and Table 1), suggesting that the presence of ethanol may cause an alteration in the reaction pathway or in the

Table 2. Water Activities of Ethanolic and Glycerolic Solutions

ethanol concentration (%, v/v)	water activity	glycerol concentration (%, v/v)
0	1	0
10.0	0.96	18.7
20.0	0.92	27.5
30.0	0.86	40.0
40.0	0.8	48.9
50.0	0.73	57.3

rates of specific reactions. Shen et al. found the pathway alteration in Maillard reaction by the presence of ethanol in previous studies.[15,20]

Effect of A_w on Browning in Ethanolic and Glycerolic Model Solutions. The oxidation of ascorbic acid (AH_2) can be simply described by the equation[6]

$$AH_2 + O_2 \rightarrow A + H_2O_2$$

where A represents dehydroascorbic acid.

Hydrogen peroxide (H_2O_2) is a major oxidant in wine.[35,36] It easily converts to a hydroxyl radical and decomposes into water and oxygen. Ethanol was reported to be a scavenger of hydroxyl radicals, which inhibits browning in an orange juice model solution.[18,33] Therefore, the scavenging effect of ethanol on hydroxyl radicals may be involved in the repression of browning at higher ethanol concentrations (Figure 1C). However, the formation of ascorbic acid oxidation product 3-hydroxy-2-pyrone increased with the increase in ethanol concentration (Table 1), suggesting that the hydroxyl radical scavenging effect of ethanol cannot be a major factor accounting for the difference in browning rates among ethanolic solutions at various concentrations.

In food systems, nonenzymatic browning may occur upon heating, dehydration, or concentration. A_w is well recognized as one of the most influential factors for nonenzymatic browning of food substances.[24,37,38] A_w values of the ethanolic solutions were evaluated in the present study by employing the freezing point depression method in dealing with the volatility of ethanol.[20−22] Aqueous solutions of glycerol were also prepared as controls. The A_w values of ethanolic and glycerolic solutions are shown in Table 2.

Figure 5 shows the changes in browning index in glycerolic model solutions during storage at 45 °C. A lag period of <7 days was found in both ethanolic and glycerolic model solutions, presumably for the production of colorless browning precursors.[6,14] After that time, no significant differences ($p > 0.05$) in browning rate were found between ethanolic and glycerolic solutions with the same A_w readings from 0.86, when the ethanol concentration in the ethanolic solution is 30%, to 1 (Figures 1C and 5), indicating that A_w is an influential factor for the difference in browning rate among ascorbic acid−catechin solutions at various ethanol concentrations and that ethanol influences the browning rate in the solution mainly via the reduction in A_w instead of behaving as a chemical additive itself.

In the solutions containing catechin alone, the browning rate and catechin degradation ratio increased as the ethanol concentration increased (Figures 1B and 3A). This phenomenon can be explained by the dehydration effect of ethanol as above-stated. However, the dehydration effect of ethanol cannot explain why the browning rate and catechin degradation ratio decreased with

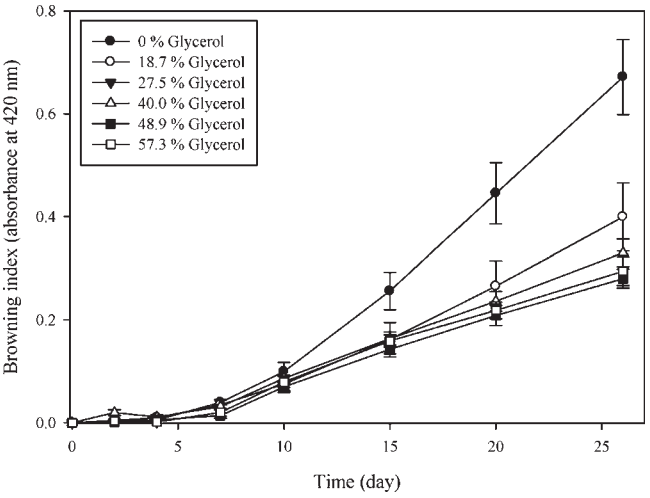

Figure 5. Browning of glycerolic model solutions in storage at 45 °C.

the increase in ethanol concentration in the solutions containing both catechin and ascorbic acid (Figures 1C and 3B). The different responses in these two solution groups to the change in ethanol concentration may be attributed to the difference between their degradation pathways. The initial step in the oxidative degradation of ascorbic acid is its conversion into dehydroascorbic acid.[14,28−30] Dehydroascorbic acid can be hydrolyzed and then decarboxylated to form an aldehyde, L-xylosone. L-Xylosone will rapidly convert to other aldehydes and ketones,[14,18,28,39] and then undergo intermolecular redox and dehydration reactions to form 2-furoic acid and 3-hydroxy-2-pyrone as final products.[28] A_w is a measure of the availability of free water molecules. We propose that higher ethanol concentrations, or lower A_w conditions, favor the dehydration reaction and accelerate the degradation of L-xylosone, therefore exhibiting higher ascorbic acid degradation ratios and producing higher total amount of the degradation products 2-furoic acid plus 3-hydroxy-2-pyrone in ascorbic acid-containing model solutions with and without the presence of catechin (Figure 2 and Table 1). On the other hand, L-xylosone is the only intermediate product in aerobic degradation of ascorbic acid that may react with catechin to accelerate browning, through the formation of brown-colored xanthylium cation pigments.[7,27] We further propose that the faster dehydration of L-xylosone in lower A_w conditions, or higher ethanol concentrations, reduces its own availability to react with catechin and, therefore, results in lower browning rates and catechin degradation ratios in the ascorbic acid−catechin model solutions (Figures 1C and 3B).

In conclusion, the present study revealed that the aerobic degradation of ascorbic acid dominates over the anaerobic one in ethanolic solutions, that the browning rate decreases as the ethanol concentration increases, and that A_w is a major factor responsible for the difference in browning rates among ascorbic acid−catechin solutions with different ethanol concentrations. The results also indicate that the presence of ethanol may reduce the browning rate in alcoholic beverages containing ascorbic acid and catechin. Further studies including the purification and identification of the browning products to elucidate the detailed mechanism of the ascorbic acid browning reaction in ethanolic solutions and the browning experiments in real alcoholic beverages containing ascorbic acid for reconfirmation will be worthwhile.

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ABBREVIATIONS USED

A_w water activity; HPLC high-pressure liquid chromatography; DSC differential scanning calorimeter.

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