Browning and Decomposed Products of Model Orange Juice

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A model solution of orange juice containing sugars, ascorbic acid, and citric acid was prepared and its browning during storage was examined. The solution gradually turned brown. Ascorbic acid (AsA) most contributed to the browning. Citric acid and such amino acids as Arg and Pro promoted the browning. DTPA, a strong chelator, inhibited the browning. 3-Hydroxy-2-pyrone (3OH2P), 5-hydroxymethylfurfural (HMF), furfural, 5-hydroxymaltol, and 2-furoic acid were identified as decomposed products in the stored solution. When 3OH2P was stored, the solution turned slightly brown. Furfural solution added with amino acids turned yellow. 3OH2P showed a positive relation with the browning of retail orange juice during storage.

Key words: orange juice; browning; ascorbic acid; 3-hydroxy-2-pyrone; furfural

Orange juice is one of the most popular beverages in the world. When orange juice is stored, it gradually turns brown. It is well known that orange juice is rich in ascorbic acid (AsA). The nutritional value of orange juice is related primarily to the content of AsA. Two of the major changes during storage of orange juice are development of off-flavor and browning.¹) AsA is an antioxidant and represses browning reaction. However, AsA is also known to contribute to browning of foods because it is easily oxidized and decomposed.¹–³) The decomposition of AsA together with non-enzymatic browning is the main deteriorative reaction that occurs during storage of orange juice.³) Tatum et al. showed several degradation products of AsA during the storage of orange juice.⁴,⁵) The factors affecting AsA degradation are pH, oxygen, AsA concentration, temperature, light, metal, citric acid, and so on. In the presence of oxygen, AsA is degraded primarily to dehydroascorbic acid via monoanion. The lactone of dehydroascorbic acid is hydolyzed to form 2,3-diketogulonic acid, which does not show vitamin C activity. Decarboxylation of 2,3-diketogulonic acid leads to xylosone, which is further degraded to reductones and furan compounds. The contribution of AsA to the browning of citrus juice has been reported.³,⁶–⁸) However, the detailed pathway of browning and the interaction between AsA or AsA degradation products and other components in the juice are still not clear. Kanner et al. reported the relationship between the browning of orange juice and the concentrations of 5-hydroxymethylfurfural (HMF) and furfural.⁹) Kacem et al.¹⁰) suggested that furfural and HMF formed by the amino-carbonyl reaction contributed to the browning of orange juice. Thus, furfural and HMF are considered important intermediates and indicators of the browning of orange juice.¹¹–¹³) However, Roig et al. said that HMF could not be used as an index of browning of citrus juice.¹⁴) Thus, the usefulness of these indicators is unclear.

The purpose of this study is to clarify the factors affecting the browning of orange juice and the interaction between AsA and other components and to find a useful indicator of the browning. The model solution is useful to analyze the browning and interaction between components in juice. Here we prepared a model solution of orange juice containing sugars, AsA, amino acids, and citric acid and stored it to examine the browning during storage and the decomposed products. We here showed the importance of degradation of AsA for the browning of orange juice that was stimulated by Arg, Pro, citric acid, and metals and was repressed by radical scavengers and a chelator, and further suggested the possibility of 3-hydroxy-2-pyrone (3OH2P) as an indicator of the browning of orange juice.

Materials and Methods

Model solution of orange juice. A model solution of orange juice consisting of sugars, AsA, citric acid, and amino acids was prepared according to the composition of Satsuma mandarins.¹⁴) Fundamental composition is

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Abbreviations: AsA, ascorbic acid; HMF, 5-hydroxymethylfurfural; 3OH2P, 3-hydroxy-2-pyreone; DTPA, diethylenetriamine-N,N,N,N,N,N-pentaacetic acid; NTA, nitriloacetic acid
shown in Table 1, that is the standard model solution. At the same time, another solution lacking in a component of the standard model solution was prepared. When sugars, citric acid, or amino acids were added to AsA solution, the concentration of each component followed Table 1. Each solution (15 ml) was put in a vial (30 ml) screwed with a plastic cap and then stored at 50°C for 2 months. Two vials were analyzed at each storage time (0–60 days). Each experiment was repeated at least twice. The browning of these solutions was estimated by OD_{420}. The changes in the components were analyzed by 3D-HPLC as described below.

**HPLC analysis for decomposed products.** The HPLC systems were as follows: pump, L-6000 (Hitachi, Tokyo, Japan); column, YMC pack R-ODS (Yamamura, Kyoto, Japan); column, YMC pack ODS (Yamamura, Kyoto, Japan); detector, photodiode array detector (Hitachi, Tokyo); for analysis, L-4200 UV-VIS detector (Hitachi; for preparation); wavelength, 250–370 nm (for analysis), 283 nm (for determination of AsA), 210 nm (for oxalic acid); flow rate, 1.0 ml/min; for analysis, and 9.99 ml/min for preparation.

**Preparation and identification of decomposed products.** HMF, furfural, and furoic acid were identified by the comparison with authentic samples (Wako Chemicals, Osaka). 3OH2P and 5-hydroxymaltol were prepared from AsA solution and identified with spectroscopic data. AsA solution (0.15%) was incubated for 3 days at 50°C, before 3OH2P was extracted with ethyl ether and isolated with a preparative HPLC as described above. The compound was obtained as white needles and was dissolved in D2O (for 1H-NMR), CD3OD (for 13C-NMR), or MeOH (for MS). 1H-NMR δH (ppm): 5.95 (1H, t, J = 6.0), 6.41 (1H, d, J = 6.0), 6.89 (1H, d, J = 6.0). 13C-NMR δC (ppm), 108.9, 119.5, 143.4, 144.5, 163.6, EI-MS (m/z): 112 (M+), 69, 43.

AsA solution (0.03%) containing 5% of fructose was incubated for 18 days at 50°C, before 3HO2P was extracted with ethylacetate and isolated with a preparative HPLC. The compound was a slightly yellowish powder and dissolved in CD3OD (for NMR), or MeOH (for MS). 1H-NMR δH (ppm): 2.20 (3H, s), 7.75 (3H, s). 13C-NMR δC (ppm), 14.6, 140.0, 142.5, 145.5, 151.5, 170.0. EI-MS (m/z): 142 (M+), 113, 69.

**Instrumental analyses.** Spectroscopic measurements were done using the following instruments; JEOL JNM-GX270 (NMR) and JEOL JMX-102/JMX-DA 6000 (EI-MS).

**Storage of retail orange juice.** Eleven samples of orange juice were purchased from a local market in 2000. Samples were stored at 50°C for 2 weeks. The value before and after storage were measured by a colorimeter (Model TC3600, Tokyo Denshoku, Tokyo), and AsA, HMF, 3OH2P, furfural, 5-hydroxymaltol, and 2-furanioc acid were analyzed by the HPLC method as the above described.

**Results and Discussion**

**Effect of each ingredient on the browning of model solution.** The model solution imitated Satsuma mandarin. The solution was stored at 50°C with headspace to promote a browning reaction. The model solution gradually turned brown during storage (Fig. 1-A). This browning curve seems to have two phases. At first, the model solution in which one component was only removed was stored, seems to have two phases. At first, the model solution in which one component was only removed was stored, and the degree of browning was compared (Fig. 1-B). When AsA was removed, the solution did not turn brown at all for 30 days and then gradually turned brown. AsA decreased during storage and completely decomposed after 3 days of storage. Degradation products seem to contribute to the browning. When citric acid or amino acids were removed, the degree of browning was reduced by 40 to 60%. On the other hand, when sugars were removed, the degree of browning had not changed for 2 weeks compared with the control. In longer storage such as 1 or 2 months, the removal of sugars reduced the browning. These results suggest that AsA most contributes to the browning within 2 weeks of storage and that an amino-carbonyl reaction between amino acids and degradation products derived of AsA contributes the browning, while sugars do only after 2 weeks of storage. This difference in origin of browning.
seems to correspond to the two phases of browning.

To ascertain the contribution of AsA to browning, AsA concentration was changed. When the AsA concentration was raised from 0.03%, 0.15%, 0.3% to 0.6%, the absorbance at 420 nm of the solution became more intensely from 0.03, 0.77, 1.07, to 1.14 at 14 days of storage and from 0.19, 2.29, 3.72, to 4.62 at 60 days of storage, respectively. This result coincided with that of Kacem et al.\textsuperscript{10} As the solution lacking in AsA did not turn brown during 2 weeks of storage, such components as sugars, amino acids, and citric acid was then added to an AsA solution to examine the interaction with AsA and other components. All of the amino acids, sugars, and citric acid stimulated the browning of AsA solution (Fig. 2-A). Among them, amino acids stimulated the browning most intensively. The degree of browning in the solution containing AsA and amino acids was more than 3 times than that of AsA solution. The stimulation of browning by amino acids such as Arg and 4-aminobutyric acid in orange juice was reported by Wolfrom et al.,\textsuperscript{16} however, they did not use AsA in the model solution. Therefore, an amino acid was added to AsA solution to examine the effects of each amino acid (Fig. 2-B). L-Ser, L-Asp, L-Pro, L-Arg, L-Ala, and L-Glu promoted the browning. Among them, L-Arg and L-Pro was the most effective on the browning. These results suggest that the interaction as the Maillard reaction between AsA degradation products and such amino acids as Arg and Pro is an important factor of the browning.

Next, the effects of metals and citric acid on the browning were examined. It is reported that citric acid\textsuperscript{3} and metals\textsuperscript{17} promote the degradation and browning of AsA. The model solution contained 0.05 ppm Fe and 0.01 ppm Cu, which might be derived from distilled water or reagents used here. As retail orange juice contained about 0.5 ppm Fe and 0.2 ppm Cu, 0.5 ppm Fe (FeCl\textsubscript{2}) or 0.2 ppm Cu (CuCl\textsubscript{2}) was added to the model solution. As a result, the browning was promoted by the addition of Fe or Cu (Fig. 3), especially in the early
was not clear, however, non-oxidative decomposition products might more contribute to browning in anaerobic condition. For example, furfural is one of the major non-oxidative degradation products of AsA.\textsuperscript{19} As described later, furfural is a reactive compound for the browning with amino acids. Another possibility is that the oxidative degradation of brown pigment might happen. Robertson and Samaniego showed that the browning in lemon juice with a high oxygen level was more intense than that with a low oxygen level,\textsuperscript{12} while Kacem \textit{et al.} reported that an orange drink with high O\textsubscript{2} showed more intense browning than that with low O\textsubscript{2}.\textsuperscript{10} The group of Sawamura reported that dehydroascorbic acid solution produced a browner color under non-aerobic conditions than under oxidative conditions.\textsuperscript{7,20}

Next the effects of radical scavengers and chelating agents on browning were examined. When 20\% (V/V) of ethanol, a radical scavenger, was added to the model solution, the browning was repressed (Fig. 4-A). The repression was more definite in the longer storage time. When 0.57 mmole/l of mannitol was added to the solution as another scavenger, the browning was repressed by 72.9\% after 14 days of storage and by 65.5\% after 60 days of storage. These results suggest that radicals participate in the browning because ethanol and mannitol are scavengers of hydroxyl radical.\textsuperscript{21} Next, the effects of chelators on the browning were examined. When diethylenetriamine-\textit{N},\textit{N},\textit{N},\textit{N}-pentacetic acid (DTPA) or nitriltriacetic acid (NTA) was added to the model solution, the degree of browning was repressed or promoted by the addition of DTPA or NTA, respectively (Fig. 4-B). AsA was more maintained in the solution added with DTPA (Fig. 4-C). The different effects of DTPA and NTA on the browning seem to be due to the difference in chelating ability between the two compounds. DTPA is a much stronger chelator than NTA.\textsuperscript{22} It was reported as a similar phenomenon that oxidative stress by active oxygen was repressed by DTPA, while stimulated by NTA.\textsuperscript{23} This result shows that metal complex with DTPA represses the AsA-degradating activity of metals, while a metal complex with NTA or citric acid promotes the degradation of AsA to lead to browning.

On the base of these results, the factors affecting the browning of the model solution of orange juice during storage was summarized in Table 3. The storage time was divided into two periods, that is, the early stage (1 or 2 weeks of storage) and the later stage (1 or 2 months of storage), on this condition that it was stored at 50°C with headspace. AsA was essential for the browning in the early stage of storage. Amino acids and citric acid stimulated the browning of AsA, while sugars had little effect on the browning. These results coincided with other reports.\textsuperscript{3,7} In the later stage, AsA contributed the most to the browning, however, the browning happened when AsA was removed from the solution. Sugars as well as amino acids and citric acid stimulated the browning. These results suggest that in the early stage of

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### Table 2. Effect of Headspace of a Vial and Degassing on the Browning of Model Orange Juice Solution during Storage

<table>
<thead>
<tr>
<th>Storage time (days)</th>
<th>Headspace (ml)</th>
<th>Degassing</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>14</td>
<td>178*</td>
<td>96.6</td>
</tr>
<tr>
<td>60</td>
<td>323*</td>
<td>96.3</td>
</tr>
</tbody>
</table>

Values show degree of browning expressed by % of control with 15 ml of headspace. n.d., not determined. \* significant difference from control (p < 0.01).
storage, the AsA degradation products are essential for browning and that the Maillard reaction between the degradation products and amino acids stimulates the formation of brown pigments. In the later stage of storage, the AsA degradation product as well as sugar degradation products also contribute to the formation of brown pigments, which is also produced by the Maillard reaction. Active oxygen species seems to be important for the degradation of AsA and browning, because metal ions stimulated the browning and DTPA, a strong chelator, and ethanol and mannitol, radical scavengers, repressed the browning.

*Decomposed products in the model solution*

Decomposed products in the model solution were analyzed by ODS-HPLC. Figure 5 shows a typical chromatogram monitored by absorbance at 283 nm. Several peaks were detected and four decomposed products (A, 3OH2P; B, HMF; C, furfural; D, 5-hydroxymaltol that is 2-methyl-3,5-dihydroxy-4H-pyran-4-one) were identified with the comparison of standards and instrumental analyses. 2-Furoic acid

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**Table 3.** Factors Affecting the Browning of Model Orange Juice Solution

<table>
<thead>
<tr>
<th>Factor</th>
<th>Effect on browning</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>7~14 days of storage</td>
</tr>
<tr>
<td>AsA</td>
<td>essential</td>
</tr>
<tr>
<td>Sugars</td>
<td>no effect</td>
</tr>
<tr>
<td>Amino acids</td>
<td>stimulation</td>
</tr>
<tr>
<td>Citric acid</td>
<td>stimulation</td>
</tr>
<tr>
<td>Metals</td>
<td>stimulation</td>
</tr>
<tr>
<td>Chelators</td>
<td>inhibition (DTPA)</td>
</tr>
<tr>
<td></td>
<td>/stimulation (NTA)</td>
</tr>
<tr>
<td>Oxygen</td>
<td>inhibition</td>
</tr>
<tr>
<td>Ethanol and</td>
<td>inhibition</td>
</tr>
<tr>
<td>mannitol</td>
<td></td>
</tr>
</tbody>
</table>

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**Fig. 4.** Effects of Ethanol (A), DTPA (B), and NTA (B) on the Browning (A and B) and Residual AsA (C) of the Model Orange Juice Solution during Storage.

Ethanol (20% v/v), DTPA (1 mM; 2) or NTA (1 mM; 3) was added to the standard model solution (control, 1) and then stored for 60 days.

**Fig. 5.** Typical Chromatogram of the Stored Model Orange Juice Solution on HPLC.

The model orange juice solution stored for 7 days at 50°C was put on ODS-HPLC (column, Mightysil RP-18 (4.6 i.d. x 250 mm); eluent, CH3CN:5%AcOH = 2:98; flow rate, 1.0 ml/min).
was also detected by another HPLC condition. Oxalic acid, which is one of decomposed products of AsA through 2,3-diketogulonic acid in physiological conditions, was not detected in this stored solution. 3OH2P, furfural and 2-furoic acid were derived of AsA through dehydroascorbic acid, while HMF and 5-hydroxymaltol were derived of fructose (data not shown). 5-Hydroxymaltol is sometimes identified as a flavor component of heated foods and formed by the Maillard reaction. However, there is no report that 5-hydroxymaltol is formed during storage of orange juice. When retail orange juice was stored, these five compounds were all detected (data not shown). 3OH2P increased till 3 days and then decreased, while HMF, furfural, 5-hydroxymaltol and 2-furoic acid gradually increased during storage (Fig. 6). This suggests that 3OH2P might be used for browning reaction. Next, the relationship between these decomposed compounds and browning was examined. When 3OH2P was stored, the solution gradually turned brown (Fig. 7-A). When amino acids were added to 3OH2P solution, the browning was a little promoted. When furfural solution was stored, the browning was not observed. However, when furfural added with amino acids turned yellow and the browning during storage was observed (Fig. 7-B). Hoffman reported a yellow compound formed by the Maillard-type reaction between furfural and proline. The solution of HMF, 5-hydroxymaltol, and 2-furoic acid did not turn brown in the absence or presence of amino acids. These results suggest that 3OH2P and furfural derived from AsA and the Maillard reaction contribute to the browning of orange juice.

Browning and decomposed products in retail orange juice

Retail orange juice was stored at 50°C, before the browning and decomposed products were measured. The browning was estimated by the difference of a value (Δa value) before and after storage. The relationship between the Δa value after 6 days of storage and 3OH2P after 14 days of storage was observed (Fig. 8), while the relation between the Δa value and HMF or furfural was

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**Fig. 6.** Changes in the Decomposed Products of the Model Orange Juice Solution during Storage.


**Fig. 7.** Browning Potential of 3OH2P (A) and Furfural (B).

3OH2P (5.0 mM) or furfural (5.7 mM) solution was stored with (■) or without (□) amino acids at pH 3.0 for 60 days. Degree of browning and residual 3OH2P or furfural was measured.

**Fig. 8.** Relationship between Δa Value after 2 Weeks Storage and 3OH2P Concentration after 7 Days of Storage.
not observed. This result suggests that 3OH2P becomes an indicator for the browning of orange juice instead of HMF and furfural.

Conclusion

A model solution of orange juice gradually turned brown during storage. AsA contributed most to the browning. Amino acids such as Arg and Pro, citric acid, and metals promoted the browning. Among five decomposed products detected here, 3OH2P and furfural contributed to the browning. 3OH2P seems to become a browning indicator of orange juice.

References

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