Thermal Degradation of IMP in Commercially Available Umami Seasoning Extract, and the Stability of IMP in the Seasoning Powder under the Influence of Various Water Activities

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Introduction

Since the early ages, extracts from dried sea tangle and dried bonito have been used for the broth of various dishes in Japan. An Umami seasoning (Dashinomoto) containing a powder of dried bonito and sea tangle with some other food components was commercially produced in 1961.1) Since then, this seasoning has been utilized widely as a substitute for natural soup stocks in home cooking and food industries, because of its convenience and economy. In recent years, most Japanese households have used the seasoning for its handiness.5) Therefore, the flavor of Dashinomoto seems to have a great influence on the acceptability of home cooking.

There have been many investigations about the thermal degradation of nucleotide seasonings, 5'-ribonucleotides.3)-7) We have reported that inosine 5'-monophosphate (IMP) and guanosine 5'-monophosphate (GMP) were degraded in a first-order reaction by heating, that the rate constants were affected by pH and temperature and that the main reaction of the thermal degradation process was hydrolysis of the phosphoric ester bond in the nucleotides.4) Mita et al. observed the decrease of IMP when the soup stock from beef was heated for more than 3 hr.9) However, sugars, proteins, lipids and salts, which are contained in Dashinomoto, are liable to influence the degradation of 5'-ribonucleotides during cooking.

The purpose of the present study was to investigate the thermal stability of 5'-ribonucleotides in an extract from Dashinomoto and the influence of water activity on the stability of the 5'-ribonucleotides in Dashinomoto powder during storage.

Materials and methods

1) Materials. Dashinomoto used in the present study was “Hondashi-Irikodashi” (made from boiled and dried anchovies, various seasonings and...
other materials) from Ajinomoto Co., Ltd., Tokyo. IMP and GMP were purchased from Nacalai Tesque Inc., Kyoto. All the other reagents were of extra grade obtained from Wako Pure Chemical Industries Ltd., Osaka and Nacalai Tesque Inc., Kyoto.

2) Extraction of 5'-ribonucleotides and the related compounds from Dashinomoto. Dashinomoto (250 mg) was suspended in 25 ml of a boiling buffer (0.01 M sodium acetate, pH 4; 0.01 M 3-(N-molpholino)-propanesulfonic acid, pH 7; 0.01 M 2-(cyclohexyl-amino)-ethanesulfonic acid, pH 9) with stirring for 5 min, before being heated at 100°C for 10 min and then cooled in an ice bath for 10 min. The resulting solution was centrifuged at 3,000 rpm and 4°C for 10 min, and the supernatant was used as the extract from Dashinomoto.

3) Thermal reaction system of Dashinomoto. The extract just described was heated in a sealed Pyrex tube at 90-100°C. After heating, the tube was immediately cooled in an ice bath to stop the thermal reaction. The amounts of 5'-ribonucleotides and the related compounds in the reacted sample were analyzed by high-performance liquid chromatography (HPLC) as subsequently described.

4) Identification of 5'-ribonucleotides. 5'-Ribonucleotides were identified by HPLC (Shimadzu LC-3A), the UV detector being set at 254 nm. The column used was a Sim-pack WAX-1 (4 x 50 mm, Shimadzu), the temperature of the column was 25-30°C, and the flow rate was 0.5 ml/min. The mobile phases were 20 mm phosphate buffer at pH 7.0 (buffer A) and 480 mm phosphate buffer at pH 6.9 (buffer B). The gradient program started from an initial composition of 100% of buffer A, before buffer B was linearly increased at the rate of 5%/min for 10 min, and then increased at the rate of 2%/min for 5 min. A C-R1A integrator (Shimadzu) was used for calculating the peak areas. Under these conditions, the retention times of IMP and GMP were 4.5 and 6.9 min, respectively.

5) Determination of 5'-ribonucleotides, nucleosides and bases. These compounds were determined by HPLC (Shimadzu LC-6A), the UV detector being set at 254 nm. The column used was a Cosmosil 5C18-P (ODS type, 4 x 250 mm, Nacalai Tesque) with a protective column (4 x 10 mm). The temperature of the column was 25-30°C, the mobile phase was methanol-0.02 M KH2PO4 (15:85 by v/v), and the flow rate was 0.5 ml/min. A C-R3A integrator (Shimadzu) was used for the calculation of peak areas. Under these conditions, the retention times of 5'-ribonucleotides, nucleosides and bases were 6.5, 10.5 and 8.5 min, respectively.

6) Influence of water activity on the stability of 5'-ribonucleotides during storage. Dashinomoto was stored at 60°C in a desiccator where the water activity (A_w) was adjusted to 0, 0.2, 0.5 or 0.8 (A_w=0, P_2O_5; A_w=0.2, saturated CH_3COOK solution; A_w=0.5, saturated Ca(NO_3)_2 solution; A_w=0.8, saturated (NH_4)_2SO_4 solution) After storage, the 5'-ribonucleotides and related compounds were extracted and determined as already described.

Results and discussion

1) Identification of 5'-ribonucleotides in the extracts from Dashinomoto

Figure 1 shows the chromatograms of the extract from Dashinomoto analyzed by HPLC (Sim-pack WAX-1 column). As shown in Fig. 1(A), many peaks were observed on the chromatogram, indicating that the extract contained various components. Since peak 1 was scaled out, the extract diluted 50 times was analyzed (Fig. 1(B)). By comparing with an authentic mixture of IMP and GMP (Fig. 1(B)), peak 1 was found to be

![Fig. 1. HPLC of a Dashinomoto extract, using a Sim-pack WAX-1 column](image-url)

(A) Chromatogram for 250 mg of Dashinomoto/ml.
(B) Chromatogram for a mixture of IMP and GMP. a, IMP; b, GMP. (C) Chromatogram for 5 mg of Dashinomoto/ml.
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IMP, whereas GMP was not detected in the extract. This result shows that the 5'-ribonucleotide in the Dashinomoto used in the present study was only IMP.

2) Thermal degradation of IMP in the extract from Dashinomoto

The contents of IMP and its degradation products were determined after heating the extract. A decrease in IMP and an increase in inosine and hypoxanthine were observed after heating for 8 hr at pH 4-9 and 90-100°C (Fig. 2).

Figure 3 shows that the semi-log plots for the thermal degradation of IMP in the extract from Dashinomoto at pH 4 and 7 gave straight lines, suggesting that the degradation of IMP in the extract proceeded according to a first-order reaction. As reported in our preceding paper, only the thermal degradation of IMP in solution is a first-order reaction, so that the present results are consistent with our previous report.

A clearly linear relationship did not exist between a logarithm of the amount of IMP and heating time at pH 9. The sample solution at pH 9 was colored brown by heating and some unidentified peaks appeared on the chromatogram. Such a phenomenon was not observed during heating an IMP solution at pH 9, suggesting that some chemical reactions of IMP with the other components in Dashinomoto occurred. The browning reaction was possibly the Maillard reaction, because this is liable to occur under alkaline conditions.

Table 1. Kinetic parameters for the thermal degradation of IMP in Dashinomoto at various pH values and temperatures

<table>
<thead>
<tr>
<th>pH</th>
<th>Temperature (°C)</th>
<th>$k_{obsd}^{b)}$ ($\times 10^{-2} \text{ hr}^{-1}$)</th>
<th>$t_{1/2}^{c)}$ (hr)</th>
<th>$E_a^{d)}$ ($\times 10^6 \text{ J} \cdot \text{mol}^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>100</td>
<td>8.5±0.36</td>
<td>8.1</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>3.7±0.10</td>
<td>19.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>2.7±0.20</td>
<td>26.0</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>100</td>
<td>4.5±0.11</td>
<td>15.0</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>2.8±0.25</td>
<td>25.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>1.2±0.12</td>
<td>58.0</td>
<td></td>
</tr>
</tbody>
</table>

* An IMP solution (0.4×10^{-3} \text{ mol} \cdot \text{dm}^{-3}) was incubated for 0, 0.5, 1.0, 1.5, 2.0, 3.0, 5.0 and 7.5 hr.
* Observed rate constant. $t_{1/2} = \frac{0.693}{k_{obsd}}$. 

(679)
In order to examine the degree of thermal degradation of IMP in the extract from Dashinomoto at pH 4 and 7, the kinetic parameters for a first-order reaction such as the observed rate constants ($k_{obsd}$), half-life periods ($t_{1/2}$) and activation energies ($E_a$) were determined (Table 1). The degradation rates were increased by lowering the pH and raising the temperature. The half-life periods were as follows: pH 4, 8.1 hr (100°C), 19 hr (95°C) and 26 hr (90°C); and pH 7, 15 hr (100°C), 25 hr (95°C) and 58 hr (90°C). The activation energy at each pH examined was about 1.5 x 10^5 J·mol⁻¹, indicating that the mechanism for the thermal degradation of IMP in the extract at pH 4 was the same as that at pH 7. These parameters were very similar to those of the thermal degradation of IMP in solution. Therefore, we presume that the main reaction in the thermal degradation of IMP in the extract from Dashinomoto was hydrolysis of the phosphoric ester bond in IMP, in analogy with the degradation in solution. These results also suggest that the components other than IMP in Dashinomoto scarcely affected the thermal degradation of IMP at pH 4 and 7. As the pH value of the broth prepared from Dashinomoto during cooking is about 6, the degradation of IMP would be affected slightly by the other components in Dashinomoto during cooking.

Nguyen and Sporns observed no particular influence from the components of soup (casein, glucose, potato starch and salts) on the stability of monosodium glutamate and 5'-ribonucleotides during heating at 124°C and various pH values for 15–60 min.

However, under actual cooking and processing conditions, Dashinomoto is mixed with various seasonings such as salts, spices and other food components. Therefore, the interaction between 5'-ribonucleotides and those components, which was not specifically observed in the present study, may have occurred.

3) Influence of water activity on the stability of IMP during storage at 60°C

Dashinomoto powder was stored at $A_w$ 0, 0.2, 0.5 and 0.8 and at 60°C. During storage for 77 days, the amounts of IMP and its degradation products were analyzed. Figure 4 shows the changes in the relative composition (on a molar basis) of IMP and its degradation products (inosine and hypoxanthine) during storage as a function of time (0–77 days). When the sample was stored at $A_w$ 0, 0.2 and 0.5, the degradation rate of IMP was very low. Even after storage for 60 days...
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days, the amount of IMP degraded was only about 5%, inosine and hypoxanthine scarcely being produced from IMP. On the other hand, when the sample was stored at $A_w$ 0.8, IMP was markedly degraded. The amounts of inosine and hypoxanthine were increased by about 10 and 5% during storage for 77 days, respectively. After 6 days, the granules of Dashinomoto liquefied and became colored brownish black. Some unidentified peaks were observed on the chromatogram and the areas increased with the passage of storage period (Fig. 5). The degree of chemical reaction of IMP with the other components increased with increasing water activity. High water activity may have caused better diffusion and thereby accelerated these reactions.

The sample stored at $A_w$ 0 had a stronger flavor than those stored at $A_w$ 0.2, 0.5 and 0.8. The increase of water activity suppressed the generation of flavor from the samples during storage, suggesting that the formed flavor compounds may have been water soluble.

Sumida reported that, when a powdered soup was moistened, the quality of the soup decreased significantly to accompany the degradation of 5'-ribonucleotides in the sample during storage. His result is consistent with the present observation. These results suggest that Dashinomoto powder should be stored under conditions with as low a water activity as possible.

References

2) T. Ueda: Shoku no Kagaku, 101, 102 (1986)
4) E. Fujita, H. Kinura, H. Nakatani, K. Ishii and E. Satani: Eiyo to Shokuryo, 18, 98 (1965)
5) C. Kuriyama, M. Fushizaki and K. Murata: Eiyo to Shokuryo, 17, 337 (1965)
13) N. Sumida: New Food Ind., 6, 78 (1964)