Note

Effects of pH and Light on the Storage Stability of the Purple Pigment, Hordeumin, from Uncooked Barley Bran Fermented Broth

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The pigment retention rate of hordeumin was higher than that of two standard anthocyanidins, cyanidin and delphinidin, when hordeumin and anthocyanidins were dissolved in Walpole buffer (pH 1.0) and stored. Moreover, when pigment solutions were stored at 15°C under light irradiation, the pigment retention rate of the hordeumin solution became higher than those of standard anthocyanidins (2 to 10 times) as the storage period increased. Comparing various pH buffers (Macllvaine buffer, pH 2.2 to 7.0), the pigment retention rate of hordeumin at pH 5.0 was highest. Furthermore, the half-life of hordeumin at pH 5.0 was increased from 9 days to 17.5 days when nitrogen gas was bubbled into the hordeumin solution. We considered that the storage stability of hordeumin is higher than standard anthocyanidins because hordeumin is a complex with anthocyanin, tannin, and protein.

Key words: hordeumin; barley bran; anthocyanin; storage stability; pH stability

Recently, many studies have been done on plant pigments such as anthocyanins as natural food additives.1-5 Anthocyanins are widely distributed among plants and used in food. However, many anthocyanins are unstable to temperature and light. Thus, stable anthocyanins were desired. It was reported that anthocyanins from purple sweet potatoes and egg plants were relatively stable.6-9

We have been trying to effectively use barley to get useful products. In our previous studies,10-12 a large amount of precipitate of the novel purple pigment, hordeumin, was formed as a secondary product in addition to ethanol in uncooked alcohol fermentation using barley bran as the fermented material. However, only a few studies on the pigment, which was produced by fermentation, have been reported. Hordeumin contains some anthocyanidins such as cyanidin and delphinidin.13 The production mechanism of hordeumin can be presumed as follows. First, barley bran is broken down by fermentation, then precursors of hordeumin such as anthocyanidins, tannin, and protein are extracted. Next, hordeumin is formed by oxidative polymerization of the precursors.14,15 Hordeumin has physiological functions that contain polyphenols such as anthocyanin and tannin.16,17 In our previous study,18 the storage stability of hordeumin was reported only below pH 1.0. However, when hordeumin is used in foods, cosmetics, etc., the storage stability must be tested between pH 1.0 and 7.0.

In this paper, we report the storage stability of hordeumin at several pHs and compare the results with standard anthocyanidins.

 Hordeumin powder (protein, 500 mg/g; polyphenol, 100 mg/g; sugar 150 mg/g) was prepared as described in a previous paper.10 The hordeumin was dissolved in buffer set several pHs (pH 1.0, Walpole buffer; pH 2.2, 5.0, 6.0, and 7.0, Macllvaine buffer), and then filtered through a membrane filter (pore size, 0.65 μm, Advantec Toyo Ltd., Tokyo, Japan). For the storage stability test, each hordeumin solution was placed in 3-ml screw vials and sealed. These vials were stored at various temperatures. Discoloration was evaluated by the residual absorbance (500 nm) of the pigment at the start of storage, which was referred to as 100%, or the pigment retention rate (PRQ), which was calculated from the continuous visible spectrum similar to our previous paper.18 The pigment retention rate (PRQ) was calculated in accordance with the following 3 formulas based on absorption spectra:

(1) Pigment quantity (h) = (h1 + h2)/2
(2) Pigment quality (Q) = h1 - h2/100
(3) Pigment retention rate (PRQ, %) = Q after fading/Q before fading × 100

The maximum and minimum values in the visible region on the absorption spectra were termed h1 and h2, respectively. Formula (1) and (2) were used to evaluate pigment quantity and quality, respectively. Formula (3) was used to evaluate of the discoloration of hordeumin. Furthermore, the formula (3) was similar to the one used by Azar et al.19 for evaluating the chromatic index. The half-life quality showed time in which the pigment lost color by 50%.
Effects of pH and Light on the Storage Stability of Hordeumin

Table 1. Color Quality of Hordeumin and Anthocyanidins

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Hunter L</th>
<th>Hunter a</th>
<th>Hunter b</th>
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<tbody>
<tr>
<td>Hordeumin</td>
<td>90.0</td>
<td>3.48</td>
<td>-2.35</td>
</tr>
<tr>
<td>Cyanidin</td>
<td>90.0</td>
<td>13.65</td>
<td>1.60</td>
</tr>
<tr>
<td>Delphinidin</td>
<td>89.9</td>
<td>17.41</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Pigments were dissolved in Walpole buffer (pH 1.0).

Figure 1 shows a comparison of hordeumin and standard anthocyanidins in storage stability. The color quality of the pigment solutions before storage are shown in Table 1. After the pigments were dissolved in Walpole buffer (pH 1.0) and stored at 30°C, the absorbance at 500 nm of the delphinidin solution rapidly decreased, then became colorless in 7 days. Discoloration of cyanidin was slower. In tested pigments, hordeumin showed the highest stability.

Furthermore, the storage stability of hordeumin and anthocyanidins were compared at 15°C under light irradiation or darkness. The delphinidin solution became rapidly discolored, while hordeumin and cyanidin were relatively stable in the dark (Fig. 2-A). Standard anthocyanidins were rapidly discolored, while hordeumin showed high stability under light irradiation (Fig. 2-B).

The storage stability of hordeumin was studied at various pH and temperatures. When hordeumin solutions were stored at -20°C, discoloration did not occur independently in every tested pH (Fig. 3). The color stability of hordeumin decreased by increasing the temperature (from -20 to 30°C) at all tested pHs. The half-life of each pH at 15°C was as follows: pH 2.2, 8.6 days; pH 5.0, 18.5 days; 6.0, 15.4; pH 7.0, 15.4 days. Thus, hordeumin had the highest storage stability at pH 5.0. Further, in lower temperatures, hordeumin was stable at all tested pHs.

Next, we studied the effects of oxygen in the storage stability of hordeumin. Each pH hordeumin solution was placed in 3-ml screw vials and sealed after bubbling with nitrogen. The vials were stored at 30°C in the dark. The hordeumin solution became discolored at pH 2.2 in spite of the presence of oxygen (Fig. 4). However, when the hordeumin solution was stored after bubbling with nitrogen at pH 5.0 to 7.0, the pigment retention rate increased. The half-lives of hordeumin, which was stored after bubbling with nitrogen, are shown in Table 2. The half-life of hordeumin in a pH 2.2 solution was not influenced by
the presence of oxygen, while those of pH 5.0–7.0 were increased by 2 times. These findings suggest that oxygen was a serious factor in the storage stability of hordeumin. Discoloration of pigment is thought to be an oxygen-mediated reaction.

It was found that hordeumin, produced from uncooked barley bran-fermented broth, has storage stability, especially against light, higher than standard anthocyanidins at pH 1.0 and shows excellent stability at pH 5.0–7.0, where standard anthocyanidins are unstable. We believe that the stability of hordeumin is higher than anthocyanidins because hordeumin is a complex with anthocyanin, tannin, and a protein. Further, the stabilization mechanism of hordeumin may be related to co-pigmentation. The structure and stabilized mechanism of hordeumin will be reported elsewhere in our paper.

Table 2. Effects of Oxygen on Half Life-Quality of Hordeumin at Various Storage pHs

<table>
<thead>
<tr>
<th>pH</th>
<th>With oxygen</th>
<th>Without oxygen</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2</td>
<td>3.9</td>
<td>4.0</td>
</tr>
<tr>
<td>5.0</td>
<td>9.0</td>
<td>17.5</td>
</tr>
<tr>
<td>6.0</td>
<td>8.9</td>
<td>14.2</td>
</tr>
<tr>
<td>7.0</td>
<td>5.7</td>
<td>13.6</td>
</tr>
</tbody>
</table>

Storage was done at 30°C in a dark room. Unit of half-life quality is days.

Fig. 4. Effects of Oxygen on Storage Stability of Hordeumin at Various pHs.

Hordeumin was dissolved in Maclinvaine buffer (pH 2.2–7.0). Storage was done at 30°C in the dark. Symbols: ○, with oxygen; ●, without oxygen.

References

15. Deguchi, T., Ohba, R., and Ueda, S., Effect of reac-
Effects of pH and Light on the Storage Stability of Hordeumin

