Effects of Increase in α-Amylase and Endo-Protease Activities during Germination on the Breadmaking Quality of Wheat

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The effects of increase in α-amylase and endo-protease activities during germination on the deterioration of the breadmaking quality of hard wheat were examined using three varieties with different strengths of gluten: VictoriaINTA, Harunoakebono, and Leader. The results are summarized as follows: (1) The degradation of the breadmaking quality of flour damaged in germination differed greatly according to the gluten strength of each variety. VictoriaINTA, which has extra-strong gluten, was more tolerant of the germination damage than the other varieties with strong gluten. (2) The degradation of specific loaf volume, which is caused by lowering the gas retention of the dough, was the result of the softening of the dough that was observed as a decrease in the mixing peak time and the breaking force of the dough. (3) The α-amylase and endo-protease activities of flours did not seem to influence the breadmaking quality, although there was a considerably high correlation between endo-protease activity of whole wheat flour and breadmaking quality. From these results, it would seem that the main reason that flour damaged during germination degrades breadmaking quality is the endo-protease in the wheat grain, which already causes partial gluten decomposition. Accordingly, it might be better to examine the endo-protease activity of whole wheat flour at harvest ripeness in order to estimate the value of breadmaking quality within the hard-wheat breeding system.

Keywords: extra-strong variety, breadmaking quality, endo-protease activity, α-amylase activity

Most Japanese hard wheat used for bread is grown in Hokkaido; however, pre-harvest sprouting often occurs as grain develops due to unfavorable weather. This problem is serious because wheat flour that is damaged during germination has a maximum viscosity (MV) of Amylograph that is below 300 B.U. and is unsuitable for industrial processing. Many studies on germination damage in wheat deal with starch degradation, i.e., MV of Amylograph, Fallin number, α-amylase activity and the quality of bread (MacGregor & Matsuo, 1972; Meredith & Pomeranz, 1985; MacGregor & Dushnicky, 1989). However, the deterioration of breadmaking quality in flour that has been damaged during sprouting cannot always be explained by low MV values (Yamauchi et al., 1998; Sato et al., 1999). Therefore, another practical index to estimate the value of breadmaking quality is required within the hard-wheat breeding system. On the other hand, there is some evidence that the degradation of storage protein also greatly contributes to the loss of breadmaking quality during germination. (Kruger, 1971; Bushuk & Lukow, 1987; Lukow & Bushuk, 1984; Janssen et al., 1996b; Weegels et al., 1996). Studies show that an increase in α-amylase and endo-protease activities in wheat may lead to degradation of endosperm starch and protein during germination and may have a deleterious effect on the quality of bread (Kiribuchi & Nakamura, 1973; Preston & Kruger, 1979; Meredith & Pomeranz, 1985; Salomonsson et al., 1989; Sun & Henson, 1991; Jones & Wrobel, 1993). However, the deterioration mechanisms of breadmaking quality during germination are not entirely clear.

The object of this study was to examine further the physical and enzymatic changes related to breadmaking quality in wheat that has been damaged during sprouting in order to develop a practical index for estimating the value of breadmaking quality within the hard-wheat breeding system. Several varieties of flour with varying strengths of gluten were used. Our primary focus was how the increase in α-amylase and endo-protease activities that occur during germination affects the breadmaking quality of flours.

Materials and Methods

VictoriaINTA with extra-strong gluten and Harunoakebono and Leader, each with strong gluten, were cultivated in a standard manner in 1997 at the Hokkaido National Agricultural Experiment Station in a Memuro test field. The three varieties were harvested at harvest ripeness and seven days later at full ripeness. The grains were dried to around 12% water content.

Germination procedure Samples of grains harvested at harvest ripeness were soaked in water at 5°C overnight, then preserved at 20°C for 24 h in an incubator. For preparation of crude α-amylase extractions, a germination treatment was carried out at 20°C for four days using Harunoakebono samples (100 g) that had been harvested at full ripeness. The samples were dried to around 12% water content and stored at 4°C. The wheat samples were milled on a Büher test mill (Büher Inc., Sennhof, Switzerland) after controlling moisture content in the grain at 16%, and a flour of 60% grade was obtained from the milling. The whole
wheat flour was prepared with a 12,000-rpm centrifuge and high-speed milling (Type ZM-1, Retch Co., Haan, Germany) with a screen of 0.5 mm.

Analytical The protein content of the flour was determined by nondestructive analysis using a near-infrared reflectance instrument (Infraromatic 8120, PerCon Co., Hamburg, Germany). The maximum viscosity (MV) value of the Amylograph was measured using an Amylograph (Brabender Co., Duisburg, Germany). Measurement was carried out using 65 g of flour with a 13.5% water content base; the starting temperature and heating rate were 25°C and 1.5°C/min, respectively. The SDS-sedimentation value (SV) was determined by the method of Takata et al. (1999), who modified the methods of Axford et al. (1979). α-Amylase activity of flour and whole wheat flour was determined by the methods of Watanabe et al. (1994) using an α-amylase kit (Ceralpha, Megazyme Co., Ltd., Wicklow, Ireland). One unit of activity was defined as the amount of enzyme releasing 1 μmol of p-nitrophenol per minute. Endo-protease activity was determined using an Azurine-crosslinked casein substrate (Protazyme AK tablet; Megazyme Co., Ltd.). Five-tenths of a gram of whole wheat flour or flour was suspended in 5.0 ml of a 100 mM sodium phosphate buffer (pH 7.0) by a stirrer for 15 min at room temperature. The suspension was then filtered through an Advantec Toyo No.2 paper filter (enzyme extract). The enzyme extract (1.0 ml) in a phosphate buffer was incubated with 1.0 ml of a Protazyme AK solution (one tablet/ml) in a 100 mM phosphate buffer (pH 7.0) with 1% SDS at 40°C for 2 to 4 h. The test tube was stirred by hand every 15 min. The reaction was terminated by the addition of 10 ml of 2% sodium biphosphate, and the tubes were stirred vigorously. The solution was filtered through an Advantec Toyo No.2 paper filter, and the absorbance (590 nm) of the filtrates was measured. One unit of enzyme activity was defined as the change in absorbance for 1 h per 1 g of flour or whole wheat flour.

Breadmaking formulation and conditions The breadmaking test was carried out following the no-time method for the formulation of standard white bread, according to Yamauchi et al. (2000), except that final proofing was done for 40 min using 5% wet yeast. The mixing peak pattern (mixing motor current value) during the mixing of the dough was measured by the method of Takata et al. (1999) using a clamp meter. The physical properties of the dough were measured by the method of Yamauchi et al. (submitted for publication) using 10 g of dough.

Preparation method of the crude α-amylase extract Crude α-amylase extract was prepared from the whole wheat flour of Harunoakebono grains that had germinated for four days (see germination procedure). Fifty grams of whole wheat flour was suspended in 100 ml of distilled water at room temperature. The suspension was centrifuged at 3000 rpm for 15 min. After centrifuging, the supernatant was filtered through an Advantec Toyo No.2 paper filter (crude α-amylase extract). The α-amylase activity of this extract was measured, and the amount of the extract to be added to the dough was determined. This crude extract contained only low levels of endo-protease activity. The breadmaking test was carried out as described above.

Results and Discussion Germination effect on starch Results of α-amylase activity and MV value are shown in Table 1. In the three varieties, the samples harvested at harvest ripeness showed a low level of α-amylase activity and a high MV value of Amylograph. However, the α-amylase activity during germination increased rapidly as the germination levels increased. After the germination treatment, the α-amylase activity of whole wheat flour increased about 17 times for Harunoakebono, 21 times for Leader, and 42 times for VictoriaINTA. The MV value decreased rapidly in parallel with an increased α-amylase activity during germination. In grain harvested at full ripeness, all varieties showed an MV value below 300 B.U., which is unsuitable for making noodles. These results indicated that the starch in the flour in each variety degraded rapidly as the α-amylase activity increased during germination.

Germination effect on protein Results of the protein content, endo-protease activity, and SDS-sedimentation (SV) value are shown in Table 2. Germination did not affect the protein content in any of the three varieties, and the endo-protease activity during germination differed little among the varieties. Although the activity in these varieties also increased with the germination levels, the ratio of increase was smaller than that of the α-amylase activity. The activity of whole wheat flour in VictoriaINTA increased the most at 1.7 times after the germination treatment. The SV value in all varieties decreased with an increase of germination levels. These results indicated that the protein in each variety of flour was degraded as the endo-protease activity in-
creased during germination. However, the tolerance for germination damage was different in each case. VictoriaINTA, an extra-strong variety, maintained a high SV value during the germination treatment compared to Harunoakebono and Leader. Axford et al. (1979) reported that there was a close relationship between the SV value and the breadmaking quality. These results indicated that the gluten quality in the VictoriaINTA, the extra-strong variety, remained comparatively good in spite of the MV value being quite low.

Germination effect on breadmaking quality The effects of germination damage on breadmaking quality are shown by the specific loaf volume, vacuum expansion of the dough, mixing peak time, and physical properties (the breaking force and breaking deformation) (Table 3). The germination effect on specific loaf volume (SLV) also differed in each variety. VictoriaINTA harvested at full ripeness expressed the highest specific loaf volume in spite of having quite a low MV value (Tables 1 and 3). In contrast, the SLV of Harunoakebono was slightly smaller, and that of Leader decreased as its MV value declined. Concerning the dough properties, the behavior of the vacuum expansion value in these varieties was linked to the changes of SLV ($r=0.77$, Fig. 1). This result agreed with the findings of Yamauchi et al. (2000), who reported that the value of the vacuum expansion of the dough correlated significantly with the SLV. Therefore, the gas retention (vacuum expansion volume) of the dough was presumed to be closely related to the SLV. Other characteristics of the dough decreased, including the peak mixing time and breaking force, and the breaking deformation increased in all varieties when the germination levels increased. However, the changes in

<table>
<thead>
<tr>
<th>Sample</th>
<th>Specific loaf volume (cm$^3$/g)</th>
<th>Expansion volume (cm$^3$)</th>
<th>Peak mixing time (min)</th>
<th>Breaking force (N)</th>
<th>Breaking deformation (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Victoria INTA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harvest ripeness</td>
<td>6.89</td>
<td>143</td>
<td>6.4</td>
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<td>Full ripeness</td>
<td>7.10</td>
<td>158</td>
<td>4.6</td>
<td>2.75</td>
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<tr>
<td>GT$^a$</td>
<td>6.26</td>
<td>149</td>
<td>3.7</td>
<td>2.67</td>
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<td>Harunoakebono</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harvest ripeness</td>
<td>5.83</td>
<td>125</td>
<td>2.9</td>
<td>2.06</td>
<td>42.2</td>
</tr>
<tr>
<td>Full ripeness</td>
<td>5.78</td>
<td>123</td>
<td>2.8</td>
<td>2.32</td>
<td>44.8</td>
</tr>
<tr>
<td>GT</td>
<td>6.10</td>
<td>130</td>
<td>2.5</td>
<td>1.98</td>
<td>42.3</td>
</tr>
<tr>
<td>Leader</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harvest ripeness</td>
<td>7.00</td>
<td>130</td>
<td>1.9</td>
<td>2.17</td>
<td>55.0</td>
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<tr>
<td>Full ripeness</td>
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<td>123</td>
<td>1.3</td>
<td>1.47</td>
<td>63.5</td>
</tr>
<tr>
<td>GT</td>
<td>5.15</td>
<td>117</td>
<td>1.2</td>
<td>0.98</td>
<td>67.8</td>
</tr>
</tbody>
</table>

$^a$Germination treatment.

![Fig. 1](image1.png)  Relationship between the dough’s vacuum expansion volume and breadmaking quality (specific loaf volume).

![Fig. 2](image2.png)  Relationship between the dough’s breaking force and specific loaf volume.

![Fig. 3](image3.png)  Relationship between the peak mixing time and specific loaf volume.
The properties of VictoriaINTA were small in comparison with those in the other varieties, and VictoriaINTA maintained relatively good breadmaking quality despite having a low MV value (Tables 1, 2, and 3). These results agreed with the findings of Lukow et al. (1984), who reported that the degradation of the breadmaking quality of flour damaged in germination differed according to the gluten strength of each variety. On the other hand, Yamauchi et al. (1998), and Sato et al. (1999) reported that hard wheat with hard gluten could maintain a relatively good breadmaking quality until full ripeness in spite of a comparatively lower MV value ranging from 100 to 300 B.U. These results indicate that a low MV value does not always explain the deterioration of breadmaking quality in flour that has been damaged during sprouting. Figures 2 and 3 show the relationship among the breaking force of the dough, peak mixing time, and specific loaf volume. The SLV, which is linked to the vacuum expansion of dough, decreased when the breaking force and mixing peak time were lowered, and there was a clearly positive correlation between these two values ($r=0.79$ and 0.66). Other studies have reported that breadmaking quality was greatly affected by the physical properties of the dough (Janssen et al., 1996a; 1996b; Weegels et al., 1996). Our present study also shows that the dough properties discussed above play an important role in the quality of breadmaking. From these results, the lowering of SLV during germination seemed to be caused by the softening of the dough, which was observed as a decrease in the breaking force of the dough and the peak mixing time.

The relationship between enzymatic activity and breadmaking quality It has been reported that the increases of α-amylase and endo-protease activities in wheat are related to the degradation of flour (Jones & Wrobel, 1993; MacGregor & Matsuo, 1972; MacGregor & Dushnicky, 1989; Prestone & Kruger, 1979). However, the relationship between the enzymatic activities and the breadmaking quality of wheat during germination is not always clear. Thus, the relationship between the activities of the enzymes α-amylase and endo-protease in flour and whole wheat flour and specific loaf volume is shown in Figs. 4 and 5. In the following experiments, VictoriaINTA with an extra strong gluten, and Harunoakebono and Leader, which both have strong gluten, are shown with different symbols because the property of the dough that is closely related to SLV (breadmaking quality) differed significantly according to the strength of the gluten. For Harunoakebono and Leader, the strong-gluten varieties, the α-amylase activity in the flours and whole wheat flour and the endo-protease activity in the whole wheat flour were negatively correlated with SLV ($r=-0.74$, –0.70, and –0.89), while the endo-protease activity in the flours hardly correlated with SLV ($r=0.07$). In contrast, the SLV in all varieties including VictoriaINTA did not correlate with the α-amylase activity in the flours (all varieties $r=-0.34$) or the whole wheat flour (all varieties $r=-0.09$), while it did correlate with the endo-protease activity of the whole wheat flour (all varieties $r=-0.77$). VictoriaINTA had a high SLV value and maintained a comparatively good dough property despite having considerably high α-amylase activity (Tables 1 and 3 and Fig. 4). In general, however, there was a negative correlation between α-amylase activity and SLV in the

<table>
<thead>
<tr>
<th>Additional level of α-amylase</th>
<th>MV&lt;sup&gt;a&lt;/sup&gt; (B.U.)</th>
<th>SLV&lt;sup&gt;b&lt;/sup&gt; (cm³/g)</th>
<th>Peak mixing time (min)</th>
<th>Expansion volume (cm³)</th>
<th>Breaking force (N)</th>
<th>Breaking deformation (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harvest ripeness</td>
<td>620</td>
<td>5.83</td>
<td>2.9</td>
<td>125</td>
<td>2.06</td>
<td>42.2</td>
</tr>
<tr>
<td>Full ripeness</td>
<td>350</td>
<td>6.16</td>
<td>2.7</td>
<td>115</td>
<td>2.22</td>
<td>44.2</td>
</tr>
<tr>
<td>GT</td>
<td>240</td>
<td>6.42</td>
<td>2.7</td>
<td>117</td>
<td>1.81</td>
<td>41.5</td>
</tr>
</tbody>
</table>

<sup>a</sup>Maximum viscosity of Amylograph, <sup>b</sup>Specific loaf volume, <sup>__</sup>Germination treatment.
strong-gluten varieties; α-amylase was not considered to have a
great effect on the quality of breadmaking (Lukow & Bushuk,
1984). Similarly, Bushuk & Lukow (1987) reported that the
damaging effects of changes that occur during germination in the
protease-protein system cause a greater loss in the quality of
breadmaking than do those in an amylase-starch system. These
results indicate that the degradation of protein in flour plays a
more important role in the deterioration of breadmaking quality
in flour damaged during germination than does starch. To clarify
the relationship between both enzymatic activities and bread-
making quality, we conducted a breadmaking experiment by
adding a crude α-amylase extract (see Materials and Methods) to
the flour of Harunoakebono, which had strong gluten and was
harvested at maturity. Table 4 shows the breadmaking quality of
ideal flour that can be created by adding various volumes of
crude α-amylase extract to the flour. Because the level of α-amyl-
ase extract was the same at full ripeness and the germination
treatment as it was during the germination procedure, the MV
value of Harunoakebono decreased to the same levels recorded at
full ripeness and the germination treatment as had been attained
during the germination procedure. In contrast, other qualities,
&beta;i.e., specific loaf volume, peak mixing time, vacuum expansion
of the dough, breaking force, and breaking deformation were the
same as those in the sample harvested at maturity. Therefore,
though the α-amylase activity of a strong-gluten variety corre-
related with SLV, the degradation of starch by increasing α-amylase
activity during germination did not seem to be a main factor in
the deterioration of the breadmaking quality of the flour. Figures
6 and 7 show the relationship among the peak mixing time,
breaking force of the dough, and endo-protease activity of whole
wheat flour. The peak mixing time and breaking force of the
dough are an index of the degradation of flour, and the endo-pro-
tease activity is closely related to the degradation of the protein
in flour. Endo-protease activity of whole wheat flour was negatively
correlated with the peak mixing time and breaking force of the
dough; there was an especially high correlation with the latter
(Harunoakebono and Leader \( r = -0.72 \), all varieties \( r = -0.66 \)).
Therefore, the degradation of protein during germination was
considered to be closely related with the deterioration of the
breadmaking quality. We concluded that a main factor in the
deterioration of the breadmaking quality of the flour during ger-
mination might be attributed to the partial decomposition of glu-
ten in the grain, which seems to be degraded by an increase in
endo-protease activity. The lowering of the mixing peak time,
breaking force, and gas retention (vacuum expansion) is gener-
ated as a result.

From the results of all experiments, it might be better to exam-
ine the endo-protease activity of whole wheat flour at harvest
ripeness to estimate the value of breadmaking quality within the
hard-wheat breeding system, because this activity was signifi-
cantly correlated with the breadmaking quality of hard wheat.

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MacGregor, A.W. and Dushnicky, L. (1989). Starch degradation in

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\begin{align*}
\text{Fig. 6. Relationship between the endo-protease activity of whole wheat} \\
\text{flour and peak mixing time. \( \text{\Delta} \), VictoriaINTA; \( \bullet \), Harunoakebono and Leader.} \\
\end{align*}
\]

\[
\begin{align*}
\text{Fig. 7. Relationship between the endo-protease activity of whole wheat} \\
\text{flour and dough’s breaking force. \( \text{\Delta} \), VictoriaINTA; \( \bullet \), Harunoakebono and Leader.} \\
\end{align*}
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