1. INTRODUCTION

Starch is an essential natural polymer and provides energy in foods such as rice, wheat, and potato. Bread is one of the most familiar processed foods containing starch and is typically made using wheat batter, which contains gluten. Gluten is a thickening agent that provides the batter with the viscoelastic properties required for bubble formation during the bread baking process.¹

Batter made from rice does not contain gluten, and thus the viscoelastic properties of rice batter must be altered in the absence of gluten to support the baking of rice bread. Possible methods for achieving this include the addition of thickening additives such as hydroxypropylmethyl cellulose², xanthan gum³, guar gum, and pectin⁴; but these compounds are not present in rice. We recently succeeded in baking pure rice bread by adding amorphous rice flour. Pure rice bread is also possible by using rice flour with a controlled particle size distribution.⁵ Therefore, it is possible to bake pure rice bread by adjusting the viscoelastic properties of rice batter by using amorphous rice flour or classifying the particle size of the rice flour.

In this study, we attempted to adjust the viscoelastic properties of rice batter by changing the molecular architecture of rice starch. Rice starch is composed of amylose, a linear polymer, and amylpectin, a branched polymer. The amylose content and the branch structure of amylpectin vary depending on the genetic background of rice. We used rice starches with different molecular architectures isolated from three rice mutant lines with different starch biosynthetic enzymes. The baking qualities of various pure rice breads were examined by measuring the expansion ratio and analyzing cross-sectional images of baked breads. For rheological assessment, we measured the storage modulus $G'$ and the loss modulus $G''$ of batter made using flour from each line. The best baking qualities was obtained using line e1. A molecular model of the swelling of amylpectin is presented to explain the storage modulus results.

Key Words: Pure rice bread / Amylose content / Amylopectin architecture / Gelatinization

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Nipponbare is a non-glutinous rice and contains at least four enzymes: adenosine diphosphate glucose pyrophosphorylase (AGPase), starch synthase (SS), starch branching enzyme (BE), and starch debranching enzyme (DBE).\(^7\) AGPase generates adenosine diphosphate (ADP)-glucose, and SS catalyzes the polymerization of this monomer into starch. BE connects starch chains through α-1,6 bonds to provide branched starch structures. DBE trims the branches to adjust the shapes of clusters of amylopectin. The line e1 rice does not contain SSIIIa, an isozyme of SS found in Nipponbare, and therefore the elongation of long chains is inhibited, resulting in an increase in short amylopectin chains.\(^6\) Line #4019 is also deficient in SSIIIa, as well as in the BE isozyme BEIIb, and thus both the elongation and branching of amylopectin are inhibited compared with e1.\(^8\) Figure 1 shows a schematic comparison of the branch structures of helical amylopectin in Nipponbare, e1, and #4019.\(^8,9,10\) Line e1 has more double helices in one cluster of branches than does Nipponbare. Among the three lines, #4019 contains the smallest number of double helices but the longest double helices. Rice flour was made from polished rice of each line using a jet mill pulverizer (MP2-350YS2; Yamamoto Co., Ltd., Yamagata, Japan).

2.2 Bread Baking Experiments

Rice batter for bread baking experiments comprised 60 g rice flour, 60 g water, 5 g sugar, 0.5 g salt, and 1 g dry yeast. These materials were stirred using a mixer (KitchenAid KSM90WW; FMI Co., Ltd., Tokyo, Japan) for 10 min. Batter (100 g) was measured and poured into a loaf pan, then fermented in an electric fermentation machine (SK-15; Taisho Electric Co., Ltd., Kusatsu, Japan) for 30 min at 40 °C. The batter was then baked for 15 min at 180 °C in a gas oven (OZ100BOEC; Ozaki Co., Ltd., Tokyo, Japan).

We evaluated the baking qualities of the batters using the expansion ratio defined as

\[
\text{Expansion ratio} (\%) = \frac{\text{Height of baked bread (mm)}}{\text{Height of fermented batter before baking (mm)}} \times 100.
\]

(1)

Height was obtained as the average of three points: the center of the sample, and the centers of the two halves of the sample.

We also analyzed cross-sectional images of the baked breads obtained using a digital camera and obtained average bubble sizes in the bread using image analysis software (MacVIEW Ver.4; Mountech, Tokyo, Japan).

2.3 Measurement of Storage and Loss Moduli

Rice batter samples comprising only rice flour/water 50/50 (wt%) were prepared by stirring with a mixer as above for 10 min, and rheological properties of the batter, the storage modulus \(G'\) and the loss modulus \(G''\), were measured by using a rotational rheometer (Physica MCR 301; Anton Paar Co., Ltd., Graz, Austria). During the measurement, the temperature was increased from 25 °C to 35 °C at 0.3 °C/min (corresponding to the increase in temperature during fermentation), and from 35 °C to 90 °C at 2.5 °C/min (corresponding to baking). The angular velocity and strain were 10 rad/s and 0.1 %, respectively. A coaxial cylinder with
an inner diameter of 24 mm and an outer diameter of 26 mm was used.

3. RESULTS AND DISCUSSION

3.1 Effect of the Molecular Architecture of Starch on Bread Baking

Figure 2 shows cross-sectional images of the baked rice breads and Table I summarizes the expansion ratios and average bubble sizes in the breads. The highest expansion ratio and the largest average bubble size were achieved using e1 and #4019, respectively. These results are discussed in Section 3.3 and compared with the results obtained for the storage modulus.

3.2 Effect of the Molecular Architecture of Starch on the Rheological Properties

Figure 3 shows the changes in the storage modulus $G'$ and the loss modulus $G''$ as the temperature increased. The initial storage modulus $G'$ and loss modulus $G''$ in the fermentation temperature range increased as the amylose content increased. At room temperature, linear amylose dissolves in water more easily than does branched crystalline amyllopectin, and we thus inferred that amylose dissolved in water increases the viscoelasticity of the batter, thus increasing $G'$ and $G''$ of the sample.

The storage modulus $G'$ and loss modulus $G''$ of rice batter typically increases gradually as the temperature increases due to gelatinization, up to their peak temperatures $T'_p$ and $T''_p$, respectively. The decreases in them at temperatures above the peak temperatures are caused by disintegration of the starch granules. Table II shows $G'$ at 25 °C as an initial value, $T'_p$, and the maximum value of $G'$ at $T'_p$. Table III is the correspondence of Table II for $G''$. The peak temperature $T'_p$

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Fig. 2. Cross-sectional photographs of rice breads, showing the dependence of bread structure on the variety of the rice flour.

Fig. 3. Changes in storage modulus (a) and loss modulus (b) as the temperature increases. Dashed line indicates 35 °C; below this temperature, the temperature rise was 0.3 °C/min, and above this temperature it was 2.5 °C/min.
of $G'$ was higher than $T'_{p}$ of $G''$. Peak temperatures $T'_{p}$ and $T''_{p}$ increased in the order e1, Nipponbare, and #4019.

3.3 Rheological Behavior Required for Bread Baking

In this section we discuss the results shown in Table I (properties of the baked breads) and the results of Fig. 3 (rheological behavior during simulated baking). Yeast generate bubbles of carbon dioxide due to fermentation up to a temperature of about 60 °C. These bubbles grow until around the peak temperatures, when the rice flour grains begin to fuse and stabilize the bubble structures. The small expansion ratio observed for #4019 (Table I) is explained by its relatively high peak temperatures, which delays stabilization of the batter.

The cross-sectional images shown in Fig. 2 and the results of Table I indicate that bread baked from Nipponbare uniformly contained small bubbles. The small storage modulus at the initial stage of baking the Nipponbare batter, as indicated in Fig. 3, could not support large bubbles and so the bubbles were small. Breads made using either e1 or #4019 contained larger bubbles than did bread made using Nipponbare. We expect that stiff batter, which is indicated by a high storage modulus, is required at the initial stage of baking to maintain large bubbles in the bread structure.

Table I. Results of bread baking experiments.

<table>
<thead>
<tr>
<th>Lines</th>
<th>Expansion ratio (%)</th>
<th>Average bubble size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nipponbare</td>
<td>230</td>
<td>0.46</td>
</tr>
<tr>
<td>e1</td>
<td>235</td>
<td>1.31</td>
</tr>
<tr>
<td>#4019</td>
<td>200</td>
<td>1.35</td>
</tr>
</tbody>
</table>

Table II. Characterization of $G'$ curves of Fig. 3 (a).

<table>
<thead>
<tr>
<th>Lines</th>
<th>$G'$ at 25 °C (Pa)</th>
<th>$T'_{p}$ (°C)</th>
<th>$G'$ at $T'_{p}$ (Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nipponbare</td>
<td>1.06×10^3</td>
<td>63.4</td>
<td>1.83×10^4</td>
</tr>
<tr>
<td>e1</td>
<td>1.96×10^4</td>
<td>60.0</td>
<td>2.10×10^4</td>
</tr>
<tr>
<td>#4019</td>
<td>2.31×10^4</td>
<td>74.5</td>
<td>2.29×10^4</td>
</tr>
</tbody>
</table>

Table III. Characterization of $G''$ curves of Fig. 3 (b).

<table>
<thead>
<tr>
<th>Lines</th>
<th>$G''$ at 25 °C (Pa)</th>
<th>$T''_{p}$ (°C)</th>
<th>$G''$ at $T''_{p}$ (Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nipponbare</td>
<td>1.91×10^7</td>
<td>61.4</td>
<td>2.39×10^4</td>
</tr>
<tr>
<td>e1</td>
<td>2.79×10^3</td>
<td>57.4</td>
<td>2.42×10^4</td>
</tr>
<tr>
<td>#4019</td>
<td>4.17×10^3</td>
<td>67.9</td>
<td>2.43×10^4</td>
</tr>
</tbody>
</table>

Figure 2 and Table I also show that bread made using #4019 contained some large bubbles, and Fig. 3 shows that the peak temperatures of #4019 was comparatively high. At about the peak temperatures, the bubble structure is stabilized and bubble growth ceases, suggesting that late stabilization resulted in large bubbles in the batter made from #4019 flour.

3.4 Molecular Architecture of Amylopectin and Rheological Behavior

The change in storage modulus $G'$ and loss modulus $G''$ shown in Fig. 3(a) are characterized by an initial value, an increasing slope, and the peak temperature. As noted in Section 3.2, the initial value of $G'$ increases as the amylose content increases.

An increase in $G'$ is caused by swelling of the starch grains, and this swelling is induced by water absorbed into the starch grains, a process that is affected by the molecular architecture of amylopectin. We propose a model of the swelling of amylopectin (Fig. 4) to explain the increasing slope of $G'$ and peak temperatures.

Figure 4 suggests that the swelling is induced by unwinding of the amylopectin double helices, which continues until the helices are unwound. Short double helices rapidly unwind, and thus the starch granules rapidly swell, resulting in a low peak temperature. The number of helices in the system determines the speed of swelling, which is connected to the increase in $G'$. Consequently, a larger number of helices causes faster swelling.

The lower content of amylose in Nipponbare results in a larger amount of amylopectin compared with e1. Nipponbare contains the largest number of unwinding double helices in the system, resulting in the steepest slope of $G'$ in Fig. 4 before the peak temperature $T'_{p}$.

Fig. 4. A model of the swelling of amylopectin. Water is indicated by gray circles.
4. CONCLUSION

Bread baking and rheological properties were examined for batters made from the three rice lines Nipponbare, e1, and #4019, which differ in amylose content and amylopectin architecture. The following new findings were obtained in this study:

(1) The batter made from e1 exhibits the largest expansion ratio of the three batters, and provided the best baking properties.

(2) We proposed a molecular model of the swelling of amylopectin that involves unwinding of the double helices.

(3) We propose the following relationship between the behavior of the storage modulus $G'$ during baking and the molecular architecture.
   a) The initial value of the storage modulus $G'$ increases as the amylose content increases.
   b) A larger number of amylopectin helices results in a more rapid increase in the storage modulus $G'$.

REFERENCES