Development of Rapid Measuring Method on Rice-cake Hardness in Waxy Rice

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The hardening of waxy (or glutinous) rice-cakes is one of the important processing properties in the manufacture of rice-cakes (mochi) and rice-crackers (Arare and Okaki), because the hardness of rice-cakes is greatly influenced by the refrigeration period before cutting (Yanase et al. 1982b). Waxy rice-cake hardness is generally evaluated by the bending degree of the rice-cake after cooling for a definite time (Egawa and Yoshii 1990, Arisaka 1994, Yamashita 1995). In addition to this method, penetration resistance measured with a tensipresser (Egawa and Yoshii 1990), hardness measured with a texturometer (Yanase et al. 1982a) and with a fruit hardness tester (Okamoto and Nemoto 1998), cutting resistance (Saito and Baba 1966), etc. have been used to measure the hardness.

However, these methods require a large amount of grains, at least 1kg of brown rice and long time, over 20 hours of refrigerating before measurement, and are unsuitable for testing the hardness in early generation breeding programs when hundreds of individuals are tested. Thus, to estimate the hardness of rice-cake more conveniently, the pasting temperature which was highly correlated with the hardness of rice-cakes (Yanase et al. 1982a, 1982b), has been measured with a viscoograph (Arisaka 1994, Matsue et al. 2002). In this method, the sample amount of 50 g is sufficient, but about 100 min is necessary to measure one sample and the vessel must be cleaned up before the next test. The Rapid Visco Analyzer (RVA-3D, Newport Scientific Pvt. Ltd., NSW, abbreviated as RVA hereafter) recently developed can measure the pasting temperature simply, easily and rapidly compared with the viscoograph.

On the other hand, Ishizaki (1994) developed a simple method to measure the hardness of rice-cake using a boiled rice-flour dough of 20 g. The validity of this method has been reported elsewhere (Kobayashi et al. 1999). However, special instruments and a great deal of skill are required for preparing the rice-cake. A more efficient method for measuring rice-cake hardness in early generation breeding is absolutely necessary for breeding a superior waxy rice variety.

In this study, a simple, rapid and easy method of preparing the rice-cake for measuring hardness was developed, and the hardness was evaluated with a tensipresser. This method is denoted as the “rapid method” hereafter. The results obtained by this method will be compared with the pasting temperature which is known to correlate with the rice-cake hardness. In addition, the method of evaluating the rice-cake hardness for individual plant will also be reported.

Plant materials

The stock seeds of Wataboushi and Koganemochi, cultivated at Niigata Crop Research Center in 2000 were used to determine measurement conditions. In order to confirm the effectiveness of the rapid method, we tested the rice-cake hardness of 39 varieties/strains cultivated in 2001 in the same field under the same crop management at the Center. Out of 39 varieties/strains, 4 varieties with different rice-cake hardness, i.e., Koganemochi (very high), Himenomochi (medium), Miyatamamochi (low) and Hiyokumochi (very low) (Ishizaki et al. 1995, 1996) were used for establishing the method for applying it to individual plant.

Preparation and manufacture of rice-cake

Brown rice kernels thicker than 1.85 mm were sampled, polished to reach the apparent pearling grade of 89±1% and milled with a sample mill (UDY Co. Ltd., Colorado). Rice-cake was prepared from 40 g of rice-flour passed through a 300 μm mesh screen. The moisture content of the flour was determined using a near-infrared spectrochemical analyzer (6250HON, Nireco Co. Ltd., Tokyo), and distilled water was added to the flour to make the moisture content 43%, which is the standard moisture of rice-cakes (Kobayashi et al. 2002b). The sample was mixed thoroughly by hand to prepare dough. The dough was flattened at a thickness of 8 mm using a rolling bar, and cut out a circle of 8 cm in diameter using a Mido-measuring frame (Toyo Rice Cleaning Machine Co. Ltd., Wakayama, denoted as “hardness-measuring frame” hereafter). This piece was put between the two attaching-plates and held with two clips (denoted as “hardness-measuring holder” hereafter), and heated in boiling water for 5 min in conformity with Saito and Baba (1966). After heating, the samples in the hardness-measuring holder were cooled in flowing water for 10 min, and water drops on the holder were wiped off by a dried towel, then
stored at 5°C. Before measurement, the hardness-measuring holder was left at 23°C for 1 hour (Fig. 1).

**Determination of measurement conditions**

A tensipresser (TTP-50BX, Takemoto Electric Inc., Tokyo) was set up under the conditions as shown in Table 1, and the hardness of rice-cake was measured at 3, 6, 12, 18 and 24 hours during refrigeration. The pressing thickness (distance between the head of a plunger of the tensipresser and the basal attaching-plate, technically termed the “clearance”) was set at 4.5 mm and the rice-cake was bit 25 times at different spots (Fig. 1).

At all refrigerating times, 3 to 24 hours, the rice-cake of Koganemochi was harder than that of Wataboushi, and the former hardened in a shorter time. Thus, it was considered that refrigerating for 6 hours was sufficient for measuring the hardness of rice-cake (Fig. 2).

For each rice-cake, the mean of the measured values, standard deviation and coefficient of variation were calculated to examine the variation of hardness at 25 different spots.
on the rice-cake in the hardness-measuring frame. The biting time required for estimating the mean value at a ±5% level (Snedecor and Cochrane 1989) was calculated as 1.1 to 4.4. This result indicated that biting at 5 different spots on the rice-cake was sufficient. The coefficient of variation for the values at 25 spots was small (Table 2). It may be considered that the rice-cake was prepared homogeneously and that almost the same value was obtained by measuring at any portion.

After the measurement, to confirm the homogeneity of individual rice-cakes, the rice-cakes were dried at 135°C for 3 hours and their moisture contents examined. The moisture content of the rice-cakes was slightly lower than the initial value of 43%, but the variation of moisture content among individual rice-cakes was small, which indicated that the rice-cakes were prepared homogeneously (Table 2).

After 6 hours of refrigeration, the hardness was examined at 5 spots on the rice-cake was measured at the pressing thickness of 2.5, 3.5, 4.5, 5.5, 6.5 and 7.5 mm, to find the optimal pressing thickness. At the pressing thickness of 2.5 mm, the rice-cake cracked, and thus no accurate value could be obtained. The largest difference in hardness between Koganemochi and Wataboushi was observed at 3.5 mm. At the pressing thickness of over 3.5 mm, penetration depth of a plunger into the rice-cake was shallower, the measured values were low, and the difference between the two varieties was decreased (Fig. 3).

Thus, the conditions for the “rapid method” was determined as follows: measure at 5 arbitrarily spots on the rice-cake after refrigerating for 6 hours with the pressing thickness of 3.5 mm.

Effectiveness of the rapid method

Studies using a conventional method revealed that the pasting temperature showed a highly significant positive correlation with the hardness of rice-cake (Yanase et al. 1982a, 1982b, Arisaka 1994, Okamoto and Nemoto 1998, Matsue et al. 2002). In this study, the pasting temperature was investigated with the RVA. Firstly, 25 ml of aqueous solution of 500 ppm of copper sulfate was added to 3.5 g each of polished rice-flour, and the mixtures were left for two hours. Then, the pasting temperature was measured with the RVA in conformity with Toyoshima et al. (1997), and the relationship between the pasting temperature and the rice-cake hardness measured by the rapid method was investigated.

Hardness of rice-cake was measured for the rice-cake of 39 waxy rice varieties/strains produced in 2001 under optimal conditions as mentioned above (Table 3). Heading date of the plant materials varied from extremely early to extremely late in Niigata prefecture, Japan. The hardness of rice-cake measured by the rapid method was higher when the heading date became earlier, except for Chugokumochi 120. It was enabled to distinguish Akunemochi, the latest maturing variety, showed extremely low hardness of rice-cake.

In this study, also, a highly significant positive correlation was observed between the pasting temperature and hardness of rice-cake determined by the rapid method (Fig. 4).

Table 1. Measuring conditions in a tensipresser

<table>
<thead>
<tr>
<th>Items</th>
<th>Set up conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plunger</td>
<td>10φ (made of aluminum)</td>
</tr>
<tr>
<td>Load cell</td>
<td>50kg</td>
</tr>
<tr>
<td>Distance</td>
<td>30.0 mm</td>
</tr>
<tr>
<td>Initial thickness</td>
<td>15.0 mm</td>
</tr>
<tr>
<td>Pressing thickness1</td>
<td>Investigating on the several conditions</td>
</tr>
<tr>
<td>Repeat time</td>
<td>1</td>
</tr>
<tr>
<td>Static time</td>
<td>0</td>
</tr>
<tr>
<td>Bite speed</td>
<td>5 mm/sec.</td>
</tr>
<tr>
<td>Multiply</td>
<td>1</td>
</tr>
<tr>
<td>Magnificant (+)</td>
<td>1.0</td>
</tr>
<tr>
<td>Magnificant (−)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

1) Technically referred to as “Clearance”.

Fig. 2. Relationship between refrigeration time and hardness of rice-cake.

Fig. 3. Relationship between pressing thickness and hardness of rice-cake.
It may be said that the hardness of the boiled rice-flour dough manufactured by the rapid method shows the same traits as that of rice-cakes produced by the conventional method. Accordingly, it is concluded that hardness of rice-cake measured by the rapid method is equal to that measured by the previous methods (Table 4).

The hardness of the rice-cake prepared from brown rice was also measured by the rapid method, and the relationship between the hardness of the rice-cakes prepared from brown and polished rice was investigated. The hardness of the brown rice-cake prepared from brown rice was about 1/2 of that prepared from polished rice (Fig. 5), but a highly significant positive correlation was observed between them ($r = 0.899, p < 0.001$). A highly significant positive correlation ($r = 0.734, p < 0.001$) was also observed between the pasting temperature of polished rice and the hardness of brown rice-cake determined by the rapid method (Table 4).

Thus, it was concluded that measurement of the rice-cake hardness by the rapid method was applicable to the brown rice-cakes. By omitting the polishing work, the time required for the preparation of rice-cake was considerably shortened, and the measurement of the hardness of rice-cake in an early generation breeding program became possible.

In the rapid method, the sample amount and the measuring time were greatly reduced compared with other methods using a rice-cake making machine (Table 4). In addition, the rice-cake is easily prepared using a hardness-measuring frame and holder (Fig.1), a tensipresser has been widely used to evaluate the texture of cooked rice, and thus many rice breeding stations in Japan are equipped with them. The results obtained by the rapid method are sufficiently precise for the test of specific character, and over 60 samples per day can be measured by this method. Therefore, this rapid method may be widely used as an efficient procedure for breeding the waxy rice varieties with outstanding processing properties.

### Table 2. Homogeneity of rice-cakes and sufficient number of biting times

<table>
<thead>
<tr>
<th></th>
<th>3 hr$^1$</th>
<th>4 hr $^2$</th>
<th>6 hr</th>
<th>12 hr</th>
<th>18 hr</th>
<th>24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB$^3$</td>
<td>KM$^3$</td>
<td>WB</td>
<td>KM</td>
<td>WB</td>
<td>KM</td>
<td>WB</td>
</tr>
<tr>
<td>Average of hardness (kg cm$^{-2}$)</td>
<td>6.6</td>
<td>9.3</td>
<td>13.3</td>
<td>19.9</td>
<td>24.0</td>
<td>27.5</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.21</td>
<td>0.37</td>
<td>0.44</td>
<td>0.91</td>
<td>0.65</td>
<td>1.25</td>
</tr>
<tr>
<td>Coefficient of variation (%)</td>
<td>3.1</td>
<td>4.0</td>
<td>3.3</td>
<td>4.6</td>
<td>2.7</td>
<td>4.6</td>
</tr>
<tr>
<td>Moisture contents of rice-cake (%)</td>
<td>42.2</td>
<td>42.4</td>
<td>42.4</td>
<td>42.0</td>
<td>42.4</td>
<td>42.5</td>
</tr>
</tbody>
</table>

Number of biting times

1) Biting frequency to presume on Average ±5% for 95% probability ($n = (1.96\sigma)^2/L^2$, Snedecor and Cochran 1989).
2) Refrigerating time (hours).
3) WB: Wataboushi, KM: Koganemochi.

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![Fig. 4](image1.png)  
**Fig. 4.** Relationship between hardness of rice-cake and pasting temperature.  
** *** Significant at 0.1% level.

![Fig. 5](image2.png)  
**Fig. 5.** Relationship between the hardness of rice-cakes prepared from polished and brown rice.  
** *** Significant at 0.1% level.
of rice-cake for the rapid method, and in some cases this amount of sample can not be obtained from individual plants. In order to evaluate the rice-cake hardness in such cases, we added the sample of Hiyokumochi with very low rice-cake hardness to the sample to be examined, and used this mixture samples for measurement. This method was applied for three varieties (Koganemochi, Himenomochi and Miyatamamochi) with different hardening characteristics (Fig. 4). The hardness of the three varieties decreased rapidly, and the difference among varieties also decreased as the percentage of Hiyokumochi increased. It was impossible to discriminate Himenomochi from Miyatamamochi after adding the sample of Hiyokumochi, but it was possible to discriminate Koganemochi due to its high hardening characteristic even after adding the sample of Hiyokumochi up to 50 % (Fig. 6). Brown rice yielded from one individual plant is usually about 20 g in the hybrid population, the rapid method is possible to distinguish individual plant with the hardening characteristic as high as Koganemochi. If Akunemochi with extremely low rice-cake hardness (Fig. 4) was added to the sample, the varietal differences may be discriminated more clearly.

It was reported that 35 g or more samples could be obtained from one individual plant under sparse planting (Hoshi et al. 1995). Therefore, obtaining as much amount of the brown rice sample as possible under sparse planting and adding at minimum percentage sample of some specific variety with extremely low hardness of rice-cake, are neces-
sary for the sure test of specific character on individual plant. Then, the scheme of the rapid method to evaluate the rice-cake hardness of individual plants is shown in Fig. 7.

Genetic and breeding research on rice-cake hardening using the rapid method

An extremely large variation of rice-cake hardness in the waxy rice varieties was demonstrated in this study (Fig. 4). In general, the higher rice-cake hardness became the higher the ripening temperature (Matsue et al. 2002). However, Chugokumochi 120 showed an extremely hard rice-cake compared with the Koganemochi regardless of its late maturity (Fig. 4, Table 3). This characteristic of Chugoku-

| Table 4. Measuring methods for hardening rate of rice-cake, and relationship between hardness of rice-cake and pasting temperature |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Literature      | Sample amount (g) | Rice-cake processing method | Measurement rice-cake size (cm) | Refrigerating conditions | Hardness measurement instrument | Pasting temperature measurement instrument | Year of study | Correlation coefficient | Multiple correlation coefficient | Sample number |
| Yanase et al. (1982b) | 1,250 | Rice-cake making machine | Diameter: 3.0, Thickness: 0.5 | 3°C, 24 hr | Texturemeter | Viscograph | 1975-77 | 0.800** | 0.640 | 18 |
| Arisaka (1994) | 4,500 | Rice-cake making machine | Thickness: 1.0 | 5°C, 24 hr | Tensipresser | Viscograph | 1994 | 0.950** | 0.903 | 12 |
| Okamoto and Nemoto (1998) | 650 | Rice-cake making machine | Diameter: 2.5, Thickness: 1.0 (52%-moisture content) | 4°C, 10 hr | Fruit hardness tester | Rapid visco analyzer | 1995 | 0.663* | 0.440 | 11 |
| Matsue et al. (2002) | 1,000 | Rice-cake making machine | Diameter: 50.0 × 5.0 × 1.5, Thickness: 2.5 | 5°C, 22 hr | Bending degree of rice-cake | Viscograph | 1999 | -0.611** | 0.373 | 18 |
| Ishizaki (1994) | 20 | Boiling rice-flour dough | Diameter: 8.0, Thickness: 0.8 (43%-moisture content) | 5°C, 6 hr | Tensipresser | Rapid visco analyzer | 2001 | 0.854*** | 0.729 | 39 |
| Ishizaki et al. (1995) | 1) | Boiling rice-flour dough | Diameter: 2.5, Thickness: 1.0 (52%-moisture content) | 5°C, 24 hr | Tensipresser | Viscograph | 1993 | 0.907*** | 0.822 | 24 |

1) In order to ensure the precision, 4 rice-cake pieces are made from 20 g kernels each.
2) Polished rice was used.
3) Brown rice was used.

* *, **, ***: Significant at 5, 1, 0.1% levels, respectively.

Fig. 6. Effect of the addition of Hiyokumochi sample on hardness of the rice-cakes of other varieties.

Fig. 7. Scheme of the rapid method to evaluate on the individual plant.
mochi 120 has already been reported by Arisaka (1994) and Kobayashi (2000). This same characteristic has already been known in Kantomochi 172 which was a progeny of the cross of African variety IRAT109 (Okamoto and Nemoto 1998, Okamoto et al. 2002) and in a mutant waxy rice variety induced from \textit{O. glaberrima} (Kobayashi 2002a).

Thus, intraspecific and interspecific variations of rice-cake hardness have been observed in waxy rice. However, the modes of inheritance and the mechanisms of rice-cake hardness have not been well-known yet. Consequently, the rapid method developed in this study may useful for the genetic or breeding research on rice-cake hardening, because it is simple, is rapidly performed and requires a smaller amount of samples than the previous methods giving the results with equal precision.

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Literature Cited


