Starch Properties of *Waxy* Rice Cultivars Influencing Rice Cake Hardening

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Hardening process in the manufacture of rice cakes is the most important process, because it is time-consuming process. We aimed to develop a new cultivar of *waxy* rice characterized by both high yield and excellent processing properties. For this purpose, the urea dissolution and X-ray diffraction (XRD) methods were employed in order to quantitatively assess the factors associated with rice cake hardness. In this study, rice starch dissolution rate and measurement of relative starch crystallinity (RSC) are discussed. Four *waxy* rice cultivars with different hardening properties were selected as test samples, and pasting properties, hardness, urea dissolution of starch and XRD properties were assessed. It is supposed that dissolution rate and RSC are closely related to rice cake hardening, are suitable parameters for quantifying rice cake hardening, and are useful for predicting the hardness of new novel cultivars. To assess rice cake hardness in novel cultivars, it is important that tests focusing on the determination of different parameters, such as pasting properties, urea dissolution and XRD, be designed.

**Key words:** *waxy* starch, rice cake, urea dissolution, starch crystallinity, hardening rank

1. INTRODUCTION

In Japan, the annual rice yield is 292,000 metric tons, and 3% of this is harvested in Akita Prefecture [1]. The cultivation area for *waxy* rice in Akita Prefecture is about 1,500 ha, with the major cultivars being “Tatsukomochi” and “Kinunohada”, which have been promoted by the Akita Agricultural Experiment Station. While these rice cultivars are characterized by high yield, they are of poor quality for rice cake production, as compared to the most well-known *waxy* rice cultivar, “Koganemochi” [1].

Hardening process in the manufacture of rice cakes is the most important process, because it is time-consuming process. For this reason, “Koganemochi” is considered to be the best *waxy* rice cultivar in Japan.

We are currently engaged in the development of a new *waxy* rice cultivar that offers processing properties similar to “Koganemochi”. Several studies related to the assessment of rice cake hardening have been reported, including the measurement of the degree of bending of rice cakes, as reported by Egawa *et al.* [2], the penetration resistance test using a compression tester or fruit hardness tester [2-4], and the thermophysical method using a viscograph or rapid visco-analyzer [4,5]. Sato *et al.* reported that rice cake hardening properties, assessed using a rapid visco-analyzer, were proportional to pasting and peak temperatures, as well as to minimum and final viscosities [6]. Igarashi *et al.* showed that it was possible to conduct analysis using amylopectin chain distribution [7]. Thus, although studies for determining rice cake hardening properties have been performed, all of the reported methods require numerous rice samples of more than several hundred grams.

In contrast, Nishi *et al.* [8] and Okamoto *et al.* [9] reported the urea dissolution method, which requires only a dozen grams of rice to determine the rate of rice cake hardening. This method has been further developed by other researchers. Sato *et al.* examined the urea dissolution method using a small quantity of rice and
reported that it could be used to assess hardness, as it shows differences among varieties and correlates with gelatinization temperature [6]. The urea dissolution method is superior to the bending and penetration resistance tests with regard to the amount of sample required. Thus, the urea dissolution method has some advantages, but it has conventionally been used to study only one condition in previous studies. For a more accurate and general assessment of rice cake hardening using the urea dissolution method, it is necessary to gather more data under multiple experimental conditions.

We aimed to develop a new cultivar of waxy rice characterized by both high yield and excellent processing properties. For this purpose, the urea dissolution reaction and XRD analysis were employed in order to quantitatively assess the rice cake hardening. In this paper, we discuss the applicability of the urea dissolution rate and relative starch crystallinity (RSC) for assessment of rice cake hardening.

2. MATERIALS AND METHODS

2.1 Materials

Four waxy rice cultivars (Koganemochi, Otonemochi, Dewanomochi and Himenomochi) harvested by the Akita Agricultural Experiment Station in 2009 were used as samples. These cultivars are popular in Akita, and were selected as a typical standard of the hardening rank. Rice grains were sieved with a 1.85-mm open sieve, and were polished up to 90 ± 0.5% by weight using a test polisher (Toyo Co., Ltd., Wakayama, Japan). Only defect-free grains were used.

EDTA · 2 Na, urea, iodine, potassium iodine and calcium fluoride were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.2 Instrumental hardness measurement

In a mechanical compression test of rice cake, rice (320 g) was soaked in water for 6 h at room temperature, steamed for 25 min and kneaded in a rice cake maker (PFC–20FK, Toshiba Corporation, Tokyo, Japan) for 13 min. Next, the rice was molded into a rectangular shape (55, 200, 15 mm, w×l×d) and chilled for 24 h at 15°C. Mechanical compression was carried out using a food rheometer (RE–33005, Yamaden, Tokyo, Japan). Samples were compressed to 50% strain by a plunger (8 mm diameter, non–sticky Teflon) at 1 mm/s. The height of the force peak was defined as hardness. The mean value of a series of 10 tests was calculated and used for analysis.

2.3 Measurement of pasting properties

Pasting properties of rice flour were analyzed using a Rapid Visco Analyzer (RVA–4, Newport Scientific Pty. Ltd., Warriewood, Australia). Milled rice flour (3.5 g) was mixed with 25.0 g of 0.01 mol EDTA · 2 Na in an RVA sample cup. A programmed heating and cooling cycle was adopted. The temperature condition during measurement was as follows: 50°C for 1 min, heated to 95°C in 4 min, maintained at 93°C for 7 min, cooled to 50°C in 4 min and maintained at 50°C for 3 min. Pasting temperature, peak viscosity (PV), minimum viscosity (MV), and final viscosity (FV) were recorded. Breakdown (BD) and consistency (CS), respectively, were derived from the following equations: BD = (PV − MV) and CS = (FV − MV). Viscosity parameters were expressed in rapid viscosity units (RVU), which are typically used in RVA tests. Tests were repeated five times.

2.4 Urea dissolution method for rice starch

Rice grains were soaked in distilled water for 1.5 h, so as to control for the time lag of the urea dissolution reaction. Twenty rice grains were placed into a φ9-cm petri dish, and 20 ml of 4.5 M urea (pH 6.0) was added. Petri dishes were placed in an incubator at 30°C, 25°C, 20°C or 15°C for 2, 10, 16, or 24 h. All tests were performed in duplicate and the results averaged. After incubation, 0.5 mL of 0.2% I2/2% KI solution was added to a petri dish and gently stirred. The reaction solution was diluted 10-fold and centrifuged at 10,000 rpm for 5 min. Absorbance at 530 nm was measured by a spectrophotometer (DU7500, Beckman Coulter Inc., CA, USA). All tests were performed in duplicate and the results averaged.

2.5 XRD analysis

Rice samples were ground into powder and passed through a 75-μm sieve. Next, 5% (w/w) calcium fluoride was added as an internal standard [10], followed by thorough mixing. Nara et al. reported that RSC was influenced by the moisture content of starch, with RSC increasing proportionally to moisture content [11]. McPherson et al. previously reported the usefulness of moisture content equilibration in starch samples [12]. In accordance with their report, our samples were hydrated to 98% relative humidity by incubation in a sealed vessel, thus equalizing the moisture content. The vessel was kept at 25°C for 24 h. Moisture contents of samples were measured using an infrared moisture tester (FD–610; Kett Electric Laboratory, Tokyo, Japan), and were found
Starch properties for rice cake hardening

XRD measurement was carried out using an ultraX 18VB2 diffractometer (Rigaku Corp., Tokyo, Japan). Each sample was packed tightly in a rectangular glass cell (50 × 35 mm, thickness 0.5 mm) and exposed to an X-ray beam at 50 kV and 27 mA. The scanning regions of the diffraction angle 2θ were 10° to 35°. The other operation conditions were as follows: scanning rate, 2°/min, sampling width, 0.02°, and divergence slit size 1°. RSC was calculated using Eq. (1)

$$RSC = \frac{(TD - BG)}{IS}$$  (1)

where TD, BG and IS are the total area of diffractogram, background area and area of internal standard, respectively. Data smoothing procedures were performed using a moving average method and backgrounds were calculated using Sonneveld-Visser’s method with a 0.10-peak width and 0.001 intensity limit. XRD analysis was carried out in triplicate.

2.5 Statistical analysis

Statistical analysis system software package (STATISTICA 06J, StatSoft Japan Inc., Tokyo, Japan) was used for analysis of variance.

3. RESULTS AND DISCUSSION

3.1 Pasting properties and instrumental hardness measurement

Table 1 describes the pasting properties and hardness of the test samples. The pasting temperature of the four cultivars ranged from 70.2 to 71.2°C; Koganemochi and Himenomochi differed significantly from the other samples (P < 0.05). The peak viscosity of Himenomochi and Dewanomochi was 542 and 589 RVU, respectively, which was significantly different from the other samples (P < 0.05). With respect to minimum viscosity, the lowest value was recorded for Himenomochi, but there were no significant differences among the other three cultivars. The highest final viscosity was observed in Otomemochi, and the lowest was observed in Himenomochi. The breakdown properties of the four cultivars showed the same numerical order as for peak viscosity.

Table 1   Pasting properties and hardness value of four waxy rice cultivars.
<table>
<thead>
<tr>
<th>Cultivar</th>
<th>PT (°C)</th>
<th>PV (RVU)</th>
<th>MV (RVU)</th>
<th>FV (RVU)</th>
<th>FV (RVU)</th>
<th>BD (RVU)</th>
<th>CS (RVU)</th>
<th>HN (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koganemochi</td>
<td>70.4±0.20</td>
<td>608±3.62</td>
<td>242±2.69</td>
<td>342±1.10</td>
<td>365±2.78</td>
<td>99±2.19</td>
<td>19.9±0.58</td>
<td></td>
</tr>
<tr>
<td>Otomemochi</td>
<td>71.0±0.32</td>
<td>618±11.46</td>
<td>247±3.70</td>
<td>360±6.56</td>
<td>370±7.98</td>
<td>113±3.34</td>
<td>18.5±0.89</td>
<td></td>
</tr>
<tr>
<td>Dewanomochi</td>
<td>71.2±0.33</td>
<td>589±2.73</td>
<td>244±2.28</td>
<td>345±0.53</td>
<td>345±2.64</td>
<td>101±2.11</td>
<td>14.7±0.58</td>
<td></td>
</tr>
<tr>
<td>Himenomochi</td>
<td>70.7±0.04</td>
<td>542±2.32</td>
<td>215±2.20</td>
<td>315±1.43</td>
<td>327±3.20</td>
<td>100±2.94</td>
<td>10.1±0.53</td>
<td></td>
</tr>
</tbody>
</table>

PT, pasting temperature; PV, peak viscosity; MV, minimum viscosity; FV, final viscosity; BD, breakdown; CS, consistency; HN, hardness. Pasting properties and HN values are the mean values of five and ten replicates, respectively. *RVU, Rapid Visco-Analyzer Unit,1 RVU=12 mPa·s; Values are means±standard deviations.

Sato et al. studied the pasting properties of a number of waxy rice cultivars and reported that hardness was positively correlated with gelatinization temperature, peak temperature, minimum viscosity, final viscosity and consistency; however, breakdown was negatively correlated with RVA properties [6]. Significant differences (P < 0.05) in hardness were observed in the four cultivars, which showed strong positive correlations with breakdown and peak viscosity, but a weak negative correlation with pasting temperature.

2.5 Statistical analysis

Statistical analysis system software package (STATISTICA 06J, StatSoft Japan Inc., Tokyo, Japan) was used for analysis of variance.
molecular conformation. The amount of dissolved starch is thought to increase in proportion to temperature and time. The dissolution reaction could be described by zero-order Eq. (2)

\[ \frac{dC}{dt} = R \]  

(2)

where, \( C \) [\( \mu g \cdot ml^{-1} \)] is concentration of dissolved starch, \( t \) [h] is time and \( R \) [\( \mu g \cdot ml^{-1} \cdot h^{-1} \)] is dissolution rate.

Figure 1 shows the time course of soluble starch concentrations at 15–30°C of Koganemochi. Coefficients of determination for regression line at 15, 20, 25 and 30°C were 0.85, 0.99, 0.99 and 0.97, respectively. We defined the regression coefficient to be the dissolution rate. Dissolution rates of the four cultivars are listed in Table 3. All coefficients of determination for the four cultivars were higher than 0.83.

The conventional urea dissolution method has been tested under only one condition for example, at room temperature for 12 h in previous studies. We believed that for a more accurate and general assessment of rice cake hardening, it was necessary to test under multiple experimental conditions. The dissolution rate defined in this study is given by the slope of regression line which consist of four data points; thus, the measurement errors can be eliminated to a certain degree.

### 3.3 XRD analysis

XRD analysis has been typically used to characterize the crystalline structure of starch. We assumed that starch crystallinity was related to its dissolution in urea and rice cake hardening. In this analysis, we controlled for the particle size and moisture content of rice powder samples, and calcium fluoride was added as the internal standard to quantify the amount of starch crystal as accurately as possible. Figure 2A shows the raw diffractionograms of four samples and 2B shows the smoothed and background-subtracted diffractionograms. RSCs were generated from the area of the background-subtracted diffractionograms. All diffractograms showed an A-type pattern and the IS peak appeared at 28.2° (2θ).

![Fig. 1](image1.png)

**Fig. 1** The time course of soluble starch concentrations at 15–30°C of Koganemochi. The regression coefficient is defined to be the dissolution rate. ◇, 15°C; □, 20°C; △, 25°C; ×, 30°C.

| Table 3 Dissolution rates for waxy rice cultivars at 15 to 30°C. |
|-------------------|----------------|---------------|----------------|----------------|
| Dissolution rate   | Koganemochi    | Otomemochi    | Dewanomochi    | Himenomochi    |
| Temperature [°C]   |                |               |                |                |
| 15                 | 0.53           | 0.53          | 0.41           | 0.68           |
| 20                 | 1.65           | 1.36          | 1.86           | 3.24           |
| 25                 | 5.66           | 5.80          | 6.79           | 7.47           |
| 30                 | 9.88           | 7.34          | 7.06           | 10.50          |

![Fig. 2](image2.png)

**Fig. 2** XRD diffractograms of four rice cultivars. Raw diffractionograms in Fig. 2A; a, Koganemochi; b, Otomemochi; c, Dewanomochi; d, Himenomochi. Smoothed and background-subtracted diffractionograms in Fig. 2B; e, smoothed diffractogram of Koganemochi; f, background-subtracted diffractogram of Koganemochi. IS refers to the internal standard (CaF₂).
RSCs of the four cultivars are listed in Table 4. The highest RSC was recorded in Koganemochi, which had excellent processing properties, especially with respect to rice cake hardening. The lowest RSC was recorded in Himenomochi, which had the lowest rice cake hardening rank in this experiment.

Table 4  RSC of four rice cultivars.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>RSC* (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koganemochi</td>
<td>745</td>
</tr>
<tr>
<td>Otomenochi</td>
<td>658</td>
</tr>
<tr>
<td>Dewanomochi</td>
<td>670</td>
</tr>
<tr>
<td>Himenomochi</td>
<td>602</td>
</tr>
</tbody>
</table>

*RSC values are the means of three replicates.

3.4 Relationship between RSC, rice cake hardness, pasting properties and urea dissolution properties

A number of reports have discussed the relationship between starch dissolution in urea and rice cake hardening; however, the rice starch dissolution rate in urea has not yet been reported. We therefore aimed to use this approach to develop a new rice cake hardening test that requires fewer than 100 grains of rice. Correlations between dissolution rate and BD, hardness and RSC are listed in Table 5. Dissolution rate at 15°C (R15) and 30°C (R30) did not show higher correlation coefficients, while dissolution rate at 20°C (R20) and 25°C (R25) showed higher correlation coefficients with BD and HN.

Sato et al. reported that BD was closely related to rice cake hardness [6], therefore BD was often used as an index of rice cake hardness. As shown in Table 5, BD showed close relationships with R25. Based on these results, it is possible that BD is estimated from R25. From the above-mentioned facts, it is considered that the most suitable temperature for the urea dissolution test is 25°C.

Rice cake hardening has been discussed from the point of view of pasting properties and/or amylopectin chain length distribution. Okamoto et al. reported that amylopectin chain length corresponded to pasting properties and rice cake hardening rate [14]. However, there are no experimental methods that can exactly determine the rice cake hardening rank via a single method.

Figure 3A shows relationship between R25 and hardness rank. Hardness rank was quoted from the literature [13], with the hardest rice cake being classified as “Rank 1”. Spearman rank correlation coefficient was 0.88 (P < 0.05). The relationship between RSC and HN is shown in

Table 5  Correlations between dissolution rate and BD, HN and RSC.

<table>
<thead>
<tr>
<th>Correlation coefficient</th>
<th>Dissolution rate</th>
<th>BD</th>
<th>HN</th>
<th>RSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>R15</td>
<td></td>
<td>-0.44</td>
<td>-0.50</td>
<td>-0.52</td>
</tr>
<tr>
<td>R20</td>
<td>-0.93*</td>
<td></td>
<td>-0.92*</td>
<td>-0.70</td>
</tr>
<tr>
<td>R25</td>
<td>-0.97**</td>
<td>-0.97**</td>
<td>-0.77</td>
<td></td>
</tr>
<tr>
<td>R30</td>
<td>-0.42</td>
<td>-0.32</td>
<td>-0.06</td>
<td></td>
</tr>
</tbody>
</table>

* and ** indicate significance at P < 0.05 and P < 0.01 levels, respectively.
Figure 3B. The strong positive correlation was observed (r = 0.92, P < 0.05). RSC is an index of crystalline region content; therefore, rice with a high RSC value contains small amounts of amorphous regions of starch. In a urea dissolution reaction, amorphous regions will dissolve more easily than crystalline regions. Our determination of the relationship between RSC and the dissolution rate is in agreement with this. Comparing the density of crystalline and amorphous regions, it is clear that crystalline regions indicate high density. We considered that RSC is proportional to rice cake hardness. The same theory applies to the result of the relationship between RSC and the dissolution rate. Liang et al. reported that the crystallinility of starches measured by XRD was related to their resistance to enzymatic digestion, and these starches were shown to have fiber-like properties in the body [15].

4. CONCLUSIONS

Hardening of rice cake could be well correlated with two physical properties of RSC and R25. The hardening of rice cake might be affected with two properties. To assess rice cake hardness in novel cultivars, it is important that tests focusing on the determination of different parameters, such as pasting properties, urea dissolution and XRD, be designed.

NOMENCLATURE

XRD : X-ray diffraction
RSC : relative starch crystallinity
C : concentration of dissolved starch, μg · ml⁻¹
R : dissolution rate, μg · ml⁻¹ · h⁻¹
t : time, h⁻¹
TD : total area of diffractogram
BG : background area of diffractogram

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モチ硬化性に影響を及ぼす糯澱粉の理化学特性

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モチの製造工程において、その硬化工程は最も長い
作業時間を要するため作業効率上重要な工程となって
いる。我々は、収率が高く加工特性にも優れた新しい
糯品種の育成を目指しており、この目的のために糊化
特性試験、尿素溶解法およびX線回折法を用いてモチ
硬化特性を定量的に評価するための方法を検討した。
本研究では、糯澱粉の尿素溶解度を測定し、糊化特
性値および相対澱粉結晶化度とモチ硬化性との関係に
ついて考察した。従来の尿素溶解法は、室温下で一定
溶解時間後の溶解度のみでモチ硬化性の評価を行なっ
てきたが、本研究では経時的に溶解度を測定しその溶
解速度を評価指標とする方法を導入した。その結果、
評価の精度と汎用性を高めることができたものと考え
ている。糯品種別溶解速度および相対澱粉結晶化度は
糯品種の硬化特性と密接な関係があり、硬化特性が明
らかになっていない糯品種の硬化ランクを推定するた
めの有効なパラメータとなりうるものと考えられる。