Iodine Absorption Scanning Analysis of the Digestibility and Gelatinization Properties of Koshitanrei Rice Cultivar for Sake Brewing

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The “Koshitanrei” rice cultivar is grown and widely used for sake brewing in Niigata Prefecture; however, the starch characteristics of this cultivar have not yet been elucidated. Therefore, we investigated the influence of starch properties on the gelatinization properties and digestibility of this cultivar, which are very important in sake production. Starches of rice samples milled to 60% yield from “Koshitanrei,” “Yamadanishiki,” “Gohyakumangoku,” and “Koshiibuki” were subjected to iodine colorimetric scanning analyses and pasting properties were investigated using a rapid viscosity analyzer. The correlation between iodine absorption and digestibility was analyzed following steaming of the rice samples. The findings of our study showed that the maximum wavelength of the iodine absorption curve (λmax), which is derived from the chain length distribution of amylopectin, of Koshitanrei was the same as for Yamadanishiki, followed by Gohyakumangoku and Koshiibuki. Further, we found that λmax was significantly correlated with enzyme digestibility. The results of our study show that enzyme digestibility can be estimated by a simple evaluation method, that is, iodine absorption scanning analysis.

Keywords: sake rice, Koshitanrei, enzyme digestibility

Introduction

Sake has long been brewed in Japan. Niigata Prefecture, in particular, is one of the major production areas of rice, and is widely known for its sake production. Given that sake is brewed using rice and water as raw materials, the selection of rice for sake brewing is critical. In sake brewing, special rice grains that are larger than the typical palatable rice grains for table rice, and shinpaku, which forms the white central part of the rice, are used.

The main rice cultivars used for sake brewing are “Yamadanishiki,” “Gohyakumangoku,” “Omachi,” “Kamenoo,” “Takanenishiki,” and “Miyamanishiki,” as each prefecture cultivates its own variety of sake rice to establish the sake’s identity. The Yamadanishiki cultivar has large grains with a central white starch core (shinpaku). This cultivar is highly prized for its quality. Yamadanishiki was registered as a new variety in 1936, and it was developed in the Hyogo Prefectural Agricultural Experiment Station by crossing “Yamadaho” and “Tankan-Wataribune” (Ikegami et al., 2005). Gohyakumangoku was registered as a new variety in 1957, and it was cultivated at Niigata Prefectural Agricultural Experiment Station by crossing “Sin200gou” and “Kikusui” (Yoshida, 2012). Unlike Yamadanishiki, Gohyakumangoku is widely cultivated throughout Japan because it can be cultivated under cold climatic conditions. However, the rice grains of Gohyakumangoku tend to break during polishing, resulting in low milling yield.

While Yamadanishiki and Gohyakumangoku are commonly used in sake brewing, many regions in Japan are developing new and improved sake rice cultivars. In sake brewing, specific cultivars of sake rice and microorganisms
Materials and Methods

**Rice samples** We used three cultivars of sake rice and a highly palatable rice cultivar. For sake brewing, we used “Koshitanrei,” “Gohyakumangoku,” and “Yamadanishiki,” and we used a highly palatable rice variety called “Koshiibuki.” Koshiibuki is used widely for brewing sake. Samples of Koshitanrei were harvested from five regions, Gohyakumangoku was harvested from eight regions, and Koshiibuki was harvested from three regions of the respective prefectures. Yamadanishiki was provided by eight sake breweries (harvested in Hyogo Prefecture). All rice samples were harvested in 2017. The brown rice was polished to 60% (w/w) using a grinding mill (Test mill TM05C; Satake Co., Ltd., Higashihiroshima, Japan). White rice flour was prepared using a vibrating sample mill (TI-100; HEIKO SEISAKUSHO LTD., Tokyo, Japan).

**Determination of gelatinization properties using RVA** The viscosity of rice samples was analyzed using RVA to obtain the RVA profiles (RVA-4D; Newport Scientific, Warriewood, Australia). According to the standard method of Toyoshima et al. (1997), 3.5 g (moisture content 14%) of rice flour was mixed with 25 mL of water. The sequential temperature curve for the 18.8 min test was as follows: (1) incubation at 50°C for 1 min; (2) increase the temperature to 95°C and hold for 7 min; (3) cool to 50°C and hold at 50°C for 3 min. The RVA profiles were characterized by peak viscosity, hot paste viscosity, cool paste viscosity, breakdown, setback, and consistency. This analysis was performed three times.

**Iodine colorimetric scanning analysis** The apparent amylose content (AAC) of rice starch was estimated using the iodine absorption method of Juliano (1971; Landers et al., 1991). Applying this iodine absorption method, Nakamura et al. (2015) developed the iodine colorimetric scanning analysis method. They measured the absorbance of iodine-starch complexes from 200 to 900 nm and reported that the wavelength of maximal absorption (λmax) and absorbance at λmax (Aλmax) vary depending on the rice varieties. Using this method, they measured not only the amylose content but also the content of resistant starch (RS) and chain length distribution of amylopectin (Nakamura et al., 2015).

In this study, in order to elucidate the impact of the starch characteristics of sake rice on the pasting properties and digestibility of steamed rice, we performed iodine colorimetric scanning analysis, rapid viscosity analyzer (RVA) measurements of pasting properties, and a digestibility test. Further, we examined the relationships of these parameters by correlation analyses and attempted to develop estimation formulas for digestibility on the basis of the iodine colorimetric scanning analysis.
Louis, MO) and waxy rice starch (fat and proteins removed from waxy rice) were used as standard amylose and standard amylpectin, respectively, for amylose content determination.

Iodine colorimetric scanning analysis was performed using the method of Nakamura et al. (2015). The iodine absorption curve of rice starch samples was measured using a UV-1800 spectrophotometer (Shimadzu Co., Kyoto, Japan). The iodine absorption spectrum was analyzed from 400 to 800 nm. The amylose content was measured at 620 nm (Juliano, 1971), and \( \lambda_{\text{max}} \) (peak wavelength on iodine absorption curve, which shows high correlation with the length of the glucan chain; molecular size of amylose), absorbance at \( \lambda_{\text{max}} \) (\( A_{\lambda_{\text{max}}} \)), New \( \lambda_{\text{max}}, \) RS, and indexes for amylpectin micro structures (Fa, Fb, F2, Fa/Fb3) were measured (Nakamura et al., 2015). New \( \lambda_{\text{max}} \) was calculated according to the following formula: New \( \lambda_{\text{max}} = A_{\lambda_{\text{max}}} / (\lambda_{\text{max}} \text{ of sample rice starch} - \lambda_{\text{max}} \text{ of glutinous rice starch}). \) RS content was calculated according to the following formula: RS = 21.312 \times A_{\lambda_{\text{max}}} - 0.030 \times \lambda_{\text{max}} + 12.251. F2 shows the area of 400 nm to \( \lambda_{\text{max}} \). Fa was calculated according to the following formula: Fa = -11.59 \times F2 value - 10.92 \times New \lambda_{\text{max}} + 34.429. Fb3 was calculated according to the following formula: Fb3 = 44.691 \times A_{\lambda_{\text{max}}} - 0.030 \times \lambda_{\text{max}} + 0.774. This analysis was performed three times.

**Digestibility test** The digestibility test was performed using the method of Commentary on Standard Analytical Methods of National Research Institute of Brewing. The rice sample (10 g) was placed in a metal mesh cage and soaked in water at 15°C for 20 h. Thereafter, the rice sample was dewatered, spread over the cooking sheet, and steamed for 45 min in a steamer. After steaming, the steamed rice was taken out, the crude heat was removed, and the steamed rice was sealed in a plastic bag and held at 15°C for 3 h. Next, the water absorption rate of the steamed rice was measured. Subsequently, the entire steamed rice was put into 50 mL of the enzyme buffer solution (0.1 M succinic acid buffer (pH: 4.3) with 60 U/mL of α-amylase). The mixture was then incubated at 15°C for 24 h digestion. After 24 h, the mixture was filtered and the amino acid content and Brix value were measured. The Brix value was measured using a refractometer (Hand Refractometer N-type series; Atago Co., Ltd., Tokyo, Japan). Incidentally, only the enzyme buffer solution (blank) was treated using the same method, and the blank value was subtracted from the sample value. This analysis was performed three times.

**Statistical Analyses** All the results, including the significance of regression coefficients, were statistically analyzed using a t-test, one-way ANOVA, Tukey’s method, and Excel Statistics (ver. 2015; Microsoft Corp., Tokyo, Japan). We used the “increase/decrease method” to choose the most suitable variables in the multi-regression analyses.

**Results and Discussion**

**Iodine absorption** The profiles of iodine absorption scan analysis are shown in Table 1. New \( \lambda_{\text{max}} \), resistant starch (RS), F2, Fa, and Fb3 were estimated according to the methods described by Nakamura et al. (2015). The AAC of Koshitanrei was comparable to that of Yamadanishiki. Sake rice has relatively high AAC (Yoshizawa et al., 1981). In addition, AAC is related to the gelatinization properties of RVA (Toyoshima et al., 1997; Asaoka et al., 1994).

The maximum of the iodine absorption curve was different in the four rice varieties. The maximum iodine absorption of Koshitanrei and Yamadanishiki was around 580 nm, while that of Gohyakumangoku was around 575 nm, and that of Koshiibuki was around 570 nm. The amylose contents of Koshitanrei and Yamadanishiki were somewhat higher than those of the other two varieties, and this affected the wavelength at which this maximum was reached. In addition, since the amylpectin side chain structures of Gohyakumangoku were different from those of Yamadanishiki and Koshitanrei, detailed analysis is necessary in the future.

There was no significant difference in the degree of polymerization in the short chain of amylpectin (Fa) between the four cultivars; however, the degree of polymerization in the long chain (Fb3) differed among the four cultivars. The Fb3 value of Koshitanrei fell between those of Gohyakumangoku and Yamadanishiki.

**RVA profiles** The RVA profile of each rice sample is shown in Table 2, and the breakdown and pasting temperatures are shown in Fig. 1. The highest breakdown was observed in Koshiibuki, while the breakdown in Koshitanrei was similar to that of Yamadanishiki but was lower than that of Gohyakumangoku. This trend is consistent with a previous report (Aramaki et al., 2004). Since the breakdown correlates with the retrogradation property, our findings suggest that the retrogradation of Koshiibuki is high and that of Koshitanrei is about the same level as Yamadanishiki (Toyoshima et al., 1997). The highest pasting temperature was observed for Gohyakumangoku, followed by Koshiibuki, Koshitanrei, and Yamadanishiki. The pasting temperature has been reported to have a negative correlation with the Brix value in digestibility (Aramaki et al., 2004), and the same tendency was observed in this study.

**Digestibility test** The moisture content of each steamed rice sample is shown in Fig. 2, and the Brix value of each rice sample is shown in Fig. 3. There was no significant difference in the moisture content of the steamed rice among the varieties; however, Koshitanrei tended to have a lower moisture content than the other rice varieties. Koshitanrei showed slower water absorption compared to the other sake rice varieties in sake making, as shown by the results of this study.

However, no significance difference was found between the amino acid contents in the rice varieties after digestion (data not shown). The Brix value after digestion showed comparable values between Koshitanrei and Yamadanishiki, and Gohyakumangoku showed about the same value as Koshiibuki.
In 2017, the temperature during the ripening period of rice was relatively low; therefore, the Brix value was slightly higher.

It was previously reported that the starch properties and structure of rice grains, such as the white-core, affect enzyme digestibility (Okuda et al., 2006). Therefore, it will be necessary for us to examine the relationship between starch structure and digestibility in the next paper.

Correlation between analysis of iodine absorption curve, digestibility test, and RVA of 22 rice samples.

The correlation between analysis of iodine absorption curve, digestibility test and RVA of 22 rice samples is shown in Table 3. The Brix value was positively correlated with AAC (0.88), λmax (0.92), Aλmax (0.77), F2 (0.73), and Fb3 (0.77) and negatively correlated with peak viscosity (-0.92), breakdown (-0.86), breakdown/consistency (-0.77), and Fa/Fb3 (-0.69) at \( p < 0.01 \).

The scatter plot of the relationship with λmax and digestibility is shown in Fig. 4. From this result, the Brix value was estimated using λmax, which was highly correlated with the Brix value as a variable, as shown in Fig. 5(A). Moreover, in order to estimate the Brix value more accurately using multiple regression analysis, this value was estimated using AAC, λmax and peak viscosity, which were highly correlated with the Brix value as variables, as shown in Fig. 5(B). In addition, Brix value, λmax, peak viscosity, AAC and estimated Brix value of each rice sample are shown in Table 4. The estimation of the Brix value using only λmax is Brix value = 0.1486 \times \lambda_{\text{max}} - 77.038, the F value of the regression analysis is 2.4 \times 10^{-9}, and the equation had multiple adjusted regression coefficients of 0.8298 based on the calibration. The estimation of the Brix value using λmax, AAC, and peak viscosity is as follows: Brix value = -0.0013 \times \text{peak viscosity} + 0.02434 \times \text{AAC} + 0.0706 \times \lambda_{\text{max}} - 27.843, the F value of the regression analysis is 1.3 \times 10^{-8}, and the equation had multiple adjusted regression coefficients of 0.8643 based on the calibration. The estimation formula using only λmax is expected to be used as a simple estimation formula; however, the estimation formula using λmax, AAC, and peak viscosity is expected to be used as a more accurate estimation formula.

Many studies have described the relationship between the molecular structure of starch and enzyme digestibility; however, the only drawback of these studies was that the methods used needed to be repeated multiple times (Okuda, 2007; Okuda, 2015). In this study, the sample was cooled for 3 h in air to permit retrogradation of steamed rice, however, under this condition, the pasting viscosities of the rice samples were inferred to be higher. Under a longer cooling time, we predict that the influence of retrogradation will be more prominent. However, for the rapid measurement of enzyme digestibility, iodine absorption scanning analysis is the ideal method. After rice is harvested in autumn, enzyme digestibility can be quickly determined by iodine absorption scanning analysis, which is very important in sake production, as this...
Effects of Rice Starch Properties on Digestibility in Sake Brewing

Table 2. RVA profiles of each rice sample

<table>
<thead>
<tr>
<th></th>
<th>Peak viscosity (cP)</th>
<th>Cold paste viscosity (cP)</th>
<th>Breakdown (cP)</th>
<th>Final viscosity (cP)</th>
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<td>Ave±SD</td>
<td>Ave±SD</td>
<td>Ave±SD</td>
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<td>a</td>
<td>1837±114</td>
<td>2933±117</td>
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<tr>
<td>Gohyakumangoku</td>
<td>3719±137</td>
<td>b</td>
<td>1990±128</td>
<td>3156±180</td>
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<td>Yamadashiki</td>
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<td>a</td>
<td>1398±72</td>
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<td>Koshiibuki</td>
<td>4242±97</td>
<td>c</td>
<td>1589±68</td>
<td>2714±63</td>
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</table>

Each value is reported as average and standard deviation (n=3). Different letters show significant differences (P < 0.05).

Fig. 1. Breakdown and pasting temperature of each rice sample. The bar graph shows the breakdown and the straight line shows the pasting temperature. The ordinate on the left shows the viscosity of the breakdown and the ordinate on the right shows the pasting temperature. Different small letters show significantly different pasting temperatures (P < 0.05). Different big letters show significantly different breakdowns (P < 0.05).

Fig. 2. Moisture content of steamed rice sample. Different letters show significant differences (P < 0.05).
### Table 3. Correlation between results of analysis of iodine absorption curve, digestibility test, and RVA test of 22 rice samples.

<table>
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<tr>
<th></th>
<th>Peak viscosity</th>
<th>Cold paste viscosity</th>
<th>Breakdown</th>
<th>Final viscosity</th>
<th>Consistency</th>
<th>Setback</th>
<th>BDACS</th>
<th>Pasting temperature</th>
<th>Moisture content of steamed rice</th>
<th>Brix</th>
<th>AAC</th>
<th>( \Delta \max )</th>
<th>( \Delta \alpha \max )</th>
<th>New( \Delta \max )</th>
<th>RS</th>
<th>F2</th>
<th>Fa</th>
<th>Fb3</th>
<th>Fa/Fb3</th>
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<tr>
<td>( \Delta \max )</td>
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<td>-0.75**</td>
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<td>( \Delta \alpha \max )</td>
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<td>F2</td>
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<td>-0.64**</td>
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<td>-0.41</td>
<td>-0.08</td>
<td>0.73**</td>
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<td>0.59**</td>
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*Correlation significance at 5 % by \( t \) test. **Correlation significance at 1 % by \( t \) test.
Fig. 3. Brix value of enzyme digestibility. Different letters show significant differences ($P < 0.05$).

Fig. 4. The scatter plot of the relationship with $\lambda_{\text{max}}$ and digestibility. ● shows Koshitanrei, ◇ shows Gohyakumangoku, ■ shows Yamadanishiki and ▲ shows Koshiibuki.

Fig. 5. Relationship predictor of Brix value and observation of Brix value. (A) shows relationships of Brix value using $\lambda_{\text{max}}$, (B) shows relationships of Brix value using $\lambda_{\text{max}}$, AAC, and peak viscosity. ● shows Koshitanrei, ◇ shows Gohyakumangoku, ■ shows Yamadanishiki and ▲ shows Koshiibuki.
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<th>Amylose content (%)</th>
<th>λmax (-)</th>
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Each value is reported as average (n=3).
contributes to the quality of sake.

As it would be necessary to confirm the adaptability of our formulae to the unknown rice samples as well as to compare the relationship between these physicochemical data and climatic conditions (Ashida et al., 2013), we plan to continue this study.

Conclusions
In this study, we evaluated the gelatinization properties and enzyme digestibility of Koshitanrei, a rice cultivar developed in Niigata Prefecture for sake brewing. Koshitanrei was bred by crossing Gohyakumangoku and Yamadanishiki, and its gelatinization properties and enzyme digestibility tended to closely resemble those of Yamadanishiki.

In addition, the enzyme digestibility was determined easily and rapidly by performing iodine absorption scanning analysis, and the pasting property was determined using RVA.

Acknowledgments We would like to express our gratitude to the prefectural sake breweries who provided the rice samples.

References

Effects of Rice Starch Properties on Digestibility in Sake Brewing
making conditions, and rapid estimation methods of digestibility by


