Abstract: Starches from three waxy rice cultivars (Hakuchoumochi, Hiyokumochi and Koganemochi) showing different physical properties were studied in order to identify the relationship between physical properties and their amylopectin structures. Pastes of Hakuchoumochi, Hiyokumochi and Koganemochi are known as soft, intermediate and hard, respectively. Pasting properties of the soft type starch showed the lowest setback (SB) and the highest breakdown (BD) among the three. The hard type showed the reverse relationship and the middle type showed an intermediate property. To follow these physical properties, partial hydrolysates of starches with debranching enzymes were prepared and the distributions of debranched short side-chains in amylopectin existing mainly in the outer layer of the amylopectin were analyzed by HPAEC-PAD. Hakuchoumochi was composed of plentiful shortish chains and scarce longish chains and suggested to be resistant to retrogradation. Koganemochi had the reverse properties and was suggested to be sensitive to retrogradation. Hiyokumochi was intermediate. From these results, it could be presumed that a feature of chain-length distribution on the outer layer of the amylopectin molecule influenced the physical properties relating to retrogradation. Key words: waxy rice, amylopectin, chain-length distribution, physical property, RVA, GPC, HPAEC-PAD

MATERIALS AND METHODS

Materials. Three waxy rice cultivars of Hakuchoumochi, Hiyokumochi and Koganemochi, cultivated in Hokkaido, Saga and Niigata in the year 2000, respectively, and a normal rice cultivar of Koshihikari, cultivated in Mie in the year 2000, were used in this study. Pseudo-omonas isoamylase and pullulanase were obtained from Hayashibara Biochemical Laboratories (Okayama).

Preparation of starches and defatted ones. Starch was isolated from rice grains by the alkali method with modification. The precipitate was washed with distilled water till the pH decreased to 7.0 and the protein could not be detected by the Bradford method. After rinsing with ethanol, the starch was dried in the air, and then passed through a 100-mesh sieve. For preparation of defatted starch, starch (2 g) dissolved in dimethyl sulfoxide (40 mL) was poured gently in 100 mL methanol and left to stand overnight. The precipitate collected with a glass-filter 3G-3 was rinsed with methanol and ether, and then dried in the air.

Starch pasting properties. The isolated starches passed through 100-mesh sieve were used for studying the pasting properties by Rapid Visco Analyzer (RVA, model RVA-3D, Newport Scientific, Australia). Moisture content was determined by oven drying at 110°C for 40 min to ensure slurry concentrations of 6, 8, 10 and 12% on dry weight basis for RVA analysis. A starch suspension was held at 30°C for 6 min, and subsequently heated to 95°C at a rate of 13°C/min, held at 95°C for 6.5 min, cooled down to 30°C at a rate of 10.8°C/min, and finally held at 30°C for 9 min. The suspension was stirred at 160 rpm
Preparation of partial and complete hydrolysates of starch by isoamylase. Partial hydrolysate of starch was prepared according to the method described previously. A 0.2% starch solution (15 mL) was digested by 15 mU of isoamylase at 30°C for approximate time. One isoamylase unit is defined as the amount of enzyme required to produce 1 μmol of reducing sugar per min from waxy maize starch. From the time course of the debranching reaction prepared in advance, the reaction time corresponding to 15% partial hydrolysis was estimated. The degree of hydrolysis (%) was calculated by dividing the value of the reducing power of partial hydrolysate with that of complete hydrolysate. The reducing power was analyzed by a modified Park-Johnson method. Complete debranching reaction was conducted with 10 mU/mL of isoamylase for 3 days under the same conditions.

Gel permeation chromatography (GPC) of the hydrolysates. Partial and complete hydrolysates (10 mL) were applied on a column (26 cm ID×100 cm) of Toyopearl HW-50S and eluted with 50 mM NaCl containing 0.01% of NaN₃ by using peristaltic pump on the flow rate of 40 mL/h, respectively. Carbohydrate in each fraction (5 mL) was estimated by the phenol-H₂SO₄ method. A reaction mixture of complete hydrolysate was prepared in advance, the reaction time corresponding to 15% partial hydrolysis was estimated. The degree of hydrolysis (%) was calculated by dividing the value of the reducing power of partial hydrolysate with that of complete hydrolysate. The reducing power was analyzed by a modified Park-Johnson method. Complete debranching reaction was conducted with 10 mU/mL of isoamylase for 3 days under the same conditions.

Distributions of debranched short side-chains in amylopectin. Debranched short side-chains in amylopectin were analyzed by HPAEC-PAD according to the method described previously. A reaction mixture of complete hydrolysis was injected directly into the HPAEC-PAD. On the other hand, partial hydrolysate was conducted with GPC and the fraction (fr. 3) eluting debranched short side-chains in amylopectin, which were concentrated by a rotary evaporator and lyophilized, was analyzed by HPAEC-PAD.

RESULTS

Starch pasting properties.

RVA viscograms of waxy rice starch (Koganemochi) at different slurry concentrations and that of Koshihikari starch at 10% slurry concentration are shown in Fig. 1. When the slurry temperature reached the pasting temperature (PT, the temperature when the viscosity reached one-twentieth of the difference between the initial viscosity and the maximum viscosity), the granules started to swell and then the maximum viscosity was recorded as P (peak viscosity). After that, the viscosity decreased quickly to H (hot paste viscosity or minimum paste viscosity) because swollen granules burst. The viscosity difference between P and H was designated as breakdown (BD: P-H). When the operation was further continued by cooling to a low temperature, the viscosity increased again to C (cold paste viscosity or final viscosity, the viscosity at the end of the run). The viscosity difference between H and C was called setback (SB; C-H).

Table 1. Pasting viscosity parameters of waxy rice starches and Koshihikari starch at 10% slurry.

<table>
<thead>
<tr>
<th>Starch</th>
<th>PT*1 (°C)</th>
<th>P*2 (RVU)</th>
<th>H*3 (RVU)</th>
<th>C*4 (RVU)</th>
<th>BD*5 (RVU)</th>
<th>SB*6 (RVU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hakuchoumochi</td>
<td>67.3</td>
<td>278</td>
<td>75</td>
<td>211</td>
<td>204</td>
<td>136</td>
</tr>
<tr>
<td>Hiyokumochi</td>
<td>67.4</td>
<td>258</td>
<td>77</td>
<td>213</td>
<td>181</td>
<td>136</td>
</tr>
<tr>
<td>Koganemochi</td>
<td>67.3</td>
<td>224</td>
<td>86</td>
<td>203</td>
<td>138</td>
<td>117</td>
</tr>
<tr>
<td>Koshihikari</td>
<td>66.3</td>
<td>402</td>
<td>118</td>
<td>308</td>
<td>284</td>
<td>190</td>
</tr>
</tbody>
</table>

Slurry concentration was adjusted by dry weight basis of starch. *1 Pasting temperature (PT), *2 peak viscosity (P), *3 hot paste viscosity (H), *4 cold paste viscosity (C), *5 breakdown (BD), *6 P-H, C-H.

Hakuchoumochi and Hiyokumochi were almost the same, implying that these data was not useful to trace the physical properties for waxy rice starches. RVA data were further collected at various slurry concentrations. The slurry concentration, H, C, BD and SB at P of 200 RVU were calculated from viscogram parameters shown in Fig. 2 (Table 2). SB values of Hakuchoumochi, Hiyokumochi and Koganemochi were 87, 90 and 101, respectively, and their BD values were 136, 129 and 119, respectively. So, it is possible to imagine that Koganemochi amylopectin has a retrogradation-susceptible structure with an abundance of longish chains and Hakuchoumochi amylopectin has a retrogradation-resistant structure with an abundance of shortish chains, respectively. Based on this expectation, distributions of debranched short side-chains in amylopectin of the three waxy rice starches were examined with Koshihikari.

GPC of partial and complete hydrolysate on Toyopearl HW-50S.

The GPC profiles of 15% hydrolysates and complete hydrolysates of the rice starches on Toyopearl HW-50S are shown in Fig. 3. The fractions eluted were classified into three fractions (fr. 1, fr. 2 and fr. 3) according to the elution patterns of the complete hydrolysate. The data for complete hydrolysates are shown in Table 3. An approximate amount of amylose could be estimated from the fr.1. The complete hydrolysate of Koshihikari showed a peak
Amylopectin of Waxy Rice and Physical Properties

Fig. 2. RVA viscogram parameters of waxy rice starches and Koshihikari starch at various concentrations.

Symbols P, H and C are explained in Fig. 1.

Table 2. Pasting viscosity parameters* of waxy rice starches and Koshihikari starch at 200 RVU of peak viscosity.

<table>
<thead>
<tr>
<th>Starch</th>
<th>Slurry (%)</th>
<th>H (RVU)</th>
<th>C (RVU)</th>
<th>BD (RVU)</th>
<th>SB (RVU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hakuchoumochi</td>
<td>8.3</td>
<td>64</td>
<td>151</td>
<td>136</td>
<td>87</td>
</tr>
<tr>
<td>Hiyokumochi</td>
<td>9.3</td>
<td>81</td>
<td>182</td>
<td>119</td>
<td>101</td>
</tr>
<tr>
<td>Koganemochi</td>
<td>9.3</td>
<td>81</td>
<td>182</td>
<td>119</td>
<td>101</td>
</tr>
<tr>
<td>Koshihikari</td>
<td>8.0</td>
<td>85</td>
<td>210</td>
<td>115</td>
<td>125</td>
</tr>
</tbody>
</table>

Symbols H, C, BD and SB are explained in Table 1. *All parameters were calculated at 200 RVU of peak viscosity by using the figures shown in Fig. 2.

Fig. 3. Gel permeation chromatography (GPC) of partial and complete hydrolysates of waxy rice starches and Koshihikari starch on a column of Toyopearl HW-50S.

Table 3. Composition of each fraction of complete hydrolysates of rice starches by isoamylase eluted on GPC Toyopearl HW-50S.

<table>
<thead>
<tr>
<th>Starch</th>
<th>Amylose</th>
<th>Amylopectin</th>
<th>fr.3/fr.2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fr.1</td>
<td>fr.2</td>
<td>fr.3</td>
</tr>
<tr>
<td>Hakuchoumochi</td>
<td>nd.*</td>
<td>25.2</td>
<td>74.8</td>
</tr>
<tr>
<td>Hiyokumochi</td>
<td>nd.*</td>
<td>25.4</td>
<td>74.6</td>
</tr>
<tr>
<td>Koganemochi</td>
<td>nd.*</td>
<td>25.9</td>
<td>74.1</td>
</tr>
<tr>
<td>Koshihikari</td>
<td>14.0</td>
<td>22.9</td>
<td>63.1</td>
</tr>
</tbody>
</table>

* Not detected.

corresponding to amylose in fr.1 because of normal starch, but those of Hakuchoumochi, Hiyokumochi and Koganemochi showed no peak in fr. 1. The values dividing fr. 3 from fr. 2 of the waxy rice starches were 2.9–3.0 and that of Koshihikari was 2.8, suggesting that waxy amylpectins have slightly more shortish chains than Koshihikari amylopectin.

Distributions of debranched short side-chains in amylopectin.

It could be thought that the physical properties of waxy rice starches were mainly influenced by the fine structures of the amylpectins because the peak of amylose was too small to detect in the fr. 1 of GPC. So, the distributions of debranched short side-chains in amylopectin were analyzed by HPAEC-PAD. To begin with, the differences between each waxy rice starch and Koshihikari and the differences among the three waxy rice starches were calculated from the data for the complete hydrolysates (Fig. 4). It was found that the three waxy rice starch amylpectins have more shortish debranched side-chains (DP 6–16) and fewer longish debranched side-chains (DP 17–34) than Koshihikari amylopectin (Fig. 4-I). Among waxy rice starches, Hakuchoumochi was an amylopectin with plenty of shortish chains and a scarcity of longish chains, Koganemochi was an amylopectin with chain-length distribution opposite that of Hakuchoumochi, and Hiyokumochi was an amylopectin of middle type (Fig. 4-II).

Moreover, a partial hydrolysis with isoamylase was conducted to obtain more detailed information about the outer layer of amylopectin. However, the bar graphs for differences of chain-length distributions concerning Hakuchoumochi showed patterns opposite to those for complete hydrolysates (Fig. 5-I). As this result was incompatible, the samples collected in fr.3 were digested further with another debranching enzyme (pullulanase, 10 mU/mL) at 30°C for 6 h and then analyzed again by...
Differences of debranched short side-chains in amylopectin between partial hydrolysates of waxy rice starches and Koshihikari starch hydrolyzed by isoamylase (I) and by isoamylase+pullulanase (II).

Symbols A, B, C and K are explained in Fig. 4.

HPAEC-PAD. The new differences of chain-length distributions shown in Fig. 5-II were compatible with complete hydrolysis, suggesting that the amylopectin of Hakuchoumochi has an unusual structure with branching points on the outer layer being weakly resistant against the action of isoamylase. This abnormal debranching pattern was not markedly observed in the partial hydrolysates of more than about 25% hydrolysis.

**DISCUSSION**

Three waxy rice starches were selected to study the relationship between physical properties and fine structures of amylopectin. Although RVA data were too complex to compare both factors simultaneously, it was found that the values of SB and BD calculated at the same peak viscosity, which was selected a value of 200 RVU in this study, provided a way to correlate the information from chemical analysis. A low SB and high BD like Hakuchoumochi has an amylopectin which is rich in shortish chains and poor in longish chains. A waxy rice starch showing high SB and low BD like Koganemochi has an amylopectin with the opposite property. According to the results of Umemoto et al. and Inouchi et al., low temperature resulted in increased amounts of short chains and decreased amounts of long chains for amylopectin. The finding that Hakuchoumochi cultivated in Hokkaido (at a lower temperature) was composed of more shortish chains agreed with that.

Koshihikari, the amylopectin of which has been confirmed to have plentiful shortish chains among normal rice starches, was also examined as representative of normal rice and it was found that its amylopectin was composed of more longish chains than Koganemochi showing the highest SB value among the three waxy rice starches tested. So, it might be considered that amylopectins of waxy rice starches and that of normal rice starches are different in detail.

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**REFERENCES**

もち米澱粉における物性とアミロペクチン構造の相関性

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異なる物性を示す3種類のもち米（はくちょうもち、ひよくちもち，こがねもち）の澱粉について，物性とアミロペクチン構造の関係を理解するために研究した。はくちょうもちの生地は柔らかく，こがねもちの生地は硬く，ひよくちもちの生地は中間的な物性を示す。この3種類のもち米のなかでは，ソフトタイプであるはくちょうもちのRVA測定結果は，最も低いセットバック値と最も高いプレークダウン値を示し，ハードタイプであるこがねもちは，はくちょうもちとは逆の傾向を示した。また，中間的な物性を示すひよくちもちのRVA測定結果は中間的な値であった。これらの特性をアミロペクチンの構造面から理解するために，枝切り酵素を澱粉に作用させ部分加水分解物を調製し，アミロペクチンの外層に主に存在している短鎖アミロースの分布をHPLC-PADで分析した。その結果，はくちょうもちは短めの鎖が多く長めの鎖が少ない構造であることから，老化に対して抵抗性がある構造であること，こがねもちは逆で老化しやすい構造であること，ひよくちもちは中間的な構造であることが明らかとなった。これらの結果から，アミロペクチン分子の外層に存在する鎖長分布の性質は，澱粉の老化に関する物性に影響することが示唆された。