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Properties of Starch and Protein of “Hattan-Type Varieties” of Rice Suitable for Brewing Original Hiroshima Sake

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Abstract : By successive crossing using Hattan-type varieties originating from “Hattanso” as a parent, “Hattan-type varieties” of rice suitable for brewing the original Hiroshima sake have been bred. In this study, the difference in the properties of starch and protein among the Hattan-type varieties was examined. Six Hattan-type varieties, Hattanso, Hattan No.10, Hattan No.35, Hattan No.40, Hattan-nishiki No.1 and Hattan-nishiki No.2, were used. As the properties of starch, amylose content, pasting properties and gelatinization properties were examined. The pasting and gelatinization properties were examined using a rapid viscoanalyzer (RVA) and a differential scanning calorimetry (DSC), respectively. As the properties of protein, the compositional ratio of two types of protein bodies (PB-II/PB-I) was analyzed. However, no significant differences in the above properties were observed among these Hattan-type varieties. The above properties of starch and protein in Hattanso seem to be retained in all of these varieties. In these varieties, breeding might not have been aimed at improvement of the properties of starch and protein.

Key words : Amylose content, Gelatinization property, Hattan-type varieties of rice, Pasting property, Protein body.

The relationship between the properties of starch of rice and suitability for sake brewing has been examined mainly in relation to amylose content of rice. It is considered that the lower the amylose content, the higher the suitability for sake brewing (Yoshizawa et al., 1981; Takayama et al., 1991a, 1991b; Wakai et al., 1997; Mizuma et al., 2003). Recently, pasting properties examined with a rapid viscoanalyzer (RVA) and gelatinization properties examined with a differential scanning calorimetry (DSC) are used to analyze the properties of starch related to the suitability for sake brewing. Pasting parameters examined with RVA are greatly influenced by the properties of starch of rice, i.e., amylose and amylopectin contents (Asaoka et al., 1994; Toyoshima et al., 1997; Jane et al., 1999; Aramaki et al., 2004a, 2004b) and side-chain structure of amylopectin (Jane et al., 1999; Aramaki et al., 2004b). Aramaki et al. (2004a) reported that peak viscosity, holding strength (minimum paste viscosity), breakdown and final viscosity measured with RVA positively correlated with the water absorptivity and digestibility, and that the pasting temperature measured with RVA negatively correlated with digestibility. The gelatinization enthalpy measured with DSC is mainly derived from the heat of fusion of crystal part of amylopectin (Tester and Morrison, 1993; Jane et al., 1999), and it is also considered to correlate positively with the water absorptivity (Aramaki et al., 2004a).

Protein is decomposed to amino acids and peptides in unrefined sake, and they are used as nutrients for propagation of koji yeast. However, they are also important taste components of sake. About 90% of protein in rice endosperm consists of the protein body (PB) (Ogawa et al., 1987; Kizaki et al., 1993). There are two types of PB, PB-I and PB-II, and prolamin is localized in PB-I and glutelin in PB-II (Tanaka et al., 1980). PB-I has a firm structure and hardly digested by koji yeast (Kizaki et al., 1990; Kizaki, 1999; Furukawa et al., 2000) and occupies about 23% of total protein in 70%-polished rice (Kizaki et al., 1991). On the other hand, PB-II occupies about 60% of total protein in 70%-polished rice (Kizaki et al., 1991). PB-II is polymerized to form a crystal structure, but is easily broken and digested by koji yeast in unrefined sake (Kizaki et al., 1990; Kizaki, 1999; Furukawa et al., 2000). Therefore, PB-II has a large effect on the amino acid content of brewed sake while PB-I has only a small effect (Wakai et al., 1997; Mizuma et al., 2002). Rice suitable for sake brewing has a higher PB-II/PB-I ratio than rice for cooking, and the suitability for sake brewing can be characterized by protein composition (Kizaki et al., 1991, 1993; Ohdoi et al., 2000; Iwano et al., 2001).

In addition to varieties of Yamada-nishiki and

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Abbreviations : DSC, differential scanning calorimetry; PB, protein body; RVA, rapid viscoanalyzer.

Omachi, Kamenoo-type varieties, Gohyakumangoku, and Miyama-nishiki, etc. are cultivated as rice varieties suitable for sake brewing in Japan. In Hiroshima Prefecture, however, quite different varieties, Hattan No.35, Hattan-nishiki No.1 and Hattan-nishiki No.2 are mainly cultivated. These varieties are cultivated only in Hiroshima Prefecture but are notable as the rice suitable for common sake brewing, and were sold to sake brewers in 32 prefectures in 2003. The evaluation value of the suitability for sake brewing of Hattan No.35, Hattan-nishiki No.1 and Hattan-nishiki No.2 is similar to that of Yamada-nishiki (Saito and Nishizawa, 1996).

The history of breeding of Hattan-type varieties, which are suitable for brewing original Hiroshima sake, started from private breeding of Hattanso, the origin of Hattan-type varieties, in 1875. Since 1907, the Hiroshima Prefecture Agriculture Experiment Station (the present Hiroshima Prefecture Agriculture Research Center) tried to improve these varieties so as to develop the white-core, which is required for rice suitable for sake brewing in addition to disease resistance, lodging resistance and yielding ability. As a result, Hattan No.35 (Takei et al., 1968) was bred in 1962, and Hattan-nishiki No. 1 (Maeshige et al., 1984a) and Hattan-nishiki No.2 (Maeshige et al., 1984b) in 1984.

Previously (Tamaki et al., 2005), we examined the suitability for sake brewing of Hattan-type varieties of rice and reported the presence of a varietal difference in the suitability for sake brewing; Hattan No.35, Hattan-nishiki No.1 and Hattan-nishiki No.2 are superior and Hattanso is inferior in the water absorptivity and digestibility. In this study we examined the involvement of the properties of starch and protein in the difference in the suitability for sake brewing among Hattan-type varieties of rice suitable for sake brewing.

Materials and Methods

Six Hattan-type varieties of rice bred from Hattanso in Hiroshima Prefecture (Hattanso, Hattan No.10, Hattan No.35, Hattan No.40, Hattan-nishiki No.1 and Hattan-nishiki No.2) were used. The seeds were sown on April 25, 2002 at Hiroshima Prefecture Agriculture Research Center, transplanted on May 21, and harvested at maturity. After harvest, rice was polished to 70%, and the water content of polished rice was adjusted to 13.5% (Research association on brewer's rice, 1996). Then they were crushed with an autocrusher (AC1A, Satake, Japan) and used for the following experiments. To examine the involvement of the properties of starch and protein in the varietal difference in the suitability for sake brewing, materials we used were the same as those used in our previous study (Tamaki et al., 2005).

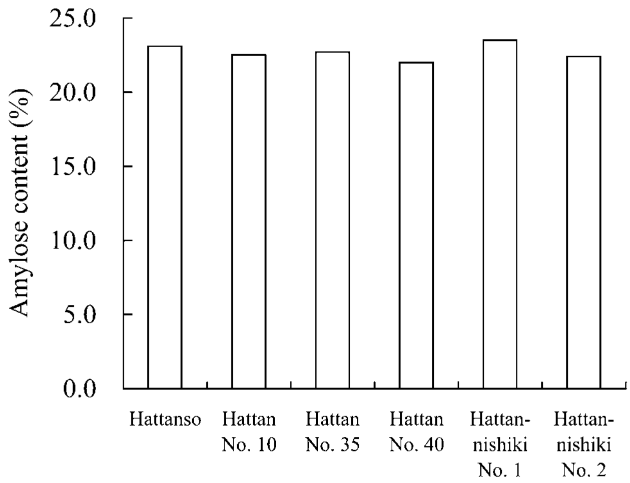

Fig. 1. Amylose content in Hattan-type varieties.
No significant difference in amylose content was observed among Hattan-type varieties.

1. Starch properties

Amylose content was measured by iodine-colorimetric method (Juliano, 1971). As the pasting properties, pasting temperature, peak viscosity, holding strength (minimum paste viscosity) and final viscosity of rice flour were measured with RVA (RVA-3D, Newport Scientific, Australia) according to the method of Aramaki et al. (2004b), using 2.8g rice flour to which distilled water was added to make the total weight 28g. As the gelatinization properties, gelatinization onset temperature, peak temperature and enthalpy were measured with DSC (Micro DSC III, Setram, France), using 200 mg rice flour to which 500 μl distilled water was added. Each experiment was repeated twice. The data were subjected to analysis of variance (ANOVA).

2. Protein properties

PB was isolated by SDS-PAGE according to the method of Kizaki et al. (1991). Then the ratio of PB components (PB-II/PB-I) was determined from the peak area of densitogram obtained using a lucino-image analyzer (LAS-1000 plus, Fuji Film, Japan). Experiments were repeated twice. The data were subjected to analysis of variance (ANOVA).

Results and Discussion

The lower the amylose content of rice suitable for sake brewing, the later was the retrogradation of steamed rice (Wakai et al., 1997), and the higher the water absorptivity, digestibility and solubility in the unrefined sake (Mizuma et al., 2003). Therefore, the rice with a lower amylose content leaves a smaller amount of sake lees, and has a higher efficiency of material use (Takayama et al., 1991a, 1991b). Aramaki et al. (2004b) found a negative correlation between amylose content and digestibility. They suggested

Table 1. RVA parameters of rice flour in Hattan-type varieties.

Variety	Peak viscosity (cP)	Holding strength (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)	Pasting temperature (°C)
Hattanso	2087	710	1378	1364	654	65.5
Hattan No. 10	2288	742	1546	1372	630	65.0
Hattan No. 35	2255	734	1521	1342	609	65.6
Hattan No. 40	2242	676	1567	1294	619	65.1
Hattan-nishiki No. 1	2076	687	1389	1316	629	67.4
Hattan-nishiki No. 2	2105	690	1436	1299	630	64.7
	ns	ns	ns	ns	ns	ns

ns : non-significant.

Table 2. DSC parameters of rice flour in Hattan-type varieties.

Variety	Onset temperature (°C)	Peak temperature (°C)	Enthalpy (-J/g)
Hattanso	60.67	66.58	10.94
Hattan No. 10	60.56	66.21	11.54
Hattan No. 35	61.02	66.83	10.67
Hattan No. 40	60.41	66.46	10.66
Hattan-nishiki No. 1	60.78	66.51	10.46
Hattan-nishiki No. 2	59.61	66.58	10.69
	ns	ns	ns

ns : non-significant.

that this was because amylose aged more easily than amylopectin and the aged starch was less digestible. Among the Hattan-type varieties, the amylose content was lowest in Hattan No.40 (22%) and highest in Hattan-nishiki No.1 (23.5%), but no significant varietal difference was observed (Fig. 1).

Table 1 shows the pasting parameters of rice flour of Hattan-type varieties measured with RVA. No varietal difference was observed in these parameters. Asaoka et al. (1994), Toyoshima et al. (1997), Jane et al. (1999) and Aramaki et al. (2004a) reported a high correlation between the pasting parameters measured with RVA and amylose content. Jane et al. (1999) and Aramaki et al. (2004b) reported that pasting parameters were correlated not only with the amylose content but also with the side-chain structure of amylopectin. Since there was no significant difference in amylose content among Hattan-type varieties (Fig. 1), there will be no varietal difference in the pasting properties.

Table 2 shows the gelatinization parameters of rice flour of Hattan-type varieties measured with DSC. There is no difference in these values among the varieties. The side-chain structure of amylopectin is highly correlated with gelatinization parameters. The higher the rate of short side-chains in the rice variety, the lower the onset, peak and concluding temperatures of gelatinization (Jane et al., 1999; Nakamura et al., 2002; Aramaki et al., 2004b). The gelatinization enthalpy measured with DSC is derived from the heat of fusion of the crystal part of amylopectin, and may be related to the content and side-chain structure of amylopectin (Tester and Morrison, 1993; Asaoka et al., 1994; Jane et al., 1999). Since we found no differences in the pasting and gelatinization properties of rice flour measured with RVA and DSC, respectively, among Hattan-type varieties, we suppose that there may be no significant difference in the side-chain structure of amylopectin among Hattan-type varieties.

In rice grains ripened at a low temperature, amylose content was higher (Inatsu, 1979; Asaoka et al., 1985; Tamaki et al., 1989; Koseki et al., 2004), and the gelatinization onset, peak and concluding temperatures and enthalpy measured with DSC were lower than those in the grains ripened at a high temperature (Asaoka et al., 1984, 1985). Although the difference in heading and maturity date among Hattan-type varieties were observed (Table 3), no significant varietal difference was observed in either amylose content or the gelatinization properties measured with DSC (Fig. 1 and Table 2). Therfore, the

Table 3. Date of heading and maturity in Hattan-type varieties.

Variety	Heading date	Maturity date
Hattanso	Aug. 11	Sep. 24
Hattan No. 10	Jul. 31	Sep. 5
Hattan No. 35	Jul. 30	Sep. 5
Hattan No. 40	Aug. 2	Sep. 10
Hattan-nishiki No. 1	Jul. 29	Sep. 5
Hattan-nishiki No. 2	Jul. 28	Sep. 5

temperature at the ripening stage is considered to have little effect on the starch properties in Hattan-type varieties.

Rice protein is decomposed to amino acids and peptides in unrefined sake. They are not only used as a nutrient for propagation of koji yeast, but also are important taste components of sake. Recently, the PB content of rice is measured as an index for judging the suitability of rice for sake brewing. In rice suitable for sake brewing, the ratio of PB-I to total protein is low, and the PB-II/PB-I ratio is high compared with rice for cooking. It is considered that the higher the PB-II/PB-I ratio, the higher the digestibility of protein by koji yeast, and the suitability of rice for sake brewing can be characterized by protein components (Kizaki et al., 1991, 1993; Ohdoi et al., 2000; Iwano et al., 2001). Among the Hattan-type varieties, the lowest PB-II/PB-I ratio was observed in Hattanso (2.30) and the highest ratio in Hattan-nishiki No.1 (2.46) (Fig. 2). Although no significant varietal difference was observed, the results were interesting because the water absorptivity and digestibility were the highest in Hattan-nishiki No.1 and the lowest in Hattanso in our previous study (Tamaki et al., 2005).

The amylose content of rice suitable for sake brewing vary with the variety, and pasting properties measured with RVA and gelatinization properties measured with DSC also vary greatly with the variety (Aramaki et al., 2004b). In addition, Aramaki et al. (2004a) reported that peak viscosity, holding strength (minimum paste viscosity), breakdown and final viscosity measured with RVA positively correlated with the water absorptivity and digestibility, that the pasting temperature measured with RVA negatively correlated with digestibility, and that the gelatinization enthalpy measured with DSC positively correlated with the water absorptivity. Previously (Tamaki et al., 2005), we reported the presence of a varietal difference in the suitability for sake brewing among Hattan-type varieties of rice. However, among Hattan-type varieties, significant differences were not observed in amylose content, pasting properties, gelatinization properties and PB-II/PB-I ratio. The results presented here showed that there was no difference in the properties of starch and protein among Hattan-type varieties.

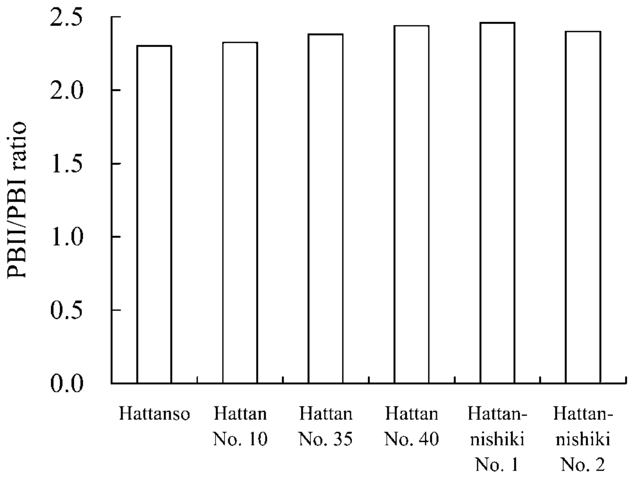

Fig. 2. PB-II/PB-I ratio in Hattan-type varieties.
No significant difference in PB-II/PB-I ratio was observed among Hattan-type varieties.

Hattanso had been selected from early varieties having large grains with white-cores. It had characteristics adapted to hilly and mountainous area in Hiroshima Prefecture, and had been cultivated for more than 50 years (Maeshige, 1987). Thereafter, the Hiroshima Prefecture Agriculture Experiment Station (the present Hiroshima Prefecture Agriculature Research Center) began breeding to isolate Hattan No.10 from Hattanso in 1921. Hattan No.10 had some inferior characteristics such as a long culm, easiness to lodge and susceptibility to rice blast, and low yielding ability. However, it was used as a basic material for breeding varieties suitable for sake brewing in Hiroshima Prefecture (Maeshige, 1993). In 1962, Hattan No.35 was bred from Hattan No.10 by introducing rice blast resistance (Takei et al., 1968), and Hattan No.40 was bred in 1965. Then, stable high-yielding ability was introduced to Hattan No.35, and Hattan-nishiki No.1 (Maeshige et al., 1984a) and Hattan-nishiki No.2 (Maeshguge et al., 1984b) were bred in 1984. Hattan-nishiki No.1 was bred as a variety for the hilly and mountainous area and Hattan-nishiki No.2 for high cold area. These varieties have yielding ability and lodging resistance similar to those of common nonglutinous rice, and improved suitability for sake brewing. Thus, the varieties suitable for sake brewing have been bred aiming to enlarge the white-core and to increase the rate of grains with a white-core. These attempts at breeding have been mostly to improve the cultivation properties. Since Hattan-type varieties have been bred by selecting for large grain, large white-core and high % of white-core grains, the water absorptivity and digestibility increased (Tamaki et al., 2005) and the evaluation value of the suitability for sake brewing of Hattan No.35, Hattan-nishiki No.1 and Hattan-nishiki No.2 was similar to that of Yamada-nishiki (Saito and Nishizawa, 1996). However, in these varieties, breeding might not have been aimed at the improvement of the properties of starch and protein. This may be why no difference in these properties was observed among the varieties used in this study.

Yanagiuchi et al. (1996) reported that white-core grains have superior water absorptivity and digestibility than non-white-core grains among the same rice varieties suitable for sake brewing, but they have similar chemical components, and suggested that the difference in the suitability for sake brewing was caused by the difference in the endosperm structure. Therefore, we consider that the difference in the suitability for sake brewing among Hattan-type varieties is related to the difference in the endosperm structure rather than the chemical properties of starch and protein. Further histological studies on the difference in the endosperm structure among Hattan-type varieties are necessary.

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