Variatel Differences in Endosperm Structure Related to High-degree Polishing Properties of “Hattan Varieties” of Rice Suitable for Brewing Original Hiroshima Sake

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Abstract: This study was conducted to clarify the effect of varietal differences in the endosperm structure on the high-degree polishing properties of Hattan varieties of rice suitable for brewing original Hiroshima sake. Four varieties were used: Hattan No.35, Hattan-nishiki No.1, Hattan-nishiki No.2, and Yamada-nishiki. Ellipsoidal-white-core grains occurred at a higher rate in Hattan-nishiki No.1 and No.2, whereas lined-white-core grains were observed at a higher rate in Hattan No.35 and Yamada-nishiki. Hattan No.35 and Yamada-nishiki showed low void polishing rates, and Hattan-nishiki No.1 and No.2 high void polishing rates after 50% polishing. Hattan-nishiki No.1 and No.2 showed inferior properties; that is, many broken and cracked grains, particularly cracked grains, after 50% polishing. The contents of broken grains and cracked grains in Hattan No.35 were higher than those in Yamada-nishiki and lower than those in Hattan-nishiki No.1 and No.2. In Hattan No.35 and Yamada-nishiki, fewer airspaces were observed between the amyloplasts at the center of the white-core as compared with Hattan-nishiki No.1 and No.2. This study showed that the differences in the endosperm structure of white-cores in the brewers’ rice grain are related to varietal differences in the tolerance to high-degree polishing.

Key words: Brewers’ rice, Endosperm structure, Hattan varieties, High-degree polishing properties.

The grains of rice suitable for sake brewing have a white opaque endosperm at their center, termed “white-core.” These white-core grains easily absorb water due to their rough structure (Nagato and Ebata, 1958; Del Rosario et al., 1968; Ando and Ichikawa, 1974; Yanagiuchi et al., 1996; Takahashi et al., 1999; Yoshii, 2000), and they are convenient for preparing malted rice (koji) since koji yeast can easily penetrate them. In addition, unrefined sake (shubo and moromi) prepared using these grains is easily solubilized and saccharized. Therefore, this characteristic of brewers’ rice has been considered indispensible for sake brewing (Nagato and Ebata, 1959; Koura, 1972; Hanamoto, 1976; Yanagiuchi et al., 1996; Yoshii, 2000; Yoshii and Aramaki, 2001).

Recently, various kinds of sake, such as ginjoshu and daiginjoshu, have been developed and the properties conferred to brewers’ rice by high-degree polishing, in addition to water absorption and digestibility, have become important factors for sake brewing. Rice for sake brewing is usually polished approximately 30% of brown rice grains; that for ginjoshu and daiginjoshu preparation is polished more than 40% and 50–70%, respectively. Rice grains with a large white-core have been considered suitable for sake brewing, and the breeding of rice for sake brewing was aimed at enlarging the white-core (Akiyama et al., 1997; Ikegami, 1997). However, there are many interstices in the white-core tissue, and it lacks physical hardness. Thus, the grains with the white-core tissues are easily broken during polishing. Since broken and unbroken grains differ in weight and are polished differently, the rate of steam absorption is not uniform when broken grains are mixed. This has an unfavorable effect on the solubility of unrefined sake and produces inferior-quality koji.

In addition to the Yamada-nishiki and Omachi varieties, the Kamenoo, Gohyakumangoku and Miyama-nishiki varieties, etc., are cultivated as rice varieties suitable for sake brewing in Japan. In Hiroshima Prefecture, however, Hattan No.35, Hattan-nishiki No.1, and Hattan-nishiki No.2, are primarily cultivated. Although cultivated only in Hiroshima Prefecture, they are notable rice varieties suitable for common sake brewing, and were sold to sake brewers in 24 prefectures in 2006. Hattan No.35 was bred in 1962, and Hattan-nishiki No. 1 and Hattan-nishiki No.2 in 1984. Hattan-nishiki No.1 was bred as a variety for the hilly area (250–350 m) and Hattan-nishiki No.2 for a higher altitude area (400 m). These two varieties have high yielding ability and lodging resistance similar to those of common nonglutinous rice, and
have improved suitability for sake brewing.

Hattan-nishiki No.1 and No.2 grains have large amounts of the white-core tissue and are easily broken by high-degree polishing. Therefore, Hattan No.35 grains whose kernels have smaller white-core tissue and are not easily broken by high-degree polishing are being used for brewing ginjōshu and dainginjōshu (Maeshige and Kobayashi, 2000). In our previous study (Tamaki et al., 2005), we clarified that Hattan No.35 has a high percentage of lined-white-core (lined white tissue on the cut surface of the grains), and it acquires superior polishing properties. On the other hand, Hattan-nishiki No.1 and No.2 have a high percentage of ellipsoidal-white-core, i.e., large, round white tissue on the cut surface of the grains, and it acquires inferior polishing properties since the grains are easily broken during polishing.

Generally, the properties of rice suitable for sake brewing are examined using rice grains after 30% polishing by using a grain-testing mill (Satake Co. Ltd., Japan) according to the National Standard Analysis Method (Research Association for Brewers’ Rice, 1996). In our previous study (Tamaki et al., 2005), we compared the polishing properties among Hattan varieties after 30% polishing by using a grain-testing mill (Satake Co. Ltd., Japan). The preparation of uniform grains polished more than 30% was difficult due to the adjustability of the mill; therefore, the examination of varietal differences using such grains was difficult. In addition, we also pointed out that further studies with grains that are polished more than 30% are necessary in order to clarify the polishing properties of Hattan varieties after high-degree polishing.

Aramaki et al. (1993) developed the compact and vertical shape-testing mill that enabled polishing to more than 50%, and now it is possible to examine the varietal differences in high-degree polishing properties. In our previous paper (Tamaki et al., 2007), we suggested that the polishing properties of brewers’ rice are closely related to the endosperm structure characterized by the density of amylloplasts. The aim of this study is to clarify the effect of varietal differences in the endosperm structure on the high-degree polishing properties of Hattan varieties after high-degree polishing.

The polishing method of Aramaki et al. (1993) was used in this study. Based on the National Standard Analysis Method (Research Association for Brewers’ Rice, 1996), polishing rate (apparent, net, and void), broken grain rate, cracked grain rate, and polishing time were analyzed. The net and void polishing rates were calculated as follows: The net polishing rate = the grain weight after 30% polishing / the grain weight of brown rice × 100. The void polishing rate = the net polishing rate – the apparent polishing rate.

The broken grain rate and cracked grain rate were calculated as follows: The broken grain rate = (the grain weight after 30% polishing – the whole-grain weight after 30% polishing) / the grain weight after 30% polishing × 100. The cracked grain rate = (the cracked grain weight after 30% polishing / the grain weight after 30% polishing) × 100.

Experiment was started after polishing the grains for 20 min with 1000 rpm to keep the fixed temperature of the testing mill during the experiment. The experiment was repeated 3 times.

The characteristics of brown rice

One hundred brown rice samples were randomly selected and cut in the vertical and longitudinal directions, and the cut surface was observed to classify the white-cores into ellipsoidal, lined, dotted, white-bellied, and non-white-core (Ebata and Nagato, 1960; Takahashi et al., 1999). The experiment was repeated 3 times.

Properties of grains after 30% polishing

The HS-4 testing mill (Chiyoda Engineering Co. Ltd., Japan) developed by Aramaki et al. (1993) was used in this study. Based on the National Standard Analysis Method (Research Association for Brewers’ Rice, 1996), polishing rate (apparent, net, and void), broken grain rate, cracked grain rate, and polishing time were analyzed. The net and void polishing rates were calculated as follows: The net polishing rate = the grain weight after 30% polishing / the grain weight of brown rice × 100. The void polishing rate = the net polishing rate – the apparent polishing rate.

The broken grain rate and cracked grain rate were calculated as follows: The broken grain rate = (the grain weight after 30% polishing – the whole-grain weight after 30% polishing) / the grain weight after 30% polishing × 100. The cracked grain rate = (the cracked grain weight after 30% polishing / the grain weight after 30% polishing) × 100.

Experiment was started after polishing the grains for 20 min with 1000 rpm to keep the fixed temperature of the testing mill during the experiment. The experiment was repeated 3 times.

Properties of grains after 50% polishing

The polishing method of Aramaki et al. (1993) was modified as follows: To achieve the polished rate of 30%, the roll rotation frequency was set at 1700 rpm. The roll rotation frequency was kept at 1300 rpm to achieve the polishing rate of 50%. Experiment was started after polishing the grains for 20 min with 1700 rpm to keep the fixed temperature of the testing mill during the experiment. Results are shown as in the section for the properties after 30% polishing.

Miniscopic observations

Since in both 2003 and 2004, grains with an ellipsoidal-white-core occurred at a higher rate in Hattan-nishiki No.1 and No.2, and those with a lined-white-core occurred at a higher rate in Hattan No.35 and Yamada-nishiki, ellipsoidal-white-core and lined-
white-core grains in the year 2003 were selected from each variety for miniscopic observation. The grains were longitudinally cut with a razor blade. The endosperm structure (×300) of the center of the white-core was observed under a miniscope (TM-1000, Hitachi Science Systems, Co. Ltd., Japan).

Results

Table 1 shows the shape of the white-core in the three Hattan varieties and Yamada-nishiki. The statistics were obtained on Hattan varieties since the aim of this study was to clarify the varietal differences in the polishing properties of Hattan varieties. In Hattan-nishiki No.1 and No.2, the percentage of ellipsoidal-white-core grains was the highest. In Hattan No.35, the percentage of lined-white-core grains was as high as that in Yamada-nishiki.

Table 2 shows the properties of grains after 30% polishing of the three Hattan varieties and the Yamada-nishiki variety. Even though the rice grain was polished 30% (apparent polishing rate), the net polishing rate varied significantly among the Hattan varieties. The polishing properties are superior in the variety with a low void polishing rate and low contents of broken and cracked grains. Yamada-nishiki had a low void polishing rate and low contents of broken and cracked grains; therefore, this variety had superior polishing properties after 30% polishing. Hattan No.35 also had a low void polishing rate. The amounts of broken grains and cracked grains in Hattan No. 35 were larger than those in Yamada-nishiki but smaller than those in Hattan-nishiki No.1 and No.2. Hattan-nishiki No.1 and No.2 had high void polishing rates and high contents of broken grains and of cracked grains, and these varieties had inferior polishing properties after 30% polishing. The polishing time required to attain 30%
polishing was not significantly different among the Hattan varieties. These results were similar to those of our previous studies, which were conducted with rice cultivated in 2002 (Tamaki et al., 2005).

Table 3 shows the properties of grain after 50% polishing of the 3 Hattan varieties and Yamada-nishiki. The void polishing rate was low in Hattan No.35 and Yamada-nishiki, but high in Hattan-nishiki No.1 and No.2. Yamada-nishiki had low contents of broken grains and of cracked grains and had superior polishing properties after 50% polishing similar to those after 30% polishing. Hattan-nishiki No.1 and No.2 had high contents of broken grains and of cracked grains, particularly cracked grains; their polishing properties after 50% polishing were inferior to those of Hattan No.35 and Yamada-nishiki. The contents of broken grains and of cracked grains in Hattan No. 35 were between those of Yamada-nishiki and Hattan-nishiki No.1 and No.2. The polishing time required to attain 50% polishing was significantly different among the Hattan varieties; the polishing time was significantly longer in Hattan No. 35.

Fig. 1 and Fig. 2 show the endosperm structure at the center of the white-core of the ellipsoidal-white-core grains. Left, dorsal side; Right, ventral side. A, amyloplast; EC, endosperm cell.

Table 3. Properties after 50% polishing in Hattan varieties and Yamada-nishiki.

| Variety               | 2003 | 2004 | 2003 | 2004 | 2003 | 2004 | 2003 | 2004 | 2003 | 2004 |
|-----------------------|------|------|------|------|------|------|------|------|------|------|
| Polishing ratio       | (%)  | (%)  | (%)  | (%)  | (%)  | (%)  | (%)  | (%)  | (%)  | (%)  |
| 2003                  |      |      |      |      |      |      |      |      |      |      |
| 2004                  |      |      |      |      |      |      |      |      |      |      |
| Net polishing rate    | 53.0 | 53.0 | 52.9 | 52.9 | 52.1 | 52.3 | 51.8 | 51.8 | 51.8 | 51.8 |
| Void polishing rate   | 3.0  | 2.9  | 3.1  | 3.1  | 2.1  | 2.1  | 1.8  | 2.1  | 2.1  | 2.1  |
| Polishing time        | 179  | 184  | 188  | 185  | 216  | 225  | 188  | 192  | 188  | 192  |
| Broken grain rate     | 12.7 | 11.0 | 11.3 | 13.0 | 7.6  | 9.2  | 6.4  | 6.0  | 6.4  | 6.0  |
| Cracked grain rate    | 27.0 | 25.6 | 25.8 | 30.1 | 17.6 | 18.8 | 9.0  | 14.3 | 9.0  | 14.3 |

Source of variation ANOVA

| V, variety | Y, year | V × Y, interaction between variety and year. |
|------------|---------|---------------------------------------------|
| V          | NS      | **                                          |
| Y          | NS      | NS                                          |
| V × Y      | NS      | NS                                          |

V, variety; Y, year; V × Y, interaction between variety and year.

**, Significant at 1% level among Hattan varieties. NS, Not significant among Hattan varieties.

Fig. 1. Endosperm structure at the center of the white-core of ellipsoidal-white-core grains. Left, dorsal side; Right, ventral side. A, amyloplast; EC, endosperm cell.

Fig. 2. Endosperm structure at the center of the white-core of lined-white-core grains. Left, dorsal side; Right, ventral side. A, amyloplast; EC, endosperm cell.
respectively. Since the endosperm structure at the center of the white-core reflects the structure of the surrounding tissues (Tamaki et al., 2007), only the center of the white-core was observed in this study. Differences in the shape of the white-core indicated different endosperm structures in the same rice variety. In all the varieties, as compared to the lined-white-core, numerous airspaces were observed between the amyloplasts in the ellipsoidal-white-core. In the Hattan No.35 and Yamada-nishiki varieties, fewer airspaces were observed between the amyloplasts in both ellipsoidal- and lined-white-core as compared to the number in the Hattan-nishiki No.1 and No.2 varieties. In addition, in the lined-white-core grains of Yamada-nishiki, fewer airspaces were observed between the amyloplasts as compared to Hattan No.35.

Discussion

The varietal differences in the numbers of broken grains and of cracked grains were higher in the 50% polished grains than in the 30% polished ones. Since the 50% polished grains bear a heavier polishing load than the 30% polished ones, varietal differences in the numbers of broken grains and of cracked grains were considered to be higher in the 50% polished grains than in the 30% polished ones. After high-degree polishing, Hattan No.35 had superior polishing properties compared to Hattan-nishiki No.1 and No.2, showing lower rates of void polishing and the presence of broken and cracked grains. Yamada-nishiki, in which the lined-white-core occurred as frequently as in Hattan No.35, showed lower occurrence rates of broken grains and of cracked grains than Hattan No.35, after 50% polishing than after 30% polishing.

The ellipsoidal-white-core grains having several interstices in the endosperm occurred frequently in the Hattan-nishiki No.1 and No.2 varieties, and polishing resulted in easy breakage of grains. Thus, these varieties were not suitable for high-degree polishing. On the other hand, lined-white-core grains having few interstices in the endosperm occurred frequently in Hattan No.35 and Yamada-nishiki, and these varieties were suitable for high-degree polishing. Both Hattan No.35 and Yamada-nishiki had more lined-white-core grains, but Yamada-nishiki had fewer interstices in the endosperm than Hattan No.35, and seemed to have fewer broken and cracked grains. One of the reasons why Yamada-nishiki is used often for brewing *ginjoshu* and *daiginjoshu* could be the extremely low rates of breaking and cracking of grains under high-degree polishing. The shape and size of white-core tissue largely influence the quality of sake (Yanagiuchi et al., 1996). Yamada-nishiki, which is used for brewing *ginjoshu* and *daiginjoshu*, has more lined-white-core grains, and sake brewers believe that the endurance to high-degree polishing is due to this type of white-core, i.e., lined-white-core. In Hiroshima Prefecture, Hattan No.35 is the highest ranked variety of rice for the brewing of sake among all the Hattan varieties. This high ranking probably depends on the higher percentage of lined-white-core.

The time required to attain 30% polishing was not significantly different among the Hattan varieties, but that required to attain 50% polishing was significantly longer in Hattan No.35. This may be related to the physical properties of the endosperm of Hattan No.35, but further studies are required to verify this.

In our previous paper (Tamaki et al., 2007), we suggested that the polishing properties of brewers’ rice might be closely related to the endosperm structure, which is characterized by the density of amyloplasts. The present experiments also clearly showed that the differences in the endosperm structure of the white-cores in the grains are related to varietal differences in the tolerance to high-degree polishing. In addition, Hattan varieties had endosperm structures different from those of Yamada-nishiki, and in particular, Hattan-nishiki No. 1 and No. 2 had an endosperm structure that easily breaks under high-degree polishing. These results support the possibility suggested by Yanagiuchi et al. (1996) that the endosperm structure of the grain affects not only the polishing properties but also the water absorption and digestibility.

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