Hardness Distribution and Endosperm Structure on Polishing Characteristics of Brewer’s Rice Kernels

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Abstract: This study was designed to determine the effects of the hardness distribution and the endosperm structure on the polishing characteristics of brewer’s rice kernels. We used four brewer’s rice cultivars, Kairyo-omachi, Hattan-nishiki No. 1, Senbon-nishiki and Yamada-nishiki. The broken kernel ratios in Kairyo-omachi and Hattan-nishiki No. 1 were significantly higher than those in Senbon-nishiki and Yamada-nishiki. Vickers hardness (VH) values in white-core tissues in kernels differed among varieties, which were significantly lower in Kairyo-omachi and Hattan-nishiki No. 1. However, no varietal differences were observed in VH values in the peripheral translucent tissues surrounding the white-core tissues. The tissues along the dorsoventral axis were softer than those along the longitudinal axis of the kernels. The tissues on the ventral side were softer than those on the dorsal side. Scanning electron microscopy (SEM) observations revealed the presence of closely arranged compound starch granules and few varietal differences in the peripheral translucent tissues surrounding the white-core tissues. However, as compared with Yamada-nishiki and Senbon-nishiki, in Hattan-nishiki No. 1 and Kairyo-omachi, the starch granules were loosely packed and the airspaces between the starch granules were more numerous in the white-core tissues. A higher number of airspaces and less starch were present in the endosperm cells along the dorsoventral axis when compared with along the longitudinal axis and on the ventral side than on the dorsal side. The present study showed that polishing characteristics are closely related with the endosperm structure, which is characterized as the density of starch granules.

Key words: Brewer’s rice, Endosperm structure, Hardness distribution, Polishing characteristics, SEM.

Brewer’s rice—large non-glutinous rice used for the brewing of rice wine (sake)—often possesses a white, opaque tissue at the centre of the kernel; this is termed as ‘white-core’. Varietal differences exist in the appearance of the parts of the white-core in the kernel, white-core ratios and the types of white-core kernels. Further, these differences are genetically controlled (Ikegami and Seko, 1995; Akiyama et al., 1997).

Nagato (1962) and Nagato and Kono (1963) studied on the hardness distribution of rice kernels. They reported that the centre (white-core tissue) of the brewer’s rice kernels is softer when compared with that of complete rice kernels. Additionally, white-core tissues are softer compared with the adjacent translucent tissues of the same brewer’s rice kernels. White-core tissues of the brewer’s rice kernels show the presence of numerous airspaces, and less starch is accumulated in these tissues (Nagato and Ebata, 1958; Del Rosario et al., 1968; Ando and Ichikawa, 1974; Yanaguchi et al., 1996; Takahashi et al., 1999; Yoshii, 2000). Del Rosario et al. (1968) and Ando and Ichikawa (1974) observed a loose arrangement of starch granules in the white-core tissues in contrast with their compact arrangement in the adjacent translucent tissues of the same brewer’s rice kernels.

It is thought that there are close relationships between the hardness distribution and the endosperm structure of brewer’s rice kernels. However, few studies were determined on the differences in the hardness distribution and the endosperm structure of brewer’s rice kernels among varieties and/or among different points in the endosperm. It is important to grasp the relationships between the hardness distribution and the endosperm structure of brewer’s rice kernels at sake production, since these are strongly related to the polishing characteristics of brewer’s rice kernels.

This study was designed to determine the effects of the hardness distribution and the endosperm structure on the polishing characteristics of brewer’s rice kernels.

Materials and Methods

Four brewer’s rice cultivars were used in this study: Kairyo-omachi, Hattan-nishiki No. 1, Senbon-nishiki and Yamada-nishiki. Rice plants were cultivated at three different areas, namely, Shobara city, Takamiya town and Miwa town in Hiroshima Prefecture in Japan in 2001.
1. Broken kernel ratio

One hundred and fifty grams of brown rice was polished to 70% by using a grain-testing mill (Satake Co. Ltd., Japan) in conformity with the National Standard Analysis Method (Research association for brewer’s rice, 1996). The broken kernel ratio was measured three times using 150 g for each replication, and the result was expressed as the mean of the three replications. The data were subjected to analysis of variance (ANOVA) and the LSD test (p < 0.05).

2. Hardness distribution of kernels

Twenty brown rice kernels, which possess a round large white tissue at the centre of the kernel along the longitudinal axis, were selected. Five kernels were set on a plate (40×17 mm) with a bonding agent along the longitudinal axis. The bonding agent was applied to the lower surface of the kernels. After 20 min, the kernels were cut longitudinally with a razor blade.

Using a microhardness tester (Akashi Co. Ltd., Japan), a preloaded (15 g) diamond pyramid was forced into the even plane of the kernel section for 15 s, and the diagonal of the quadrate impression was measured using a microscope. The Vickers hardness (VH) was then calculated as follows:

\[ \text{VH} = 2P\sin \frac{\theta}{2}d^2 \times 1000 = 1854.4Pd^2 \text{ (Kg mm}^{-2} \text{)} \]

where ‘P’ is the load (15 g), ‘\( \theta \)’ is the angle of the diagonal phases of the diamond pyramid and ‘d’ is the length of the diagonal of the quadrate impression (\( \mu \text{m} \)).

The VH values were measured at nine points in the longitudinal section of the same kernel; these included five points along the area from the apical end to the basal end and four points along the area from the dorsal side to the ventral side (Fig. 1). The former points were at the centre of the kernel (C), the edges of the white-core tissue close to the apical end and the basal end respectively; A and E, centres of the areas from the apical end to the edge of the white-core tissue and from the basal end to the edge of the white-core tissue respectively; G and H, edges of the white-core tissue close to the dorsal and ventral sides respectively; F and I, centres of the area from the dorsal side to the edge of white-core tissue and from the ventral side to the edge of the white-core tissue respectively.

The VH values were measured at nine points in the longitudinal section of the same kernel; these included five points along the area from the apical end to the basal end, and four points along the area from the dorsal side to the ventral side (Fig. 1). The former points were at the centre of the kernel (C), the edges of the white-core tissue close to the apical and the basal ends (B and D respectively), and the centre of the area from the apical end to the edge of the white-core tissue and that from the basal end to the edges of the white-core tissue (A and E respectively). The latter points were at the edge of the white-core tissue close to the dorsal and ventral sides (G and H respectively) and the centre of the area from the dorsal side to the edge of white-core tissue and from the ventral side to the edge of the white-core tissue (F and I respectively).

The effects of the bonding agent on VH were not...
studied; however, we thought that there were no changes in VH because the time interval between the application of the bonding agent until it dried was brief.

The moisture content of brown rice was adjusted to 13.8% in conformity with the National Standard Analysis Method (Research association for brewer’s rice, 1996). Experiments were conducted at a temperature of 20°C and 70% RH with two replications, i.e. a total of eight replications with five kernels each. The data were subjected to analysis of variance (ANOVA) and the LSD test (p < 0.05).

3. Scanning electron microscopy

Kernels were cut longitudinally with a razor blade. The specimens were observed using a SEM (S-2460N, Hitachi Co. Ltd., Japan) at 15 KV, 30 Pa and −10°C according to the method proposed by Yoshii and Aramaki (2001).

The endosperm structure was observed at nine points. These points were the same points, at which we measured the VH in the kernels. The results of kernels cultivated in Shobara city were shown since the differences did not exist in the endosperm structure among cultivated areas.

Results

Fig. 2 shows the broken kernel ratios at 70% polishing in each variety at three different cultivated areas. In all the cultivated areas, the broken kernel ratios in Kairyo-omachi and Hattan-nishiki No. 1 were significantly higher than those in Senbon-nishiki and Yamada-nishiki.

Fig. 3 shows the VH values of the kernels at the different points along the area from the apical end to the basal end in the longitudinal section at three different cultivated areas.
(C) was the softest part of the kernel in all the varieties at all the cultivated areas. The white-core tissues were significantly softer than the translucent tissues in all the varieties at all the cultivated areas. No varietal differences were observed in the VH values of the translucent tissues (A and E) at all the cultivated areas. However, the VH values of the white-core tissues (B, C and D) were lower in Kairyo-omachi and Hattan-nishiki No. 1 than in Senbon-nishiki and Yamada-nishiki at all the cultivated areas.

Fig. 4 shows the VH values of the kernels at the different points along the area from the dorsal side to the ventral side in the longitudinal section. The centre (C) was the softest part of the kernel in all the varieties at all the cultivated areas. The white-core tissues were significantly softer than the translucent tissues in all the varieties at all the cultivated areas. No varietal differences were observed in the VH values of the translucent tissues (F and I) at all the cultivated areas. However, the VH values of the white-core tissues (G, C and H) were lower in Kairyo-omachi and Hattan-nishiki No. 1 than in Senbon-nishiki and Yamada-nishiki at all the cultivated areas.

The tissues along the dorsoventral axis were softer, however not significantly, when compared with those along the longitudinal axis of the kernels in all the varieties (Table 1). Further, the tissues on the ventral side were softer, however not significantly, than those on the dorsal side (Fig. 4).

Fig. 5 shows the SEM micrographs at the centre of the kernels (C in Fig. 1) of each variety. In all the varieties, the white-core tissues located at the centre of the kernels were characterized by loosely packed starch granules and by the presence of numerous airspaces between the starch granules. As compared with Hattan-nishiki No. 1 and Kairyo-omachi, in Yamada-nishiki and Senbon-nishiki, a lesser number of airspaces were observed between the starch granules. Cracks and fissures were observed along the dorsoventral axis in all the provided varieties. However, the VH analysis was not affected by these cracks and fissures.

Fig. 6 shows the SEM micrographs of the edge of the white-core tissue close to the apical end (B in Fig. 1). As compared with Yamada-nishiki and Senbon-nishiki, in Hattan-nishiki No. 1 and Kairyo-omachi, starch granules were loosely arranged and numerous airspaces were observed between the starch granules. The SEM micrographs of the edge of the white-core tissue close to the basal end (D in Fig. 1) seemed like those close to the apical end (Date not shown).

Fig. 7 shows the SEM micrographs of the centre of the area from the apical end to the edge of the white-core tissue (A in Fig. 1). In all the varieties, the compound starch granules were closely arranged in this tissue compared with in the white-core tissue. Few structural differences existed in the kernels among

| Variety            | Dorsoventral axis  | Longitudinal axis |
|--------------------|--------------------|-------------------|
| Kairyo-omachi      | 11.26              | 11.59             |
| Hattan-nishiki No.1| 11.27              | 11.65             |
| Senbon-nishiki     | 11.50              | 11.83             |
| Yamada-nishiki     | 11.60              | 11.93             |

1) Means of VH values including C, F, G, H and I of four varieties and three cultivated areas in Fig. 4.
2) Means of VH values including A, B, C, D and E of four varieties and three cultivated areas in Fig. 3.

Fig. 5. SEM micrographs at the centre of the kernels (C in Fig. 1).

Fig. 6. SEM micrographs of the edge of the white-core tissue close to the apical end (B in Fig. 1).
varieties. The SEM micrographs of the centre of the area from the basal end to the edge of the white-core tissue (E in Fig. 1) seemed like those from the apical end to the edge of the white-core tissue (Date not shown).

Figs. 8 and 9 show the SEM micrographs of the edge of the white-core tissues close to the dorsal side (G in Fig. 1) and those close to the ventral side (H in Fig. 1), respectively. As compared with Yamadannishiki and Senbon-nishiki, in Hattan-nishiki No. 1 and Kairyo-omachi, starch granules were loosely arranged and numerous airspaces were observed between the starch granules in both the tissues. A lesser number of airspaces were observed between the starch granules at the edge of white-core tissues close to the dorsal side (Fig. 8) as compared with those close to the ventral side (Fig. 9).

Figs. 10 and 11 show the SEM micrographs of the centre of the area from the dorsal side to the edge of the white-core tissues (F in Fig. 1) and those of the centre of the area from the ventral side to the edge of the white-core tissues (I in Fig. 1), respectively. In all the varieties, the compound starch granules were closely arranged in these tissues as compared with in the white-core tissues. Few structural differences in these tissues existed in the kernels among varieties. At the centre of the area from the dorsal side to the edge of the white-core tissues (Fig. 10), a lesser number of airspaces were observed between the starch granules as compared with that from the ventral side to the
The hardness distribution and the endosperm structure of brewer’s rice kernels had the close relationships, and they differed among varieties, and between the longitudinal axis and the dorsoventral axis and between the ventral side and the dorsal side.

Discussion

Cracks and fissures were observed along the dorsoventral axis of the white-core tissues at the centre of brewer’s rice kernels, as reported by Ueda and Ota (1958) and Nagato (1962). As suggested by Del Rosario et al. (1968), these cracks and fissures may be regarded as artifacts that resulted from sample preparation because the centre of the kernels are softer than the other tissues present in the white-core kernels. Watanabe et al. (1997) reported that cracks and fissures were difficult to appear by SEM with cooling stage. In this study, we used the SEM with cooling stage, however, cracks and fissures were observed. Further studies should be needed to investigate the several factors affecting cracks and fissures.

Nagato (1962) and Nagato and Kono (1963) measured the VH values of brewer’s rice kernels at different points in the transection. The longitudinal section of the rice kernels has been evaluated for the breeding selection or for the observation of the apparent quality of rice kernels. Therefore, in this study, VH was studied in the longitudinal section of the kernels. This study showed that the white-core tissues are softer when compared with the surrounding peripheral translucent tissues, as reported by Nagato (1962) and Nagato and Kono (1963). White-core tissues were characterized by loosely packed starch granules and by the presence of numerous airspaces between the starch granules, as reported by Nagato and Ebata (1958), Del Rosario et al. (1968), Ando and Ichikawa (1974), Yanaguchi et al. (1996), Takahashi et al. (1999) and Yoshii (2000).

The VH values in the white-core tissues in kernels of Hattan-nishiki No. 1 and Kairyo-omachi were lower than those of Yamada-nishiki and Senbon-nishiki. From SEM observation, in Hattan-nishiki No. 1 and Kairyo-omachi, more airspaces were observed between the starch granules in the white-core tissues of the kernels than in Yamada-nishiki and Senbon-nishiki. No varietal differences were observed in the VH analysis in the peripheral translucent tissues surrounding the white-core tissues. From SEM observation, few structural differences existed in these tissues among varieties. The kernels of Kairyo-omachi and Hattan-nishiki No. 1 were broken easily during the process of polishing when compared with those of Senbon-nishiki and Yamada-nishiki. Therefore, we can conclude that the kernels which possess more loosely packed starch granules in the white-core tissues tend to break more easily while being polished to 70%. Structural properties of white-core kernels could affect not only the VH values of the kernels but also the broken kernel ratios.

Ueda and Ota (1958) and Nagato (1962) reported that the tissues along the dorsoventral axis were softer when compared with those along the lateral axis, as observed in the transection of the kernels. From VH analysis in this study, the tissues along the dorsoventral axis were softer when compared with those along the longitudinal axis in the longitudinal section of the kernels. From SEM observation, a higher number of airspaces and less starch were present in the endosperm cells along the dorsoventral axis when compared with along the longitudinal axis. Ueda and Ota (1958) suggested that the differences in the hardness between the tissues along the dorsoventral axis and the lateral axis are due to the differences in the strength of cells, which constitute the tissues along both axes. However, from SEM observation in this study, the starch accumulation could be related strongly to the differences in the hardness between the tissues along the dorsoventral axis and the longitudinal axis. Utsunomiya et al. (1974) also observed less starch accumulation along the dorsoventral axis than along the longitudinal axis by SEM. It is suggested that insufficient filling of starch granules in the kernels could be attributed to the extreme cell expansions, which caused the decline of hardness of those kernels.

The new and important finding of this study is that the tissues on the ventral side were softer than those on the dorsal side of the kernels from VH analysis. From SEM observation, a higher number of airspaces and less starch were present in the endosperm cells on the ventral side than on the dorsal side. Hoshikawa (1968) and Matsuda et al. (1979) reported that the starch accumulation on the dorsal side finished earlier.
than that on the ventral side of non-glutinous rice kernels. Further, Hoshikawa (1968) reported that the vigorous development of the ventral side results in the less starch accumulation on the ventral side. Ebata and Nagato (1960) also reported that the ventral side of white-core kernels develop vigorously during the early ripening period and the starch is not sufficiently accumulated on the ventral side. Those previous reports suggested that less starch accumulation in the ventral side appear in both non-glutinous rice and brewer’s rice. In this study, we could confirm the differences of hardness and starch accumulation between ventral side and dorsal side of rice kernels through VH analysis and SEM observation.

Another new finding in this study is that brewer’s rice kernels possess white-core-like conformation, such as airspaces between starch granules and insufficient starch accumulation, in the peripheral translucent tissues surrounding white-core tissues in some varieties. However, the number of airspaces in the peripheral translucent tissues is lesser than that in the white-core tissues. Furthermore, the ventral peripheral translucent tissues of brewer’s rice kernels possess intermediate characteristics between white-core tissues and the dorsal peripheral translucent tissues. Takahashi et al. (1999) reported that the brewer’s rice cultivar “Gin’nosei” contains fewer white-core kernels and is superior in water absorption and digestibility. Additionally, the transparent centre tissues of kernels in this cultivar possess intermediate characteristics between the white-core tissues and the peripheral translucent tissues surrounding the white-core tissues. We can conclude that those properties of endosperm structure are not specific in “Gin’nosei”, but generally found in brewer’s rice endosperm to some extent.

Polishing characteristics are important traits in sake production, and those varieties, which are difficult to be broken while being polished, are preferable. The present study showed that polishing characteristics are closely related with the endosperm structure, which is characterized as the density of starch granules. Those new findings can be useful for brewer’s rice breeding.

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