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Note

Polishing Properties of Sake Rice *Koshitanrei* for High-Quality Sake Brewing

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The Japanese high-quality sake *Daiginjo-shu* is made from highly polished rice (polishing ratio, less than 50%). Here we showed that the sake rice *Koshitanrei* (KOS) has an excellent polishing property. Rice grains of KOS had the same lined white-core region as the sake rice *Yamadanishiki* (YAM). The grain rigidity/hardness of KOS was higher than that of the sake rice *Gohyakumangoku* (GOM). The loss ratio of KOS after high polishing by an industrial polishing machine was lower than that of GOM. Further, a clear taste of sake produced from KOS was confirmed by sensory evaluation.

Key words: *daiginjo-shu*; *koshitanrei*; polishing property; sake brewing; sake rice

The nature of the raw materials is one of the important factors determining the quality of a final product. The Japanese traditional alcoholic beverage sake is produced from rice and water by a unique process using 2 microorganisms, a fungus (*Aspergillus oryzae*, so-called *koji-mold*) and yeast (*Saccharomyces cerevisiae*).¹ In sake brewing, 2 processes, the hydrolysis of starch to glucose by enzymes from *A. oryzae*, and the production of ethanol from glucose by *S. cerevisiae*, proceed simultaneously.¹ Brown rice is polished, and the steamed rice is used in 2 ways: direct addition of it to sake mash (as *kakemai*), and the production of malted rice (*koji*). The germ and the surface fraction of rice grains are abundant in proteins, lipids, and inorganic substances such as potassium and phosphate.^{1–3} An excess of these ingredients, especially proteins (total amino acids), becomes a negative factor (producing a rough taste and deepening the color) in sensory evaluation by sake tasting,^{1,4,5} and further accelerates deterioration during storage.¹ Therefore, highly polished rice of less than 50% polishing ratio (polishing ratio, percentage of white/polished rice weight to the original brown rice weight) is used in brewing the high-quality sake *Daiginjo-shu*.⁶

The rice suitable for sake brewing is called sake rice. Several features are desired in such rice. Especially, the strength of the rice grain is an important feature for high-quality sake brewing. The grain size and the

contents of proteins/lipids of sake rice are larger and less, respectively, than those of the general rice eaten in Japan. Sake rice has a characteristic white opaque-core region (called *shin-paku*), which has a low content of starch, in the center of the grain.⁷ This white-core region contributes not only to the invasion of *koji*-mold to the inside of the steamed rice grain, but also to the digestibility and gelatinization of the steamed rice during the sake brewing.^{7–9}

Breeding of sake rice is done in many areas of Japan.¹⁰ In Niigata Prefecture, the originally developed sake rice *Gohyakumangoku*¹¹ (GOM) is used widely.¹² GOM produces a high-quality sake with a clear taste (“*Tanrei*” one of the perceivable attributes of the refined sake), but the hardness of its grains is insufficient for high polishing (especially to less than the 50% polishing ratio for *daiginjo-shu*). This disadvantage of GOM is due to the shape and size of its white-core region. This region of GOM is larger than that of the sake rice *Yamadanishiki* (YAM), which was developed originally in Hyogo Prefecture¹¹ and has been used widely for *daiginjo-shu* brewing in Japan. To improve the polishing property of GOM, the sake rice *Koshitanrei* (KOS) was originally developed in Niigata Prefecture by genetically crossing GOM with YAM.¹³ Recently, KOS has been used widely for high-quality sake brewing in Niigata Prefecture,¹² but analysis of the characteristics of KOS has been limited.¹³ No comparative analysis of the polishing property among these 3 rice cultivars (GOM, YAM, KOS) has been reported.

In this study, to clarify the suitability of KOS for high-quality sake brewing, we examined the polishing property of KOS using a wide range of polished rices (up to a polishing ratio of 30%). First, using a laboratorial polishing machine, we compared the polishing property of KOS with that of GOM and YAM. Then, using an industrial polishing machine, we focused on KOS and GOM, because KOS was developed to improve the polishing property of GOM. We confirmed that KOS had an excellent polishing property by comparing this property with that of GOM. Finally, we demonstrated KOS to be suitable for the brewing of high-quality sake with a clear taste.

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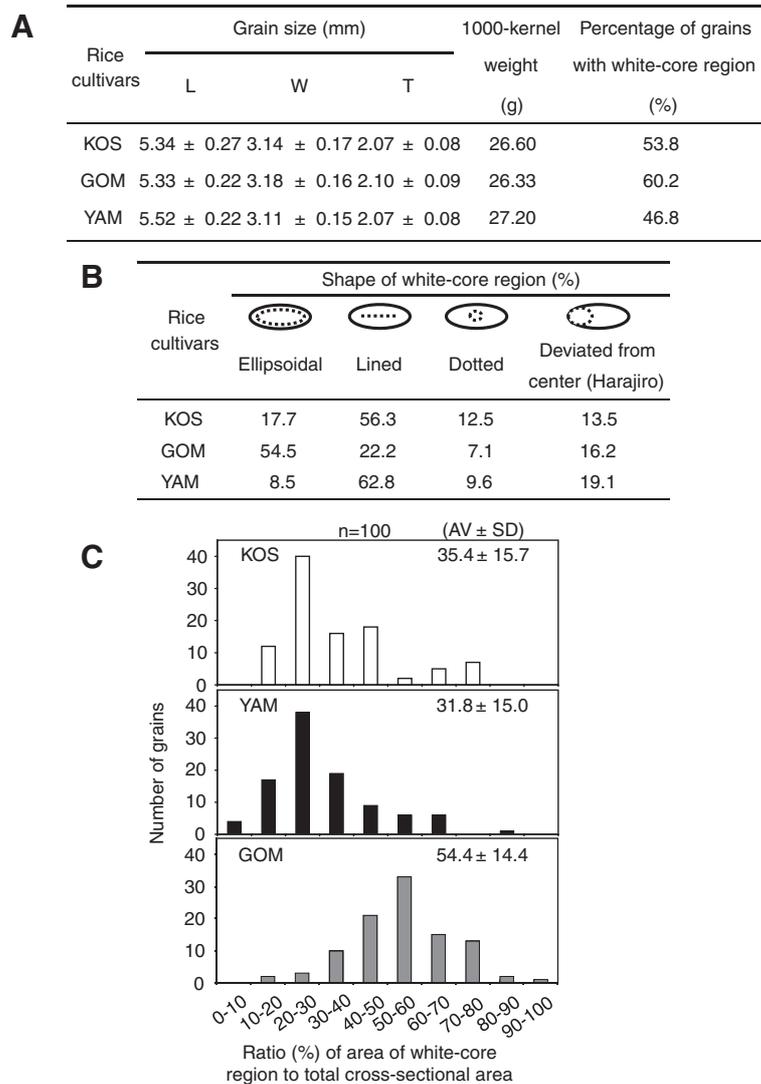


Fig. 1. Analysis of Grain Size and White-Core Region of *Koshitanrei*.

A, Grain size (L, length; W, width; T, thickness) and percentage of grains with a white-core region. Values indicate averages for 2 independent experiments. All sake rice cultivars used in this study were harvested in 2012: *Koshitanrei* (KOS, grown in Niigata Prefecture), *Gohyakumangoku* (GOM, grown in Niigata Prefecture), and *Yamadanishiki* (YAM, grown in Hyogo Prefecture). B, Classification of shapes of the white-core region. Values indicate averages for 2 independent experiments (n = 100). C, Ratio of area of white-core region to total cross-sectional area of brown rice grains (n = 100). Values indicate AV (average) ± SD (standard deviation). In analysis of the white-core region, we followed the Official Methods of Analysis of the National Tax Administration Agency.¹⁶⁾ For observation of sections and classification of the white-core regions, basically we followed the method reported by Takahashi *et al.*¹⁷⁾ First we applied liquid paraffin to cross-sections of the top side of brown rice. Next we acquired images of cross-sections of brown rice by microscopy (BX51, Olympus, Tokyo) and binarized the white-core region by the use of an image analysis processor (WinROOF, Mitani, Tokyo). Finally, we calculated the ratio of the area of white-core region to the total cross-sectional area of brown rice grains.

First we analyzed the grain size and white-core region of KOS, and then we compared KOS with GOM and YAM in respect to these features. We measured the size of brown rice grains and the percentage of grains with a white-core region in 3 rice cultivars (Fig. 1A). The grain size (L, W, T) of KOS was similar to that of GOM, but the grain length of KOS appeared to be shorter than that of YAM. The 1,000 kernel weight of KOS had a value intermediate between those of GOM and YAM. The percentage of KOS grains with a white-core region was also intermediate between those for GOM and YAM.

As mentioned above, sake rice has a characteristic white opaque-core region (called *shin-paku*), which contributes not only to the invasion of the *koji*-mold to the inside of the steamed rice grain but also to the digestibility/gelatinization of the steamed rice during

the sake brewing.⁷⁻⁹⁾ Next we examined the shape of the white-core region in brown rice grains of the 3 sake rice cultivars. The shapes of the white-core regions were classified into 4 groups: ellipsoidal, lined, dotted, and deviated from the center (Fig. 1B). In GOM, the percentage of ellipsoidal white-cores was more than half (54%). On the other hand, in YAM, the lined white-core was the major one (62%). The distribution of the shape of the white-core in KOS was similar to that in YAM. Further, we measured the area ratio of the white-core region to the total cross-sectional area of 100 brown rice grains (Fig. 1C). The average values of the area ratio for KOS, YAM, and GOM were 35.4%, 31.8%, and 54.4% respectively. The peaks of the area ratios of YAM and GOM were 20–30% and 50–60% respectively. The peak and the distribution of the area ratio of KOS were very similar to those of YAM. Thus the shape and area

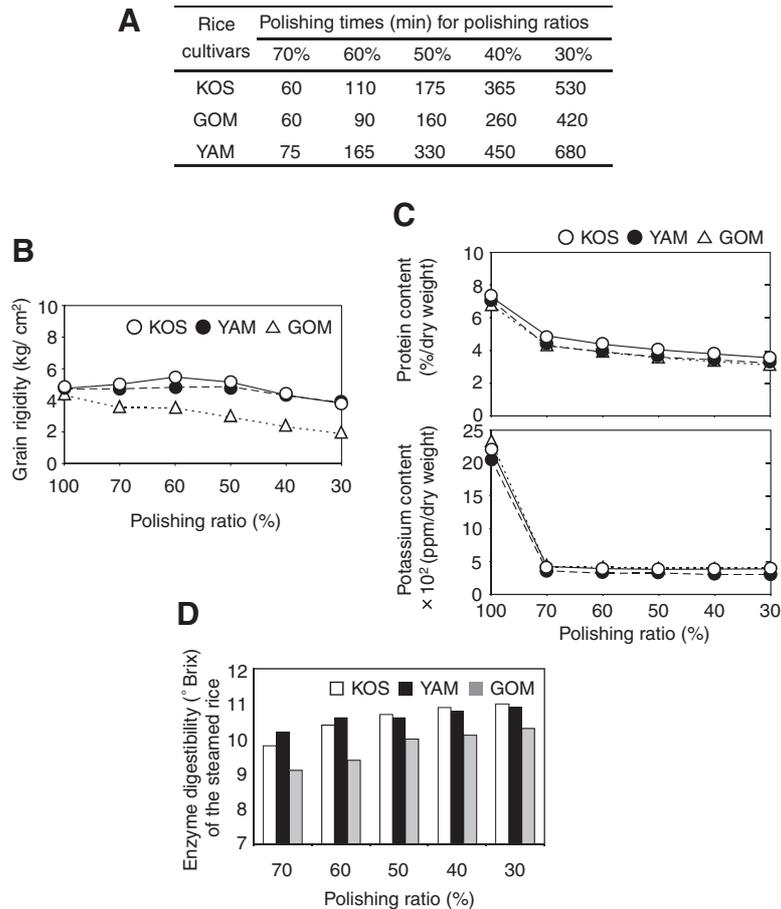


Fig. 2. Polishing Property of *Koshitanrei* Assessed by Laboratorial Polishing Machine.

A, Polishing time for the polishing ratio obtained with the laboratorial polishing machine. Brown rice was polished to white rice (from 70% to 30% polishing ratio) by the use of a laboratorial polishing machine (HS-4; Chiyoda Engineering, Hiroshima, Japan; input of rice, 70 g) at a speed of 1,300 rpm. B, Grain rigidity. The strength of the brown and polished rice was measured by crushing the grains ($n = 100$) with a grain rigidity tester (Kiya, Tokyo). Values indicate averages. The moisture contents of the rice grains were adjusted as follows: brown rice = 14.5%, polished rice 70% = 11.5%, 60% = 10.0%, 50% = 9.5%, 40% = 9.0%, 30% = 8.5%. C, The protein and potassium levels of polished rice. Values indicate averages for 2 independent experiments. D, Enzymatic digestibility (Brix value) of the steamed rice. Values indicate averages for 2 independent experiments. We followed the National Standard Analysis Method of Materials.¹⁸⁾

ratio of the white-core region of KOS were similar to those of YAM, although the grain size of KOS was almost the same as that of GOM.

Next, to clarify the polishing property of KOS, we polished the 3 sake rice cultivars by using a laboratorial polishing machine (HS-4; Chiyoda Engineering, Japan; input of rice, 70 g) at a constant rate of 1,300 rpm, and compared polishing times among them (Fig. 2A). The polishing times for polishing ratios from 70% to 50% for KOS were similar to those for GOM. However, the polishing times for polishing ratios from 40% to 30% for KOS were much longer than those for GOM. On the other hand, the polishing times for polishing ratios from 60% to 30% for YAM were much longer than those for the other cultivars. The polishing time for the 30% polishing ratio for KOS was intermediate between those for GOM and YAM.

To explore the causes of the differences in the polishing time among the rice cultivars, we measured the strength of the brown and polished rice by crushing grains ($n = 100$) with a grain rigidity tester (Fig. 2B). Grain rigidity indicates the strength of the rice grain. The grain rigidities of the brown rice grain for the 3 sake rice cultivars showed almost the same values (4.32–4.75 kg/cm²). For GOM, the grain rigidity/hardness

decreased from 4.32 kg/cm² (100% brown rice) to 1.88 kg/cm² (30% polishing ratio) with the decrease in the polishing ratio. On the other hand, in the case of KOS and YAM, the grain rigidity of the brown rice was maintained up to the 50% polishing ratio, and then decreased slightly with further decrease in this ratio (40–30% polishing ratio). The change in grain rigidity with decreasing polishing ratios in KOS was very similar to that in YAM, indicating that the KOS and YAM rice grains had almost the same strength.

The shape of the white-core region and the grain rigidity of KOS were very similar to those of YAM, but the polishing time for these rices were different. The frequency of the lined white-core region for the 3 sake rice cultivars (Fig. 1B) might be positively correlated with grain rigidity (Fig. 2B), and the polishing time for the 3 sake rice cultivars (Fig. 2A) might be negatively correlated with the percentage of grains with a white-core region (Fig. 1A). It has been reported that physical properties between grains with and without a white-core region in sake rice are related to the structures of the endosperm cells.¹⁴⁾ Further analysis is necessary to clarify this point.

To investigate the suitability of KOS for high-quality sake brewing, we measured the protein and potassium

contents of the polished rice (polishing ratios from 70 to 30% obtained by the use of the laboratorial polishing machine; Fig. 2C). The changes in the crude protein (Fig. 2C, upper panel) and potassium contents (Fig. 2C, lower panel) of KOS were very similar to those of the other 2 rice cultivars. The protein contents (6.7–7.2%) of the brown rice decreased gradually with decreasing polishing ratios up to the 50% polishing ratio, and then decreased further slightly at the 40–30% polishing ratio (3.1–3.5% for 30% polished rice). The potassium content decreased sharply with decreasing

polishing ratios to the 70% polishing ratio, and then did not change further (brown rice, 2,064–2,339 ppm; 30% polishing ratio, 299–401 ppm). The changes in protein and potassium contents of KOS were consistent with those previously reported for other sake rice cultivars.^{2,3)}

Next we measured the enzymatic digestibility of the steamed rice (Fig. 2D). The Brix value indicates the solubility of unrefined rice in sake mash. For the 3 sake rice cultivars, the Brix values increased from 9.1–10.2 (70% polishing ratio) to 10.3–11.0 (30% polishing

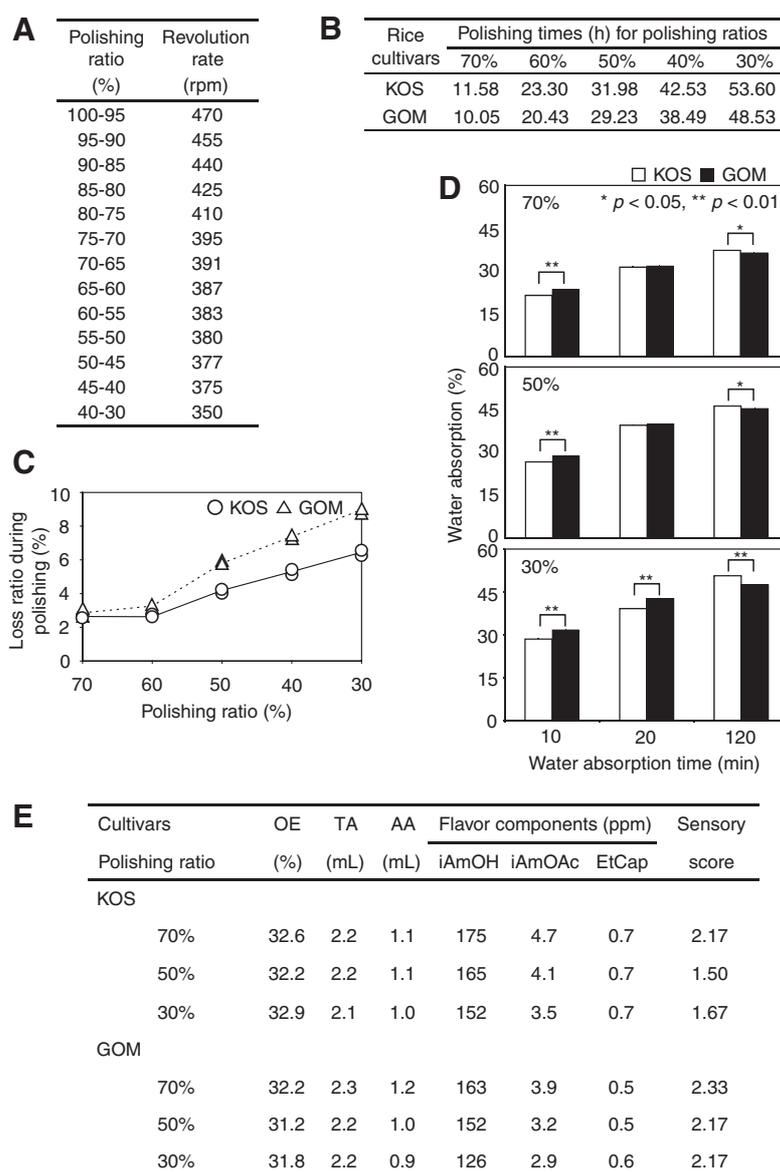


Fig. 3. Polishing Property of *Koshitanrei* Determined by the Industrial Polishing Machine.

A, Polishing condition. Brown rice of 2 sake rice cultivars (means of moisture contents of KOS and GOM were 14.94% and 15.08% respectively) was polished to white rice (to a 30% polishing ratio) with an industrial polishing machine (NF-26FA, Shin-Nakano, Okayama, Japan; input of rice, 1,020 Kg). B, Polishing time. C, Loss ratio during polishing. Two sets of independent experimental data are plotted. Lines indicate averages. D, Water-absorbing ratio of the polished rice. Values indicate $AV \pm SD$ for 5 independent experiments. The SDs were sufficiently small (0.09–0.33). Single and double asterisks indicate $p < 0.05$ and $p < 0.01$ respectively for the ratio (%) between KOS and GOM. E, Properties of the sake produced from the rice. General properties (OE, original extract; TA, total acidity; AA, amino acidity), and flavor components (iAmOH, isoamyl alcohol; iAmOAc, isoamyl acetate; EtCap, ethyl caproate) of the sake are indicated as averages for 2 independent experiments. General properties and flavor components were measured according to the Official Methods of Analysis of the National Tax Administration Agency¹⁹⁾ and gas chromatography (GC-2014, Shimadzu, Kyoto, Japan), respectively. Sake samples adjusted the 20 °C were evaluated by 7 experienced panelists. Sake quality was graded by sensory scores from 1 to 4, 1 indicating the highest quality. The sake was produced as follows (small-scale sake brewing test, total rice, 300 g): 240 g of polished rice (polishing ratios, 70, 50, and 30%) was used as *kakemai* (addition to sake mash as steamed rice).²⁰⁾ 60 g of the same *koji*-mold made from 50% polished GOM by an industrial *koji*-manufacturing system was used. The sake yeast G9NF strain, which was developed originally at the Niigata Prefectural Sake Research Institute,²¹⁾ was used. Sake fermentation was done at 12 °C for 16 d, and clear sake liquid was obtained by centrifugation.

ratio). The enzymatic digestibility of KOS and YAM was higher than that of GOM. The change in the Brix values of KOS was similar to that of YAM.

To confirm the polishing property of KOS, we investigated the polishing properties of KOS and GOM with an industrial polishing machine (NF-26FA, Shin-Nakano Industry, Japan; input of rice, 1,020 Kg) under the polishing conditions shown in Fig. 3A. As shown in Fig. 3B, the polishing time (11.58 h) for the 70% polishing ratio in the case of KOS was slightly longer than that for GOM (10.05 h). The polishing time (31.98 h) for the 50% polishing ratio for KOS was 2.7 h longer than that for GOM (29.23 h). Further, the polishing time (53.60 h) for the 30% polishing ratio for KOS was 5.0 h longer than that for GOM (48.53 h). These results obtained with the industrial polishing machine showed the same tendency as those obtained with the laboratorial polishing machine (Fig. 2A).

We measured the loss ratio during polishing (Fig. 3C), which is calculated by subtracting the apparent polishing ratio (percentage of total polished-rice weight to the original total brown rice weight) from the true polishing ratio (percentage of 1,000 polished-rice kernel weight to 1,000 brown rice kernel weight). The loss ratio of KOS increased with decreasing polishing ratios, from 2.64% (70% polishing ratio) to 6.45% (30% polishing ratio). On the other hand, the loss ratio of GOM also increased with decreasing polishing ratios, from 2.86% (70% polishing ratio) to 8.96% (30% polishing ratio), but the loss ratio of KOS was lower than that of GOM. These results indicate that KOS has an excellent polishing property for high-quality sake brewing with highly polished rice. It is possible that this excellence is due to the high frequency of the lined white-core of KOS (Fig. 1B).

Using polished rice (polishing ratios, 70, 50, 30%) prepared by the use of the industrial polishing machine, we further examined the water-absorbing ratios of KOS and GOM (Fig. 3D). The amounts of water absorbed by KOS and GOM increased with decreasing polishing ratios. In the case of water absorption for a short time (especially for 10 min), the water-absorbing ratio of KOS was lower than that of GOM. On the other hand, when water was absorbed for 120 min, the water-absorbing ratio of KOS was higher than that of GOM. These results indicate that the maximum water absorption of KOS for 120 min of water absorption was higher than that of GOM. It has been reported that rice grains of YAM with a lined white-core show a high water-absorbing ratio in the case of water absorption for 120 min.¹⁵⁾ KOS had the same lined white-core as YAM (Fig. 1B).

To confirm further the suitability of KOS for high-quality sake brewing, we carried out a small-scale sake brewing test using 300 g (total rice) of polished rice (70, 50, and 30% polishing ratios) of KOS and GOM (Fig. 3E). In general, sake rice grains with a white-core region, which have a high water absorption rate and a high Brix value, exhibit good digestibility or saccharification in sake mash. The original extract of sake from KOS was higher than that from GOM, indicating that KOS had good digestibility in sake mash. This result is consistent with the results for the Brix value (Fig. 2D) and the water-absorbing ratio for 120 min (Fig. 3D).

There was no difference in the total acidity or amino acidity of the sakes produced from KOS and GOM. The ethyl caproate (EtCap) content of sake produced from KOS was slightly higher than that from GOM, and the isoamyl acetate (iAmOAc) content of sake produced from KOS was 1.2 times higher than that from GOM. Indeed, the clear taste of the sake produced from KOS was evaluated to be high by 7 experienced panelists. In sake tasting, appropriate levels of these esters (EtCap and iAmOAc) correlate positively with the sensory evaluation scores. One of the reasons for a high evaluation of sake from KOS is high contents of the esters. Further analysis of the relationship between sake taste and the conversion ratio from raw materials to ethanol is important to clarify the character of sake rice cultivars.

In this study, with the laboratorial polishing machine, we compared the polishing property of KOS with those of GOM and YAM. Then, with an industrial polishing machine, we focused on KOS and GOM and compared their polishing and brewing properties, because KOS was originally developed to improve the polishing property of GOM. As the next step, it is necessary to compare polishing and brewing properties between KOS and YAM, with an industrial polishing machine.

Taken together, our data indicate that KOS has an excellent polishing property for the production of high-quality sake from highly polished rice. Our results provide information that should contribute to the improvement of high-quality sake brewing using KOS and stimulate further breeding and characteristic analysis of sake rice in Japan.

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