博　士　論　文

Studies on the brewing properties of rice for high-quality sake brewing

（高品質清酒醸造のための原料米の酒造適性に関する研究）

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主論文

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Introduction

Alcoholic beverages are produced from raw materials containing sugar and/or starch. The raw material is one of the important factors that determine the quality of the final product. In the Liquor Tax Act of Japan, alcoholic beverages are classified into 2 categories, i.e., fermented alcoholic beverages and distilled liquors. The Japanese traditional alcoholic beverage sake belongs to the fermented alcoholic beverage group, as are beer and wine; and alcohol is obtained by fermentation of the raw materials. The process to produce wine from raw materials/grapes, in which glucose is already included, is called *tan-hakko* (single fermentation) since it does not require the saccharification process. However, in contrast to wine, in the case of beer and sake, this process of saccharification is necessary because no sugar is included in the raw materials. In beer brewing, the process of alcoholic fermentation is accomplished after saccharification (so called *tanko-fuku-hakko*). On the other hand, in sake brewing, 2 conversion processes, saccharification and fermentation, are carried out in parallel. This simultaneous change is called *heiko-fuku-hakko* (parallel multiple fermentation), which is a unique process in the sake brewing (Yoshizawa, 1999).

Sake is produced from rice (*Oryza sativa* L.) and water by a unique process using two microorganisms, a fungus (*Aspergillus oryzae*) and yeast (*Saccharomyces cerevisiae*; Yoshizawa, 1999). In sake brewing, brown rice is polished; and the steamed rice is then used in 2 ways, In one it is directly added to sake mash (as *kakemai*); and in the other, it is used to make malted rice (*koji*), in which *A. oryzae* spores are inoculated onto the steamed rice, which is then incubated about 2 days to generate the *koji*. During the *koji* making process, *A. oryzae* produces a number of enzymes, such as saccharification enzyme, proteolytic enzymes, and others. The first mash (*shubo*) is prepared from *koji*, steamed rice, water, and yeast. After 2 weeks of the *shubo*-making process, the sake mash is expanded to become the main mash (*moromi*) by adding *koji*, steamed rice, and water. After about 1 month of sake fermentation, the clear sake liquid is obtained by filtration. The sake brewing method is peculiar to sake among alcoholic beverages (Yoshizawa, 1999).

Sake contains many components (glucose, esters, amino acids, organic acids, etc.) that influence its flavor and taste, other than ethanol: and many of them are derived from the rice (Yoshizawa, 1999). Therefore, the selection and breeding of the rice are very important for the sake brewing. Several features are desired for the rice used for the production of sake. A low protein content, low crush rate (sufficient grain rigidity/hardness), and high water absorption/enzymatic digestibility are desired properties for the rice used in high-quality sake brewing. An appropriate amount of inorganic salts such as potassium and phosphate in the rice used for sake brewing is also necessary for cell growth of the *koji*-mold (*A. oryzae*) and yeast (*S. cerevisiae*).

In general, the rice as raw material for sake brewing has been used 2 ways, i.e., at a ratios of 80% as *kakemai* (steamed rice added to the sake mash) or at one of 20% as *koji-mai* (malted rice) with respect to the weight of the total rice (100 %). Two kinds of rice (*Oryza sativa* L.), sake rice (SR) and cooking rice (CR) have been used for sake brewing. Officially, SR has been defined as *shuzo-koteki-mai* in the Agricultural Products Inspection Act. SR but not CR 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 but also to the gelatinization of the steamed rice during the sake brewing. It has been thought that the white-core region of the sake rice is important for the sake brewing (Yanagiuchi *et al*., 1996; Tamaki *et al*., 2006; Mizuma *et al*., 2008). On the other hand, usually CR is used as *kakemai*, although SR can also be used as *kakemai*. However, analyses of CR for sake brewing have been limited.

Highly polished rice (polishing ratio, less than 50%) is used for the high-quality sake *Daiginjo-shu*. The germ and the surface fraction of the rice grain are rich in proteins, lipids, and inorganic substances such as potassium and phosphate (Yoshizawa, 1999; Okuda *et al*., 2007; Aramaki *et al*., 2011). An excess amount of these ingredients, especially proteins (total amino acids), becomes a negative factor in the sensory evaluation of the sake tasting (Yoshizawa, 1999; Hashizume *et al*., 2007; Sugimoto *et al*., 2010). Furthermore, the aroma of sake containing a high content of amino acids appears to deteriorate during storage (Yoshizawa, 1999). Therefore, the high polishing of the rice kernel is an important step for removing/reducing these ingredients for the high-quality sake brewing. Indeed, the high-quality sake is produced from high-polished rice (polishing ratio of *Ginjo-shu* or *Daiginjo-shu* is less-than 60% or 50%, respectively). Therefore, sufficient grain rigidity resisting high-polishing is also required for the rice used in high-quality sake brewing.

Recently, the high-quality sake (*Ginjo-shu*, *Daiginjo-shu*, etc) with a clear taste ("*Tanrei*", one of the perceivable attributes of refined sake) has been preferred by many consumers. To improve the refined sake and to establish a sake brewing process suitable for individual rice cultivars, the breeding and the research/characterization of rice have been performed in many areas of Japan (Maeshige and Kobayashi, 2000). In Japan, approximately 100 types of SR have been used in sake brewing (http://www.maff.go.jp/j/

seisan/syoryu/kensa/kome/index.html). In particular, *Yamadanishiki* (YAM, grown in Hyogo Prefecture), *Gohyakumangoku* (GOM, grown in Niigata Prefecture), and *Miyamanishiki* (MYN, grown in Nagano Prefecture) have been widely used as the major SR for high-quality sake brewing.

In addition to varietal characteristics/genetic background of rice, the cultivation conditions affect the protein content of brown rice. Previously, it was reported that the protein content is negatively correlated with the period of ear emergence and that the protein content of late-maturing rice cultivars tends to be lower than that of early-maturing rice cultivars (Higashi and Kushibuchi, 1976). Indeed, it has been suggested that the protein content of rice is affected by the number of hours of sunlight and by the temperature during the cultivation period (Koseki *et al*., 2004). Further, the pasting properties, as investigated with a Rapid Visco Analyzer (RVA), have been used to analyze the properties of the endosperm starch (amylose/amylopectin content and side-chain structure of amylopectin) related to the suitability of the rice for sake brewing (Aramaki *et al*., 2004; Okuda *et al*., 2005; Okuda *et al*., 2006; Okuda *et al*., 2007).

In Niigata Prefecture, 2 SRs, GOM and *Takanenishiki* (TAK), have been used (http://www.maff.go.jp/j/seisan/syoryu/kensa/kome/index.html) and have contributed to the production of high-quality sake with a clear taste ("*Tanrei*", one of the perceivable attributes of refined sake). In addition to these SRs, 2 CRs, *Yukinosei* (YUK) and *Koshi-ibuki* (KSI), also have been used widely for sake brewing in Niigata Prefecture (http://www.maff.go.jp/j/ seisan/syoryu/kensa/kome/index.html).

In 2004, a new original sake rice, known as *Koshitannrei* (SR KOS), was developed in Niigata Prefecture by genetically crossing sake rice *Gohyakumangoku* (SR GOM) with sake rice *Yamadanishiki* (SR YAM) to improve the polishing and brewing properties of GOM; and it has been registered as a new SR (Kobayashi *et al*., 2006). GOM has contributed to the brewing of the high-quality sake with a clear taste. However, this rice cultivar is unsuitable for high-polishing (less-than 50% of polishing ratio for *Daiginjo-shu*), because the strength of the rice grain of this cultivar is insufficient for the high-polishing procedure. Therefore, YAM was brought from other prefectures for the production of *Daiginjo-shu*. Based on such facts, the development had been carried out with the concept of *Daiginjo-shu* with 100% rice from Niigata Prefecture. In recent years, the sake produced from KOS has been extensively evaluated, and KOS has been used widely for the brewing of high-quality sake in Niigata Prefecture. Characterization of the brown rice grain and 70% polished rice of KOS has been reported (Kobayashi *et al*., 2006). However, the polishing and brewing properties of KOS over a wide range of polishing ratios from 70% to 30% using the industrial polishing machine have not been reported yet.

In 1996, the late-maturing cooking rice *Sensyuraku* (CR SEN) was selected again as a rice with a low protein content and was revived as *fukkoku-mai* (revived rice). The following points can be given as reasons: First, its cultivar is a low protein content rice, and produces high-quality sake with a clear taste (suitable for quality of sake of Niigata). Next, the harvest time of this rice cultivar does not overlap that of the major SR GOM. Further, I cannot consider that the only "rice for making sake" is "SR," because there are cultivars that are not classified as SR but are well known as raw materials for sake, e.g., *Kamenoo*, etc. In 1999, through analysis and selection of properties for rice cultivars, I used the late-maturing CR SEN for sake brewing. SEN was originally developed in Niigata Prefecture by crossbreeding between *Kanto-53* and *Norin-36* in 1960, and it was classified as cooking rice in the Agricultural Products Inspection Act of Japan. However, in 1971, due to the increase in consumption of CR *Koshihikari* (KSH), SEN was omitted from the recommended list of rice cultivars. Currently, SEN is being used for high-quality sake brewing. However, analysis of the brewing properties of SEN has been limited. Further, compared with that of SRs, the analysis of CRs for sake brewing has been limited.

In this study, I analyzed the suitability of rice for high-quality sake brewing, 2 rice cultivars (*Oryza sativa* L.), i.e., sake rice *Koshitanrei* (SR KOS) and cooking rice *Sensyuraku* (CR SEN) for suitability as cultivars for the production of high-quality sake. In Chapter I, in order to clarify the suitability of KOS for high-quality sake brewing, I examined the polishing/brewing properties of KOS by using a wide range of polished rice (up to a polishing ratio of 30%). In Chapter II, in order to clarify the suitability of SEN for high-quality sake brewing, I described the original screening of late-maturing CR SEN as a rice with a low protein content and characterized its properties for high-quality sake brewing.

Chapter I

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

1.1. Abstract

Sake produced from sake rice *Koshitanrei* (SR KOS) has been extensively evaluated and KOS has been used widely for the brewing of high-quality sake in Niigata Prefecture. Characterization of the brown rice grain and polished rice (only 70% polishing ratio) of KOS has been reported (Kobayashi *et al*., 2006). However, the polishing and brewing properties of KOS over a wide range of polishing ratios from 70% to 30%, determined by use of an industrial polishing machine, has not been reported yet. To clarify the suitability of KOS for high-quality sake brewing, I examined its polishing/brewing properties by using rice with a wide range of polishing ratio (up to 30%) and compared KOS with 2 SRs, *Yamadanishiki* (YAM) and *Gohyakumangoku* (GOM). 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, I demonstrated that KOS is suitable for the brewing of high-quality sake with a clear taste. These results provide information that should contribute not only to the improvement of high-quality sake brewing using KOS but also to analysis of breeding and characteristics of sake rice in Japan.

1.2. Introduction

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*) (Yoshizawa, 1999). 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 (Yoshizawa, 1999). 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 (Yoshizawa, 1999; Okuda *et al*., 2007; Aramaki *et al*., 2011). 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 (Yoshizawa, 1999; Hashizume *et al*., 2007; Sugimoto *et al*., 2010) and further accelerates deterioration during storage (Yoshizawa, 1999). 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* (http://www.nta.go.jp/shiraberu/senmonjoho/sake/hyoji/seishu/gaiyo/02.htm).

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 (Yanagiuchi *et al*., 1996). 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 (Yanagiuchi *et al*, 1996; Tamaki *et al*., 2006; Mizuma *et al*., 2008).

Breeding of sake rice is done in many areas of Japan (Maeshige and Kobayashi, 2000). In Niigata Prefecture, the originally developed sake rice *Gohyakumangoku* (GOM) (http://www.gene.affrc.go.jp/databases-plant_search_en.php) is used widely (http://www.maff.go.jp/j/seisan/syoryu/kensa/kome/index.html). 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 Prefecture (http://www.gene.affrc. go.jp/databases-plant_search_en.php) and has been used widely for *Daiginjo-shu* brewing in Japan. To improve the polishing property of GOM, the sake rice *Koshitannrei* (KOS) was originally developed in Niigata Prefecture by genetically crossing GOM with YAM (Kobayashi *et al*., 2006). Recently, KOS has been used widely for high-quality sake brewing in Niigata Prefecture (http://www.maff.go.jp/j/seisan/syoryu/kensa/kome/index.html), but analysis of the characteristics of KOS has been limited (Kobayashi *et al*., 2006). 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, I 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, I compared the polishing property of KOS with that of GOM and YAM. Then, using an industrial polishing machine, I focused on KOS and GOM, because KOS was developed to improve the polishing property of GOM.

I confirmed that KOS had an excellent polishing property by comparing this property with that of GOM. Finally, I demonstrated KOS to be suitable for the brewing of high-quality sake with a clear taste.

1.3. Materials and Methods

1.3.1 Rice cultivars

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). Brown rice of 3 cultivars (KOS, GOM, and YAM) 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. Further, brown rice of 2 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) using an industrial polishing machine (NF-26FA, Shin-Nakano, Okayama, Japan; input of rice, 1,020 Kg) with the polishing conditions shown in Table 4.

1.3.2 General analysis of rice grain

I followed the National Standard Analysis Method of Materials for sake brewing (http://www.sakamai.jp/bunseki.html). The moisture contents of the rice grains of the brown and polished rice were adjusted as follows: brown rice = 14.5 %, 70% polished rice = 11.5 %, 60 % = 10.0 %, 50 % = 9.5 %, 40 % = 9.0 %, 30 % = 8.5 %. The strength of the brown and polished rice was measured by crushing the grains (n = 100) with a grain rigidity tester (Kiya, Tokyo).

1.3.3 Analysis of shape and size of white-core region in rice

In analysis of the white-core region of rice grain, I followed the Official Methods of Analysis of National Tax Administration Agency (http://www.nta.go.jp/shiraberu/zeiho-kaishaku/tsutatsu/kobetsu/sonota/070622/01.htm). For observation of sections and classification of the white-core regions, basically I followed the method reported by Takahashi *et al*., 1999. First I applied liquid paraffin to cross-sections of the top side of brown rice. Next I 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, I calculated the ratio of the area of white-core region to the total cross-sectional area of brown rice grains.

1.3.4 Small-scale sake brewing test

For the small-scale sake brewing test, the sake rice (KOS and GOM) polished by industrial polishing machine (NF-26FA, Shin-Nakano, Okayama, Japan; input of rice, 1,020 Kg) was used. 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) (Aramaki *et al*., 2004b). Sixty gram 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 (Sato *et al*., 2005), was used. Sake fermentation was done at 12°C for 16 d, and clear sake liquid was obtained by centrifugation.

1.3.5 General properties, flavor components, and sensory evaluation of sake

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 Agency (http://www.nta.go.jp/shiraberu/zeiho-kaishaku/tsutatsu/kobetsu/sonota/070622/01.htm) 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.

1.4. Results and Discussion

1.4.1 Analysis of grain size and white-core region of rice cultivars

First I analyzed the grain size and white-core region of KOS, and then I compared KOS with GOM and YAM in respect to these features. I measured the size of brown rice grains and the percentage of grains with a white-core region in 3 rice cultivars (Table 1). 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 (Yanagiuchi *et al*., 1996; Tamaki *et al*., 2006; Mizuma *et al*., 2008). Next I 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 (Table 2). 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, I measured the area ratio of the white-core region to the total cross-sectional area of 100 brown rice grains (Fig. 1). 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 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.

1.4.2 Polishing properties of rice cultivars by the use of a laboratorial polishing machine

Next, to clarify the polishing property of KOS, I 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 (Table 3). 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, I measured the strength of the brown and polished rice by crushing grains (n = 100) with a grain rigidity tester (Fig. 2). 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/cm^2). For GOM, the grain rigidity/hardness decreased from 4.32 kg/cm^2 (100% brown rice) to 1.88 kg/cm^2 (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 (Table 2) might be positively correlated with grain rigidity (Fig.2), and the polishing time for the 3 sake rice cultivars (Table 3) might be negatively correlated with the percentage of grains with a white-core region (Table 1). 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 (Tamaki *et al*., 2005). Further analysis is necessary to clarify this point.

1.4.3 Change of protein content and enzymatic digestibility by polishing ratio in rice cultivars

To investigate the suitability of KOS for high-quality sake brewing, I 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. 3). The changes in the crude protein (Fig. 3A, upper panel) and potassium contents (Fig. 3B, 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 (Okuda *et al*., 2007; Aramaki *et al*., 2011).

Next I measured the enzymatic digestibility of the steamed rice (Fig. 4). 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 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.

1.4.4 Polishing and brewing properties of rice cultivars by the use of an industrial polishing machine

To confirm the polishing property of KOS, I 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 Table 4. As shown in Table 5, 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 (Table 3).

I measured the loss ratio during polishing (Fig. 5), 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 rice), 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 (Table 2).

Using polished rice (polishing ratios, 70, 50, 30%) prepared by the use of the industrial polishing machine, I further examined the water-absorbing ratios of KOS and GOM (Fig. 6). 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 (Aramaki *et al*., 2004a). KOS had the same lined white-core as YAM (Table 2).

To confirm further the suitability of KOS for high-quality sake brewing, I 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 (Table 6). 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. 4) and the water-absorbing ratio for 120 min (Fig. 6). 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, I compared the polishing property of KOS with those of GOM and YAM. Then, with an industrial polishing machine, I 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, those data indicate that KOS has an excellent polishing property for the production of high-quality sake from highly polished rice. Those 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.

Table 1 Grain size (L, length; W, width; T, thickness) and percentage of grains with a white-core region.

Rice cultivars [a]	Grain size (mm) [b]			1000-kernel weight [b] (g)	Percentage of the grains with white-core region [b] (%)
	Length	Width	Tickness		
KOS	5.34 ± 0.27	3.14 ± 0.17	2.07 ± 0.08	26.60	53.78
GOM	5.33 ± 0.22	3.18 ± 0.16	2.10 ± 0.09	26.33	60.20
YAM	5.52 ± 0.22	3.11 ± 0.15	2.07 ± 0.08	27.20	46.80

[a] 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] Values indicate averages for 2 independent experiments.

Table 2 Classification of shapes of the white-core region.

Rice cultivars	Shape of white-core region (%)			
	Ellipsoidal	Lined	Dotted	Deviated from center (Harajiro)
KOS	17.7	56.3	12.5	13.5
GOM	54.5	22.2	7.1	16.2
YAM	8.5	62.8	9.6	19.1

Values are averages from 2 independent experiments (n = 100).

Table 3 Polishing time for the polishing ratio obtained with the laboratorial polishing machine.

Rice cultivars	Polishing times (min) for polishing ratios				
	70%	60%	50%	40%	30%
KOS	60	110	175	365	530
GOM	60	90	160	260	420
YAM	75	165	330	450	680

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.

Table 4 Polishing condition.

Polishing ratio (%)	Revolution rate (rpm)
100 - 95	470
95 - 90	455
90 - 85	440
85 - 80	425
80 - 75	410
75 - 70	395
70 - 65	391
65 - 60	387
60 - 55	383
55 - 50	380
50 - 45	377
45 - 40	375
40 - 30	350

Brown rice of 2 sake rice cultivars (means of moisture contents of KOS and GOM were 14.9% and 15.1% 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).

Table 5 Polishing time.

Rice cultivars	Polishing times (h) for polishing ratios				
	70%	60%	50%	40%	30%
KOS	11.58	23.30	31.98	42.53	53.60
GOM	10.05	20.43	29.23	38.49	48.53

The polishing was done with an industrial polishing machine (NF-26FA, Shin-Nakano, Okayama, Japan; input of rice, 1,020 Kg) under the polishing conditions showed in Table 4.

Table 6 Properties of the sake produced from the rice.

Cultivars polishing ratio	OE [a] (%)	TA [a] (mL)	AA [a] (mL)	Flavor components [a] (ppm) iAmOH	iAmOAc	EtCap	Sensory score [b]
KOS							
70%	32.6	2.2	1.1	175	4.7	0.7	2.17
50%	32.2	2.2	1.1	165	4.1	0.7	1.50
30%	32.9	2.1	1.0	152	3.5	0.7	1.67
GOM							
70%	32.2	2.3	1.2	163	3.9	0.5	2.33
50%	31.2	2.2	1.0	152	3.2	0.5	2.17
30%	31.8	2.2	0.9	126	2.9	0.6	2.17

[a] 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.

[b] Sake quality was graded by sensory scores from 1 to 4, 1 indicating the highest quality.

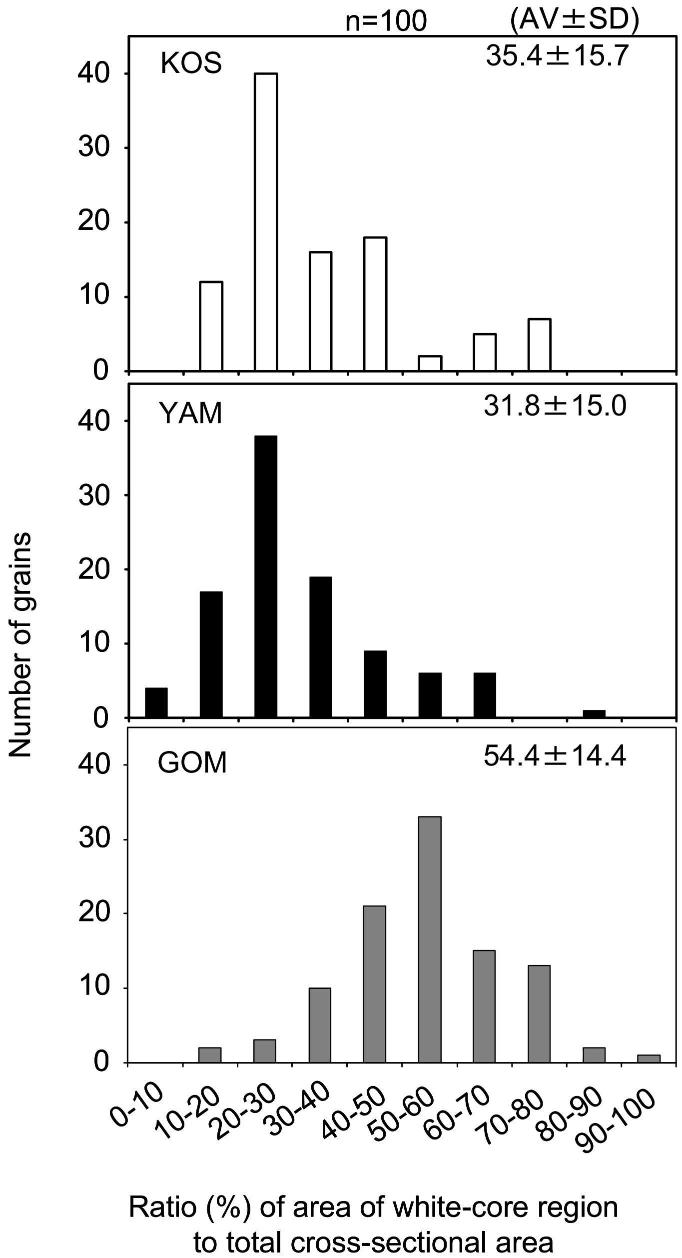

Figure 1 Ratio of area of white-core region to total cross-sectional area of brown rice grains (n = 100).

Values indicate AV (average) ± SD (standard deviation).

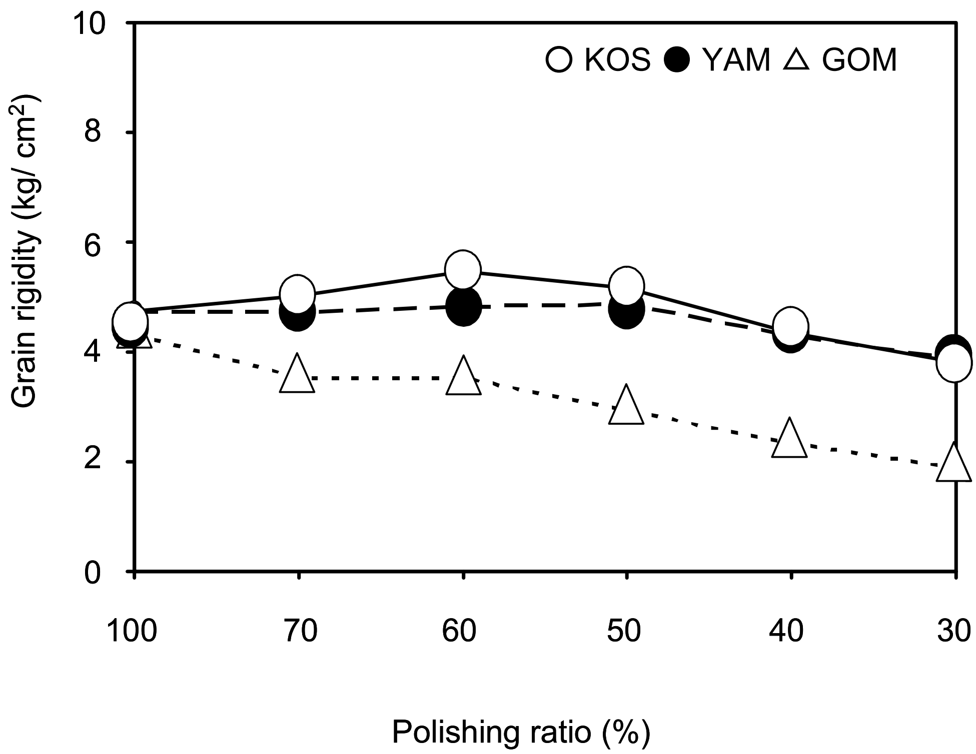

Figure 2 Grain rigidity.

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%.

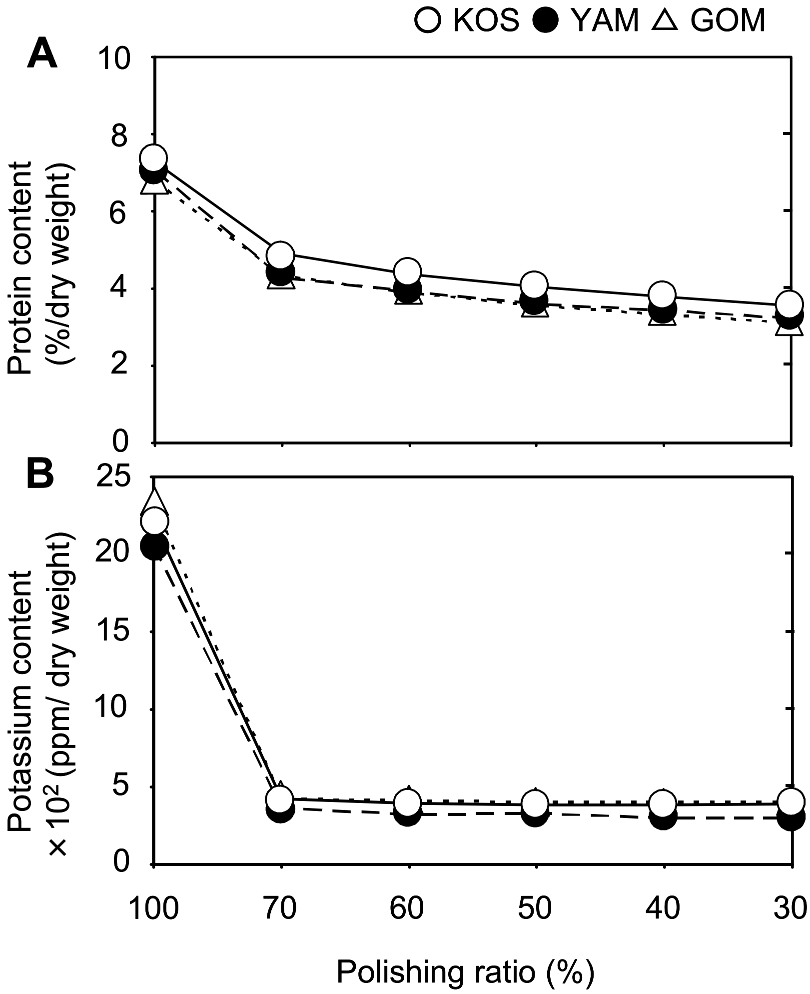

Figure 3 Protein (A) and potassium (B) levels of polished rice.

Values indicate averages for 2 independent experiments.

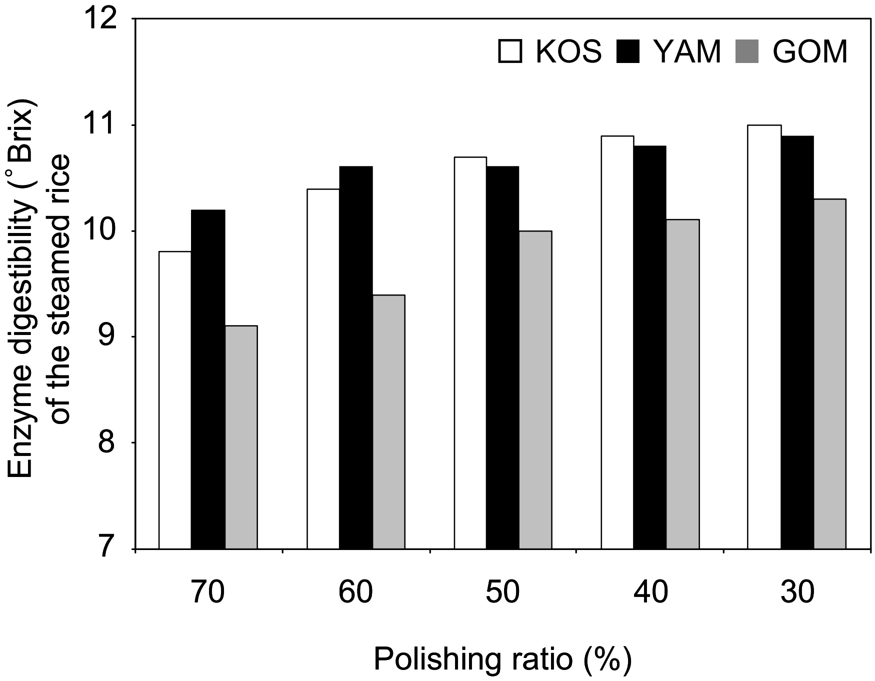

Figure 4 Enzymatic digestibility (Brix value) of the steamed rice.
Values indicate averages for 2 independent experiments.

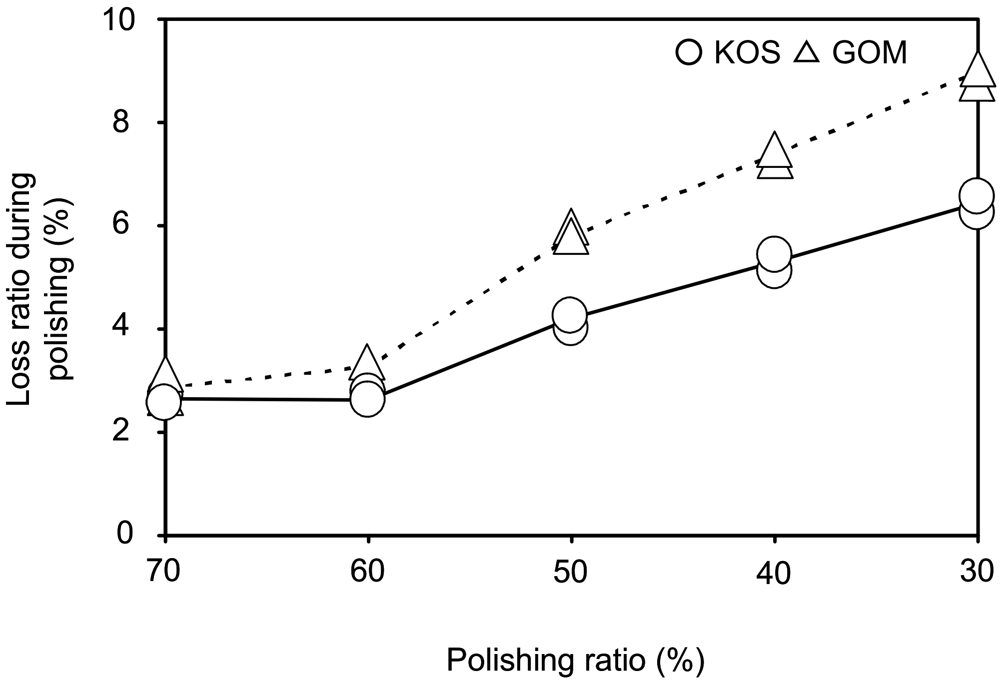

Figure 5 Loss ratio during polishing.

Two sets of independent experimental data are plotted. Lines indicate averages.

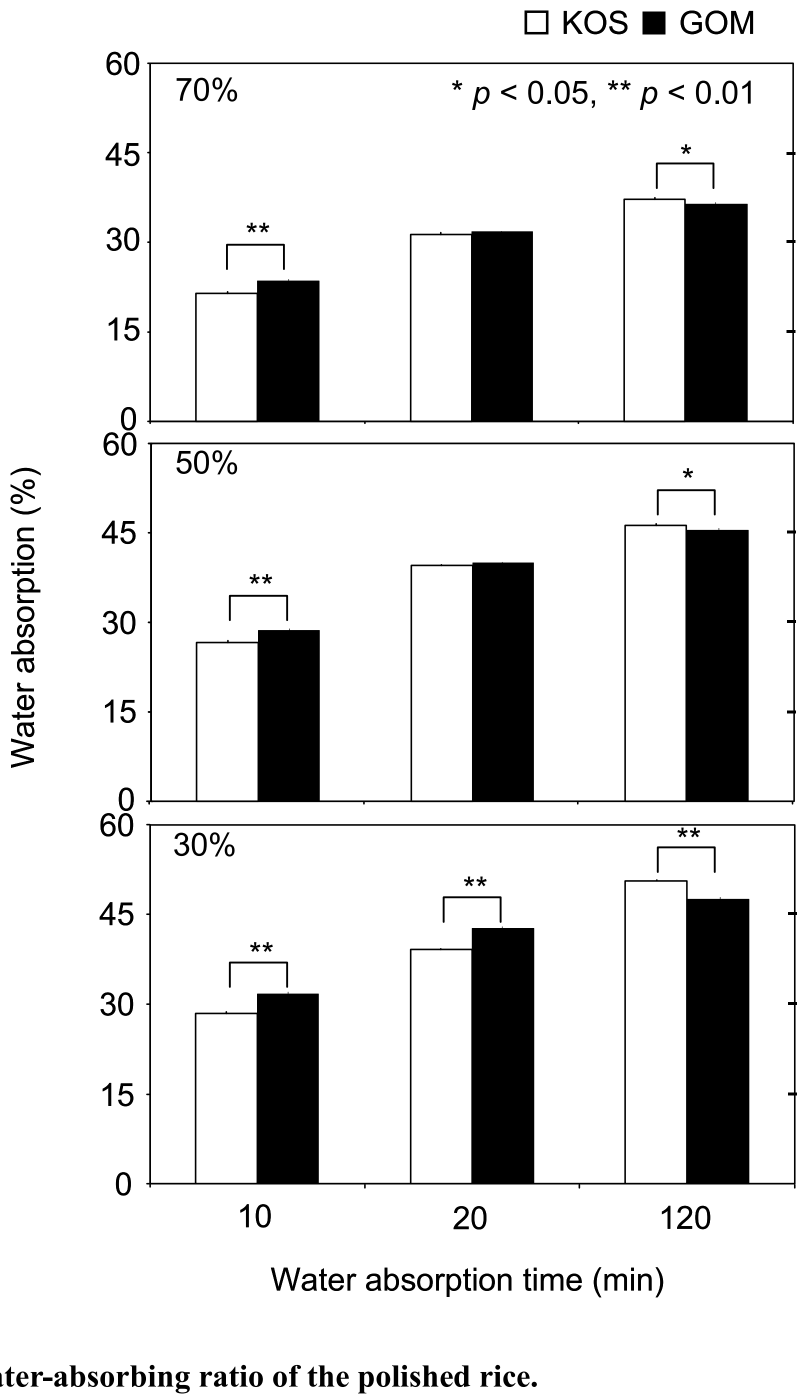

Figure 6　Water-absorbing ratio of the polished rice.

Values indicate AV ± 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.

Chapter II

Brewing Properties of Late-Maturing Cooking Rice *Sensyuraku* for High-Quality Sake Brewing

2.1. Abstract

The late-maturing cooking rice *Sensyuraku* (CR SEN), which was revived as *fukkoku-mai* (revived rice), has been used for high-quality sake brewing. However, the screening methods for SEN in the revival have not been reported yet. Additionally, characterization of SEN for high-quality sake brewing using highly polished rice has been limited. Here I described the original screening for SEN and characterization of its properties for high-quality sake brewing. SEN was selected again as a rice with a low protein content. To clarify the brewing properties of SEN for high-quality sake brewing, I examined its brewing properties using highly polished rice and compared them with those of 2 SRs, early-maturing sake rice *Gohyakumangoku* (SR GOM) and late-maturing sake rice *Koshitanrei* (SR KOS), and 1 CR, early-maturing cooking rice *Yukinosei* (CR YUK). The protein content of SEN was lower than that of SR GOM or CR YUK, and its grain rigidity was higher than that of GOM. The excellent properties of SEN with respect to both water-adsorption and enzyme digestibility were confirmed by use of a Rapid Visco Analyzer (RVA). Further, based on sensory evaluation I confirmed a clear taste of sake produced from SEN. Thus, SEN has excellent properties, equivalent to those of SR, for high-quality sake brewing. These results provide information that should shed light on the breeding and characteristics of CRs for high-quality sake brewing.

2.2. Introduction

The Japanese alcoholic beverage sake is produced from 2 raw materials, rice and water, by 2 simultaneous bioconversions, i.e., the hydrolysis of starch in the steamed rice to glucose by saccharification enzymes from the fungus *Aspergillus oryzae* (*koji*-mold) and the production of ethanol from glucose by the yeast *Saccharomyces cerevisiae* (Yoshizawa, 1999). Brown rice is polished, and the steamed rice is used in 2 ways: one is direct addition of it to sake mash (as *kakemai*), and the other is the production of malted rice (*koji*), in which the *A. oryzae* spores are inoculated to the steamed rice and incubated about 2 days to generate *koji*. During the *koji* making process, *A. oryzae* produces a number of enzymes, saccharification enzyme, proteolytic enzyme, and others. The first mash (*shubo*) is prepared from *koji*, steamed rice (*kakemai*), water, and yeast (*S. cerevisiae*). After 2 weeks of the *shubo* making process, sake mash is expanded to main mash (*moromi*) by adding *koji*, steamed rice (*kakemai*), and water. After about 1 month of sake fermentation, the clear sake liquid is obtained by filtration. The rice used in sake brewing has attracted attention as an important determinant for the quality of the final sake product.

In general, 2 kinds of rice (*Oryza sativa* L.), sake rice (SR) and cooking rice (CR), have been used for sake brewing. Officially, SR has been defined as *shuzo-koteki-mai* in Agricultural Products Inspection Act. SR but not CR has a characteristic white opaque-core region (called *shin-paku*), which contributes to the invasion of the *koji*-mold to the inside of the steamed rice grain. Therefore, SR is used mainly for making *koji*. On the other hand, usually CR is used as *kakemai*, although SR is also used as *kakemai*. However, compared to those of SR, analyses of CR for high-quality sake brewing, such as *Daiginjyo-shu* produced from highly polished rice of less than 50% polishing ratio (polishing ratio, percentage of polished rice weight to the original brown rice weight), have been limited.

Several features of the rice are desired for high-quality sake brewing, e.g., high water absorption rate, high digestibility/saccharification in sake mash, low protein content, and sufficient grain rigidity/hardness for polishing. Especially, a low protein content is an important feature for high-quality sake brewing, because an excess amount of proteins (amino acids) causes a rough taste and deepening of the color of the refined sake, which are negative factors in terms of sensory evaluation (Yoshizawa, 1999; Hashizume *et al*., 2007; Sugimoto *et al*., 2010). Furthermore, the aroma of sake containing a high content of amino acids appears to deteriorate during storage (Yoshizawa, 1999). As the germ and the surface fraction of the rice grain are abundant in proteins and lipids, the rice used for high-quality sake brewing is highly polished to reduce these negative factors for sake taste (Yoshizawa, 1999; Okuda *et al*., 2007; Anzawa *et al*., 2013). Therefore, sufficient grain rigidity to resist high-polishing is also required for the rice used in high-quality sake brewing (Anzawa *et al*., 2013).

In addition to varietal characteristics/genetic background of the rice, the cultivation conditions affect the protein content of brown rice. Previously, it was reported that the protein content is negatively correlated with the period of ear emergence and that the protein content of late-maturing rice cultivars tends to be lower than that of the early-maturing rice ones (Higashi and Kushibuchi, 1976). Compared with those of early-maturing rice, temperatures during the ripening period and nighttime of late-maturing rice are relatively low. This temperature difference is thought to be one of the causes of the lower protein content of the late-maturing rice grain. Indeed, it has been suggested that the protein content of rice is affected by the number of hours of sunlight and by the temperature during the cultivation period (Koseki *et al*., 2004). Crude protein contents of several rice cultivars tend to be increased by a high temperature during the ripening period (Koseki *et al*., 2004), although no statistically-significant difference between crude protein content and mean air temperature during 1 month after heading has been reported in the analysis of 51 across-the-country rice cultivars (Okuda *et al*., 2009).

To establish a process of sake brewing suitable for individual rice cultivars (*Oryza sativa* L.), research on and characterization of SR have been conducted in various regions of Japan. The late-maturing SR *Yamadanishiki* (YAM) developed in Hyogo Prefecture (http://www.gene.affrc.go.jp/databases-plant_search_en.php) has been used widely in Japan. In Niigata Prefecture, 2 early-maturing SRs, *Gohyakumangoku* (GOM) and *Takanenishiki* (TAK) (http://www.gene.affrc.go.jp/databases-plant_search_en.php), have been used and have contributed to the production of high-quality sake with a clear taste ("*Tanrei*" one of the perceivable attributes of the refined sake). In 2004, another late-maturing SR, *Koshitanrei* (KOS), was developed in Niigata Prefecture; (http://www.gene.affrc.go.jp/databases-plant_search_en.php; Kobayashi *et al*., 2006) and recently its excellent polishing property was reported (Anzawa *et al*., 2013).

The late-maturing CR *Sensyuraku* (SEN) was originally developed in Niigata Prefecture by crossbreeding between *Kanto-53* and *Norin-36* in 1960 (http://www.gene.affrc.go.jp/databases-plant_search_en.php). However, in 1971, due to the increase in consumption of CR *Koshihikari* (KSH), SEN was omitted from the recommended rice cultivar list. In 1996, SEN was selected again as a rice with a low protein content and was revived as *fukkoku-mai* (revived rice). SEN has an advantage in cultivation, because the harvest time of SEN does not overlap that of major SR GOM. Currently, SEN is being used for high-quality sake brewing. However, analysis of brewing properties of SEN has been limited. In this report, I describe the original screening results and brewing properties of SEN for high-quality sake brewing.

2.3. Materials and Methods

2.3.1 Rice cultivars

In this study, I used 3 cooking rice (CR) cultivars (*Oryza sativa* L.), SEN, *Yukinosei* (YUK, crossbreeding between *Huko*-No.101 and *Niigata*-No.8) (http://www.gene.affrc.go.jp/databases-plant_search_en.php), and *Koshi-ibuki* (KSI, crossbreeding between *Hitomebore* and *Doman-naka*) (http://www.gene.affrc.go.jp/databases-plant_search_en.php), and 5 sake rice (SR) cultivars (*Oryza sativa* L.), GOM, KOS, Niigata Sake-No.36 (crossbreeding between GOM and *Sei-kei* Sake-No.94), *Cyou*-No.884 (crossbreeding between *Cyou*-No.460

and GOM), and *Cyou*-No.1162 (crossbreeding between *Yukinosei* and Niigata Sake-No.28). To polish the brown rice, I used 2 polishing machines, a laboratory machine (HS-4; Chiyoda Engineering, Hiroshima, Japan) and an industrial one (NF-26FA, Shin-Nakano, Okayama, Japan).

2.3.2 General analysis of rice grain

For general analysis of rice grains, I followed the National Standard Analysis Method of Materials for Sake Brewing (http://www.sakamai.jp/bunseki.html). The strength of the brown and polished rice was measured by crushing the grains (n = 100) with a grain rigidity tester (Kiya, Tokyo).

2.3.3 Analysis of protein body in rice

For analysis of the protein body (PB), I followed the method reported by Kizaki *et al*., 1991. The PB was detected by SDS-PAGE, and PB composition (%) was calculated from the peak area on densitograms by use of an imaging system (Gel Doc EZ Imager, Bio-Rad Laboratories, Inc., USA).

2.3.4 Pasting properties of starch in rice

The pasting properties/parameters (Fig. 9), including peak viscosity (PV), breakdown (BD), set back (SB), and pasting temperature (PT: start of gelatinization), in the heat-induced gelatinization of rice flour were measured by use of Rapid Visco Analyzer (RVA; Model RVA-4500, Newport Scientific, Australia) according to the method of Aramaki *et al*.,2004 and Okuda *et al*., 2005: distilled water was added to 2.8 g (10%, w/w, dry weight basis) of rice flour to make the total weight to 28 g. Each suspension was equilibrated at 50ºC for 1 min, heated at a rate of 5ºC/min to 95ºC, maintained at 95ºC for 5 min, cooled to 50ºC at a rate of 5ºC/min, and then maintained at that temperature for 6 min. A constantly rotating paddle (160 rpm) was used.

2.3.5 *Koji*-making on industrial scale

The malted rice (*koji*) was produced from 600 kg of the polished rice (polishing ratios, 60 or 55%) using 4 rice cultivars, 2 CRs (SEN and *Koshi-ibuki*, KSI) and 2 SRs (GOM and KOS), by an industrial *koji*-manufacturing system (Churitsu Industry Co., Ltd., Tokyo) under the programed conditions (maximum temperature, 42°C; culture time, 50 h). Enzyme activity (α-amylase and glucoamylase) of the produced *koji*-mold (*Aspergillus oryzae*) was measured by the assay kit (Kikkoman Biochemifa Company, Tokyo) (Imai *et al*., 1996; Shirokane *et al*., 1996).

2.3.6 Small-scale sake brewing test

The sake from a small-scale brewing test (total rice, 300 g) was produced as follows: 60 g of the identical malted rice (*koji*) made from polished GOM (50% polishing ratio) by an industrial *koji*-manufacturing system was used for individual small-sacle sake brewing test. Two hundred forty grams of polished rice (polishing ratios, 60 and 40%) of 4 rice cultivars, 2CRs (SEN and YUK) and 2SRs (KOS and GOM), was used only as *kakemai* (steamed rice added to the sake mash). The sake yeast G9NF strain (*Saccharomyces cerevisiae*), developed originally at the Niigata Prefectural Sake Research Institute (Sato *et al*., 2005), was used for sake fermentation, which was carried out at 12°C for 16 days; and the clear sake liquid was obtained by centrifugation

2.3.7 General properties, flavor components, and sensory evaluation of sake

General properties (OE, original extract; TA, total acidity; AA, amino acidity), and flavor components (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 Agency (http://www.nta.go.jp/shiraberu/zeiho-kaishaku/tsutatsu/kobetsu//sonota/070622/01.htm) and gas chromatography (GC-2014, Shimadzu, Kyoto, Japan), respectively. Sake samples adjusted to a temperature of 20°C were evaluated by 7 experienced panelists. Sake quality was graded by sensory scores from 1 to 4, 1 indicating the highest quality.

2.4 Results and Discussion

2.4.1 Original selection of the late-maturing cooking rice *Sensyuraku* (SEN) as a rice with low-protein content

In 1996, to screen for a rice cultivar(s) with a low-protein content suitable for high-quality sake brewing, I obtained seed rice of 4 rice cultivars (Table 7), late-maturing CR SEN, and 3 early-maturing SRs (A/Niigata Sake-No.36, B/*Cyou*-No.884, and C/*Cyou*-No.1162), recommended from the Crop Research Center of Niigata Agricultural Research Institute, and cultivated them in a compartment of 15 m^2 within the same field under the same fertilization conditions. I examined the protein content of the brown rice and the crush rate of the polished rice during polishing by using a laboratory polishing machine (HS-4; Chiyoda Engineering, Japan; input of rice, 70 g; at a constant rate of 1,300 rpm) from 70% to 40% polishing ratio of 4 rice cultivars (SEN, A, B, and C), and compared the results with those of SR GOM and CR *Yukinosei* (YUK) as the control (Table 7). The early-maturing YUK is being used as *kakemai*. As the result, the crude protein of the SEN brown rice and the crush rate of the SEN polished rice tended to be lower than those of the other cultivars, thus showing SEN to have a low protein content and excellent polishing properties. Consistent with low crush rate, the polishing time of 2 CRs (SEN and YUK) was longer than that for GOM (Table 7). In this first screening, SEN was selected as the rice cultivar with the lowest protein content among 4 rice cultivars.

2.4.2 Follow-up investigation of properties of *Sensyuraku*

For further investigation of the properties of SEN for high-quality sake brewing, follow-up investigations on SEN were performed in 1998 and 1999. SEN and 2 rices (SR GOM and CR YUK) were cultivated, each in a compartment of 3,000 m^2, within the same field, and the properties of SEN were compared with those of 2 others (Table 8). The 1,000-grain weight of SEN brown rice was larger than that of YUK and was similar to that of GOM, indicating that CR SEN belonged to the same large-grain rice group as SR GOM. Also, the protein and amylose contents of SEN brown rice were lower and higher, respectively, than those of 2 others. Using a laboratory polishing machine, I polished the 3 rice cultivars and compared their polishing properties (Table 8). The polishing time spent for a 70% polishing ratio of 2 CRs (SEN and YUK) was longer than that for GOM, and the crush rate for SEN or YUK with a 70% polishing ratio was lower than that for GOM. These results suggested that the hardness of the SEN grain was greater than that of the grain of GOM. This result is also consistent with a previous report indicating that the crush rate of CR is generally lower than that of SR with a white-core region (*shin-paku*) (Iemura *et al*., 1996).

Next I examined 2 basic brewing properties, water absorbability of the polished rice (70% polishing ratio) and enzymatic digestibility (Brix value) of the steamed rice (Table 8). Water absorbability for 20 min for SEN tended to be lower than that for GOM, but the absorbability for 120 min for the former tended to be higher than that for the others. The Brix value of SEN was higher than those of 2 others. These results suggested that SEN has excellent properties in terms of both water absorbability and enzymatic digestibility. To further investigate the starch retrogradation rate for the steamed rice (60% polishing ratio), I measured the Brix value of the steamed rice after cooling at 15°C for 1 to 3 h after steaming (Fig. 7). The starch retrogradation rate (decrease in the Brix value after cooling) is positively correlated with the decreasing rate of enzymatic digestibility (Okuda *et al*., 2006). The rate of decrease in the Brix value for SEN was lower than that for GOM or YUK (Fig. 7), indicating that the starch retrogradation rate of SEN was relatively low compared with that for 2 others. The starch retrogradation rate of YAM is relatively low (Okuda *et al*., 2006), and this feature is thought to be one of the reasons for the excellent brewing properties (enzymatic digestibility, etc.) of YAM. It is possible that the structure of endosperm starch in SEN might be similar to that in YAM. To investigate this point, it is necessary to compare starch gelatinization/retrogradation between SEN and YAM.

Taken together, these results (Table 7, 8 and Fig. 7) suggested that SEN had excellent properties for high-quality sake brewing, low protein content, low crush rate (excellent polishing properties), and high water adsorption/enzymatic digestibility.

2.4.3 Analyses of polishing/brewing properties of rice cultivars for high-quality sake brewing

In 2012, I conducted an exhaustive investigation of the polishing/brewing properties of the late-maturing CR SEN with 3 rice cultivars (late-maturing SR KOS, and 2 early-maturing rices SR GOM and CR YUK) (Table 9). To first investigate the strength of SEN during polishing, I measured the grain rigidity of the brown and polished rice (60% and 40% polishing ratios) of SEN by crushing the grains (n = 100) with a grain rigidity tester (Table 10), and compared the results with that for GOM. GOM produces high-quality sake with a clear taste, but the hardness of its grains is insufficient for high polishing (especially to less than the 50% polishing ratio for *Daiginjo-shu*). The grain rigidity of the SEN brown rice was similar to that of others, but the grain rigidity of the SEN polished rice was higher than that of GOM. Thus, SEN showed excellent polishing properties.

The crude protein contents of the brown rice of 2 late-maturing rice (CR SEN and SR KOS) were lower than those of 2 early-maturing rice (SR GOM and CR YUK) (Table 10). The previous report suggested that the protein content of rice is affected by the number of hours of sunlight and by the temperature during the cultivation period (Koseki *et al*., 2004). Mean air temperature during 1 month after ripening of late-maturing rice (SEN and KOS) tended to be lower than those of early-maturing rice (GOM and YUK) (Table 9), indicating the positive correlation between the protein content and the mean air temperature. On the other hand, there was no significant correlation between the protein content and mean hours of sunlight during 1 month after ripening in this year (Table 9 and 10). The cultivation time (days after transplanting) of late-maturing rice was longer than that of early-maturing rice (Table 9). It is possible that the nitrogen source fertilized in soil might be used in the growth of plant body rather than the accumulation in rice grain during long cultivation time. Crude protein content in rice grain would be affected by many factors; genetic background, cultivation temperature, fertilization conditions, etc. Further analysis is necessary to clarify this point.

Previously, it was reported that storage proteins in the rice endosperm consist of 20-25% PB-I (protein body type I), 60-65% PB-II (protein body type II), and 10-15% albumin and globulin in the cytoplasm (Ogawa *et al*., 2006). Approximately 90% of the total proteins of rice endosperm are represented by 2 types of proteinaceous particles, PB-I and PB-II. PB-I is spherical with lamellar structure and contains prolamin; on the other hand, PB-II is non lamellar and is rich in glutelin (Tanaka *et al*., 1980). To investigate the protein and the brewing properties of SEN, I examined the PB composition of brown rice (100% polishing ratio) and polished rice (60% and 40% polishing ratios) of 4 rice cultivars, 2 late-maturing rice (CR SEN and SR KOS) and 2 early-maturing rice (CR YUK and SR GOM) (Table 10). For brown rice, the PB composition (I+II) of late-maturing SEN was similar to that of late-maturing KOS, and lower than that of the early-maturing rice (GOM and YUK). This result is consistent with a previous report indicating that the protein content of late-maturing rice cultivars tends to be lower than that of early-maturing ones (Higashi and Kushibuchi, 1976).

It has been reported that PB-II, but not PB-I, in sake mash (*moromi*) is dissolved by the enzymes from the *koji*-mold and that PB-I thus remains in the late-stage sake mash (Furukawa *et al*., 2000). Also, the PB-I content and the ratio of PB-II/PB-I of SR tend to be lower and higher, respectively, than those of CR (Kizaki *et al*., 1993). However, the analysis of PBs has been limited (polished rice of 70% polishing ratio) (Kizaki *et al*., 1993). As shown in Table 10, the ratio of PB-II/PB-I for SEN brown rice was lower than that for KOS or GOM; and for the SEN polished rice (60% polishing ratio), the ratio of PB-II/PB-I was similar to those for the SRs. The PB-I content and the ratio of PB-II/PB-I for SEN were similar to that for SR, suitable for sake brewing.

Further, I measured the enzymatic digestibility of the steamed rice of 4 rice cultivars (Fig. 8). The Brix value of SEN was similar to that of KOS and was higher than those of GOM and YUK, indicating that CR SEN had good digestibility, equivalent to SR KOS, in the sake mash.

2.4.4 Confirmation of brewing properties of rice cultivars by Rapid Visco Analyzer

It has been reported that the brewing properties of rice are correlated significantly with the physical properties/parameters (Fig. 9), including peak viscosity (PV), breakdown (BD), set back (SB), and pasting temperature (PT: start of gelatinization), in the heat-induced gelatinization of rice flour determined by use of a Rapid Visco Analyzer (RVA) (Aramaki *et* *al*., 2004; Okuda *et al*., 2005). The reported correlations are as follow (Aramaki *et al*., 2004): the crude protein content of rice is correlated negatively with PV and BD, and positively with PT. Water absorption of rice is correlated positively with PV and BD. Enzyme digestibility (Brix value) of steamed rice is correlated positively with PV and BD, and negatively with PT.

To estimate/confirm the brewing properties of SEN, we measured the pasting/viscosity parameters (PV, BD, SB, and PT) of 4 rice cultivars, 2 CRs (SEN and YUK) and 2 SRs (KOS and GOM) by using the RVA (Table 11). The PV and BD of SEN were higher than those of 2SRs (KOS and GOM) or CR YUK (Table 11). This result suggested that the crude protein content of SEN was lower than those of 2 SRs (KOS and GOM) or CR YUK and that the enzyme digestibility and the water absorption of SEN were higher than those of the others. Further, this suggestion supports the previous results (Table 7, 8, 10, and Fig. 8) showing the excellent properties of SEN for high-quality sake brewing, i.e., low protein content and good water adsorption/enzymatic digestibility. Additionally, the PV and BD of the 4 rice cultivars increased with decreasing polishing ratios; on the other hand, their PT decreased with decreasing polishing ratios. As the crude protein content of rice is correlated negatively with PV and BD, and positively with PT, these results are consistent with the fact that the rice protein content decreased with decreasing polishing ratios. Further, it has been reported that the ratio of PB-II/PB-I and PT increase as the temperature during the ripening period of rice cultivars becomes higher (Ashida *et al*., 2013). The present results for the late-maturing CR SEN are also consistent with this previous report (Ashida *et al*., 2013).

Previously, it was reported that the total starch content of rice increase with decrease in polishing ratios, and are correlated positively with PV (Okuda *et al*., 2007), suggesting that pasting parameters (PV, etc.) examined by RVA are affected by not only the crude protein content but also the properties of endosorm starch of rice, i.e., amylose/amylopectin content and side-chain structure of amylopectin (Aramaki *et al*., 2004; Okuda *et al*., 2005; Okuda *et al*., 2006; Okuda *et al*., 2007). Thus, the previous reports indicate a strong correlation between the RVA parameters and brewing properties (water absorption and enzyme digestibility). Further, the side-chain of amylopectin in late-maturing YAM tends to be shorter than that in early-maturing GOM, and one of the reasons of this phenomenon is thought to be caused by the low temperature during the ripening period (Okuda, 2007). To further analyze the RVA parameters of SEN, I need to investigate the properties/structure of starch in the rice.

2.4.5 Polishing and *koji*-production on an industrial scale

To further investigate the polishing/brewing properties of SEN, I polished 4 rice cultivars (polishing ratios, 60, 40%) by use of the industrial polishing machine (NF-26FA, Shin-Nakano Industry, Japan; input of rice, 1,020 Kg). The polishing time spent for a 40% polishing ratio of 2 CRs (SEN and YUK) was longer than those for 2 SRs (KOS and GOM) (Table 12). I further investigated the water-absorbing ratios of 4 rice cultivars (Fig. 10). The amounts of water absorbed by 4 rice cultivars increased with decreasing polishing ratios. Compared with those of 2 SRs (KOS and GOM), the water-absorbing ratio of SEN for a short time (10, 20 min) was low, on the other hand, in the case of water absorption for 120 min (the maximum water absorption) of SEN was high. The water absorption (for either 20 or 120 min) of SEN was higher than that of YUK.

To further investigate the suitability of SEN for sake brewing, I produced a malted rice (*koji*) using 600 kg of polished rice (polishing ratios, 60 or 55%) using 4 rice cultivars, 2 CRs (SEN and *Koshi-ibuki*, KSI) and 2 SRs (GOM and KOS) by an industrial *koji*-manufacturing system (Churitsu Industry Co., Ltd., Tokyo), and measured the enzyme activities of α-amylase (α-A) and glucoamylase (GA) in the *koji*. As shown in Table 13, the enzyme activities of these amylases in the *koji* of SEN were higher than that in the CR KSI *koji*, and were similar level to those in the SR *koji*. These results indicated that SEN is suitable for not only *kakemai* but also *koji*-making, as SR. Further analysis is necessary to clarify this point.

2.4.6 Quality of sake produced from *Sensyuraku*

Furthermore, to confirm the suitability of SEN for high-quality sake *Ginjyo-shu* or *Daiginjyo-shu* (less than 60% or 50% polishing ratio, respectively), I carried out a small-scale sake brewing test using 300 g (total rice) of polished rice (60 and 40% polishing ratios) of 4 rice cultivars (Table 14). The original extract (OE) of sake from SEN was similar to that from KOS, and was slightly higher than that from GOM and YUK (alcohol contents (%) were as follows: sake using polished rice of 60% polishing ratio = 14.4-14.8%, that of 40% polishing ratio = 13.1-13.6%). This result is consistent with the high digestibility (Brix value in Table 8, Fig. 7 and 8) and the maximum water absorption (the water-absorbing ratio for 120 min in Table 8 and Fig. 10), indicating that SEN had good digestibility in the sake mash. The amino acidity (AA) of the sake produced from SEN appeared to be slightly lower than that of the YUK sake. Flavor components (isoamyl acetate and ethyl caproate) of the sake produced from SEN was higher than that of YUK sake and was similar level to those of

SR sake (KOS and GOM). Indeed, the clear taste of the sake produced from SEN was evaluated and gave a higher sensory score than that for YUK by 7 experienced panelists. As to sake tasting, an excess amount of AA and appropriate levels of flavor components correlated negatively and positively, respectively, with the sensory evaluation scores. One of the reasons for a high evaluation of sake from SEN will be the low level of AA and high contents of the esters, similar to that of SR sake.

In this study, I described the original screening of SEN and properties of SEN for high-quality sake brewing. The late-maturing CR SEN was selected as a rice with a low protein content. These results indicated that SEN had excellent properties, equivalent to SR, for the production of high-quality sake from highly polished rice such as *Daiginjo-shu*. In this study, I confirmed the excellent properties of SEN, which was originally developed in Niigata Prefecture, by comparing with that of SR GOM that has been widely used in Niigata Prefecture. As the next step, it is necessary to compare brewing properties between SEN and SR YAM. These results provide information that should contribute to the improvement of high-quality sake brewing using SEN and shed light on the breeding and characteristics of CR for high-quality sake brewing.

Table 7 Protein content and polishing properties of *Sensyuraku*.

Rice cultivars [a]	Brown rice: 1000-kernel weight (g)	Brown rice: Crude protein (%)	Crush rate (%) of the polished rice [b], polishing ratio of: 70%	60%	50%	40%	Polishing times (min) for polishing ratios [b]: 60%	50%	40%
harvested/examined in 1996									
SEN	25.1	6.8	0.8	2.5	5.1	6.0	120	200	335
A	25.8	7.7	2.1	2.9	5.0	6.5	80	115	180
B	32.1	8.5	6.4	13.6	25.4	35.9	115	165	265
C	27.0	7.7	1.5	2.9	6.7	8.5	105	175	270
GOM	26.7	7.2	6.5	5.8	12.6	13.2	90	140	230
YUK	23.4	7.0	3.0	3.9	6.3	8.3	110	180	330

Values indicate averages for 2 independent experiments.

[a] Rice cultivars were cultivated in the same field of Niigata Prefecture and harvested/examined in 1996: SEN, GOM, YUK, and 3 sake rice cultivars, A (Niigata Sake-No.36), B (*Cyou*-No.884), and C (*Cyou*-No.1162).

[b] Brown rice was polished to white rice (polishing ratio from 70% to 40%) by using a laboratory polishing machine.

Table 8 Follow-up investigation of *Sensyuraku* for sake brewing.

Rice cultivars [a]	Brown rice [b]			Polished rice (polishing ratio of 70%) [b]				
	1000-kernel weight	Crude protein	Amylose content	Polishing time	Crushed rice rate	Absorbed water for 20 min	Absorbed water for 120 min	Enzyme digestibility (Brix) of steamed rice
	(g)	(%)	(%)	(min)	(%)	(%)	(%)	(%)
harvested/examined in 1998								
SEN	26.4	6.8	20.8	67	1.9	24.8	27.5	10.9
GOM	25.9	7.4	19.2	60	2.4	25.2	27.2	10.6
YUK	22.7	7.4	16.0	60	2.5	22.8	26.1	10.0
harvested/examined in 1999								
SEN	25.6	6.8	19.9	65	3.2	26.2	30.9	11.4
GOM	25.8	7.9	19.6	58	5.0	26.6	30.2	10.9
YUK	23.1	7.3	17.8	63	5.0	25.9	29.2	10.4

[a] Three rice cultivars, SEN, GOM, and YUK, were cultivated in the same field of Niigata Prefecture and harvested/examined in 1998 and 1999.

[b] The moisture content of the rice grains of the brown and polished rice was adjusted as follows: brown rice = 13.8%, 70% polished rice = 13.5%.

Table 9 Cultivation record of rice cultivars in 2012.

Rice cultivar	Date (days after transplanting)			1 month after ripening	
	Transplanting	Ripening	Maturity/ Harvest	Mean air temperature (℃)	Mean hours of sunlight (h)
harvested/examined in 2012					
late-maturing rice					
SEN	May. 22 (1)	Aug. 16 (86)	Sep. 22 (123)	27.2	8.8
KOS	May. 19 (1)	Aug. 18 (91)	Sep. 22 (126)	26.5	8.1
early-maturing rice					
GOM	May. 10 (1)	Jul. 26 (77)	Sep. 2 (115)	27.9	9.1
YUK	May. 21 (1)	Jul. 31 (71)	Sep. 12 (114)	27.6	8.4

The rice cultivars used in Table 10-14 and Fig. 7, 8, 10 were harvested/examined in 2012.

Table 10 Grain rigidity and composition of protein body in rice cultivars.

Rice cultivars	Grain rigidity [a] (kg/ cm^2) (AV ± SD)	Crude Protein [b] (%)	Protein body composition [b] (%) PB-I	PB-II	(I+II)	other	PB-II/PB-I [b]
Brown rice [c]							
SEN	4.82 ± 1.05	6.9	22.5	42.5	(65.0)	35.0	1.89
KOS	4.75 ± 1.67	6.8	21.7	44.5	(66.2)	33.8	2.05 *
GOM	4.32 ± 1.51	7.3 *	22.9	47.0 *	(69.9) *	30.1 *	2.05 *
YUK	4.61 ± 1.61	7.4 *	24.7 *	44.6 *	(69.3) *	30.7 *	1.80 *
Polished rice [c] polishing ratio of							
60%							
SEN	7.51 ± 1.37	4.0	24.6	52.4	(77.0)	23.0	2.13
KOS	5.47 ± 2.18	3.9	24.2	50.2	(74.4)	25.6 *	2.07
GOM	3.52 ± 2.18	4.4 *	25.2	53.2	(78.4)	21.6	2.11
YUK	7.34 ± 1.53	4.5 *	25.5 *	51.4	(76.9)	23.1	2.01 *
40%							
SEN	6.04 ± 1.01	3.5	22.7	47.7	(70.4)	29.6	2.10
KOS	4.33 ± 1.76	3.3	22.2	49.4	(71.6)	28.4	2.23 *
GOM	2.33 ± 0.74	3.8 *	23.1	49.4	(72.5)	27.5	2.14
YUK	5.95 ± 1.89	3.8 *	25.4 *	51.9 *	(77.3) *	22.7 *	2.04 *

[a] Values (grain rigidity) indicate AV (average) ± SD (standard deviation, n = 100).

[b] Values (crude protein and protein body composition) indicate averages for 3 independent experiments.

[c] Brown rice was polished to white rice (from 60% to 40% polishing ratio) by using a laboratory polishing machine (HS-4; Chiyoda Engineering, Hiroshima, Japan; input of rice, 70g) operated at a constant rate of 1,300 rpm and the moisture content of the rice grains was adjusted as follows: brown rice = 14.5%, polished rice 60% = 10.0%, 40% = 9.0%.

[*] Single asterisks indicate $p < 0.05$ for the significance of the difference in ratio (%) between SEN and others.

Table 11 Rapid Visco Analyzer (RVA) parameters of the rice cultivars.

Rice cultivars	RVA parameters (AV ± SD) [a]			
	PV (cp)	BD (cp)	SB (cp)	PT (℃)
Brown rice				
SEN	2,803 ±1	1,872 ±18	1,024 ±11	67.5 ±0.1
KOS	2,745 ±4*	1,834 ±12*	956 ±21*	67.6 ±0.1
GOM	2,356 ±5*	1,302 ±14*	918 ±6*	67.9 ±0.1*
YUK	2,748 ±25*	1,792 ±13*	878 ±13*	67.5 ±0.2
Polished rice polishing ratio of 60%				
SEN	4,013 ±21	2,669 ±7	951 ±1	65.6 ±0.1
KOS	3,934 ±33*	2,512 ±29*	961 ±27	65.6 ±0.1
GOM	3,697 ±36*	2,279 ±9*	947 ±27	65.3 ±0.1*
YUK	3,549 ±39*	2,137 ±36*	913 ±55*	65.9 ±0.1*
40%				
SEN	4,131 ±28	2,782 ±24	956 ±2	64.2 ±0.1
KOS	3,974 ±30*	2,507 ±49*	986 ±24*	64.5 ±0.1*
GOM	3,835 ±23*	2,412 ±24*	939 ±35	65.1 ±0.2*
YUK	3,794 ±37*	2,225 ±31*	936 ±35	65.3 ±0.2*

[a] Values indicate AV ± SD for 4 independent experiments.

* Single asterisks indicate $p < 0.05$ for the significance of the difference in value between SEN and others.

Table 12 Polishing time for the polishing ratios.

Rice cultivars	Polishing times (h) for polishing ratios	
	60%	40%
SEN	23.91	49.76
KOS	23.30	42.53
GOM	20.43	38.49
YUK	23.90	50.92

Brown rice of 4 rice cultivars (means of moisture contents of SEN, KOS, GOM, and YUK were 15.3%, 15.2%, 15.2%, and 15.2%, respectively) was polished to white rice.
The polishing was done with an industrial polishing machine (NF-26FA, Shin-Nakano, Okayama, Japan; input of rice, 1,020 Kg) under the polishing conditions reported previously (Anzawa *et al*., 2013).

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Table 13 Enzyme activity (α-amylase/α-A, glucoamylase/GA) of the malted rice (*koji*).

Rice cultivars	Polishing ratio (%)	Enzyme activity of *Koji* (U/*koji*-g) [a]	
		α-A	GA
SEN	60	1,198 ± 30	261 ± 31
GOM	60	1,125 ± 57	214 ± 26
KSI	60	791 ± 29 *	153 ± 22 *
KOS [b]	55	1,332 ± 26	251 ± 21

[a] Values indicate AV ± SD for 3 independent experiments.

[b] Value of KOS was indicated as reference.

* Single asterisks indicate $p < 0.05$ for the significance of the difference in value between SEN and GOM or KSI.

Table 14 Properties of the sake produced from the rice.

Rice cultivars	OE [a]	TA [a]	AA [a]	Flavor components [a] (ppm)		Sensory
Polishing ratio	(%)	(mL)	(mL)	iAmOAc	EtCap	Score [b]
SEN						
60%	32.2	2.2	1.0	4.0	0.7	2.33
40%	31.9	2.1	0.9	3.4	0.6	1.83
KOS						
60%	32.5	2.2	1.1	4.4	0.7	2.17
40%	32.3	2.2	1.1	3.8	0.7	1.67
GOM						
60%	31.6	2.3	1.1	3.4	0.6	2.33
40%	31.3	2.2	1.0	3.0	0.6	2.17
YUK						
60%	31.5	2.2	1.2	3.5	0.5	2.50
40%	31.3	2.1	1.0	3.0	0.5	2.50

[a] General properties (OE, original extract; TA, total acidity; AA, amino acidity), and flavor components (iAmOAc, isoamyl acetate; EtCap, ethyl caproate) of the sake are indicated as averages for 2 independent experiments.

[b] Sake quality was graded by sensory scores from 1 to 4, with 1 indicating the highest quality.

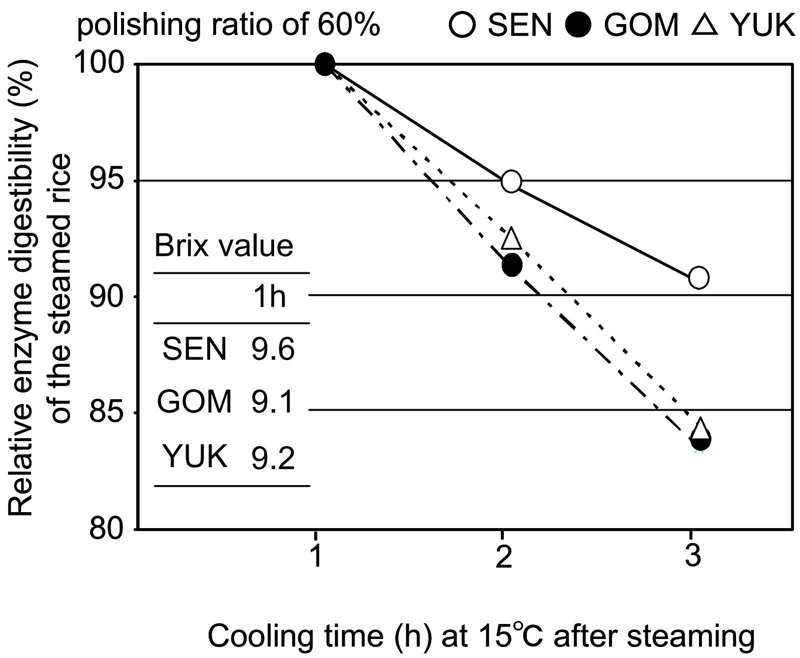

Figure 7　Relative enzymatic digestibility (Brix value) of the steamed rice after cooling at 15 °C for the indicated time (h) to the Brix value of the steamed rice after cooling for 1 h.

The rice cultivars were harvested/examined in 1999, and white rice with a 60% polishing ratio was used in this study.　Values indicate averages for 2 independent experiments.

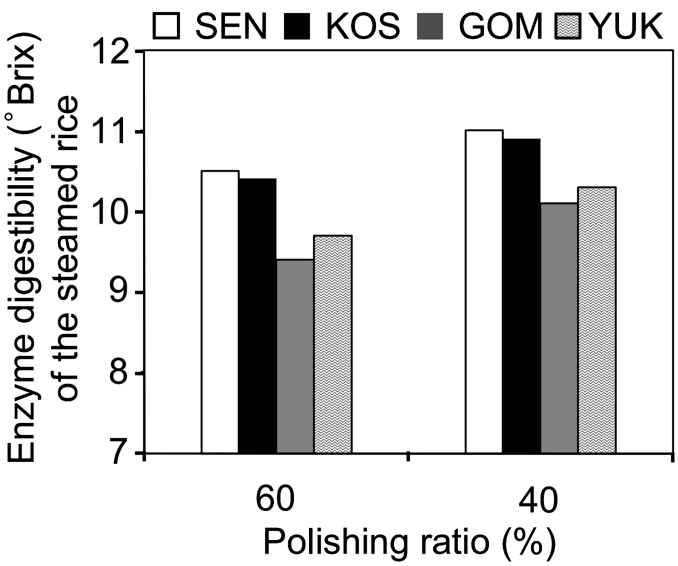

Figure 8 Enzymatic digestibility (Brix value) of the steamed rice.
Values are averages from 2 independent experiments.

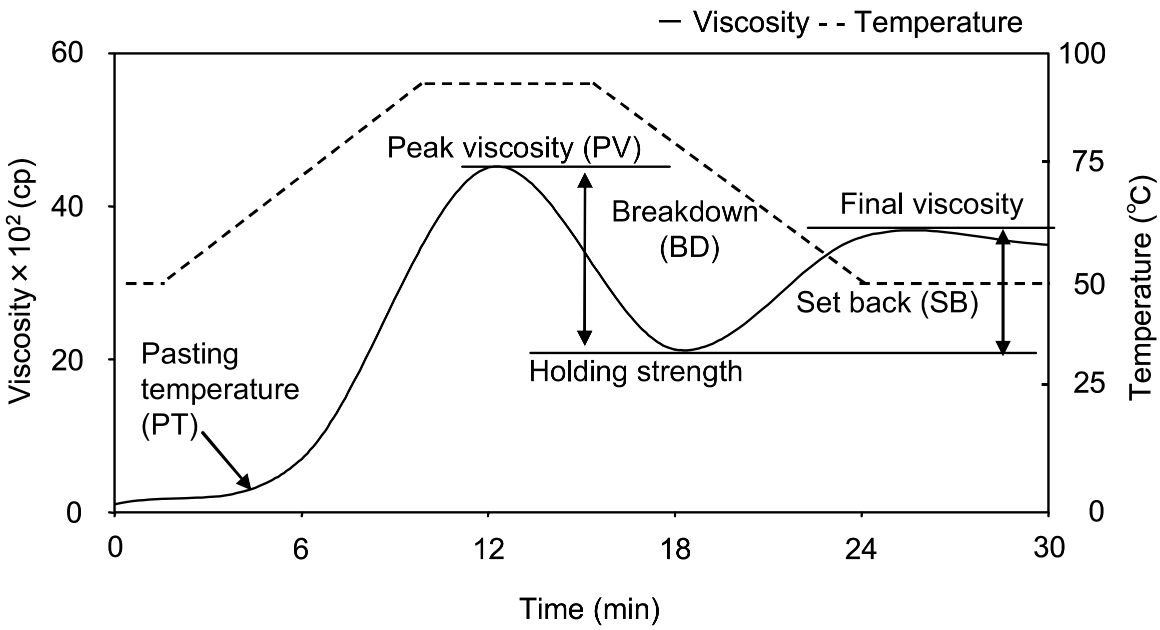

Figure 9 A representative rapid viscogram pattern of a rice flour generated by the Rapid Visco Analyzer (RVA).

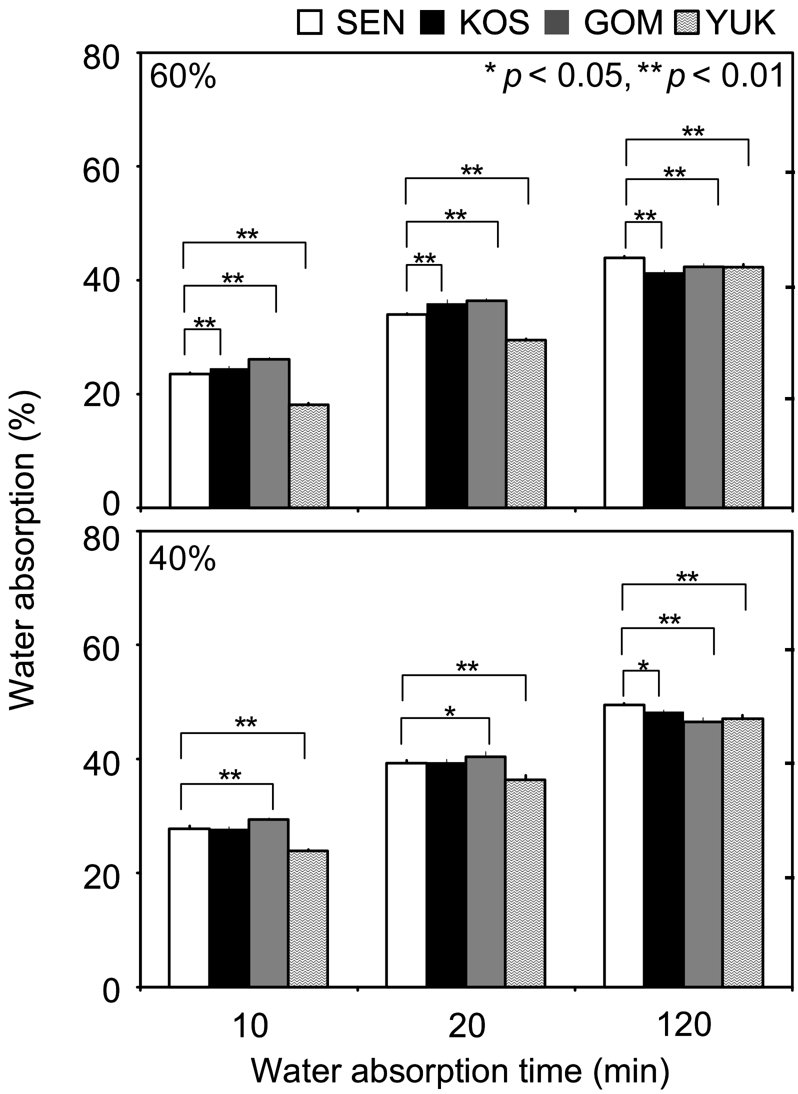

Figure 10 Water-absorption ratio of the polished rice

Values indicate AV ± SD for 5 independent experiments. The SDs were sufficiently small (0.19-0.82). Single and double asterisks indicate $p < 0.05$ and $p < 0.01$, respectively, for the significance of the difference in the ratios (%) indicated by the brackets. For the polished rice with a polishing ratio of 60%, the means of moisture contents of 4 rice cultivars (SEN, KOS, GOM, and YUK) were 9.9%; and for polished rice with a 40% polishing ratio, they were 8.8%, 8.9%, 8.9%, and 8.9%, respectively.

Concluding remarks

Sake is produced from rice (*Oryza sativa* L.) and water by a unique process using two microorganisms, fungi (*Aspergillus oryzae*) and yeast (*Saccharomyces cerevisiae*). The nature of the raw materials is one of the important factors determining the quality of the final product. Therefore, for the production of high-quality sake such as *Daiginjo-shu* (polishing ratio, less than 50%), it is necessary to choose excellent rice cultivars for control of the process. In this study, I examined the brewing properties of rice for high-quality sake brewing, i.e., sake rice *Koshitanrei* (SR KOS) and cooking rice *Sensyuraku* (CR SEN) developed in Niigata Prefecture.

In Chapter I, to clarify the brewing properties of KOS for high-quality sake brewing, I examined the polishing/brewing properties of KOS by using a wide range of polished rice (up to a polishing ratio of 30%) and compared them with those of 2 SRs, *Yamadanishiki* (YAM) and *Gohyakumangoku* (GOM). To improve the polishing property of GOM, the SR KOS was originally developed in Niigata Prefecture by genetically crossing GOM with YAM. However, analysis of the polishing/brewing properties of KOS has been limited. I compared the polishing property of KOS with that of GOM and YAM as determined by use of a laboratory polishing machine. The shape of the white-core region and the grain rigidity of KOS were very similar to those of YAM. The loss ratio of KOS after high polishing by an industrial polishing machine was lower than that of GOM. These results indicate that highly polished KOS has an excellent properties for high-quality sake brewing. It is possible that this excellence is due to the high frequency of the lined white-core grains of KOS. Further, a clear taste of sake produced from KOS was confirmed by sensory evaluation.

In Chapter II, I described the original screening for late-maturing cooking rice *Sensyuraku* (CR SEN) and characterization of its properties for high-quality sake brewing. SEN was revived as *fukkoku-mai* (revived rice) and has been used for high-quality sake brewing. However, the screening methods of SEN in the revival have not been reported yet. Additionally, characterization of SEN for high-quality sake brewing using highly polished rice has been limited. SEN was selected again as a rice with a low protein content. To clarify the brewing properties of highly polished SEN for high-quality sake brewing, I examined them and compared them with those of 2 SRs, early-maturing GOM and late-maturing KOS, and 1 CR, early-maturing cooking rice *Yukinosei* (CR YUK). The protein content of SEN was lower than that of SR GOM or CR YUK, and its grain rigidity was higher than that of GOM. Recently, pasting properties investigated with a Rapid Visco

Analyzer (RVA) were used to analyze the properties of endosperm starch related to the suitability for sake brewing (Aramaki *et al*., 2004; Okuda *et al*., 2005; Okuda *et al*., 2006; Okuda *et al*., 2007). The excellent properties of SEN with respect to both water-adsorption and enzyme digestibility were confirmed by use of the RVA. Additionally, the levels of enzyme activities of the amylases in the *koji* of SEN were similar to those in the *koji* of SR. These results indicate that CR SEN, like SRs, is suitable for not only *kakemai* but also *koji*-making. Thus, SEN has excellent properties, equivalent to those of SR, for high-quality sake brewing.

In sake brewing, the quality of rice is important not only for the quality of the sake but also for control of the process of sake brewing. Therefore, breeding/selection of the rice is an important step for the brewing of high quality sake. In this study, I investigated the polishing/brewing properties of 2 rice cultivars (SR KOS and CR SEN) for the brewing of high-quality sake, in particular *Daiginjo-shu* (polishing ratio, less than 50%). This study not only should stimulate further analysis of the breeding and characteristics of SRs but also shed light on the breeding and characteristics of CRs for high-quality sake brewing. In addition, it result provides useful information for not only control of process of sake brewing but also the production of relevant foods produced from rice. Finally, I propose that these studies/efforts should be continued for high-quality sake brewing.

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Running title: Suitability of *Koshitanrei* for High-Quality Sake Brewing

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 Prefecture[11)] and has been used widely for *daiginjo-shu* brewing in Japan. To improve the polishing property of GOM, the sake rice *Koshitannrei* (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.

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.

Fig.1

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 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

Fig. 2

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/cm^2). For GOM, the grain rigidity/hardness decreased from 4.32 kg/cm^2 (100% brown rice) to 1.88 kg/cm^2 (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 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).

Fig. 3

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 rice), 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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Figure legends

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.

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]

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 Agency[19] 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 days, and clear sake liquid was obtained by centrifugation.

A

Rice cultivars	Grain size (mm)			1000-kernel weight (g)	Percentage of grains with white-core region (%)
	L	W	T		
KOS	5.34 ± 0.27	3.14 ± 0.17	2.07 ± 0.08	26.60	53.8
GOM	5.33 ± 0.22	3.18 ± 0.16	2.10 ± 0.09	26.33	60.2
YAM	5.52 ± 0.22	3.11 ± 0.15	2.07 ± 0.08	27.20	46.8

B

Rice cultivars	Shape of white-core region (%)			
	Ellipsoidal	Lined	Dotted	Deviated from center (Harajiro)
KOS	17.7	56.3	12.5	13.5
GOM	54.5	22.2	7.1	16.2
YAM	8.5	62.8	9.6	19.1

C

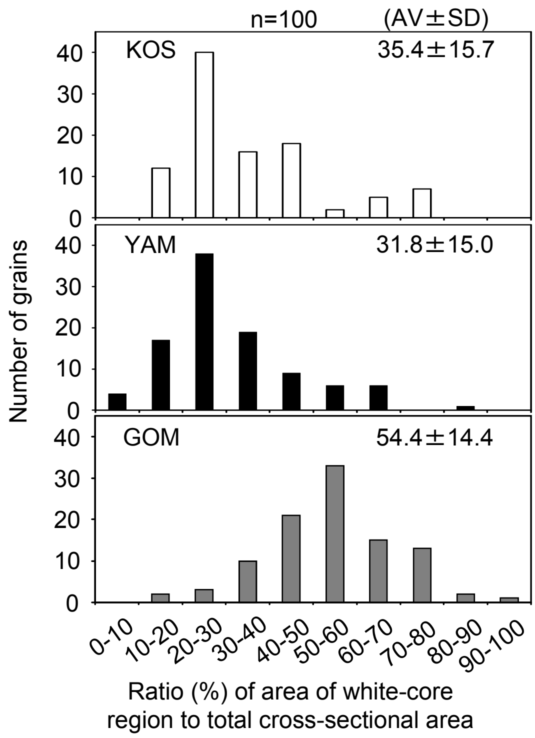


Fig. 1, Anzawa *et al.*

A

Rice cultivars	Polishing times (min) for polishing ratios				
	70%	60%	50%	40%	30%
KOS	60	110	175	365	530
GOM	60	90	160	260	420
YAM	75	165	330	450	680

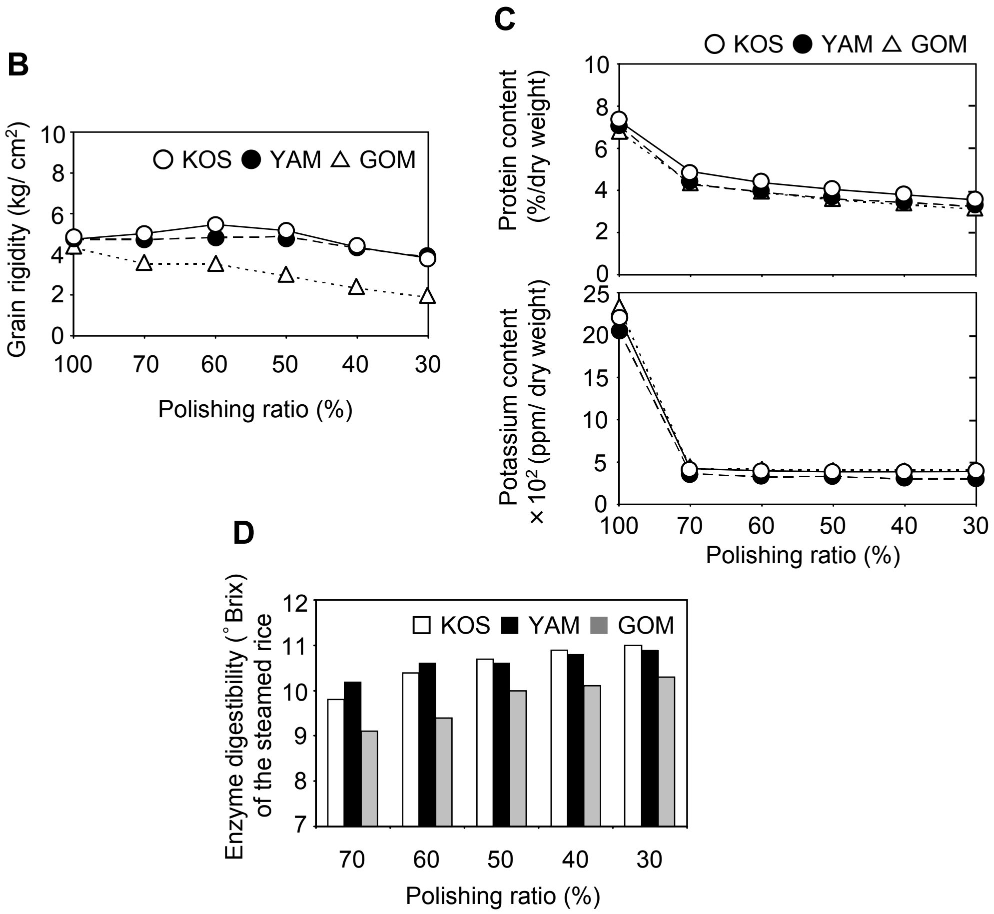


Fig. 2, Anzawa *et al.*

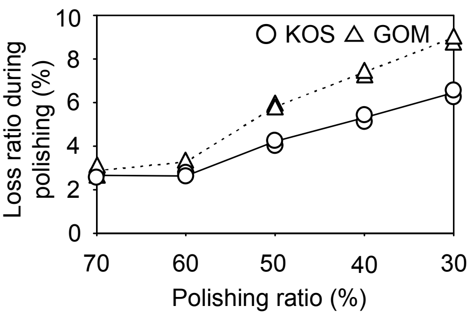
A

Polishing ratio (%)	Revolution rate (rpm)
100-95	470
95-90	455
90-85	440
85-80	425
80-75	410
75-70	395
70-65	391
65-60	387
60-55	383
55-50	380
50-45	377
45-40	375
40-30	350

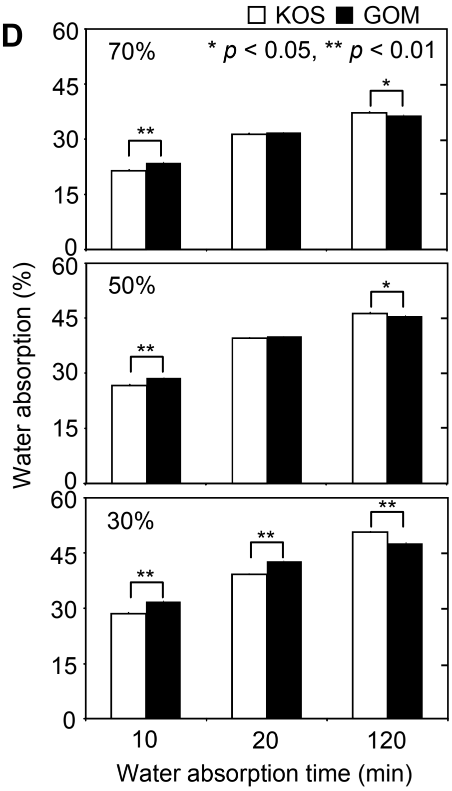
B

Rice cultivars	Polishing times (h) for polishing ratios				
	70%	60%	50%	40%	30%
KOS	11.58	23.30	31.98	42.53	53.60
GOM	10.05	20.43	29.23	38.49	48.53

C



D



E

Cultivars Polishing ratio	OE (%)	TA (mL)	AA (mL)	Flavor components (ppm) iAmOH	iAmOAc	EtCap	Sensory score
KOS							
70%	32.6	2.2	1.1	175	4.7	0.7	2.17
50%	32.2	2.2	1.1	165	4.1	0.7	1.50
30%	32.9	2.1	1.0	152	3.5	0.7	1.67
GOM							
70%	32.2	2.3	1.2	163	3.9	0.5	2.33
50%	31.2	2.2	1.0	152	3.2	0.5	2.17
30%	31.8	2.2	0.9	126	2.9	0.6	2.17

Fig. 3, Anzawa *et al.*

Running title: Characterization of *Sensyuraku* for High-Quality Sake Brewing

Late-Maturing Cooking Rice *Sensyuraku* Has Excellent Properties, Equivalent to Sake Rice, for High-Quality Sake Brewing

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Abstract

Low protein content and sufficient grain rigidity are desired properties for the rice used in high-quality sake brewing, such as *Daiginjo-shu* (polishing ratio of the rice, less than 50%). Two kinds of rice, sake rice (SR) and cooking rice (CR), have been used for sake brewing. Compared to those of SR, analyses of CR for high-quality sake brewing using highly polished rice have been limited. Here we described the original screening of late-maturing CR *Sensyuraku* (SEN) as a rice with low protein content and characterization of its properties for high-quality sake brewing. The protein content of SEN was lower than those of SR *Gohyakumagoku* (GOM) and CR *Yukinosei* (YUK), and its grain rigidity was higher than that of GOM. The excellent properties of SEN with respect to both water-adsorption and enzyme digestibility were confirmed by use of a Rapid Visco Analyzer (RVA). Further, we confirmed a clear taste of sake produced from SEN by sensory evaluation. Thus, SEN has excellent properties, equivalent to those of SR, for high-quality sake brewing.

Key words: late-maturing rice; low protein content; polishing property; *sensyuraku*; sake brewing

Introduction

The Japanese alcoholic beverage sake is produced from 2 raw materials, rice and water, by 2 simultaneous bioconversions, i.e., the hydrolysis of starch in the steamed rice to glucose by saccharification enzymes from the fungus *Aspergillus oryzae* (*koji*-mold) and the production of ethanol from glucose by the yeast *Saccharomyces cerevisiae*.[1] Brown rice is polished, and the steamed rice is used in 2 ways: one is direct addition of it to sake mash (as *kakemai*), and the other is the production of malted rice (*koji*), in which the *A. oryzae* spores are inoculated to the steamed rice and incubated about 2 days to generate *koji*. During the *koji* making process, *A. oryzae* produces a number of enzymes, saccharification enzyme, proteolytic enzyme, and others. The first mash (*shubo*) is prepared from *koji*, steamed rice (*kakemai*), water, and yeast (*S. cerevisiae*). After 2 weeks of the *shubo* making process, sake mash is expanded to main mash (*moromi*) by adding *koji*, steamed rice (*kakemai*), and water. After about 1 month of sake fermentation, the clear sake liquid is obtained by filtration. The rice used in sake brewing has attracted attention as an important determinant for the quality of the final sake product.

In general, 2 kinds of rice (*Oryza sativa* L.), sake rice (SR) and cooking rice (CR), have been used for sake brewing. Officially, SR has been defined as *shuzo-koteki-mai* in Agricultural Products Inspection Act. SR but not CR has a characteristic white opaque-core region (called *shin-paku*), which contributes to the invasion of the *koji*-mold to the inside of the steamed rice grain. Therefore, SR is used mainly for making *koji*. On the other hand, usually CR is used as *kakemai*, although SR is also used as *kakemai*. However, compared to those of SR, analyses of CR for high-quality sake brewing, such as *Daiginjo-shu* produced from highly polished rice of less than 50% polishing ratio (polishing ratio, percentage of polished rice weight to the original brown rice weight), have been limited.

Several features of the rice are desired for high-quality sake brewing, e.g., high water absorption rate, high digestibility/saccharification in sake mash, low protein content, and sufficient grain rigidity/hardness for polishing. Especially, a low protein content is an important feature for high-quality sake brewing, because an excess amount of proteins (amino acids) causes a rough taste and deepening of the color of the refined sake, which are negative factors in terms of sensory evaluation.[1-3] Furthermore, the aroma of sake containing a high content of amino acids appears to deteriorate during storage.[1] As the germ and the surface fraction of the rice grain are abundant in proteins and lipids, the rice used for high-quality sake brewing is highly polished to reduce these negative factors for sake taste.[1,4,5] Therefore, sufficient grain rigidity to resist high-polishing is also required for the rice used in high-quality sake brewing.[5]

In addition to varietal characteristics/genetic background of the rice, the cultivation conditions affect the protein content of brown rice. Previously, it was reported that the protein content is negatively correlated with the period of ear emergence and that the protein content of late-maturing rice cultivars tends to be lower than that of the early-maturing rice ones.[6] Compared with those of early-maturing rice, temperatures during the ripening period and nighttime of late-maturing rice are relatively low. This temperature difference is thought to be one of the causes of the lower protein content of the late-maturing rice grain. Indeed, it has been suggested that the protein content of rice is affected by the number of hours of sunlight and by the temperature during the cultivation period.[7] Crude protein contents of several rice cultivars tend to be increased by a high temperature during the ripening period,[7] although no statistically-significant difference between crude protein content and mean air temperature during 1 month after heading has been reported in the analysis of 51 across-the-country rice cultivars.[8]

To establish a process of sake brewing suitable for individual rice cultivars (*Oryza sativa* L.), research on and characterization of SR have been conducted in various regions of Japan. The late-maturing SR *Yamadanishiki* (YAM) developed in Hyogo Prefecture[9] has been used widely in Japan. In Niigata Prefecture, 2 early-maturing SRs, *Gohyakumangoku* (GOM) and *Takanenishiki* (TAK),[9] have been used and have contributed to the production of high-quality sake with a clear taste ("*Tanrei*" one of the perceivable attributes of the refined sake). In 2004, another late-maturing SR, *Koshitanrei* (KOS), was developed in Niigata Prefecture;[9,10] and recently its excellent polishing property was reported.[5]

The late-maturing CR *Sensyuraku* (SEN) was originally developed in Niigata Prefecture by crossbreeding between *Kanto-53* and *Norin-36* in 1960.[9] However, in 1971, due to the increase in consumption of CR *Koshihikari* (KSH),[9] SEN was omitted from the recommended rice cultivar list. In 1996, SEN was selected again as a rice with a low protein content and was revived as *fukkoku-mai* (revived rice). SEN has an advantage in cultivation, because the harvest time of SEN does not overlap that of major SR GOM. Currently, SEN is being used for high-quality sake brewing. However, analysis of brewing properties of SEN has been limited. In this report, we describe the original screening results and brewing properties of SEN for high-quality sake brewing.

Materials and Methods

Rice cultivars. In this study, we used 3 cooking rice (CR) cultivars (*Oryza sativa* L.), SEN, *Yukinosei* (YUK, crossbreeding between *Huko*-No.101 and *Niigata*-No.8),[9] and *Koshi-ibuki* (KSI, crossbreeding between *Hitomebore* and *Doman-naka*),[9] and 5 sake rice (SR) cultivars (*Oryza sativa* L.), GOM, KOS, Niigata Sake-No.36 (crossbreeding between GOM and *Sei-kei* Sake-No.94), *Cyou*-No.884 (crossbreeding between *Cyou*-No.460 and GOM), and *Cyou*-No.1162 (crossbreeding between *Yukinosei* and Niigata Sake-No.28). To polish the brown rice, we used 2 polishing machines, a laboratory machine (HS-4; Chiyoda Engineering, Hiroshima, Japan) and an industrial one (NF-26FA, Shin-Nakano, Okayama, Japan).

General analysis of rice grain. For general analysis of rice grains, we followed the National Standard Analysis Method of Materials for Sake Brewing.[11] The strength of the brown and polished rice was measured by crushing the grains (n = 100) with a grain rigidity tester (Kiya, Tokyo).

Analysis of protein body in rice. For analysis of the protein body (PB), we followed the method reported by Kizaki *et al.*[12] The PB was detected by SDS-PAGE, and PB composition (%) was calculated from the peak area on densitograms by use of an imaging system (Gel Doc EZ Imager, Bio-Rad Laboratories, Inc., USA).

Pasting properties of starch in rice. The pasting properties/parameters (Fig. 3A), including peak viscosity (PV), breakdown (BD), set back (SB), and pasting temperature (PT: start of gelatinization), in the heat-induced gelatinization of rice flour were measured by use of Rapid Visco Analyzer (RVA; Model RVA-4500, Newport Scientif c, Australia) according to the method of Aramaki *et al.*[13] and Okuda *et al.*[14]: distilled water was added to 2.8 g (10%, w/w, dry weight basis) of rice flour to make the total weight to 28 g. Each suspension was equilibrated at 50ºC for 1 min, heated at a rate of 5ºC/min to 95ºC, maintained at 95ºC for 5 min, cooled to 50ºC at a rate of 5ºC/min, and then maintained at that temperature for 6 min. A constantly rotating paddle (160 rpm) was used.

Koji-making on industrial scale. The malted rice (*koji*) was produced from 600 kg of the polished rice (polishing ratios, 60 or 55%) using 4 rice cultivars, 2 CRs (SEN and *Koshi-ibuki*, KSI) and 2 SRs (GOM and KOS), by an industrial *koji*-manufacturing system (Churitsu Industry Co., Ltd., Tokyo) under the programed conditions (maximum temperature, 42°C; culture time, 50 h). Enzyme activity (α-amylase and glucoamylase) of the produced *koji*-mold (*Aspergillus oryzae*) was measured by the assay kit (Kikkoman Biochemifa Company, Tokyo).[15,16]

Small-scale sake brewing test. The sake from a small-scale brewing test (total rice, 300 g) was produced as follows: 60 g of the identical malted rice (*koji*) made from polished GOM (50% polishing ratio) by an industrial *koji*-manufacturing system was used for individual small-sacle sake brewing test. Two hundred forty grams of polished rice (polishing ratios, 60 and 40%) of 4 rice cultivars, 2CRs (SEN and YUK) and 2SRs (KOS and GOM), was used only as *kakemai* (steamed rice added to the sake mash). The sake yeast G9NF strain (*Saccharomyces cerevisiae*), developed originally at the Niigata Prefectural Sake Research Institute,[17] was used for sake fermentation, which was carried out at 12°C for 16 days; and the clear sake liquid was obtained by centrifugation.

General properties, flavor components, and sensory evaluation of sake. General properties (OE, original extract; TA, total acidity; AA, amino acidity), and flavor components (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 Agency[18] and gas chromatography (GC-2014, Shimadzu, Kyoto, Japan), respectively. Sake samples adjusted to a temperature of 20°C were evaluated by 7 experienced panelists.

Sake quality was graded by sensory scores from 1 to 4, 1 indicating the highest quality.

Results and Discussion

Original selection of the late-maturing cooking rice Sensyuraku (SEN) as a rice with low-protein content

In 1996, to screen for a rice cultivar(s) with a low-protein content suitable for high-quality sake brewing, we obtained seed rice of 4 rice cultivars (Fig. 1A), 1 late-maturing CR SEN, and 3 early-maturing SRs (A/Niigata Sake-No.36, B/*Cyou*-No.884, and C/*Cyou*-No.1162), recommended from the Crop Research Center of Niigata Agricultural Research Institute, and cultivated them in a compartment of 15 m^2 within the same field under the same fertilization conditions. We examined the protein content of the brown rice and the crush rate of the polished rice during polishing by using a laboratory polishing machine (HS-4; Chiyoda Engineering, Japan; input of rice, 70 g; at a constant rate of 1,300 rpm) from 70% to 40% polishing ratio of 4 rice cultivars (SEN, A, B, and C), and compared the results with those of SR GOM and CR *Yukinosei* (YUK) as the control (Fig. 1A). The early-maturing YUK is being used as *kakemai*. As the result, the crude protein of the SEN brown rice and the crush rate of the SEN polished rice tended to be lower than those of the other cultivars, thus showing SEN to have a low protein content and excellent polishing properties. Consistent with low crush rate, the polishing time of 2 CRs (SEN and YUK) was longer than that for GOM (Fig. 1A). In this first screening, SEN was selected as the rice cultivar with the lowest protein content among 4 rice cultivars.

Fig. 1

Follow-up investigation of properties of Sensyuraku

For further investigation of the properties of SEN for high-quality sake brewing, follow-up investigations on SEN were performed in 1998 and 1999. SEN and 2 rices (SR GOM and CR YUK) were cultivated, each in a compartment of 3,000 m^2, within the same field, and the properties of SEN were compared with those of 2 others (Fig. 1B). The 1,000-grain weight of SEN brown rice was larger than that of YUK and was similar to that of GOM, indicating that CR SEN belonged to the same large-grain rice group as SR GOM. Also, the protein and amylose contents of SEN brown rice were lower and higher, respectively, than those of 2 others. Using a laboratory polishing machine, we polished the 3 rice cultivars and compared their polishing properties (Fig. 1B). The polishing time spent for a 70% polishing ratio of 2 CRs (SEN and YUK) was longer than that for GOM, and the crush rate for SEN or YUK with a 70% polishing ratio was lower than that for GOM. These results suggested that the hardness of the SEN grain was greater than that of the grain of GOM. This result is also consistent with a previous report indicating that the crush rate of CR is generally lower than that of SR with a white-core region (*shin-paku*).[19)]

Next we examined 2 basic brewing properties, water absorbability of the polished rice (70% polishing ratio) and enzymatic digestibility (Brix value) of the steamed rice (Fig. 1B). Water absorbability for 20 min for SEN tended to be lower than that for GOM, but the absorbability for 120 min for the former tended to be higher than that for the others. The Brix value of SEN was higher than those of 2 others. These results suggested that SEN has excellent properties in terms of both water absorbability and enzymatic digestibility. To further investigate the starch retrogradation rate for the steamed rice (60% polishing ratio), we measured the Brix value of the steamed rice after cooling at 15°C for 1 to 3 h after steaming (Fig.1C). The starch retrogradation rate (decrease in the Brix value after cooling) is positively correlated with the decreasing rate of enzymatic digestibility.[20)] The rate of decrease in the Brix value for SEN was lower than that for GOM or YUK (Fig. 1C), indicating that the starch retrogradation rate of SEN was relatively low compared with that for 2 others. The starch retrogradation rate of YAM is relatively low,[20)] and this feature is thought to be one of the reasons for the excellent brewing properties (enzymatic digestibility, etc.) of YAM. It is possible that the structure of endosperm starch in SEN might be similar to that in YAM. To investigate this point, it is necessary to compare starch gelatinization/retrogradation between SEN and YAM.

Taken together, these results (Fig. 1) suggested that SEN had excellent properties for high-quality sake brewing, low protein content, low crush rate (excellent polishing properties), and high water adsorption/enzymatic digestibility.

Analyses of polishing/brewing properties of rice cultivars for high-quality sake brewing

In 2012, we conducted an exhaustive investigation of the polishing/brewing properties of the late-maturing CR SEN with 3 rice cultivars (late-maturing SR KOS, and 2 early-maturing rices SR GOM and CR YUK) (Fig. 2A). To first investigate the strength of SEN during polishing, we measured the grain rigidity of the brown and polished rice (60% and 40% polishing ratios) of SEN by crushing the grains (n = 100) with a grain rigidity tester (Fig. 2B), and compared the results with that for GOM. GOM produces high-quality sake with a clear taste, but the hardness of its grains is insufficient for high polishing (especially to less than the 50% polishing ratio for *Daiginjo-shu*). The grain rigidity of the SEN brown rice was similar to that of others, but the grain rigidity of the SEN polished rice was higher than that of GOM. Thus, SEN showed excellent polishing properties.

Fig. 2

The crude protein contents of the brown rice of 2 late-maturing rice (CR SEN and SR KOS) were lower than those of 2 early-maturing rice (SR GOM and CR YUK) (Fig. 2B). The previous report suggested that the protein content of rice is affected by the number of hours of sunlight and by the temperature during the cultivation period.[7)] Mean air temperature during 1 month after ripening of late-maturing rice (SEN and KOS) tended to be lower than those of early-maturing rice (GOM and YUK) (Fig. 2A), indicating the positive correlation between the protein content and the mean air temperature. On the other hand, there was no significant correlation between the protein content and mean hours of sunlight during 1 month after ripening in this year (Fig. 2A and 2B). The cultivation time (days after transplanting) of late-maturing rice was longer than that of early-maturing rice (Fig. 2A). It is possible that the nitrogen source fertilized in soil might be used in the growth of plant body rather than the accumulation in rice grain during long cultivation time. Crude protein content in rice grain would be affected by many factors; genetic background, cultivation temperature, fertilization conditions, etc. Further analysis is necessary to clarify this point.

Previously, it was reported that storage proteins in the rice endosperm consist of

20-25% PB-I (protein body type I), 60-65% PB-II (protein body type II), and 10-15% albumin and globulin in the cytoplasm.[21] Approximately 90% of the total proteins of rice endosperm are represented by 2 types of proteinaceous particles, PB-I and PB-II. PB-I is spherical with lamellar structure and contains prolamin; on the other hand, PB-II is non lamellar and is rich in glutelin.[22] To investigate the protein and the brewing properties of SEN, we examined the PB composition of brown rice (100% polishing ratio) and polished rice (60% and 40% polishing ratios) of 4 rice cultivars, 2 late-maturing rice (CR SEN and SR KOS) and 2 early-maturing rice (CR YUK and SR GOM) (Fig. 2B). For brown rice, the PB composition (I+II) of late-maturing SEN was similar to that of late-maturing KOS, and lower than that of the early-maturing rice (GOM and YUK). This result is consistent with a previous report indicating that the protein content of late-maturing rice cultivars tends to be lower than that of early-maturing ones.[6]

It has been reported that PB-II, but not PB-I, in sake mash (*moromi*) is dissolved by the enzymes from the *koji*-mold and that PB-I thus remains in the late-stage sake mash.[23] Also, the PB-I content and the ratio of PB-II/PB-I of SR tend to be lower and higher, respectively, than those of CR.[24] However, the analysis of PBs has been limited (polished rice of 70% polishing ratio).[24] As shown in Fig. 2B, the ratio of PB-II/PB-I for SEN brown rice was lower than that for KOS or GOM; and for the SEN polished rice (60% polishing ratio), the ratio of PB-II/PB-I was similar to those for the SRs. The PB-I content and the ratio of PB-II/PB-I for SEN were similar to that for SR, suitable for sake brewing.

Further, we measured the enzymatic digestibility of the steamed rice of 4 rice cultivars (Fig. 2C). The Brix value of SEN was similar to that of KOS and was higher than those of GOM and YUK, indicating that CR SEN had good digestibility, equivalent to SR KOS, in the sake mash.

Confirmation of brewing properties of rice cultivars by Rapid Visco Analyzer

It has been reported that the brewing properties of rice are correlated significantly with the physical properties/parameters (Fig. 3A), including peak viscosity (PV), breakdown

Fig. 3

(BD), set back (SB), and pasting temperature (PT: start of gelatinization), in the heat-induced gelatinization of rice flour determined by use of a Rapid Visco Analyzer (RVA).[13,14] The reported correlations are as follow:[13] the crude protein content of rice is correlated negatively with PV and BD, and positively with PT. Water absorption of rice is correlated positively with PV and BD. Enzyme digestibility (Brix value) of steamed rice is correlated positively with PV and BD, and negatively with PT.

To estimate/confirm the brewing properties of SEN, we measured the pasting/viscosity parameters (PV, BD, SB, and PT) of 4 rice cultivars, 2 CRs (SEN and YUK) and 2 SRs (KOS and GOM) by using the RVA (Fig. 3B). The PV and BD of SEN were higher than those of 2SRs (KOS and GOM) or CR YUK (Fig. 3B). This result suggested that the crude protein content of SEN was lower than those of 2 SRs (KOS and GOM) or CR YUK and that the enzyme digestibility and the water absorption of SEN were higher than those of the others. Further, this suggestion supports the previous results (Fig. 1, 2B, and 2C) showing the excellent properties of SEN for high-quality sake brewing, i.e., low protein content and good water adsorption/enzymatic digestibility. Additionally, the PV and BD of the 4 rice cultivars increased with decreasing polishing ratios; on the other hand, their PT decreased with decreasing polishing ratios. As the crude protein content of rice is correlated negatively with PV and BD, and positively with PT, these results are consistent with the fact that the rice protein content decreased with decreasing polishing ratios. Further, it has been reported that the ratio of PB-II/PB-I and PT increase as the temperature during the ripening period of rice cultivars becomes higher.[25] The present results for the late-maturing CR SEN are also consistent with this previous report.[25]

Previously, it was reported that the total starch content of rice increase with decrease in polishing ratios, and are correlated positively with PV,[4] suggesting that pasting parameters (PV, etc.) examined by RVA are affected by not only the crude protein content but also the properties of endosorm starch of rice, i.e., amylose/amylopectin content and side-chain structure of amylopectin.[4,13,14,20] Thus, the previous reports indicate a strong correlation between the RVA parameters and brewing properties (water absorption and enzyme digestibility). Further, the side-chain of amylopectin in late-maturing YAM tends to be shorter than that in early-maturing GOM, and one of the reasons of this phenomenon is thought to be caused by the low temperature during the ripening period.[26)] To further analyze the RVA parameters of SEN, we need to investigate the properties/structure of starch in the rice.

Polishing and koji-production on an industrial scale

To further investigate the polishing/brewing properties of SEN, we polished 4 rice cultivars (polishing ratios, 60, 40%) by use of the industrial polishing machine (NF-26FA, Shin-Nakano Industry, Japan; input of rice, 1,020 Kg). The polishing time spent for a 40% polishing ratio of 2 CRs (SEN and YUK) was longer than those for 2 SRs (KOS and GOM) (Fig.4A). We further investigated the water-absorbing ratios of 4 rice cultivars (Fig. 4B). The amounts of water absorbed by 4 rice cultivars increased with decreasing polishing ratios. Compared with those of 2 SRs (KOS and GOM), the water-absorbing ratio of SEN for a short time (10, 20 min) was low, on the other hand, in the case of water absorption for 120 min (the maximum water absorption) of SEN was high. The water absorption (for either 20 or 120 min) of SEN was higher than that of YUK.

Fig. 4

To further investigate the suitability of SEN for sake brewing, we produced a malted rice (*koji*) using 600 kg of polished rice (polishing ratios, 60 or 55%) using 4 rice cultivars, 2 CRs (SEN and *Koshi-ibuki*, KSI) and 2 SRs (GOM and KOS) by an industrial *koji*-manufacturing system (Churitsu Industry Co., Ltd., Tokyo), and measured the enzyme activities of α-amylase (α-A) and glucoamylase (GA) in the *koji*. As shown in Fig. 4C, the enzyme activities of these amylases in the *koji* of SEN were higher than that in the CR KSI *koji*, and were similar level to those in the SR *koji*. These results indicated that SEN is suitable for not only *kakemai* but also *koji*-making, as SR. Further analysis is necessary to clarify this point.

Quality of sake produced from Sensyuraku

Furthermore, to confirm the suitability of SEN for high-quality sake *Ginjo-shu* or *Daiginjo-shu* (less than 60% or 50% polishing ratio, respectively), we carried out a small-scale sake brewing test using 300 g (total rice) of polished rice (60 and 40% polishing ratios) of 4 rice cultivars (Fig. 4D). The original extract (OE) of sake from SEN was similar to that from KOS, and was slightly higher than that from GOM and YUK (alcohol contents (%) were as follows: sake using polished rice of 60% polishing ratio = 14.4-14.8%, that of 40% polishing ratio = 13.1-13.6%). This result is consistent with the high digestibility (Brix value in Fig. 1B, 1C, 2C) and the maximum water absorption (the water-absorbing ratio for 120 min in Fig. 1B and 4B), indicating that SEN had good digestibility in the sake mash. The amino acidity (AA) of the sake produced from SEN appeared to be slightly lower than that of the YUK sake. Flavor components (isoamyl acetate and ethyl caproate) of the sake produced from SEN was higher than that of YUK sake and was similar level to those of SR sake (KOS and GOM). Indeed, the clear taste of the sake produced from SEN was evaluated and gave a higher sensory score than that for YUK by 7 experienced panelists. As to sake tasting, an excess amount of AA and appropriate levels of flavor components correlated negatively and positively, respectively, with the sensory evaluation scores. One of the reasons for a high evaluation of sake from SEN will be the low level of AA and high contents of the esters, similar to that of SR sake.

In this study, we described the original screening of SEN and properties of SEN for high-quality sake brewing. The late-maturing CR SEN was selected as a rice with a low protein content. Our results indicated that SEN had excellent properties, equivalent to SR, for the production of high-quality sake from highly polished rice such as *Daiginjo-shu*. In this study, we confirmed the excellent properties of SEN, which was originally developed in Niigata Prefecture, by comparing with that of SR GOM that has been widely used in Niigata Prefecture. As the next step, it is necessary to compare brewing properties between SEN and SR YAM. Our results provide information that should contribute to the improvement of high-quality sake brewing using SEN and shed light on the breeding and characteristics of CR for high-quality sake brewing.

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Figure legends

Fig.1. Selection of the cooking rice *Sensyuraku* as a rice with low protein content.
A, Protein content and polishing properties of SEN. Values indicate averages for 2 independent experiments. Rice cultivars were cultivated in the same field of Niigata Prefecture and harvested/examined in 1996: SEN, GOM, YUK, and 3 sake rice cultivars, A (Niigata Sake-No.36), B (*Cyou*-No.884), and C (*Cyou*-No.1162). Brown rice was polished to white rice (polishing ratio from 70% to 40%) by using a laboratory polishing machine. B, Follow-up investigation of SEN for sake brewing. Three rice cultivars, SEN, GOM, and YUK, were cultivated in the same field of Niigata Prefecture and harvested/examined in 1998 and 1999. The moisture content of the rice grains of the brown and polished rice was adjusted as follows: brown rice = 13.8%, 70% polished rice = 13.5%. C, Relative enzymatic digestibility (Brix value) of the steamed rice after cooling at 15 °C for the indicated time (h) to the Brix value of the steamed rice after cooling for 1 h. The rice cultivars were harvested/examined in 1999, and white rice with a 60% polishing ratio was used in this study. Values indicate averages for 2 independent experiments.

Fig.2. Analysis of protein and starch contents of rice cultivars.
A, Cultivation record of rice cultivars in 2012. The rice cultivars used in Fig. 2, 3, and 4 were harvested/examined in 2012. B, Grain rigidity and composition of protein body in rice cultivars. The strength (grain rigidity) of the brown and polished rice was measured by crushing the grains (n = 100) with a grain rigidity tester (Kiya, Tokyo). Values indicate AV (average) $\pm$ SD (standard deviation, n = 100). The moisture content of the rice grains was adjusted as follows: brown rice = 14.5%, polished rice 60% = 10.0%, 40% = 9.0%. Values (crude protein and protein body composition) indicate averages for 3 independent experiments. Single asterisks indicate $p < 0.05$ for the significance of the difference in ratio (%) between SEN and others. Brown rice was polished to white rice (from 60% to 40% polishing ratio) by using a laboratory polishing machine (HS-4; Chiyoda Engineering,

Hiroshima, Japan; input of rice, 70g) operated at a constant rate of 1,300 rpm. C, Enzymatic digestibility (Brix value) of the steamed rice. Values indicate averages for 2 independent experiments.

Fig.3. Pasting parameters of rice cultivars by Rapid Visco Analyzer

A, A representative rapid viscogram pattern of a rice flour generated by the Rapid Visco Analyzer (RVA). B, RVA parameters of the rice cultivars. Values indicate AV $\pm$ SD for 4 independent experiments. Single asterisks indicate $p < 0.05$ for the significance of the difference in value between SEN and others.

Fig.4. Brewing properties of rice cultivars for high-quality sake brewing.

A, Polishing time for the polishing ratios. Brown rice of 4 rice cultivars (means of moisture contents of SEN, KOS, GOM, and YUK were 15.3%, 15.2%, 15.2%, and 15.2%, respectively) was polished to white rice. The polishing was done with an industrial polishing machine (NF-26FA, Shin-Nakano, Okayama, Japan; input of rice, 1,020 Kg) under the polishing conditions reported previously.[5)] B, Water-absorption ratio of the polished rice. Values indicate AV $\pm$ SD for 5 independent experiments. The SDs were sufficiently small (0.19-0.82). Single and double asterisks indicate $p < 0.05$ and $p < 0.01$, respectively, for the significance of the difference in the ratios (%) indicated by the brackets. For the polished rice with a polishing ratio of 60%, the means of moisture contents of 4 rice cultivars (SEN, KOS, GOM, and YUK) were 9.9%; and for polished rice with a 40% polishing ratio, they were 8.8%, 8.9%, 8.9%, and 8.9%, respectively. C, Enzyme activity (α-amylase/α-A, glucoamylase/GA) of the malted rice (*koji*). Values indicate AV $\pm$ SD for 3 independent experiments. Single asterisks indicate $p < 0.05$ for the significance of the difference in value between SEN and GOM or KSI. Value of KOS was indicated as reference. D, Properties of the sake produced from the rice. General properties (OE, original extract; TA, total acidity; AA, amino acidity), and flavor components (iAmOAc, isoamyl acetate; EtCap, ethyl caproate) of the sake are indicated as averages for 2 independent experiments. Sake quality was graded by sensory scores from 1 to 4, with 1 indicating the highest quality.

A

Rice cultivars	Brown rice: 1000-kernel weight (g)	Brown rice: Crude protein (%)	Crush rate (%) of the polished rice, polishing ratio of 70%	60%	50%	40%	Polishing times (min) for polishing ratios 60%	50%	40%
harvested/examined in 1996									
SEN	25.1	6.8	0.8	2.5	5.1	6.0	120	200	335
A	25.8	7.7	2.1	2.9	5.0	6.5	80	115	180
B	32.1	8.5	6.4	13.6	25.4	35.9	115	165	265
C	27.0	7.7	1.5	2.9	6.7	8.5	105	175	270
GOM	26.7	7.2	6.5	5.8	12.6	13.2	90	140	230
YUK	23.4	7.0	3.0	3.9	6.3	8.3	110	180	330

B

Rice cultivars	Brown rice: 1000-kernel weight (g)	Brown rice: Crude protein (%)	Brown rice: Amylose content (%)	Polished rice (polishing ratio of 70%): Polishing time (min)	Crushed rice rate (%)	Absorbed water for 20 min (%)	Absorbed water for 120 min (%)	Enzyme digestibility (Brix) of steamed rice (%)
harvested/examined in 1998								
SEN	26.4	6.8	20.8	67	1.9	24.8	27.5	10.9
GOM	25.9	7.4	19.2	60	2.4	25.2	27.2	10.6
YUK	22.7	7.4	16.0	65	2.2	22.8	26.1	10.0
harvested/examined in 1999								
SEN	25.6	6.8	19.9	65	3.2	26.2	30.9	11.4
GOM	25.8	7.9	19.6	58	5.0	26.6	30.2	10.9
YUK	23.1	7.3	17.8	63	4.0	25.9	29.2	10.4

C

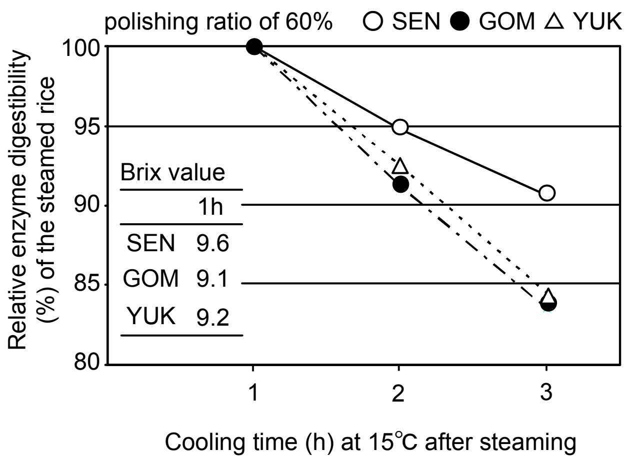


Fig.1, Anzawa *et al.*

A

Rice cultivar	Date (days after transplanting)			1 month after ripening	
	Transplanting	Ripening	Maturity/ Harvest	Mean air temperature (°C)	Mean hours of sunlight (h)
harvested/examined in 2012					
late-maturing rice					
SEN	May. 22 (1)	Aug. 16 (86)	Sep. 22 (123)	27.2	8.8
KOS	May. 19 (1)	Aug. 18 (91)	Sep. 22 (126)	26.5	8.1
early-maturing rice					
GOM	May. 10 (1)	Jul. 26 (77)	Sep. 2 (115)	27.9	9.1
YUK	May. 21 (1)	Jul. 31 (71)	Sep. 12 (114)	27.6	8.4

B

Rice cultivars	Grain rigidity (kg/ cm^2) (AV ± SD)	Crude protein (%)	Protein body composition (%)				PB-II/PB-I
			PB-I	PB-II	(I+II)	other	
Brown rice							
SEN	4.82 ± 1.05	6.9	22.5	42.5	(65.0)	35.0	1.89
KOS	4.75 ± 1.67	6.8	21.7	44.5	(66.2)	33.8	2.05 *
GOM	4.32 ± 1.51	7.3 *	22.9	47.0 *	(69.9) *	30.1 *	2.05 *
YUK	4.61 ± 1.61	7.4 *	24.7 *	44.6 *	(69.3) *	30.7 *	1.80 *
Polished rice polishing ratio of 60%							
SEN	7.51 ± 1.37	4.0	24.6	52.4	(77.0)	23.0	2.13
KOS	5.47 ± 2.18	3.9	24.2	50.2	(74.4)	25.6 *	2.07
GOM	3.52 ± 2.18	4.4 *	25.2	53.2	(78.4)	21.6	2.11
YUK	7.34 ± 1.53	4.5 *	25.5 *	51.4	(76.9)	23.1	2.01 *
40%							
SEN	6.04 ± 1.01	3.5	22.7	47.7	(70.4)	29.6	2.10
KOS	4.33 ± 1.76	3.3	22.2	49.4	(71.6)	28.4	2.23 *
GOM	2.33 ± 0.74	3.8 *	23.1	49.4	(72.5)	27.5	2.14
YUK	5.95 ± 1.89	3.8 *	25.4 *	51.9 *	(77.3) *	22.7 *	2.04 *

* $p < 0.05$

C

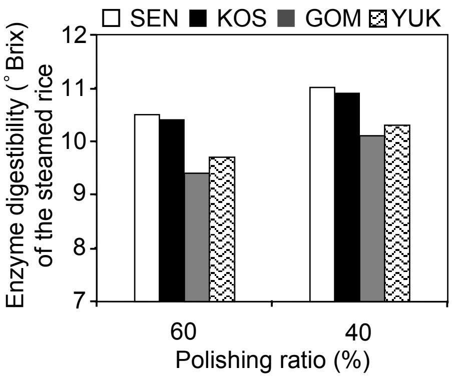
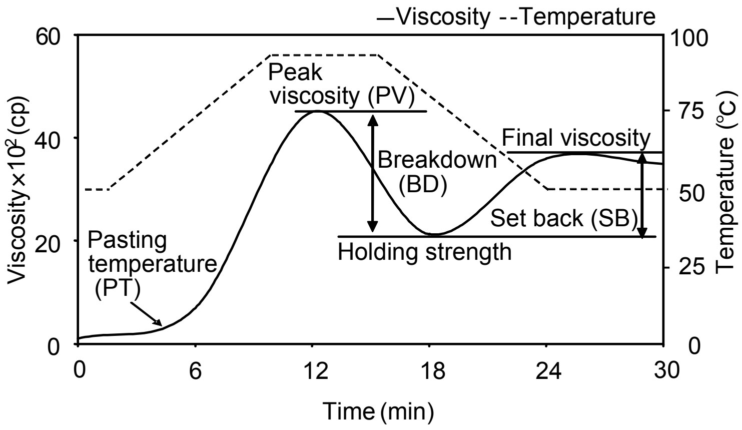


Fig.2, Anzawa *et al.*

A



B

Rice cultivars	RVA parameters (AV ± SD)			
	PV (cp)	BD (cp)	SB (cp)	PT (℃)
Brown rice				
SEN	2,803 ± 1	1,872 ± 18	1,024 ± 11	67.5 ± 0.1
KOS	2,745 ± 4 *	1,834 ± 12 *	956 ± 21*	67.6 ± 0.1
GOM	2,356 ± 5 *	1,302 ± 14*	918 ± 6 *	67.9 ± 0.1*
YUK	2,748 ± 25*	1,792 ± 13 *	878 ± 13*	67.5 ± 0.2
Polished rice polishing ratio of				
60%				
SEN	4,013 ± 21	2,669 ± 7	951 ± 1	65.6 ± 0.1
KOS	3,934 ± 33*	2,512 ± 29*	961 ± 27	65.6 ± 0.1
GOM	3,697 ± 36*	2,279 ± 9 *	947 ± 27	65.3 ± 0.1*
YUK	3,549 ± 39*	2,137 ± 36 *	913 ± 55*	65.9 ± 0.1*
40%				
SEN	4,131 ± 28	2,782 ± 24	956 ± 2	64.2 ± 0.1
KOS	3,974 ± 30*	2,507 ± 49*	986 ± 24*	64.5 ± 0.1*
GOM	3,835 ± 23*	2,412 ± 24*	939 ± 35	65.1 ± 0.2*
YUK	3,794 ± 37*	2,225 ± 31 *	936 ± 35	65.3 ± 0.2*

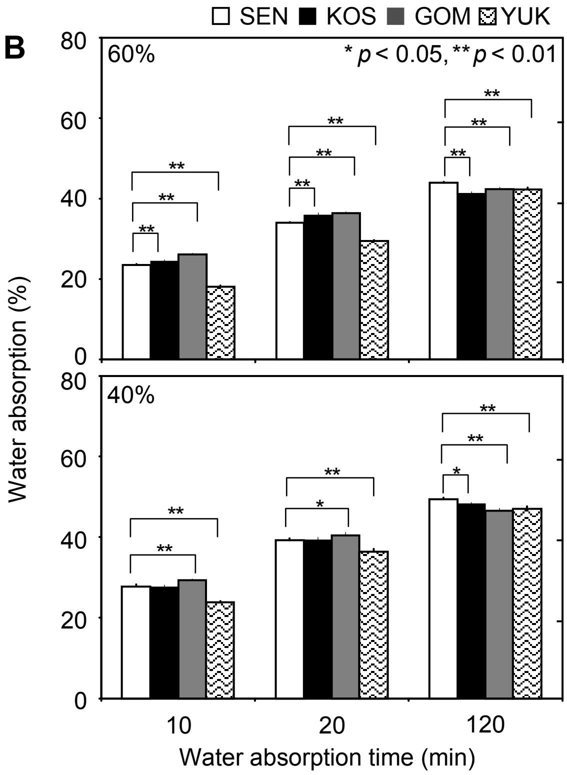
* $p < 0.05$

Fig.3, Anzawa *et al.*

A

Rice cultivars	Polishing times (h) for polishing ratios	
	60%	40%
SEN	23.91	49.76
KOS	23.30	42.53
GOM	20.43	38.49
YUK	23.90	50.92

B



C

Rice cultivars	Polishing ratio (%)	Enzyme activity of *Koji* (U/*koji*-g)	
		α-A	GA
SEN	60	1,198 ± 30	261 ± 31
GOM	60	1,125 ± 57	214 ± 26
KSI	60	791 ± 29*	153 ± 22*
KOS	55	1,332 ± 26	251 ± 21

* $p < 0.05$

D

Rice cultivars Polishing ratio	OE (%)	TA (mL)	AA (mL)	Flavor components (ppm) iAmOAc	EtCap	Sensory score
SEN						
60%	32.2	2.2	1.0	4.0	0.7	2.33
40%	31.9	2.1	0.9	3.4	0.6	1.83
KOS						
60%	32.5	2.2	1.1	4.4	0.7	2.17
40%	32.3	2.2	1.1	3.8	0.7	1.67
GOM						
60%	31.6	2.3	1.1	3.4	0.6	2.33
40%	31.3	2.2	1.0	3.0	0.6	2.17
YUK						
60%	31.5	2.2	1.2	3.5	0.5	2.50
40%	31.3	2.1	1.0	3.0	0.5	2.50

Fig.4, Anzawa *et al.*