

Morphological and starch characteristics of the *Japonica* rice mutant variety Seolgaeng for dry-milled flour

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Abstract Producing good-quality, fine rice flour is more difficult than wheat flour because the rice grain is harder. The non-glutinous *Japonica*-type variety Seolgaeng, derived from N-methyl-N-nitrosourea (MNU) mutagenesis, and four other varieties, representing a range of amylose contents, were evaluated in this study. Dry-milled Seolgaeng rice flour exhibited an average particle size that is <70 μm, a more uniform particle-size proportion than other varieties. Moreover, we noted significant differences in the damaged starch content in flour from Seolgaeng compared to the other varieties ($p<0.05$). Seolgaeng flour showed a round starch structure, which would lead to better friability, finer particle size, and less damage to the endosperm during dry milling. Indeed, among all varieties evaluated in this study, dry-milled Seolgaeng flour had the finest particle size (averaging <70 μm) and exhibited less damaged starch. With its round starch granules, Seolgaeng is a suitable candidate for dry-milled rice flour.

Keywords: *Japonica* mutant variety, Seolgaeng rice, dry-milled rice flour, round starch granule

Introduction

Rice (*Oryza sativa* L.) is one of the main agricultural crops in Asian countries, including Korea, as well as the most important staple food in the world. Rice kernels are generally eaten in a cooked form in order to obtain various nutrients. Rice varieties that produce kernels that are firm and fluffy after cooking are generally favored in countries such as India, Pakistan, and Indonesia, whereas varieties with kernels that maintain their shape, glossiness, savory odor, stickiness, and tenderness when cooked are preferred in Korea. A number of studies have reported that rice quality is highly dependent on the rice variety (1,2). Rice can also be processed into flour, which can then be consumed via various foods such as cakes, noodles, breads, and confectionaries. Moreover, rice flour is a useful substitute for wheat flour (*Triticum aestivum* L.), particularly for people with wheat hypersensitivity. Specialty rice varieties have been developed in Korea through mutation breeding approaches using mutagens such as N-methyl-N-nitrosourea (MNU), and a wide range of useful grain traits have been obtained and developed into varieties including altered amylose (e.g., dull, low, and high), floury endosperm, and giant embryo (3–5). Several studies have been conducted to develop and improve the quality of multi-purpose rice,

particularly those intended for use as raw materials for industrial uses (2,6).

Producing rice flours having fine particles is more difficult than wheat flour because of the hardness of the rice grain. Rice flour quality is highly dependent on the rice variety and milling conditions. Rice flour is usually prepared using one of three milling methods: wet, semi-dry, or dry. During wet milling, rice is soaked in water, drained, and ground with water; during dry milling, rice is ground without the soaking process (7). During semi-dry milling, rice is soaked, drained, and ground without adding any water. In order to produce high-quality rice flour of fine particle size that is equivalent to wheat flour, a number of studies have been performed on the wet milling method despite its high costs (8). Given the considerable differences in the milling costs between dry and wet milling, the production of superior rice flour via dry milling of the most suitable rice cultivars would significantly reduce milling costs.

At present, there is very little research on the relationship between the morphological and starch characteristics of rice kernels and the appropriate varieties for producing good-quality, dry-milled rice flour. Research on the effects of dry milling on rice flour quality is also required. In this study, we examined a number of rice varieties with different genetic backgrounds in order to determine the morphologies

and starches that are suitable for producing high-quality, dry-milled rice flour.

Materials and Methods

Materials and sample preparation Five Korean rice varieties (Boseogchal, Seolgaeng, Ilpum, Hanareum, and Goami), representing different amylose contents, were selected for this study (Table 1) after screening a larger set of varieties that included waxy (Boseogchal) and semi-waxy (Baegjinju 1), non-waxy *Japonica*-type (Ilpum, Seolgaeng, Samkwang, Hwangmi 1, Deuraechan, and Daerip 1), non-waxy *Tongil*-type (Hanareum and Dasan 1), and high-amylose *Japonica*-type (Goami and Saegoami) varieties. Rice plants of the selected varieties were cultivated in an experimental paddy field at the National Institute of Crop Science (NICS), Suwon, Korea in 2011. Seeds were sown on April 25, 2011, and seedlings were transplanted on May 25, 2011. The planting density was 22.22 m⁻² (15 cm×30 cm), and N–P₂O₅–K₂O fertilizers were applied at the level of 90–45–57 kg/ha. All cultivars were harvested 45 days after heading. After harvesting, the paddy rice samples of each variety were air-dried in a shaded greenhouse until the moisture content (15%) suitable for storage under natural conditions was achieved. The hulls were removed from the paddy rice using a roller husking machine (SY88-TH; Ssangyong Ltd., Incheon, Korea), and the brown rice was polished using a laboratory polishing machine (MC-90A; Toyo Co., Wakayama, Japan) in order to analyze the chemical properties of the rice grains.

Morphological and physicochemical characteristics of grains The length, width, and thickness of each rice grain were determined by measuring 10 randomly selected head rice kernels using a Mitutoyo Digimatic Caliper (CD-15CP; Mitutoyo Co., Kawasaki, Japan). The 1,000-grain weight of the milled kernels was measured in grams in triplicate, and the average weight was determined.

The hardness of the rice kernels was determined by measuring the pressure at the grain breakage point. A 5-mm probe, 0.4-mm/s test speed, and 40.0-g trigger force were applied using a Texture Analyzer (Stable Micro Systems Ltd., Godalming, UK). The moisture content was calculated as weight loss after drying at 105°C according to Approved AACC Method 44-15 (9).

For observation using scanning electron microscopy (SEM), the rice grains were transversely broken at the mid region using a razor blade, and the pieces were mounted onto a circular aluminum

specimen stub and gold-coated in a vacuum using a PELCO SC-4 sputter coater (Ted Pella, Inc., Redding, CA, USA). The endosperm structures of the rice were examined using SEM (LEO 440; Leica and Zeiss Co., Cambridge, England) at a 20 kV accelerating voltage.

The amylose content of each rice sample was determined using the iodine-colorimetric method described by Juliano (10), and the amylose-iodine blue color was determined at 620 nm using a UV/visible spectrometer (Evolution 600 series; Thermo Fisher Scientific, Waltham, MA, USA).

The chain length distribution of amylopectin was characterized using high-performance anion-exchange chromatography (HPAEC) and a pulsed amperometric detector (PAD) according to the method described by Hanashiro *et al.* (11) with slight modifications. Briefly, enzymatically debranched starches were prepared, and 6 mg starch was suspended in 5 mL of 100% methanol in a screw cap tube. The samples were then heated to 100°C in a water bath. Ten microliters of 2% (w/v) sodium azide and 50 µL of 600 mM sodium acetate buffer were added to 1 mL of the gelatinized sample. An isoamylase solution obtained from *Pseudomonas amylofermosa* was used to debranch the sample over 24 h at 37°C. The debranched samples were then centrifuged and filtered through a 0.2-µm Millipore FH membrane. The samples were then analyzed using a HPAEC-PAD system (DX500; Dionex Co., Sunnyvale, CA, USA) equipped with a GP50 gradient pump, ED40 electrochemical detector, 50-mm CarboPac PA1 guard column, and 250-mm CarboPac PA1 analytical column. Sugars with degree of polymerization (DP) 7 were used to identify the chromatographic peaks.

Determination of the damaged starch content and particle-size distribution For dry milling, the moisture content of the rice grain was equilibrated for >24 h in an air-conditioned room. Rice flours were prepared using an air classification mill (ACM185; Hankook Crusher Co., Ltd., Incheon, Korea) under the milling conditions from two classification speed control of 5 and 15 Hz, respectively.

The damaged starch content of the rice flour was determined according to Approved AACC Method 76-31 (9) using a Megazyme starch damage assay kit (Megazyme International Ireland Ltd., Co., Wicklow, Ireland). The particle-size distribution of the rice flour was measured as the volume-base distribution using a laser-diffraction particle size analyzer (Malvern Mastersizer 2000; Malvern Instruments Ltd., Worcestershire, UK). The particle size at 50% cumulative distribution was considered as the mean particle size.

Table 1. Cross combinations and selected characteristics of the rice varieties used in this study

Variety	Cross combination	Subspecies (eco-type)	Year	Characteristics
Boseogchal	Hwayeong//Tamjin/2*Sinseonchal	<i>Japonica</i>	2004	Waxy
Seolgaeng	Ilpum (MNU) mutant	<i>Japonica</i>	2001	Medium amylose
Ilpum	Suweon295-sv3/Inabawase	<i>Japonica</i>	1990	Medium amylose
Hanareum	Milyang103/Suweon405	<i>Tongil</i>	2002	Medium amylose
Goami	Milyang95//Gimcheonaengmi/Ilpum*2	<i>Japonica</i>	2000	High amylose

Statistical analysis All experiments were performed ≥ 3 times, and the results were statistically analyzed using the SPSS 15.0 (SPSS Inc., Chicago, IL, USA). The statistical significance was determined using 1-way analysis of variance and Duncan's multiple range test ($p < 0.05$). The significance of each group between rice for flour and the other varieties was verified using the Student's *t*-test ($p < 0.001$, 0.01, and 0.05).

Results and Discussion

Agronomical, morphological, and starch characteristics of the rice cultivars The five rice varieties used in this study represent a range of amylose contents: waxy (Boseogchal), medium (Seolgaeng, Ilpum, and Hanareum), and high (Goami). More than 90% of the milled rice (dry weight) is starch, comprising two polymeric forms of glucose: amylose and amylopectin (12). Thus, the functionality of rice flour depends on the starch characteristics (13). Boseogchal, Seolgaeng, Ilpum, and Goami are *Japonica* varieties developed by the Rural Development Administration (RDA), Korea, in 2004, 2001, 1990, and 2000, respectively. Hanareum is a high-yield, *Tongil*-type, rice cultivar (14) that was developed from a 3-way cross between *Indica*-type Suwon 295-SV3 and *Japonica*-type Inabawase. The induced mutant variety, Seolgaeng, was developed from Ilpum, a high-quality *Japonica* variety, using N-methyl-N-nitrosourea (MNU) treatment.

As shown Fig. 1A, non-glutinous Seolgaeng rice has an entirely opaque endosperm. For various genetic reasons (15,16), the grains of the glutinous Boseogchal and non-glutinous Seolgaeng appear similarly opaque. Boseogchal kernels, which have opaque endosperms, are not chalky. The starch granules of waxy rice endosperms are tightly packed, but their outer surface has many micro-pores and hollows (15,17). Temperature affects the development of chalky kernels, and the white and opaque appearance of chalky areas is caused by the loose packaging of the starch granules in the rice grain. Consequently, the grains appear opaque because numerous air spaces in the starchy endosperm diffusely reflect light. Floury rice mutants have been developed, and their opaque endosperms are expected to be easily milled because of the numerous air spaces in the grain (16).

The morphological properties and hardness values of the kernels from each variety are shown in Table 2. The length and width of the rice grains ranged from 4.98 to 5.72 mm and 2.57 to 2.97 mm, respectively. The length/width ratios of Seolgaeng and Ilpum were significantly lower than those of other varieties. Boseogchal, Seolgaeng, Ilpum, and Goami (all considered as short grains) had length/width ratios < 2 . The 1,000-grain weights ranged from 19.7 to 20.8 g. The 1,000-grain weights of Seolgaeng and Hanareum were significantly lower than those of the other rice cultivars. The moisture content ranged from 10.9 to 11.8%, but there were no differences in the moisture contents among the varieties. Significant differences were observed in the hardness values of the rice kernels ($p < 0.05$).

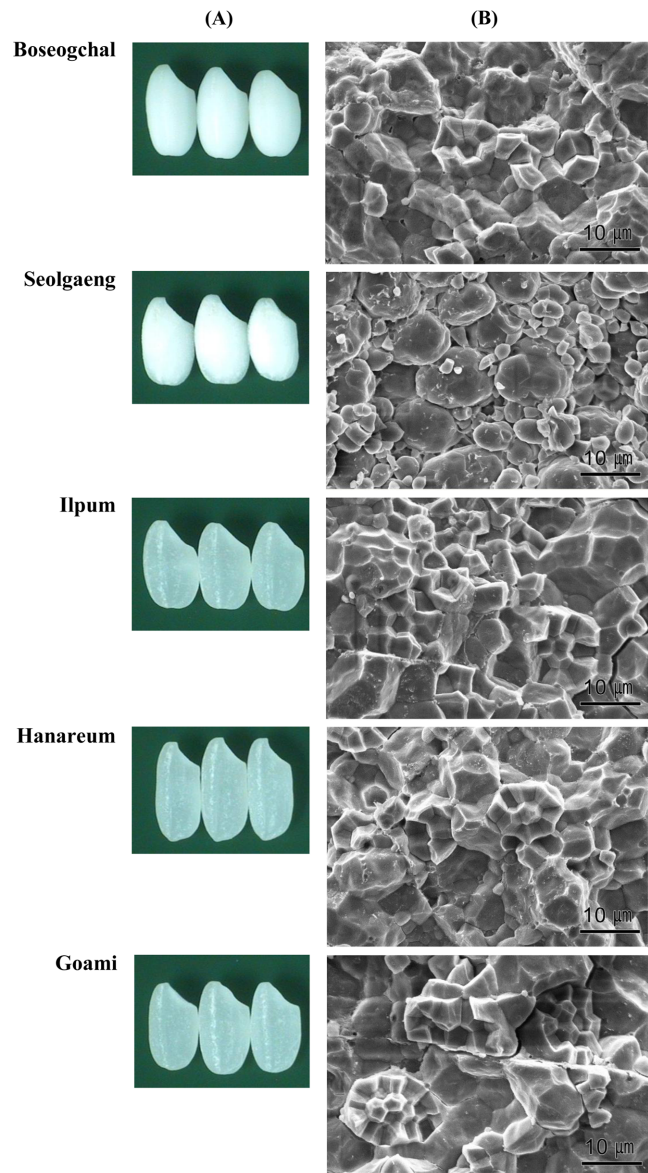


Fig. 1. Grain appearance (A) and scanning electron microscope (SEM) photographs of the cross section of rice endosperm (B) (5,000 \times magnification)

Seolgaeng demonstrated a significantly lower hardness (2.95 kg) than the other varieties (3.98–5.08 kg).

Starch is the major component of rice grains, and the ratio of amylose to amylopectin is a major determinant of eating quality, cooking, and processing properties (1,10,18). According to colorimetric starch-iodine color absorption studies (10), the amylose contents of the five varieties are significantly different (Table 2). The amylose content of Boseogchal, the glutinous variety, is the lowest at 5.4%. Small differences were noted between Seolgaeng (19.9%), Ilpum (20.3%), and Hanareum (19.4%). Goami had the highest amylose content (27.7%), which was significantly higher than the others.

The amylopectin chain length was analyzed using HPAEC-PAD after debranching the starch fraction. Seolgaeng exhibited 32.98% short

Table 2. Morphological and physicochemical characteristics of the rice grain varieties in this study

Variety	Length (mm)	Morphological properties				Moisture content (%)	Hardness (kg)	Amylose content (%)	Amylopectin chain length distribution (%)			
		Width (mm)	Thickness (mm)	Length/width ratio	1,000-Grain weight (g)				DP≤12 (A, %)	12<DP≤24 (B ₁ , %)	24<DP≤36 (B ₂ , %)	36<DP ¹⁾ (B ₃ , %)
Boseogchal	5.02 ^{c2)}	2.86 ^b	2.07 ^b	1.76 ^b	20.6 ^b	11.3 ^a	4.41 ^b	5.4 ^c	32.56 ^b	51.19 ^b	11.50 ^a	4.74 ^{ab}
Seolgaeng	4.98 ^c	2.97 ^a	2.07 ^b	1.68 ^c	19.7 ^d	11.0 ^a	2.95 ^d	19.5 ^b	32.98 ^b	51.15 ^b	11.12 ^{bc}	4.75 ^{ab}
Ilpum	4.98 ^c	2.97 ^a	2.11 ^a	1.67 ^c	20.8 ^a	11.8 ^a	3.98 ^c	20.3 ^b	33.30 ^a	51.12 ^b	11.01 ^c	4.57 ^c
Hanareum	5.72 ^a	2.57 ^c	1.86 ^d	2.22 ^a	19.8 ^d	11.3 ^a	4.42 ^b	19.4 ^b	32.00 ^c	51.87 ^a	11.29 ^{ab}	4.84 ^a
Goami	5.18 ^b	2.87 ^b	1.94 ^c	1.81 ^b	20.1 ^c	10.9 ^a	5.08 ^a	27.7 ^a	33.19 ^{ab}	50.83 ^c	11.33 ^a	4.64 ^{bc}

¹⁾Degree of polymerization

²⁾Different lower case letters within the column indicate significant difference at $p < 0.05$.

chains (A chains, $6 \leq DP \leq 12$), 51.15% medium-length chains (B1 chains, $13 \leq DP \leq 24$), 11.12% long chains (B2 chains, $25 \leq DP \leq 36$), and 4.75% very long chains (B3 chains, $DP \geq 37$). The five varieties exhibited few differences in the chain length distribution ratio within the amylopectin cluster.

SEM analysis of the endosperm structure To examine the structural differences in the endosperms of the varieties, the cross-sectional appearances of the rice kernels were analyzed (Fig. 1B). As shown in the representative SEM images of the internal grain structures, the starch granules of Boseogchal, Ilpum, Hanareum, and Goami appear polygonal and angular. Polygonal granules are more densely packed, and no major differences are noted in the arrangement of the starch granules within the endosperms of these four varieties. In contrast, the starch granules in Seolgaeng kernels are round, ellipsoidal, and irregular, and they appear similar to wheat with round starch (19). Seolgaeng is also opaque, which is similar to waxy rice grains (Fig. 1A), and the internal structure of its endosperm is similar to round starch granules, unlike the angular shapes of the other varieties. The white opaque area of Seolgaeng is caused by the round starch granules, which result in more air spaces in the starchy endosperm that diffusely reflect light (6).

Dry-milled rice flour properties, particle-size distribution, and damaged starch content The use of rice grains is limited to cooked rice; however, rice flour can serve as intermediate material in various processed foods. During the dry milling process, producing rice flour with fine particle size is more difficult than wheat owing to higher grain hardness (20). All rice flours were prepared under 2 types of dry milling conditions according to classification control using Air Classification Mill (ACM185; Hankook Crusher Co., Korea). As such, research on rice varieties with different amylose contents has been used to identify varieties suitable for dry milling (Fig. 2). One milling process provided low-speed, 5 Hz air classification speed, and the resulting rice flours demonstrated considerably lower starch damage regardless of rice variety. Using the second process, which used a high-speed, 15 Hz air classification speed, fine flour particles were finished to $< 70 \mu\text{m}$ and highly damaged starch (9.4–13.8%) was

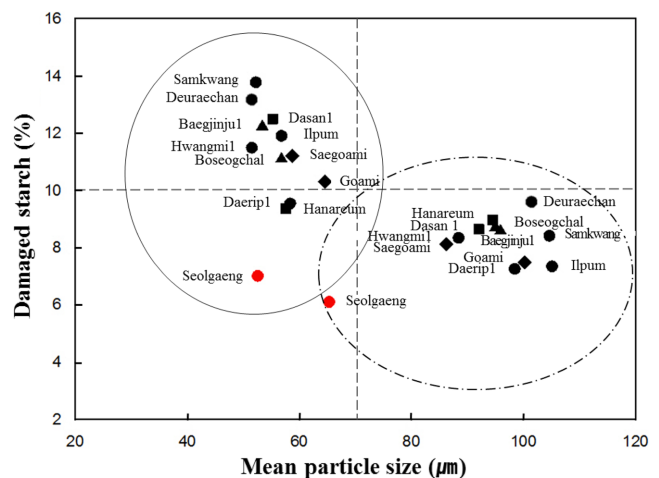


Fig. 2. Comparison of mean particle size and damaged starch content in the dry-milled flours according to the Air Classification Mill. Rice flours were ground at an air classification speed of 5 Hz (a dotted circular line) and 15 Hz (a solid circular line). ▲: waxy and semi-waxy varieties; ●: non-waxy *Japonica*-type varieties; ■: non-waxy *Tongil*-type varieties; ◆: high amylose *Japonica*-type varieties. The five varieties used in this study were selected from the other varieties shown here.

noted in all rice varieties, except Seolgaeng (Fig. 2). Dry-milled rice flours made from generic rice varieties showed an average particle size of $> 100 \mu\text{m}$ and the damaged starch content of $> 10\%$. Using rice flour of this quality can reduce the expansion of surface-absorbing moisture and the degree of absorption in the course of processing for bread, thereby preventing the deformation of structure or color of the processed food and maintaining product quality (21,22). In contrast, dry-milled rice flour produced using Seolgaeng kernels showed an average particle size that is $< 70 \mu\text{m}$ and $< 10\%$ of the starch is damaged.

According to particle-size distribution, the opaque, non-glutinous Seolgaeng rice demonstrated a narrow peak at the fine size, whereas the entire particle-distribution range for the other varieties was wide (Fig. 3A). Moreover, the dry-milled Seolgaeng rice flour demonstrated more uniform particle-size distribution than the other varieties, similar to commercial wheat flour. As shown in Table 3, the mean particle size of the dry-milled flours obtained was between 65.3 and

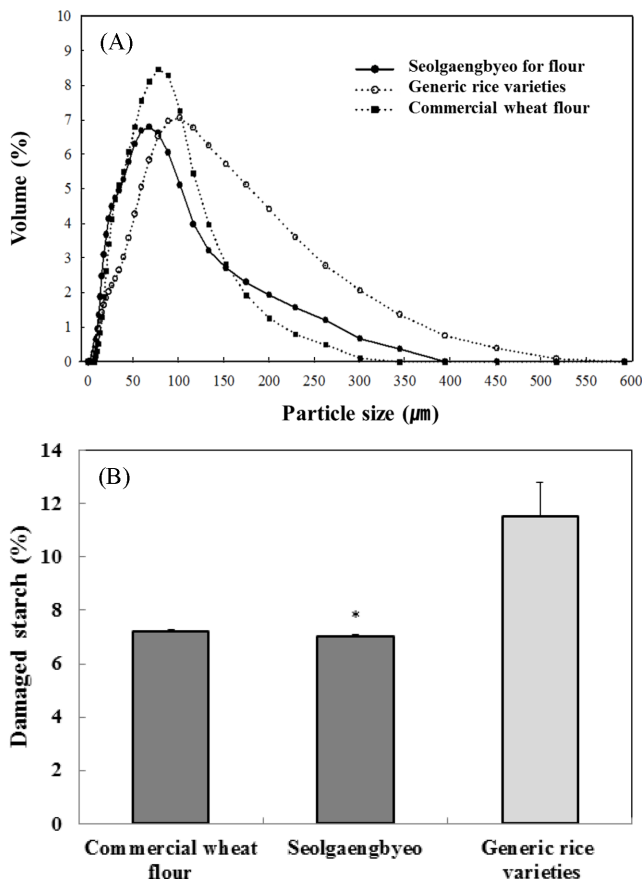


Fig. 3. Comparison of particle-size distributions (A) and damaged starch content (B) in dry-milled rice flours of Seolgaeng and generic rice varieties. *Indicates significant differences between Seolgaeng and other rice varieties at the 5% level according to Student's *t*-test

105.1 µm. In particular, dry-milled Seolgaeng demonstrated the highest proportion (50.7%) of fine particles (<56 µm), as well as the lowest average particle size. Consequently, it is possible to produce fine, dry-milled rice flour using Seolgaeng.

A high percentage of damaged starch has been reported to be associated with the presence of fine particles during dry milling (23), and similar results were observed in our study (Fig. 2). We observed significant differences in the damaged starch content between Seolgaeng and the other rice varieties. Seolgaeng, which provided the finest rice flour, exhibited significantly lower damaged starch content than the other varieties ($p < 0.05$) (Fig. 3B). The effects of

damaged starch on particle size may be owing to the tightly packed starch structure and the hardness of the rice kernels. The endosperm of the rice grain is a very dense and hard structure comprising complex starches (20). Because rice grains are harder than wheat, dry milling of rice under the same conditions as wheat results in higher damaged starch content and fewer fine particles, which in turn degrades the quality of rice flour (8,20,24). In order to produce high-quality rice flour that is equivalent to wheat flour, a number of studies have been performed on the wet milling method despite its higher costs (7). In addition, rice mutants with a white core, milky white, complete chalkiness, or floury are being studied to determine their suitability for use in producing dry-milled rice flour (6,25).

Consistent with the round starch granules (Fig. 1B), we observed that Seolgaeng kernels are more friable and produce finer particles with less damaged starch under dry milling conditions than the other varieties examined here. Our results indicate that processing Seolgaeng using dry milling technology will yield finer rice flour (19,26) and that the processing costs for rice flour milling would be considerably reduced using Seolgaeng rice as the commercial raw material in mass dry milling operations. Alternatively, Seolgaeng may be a useful germplasm for the development of a more suitable rice variety by crossing with *Tongi*-type materials in order to produce a higher-yielding variety with round starch granules.

Comparison of the dry-milled rice flour characteristics of the varieties with different amylose content in this study shows that the physicochemical properties did not affect the flour characteristics of dry-milled rice. However, the hardness of the rice kernel appears to influence the damaged starch content after milling.

In conclusion, the non-glutinous *Japonica* Seolgaeng rice had an entirely opaque endosperm. Seolgaeng rice showed a round starch structure, which would lead to better friability, finer particle size, and less damage to the endosperm during dry milling. Consequently, Seolgaeng appears to be suitable for producing fine dry-milled rice. Accordingly, we can expect to produce dry-milled rice flour that is similar to wheat flour, and this would considerably reduce milling costs.

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Table 3. Mean particle sizes and particle-size distributions of dry-milled flours made from the five rice varieties

Variety	Particle size distribution (µm, %)					Mean particle size (µm)
	<56 µm	75 µm	100 µm	150 µm	>150 µm	
Boseogchal	32.1 ^{b1)}	11.1 ^c	13.8 ^b	19.4 ^b	23.5 ^c	95.0 ^c
Seolgaeng	50.7 ^a	13.5 ^a	12.7 ^c	12.3 ^c	10.8 ^d	65.3 ^d
Ilpum	24.6 ^d	9.9 ^d	12.3 ^c	20.8 ^a	32.4 ^a	105.1 ^a
Hanareum	31.2 ^b	11.9 ^b	14.9 ^a	19.4 ^b	22.5 ^c	94.5 ^c
Goami	28.7 ^c	10.8 ^c	12.9 ^c	20.8 ^a	26.8 ^b	100.2 ^b

¹⁾Different lower case letters within the column indicate significant difference at $p < 0.05$.

Disclosure The authors declare no conflict of interest.

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