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Comparative Structure and Physicochemical Properties of Ilpumbyeo, a High-Quality Japonica Rice, and Its Mutant, Suweon 464

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A new rice mutant Suweon 464 (S-464) derived from a high-quality rice, Ilpumbyeo (IP), revealed a striking difference in cooking quality from IP. The physicochemical properties of S-464 and IP were compared. S-464 was unusually high in amylose and fiber contents, had B-type crystallinity of starch, and had a markedly lower proportion of short chains in the distribution of glucan-chain fractions of debranched starch as compared with IP. Scanning electron microscopy revealed that starch granules of S-464 were much smaller in size than those of IP and that many of them were not separated from amyloplasts. The physicochemical properties of S-464 would contribute to poor gelatinization, lower swelling power, higher hardness, and less stickiness when cooked. Although S-464 may not be desirable for cooked rice, the mutant could be an excellent candidate for other processed food products on the basis of its unusual properties of starch and high fiber, protein, and lipid contents.

KEYWORDS: New rice mutant; physicochemical properties; X-ray pattern; amylopectin structure; pasting property; SEM; texture

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food for more people than any other crop species. A major portion of the world population obtains more than half of their daily dietary calories from rice. Rice is consumed mostly in the boiled form of whole (milled) grain. Various factors affecting the quality of rice food products, including cooked rice, have been studied extensively. Interaction among starch, protein, and lipid, the main rice grain components, has been recognized to dictate the quality measures of rice such as pasting properties, texture, and taste of rice products (*1*). Starch structure and protein functionality in rice have been well documented (*2*−*4*).

The per capita consumption of rice has steadily decreased since the 1980s, presumably due to the increased consumption of fast food products, which incorporate the use of wheat and maize. However, the hypoallergenic property of rice and rice products has received increased interest in Korea. The interest in rice has led to the development of new rice varieties suitable for incorporation into various processed "healthy" food products. A new rice variety, Suweon 464 (S-464), was developed by mutation breeding via *N*-methyl-*N*-nitrosourea (MNU) treatment of Ilpumbyeo (IP), a high-quality japonica rice variety (*5*), at the National Crop Experiment Station, RDA. However, S-464 has unsuitable properties for traditional cooking due to its high amylose content and difficulty of gelatinization, but it has promising qualities from the nutritional point of view such as a high fiber content.

Recently, a series of studies has been initiated on the comparison of various aspects of physicochemical, cytochemical, and ultrastructural characteristics of IP and S-464 to elucidate the factors involved in dictating the quality of cooked rice and to exploit the possible usage of S-464 in other processed food products. This paper reports significant differences between IP and S-464 in physicochemical and structural properties of starch including gelatinization, crystal type, fine structure of starch, isolated starch granule morphology, and texture of cooked rice. The detailed information from the new rice mutant should be useful for various applications of S-464 in the rice food industry.

MATERIALS AND METHODS

Materials. A mutant rice, S-464, and its original variety, IP, were cultivated in the experiment field of the National Crop Experiment Station, Suwon, South Korea, in 2001. The milled rice grain was used to analyze various physicochemical properties of raw and cooked rice.

Starch Isolation. Starch was isolated using a method described by Hoover and Sosulski (*6*) involving repeated steeping of rice flour in 0.2% aqueous NaOH. The isolated starch was dried in an oven at room temperature, ground into powder, and passed through a 100-mesh sieve.

Physicochemical Analyses. Moisture, ash, and crude protein, fat, and fiber were determined using the standard AOAC methods (*7*). The

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amylose content was determined using the simplified assay method of Juliano (*8*).

X-ray patterns of starch were obtained with copper (nickel foil-filtered) Kα radiation using powder X-ray diffractometry (D 5005, Germany, Bruker). The operation setting for the diffractometry was 40 kV and 40 mA. The angle of diffraction (2θ) scanned was from 3 to 40°, with 2 s count time. The degree of crystallinity was quantitatively estimated following the method of Nara and Komiya (*9*).

The pasting properties of rice flours were measured by AACC approved method 61-02 (*10*) with a Rapid Visco-Analyzer (RVA; Newport Scientific Pty, Ltd., Warriewood, NSW, Australia).

Swelling power and solubility of isolated starches were determined by heating starch−water slurries in a water bath at temperatures ranging from 65 to 95 °C in 15 °C intervals according to the procedures of Walter et al. (*11*). Briefly stated, the 0.5 g starch sample was heated in 30 mL of water at designated temperatures for 30 min. The slurries were then centrifuged at 8000 rpm for 20 min, and the supernatant was removed and dried by evaporation at 130 °C. The residue obtained was used for calculating the starch solubility. The swelling power was determined by measuring the amount of original precipitate from the centrifugation and calculating the amount of water absorbed by the starch (percent weight increase) after subtraction of the amount of solubilized starch (*12*).

The fine structure of starch, especially amylopectin, was determined by measuring the length and distribution of α-1,4-glucan-chain fractions in debranched starch using a high-performance anion-exchange chromatography system equipped with a pulsed amperometric detector (HPAEC-PAD), a modification of the method described by Nishi et al. (*13*). One milliliter of the gelatinized starch was placed in 50 μL of 0.6 M sodium acetate (pH 4.4) containing 10 μL of 2% NaN_3. Debranching of the starch was achieved by the addition of 0.5 μL of isoamylase from *Pseudomonas amyloderamosa* (Sigma I2758, 1,250,000 units) prior to incubation at 40 °C for 24 h. The pH of the hydroxyl groups was adjusted to 9.0 with an ammonium solution, and 50 μL of sodium borohydride was added to the samples. After standing for 24 h at room temperature, the mixture was freeze-dried, dissolved in 0.05 mL of 1 M NaOH for 1 h, and diluted with 0.45 mL of water. An aliquot of 25 μL of the preparation was injected into a BioLC (system model DX-300, Dionex, Sunnyvale, CA) equipped with a PAD and Carbopac PA1 column (Dionex 4 × 250 mm, P/N 35391). Eluents A (0.5 M sodium hydroxide), B (0.5 M sodium acetate), and C (distilled water) were operated at a flow rate of 1 mL/min. The separation gradient of eluents was as follows: 0 min, 20% A, 10% B, and 70% C; 0−40 min, linear gradient to 20% A and 80% B.

Scanning Electron Microscopy (SEM). Isolated rice starch powders were sprayed directly onto aluminum specimen stubs. The specimens were sputter coated with gold and viewed with a JSM 5410LV (JEOL) scanning electron microscope at 15 kV.

Granule size distribution of starch was evaluated by a Camscope video microscope IT system equipped with Kan Scope 3.0 image acquisition software (Sometech, Korea). Fifty starch granules on each SEM image were measured per sample, and granule size was expressed in terms of the diameter of image surface. Diameters of irregular polygons were averaged.

Texture Analysis of Cooked Rice. Thirty grams of milled rice was placed into a stainless steel cup (60 mm diameter and 70 mm deep), and water was added to give a weight ratio of 1:1.25. After 20 min of soaking at room temperature, containers with water and rice were placed in an electronic rice cooker (LG Co.) holding ~200 mL of water to conduct to the cup. The rice in the cup was then steamed for 30 min in the cooking mode followed by 10 min postcooking (warming) periods.

Texture profile analysis (TPA) was conducted with a texture analyzer (TA-XT2, Stable Micro Systems, Godalming U.K.) by placing a single kernel of cooked rice on the base plate of the analyzer at room temperature. A two-cycle compression, force-versus-time program was used with a test speed of 2 mm/s and a rate of 80% strain using a cylinder plunger having a 20 mm diameter.

Parameters recorded from the test curves were hardness (H), adhesiveness (A3), cohesiveness (A2/A1), and springiness (D2/D1) (**Figure 1**). Gumminess was expressed by multiplying hardness by cohesiveness values and chewiness by multiplying gumminess by springiness. Values of standard calculations of curve attributes to TPA were as described by Bourne (*14*) and defined by Munoz (*15*).

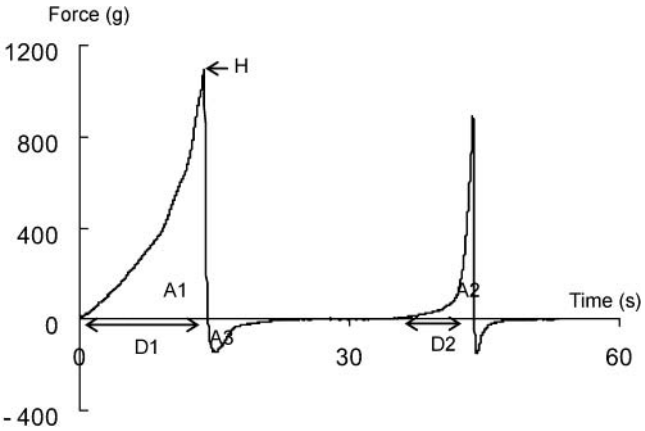

Figure 1. Typical curve of texture profile analysis for a kernel of cooked rice. TPA parameters: H, hardness (g) = peak force of first compression; A3, adhesiveness = area of negative force curve; A2/A1, cohesiveness = ratio of area under curves A2/A1; D2/D1, springiness = ratio of D2 to D1; gumminess = hardness × cohesiveness; and chewiness = gumminess × springiness.

Table 1. Proximate Composition and Amylose Content of Milled Rice

variety	moisture (%)	ash (%)	protein (%)	lipid (%)	fiber (%)	amylose (%)
IP	9.85 ± 0.10[a]	0.37 ± 0.01	6.66 ± 0.16	0.44 ± 0.01	0.39 ± 0.00	18.63 ± 0.67
S-464	11.69 ± 0.92	0.87 ± 0.02	7.61 ± 0.03	1.89 ± 0.03	0.72 ± 0.05	33.96 ± 1.16

[a] Standard deviation.

Data Analysis. Physicochemical analyses were done in triplicate, and texture analysis of cooked rice was repeated on 20 replicate samples. The results are presented as means ± standard deviation (SD). Statistical data were analyzed using the data analysis tool pack of Microsoft Excel 2000.

RESULTS AND DISCUSSION

Proximate Composition and Amylose Content. S-464 showed higher values than IP in all of the results including proximate composition and amylose content (**Table 1**). Amylose content, one of the most important components characterizing rice quality, in S-464 was almost twice that in IP. On the basis of the category classified by the International Rice Research Institute (IRRI, Manila, Philippines), IP and S-464 should be classified as low- and high-amylose rices, respectively (*16*). Crude fiber, one of the basic components for a number of processed foods, was also considerably higher in S-464: 0.72% compared with 0.39% for IP (**Table 1**). Fiber contents in other ordinary milled rices have been reported to range from 0.2 to 0.5% (*16*).

X-ray Diffraction Pattern and Relative Cystallinity of Rice Starch. As shown in **Figure 2**, the X-ray diffraction patterns of isolated starch in IP and S-464 were quite different. Isolated starch from IP showed a typical A-type of X-ray diffraction pattern, which is the same as that of most ordinary rice starches (*17*). The starch of S-464, however, had a B-type X-ray pattern, indicating a different starch composition compared with IP as well as that of other ordinary rices. The relative crystallinity of S-464 was 21.97%, whereas that of IP was 26.88% (**Table 2**). The differences in the X-ray diffraction type and degree of crystallinity between IP and S-464 reflect the differences in amylose contents; S-464 contained twice as much amylose as IP (**Table 1**), and the ratio of mole percentage of short-chain

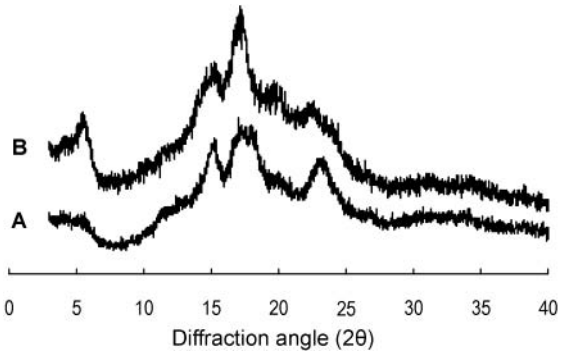

Figure 2. X-ray diffraction patterns of rice starch of IP (A) and S-464 (B).

Table 2. Relative Crystallinity and X-ray Pattern in Rice Starch of IP and S-464

variety	relative crystallinity (%) $[100A_c/A_c + A_a]$[a]	relative crystallinity ratio[b]	crystal pattern
IP	26.88 ± 0.01	1.00	A
S-464	21.97 ± 0.02	0.82	B

[a] A_c and A_a are crystalline area and amorphous area, respectively. [b] Ratio means relative crystallinity/relative crystallinity of IP.

Table 3. Distributions of Glucan-Chain Fractions in Debranched Rice Starch of IP and S-464

debranched fraction[a] (%)	IP	S-464
a ($6 \leq DP \leq 12$)	33.75 ± 1.18	13.62 ± 0.97
b1 ($12 < DP \leq 24$)	58.42 ± 1.75	75.82 ± 2.44
b2 ($24 < DP \leq 36$)	6.80 ± 1.39	10.35 ± 0.58
b3 ($DP \geq 37$)	0.12 ± 0.02	0.21 ± 0.01
a/b1	57.77	17.96

[a] Grouping of degree of polymerization (DP) numbers followed that of Hanashiro et al. (*2*).

fractions in debranched starch was much richer in IP than in S-464 (**Table 3**). Similar results were obtained by Cheetham and Tao (*18*), who found that in maize starches the degree of starch crystallinity decreased with the increase in amylose content and average chain length in amylopectin and that it was almost directly proportional to the mole percent of short-chain fraction, especially that with the degree of polymerization (DP) ranging from 10 to 13. The low ratio of short chains with $6 \leq DP \leq 12$ in S-464 apparently decreases crystalline structure. Imberty et al. (*19*, *20*) have shown that double helices of A- and B-type starches are packed in a pseudohexagonal array. The lattices of B-type starches have a large void (channel) in which 36 water molecules can be accommodated. However, in A-type starches, the lattices contain a helix in the center of the molecule rather than a column of water.

Distribution of α-1,4-Glucan-Chain Fractions. Figure 3 shows the distribution of glucan chains shorter than DP 42 in the debranched rice starches by isoamylase from *P. amylodera-mosa*, determined using the HPAEC. Because the column used in this system can detect only the glucan chains shorter than DP 42, most of the branch chains should have been separated from the amylopectin structure in normal rice starch. There is a possibility of some glucan branches separated from amylose molecule, especially from S-464 starch. The proportion of the short chains in S-464 starch was dramatically lower, although it has a relatively higher long-chain frequency than IP. The ratio of fraction a ($6 \leq DP \leq 12$) and fraction b1 ($13 \leq DP \leq 24$) was, however, significantly lower in S-464 than in IP, 17.96: 57.55 (**Table 3**). The peaks in chain length distribution of amylopectin branches were DP 12 for IP and DP 15 for S-464 (**Figure 3**). Our results were in agreement with those reported by Jane et al. (*21*), which showed that A-type starches had peaks at shorter chain length (DP 12−14) than B-type starches (DP 14−16) and that A-type starches had larger proportions of short chains than B-type starches. In addition, Jane et al. (*21*) reported that the chain length distribution of amylopectin branches determined the gelatinization temperature of starch, enthalpy changes, and pasting properties. The gelatinization temperature of starch increased with increasing amounts of long-chain amylopectin fractions. In agreement with Jane et al. (*21*), our results showed that S-464, which had a higher proportion of long-chain debranched amylopectin fractions, had a higher initial pasting temperature (**Table 4**) and a higher gelatinization temperature (76.41 °C) than IP (65.27 °C, data not shown) as determined by differential scanning calorimetry (DSC).

Pasting Properties. There were significant differences in pasting properties between the rice flours and starches (**Table 4**). Flour and starch components from S-464 had markedly higher initial pasting temperature, lower viscosities in all viscogram components, and higher setback viscosity compared with IP. Furthermore, viscosities of flour and starch of S-464 continued to increase with time, whereas viscosities of flour and starch of IP decreased over time. Setback viscosity, which was closely associated with the degree of retrogradation (*22*), suggests that the milled rice of S-464 would be slow to gelatinize during cooking and quick to retrograde after cooking. The pasting properties of rice starches of IP and S-464 showed tendencies more similar to those of the flours with higher viscosities than to those of rice flours. The higher viscosities noted in the starches compared to flours are most likely the result of the removal of protein.

Swelling Power and Solubility of Rice Starch. The differences in swelling power and solubility of the rice starch between IP and S-464 at different temperatures are shown in **Table 5**. Swelling power increased as water temperature increased both in IP and in S-464. The swelling power ranged from 6.84 to 16.86% in IP and from 3.07 to 9.95% in S-464, indicating that the S-464 starch had a much lower swelling power throughout the range of temperatures (65−95 °C) examined compared to that of the IP starch. According to Hoover et al. (*23*), rice starch with a greater swelling power had a weaker bonding force within the granular interior than starches of lower swelling power. The enthalpy of gelatinization (ΔH) determined by DSC of IP (10.76 J/g) was lower than that of S-464 (11.91 J/g, data not shown), suggesting that bonding forces within IP starch granules would be of a lower order of magnitude than that of S-464 starch. The lower swelling power of S-464 starch may also be related to its poor gelatinization properties in that the S-464 starch required a much higher pasting temperature, resulting in a lower viscosity compared to IP starch (**Table 4**).

The water solubility of both IP and S-464 starches increased with an increase in temperature (**Table 5**). At 65 °C, S-464 starch showed a slightly lower water solubility than IP starch. However, at temperatures >80 °C, the solubility of S-464 starch became markedly higher than that of IP starch. The higher water solubility of S-464 starch at higher temperature may be related to its unusually higher amylose content (~15%), which would have been leached out from starch granules into the water, especially above the gelatinization temperature.

SEM of Rice Starch. The rice starch granules of IP and S-464 differed in size and morphology when viewed with SEM

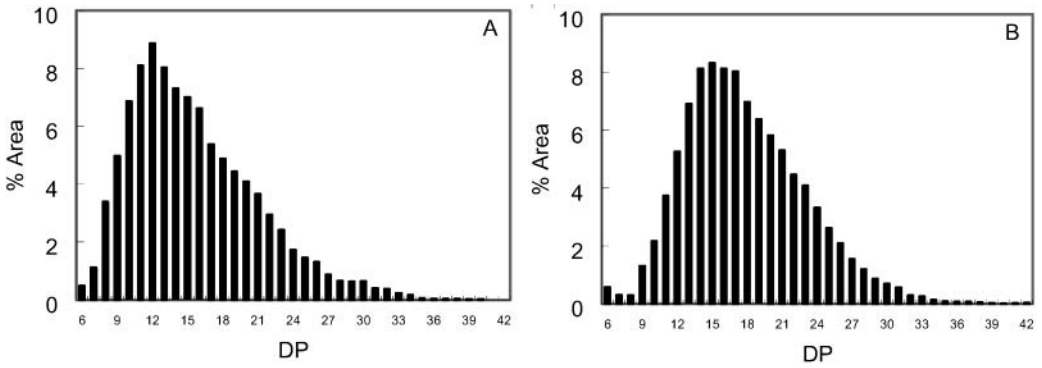

Figure 3. Distributions of glucan-chain fractions in amylopectin molecules of debranched rice starch of IP (A) and S-464 (B).

Table 4. Pasting Properties of Rice Flour and Isolated Starch of IP and S-464

sample	variety	initial pasting temp (°C)	peak (RVU[a])	hot (RVU)	breakdown[b] (RVU)	final (RVU)	setback[c] (RVU)
flour	IP	69.20 ± 0.12	205.00 ± 18.81	86.00 ± 4.22	119.00 ± 22.00	172.00 ± 5.61	86.00 ± 1.41
	S-464	79.80 ± 1.23	55.50 ± 2.10	59.50 ± 2.13	−4.00 ± 0.08	156.50 ± 0.72	97.00 ± 1.41
starch	IP	68.85 ± 0.28	273.42 ± 3.53	139.46 ± 1.47	133.96 ± 2.06	233.38 ± 2.06	93.92 ± 0.58
	S-464	80.90 ± 0.07	120.54 ± 3.12	101.08 ± 2.35	19.46 ± 0.76	202.54 ± 6.54	101.46 ± 0.47

[a] Rapid visco analyzer units. [b] Peak viscosity minus hot viscosity. [c] Final viscosity minus hot viscosity.

Table 5. Swelling Power and Water Solubility of Starch of IP and S-464

	swelling power (g/g)			water solubility (%)		
variety	65 °C	80 °C	95 °C	65 °C	80 °C	95 °C
IP	6.84 ± 0.11	9.59 ± 0.23	16.86 ± 0.34	0.33 ± 0.06	1.47 ± 0.27	9.80 ± 0.20
S-464	3.07 ± 0.13	6.04 ± 0.43	9.95 ± 1.30	0.13 ± 0.07	5.07 ± 0.61	14.2 ± 2.03

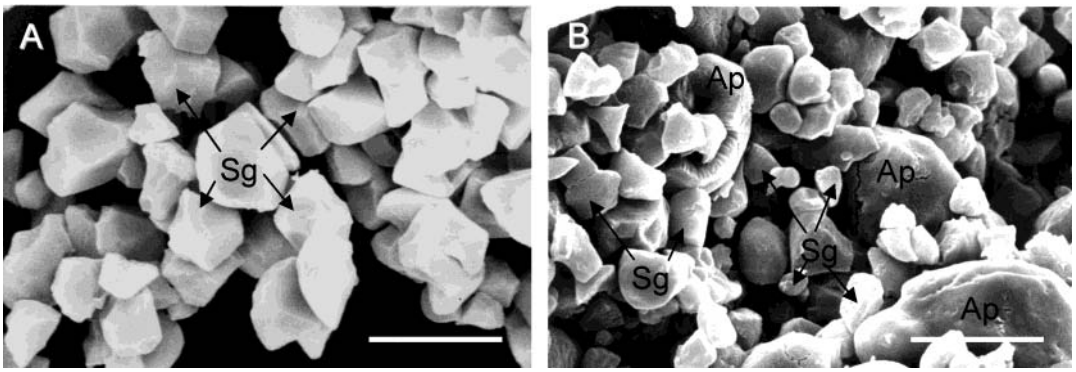

Figure 4. Scanning electron micrographs of starch granules of IP and S-464 at 3000× (scale bar = 10 μm) (A) from IP [individual starch granules (Sg) having polygonal shape with various angles are well separated; no amyloplasts (Ap) shown in S-464 in (B) are present] and (B) from S-464 [individual starch granules (Sg) are much smaller than those of IP shown in (A) and are somewhat rounded in appearance; in addition, a number of amyloplasts (Ap), which appear as large elongated bodies with smooth surfaces, containing apparently unseparated compound starch granules, are present].

Table 6. Texture Properties of Cooked Rice of IP and S-464

variety	hardness (g)	adhesiveness	springiness	cohesiveness	gumminess	chewiness
IP	1657.68 ± 107.20	22.24 ± 2.60	1.61 ± 0.14	0.34 ± 0.03	561.28 ± 45.72	902.93 ± 45.51
S-464	2425.00 ± 102.48	0.43 ± 0.09	1.55 ± 0.12	0.27 ± 0.02	666.18 ± 63.39	1032.84 ± 97.96

(**Figure 4**). Whereas the starch granules of IP were polygonal with various angles, those of S-464 were partly rounded and less angular. In addition, individual starch granules of S-464 were mixed with large voluminous, nonangular rounded bodies, which were often elongated in shape (**Figure 4B)**. On the basis of their sizes, which were greatly larger than those of individual starch granules, and general morphology, it is apparent that they are compound starch granules from which smaller individual starch granules are separated. Hoshikawa (*24*) clearly demonstrated that the compound starch granules of rice, which had been thought to consist of several amyloplasts, consist of a single amyloplast that contains many individual starch granules. It is believed, therefore, that the large voluminous bodies mixed with smaller individual granules shown in **Figure 4B** represent the morphological entity of amyloplasts that survived the harsh treatments received during the starch isolation processes (see Materials and Methods). These amyloplasts were covered by a coating, apparently the amyloplast

membrane, which could have prevented the separation of individual starch granules. Amyloplasts were not observed in IP (**Figure 4A**), indicating that the coating (amyloplast membrane) had been broken completely during starch isolation processes, freeing entire individual starch granules within them. The mean size of starch granules in S-464 was about half that of IP, determined by measuring diameters of randomly selected granules. The average starch granule diameters of S-464 and IP were approximately 2.6 and 5.2 μm, respectively. The lower swelling power of S-464 compared to that of IP can be explained, at least in part, by the fact that many of the starch granules in S-464 were confined to the amyloplasts, limiting the entrance of water and consequent absorption (**Table 5**). The numbers of amyloplasts containing mature, structurally and mechanically intact starch granules following starch isolation is unknown.

Starch granules of S-464, which have physicochemical properties very different from those of IP, might have tightly bonded to the amyloplast membranes so as to escape from morphological and physical disruption during endosperm maturation and isolation from the milled rice.

Texture Analysis of Cooked Rice. Individual cooked rice grains of S-464 had higher hardness, gumminess, and chewiness values but lower adhesiveness, springiness, and cohesiveness values compared to those of IP (**Table 6**). Higher hardness and lower stickiness in cooked rice of S-464 might be due to the higher contents of amylose, fiber, and protein and higher proportion of long glucan-chain fractions in amylopectin compared to values obtained from IP. Insolubility of protein may interrupt moisture absorption and swelling of starch granules during rice cooking (*25*, *26*). An investigation by Juliano et al. (*27*) and Reddy et al. (*28*) confirmed that a positive correlation existed between the longer exterior amylopectin chains and the firm, dry, and nonsticky texture of cooked rice.

Conclusions. Milled IP rice, one of the most highly valued, high-quality rices for cooking in Korea, and its mutant, S-464, were greatly different in all aspects of physicochemical properties and the ratio of basic components analyzed. The results shown in S-464 are similar to biochemical analysis of the amylose-extender mutant of rice (*Oryza sativa* L.), which had reduced activity of starch-branching enzyme IIb (BE IIb) and in the proportion of short-chain fractions (*13*). The starch structure of S-464 might be altered by genetic modification in BE IIb, but we need to identify the change of starch-branching enzyme activity in S-464.

In contrast to IP, the milled grains of S-464 were unsuitable for ordinary cooking primarily because of the hardness they retained after cooking. The hardness of S-464 grains remained even after the cooked grains were destarched with malt treatment (data not shown). Hardness retention may be attributed to structural and physicochemical properties of the starch, the major constituent of the rice grain. The markedly higher contents of amylose, protein, lipid, and fiber and looser amylopectin structure of rice grain in S-464 compared to IP may also contribute to the hardness or other unsuitability of S-464 for cooking.

Although S-464 rice may not be suitable for standard cooking, it has many desirable properties for other processed foods or special industrial products in terms of health or functional utility.

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