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Physicochemical Properties of *Japonica* Non-Waxy and Waxy Rice during Kernel Development

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Abstract In this study we examined the changes in weight and dimension, protein and amylose contents, and pasting properties of brown rice flour, as well as the gelatinization properties of starch, from two non-waxy *japonica* cultivars and one waxy *japonica* cultivar planted in an experimental field in 2002 under the same fertilizer conditions. The weight of both rough and brown rice increased consistently up to 42 days after flowering (DAF) for the non-waxy rice and to 35 DAF for the waxy rice. The changes in dimension of the brown rice kernel indicated that the length was maximized first, followed by breadth and then thickness. The protein content of the non-waxy rice remained fairly constant, but that of the waxy rice decreased by about 1% after 14 DAF. The amylose content of the non-waxy brown rice flour increased, but that of the waxy brown rice flour decreased during kernel development. As the kernel developed, the peak viscosity of the non-waxy rice flour increased up to 35 DAF, after which it decreased, whereas that of the waxy brown rice flour increased consistently. The gelatinization temperature of starch also increased in the waxy rice during kernel development up to 21 DAF. The gelatinization enthalpy of starch, however, increased in all rice cultivars throughout the kernel development.

Keywords: rice, waxy rice, kernel development, gelatinization, starch

Introduction

Rice (*Oryza sativa* L.) is the staple food in Korea, with the standard variety being low amylose *japonica* type at different maturities. With the rising living standards for all consumers, improved rice quality has become one of the most important goals in rice breeding programs as well as in rice production practice in Korea. Rice is harvested as rough rice and stored before consumption. The hull of rough rice is removed to produce brown rice which is then milled to remove the bran layer and germ from brown rice to produce milled rice, which is eaten as intact cooked grains.

The major constituent of endosperm is the starch that is accumulated by assimilates transport during kernel development (1, 2). The obstruction of assimilates transport during grain development causes imperfections in the morphological structure of the endosperm, which may affect the quality of cooked rice (3). The morphological structure of fully ripened rice grains provide fundamental information on rice quality with respect to milling, processing, and consumption. The morphological structure of the *japonica* rice endosperm was hypothesized to play an important role in the formation of internal hollows in well-developed grains during cooking (4, 5). These hollows resulted from cracks and fissures that developed during grain soaking and were sealed in the peripheral layer by gelatinized starch during cooking as the grain expanded mainly in the longitudinal direction (4). The final hollow volume and shape of the cooked rice had no correlation with amylose content or flour gelatinization properties (5).

The morphological structure of ripened rice has been well documented (6). The ultrastructure of starch granules and protein bodies in a Korean rice endosperm cell was reported by Kim *et al.* (7). Evers and Millar (1) reviewed the cereal grain structure and development. However, there are fewer reports on developing rice grains, although it is equally important to understand changes in the grain structure during development. Nagato and Chaudhry (8) reported a ripening process and kernel development in *japonica* and *indica* rice. Horigane *et al.* (3) visualized the moisture distribution during development of *japonica* rice caryopses by nuclear magnetic resonance micro imaging. Hwang (2) reported the expression of starch biosynthetic genes of Korean non-waxy and waxy rice during kernel development. Nanda *et al.* (9) compared the proteins, soluble amino acids, glutamate dehydrogenase (GDH) activity, GDH isoenzymes, and dry matter accumulation during kernel development of high and low protein, *indica* rice cultivars. Shi *et al.* (10) reported the genetic effects on brown rice thickness of *indica* rice at various rice filling stages.

The maturity of rice may also be an important factor controlling the final rice quality, by affecting the color and hot paste viscosity of the milled rice (11), yield (12), and the gloss of cooked rice (11, 12).

The objective of the present study was to compare the changes in weight and shape, and pasting and gelatinization properties of *japonica* non-waxy and waxy rice during the kernel development.

Materials and Methods

Materials Two non-waxy *japonica* cultivars, Dongjinbyeo and Ilpumbyeo, of medium and medium-late maturity, and one waxy *japonica* cultivar, Shinsunchalbyeo, of medium maturity, were used. Rice cultivars were planted in an experimental field of the National Yeongnam Agricultural Experiment Station, Rural Development Administration, Daegu, Korea, in 2002, under the same fertilizer conditions.

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The panicles were collected between 7 to 42 days after flowering (DAF) at 7-day intervals. The panicles were immediately frozen in liquid nitrogen and transported to the laboratory. The frozen samples were thawed in a refrigerator for 2 days and dried at room temperature for 10 days. The dried rough rice was separated from the panicles and stored in a refrigerator until used. The hull of the rough rice was removed by hand to obtain the brown rice before the experiments.

Measurement of kernel weight One hundred rough rice or brown rice kernels were randomly collected and weighed individually with an accuracy of 0.1 mg. The brown rice samples were used for further analyses of kernel color and dimension measurements.

Observation of brown rice kernel color The color of the brown rice kernel was observed visually and divided into four groups: green, green with some brown, brown with some green, and brown. The colors of 100 kernels were recorded.

Measurements of length, breadth, and thickness of brown rice kernel The length, breadth, and thickness of each of the 100 kernels of brown rice were measured using a micrometer caliper (Mitutoyo Corp., Kanagawa, Japan) with an accuracy of 0.05 mm.

Determination of protein and amylose contents of brown rice flour Brown rice was ground with a mill (Super Mill 1500; New Port Scientific Pty. Ltd., Warriewood, NSW, Australia) with a gap setting of 0.2. The coarse flour was further ground with mortar to pass through a 60-mesh sieve.

Protein content was determined by the micro-Kjeldahl method (13) using a nitrogen conversion factor of 5.95. Amylose content was determined by using the method of Juliano (14). The fat of the brown rice flour was extracted with ethyl ether for 24 hr using a Soxhlet, after which 4.5 mL of 1 N NaOH was added to 50 mg of the defatted sample. The suspension was thoroughly mixed and distilled water added to make up a 50 mL solution. To 5 mL of this sample solution, 1 mL of 1 N acetic acid and 2 mL of iodine reagent (0.2 g KI and I_2 in 100 mL) were added. The volume was diluted to 100 mL and the absorbance was measured at a wavelength of 620 nm using a spectrophotometer (Genesys 5; Spetronic Instruments Inc., Rochester, NY, USA). The amylose content was estimated from a standard curve, which was constructed using a mixture of potato amylose (type III, 9005-82-7; Sigma Chemical Co., St. Louis, MO, USA) and potato amylopectin (9037-22-3; Sigma Chemical Co.).

Measurement of hot paste viscosity of brown rice flour The hot paste viscosity of the brown rice flour was measured using the Rapid Visco Analyzer (RVA model-4D; Newport Scientific Pty. Ltd., Warriewood, NSW, Australia) following profile SDT 1 of ICC-Standard Method (15). Flour (3 g, db) was suspended in distilled water (25 g) and placed in an aluminum can containing a plastic paddle. The suspension was first held at 50°C for 1 min, and then heated to 95°C at a rate of 11.84°C/min. The sample was held at 95°C for 2.5 min, then cooled to 50°C at a rate of 11.84°C/min, and held for 1.4 min.

Peak viscosity, trough, breakdown (peak viscosity minus trough), final viscosity, and setback (final viscosity minus trough) were obtained from the RVA viscogram and expressed as rapid visco unit (RVU). Degree of collapse (DC) was termed as (breakdown/peak viscosity) × 100 (16).

Starch isolation Starch was isolated from the brown rice flour by the alkaline treatment. Flour (1 g) was mixed in 50 mL of 0.2 % NaOH, blended in a stirrer at 4°C for 5 hr, and passed though a 400-mesh sieve. The alkali treatment was repeated once again with 100 mL of 0.2 % NaOH. It was allowed to stand at 4°C for 27 hr and centrifuged at 8000×g for 30 min. The supernatant was carefully removed and the upper, non-white layer was scraped off. The white layer was washed with distilled water until the sediment became neutral. The starch was then collected and dried in an oven at 40°C for 48 hr and passed though a 60-mesh sieve.

Differential scanning calorimetry of starch Differential scanning calorimetry (DSC) measurement was conducted with a differential scanning calorimeter (DSC-7; Perkin-Elmer Co., Norwalk, CT, USA) fitted with a thermal analysis data station. Starch samples (10 mg, db) were weighed into a stainless steel capsules (No. 0319-0218, Perkin-Elmer) to which 30 mg of distilled water was added. Capsules were hermetically sealed by a quick press and allowed to stand for 1 hr at room temperature. The samples were heated from 30 to 100°C at a rate of 10°C/min. The DSC analyzer was calibrated using indium and an empty capsule was used as a reference.

Onset (To), peak (Tp), and conclusion (Tc) temperatures were reported. The endothermic enthalpy (ΔH) was calculated by integrating the area between the thermogram and a base line under the peak, and was expressed in terms of joules per unit weight of dry starch (J/g).

Results and Discussion

Weight of rough rice and brown rice The single kernel weights of the rough rice and brown rice in the non-waxy and waxy rice during development are presented in Table 1. The weight distribution patterns of the rough rice and brown rice were similar, in that the weight increased consistently up to 42 DAF for the non-waxy rice and to 35 DAF for the waxy rice. The weight of the majority of the rough rice was in the range of 5-14.9 mg at 7 DAF for the non-waxy rice, whereas that of the waxy rice was more broadly distributed from light (less than 5 mg) to heavy (15.0-19.9 mg) (Ed- there is data missing here and I don't know what the range should be) at 7 DAF. The weight of the rough rice for the non-waxy Dongjinbyeo at 42 DAF was 20.0-29.9 mg and that for the non-waxy Ilpumbyeo was 15.0-29.9 mg at the same time. The waxy rice showed the same range in weight as Ilpumbyeo at 35 and 42 DAF.

The weight change of the rough rice is shown in Fig. 1. The weight of the non-waxy rough rice rapidly increased from 7 to 28 DAF and then plateaued, while that of the waxy rice continuously increased up to 35 DAF. The

Table 1. Distribution of rough rice and brown rice weight during kernel development

Weight (mg)	Non-waxy rice												Waxy rice					
	Dongjinbyeo						Ilpumbyeo						Shinsunchalbyeo					
	7[1]	14	21	28	35	42	7	14	21	28	35	42	7	14	21	28	35	42
<5.0	1(36)[2]	4(22)	-	-	-	-	4(26)	5(27)	(11)	-	-	-	24(41)	5(17)	(13)	-	-	-
5.0-9.9	55(64)	36(63)	(29)	-	-	-	42(71)	40(61)	14(18)	(5)	-	-	37(51)	38(60)	16(21)	-	-	-
10.0-14.9	44	46(15)	40(41)	1(14)	-	-	52(3)	43(12)	18(36)	10(12)	4(14)	(10)	34(8)	44(23)	22(30)	4(19)	(1)	-
15.0-19.9	-	14	30(27)	16(45)	6(46)	(38)	2	12	32(229)	11(40)	13(42)	13(42)	5	13	27(28)	23(61)	4(44)	5(45)
20.0-24.9	-	-	27(3)	48(41)	53(54)	58(62)	-	-	28(6)	39(43)	42(44)	42(48)	-	-	28(8)	55(20)	44(55)	44(55)
25.0-29.9	-	-	3	35	41	42	-	-	8	40	41	45	-	-	7	18	52	51

[1] Days after flowering (DAF).
[2] Values are the numbers of kernels out of 100 kernels; those in parentheses are the numbers of brown rice kernels.

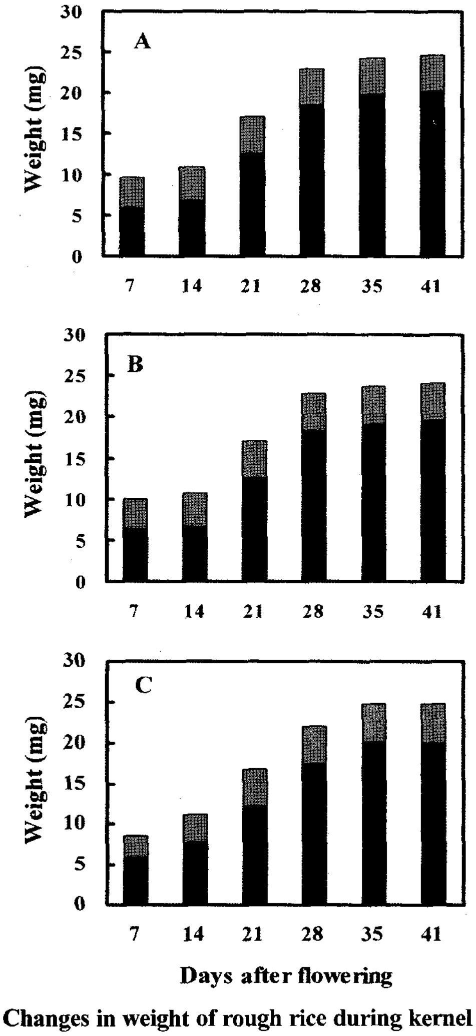

Fig. 1. Changes in weight of rough rice during kernel development for non-waxy (A: Dongjinbyeo; B: Ilpumbyeo) and waxy (C: Shinsunchanlbyeo) rice. ■ : Brown rice kernel; ▒ : Hull.

weight of Dongjinbyeo increased from 9.5 to 23.1 mg during 7 to 28 DAF and reached 24.7 mg at 42 DAF. In the case of Ilpumbyeo, its weight of 9.9 mg at 7 DAF increased to 22.9 mg at 28 DAF and to 24.2 mg at 42 DAF. The weight of the waxy rice was 5.9 mg at 7 DAF, which was continuously increased to reach the maximum weight at 35 DAF. The hull weight at 7 and 42 DAF for a kernel of the non-waxy rice was 3.7 and 4.5 mg, and that of the waxy rice was 2.6 and 4.7 mg, respectively, indicating the weight of the rough rice is mainly governed by the weight of the brown rice. These results are in good agreement with the report of Horigane *et al.* (3), who reported that the changes in *japonica* rough rice grain weight during development increased up to 30 days after anthesis but decreased thereafter. The optimum harvest time of the rough rice in Korea, based on yield and milling property, is recommended to be 45-50 days after heading for medium maturity cultivars and 50 days after heading for medium-late maturity cultivars (12). Chae and Jun (12) reported that the gloss of cooked rice was best for rice harvested at 40 days after heading, which was not affected by the maturity of rice cultivars.

It is clear from Fig. 1 that as the kernels developed the weight proportion of the brown rice increased while and that of the hull decreased. At 7 DAF, the proportions of the non-waxy brown rice and hull were 61.6 and 38.4% for Dongjinbyeo, 62.7 and 37.3% for Ilpumbyeo, and 69.1 and 30.9% for the waxy rice, respectively.

The proportion of hull continuously decreased as the kernel developed. At 42 DAF, the proportions of the non-waxy brown rice and hull were 81.8 and 18.2% for Dongjinbyeo, 81.5 and 18.5% for Ilpumbyeo, and 81.1 and 18.9% for the waxy rice, respectively.

The weight changes of the brown rice followed a similar pattern with the rough rice (Fig. 1). There was a linear relationship between the weights of the rough and brown rice in all cultivars during development. As an example, the relationship at 42 DAF is shown in Fig. 2. These results indicated that the dry weight of the brown rice can be estimated from that of the dry rough rice. In a breeding program in which a small quantity of sample is handled, it is possible to predict the weight of the brown rice by measuring the weight of the rough rice, thus saving labor and time.

At 28 and 42 DAF, the weight of the non-waxy Dongjinbyeo brown rice increased by 3.15- and 3.42-fold, and that of the non-waxy Ilpumbyeo by 2.97- and 3.18-fold, respectively, compared with the weight at 7 DAF. At 28 and 35 DAF, the weight of the waxy rice increased by 2.97- and 3.39-fold, respectively, compared with that at 7 DAF. Nanda *et al.* (9) reported that the dry weight of

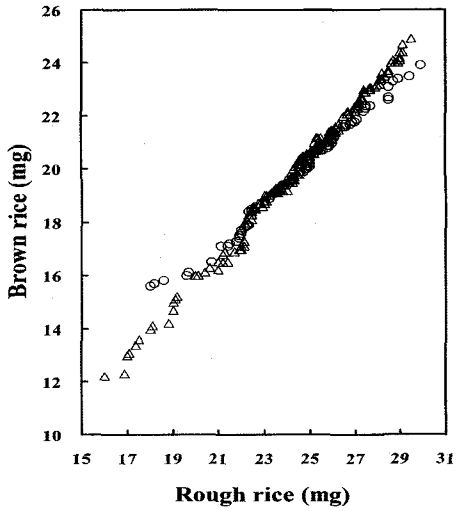

Fig. 2. Relationship between rough and brown rice weight for non-waxy (△ , R^2=0.993) and waxy (○ , R^2=0.987) rice.

dehulled low protein grains in *indica* rice variety increased progressively till maturity (30 days after anthesis), and that the dry weight of the developing kernel increased rapidly by 4.50-fold up to 15 days after anthesis. Thereafter, however, the accumulation of dry matter was slow, reaching a 4.77-fold increase in weight at maturity. These results may explain the genetic differences in *japonica* and *indica* rice: the former has a long kernel development time, and accumulates the dry weight at a slower rate (8).

The rate of increase of the hull weight during kernel development was higher at an early stage of development from 7 to 21 DAF for both the non-waxy and waxy rice cultivars, and was practically negligible thereafter (Table 2). This indicates that the hull biosynthesis is completed earlier than that of caryopsis, and that the increase in weight of the rough rice during kernel development is largely influenced by the weight of the brown rice kernel.

The accumulation of single kernel weight of the non-waxy brown rice indicated that the rate of weight accumulation of Dongjinbyeo was about 2-fold higher than that of Ilpumbyeo between 7 and 14 DAF. However, both cultivars showed the greatest accumulation of weight at a rate of about 0.73 mg/day from 14 to 28 DAF, and then decreased to about 0.1 during 28-35 DAF and further to 0.05 mg/day during 35-42 DAF. The accumulation of weight of the waxy rice at 1-14 DAF was 0.24 mg/day, which was much higher than that of the non-waxy rice, and continued to accumulate the weight until 28 DAF and then decreased by 0.32 mg/day at 28-35 DAF. The total mean rate of single kernel weight increase of the non-waxy brown rice for Dongjinbyeo and Ilpumbyeo during 7-42 DAF was 0.40 and 0.38 mg/day, respectively, and that of the hull was 0.02 mg/day for both cultivars, while those for the waxy rice were 0.39 and 0.06 mg/day, respectively.

Appearance The color of the kernel also significantly changed during development (Table 3). The kernel color of the non-waxy brown rice was green up to 21 DAF, after which it started to change from green to brown. Although there were some differences in kernel color during 28-35 DAF, the kernel color of the two non-waxy cultivars at 42 DAF was essentially the same. Some green kernels and brown with green kernels were found for both cultivars at 42 DAF. Choi *et al.* (17) reported that the rice grains (cultivar, Dongjinbyeo) harvested at full maturity contained 9.8% of incompletely ripened grains, which is in agreement with the data in Table 3.

The color of the waxy rice started to change from green to brown after 14 DAF. The change in color was more rapid during 15-35 DAF compared with the non-waxy rice, reaching 94% conversion to brown at 35 DAF and 100% at 42 DAF (Table 3).

Dimension Changes in the brown rice kernel dimension during kernel development are shown in Fig. 3. The kernel length of the non-waxy Dongjinbyeo was predominantly in the range of 4.60-5.35 mm: 72% at 7 DAF, 81% at 14 DAF, 84% at 21 DAF, 91% at 28 DAF, 92% at 35 DAF, and 100% at 42 DAF. The kernel length of the non-waxy Ilpumbyeo was somewhat more broadly distributed at the same DAF with the majority in the range of 4.20-4.95 mm: 83% at 7 DAF and 92% at 14-42 DAF. Most kernels (99%) of the waxy rice at 7 DAF were in the range of 4.20-4.95 mm in length which was increased to 4.60-5.35mm up to 28 DAF (94%) and remained constant

Table 2. Rate of weight increase in a single kernel of brown rice and hull

DAF[1]	Non-waxy rice				Waxy rice	
	Dongjinbyeo		Ilpumbyeo		Shinsunchalbyeo	
	Brown rice	Hull	Brown rice	Hull	Brown rice	Hull
7-14	0.12±0.14[2]	0.04±0.08	0.06±0.13	0.03±0.07	0.24±0.04	0.10±0.08
14-21	0.72±0.19	0.05±0.08	0.74±0.33	0.07±0.09	0.57±0.37	0.13±0.08
21-28	0.75±0.20	0.00±0.07	0.72±0.18	0.01±0.06	0.66±0.34	0.00±0.05
28-35	0.15±0.11	0.01±0.06	0.10±0.11	0.00±0.05	0.32±0.13	0.03±0.03
35-42	0.05±0.04	0.00±0.03	0.07±0.05	-0.01±0.07	-0.02±0.04	-0.01±0.04
Mean	0.40±0.01	0.02±0.01	0.38±0.04	0.02±0.02	0.39±0.03	0.06±0.03

[1] Days after flowering.

[2] Unit is mg/day. Data are the means of single kernel weight±standard deviation.

Table 3. Changes in kernel color of brown rice during kernel development

DAF1)	Non-waxy rice		Waxy rice
	Dongjinbyeo	Ilpumbyeo	Shinsunchalbyeo
7	g(100)2)	g(100)	g(100)
14	g(100)	g(100)	g(100)
21	g(100)	g(98)g/b(2)	g(78)g/b(12)
28	g(49)g/b(33)b/g(18)	g(46)g/b(22)b(32)	g(62)g/b(24)b(14)
35	g/b(9)b/g(10)b(81)	g(22)g/b(13)b(65)	g/b(6)b(94)
42	g(1)b/g(7)b(92)	g(2)b/g(8)b(90)	b(100)

1)Days after flowering.
2)g: green; g/b: green with brown; b/g: brown with some green; b: brown. The numbers in parentheses are the numbers of kernels out of 100 kernels.

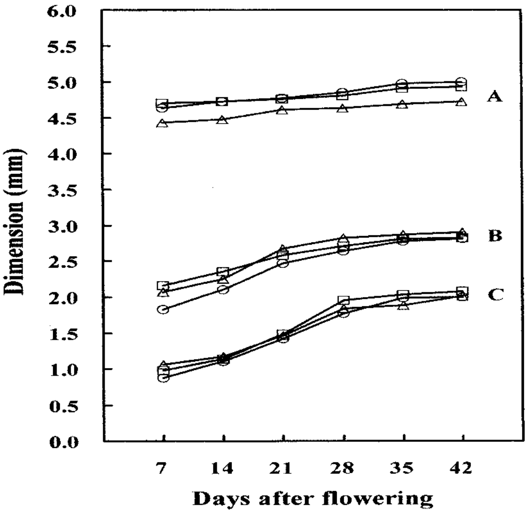

Fig. 3. Changes in brown rice kernel length(A), breadth(B) and thickness(C) during kernel development for non-waxy (□ : Dongjinbyeo; △ : Ilpumbyeo) and waxy (○ : Shinsunchalbyeo) rice.

thereafter.

The kernel breadth of the majority of the non-waxy cultivars was in the range of 1.80-2.55 mm at 7 DAF and increased to 2.60-3.35 mm as the kernels matured. Most kernels of the waxy rice had the breadth in the range of 1.40-2.55 mm at 7-14 DAF, which was increased to 2.60-3.35 mm during kernel development. The non-waxy Ilpumbyeo had the highest proportion of kernels having breadth of 3.00- 3.35 mm, followed by the waxy rice and then the non-waxy Dongjinbyeo. At 42 DAF the proportion of the kernels having breadth of 2.60-2.95 mm was 87% for Dongjinbyeo, 65% for Ilpumbyeo, and 73% for the waxy rice.

All rice cultivars became thicker throughout the entire development period, reaching a maximum thickness of 1.80-2.15 mm at 42 DAF.

The dimension change patterns of the non-waxy brown rice between Dongjinbyeo and Ilpumbyeo were similar, although the length was longer in the former and breadth was slightly greater in the latter. The length of the waxy rice changed similar to that of Dongjinbyeo; however, the breadth and thickness were lower than those of the non-waxy rice.

The length increased from 7 to 42 DAF by 1.05-fold for the non-waxy Dongjinbyeo, 1.07-fold for the non-waxy Ilpumbyeo, and 1.08-fold for the waxy rice. The ratio of breadth at 42 DAF compared with that at 7 DAF was higher by 1.31-, 1.40-, and 1.54-fold for Dongjinbyeo, Ilpumbyeo, and Shinsunchalbyeo, respectively. The thickness of rice kernel at 42 DAF (Ed- it is acceptable to carry over the respective comparison from the previous sentence) was respectively 2.12-, 1.91-, and 2.27-fold greater than that at 7 DAF. Nagata and Chaudhry (8) reported that the maximum length, breadth, and thickness of *japonica* rice caryopsis during kernel development were reached in descending order. Using nuclear magnetic resonance microimaging technique, Horigane *et al.* (3) observed the developing Japanese rice spikelets (cultivar, *japonica* Koshihikari) and reported that the fertilized caryopsis began to elongate rapidly after anthesis and reached the top of the hull at a maximum length of 5-7 days after anthesis. In the next stage, the girth began to increase, and the caryopsis reached its maximum breadth 15 days after anthesis and its maximum thickness (lateral side) 20-25 days after anthesis. These results support the previous work for Japanese rice (8). They are, however, somewhat different from the results of the present study in which length, breadth, and thickness increased throughout the development stage. It remains to be elucidated whether these differences reflect the varietal differences between Korean and Japanese rice.

The dimension change data clearly show the differences in kernel shape between the non-waxy rice cultivars, as well as between the non-waxy and waxy rice. Tan *et al.* (18) reported that the appearance of rice was mainly determined by the grain shape specified by grain length, grain breadth, and the translucency of the endosperm. Genetic analyses of grain length and breadth of rice grains demonstrated that grain shape was quantitatively inherited (19). Shi *et al.* (20) found that the shape of *indica* rice grain was simultaneously controlled by triploid endosperm genes, cytoplasmic genes, embryo and maternal plant genes, and their genotype × environment interaction effects. It was shown (10) that the *indica* brown rice thickness was genetically controlled by the net genetic effects of genes expressed at the early (1-7 DAF) and late (15-21 DAF) filling stages, and that net genotype × environment interaction effects were more important to the brown rice thickness at the early filling and mature stages of rice.

The relationship between weight and thickness of the brown rice kernels was linear for both the non-waxy and waxy rice (Fig. 4). For the brown rice kernels lighter than 10 mg, changes in thickness by 0.1 mm corresponded to an increase in weight of 0.88 mg for the non-waxy rice, and 0.95 mg for the waxy rice. However, for the brown rice kernels heavier than 10 mg, the changes in thickness by 0.1 mm corresponded to an increase in weight of 1.47 mg for the non-waxy Dongjinbyeo, 1.69 mg for the non-waxy Ilpumbyeo, and 1.51 mg for the waxy rice. These results may explain the differences in varietal characteristics between the non-waxy rice, and the non-waxy and waxy rice.

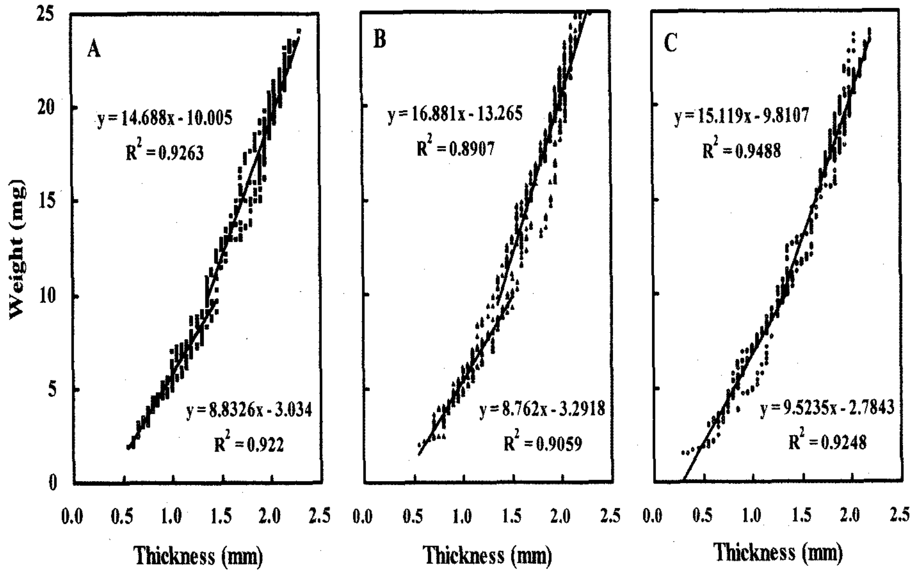

Fig. 4. Relationship between weight and thickness of non-waxy (A: Dongjinbyeo; B: Ilpumbyeo) and waxy (C:Shinsunchalbyeo) brown rice.

The ratios of length-to-breadth (L/B), length-to-thickness (L/T), and breadth-to-thickness (B/T) rapidly decreased during kernel development up to 28 DAF but then remained fairly constant after 35 DAF for both the non-waxy and waxy rice (Fig. 5). In Europe, the values of length and the L/B ratio are used for commercial classifi-cation of rice for grain dimensions (21). According to the standard of the European Union, rice grains with a length of less than 5.5 mm and an L/B ratio of less than 2 are classified as round grain *japonica* (22). Based on this, the data in Fig. 3 and 4 indicate that the non-waxy and waxy rice employed in this study belong to round grain.

Koutroubas *et al.* (21) found that grain length, grain breadth, and grain L/B ratio of the brown rice were strongly correlated with the corresponding traits of the rough rice. These associations indicate that grain size and shape for the brown rice could be predicted by measurements of the rough rice to reduce the time and effort that is required for removing the hull in a breeding program. Evers and Millar (1) stated that the L/B ratio is a useful guide to the nature of the endosperm and more significantly the starch type present, and that the shorter grains tend to be associated with stickier cooking quality.

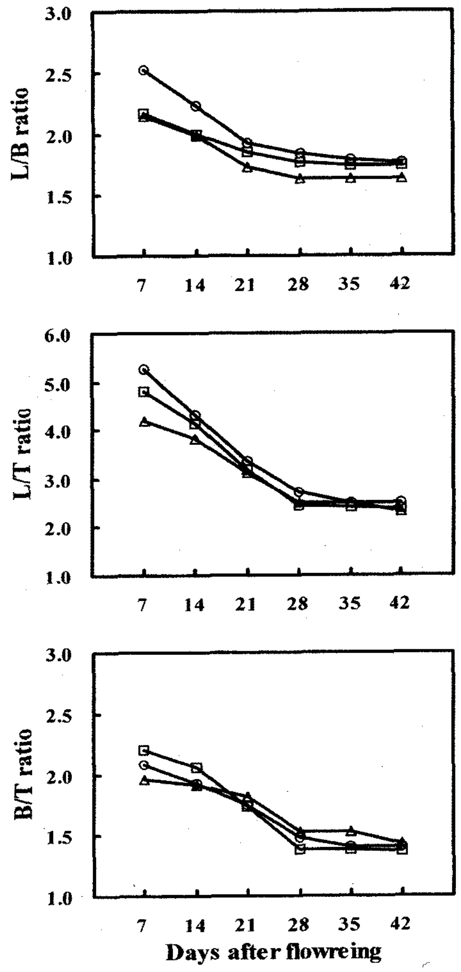

Fig. 5. Changes of the ratio of length-to-breadth (L/B), length-to-thickness (L/T), and breadth-to-thickness (B/T) in brown rice kernels during kernel development for non-waxy (□: Dongjinbyeo; △ : Ilpumbyeo) and waxy (○ : Shinsunchalbyeo) rice.

Protein and amylose contents Protein and amylose contents of the brown rice are presented in Table 4. Protein content of the non-waxy Dongjinbyeo was higher at 7 DAF, and slightly decreased as the kernel developed, whereas that of Ilpumbyeo remained fairly constant. The waxy rice showed higher protein content up to 14 DAF and somewhat lower protein content thereafter. Nanda *et al.* (9) reported that Indian brown rice accumulated more protein during the early period of development, and sharply decreased as the kernel developed. Their results showed that the protein content of one rice variety (cultivar, Ranta) was 172.87 mg/g of kernel at 5 days after anthesis, but that this dropped to 71.93 mg/g at 10 days after anthesis and then to 64.65 mg/g at 30 days after

Table 4. Changes in protein and amylose contents of brown rice during kernel development

DAF$^{1)}$	Non-waxy rice				Waxy rice	
	Dongjinbyeo		Ilpumbyeo		Shinsunchalbyeo	
	Protein (%)	Amylose (%)	Protein (%)	Amylose (%)	Protein (%)	Amylose (%)
7	$8.8^{a2)}$	14.9^{e}	9.1^{a}	17.2^{c}	10.1^{a}	6.7^{a}
14	$8.6^{a,b}$	16.2^{d}	8.8^{c}	18.0^{b}	10.3^{a}	5.9^{b}
21	$8.4^{b,c}$	18.3^{c}	8.7^{d}	18.3^{b}	9.0^{b}	5.5^{c}
28	$8.4^{b,c}$	19.4^{b}	9.0^{b}	$18.5^{a,b}$	8.9^{b}	5.5^{c}
35	8.3^{c}	20.1^{a}	9.0^{b}	19.2^{a}	9.0^{b}	5.4^{c}
42	8.2^{c}	20.4^{a}	9.1^{a}	19.2^{a}	9.2^{b}	5.4^{c}

$^{1)}$Days after flowering.
$^{2)}$Mean value of two determinations. The same letters in the same column are not significantly different at $p<0.05$.

anthesis (maturity). The low accumulation of protein in the latter part of the grain development was attributed to rapid carbohydrate synthesis (9). Chae and Jun (12) reported that the protein content of Korean *japonica* milled rice increased significantly as the ripening period was increased from 40 to 70 days after heading. The timing of the onset of protein accumulation in rice during kernel development is not clear at present. It was demonstrated (23, 24) that the major gluten proteins accumulated slightly earlier than starch in wheat.

Amylose content of the non-waxy brown rice increased during grain development (Table 4). The amylose content of Dongjinbyeo increased from 14.9% at 7 DAF to 20.4% at 42 DAF, while that of Ilpumbyeo increased from 17.2% at 7 DAF to 19.2% at 42 DAF. The amylose content of the waxy rice decreased. It was reported that the amylose content of the non-waxy *japonica* and *indica* rice increased (25 - 27) while that of the waxy *japonica* and *indica* rice decreased (26, 27) during development from DAF to maturity. It was shown (12) that the amylose content of milled rice remained constant during the development period of 40-70 days after heading.

RVA viscosity RVA viscosity results of the brown rice flour are summarized in Table 5. Due to the limited number of the samples, data of 7-21 DAF are not given. As kernel developed, the peak viscosity of the non-waxy rice increased up to 35 DAF, and then decreased. In contrast, the peak viscosity of the waxy rice increased consistently throughout the development. The minimum and final viscosity showed a similar trend to the peak viscosity. The breakdown was the highest for the non-waxy Dongjinbyeo, followed by the non-waxy Ilpumbyeo and the waxy rice. DC decreased between 21-28 DAF and remained unchanged thereafter in all cultivars. DC was the highest for the waxy rice. For the non-waxy rice, Ilpumbyeo showed a DC value lower than that of Dongjinbyeo.

Takami *et al.* (16) reported highly negative relationships between the hardness of cooked rice measured by the Tensipresser and the DC and breakdown as measured by RVA. Sasaki *et al.* (28) reported a significant positive correlation between amylose content and final viscosity and setback of wheat starch. However, there were no such relationships in this study as reported by Sasaki *et al.*(28).

DSC DSC data of the rice starch are presented in Table 6. The gelatinization temperature, the difference between Tc and To, increased in the non-waxy Dongjinbyeo and the waxy rice during kernel development up to 21 DAF, but that of the non-waxy Ilpumbyeo decreased during kernel development. Gelatinization enthalpy (ΔH), however, increased in all rice cultivars during kernel

Table 5. RVA data of brown rice flour during kernel development

Rice	DAF$^{1)}$	Peak viscosity (RVU)	Trough (RVU)	Final viscosity (RVU)	Setback$^{2)}$ (RVU)	Breakdown$^{3)}$ (RVU)	Degree of collapse$^{4)}$ (%)
Dongjin-byeo	21	$115.3^{c5)}$	58.3^{c}	137.7^{d}	79.4^{c}	57.0^{a}	49.5^{a}
	28	140.6^{a}	87.6^{a}	185.9^{b}	98.3^{b}	52.9^{b}	37.7^{c}
	35	141.3^{a}	87.7^{a}	188.6^{a}	100.9^{a}	53.5^{b}	$37.9^{b,c}$
	42	133.6^{b}	82.2^{b}	183.3^{c}	101.1^{a}	51.4^{c}	38.4^{c}
Ilpum-byeo	21	92.3^{d}	62.1^{d}	156.9^{c}	94.8^{b}	30.2^{c}	32.7^{a}
	28	132.5^{c}	98.9^{c}	198.6^{a}	99.7^{a}	33.6^{b}	25.4^{b}
	35	142.4^{a}	108.1^{a}	199.3^{a}	91.2^{c}	$34.3^{a,b}$	24.1^{c}
	42	138.3^{b}	103.5^{b}	194.5^{b}	91.0^{c}	34.8^{a}	25.2^{b}
Shinsun-chalbyeo	21	45.3^{d}	22.7^{d}	33.7^{d}	11.0^{c}	22.6^{d}	49.9^{a}
	28	61.6^{c}	34.6^{c}	46.3^{c}	11.6^{b}	27.0^{c}	43.8^{b}
	35	67.2^{b}	37.9^{b}	49.5^{b}	11.6^{b}	29.3^{b}	43.6^{b}
	42	68.9^{a}	39.1^{a}	51.8^{a}	12.7^{a}	29.8^{a}	43.3^{b}

$^{1)}$DAF: days after flowering.
$^{2)}$Difference in final viscosity and trough.
$^{3)}$Difference in peak viscosity and trough.
$^{4)}$(Breakdown / peak viscosity)×100.
$^{5)}$Mean value of two determinations. The same letters in the same column are not significantly different at $p<0.05$.

Table 6. DSC data of rice starch during kernel development

Rice	DAF$^{1)}$	Temperature (°C)$^{2)}$				ΔH$^{3)}$ (J/g)
		To	Tp	Tc	Tc-To	
Dongjin byeo	7	$62.2^{a4)}$	$71.3^{a,b}$	80.1^{b}	17.9^{d}	8.2^{c}
	14	62.2^{a}	$71.2^{b,c}$	80.8^{a}	$18.6^{a,b}$	$9.5^{a,b}$
	21	62.1^{a}	71.4^{a}	80.8^{a}	18.7^{a}	9.7^{a}
	28	62.0^{a}	71.1^{c}	80.2^{b}	$18.2^{c,d}$	$9.4^{a,b}$
	35	61.5^{c}	71.0^{c}	79.8^{c}	$18.3^{b,c}$	9.2^{b}
	42	61.8^{b}	71.0^{c}	$79.9^{b,c}$	$18.1^{c,d}$	9.6^{a}
Ilpum byeo	7	55.7^{d}	66.9^{c}	78.7^{d}	23.0^{a}	5.1^{c}
	14	61.6^{b}	70.7^{b}	$80.7^{a,b}$	19.1^{b}	8.4^{b}
	21	61.0^{c}	70.1^{b}	$80.3^{b,c}$	19.3^{b}	9.0^{a}
	28	61.4^{b}	71.5^{a}	80.9^{a}	19.5^{b}	9.0^{a}
	35	61.9^{a}	71.4^{a}	$80.4^{a,b,c}$	18.5^{c}	8.8^{a}
	42	61.5^{b}	71.4^{a}	80.1^{c}	18.6^{c}	8.9^{a}
Shinsun chalbyeo	7	60.2^{d}	70.8^{e}	80.3^{d}	20.1^{b}	7.3^{e}
	14	62.6^{a}	71.3^{c}	$82.1^{b,c}$	19.5^{c}	9.1^{c}
	21	61.8^{c}	71.7^{b}	82.4^{a}	20.6^{a}	11.4^{a}
	28	62.2^{b}	$71.5^{b,c}$	$82.4^{a,b}$	20.2^{b}	10.8^{b}
	35	61.6^{c}	71.0^{d}	81.9^{c}	20.2^{b}	$11.2^{a,b}$
	42	62.2^{b}	72.1^{a}	$82.3^{a,b}$	20.2^{b}	8.5^{d}

$^{1)}$DAF: days after flowering.
$^{2)}$To: onset temperature; Tp: peak temperature; Tc: completion temperature; Tc-To: difference in completion temperature and onset temperature.
$^{3)}$ΔH: gelatinization enthalpy.
$^{4)}$Mean value of two determinations. The same letters in the same column are not significantly different at $p<0.05$

development. The gelatinization enthalpy of the non-waxy Dongjinbyeo was higher than that of the non-waxy Ilpumbyeo in all development stages. It should be noted that the gelatinization enthalpy for the waxy rice at 21-35 DAF was significantly higher than that of the non-waxy rice.

The differences in gelatinization temperature and gelatinization enthalpy in starches may be attributed to differences in amylose content and granular structure (29). Because amylopectin plays a major role in starch granule crystallinity, the presence of amylose lowers the melting point of crystalline regions and the energy for starting gelatinization (30). More energy is needed to initiate the melting in the absence of amylose-rich amorphous regions (31). Sodhi *et al.* (29) reported that Indian rice starches with lower amylose contents had higher transition temperatures and enthalpies of gelatinization than the starches with higher amylose contents. Endothermic enthalpy and final gelatinization temperature correlated negatively with amylose content of wheat starch (28). However, Sodhi *et al.* (29) found that To and Tp values of Indian rice starch were lower that those reported for Taiwan rice starch by Li and Yeh (32) at a similar amylose content, and postulated that these differences may be due to differences in granular structure of rice.

The differences in transitional temperatures in the rice starches may also have arisen due to differences in the length of the amylopectin (33). Starch gelatinization temperature increases with increasing branch chain-length of amylopectin (33). The average chain-length of amylopectin of the *japonica* non-waxy rice starch tends to increase, whereas that of the *japonica* waxy rice starch decreases as rice ripens (27).

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References

1. Evers T, Millar S. Cereal grain structure and development: Some implications for quality. J. Cereal Sci. 36: 261-284 (2002)
2. Hwang JW. Relationship between expression of starch biosynthetic genes and physicochemical properties in different rice cultivars. MS thesis, Kyungpook National University, Daegu, Korea (2003)
3. Horigane AK, Engelaar WMHG, Maruyama S, Yoshida M, Okubo A, Nagata T. Visualisation of moisture distribution during development of rice caryopses (*Oryza sativa* L.) by nuclear magnetic resonance microimaging. J. Cereal Sci. 33: 105-114 (2001)
4. Horigane AK, Toyoshima H, Hemmi H, Engelaar WMHG, Okubo A, Nagata T. Internal hollows in cooked rice grains (*Oryza sativa* cv. Koshihikari) observed by NMR Micro imaging. J. Food Sci. 64: 1-5 (1999)
5. Horigane AK, Engelaar WMHG, Toyoshima H, Ono H, Sakai M, Okubo A, Nagata T. Difference in hollow volumes in cooked rice grains with various amylose contents as determined by NMR Micro imaging. J. Food Sci, 65: 408-412 (2000)
6. Champagne ET, Wood DF, Juliano BO, Bechtel DB. The rice grain and gross composition. pp. 77-107. In: Rice Chemistry and Technology. Champagne ET(ed). American Association of Cereal Chemists, St. Paul, MN, USA (2004)
7. Kim SK, Chang BS, Lee SJ. Ultrastructure of compound starch granules and protein bodies of starchy endosperm cell in rice. Agric. Chem. Biotechnol. 39: 379-383 (1996)
8. Nagata K, Chaudhry FM. A comparative study of ripening process and kernel development in japonica and indica rice. Proc. Crop Sci. Soc. Japan. 38: 425-433 (1969)
9. Nanda BB, Mali PC, Lodh SB. Glutamate dehydrogenase activity and isoenzymes, protein, and soluble amino acids in developing grains of high and low protein rice. Cereal Chem. 68: 351-353 (1991)
10. Shi CH, Wu JG, Wu P. Developmental behavior of gene expression for brown rice thickness under different environments. Genesis 33: 185-190 (2002)
11. Yoon SH, Kim SK. Physicochemical properties of rice differing in milling degrees. Food Sci. Biotechnol. 13: 57-62 (2004)
12. Chae GC, Jun DK. Effect of harvest time on yield and quality of rice. Korean J. Crop Sci. 47: 254-258 (2002)
13. AACC. Approved Method of the AACC. 10th ed. Method 46-12. The American Association of Cereal Chemists International, St. Paul, MN, USA (2000)
14. Juliano BO. IRRI Annual Report for 1987. International Rice Research Institute, Los Baños, Philippines. p. 43 (1988)
15. ICC. Rapid pasting method using the Rapid Visco Analyses. International Association for Cereal Science and Technology, Vienna, Austria (1994)
16. Takami K, Nakaru Y, Horibata T, Koriyama T, Inouchi N. Relationship between physical properties of cooked rice and properties of endosperm starches of new types of rice grains. J. Appl. Glycosci. 51: 355-362 (2004)
17. Choi HC, Chi JH, Lee CS, Kim YB, Cho SY. Varietal and locational variation of grain quality components of rice produced in middle and southern plain areas in Korea. Korean J. Crop Sci. 39: 15-26 (1994)
18. Tan YF, Xing YZ, Li JX, Yu SB, Xu CG. Zhang Q. Genetic bases of

appearance: quality of rice grains in Shanyou 63, an elite rice hybrid. Theo. Appl. Genet. 101: 823-829 (2000)

19. Chen JG, Song GQ, Qu SH, Zhou Y. Direct and maternal genetic effects on grain quality characters in early hybrid rice. Chinese. J. Rice Sci. 12: 79-84 (1998)
20. Shi CH, Zhu G, Wu G, Fan L. Genetic and genotype × environment interaction effects from embryo, endosperm, cytoplasm and maternal plant for rice grain shape traits of Indica rice. Field Crop Res. 68: 191-198 (2000)
21. Koutroubas SD, Mazzini F, Pons B, Ntanos DA. Grain quality variation and relationships with morpho-physiological traits in rice (*Oryza sativa* L.) genetic resources in Europe. Field Crops Res. 86: 115-130 (2004)
22. Council Regulation (EC) No. 3877/1987. Off. J. Eur. Communo. 365/1-2 (1987)
23. Altenbach SB, Kothari KM, Lieu D. Environmental conditions during wheat grain development alter temporal regulation of the major gluten protein genes. Cereal Chem. 79: 279-285 (2002)
24. Altenbach SB, DuPont FM, Kothari KM, Chan R, Johnson EL, Lieu D. Temperature, water and fertilizer influence the timing of key events during grain development in a US spring wheat. J. Cereal Sci. 37: 9-20 (2003)
25. Briones VP, Magbauna LG, Juliano BO. Changes in physicochemical properties of starch of developing rice grain. Cereal Chem. 45: 351-357 (1968)
26. Blakeney AB. Studies on rice starch. The accumulation and properties of starches in the stem, pollen and grain of some rice varieties grown in southern New South Wales. MS thesis, University of Sydney, Australia (1980)
27. Juliano BO. Physicochemical data on rice grain. Technical Bulletin 6. The International Rice Research Institute, Los Baños, Philippines (1966)
28. Sasaki T, Yasui T, Matsuki J. Effect of amylose content on gelatinization, retrogradation, and pasting properties of starches from waxy and nonwaxy wheat and their F1 seeds. Cereal Chem. 77: 58-63 (2000)
29. Sodhi NS, Singh N. Morphological, thermal and rheological properties of starches separated from rice cultivars grown in India. Food Chem. 80: 99-108 (2003)
30. Flipse E, Keetels CJAM, Jacobson E, Visser RGF. The dosage effect of the wildtype GBSS allele is linear for GBSS activity, but not for amylose content: absence of amylose has a distinct influence on the physico-chemical properties of starch. Theor. Appl. Genet. 92: 121-127 (1996)
31. Krueger BR, Knutson CA, Inglett GE, Walker CE. A differential scanning calorimetry study on the effect of annealing on gelatinization behavior of corn starch. J. Food Sci. 52: 715-718 (1987)
32. Li JY, Yeh AI. Relationships between thermal, rheological characteristics and swelling power of various starches. J. Food Eng. 50: 141-148 (2001)
33. Jane J, Chen YY, Lee LF, McPherson A, Wong KS. Radosavljevic M, Kasemsuwan T. Effect of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. Cereal Chem. 76: 827-832 (1999)