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Physicochemical Characteristics of Starches in Rice Cultivars of Diverse Amylose Contents

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ABSTRACT Through the sampling four rice cultivars with differing amylose contents, the relationship between the structural and gelatinization properties of endosperm starches was analyzed. These rice varieties exhibited different chain length distribution ratio within the amylopectin cluster as well as varing amylose levels. The proportion of amylopectin short chains of in Goami cutlivars was higher than the other varieties, whereas the Goami 2 which shows amylose extender mutant properties in the endosperm showed the highest proportion of long chains. In X-ray diffraction analysis of rice starches, the Goami 2 variety displayed a B-type pattern whereas the other varieties were all A-type. Among the cultivars with high and normal rice starch levels, those with the higher amylose contents showed distinctly lower swelling. Goami 2 rice was found to have the highest onset and peak gelatinization temperature from the differential scanning calorimetry results. The four rice cultivars under analysis also showed different rates of hydrolysis by amyloglucosidase. These findings suggest that the composition and chemical structure of the starch content is a major determinant of both the gelatinization and functional properties of rice.

Keywords : rice cultivars, endosperm starch, structural properties, gelatinization

Rice (*Oryza sativa* L.) is one of the most important commercial crops in Korea and other Asian countries. However, the consumption of rice in Korea is decreasing every year due to the improved economic status of many people leading to changes in their eating habits. For rice producers, it is therefore imperative to improve the rice varieties on offer and thereby attract more rice consumption. Starch is the major component of rice grains, which are widely recognized as an important source of this carbohydrate in the food industry. Numerous research activities are currently in progress to develop new food materials through structural analyses of carbohydrates and identification of new glycosyl transferases (Vikso-Nielsen and Blennow, 1998; Fang *et al*., 2002). To date, many studies on starch have reported on its the iodine-binding capacity, viscometry, optical rotation, surface tension, nuclear magnetic resonance spectroscopic characteristics, and other properties using enzyme hydrolysis and differential scanning calorimetry. Starch complexes upon the addition of monoglyceride and cyclodextrin have also been analyzed (Blennow *et al*., 2000; Vandeputte and Delcour, 2004; Singh *et al*., 2006; Yu and Wang, 2007; Chung *et al*., 2008). In addition, diverse studies are currently underway to determine the bioactive efficiency of non-starch functional materials generated using giant embryonic rice or colored rice and thereby develop healthy foods using such material (Kang *et al*., 2011).

Starch is composed of two α-polyglucans, amylose and amylopectin, the ratios of which differ between rice cultivars (Kang *et al*., 1995). Of note in particular, the ratio of amylose and amylopectin has been revealed to be an important determinant of the cooking and eating quality of milled rice as well as the outcomes of processing (Choi, 2002; Nakamura *et al*., 2002; Noda *et al*., 2003; Qi *et al*., 2003). To develop high-value functional starchy rice, knowledge of the physical and chemical characteristics and

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fine structures of the rice starch is required, as is a biochemical index to enable the resulting data to be effectively used.

Hence, in our current study we focused on elucidating the structures and physicochemical characteristics of starch contents of four rice cultivars with differing amylose levels with the aim of obtaining useful basic data that will assist in the selection of starch materials and thereby the selection of new functional rice varieties.

MATERIALS AND METHODS

Materials and starch samples preparation

Four rice varieties with normal (Ilpum and Seolgaeng) and high (Goami and Goami 2) amylose contents were selected for analysis in this study. All cultivars were grown and harvested (2010 season) under the same environmental conditions at the National Institute of Crop Science (NICS), RDA, Korea. Rice starches were isolated using the alkali steeping method of Yamamoto *et al*. (1973). Briefly, rice was steeped in 3~4 volumes of 50 mM lithium hydroxide solution at 4℃ for 12 h. Isoamyl alcohol, acetone and ethyl alcohol were added to remove endosperm proteins and lipids and this was followed by washing with distilled water. The precipitates were then collected and dried at room temperature. The starch samples were stored in a desiccator prior to analysis.

Determination of amylose contents

Twelve milligrams of starch was gelatinized by treatment with sodium hydroxide solution (2N) for 12 h at room temperature. After the addition of 1/9 N acetic acid, an aliquot of the solution was added to 0.2 ml of 1% (w/v) I_2, 10% (w/v) KI. Measurements at A_{680} (the blue value) and the λ_{max} were determined from 500 to 700 nm using UV/visible spectrometer (DU 800 series; Beckman Coulter Inc., Fullerton, CA). The amylose content of the rice starches was determined using the calibration line, which was obtained from the blue value at 680 nm by changing the ratio of pure amylose (Sigma, St. Louis, MO).

Analysis of the amylopectin chain length distribution

The chain length distribution of the amylopectin was characterized by high-performance anion-exchange chromatography with a pulsed amperometric detector (HPAEC-PAD) according to the method of Hanashiro *et al*. (1996) with slight modifications. Briefly, enzymatically debranched starches were prepared, and 5 mg amounts were suspended in 5 ml 100% methanol in a screw cap tube. The samples were then heated at 100℃ in a water bath. To 1 ml of gelatinized sample, 10 ul of 2% (w/v) sodium azide and 50 ul of 600 mM sodium acetate buffer were added. Isoamylase solution from *Pseudomonas amylodermosa* was used to debranch the sample over 24 h at 37℃. The debranched samples were then centrifuged and filtered through a Millipore 0.2 µm FH membrane. The HPAEC-PAD (Bio LC, Dionex Co., Sunnyvale, CA) system was then used to analyze the samples and was equipped with a GP50 gradient pump, ED40 electrochemical detector, and Carbopac PA1 Column (250 × 2 mm). Sugars with DP 7 were used to identify the chromatographic peaks.

Enzymatic hydrolysis of starches

Enzymatic hydrolysis was performed using 100 mg of each starch sample which had been accurately weighed into 15 ml screw capped tubes and to which 1 ml of a 5% enzyme solution (amyloglucosidase, Fluka, Switzerland) was added. The sealed tubes were then incubated at 37℃ for 1 h, after which aliquots were transferred to clean tubes, mixed with distilled water and boiled for 10 min. The total sugar contents were used to determine the carbohydrate levels using the phenol-sulfuric acid method and measurement of the absorbance at 490 nm. The glucose content was assayed using the glucose-oxidase peroxidase method and absorbance measurements at 525 nm (*Dubois et al*., 1956; Loyd and Whelan, 1969).

X-ray diffraction analysis

Different starch preparations were analyzed with an X-ray diffractometer (X′pert APD, Philips, Eindhoven, Netherland). Briefly, starch powders were packed into aluminum cells and were exposed to X-ray beams with the generator running at 40 kV and 30 mA. The total diffraction intensity was then measured in each case over the angular range 5° to 40°. The overall degree of crystallinity was quantified as the ratio of the area of crystalline reflections to the overall diffraction.

Thermal properties and swelling-solubility

The thermal behaviors of the different starch samples were determined using a differential scanning calorimetry (DSC) (Model Q200 calorimeter, TA Instruments, Inc., New Castle, DE). The starch samples were weighed directly into DSC pans and distilled water was added to obtain a flour-to-water ratio of 1:2 (w:w). The pan was then hermetically sealed and allowed to stand for 1 hr prior to thermal analysis. Thermal scanning was undertaken from 30 to 120℃ at a heating rate of 10℃/min. The gelatinization onset (T_o), peak (T_p) and conclusion (T_c) temperatures, and the transition enthalpies (△H) were determined from the peak area of the DSC endotherm.

The swelling power and water solubility indices were determined using the method of Li and Yeh (2001) with some modifications. Briefly, the starch samples were placed in a 15 ml tune to which, distilled water was added slowly, and incubated at 55~95℃ for 1 h. The samples were then quickly cooled in an ice water bath and centrifuged at 8000 g for 20 min. The supernatant of the centrifuged portion was carefully separated from the residue.

Statistical analysis

All experiments were performed in triplicate (n=3) and the data were statistically analyzed using the SPSS 15.0 program (SPSS Inc., Chicago, IL). Statistical significance was determined by one-way analysis of variance using Duncan's multiple range test ($P < 0.05$).

RESULTS AND DISCUSSION

Amylose content and amylopectin chain length distribution in different rice varieties

It has been demonstrated that the amylose-to-amylopectin ratio in rice can be used as a major index of palatability, cooking and processing (Hizukuri *et al*., 1983). In our present study, the starch granules was isolated from four different rice cultivars, and the amylose content was then determined through absorbance measurements at 680 nm (blue value) that reflect the levels of starch-I_2 complex formation (Table 1). Significant differences in the amylose content and maximum absorption wavelength (λ_{max}) were observed among the four rice cultivars. The differences in the wavelength for maximum absorbance are a measure of amylose chain length, and high absorbance value at the maximum absorption wavelength indicates the duplication of amylose molecules with similar chain lengths. The Goami 2 cultivar showed the highest absorbance values at the maximum absorption wavelength as well as the most abundant amylose content.

The amylopectin chain length distribution in the starches of all of the rice cultivars under analysis was measured using high performance anion exchange chromatograph, and was classified into four groups according to the DP (degree of polymerization) level. These groups were very short chains with a DP of 6 to 12 (A chains), medium length chains with a DP of 13 to 24 (B_1 chains), long chains with a DP of 25 to 36 (B_2 chains), and very long chains with a DP greater than 36 (B_3 chains) (Hanashiro *et al*., 1996). The starches from these four rice cultivars were thereby found to exhibit significantly different amylopectin chain length distributions. The proportion of short chains in the Goami cultivar, which has a high amylose content, was higher than in any of the other cultivars, whereas the proportion of long chains with a DP ≥ 37 was the lowest in this variety. The Goami 2 cultivar was developed by mutation breeding involving *N*-methyl-*N*-nitrosourea (MNU) treatment in the high eating quality Ilpum rice, and has

Table 1. Amylose contents and λmax values of iodine absorption of the indicated rice starches.

Variety	Amylose content (%)	Wavelength of maximum absorbance	
		λ_{max} (nm)	Optical density
Ilpum	21.37^b	580^a	0.37^b
Seolgaeng	18.91^a	579^a	0.34^a
Goami	30.61^c	603^c	0.44^c
Goami 2	40.02^d	587^b	0.67^d

Different letters within the column indicate statistically significant differences ($P < 0.05$).

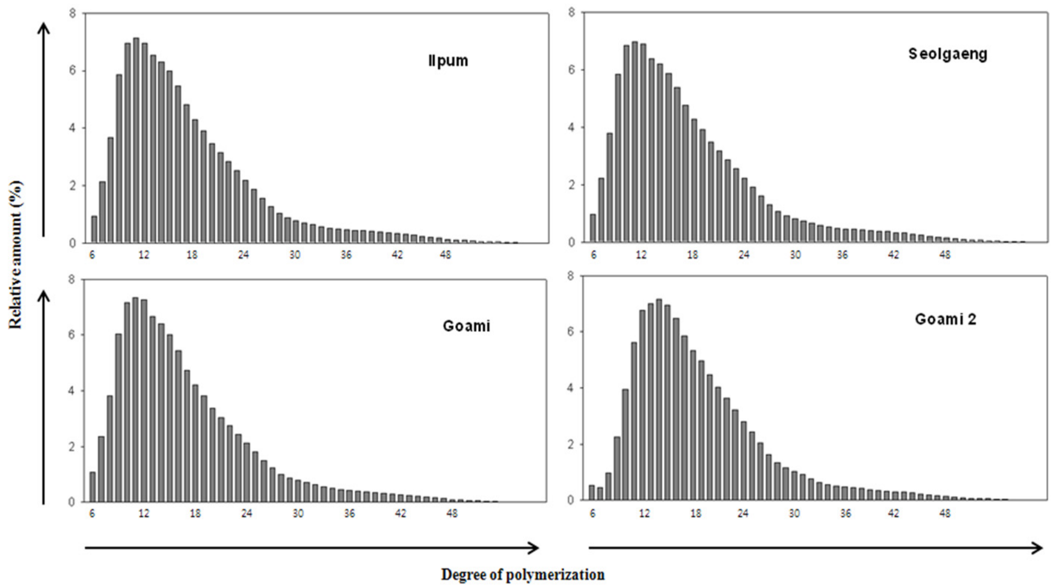

Fig. 1. Amylopectin chain length distributions in different rice starches.

been reported to have distinct physicochemical characteristics (Kim *et al*., 2005). Furthermore, Goami 2 has a mutant amylose extender in its endosperm and was found in our present analysis in terms of the amylopectin profile to have the highest levels of medium length chains with a DP of 13 to 24, long chains with a DP of 25 to 36 and very long chains, but the lowest levels of short chains. This result is similar to the previous findings of Yoshimoto *et al*. (2000) who demonstrated that amylopectin molecules from high amylose starches contain a relatively high proportion of very long chain products. The study of Radhika *et al*. (1993) on rice starch has shown that a higher level of long chain, amylopecins is associated with firmer rice after cooking and that retrogradation is also more advanced in rice with more long chain amylopectins.

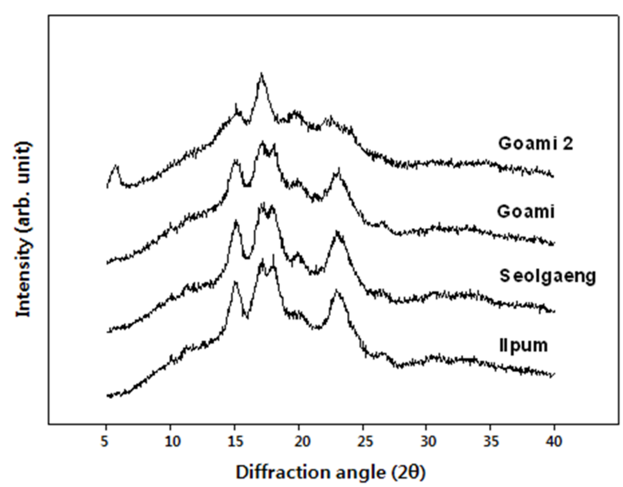

Fig. 2. X-ray diffraction patterns of starches from rice cultivars with diverse amylose contents.

X-ray diffraction crystallinity

The starch fractions from our four rice cultivars were tested using X-ray diffraction, as shown in Fig. 2. These X-ray diffraction patterns were A-type in all of strains except Goami 2, with the main diffraction peaks observed at 15, 17, 18 and 23 degrees 2θ. The Goami 2 variety displayed B-type pattern however, which is characterized by tube and root starches. Compared with the other cultivars, Goami 2 also showed longer amylopectin chains as well as a higher amylose content in its starch granules. This result is similar to that of Hizukuri *et al*. (1983), who reported that the chain length of amylopectin is a major determinant of crystalline polymorphism, and that B-type starches have higher proportions of longer amylopectin chains than their A-type counterparts. In addition, the structure of the A-type starches is denser than that of the B-type, which affects their behavior during processing. The X-ray diffraction, peak heights of the indicate the degree of crystallinity, and in this regard the Ilpumbyeo cultivar displayed better defined peaks than the other rice varieties.

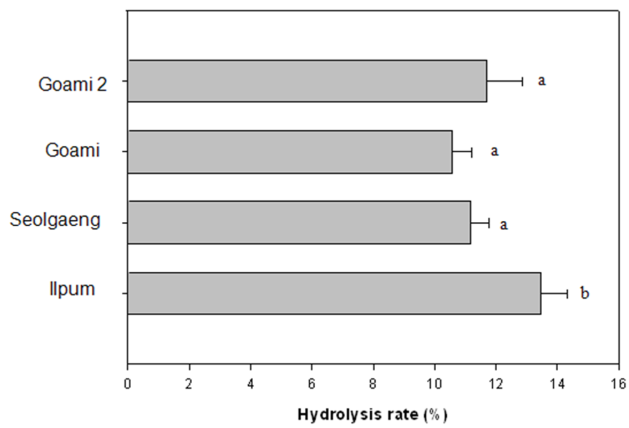

Fig. 3. Hydrolysis rate of different rice starches using glucoamylase.

Extent of starch hydrolysis

Generally, starchy foods have different digestion rates which accord with diverse factors. In our present study, as method of inferring the fine structure of starch, the rate of *in vitro* starch digestion among the four rice cultivars was determined (Fig. 3). The hydrolysis of glucoamylase from starch granules after 60 minutes was found to range from 10.5 to 13.5% among the four cultivars under analysis. The Ilpum cultivar showed the highest hydrolysis activity levels, which reached 13.5%, whereas starches in the other cultivars were digested at a 10.5~11.7% rate within 60 minutes of incubation, the lowest being Goami. Goami rice grains do not swell or gelatinize as readily upon cooking under the same conditions as the other cultivars and, consequently, may be digested more slowly upon consumption. Many studies have reported that the amylose levels are not correlated with the starch digestion rates in different rice varieties (Srinivasa, 1971; Juliano and Goddard, 1986; Panlasigui *et al.*, 1991). However, these analyses also indicate that the amylose content alone may not be the principal predictor of the starch digestion rate, and hence that other physicochemical properties of starch exert an influence on this process.

The gelatinization and swelling of rice starches

Gelatinization is regarded as one of the most important characteristics in the evaluation of rice starch properties (Núñez-Santiago *et al.*, 2004). In particular, it is one of the most important rheological indicators of the cooking quality and processing characteristics of rice. The amylose content and branch chain length distribution have also been found to have a substantial impact on the swelling and pasting properties of rice starches (Tsai *et al.*, 1997; Jane *et al.*, 1999). To examine the gelatinization characteristics of the four rice cultivars under analysis in this study, the swelling and thermal properties of their starches were measured using method of Li and Yeh (2001) and a differential scanning calorimeter, respectively. As shown in Fig. 4, the SP (swelling power) and WSI (water solubility index) of all rice starches increased with the temperature. Varietal differences in the swelling power and water solubility indices of the starches during heating were also observed. Amylose and some amylopectins leach out during swelling which was found to increase dramatically at temperatures greater than 85℃ in the Ilpum and Seolgaeng but not in the high-amylose Goami and Goami 2 cultivars. This appears to be the result of the restricted swelling power of rice, which is affected by the rigidity of the starch granules. Rice starch granules with a high-amylose content act as an inhibitor of swelling (Morrison *et al.*, 1993). Tester and Morrison (1990) have reported in this regard that amylopectins contribute to swelling, whereas amylose and amylose-lipid complexes inhibit swelling. In the Goami cultivars, the swelling power at 85℃ was considerably lower than in the other varieties. In contrast, the water solubility index was the highest in the Goami. It might be inferred from this that the swollen granular structure of the Goami rice is more rigid than those of the other cultivars. Hence, Goami cultivars have higher amylose contents and stronger inner structures than other varieties, which affect gelatinization. Despite a lower level of swelling, solubility is considered to rise as the temperature of the amylose molecules remaining in the rice starch increases.

The thermal properties (DSC results) of the starches separated from the rice cultivars are shown in Table 2. The gelatinization temperature is the temperature at which the starch crystals melt to begin the process of cooking. The initial temperatures ranged from 61.4 to 72.5℃, and from 71.0 to 83.8℃ at the peak, and from 88.6 to 99.5℃ at the conclusion of gelatinization. There were significant differences found in the initial, peak and conclusion gelatinization temperatures. Most notably, those of the Goami cultivars were lower. These data show that the gelatinization of Goami

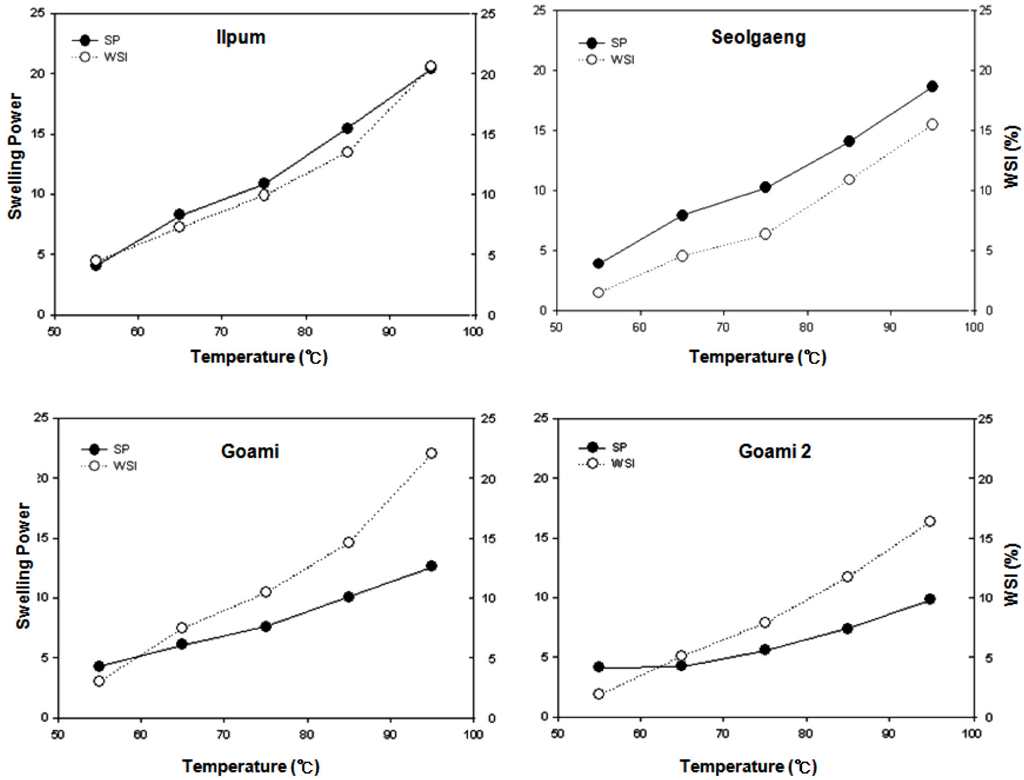

Fig. 4. Swelling power and solubility indices of rice starches from the indicated cultivars.

Table 2. DSC characteristics of starches from rice cultivars.

Variety	T_o (℃)	T_p (℃)	T_c (℃)	△H (J/g)
Ilpum	65.4[b]	74.7[b]	92.1[b]	8.8[b]
Seolgaeng	65.4[b]	74.2[b]	90.7[b]	7.5[a]
Goami	61.4[a]	71.0[a]	88.6[a]	7.5[a]
Goami 2	72.5[c]	83.8[c]	99.5[c]	7.4[a]

Different letters within the column indicate statistically significant differences ($P < 0.05$).
T_o, onset of gelatinization; T_p, peak temperature; T_c, conclusion temperature; $\triangle H$, enthalpy of gelatinization.

starch requires a smaller amount of energy compared with the other rice starches. Goami 2, with a high amylose and longer amylopectin chain ratio, showed the highest onset and peak gelatinization temperatures. Moreover, gelatinization in this cultivar was complete at about 100℃ and occurred over a range that is 10℃ higher than that of the other starches. Jane *et al*. (1999) have reported that the chain length and distribution of amylopectin branches determines the gelatinization temperature of starch, in addition to the enthalpy changes, and pasting properties, and that this temperature increases with the interactions between longer chains within the amylopectin cluster. The gelatinization enthalpy (△H), which is the energy required to melt rice starches, showed energy content differences ranging between 7.4 and 8.8 J/g, and was found to be highest in Ilpum.

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