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Impact of molecular and crystalline structures on *in vitro* digestibility of waxy rice starches

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ABSTRACT

The *in vitro* digestibility, molecular structure and crystalline structure of waxy rice starches isolated from six Korean cultivars (Shinsun, Dongjin, Baekok, Whasun, Chungbaek, and Bosuk) were investigated. The molecular weight (M_w) of waxy rice starches ranged from 1.1×10^8 g/mol to 2.2×10^8 g/mol. Chungbaek waxy rice starch had the highest average chain length (24.3) and proportion (20.7%) of long branch chains (DP ≥ 37), and the lowest proportion (19.0%) of short branch chains (DP 6–12) among the tested six waxy rice starches. The relative crystallinity and intensity ratio of 1047/1022 ranged from 38.9% to 41.1% and from 0.691 to 0.707, respectively. Chungbaek had the highest gelatinization temperature and enthalpy. Chungbaek had the highest pasting temperature (70.7 °C), setback (324 cP) and final viscosity (943 cP), whereas Baekok showed the highest peak viscosity (1576 cP) and breakdown (1031 cP). Chungbaek had lower rapidly digestible starch (RDS) content and expected glycemic index (eGI), and higher resistant starch (RS) content, whereas Whasun exhibited higher RDS content and eGI. The slowly digestible starch (SDS) content of Shinsun (38.3%) and Bokok (32.0%) was significantly higher than that of other cultivars (11.3–22.0%).

1. Introduction

Rice is one of the important crops served as a staple food around the globe. Different cultivars of waxy and non-waxy rices are usually classified according to their amylose content in starch. Rice has been used in various foods and industrial applications as an ingredient (Bao, Corke, & Sun, 2004). Waxy rice, also called sticky or glutinous rice, is considered to be excellent for thickening soups, sauces, gravies, baby foods and puddings due to its stickiness characteristic, a more porous texture, and good water retentions (Bao et al., 2004). In Korea, waxy rice is widely used in food products including glutinous rice cakes (*Injeolmi*) and rice snacks (*Hangwa*) (Choi, Kim, Lee, & Shin, 2001). Waxy rice starch consists almost entirely of amylopectin. An understanding of amylopectin molecular structure is very important for optimizing the industrial applications of waxy rice cultivars.

A number of reports have suggested that the functional properties of waxy rice starch are influenced by the fine structure of amylopectin (Jane et al., 1999; Ong & Blanshard, 1995; Vandeputte, Vermeylen, Geeroms, & Delcour, 2003). Gelatinization temperature negatively correlated with the amount of amylopectin short branch chains (DP 6–12) and positively with amylopectin long branch chains (DP ≥ 37) (Park, Ibanez, Zhong, & Shoemaker, 2007). The degree of polymerization (DP) of amylopectin negatively correlated with the hardness of staled cooked waxy rice (Villareal, Juliano, & Hizukuri, 1993). Very short chains (DP 6–9) of amylopectin caused a decrease in the pasting temperature of waxy rice starches (Hanashiro, Abe, & Hizukuri, 1996), whereas a higher proportion of long branch chains in amylopectin increased the pasting temperature and setback, and decreased peak viscosity and shear thinning (Jane et al., 1999; Park et al., 2007; Patindol, Gu, & Wang, 2009).

Starch digestion is one of the important metabolic responses following a meal (Jenkins et al., 1982). Dietary starch has been classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst, Kingman, & Cummings, 1992).

Digestibility of waxy rice starch is influenced by interplay of various factors including granule size, granule porosity, amylopectin chain length distribution, and degree of crystallinity (Singh, Dartois, & Kaur, 2010). The digestion characteristics of waxy rice starches may provide useful information for consumers to select suitable rice varieties for processing and health benefits. Because the amylopectin is the organizer of starch granules, its fine molecular

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structure and related crystalline structures are keys to understanding the starch digestion process. A few studies have reported on the relationship between molecular and crystalline structures and starch nutritional fractions of waxy rice starch (Sasaki et al., 2009). Furthermore, there is no information available on *in vitro* digestibility and molecular or crystalline structure of waxy rice starches for the rice varieties widely grown in Korea.

The aim of the present study was to assess the genetic differences among the six Korean waxy rice varieties grown under relatively similar environmental conditions through measurement of the molecular and crystalline structures and properties of the respective starches. The following properties of waxy rice starches in six Korean varieties were determined: the molecular structure (apparent amylose content, molecular weight of amylopectin, and amylopectin branch chain length distribution), crystalline structure (X-ray diffraction pattern and relative crystallinity for long-range order, gelatinization properties from DSC, and secondary ordered structure in starch granule from FT-IR), physicochemical properties (swelling factor and pasting properties) and *in vitro* starch digestibility (RDS, SDS, RS and eGI).

2. Materials and methods

2.1. Materials

Six waxy rice varieties in Korea, Shinsun, Dongjin, Baekok, Whasun, Chungbaek, and Bosuk, were obtained from the Rural Development Administration (Suwon, Korea) in 2012. Rice grains were wet-milled according to the procedure of Chiang and Yeh (2002). Rice starch was isolated according to the alkaline steeping method described by Lim, Lee, Shin, and Lim (1999).

2.2. Molecular structure analysis

2.2.1. Amylose content

Amylose content of waxy rice starches was determined using a commercial assay kit (Concanavalin A method) from Megazyme (Megazyme International Ireland Ltd., Bray, Ireland) according to the procedure described by Gibson, Solah, and McCleary (1997).

2.2.2. Molecular weight analysis

The average molecular weight (M_w) of waxy rice starches was analyzed using high performance size exclusion chromatography (HPSEC), coupled to a multi-angle laser light scattering and refractive index detection (HPSEC–MALLS–RI) system. Waxy rice starch was purified following the method described by Han and Lim (2004). The purified starch (12 mg, db) was dissolved in 0.1 M NaOH (1 mL) at 50 °C for 10 min, then added 3 mL distilled water and neutralized by 0.1 M HCl. The solution was heated in a microwave oven (RE-552W, Samsung, Seoul, Korea) using a microwave bomb (no. 4872, Parr Instrument Co., Moline, IL, USA) for 30 s and injected into system. The HPSEC–MALLS–RI system consisted of a pump (model 321, Gilson, Middleton, WI, USA), an injector valve with a 200 μL sample loop (model 7072, Rheodyne), SEC columns (TSK G5000 PW, 7.5 mm × 600 mm, TosoBiosep, Mongomeryville, PA, USA), a multi-angle laser light scattering detector (HELEOS, Wyatt Technology Corp., Santa Barbara, CA, USA) and a refractive index detector (Waters 2414). An aqueous solution of 0.15 M $NaNO_3$ and 0.02% NaN_3 was used for the mobile phase at a flow rate of 0.4 mL/min. Molecular weight was calculated using ASTRA 5.3 software (Wyatt Technology Corp.).

2.2.3. Amylopectin chain length distribution

Waxy rice starch (10 mg, db) was dispersed in 2 mL of 90% dimethyl sulfoxide (DMSO) and heated in a boiling water bath with stirring for 20 min. Starch solution was mixed with absolute ethanol (6 mL) and centrifuged at 3000 g for 15 min. The precipitate was dissolved with 2 mL of 50 mM sodium acetate buffer (pH 3.5) by stirring in a boiling water bath for 20 min. After equilibration of the solution at 37 °C, isoamylase (5 μL) was added (68,000 unit/mg of protein, Hayashibara Biochemical Laboratories, Okayama, Japan) and the starch solution was incubated at 37 °C with slow stirring for 24 h. The enzyme was inactivated by boiling for 10 min. An aliquot (200 μL) of the debranched sample was diluted with 2 mL of 150 mM NaOH. The sample was filtered (0.45 μm nylon syringe filter) and injected into high-performance anion-exchange chromatography (HPAEC) with a pulse amperometric detector (PAD) system. The HPAEC system consisted of a Dionex ICS-5000 (Dionex Corporation, Sunnyvale, CA), an ED50 electrochemical detector, and a CarboPac PA100 column (4 × 25 mm, Dionex Corporation, Sunnyvale, CA). Separation was achieved using a gradient eluent with 150 mM NaON and 500 mM sodium acetate in 150 mM NaON, at a flow rate of 1 mL/min.

2.3. Crystalline structure analysis

2.3.1. X-ray diffraction and relative crystallinity

The X-ray diffraction patterns of waxy rice starches were obtained with an X-ray diffractometer (PANalytical, X'pert MPD high resolution XRD, Almelo, Netherlands) operated at 40 kV and 40 mA. The scanning range and rate were 3–40° (2θ) and 2.0°/min, respectively. The crystallinity of starch was quantitatively calculated following the method described by Nara and Komiya (1983) using a peak-fitting software (Origin 6.0, Microcal, Northampton, MA).

2.3.2. Fourier transform infrared (FT-IR) spectroscopy

FT-IR spectra of waxy rice starches were obtained with a Digilab FTS 7000 spectrometer (Digilab USA, Randolph, MA), equipped with a thermoelectrically cooled deuterated triglycine sulfate (DTGS) detector using an attenuated total reflectance (ATR) at a resolution of 4 cm^{-1} by 128 scans. Spectra were baseline-corrected, and then deconvoluted by drawing a straight line between 1200 and 800 cm^{-1}. A half-band width of 15 cm^{-1} and a resolution enhancement factor of 1.5 with Bessel apodization were employed. Intensity measurements were performed on the deconvoluted spectra by recording the height of the absorbance bands from the baseline.

2.3.3. Differential scanning calorimetry (DSC)

Thermal properties of waxy rice starches were measured using a differential scanning calorimeter (DSC6100, Seiko Instruments, Chiba, Japan). Starch (3 mg, db) was weighted into an aluminum pan (Seiko Instruments, Chiba, Japan) with 6 μL of distilled water. The sample pan was sealed, equilibrated at room temperature for 12 h and then heated from 10 to 130 °C at a heating rate of 5 °C/min. An empty pan was used as a reference.

2.4. Rapid visco-analyzer (RVA)

Pasting properties of waxy rice starches were analyzed by using a Rapid Visco-Analyzer (RVA-TecMaster, Newport Scientific Pty. Ltd., Warriewood, Australia). Starch slurries (7% w/w db, 30 g of total weight) were equilibrated at 50 °C for 1 min, heated to 95 °C at a rate of 6 °C/min, held at 95 °C for 5 min, cooled to 50 °C at 6 °C/min, and held at 50 °C for 2 min. A constant rotating speed of paddle at 160 rpm was used.

2.5. Swelling factor (SF)

SF at 65 °C was measured according to the method of Tester and Morrison (1990).

2.6. *In vitro* starch digestibility

Starch digestibility was analyzed according to the method described by Englyst et al. (1992) with modifications. Porcine pancreatic α-amylase (0.45 g, P-7545, Sigma, St. Louis, MO) was dispersed in distilled water (4 mL) and centrifuged at 1500 g for 12 min. The supernatant (2.7 mL) was transferred to a beaker, and 0.3 mL of amyloglucosidase (A-9913, Sigma) and 0.2 mL of invertase (I-4504, Sigma) were added to the solution. This enzyme solution was freshly prepared for each digestion analysis. Starch (100 mg) and 4 mL of 0.5 M sodium acetate buffer (pH 5.2) were added to each test tube. The enzyme solution (1 mL) and 15 glass beads (4 mm diameter) were added to each tube, followed by incubation in a shaking water bath (37 °C, 170 rpm). Aliquots (0.1 mL) were taken at intervals and mixed with 1 mL of 80% ethanol. The hydrolyzed glucose content was measured using glucose oxidase and peroxidase assay kits (GAGO-20, Sigma). The three starch nutritional fractions as based on the rate of hydrolysis were follows: rapidly digestible starch (RDS, digested within 20 min); slowly digestible starch (SDS, digested between 20 min and 120 min); and resistant starch (RS, undigested after 120 min).

The expected glycemic index (eGI) of waxy rice starches was calculated in accordance with the procedure established by Granfeldt, Bjorck, Drews, and Tovar (1992). The hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve (0–180 min) of the waxy rice starches by the area obtained for a standard material (white bread). The expected glycemic index (eGI) was calculated using the equation of Granfeldt et al. (1992): eGI = 8.198 + 0.862 HI.

2.7. Statistical analysis

The data reported were the means of triplicate measurements. Statistical analysis with Duncan's multiple test ($P < 0.05$) was carried out using SPSS V. 8.2 software (SPSS Institute Inc., Cary, NC).

3. Results and discussion

3.1. Amylose content

The amylose contents of starches isolated from the six waxy rice varieties, as determined by Con A method, ranged from 1.1% in Chungbaek to 2.4% in Bosuk. The amylose content of Chungbaek was slightly lower than that of the other cultivars (Table 1). Similarly, it has been suggested that the amylose content in waxy rice starch is usually below 2% (Wang & Wang, 2002; Zhu, Liu, Sang, Gu, & Shi, 2010).

3.2. Structural characterization of amylopectin

Molecular weight distribution of the waxy rice starches is shown as example in Fig. 1A. A single peak for amylopectin was achieved

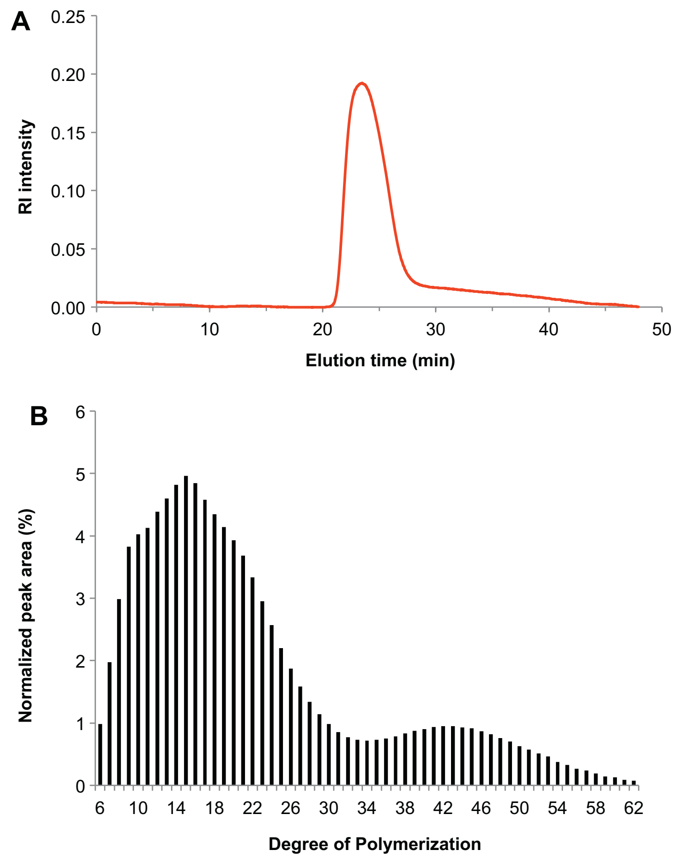

Fig. 1. HPSEC–RI chromatogram (A) and amylopectin branch chain length distribution (B) of waxy rice starches (Shinsun).

using the HPSEC–MALLS–RI system (Fig. 1A). The weight average molecular weights (M_w) of amylopectin for different waxy rice starches are presented in Table 1. Molecular weight of waxy rice starches ranged from 1.1×10^8 g/mol (Whasun) to 2.2×10^8 g/mol (Bosuk), which was comparable to those reported in other studies of rice starches (Park et al., 2007).

Normalized HPAEC–PAD chromatogram of amylopectin branch chain length distribution is shown as example in Fig. 1B. Two distinct groups with DP (DP 6–33 and DP 34–62) in the chromatogram were observed. The first group of short chains showed a maximum at DP 15 with a shoulder at DP 19–22. The second group of long chains had a maximum at DP 42, which was in accordance with a previous report (Bertoft & Koch, 2000). The normalized chromatogram calculated from HPAEC–PAD results are grouped into chain types with the corresponding DP as follows: A chains (DP 6–12), B1 chains (DP 13–24), B2 chains (DP 25–36), and B3+ chains (DP ≥ 37) (Hanashiro et al., 1996). The peak area ratios of each chain type are summarized in Table 1. The HPAEC system used

Table 1
Amylose content, molecular weight of amylopectin, and amylopectin branch chain length distribution of waxy rice starches[A].

Rice starch	Amylose content (%)	Molecular weight ($\times 10^8$ g/mol)	Average chain length	Distribution (%)			
				DP 6–12	DP 13–24	DP 25–36	DP ≥ 37
Shinsun	2.0 ± 0.2^{ab}	1.6 ± 0.1^{c}	21.9 ± 0.3^{d}	21.8 ± 0.7^{a}	48.4 ± 0.5^{a}	14.1 ± 0.6^{c}	15.6 ± 0.5^{d}
Dongjin	1.5 ± 0.1^{bc}	1.5 ± 0.1^{c}	23.9 ± 0.1^{a}	18.8 ± 0.2^{c}	45.1 ± 0.4^{cd}	16.9 ± 0.1^{a}	19.2 ± 0.3^{b}
Baekok	1.2 ± 0.2^{c}	1.5 ± 0.0^{c}	23.2 ± 0.0^{b}	20.3 ± 0.0^{b}	46.0 ± 0.1^{c}	15.7 ± 0.1^{b}	18.0 ± 0.0^{bc}
Whasun	1.9 ± 0.3^{b}	1.1 ± 0.0^{d}	22.1 ± 0.3^{d}	22.5 ± 0.1^{a}	47.6 ± 0.6^{b}	14.0 ± 0.1^{c}	15.9 ± 1.0^{d}
Chungbaek	1.1 ± 0.1^{c}	2.0 ± 0.0^{b}	24.3 ± 0.3^{a}	19.0 ± 0.8^{c}	44.3 ± 0.4^{d}	16.1 ± 0.5^{ab}	20.7 ± 0.6^{a}
Bosuk	2.4 ± 0.0^{a}	2.2 ± 0.1^{a}	22.6 ± 0.0^{c}	21.5 ± 0.1^{a}	46.7 ± 0.2^{b}	14.5 ± 0.1^{c}	17.3 ± 0.2^{c}

DP, degree of polymerization.
[A] Values followed by the different superscripts in the same column are significantly different ($P < 0.05$).

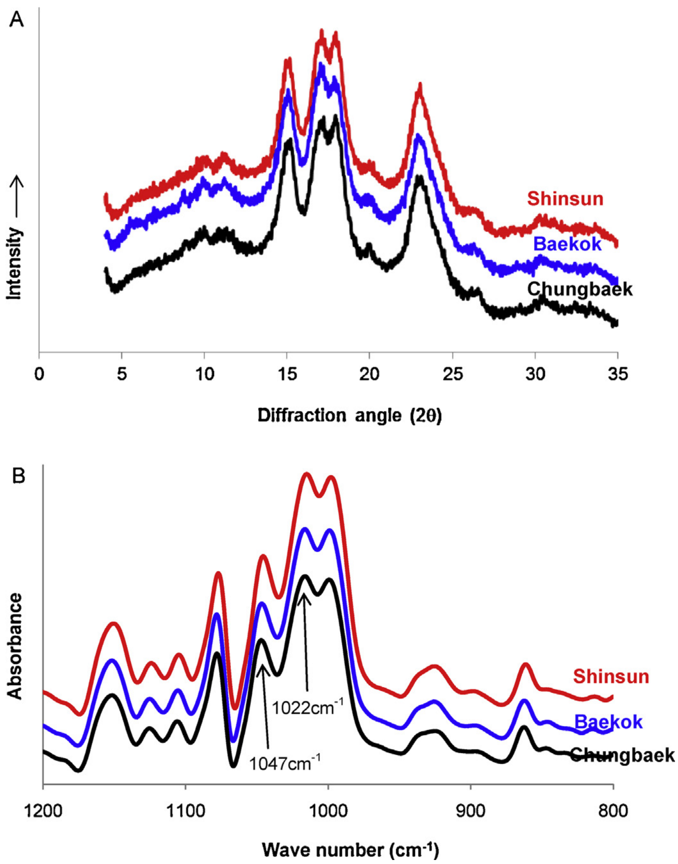

Fig. 2. X-ray diffraction patterns (A) and deconvoluted Fourier transform infrared (FT-IR) spectra (B) of waxy rice starches.

in this study was sensitive to resolve glucans with DP ≤ 62. The average branch chain length of amylopectin of the six waxy rice starches ranged between 21.9 (Shinsun) and 24.3 (Chungbaek). Jane et al. (1999) reported that the average branch chain length of cereal amylopectin ranged from 18.8 to 23.5. Wang and Wang (2002) found that the average branch chain length of four waxy rice starches ranged between 20.0 and 20.7. Chungbaek had a smaller proportion (19.0%) of short A chains (DP 6–12) and a larger proportion (20.7%) of longer B3+ chains (DP ≥ 37) than the other waxy rice starches. The opposite trend was observed for Shinsun and Whasun, which displayed a larger proportion (21.8% and 22.5%, respectively) of short A chains and a smaller proportion (15.6% and 15.9%, respectively) of long B3+ chains (Table 1). The proportion of short A + B1 chains followed the order among the tested six waxy rice starches: Shinsun (70.2%) ~ Whasun (70.1%) > Bosuk (68.2%) > Baekok (66.3%) > Dongin (63.9%) ~ Chungbaek (63.3%). This result indicates that the amylopectin of Dongin and Chungbaek contained fewer short chains and more long chains than the other cultivars, whereas Shinsun and Whasun consisted of more short chains and fewer long chains. It was reported that the waxy cereal starches contained about 70–80% of short chains of amylopectin with DP 6–24 (Jane et al., 1999; Shi & Seib, 1992). Our results suggest that the fine molecular structure of starches isolated from the six Korean waxy rice cultivars varied.

3.3. Crystalline structure

The X-ray diffraction patterns of waxy rice starches are presented as examples in Fig. 2A. The waxy rice starches displayed the typical A-type polymorphic form with an unresolved peak at 17 and 18° and individual peaks at 15, 20 and 23° in the X-ray diffractograms (Fig. 2A). The relative crystallinity (Table 2) calculated from X-ray diffraction pattern ranged from 38.9% (Dongjin) to 41.1% (Chungbaek), which was relatively higher than the reported range of 29.2–39.3% for rice starches (Ong & Blanshard, 1995). Cheetham and Tao (1998) reported that the proportion of short chains (DP 10–13) in maize amylopectin played an important role in the formation of crystallinity, a higher proportion of DP 10–13 having lower crystallinity. Gidley and Bulpin (1987) also suggested that the presence of short chains (DP < 10) in amylopectin resulted in a decrease in the stability of double helix. On the other hand, Sanders, Thompson, and Boyer (1990) claimed that the higher proportion of longer chains might form longer crystallites, resulting in a more perfect crystalline structures. In this study, Chungbaek with higher M_w and average chain length, with a greater proportion of long chains and fewer short chains of amylopectin, exhibited higher relative crystallinity. The opposite trend was observed for Whasun having lower relative crystallinity, as it had lower M_w and average branch chain length of amylopectin.

Table 2
Relative crystallinity and intensity ratio of 1047 cm^{-1} to 1022 cm^{-1} of waxy rice starches[A].

Rice starch	Relative crystallinity (%)	Ratio of 1047 cm^{-1}/1022 cm^{-1}
Shinsun	40.1 ± 0.1^{b}	0.701 ± 0.001^{ab}
Dongjin	38.9 ± 0.1^{c}	0.691 ± 0.005^{c}
Baekok	40.7 ± 0.0^{a}	0.698 ± 0.001^{bc}
Whasun	39.0 ± 0.2^{c}	0.691 ± 0.004^{c}
Chungbaek	41.1 ± 0.2^{a}	0.707 ± 0.002^{a}
Bosuk	41.0 ± 0.3^{a}	0.699 ± 0.001^{b}

[A] Values followed by the different superscripts in the same column are significantly different (P < 0.05).

The deconvoluted FT-IR spectra of waxy rice starches are presented as examples in Fig. 2B. The FT-IR band at 1047 cm^{-1} and 1022 cm^{-1} have been known to be sensitive to changes in the crystalline and amorphous regions near the granule surface of starch, respectively (van Soest, Tournois, de Wit, & Vliegenthart, 1995). The intensity ratio of bands at 1047 cm^{-1} and 1022 cm^{-1} (1047/1022) expresses the ratio of ordered crystalline regions to amorphous regions in starch (Table 2). Among the tested waxy rice starches, the ratio 1047/1022 was the highest in Chungbaek and the lowest in Dongjin and Whasun, which were in agreement with the results of relative crystallinity.

The crystalline structures as determined by X-ray diffraction and FT-IR spectroscopy showed relatively good relationships with amylopectin molecular structures in Baekok, Whasun, Chungbaek, and Bosuk.

3.4. Thermal properties

Thermal properties of waxy rice starches, as determined using DSC, are presented in Table 3. The onset (T_o), peak (T_p), and conclusion (T_c) gelatinization temperatures and gelatinization enthalpy (ΔH) of the six waxy rice starches ranged from 57.2 °C to 61.4 °C, from 62.7 °C to 68.5 °C, from 73.4 °C to 79.6 °C, and from 13.2 J/g to 16.8 J/g, respectively (Table 3). T_o, T_p, T_c, and ΔH varied significantly among the six waxy rice starches. Chungbaek had the highest gelatinization temperatures (T_o, T_p, and T_c) among the tested waxy rice starches, whereas Whasun had the lowest T_o, and Baekok showed the lowest T_p and T_c. Gelatinization temperature has been considered as a parameter of crystalline perfection (Tester & Morrison, 1990). Gelatinization temperatures are influenced by the molecular structure of crystalline regions, which corresponds to the distribution of amylopectin chains (Gidley & Bulpin, 1987). Gidley and Bulpin (1987) suggested that the short chains (DP < 10) in amylopectin caused a decrease in the stability of double helix, which may result in lower gelatinization temperatures. Similarly Noda,

Table 3
Thermal properties of waxy rice starches[A].

Rice starch	T_o (°C)	T_p (°C)	T_c (°C)	T_c–T_o (°C)	ΔH (J/g)
Shinsun	60.7 ± 0.1^b	67.5 ± 0.1^b	78.1 ± 1.1^{ab}	17.4 ± 1.2^d	15.4 ± 0.0^c
Dongjin	57.2 ± 0.1^d	65.1 ± 0.3^d	76.5 ± 0.2^{bc}	19.3 ± 1.1^{bc}	16.6 ± 0.2^{ab}
Baekok	56.0 ± 0.3^e	62.7 ± 0.3^e	73.4 ± 0.3^d	17.4 ± 0.6^d	13.2 ± 0.0^d
Whasun	55.1 ± 0.1^f	67.4 ± 0.3^b	76.1 ± 0.1^c	21.0 ± 0.0^{ab}	16.3 ± 0.2^b
Chungbaek	61.4 ± 0.1^a	68.5 ± 0.3^a	79.4 ± 0.1^a	18.0 ± 0.3^{cd}	16.8 ± 0.2^a
Bosuk	58.3 ± 0.1^c	66.3 ± 0.2^c	79.6 ± 0.1^a	21.3 ± 0.3^a	16.3 ± 0.3^b

T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔH, gelatinization enthalpy.
[A] Values followed by the different superscripts in the same column are significantly different ($P < 0.05$).

Nishiba, Sato, and Suda (2003) reported a negative correlation between the proportion of amylopectin short chains (DP 6–12) and gelatinization temperatures. In the present study, Chungbaek had a smaller proportion of DP 6–12, which could explain its higher gelatinization temperature. On the other hand, Yuan, Thompson, and Boyer (1993) claimed that a higher proportion of long chains contributed to higher gelatinization temperatures, because the longer chains may form longer crystallites, resulting in increased crystalline order. Chungbaek had the highest average chain length and the greatest proportion of long chains (DP ≥ 37), which could also explain the highest gelatinization temperature (Table 3). Gelatinization temperature range (T_c–T_o) varied from 17.4 °C (Shinsun and Baekok) to 21.0 °C (Whasun), indicating a wide range of heterogeneity in starch crystallites within the granules of the tested waxy rice.

Gelatinization enthalpy (ΔH) of waxy rice starches followed the order: Chungbaek > Dongjin > Bosuk ~ Whasun > Shinsun > Baekok. Gelatinization enthalpy represents the amount of energy required for disrupting double helices, which corresponds to the distribution of amylopectin chains (Cooke & Gidley, 1992). A larger proportion of long chains may form a greater amount of ordered structures, requiring more thermal energy to break the crystalline order (Jane et al., 1999). Consequently, the greater gelatinization enthalpy of waxy rice starches from Chungbaek could be attributed to its higher proportion of long chains and greater average chain length (Table 1). Marginally present linear fractions, which are detected as apparent amylose content (Table 1), may also play a role in the formation of crystallinity. Amylose has been generally considered to disrupt the crystalline packing (Cheetham & Tao, 1998). The lower amount of linear fractions in Chungbaek could also explain its greater gelatinization enthalpy.

3.5. Pasting properties

Pasting properties of the waxy rice starches are summarized in Table 4. Pasting temperature, peak viscosity, breakdown, setback and final viscosity of the six waxy rice starches ranged 65.5–70.7 °C, 1281–1576 cP, 653–1031 cP, 243–325 cP, and 869–943 cP, respectively (Table 4). Chungbaek waxy rice starch exhibited the highest pasting temperature (70.7 °C) among the tested waxy rice starches. Pasting properties are associated with amylopectin branch chain length distribution (Jane et al., 1999). Koroteeva et al. (2007) claimed that a higher proportion of long chains (DP ≥ 37) in amylopectin facilitated the stabilization of starch granules, resulting in higher pasting temperature as well as gelatinization temperature. Chung, Liu, Lee, and Wei (2011) suggested that amylopectin with more short chains (DP 6–12) displays lower pasting temperature, as the short branch chains do not provide strong interactions to hold the integrity of the swollen granules. Chungbaek consisted of more long branch chains (DP ≥ 37) and less short chains (DP 6–12), which may explain the highest pasting temperature. The order of pasting temperature was similar to that of gelatinization peak temperature (T_p) in this study. Amylopectin is generally considered to be responsible for starch crystallinity, while amylose disrupts the crystalline packing (Cheetham & Tao, 1998). Gelatinization temperature is the point where starch granules begin to swell and lose crystallinity, while pasting temperature is the temperature of the phenomenon following gelatinization. Thus the temperature between gelatinization and pasting usually shows variances (Thomas & Atwell, 1997). A similar trend of temperature between pasting and gelatinization among the tested waxy rice starches could be attributed to the marginal disruption in crystalline packing by a few amyloses in the waxy rice starches.

Baekok waxy rice starch had the lowest pasting temperature (65.5 °C) and the highest peak viscosity (1576 cP) and breakdown (1031 cP). These results indicated that the starch granule in Baekok cultivar swelled rapidly and greatly, and that the swollen granules degraded with a greater decrease in viscosity. This could be partially associated with its molecular structure. Studies have shown that a lower amylose content and molecular weight correlate with higher peak viscosity (Reddy, Subramanian, Ali, & Bhattacharya, 1994; Park et al., 2007). This is in agreement with the results of our present study.

Chungbaek waxy rice starch exhibited higher setback and final viscosity than other waxy rice starches (Table 4). The setback and final viscosity are attributed to a re-ordering of longer linear chains of amylopectin in waxy rice starch. Suzuki, Takeda, and Hizukuri (1985) reported that a higher proportion of long chains contributed to a more recrystallized domains, resulting in a greater extent of retrogradation. Therefore, the greater setback and final viscosity of Chungbaek could be explained by its longer amylopectin branch chain length.

Table 4
Pasting properties of waxy rice starches[A].

Rice starch	Pasting temperature (°C)	Peak viscosity (cP)	Breakdown (cP)	Setback (cP)	Final viscosity (cP)
Shinsun	69.1 ± 0.1^b	1281 ± 9^d	692 ± 6^c	280 ± 1^b	869 ± 2^c
Dongjin	66.5 ± 0.3^d	1390 ± 16^c	819 ± 15^b	315 ± 11^a	887 ± 12^c
Baekok	65.5 ± 0.0^e	1576 ± 8^a	1031 ± 15^a	325 ± 4^a	870 ± 11^c
Whasun	68.4 ± 0.0^c	1302 ± 15^d	677 ± 8^c	287 ± 4^b	911 ± 3^b
Chungbaek	70.7 ± 0.6^a	1438 ± 6^b	818 ± 0^b	324 ± 2^a	943 ± 4^a
Bosuk	67.9 ± 0.0^c	1300 ± 3^d	653 ± 4^d	243 ± 13^c	890 ± 12^c

[A] Values followed by the different superscripts in the same column are significantly different ($P < 0.05$).

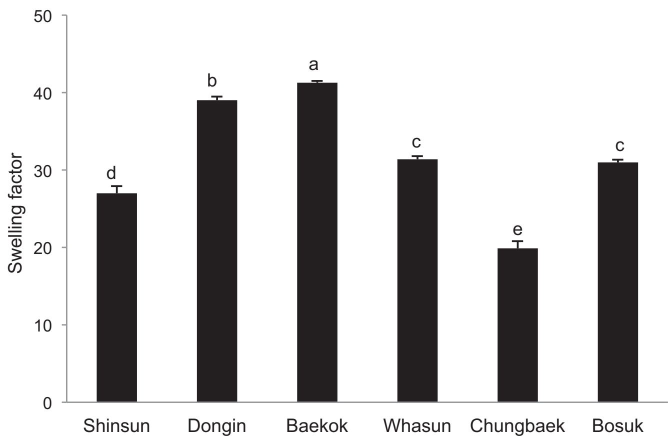

Fig. 3. Swelling factor at 65 °C of waxy rice starches. Values with different letters above each bar among cultivars are significantly different ($P < 0.05$).

Table 5
The amount of starch nutritional fractions (RDS, SDS and RS) and expected glycemic index (eGI) of waxy rice starches[A].

Rice starch	Starch nutritional fractions			eGI
	RDS (%)	SDS (%)	RS (%)	
Shinsun	55.8 ± 0.1[e]	38.3 ± 0.2[a]	5.9 ± 0.3[ab]	84.0 ± 0.2[d]
Dongjin	74.9 ± 0.2[c]	18.3 ± 1.1[c]	6.8 ± 0.9[a]	88.7 ± 0.5[c]
Baekok	68.0 ± 0.6[d]	32.0 ± 0.6[b]	0.0 ± 0.0[c]	91.9 ± 0.4[ab]
Whasun	84.5 ± 1.6[a]	11.3 ± 1.2[e]	4.1 ± 1.0[b]	93.0 ± 1.6[a]
Chungbaek	70.5 ± 0.6[d]	22.0 ± 1.3[c]	7.5 ± 1.1[a]	88.0 ± 0.8[c]
Bosuk	80.5 ± 0.3[b]	13.3 ± 0.0[d]	6.2 ± 0.2[ab]	90.6 ± 0.2[b]

RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch; eGI, expected glycemic index.
[A] Values followed by the different superscripts in the same column are significantly different ($P < 0.05$).

3.6. Swelling factor

Swelling factor (SF) of the waxy rice starches at 65 °C is presented in Fig. 3. SF of waxy rice starches ranged from 19.9 (Chungbaek) to 41.3 (Baekok). It is generally considered that the swelling behavior of starch is primarily related to amylopectin, while amylose acts as inhibitor of swelling (Tester & Morrison, 1990). The order of SF was inversely correlated with that of amylose content and similar to that of peak viscosity except Chungbaek had the lowest SF. SF is also influenced by amylopectin molecular structure (Hoover & Ratnayake, 2002). Longer amylopectin branch chain lengths in Chungbaek would suppress swelling of starch.

3.7. In vitro starch digestibility

The amount of starch nutritional fractions, for rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS), are presented in Table 5. *In vitro* digestibility of native waxy rice starches is influenced by degree of crystallinity (Chung, Lim, & Lim, 2006) and molecular structure of amylopectin (Srichuwong & Jane, 2007). RS contents of waxy rice starches ranged from 0% (Baekok) to 7.5% (Chungbaek). Similarly, a higher level of hydrolysis is reported in waxy rice starches with smaller granules and greater relative surface area, which leads to greater susceptibility to enzymatic hydrolysis (Chung et al., 2011). The highest RS content was obtained in Chungbaek, indicating a lower degree of enzyme hydrolysis. This result could be influenced by the crystalline and molecular structures. Chungbaek waxy rice starch had the highest amount of crystalline structures in relative crystallinity and ratio of 1047/1022 (Table 2) and the greatest crystalline rigidity in the gelatinization temperatures (Table 3). The crystallites have been considered to impose a physical limit on the accessibility of the enzymes and thus the greater rigidity and extent of crystalline regions induce a decrease in enzyme susceptibility (Chung et al., 2006). Chungbaek had the lowest proportion of short A chains (DP 6–12) and the highest proportion of long branch chains (DP ≥ 37) and average chain length (Table 1). Jane, Wong, and McPherson (1997) claimed that the short chains with DP 6–12 in amylopectin could induce weak points in the starch crystalline structure, which caused a greater susceptibility to enzymatic hydrolysis. On the other hand, Sasaki et al. (2009) suggested that long chains of amylopectin could make for stable and higher crystalline structures due to the increased hydrogen bonds between chains, resulting in a higher resistance to enzymatic digestion. Baekok waxy rice starch was completely hydrolyzed within 2 h, displaying 0% of RS content. Baekok had substantially lower gelatinization temperature, indicating lower stability and perfection of crystalline regions which lead to increased enzyme hydrolysis.

The RDS content was in the following order: Whasun (84.5%) > Bosuk (80.5%) > Dongjin (74.9%) > Chungbaek (70.5%) ≥ Baekok (68.0%) > Shinsun (55.8%). The highest RDS content of Whasun could be attributed to its lower proportion of long branch chains (DP ≥ 37), a higher proportion of short A chains (DP 6–12), lower molecular weight in molecular structure and lower onset temperature of gelatinization and lower crystallinity as dictated by its relative crystallinity and ratio of 1047/1022 in crystalline structure. Shinsun displayed the lowest RDS content, implying a resistance to enzymatic attack at the early stages of digestion. Although the significantly lower RDS content of Shinsun could not explain its molecular feature since it had lower molecular weight and average chain length, we assumed that the relatively higher gelatinization temperature and greater surface crystalline structure in ratio of 1047/1022 may influence its enzyme resistance during the initial stages of digestion.

SDS content of waxy rice starches ranged from 13.3% (Bosuk) to 38.3% (Shinsun). It was interesting that Shinsun and Baekok waxy rice starch had over 30% of SDS content, which was much higher than the other cultivars (18.3–22.5%). The crystalline structure could influence the SDS content, as Shinsun and Baekok had intermediate amounts of crystalline structures among the tested cultivars, as found in the relative crystallinity and ratio of 1047/1022 (Table 2). The large fraction of these crystalline structures could be hydrolyzed during the intermediate and late stages of digestion, leading to increased SDS content. The greater amount of SDS content may be beneficial, as SDS is generally considered to be the most desirable of dietary starches for its complete but slow digestion in the small intestines (Lehmann & Robin, 2007).

3.8. Expected glycemic index (eGI)

The eGI calculated from digestion curve ranged between 84.0 (Shinsun) and 93.0 (Whasun) (Table 5). Comparable value (87.6) of *in vitro* glycemic index in waxy rice starch has been reported (Chung et al., 2006). Among the tested waxy rice starches, Shinsun had the lowest eGI value, which was related to its low RDS content and high SDS content. Chungbaek showed a relatively lower eGI and Whasun had the highest eGI value, which may be due to their molecular and crystalline structures as explained above. The difference in eGI among the cultivars could be used to effectively predict blood glucose response of waxy rice foodstuffs.

4. Conclusions

Significant differences in the physicochemical properties and digestibility of waxy rice starches were observed among six cultivars grown in Korea. The molecular and crystalline structures of waxy rice starches had a substantial impact on the *in vitro* starch digestibility. The waxy rice starch of Chungbaek cultivar had a significantly higher gelatinization temperature, relative crystallinity, intensity ratio of 1047/1022, average chain length of amylopectin and proportion of long branch chains of amylopectin, and lower proportion of short branch chains (DP 6–12) of amylopectin, which could be attributed to its higher RS content and lower RDS content and eGI. Whasun waxy rice starch, exhibiting higher RDS content and eGI, showed a higher proportion of short branch chains of amylopectin and lower relative crystallinity, intensity ratio of 1047/1022, onset gelatinization temperature, molecular weight and proportion of long branch chains of amylopectin. Our experimental results suggest that crystalline and molecular structures are key factors in controlling starch digestibility.

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