

# Structural and physicochemical properties of native starches and non-digestible starch residues from Korean rice cultivars with different amylose contents

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## ABSTRACT

In this study, rice starches from four cultivars: Baegokchal (BOC), Ilmi (IM), Mimyeon (MM), and Dodamssal (DDS), were studied in terms of their physicochemical and structural features. Native starches (NS) from MM and DDS showed high amylose content and low rapidly digestible starch, as well as high slowly digestible starch and resistant starch (RS) ratios. To elucidate the characteristics of RS in rice, non-digestible starches (NDS) were isolated from NS from each cultivar. The starch crystallinity of BOC, IM, and MM showed an A-type X-ray diffractometry pattern; however, DDS granules displayed a C-type crystallinity pattern with a predominant B-type. DDS starch granules had a convex spherical shape, whereas BOC, IM, and MM starch granules had a polygonal shape. All starches from IM and BOC were hydrolyzed, with no NDS residues remaining. The NDS from MM and DDS, which are high-amylose cultivars, showed a lower molecular weight, longer average amylopectin chain length, and lower viscosity than NS. DDS had the lowest digestibility, highest RS content, and showed potential for use as a source of starch for weight loss and hypoglycemic effects owing to its low glycemic index. The low viscosity of DDS can potentially be exploited for its use as a daily dietary component through the development of suitable processing methods for products such as rice noodles.

## 1. Introduction

Rice (*Oryza sativa* L.) is a major source of carbohydrates and plays an important role in providing energy to the body (Hu, Zhao, Duan, Linlin, & Wu, 2004). The most common storage form of carbohydrates in plants is starch, which is one of the most important components of the human diet (Amagliani, O'Regan, Kelly, & O'Mahony, 2016). Starch accounts for at least 35% of daily calorie intake in developed countries and 80% of the daily calorie intake comes from a single source, such as rice, in many Asian and African countries.

Starch of cereals including rice is composed of two polysaccharides: amylose and amylopectin. Studies have shown that the crystal structure, amylopectin structure, and amylose content of rice starch affect its functional properties. Understanding the structure and functional properties of rice starch is industrially useful, and is very important for

selecting suitable rice cultivars (Chung, Liu, Lee, & Wei, 2011).

Health concerns related to cholesterol levels, cancer causing factors, and obesity have influenced consumers to change their dietary demands, and the increased demand for high-quality foods has caused an increase in research on new technologies and ingredients (Pérez-Alvarez, 2008, pp. 1–17). Starches have been classified into various categories based on their digestive characteristics: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst, Kingman, & Cummings, 1992a). The fraction of starch that is not hydrolyzed to D-glucose in the small intestine or fermented by microorganisms in the large intestine is defined as RS that has various physiological advantages. RS is composed of a linear chain of  $\alpha$ -1,4-D-glucan; it can be obtained from the retrograded amylose fraction and is reported to have a relatively low molecular weight ( $\sim 1.2 \times 10^5$  g/mol) (Tharanath, 2002).

Studies on corn starch with various amylose concentrations have

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reported a strong correlation between RS and amylose content (Morita, Ito, Brown, Ando, & Kiriya, 2007). Based on this research, a Chinese research team has developed a strain of rice with 55% amylose content in starch with B-type crystallinity, and reported its starch characteristics and digestibility (Zhu, Liu, Wilson, Gu, & Shi, 2011). The Korean Rural Development Administration has made efforts to develop rice processing capabilities in response to the increasing consumer demand for functional rice. Among the products developed, Dodamssal (DDS) and Mimyeon (MM), two cultivars of high-amylose rice, were released in 2013 and 2012, respectively.

The goal of this study was to isolate rice starches with different amylose contents and to compare their structural characteristics and digestibility. Non-digestible starch (NDS) in particular was isolated from the native starch (NS) of high-amylose rice cultivars to identify the differences between NDS and NS from the same cultivars and to investigate their structural characteristics. In addition, the morphology, molecular weight, amylopectin molecular chain distribution, and pasting properties of NS and NDS were investigated to generate basic data with the aim of developing new food production strategies.

## 2. Materials and methods

### 2.1. Preparation of rice flour samples

Four rice cultivars were harvested from the National Institute of Crop Science (Suwon, Gyeonggi-do, South Korea) in 2015. The Baegokchal (BOC) and Ilmi (IM) cultivars are both *Japonica* type, and waxy rice and normal rice, respectively (Lim et al., 1996; Song et al., 2013). MM is a tongil-type, high-amylose hybrid variety of *Japonica* and *Indica* (RDA, 2013). Dodamssal (DDS) is a *Japonica*-type high-amylose breed developed using traditional breeding from Goami (a breed with an amylose content of 26.7%) and Goami2 (a mutant breed developed using *N*-methyl-*N*-nitrosourea (MNU) treatment and containing resistant starch and about 33% of amylose) (Kang, Hwang, Kim, & Choi, 2003; RDA, 2014; Song et al., 2008; Yoon et al., 2013). After the mature rice seeds from each cultivar were harvested and naturally dried, they were dehulled using a huller (SY88-TH, Ssangyong, Incheon, Korea), polished by a rice miller (MC-90A, Toyo, Chiba, Japan), ground into rice flour, and prepared after being passed through a 100-mesh sieve.

### 2.2. Composition analysis of moisture, crude protein, fat, amylose content, and resistant starch

The moisture content was measured after incubation at 105 °C for 2 h. Crude ash was determined after incubation at 600 °C for 5 h. Crude protein was measured using the semimicro-Kjeldahl method (Kjeltec 2400 AUT; Foss Tecator, Hilleroed, Denmark). These were analyzed following methods described by Zhu et al. (Zhu et al., 2011). Crude fat content was determined by the Soxhlet extraction method using the Soxtec System HT 1043 extraction unit (Foss Tecator). The apparent amylose content of the rice flours, NS, and NDS was determined by iodine colorimetry, as described previously (Juliano, 1985).

The amylose content of NS and NDS was analyzed by an amylose/amylopectin assay using the Megazyme kit (Megazyme International, Ltd., Wicklow, Ireland). RS and soluble starch contents were analyzed using the AOAC method (McCleary et al., 2002).

### 2.3. Starch isolation from rice cultivars

Starches were isolated from mature rice grains following the method described by Wang and Wang (Wang & Wang, 2001). The isolated starches were dried at 40 °C, ground into powder, and prepared as rice flour.

### 2.4. NS hydrolysis and isolation of NDS

NS from the samples was hydrolyzed according to both the AOAC Official Method (2002.02) and the AACC Method (32–40), with some modifications (Man et al., 2013). Briefly, 1 g starch was suspended in 50 mL enzyme solution (100 mM maleate buffer, pH 6.0, 1500 U PPA, 150 U AMG). Amylolysis was conducted in a constant temperature shaking water bath with continuous shaking (150 rpm) at 37 °C for 16 h. After hydrolysis, undissolved residue was obtained by centrifugation (1500 × g, 10 min) at 4 °C. The remaining solid content was defined as NDS. The residue was subsequently washed in 50% ethanol (v/v) to remove residual enzymes. After removing supernatants, the residue was dried at 40 °C for 2 days. The dried starch was finally ground into a powder and passed through a 100-mesh sieve for further structural analysis as NDS.

### 2.5. Microscopic observation of NS and NDS

The granule surfaces of NS and NDS were observed by a scanning electron microscope (SEM; SEM-3000, Hitachi, Japan). The samples were treated following the method of You et al. (You et al., 2016).

### 2.6. Molecular weight distribution of NS and NDS

The average molecular weight (MW) of NS and NDS was analyzed using high-performance size exclusion chromatography (HPSEC) with the Pullulan Shodex standard (P-82, Showa Denko K.K., Tokyo, Japan). NS and NDS samples (1.0 g) were prepared in 100 mL 90% DMSO for 1 h at 95 °C, and then stirred for 24 h at 25 °C. The solubilized starch molecules were precipitated by adding ethanol (500 mL), centrifuged, and then dried at 25 °C for 24 h. Next, 0.5% solubilized starch was prepared in 50 mM sodium acetate buffer (pH 4.5) by heating the solution for 30 min at 95 °C in a water bath, filtering it with a 0.45-μm syringe filter, and injecting it into the HPSEC analyzer.

HPSEC equipment consisted of a pump (Prostar 210, Varian, Inc., Palo Alto, CA, USA), an injector (7725i, Rheodyne, Rohnert Park, CA, USA), SEC columns (Shodex OHPak SB-806 HQ, 8.0 mm ID × 300 mm, Showa Denko K.K.; and OHPak SB-804 HQ, 8.0 mm ID × 300 mm, Showa Denko K.K.), and an RI detector (Prostar 355 refractive index, Varian, Inc., Palo Alto, CA, USA). The mobile phase was deionized water (HPLC grade, flow-rate: 0.4 mL/min).

### 2.7. Chain length distribution of amylopectin in NS and NDS

The amylopectin chain length (CL) distribution of NS and NDS was analyzed using the High-Performance Anion-Exchange Chromatography Coupled with Pulsed Electrochemical Detection (HPAEC-PAD) system. Solutions of 1% NS and NDS were prepared as described in section 2.6, followed by the addition of 1 U/g isoamylase starch (megazyme), and incubation for 2 h at 40 °C. The debranched starch solution was boiled for 10 min, filtered with a 0.45-mm syringe filter, and injected into the HPAEC-PAD system. The DX-500 system (Dionex, Sunnyvale, CA, USA) was equipped with a pulsed amperometric detector (ED40, Dionex) using a CarboPOac PA-1 column (4 × 250 mm, Dionex). The mobile phase was an aqueous solution of 50 mM sodium hydroxide and 600 mM sodium acetate, and the flow rate was 1.0 mL/min.

### 2.8. X-ray diffractometry (XRD) of NS and NDS

XRD analyses of NS and NDS were performed using an X-ray diffractometer (D8 ADVANCE with DAVINCI, Bruker, Billerica, MA, USA)—detector: LYNXEYE XE, generator: 40 kV, 40 mA - (2θ) 3–50°, scanning speed: 5.0 s/step, wavelength (λ): Cu Kα1 – 1.5418 Å.

## 2.9. In vitro digestion properties of NS

In vitro digestion of NS was analyzed following the method of Englyst et al. (Englyst, Kingman, & Cummings, 1992b). A total of 30 mg NS was incubated in 1.5 mL enzyme solution (10 mM sodium acetate buffer, pH 5.2, pancreatic  $\alpha$ -amylase (PPA; P7545, Sigma Aldrich, St. Louis, MO, USA), and amyloglucosidase (AMG; Novozymes, Bagsværd, Denmark)), prepared immediately before use. Briefly, 2 g PPA was added to 24 mL distilled water, and the mixture was stirred for 10 min. An aliquot of 20 mL supernatant was removed, and 3.6 mL distilled water and 0.4 mL AMG were added, followed by stirring at 37 °C for 10 min. After the digestion was complete, glucose content was determined as described by Cai et al. (2015).

## 2.10. Pasting properties of NS and NDS

The pasting properties of NS and NDS were analyzed using a Rapid Visco-Analyzer (RVA-4, Newport Scientific, Warriewood, Australia). The samples were prepared as described by Hong, Gomand, & Delcour (2015).

## 2.11. Statistical analysis

The data were statistically analyzed by one-way ANOVA and Duncan's multiple range test using SAS 9.2 (SAS Institute, Cary, NC, USA). A value of  $p < 0.05$  was considered statistically significant.

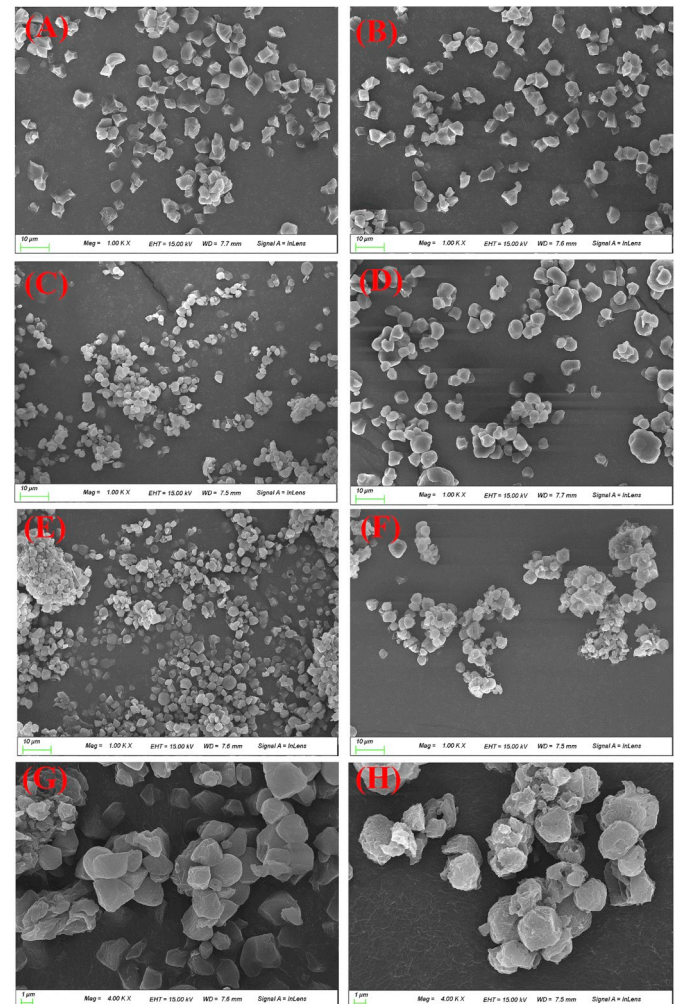
## 3. Results and discussion

### 3.1. Proximate composition of rice samples

Four rice cultivars with different amylose contents were used for proximate analysis of the nutritional factors of rice, including moisture, ash, fat, crude protein, and starch. The results of these chemical composition analyses are shown in Table 1. The apparent amylose contents of the rice flours in this study ranged from 4.5% to 40.6%. The values for BOC, IM, MM, and DDS were 4.5%, 20.6%, 27.6%, and 40.6%, respectively. The amylopectin chains of BOC interacted with iodine, which resulted in a higher apparent amylose content than that of most waxy rice cultivars. The cultivars in this study were categorized as follows: IM, intermediate; BOC, waxy; MM and DDS, high (Juliano, 1985; Vlachos & Arvanitoyannis, 2008).

The moisture contents of DDS and MM, the high-amylose rice cultivars, were higher than those of the waxy and intermediate rice cultivars. The fat contents of BOC, IM, MM, and DDS were 0.45%, 0.41%, 0.30%, and 1.17%, respectively. The fat content of DDS rice flour was significantly higher than that of the other cultivars, while the total starch content was lower. Starch content was defined as both RS and soluble starch. DDS rice flour showed an RS content of 10.84%, while the other cultivars contained <1% RS. The content of soluble starch that was broken down easily by hydrolytic enzymes was 81.04%, 78.57%, 78.11%, and 65.22% for IM, MM, BOC, and DDS, respectively. The total starch content of the four kinds of rice flour ranged from 76.06% to 81.66%, with DDS having the lowest total starch content, and the

highest contents of moisture, crude fat, crude protein, and ash. These results suggest that the starch composition of DDS was different from that of the other three rice cultivars. According to Zhu et al. (2011), the protein contents of medium-amylose and high-amylose rice are higher than those of waxy or low-amylose rice; the ash content is also directly proportional to the amylose content. In the case of fat content, higher values were recorded in mutated high-amylose corn (Pérez, Baldwin, & Gallant, 2009, pp. 149–192); long-chain amylose has shown a closer relationship with fat content than the shorter-chain amylopectin.



**Fig. 1.** Scanning electron micrographs of native starch (NS) from the Ilmi (IM) (A), Baegokchal (BOC) (B), Mimyeon (MM) (C), and Dodamssal (DDS) (D) rice cultivars, as well as non-digestible starch (NDS) from MM (E) and DDS (F) at 1000 $\times$  magnification. NDS from MM and DDS are shown in (G) and (H), respectively, at 4000 $\times$  magnification.

**Table 1**

Proximate composition of rice flour obtained from four different cultivars.

Sample	Apparent amylose content (%)	Moisture (%)	Crude Protein (%)	Fat (%)	Ash (%)	Starch content (%)		
						RS	SS	TS
Baegokchal (BOC)	4.5 $\pm$ 0.1 <sup>d1</sup>	10.22 $\pm$ 0.02 <sup>d</sup>	5.66 $\pm$ 0.07 <sup>c</sup>	0.45 $\pm$ 0.10 <sup>b</sup>	0.34 $\pm$ 0.00 <sup>c</sup>	0.18 $\pm$ 0.01 <sup>c</sup>	78.11 $\pm$ 0.78 <sup>a</sup>	78.47 $\pm$ 0.80 <sup>ab</sup>
Ilmi (IM)	20.6 $\pm$ 0.7 <sup>c</sup>	10.72 $\pm$ 0.07 <sup>c</sup>	5.87 $\pm$ 0.02 <sup>c</sup>	0.41 $\pm$ 0.04 <sup>b</sup>	0.35 $\pm$ 0.00 <sup>c</sup>	0.31 $\pm$ 0.04 <sup>bc</sup>	81.04 $\pm$ 1.82 <sup>a</sup>	81.66 $\pm$ 1.86 <sup>a</sup>
Mimyeon (MM)	27.6 $\pm$ 0.5 <sup>b</sup>	11.78 $\pm$ 0.11 <sup>b</sup>	6.50 $\pm$ 0.01 <sup>b</sup>	0.30 $\pm$ 0.06 <sup>b</sup>	0.43 $\pm$ 0.00 <sup>b</sup>	0.44 $\pm$ 0.11 <sup>b</sup>	78.57 $\pm$ 0.29 <sup>a</sup>	79.01 $\pm$ 0.41 <sup>ab</sup>
Dodamssal (DDS)	40.6 $\pm$ 0.4 <sup>a</sup>	13.22 $\pm$ 0.12 <sup>a</sup>	7.17 $\pm$ 0.02 <sup>a</sup>	1.17 $\pm$ 0.12 <sup>a</sup>	0.72 $\pm$ 0.00 <sup>a</sup>	10.84 $\pm$ 0.14 <sup>a</sup>	65.22 $\pm$ 3.02 <sup>b</sup>	76.06 $\pm$ 3.17 <sup>b</sup>

<sup>1</sup> Values with different letters (a–c) within a column are significantly different ( $P < 0.05$ ) by Duncan's multiple range test.

RS: resistant starch; SS: soluble starch; TS: total starch. Data are shown as the mean  $\pm$  SD from three independent experiments.



### 3.2. Microscopic observation of NS and NDS

Scanning electron micrographs of NS and NDS are presented in Fig. 1. Starch is composed of individual particles called granules. The granules from the four cultivars is displayed in Fig. 1A–D. It was found that the size and shape of rice starch particle structure varied according to the cultivar (Wani et al., 2012). While the starch granule sizes in the four rice cultivars in Fig. 1A–D were mostly below 10  $\mu\text{m}$ , their particle size and morphology varied. The largest variation in starch granule size was observed in DDS. Waxy rice, BOC (Fig. 1A), and non-waxy rice, IM (Fig. 1B), containing intermediate amylose showed similar sizes and concave polygons. Among the four varieties, MM (Fig. 1C), a high-amylose variety, had irregular but generally smaller starch size than other varieties. MM also showed flat or slightly concave polyhedrons and some convex or round polyhedrons. The granules from the DDS cultivar with a high RS content (Fig. 1D) were aggregated and appeared as larger lumps; there were many round starch particles in addition to the convex polygonal starch granules.

While both BOC and IM were hydrolyzed not to obtain NDS, photomicrographs of NDS particles from MM and DDS, high amylose rice cultivar, are shown in Fig. 1E and F; the NDS residues were lumpy and smaller than those of NS (Fig. 1A–D). The NDS from MM and DDS (Fig. 1E and F) was mainly composed of soft polygons and rounded starch granules, in contrast to the convex polygonal and irregular angled starch particles observed in Fig. 1C and D. The NDS residues of MM were smaller than the NDS residues from DDS. The NDS residues of DDS, which showed the lowest degree of hydrolysis by enzymes, are shown in Fig. 1F. Starch granules appeared as lumps, and thin membranes between the starch granules, and attached to the granules, were observed. Starch granules contain fat, protein, minerals, and moisture, as well as amylose and amylopectin, which are  $\alpha$ -glucans (Amagliani et al., 2016). The combination of starch and lipids reduces the effectiveness of enzymatic hydrolysis (Crowe, Seligman, & Copeland, 2000). In this study, NDS content was the highest in DDS, which also had a high fat content.

The hydrolyzed starch patterns were similar regardless of granule size or crystallinity. However, the degree of hydrolysis is not uniform among the starch particles, and small starch particles show high digestibility (Dhital, Butardo Jr, Jobling, & Gidley, 2015). In this study, MM with small starch particles had a higher digestibility than DDS, and thus the amount of NDS remaining after hydrolysis was small (Table 3). The granule surface of NDS remaining after hydrolysis in MM and DDS is shown in Fig. 1G and H at 4000 $\times$  magnification. The granule surface was rugged, and holes were observed. The surface of starch is damaged by hydrolase, and the hole is connected to the interior channels. The action of  $\alpha$ -amylase continues in the internal space, and hydrolysis progresses from the inside to the outside, causing the starch to be split

**Table 3**

Starch classification and content in four rice cultivars according to digestion rate.

Sample	RDS (%)	SDS (%)	RS (%)	NDS residues (%)
Baegokchal (BOC)	61.31 $\pm$ 4.30 <sup>a1</sup>	35.29 $\pm$ 3.31 <sup>c</sup>	3.39 $\pm$ 0.99 <sup>c</sup>	n. d. <sup>c</sup>
Ilmi (IM)	47.21 $\pm$ 2.64 <sup>b</sup>	48.49 $\pm$ 3.69 <sup>b</sup>	4.30 $\pm$ 1.63 <sup>c</sup>	tr <sup>c6</sup>
Mimyoon(MM)	24.30 $\pm$ 0.22 <sup>c</sup>	65.84 $\pm$ 2.67 <sup>a</sup>	9.86 $\pm$ 2.60 <sup>b</sup>	2.76 $\pm$ 0.59 <sup>b</sup>
Dodamssal (DDS)	20.54 $\pm$ 1.13 <sup>c</sup>	61.24 $\pm$ 3.93 <sup>a</sup>	18.22 $\pm$ 2.92 <sup>a</sup>	16.67 $\pm$ 1.28 <sup>a</sup>

<sup>1</sup> Values with different letters (a–c) within a column are significantly different ( $P < 0.05$ ) by Duncan's multiple range test.

RDS: rapidly digestible starch, hydrolyzed within 20 min. SDS: slowly digestible starch, hydrolyzed within 20–120 min; RS: resistant starch, not hydrolyzed after 120 min; NDS: non-digestible starch; Tr: trace; n.d: not detected. Data are shown as the mean  $\pm$  SD from three independent experiments.

and decomposed (J. Li, Vasanathan, Hoover, & Rossnagel, 2004). The hydrolyzed starch surface of DDS (Fig. 1H) was rougher and had many holes compared to that of MM (Fig. 1G). It was assumed that the granule size of NDS was much smaller than that of NS of DDS because the starch particles were broken by hydrolysis through the internal channel connected to the pore. Fig. 1D shows many subgranules. The rice starch with A-type crystallinity is easily hydrolyzed overall. The rice starch with C-type crystallinity has a subgranule of A-type crystallinity and a surrounding region with granules of B-type crystallinity. Thus, the peripheral region having B-type granules has higher resistance to hydrolase (Man et al., 2014). The yield of NDS of DDS was higher than that of MM, but a hydrolyzed surface was observed more frequently in DDS than in MM. However, many granules in the internal region, which have A-type crystallinity of DDS, were hydrolyzed. In conclusion, the relative crystallinity of DNS was more greatly reduced than that of MM compared to NS of DDS (Table 2). In the case of MM with A-type crystallinity, NDS had less surface damage or pores than NDS of DDS, which should be further investigated.

### 3.3. Amylose content of NS and NDS

Table 2 shows the amylose content of NS and NDS in four cultivars, including the changes in amylose content before and after starch-hydrolyzing enzyme treatment. With the exception of BOC, the measured amylose content of NS was 2–10% higher than that of the rice flours before enzyme treatment (Table 1). Rice flour contains other macromolecules in addition to starch. In particular, DDS was higher in

**Table 2**

Amylose content, amylopectin chain length distribution, average chain length (CL) and relative crystallinity of native starch (NS) and non-digestible starch (NDS) obtained from four rice cultivars.

Cultivar	Type of starch	Amylose content(%)		Amylopectin chain length distribution (%)					Average CL (DP)	Relative crystallinity (%)
		Apparent	Con A method	DP < 6	DP 6–12	DP 13–24	DP 25–36	DP $\geq$ 37		
BOC	NS	4.4 $\pm$ 0.1 <sup>f</sup>	2.0 $\pm$ 0.2 <sup>f</sup>	n.d.	35.5 $\pm$ 0.3 <sup>a</sup>	47.5 $\pm$ 0.5 <sup>c</sup>	9.8 $\pm$ 0.2 <sup>d</sup>	7.3 $\pm$ 0.2 <sup>d</sup>	17.7 <sup>c</sup>	42.2 $\pm$ 0.7 <sup>a1</sup>
IM	NS	22.8 $\pm$ 0.3 <sup>e</sup>	20.8 $\pm$ 0.1 <sup>e</sup>	n.d. <sup>2</sup>	35.6 $\pm$ 0.4 <sup>a</sup>	47.9 $\pm$ 0.3 <sup>c</sup>	9 $\pm$ 0.1 <sup>d</sup>	7.5 $\pm$ 0.1 <sup>d</sup>	17.7 <sup>c</sup>	39.5 $\pm$ 0.1 <sup>b</sup>
MM	NS	29.4 $\pm$ 0.1 <sup>c</sup>	25 $\pm$ 0.2 <sup>d</sup>	n.d.	30.7 $\pm$ 0.2 <sup>b</sup>	52.6 $\pm$ 0.2 <sup>b</sup>	9.1 $\pm$ 0.2 <sup>d</sup>	7.6 $\pm$ 0.2 <sup>d</sup>	18.1 <sup>c</sup>	40.0 $\pm$ 0.8 <sup>b</sup>
DDS	NS	51.7 $\pm$ 1.1 <sup>a</sup>	44.7 $\pm$ 0.5 <sup>b</sup>	n.d.	22.9 $\pm$ 0.1 <sup>c</sup>	54.6 $\pm$ 0.3 <sup>a</sup>	11.1 $\pm$ 0.1 <sup>c</sup>	11.4 $\pm$ 0.1 <sup>c</sup>	20.3 <sup>b</sup>	31.5 $\pm$ 1.2 <sup>d</sup>
MM	NDS	24.0 $\pm$ 0.2 <sup>d</sup>	28.4 $\pm$ 0.1 <sup>c</sup>	5.4 $\pm$ 0.1 <sup>a</sup>	20.3 $\pm$ 0.2 <sup>d</sup>	47.9 $\pm$ 0.2 <sup>c</sup>	14.1 $\pm$ 0.3 <sup>b</sup>	12.3 $\pm$ 0.2 <sup>b</sup>	20.3 <sup>b</sup>	36.8 $\pm$ 0.1 <sup>c</sup>
DDS	NDS	34.3 $\pm$ 0.9 <sup>b</sup>	63.3 $\pm$ 0.3 <sup>a</sup>	2.1 $\pm$ 0.1 <sup>b</sup>	12.7 $\pm$ 0.2 <sup>e</sup>	55.2 $\pm$ 0.4 <sup>a</sup>	16.0 $\pm$ 0.2 <sup>a</sup>	14.0 $\pm$ 0.3 <sup>a</sup>	23.0 <sup>a</sup>	26.9 $\pm$ 0.1 <sup>e</sup>

<sup>1</sup> Values with different letters (a–f) within a column are significantly different ( $P < 0.05$ ) by Duncan's multiple range test.

<sup>2</sup> n.d.: not detected. Values are shown as the mean  $\pm$  SD from three independent experiments.

fat content than the other rice cultivars, which resulted in a greater difference in the apparent amylose content of rice flour and rice starch.

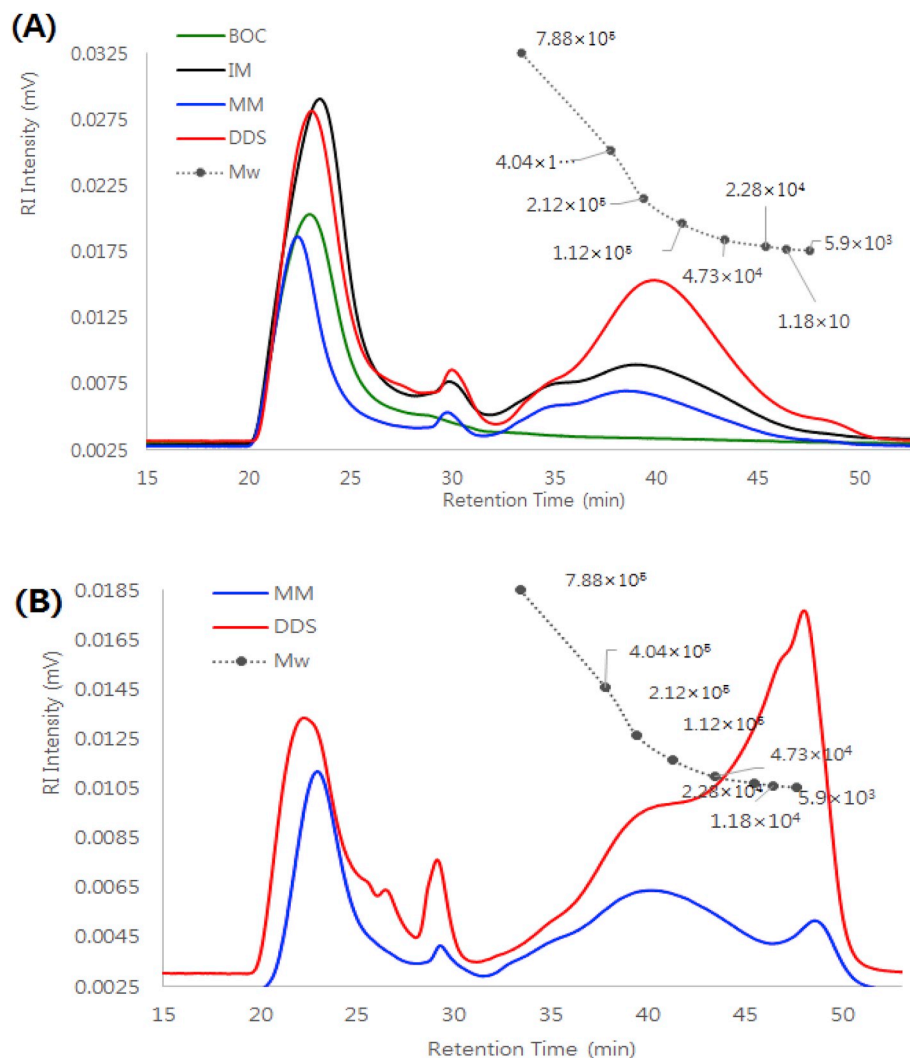
However, the apparent amylose content of NDS from MM and DDS was 5.4% and 17.4%, lower than that of NS. Unlike the margin for error of the colorimetric method before hydrolysis, which generally overestimates amylose content due to the formation of complexes between iodine and long amylopectin chains, the remaining linear chains after hydrolysis were too short to form an iodine complex and could not be detected by iodine bonding (S. Wang, Blazek, Gilbert, & Copeland, 2012).

Complicating the hypothesis that NDS would be higher in amylose than NS, the structure of hydrolyzed starch affects the detection of amylose content. For that reason, the values obtained here were compared with those obtained from other analytical methods. Analysis of amylose using the Concanavalin A (Con A) method showed that the amylose contents of NS from BOC, IM, MM, and DDS were 2%, 20.8%, 25%, and 44.7%, respectively—at least 2% lower than the values found by the iodine-binding method (4–7% lower for high-amylose cultivars). On the other hand, the amylose contents of NDS from MM and DDS were 28.4% and 63.3%, respectively—4% and 29% higher than those measured by the iodine-binding method, respectively. In this Con A precipitation method, amylose content was high due to removal of amylopectin side branch by hydrolysis, which is similar to other study (S. Wang & Copeland, 2015). The hydrolyzed amylopectin was transformed into a structure that could not bind Con A, and therefore the amylose

content was overestimated. Therefore, in the Con A precipitation method, NDS of MM and DDS showed lower amylopectin and higher amylose content than NS.

#### 3.4. Molecular weight distribution of NS and NDS

NS MW distribution of four rice cultivars with different amylose contents, as well as the NDS MW distribution of MM and DDS, are shown in Fig. 2 as RI profiles. The first peak in Fig. 2A shows amylopectin, the second shows intermediate materials present between amylopectin and amylose, and the third shows amylose at a MW of  $5.9 \times 10^3$ – $7.88 \times 10^5$  g/mol, the molecular weight of the pullulan standard. In a previous study, the weight-average MW of amylose content in six rice cultivars analyzed by scattering and refractive index detectors was  $5.1$ – $6.9 \times 10^5$  g/mol (Chen & Bergman, 2007). The amylose content of rice starch for the cultivars investigated here peaked at  $1.12$ – $2.12 \times 10^5$  g/mol and showed a somewhat lower MW. BOC is a glutinous rice cultivar, and only one amylopectin peak was found in the RI profile from this cultivar, while no amylose peaks were observed; furthermore, the intensity of the RI peak for amylose from DDS was higher than those of IM and MM. The molecular weight of NDS from MM and DDS, which was lower than the molecular weight of NS (Fig. 2A), is shown in Fig. 2B. The peak of NDS from DDS showed MW  $5.9 \times 10^3$ – $1.18 \times 10^4$  g/mol, and a new peak of MW  $\leq 5.9 \times 10^3$  g/mol, which was not identified in NS, was observed in NDS from MM. It was found that the MW decreased after treating NS



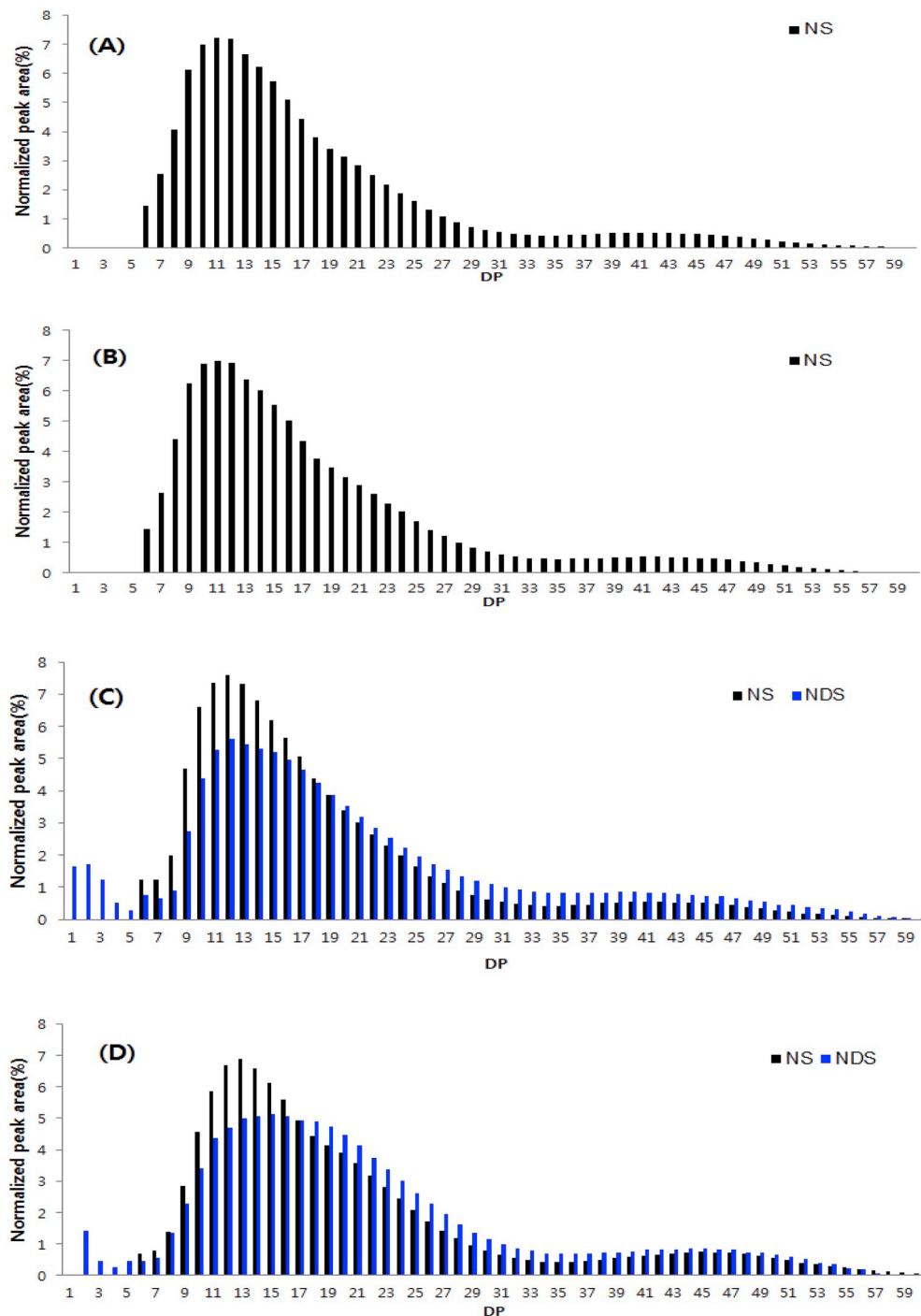
**Fig. 2.** HPSEC chromatogram of rice starches obtained from four different cultivars (A) and non-digestible starch (NDS) residues from Dodamssal (DDS) and Mimyeon (MM) (B). IM: Ilmi; BOC: Baegokchal; Mw: average molecular weight (g/mol).

from DDS and MM with digestive enzymes.

RS exists in the form of a linear chain of  $\alpha$ -1,4-o-glucan molecules. In previous studies, the MW of RS (RS3 type) obtained from the retrograded amylose layer was reported to be relatively low, at  $1.2 \times 10^5$  g/mol (Tharanath, 2002). The starch used in this study was NS (RS2 type), and the result was the same as that observed in retrograded resistant starch, although the type of starch differed. RS isolated from the NS of high-amylose rice is shown in Fig. 2B, and showed a peak at MW  $1.2 \times 10^5$  g/mol, as mentioned in the previous study, and the molecular weight of NDS from DDS was lower than that of RS3.

### 3.5. Amylopectin CL distribution in NS and NDS

Normalized chromatograms of the amylopectin CL distribution of NS and NDS from BOC, IM, MM, and DDS is shown in Fig. 3. The normalized peak area (%) of DP6-18 and DP6-17 of NS from MM (Fig. 3C) and DDS (Fig. 3D) was higher than that of DP6-18 and DP6-17 from NDS. The normalized peak area (%) of DP > 18 and DP > 17 of NDS from MM and DDS was high compared to that of NS. The CL of amylopectin is classified according to the type of chain: A chains (DP 6–12), B1 chains (DP 13–24), B2 chains (DP 25–36), and B3 chains (DP > 37) (Hanashiro, Abe, & Hizukuri, 1996). A chains are the shortest and outermost chains, and they are connected to B chains by  $\alpha$ -1,6 bonds; B chains are divided



**Fig. 3.** HPAEC-PAD profiles of native starch (NS, black) and non-digestible starch (NDS, blue) obtained from the Ilmi (IM) (A), Baegokchal (BOC) (B), Mimyeon (MM) (C), and Dodamssal (DDS) (D) rice cultivars.

into B1, B2, and B3 according to the number and length of the connected cluster (Donald, 2004). The peak area ratios of A, B1, B2, and B3 chains are shown in Table 3, and glucans with  $DP \leq 60$  were analyzed with the HPAEC-PAD system. IM, which displayed an intermediate amylose content, showed a statistically non-significant distribution of amylopectin CL compared to the glutinous rice cultivar BOC. However, the NS of the high-amylose cultivars MM and DDS showed a lower A chain and higher B1 chain content. B2 and B3 chains were more abundant in DDS than in the other rice cultivars. It has been reported that A and B1 chains form a single cluster, while B2, and B3 chains form two, three, or more clusters (Wani et al., 2012). According to previous studies (Man et al., 2014), high-amylose rice starch has the lowest content of short branch-chains, and the lowest amylopectin branching degree. In the present study, amylopectin A chains from the high-amylose DDS were the least abundant, but a high percentage of long chains ( $DP > 13$ ) were identified, as well as high B2 and B3 ratios within more than two clusters.

As shown in Table 2, NDS from MM and DDS contained 5.4% and 2.1% of short-chains ( $DP < 6$ ), respectively; these were not present in NS. The average CL value of amylopectin was previously reported to be DP 17–24 in rice starch (Morrison & Karkalas, 1990). In this study, the amylopectin CL of NS from BOC, IM, MM, and DDS was 17.7, 17.7, 18.1, and 20.3, respectively, consistent with previous studies. The average CL value of amylopectin in NDS from MM and DDS was 20.3 and 23.0, respectively, higher than that in NS.

### 3.6. Crystal structure of NS and NDS

The crystal structure of four NS and two NDS isolated from rice cultivars with different amylose contents were investigated by XRD analysis, as shown in Fig. 4. Starches are classified into A-, B-, and C-types, the latter being a hybrid of types A and B based on their XRD patterns. In A-type starch, unseparated doublets were shown at 17 and

18° 2 $\theta$ , and strong diffraction peaks were observed at 15° and 23° 2 $\theta$ . B-type starch had a characteristic peak near 5.6° 2 $\theta$ , one distinct peak at 17° 2 $\theta$ , and small peaks at 15°, 20°, 22°, and 24° 2 $\theta$ . C-type starch was classified into CA-type, which was close to A-type, typical C-type, and CB-type, which was close to B-type, depending on the ratio of A-type and B-type polymorphs. Typical C-type starches had strong diffraction peaks at 17° and 23° 2 $\theta$ , which are characteristic of A-type, and small peaks at 5.6° and 15° 2 $\theta$ , which are characteristic of B-type starch. CA-type starch showed a small shoulder peak at 18° 2 $\theta$  and strong and distinct peaks at 15° and 23° 2 $\theta$ , similar to the A-type starch crystal pattern. However, CB-type starch showed peaks at 22° and 24° 2 $\theta$  and a weak peak at 15° 2 $\theta$ , which is much stronger in A-type starch (Cheetham & Tao, 1998; Man et al., 2012).

A-type XRD patterns for BOC, IM, and MM, but not DDS, were observed, with strong reflections at approximately 15° and 23° 2 $\theta$ , and an unresolved doublet at 17° and 18° 2 $\theta$  (Fig. 2A). These results are consistent with previous studies showing A-type starch patterns, such as waxy rice and normal starch rice cultivars (Cai et al., 2015; Chung et al., 2011). The NS of DDS had a strong peak at 17° 2 $\theta$  and small peaks around 5.6°, 15°, 20°, and 23° 2 $\theta$ . One of the crossing parents of DDS, Goami 2 (known as S-464 in terms of rice line), had a B-type XRD pattern, which showed a peak similar to the NS diffraction pattern of DDS. There was a slight difference, though, in that a very small shoulder peak was observed at 24° 2 $\theta$  where the 22–23° 2 $\theta$  peak ended (Kang et al., 2003). Other studies also showed a small diffraction peak at 24° 2 $\theta$  in Goami 2, and rice varieties such as Goami 3 and Goami 4, which have been developed since then, have also been reported as of B-type (Yoon et al., 2013). DDS flour had a weak shoulder peak at 24° 2 $\theta$ , where the peak at 22–23° 2 $\theta$  ended, as shown in the starch XRD pattern in Goami 2 (data not shown). However, in terms of the starch pattern of DDS, the diffraction peak at 20° 2 $\theta$  in an amylose and lipid complex is connected to a broad peak at 22–23° 2 $\theta$ . Therefore, the starch in this study was of C-type, characterized by broad peaks at 22–23° 2 $\theta$ , but it did not show a shoulder peak at 18° 2 $\theta$ . Thus, it was not considered as CA-type. On the other hand, although two weak peaks at 22° and 24° 2 $\theta$ , which are reported to be present in CB-type, were not observed, the starch was determined to be a C-type starch with a dominant B-type based on the B-type characteristic peak at 5.6° 2 $\theta$  and the weak peak at 15° 2 $\theta$ . In China, a transgenic rice line was produced by inhibiting the expression of starch branching enzyme (SBE) in Te-qing, a wild-type Indica rice cultivar. Thus, the study of high amylose C-type starch containing 14% of resistant starches was carried out first. This starch is a CA-type starch with a strong peak at 17° 2 $\theta$  and a shoulder peak at 18° 2 $\theta$ , and a peak at 23° 2 $\theta$  was broken into 22° and 24° 2 $\theta$  peaks through acid hydrolysis and germination process, changing the XRD pattern (Pan, Lin, Wang, Liu, & Wei, 2018; Wei et al., 2010). In our study, the XRD pattern of DDS starch did not change from C- to B-type after a 16-h enzymatic hydrolysis, thereby requiring further studies.

In addition, no significant differences in XRD pattern were observed for NS and NDS from MM, consistent with previous studies showing that the A-type pattern does not change during digestion (Huang et al., 2016). The crystallinity of NS and NDS from MM was 40.0% and 36.8%, respectively. Although there were no differences between the NS and NDS XRD patterns within DDS rice, the crystallinity of the remaining NDS after the hydrolysis of NS was reduced by 4.6%, from 31.5% to 26.9%. In the present study, the crystallinity of BOC and IM were 42.2% and 39.5%, respectively, and those of the high-amylose MM and DDS cultivars were 40.0% and 31.5%, respectively. The amylopectin branching cluster forms a crystalline region in rice starch, whereas the amylopectin branching point and amylose form amorphous regions (Amagliani et al., 2016). Starch crystallinity was highest in BOC, which was mainly amylopectin, and lowest in DDS with high amylose rice and C<sub>B</sub>-type pattern. Although amylose content and crystallinity were not significantly correlated in different amylose rice cultivars, amylose and crystal pattern had a significant effect on the crystallinity of high-amylose starch. Similar results were obtained in previous studies

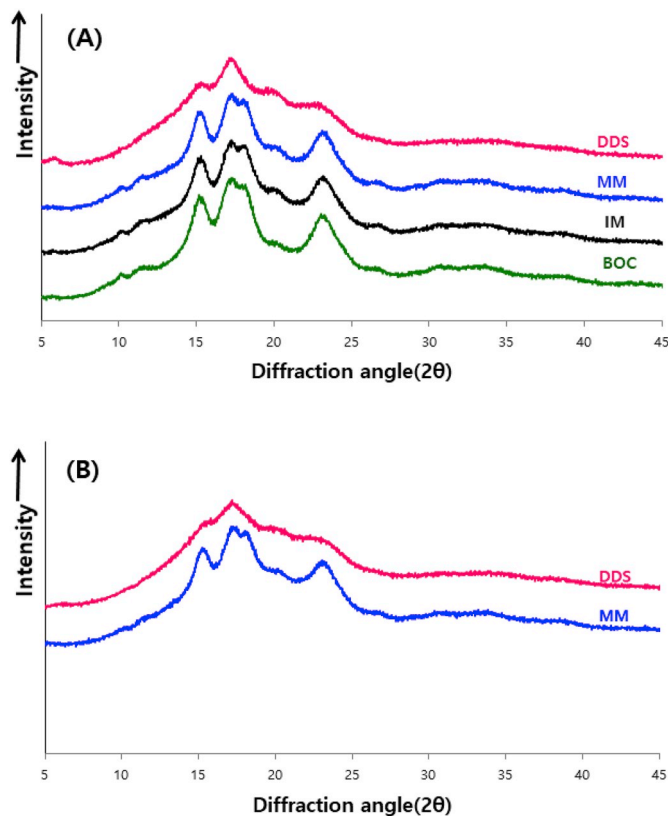


Fig. 4. X-ray diffraction pattern of native starch (NS) (A) and non-digestible starch (NDS) (B). IM: Ilmi, BOC: Baegokchal; MM: Mimyeon; DDS: Dodamssal.



(Tester, Karkalas, & Qi, 2004; Zhu et al., 2011).

### 3.7. *In vitro* digestion properties of NS and relative yield of NDS

Starch was isolated from four rice cultivars by the alkaline extraction method. The extracted starch was treated with hydrolysis digestion enzymes to classify it according to digestion rate and to investigate its hydrolytic characteristics (Table 3). The proportion of RDS (digested within 20 min) was 20.54–61.31%; higher amylose content was associated with a lower proportion of RDS.

Analysis of waxy and normal starch digestion rates showed that the range of RDS content in IM and BOC was similar to that found in previous studies. BOC had a higher RDS content than that of IM, which is in line with the finding that waxy starch has a higher digestion rate than normal starch owing to hydrolases (Chung et al., 2011; Srichuwong & Jane, 2007). Amylopectin chain length distribution was also reported to affect RDS, and in our study, MM and DDS, both of which are high-amylose rice with a small percentage of short chains and a large percentage of long chains, had low RDS content. However, the fact that BOC has a greater RDS content than that of IM, which had similar average amylopectin chain length (Table 2), shows that IM consists of both intermediate components and amylose, whereas BOC only consists of amylopectin (Fig. 3A). The long chains of amylose and amylopectin have been reported to increase resistance to hydrolysis by strengthening the crystal by forming a double helix, and the results were in line with that of Lin, Zhang, Zhang, and Wei (2017) that RDS is negatively correlated with intermediate component peak and amylose. Furthermore, previous studies showing a high percentage of long chain amylose in high-amylose rice confirmed that waxy rice varieties have a low branched amylose content (H. Li & Liu, 2019). Thus, RDS content in BOC and IM is presumed to have been influenced not only by amylose content but also by molecular structure, such as branched amylose length.

The content of SDS (digested within 20–120 min) was 35.29–65.84%, and was highest for MM, followed by DDS, IM, and BOC, in that order. MM had a higher SDS content than that of DDS but not to a statistically significant extent, and high-amylose rice varieties had significantly higher SDS content than that of normal and waxy rice. One study reported that amylose and RS are positively correlated. The content of RS, which did not digest within 120 min, was the highest for DDS, followed by MM, IM, and BOC, in that order.

Lin et al. (2016) discovered that amylose and amylopectin chain lengths are positively correlated in high-amylose varieties, where hydrolysis decreases with increasing amylopectin chain length, thus resulting in a greater RS content (Lin et al., 2017, 2019; Zhu et al., 2011).

In our study, IM had a greater RS content compared to BOC, though statistically insignificant, but RS content was significantly greater in high-amylose rice compared to that in normal rice and waxy rice, similar to previous findings (Table 3). On the other hand, starch granules with B- or C-type XRD patterns were reported to have greater resistance to hydrolytic enzymes compared to those with A-type pattern (Pan et al., 2018; Wei et al., 2010). In our study, the higher RS content in DDS than in MM is believed to have been influenced by various factors, including high amylose content, percentage of long branch-chain of amylopectin, dominance of B-type starch crystallinity, and amylose fatty acid complex (Table 2, Fig. 2A). All starches in IM and BOC were hydrolyzed, with no NDS residues remaining; in contrast, 2.76 g and 16.67 g NDS were obtained from 100 g NS from MM and DDS, respectively.

Zhu et al. (2011) reported that high-amylose rice have low crystallinity, which in turn leads to high RS content; they stated that amylose molecules in the amorphous regions are hydrolyzed and the resulting smaller molecules have resistance to zymolysis. In support of this, we found in the present study that DDS, which has the lowest starch crystallinity, had the lowest hydrolysis rate. In conclusion, we observed that the starch digestibility of four cultivars in relation to their RDS, SDS, and RS ratios were affected by amylose content and the ratio of short chain

and long chain of amylopectin, whereas the relative crystallinity did not appear to be relevant.

In particular, differences were observed in starch crystallinity, average chain length, starch particle size, and shape of high-amylose MM and DDS rice starch. Even as powdered rice, the high-amylose DDS cultivar with low digestibility and high RS showed higher values of components that influence starch digestion, such as fat, ash, and protein contents, than other cultivars, while showing lower starch content. The properties of extracted starch were larger relative granule size, C-type crystallinity, longer average chain length, smaller short-chain ratio, and higher long-chain ratio. As the NDS of MM and DDS is hydrolyzed starch, amylose content was measured to be lower than that of NS and the short chain was even shorter owing to hydrolysis. However, NDS showed higher ratio of amylopectin long chain and average chain length. Therefore, it is believed to be a major factor in low digestibility.

### 3.8. Pasting properties

The pasting properties of starch are the main factor affecting the taste and quality of rice and processed foods; this includes amylose content and amylopectin structure (Pang et al., 2016). It was therefore important to identify these characteristics using a rapid viscosity analyzer in order to apply our findings to the food industry.

Table 4 and Fig. 5 show the gelatinization characteristics of NS and NDS from different rice cultivars. The peak viscosity (PV) and through viscosity (TV) of MM, a high-amylose rice cultivar, were the highest. In addition, for BOC, IM, and DDS, in that order, lower amylose content was associated with higher breakdown values of PV and TV. BOC, which displayed high amylopectin content, had a high viscosity. The high amylose content in the DDS cultivar seemed to be related to its low viscosity.

In rice varieties with similar amylose content, the higher the proportion of long chains of amylopectin, the lower the viscosity. PV and TV of low amylose varieties are high when the ratio of long to short branch-chains in amylopectin is similar (Lin et al., 2019). Low viscosity may have resulted from the ratio of long to short chains in amylopectin of DDS in this study. There was no difference in amylopectin CL distribution between BOD and IM (Table 2). However, high PV, TV, and BD and low FV and SB are thought to be related to amylose content. Furthermore, high-amylose varieties have high gelatinization temperature owing to the low percentage of short chain amylopectin, and the amylopectin microstructure as well as amylose content were found to influence the viscoelasticity of rice starch (Takahashi & Fujita, 2017). In the present study, the low percentage of short-chain amylopectin in DDS, a high-amylose rice variety, was in line with the gelatinization properties.

Previous research has shown that Indica rice requires a high temperature for gelatinization, PV, and TV, with a higher final viscosity and breakdown result than Japonica Rice (Kang, Hwang, Kim, & Choi, 2006). Thus, when the MM cultivars were developed, these exceptional characteristics were carried over by crossing the parent strains; the resultant cultivar was named Hyangmi2, and displays characteristics of both basmati and Indica rice. This suggests that MM is higher in PV and TV than in BOC and IM, even though the amylose content of MM is higher than that of BOC and IM. Additionally, Gonzalez and Pérez (2002) reported an inversely proportional relationship between margins and PV in high-amylose starch. However, in this study, MM starch granules with their higher relative crystallinity exhibit higher PV and TV values. Further, compared with starch granules of other grain types, smaller starch granules, lower fat content, hydration, and swelling are all believed to effect higher PV values. As shown in Table 2, the short chain in the region of DP6–24 shows a higher ratio with higher amylose grains. However, above DP25, the amylopectin chain length of MM did not show differences compared with BOC, IM but shows a lower ratio relative to DDS. It is believed that the long-chain ratio of amylopectin



**Table 4**

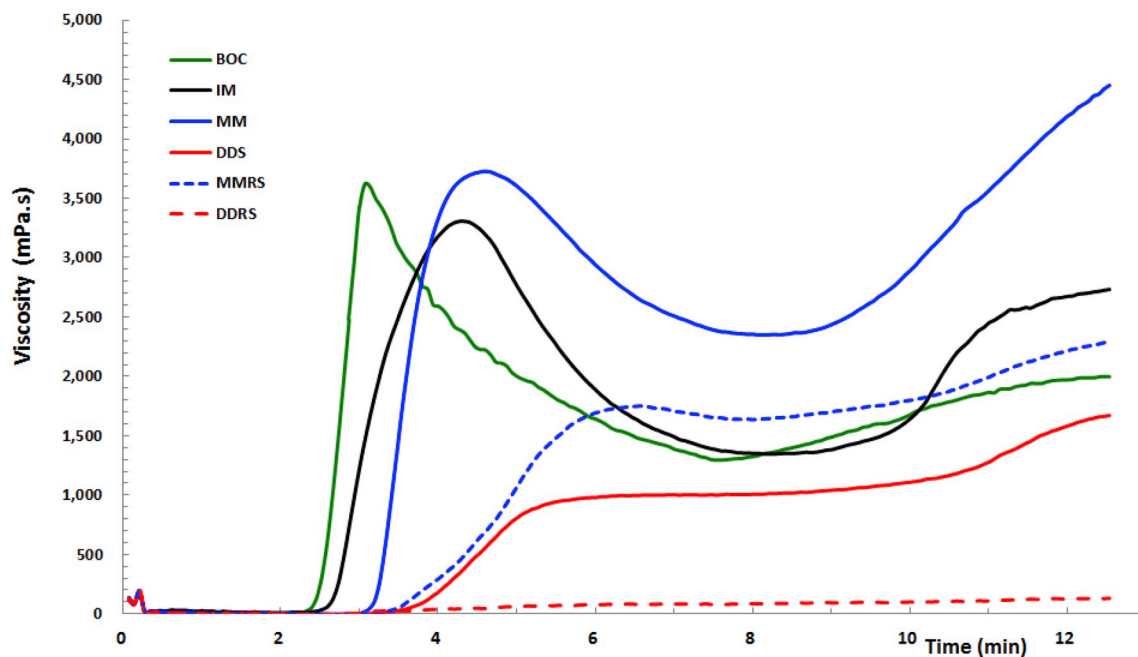
Pasting properties of native starch (NS) and non-digestible starch (NDS) obtained from four rice cultivars.

Cultivar	Type of starch	Viscosity (mPa.s)					PT (min)
		PV	TV	BD	FV	SB	
BOC	NS	3616 ± 6 <sup>b</sup>	1376 ± 115 <sup>c</sup>	2240 ± 120 <sup>a</sup>	2033 ± 52 <sup>d</sup>	-1583 ± 57 <sup>f</sup>	3.1 ± 0.0 <sup>e</sup>
IM	NS	3241 ± 87 <sup>c1</sup>	1317 ± 38 <sup>c</sup>	1923 ± 50 <sup>b</sup>	2702 ± 38 <sup>b</sup>	-539 ± 49 <sup>e</sup>	4.3 ± 0.0 <sup>d</sup>
MM	NS	4074 ± 38 <sup>a</sup>	2236 ± 49 <sup>a</sup>	1839 ± 11 <sup>b</sup>	4493 ± 66 <sup>a</sup>	418 ± 28 <sup>c</sup>	4.2 ± 0.0 <sup>d</sup>
DDS	NS	945 ± 80 <sup>e</sup>	942 ± 81 <sup>d</sup>	3 ± 1 <sup>c</sup>	1604 ± 90 <sup>e</sup>	658 ± 10 <sup>a</sup>	7.0 ± 0.0 <sup>a</sup>
MM	NDS	1747 ± 14 <sup>d</sup>	1631 ± 22 <sup>b</sup>	116 ± 14 <sup>c</sup>	2284 ± 17 <sup>c</sup>	536 ± 44 <sup>b</sup>	6.6 ± 0.0 <sup>b</sup>
DDS	NDS	82 ± 3 <sup>f</sup>	78 ± 2 <sup>e</sup>	4 ± 2 <sup>c</sup>	129 ± 4 <sup>f</sup>	48 ± 2 <sup>d</sup>	6.4 ± 0.0 <sup>c</sup>

<sup>1</sup> Values with different letters (a–e) within a column are significantly different ( $P < 0.05$ ).

mPa.s (millipascal-second): unit of rapid viscosity; PV: peak viscosity; TV: trough viscosity; BD: breakdown viscosity; FV: final viscosity; SB: setback viscosity; PT: peak time.

Data are shown as the mean ± SD from three independent experiments.

**Fig. 5.** RVA pasting profiles of native starch (NS) and non-digestible starch (NDS) obtained from four rice cultivars. IM: Ilmi; BOC: Baegokchal; MM: Mimyeon; DDS: Dodamssal.

has a greater influence on the pasting property than short-chain ratio. Fig. 5 shows that a higher amylose content was associated with a higher temperature of gelatinization. Table 4 shows that the peak time (PT) of BOC was the fastest, at 3.1 min, while DDS, which had the highest amylose content, took more than 7 min. The setback (SB) value was in the order of BOC < IM < MM < DDS, and higher amylose content was associated with a higher value. The gelatinization properties of NDS from MM and DDS showed lower PV, TV, BD, and FV values compared to those of NS. Consistent with previous research, in acid-hydrolyzed starches, viscosity was low because the low-MW dextrin did not swell with water, but rather dissolved (S. Wang & Copeland, 2015). In Fig. 3B, it can be seen that the results of this study were similar, as the MW was reduced by starch decomposition. The NDS gelatinization temperature of MM was 81.3 °C, which was higher than that of NS (75.8 °C). NDS from DDS rarely showed gelatinization under the experimental conditions.

#### 4. Conclusions

We compared the structural and physicochemical characteristics of four rice cultivars with different amylose contents by isolating NS. The rice cultivars with high amylose content showed low RDS, as well as high SDS and RS ratios. Additionally, we analyzed the structural

properties of NDS from two high-amylose cultivars to investigate the properties of RS. DDS starch with the highest amylose content showed a C type crystal pattern, a low relative crystallinity, a high content of RS, and a high ratio of long chain length in amylopectin. There was no change in the crystal pattern of NDS compared to NS, but the crystallinity was lowered and showed a low molecular weight and high amylopectin long chain length ratio. DDS is considered to be useful as a health food, and in the future these results may be used as basic data for the development of food products such as meal powders and gluten-free noodles. Further research is required to understand the effects of various processing methods on the digestibility, RS content, and starch structure of the product.

#### Acknowledgements

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