

Molecular structures of rice starch to investigate the differences in the processing quality of rice flours

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Abstract The molecular structures of non-waxy rice starches purified from three Korean rice cultivars, Dongjin1, Hopyeong, and Ilmi, were investigated to determine the main reasons why it is difficult to make manju with Dongjin1 flour compared to other rice flours with similar processing qualities. The molecular structural characteristics of the three rice starches were analyzed by high-performance size-exclusion chromatography and high-performance anion-exchange chromatography. The Dongjin1 starch had lower molecular weight (MW) amylose, a smaller proportion of short a chains, and higher average amylopectin branch chain length than the other two starches. These properties of Dongjin1 starch resulted in a lower water binding capacity, lower swelling power at 80 °C, and lower peak viscosity than those of the other two starches. It is suggested that the MW of amylose and degree of polymerization of amylopectin are important factors to make gluten-free bakery products using non-waxy rice flours.

Keywords Processing quality · Rice starch · Molecular weight of amylose · Degree of polymerization

Introduction

Rice is an important cereal crop in the world, and it is the most consumed staple food in Asian countries. According to the Statistics Korea, per capital rice consumption in Korea has been continuously decreasing, from 72.8 kg in 2010 to 61.9 kg in 2016. In light of such trend, there have been numerous efforts worldwide to increase rice consumption for making processed foods. The processing properties of rice and rice flour depend on rice cultivar, amylose (AM) and protein contents, particle size distributions of the flour, water absorption power, molecular structure of starch, and a milling method (Kim and Shin, 2014; Shin et al., 2010; Suwannaporn et al., 2013). In recent years, the use of rice flour in various foods, including bakery products, tortillas, noodles, processed meats, beverages, puddings, salad dressing, and gluten-free foods, has increased because of its superior quality compared to wheat flour (e.g., white color, bland taste, small starch granules, ease of digestion, and hypoallergenic properties) (Kadan et al., 2008; Kim and Shin, 2014; Shin et al., 2010).

As the use of rice flour in various processed food products has increased, determining the properties of rice flour suitability for each processed food product has become necessary and standard indices are required to define the quality of rice flour. Although many scientists have studied rice flour properties using different rice cultivars, degrees of milling, particle sizes, amylose content, and a milling method, factors that affect the processing quality of rice flour are yet unknown. However, as it is difficult to substitute wheat flour with rice flour ground by dry and wet milling processes, a new method was developed (Kim and Shin, 2014; Shin et al., 2010). Dongjin1, Hopyeong, and Ilmi are japonica-type rice cultivars grown to breed rice cultivars in the Honam region of Korea and are known to have good tastes, textures, and health benefits. While most of bakery

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products can be made from the newly developed rice flour, it may be difficult to make manju, which is a popular traditional Japanese confection, depending on rice cultivars. Manju was prepared using three different kinds of rice cultivars, Dongjin1, Hopyeong, and Ilmi, as part of the development of gluten-free bakery products. The three rice cultivars used in the present study are low (12–20%) amylose varieties (Lee et al., 2017). Manju made using Dongjin1 rice flour has a cracked shell at the bottom and is of a lower quality than manju made using the Hopyeong and Ilmi rice flour (Fig. 1). All rice flours were made using the same method, which was newly developed to substitute for wheat flour (Kim and Shin, 2014; Shin et al., 2010). Nevertheless, manju made from Dongjin1 rice flour showed different behavioral patterns from manju prepared from the other rice flours.

As such, it is important to know the properties of starches isolated from various rice cultivars to select appropriate rice flour for specific industrial applications (Han and Hamaker, 2001; Noosuk et al., 2005; Park et al., 2007). Because starch is a major component of rice flour, the physicochemical properties and molecular structure of starch should be assessed. The previous research has shown that the properties of starch, including granular size, structure, type of crystal polymorph, AM/amylopectin (AP) ratio, gelatinization, amylose lipid complex and presence of non-carbohydrate content, affect the quality of rice flour and its products (Cornejo and Rosell, 2015; Gani et al., 2013; Gani et al., 2017).

Therefore, our objectives were to examine the physical, pasting, thermal, and structural properties of starches isolated from the three non-waxy rice cultivars to identify the major factors that affect the properties of rice flour and render manju with different properties. In addition, the relationship between the starch properties and rice processing adaptability was elucidated.

Materials and methods

Materials

Three japonica-type rice cultivars (Dongjin1, Hopyeong, and Ilmi) grown in the Jeonnam region of Korea were used



Fig. 1 The surface shape of Manju prepared from Dongjin 1, Hopyeong, and Ilmi rice flours

in this study. Dongjin1 and Hopyeong were purchased from Jukam Farms (Goheung, Korea) and Bannam Non-ghup (Naju, Korea), respectively. Ilmi was obtained from Jeollanam-do Agricultural Research & Extension Services (Naju, Korea). The rice was cultivated in 2013. Starches used for this study were isolated from white rice grains using an alkaline steeping method (Kim et al., 2010).

Analysis of general components

The moisture (Method 44–15.02), lipid (Method 30–10.01), protein (Method 46–13.01), and ash (Method 08–01.01) contents of the isolated starches were analyzed using AACCI methods (2012).

General physicochemical properties

Apparent amylose content and water binding capacity

The apparent amylose (AAM) contents of rice starches were analyzed using the method of Williams et al., (1970). Briefly, rice starch (20 mg, dry basis, db) was dissolved in a 0.5 N KOH solution (10 mL) for 1 h. The starch solution was then neutralized with 0.5 N HCl (5 mL), and then 0.5 mL of an iodine reagent (0.2% I₂ + 2% KI) was added. A UV/Vis spectrophotometer (OPTIZEN POP, Mecasys Co., Ltd, Daejeon, Korea) was used to measure the absorbance at 680 nm. The amylose content was calculated using the following equation: $y = 0.009x + 0.0383$ ($R^2 = 0.992$).

The water binding capacity (WBC) was evaluated using the method of Medcalf and Gilles, (1965). Starch dispersion (2.5%, w/v) was stirred for 1 h and then centrifuged (Supra 22 K, Hanil Science Industrial Co., Gimpo, Korea) at 3000×g for 30 min. The WBC was obtained using the following equation:

$$\text{WBC (\%)} = \frac{(\text{sediment weight (g)} - \text{sample weight (g)})}{\text{sample weight (g)}} \times 100$$

Pasting properties

Rapid Visco Analyzer (RVA-TecMaster, Perten Instruments AB, Hägersten, Sweden) was used to measure the properties of starch paste. Starch (3 g, 12% moisture basis, mb) was dispersed with distilled water (25 mL) in an aluminum canister. Starch slurries were then equilibrated at 50 °C for 1 min, increased to 95 °C for 3.7 min, held at 95 °C for 2.5 min, decreased to 50 °C for 3.8 min, and held at 50 °C for 2 min. The constant rotating speed of the paddle was set at 160 rpm.

Determination of molecular structure

Molecular weight of starch was analyzed using a HPSEC system (ProStar, Varian, Inc. Palo Alto, USA). In addition, distribution of the AP chain length was analyzed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD; Dionex ICS-5000, Dionex Co., Sunnyvale, CA, USA). A gradient eluent of NaOH (150 mM) and sodium acetate (500 mM) in NaOH (50 mM) were used as the mobile phase. Samples for injection were prepared according to the previous study (Do et al., 2012).

Determination of crystalline structure

To determine the crystalline structure of rice starches, FTIR and XRD were used. FTIR spectra of the three rice starches were collected using an FTIR/NIR spectrometer (PerkinElmer, Waltham, MA, USA) at 25 °C (You et al., 2004). An X-ray diffractometer (D/Max 1200; Rigaku Co., Tokyo, Japan) was used to investigate the crystalline type of the starch samples. The XRD was used under the following conditions: target, Cu-K α ; filter, Ni; full-scale range, 3000 cps; scanning speed, 8°/min; diffraction angle (2 θ), 5–40°; current, 20 mA; voltage, 40 kV.

Morphological properties

The sizes and shapes of the starch granules were observed using a SEM (JEOL JAM-540, Tokyo, Japan). The sizes of the starch granules were measured by repeatedly analyzing the shape of the granules from the SEM images and calculating the average size from approximately 10 granules.

Thermal properties

The thermal properties were measured using a DSC (DSC-Q1000, Universal V.3.6C TA Instruments, Olivia Gibson, UK). The temperature was calibrated using indium. Samples and water were placed in an aluminum pan, sealed, and allowed to equilibrate for 12 h. The sample pan was heated at a rate of 10 °C/min from 30 to 130 °C. The onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and gelatinization enthalpy change (ΔH) were then measured.

Rheological properties

The dynamic viscoelastic properties of the samples were examined using a rheometer (AR 1500ex, TA Instruments Ltd, New Castle, DE, USA) operating in an oscillatory mode with parallel plate geometry (20-mm diameter, 1-mm gap). Rice starch powder was dispersed into distilled water

and then heated at 95 °C for 15 min. The resulting hot paste was placed between the parallel plates of the rheometer (25 °C). A dynamic frequency sweep test was performed at a constant strain (1.0%), over a frequency range between 0.63 and 63 rad/s. During the measurement, the samples were wrapped with a thin oil layer to prevent any dehydration, and the dynamic rheological measurements were performed in triplicate.

Statistical analysis

All samples were analyzed in triplicate. Means and standard deviations are reported. Duncan's multiple range tests were performed using SPSS 12.0 K (SPSS Inc., Chicago, IL, USA).

Results and discussion

General composition and physicochemical properties

The crude protein, lipid, and ash contents were, respectively, 0.29, 0.11, and 0.13% in case of Dongjin1 starch, 0.76, 0.13, and 0.14% in case of Hopyeong starch and 0.28, 0.12, and 0.14% in case of Ilmi starch. The crude protein content of Hopyeong starch was remarkably higher than those of the other rice starches ($p < 0.05$). The AAM contents of the starches isolated from Dongjin1, Hopyeong, and Ilmi rice cultivars were 17.68, 16.87, and 16.66%, respectively. All rice cultivars used in this study are low amylose cultivar (Lee et al., 2017). However, Dongjin1 starch had a slightly higher amylose content than Hopyeong and Ilmi starches. The WBCs of Dongjin1, Hopyeong, and Ilmi rice starches were 107.52, 113.52, and 121.96%, respectively. The lowest value of WBC was obtained from Dongjin1, which had the highest AAM content, whereas the highest value of WBC was obtained from Ilmi, which had the lowest AAM content. It shows that this result corresponds to the results of previous studies, i.e., that an increase in AM content decreases the WBC of the starch (Gani et al., 2013; Iturriaga et al., 2004). However, another study reported that other factors, i.e., protein conformation, particle size, lipid-amylose complex, and lipid and protein content, could affect the value of the WBC (Cornejo and Rosell, 2015).

Molecular structural characteristics

The MW distribution of rice starches isolated from the three rice cultivars (analyzed by HPSEC) are shown in Fig. 2. A first peak after 35 min of elution time represents AP. The MW values of the three rice APs were $> 1 \times 10^6$,

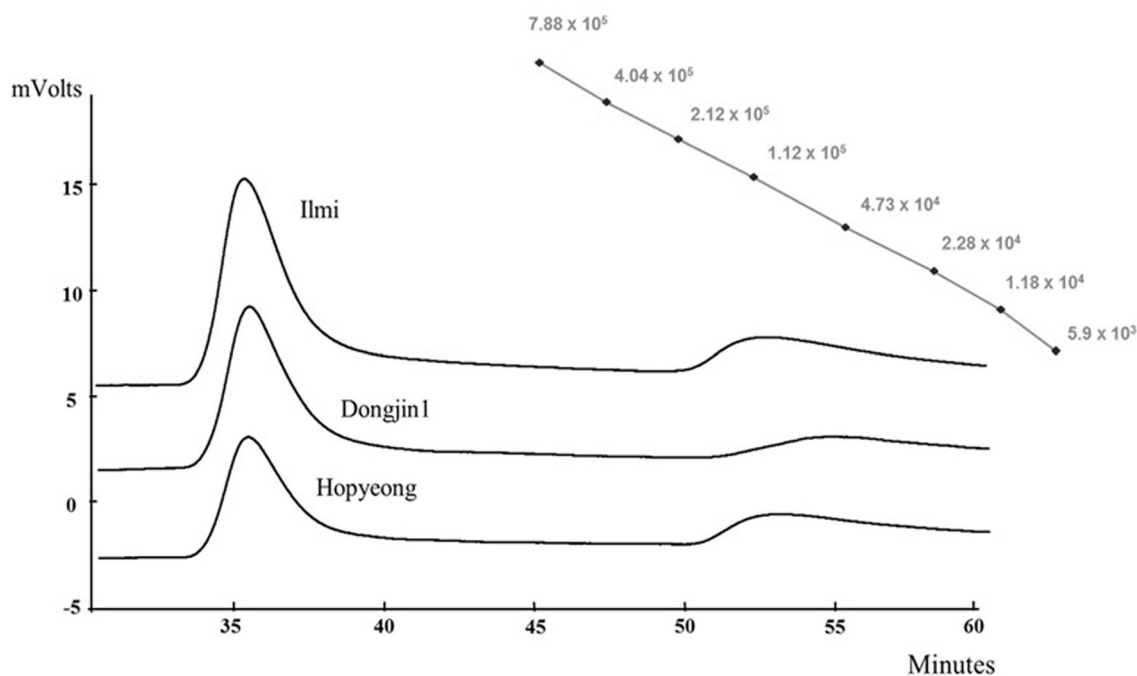


Fig. 2 Molecular weight distributions of rice starches (Ilmi, Dongjin1, and Hopyeong) determined by an HPSEC system

which are within the range of values reported in other studies (Park et al., 2007; You et al., 2004).

A second peak at 53–55 min of elution time represents AM, and the MW values of AM from Dongjin1, Hopyeong, and Ilmi starches are within the range of $2.12 \times 10^5 < MW < 2.28 \times 10^4$. It has been reported that the weight-averaged molar masses of amylose ranged from 2.20 to 8.31×10^5 g/mol (Noosuk et al., 2005), and the MW of amylose from various starches was $0.5\text{--}3.0 \times 10^6$ g/mol (Suortti et al., 1998). These references suggested that the second peak corresponds to amylose.

Clark et al., (1989) reported the effects of chain length and concentration on amylose gel properties such as modulus and turbidity. Whenever some non-crosslinking aggregation occurs, or short chains (DP = 250 and 300, $4.5\text{--}5.4 \times 10^4$), turbidity increases preceding the modulus development, for longer chains (DP > 1100, 1.98×10^5), modulus increases occur before significant turbidity becomes apparent. Compared to the MW of AM of the three rice cultivars, the maximum MW of Dongjin1 AM (6.62×10^4) is much lower than that of the other two rice AM ($1.12\text{--}1.15 \times 10^5$), even though all three rice cultivars are low AM varieties. It is suggested that the shorter chain length of Dongjin1 amylose could have resulted in a weaker gel network. The relatively higher AM content of Dongjin1 starch may be attributed to the different AP structural characteristics of Dongjin1. The AM content was obtained using the iodine reagent method in the present study; hence, these values provide the AAM content instead of the exact AM content because long-branch

chains of AP can bind iodine to form a single helical complex, like AM, and develop a blue color (Jane et al., 1999).

To confirm this possibility, the branch chain length distributions of AP for the three rice starches were determined using HPAEC-PAD, and the results are presented in Table 1. The chain length of debranched AP has four fractions: A chain (DP 6–12), B1 chain (DP 13–24), B2 chain (DP 25–36), and B3 + chain (DP ≥ 37) (You et al., 2004), and the branch chain distribution (%) is the highest in DP 13–24 in all three rice cultivars.

The average branch chain length of the rice starches ranges from 20.34 (Ilmi) to 20.71 (Dongjin1), and Dongjin1 has a higher average branch chain length than Hopyeong and Ilmi. This value for the average branch chain is similar to that from another study reported for starches isolated from rice cultivars grown in Korea (Park et al., 2007). Jane et al., (1999) reported that the average branch chain length of cereal AP ranges from 18.8 to 23.5. According to another study, it ranges from 20.0 to 20.7 for four Chinese waxy rice starches (Wang and Wang, 2002). Moreover, it ranges from 21.9 to 24.3 for six Korean waxy rice starches (You et al., 2004).

Dongjin1 has a smaller proportion (29.65%) of short A chains (DP 6–12) than the other two starches and a higher average branch chain length proportion. However, the AP of Hopyeong and Ilmi show similar proportions of short chains and long chains. It was reported that the difference in the MW of the starch and chain length distribution of AP could affect the physicochemical properties of the starch

Table 1 Average chain length and branch chain distribution of amylopectin from three rice starches

Rice starches	Average chain length	Peak DP	Distribution (%)			
			DP 6–12	DP 13–24	DP 25–36	DP ≥ 37
Dongjin1	20.71 ± 0.10 ^b	12	29.65 ± 0.09 ^{ab}	45.06 ± 0.34	11.36 ± 0.15	13.94 ± 0.28
Hopyeong	20.45 ± 0.16 ^{ab}	12	31.28 ± 0.15 ^a	43.59 ± 0.34	11.47 ± 0.11	13.56 ± 0.38
Ilmi	20.34 ± 0.13 ^a	12	32.13 ± 0.49 ^a	43.07 ± 0.11	11.20 ± 0.12	13.52 ± 0.27

Data represents mean ± SD

^{a,b}Values in the same column marked with a different letter are significantly ($p < 0.05$) different by Duncan's multiple range test

(Wang et al., 2010). Thus, the smaller amylose MW and different structural properties of Dongjin1 AP (that is, a higher average branch chain length of AP and a smaller proportion of short A chain) would provide the rice flour with different processing properties for making manju. In the case of manju made from rice flour, the AM gel network of rice flour and egg gelation would contribute to the formation of the manju shell (Lee and Shin, 2009). Thus, the properties of starch affect the quality of manju, and, in this sense, the properties of the above-mentioned starch of Dongjin1 would probably influence the quality of manju.

Crystalline structure and morphological properties

Fourier-transform infrared spectroscopy (FTIR) spectra of the three rice starches are shown in Fig. 3A. The FTIR absorbance band at 1047 cm^{-1} represents the amount of crystalline starch, whereas the band at 1022 cm^{-1} is a characteristic of amorphous starch (You et al., 2004). The intensity ratio of bands at 1047 and 1022 cm^{-1} (1047/1022) expresses the ratio of ordered crystalline regions to amorphous regions in the starch (Table 2). The 1047/1022 ratio of rice starches ranges from 0.6816 to 0.6907. The different values 1047/1022 among the rice flours of the three cultivars are not statistically significant ($p \geq 0.05$).

XRD patterns of the three rice starches are displayed in Fig. 3B. These rice starches showed the typical A-type crystal and diffraction angle (2θ) at 15°, 17°, and 23° on X-ray diffractograms with individual peaks (Fig. 3B). The relative crystallinities of Dongjin1, Hopyeong, and Ilmi starches are 38.74, 36.31, and 39.38%, respectively (Table 2), and this result showed a similar range of 37–40% for non-waxy rice starches reported (Iturriaga et al., 2004; Park et al., 2007). According to a previous study, the chain length distribution of AP is related to the degree of crystallinity of the starch. For example, the presence of short chains (DP < 10) of AP might be detrimental to the formation of a crystalline structure (Park et al., 2007). However, a noticeable relationship between the crystallinity and molecular structure of starch in the three rice cultivars was not observed in the current study.

All starch granules are polygonal in shape and of different sizes (Fig. 3C). The granular sizes of Dongjin1, Hopyeong, and Ilmi rice starches are 4.90 ± 0.58 , 5.50 ± 0.78 , and 5.58 ± 1.05 μm , respectively. Dongjin1 starch granules are the smallest among them. The size of granules plays a key role in processes, such as milling, mixing, and pasting (Li et al., 2016).

Pasting and thermal properties

The initial pasting temperature, peak, breakdown, setback, and final viscosities of the rice starches are shown in Table 2. Dongjin1 has the highest initial pasting temperature, lowest peak viscosity, highest final viscosity, and lowest breakdown viscosity among the three rice starches. The pasting temperature, final, breakdown, and setback viscosity values of Hopyeong and Ilmi starches were not significantly different ($p \geq 0.05$). Dongjin1 starch showed a different aspect of the pasting properties from the other two starches. It was reported the pasting properties of starch were influenced by AM and lipid contents, molecular size, and AP branch chain distribution (Jane et al., 1999; Park et al., 2007). In our study, it was discovered that because Dongjin1 starch had a relatively higher AM content, lower MW of AM, and lower DP 6–12 of AP, it was difficult for Dongjin1 rice flour to form the network. However, as described in a previous study of *du waxy* and *ae waxy* maize, the longer branch chains of AP could form helical complexes with lipids and affect the integrity of the starch granules when measuring the pasting properties (Jane et al., 1999).

The values of T_o and T_p are significantly different for each rice cultivar, but the T_c , the differences in temperature (ΔT), and ΔH are not significantly different (Table 2). Dongjin1 starch displayed higher T_o and T_p values than the other starches. Jang et al. (Jang et al., 2016) reported that the japonica waxy starch, which contained the highest protein content, showed the highest T_o and T_p values among the tested samples, except for the high amylose starch. The short AP chains of DP 6–9 and longer chains of DP 12–22 led to lower and higher DSC T_o , T_p , and T_c ,

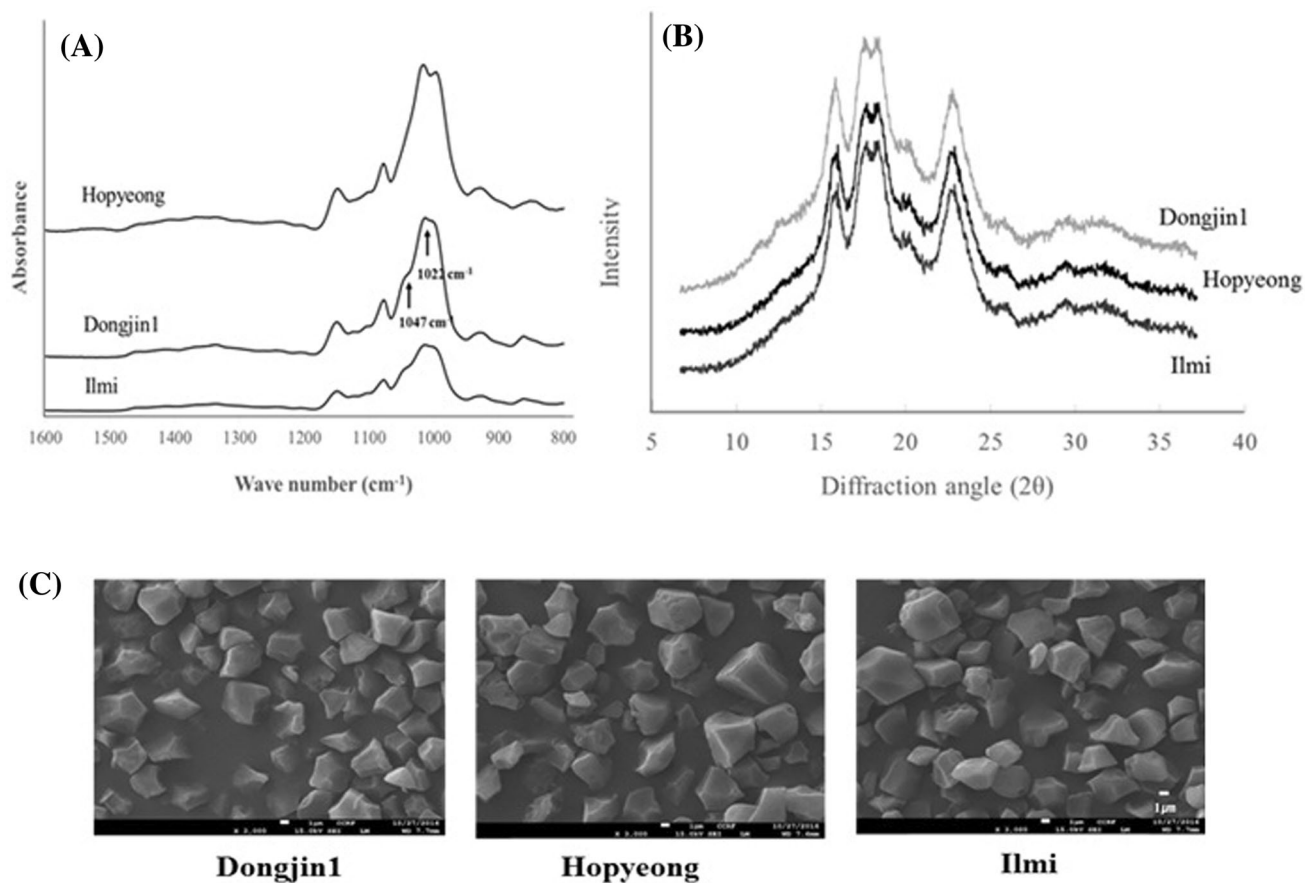


Fig. 3 Deconvoluted FT-IR (A), X-ray diffractograms (B), and SEM images (C) of three rice starches

Table 2 Crystalline structure, pasting properties and thermal properties of rice starches

	Rice starches		
	Dongjin1	Hopyeong	Ilmi
Crystal type	A	A	A
Relative crystallinity (%)	38.68 ± 0.08 ^a	36.58 ± 0.39 ^b	38.76 ± 0.88 ^a
Abs. ratio of 1047/1022 cm ⁻¹	0.6816 ± 0.002 ^a	0.6901 ± 0.005 ^a	0.6907 ± 0.008 ^a
Initial pasting temp. (°C)	78.23 ± 0.04 ^a	75.88 ± 0.04 ^b	75.83 ± 0.04 ^b
Peak viscosity (P)	255.21 ± 3.24 ^c	266.04 ± 1.94 ^b	294.33 ± 1.06 ^a
Trough viscosity (T)	193.00 ± 1.30 ^a	157.46 ± 4.18 ^c	180.38 ± 0.88 ^b
Final viscosity (F)	310.92 ± 0.71 ^a	270.75 ± 9.55 ^b	288.79 ± 3.01 ^b
Breakdown viscosity (P-T)	62.21 ± 1.94 ^b	108.58 ± 6.13 ^a	113.96 ± 0.18 ^a
Setback viscosity (F-T)	117.92 ± 0.59 ^a	113.29 ± 13.73 ^a	108.42 ± 2.12 ^a
Onset temp (T _o , °C)	64.61 ± 0.01 ^a	59.95 ± 0.04 ^b	62.09 ± 1.56 ^{ab}
Peak temp (T _p , °C)	69.57 ± 0.36 ^a	67.22 ± 0.11 ^c	68.41 ± 0.11 ^b
Conclusion temp (T _c , °C)	75.44 ± 0.08	73.56 ± 0.16	75.84 ± 2.37
ΔT (T _c - T _o)	10.83 ± 0.08	13.61 ± 0.20	13.75 ± 3.93
ΔH (J/g)	9.92 ± 0.28	9.83 ± 0.49	9.74 ± 0.15

Data represents mean ± SD

^{a-c}Values with different letters in the same row are significantly ($p < 0.05$) different by Duncan's multiple range test

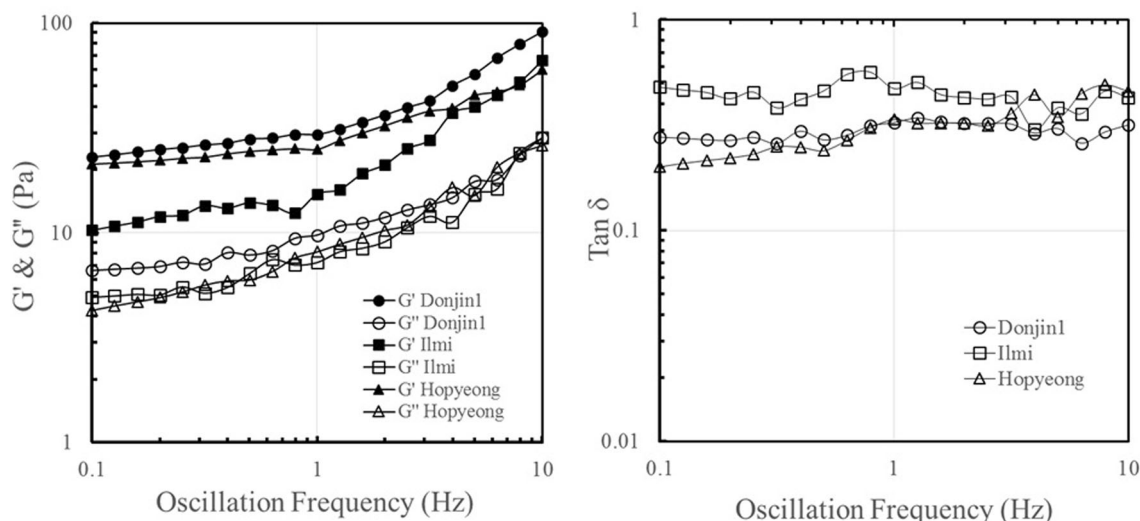


Fig. 4 Changes in the rheological properties (G' , G'' , and $\tan \delta$) of 0.1% native rice starch gels during a frequency sweep

respectively (Sodhi and Singh, 2003). Thus, the different structural characteristics of AP molecules of Dongjin1 starch might have caused different thermal properties and pasting properties (Park et al., 2007).

Dynamic oscillatory rheometry

The variations of the storage modulus (G'), loss modulus (G''), and loss tangent ($\tan \delta$), as a function of frequency at 25 °C, are illustrated in Fig. 4. The G' values for all samples were much larger than the G'' values across the entire frequency range tested, indicating that the starch gels were elastic rather than fluidic (Carrillo-Navas et al., 2014; Chaisawang and Supphantharika, 2006). This elastic and solid-like behavior could be attributed to the formation of a starch gel network. G' increases with increasing frequency. The starch gel needs to become increasingly elastic to avoid complete fracture against high-frequency disturbances.

The $\tan \delta$ (G''/G') values of all starch samples were less than one, suggesting that they were more elastic than viscous. There was no difference between Dongjin1 and Hopyeong with respect to $\tan \delta$; however, the value for Ilmi was larger than that of the other two rice starches, indicating an increase in the viscous characteristics of the Ilmi starch gel.

The basic structure of the starch gel plays the most important role in the gel's behavior (Jang et al., 2016). Starch gels are composite materials consisting of swollen AP embedded in and reinforced by an interlaced amylose gel matrix (Miles et al., 1985). The relative contents of short and long amylose chains in the gel matrix and the shape and size of the swollen granules determine the mechanical properties of the gel (Biliaderis and Juliano,

1993). Therefore, Dongjin1 and Hopyeong starch gel having more elastic properties than Ilmi starch may be attributed to the different AP structure and amylose and protein contents. With weak gel prepared from the lower MW of Dongjin1 amylose, manju shell made from Dongjin1 flour could be cracked easily.

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Compliance with ethical standards

Conflict of interest The authors declared that they have no conflict of interest.

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