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Impact of diverse cultivars on molecular and crystalline structures of rice starch for food processing

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ABSTRACT

The objective of this study was to determine the molecular and crystalline structures of starches from diverse rice cultivars for three major food processing in Korea (cooked rice, brewing and rice cake). Rice starches were isolated from 10 different rice varieties grown in Korea. Apparent amylose contents of rice starches from cooked rice, brewing and rice cake varieties were 21.1–22.4%, 22.9–24.6%, and 20.1–22.0%, respectively. Rice starches from rice cake varieties showed higher peak viscosity but lower pasting temperature than those from cooked rice and brewing varieties. Swelling factor at 80 °C of rice starches from cooked rice, brewing and rice cake varieties was 16.6–19.0, 17.8–19.3, and 17.8–19.2, respectively. Based on structure and physicochemical properties of rice starches extracted from different rice varieties, principal component analysis (PCA) results showed that these rice varieties could be clearly classified according to processing adaptability for cooked rice and rice cake.

1. Introduction

Rice (*Oryza sativa* L.) is a principal cereal grain consumed as cooked rice. It is a staple food in most Asian countries (Xu, Zhang, Guo, & Qian, 2011). In Korea, annual consumption of rice for food processing was increased from 6.0 kg in 2010 to 8.9 kg in 2014, suggesting that it is possible to increase rice consumption through processing (Yoon et al., 2015). There are three major rice processing applications in Korea: cooked rice, brewing, and rice cake. A wide variety of rice cultivars have been used for different food products. However, the rice food industry is suffering from inefficient information to validate the categorization of the major rice processing applications.

Starch is the most important constituent of rice that affects the processability of rice. Characteristics of rice starch will determine the processability of rice cultivars. Normal and waxy rice cultivars are generally classified according to their amylose contents in starch. Normal rice starch is composed of 20–30% linear amylose and 70–80% highly branched amylopectin, while waxy starch consists of almost amylopectin. Amylose is fundamentally long linear chains consisted of α-(1-4)-linked D-glucose units with a few branches, whereas amylopectin has much shorter chains of α-(1-4)-linked D-glucan which is highly branched through additional α-(1-6)-linked D-glucose linkages with a larger molecular weight. Amylose content has been used to distinguish rice starch characteristics (Vandeputte & Delcour, 2004). In many food and other industrial products, the pasting properties of starch are used to evaluate the suitability for processing. Lee et al. (2012) have suggested that hydration and pasting properties of rice starches could be used as indicators of processability for specific application purposes.

Various studies have suggested that the molecular and crystalline structures of rice starches can affect their physicochemical properties (Chávezmurillo, Méndezmontealvo, Wang, & Bellopérez, 2012; Chung, Liu, Wang, Yin, & Li, 2010; Kowittaya & Lumdubwong., 2014; Pantidol, Gonzalez, Wang, & McClung, 2007). However, the relationship between the characterization (molecular and crystalline structures, physicochemical properties and *in vitro* digestibility) of starches and processing adaptability has not been reported yet.

Therefore, the objective of this study was to determine the molecular structure (amylose content, molecular weight, and branch chain length distribution of amylopectin), crystalline structure (relative crystallinity and X-ray diffraction pattern), thermal characteristics (gelatinization properties), physicochemical properties (pasting properties) and *in vitro* digestibility of rice starches from different cultivars for three major rice processing applications

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in Korea. In addition, the relationship between starch properties and rice processing adaptability was determined.

2. Materials and methods

2.1. Materials

Ten rice cultivars grown in Korea including purpose-specific cultivars for three major rice-processed foods; cooked rice (Samgwang, Hopum, Chilbo, and Hiami), brewing (Seolgaeng and Daerib1) and rice cake (Dasan, Saegayejinmi, Hyangmi1 and Mihyang) were obtained from Rural Development Administration (Suwon, Korea) in 2015. All rice grains were dehulled and polished.

2.2. Starch isolation

Rice starch was isolated from polished rice according to the alkaline steeping method (Lim, Lee, Shin, & Lim, 1999).

2.3. Apparent amylose content

Apparent amylose content of isolated rice starches was analyzed using the iodine reagent method (Williams, Kuzina, & Hlynka, 1970).

2.4. Molecular weight analysis

The average molecular weight (M_w) was analyzed by high performance size exclusion chromatography (HPSEC) and multi-angle laser light scattering (MALLS) with refractive index detector (RI) system. Rice starch was purified following the method described by Han and Lim (2004). The purified starch (12 mg) was dissolved in 0.1 M NaOH (1 mL) at 50 °C for 10 min followed by the addition of 3 mL distilled water. The solution was then neutralized by 0.1 M HCl (1 mL). After heating in a microwave oven (RE-552W, Samsung Co., Seoul, Korea) using a microwave bomb (#4872, Parr Instrument Co., Moline, IL, USA) for 30 s, the solution was filtered through a nylon filter (0.45 μm) before injecting it into the HPSEC 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, SEC column (TSK G5000 PW, TosoBiosep, Mongomeryville, PA, USA), a MALLS (HELEOS, Wyatt Technology Corp., Santa Barbara, CA, USA) and a RI 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. The specific RI increment value (dn/dc) of 0.146 mL/g was used for calculation of starch molecular weight (Han & Lim, 2004).

2.5. Amylopectin chain length distribution

Rice starch (10 mg) was dispersed in 2 mL of 90% dimethyl sulfoxide (DMSO) and boiled with continuous stirring for 20 min. Starch solution was mixed with absolute ethanol (6 mL) and centrifuged (2700 rpm for 12 min). The precipitate was dissolved with 2 mL of 50 mM sodium acetate buffer (pH 3.5) and heated in a boiling water bath with continuous stirring for 20 min. After the solution was equilibrated to 37 °C, isoamylase (5 μL, E-ISAMY, Megazyme International Ireland Ltd., Bray, Ireland) was added and the starch solution was incubated at 37 °C with stirring for 24 h. The enzyme was inactivated by boiling for 10 min. An aliquot (200 μL) of the debranched rice 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) equipped 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 (4×250 mm, Dionex Corporation, Sunnyvale, CA). Separation was achieved using a gradient eluent of 150 mM NaON and 500 mM sodium acetate in 150 mM NaON at a flow rate of 1 mL/min.

2.6. X-ray diffraction and relative crystallinity

X-ray diffraction analysis was performed with an X-ray diffractometer (PANalytical, X'pert MPD high resolution XRD, Almelo, Netherlands) operated at 40 kV and 40 mA. Diffractograms were obtained from 4 to 30° (2θ) at a scan rate of 2.0°/min. Relative crystallinity was quantitatively calculated following the method described by Lopez-Rubio, Flanagan, Gilbert, and Gidley (2008).

2.7. Thermal properties

Gelatinization properties of rice starch were determined using differential scanning calorimeter (DSC6100, Seiko Instruments, Chiba, Japan). Starch (3 mg) was weighted into an aluminum pan (Seiko Instruments) and 6 μL of distilled water was added. The pan was hermetically sealed, equilibrated at room temperature for 1 h, and then heated from 20 to 130 °C at a heating rate of 5 °C/min.

2.8. Pasting properties

Pasting properties of rice starches were analyzed using a Rapid Visco-Analyzer (RVA-TecMaster, Newport Scientific Pty. Ltd., Warriewood, Australia). Starch (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 then held at 50 °C for 2 min.

2.9. Swelling factor (SF)

Swelling factor of rice starches at 80 °C was measured by blue dextran dye exclusion according to the method of Tester and Morrison (1990). Briefly, starch (200 mg) was weighed in replicates of 15 mL of screw cap tube, 10 mL of water added, and the sealed tubes incubated in water bath at 80 °C for 30 min. These tubes were then cooled rapidly to 20 °C, 1 mL of blue dextran (5 mg/mL, D5751, Sigma, St. Louis, MO, USA) was added, and the contents mixed by gently inverting the closed tubes several times. After centrifuging at 1500 × g for 5 min, the absorbance of the supernatant was measured. The absorbance of reference tubes containing no starch was also measured.

2.10. In vitro starch digestibility

The amount of rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) were determined by a procedure of Englyst method (Englyst, Kingman, & Cummings, 1992) with slight modifications. Porcine pancreatic α-amylase (0.45 g, P-7545, Sigma, St. Louis, MO) was dissolved in distilled water (4 mL), and centrifuged at 1500 × g for 12 min. The enzyme solution containing 2.7 mL of the supernatant and 0.3 mL of amyloglucosidase (A-9913, Sigma) was prepared immediately before use. Starch (100 mg) was dispersed in 4 mL of sodium acetate buffer (0.5 mol/L, pH 5.2). 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) of hydrolyzed solution were taken at 20 and 120 mins after incubation and mixed with 1 mL of 80% ethanol. Glucose content in the supernatant was measured using glucose assay kit (GAGO-20, Sigma). RDS, SDS, and RS contents were defined as fractions digested within