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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).