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Influence of molecular structure on physicochemical properties and digestibility of normal rice starches

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ABSTRACT

Normal rice starches were isolated from six different rice varieties grown in Korea and their molecular structure, crystalline structure, and *in vitro* digestibility were investigated. Apparent amylose content was the highest in starch from Junam cultivar (25.5%) and lowest in Hopum (22.4%). Starch from Hiami cultivar had the lowest molecular weights of amylose and amylopectin, average amylopectin chain length, proportion of short chains (DP 6–12), and proportion of long chains (DP ≥ 37) among the tested rice starches. The relative crystallinity and ratio of 1047/1022 ranged from 30.2 to 36.7% and from 0.638 to 0.652, respectively. Hiami had the lowest gelatinization temperatures and the highest gelatinization enthalpy. Hiami had the highest pasting temperature (92.1 °C), the lowest setback (515 cP) and final viscosity (876 cP), whereas Hanareum had the lowest pasting temperature (82.7 °C), the highest setback (1002 cP), and final viscosity (1580 cP). The rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) content ranged from 43.9–53.5%, 35.5–52.6%, and 0.5–15.6%, respectively. The Junam cultivar had the lowest RDS content, whereas Hiami had the highest RDS content. The RS content of Hanareum (16.2%) and Boramchan (14.5%) was significantly higher than that of other normal rice cultivars (3.0–6.5%).

1. Introduction

Rice is one of the most widely consumed basic foods in the world. The genetic diversity of rice cultivars greatly affects their physical properties, composition, and cooking properties [1]. Starch is one of the main components in rice grain so that its physicochemical properties determine the acceptability of the rice cultivar. Rice starch is primarily composed of linear amylose and branched amylopectin. Amylose is long linear chains consisting of α-(1-4)-linked D-glucose units with a few branches, while amylopectin has much shorter chains of α-(1-4)-linked D-glucan with branches of α-(1-6)-linked D-glucose linkages [2]. Rice cultivars can be sorted according to their amylose content in starch. Normal rice starch contains 20–30% amylose and 70–80% amylopectin, whereas the amylose content of waxy rice is less than 2% [3].

In Korea, rice is traditionally consumed as cooked rice and is also used in making alcohol and soybean paste. Rice starch is used in various processed foods as an adhesive, thickener, extending agent, and inflating agent. The functional properties of rice starch are influenced by the crystalline structure, the amylose–amylopectin ratio and the fine structure of amylopectin [4–6]. An understanding of the functional properties of rice starch is very important for optimizing industrial applications and allowing consumers to select suitable rice varieties. The gelatinization temperature of rice starch was inversely correlated with the amount of amylopectin short branch chains (DP 6–12) and was positively correlated with the proportion of amylopectin long branch chains (DP ≥ 37) [7]. Higher amylose content in rice starch increased starch pasting temperature and decreased peak viscosity [4].

Starch digestion is one of the significant metabolic responses. Dietary starch has been classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) [8]. RDS is linked to elevated plasma glucose and insulin and SDS is related to a slow increase in postprandial blood glucose levels, which is advantageous for satiety and diabetes management [9]. RS is defined as the portion of starch not digested in the small intestine [8]. Although many studies have been conducted on the physicochemical and functional properties of rice starches according to genetic diversity [3,10,11], few studies have examined the relationship between molecular structure and starch nutritional fraction.

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Furthermore, no information is available on the molecular structures and *in vitro* digestibility of rice starches obtained from Korean normal rice cultivars.

The purpose of this study was to investigate the molecular structure (apparent amylose content, molecular weight, and amylopectin branch chain length distribution), crystalline structure (X-ray diffraction pattern relative crystallinity, ratio of 1047 cm^{-1}/1022 cm^{-1} from FT-IR), physicochemical properties (pasting properties, and thermal characteristics) and *in vitro* digestibility (RDS, SDS, RS, and eGI) of rice starches from six Korean normal rice cultivars.

2. Materials and Methods

2.1. Materials

Six normal rice varieties (Junam, Samgwang, Hiami, Hopum, Hanareum, and Boramchan) grown in 2013 were obtained from the National Institute of Crop Science, Rural Development Administration (Suwon, Korea). Rice grains were dehulled and milled to white rice.

2.2. Starch isolation

Rice starch was isolated using the alkaline steeping method described by Lim et al. [12]. Rice flour (50 g, db) was dispersed in the 150 ml of 0.2% NaOH with constant stirring in room temperature for 2 h and then centrifuged at 1500 × *g* for 10 min. The supernatant was discarded and the fresh 0.2% NaOH solution (150 ml) was added to the precipitate and then the mixture was stirred. The slurry was then centrifuged again and the same procedure was repeated twice. After the final extraction, the starch precipitate was blended with 200 ml of distilled water and neutralized to pH 7.0 by adding 0.1 N HCl solution. The starch dispersion was centrifuged and the starch residue was washed twice with distilled water. The purified starch was dried at 40 °C, ground and passed through a 100 mesh sieve.

2.3. Chemical composition

The crude protein, lipid, and ash contents were determined using the standard AACC [13] methods.

2.4. Apparent amylose content

Apparent amylose content of normal rice starches was determined by the colorimetric method [14]. The rice starch (20 mg, db) was mixed with 0.5 N KOH solution (10 ml) and the starch suspension was heated for 10 min in a boiling water bath. After cooling, an aliquot (1 ml) of the solution was mixed with 1 ml of 0.5 N HCl and 0.5 ml of iodine reagent (0.2% I^2 + 2.0% KI) and diluted to 50 ml with distilled water. The absorbance of color-developed starch solution was measured at 640 nm using a spectrophotometer (Optizen Pop, Mecasys Co., Daejeon, Korea). The amylose content of starch sample was determined from a standard curve prepared with potato amylose (Sigma, St. Louis, MO, USA).

2.5. Molecular weight

The average molecular weight (M_w) of normal rice starches was measured by high performance size exclusion chromatography coupled to a multi-angle laser light scattering and refractive index detection (HPSEC-MALLS-RI) system. Normal rice starches were purified by dissolving them in 90% dimethyl sulfoxide (DMSO), heating in a boiling water bath with gentle stirring and precipitating with ethanol [15]. The purified starch (12 mg, db) was dissolved in 0.1 M NaOH (1 ml) at 50 °C for 10 min, then 3 ml of distilled water was added and the mixture was neutralized with 0.1 M HCl. The solution was heated in a microwave oven (RE-552 W, Samsung, Seoul, Korea) using a microwave bomb (no.4872, Parr Instrument Co., Moline, IL, USA) for 30 s. The heated starch solution was injected into the HPSEC-MALLS-RI system consisting 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 × 600 mm, TosoBiosep, Mongomeryville, PA, USA), a multi-angle laser light scattering detector (HELEOS, Wyatt Technology, Santa Barbara, CA, USA) and a refractive index detector (Waters 2414). The mobile phase was an aqueous solution of 0.15 M $NaNO_3$ and 0.02% NaN_3 and the flow rate was 0.4 ml/min. The M_w was calculated using ASTRA 5.3 software (Wyatt Technology).

2.6. Amylopectin chain length distribution

Normal rice starches (10 mg, db) were dispersed in 2.0 ml of 90% DMSO and heated in a boiling water bath for 20 min with stirring. The solution was mixed with absolute ethanol (6 ml) and centrifuged at 2700 rpm for 12 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, 10 μl of isoamylase (59,000 unit/mg of protein, Hayashibara Biochemical Laboratories, Okayama, Japan) was added and the solution was incubated at 37 °C for 20 h with slow stirring (200 rpm). The enzyme was inactivated by boiling for 10 min and an aliquot (400 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 with a pulse amperometric detector (HPAEC-PAD) system. The HPAEC system consisted of a Dionex ICS-5000 (Dionex Corporation, Sunnyvale, CA, USA), a Bio-LC gradient pump and an ED50 electrochemical detector. Separation was obtained using a CarboPac PA100 column (4 × 250 mm, Dionex Corporation) with a guard column and a gradient eluent with 150 mM NaOH and 500 mM sodium acetate in 150 mM NaOH at a flow rate of 1 ml/min.

2.7. X-ray diffraction and relative crystallinity

The crystalline structure of the normal rice starches were analyzed using an X-ray diffractometer (PANalytical, X'pert MPD high resolution XRD, Almelo, Netherlands). The diffractometer was operated at 40 kV and 40 mA with a scanning range of 3–40° (2θ) and a scan rate of 2.0 min. The moisture content of normal rice starches was adjusted to ~20% by being stored in a desiccator over saturated solution of K_2SO_4 (relative humidity = 97%) at room temperature for 5 days. The starch crystallinity was quantitatively calculated according to the method described by Lopez-Rubio et al. [16] using peak-fitting software (Origin 6.0, Microcal, Northampton, MA, USA).

2.8. Fourier transform infrared (FT-IR) spectroscopy

Infrared spectra of the normal starches were analyzed on a Digilab FTS 7000 spectrometer (Digilab USA, Randolph, MA, USA) equipped with a thermoelectrically cooled deuterated triglycine sulfate (DTGS) detector using an attenuated total reflectance (ATR) accessory following the method described by You et al. [17].

2.9. Thermal properties

Gelatinization properties of normal rice starches were measured using a differential scanning calorimeter (DSC6100, Seiko Instruments, Chiba, Japan). Starch (3 mg, db) was weighed into an aluminum pan (15 μl, Seiko Instruments) with 6 μl of distilled