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Molecular structure and physicochemical properties of starch isolated from hydrothermally treated brown rice flour

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ABSTRACT

The impact of single and dual hydrothermal treatments on the molecular structure, crystalline structure, *in vitro* digestibility, and physicochemical properties of starches isolated from hydrothermally treated brown rice flour was investigated. Annealed rice starch (ANN) exhibited no significant change in molecular structure, whereas heat-moisture treated rice starch (HMT) showed decreased apparent amylose content, molecular weight of amylopectin, average chain length of amylopectin, and long amylopectin branch chains (DP $\geq$ 37). The relative crystallinity, intensity ratio of 1045 cm^{-1}–1015 cm^{-1}, and gelatinization enthalpy remained unchanged in ANN, but significantly decreased in HMT. In dually modified starches, the HMT-ANN had higher gelatinization temperatures, gelatinization enthalpy, relative crystallinity and ratio of 1045 cm^{-1}–1015 cm^{-1} than the ANN-HMT. Hydrothermal treatment substantially increased starch digestibility in the granular state, showing increased rapidly digestible starch (RDS) content and decreased resistant starch (RS) content. However, in the gelatinized starches, ANN and HMT showed decreased RDS level and increased slowly digestible starch (SDS) and RS levels. The changes in amounts of RDS, SDS, and RS in ANN, HMT and dually modified starches could be attributed to the alteration of crystalline and molecular structures.

1. Introduction

Rice is one of the most important foods in the world and supplies as much as half of the daily calories for the world's population. Brown rice is a rice kernel obtained by hulling rough rice and consists of the embryo (2–3%), endosperm (92%), and bran (5–6%). The milling product of brown rice is white rice, which is composed entirely of starchy endosperm (~90% starch) (Marshall & Wadsworth, 1997). Compared to white rice, brown rice contains bran layers with a variety of nutritional and bio-functional components, such as dietary fibers, γ-oryzanol, vitamins, and minerals (Cho & Lim, 2016). These components show physiological activities against diabetes, obesity, hypertension, and high cholesterol (Kahlon, Chow, & Sayre, 1994).

Heat–moisture treatment (HMT) and annealing (ANN) are often used to modify the physicochemical properties of starch without destroying its granular structure. ANN involves the incubation of starch in an excess water (>65%) at temperatures below gelatinization but above the glass transition, whereas HMT uses relatively lower moisture levels (usually <35%) but higher temperatures (Jacobs & Delcour, 1998). Both ANN and HMT influence granular swelling, amylose leaching, pasting properties, gelatinization behavior, molecular structure, crystalline structure, and *in vitro* digestibility of starch (Chung, Liu, & Hoover, 2009a; Jacobasch, Dongowski, Schmiedl, & Schmehl, 2006; Lim, Chang, & Chung, 2001). These hydrothermal treatments are considered to be more natural and safe compared to chemical treatment, whose starches can be safely used in various food products.

Starch is classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) based on its rate of glucose release and absorption in the gastrointestinal tract (Englyst, Kingman, & Cummings, 1992). Changes in the structure and physicochemical properties by ANN and HMT highly influence starch digestibility (Chung et al., 2009a).

Numerous studies have been performed to determine the effect of ANN and HMT on the physicochemical properties of starches (Chung et al., 2009a, Chung, Liu, & Hoover, 2009b; Gunaratne & Hoover, 2002; Hoover & Manuel, 1996; Jacobs & Delcour, 1998). However, a few studies have reported the effect of the combination of HMT and ANN on starch properties (Chung et al., 2009b; Chung,

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Liu, & Hoover, 2010; Stute, 1992; Zang, Ma, Kong, Gao, & Yu, 2015). ANN and HMT are commonly used for modification of starch. However, in this study, the hydrothermal treatments were applied to brown rice flour and the changes in molecular structure analysis (the apparent amylose content, molecular weight analysis, and amylopectin chain length distribution), color properties, crystalline structure analysis (X-ray diffraction and relative crystallinity, crystalline structure in surface of starch granule by FT-IR, and the gelatinization properties by DSC), pasting properties and *in vitro* digestibility (RDS, SDS, and RS) of starch isolated from hydrothermally treated brown rice flour were investigated. The results of this study may provide an alternative method for improving food quality by the addition of brown rice to various processed foods.

2. Materials and methods

2.1. Materials

Brown rice (*Dasan* cultivar) harvested in 2013 was obtained from the Rural Development Administration (Suwon, Korea). Brown rice (~10% moisture content) was ground into fine flour using a mechanical grinder (DA5500, Daesung Artlon Co., Seoul, Korea) and then passed through a 100 mesh sieve.

2.2. Hydrothermal treatment

For annealing (ANN), brown rice flour slurry (70% moisture content) was incubated in a water bath at 50 °C for 24 h. After incubation, the sample was centrifuged (2000 rpm, 10 min) and the supernatant was decanted. The annealed brown rice flour was washed with deionized water and dried at 40 °C. For heat-moisture treatment (HMT), brown rice flour was weighed into glass containers. The moisture content of brown rice flour was adjusted to 20% by adding appropriate amounts of distilled water. The container was sealed, kept for 24 h at ambient temperature, and then placed in an oven at 110 °C for 2 h. The container was opened, and the treated rice flour was dried at 40 °C. For dual modification, brown rice flour was subjected to annealing followed by heat moisture treatment (ANN-HMT) or heat-moisture treated brown rice flour treated by annealing (HMT-ANN).

2.3. Starch isolation

The isolation of rice starch from hydrothermally treated brown rice flours was performed according to the method described by Lim, Lee, Shin, and Lim (1999). Brown rice flour was dispersed in NaOH solution (0.2%) with constant stirring in room temperature for 2 h, centrifuged and the supernatant was discarded. The process was repeated three times. After the final extraction, the starch precipitate was neutralized by adding 0.1 N HCl solution, 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.

2.4. Scanning electron microscopy (SEM)

The granule morphology of rice starches was observed using a scanning electron microscope (JAM-540, JEOL Ltd., Tokyo, Japan). The starch samples were mounted on a metal plate with double-sided adhesive tape, coated with a thin film of gold and palladium and the examined an accelerating voltage of 15 kV and 5000× magnification.

2.5. Apparent amylose content

The apparent amylose content of rice starches was determined by a colorimetric method (Williams, Kuzina, & Hlynka, 1970). The rice starch was mixed with 0.5 N KOH solution and the starch suspension was heated for 10 min in a boiling water bath. After cooling, an aliquot of the solution was mixed with 0.5 N HCl and iodine reagent (0.2% I_2 + 2.0% KI). The absorbance of color-developed starch solution was measured using a spectrophotometer (Optizen Pop, Mecasys Co., Daejeon, Korea).

2.6. Molecular weight analysis

The molecular weight (M_w) of rice starches was analyzed by high-performance size exclusion chromatography coupled with multi-angle laser light scattering and refractive index detectors (HPSEC-MALLS-RI). The rice starch (1 g) was dispersed in 100 mL of 90% dimethyl sulfoxide (DMSO) with heating in a boiling water bath while stirring for 1 h followed by stirring at room temperature for 24 h. The starch sample was precipitated by adding ethanol, centrifuged, and then dried. The purified starch (12 mg) was dissolved in 0.1 M NaOH (1 mL) at 50 °C for 10 min and neutralized by adding 0.1 M HCl. The solution was heated in microwave oven (RE-552 W, Samsung, Seoul, Korea) using a microwave bomb for 30 s. The solution was the filtered through a 0.45 μm nylon syringe filter and injected into the chromatography system. The HPSEC-MALLS-RI system consisted of an SEC column (TSK G5000 PW, 7.5 mm × 600 mm, TosoBiosep, Mongomeryville, PA, USA), MALLS detector (HELEOS, Wyatt Technology Corp., Santa Barbara, CA, USA), and a RI detector (Waters 2414, Bedford, MA, USA). 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.

2.7. Amylopectin chain length distribution

Amylopectin chain length distribution of the rice starches was determined by high performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD, Dionex ICS-5000, Dionex Corporation, Sunnyvale, CA, USA) (Liu, Gu, Donner, Tetlow, & Emes, 2007). Rice starch (10 mg, db) was dispersed in 2 mL of 90% DMSO and heated in a boiling water bath with stirring for 20 min. After cooling, the starch solution was precipitated with absolute ethanol (6 mL) and centrifuged (2700 rpm for 12 min). The precipitated starch was dissolved in 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 (10 mg) was added (68,000 unit/mg of protein, Hayashibara Biochemical Laboratories, Okayama, Japan) and the starch solution was incubated at 37 °C for 20 h with slow stirring (100 rpm). After the incubation, the starch solution was placed in a boiling water bath for 10 min to inactivate the enzyme. An aliquot (400 mL) of the debranched starch solution was diluted with 2 mL of 150 mM NaOH, filtered (0.45 μm nylon syringe filter) and injected into the HPAEC system. The HPAEC system consisted of an ED50 electrochemical detector and the CarboPac PA200 column (3 × 250 mm, Dionex Corporation, Sunnyvale, CA, USA). The mobile phase was a gradient eluent with 150 mM NaOH and 500 mM sodium acetate in 150 mM NaOH and the flow rate was 0.5 mL/min.

2.8. Color properties

The color of the rice starches was measured by using a colorimeter (Minolta JP/CM-3500D, Tokyo, Japan). The instrument was calibrated with a standard-white reference plate and L^* (whiteness), a^* (greenness) and b^* (yellowness) values were determined.