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Effects of amylosucrase treatment on molecular structure and digestion resistance of pre-gelatinised rice and barley starches

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ABSTRACT

Structural modification of rice and barley starches with *Neisseria polysaccharea* amylosucrase (*Np*AS) was conducted, and relationship between structural characteristics and resistant starch (RS) contents of *Np*AS-treated starches was investigated. Pre-gelatinised rice and barley starches were treated with *Np*AS. *Np*AS-treated starches were characterised with respect to morphology, X-ray diffraction pattern, amylopectin branch-chain distribution, and RS content, and their structural characteristics were correlated to RS contents. Regardless of amylose contents of native starches, *Np*AS-treated (relative to native) starches possessed lower and higher proportions of shorter (DP 6–12) and intermediate (DP 13–36) amylopectin (AP) branch-chains, respectively. RS contents were higher for *Np*AS-treated starches relative to native starches, and maximum RS contents were obtained for *Np*AS-treated starches of waxy rice and barley genotypes. Amylose contents were not associated with RS contents of *Np*AS-treated starches. However, shorter and intermediate AP branch-chain portions were negatively and positively correlated to RS contents of *Np*AS-treated starches, respectively.

1. Introduction

Native starch granules, consisting of essentially linear amylose (AM) and highly-branched amylopectin (AP), are used as main or minor ingredients for the processed foods (Perera, Meda, & Tyler, 2010). In many cereal-based and starch-based food systems, starch is present in the gelatinised or retrograded forms. Starches which completely and/or partially rupture their granular forms mainly by heat treatments (e.g., boiling, baking, deep-fat and pan frying, and sterilisation) facilitate accessibility of starch digestive enzymes to starch molecules. Thus, starch molecules are rapidly and completely digested, resulting in an increase in the magnitude and duration of glycemic response following greater glucose intake (Perera et al., 2010). Consequently, persons suffering from Type 2 diabetes and obesity have tried to limit intake of high glycemic index (GI) foods generally containing high amounts of starches and starch products.

There has been great attention to resistant starch (RS) as a way of improving and resolving highly-digestible characteristics of starch by starch digestive enzymes. RS refers to the indigestive portions of starches and starch products by starch digestive enzymes during their passage through the gastrointestinal tract (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010). RS was classified as four types: physically enzyme-inaccessible starch granules (RS type 1), raw starch granules (ungelatinised and undamaged) isolated from potato (RS type 2), recrystallised and retrograded starches (RS type 3), and chemically-modified starches (RS type 4) (Kim et al., 2010; Ryu et al., 2010). RS types 1, 2, and 3 still exhibit poor resistance to starch digestive enzymes by heat treatment in an aqueous medium, while chemical modification of native starch with various reacting agents (RS type 4) enhances slowly-digestible and resistant starches (Han & BeMiller, 2007). Among chemically-modified starches, phosphorylated cross-linking reaction of native starch granules with phosphorus oxychlroide and a mixture of sodium trimetaphosphate and sodium tripolyphosphate are reported to be most effective for RS production (Woo & Seib, 2002). Due to repulsion of consumers against chemical derivatives of starch, however, there are recent trends to avoid utilisation of chemically-modified starch products in the processed foods (Kim & BeMiller, 2012).

Thus, a few attempts to modify partially and completely gelatinised starches with amylosucrase (*Np*AS) from *Neisseria polysaccharea* have been to replace RS type 4 to RS type 3 (free to toxic

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chemicals and acceptable by consumers) and to enhance resistance of cooked RS type 3 to starch digestive enzymes (Ryu et al., 2010; Shin, Choi, Park, & Moon, 2010). *Np*AS is a bacterial glucosyltransferase that catalyses the sequential translocation of glucosyl units from sucrose onto acceptor molecules. In the presence of starch molecules as an acceptor, *Np*AS is known to elongate starch molecules at their non-reducing ends by α-(1,4) glycosidic bond (Ryu et al., 2010; Shin et al., 2010). Ryu et al. (2010) investigated effects of acceptor (starch) and donor (sucrose) concentrations in *Np*AS reaction on yield and RS content of *Np*AS-treated corn starches (waxy, normal, high-amylose). They reported that the highest RS yields and of *Np*AS-treated corn starches were obtained from *Np*AS reaction at 3% (w/v) starch and 0.3 M sucrose (Ryu et al., 2010). Shin et al. (2010) also conducted modification of waxy and normal starches of rice and potato with *Np*AS. In their study, the increases in slowly-digestible and resistant starches of *Np*AS-modified starches were observed when their digestibilities in an aqueous medium were determined by Englyst's method without heat treatment. Though both studies observed elongation of AP branch-chains by *Np*AS, different conclusions were drawn from their respective results. Ryu et al. (2010) suggested that the increases in RS contents of *Np*AS-treated corn starches were the result of AP branch-chain elongation by *Np*AS. However, Shin et al. (2010) suggested that the enhanced slowly-digestible starch fractions were due to elongation of AP branch-chains by *Np*AS, and the increased RS contents were the result of retrogradation of amylose molecules. The inconsistency between both studies may be due to the treatment of *Np*AS-treated starches for RS determination. Ryu et al. (2010) and Shin et al. (2010) treated fully-cooked and uncooked *Np*AS-treated starches with starch digestive enzymes, respectively. To the best of our knowledge, there is lack of information on the relationship between RS production and structural modification of starch molecules with *Np*AS.

To further resolve these conflicting reports, therefore, this study investigated structural characteristics and RS contents of *Np*AS-treated rice and barley starches (possessing various amylose contents). They were compared to RS contents of *Np*AS-treated starches to those of physically-modified starches (subjected to repeated autoclaving-cooling cycles) to assess starch retrogradation effects on RS contents, and correlated AP structural characteristics and AM contents of *Np*AS-treated starches to their RS contents to identify major factors influencing RS formation within *Np*AS-treated starches.

2. Materials and methods

2.1. Materials and chemicals

Rice (Goamy, Dongjin, Manmi, and Sinsunchal varieties) and barley (Gwangan, Gang, Ohl, and Chal varieties) grains were sources of rice and barley starches used in this study. Goamy and Manmi rice grains, and four barley grains were obtained from Yeongnam Agricultural Research Institute (NICS) (Milyang, Korea). Dongjin and Sinsunchal rice grains were kindly gifted from Jukam Farms (Goheung, Korea). Other chemicals and reagents were of analytical grades.

2.2. Rice starch isolation

Rice starches were isolated by an alkaline steeping method. Rice grain was soaked in double distilled water (DDW) for 4 h and subsequently in an aqueous solution (0.2%, w/v) of NaOH for 1 h. The softened grain was blended with the aqueous NaOH solution for 2 min, after which the slurry was passed through two serial sieves of 100 and 270 mesh. While the sieve throughs (starch fraction) were set aside, the sieve overs (rice grain residue) were blended again. These procedures were repeated a total of three times. The pooled sieve throughs were centrifuged at 1100*g* for 10 min, and the supernatant was discarded. The obtained starch pellet was re-suspended in the aqueous NaOH solution, and left at 4 °C for 24 h, after which the supernatant was discarded. These purification procedures were repeated until a yellow layer (protein residue) was not observed. The purified starch was washed with DDW, neutralised with 1 N HCl, washed again with DDW, and recovered by centrifugation (1100*g*, 10 min). The resultant starch pellet was air-dried at room temperature (approximately 24 °C), ground, and passed through the 100-mesh sieve. The final rice starches were stored in a desiccator at room temperature for further analyses.

2.3. Barley starch isolation

Barley grain (100 g) was crushed in a laboratory mixer (M20, IKA-Werke GmbH & Co. KG, Staufen, Germany) at setting 1, and steeped in tap water (400 ml) at 4 °C for 16 h. Wet crushed grain was wet-milled using a laboratory mixer at setting 7 for 7 min, after which the slurry passed through 200-mesh sieve. While sieve throughs (barley starch and soluble protein fractions) were set aside, sieve overs (barley bran, and grain residues) were discarded. The pooled sieve throughs were centrifuged at 3000*g* for 4 min, and the recovered pellet (starch and protein mixture) was suspended in tap water, following a removal of supernatant. The suspension was adjusted to pH 11.5 with 1 M NaOH, passed through 300-mesh sieve, and centrifuged at 3,000*g* for 4 min. The pellet was carefully scraped off with a spatula to remove yellowish brown layer (protein fraction). The resultant pellet was suspended again in tap water and centrifuged at 3000*g* for 4 min. These procedures (i.e., pellet suspension, centrifugation, protein layer scrapping) were repeated until the yellowish brown layer was not observed. The pellet of the final step was re-suspended in tap water, adjusted to pH 5.0 with 1 M HCl, and recovered by centrifugation (3000*g*, 20 min). The isolated starch was air-dried at room temperature, ground, and passed through the 100-mesh sieve. The final barley starch sample was stored in a desiccator at room temperature for further analyses.

2.4. Determination of amylose content

Apparent AM contents of isolated rice and barley starches were determined according to a colourimetric method outlined by AACC Approved Method 32-40 (AACC International, 2000). Native starch sample (20 mg, d.b.) was dispersed in absolute ethanol (0.2 ml), and then mixed with 1 M NaOH (1.8 ml) with vigorous vortexing. The starch suspension was heated for 10 min in a boiling water bath, and cooled to room temperature (≈22 °C). The resultant starch solution (1 ml) was diluted to 10 ml with deionised water. An aliquot (0.5 ml) of the diluted starch solution was combined with 1 M acetic acid (0.1 ml) and Lugol's solution (0.2 ml; 0.2% I_2 + 2.0% KI), and diluted again to 10 ml with deionised water, followed by holding for 20 min in the dark. The absorbance of the colour-developed starch solution was measured at 620 nm using a spectrophotometer (DU 730, Beckman, CA, USA). The AM content of the starch sample was determined from a standard curve prepared with potato AM Type III (Sigma–Aldrich Chemical Co., St. Louis, MO, USA).

2.5. Preparation of retrograded rice and barley starches by autoclaving-coolingtreatment

Retrograded rice and barley starches were prepared through autoclaving-cooling (AC) treatment outlined by Sievert and