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Structural and physicochemical properties of granular starches after treatment with debranching enzyme

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ABSTRACT

The present study modified maize and potato granular starches by partial debranching treatment below the gelatinization temperature, and investigated their structural and physicochemical properties. Pullulanase was much effective (more than three times) on hydrolyzing potato starch compared to maize starch as measured from total carbohydrate values in the supernatant. The pullulanase hydrolysis decreased the amount of double helices as observed from DSC measurement. These effects were dependent upon the time of enzyme hydrolysis (24 h > 8 h > 1 h) as well as type of starch (potato > maize). The pullulanase hydrolysis decreased the peak viscosity of the potato starch paste, whereas the effect was very less pronounced for maize starch. The current results showed that it is possible to achieve the starches with desired physicochemical properties by varying the starch type as well as modification process.

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1. Introduction

Starch, the second most abundant biomass in nature, is biosynthesized as semi-crystalline granules in higher plants, and generally consists of two polymers, amylose and amylopectin. Amylose is a slightly branched molecule (Takeda, Maruta, & Hizukuri, 1992), whereas amylopectin is a much larger molecule with highly branched structure consisting of ca. 95% α- (1,4) linkages and ca. 5% α- (1,6) linkages (Tester, Karkalas, & Qi, 2004a). Starches obtained from different botanical origins vary in granular morphology, crystalline organization and molecular structure, thus their physicochemical and nutritional properties are origin dependent (Lehmann & Robin, 2007). In order to achieve the desired properties and meet the requirement of food and industrial applications, starches are modified using physical, chemical and enzymic techniques (Chung, Liu, & Hoover, 2009; Jacobs, Eerlingen, Charwart, & Delcour, 1995; Tester, Karkalas, & Qi, 2004b; Zhang, Huang, Luo, Fu, Jiang & Jane, 2011).

To expand the industrial applications of native starches, enzyme modification has been widely used to meet the requirement of the clean labeled food. The enzymatic modification utilizes the ability of enzyme to hydrolyze/synthesize α-(1,4) and/or α-(1,6) linkages of starch molecules. For example, the branch chains of amylopectin and amylose can be selectively cleaved at α-(1,6) linkages by either isoamylase or pullulanase (Cai, Shi, Rong, & Hsiao, 2010; Manners, 1989). The pullulanase debranched starches contained a large number of short branch chains (Liu, Hong, & Gu, 2013), showing a strong retrogradation tendency in an aqueous system (Cai & Shi, 2010). Thus, the combined debranching method with controlled crystallization could be used to alter molecular and supramolecular structure of starches as well as diverse functionality. Furthermore, partially debranched starches had greater capacity to form complex with iodine and fatty acids, and possessed higher solubility but lower viscosity compared with their native counterparts (Klaochanpong, Puttanlek, Rungsardthong, Puncha-arnon, & Uttapap, 2015). The debranching hydrolysis could be a novel technique to alter the functionality of native starches without destroying the granular structure. In this study, we compared debranching treatment of A-(maize) and B-(potato) type polymorphic starch granules below the gelatinization temperature, and investigated the structural and physicochemical properties of partially debranched granular starches.

Abbreviations: DMS, maize starch with debranching treatment; DPS, potato starch with debranching treatment; DSC, differential scanning calorimeter; HMS, maize starch with hydrothermal treatment; HPS, potato starch with hydrothermal treatment; NMS, native maize starch; NPS, native potato starch; SEM, scanning electron microscopy; XRD, X-ray diffraction.

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2. Materials and methods

2.1. Materials

Maize and potato starches were obtained from Tiancheng Company (Jilin, China). Pullulanase (EC 3.2.1.41, 405units/g) was provided by Amano Enzyme Company (Shanghai, China). One unit is defined as the amount of pullulanase that catalyzes the increase of reduction power equivalent to 1 μM of glucose per minute. All other chemicals used in this study were of analytical grade.

2.2. Preparation of debranched granular starches

Starch (30 g, dry starch basis, dsb) was mixed with 295 mL of sodium acetate buffer (0.01 M, pH 5.0), and incubated in a water bath at 60 °C for 30 min. For enzymatic modification, pullulanase (10 units per dry starch basis) was added and the mixture was kept at 60 °C for different time intervals (1, 8, and 24 h) with constant stirring (250 rpm). These starches treated at 60 °C without pullulanase hydrolysis are regarded as hydrothermal samples. All hydrothermal and debranched starches were recovered by 3000 *g* centrifugation for 10 min followed by washing with ethanol for three times. The precipitate was oven-dried at 37 °C overnight.

2.3. Determination of hydrolysis rate

All hydrothermally treated and debranched starches were recovered by 3000 *g* centrifugation for 10 min. The hydrolysis rate was measured by total carbohydrate values in the supernatant through the phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956; Huang et al., 2010). The total carbohydrate value was calculated as follows.

$$T_C = C \times N \times V \quad (1)$$

where T_C is the total carbohydrate content of hydrolysate; C is absorbance of diluted hydrolysate according to the regression equation; N is the diluted multiples of sample solution; V is total volume of hydrolysate. The hydrolysis rate was calculated as follows.

$$\text{Hydrolysisrate}(\%) = \frac{T_C \times 0.9}{M_S} \times 100 \quad (2)$$

where M_s is the mass of native starch.

2.4. Swelling power

Swelling power (SP) was determined by using 10% starch suspension according to a method reported elsewhere (Singh, Singh, Isono, Noda, & Singh, 2009). The suspension was heated at 60 °C with mechanical stirring for 30 min, and centrifuged at 800*g* for 10 min. The supernatant was discarded and the wet starch residue was weighed. The swelling power was calculated as the weight of starch residue per gram of starch.

$$SP = \frac{M_r}{M_w} \quad (3)$$

where M_r is the mass of starch residue after suspension was centrifuged (g), and the M_w is the mass of the dry weight (g) of the hydrothermally treated starch.

2.5. Apparent amylose content

Apparent amylose content of starches was determined by measuring iodine affinities of defatted whole starch using a potentiometric autotitrator (888 Titrando, Brinkmann Instrument, Westbury, NY, USA) following the method reported elsewhere (Stevenson, Domoto, & Jane, 2006; Takeda, Hizukuri, & Juliano, 1987). Starch samples were dissolved and defatted in 90% dimethyl sulfoxide (DMSO) solution, and followed by alcohol precipitation. An appropriate amount of precipitated sample (100 mg) is weighed and transferred to a dry beaker. The water (1 mL) and KOH solution (1 M, 5 mL) were added to suspend the sample with occasional stirring. HCl solution (0.5 M) was used to neutralize the mixture and then KI (0.5 M, 10 mL) was added. Sufficient water is added to give a total weight of 100.9 g over the weight of the empty beaker. Then the mixture is potentiometrically titrated with iodine at 30 °C with continuous mechanical agitation. Apparent amylose content was calculated by dividing the iodine affinity of the starch by 19.0%, the typical value of iodine affinity for purified maize amylose (Lu, Jane, Keeling, & Singletary, 1996).

2.6. Light microscopy

Polarized light microscopy was performed on a BX-51 microscope (Olympus, Tokyo, Japan). One drop of starch suspension was placed on the microscope slide before covering with a cover slip, and the images were recorded at 500× magnification.

2.7. Scanning electron microscopy (SEM)

Starch granules were mounted on an aluminum stub using double-sided tape, coated with a thin film of gold. The images were examined under scanning electron microscope (TM3000, Hitachi, Tokyo, Japan) at an accelerating voltage of 10 kV.

2.8. Wide angle X-ray diffraction (XRD)

Starch samples were equilibrated in a chamber with 100% relative humidity at 25 °C for 24 h (Jane, Wong, & Mcpherson, 1997). X-ray diffractometer (D8 Advance, Bruker, Germany) was operated at 40 kV and 40 mA with Cu Kα radiation (λ=0.154 nm). The starch powder was packed tightly in a rectangular glass cell, and scanned over the range 5–35 Bragg angles at a rate of 2°/min at room temperature. Relative crystallinity of the starches was calculated using the following equation.

$$\text{Relativecrystallinity}(\%) = 100 \times A_c/(A_c + A_a) \quad (4)$$

where A_c is the crystalline area on the X-ray diffractogram, and A_a is the amorphous area.

2.9. Thermal properties

A differential scanning calorimeter (DSC-8000, PerkinElmer, Norwalk, CT, USA) with an intra cooler was used to examine the thermal properties of starch samples. Starch samples (~3 mg) were mixed with deionized water (moisture level 70%), and hermetically sealed in high-pressure stainless steel pans (PE No.BO182901) with a gold-plated copper seal (PE No. 042-191758).After equilibrating for 24 h at room temperature, samples were scanned at a heating rate of 5 °C/min from 30 to 150 °C. The enthalpy change (ΔH), onset (T_o), peak (T_p) and conclusion (T_c) temperatures were calculated by using a Pyris software (Perkin Elmer, Norwalk, CT, USA).

2.10. Pasting properties

The pasting properties of starch samples were measured using a Micro Visco Amylo-Graph (Brabender, Germany). Starch dispersion (6%, w/w, dsb) was directly transferred into a stainless steel canister. The dispersion was heated from 30 to 95 °C at a rate of 7.5 °C/min, held at 95 °C for 5 min, cooled to 50 °C at a rate of 7.5 °C/min, then held at 50 °C for another 5 min. The pasting tem-

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