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Partial branching enzyme treatment increases the low glycaemic property and α-1,6 branching ratio of maize starch

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ABSTRACT

Partial branching enzyme treatment was used to modulate the starch fine chain structure responsible for a high content of slowly digestible starch fraction. Normal maize starch modified using branching enzyme for 4 h showed a maximum slowly digestible starch content of 23.90%. The branching enzyme hydrolysis decreased the amylose content from 32.8% to 12.8%. The molecular weight distribution of enzyme-treated starches showed a larger proportion of low molecular weight fractions appeared in the enzyme treated starch sample compare to native starch. The number of shorter chains (DP < 13) increased from 18.71% to 28.23.1%, accompanied by a reduction of longer chains (DP > 30) from 20.11% to 11.95%. ^{1}H NMR spectra showed an increase of α-1,6 branching ratio from 4.7% to 9.4% during enzyme treatment. The increase in the amount of shorter chains and more α-1,6 linkages likely contribute to their slow digestion property. These results suggest that starches treated with partial branching enzyme synthesis a novel branched structure with slowly digestible character.

1. Introduction

Starch is one of the most important glycaemic carbohydrates in staple foods and the glucose generated from starch digestion plays an important role in energy metabolism and glucose homeostasis (FAO/WHO, 2002; Manuel-y-Keenoy & Perez-Gallardo, 2012; Miao, Jiang, Cui, Zhang, & Jin, 2013; Semjonous et al., 2009). According to the rate and extent of digestion, starch has been classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS), in relation to its physiological response and health effect after consumption (Englyst, Kingman, & Cummings, 1992). RDS is rapidly digested and absorbed in the duodenum and proximal regions of the small intestine, which leads to fast elevation of blood glucose and insulin levels, whereas SDS is digested slowly throughout the entire small intestine to provide sustained glucose release with low initial glycaemia and a subsequent slow and prolonged release of glucose (Ludwig, 2002; Miao et al., 2013; Seal et al., 2003). Recent studies have shown that the continued increase in incidence of diabetes, cardiovascular diseases and obesity has been associated with long-term consumption of foods containing a high mount of RDS (Manuel-y-Keenoy & Perez-Gallardo, 2012; O'Keefe, Abuannadi, Lavie & Bell, 2011; Semjonous et al., 2009). Large fluctuation in blood glucose level generates stress on the regulatory system of glucose homoeostasis, leading to cell, tissue and organ damage (Ludwig, 2002). Therefore, improving food quality with a higher amount of SDS is becoming an area of interest for researchers from academia and industry, due to the scarcity of SDS in processing diet. Moreover, the earlier research on producing SDS was done using physical, chemical or enzymatic methods (Ao et al., 2007; Han & BeMiller, 2007; He, Liu, & Zhang, 2008; Miao, Jiang, & Zhang, 2009; Miao, Jiang, Zhang, Jin, & Mu, 2011; Miao et al., 2013).

Branching enzyme (1,4-α-D-glucan:1,4-α-D-glucan 6-α-D-(1,4-α-D-glucano)-transferase, EC 2.4.1.18) is a member of the α-amylase super-family, and also known to catalyse transglycosylation to form α-1,6-branching points in amylopectin or glycogen, by cleaving an α-1,4-glycosidic bond of substrate and transferring the non-reducing end terminal fragment of the chain to C-6 hydroxyl position of an internal glucose residue (Abad et al., 2002; Palomo et al., 2011; Praznik, Rammesmayer, Spies, & Huber, 1992). In the earlier studies, branching enzyme was widely used in starch processing, such as highly branched cyclic α-glucan (Kawabata, Toeda, Takahashi, Shibamoto, & Kobayashi, 2002;

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Kim, Ryu, Bae, Huong, & Lee, 2008; Takata, Kato, Takagi, & Imanaka, 2005; Takata, Takaha, Okada, Hizukuri, et al., 1996; van der Maarel, Vos, Sanders, & Dkjkhuizen, 2003), cluster dextrin (Lee et al., 2013; Song, Min, Hwang, & Lee, 2008; Takata et al., 2010; Takii, Ishihara, Kometani, Okada, & Fushiki, 1999) and glycogen (Kajiura, Kakutani, Akiyama, Takata, & Kuriki, 2008; Ryoyama, Kidachi, Yamaguchi, Kajiura, & Takata, 2004). Also, branching enzyme has potential use in the baking industry as an anti-staling agent and for increasing loaf volume (Kawabata et al., 2002; Kim et al., 2008; van der Maarel & Leemhuis, 2013). Yet little work has been reported on the structural modification using branching enzyme for understanding of the slow digestion property of starch. The objective of the current study was to investigate the impacts of partial branching enzyme treatment of maize starch on the slow digestible property, to study the branched structure and determine whether there is any relationship to produce tailor-made SDS.

2. Materials and methods

2.1. Materials

Normal maize starch was purchased from Shanghai Yuanju Biological Technology Co., Ltd. (Shanghai, China). Alpha-amylase (type VI-B) from porcine pancreas and amyloglucosidase (Dextrozyme® GA) from *Aspergillus niger* were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO) and Novozymes (Tianjin, China), respectively. Isoamylase, the amylose assay kits and the glucose oxidase–peroxidase assay kits were purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland). The pET-22b(+) expression vector was obtained from Novagen (Darmstadt, Germany). Oligonucleotides were synthesised by Generay Biotech Co., Ltd. (Shanghai, China). The resin for protein purification and the Chelating Sepharose Fast Flow were obtained from GE (Uppsala, Sweden). All chemicals were reagent grade and were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Cloning and preparation of branching enzyme

The complete genome sequence of *Acidothermus cellulolyticus* 11B has been released in GenBank (NCBI accession number: CP000481.1). The full-length nucleotide sequence of branching enzyme gene with locus_tag Acel_0676 from the strain was synthesised and incorporated with *Nde*I and *Xho*I sites in 5′- and 3′-terminal of the gene, and then was cloned into the expression vector pET-22b(+) by Generay Biotech Co, Ltd. (Shanghai, China). An in-frame 6× histidine-tag sequence at the C-terminus was provided in the recombinant plasmid.

The recombinant *Escherichia coli* BL21 (DE3) harbouring branching enzyme gene was cultivated with shaking (200 rpm) in Luria–Bertani medium (1% Bacto-tryptone, 0.5% yeast extract, 0.5% NaCl) containing ampicillin (50 μg/ml) at 37 °C. The extracellular expression was performed at 28 °C for 8 h by adding isopropyl-β-D-thiogalactopyranoside (IPTG) to 0.5 mM when the absorbance at 600 nm was 0.6. After induction, the culture supernatant was collected by centrifugation (10,000*g* for 10 min) at 4 °C for protein purification. The target protein was expressed as 6× his-tagged fusion protein, and purified by Ni–NTA affinity chromatography according to manufacture's protocol (pET His Taq System, Novagen). Purity of the protein was confirmed electrophoretically using sodium dodecyl sulphate–polyacrylamide gel electrophoresis and the optimum reaction temperature and pH of the enzyme were determined as 50 °C and 7.5, respectively. Branching enzyme activity was determined using the iodine assay as suggested by Takata, Takaha, Okada, Hizukuri, et al. (1996). One unit of branching enzyme activity was defined as the amount of enzyme that can decrease the absorbance of the amylose–iodine complex by 1% per min at 660 nm.

2.3. Enzymatic modification of maize starch

The maize starch slurry (8% w/v in diluted pH 7.5, 50 mM sodium phosphate buffer solution) was cooked in a water bath at 95 °C for 60 min. The temperature of the starch sample was adjusted to 50 °C, and branching enzyme (5000 U/g dry weight of starch) was added to the solution. The enzymatic reaction was incubated for 0.5, 1, 2, 4, 6 or 12 h. Immediately after the reaction, the solutions were autoclaved at 121 °C for 30 min to stop reaction and cooled to room temperature, and 1 volume of 90% ethanol (v/v) was added to facilitate the precipitation of the reactant. The precipitated starch was collected by centrifugation at 5000*g* for 10 min, washed with deionised water and collected by centrifugation twice, then freeze-dried. The collected material was ground to form a powder (120 mesh) and stored in a desiccator for further analysis. The enzymatically hydrolysed samples were named BS1, BS2, BS3, BS4, BS5 and BS6, respectively, based on the enzymatic reaction times listed above. The supernatant of the hydrolysed products, with the enzymatic reaction stopped at different time points, was collected for the determination of the degree of hydrolysis using the phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). To 1.0 ml of the supernatant, 1.0 ml of phenol solution (6%) and 5.0 ml of concentrated sulphuric acid (95.5%) were added and then mixed well. After cooling to room temperature, the solution was put into a quartz cuvette and its absorbance was determined at 490 nm. The control sample was prepared using the same method used for BS-modified starches except without the addition of enzyme.

2.4. Amylose content and iodine binding analysis

The amylose content of the starch sample was determined by the amylose assay kit using the Concanavalin A precipitation procedure (Gibson, Solah, & McCleary, 1997). The iodine binding analysis was measured using a UV/visible Spectrophotometer (UV-2102PC, Unico Instrument Co., Ltd., Shanghai, China) according to the method described by Shen, Bertoft, Zhang, and Hamaker (2013). An iodine reagent was prepared by adding 2 mg of I_2 and 20 mg of KI to 1 ml of deionised water. The reagent was stored in a non-actinic bottle at room temperature. The dried starch sample (50 mg) was dissolved in deionised water in a 10 ml screw-cap vial. The diluted solution (0.5 ml) was mixed with the iodine solution (1.5 ml) and then adjusted to a final volume of 2 ml with water. The absorbance spectra and the wavelength of maximum absorption were analysed over a wavelength scan of 400–800 nm.

2.5. High-performance size-exclusion chromatography analysis

The molecular weight distribution of starch was measured using a high performance size exclusion chromatography system with a multi-angle laser light scattering detector and a refractive index detector (HPSEC-MALLS-RI) (Wyatt Technology, Santa Barbara, CA, USA). The starch samples (10 mg) were added to 5 ml of deionised water and boiled with stirring for 15 min to completely dissolve the samples using DMSO with 50 mM LiBr at a concentration of 2% (w/v). The dissolved sample was filtered through 5 μm cellulose acetate filters (Whatman, Maidstone, UK) and injected into the HPSEC-MALLS-RI system. Two series tandem columns (300 × 8 mm, Shodex OH-pak SB-806 and 804, Showa Denko K.K., Tokyo, Japan) with an OH-pak SB-G guard column, a DAWN HELEOS II laser photometer fitted with a He–Ne laser (λ = 632.8 nm) with a K-5 flow cell, and an OPTILAB® T-rEX Interferometric Refractometer were used. The flow rate was set at