



Pullulanase hydrolysis behaviors and hydrogel properties of debranched starches from different sources



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ABSTRACT

In the present study, debranched starches (DBS) from different sources were prepared by treatment with pullulanase with an aim to investigate the differences in pullulanase hydrolysis and the hydrogel properties of the resulting DBS. The results indicated that all DBS were V-type starches with hollow, single-helical structures. Debranched maize starch (DBMS) and wheat starch (DBWS) performed similarly. Pullulanase treatment resulted in an obvious reduction in viscosity for all tested starches except debranched pea starch (DBPeS). The average molecular weight of small fragments of debranched potato starch (DBPoS) was higher than that of the other samples, and longer lateral chains tended to be cleaved by pullulanase for DBPoS. The results of the solubility and water-holding capacity analyses indicated the DBS starches were capable of capturing water to form hydrogels. DBPoS was more easily digested by gastrointestinal amylase, whereas its pea-source counterpart showed the opposite trend. The results confirmed that generation of short linear glucan chains by pullulanase hydrolysis effectively improved solubility and water-holding capacity, which contributed to their hydrogel-forming properties.

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1. Introduction

Starch is the second most abundant biomass material in nature, second only to cellulose (Le Corre et al., 2010). Due to its natural, renewable, biodegradable properties, starch and its derivatives have been widely used in many industries, such as food, paper, textile, and plastic (Du, Zang, & Du, 2011; Meshram, Patil, Mhaske, & Thorat, 2009). Starch is widely distributed in seeds (maize, wheat, rice, and pea), fruits (green banana and plantain), roots (potato, cassava, and sweet potato), and leaves (tobacco) as a source of stored energy.

Starch contains two α -D-glucose polymers, namely linear amylose and branched amylopectin. Each starch chain contains 7–60 glucose units linked by 1,4- α -D-glucosidic bonds, and different starch chains are bound to each other by 1,6- α -D-glucosidic bonds. Starches obtained from different botanical origins vary

mainly in their morphology and crystalline organization, as well as their amylose/amylopectin composition, which strongly affects their physicochemical properties and their susceptibility to pullulanase hydrolysis (Lehmann & Robin, 2007; Onofre & Wang, 2010b; Tester, Karkalas, & Qi, 2004).

According to their crystalline structure, native starches can be grouped into three types: A-type (cereal starches: maize, wheat, etc.), B-type (fruit and tuber starches: potato, etc.), and C-type starches (leguminous starches: pea, etc.). Amylopectin in A-type starch has more branching points and shorter side chains, whereas B-type starch is favored by longer lateral chains, and its branching points are more distant from each other (Barsby, Donald, & Frazier, 2001; Buléon, Colonna, Planchot, & Ball, 1998). Thus, the density of the crystalline domains in A-type starch is higher than that in B-type starch. C-type starch is a combination of A-type and B-type crystalline structures. In some modified starches, V-type crystalline structures can be found. The formation of V-type crystalline structure is probably caused by the formation of inclusion complexes between amylose and small organic polar molecules, like fatty acids and alcohol (Thérien-Aubin & Zhu, 2009). Compared to the double helices in A-type and B-type starches, V-type contains single helices, and this larger hollow helix is beneficial for encapsulating small molecules and targeted delivery.

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In this paper, four starches from maize (A-type), wheat (A-type), potato (B-type), and pea (C-type) were modified by pullulanase treatment. After pullulanase debranching, the 1,6- α -D-glucosidic bonds could be selectively cleaved and many short linear glucan chains were obtained. This modification likely causes significant changes in both the overall structure and physicochemical properties. This study aimed to investigate the behaviors of starches from different sources following incubation with pullulanase and the physicochemical properties of their debranched derivatives. The contributions of starch fine structure, crystalline structure and molecular weight distribution to the properties of the DBS were also evaluated.

2. Materials and methods

2.1. Materials

Starches were provided by Zhucheng Xingmao Corn Development Co., Ltd. (Shandong, P. R. China). Pancreatin (batch No. SLBC2100V) and HPLC-grade DMSO (batch No.75927E) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA) and Adamas-beta Co., Ltd. (Shanghai, China), respectively. Promozyme D2 (EC 3.2.1.41, Enzyme activity: 600 U/mL) was obtained from Novozymes (batch No. ATS20036; Tianjin, P. R. China). Isoamylase (EC 3.2.1.68, batch No. BCBK8690V) were purchased from Sigma–Aldrich Chemical Co. All the other chemicals used in this study were of analytical grade.

2.2. Preparation of DBS

DBS was prepared based on the method of Cai et al. (Cai, Bai, & Shi, 2012). A starch slurry (5% w/v, based on the dry weight of starch) in acetic acid buffer (0.01 mol/L, pH 5.5) was boiled in a 500-mL pressure tube (ACE Glass, Vineland, NJ, USA) with constant stirring for approximately 15 min. Then, the tube was transferred to an oven (DHG-9055A; Yiheng Scientific Instrument Co., Ltd., Shanghai, P. R. China) and incubated for 1 h at 130 °C to thoroughly gelatinize the starch. Once the starch paste was cooled to 55 °C, pullulanase (10 μ L/mg dry basis, Promozyme D2; Novozymes) was added and incubated for 4 h at 55 °C. Then, the enzymolysis liquid was precipitated by slowly adding it to 1000 mL of anhydrous ethanol with continuous stirring. After the mixture was stored at room temperature overnight, the precipitate was collected by centrifugation at 4500 rpm for 15 min (RJ-LD-IIB; Ruijiang Instruments Co., Ltd., Jiangsu, P. R. China), and then washed three times with 200 mL of anhydrous ethanol. Finally, the modified starch was vacuum dried (DZG-6050; Senxin Experimental Instrument Co., Ltd., Shanghai, P. R. China) at 40 °C, and DBS was obtained after grinding and sieving.

2.3. High performance anion exchange chromatograph (HPAEC)

Chain length distributions of native starches from different sources were determined by a HPAEC instrument equipped with pulsed amperometric detection (HPAEC-PAD) (Dionex ICS-5000, Dionex Corp., Sunnyvale, CA, USA). A CarboPac™ PA200 (3 $\times$ 250 mm) column was eluted at a flow rate of 0.5 mL/min according to a gradient program: 40% eluent B at 0 min, 50% at 2 min, 60% at 10 min, and 80% at 40 min for 20 min (Eluent A: 150 mmol/L NaOH; Eluent B: 150 mmol/L NaOH containing 500 mmol/L sodium acetate). The column temperature was kept at 25 °C.

50 mg starch (based on dry starch) was mixed with 10 mL citrate buffer (0.01 M, pH 3.5) followed by gelatinization in boil water for 30 min. 2 mL starch slurry was added into a 5 mL centrifuge tube when the temperature of the starch paste cooled down to 37 °C.

Isoamylase (100 μ L, Enzyme activity: 10,000 U/mL) was added into the tube, and the reaction was maintained for 20 h until the debranching hydrolysis was thoroughly conducted. Then, the solution was heated in boiled water for 15 min in order to inactivate the pullulanase. After centrifugation at 10,000 rpm for 10 min, 0.5-mL supernatant was diluted in 4.5 mL deionized water. The diluted starch solution was filtered through a 0.45 μ m membrane filter, and 50 μ L sample was injected into the HPAEC-PAD system. Maltotriose and maltoheptaose (Sigma–Aldrich, Inc., St. Louis, MO, USA) were used as standards.

2.4. Viscosity analysis

The viscosity changes during pasting and pullulanase hydrolysis were analyzed by a Brabender® viscosograph (803200 series Brabender® OHG; Duisburg, Germany). Native starches (36 g, dry basis) were dispersed in HAc-NaAc (0.01 mol/L, pH 5.5). The concentration of starch slurries was 8% (w/w), and the total mass was 450 g. The slurries were gradually heated from 50 °C to 95 °C at a rate of 1.5 °C/min, and then the mixture was held at 95 °C for 30 min. During the cooling phase, the temperature was reduced from 95 °C to 55 °C at a rate of 1.5 °C/min. When the temperature reached 55 °C, 100 μ L of pullulanase was added into the mixture, and the temperature was held at 55 °C for 60 min.

2.5. Particle size analysis

A particle size analyzer (S3500; Microtrac Inc., Largo, FL, USA) was used to analyze the particle size distribution of the starch samples. Dry particles of different samples were directly measured. Microtrac Flex software (version 10.3.3) was used to analyze the data.

2.6. X-ray diffraction (XRD)

X-ray diffraction analysis was conducted by using an X-ray diffractometer (D8 Advance; Bruker AXS Co., Karlsruhe, Germany). Starch samples were scanned from 5° to 35° (2 θ) at 3°/min, 35 kV, and 20 mA with Cu-K α radiation (λ = 1.5406 Å). The data were analyzed with MDI Jade 5.0 software (Materials Data Inc., Livermore, CA, USA).

2.7. Gel permeation chromatography (GPC)

GPC was employed to investigate the molecular weight distribution (MWD) of the starch samples. Measurements were made according to the method reported by Cai (Cai & Shi, 2010) with some modifications. Briefly, starch samples (30 mg, dry basis) were thoroughly dissolved in 3 mL of 90% DMSO with 50 mmol/L NaNO₃ in boiling water with constant stirring for 24 h. The sample was filtered through a 0.45- μ m organic filter, and then injected into a Shimadzu HPLC/GPC instrument (CTO-20A; Shimadzu Corporation, Kyoto, Japan) equipped with an RID-10A refractive index detector and three Phenogel columns, namely a Styragel HR3 (M_w: 500–30,000), Styragel HR4 (M_w: 5000–600,000), and Styragel HMW7 (M_w: 500,000–1 $\times$ 10⁸) (Waters, Inc., Torrance, CA, USA), and a differential refractive index detector. The samples were eluted with 90% (v/v) DMSO containing 0.5 mmol/L NaNO₃ at a flow rate of 0.8 mL/min. The column oven temperature was set to 80 °C. Standard dextrans (Sigma–Aldrich, St. Louis, MO, USA) with different molecular weights (M_w) were used for M_w calibration. Data processing was performed with the help of XPS Peak fit software. After peak fitting, the GPC data were used to characterize the MWD of the starch samples.

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