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Preparation and properties of RS III from waxy maize starch with pullulanase

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ABSTRACT

Waxy maize starch was treated by pullulanase debranching and retrogradation at room temperature to produce resistant starch (RS). Physicochemical properties, crystalline structure and in-vitro digestibility of starch samples with different RS content were investigated. Compared with native starch, apparent amylose content of RS products increased. Based on Gel Permeation Chromatography (GPC) the Molecular Weight Distribution (MWD) of resistant starches significantly changed. Scanning Electron Microscopy (SEM) showed that upon pullulanase debranching and retrogradation treatment the granular structure of native starch was destroyed and all RS samples exhibited irregular shaped fragments. Crystal structure of samples changed from A–type to a mixture of B and V–type. The crystallinity of resistant starch also improved as compared with native starch. Moreover, samples with higher resistant starch showed higher relative crystallinity. Differential Scanning Calorimetry (DSC) determination showed that *To*、*Tp*、*Tc* and ΔH all increased which was in agreement with RS content. The resistance of waxy maize starch with Pullulanase treatment to α-amylase digestibility also increased, while the in-vitro digestibility of products decreased.

1. Introduction

Starch, composed of essentially linear amylose and highly branched amylopectin with α-D-glucopyranose as structural unit, is the main glycemic carbohydrate material in cereal- and tuber-based food products (Zhang, Sofyan, & Hamaker, 2008). Starch industries are interested in Rapidly Digestible Starch (RDS), Slowly Digestible Starch (SDS) and Resistant Starch (RS) as a nutrition-valued food product (Pongjanta, Utaipattanaceep, Naivikul, & Piyachomkwan, 2009). Resistant Starch (RS) is defined as starch and products of starch degradation that cannot be absorbed in the small intestine of healthy individuals and, hence, might be fermented in the colon (Englyst, Kingman, & Cummings, 1992). RS presents an exciting new potential as a food ingredient, since it is generally stable to heat treatments and survives most food processes (Ozturk, Koksel, Kahraman, & Ng, 2009). RS has been shown to have physiological benefits resembling those of soluble dietary fibers, including prebiotic effect on colon micro flora, altering metabolism, improving cholesterol metabolism, and reducing the risk of ulcerative colitis and colon cancer (Ozturk, et al., 2009; Pongjanta et al., 2009).

There are four types of RS: RS1, physically inaccessible starch to digestion; RS2, native granule or ungelatinized starch; RS3, retrograded or crystalline starch, and; RS4, chemically modified starch (Eerlingen, Deceuninck, & Delcour, 1993; Guraya, James, & Champagne, 2001). RS3 is of particular interest, because of its thermal stability (Shi & Gao, 2011). RS3 is primarily composed of retrograded amylose because of its strong tendency to reassociate. Therefore, amylose content is the main factor governing the formation of RS3 (Luckett & Wang, 2012). However, the most common way to further increase RS3 formation is by enzymatic debranching, which results in all linear glucans that can more readily reassociate. Pullulanase (pullulan 6-glucanohydrolase, EC 3.2.1.41) is a debranching enzyme, which has been gaining importance in starch conversion processes. It cleaves α-1, 6 linkages in pullulan, amylopectin and other related polysaccharides (Lin & Chang, 2006). Waxy maize starch consists almost entirely of amylopectin (Cai & Shi, 2010). After treatment with pullulanase, amylopectin is transformed into a population of short linear glucans which can promote the formation of resistant starch.

To exacerbate the formation of RS3, debranched starches can be stored at a specified temperature to promote retrogradation which can lead to a new and strong crystalline structure. Amylose degree of polymerization (DP) has been shown to affect RS3 formation (Eerlingen et al., 1993). An appropriate chain length is required for crystallization and formation of double helices and is suitable to form RS3. In a short, there are several factors that affect the formation of RS.

The digestion of starch has been the subject of many investigations, mostly involving in-vitro measurement by different

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enzymes, rather than measuring actual digestibility in-vivo (Blazek & Gilbert, 2010). The digestibility of native starch depends on granular size, amylose/amylopectin ratio, amylopectin chain length, extent of intermolecular associations within the granule and the degree of crystallinity (Hoover, 2001). It means that structural modification of starch may change its digestibility. The enzymatic hydrolysis changes the granular and crystalline structures of starch. In addition, the effects of enzyme concentration on the digestion behavior of waxy maize starch are not fully understood. It is meaningful to investigate the digestibility of enzymatic hydrolysis of starch.

The objective of this study was to investigate the effects of pullulanase enzyme concentrations on physicochemical properties and α-amylase hydrolysis rate of RS3 samples from waxy maize starch. The amylose content, morphology, crystalline properties, thermal properties, molecular weight distribution and in-vitro digestibility of resistant starch samples were investigated penetratingly. This research can provide important information about properties of waxy maize resistant starches.

2. Materials and methods

2.1. Materials

Waxy maize starches were purchased from Gansu Xue Jing Biochemical Co., Ltd., China. Pullulanase (E.C.3.2.1.41, 1000 ASPU/g, 1.15 g/mL) (ASPU is defined as the amount of enzyme that liberates 1.0 mg glucose from starch in 1 min at pH 4.4 and 60 °C) was obtained from Danisco Company (Diazyme® P10, USA). Resistant starch assay kit was purchased from Megazyme International Ireland Limited. Pancreatic α-amylase (E.C.3.2.1.1, 260 units/g) (One unit will liberate 1.0 mg of maltose from starch in 3 min at pH 6.9 at 20 °C) was sought from Sigma Chemical Company (St. Louis, MO, USA). Amylose and amylopectin standards were obtained from Sigma Chemical Company. Chemicals and solvents used in this work were of analytical grade.

2.2. Preparation of resistant starch samples with pullulanase

Waxy maize starch slurry (8% w/w in diluted pH4.5 buffer solution) was cooked in water bath at 95 °C for 30 min. The temperature of cooked waxy maize starch was adjusted to 58 °C and different concentrations of pullulanase enzyme ranging from 5 to 30 ASPU/g of dry starch were added. After a 24 h incubation period, the reaction was stopped by heating the samples at 100 °C for 30 min and then cooled to room temperature. The solutions were stored at 25 °C for 24 h to form retrograded starch. The precipitated starch was dried at 45 °C overnight. All the samples were ground and screened through 80 mesh sieve.

Based on the different pullulanase concentrations, the samples treated by pullulanase at 5, 10, 15, 20 and 30 ASPU/g of dry starch will be referred to as M1, M2, M3, M4 and M5.

2.3. Resistant starch determination

Resistant starch content was determined using a Megazyme Resistant Starch Assay Kit considering the Method of 2002.02 of the Association of Official Analytical Chemists (AOAC) (McCleary et al., 2002). The samples were incubated in a shaking water bath with pancreatic α-amylase and amyloglucosidase for 16 h at 37 °C to hydrolyze digestible starch to glucose. The reaction was terminated with 4 mL ethanol and the indigested RS3 was recovered by centrifugation (5000 *g*, 10 min). The supernatant was then decanted and the pellet washed twice with 50% ethanol (w/w) to remove the digested starch. The sediment, or RS3, was solubilized in 2 mL of 2 M KOH in an ice bath, neutralized with 8 mL sodium acetate (1.2 M). Then the RS hydrolyzed to glucose with of amyloglucosidase (0.1 mL, 3300 U/mL). The glucose oxidase/peroxidase reaction was used to measure the glucose released from the digested and resistant starches. Absorbance was read at 510 nm after a 20 min incubation period at 50 °C. Resistant starch was calculated as glucose ×0.9.

2.4. Apparent amylose contents

Apparent amylose contents in starches were assessed according to the colorimetric procedure of Kumari, Urooj, & Prasad (2007).

2.5. Gel Permeation Chromatograph (GPC)

Each starch sample (4 mg) was mixed with DMSO (4 mL) and stirred in a boiling water bath for 24 h. The sample was filtered through a 2 μm filter and then injected by an autosampler into a PL-GPC 220 instrument (Polymer Laboratories, Inc., Amherst, MA, USA) with three Phenogel columns (Phenomenex, Inc., Torrance, CA, USA), a guard column (Phenomenex, Inc., Torrance, CA, USA), and a differential refractive index detector. The eluant system was DMSO containing 0.5 mM $NaNO_3$ at a flow rate of 0.8 mL/min. The column oven temperature was controlled at 80 °C. Standard dextrans (American Polymer Standards Co., Mentor, OH, USA) with different molecular weights (MW) were used for MW calibration.

2.6. Scanning Electron Microscopy (SEM)

Starch samples were prepared by sprinkling the starch on double-sided adhesive tape attached to a circular aluminum stub, and then coated with 20 nm gold under vacuum. The samples were viewed and photographed in a scanning electron microscope (model S-3700N, Hitachi, Japan) at an accelerating potential of 20 kV.

2.7. X-ray Diffraction (XRD)

X-ray diffractograms were obtained with a D/Max-2200 X-ray diffractometer (Rigaku Denki Co., Tokyo, Japan) using Cu Kα radiation at 44 kV and 26 mA. Moisture content of the samples was equilibrated in a sealed dessicator at room temperature before analysis. The diffractograms were scanned between 4° and 35° (2θ) at the rate of 5°/min. Relative crystallinity was estimated by the ratio of the crystalline area to the total diffractogram area (Chen, Zhang, Huang, & Lu, 2010).

2.8. Differential Scanning Calorimetry (DSC)

Gelatinization temperatures were measured and recorded on a Perkin–Elmer DSC8000 (Norwalk, CT, USA) differential scanning calorimeter (DSC), equipped with a thermal analysis software, Pyris window (Perkin–Elmer). A 30% (w/w) suspension of solid sample in water was prepared and sealed in a DSC pan. Samples were allowed to equilibrate during 2 h at room temperature and then heated from 30 °C to 150 °C at 10 °C/min. An empty pan was used as a reference.

2.9. In-vitro digestibility of starch samples

In-vitro digestibility of the RS3 starch was prepared according to the methods of Jenkins et al. (1981) and Wen, Lorenz, Martin, Stewart, & Sampson (1996) with some modifications. 10 mL of pancreatic α-amylase solution (520 U/mL) was added to RS sample in a 13 cm dialysis bag (width 4.5 cm, molecular weight cutoff