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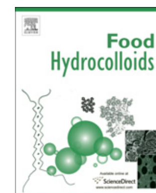

Structural characterizations of waxy maize starch residue following *in vitro* pancreatin and amyloglucosidase synergistic hydrolysis

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ABSTRACT

The objective of this work was to determine if *in vitro* digestion altered the molecular structure and slow digestion property of waxy maize starch. The Englyst testing on partially hydrolysis residual starches showed an increase of RDS accompanied a reduction of RS with increasing time of digestion, while SDS was almost constant. Scanning electron micrographs showed that the pattern of enzymatic hydrolysis was inside-out layer-by-layer digestion. A threefold decrease in the average molecular weight of starch components was observed after α-amylolysis for 120 min. There were increases in the onset temperature, peak temperature and ratio of absorbance 1047/1022 cm^{-1}, while the enthalpy of gelatinization, crystal structure, and crystallinity invaried. These changes suggest simultaneously enzymatic hydrolysis of both crystalline and amorphous regions of starch granule during *in vitro* digestion. SDS of starch residue also may consists of layered structure of amorphous and crystallite regions and located periphery of starch granules.
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1. Introduction

Among food carbohydrates, starch occupies a unique position. It is a major reserve polysaccharide of green higher plants and probably the second most abundant natural biopolymer on earth, next to cellulose. It is also the basic source of energy for the majority of the world's population. In human nutrition, starch plays a major part in supplying the metabolic energy that enables the body to perform its different functions. According to the rate and extent of starch digestibility, starch is generally classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) related to its physiological effect after consumption for nutritional purpose (Englyst, Kingman, & Cummings, 1992). Recent studies have shown that diabetes, cardiovascular diseases, and obesity may be associated with long-term consumption of food with high glycemic index (GI), which is positively correlated with the content of RDS (Englyst et al., 1992; Ludwig, 2002). A moderate postprandial glycemic and insulinemic response of SDS or RS implies that SDS/RS-rich foods may provide wide health benefits in reducing physiology and metabolic disorders involved in chronic non-transmissible diet-related diseases (Ludwig, 2002; Miao, Jiang, & Zhang, 2009; Rose et al., 2009).

Currently, two primary RS type products namely resistant starch granules (RS_2, Hi-Maize™ and Hylong® VII) and retrograded starch (RS_3, Novelose® and Crystalean®) manufactured by National Starch and Chemical Corporation or Opta Food Ingredients are being sold to food industries, whereas there is no commercially available SDS, but the process for making it based on physical, chemical, or enzymatic treatments has been reported (Miao, Jiang, et al., 2009). Shin, Kim, Ha, Lee, and Moon (2005) reported that the granular sweet potato starch with 50% moisture content heated at 55 °C increased by 200% in terms of heat-stable SDS compared to raw starch. Using encapsulation of starch with biopolymer to reduce digestibility, Rose et al. (2009) showed that dropping a homogeneous mixture of sodium alginate and starch into a $CaCl_2$ solution can be obtained starch-entrapped microspheres as novel slowly digestible carbohydrate ingredients that lead to a moderate and extended glycemic response. Miao, Jiang, et al. (2009) attempted the production of SDS based on partially hydrolysis of gelatinized waxy maize starch with pullulanase followed by controlled retrogradation. High enzyme concentration and less debranching time increased the amount of SDS, whereas longer times accelerated the production of RS, which is consistent of these studies of Guraya, James, and Champagne (2001) and Shin et al. (2004). According to Han et al. (2006), a low GI maize starch with some branched structure can be developed by partial α-amylase treatment and cooling. Ao et al. (2007) showed that both the increase in branch density and crystalline structure of starch enhance its slow digestibility through the partial shortening of amylopectin A and B1 chains (exterior chains), as well as linear chains of amylose, through the action of either β-amylase or maltogenic α-amylase with transglucosidase.

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A process for making SDS from citric acid-treated rice starch was studied by Shin et al. (2007), who reported that the optimum conditions for producing heat-stable SDS (54.1%) involved adding 2.62 mmol citric acid into 20 g starch and then reacting at 128.4 °C for 13.8 h. Esterification with octenyl succinic anhydride (OSA) was shown to be the more effective of waxy starch modifications used for making SDS, followed by crosslinking-hydroxypropylated or crosslinking-acetylated starch than cross-linking starch (Han & BeMiller, 2007). Dry heating (130 °C) of OSA-starch increased the SDS content and decreased RS content, which was in agreement with the acid-treated rice starch reported by Shin et al. (2007). In a study of Sang and Seib (2006), simultaneous heat-moisture treatment and phosphorylation (cross-linking) of high-amylose (~70%) corn starch created higher SDS levels, which retained after cooking compared to raw starch. Similarly, He, Liu, and Zhang (2008) showed SDS (42.8%) in heat-moisture treatment of OSA-starch (10% moisture, at 120 °C for 4 h) was higher than that of OSA-starch (28.3%). As far as SDS is concerned, the main focus is on SDS preparation, whereas there are limited reports on mechanisms of slow digestion and structural properties of SDS are not well understood.

The susceptibility of starch granules to degradation by amylolytic enzymes is a solid-solution two-phase in which the enzyme needs first to diffuse toward and absorb the solid substrate, and then catalyses for cleaving the glycosidic linkages (Colonna, Leloup, & Buléon, 1992). The enzymatic hydrolysis of starch granules, which has long been used for industrial process of making sweeteners, syrups and chemical (e.g. ethanol, acetone and lactic acid), or as a tool to study the starch ultrastructure, was effected by both starch botanical origin and α-amylase source (Gallant, Bouchet, Buléon, & Pérez, 1992; Nigam & Singh, 1995). However, little information exists regarding starch digestibility and structural properties during enzymatic treatment *in vitro*. In this study, structural changes and the digestibility of residual waxy maize starch following *in vitro* pancreatic α-amylase and amyloglucosidase hydrolysis were investigated. It is demonstrated that the relationship between slow digestion property and molecular structure and a theoretical mechanism for practical preparations of SDS may be achieved.

2. Materials and methods

2.1. Materials

Waxy maize starch was obtained from Changchun Dacheng Industrial Group Co. Ltd. (Changchun, Jilin, China). Type VI-B porcine pancreas α-amylase and amyloglucosidase Dextrozyme® GA from *Aspergillus niger* were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO) and Novozymes (Tianjin, China), respectively. Glucose assay reagents and isoamylase were from Megazyme International Ireland Ltd. (Wicklow, Ireland). All chemicals were of reagent grade and were obtained from Sinopharm Chemical Reagent Co. Ltd., Shanghai, China.

2.2. Preparation of starch samples

Starch (1 g) was dissolved in phosphate buffer (100 ml, 0.2 mol/l, pH 5.2) and equilibrated at room temperature for 5 min 25 ml of pancreatic α-amylase (290 U/ml) and amyloglucosidase (15 U/ml) were then added and followed by incubation in a water bath at 37 °C with shaking at a speed of 160 rpm. After 20, 40, 80, and 120 min, absolute ethanol was used to stop the reaction and then centrifuged at 3000 × g for 15 min. The precipitates were dried overnight in an air oven at 50 °C. These starch residuary samples were named WM20, WM40, WM80 and WM120, respectively.

2.3. In vitro digestibility of starch samples

The digestibility of starch was analyzed according to the procedure of Englyst et al. (1992) with a slight modification. To prepare enzyme solution I, amyloglucosidase solution (0.14 ml) was diluted to 6.0 ml with deionized water. Enzyme solution II was prepared by suspending porcine pancreatic α-amylase (12.0 g) in water (80.0 ml) with magnetic stirring for 10 min, centrifuging the mixture for 10 min at 1500 × g, then transferring a portion (54.0 ml) of the supernatant into a beaker. Enzyme III was prepared immediately before use by mixing water (4.0 ml), enzyme solution I (6.0 ml), and enzyme solution II (54.0 ml).

A starch sample (200 mg) was dissolved in 15 ml of phosphate buffer (0.2 mol/l, pH 5.2) by vortexing. After equilibrated at 37 °C for 5 min, seven glass balls (10 mm diameter) and enzyme solution III (5.0 ml) were then added, followed by incubation in a water bath at 37 °C with shaking (150 rpm). Aliquots of hydrolyzed solution (0.5 ml) were taken at different time intervals and mixed with 4 ml of absolute ethanol to deactivate the enzymes. The glucose content of the hydrolyzates was determined using glucose oxidase/peroxidase (GOPOD) assay kits (Wicklow, Ireland). Percentage of hydrolyzed starch was calculated by multiplying a factor of 0.9 with the glucose content. Each sample was analyzed in triplicate.

The values of different starch fractions of RDS, SDS and RS were obtained by combining the values of G20 (glucose released after 20 min), G120 (glucose released after 120 min), FG (free glucose) and TG (total glucose) and using the following formulas:

$$\%RDS = (G120 - FG) \times 0.9 \times 100$$

$$\%SDS = (G120 - G20) \times 0.9 \times 100$$

$$\%RS = (TG - FG) \times 0.9 \times 100 - (RDS + SDS)$$

2.4. Scanning electron microscopy

Starch granule images were observed at 1500 or 5000 × magnification under a scanning electron microscopy (Quanta-200, FEI company, Eindhoven, The Netherlands). Dried, finely ground samples were mounted on an aluminum stub using double-sided stick tape and coated with a thin film of gold (10 nm), then examined at an accelerating voltage of 5 kV.

2.5. Molecular weight distribution profiles

Starch samples (20 mg) were added to 5 ml of deionized water and boiled with stirring for 15 min to completely dissolve the samples. The dissolved samples, filtered through 5 μm cellulose acetate filters (Whatman, Maidstone, UK), were injected into a high performance size exclusion column chromatography system (HPSEC) with multi-angle laser light scattering detector (MALS) and a refractive index detector (RI), a pump (LC-10AT, Shimadzu Corporation, Kyoto, Japan), an injector valve with a 100 μl loop (Rheodyne, Cotati, CA). Two series tandem columns (300 × 7.5 mm, PL aquagel-OH MIXED 8 μm, Polymer Laboratories Ltd., Shropshire, UK), a DAWN DSP-F laser photometer fitted with He–Ne laser ($\lambda = 623.8$ nm) with a K-5 flow cell (Wyatt Technology, Santa Barbara, CA), and a RID-10A differential refractive detector (Shimadzu Corporation, Kyoto, Japan) were used. The flow rate was set at 0.6 ml/min with a mobile phase of $NaNO_3$ solution (0.1 M) containing 0.02% NaN_3 that had been passed through a 0.45 μm

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