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Multi-scale structural and digestion resistibility changes of high-amylose corn starch after hydrothermal-pressure treatment at different gelatinizing temperatures

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ABSTRACT

The effects of gelatinizing temperature on the starch enzymatic digestion resistibility and the changes in the multi-scale structures (molecular molar mass and its distribution, molecular ordered structure, crystalline structure, lamellar structure, and granular fractal structure) of a high-amylose corn starch induced by hydrothermal-pressure (HP) processing were investigated, and the gelatinizing temperature – starch structure – digestion resistibility relationships were revealed by the combination of GPC-MALS, ATR-FTIR, XRD, DSC, and SAXS techniques. The results indicate that the short-range orders on the granular surface, the crystallinity, and the total enthalpy should not be the ultimate determinants of the resistibility against enzymatic digestion of the HP-treated starch. The degraded starch molecules with the molecular molar mass between 4×10^5 to 4×10^6 g/mol, a higher degree of the helical ordered structures of the complexes between the degraded amylose molecules and the lipid and of the complexes between the degraded amylose molecules, a newly formed repeat aggregation structure with about 18–15 nm thickness, a great amount of the ordered amylopectin crystalline lamellae, and a mass fractal structure in the length scale of $18.5 < d < 78.5$ nm, all contributed to the greater resistibility against enzymatic digestion of the HP-treated starch. It was the more ordered starch molecular aggregation architecture which came from the multi-scale structural changes after the HP treatment that could be more difficult for starch enzymes to attack the active sites of starch molecules, resulted in a higher content of resistant starch.

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1. Introduction

Numerous scientific evidences have demonstrated that foods increasingly affect human health and that providing healthy delicious foods is the real challenge for food industry (Palzer, 2009; Zúñiga & Troncoso, 2012). Controlling food digestibility and nutrient bioavailability is an important approach in food design to avoid the diet-related diseases such as obesity and type II diabetes mellitus. Food digestibility and bioavailability strongly depend on the food composition and microstructure formed during food processing. Thus, to create new functional healthy food products and new functional properties of food ingredients depends on the knowledge of not only the structures of native materials but also the changes in their structures across a wide range of length scales brought about by food processing (Ubbink & Mezzenga, 2006).

Polysaccharides constitute the most heterogeneous group among the major food elements and play an important role in the nutritional value of food and in human nutrition. Starch, one of the most important polysaccharides widely used as a raw material in foods, has become a significant source of energy in human diets. For beneficial physiological effects and nutritional purposes, starch, depending on the rate and extent of digestion, can be classified into three categories, namely rapidly digested starch (RDS), slowly digested starch (SDS), and resistant starch (RS) (Englyst, Kingman, & Cummings, 1992). Many health benefits and functional properties of RS have been mentioned including the pre-biotic effect on colon microbes, the alteration to lipid metabolism, the improvement in cholesterol metabolism, and the reduction in the risk of ulcerative colitis and colon cancer (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010). Due to its tendency to decrease the plasma insulin and glucose response by reducing the glycemic index of food, RS is acknowledged as an important contributor to the prevention and management of diabetes and obesity (Topping et al., 2008). At present, RS, the total of starch and its degradation products that escape digestion in the small intestine of a healthy human and may be subject to bacterial fermentation in the large intestine (Asp & Björck, 1992), has been classified into five categories in foods (Fuentes-Zaragoza et al., 2011): RS1 is the physically inaccessible starch; RS2 is the raw starch granules from potato, pea, or high-amylose corn starch, which has the B- or C-type polymorphism; RS3 is the retrograded starch after cooking; RS4 is the chemically modified starch; and RS5 is the amylose-lipid complexed starch.

Actually, the digestion resistibility of starch is controlled by the starch microstructure. The structure of starch is complex and now thought to be organized in multiple scales including the granule

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(~0.5–100 μm), growth rings (~120–500 nm), blocklets (~20–50 nm), amorphous and crystalline lamellae (~9 nm), and amylopectin and amylose chains (~0.1–1 nm) (Cardoso & Westfahl, 2010; Pérez & Bertoft, 2010; Waigh, Gidley, Komanshek, & Donald, 2000). Many researches have revealed that the different crystal polymorph (Shamai, Bianco-Peled, & Shimoni, 2003), crystallinity (Singh, Dartois, & Kaur, 2010), amylopectin/amylose ratio (Lopez-Rubio, Flanagan, Shrestha, Gidley, & Gilbert, 2008), amylopectin fine structures (Benmoussa, Moldenhauer, & Hamaker, 2007), and molecular molar mass (Syahariza, Sar, Hasjim, Tizzotti, & Gilbert, 2013) can affect the digestion resistibility of starch. However, the mechanism of RS formation has not been clarified so far and the relationship between the starch structure and the resistibility against digestion needs to be further studied.

Furthermore, food processing alters the starch structure and also influences the starch resistibility against digestion. Depending on the processing conditions such as heat-moisture treatment and annealing (Chung, Liu, & Hoover, 2010; Juansang, Puttanlek, Rungsardthong, Puncha-arnon, & Uttapap, 2012), autoclaving-cooling cycles (Zhao & Lin, 2009), acid hydrolysis (Mutungi et al., 2010), drying method (Ozturk, Koksel, Kahraman, & Ng, 2009), and enzymatic debranching (Cai, Shi, Rong, & Hsiao, 2010), different degrees of starch digestion resistibility were obtained. With the advantages of prolongation of the shelf-life, improvement in the palatability, taste, texture and flavor, and enhancement in the functional properties, thermal processing has become one of the most important food processing methods. Thermal processing results in gelatinization and retrogradation, which are two of the most important thermal behaviors related to starchy foods. Gelatinization breaks up the starch structure and disrupts the inter- and intra-molecular hydrogen bonds between starch chains, allowing the separation of the chains which thus become more accessible by enzymes, and therefore increasing the susceptibility of starch to digestive enzymes (Chung, Lim, & Lim, 2006). When the gelatinized starch molecules retrograde upon cooling or dehydration (Shamai et al., 2003), the starch digestion resistibility could be increased. High-amylose starch shows good enzymatic digestion resistibility due to the higher amylose content and its internal structure and B-type crystal (Margareta Leeman, Karlsson, Eliasson, & Björck, 2006). However, its gelatinization temperature is higher than normal starch, which causes the difficulty in processing. Regarding this, high temperature and higher pressure could be useful to assist the gelatinization of high-amylose starch. Nevertheless, few studies have involved the influence of the hydrothermal-pressure (HP) processing on the multi-scale structures and subsequently on the digestion resistibility.

Thus, the purpose of this study was to investigate the effect of different gelatinizing temperature during the HP-treatment on the digestion resistibility of a high-amylose corn starch, as well as the relationship between the HP-treatment induced changes in the starch multi-scale structures (molecular molar mass and its distribution, molecular ordered structure, crystalline structure, lamellar structure, granular fractal structure) and the change in the digestion resistibility.

2. Materials and Methods

2.1. Materials

A high-amylose corn starch, Gelose 80 (G80), with the amylose content of about 80 % from Penford (Australia) was used and its original moisture content is about 15.0 %. Termamyl α-amylase (120 KNU/g) from *Bacillus Licheniformis* and Amyloglucosidase (300 AGU/g) from *Aspergillus Niger* were purchased from Novo Nordisk Bioindustrials (Guangzhou, China).

2.2. Gelatinized Starch by the HP-treatment at Different Temperatures

About 50 g of dry starch was dispersed in 250 mL of water and cooked in a sealed pressure reactor (Parr 4545, Parr Instrument Co., USA) at a specific temperature (80, 90, 100, 110, 120, 130, or 140 °C) under the pressure of 15 MPa with stirring at 100 rpm for 2 h. And then the gelatinized starch dispersion was cooled and stored at 4 °C for 24 h. Then all samples thus obtained, which were referred to as T-80, T-90, T-100, T-110, T-120, T-130, and T-140, were air dried at 60 °C and ground to pass through an 80 μm sieve for further analyses.

2.3. Digestion Resistibility of the HP-treated Starches at Different Temperatures

The RS content was determined according to the Method 991.43 total dietary fiber of the Association of Official Analytical Chemists (AOAC, 2003), following the reason provided by Li, Jiang, Campbell, Blanco, and Jane (2008).

2.4. Structural Characteristics of the HP-treated Starches at Different Temperature

2.4.1. Gel Permeation Chromatography (GPC) Coupled with Multi Angle Light Scattering (MALS) Analysis

A Waters GPC system, equipped with a Waters 1515 Isocratic HPLC pump and a Waters 717 auto sampler followed by a DAWN HELEOS 18-angle MALS detector (Wyatt Technology Co., USA) and an Optilab rEx refractive index detector (Wyatt Technology Co., USA), was used to measure the weight-average molecular molar mass (M_w), mean square radius of gyration (R_g) and the molecular molar mass (M) distribution of the samples. The mobile phase was DMSO with LiBr (50 mmol/L) filtered through a 0.22 μm PTFE filter and then degassed with ultrasound treatment. A certain amount of the sample (~5 mg) was suspended in 10 mL of the mobile phase and heated in boiling water for 1 h, and then shaken at 60 °C for 12 h to ensure the full dissolution in the mobile phase. All solutions were filtered through a 5 μm PTFE filter film (Millipore Co., USA) before injected into the GPC column (Styragel HMW 6E, Waters Co., USA). The column temperature was controlled at 40 °C and a wavelength of 658 nm was used in the experiment. The flow rate and total injected volume were 1.0 mL/min and 0.1 mL respectively. M_w, R_g and M were calculated using the Astra V software according to the Zimm model.

2.4.2. Attenuated Total Reflectance–Fourier Transform Infrared Spectroscopy (ATR-FTIR)

ATR-FTIR analyses of the starch samples were carried out on a Bruker Tensor 37 FTIR spectrometer with a DTGS detector equipped with a ATR single-reflectance cell containing a germanium crystal (45° incidence angle) (PIKE Technologies, USA) following the method of (Chung & Liu, 2009) with minor modification. The samples were oven-dried at 45 °C to a similar moisture content (~10 %) in order to minimize the effect of water, and measured directly after pressing the samples on the crystal. The spectra, recorded against an empty cell as the background, were acquired at wavelength between 800 and 1200 cm^{-1} with 4 cm^{-1} resolution. All spectra, the averages of 128 scans, were baseline corrected by drawing a straight line, and deconvoluted with the Lorentzian peak type with a deconvolution factor of 40 cm^{-1} and a noise reduction factor of 0.2 by the OPUS 6.5 software. The peak intensities in regions 1047 cm^{-1} and 1022 cm^{-1} of the deconvoluted spectra were calculated by recording the heights of the absorbance bands from the baseline.

2.4.3. X-ray Diffraction (XRD)

XRD analyses were performed with a diffractometer (Xpert PRO, Panlytical, Netherlands). The samples were layered in a sample pool and scanned using Cu-Kα radiation with a wavelength of 0.1542 nm as the X-ray source. The operation setting for the diffractometer was 40 mA and 40 kV. Data were collected from 2θ of 4.00 to 30.00 (θ being the angle of diffraction) using sequential scanning with a step width of 0.033° and a scanning speed of 10°/min. The moisture

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