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Understanding the structure and digestibility of heat-moisture treated starch

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ABSTRACT

To rationalize the effects of heat-moisture treatment (HMT) on starch digestibility, the HMT-induced alterations in the mesoscopic and molecular scale structures of regular and high-amylose maize starches, as well as in their digestibility, were evaluated. Accompanying the supramolecular structural disorganizations and certain molecular degradation induced by HMT, somewhat molecular rearrangements occurred to probably form densely packed starch fractions, which eventually weakened starch digestion and thus transformed RDS into SDS and RS for regular and high-amylose starches. Interestingly, due to its larger amount of inter-helical water molecules that could be induced by HMT, B-polymorphic high-amylose starch was more susceptible to HMT (relative A-polymorphic regular starch), causing more prominent structural evolutions including molecular re-assembly and thus increasingly slowed digestion. In particular, the treated high-amylose starch with 30% moisture content showed a high SDS + RS content (48.3%). The results indicate that HMT-treated starch may serve as a functional ingredient with adjustable enzymatic digestibility for various food products.

1. Introduction

Starch is the major storage carbohydrate in many plants and is one main energy source for humans. Normally, starch is a mixture of two biological macromolecules [1], i.e., amylose, mostly a linear 1,4-α-D-glucan with a small number of long branches, and amylopectin, a highly branched polymer made up of mainly α-1,4 linkages and ca. 5% α-1,6 linkages at the branching points. In the starch granule, these two biopolymers are organized on different scales to form starch's hierarchical structure, mainly including the growth rings, the amorphous-crystalline (semicrystalline) lamellae, the crystallites and the molecular chains [2–5]. Amylose/amylopectin ratio varies among starches from different botanical origins. While high-amylose starch possesses an amylose content up to 85%, waxy starch may contain no amylose [6]. The amylose/amylopectin ratio plays a key role in affecting the structural features (crystallinity, polymorphic type, etc.) of starch and thus altering its properties such as digestibility, thermal behaviors, and rheological property.

Enzymatic digestion of starch is a heterogeneous reaction in which the enzyme firstly diffuses toward and binds starch substrate, followed by adsorption and catalytic events. To eliminate complex intrinsic host factors and individual diversity, starch digestibility is most often characterized by using in vitro methods that simulate in vivo conditions of starch digestion. According to the digestion rate, starch is generally classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) [7]. RS has been found to be related to improvements of lipid and cholesterol metabolisms, prebiotic effects on colon microorganism, and reduction in the risk of ulcerative colitis and colon cancer [8,9]. Again, SDS shows potential health benefits regarding low glycemic response, causing satiety and improved physical performance and glucose tolerance, as well as reduced blood lipid level and insulin resistance [8,10,11]. Therefore, slowing starch digestion has gained huge interest in the design and development of novel functional foods with enhanced health benefits.

For obtaining desired digestion behaviors of starch, various (physical, chemical and enzymatic) modifications have been applied to regulate the digestibility through changing the structural features [9,12–15]. Among these methods, physical modification has attracted increasing attention due to the interest in producing starch products and reducing generated wastes during modification. Heat-moisture treatment (HMT), as a typical starch modification method, refers to the exposure of starch granules to

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high temperature (90–120 °C) above the glass transition temperature but below the gelatinization temperature for a certain time period ranging from 15 min to 16 h at a restricted moisture content (10%–35%) [16–20]. HMT has been reported to alter the structure of the starch granule and eventually affect the physicochemical properties, such as chemical reactivity, gelatinization, and retrogradation [14,19,21–23]. Also, HMT-treated starch may display higher enzymatic resistance than the native counterpart, probably due to HMT induced between starch molecular interactions [14,22,24–26]. This raises the possibility of using HMT to increase the total content of RS and SDS in starch products. As discussed above, amylose content greatly influences the polymorph and other structural characteristics of starch. Understanding the digestibility of starches with different amylose/amylopectin ratios as affected by HMT from a structural view would help us in comprehensively exploring the underlying mechanism and thus rationalizing HMT effects on starch digestion. Nevertheless, this understanding has not yet been well disclosed.

Therefore, using two maize starches with different amylose contents, this work attempted to evaluate the how HMT modulates the digestion behaviors of starch by altering the mesoscopic and molecular structures (lamellae, crystallites and molecular chains). The findings from present study enable us to well understand HMT effects on starch structure and digestion, as well as the related mechanism.

2. Materials and methods

2.1. Materials

Regular maize starch (RMS) (19.1% amylose content) and Gelose 50 high-amylose maize starch (G50) (51.6% amylose content) were obtained from Penford (Australia). The moisture content (*MC*) for starch samples was determined using a MA35 moisture analyzer (Sartorius Stedim Biotech GmbH, Germany). As measured using the method described by Chang et al. [27], the free lipid and bound lipid contents for RMS were 0.23 and 0.12 (g/100 g dry starch), respectively, and those contents for G50 were 0.25 and 0.39 (g/100 g dry starch), respectively. Porcine pancreatic α-amylase (P-7545, activity 8 USP/g) and amyloglucosidase (A3306, 318 u/mL) were purchased from Sigma-Aldrich (USA). The glucose oxidase-peroxidase assay kit (K-GLUC) was supplied by Megazyme (Ireland). Amylose (A0512) and amylopectin (A8515) were obtained from Sigma-Aldrich (USA).

2.2. Heat-moisture treatment

The moisture content of native starch was adjusted to a certain value (20%, 25% or 30%). Each starch sample was placed in a 500 mL container and then rotated in the oil bath at 120 °C for 2 h, followed by cooling to room temperature. Afterwards, the samples were removed from the containers and dried at 40 °C for 12 h. The samples were ground and sieved using a 100-mesh sieve for further analyzes. In the following, code typically as "RMS-HMT-20" was used, where "RMS" represents the type of starch, "HMT" means the heat-moisture treatment, and "20" indicates the moisture content of a specific sample.

2.3. In vitro digestibility and apparent amylose content

For native and modified starches, the in vitro digestibility was determined using a previous method with proper modifications [7]. In brief, 3 g of porcine pancreatic α-amylase was dispersed in 20 mL of deionized water and centrifuged at 3000*g* for 15 min. The supernatant (13.5 mL) was transferred into a beaker, and then 225 U of amyloglucosidase and 1 mL of deionized water were added to the solution. The enzymatic solution should be freshly prepared for each digestion trial. Starch (1 g) and 20 mL of 0.1 M sodium acetate buffer (pH = 5.2) were added to a test tube, and then the tube was cooked in a boiling water bath for 30 min. The starch dispersion was cooled to 37 °C, mixed with an enzyme solution (5 mL) containing pancreatic α-amylase and amyloglucosidase, and incubated in a 37 °C water bath. The aliquots (0.5 mL) were taken at certain time intervals and mixed with 20 mL of 70% ethanol. The mixed solution was centrifuged at 3000*g* for 10 min, and the hydrolyzed glucose content was measured by using the glucose oxidase-peroxidase reagent. Based on the hydrolysis rate, starch was classified as RDS (digested within 20 min), SDS (digested between 20 and 120 min), and RS (undigested within 120 min), respectively.

The apparent amylose content (*AAC*) of each sample was determined according to the method by McGrance with modifications [28]. 20 mg of dry starch was dissolved in 90% dimethylsulfoxide (8 mL) in a vial. All vials were shaken for 15 min and then heated in a water bath with shaking at 100 °C for 2 h. The vials were cooled to room temperature, and the starch solutions were diluted with water to 25 mL in flasks. The diluted solutions (1.0 mL) were mixed with 40 mL of deionized water and 5 mL of iodine (I_2)/potassium iodide (KI) solutions (0.0025 M I_2 and 0.0065 M KI). The UV absorbance for the solutions was measured at 600 nm using a Unico UV-3802 spectrophotometer (China). The *AAC* values were calculated from a standard curve established using mixture solutions of amylose and amylopectin.

2.4. Small angle X-ray scattering (SAXS)

SAXS experiments were performed according to our previously reported method [29,30]. The samples were measured using a Anton-Paar SAXS instrument (Austria) equipped with a PW3830 X-ray generator (PANalytical), which was operated at 50 mA and 40 kV using Cu Kα radiation with a wavelength of 0.1542 nm as the X-ray source. Starch slurries with certain moisture content (approximately 60%) were prepared for this experiment and equilibrated at 20 °C for 24 h before using. Each sample was placed in a paste sample cell and exposed to the incident X-ray monochromatic beam for 10 min. The data were recorded using an image plate and collected by the IP Reader software with a PerkinElmer storage phosphor system. All data were normalized, and the background intensity and smeared intensity were removed using SAXSquant 3.0 software for further analysis.

2.5. Polarized light microscopy

Starch granules were observed using a polarized optical microscope (Axioskop 40 Pol/40A Pol, ZEISS, Germany) equipped with a 35 mm SLA camera (Power Shot G5, Canon, Japan). All samples were dispersed as 10 mg of starch in 1 mL of distilled water in glass vials, and the images were recorded at 500× magnification [1].

2.6. X-ray diffraction (XRD)

XRD measurements were performed using an Xpert PRO diffractometer (PANalytical B.V., Netherlands), using Cu Kα radiation with a wavelength of 0.1542 nm as the X-ray source operated at 40 mA and 40 kV. The diffraction angle (2θ) range scanned was from 4° to 40° with a scanning speed of 10°/min and a scanning step of 0.033°. The *MC* of each sample was ca. 12% before analysis. The relative crystallinity (*RC*) of each sample was calculated by the method of Vermeylen [31].