Insights into the multi-scale structure and digestibility of heat-moisture treated rice starch

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ABSTRACT
The digestibility and structural changes of rice starch induced by heat-moisture treatment (HMT) were investigated, and the relationships among the moisture content–starch structure–starch digestibility were revealed. HMT could simultaneously disorder and reassemble the rice starch molecules across multi-scale lengths and convert some fractions of rapidly-digestible starch (RDS) into slowly-digestible starch (SDS) and resistant starch (RS). In particular, the HMT rice starch with less than 30% moisture content showed a higher SDS + RS content (25.0%). During HMT, SDS and RS were preferably formed by the degraded starch molecules with $M_w$ between $4 \times 10^3$ and $4 \times 10^6$ g/mol, single helices and amylose-lipids complexes that were formed by degraded starch chains with higher thermal stability and crystalline lamellae with greater thicknesses. Thus, our research suggests a potential approach using HMT to control the digestion of starch products with desired digestibility.

1. Introduction
Rice is the most important cereal crop and the staple food in Asia. However, compared with other starch foods, rice is commonly known as food with a higher glycemic index (GI), ranged from 54 to 121 (Edes & Shah, 1998; Hu, Zhao, Duan, Linlin, & Wu, 2004). The consumption of large amounts of high-GI food may lead to obesity, diabetes, and cardiovascular disorders (Shu, Jia, Ye, Li, & Wu, 2009; Yang et al., 2006). Thus, rice with a lower GI should be helpful for avoiding the diet-related diseases.

Starch is the major component (ca. 90%) of rice and an important part of human nutrition (Zhou, Robards, Helliwell, & Blanchard, 2002). According to the rate and extent of starch digestion in vitro, starch is classified into three major fractions: rapidly-digestible starch (RDS), slowly-digestible starch (SDS) and resistant starch (RS) (Englyst, Vinoy, Englyst, & Lang, 2007). The rate of starch digestion directly relates to the glycemic and insulin responses, and a higher amount of RDS in food leads to a lower GI (Englyst et al., 2007). On the contrary, SDS and RS may result in slower glucose release and lower glycemic response, suppressing the occurrence of those metabolic diseases (Zhang, Li, Chen, & Situ, 2016). Thus, it is promising to modulate the starch digestibility for the design of low-GI foods with health benefits.

Recently, various approaches (e.g., chemical, physical, genetic, and multiple modifications) have been explored to produce high amounts of SDS and/or RS (Lee & Moon, 2015; Miao, Zhang, Mu, & Jiang, 2010; Shin et al., 2007). In particular, there is considerable interest in the use of physical methods to improve starch properties while maintaining food safety. Heat-moisture treatment (HMT) commonly occurs at a low moisture content (MC) (< 35% H2O, w/w) and at a temperature above the glass transition temperature ($T_g$) but below gelatinization temperature for a fixed period (15 min to 16 h). HMT has been reported as a green technique to alter the molecular, crystalline and granule structure and thus to regulate the granule swelling, amylase leaching, gelatinization parameters, viscosity, and enzyme susceptibility (Hoover, 2010; Jiranuntakul, Puttanlek, Rungrsdthong, Puncharon, & Uttapap, 2011; Zavareze & Dias, 2011). Generally, HMT-treated starches tended to have a higher gelatinization temperature, lower paste viscosity, decreased granule swelling degree, and increased thermal stability (Huang, Zhou, Jin, Xu, & Chen, 2015; Jacobs & Delcour, 1998; Jiranuntakul et al., 2011). However, the change in enzyme susceptibility after HMT could depend on the starch source and treatment conditions (e.g., temperature, moisture, and time) (Chung, Liu, & Hoover, 2009; Gunaratne & Hoover, 2002; Hoover, 2010; Kweon, Haynes, Slade, & Levine, 2000). Furthermore, while the digestibility of corn, potato and legume starches subjected by HMT has been extensively reported, few studies have been focused on the...
changes of digestibility of rice starch during HMT. Zavareze, Storck, de Castro, Schirmer, and Dias (2010) reported that the digestibility of rice starches with different amylose contents increased when HMT (110 °C and 1 h) was conducted with the moisture ranged from 15 to 25%. Besides, all of the above studies showed that the impact of HMT on starch digestibility depends on the HMT parameters, which can be linked to the changes of starch crystalline structure (crystallities disruption and changes in the polymorphic form) and morphology (formation of fissures and cracks on the granule surface).

The structure of starch is complex and considered to be organized on different length scales, comprised of the granule, the growth rings, and the semi-crystalline lamellae system (Pérez & Bertoft, 2010). This multi-scale packing of starch molecules has varying degrees of compactness, which with prominently different susceptibilities to enzyme hydrolysis (Zhang, Wang, et al., 2014). However, no systematic study has been undertaken to investigate the relationship between the starch multi-scale structure and the susceptibility of starch towards enzyme subjected to heat-moisture treatment (HMT). In particular, there is limited understanding of how the lamellar structure, helical structures, and short-range molecular orders regulate the starch digestion rate. Thus, from the view of the molecular level and molecular interactions, the mechanism regarding the structurally-modulated digestibility of rice starch was discussed. It is important to study the relationships between the specific structures of starch as varied by HMT and its digestion rate, which is crucial for further understanding the health effects of resistant starch.

In this work, the effect of HMT on the granule morphology, semi-crystalline lamellae, crystallites, short-range molecular orders, helical conformations, and chain length and distribution of rice starch were investigated. Also, the related changes in the digestion rate were evaluated. In this way, the mechanism of the regulation of starch digestibility by HMT was revealed from the view of hierarchical structural changes. Moreover, the findings from the present study are crucial for the rational development of starch-based foods with tailored digestibility using HMT.

2. Materials and methods

2.1. Materials

The paddy rice was kindly supplied by South China Agricultural University. Standard amylose (A5012) and amylopectin (A8515) were obtained from Sigma-Aldrich (USA). Porcine pancreatic α-amylase (Cat. No.: P-7545, activity 8 × USP/g) and amyloglucosidase (A3306, > 300 U/mL) were purchased from Sigma-Aldrich (USA). The glucose oxidase–peroxidase assay kit (Cat. No.: K-GLUC) was obtained from Megazyme (Ireland).

2.2. Starch isolation

The paddy rice was soaked in distilled water at 4 °C for 24 h. The soaked rice grain was ground with a laboratory blender and passed through a 63 μm sieve, followed by standing at 4 °C for 48 h. The supernatant solution was drained off, and the residue was diluted to the original volume with 0.4% sodium hydroxide solution, then stored at 4 °C for 48 h. The re-slurried starch was passed through a 63 μm sieve again and kept at 4 °C for 24 h. The process was repeated three times, neutralized with 1 M HCl to pH 7, washed with deionized water and dried overnight at 40 °C (Wang & Wang, 2004). The moisture, protein and free lipid contents of rice starch were 10.32, 0.65 and 0.20 (g/100 g dry starch), respectively.

2.3. Heat-moisture treatment (HMT)

The HMT of rice starch was conducted under different moisture contents (MC) (10%, 20%, and 30%; coded as HMT-10, HMT-20, and HMT-30, respectively). The samples were equilibrated at 4 °C for 24 h. Then, they were placed in a 500 mL screwed stainless steel reactor with continuous rotation and heated with oil at 110 °C for 4 h, followed by cooling to room temperature. During the HMT, the starch granules can be stirred by the paddle when the reactor was continuously rotated. In this way, the starch granules can be uniformly heated under a different MC. The treated samples were subsequently dried at 40 °C until the MC of the samples reached about 11% and then ground. The apparent amylose content (AAC) of starch samples were determined according to the method of Juliano et al. (1981).

2.4. In vitro digestibility

In vitro starch digestibility was analyzed with a properly modified Englyst procedure (Englyst, Kingman, & Cummings, 1992). The enzyme solution was prepared as follows: 3 g of porcine pancreatic α-amylase was dispersed in 20 mL of deionized water with magnetic stirring and centrifuged at 3000g for 25 min. The supernatant (13.5 mL) was transferred into a beaker and mixed with 0.7 mL of amylglucosidase and 0.8 mL of deionized water. The enzyme solution was freshly prepared before use.

Starch (ca. 1 g) sample was placed into 100 mL flasks. Acetate buffer (0.1 M, pH 5.2, 20 mL) was added using a pipette. Then, the flask was heated to boiling for 30 min. The boiled samples were cooled to 37 °C, and each was mixed with 5 mL of the enzyme solution, followed by incubation in a water bath at 37 °C with shaking (180 rpm). At different time intervals, 0.5 mL of hydrolysate was collected, and 20 mL of 67% ethanol was added to deactivate the enzyme. The glucose concentration was calculated using the GOPOD assay. According to the rate of hydrolysis, starch was classified into rapidly-digestible starch (RDS, digested within 20 min), slowly-digestible starch (SDS, digested between 20 and 120 min), and resistant starch (RS, undigested within 120 min), respectively.

2.5. Structural characterization

2.5.1. Gel permeation chromatography (GPC) coupled with multi-angle light scattering (MALS)

The weight-average molecular molar mass (Mw) and molecular molar mass distribution of starch samples were analyzed using a GPC (Waters, USA) system equipped with a MALS detector (Wyatt, USA) and a refractive index detector. Three chromatographic columns (Styragel HR 3, Styragel HMW 6E, and Styragel HMW 7, Waters, USA) and a laser with a wavelength of 658 nm were used. Starch (ca. 5 mg) was dispersed in 10 mL of DMSO containing LiBr (50 mM) with heating in a boiling water bath for 1 h. The mobile phase was DMSO with LiBr (50 mmol/L) filtered through a 0.22 μm PTFE filter and degassed by ultrasound before use. Then, the sample was shaken at 60 °C for 12 h to ensure full dissolution of the starch in the mobile phase (Liu, Halley, & Gilbert, 2010). Before injection, the starch sample solutions were filtered using a 5 μm membrane filter (Millipore Co., USA). The flow rate and total injected volume were 1.0 mL/min and 0.1 mL, respectively. The light scattering data were collected and analyzed using the Astra V software program.

2.5.2. Fourier transform Raman (FT-Raman) spectrometry

The FT-Raman spectra of starch samples were obtained by using a Nicolet iS50 instrument (ThermoFisher, USA) with a near-infrared YAG laser with a wavelength of 1064 nm. Spectra were recorded from the same spot size of each sample in the range of 4000–100 cm⁻¹, with a resolution of approximately 8 cm⁻¹. The full width at half height (FWHH) of the band at 480 cm⁻¹ was used to characterize the molecular order of starch. All measurements were performed at least five times to obtain the stable spectra.
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