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A further understanding of the multi-scale supramolecular structure and digestion rate of waxy starch

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ABSTRACT

This work concerns how the multi-scale supramolecular structure of starch relates to its digestion rate from a view of structural heterogeneity. The untreated granule starch displayed a dual-phase digestion pattern, ascribed to two digestible fractions within the heterogeneous multi-scale structure of starch, which had prominently different digestion rates. Not only amorphous starch but also part of molecular orders (crystallites with flaws) were digested at a same rate k_1 at the first phase; densely-assembled starch including orders with fewer flaws was digested at a rather slow rate k_2 (*ca.* 2/5 of k_1) at the second phase. When alkali altered the heterogeneous supramolecular structure of starch, the digestion behaviors were also changed. The 0.1% (w/v) alkali solution slightly disrupted the starch multi-scale structure, which reduced the molecular orders, disrupted the lamellae, weakened the molecular organization within growth rings, and enlarged the granule pores. Then, part of resistant starch was transformed into slowly-digestible fraction with a digestion rate close to k_2. In contrast, when stronger (0.5% w/v) alkali was used, the starch multi-scale structure was more apparently disrupted, causing even granule swelling. This structural change resulted in a triple-phase digestion with three different digestion rates. Moreover, especially with stronger alkali, along with the structural disruption, some orders with a higher thermal stability emerged and reduced the accessibility of starch molecules to the enzyme. In this case, the digestion rate decreased with the treatment time.

1. Introduction

The digestion of food biopolymers, *e.g.*, starch, and protein, always involves enzymes that depolymerize the macromolecular substances into oligomer/monomer units under certain kinetics. Starch, as a storage biopolymer in higher plants, is a key carbohydrate providing energy for humans (Juansang, Puttanlek, Rungsardthong, Puncha-arnon, & Uttapap, 2012). Starch contains two major D-glucans, *i.e.*, amylose and amylopectin (Liu, Halley, & Gilbert, 2010). These two polymers assemble on different scales in the starch granule to form a multi-scale supramolecular structure with heterogeneity, mainly including the whole granule (<1 μm-100 μm), the growth rings (100–400 nm), and the semi-crystalline lamellae (9–10 nm) (Perez & Bertoft, 2010; Zhang, Chen, Xie, et al., 2015; Zhang, Xiong, et al., 2014). The digestion of starch releases glucose, which relates to metabolic diseases, *e.g.*, Type II diabetes, obesity and cardiovascular diseases (Robertson, Currie, Morgan, Jewell, & Frayn, 2003; Zou, Sissons, Gidley, Gilbert, & Warren, 2015). Thus, to maintain people's health, considerable attention has been paid to the modulation of starch digestibility (*e.g.*, digestion rate and degree) (Chen et al., 2016).

Despite for human diets starch is usually consumed after processing, granule starch is also used widely, for example for low-

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moisture foods (*e.g.*, biscuits) (Blazek & Gilbert, 2010), fruits, vegetables, animal feeds, and industrial conversions. For the granule starch digestion, the enzyme firstly diffuses toward and binds the substrate, followed by the adsorption and catalytic events (Bertoft & Manelius, 1992). The tight assembly of starch molecular chains in the multi-scale supramolecular structure of granule starch can suppress the enzyme diffusion/absorption and hydrolysis (Bertoft & Manelius, 1992). The digestion rate of untreated granule starch by amylase is normally several times lower than that of starch after processing such as cooking (Blazek & Gilbert, 2010; Noda et al., 2008; Zhang, Dhital, & Gidley, 2013). This lower digestion rate was proposed due to the existence of ordered structure in granule starch before processing, which reduces the accessibility of starch molecules to the digestive enzymes. However, an increasing number of studies have shown contradictory results, revealing that the degree of molecular disassembly (structural disorganization) of granule starch during processing, such as gelatinization, only has little effects on starch digestibility including digestion rate (Chung, Lim, & Lim, 2006; Tamura, Singh, Kaur, & Ogawa, 2016; Wang, Sun, Wang, Wang, & Copeland, 2016). Hence, it is still inconclusive how the multi-scale structure of granule starch governs the digestion rate of starch.

As mentioned above, the multi-scale supramolecular structure of granule starch is heterogeneous. That is, starch molecules packed in the structures on multiple scales have varied degrees of compactness and thus show structural heterogeneity. This heterogeneity probably endows starch molecules assembled in granule starch with prominently different susceptibilities to enzyme hydrolysis (*i.e.*, digestion rates). However, though numerous reports evaluate the effect of starch structure, such as crystallites and helices, on starch digestibility (Zhang, Chen, Zhao, & Li, 2013; Zhang, Wang et al., 2014), there is limited understanding of how the multi-scale supramolecular structure of granule starch relates to its digestion rate from a structural heterogeneity view. Therefore, based on this view, we may better explore the links between specific structures of granule starch and starch digestion rate, which is crucial for the rational development of starchy foods with tailored digestibility.

This work was aimed at disclosing the relationship between the multi-scale supramolecular structure of granule starch and its digestion rate from a view of structural heterogeneity. Regarding this, the multi-scale structural features of the starch were interrogated by different techniques. The digestibility (digestion rate) of the starch was evaluated using a modified *in vitro* method (Zou et al., 2015). Besides, alkali was used to vary the multi-scale supramolecular structure of starch with heterogeneity. Intense alkali can quickly disrupt the starch structure and prominently degrades starch molecules (Han & Lim, 2004) due to the β-elimination of reducing semi-acetal groups. In this work, we chose to use moderate alkali treatment (alkali concentrations: 0.1% w/v and 0.5% w/v) with long-term periods (6 and 12 days) to modify the starch structure without degradation of starch molecules (Cai et al., 2014; Jiang et al., 2014; Nadiha, Fazilah, Bhat, & Karim, 2010; Praznik, Buksa, Ziobro, Gambuś, & Nowotna, 2012; Wang & Copeland, 2012). Also, waxy starch has advantages for this study, as its granule has a loose surface (typically with pores) and an interior structure with weak-points. This makes any evolutions in the supramolecular structure and thus in the digestion rate of starch induced by alkali more apparent.

2. Materials & methods

2.1. Materials

Waxy maize starch was purchased from Penford Australia Pty Ltd. (Lane Cove, NSW, Australia). The starch has an amylose content of *ca.* 3%, as measured using the iodine colorimetric method (Tan, Flanagan, Halley, Whittaker, & Gidley, 2007). A moisture analyzer (MA35, Sartorius Stedim Biotech GmbH, Germany) was used to measure the moisture content of each starch sample. Sodium hydroxide, sodium azide, and ethanol were of analytical grade, and were purchased from Tianjin Kemeou Chemical Reagent Co., Ltd. (China). α-Amylase from porcine pancreas (A-3176; 23 unit amylase/mg solid; one unit liberates 1.0 mg of maltose from starch in 3 min at 37 °C), phosphate buffered saline tablet (P4417-100TAB), 4-hydroxybenzhydrazide (PAHBAH, H9882) and maltose (M-9171) were supplied by Sigma-Aldrich Pty Ltd. (Castle Hill, NSW, Australia).

2.2. Preparation of alkali-treated starch

Approx. 10.0 g of the starch was added into 150 mL sodium hydroxide aqueous solution at a concentration of 0.1% (w/v) or 0.5% (w/v), together with 0.1% (w/v) sodium azide as a chemical preservative. The starch slurries were kept at 35 °C for 6 or 12 days with intermittent shaking to fully re-suspend the starch. After the treatment, the starch was washed with deionized water, followed by 95% ethanol (Jiang et al., 2014; Wang & Copeland, 2012), and centrifuged for 3–5 times until the slurry became neutral. The starch sediment was dried in an oven at 35 °C for 48 h. In the following, codes typical as "S-0.5-12" was used, where "S" indicates the starch, "0.5" denotes the concentration (%) of sodium hydroxide, and "12" means the days of treatment. Also, "S" represents the native (*i.e.*, untreated) starch.

2.3. Scanning electron microscopy (SEM)

The granule morphology of the starch was observed using a Zeiss Merlin Ultra Resolution SEM (Carl Zeiss AG, Oberkochen, Germany). The samples were mounted on a metal stage and then coated with iridium. The images were obtained at an accelerating voltage of 2 kV.

2.4. Laser diffraction analysis

Granule size distribution was evaluated by a Malvern Mastersizer 2000 laser diffraction analyzer (Version 5.22, Malvern, UK). The obscuration value was 12%–17%, with a pump speed 2050 r/min. The refractive index of the starch and the dispersing reagent ethanol was 1.54 and 1.36, respectively.

2.5. Synchrotron small-angle X-ray scattering (SAXS)

SAXS measurements were performed on the SAXS/WAXS beamline (flux, 1013 photons/s) at the Australian Synchrotron (Clayton, Vic, Australia), at a wavelength $\lambda = 1.47$ Å. The starch suspensions with a starch concentration of 40 wt% were placed on a multi-well stage, and then the SAXS data were recorded for an acquisition time of 1 s. The scattering of pure water with Kapton tape (5413 AMBER 3/4IN X 36YD, 3M, USA) on the stage window was used as the background data. All the data were background subtracted and normalized. The data in the range of $0.0020 < q < 0.20$ Å^{-1} were used as the SAXS pattern, where $q = 4\pi\sin\theta/\lambda$, in which 2θ is the scattering angle and λ the X-ray wavelength.

For the SAXS patterns, the data in the range of $0.0020 < q < 0.04$ Å^{-1} were fitted using a unified model Eq. (1) (Zhang, Chen, Xie, et al., 2015).

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