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Insights into the hierarchical structure and digestion rate of alkali-modulated starches with different amylose contents

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ABSTRACT

Combined analytical techniques were used to explore the effects of alkali treatment on the multi-scale structure and digestion behavior of starches with different amylose/amylopectin ratios. Alkali treatment disrupted the amorphous matrix, and partial lamellae and crystallites, which weakened starch molecular packing and eventually enhanced the susceptibility of starch to alkali. Stronger alkali treatment (0.5% w/w) made this effect more prominent and even transformed the dual-phase digestion of starch into a triple-phase pattern. Compared with high-amylose starch, regular maize starch, which possesses some unique structure characteristics typically as pores and crystallite weak points, showed evident changes of hierarchical structure and in digestion rate. Thus, alkali treatment has been demonstrated as a simple method to modulate starch hierarchical structure and thus to realize the rational development of starch-based food products with desired digestibility.

1. Introduction

Starch is the main storage carbohydrate in most higher plants and is one of the most important energy sources for humans and animals. Starch contains two D-glucan biopolymers, *i.e.*, amylose, a relatively linear 1,4-α-D-glucan with a small number of long branches; and amylopectin, mainly a 1,4-α-D-glucan containing high-density branches (*ca.* 5% of glycosidic bonds are α-1,6) (Zhang, Zhao, Li, Li et al., 2014). These two macromolecules are organized on different length scales in the starch granule to form the supramolecular structure of starch, from the whole granule (<1 μm–100 μm), growth ring (100–400 nm), semi-crystalline lamellae (9–10 nm), to crystalline structure (>0.1 nm–*ca.* 1 nm) (Pikus, 2005; Zhang, Li, Liu, Xie, & Chen, 2013). In addition, depending on the packing of amylopectin side chains into double helices and amylose single helices, the crystalline structure (polymorph) of starch has been classified as A-, B-, C- or V-type. All this multi-scale structure of starch depends on the botanical origin.

In addition, starch structure may be altered by various treatments, resulting in changes in the digestibility and other properties of starch. As a main food ingredient, starch is usually processed for

Abbreviation: RMS, regular maize starch; G50, Gelose 50 starch.

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consumption, in which case native starch in low-moisture foods (Blazek & Gilbert, 2010) (*e.g.*, biscuits and muesli), fruits, vegetables, and animal feeds can be ingested directly. Since the digestibility of starch may be associated with metabolic diseases such as Type II diabetes, obesity, and cardiovascular diseases, it plays a key role in determining the health benefits of starch-based foods (Zou, Sissons, Gidley, Gilbert, & Warren, 2015). Hence, there has been huge interest in understanding the digestion behavior (reflected mainly by hydrolysis rate and digested proportion) of starch and starch-based foods, which is crucial for rational development of starch-based food products with desirable digestibility.

The Englyst method (Englyst, Kingman, & Cummings, 1992) is extensively employed to assess the *in vitro* digestibility of starch. It characterizes the proportion of starch digested within a certain time period, based on which starch can be classified into three different types, *i.e.*, rapidly-digestible starch (RDS), slowly-digestible starch (SDS), and resistant starch (RS). The digestion rate of starch is further characterized by the hydrolysis indices, equal to the areas under the digestibility curve between the start and selected completion time points (Aravind, Sissons, & Fellows, 2011; Butterworth, Warren, Grassby, Patel, & Ellis, 2012; Edwards, Warren, Milligan, Butterworth, & Ellis, 2014). Nonetheless, these methods cannot allow for a rigorous, quantitative comparison between digestion rates and digested proportions, and are incapable of quantifiably determining the digestion rate constant as starch digestion proceeds. To offset this limitation, a novel method, termed as a logarithm of the slope (LOS) plot, (Poulsen, Ruiter, Visser, & Iversen, 2003) by modelling the hydrolytic process using first-order enzyme kinetic principles (Goñi, Garcia-Alonso, & Saura-Calixto, 1997), has been applied to acquire the hydrolysis rate of starch. The LOS plot enhances the differences in starch digestion rate constant and visibly reveals the number of starch digestion steps throughout the whole enzymatic hydrolysis period. This method based on the LOS plot has been used to identify and quantify the digestion rates and digested proportions in purified starches, homogeneous foods, and edible starch-rich plant tissues (Edwards et al., 2014).

For the production of starch and starch-based food products, alkali treatment has been widely used. This treatment has not only been confirmed effective at isolating starches from agro-products with high yields and purity, especially for starch tightly associated with protein (Correia & Beirão-da-Costa, 2012; Han & Hamaker, 2002), it has also been used for preparation of diverse starch-based foods, such as tortillas, yellow alkaline noodles and dumplings (Campus-Baypoli, Rosas-Burgos, Torres-Chavez, Ramírez-Wong, & Serna-Saldivar, 1999; Lai, Karim, Norziah, & Seow, 2002). Moreover, the treatment with reagents such as sodium hydroxide and sodium carbonate often imparts the foods with a characteristic aroma and flavor, as well as a firm and elastic texture (Karim et al., 2008). Therefore, it is necessary to understand how alkali treatment changes the structure and digestion behavior of starch.

Alkali treatment has been shown capable of altering the structures (morphology(Cai et al., 2014; Cardoso, Putaux, Samios, & da Silveira, 2007; Jiang et al., 2014; Nor Nadiha, Fazilah, Bhat, & Karim, 2010; Paredes-Lópezb & Bello-Péreza, 2007; Wang & Copeland, 2012; Wang et al., 2014), short- and long-range orders (Cai et al., 2014; Cardoso et al., 2007; Jiang et al., 2014; Wang & Copeland, 2012), and semi-crystalline lamellae (Cai et al., 2014)) and properties (*e.g.*, swelling(Jiang et al., 2014; Paredes-Lópezb & Bello-Péreza, 2007; Wang & Copeland, 2012; Wang et al., 2014), solubility (Jiang et al., 2014; Paredes-Lópezb & Bello-Péreza, 2007; Wang et al., 2014), pasting(Han & Tyler, 2003; Karim et al., 2008; Lai et al., 2002; Nor Nadiha et al., 2010; Nor Nadiha et al., 2010; Wang, Li, Wang, Liu, & Adhikari, 2012), rheological (Shiau & Yeh, 2001), and thermal properties (Cai et al., 2014; Cardoso et al., 2007; Lai et al., 2002; Nor Nadiha et al., 2010; Paredes-Lópezb & Bello-Péreza, 2007; Wang & Copeland, 2012; Wang et al., 2014)) of starches of various botanical origins (*e.g.*, corn (Paredes-Lópezb & Bello-Péreza, 2007; Wang et al., 2012) rice, (Cai et al., 2014; Cardoso et al., 2007), potato (Nor Nadiha et al., 2010), sago (Karim et al., 2008), and pea (Han & Tyler, 2003; Wang & Copeland, 2012)). However, the effect of alkali treatment on the digestion behavior has not been extensively studied as on other properties of starch. To date, only the changes in *in vitro* digestibility of alkali-treated starches, based on the Englyst classification method, have been investigated, which has shown that alkali treatment can increase the digested proportion (Wang & Copeland, 2012; Wang et al., 2014). Yet, alkali-induced changes in digestion rate of starch have not been understood, in particular from a hierarchical structural view. The lack of this understanding prevents us from exploring the mechanism of how alkali treatment changes the digestion behavior of starch and the rational application of alkali-treated starch for desired digestion patterns.

Although strong alkali can quickly disrupt starch structure, the degradation of starch molecules is often induced (Han & Lim, 2004), due to the β-elimination of reducing semiacetal groups. Considering this fact, low alkali concentrations (*e.g.*, 0.1%, 0.2%, and 0.4%), moderate temperatures (*e.g.*, 30 °C and 35 °C) and long time periods (up to 30 days) (Cai et al., 2014; Jiang et al., 2014; Nor Nadiha et al., 2010; Praznik, Buksa, Ziobro, Gambuś, & Nowotna, 2012; Wang & Copeland, 2012) have been widely used for a slow disruption of starch supramolecular structure without molecular degradation. This study is mainly focused on how the starch supramolecular hierarchical structure and alkali treatment affect the digestion behavior of starch. Thus, moderate alkali treatment for long periods should be used in the present work, although further investigations are still needed to reduce the treatment time and thus to better meet practical requirements

On the other hand, starches of different origins can have different amylose contents (up to 85%). The amylose content has a prominent influence on starch hierarchical structure. In particular, while regular starches (with 10–30% amylose content) normally contain a large amount of A-type crystallites, high-amylose starches (with >50% amylose content) usually exhibit a B-type polymorph with lower crystallinity and are less susceptible to various physicochemical treatments such as acid hydrolysis and hydrothermal treatment (Kim & Huber, 2010; Zhang, Zhao, Li, Li et al., 2014). Thus, starches with different amylose contents can be ideal models to probe the alkali treatment-digestion mechanism. Regarding this, the current work involved the use of regular and high-amylose maize starches to study the effect of mild alkali on the lamellar and crystalline structures of starch as well as thermal and digestion behaviors (especially digestion rate). This resulted in ultimate understanding of the chemistry/structure–digestibility relationship for alkali-treated starch. More importantly, the understanding from this work could be helpful in developing starch-based products with tailored digestibility using alkali and more similar chemical treatments.

2. Materials and methods

2.1. Materials

Regular maize starch (RMS) and high-amylose maize starch (Gelose 50 or G50) were purchased from Penford Australia Pty Ltd. (Lane Cove, NSW, Australia). RMS and G50 have amylose/amylopectin ratios of 23/77 and 50/50, respectively, as measured using the iodine colorimetric method (Tan, Flanagan, Halley, Whittaker, & Gidley, 2007). The moisture content of each starch sample was determined using a MA35 moisture analyzer (Sartorius Stedim Biotech GmbH, Germany). Sodium hydroxide, sodium azide, and ethanol, purchased from Tianjin Kemeou Chemical Reagent Co., Ltd. (China), were of analytical grade. α-