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Slowly digestible starch in fully gelatinized material is structurally driven by molecular size and A and B1 chain lengths

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ABSTRACT

The objective of this study was to obtain structure-digestion relationships of fully gelatinized starch. Twelve starch samples with marked fine structural differences (HPLC-SEC) were studied for their retrogradation behavior (thermal and rheological properties of starch gels) and *in vitro* digestibility. A reduction in the digestion rate during storage for 7 days was observed in all samples and, interestingly, this reduction was particularly evident in sago (64.7%), potato (57.3%), pea (55.1%) and acid-converted maize (ACM, 51.6–51.8 %) starches. Results indicated two potential interactions that may result in slowly digestible supramolecular structures: 1) double helices between external A and B1 chains of DP at peak maximum ≥ 15.5 Glucose Units (perhaps involving internal long chains) that also are prone to forming intermolecular associations [high relative drop in the storage modulus (G') during heating of 7 days-stored gels] and; 2) interactions of small molecular size acid-hydrolyzed starch molecules that may be more mobile and easily aligned.

1. Introduction

Starch, being the principal component in cereals, tubers and pulses, is the major polysaccharide related to postprandial glycaemia and glycemic index of foods. Glucose response is frequently described by its glycemic index (GI) or glycemic response, *i.e.*, rapid increase of the postprandial blood glucose, and many studies have linked low GI diets with reduced risk of developing type 2 diabetes and cardiovascular disease (Jenkins et al., 2002). In humans, starch is successively hydrolyzed by salivary and pancreatic α-amylase in the mouth and small intestine, and then to glucose by the mucosal α-glucosidases, and differences in the glycemic response depend highly on the rate and extent to which starch is digested.

Native starch naturally exists in the form of starch granules with different properties, including surface porosity and semi-crystalline structure. Based on their X-ray diffraction pattern, starches can be classified into three types: A-, B- and C- (combination of A and B-allomorphs) type. A-type starches encompass those from cereals, and many possess surface pores and channels, whereas such pores do not exist in B-type starches, which include those from tubers. This macrostructural difference is important during digestion as digestive enzymes enter the channels and digest starches gradually in an inside-out manner. In this way, A-type starches are slowly digestible (Zhang, Ao, & Hamaker, 2006; Zhang, Venkatachalam, & Hamaker, 2006) and B-type starches are inherently resistant. This native starch macromolecular structure, acting as a natural physical barrier to enzyme digestion, is lost in many foods because of hydrothermal processes, such as cooking, boiling and baking, among others, which lead to partial or complete starch gelatinization (Martinez, Roman, & Gomez, 2018; Varriano-Marston, Ke, Huang, & Ponte, 1980). An exception to this is high amylose starch (HAMS), which have been reported to retain its native structure (assessed by X-ray diffraction pattern) during baking (Hoebler, Karinthi, Chiron, Champ, & Barry, 1999). The amorphous structure of gelatinized starch results in far greater availability of α-amylase binding sites, which makes the substrate more susceptible to enzyme hydrolysis (Baldwin et al., 2015) and results in a wide array of food products with high GI (Foster-Powell, Holt, & Brand-Miller, 2002).

A great deal of effort has been put forth to decrease starch bioaccessibility using a variety of methods, although many entail the use of expensive enzymes (Ao et al., 2007; Guraya, James, & Champagne, 2001; Han et al., 2006; Shi, Cui, Birkett, & Thatcher, 2005; Shin et al., 2004), or physical modifications such as heat-moisture treatment (Lee

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& Moon, 2015), where the positive effects on digestion are often lost during further thermal processing unless using HAMS (Hoebler et al., 1999).

Starch retrogradation, defined as the re-association of amylose and amylopectin chains into ordered structures that are different from the original, is known to result in a reduction of the rate and extension of starch digestion, depending on the main constituent involved. Amylose double helices (retrograded amylose) are known to be enzymatically resistant and yield resistant starch (RS) (Haralampu, 2000; Patel et al., 2017), whereas retrograded amylopectin has been attributed to the formation of slowly digestible starch (SDS) [Cui & Oates, 1997; Farhat et al., 2001; Zhang, Sofyan, & Hamaker, 2008; Borah, Deka, and Duary, (2017). In a study on rice starch digestibility and amylopectin fine structure parameters, Benmoussa, Moldenhauer, and Hamaker, (2007) showed that the lowest content of rapidly digestible starch (RDS) was found in rice cultivars with high proportions of amylopectin with long chains. Zhang et al. (2008) found that higher content of amylopectin with longer chains promoted formation of SDS through retrogradation in starches from maize mutants. Several authors have reported, albeit with no digestion analysis, that longer amylopectin branches are more prone to form intra- and intermolecular associations during cooling (Jane & Chen, 1992; Kohyama, Matsuki, Yasui, & Sasaki, 2004; Matalanis, Campanella, & Hamaker, 2009). However, the combined roles and synergies of multiple starch molecular features, including amylopectin fine structure and amylose length, for controlling amylopectin and amylose retrogradation and the rate of digestion are not evident. For example, Jane and Chen (1992), working with reconstituted slurries of different amylopectins and amyloses, found that mixtures of amylopectin with amylose of intermediate length (667 DP) resulted in the greatest gel strength; yet, the structure-digestibility relationship was not investigated.

Despite extensive research conducted on starch structure-digestion relationships, a mechanistic understanding of how SDS structurally forms in a fully gelatinized material is still unclear. We hypothesized that certain starches found in nature inherently, or after acid-conversion commonly used in some food applications, lead to the formation of SDS after gelatinization. In this study, 12 starches with different structural features served as a basis to elucidate structure-re-association relationships and their effect on starch digestibility. The slow digestion property of these starches was investigated after full gelatinization as determined by DSC, except for high amylose maize (HAM) starches, whose slow digestion property could also be derived from ungelatinized material. HAM starches have been reported to have conclusion temperatures of starch gelatinization (T_c) up to 130 °C (Jane et al., 1999), which is not attainable with the conventional RVA used in this study. Then, select starches were tested in a cake system to demonstrate this effect in a real product.

2. Materials and methods

2.1. Materials

Maize, acid-converted maize, waxy maize, potato, waxy potato, rice, waxy rice, high amylose maize and sago starches were generously supplied by Ingredion Inc. (Bridgewater, NJ, USA), pea starch by Roquette (Lestrem, France) and wheat starch by ADM (Chicago, IL, USA). α-Amylase [(1→4)-α-glucan-4-glucanohydrolase] from porcine pancreas (type VI-B pancreatic α-amylase, Sigma A3176) was purchased from Sigma-Aldrich (St Louis, MO, USA). Dimethyl sulfoxide (DMSO) and lithium bromide (ReagentPlus) were purchased from VWR (Radnor, PA, USA) and Beantown Chemical (Hudson, NH, USA), respectively. *Pseudomonas* sp. isoamylase (glycogen 6-glucanohydrolase; EC 3.2.1.68) was purchased from Megazyme International Ltd. (Co. Wicklow, Ireland).

2.2. Methods

2.2.1. Molecular size and unit chain length distribution of amylose and amylopectin

The molecular size distributions of fully branched and debranched starches were analyzed in duplicate using a size exclusion chromatography (SEC) system (Agilent 1260 series, Agilent Technologies, Waldbronn, Germany) equipped with a refractive index detector (RID, 1260 RID, Agilent, Agilent Technologies, Waldbronn, Germany) following the method of Cave, Seabrook, Gidley, and Gilbert, (2009) with minor modifications. Starch extraction was performed as in Syahariza, Li, and Hasjim, (2010) with minor modifications, involving the incubation of samples with protease (0.9 U/mL) in tricine buffer (20 mg/mL, pH 7.5, 250 mM) at 37 °C for 30 min.

For the fully branched size distribution (whole molecule), starch (8 mg) was dissolved in 1 mL DMSO solution containing 0.5% (w/w) lithium bromide (DMSO/LiBr) at 80 °C in a thermomixer (Eppendorf, Hamburg, Germany) for 24 h. Samples were then precipitated two times with absolute ethanol (10 mL) and re-dissolved in 0.05% DMSO/LiBr as in the previous step. Samples were mixed and centrifuged at 4000 *g* for 10 min. Supernatant was transferred into a SEC vial and then injected into GRAM 30 and 3000 columns (PSS GmbH, Mainz, Germany) connected in series. These columns provided separation in the range of 5×10^3 to $\sim 5 \times 10^6$ Da, corresponding to hydrodynamic radius (R_h) from ~0.5 to ~50 nm. The injection volume, flow rate, and temperature were 100 µL, 0.3 mL/min, and 80 °C, respectively. For the molecular size distribution of individual branches of starch molecules, starch was dissolved in 1.5 mL DMSO/LiBr and treated in the same way as fully branched samples. The recovered starch pellet was dissolved in 0.9 mL of warm deionized water in a boiling water bath for 15 min, cooled down to room temperature, and mixed with 5 µL sodium azide solution (40 mg/mL), 0.1 mL acetate buffer (0.1 M, pH 3.5), and 2.5 µL isoamylase in sequence. The debranching reaction was carried out at 37 °C for 3 h. The debranched starch dispersion was neutralized to pH ~7 with 0.1 M NaOH solution and then heated at 80 °C in a thermomixer for 1 h to inactivate the enzyme. Debranched samples were freeze-dried, dispersed in 1 mL DMSO/LiBr, and injected into PSS GRAM 100 and 1000 columns connected in series, which had a separation range of 100 to $\sim 10^6$ Da.

Data analysis and calculations to obtain SEC plots were performed as reported previously by Wang, Hasjim, Wu, Henry, and Gilbert, (2014) and Liu, Halley, and Gilbert, (2010). The amylose content of the different starches was determined from the SEC molecular size distribution of debranched starch as the ratio of the area under the curve (AUC) of amylose branches to the AUC of overall amylopectin and amylose branches (International Standardization Organization, 2011; Vilaplana, Hasjim, & Gilbert, 2012).

The degree of branching (DB) of whole starch molecules, defined as the percentage of α-(1→6) glycosidic linkages (branching points) to the total of both α-(1→4) and α-(1→6) glycosidic linkages, was determined as the study performed by Wu, Morell, and Gilbert, (2013).

2.2.2. Thermal properties of retrograded gels

Starch (8 mg) and distilled water (13 µl) were hermetically sealed in high volume differential scanning calorimetry (DSC) pans. Sample pans were equilibrated for 1 h before testing. Starch suspensions were analyzed in triplicate by DSC (TA Instruments DSC 2920 Modulated DSC, New Castle, DE, USA) from 30 to 100 °C at a heating rate of 10 °C/min (1^{st} heat). An empty pan was used as a reference, and the system was calibrated with indium. Pans were rapidly cooled to room temperature and then subjected to the temperature cycle of 4 °C for 24 h followed by 25 °C for 24 h (2^{nd} heat). This temperature cycle was repeated for 7 days. Samples were then rescanned from 20 to 200 °C at 5 °C/min. An example of a thermogram showing an endotherm corresponding to retrograded amylopectin is shown in supplementary material.