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Polymeric tannins significantly alter properties and *in vitro* digestibility of partially gelatinized intact starch granule

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ABSTRACT

Excess calorie intake is a growing global problem. This study investigated effect of complexing partially gelatinized starch with condensed tannins on *in vitro* starch digestibility. Extracts from tannin and non-tannin sorghum, and cellulose control, were reacted with normal and waxy maize starch in 30% (30E) and 50% ethanol (50E) solutions at 70 °C/20 min. More tannins complexed with the 30E than 50E starches (mean 6.2 vs 3.5 mg/g, respectively). In the 30E treatments, tannins significantly increased crystallinity, pasting temperature, peak viscosity, and slow digesting starch (from 100 to 274 mg/g) in normal, but not waxy starch, suggesting intragranular cross-linking with amylose. Tannins doubled resistant starch (RS) to approx. 300 mg/g in both starches. In 50E treatments, tannins made both maize starches behave like raw potato starch (>90% RS), suggesting granule surface interactions dominated. Non-tannin treatments generally behaved similar to cellulose. Condensed tannins could be used to favorably alter starch digestion profile.

1. Introduction

The rising prevalence of chronic diseases related to excess caloric intake, such as diabetes and obesity, is a critical public health problem facing both developed and developing countries. Strategies that lower caloric impact of foods without negatively affecting their sensory properties are necessary. Carbohydrates are the major source of metabolic energy in foods, accounting for approximately 52% of calories derived from food in the US, and up to 80% in the developing countries (Awika, 2011; USDA-ERS, 2014). Among the dietary carbohydrates, starch contributes most of the calories and is thus a prime target for favorably altering caloric profile of foods.

Starch is nutritionally classified as rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst, Kingman, & Cummings, 1992; Englyst, Hudson, & Englyst, 2000). RDS leads to rapid spike in blood glucose level after ingestion, whereas the SDS results in a slower sustained postprandial glucose response and is thus thought to help improve satiety (Aller, Abete, Astrup, Martinez, & van Baak, 2011; Zhang & Hamaker, 2009). The RS fraction escapes enzyme hydrolysis in the small intestine and functions as dietary fiber. Increasing SDS and RS in starchy foods is therefore of great interest to the food industry.

Interest in polyphenols as potential regulators of glucose uptake and metabolism has grown (Hanhineva et al., 2010; Zhu, 2015). However, evidence suggests that direct interaction of monomeric polyphenols with starch, has limited practical impact on starch digestibility profile (Liu, Wang, Peng, & Zhang, 2011). On the other hand, Barros et al. (Barros, Awika, & Rooney, 2012, 2014) recently showed that the high molecular weight proanthocyanidins, PA, (condensed tannins) from sorghum interact with amylose to form RS in completely gelatinized and dispersed starch (no intact granules); interaction of the tannins with amylopectin did not form RS. The evidence suggests that the structure of amylose affords a more efficient interaction with the high MW tannins, suggesting the interactions involve extensive hydrogen-bonding, along with hydrophobic interactions, as was demonstrated for other carbohydrates (Le Bourvellec, Bouchet, & Renard, 2005; Soares, Mateus, & de Freitas, 2012), and well documented for proteins (Hagerman & Butler, 1981). Thus there is opportunity to utilize the tannins to directly reduce starch digestibility. However, given that starch is only partially gelatinized in most starchy foods (e.g., cookies – 2–11%, crackers – 3%, bread – 33–71%, cereal flakes – 24–27% (Varriano-Marston, Ke, & Huang, 1980; Wootton & Chaudhry, 1980), and granule integrity is largely retained, it is not clear how above observations would translate into a typical food system.

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Dunn, Yang, Girard, Bean, and Awika (2015) reported that tortilla (in which starch is partially gelatinized) processed from wheat flour with added high tannin sorghum bran (rich in high MW PA), increased SDS from 13 to 21% (compared to control and non-tannin brans) without an increase in RS formation. Extensive interaction of PA with gluten proteins during the dough mixing stage was observed; thus it was not clear whether the protein-PA interactions influenced the observed changes in starch digestibility. Other authors also found limited impact of PA on RS formation in heterogeneous food matrix (Lemlioglu-Austin, Turner, McDonough, & Rooney, 2012; Mkandawire et al., 2013), but significant increase in SDS and reduced glycemic index (Lemlioglu-Austin et al., 2012). This study thus investigates how the degree starch swelling and gelatinization affects tannin-starch interactions, and effect of the interactions on starch properties and *in vitro* digestibility.

2. Materials and methods

2.1. Materials

2.1.1. Sorghum phenolic extracts

Two sorghum varieties grown in College Station, TX were chosen based on their different polyphenol concentration and profiles. High tannin sorghum (high in polymeric tannins) and a white pericarp sorghum (with no tannins) were used. Sorghum brans were obtained by decorticating 1 kg batches in a PRL mini-dehuller (Nutama Machine Company, Saskatoon, Canada) and were separated with a KICE grain cleaner (model 6DT4-1, KICE Industries Inc., Wichita, KS). The brans (approximately 10% yield) were milled to pass through a 0.5 mm screen using a UDY cyclone mill (model 3010−030, UDY Corporation, Fort Collins, CO). They were kept at −20 °C until used. Brans (100 g) were extracted in 70% acetone (400 mL) with stirred for 2 h. The mixture was then filtered, and the residue re-extracted twice as described above for 1-h each time. The acetone was immediately removed from the combined supernatant under vacuum at 40 °C and stored at −20 °C until used. Portions of the aqueous extracts were also freeze-dried.

2.1.2. Starch and reagents

Normal (amylose content = 23.9%) and waxy (amylose content = 0.36%) maize starches were obtained from National Starch Food Innovation (Bridgewater, NJ). Potato starch (amylose content = 21.9%) was obtained from Penford Food Ingredients (CO, USA). Total starch was determined using the total starch kit (AACC method 76-13, AACC-International, 2010), and the amylose content was determined using the amylose/amylopectin ratio kit, both from Megazyme. All solvents (HPLC or analytical grade) and reagents were obtained from Sigma (St. Louis, MO). Porcine pancreas α-amylase (EC 3.2.1.1) was also purchased from Sigma–Aldrich Chemical Co., Ltd (St. Louis, MO), whiles D-Glucose (GOPOD format) assay was purchased from Megazyme (Ireland).

2.2. Methods

2.2.1. Preparation of phenolic-extract-treated starch products

Tannin extract (2.5 g total solids) from high-tannin sorghum was incubated separately with normal and waxy maize starch (25 g), in 30% and 50% aqueous ethanol solutions (v/v) at 70 °C for 20 min. Ethanol solution and tannin extract made up a total volume of 75 mL. Samples were then centrifuged to remove the supernatant, and the sediments collected was oven dried at 40 °C overnight to remove residual ethanol and water. The sediments were then gently dispersed with pestle and mortar to obtain powdered samples, which were stored at 4 °C until use. Two other treatments were included, replacing the tannin extract with non-tannin extract from white sorghum (2.5 g total solids) for comparison, and cellulose powder (2.5 g) as a control treatment.

2.2.2. Phenolic extract characterization

Phenol content of the sorghum extracts was estimated according to the Folin–Ciocalteu method described by (Kaluza, Mcgrath, Roberts, & Schroder, 1980). Monomeric phenolic profile of the extracts was determined following the HPLC method previously described by Awika, Yang, Browning, and Faraj (2009).

The tannin extract was also profiled for PA content and MW distribution by the normal-phase HPLC-FLD method of (Langer, Marshall, Day, & Morgan, 2011) using conditions described by Barros et al. (Barros et al., 2012). Catechin and procyanidin B1, and C1 were used to quantify monomers, dimers, and trimers, respectively. Quantitative data for PA with a DP greater than or equal to four were based on procyanidin C1 (DP 3) peak response as previously described by Ojwang, Yang, Dykes, and Awika (2013).

2.2.3. Quantifying proportion of proanthocyanidins that reacted with starch

The normal-phase HPLC-FLD method described above was used to profile and quantify PA in both supernatant collected after incubating the starch with tannin extract, as well as the methanolic extract (400 mg starch: 1.2 mL MeOH, vortexed low speed at 20 °C/2 min) of the final dried tannin-treated starches. Samples were filtered (0.45 μm, nylon) and then injected (10 μL) into HPLC. Proportion of PA that reacted with starch (mg PA/g of starch) was calculated as:

Total mg PA in starting extract

− (mg PA in supernatant after starch-PA incubation

+ mg PA in methanolic extract from dry PA-treated starch).

2.2.4. Starch swelling properties

Solubility (%S) and swelling power (SP) were determined for starch treatments following a method described by Kibar et al. (2010), with some modifications. Starch suspension (1:15 w/v) was incubated for 30 min at room temperature (25 °C) with horizontal shaking in a reciprocating shaker set at low speed (160 rpm). The suspension was then centrifuged at 8000*g* for 20 min, and the supernatant decanted into previously tared aluminum tin. The tin was dried for 24 h at 105 °C, and the soluble solids weighed and used to measure %S. The sediment left after decanting was weighed and used to calculate the SP. Calculations were done as follows:

$$\%S = [(\text{mass of solubles})/(\text{mass of dry starch})] \times 100$$

$$SP = (\text{mass of sediment})/[(\text{mass of dry starch}) \times (1 - (\%S/100))]$$

2.2.5. Thermal properties of starch samples

Differential Scanning Calorimetry (DSC) measurements were done using a Perkin-Elmer DSC-6 (Boston, USA). The calorimeter was calibrated with indium, and the DSC runs were operated under ultra-high purity nitrogen (30 mL/min) using a sealed empty aluminum pan as reference. Starch samples (3 mg, db) were each weighed into an aluminum pan, and distilled water added to get to 12 mg total weight (1:3 starch:water ratio, w/w). The pan was then hermetically sealed and equilibrated at room temperature overnight to allow adequate starch hydration. Samples were heated from 20 °C to 95 °C at a scanning speed of 10 °C/min and a heat flow rate of 20 mW/g. The raw data was processed with Pyris 5 software (Perkin-Elmer) to obtain the onset (T_o), peak (T_p), and conclusion (T_c) temperatures and gelatinization