**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 – 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.

Keywords: Tannins; Proanthocyanidins; Starch digestibility; Amylose; Resistant starch

40 1. Introduction

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

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

56 Interest in polyphenols as potential regulators of glucose uptake and metabolism has grown
57 (Hanhineva, Torronen, Bondia-Pons, Pekkinen, Kolehmainen, Mykkanen, et al., 2010; Zhu,
58 2015). However, evidence suggests that direct interaction of monomeric polyphenols with starch,
59 has limited practical impact on starch digestibility profile (Liu, Wang, Peng, & Zhang, 2011). On
60 the other hand, Barros et al (Barros, Awika, & Rooney, 2012; Barros, Awika, & Rooney, 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.

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, Kaufman, Bean, Weller, Jackson, & Rose, 2013), but significant increase in SDS and reduced glycemic index (Lemlioglu-Austin, Turner, McDonough, & Rooney, 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.

84

85 **2. Materials And Methods**

86 *2.1. Materials*

87 *2.1.1 Sorghum phenolic extracts*

88 Two sorghum varieties grown in College Station, TX were chosen based on their different
89 polyphenol concentration and profiles. High tannin sorghum (high in polymeric tannins) and a
90 white pericarp sorghum (with no tannins) were used. Sorghum brans were obtained by
91 decorticating 1 kg batches in a PRL mini-dehuller (Nutama Machine Company, Saskatoon,
92 Canada) and were separated with a KICE grain cleaner (model 6DT4-1, KICE Industries Inc.,
93 Wichita, KS). The brans (approximately 10% yield) were milled to pass through a 0.5 mm screen
94 using a UDY cyclone mill (model 3010–030, UDY Corporation, Fort Collins, CO). They were
95 kept at –20 °C until used. Brans (100 g) were extracted in 70% acetone (400 mL) with stirred for
96 2 h. The mixture was then filtered, and the residue re-extracted twice **as described above** for 1-h
97 each time. The acetone was immediately removed from the combined supernatant under vacuum
98 at 40 °C and stored at –20 °C until used. Portions of the aqueous extracts were also freeze-dried.

99 *2.1.2 Starch and reagents*

100 Normal (amylose content = 23.9%) and waxy (amylose content = 0.36%) maize starches were
101 obtained from National Starch Food Innovation (Bridgewater, NJ). **Potato starch (amylose**
102 **content = 21.9%) was obtained from Penford Food Ingredients (CO, USA). Total starch was**
103 **determined using the total starch kit (AACC method 76-13, AACC-International, 2010), and the**
104 **amylose content was determined using the amylose/amylopectin ratio kit, both from Megazyme.**
105 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), while 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 minutes. 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 were 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, Awika, & Rooney, 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 $8,000 \times g$ 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 enthalpies (ΔH). The degree of gelatinization (DG) of each starch treatment sample was determined by comparing the gelatinization enthalpy of the treatment sample (ΔH_s) to its native form (ΔH_n) (Baks, Ngene, Van Soest, Janssen, & Boom, 2007):

$$DG = [1 - (\Delta H_s / \Delta H_n)] \times 100$$

2.2.6 Starch Pasting Properties

A Rapid Visco Analyzer (RVA) was used to record starch pasting properties of the treatments as detailed by Barros et al (Barros, Awika, & Rooney, 2012). Distilled water was added to each of the prepared samples (3.0 g, dry basis) in the RVA canister to obtain a total constant sample weight of 28 g. An RVA model 4 (Newport Scientific PTY Ltd., Warriewood, Australia) was used. Initial sample equilibration at 50°C for 1 min followed by a linear temperature increase from 50–95 °C in 7.5 min, and then a holding step at 95 °C for 5 min, cooling to 50°C within 7.5 min, and another holding step at 50 °C for 2 min, for a total of 23 min.

2.2.7 Starch crystallinity

Starch crystallinity determination was carried out using a method described by Mutungi, Passauer, Onyango, Jaros, and Rohm (2012) with some modifications. A Bruker D8 Advanced X-ray diffractometer was used. Samples were scanned with Cu K α radiation ($\lambda = 0.154$ nm), and reflections were detected in the angular range $2\theta = 2\text{--}32^\circ$ with a step intervals of 0.05° . Scanning duration at each step was 5 s. The X-ray generator operating conditions were 35 kV and 45 mA. Raw waxy and normal corn starch samples were also scanned for comparison. All samples were conditioned at room temperature (25°C) for 48 h before scanning. Percent crystallinity was calculated using DIFFRAC^{plus} TOPAS software following recommendations by Lopez-Rubio, Flanagan, Gilbert, and Gidley (2008).

2.2.8 In-vitro starch digestibility

In vitro digestibility of samples was measured as previously described by Englyst, Hudson, and Englyst (2000). In summary, the starch treatments (400 mg) were digested by α -amylase (300 U/mg, A3176 Sigma-Aldrich, St. Louis, MO) and amyloglucosidase (95 U/mL, Cat. No. E-AMGDF, Megazyme International) mixture. Glucose released after 20 and 120 min was determined by reacting an aliquot with glucose oxidase reagent (K-GLUC GOPOD format assay kit, Megazyme, Ireland) and the absorbance at 510 nm read on a UV-vis spectrophotometer (Shimadzu Scientific Instruments) using a glucose standard assay kit (K-GLUC GOPOD format assay kit, Megazyme, Ireland). Raw potato starch was used for comparison. The content of the hydrolyzed starch was calculated by multiplying a factor of 0.9 with the glucose content. The percentage of SDS and RS in the products were obtained by the following equations:

$$\text{SDS\%} = [(G_{120} - G_{20}) \times 0.9 / \text{TS}] \times 100;$$

192 $RS\% = TS - (RDS + SDS);$

193 Where, G_{20} and G_{120} are glucose content released after 20 and 120 min, respectively; **RDS is (G_{20}**
194 **X 0.9)**; and TS is the weight of starch (**excluding added cellulose or extracts**) in sample used for
195 each test. Results were expressed on db.

196 *2.3 Statistical Analysis*

197 Data were analyzed using a one-way analysis of variance (ANOVA) to determine significant
198 differences among them. Tukey's HSD ($P \leq 0.05$) was used to separate means. The software
199 used was SAS version 9.4 for windows. All tests were replicated at least twice.

201 **3. Results And Discussion**

202 *3.1 Phenolic profile of the sorghum extracts*

203 Phenol content (mg GAE/g) of the sorghum phenolic extracts were 255.3 ± 0.7 (tannin) and 23.6
204 ± 0.3 (white, non-tannin). Phenolic acid acyl-glycerides were identified as the major monomeric
205 polyphenols in both sorghum extracts, similar to previous observation (Yang, Allred, Geera,
206 Allred, & Awika, 2012) (data not shown). The tannin sorghum extract contained 111 ± 3.0 mg
207 PA per g. More than 99% of this was made up of polymeric tannins ($DP > 3$) (Fig 1). The PA
208 profile is consistent with that reported previously by Barros, Awika, and Rooney (2012).

209 *3.2 Starch swelling and gelatinization properties*

210 **Ethanol competes with starch for water, and was therefore included in the treatments to control**
211 **starch swelling and gelatinization. It also increases the solubility of PA, which can facilitate PA-**

starch interactions. Starches treated with tannin extract in 30% ethanol (30E) had higher amounts of tannins (average 6.2 mg PA/g starch) interacting with starch compared to 50% ethanol (50E) treatments (average 3.5 mg PA/g of starch) (Table 1 Supplement). This is likely due to greater extent of swelling of 30E starches compared to the 50E starches (Table 1). When the starch swells substantially, pores on the granule surface become easy channels for tannins to migrate into the interior of the granule to interact with starch polymers. **Interestingly, the amount of PA that interacted with the starches was only 3.1 – 5.8% of what was added (Table 1 Supplement).** This suggests a relatively low affinity of granular starch for PA, probably due to the tightly packed nature of starch polymers in the granule, limiting binding sites. The fact that the normal starch bound slightly more PA than the waxy starch in the 30E treatment suggests a stronger affinity of amylose for PA than amylopectin. The tannins that interacted with starch were essentially unextractable in methanol (Fig 1), **which suggests specific binding of the tannins to starch.** As expected, the 30E starches were also more gelatinized (67.2-97%) compared to 50E starches (1.3-45.3%) (Table 2).

The 30E starches had lower melting/gelatinization enthalpies (ΔH) and narrower melting temperature range ($T_c - T_o$), than the 50E treatments. This confirms that the 30E starches underwent a higher degree of gelatinization. The 30E starches exhibited higher DSC onset (T_o) and peak (T_p) temperatures than the 50E starches (Tables 2). The higher T_o and T_p is an indication of greater resistance of the starch to gelatinization; which may have resulted from a modification/ rearrangement of the internal amorphous structure of the starch into a more rigid form, more resistant to gelatinization; a phenomenon common in heat treated starches (Barichello, Yada, Coffin, & Stanley, 1990; Wongsagonsup, Varavinit, & BeMiller, 2008). Mutungi, Passauer, Onyango, Jaros, and Rohm (2012) explain that, different combinations of

thermal treatments induce ordering effects in starch granule structure that result in new products of different crystalline orientations, some of which have high resistance to gelatinization.

An interesting observation was that in the 30E treatments, both sorghum extracts had similar effect on the extent of normal starch gelatinization (Table 2). However, in the 50E treatment, the tannin extract completely inhibited gelatinization of the normal starch, and had a melting enthalpy similar to raw starch (Table 2). On the contrary, in waxy starch, the 50E tannin treatment had similar degree of gelatinization as the cellulose control (almost 30%) and higher than the white sorghum treatment (10%).

The high MW tannins are relatively hydrophobic, and are thus more soluble in 50% ethanol compared to 30% ethanol. Thus, besides inhibiting starch swelling to a higher degree than in 30E, the 50E treatment would likely allow tannins to solubilize more freely and thus interact better with starch molecules. The fact that there was such a sharp contrast in behavior of normal vs waxy starch in presence of tannins in 50E treatment despite similar tannin inclusion (approx. 3.5 mg/g starch) (Table 1 Supplement) suggests different chemical interactions are involved. The data suggests strong specific interaction of the tannins with the normal starch, but not waxy starch, in the 50E, which supports the specificity of amylose-tannin interactions hypothesis (Barros, Awika, & Rooney, 2012).

Amylose structure is more likely to allow for a high degree of hydrogen bonding and hydrophobic interactions with tannins, compared to the highly branched amylopectin which is sterically unfavorable for such interactions. Thus it is plausible that tannins acted as cross-linkers of amylose polymers near the granule surface in the 50E treatments. The fact that the non-tannin waxy starch treatments had lower degree of gelatinization compared to the tannin and cellulose

control may be due to the highly branched amylopectin undergoing a less-hindered interaction with monomeric phenolic compounds, especially in their amorphous regions. This would conceivable strengthen the amorphous regions (reduce mobility) and make them more resistant to gelatinization. Chai et al (Chai, Wang, & Zhang, 2013) reported that relatively high levels of monomeric tea polyphenols at 100 mg/g starch slightly (though not significantly) reduced postprandial glycemic response to waxy starch, but significantly increased the response in a high amylose starch.

3.3 Starch pasting properties

In general, the pasting temperatures and peak times were higher for the 30E normal starches than their control, whereas the 50E normal starches were generally similar to the control (Table 3). This suggests a higher degree of crystallinity of the 30E normal starches, likely due to reassociation of amylose polymers in the granule, which agrees with the DSC data (Table 2). For the waxy starches, there were no clear differences between the 30E and 50E treatments, and both groups were similar to control (Table 3). This is likely because the lack of amylose in the waxy starch limits available regions of starch polymers that can reassociate after hydrothermal treatment. By contrast, the 30E normal starch tannin treatment had a markedly higher pasting temperature compared to all other normal starch treatments and control (Table 3). A possible explanation is that the 30E treatment resulted in enough starch swelling to allow the tannin polymers to migrate into the granule interior and partially cross-link the amylose polymers inside the granule thus making the granule more resistant to swelling. The interaction of tannins with the 50E normal starch likely occurred mostly close to the surface thus did not have as much

279 impact on pasting temperature. Within the waxy starch treatments, the non-tannin extract
280 treatments tended to have higher pasting temperatures than comparable tannin and cellulose
281 treatments, which may be due to the association of monomeric phenolics with amylopectin
282 discussed in Section 3.2 above.

283 The peak viscosity for the heat treated starches were generally lower than corresponding
284 untreated starches (Table 3), which indicates the heat treatments reduced swelling capacity of the
285 starches. However, the tannin treatments consistently had higher peak viscosities than
286 comparable treatments for both normal and waxy starches, and actually produced higher peak
287 viscosities than untreated waxy maize starch (Table 3). A similar phenomenon was reported for
288 sorghum starch in presence of tannins (Beta, Corke, Rooney, & Taylor, 2001). This suggests
289 cross-linking and/or granule stabilizing effect of the polymeric tannin. The similarity of pasting
290 properties of the tannin treated 50E normal starch to non-treated starch also agreed with the DSC
291 data (Table 2) which showed the 50E normal tannin treatment behaved similar to the raw starch.

293 *3.4 Starch crystallinity*

294 All treatments retained the typical A-type polymorph diffraction patterns of their native/raw
295 starches (data not shown), which indicates no inclusion complexes between amylose and
296 polyphenols were formed. The monomeric polyphenols are too hydrophilic to interact with
297 amylose polymers in this manner, whereas the polymeric tannins are too bulky. This may suggest
298 that the interaction of tannins with starch is most strongly influenced by hydrogen-bonding, with
299 the close proximity of hydroxyl groups in the polymeric tannins ensuring stronger interactions
300 with amylose. However, formation of inclusion complexes have been reported between amylose

and the monomeric flavonoid, genistein (Cohen, Orlova, Kovalev, Ungar, & Shimoni, 2008), though such complexes are likely weak due to flavonoid bulk (Ya, Gaffney, Lilley, & Haslam, 1989) and limited hydrogen bonding sites. Clear evidence with other carbohydrates consistently demonstrate that the carbohydrate polymers with hydrophobic regions bind much more strongly to polymeric tannins than carbohydrates with similar structures but no hydrophobic regions (Soares, Mateus, & de Freitas, 2012). This indicates that hydrophobic interactions likely play an important role in stabilizing amylose-tannin complexes as well.

The X-ray crystallinity of the starch treatments present some interesting results (Table 4). The 50E treatments generally had higher crystallinity than raw starches and 30E treatments. This observation was expected, considering the fact that starch gelatinization results in loss of starch crystallinity. Also, the limited moisture in the 50E treatments restricted starch swelling while allowing for some internal molecular rearrangement/association within the granule during the heat treatment, which may explain their higher crystallinity than raw starches. Heat treatment of starches can result in the rearrangement of starch molecules, which can effect double helical chains shifting within the crystallites, resulting in a more ordered, hence more crystalline structure (Hoover & Vasanthan, 1994; Mutungi, Passauer, Onyango, Jaros, & Rohm, 2012). Wongsagonsup, Varavinit, and BeMiller (2008) made similar observations in temperature cycled (TC) normal maize starch.

The 30E normal starch tannin treatment was a lot more crystalline (51.4%) than comparable non-tannin extract and cellulose treatments (16.0 – 22.8%), which explains its much higher pasting temperature than the other treatments (Table 3). The fact that such a difference was not observed in the 30E waxy treatments confirms that specific tannin-amylose interactions are responsible for the apparent anomalous observations. Thus tannin-amylose interactions appear to

relatively specific with consistent effect on starch properties. Also worth noting was the fact that the E50 waxy non-tannin extract treatment had a slightly (but significantly) higher crystallinity than comparable tannin and cellulose treatments (Table 4), which also confirms the RVA data.

3.5 In-vitro starch digestibility

Of particular interest is how the observed effects of tannins on physical properties of starch affect starch digestibility. This would provide insight on how starch-tannin interactions can be utilized to produce nutritionally desirable starches. The tannins had marked effect on starch digestibility compared to other treatments (Figures 2 & 3). In the 30E starches, tannins reduced RDS in normal starch (from 735 mg/g to 397 mg/g); representing a 46% decrease (Fig. 2). The white sorghum extract had no effect on RDS (was similar to cellulose). The tannin treated normal 30E starch also had much higher SDS (274 mg/g) and RS (299 mg/g) compared to non-tannin treatments, which were similar (SDS average = 96.7 mg/g, RS average = 148 mg/g). Thus the tannin-starch interactions produced a net SDS and RS increase of 177 and 151 mg/g, respectively in the normal 30E maize starch. This indicates that the tannins formed non-digestible and slow-digesting complexes with starch. The effects are especially remarkable given there was only 6.5 mg tannins/g of starch in the complexes (Table 1 Supplement). Barros et al (Barros, Awika, & Rooney, 2012) observed up to a maximum 86 mg RS/g starch when completely gelatinized and dispersed normal maize starch was reacted with approx. 24 mg tannins/g starch (Barros, Awika, & Rooney, 2014). The complete disruption of granule integrity may be a factor in their lower values. However, they used an aqueous matrix for reacting starch

with tannins; this likely limited starch-tannin interactions due to tannins readily precipitating in water.

In the waxy 30E starch, tannins had a smaller but still substantial reduction in RDS, from 754 mg/g to 516 mg/g, representing a 31.6% reduction. The change in RDS was mostly explained by a concomitant increase in RS; the tannin effect on the SDS (98 mg/g vs control cellulose 65 mg/g) was small and similar to the non-tannin extract (105 mg/g) (Fig 2). The observation was somewhat unexpected, because Barros et al (2012, 2014) reported that tannin interaction with amylopectin did not produce any change in RS in fully gelatinized waxy starch and isolated amylopectin. Thus it appears from our data that starch gelatinization and granule integrity has a marked effect on ability of tannins to interact with starch in a way that impedes enzyme digestion. This is highly relevant because starch granule integrity is largely retained in most starchy foods. We theorize that the effect of the tannins on RS formation in waxy maize starch may be due to formation of sufficient hydrogen bonding to partially block enzyme access to the starch hydrolysis sites. The non-tannin extract had no effect on RS formation in the 30E waxy maize starch, further highlighting limited impact of monomeric polyphenols on starch digestibility (Barros, Awika, & Rooney, 2014). However, it produced a slight, but significant increase in SDS compared to cellulose control, supporting the theory that the monomeric polyphenols interacted mainly with the amorphous regions of amylopectin, which are usually more readily hydrolyzed by α -amylases.

Based on the degree of gelatinization and starch properties, we expected the 50E treatments to have significantly different starch digestibility profiles compared to the 30E treatments. In the 50E samples, the cellulose and white sorghum treatments had digestibility profiles near identical to raw starches (Fig 2), indicating the starches did not swell enough to enhance their digestibility

during the heat treatment. Interestingly, both normal and waxy 50E starches were almost non-digestible when reacted with tannins (both had approx. 90% RS) (Fig 2). This was remarkable, given the low level of tannins that reacted with these starches (3.4 mg/g starch, Table 1 Supplement). In fact the 50E tannin treatments had much lower digestibility than corresponding raw normal and waxy maize starches; and behaved more like raw potato starch (Fig 2).

Considering the fact that the 50E starches underwent minimal swelling/gelatinization (Table 1), interactions between tannins and starch likely occurred largely close to the granule surface. The interactions would most likely be concentrated in/near granule pores where enzymes initially access starch. This would in turn essentially block the pores, making enzyme access difficult. Raw cereal starches are susceptible to enzyme hydrolysis due to the presence of these surface pores. By blocking these pores, tannins made the maize starches behave like native raw potato starch, in which the lack of surface pores on granules gives it a low digestibility (RS > 90%) (Juszczak, Fortuna, & Krok, 2003).

4. Conclusion

Our findings clearly demonstrate that polymeric tannins, even at relatively low levels, interact with starch in a way that alter starch properties, and dramatically affect starch digestibility profile. The degree of granule swelling and gelatinization has a big impact on the starch-tannin interactions. Obviously the interactions are enhanced in amylose-containing starch, due to the more likely strong amylose-tannin interaction through extensive hydrogen bonding and hydrophobic interactions. It appears that conditions that allow for complete solubilization of tannins (i.e., take advantage of their partition coefficient) can significantly improve the

efficiency of their interactions with starch. This study provides a compelling evidence for the potential of natural high molecular weight polyphenols as new ingredients to produce nutritionally beneficial starches. The fate of the tannin-starch complexes *in vivo* and interaction with gut microbiota will be of particular interest.

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Figure captions

Figure 1. Normal phase HPLC-FLD of sorghum proanthocyanidin (tannin) profile of high-tannin sorghum used in the study (A), and profile obtained from methanolic extract of dried tannin-reacted normal starch from 30% ethanol treatment (B). Numbers on peaks denote degree of polymerization (DP). P = polymers with DP > 10. Fig 1 A inset represents generic structure of sorghum proanthocyanidin polymer.

Figure 2. Effect of starch-tannin interactions on digestibility of partially gelatinized normal and waxy maize starches treated in 30% (30E) and 50% (50E) aqueous ethanol solution. Samples were incubated in respective solutions at 70°C/20 min. RDS, SDS, and RS represent rapidly digesting, slow digesting, and resistant starch, respectively. Raw starch denotes native starch not subjected to the solvent/heat treatments. Native (untreated) raw potato starch digestibility included for comparison; the potato starch was digested under similar conditions as the maize starches. Error bars indicate $\pm$ standard deviation. Same letters within starch digestibility types are not significantly different ($P \leq 0.05$).

Table 1: Swelling properties of maize starches treated in ethanol solutions with sorghum phenolic extracts

Starch treatment	Normal starch (23.9% amylose)		Waxy starch (0.36% amylose)	
	Solubility (%)	Swelling power (%)	Solubility (%)	Swelling power (%)
Native starch ^A	0.02 ± 0.00 ^e	1.94 ± 0.04 ^c	0.11 ± 0.10 ^c	2.11 ± 0.01 ^e
<i>30% Aqueous ethanol</i>				
Control Starch	2.07 ± 0.10 ^{bc}	4.70 ± 0.05 ^a	6.19 ± 0.86 ^a	5.05 ± 0.13 ^c
Starch + cellulose	1.7 ± 0.17 ^c	4.72 ± 0.13 ^a	4.06 ± 0.59 ^b	6.66 ± 0.15 ^a
Starch + non-tannin extract	4.97 ± 0.09 ^a	4.87 ± 0.01 ^a	7.46 ± 0.14 ^a	5.44 ± 0.09 ^b
Starch + tannin extract	0.31 ± 0.01 ^e	4.09 ± 0.06 ^b	3.01 ± 0.06 ^b	4.21 ± 0.01 ^d
<i>50% Aqueous ethanol</i>				
Control Starch	0.34 ± 0.03 ^e	2.06 ± 0.06 ^c	0.28 ± 0.00 ^c	2.26 ± 0.05 ^e
Starch + cellulose	0.28 ± 0.08 ^e	2.09 ± 0.02 ^c	0.47 ± 0.01 ^c	2.27 ± 0.02 ^e
Starch + non-tannin sorghum extract	2.54 ± 0.26 ^b	2.01 ± 0.07 ^c	2.88 ± 0.13 ^b	2.22 ± 0.06 ^e
Starch + tannin extract	0.89 ± 0.18 ^d	2.10 ± 0.01 ^c	1.24 ± 0.00 ^c	2.18 ± 0.00 ^e

Starch treatments were heated at 70°C/20 min in specified solutions, rinsed and dried before analysis. ^ANative starch = starch not subjected to the thermal treatment. Means followed by the same letter within column are not significantly different ($P \leq 0.05$).

Table 2: Thermal properties of starch treated in aqueous ethanol solutions with sorghum phenolic extracts

Sample	T _o (°C)	T _p (°C)	T _c (°C)	T _c – T _o (°C)	ΔH (J/g dry starch)	Degree of Gelatinization (%) ^C
Normal Maize Starch						
NS ^A	66.0 ± 0.1 ^b	71.9 ± 0.2 ^c	78.3 ± 0.3 ^{bc}	12.3 ± 0.4 ^a	12.2 ± 1.1 ^a	0.0 ^e
<i>30% Aqueous ethanol</i>						
Cellulose	73.2 ± 0.5 ^a	76.4 ± 0.1 ^a	80.1 ± 0.6 ^a	6.9 ± 0.3 ^{cd}	0.4 ± 0.1 ^e	97.1 ± 0.6 ^a
White ^B	73.4 ± 0.0 ^a	76.6 ± 0.1 ^a	80.1 ± 0.0 ^a	6.7 ± 0.0 ^d	1.6 ± 0.2 ^d	87.2 ± 1.3 ^b
Tannin ^B	72.4 ± 0.0 ^a	74.9 ± 0.2 ^b	78.8 ± 0.1 ^b	6.4 ± 0.1 ^d	1.5 ± 0.0 ^d	87.8 ± 0.3 ^b
<i>50% Aqueous ethanol</i>						
Cellulose	65.6 ± 0.9 ^b	69.9 ± 0.9 ^d	74.7 ± 0.2 ^e	9.1 ± 0.7 ^{cb}	4.7 ± 2.6 ^c	45.0 ± 1.6 ^c
White ^B	67.0 ± 0.8 ^b	72.1 ± 0.2 ^c	77.4 ± 0.9 ^d	10.4 ± 1.4 ^{ab}	9.1 ± 0.8 ^b	29.7 ± 1.2 ^d
Tannin ^B	66.8 ± 0.3 ^b	72.1 ± 0.3 ^c	77.7 ± 0.0 ^{dc}	10.9 ± 0.3 ^{ab}	12.0 ± 0.1 ^a	1.3 ± 1.0 ^e
Waxy Maize Starch						
NS ^A	66.2 ± 0.4 ^c	72.3 ± 0.2 ^d	79.1 ± 0.1 ^b	12.9 ± 0.4 ^{ab}	15.3 ± 1.0 ^a	0.0 ^f
<i>30% Aqueous ethanol</i>						
Cellulose	74.8 ± 0.4 ^a	77.5 ± 0.3 ^a	81.2 ± 0.3 ^a	6.4 ± 0.2 ^d	1.5 ± 0.1 ^f	90.5 ± 0.8 ^a
White ^B	73.9 ± 0.4 ^{ab}	77.3 ± 0.4 ^a	82.0 ± 0.3 ^a	8.2 ± 0.2 ^c	5.0 ± 0.5 ^d	67.2 ± 3.1 ^c
Tannin ^B	72.7 ± 0.6 ^b	75.9 ± 0.5 ^b	79.9 ± 0.2 ^b	7.2 ± 0.4 ^{cd}	3.0 ± 0.3 ^e	80.4 ± 1.7 ^b
<i>50% Aqueous ethanol</i>						
Cellulose	66.7 ± 0.4 ^c	73.3 ± 0.7 ^{cd}	79.9 ± 0.1 ^b	13.2 ± 0.3 ^{ab}	11.1 ± 0.7 ^c	30.0 ± 0.5 ^d
White ^B	67.2 ± 0.2 ^c	73.6 ± 0.1 ^c	79.6 ± 0.5 ^b	12.4 ± 0.3 ^b	14.3 ± 0.9 ^b	10.3 ± 0.4 ^e
Tannin ^B	66.1 ± 0.5 ^c	72.4 ± 0.0 ^d	79.6 ± 0.4 ^b	13.5 ± 0.2 ^a	10.9 ± 0.1 ^c	28.6 ± 0.4 ^d

Starch treatments were heated at 70°C/20 min in specified solutions, rinsed and dried before analysis..^ANative starch not subjected to the thermal treatment. ^BSorghum extracts (White = non-tannin extract). To, onset temperature; Tp, peak temperature; Tc, Conclusion temperature; Tc – To, melting temperature range; ΔH, melting enthalpy change. ^CDegree of gelatinization calculated as: $1 - [(\Delta H_{\text{sample}} / \Delta H_{\text{native starch}})] \times 100$. Means followed by the same letter within column and starch type are not significantly different ($P \leq 0.05$).

Table 3: Pasting properties of maize starches treated in ethanol solutions with sorghum phenolic extracts

Treatment	Pasting properties				
	Peak time (min)	Pasting temp. (°C)	Peak viscosity (cP)	Final viscosity (cP)	Breakdown (cP)
Normal Maize starch					
NS ^A	8.3 ^e	72.5 ^d	4370 ^a	4411 ^a	1822 ^a
<i>30% Aqueous ethanol</i>					
Cellulose	10.2 ^a	75.7 ^b	3086 ^c	3981 ^{bcd}	606 ^d
White ^B	9.9 ^b	74.2 ^{bc}	3020 ^c	3741 ^d	706 ^d
Tannin ^B	9.6 ^c	84.8 ^a	3220 ^{bc}	4032 ^{bc}	884 ^c
<i>50% Aqueous ethanol</i>					
Cellulose	8.5 ^e	72.5 ^d	3329 ^{bc}	3767 ^{cd}	1102 ^b
White ^B	8.3 ^e	72.8 ^{cd}	3543 ^b	3863 ^{cd}	1228 ^b
Tannin ^B	8.7 ^d	74.0 ^{cd}	4300 ^a	4150 ^{ab}	1833 ^a
Waxy Maize Starch					
NS ^A	5.7 ^b	69.5 ^b	4826.0 ^c	2216 ^a	3109 ^c
<i>30% Aqueous ethanol</i>					
Cellulose	5.7 ^b	50.0 ^d	3697 ^g	1595 ^e	1863 ^e
White ^B	6.1 ^a	71.4 ^a	4252 ^f	1830 ^d	2660 ^d
Tannin ^B	6.0 ^{ab}	68.9 ^c	5571 ^a	1772 ^d	4034 ^a
<i>50% Aqueous ethanol</i>					
Cellulose	5.8 ^{ab}	70.1 ^{abc}	4506 ^e	1983 ^c	2913 ^c
White ^B	5.9 ^{ab}	70.8 ^{ab}	4675 ^d	2193 ^{ab}	2910 ^c
Tannin ^B	5.8 ^{ab}	70.1 ^{abc}	5157 ^b	2078 ^{bc}	3504 ^b

Starch treatments were heated at 70°C/20 min in specified solutions, rinsed and dried before analysis. ^ANative starch not subjected to the thermal treatment. ^BSorghum extracts (White = non-tannin extract). Means followed by the same letter within column and starch type are not significantly different ($P \leq 0.05$).

Table 4: X-Ray diffraction crystallinity (%) of maize starches treated in ethanol solutions with sorghum phenolic extracts

Starch treatment	% Crystallinity (A-Type polymorph) ^a	
	Normal starch	Waxy starch
Native starch ^A	27.63 ± 1.62 ^{bc}	45.82 ± 0.71 ^c
<i>30% Aqueous ethanol</i>		
Cellulose	22.83 ± 2.65 ^{dc}	24.35 ± 0.28 ^e
Non-tannin extract	15.97 ± 1.08 ^d	26.86 ± 2.68 ^{de}
Tannin extract	51.36 ± 2.22 ^a	29.25 ± 0.93 ^d
<i>30% Aqueous ethanol</i>		
Cellulose	33.67 ± 0.25 ^b	52.26 ± 0.21 ^b
Non-tannin extract	46.13 ± 0.32 ^a	63.32 ± 0.51 ^a
Tannin extract	45.12 ± 2.58 ^a	56.43 ± 0.23 ^b

Starch treatments were heated at 70°C/20 min in specified solutions, rinsed and dried before analysis. ^aPercent crystallinity was calculated using DIFFRAC^{plus} TOPAS software following recommendations by Lopez-Rubio, Flanagan, Gilbert, and Gidley (2008). ^ANative starch not subjected to the thermal treatment. Means followed by the same letter within column are not significantly different ($P \leq 0.05$).

Figure(s)

Figure 1

Figure 2