Research Article

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# Variability in tannin content, chemistry and activity in a diverse group of tannin containing sorghum cultivars†

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## Abstract

**BACKGROUND: Tannins are large polyphenolic polymers and are known to bind proteins, limiting their digestibility, but are also excellent antioxidants. Numerous studies investigating the functional properties of sorghum tannin have been conducted by comparing grain samples from different sorghum lines without considering the other intrinsic characteristics of the grain. The purpose of this study was to remove the confounding intrinsic factors present in the endosperm so the effect of the tannins could be evaluated utilizing a unique decortication/reconstitution procedure.**

**RESULTS: The tannin content of the 14 cultivars tested ranged from 2.3 to 67.2 catechin equivalents. The bran fractions were studied for their impact on protein binding and antioxidant capacity. Protein digestibility by pepsin ranged from 8% to 58% at the highest tannin level addition. Protein binding ranged from 3.11 to 16.33 g blue bovine serum albumin $kg^{-1}$ bran. Antioxidant capacity ranged from 81.33 to 1122.54 μmol Trolox equivalents $g^{-1}$ bran. High-performance size-exclusion chromatography detailed molecular size distributions of the tannin polymers and relationship to tannin functionality.**

**CONCLUSION: The tannin content and composition play a significant role in determining tannin functionality. These differences will allow for selections of high-tannin sorghums with consideration of the biological activities of the tannins.**


**Keywords:** sorghum; tannin; protein digestibility; kafirin

## INTRODUCTION

Sorghum (*Sorghum bicolor*) is fifth in worldwide cereal production, with over 59 million metric tons produced in 2009.[1] Sorghum is currently grown in over 100 countries on over 39 million ha.[1] Sorghum has broad application throughout the world, playing a key role as a food staple, essential animal feed, and as raw material for the biofuel and adhesives industries.[2]

Sorghum may also be a source of unique phytochemical constituents that have important human health attributes.[3] Perhaps the most widely studied phytochemical constituents are the tannins found in sorghum cultivars with a pigmented testa layer. Tannins, secondary metabolites found in many plant species, are polymeric phenolic compounds that often serve as a defense mechanism against pathogens and predators. Tannins are known to bind to proteins, carbohydrates and other nutrients, limiting the nutritional value of the food or feed.[4–8]

High-tannin sorghums fed to rats and chicks were shown to significantly lower growth rates, feeding efficiency and overall nutritional value.[9,10] Elkin *et al.*[11] showed that sorghum cultivars with similar tannin content levels had differing effects on protein digestibility, concluding that fundamental differences in the protein content and composition could account for some of the differences.

Protein binding assays have been used to investigate the role of tannins in limiting protein digestibility. Tannins are known to have an affinity toward proline residues of salivary proteins.[12,13] Simon *et al.*[14] stated that the interaction between tannins and proteins is probably dependent on the size and stereochemistry of the tannin as well as the type of protein and tannin. Researchers using crude extracts from canola hulls found that the ratio of protein to tannin can influence the binding between protein and tannin.[15] Crude tannin extracts were diluted to approximately equal tannin content levels and the protein concentration was held constant to reduce this effect. This eliminates bias from samples having large differences in the protein:tannin ratio of the crude extracts.

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Conversely, not all biological activities mediated by tannins are negative. Tannins are considered to be excellent antioxidants. Hagerman *et al.*[16] showed that tannins were 15–30 times more effective than simple phenolics in radical scavenging ability. Other studies have shown that, as the phenolic levels in apples and cocoa increase, the antioxidant capacity increases.[17,18] Awika and Rooney[3] showed that bran from high-tannin sorghums had an antioxidant capacity nearly 30 times higher than grapes.

Grain structure, protein cross-linking, protein hydrophobicity and many other factors have been reported to impact protein digestibility in sorghum.[19] Numerous studies investigating the functional properties of sorghum tannin have been conducted by comparing grain samples from different sorghum lines without considering the other intrinsic characteristics of the grain. Hence the effects of tannin on digestibility and feed values are often confounded with differences in other grain traits, especially differences in the endosperm. The purpose of this study was to evaluate the effect of the tannins without the confounding intrinsic factors. Thus the objectives of this study were to (i) utilize a novel bran–endosperm reconstitution procedure to isolate the influence of sorghum tannins on protein digestibility, (ii) compare tannins isolated from multiple sorghum cultivars for differences in protein binding ability and antioxidant capacity, and (iii) relate the chemical structure characteristics to the functionality of sorghum tannins.

## EXPERIMENTAL

### Sorghum cultivars

Seven high-tannin lines – Shanqui Red, Ajabsido, Koro Kollo, IS8525, Sumac, SC103-12E and SC599 – were grown over 2 years at the Kansas State University Ashland Bottoms Research Farm. Samples were grown over 2 years to verify that effects seen were not artifacts from one growing season since little is known about how the environment effects tannin content in sorghum. A non-tannin hybrid, NC213#9, was used as a control sample for the reconstitution study. Mycogen 627, a non-tannin sorghum hybrid, produced in the same crop year, was utilized to create the 'base' endosperm used in the reconstitution study. The intention in using a single non-tannin endosperm and bran as the base was to limit intrinsic factors associated with endosperms from each of the tannin-containing cultivars.

### Processing of sorghums

All sorghum samples were mechanically cleaned using a Clipper office tester (AT Ferrell Co., Bluffton, IN, USA) to remove unwanted plant residue, with additional hand cleaning as necessary. Sorghum kernel hardness and size attributes were measured using the single-kernel characterization system (SKCS) (Perten, Springfield, IL, USA), specifically calibrated for use with sorghum.[20] The samples were decorticated using a tangential abrasive dehulling device (TADD; Venables Machine Works, Saskatoon, Canada) equipped with an 80-grit abrasive disk to remove the outer layers of the grain containing the testa layer and tannins.[21] Owing to differences in sample characteristics, the decortication times varied for all samples to ensure that a removal of 20% of the grain by weight was achieved. This level of decortication to 20% was determined in preliminary research on three tannin-containing sorghum lines to remove ~90% of the measurable tannins. Bran fractions from the decortication process were collected and stored at −20 °C. The decorticated grain from the non-tannin sample was ground using an Udy Cyclone mill (Udy Corp., Fort Collins, CO, USA) to pass through a 0.5 mm screen and stored as above.

**Table 1.** Selected sorghum bran reconstitution formulations

| Treatment name (%RF) | Base endosperm (g) | High-tannin bran (g) | Non-tannin bran (g) |
|---|---|---|---|
| 0 | 8.0 | 0.0 | 2.0 |
| 25 | 8.0 | 0.5 | 1.5 |
| 50 | 8.0 | 1.0 | 1.0 |
| 75 | 8.0 | 1.5 | 0.5 |
| 100 | 8.0 | 2.0 | 0.0 |

The bran fraction from the individual tannin lines and the control non-tannin were combined with the bran from the non-tannin base in selected ratios (Table 1) and added back to the 'base' endosperm of the non-tannin sample, which will be referred to as reconstituted flour (RF). The ratio of tannin bran to non-tannin bran was specified as a percentage, i.e. a sample of RF with 50% tannin bran and 50% non-tannin is considered 50% RF. The original grains will be referred to as whole grain (WG).

### Analytical procedures

*Proximate analysis*

Protein content of the whole grain and bran fractions was determined with the LECO FP-528 Nitrogen Determinator (St Joseph, MI, USA) using a nitrogen combustion method (AACC method 46–30).[22] Nitrogen values were converted to total protein by multiplying by 6.25.

Tannin content in the WG, RF and bran fractions was determined using the modified vanillin hydrochloric acid (MV-HCl) and reported as catechin equivalents (CE) in g $kg^{-1}$ of sample.[23]

*Functionality analysis*

Protein digestibility was determined using the modified pepsin method described by Mertz *et al.*[24] The RF treatments were incubated at 37 °C in a 0.1 mol $L^{-1}$ phosphate buffer (pH 2.0) containing 1.5g $L^{-1}$ pepsin for 2 h with constant shaking. The samples were centrifuged and washed with 0.1 mol $L^{-1}$ phosphate buffer twice. Residues were freeze-dried and analyzed by nitrogen combustion.

Protein binding capacity was determined using the blue bovine serum albumin (BSA) method described by Asquith and Butler.[25] The bran fractions were extracted with 1% HCl in methanol for 20 min at 30 °C, followed by subsequent vortex agitation for 10 s at 0, 10 and 20 min time intervals. The samples were then centrifuged at 805 × *g* for 4 min. The MV-HCl method was used to determine the tannin content of the extract and 3 mL aliquots were dried under vacuum. The dried samples were resuspended in the appropriate amount of absolute methanol to achieve similar tannin contents in all samples (approximately 20 g CE $kg^{-1}$ of bran).

Antioxidant capacity was measured on bran fractions using the oxygen radical absorbance capacity (ORAC) method as described by Huang *et al.*[26] Samples were read using a Synergy 2 multi-detection microplate reader (BioTek Instruments, Inc., Winooski, VT, USA) at 37 °C. The ORAC values were calculated as described by Cao *et al.*[27] The area under the curve (AUC)

and the net AUC ($AUC_{sample} - AUC_{blank}$) of the standards and samples were determined using Gen5 data reduction software.

A series of indices were created to compare the effects of the tannin containing cultivars on a per unit tannin basis:

$$\text{digestibility index (DI)} = (0\%\ \text{RF} - 100\%\ \text{RF}) \times (\text{CE of } 100\%\ \text{RF})^{-1} \quad (1)$$

$$\text{protein binding index (BI)} = (\text{bound protein}) \times (\text{CE of extracts})^{-1} \quad (2)$$

$$\text{antioxidant index (AI)} = \text{ORAC} \times (\text{CE of bran fraction})^{-1} \quad (3)$$

*Chromatographic analysis*

Molecular weight distribution of sorghum tannins was analyzed using high-performance size-exclusion chromatography (HP-SEC), as previously described by Kaufman *et al*.[28] Samples were extracted using the same extraction procedure as for the MV-HCl assay. Kafirins were extracted and analyzed via reverse-phase high-performance liquid chromatography (RP-HPLC), as previously described in Bean *et al*.,[29] using a 150 mm × 2.0 mm Jupiter 300Å $C_{18}$ column (Phenomonex, Torrance, CA, USA).

### Statistical analysis

Measurements for the protein binding and protein digestibility assays were conducted on two replicate samples. The tannin content and antioxidant assays were conducted over three replicate samples. Data were corrected for moisture and presented on a dry mass basis. Analysis of variance was performed using SAS 2005 (SAS Institute, Version 9.1, Cary, NC, USA). Mean separations were determined using least significant difference (LSD) testing at the $P < 0.05$ level. Correlations were determined using the Pearson correlation coefficient.

## RESULTS AND DISCUSSION

### Physical grain characteristics

The genetic diversity of the sorghum seeds could be visually observed by differences in pericarp color. The size and hardness of the samples also varied. Of the tannin-containing sorghums, SC599 exhibited the highest hardness value, whereas SC103-12E was the least hard (Table 2). The SKCS hardness values were higher in year 2 than in year 1. This was very apparent with Ajabsido as year 2 was approximately 37% harder than year 1. The Ajabsido samples exhibited the largest average single kernel diameter (2.9 mm) and weight (37.6 mg), whereas Sumac exhibited the smallest kernel diameter (1.7 mm) and lowest kernel weight (16.4 mg). The kernel attributes and chemical composition may be influenced by differing genetic backgrounds and changes in environmental conditions between the two crop years.

**Table 2.** Whole-grain physical characteristics of the seven tannin-containing sorghum cultivars and non-tannin hybrid grown in two crop years at the same location

| | | | SKCS[a] | | |
|---|---|---|---|---|---|
| Cultivar | Year | Pericarp color | Hardness | Diameter (mm) | Weight (mg) |
| Shanqui Red | 1 | Red | 85.1 | 2.2 | 24.4 |
| | 2 | Red | 87.8 | 2.3 | 27.0 |
| Ajabsido | 1 | White | 48.5 | 2.9 | 35.7 |
| | 2 | White | 72.5 | 2.7 | 37.6 |
| Koro Kollo | 1 | Red | 59.9 | 2.7 | 34.0 |
| | 2 | Red | 71.4 | 2.7 | 34.7 |
| IS 8525 | 1 | Red | 49.2 | 2.1 | 23.5 |
| | 2 | Red | 64.6 | 2.2 | 25.9 |
| Sumac | 1 | Red | 52.0 | 1.7 | 16.4 |
| | 2 | Red | 75.5 | 2.0 | 17.4 |
| SC103-12E | 1 | Red | 32.8 | 2.0 | 22.7 |
| | 2 | Red | 39.3 | 2.0 | 23.3 |
| SC599 | 1 | White | 87.0 | 1.8 | 18.1 |
| | 2 | White | 91.5 | 2.0 | 21.3 |
| Mycogen 627 | Base | Red | 89.2 | 2.3 | 26.3 |

[a] Single-kernel characterization system.

### Protein digestibility

A reconstitution series using an RF from 0 to 100 was employed to investigate the effect of tannin content on protein digestibility. There was a distinct effect of tannin content on protein digestibility (Fig. 1). Since the tannin content of the 100% RF ranged from 1.8 to 50.6 g $kg^{-1}$ the treatments were normalized to the highest tannin content of each cultivar. The normalization allowed the *x*-axis to have a scale of 0–1 for all cultivars; thus the cultivars could be compared effectively. The regression equation for the non-normalized digestion treatments can be found in Table 3. A 0% RF treatment was created for all treatments, so slight variation due to experimental error is shown in Fig. 1; the mean for the 0% RF treatments was 63.28% digestible with a standard deviation of 3.15. The mean of the 0% RF treatments was used as the zero value for all cultivars in Fig. 1. The whole-grain protein digestibility of the base sorghum was 63.3%, which demonstrated that no effects due to reconstitution were observed. The control non-tannin sorghum also exhibited negligible effects on digestibility; therefore other differences in the bran fractions show little effect on protein digestibility. As the tannin level increased in the RF samples, the protein digestibility decreased. This was not unexpected and has been reported by previous researchers.[11,30] However, there were differences in the rate of decrease; i.e. the slope of the regression lines for the normalized data shown in Fig. 1 may be attributed to both genetic and environmental factors. The sample reconstituted with bran from cultivar SC103-12E strongly showed the environmental influence of the tannin's effect on digestibility; the sorghum grown in year 1 had a 38% faster rate of decrease than the sample grown in year 2. Conversely, the cultivar Ajabsido exhibited a 57% slower rate of decrease from year 1 to year 2. Interestingly, RF samples with bran from Ajabsido and SC103-12E showed the greatest differences in slopes in Fig. 1 and also had large differences in physical grain traits between years (Table 2), suggesting a higher degree of environmental influences on these cultivars.

The protein contents of the 100% RF samples varied from 91 to 103 g $kg^{-1}$ due to differences in the bran fractions (Table 3); however, differences in protein content were not correlated to the digestible protein ($R^2 = 0.06$). Thus residual protein in the bran had no impact on the overall digestibility of the RF samples. With

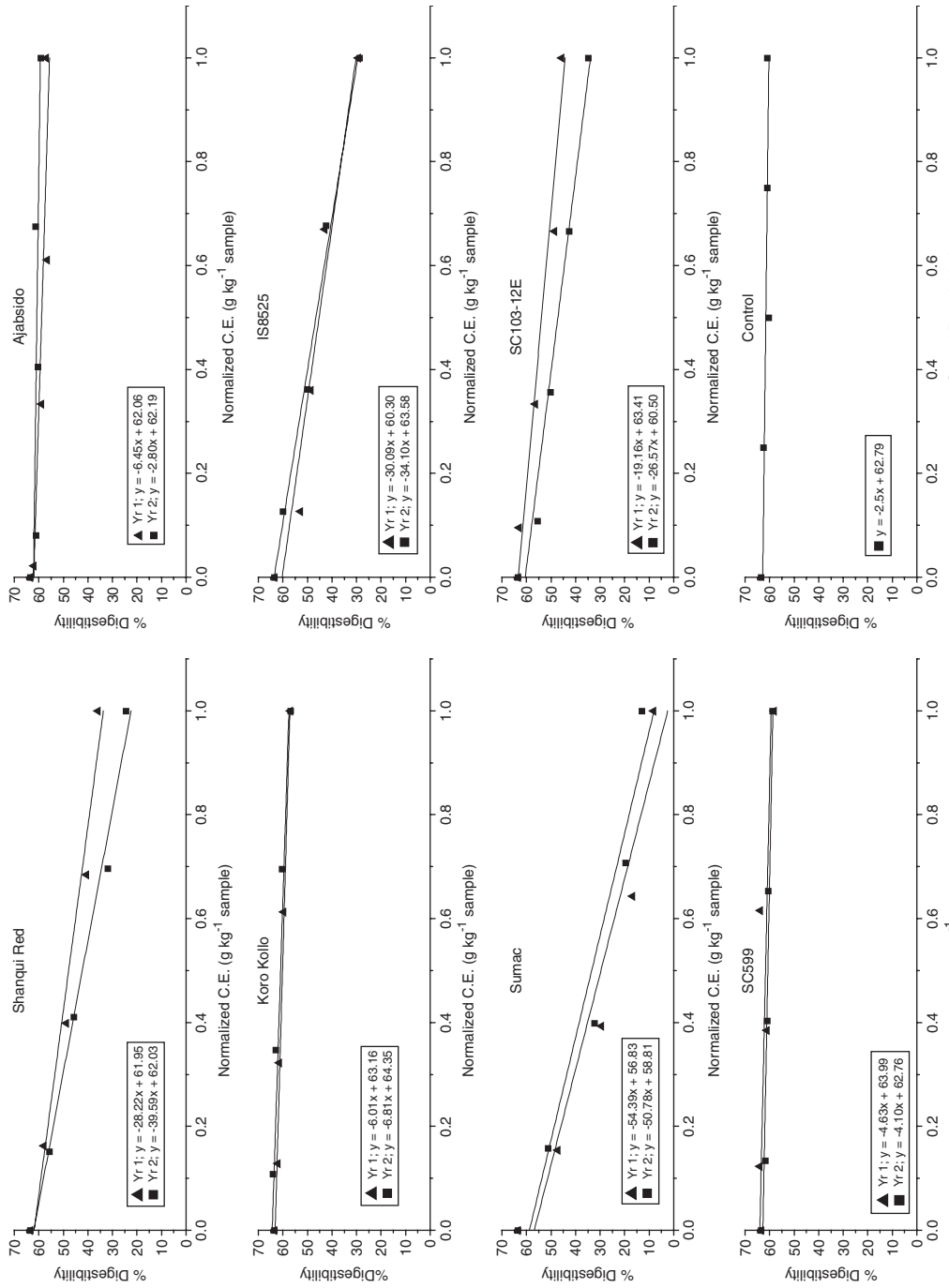


**Figure 1.** Protein digestibility of reconstituted sorghum treatments for seven high-tannin cultivars grown in two crop years. Due to large differences in tannin content, note that the tannin content data were normalized by dividing by the highest content level.

**Table 3.** Effect of tannin content on the protein digestibility of reconstituted flours[a]

| Cultivar | Year | 100% reconstituted flour: Protein (g kg$^{-1}$) | Tannin content CE[b] (g kg$^{-1}$ sample) | Digestible protein (%) | Digestibility index ($\Delta D^c$CE$^{-1}$) | Regression[d] equation |
|---|---|---|---|---|---|---|
| Shanqui Red | 1 | 94*hi | 20.3f | 35.8c | 1.13 | $y = -1.23x + 59.59$ |
| | 2 | 96gh | 29.7c | 24.4d | 1.31 | $y = -1.34x + 62.11$ |
| Ajabsido | 1 | 100cde | 1.8m | 56.9a | 1.44 | $y = -2.42x + 60.48$ |
| | 2 | 103a | 3.7kl | 59.1a | 0.96 | $y = -0.65x + 61.89$ |
| Koro Kollo | 1 | 101bc | 3.1l | 57.0a | 2.43 | $y = -2.20x + 63.74$ |
| | 2 | 102ab | 4.6jk | 56.8a | 1.60 | $y = -1.60x + 64.75$ |
| IS 8525 | 1 | 97fg | 23.6e | 29.3d | 1.37 | $y = -1.23x + 59.52$ |
| | 2 | 98def | 27.6d | 28.6d | 1.38 | $y = -1.23x + 63.50$ |
| Sumac | 1 | 95hi | 43.4b | 8.2e | 1.27 | $y = -1.25x + 56.92$ |
| | 2 | 98efg | 50.6a | 12.9e | 1.09 | $y = -1.07x + 61.30$ |
| SC103-12E | 1 | 99def | 6.3hi | 45.7b | 2.93 | $y = -3.12x + 63.80$ |
| | 2 | 100cd | 12.9g | 34.7c | 1.98 | $y = -1.91x + 59.10$ |
| SC599 | 1 | 91j | 6.5h | 58.1a | 1.58 | $y = -1.23x + 66.43$ |
| | 2 | 94i | 5.2ij | 58.8a | 0.25 | $y = -0.37x + 61.18$ |
| LSD | | 2 | 1.2 | 4.9 | NA | |

[a] Data reported on dry mass basis.
[b] Catechin equivalents.
[c] Change in digestibility (digestibility of 0% high-tannin bran treatment − digestibility of 100% high-tannin bran treatment).
[d] Regression of digestibility treatments, not normalized data.
*Samples with identical letters are not different ($P < 0.05$).

**Table 4.** Protein binding and antioxidant capacity of tannins derived from seven sorghum cultivars grown in two different crop years at the same location

| Cultivar | Year | Bound protein (g blue-BSA[a] kg$^{-1}$ sample) | Binding index (bound protein[b] CE$^{c-1}$) | Antioxidant capacity (μmol TE[d] g$^{-1}$) | Tannin content CE (g kg$^{-1}$ bran) | TEAC index (μmol TEAC CE$^{-1}$) |
|---|---|---|---|---|---|---|
| Shanqui Red | 1 | 3.53*fgh | 0.32 | 682.06*c | 130.7e | 5.22 |
| | 2 | 3.11h | 0.25 | 853.51b | 166.0c | 5.14 |
| Ajabsido | 1 | 16.63a | 1.04 | 92.58hi | 12.1j | 7.65 |
| | 2 | 5.02de | 0.75 | 95.99hi | 22.5i | 4.27 |
| Koro Kollo | 1 | 12.29b | 0.67 | 103.26h | 16.3j | 6.33 |
| | 2 | 6.98c | 0.89 | 81.33i | 23.4i | 3.48 |
| IS 8525 | 1 | 3.28gh | 0.27 | 559.81d | 145.4d | 3.85 |
| | 2 | 3.88fgh | 0.26 | 686.59c | 179.0b | 3.84 |
| Sumac | 1 | 4.08fg | 0.24 | 1122.54a | 169.7c | 6.61 |
| | 2 | 4.33ef | 0.20 | 1084.74a | 183.8a | 5.90 |
| SC103-12E | 1 | 5.64d | 0.47 | 330.79f | 41.2h | 8.03 |
| | 2 | 3.74fgh | 0.39 | 493.08e | 83.6f | 5.90 |
| SC599 | 1 | 3.84fgh | 0.34 | 157.34h | 51.5g | 3.06 |
| | 2 | 4.18ef | 0.40 | 254.81g | 49.4g | 5.16 |
| LSD | | 0.82 | NA | 65.17 | 4.7 | NA |

[a] BSA, bovine serum albumin.
[b] g blue-BSA kg$^{-1}$ sample.
[c] Catechin equivalents.
[d] Trolox equivalents.
*Samples with identical letters are not different ($P < 0.05$).

the exception of RF sample with SC599 bran, the year 2 samples were higher in tannin content. Samples reconstituted with bran from Ajabsido exhibited the lowest tannin content, whereas those from Sumac bran exhibited the highest. The protein digestibility of RF Sumac sample was significantly lower during both crop years compared to the other samples. RF samples made from the bran of Ajabsido, Koro Kollo and SC599 were significantly higher in protein digestibility compared to the other samples but did not differ among themselves. A digestibility index (DI) was used to distinguish differences in the effect of tannin content on digestibility. The indices ranged from 0.25 to 2.93 for all the samples tested (Table 3). The DI shows the effect on digestibility on a per unit tannin content basis, which allows for comparison between high and low tannin levels. RF sample produced with bran from SC103-12E showed the greatest impact on protein digestibility, with both the largest slope on the regression lines and the highest DI (Table 3). The highest indices were found in the cultivars, Koro Kollo and SC103-12E, which exhibited relatively low tannin content. This may be due to the molecular weight distribution of the tannin oligomers and polymers. A correlation between the DI and the slopes of the regression lines ($r = -0.9$) suggests another component influencing protein digestibility in addition to tannin content. Naczk *et al.*[31] reported a similar effect in canola hulls; varieties with lower total tannin levels bound more protein than varieties with higher tannin levels. Since the protein content and composition were controlled by using the reconstituted treatment series, compositional differences in the tannins may be responsible for the variation between cultivars, as evident in the slopes of the regression lines and DI.

## Protein binding

A series of experiments were conducted to study the protein binding ability of the samples used in this study. Since the tannin extracts used in the protein binding assay contained varying amounts of tannin, the samples were diluted to a constant level. The purpose of comparing equal values was to determine whether there were differences in the binding due to composition changes rather than just total tannin content. The amount of bound blue-BSA protein ranged from 3.11 to 16.63 g $kg^{-1}$ of bran (Table 4). The binding index (BI) values ranged from 0.2 to 1.04. A similar trend to protein digestibility was observed in the protein binding assay; i.e. RF samples from cultivars that were low in tannin content – Ajabsido and Koro Kollo – had the highest protein binding per unit tannin content. Bran from SC103-12E grown in year 2 ranked fifth in BI but exhibited the greatest impact on protein digestibility.

The protein binding/precipitation ability of tannins has been correlated with the nutritional value of high tannin feeds.[32] Tannins bind to the proteins primarily by both hydrogen bonding and hydrophobic interactions and typically involve large, proline-rich proteins.[5,13,33,34]

RP-HPLC was used to further investigate protein–tannin interactions in the RF samples used in this study. Chromatograms of kafirins extracted from the RF samples revealed that as the amount of tannin bran increased a gradual disappearance of the $\gamma$-kafirins was seen (Fig. 2). This is presumably due to binding of the $\gamma$-kafirins to tannins, which rendered them insoluble. Using purified kafirins and tannins, Taylor *et al.*[35] observed a similar effect. The results in Fig. 2 show that binding of the $\gamma$-kafirins in the presence of tannins can occur in sorghum flour. An important finding, however, was that bran from all the tannin-containing sorghum cultivars used in this study did not all bind

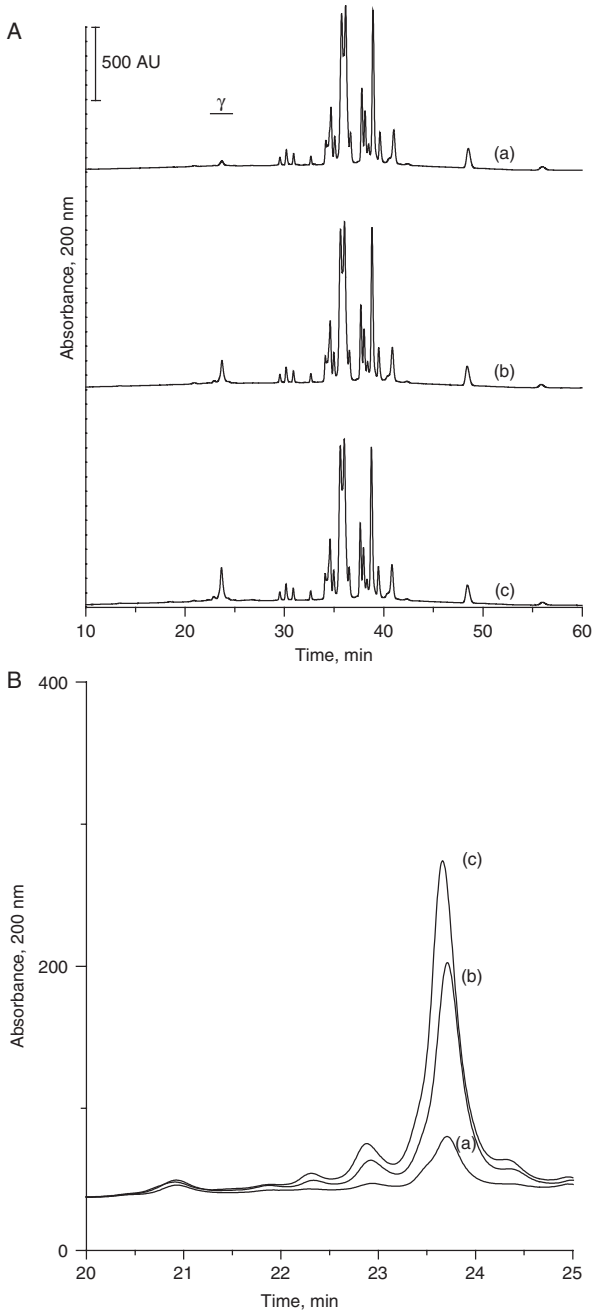


**Figure 2.** (A) RP-HPLC protein profile with (a) 100% high-tannin bran added, (b) 50% high-tannin bran added and (c) 0% high-tannin bran added. (B) An expanded view of the effect of increasing tannin content on the $\gamma$-kafirin peak.

$\gamma$-kafirins equally (Fig. 3). The chromatograms of kafirins extracted from RF samples containing bran from the cultivars Ajabsido, Koro Kollo and SC599 still showed the presence of $\gamma$-kafirins, indicating that the tannins in these samples did not bind the $\gamma$-kafirins to the same degree as the other samples. In the case of Ajabsido and Koro Kollo this may have been due to overall tannin levels in the samples as these two cultivars produced RF samples with the lowest tannin content. However, the RF sample produced from SC599 had overall tannin content similar to other samples (e.g. SC103-12E and Shanqui Red) used in this study. SC 599 also had a relatively high protein digestibility among

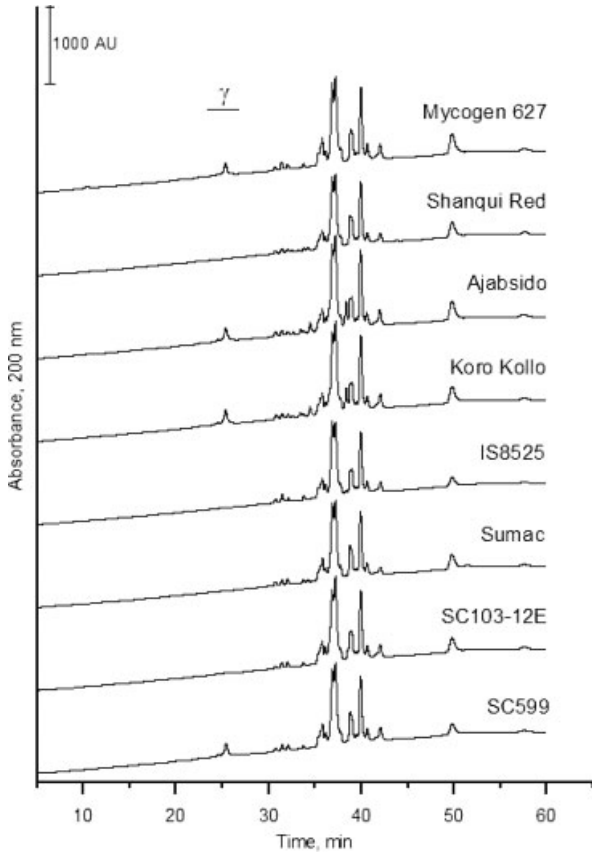


**Figure 3.** RP-HPLC protein profiles of reconstitution treatment samples with 100% high-tannin bran added. The $\gamma$-kafirin peak elutes at approximately 25 min.

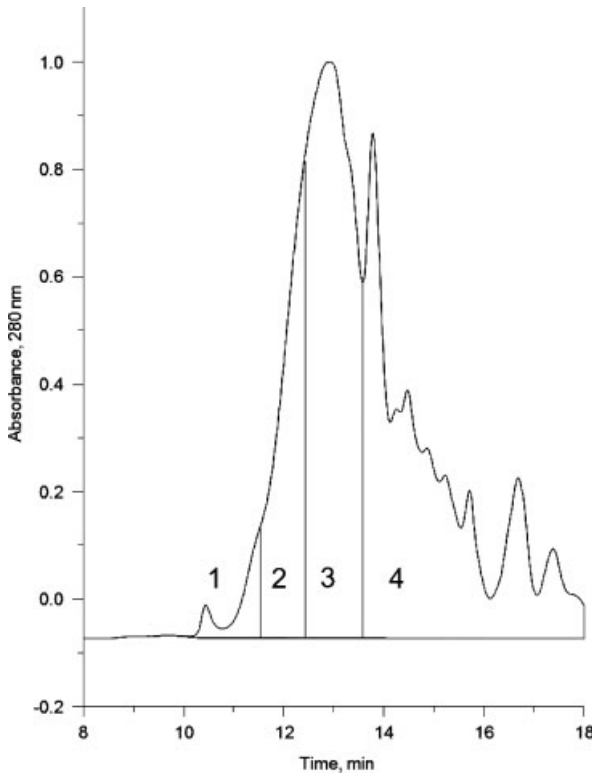


**Figure 4.** Sample SEC chromatograms showing the peak times used during integration analysis.

**Table 5.** SEC composition of high-tannin sorghums, showing peak area %[a] corresponding to the peaks in Fig. 4

| Cultivar | Year | Peak 1 | Peak 2 | Peak 3 | Peak 4 |
|---|---|---|---|---|---|
| Shanqui Red | 1 | 8.44*c | 25.79ab | 38.15a | 27.62f |
| | 2 | 3.53h | 19.83efg | 34.19bcd | 42.45ab |
| Ajabsido | 1 | 4.10fgh | 21.38cdef | 32.79cd | 41.72b |
| | 2 | 10.10b | 25.98a | 36.67ab | 27.25f |
| Koro Kollo | 1 | 7.87c | 23.93abc | 33.01bcd | 35.19e |
| | 2 | 5.01e | 22.57bcde | 36.44abc | 35.98de |
| IS 8525 | 1 | 4.69ef | 24.88ab | 34.18bcd | 36.24de |
| | 2 | 6.22d | 21.16cdef | 32.94cd | 39.68c |
| Sumac | 1 | 5.82d | 17.08g | 34.99abc | 42.11b |
| | 2 | 3.94gh | 18.73gf | 33.08bcd | 44.25a |
| SC103-12E | 1 | 11.69a | 26.58a | 34.01bcd | 27.73f |
| | 2 | 7.92c | 23.39abcd | 30.96d | 37.73d |
| SC599 | 1 | 4.24fg | 24.12abc | 34.18bcd | 37.46d |
| | 2 | 3.91gh | 20.22defg | 33.53bcd | 42.33b |
| LSD | | 0.63 | 3.30 | 3.70 | 1.90 |

[a] Peak area % is the peak area/total peak area of the SEC chromatograms.
* Samples with identical letters are not different ($P < 0.05$).

the cultivars used in this study. Further research is needed to determine the cause and mechanism of these interactions and to explain why the tannins from these three cultivars do not affect the $\gamma$-kafirins in the same way as the other samples and whether this was related to protein digestibility of these samples.

### Antioxidant capacity

Antioxidant activity using the ORAC assay was reported in Trolox equivalent absorbance capacity (TEAC). The ORAC values of the sorghum bran can be found in Table 4. Antioxidant activity levels ranged from 81.33 to 1122.54 μmol TEAC $g^{-1}$ sample. As was done for protein digestibility and protein binding, an index of antioxidant capacity (AI) per unit CE was calculated (TEAC value divided by CE content of the bran). These values ranged from 3.06 to 8.03 μmol TEAC $CE^{-1}$. The seven sorghum cultivars used in this study all had comparable antioxidant values to those reported by Awika *et al.*[36] The cultivars that had lower tannin content generally had lower raw antioxidant activity, but when compared on a per unit tannin basis by the AI the tannins in the low-tannin lines were the most powerful antioxidants (Table 4). This suggests that there may be a compositional effect on antioxidant activity. Since crude extracts were used in this assay, other phenolic compounds may influence the antioxidant capacity and this factor should be considered when interpreting these results.

### HP-SEC analysis

Tannins from diverse sorghum lines were analyzed by HP-SEC to discriminate differences in tannin molecular weight, distribution and composition. Peak analysis of the SEC chromatograms from the seven high-tannin cultivars was used to determine differences in composition. Figure 4 shows a typical SEC chromatogram of a sorghum bran fraction; the chromatogram was integrated using the four peaks shown in the figure. The peak positions were

**Table 6.** Correlation analysis between and among the biological activity of tannins from seven sorghum cultivars

| | Tannin content | Raw digestibility | Δ digestibility[a] | Digestibility index | Raw binding | Binding index | Raw antioxidant |
|---|---|---|---|---|---|---|---|
| Tannin content | 1.00 | | | | | | |
| Raw digestibility | −0.93 | 1.00 | | | | | |
| Δ digestibility | 0.93 | −0.99 | 1.00 | | | | |
| Digestibility index | n.s. | n.s. | n.s. | 1.00 | | | |
| Raw binding | −0.60 | n.s. | n.s. | n.s. | 1.00 | | |
| Binding index | −0.82 | 0.72 | −0.74 | n.s. | 0.80 | 1.00 | |
| Raw antioxidant | 0.94 | −0.98 | 0.96 | n.s. | n.s. | −0.78 | 1.00 |
| Antioxidant index | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| Peak 1 area %[b] | n.s. | n.s. | n.s. | 0.54 | n.s. | n.s. | n.s. |
| Peak 2 area % | n.s. | 0.54 | −0.55 | n.s. | n.s. | n.s. | −0.59 |
| Peak 3 area % | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| Peak 4 area % | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |

[a] Change in digestibility (digestibility of 0% high tannin bran treatment − digestibility of 100% high-tannin bran treatment).
[b] Peak area % is the peak area/total peak area of the SEC chromatograms.
n.s., not significant ($P < 0.05$).

assigned to common peaks and shoulders present across the 14 samples, with integration times held constant for comparison across samples. Utilizing the same separation protocol, Kaufman *et al.*[28] and Taylor *et al.*[37] reported that tannins with a degree of polymerization (DP) of 22 eluted at approximately 11 min, DP 10 eluted at approximately 12.5 min, DP 2 standards eluted at approximately 13.5 min and monomeric epicatechin eluted at approximately 15 min. Therefore peak 1 would consist of tannins with a DP of 22 and larger, peak 2 ranges from DP 10 to 22, peak 3 ranges from DP 3 to 9 and peak 4 consists of dimers and monomers. Peak area percent was calculated by dividing the peak area by the total area of the chromatogram from 10 to 18 min and then multiplying by 100.

Comparison of the peak area percent can be found in Table 5. SC103-12E (year 1) exhibited a much higher proportion of peak 1 than the other cultivars. Across all sorghum cultivars tested, peak 2 makes up 17–26% of the total peak area. Peak 3 ranges from 31% to 38% of the total peak area and appears to be relatively stable across the two years, with only Shanqui Red and Ajabsido exhibiting any environmental variance for both peaks. Sumac (year 2) has the highest relative portion of peak 4 with 44.25% of total peak area. SC103-12E (year 1), Shanqui Red (year 1) and Ajabsido (year 2) exhibited the lowest proportion, at around 27.5%. The dimer and monomeric portion of the molecular weight distribution (peak 4) exhibited a large year-to-year variance across most of the cultivars, showing that the effect of environment played a role in the synthesis of this molecular weight range of polyphenols.

### Relationships between factors

A correlation table was created to find relationships between the factors in this study (Table 6). Tannin content was negatively correlated to protein digestibility and protein binding as well as positively correlated with antioxidant capacity. Protein digestibility scores were negatively correlated with antioxidant capacity, but positively correlated with the protein binding index. The change in digestibility (digestibility of 0% tannin bran treatment − digestibility of 100% tannin bran treatment) showed an inverse of the raw digestibility positive correlations, with a positive correlation to antioxidant capacity and a negative correlation to the protein binding index. However, the digestibility and antioxidant indices were not significantly correlated with any other factor. It appears that the biological activity of sorghum tannins are not only related to tannin content but also associated with tannin composition.

Because previous results showed that tannin composition was significantly related to tannin functionality, tannins from all the bran samples used in this study were characterized by SEC. Correlations were then made between peak area percent (a reflection of tannin composition) of the samples and biological activity (Table 6).

BI and AI were not correlated with any of the peak areas; however, DI was positively correlated with peak area from peak 1. The peak 2 region was positively correlated with the raw digestibility (i.e. as peak area increased the protein digestibility increased); however, peak 2 was negatively correlated with antioxidant capacity. These results support findings suggesting that biological activity may be related to $M_w$ differences.[31] Previous research has also shown that high-$M_w$ tannins have the most potent *in vitro* antioxidant activity.[16,38] Future research is needed to further evaluate the $M_w$ range that is the most biologically active.

## CONCLUSIONS

There are significant differences in the tannin content and composition due to genetic and environmental factors. These differences influence the behavior of tannin oligomers and polymers and their impact on the nutritional value of sorghum. The amount of tannin present does affect the extent of protein digestibility, protein binding or antioxidant capacity. The novel reconstitution treatment study found that tannin content was only partially responsible for that effect; differences in the chemical composition may also contribute. Differences in molecular weight distribution showed a significant impact on protein digestibility, protein binding and antioxidant capacity. Therefore it may be possible to have a high-tannin sorghum, consisting of mostly large polymers that may not have significant impacts on tannin functionality. The reverse of that would also hold true: a relatively low tannin sorghum could have drastic effects on protein digestibility, protein binding and antioxidant capacity if the tannins present consisted mainly of the very active molecular weight range.

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