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Assessing Sorghum Protein Digestibility

Comparative study of tannin and non-tannin sorghums

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Abstract

Sorghum is the fifth most important grain crop worldwide and is becoming increasingly important as a bio-fuel feedstock due to its superior tolerance to water-deficit stress and warm temperatures. Although not commonly consumed as a human food in the U.S., in parts of the world, *e.g.*, Africa and India, it is an important source of nutrition. Despite its advantages as a crop, as a food sorghum's nutritional value is considerably lower co mpared to other major staples due to its low digestibility of protein and starch. Moreover, sorghum protein digestibility further decreases when it is cooked for human consumption.

To address these nutritional problem, factors affecting low sorghum protein digestibility were examined, mainly the effects of tannin. Tannin is a polyphenolic compound found in some of the more indigestible sorghum lines. A review of the literature suggests that tannins preferentially bind to a certain type of sorghum storage proteins, γ-kafirins, forming high molecular aggregates that prohibit the proteases from accessing proteins during the process of digestion (Taylor, Bean et al. 2007). To examine in more detail how the presence of tannin reduces sorghum protein digestibility, we analyzed the molecular properties of six different sorghum lines that contain or do not contain tannin. Interestingly, we discovered that tannin- containing lines do not always have lower digestibility compared to non-tannin lines. The comparison of sorghum varieties, Macia and Seredo, non-tannin and tannin-containing lines, respectively, gave results consistent with the literature that states that tannin prohibits protein digestibility (Duodu, Taylor et al. 2003).

However, similar indigestibility patterns seen in both 296B and RIA19, non-tannin and tannin lines, respectively, and the differential digestibility seen when comparing P898012/T4 lines and RIA19, indicate that presence of tannin is not the only factor that determines digestibility. Hence, our current findings suggest that the presence of tannin works in combination with other factors to affect the overall protein digestibility of sorghum.

Introduction

Sorghum (*Sorghum bicolor* L. Moench), a tropical plant belonging to the family, Poaceae, is ranked as the 5th most important crop among world cereal grains, after wheat, rice, corn and barley (Schober, Bean et al. 2008). Its ability to tolerate conditions of limited moisture and therefore able to grow during periods of extended drought has allowed it to become widely consumed as the major staple food crop for millions of poor in semi-arid tropics of Africa and India. However, published literature indicates that sorghum, especially when it is cooked for human consumption, has a markedly poor protein digestibility compared to other available food crops such as corn, one of its close relatives. The unique organizational structure and composition of its grain lowers susceptibility of its proteins to proteolysis, generating a nutritional constraint. Moreover, it contains inadequate amounts of some amino acids, especially lysine. Therefore, its poor protein digestibility and lack of certain essential amino acids generate severe nutritional problems, including malnutrition and starvation, for those who eat it as their staple food.

The proteins of the sorghum grain are classically divided into classes,

based on solubility in different solvents: albumins, globulins, kafirins, cross-linked kafirins, cross-linked glutelins, and un-extracted structural protein residue. Among the different types of proteins, kafirins (prolamins) are aqueous alcohol-soluble proteins that make up the majority of storage proteins in sorghum. The prolamins comprise 50-60% of the total protein of endosperm and are classified into three main groups, according to their molecular weight, extractability, and structure (El Nour, Peruffo et al. 1998). Currently, four different types of sorghum kafirins are sub-classified as α, β, γ and δ kafirins, which structurally interact with each other. Among the four different types, α kafirins make up 81% of total kafirins, hence constituting the majority. β kafirins and γ kafirins make up 13% and 5.6% respectively (Chamba, Halford et al. 2005). Amino acid sequence comparisons demonstrate that these are all members of the prolamin superfamily, but β and γ kafirins are both unusually rich in cysteine residues compared to α kafirins (Belton, Delgadillo et al. 2006). The gene coding for γ kafirin has been sequenced, revealing the presence of 14 cysteine residues (Shull et al., 1991). The resulting disulfide bonds formed between cysteine residues of β and γ kafirins inhibit digestibility of β and γ kafirins. Furthermore, the more digestible α kafirins are enclosed within a tightly knit network of disulfide bonds formed among cysteine residues of β and γ subunits that inhibit proteolytic enzymes from accessing these kafirins, therefore further reducing overall digestibility.

Sorghum protein digestibility is highly affected, not only by the structure of the grain, but also by the extent of protein-starch interaction. In sorghum, protein and starch granules are in close association with each other. The protein inside the endosperm exists as a matrix with numerous, spherical protein bodies tightly

clustered with starch granules. Scanning electron microscopy (SEM) of the sorghum endosperm reveals the dense physical interaction network between starch and protein to the digestibility of both components. Microscopic examination of iodine-stained sections of sorghum grain confirms that sorghum grains indeed are composed of numerous protein bodies tightly associated with starch granules (Figure 1;(Wong, Lau et al. 2009). This type of physical interaction network between the two components contributes to the low digestibility of protein as well as starch in sorghum, as they prohibit the digestive enzymes to access to each other. The corneous endosperm which constitutes the outer portion of endosperm contains more protein body complexes that have poorer digestibility compared to the floury endosperm, which is the inner portion of the endosperm with fewer protein body complexes. The implication of such a close physical association between starch and protein may be that the starch, especially when gelatinized after cooking, could severely reduce the accessibility of proteolytic enzymes to the protein bodies and therefore reduce protein digestibility (Duodu, Taylor et al. 2003). Furthermore, data in other literature suggests that cooking can also result in an enhanced formation of enzyme-resistant, disulphide-bonded oligomers, which may be one of the main causes of the low digestibility (Duodu, Nunes et al. 2002).

Protein digestibility inhibitors, such as tannin, are another factor that can influence sorghum protein digestibility. The tannins, present in brown and red sorghums, are polyphenolic compounds found in some sorghum lines, and their interaction with sorghum protein is known to lower the protein digestibility. Because proteins rich in proline can bind more readily to tannins than other proteins, sorghum tannin preferentially binds to the γ-kafirins that are richer in proline,

making γ-kafirin-bound tannins form high molecular weight aggregates (Taylor, Bean et al. 2007). The formation of aggregates prevents proteases from accessing the more digestible α-kafirins and this leads to a further drop of overall protein digestibility in tannin-containing sorghum lines.

In this study, we focused on examining the properties of different types of kafirins and identifying molecular characteristics of tannin and non-tannin sorghum lines in order to understand the differential protein digestibility of various tannin and non-tannin sorghums. To visualize the fates of different kafirins during pepsin digestion, we performed time course assays of *in vitro* pepsin digestion of two selected tannin sorghum lines. For monitoring their extractability and digestibility, we executed gel-based extraction assays and western blot analysis. The gel-based system allows a fast and simple analysis of protein digestibility by pepsin. It not only reveals what types of kafirins are left undigested after pepsin treatment, but also allows an estimate of the percent digested over time (Wong, Lau et al. 2009).

Through continued analyses of kafirins using the gel-based assay, we seek to achieve a more comprehensive understanding of the various factors that contribute to poor digestibility of sorghum. This could eventually lead to development of a nutritious and digestible transgenic sorghum line that could provide food for starving people around the world.

Materials and methods

2.1 Grain and preparation of materials

Sorghum lines studied in this report, Macia, Seredo, 296B, RIA19, P898012

(P8), and P898012 T4 (T4) were selected to comparatively study the different digestibilities of tannin and non-tannin sorghum lines. Meal of Macia (non-tannin) and Seredo (tannin) are from African sorghum lines and were gifts from Professor John Taylor (University of Pretoria, Pretoria, South Africa). Grain of 296B (non-tannin) and RIA19 (tannin) were obtained from Dr. Jeff Pedersen (University of Nebraska Agricultural Research Development Center, Ithaca, NE). Grain from P8, (nonGMO), T4 (GMO) are tannin-containing lines from Pioneer HiBred (??) through the African Biofortifized Sorghum Project. To mill grain to produce meal for all analyses, seeds were ground in a Wiley mill (??) to pass a 40-mesh screen. Ground meal was stored at room temperature until used in digestion assays. Pepsin (porcine stomach mucosa, P-7000, Sigma-Aldrich, St. Louis, MO) was used in protein digestibility assays.

2.2 *In vitro* pepsin digestion

Meal (50 mg) was incubated with pepsin (1ml at 20mg/ml, pH 2.0) for various times in time course experiments or for a fixed period of 2 hours at 37^{o}C with shaking following by neutralization with 0.1ml 2N NaOH to pH 7. The suspension was then centrifuged for 10 min at 14,000 rpm in a desktop micro-centrifuge, and the pellet was washed twice sequentially, with 1 ml KH_2PO_4 buffer (pH 7.0) followed by 1ml of dd H_2O. The undigested protein in the pellet remaining after pepsin digestion was then extracted according to the method described in *Materials and Methods 2.3* of this paper.

2.3 Extraction of protein undigested by pepsin

2.3.1 One-solvent system.

Protein remaining in pellets after various pepsin digestion regimes was extracted with 0.5ml borate-SDS-ME (0.0125 M borate, 1% sodium dodecyl sulfate, 2% β-mercaptoethanol, pH 10) twice sequentially in 1.5ml Eppendorf tubes. Extracts were combined and saved for gel and western analyses.

2.3.2 Three-solvent extraction system.

Extraction of undigested protein was further modified in the “Premier extraction method” (unpublished,data, J. Wong, University of California, Berkeley), where three sequential extractions were performed with 0.5ml each of 60% t-butanol, 60% t-butanol with ME, and finally twice with borate-SDS-ME buffer. Each extraction was for 1 h at room temperature. Extracts from each solvent extraction were saved for future western blot analysis. Tested using western blot analysis, this method was designed to selectively extract different types of kafirins at each sequential step: non-cross linked kafirins with 60% t-butanol, cross-linked kafirins and α kafirins with 60% t-butanol with ME, and finally glutelins and γ-kafirins with borate-SDS-ME. The amounts of proteins in each step of the extractions and residual protein after the end of the final extraction (N * 6.25) were determined by the Dumas combustion method (AACC Standard Method 46-30, 2000).

2.4 *SDS-PAGE separation of protein undigested by pepsin*.

Use of NuPAGE Bis-Tris gels (Invitrogen, Carlsbad, CA) of 12% with MOPS buffer was chosen to obtain optimal resolution of small- to medium-sized proteins. Protein extracts (200 uL) were concentrated with acetone (1:5, v/v) at -20°C

overnight. Protein pellets were collected by centrifugation and then re-dissolved in 1X NuPAGE Sample Buffer plus 0.2% ME (50 uL) by shaking for 1 hr at room temperature. Aliquots (20 uL) of re-dissolved protein were boiled for 5 min, centrifuged and ~5 uL of each sample were applied to 12% Bis-Tris gels with MOPS buffer and electrophoresed for 1 hr 20 min at 150 V. Protein gels were stained with colloidal Commassie Blue G-250 overnight and destained with several changes of ddH_2O.

2.5 *Western blot analysis*

After electrophoresis, proteins in the gel were transferred to nitrocellulose membrane at 4ºC for 75 minutes at 70V constant voltage. Then the membrane was processed for blotting with antibody against various zeins (α, β, γ, and δ-) at a 1:6250 dilution in 5% powdered milk in TBS overnight at 4ºC. Secondary anti-body was goat anti-rabbit IgG (H+L)-HRP Conjugate at a 1:2500 dilution in 5% powder milk for 1 hr at 25ºC. Between the two antibody treatments, the blot was washed twice with TBS containing 0.05% Nonidet. P40. A TMB substrate kit for peroxidase (Vector Laboratories, Inc., Burlingame, CA, USA) was used to develop the activity staining of the HRP conjugate on the blot according to manufacturer's instruction.

2.6 Quantification of proteins in gels and western blots.

Stained gels and blots were scanned with a UMAX PowerLook 1100 scanner using UMAX MagicScan version 4.4 within Adobe Photoshop, v.6 (Adobe Systems, San Jose CA). Amount of kafirin protein, expressed as volume (intensity x area), in bands

from undigested extracts and/or in immuno-crossreactive bands, was quantified with Quantity One software (Bio-Rad, Hercules, CA). Higher protein band volume indicates more protein in the gel, and thus reduced digestibility (Aboubacar et al., 2001).

Results and Discussion

3.1 Time course of *in vitro* pepsin digestion of P8 and T4 sorghum lines

Analyzing the time course of *in vitro* pepsin digestion with western blots using antibodies for different kafirins allows us to track the fate of kafirins during pepsin digestion analysis. In this experiment, because grain storage proteins of sorghum and maize, the kafirins and zeins, respectively, have similar chemical properties and reactivities (Oom, Pettersson et al. 2008), the more widely available zein antibodies were used as kafirin antibodies to detect α, γ and δ kafirins. P8 and T4 are tannin-containing sorghum lines that are in a parent-and-offspring relationship. T4 is known as a fourth generation transgenic of a line that derived from P8 parentage crossed with another sorghum line. Extraction of the kafirins from the two sorghum lines for gel analysis was performed first with borate-SDS-ME buffer. Figure 2 shows an image of a NuPAGE gel (Figure 2A) and its western blot analyses (Figure 2 C-G) probed with α-kafirin (Figure 2E-F), γ-kafirin (Figure 2C-D) and δ-kafirin (Figure 2G) antibodies. Strikingly, these result indicate that the major band, with molecular weight of 19-20kD, contains γ-kafirin (Figure 2C). This result contradicts claims from the previous research that the major protein band

represents α-kafirin, since α-kafirin is believed to be the dominant type of kafirin in sorghum (Belton, Delgadillo et al. 2006). However, when probed with anti-19kD α-zein antibody, a portion of the major band also cross-reacted, suggesting that α-kafirin makes up a component of the major band at 19kD (Figure 2E).

Meanwhile, results from probing the blots with 27kD γ-zein antibody (Figure 2C) and 50kD γ–zein antibody (Figure 2D) show cross-reacting high molecular weight protein components. This comparative analysis of the western blots with the high and low molecular weight antibodies available (27kD, 50kD γ-zein antibodies) suggests that the γ-kafirins may form oligomers by cross-linking its subunits. Especially, figure 2C probed with 27kD γ-zein antibody shows proteins with multiple two folds of molecular weights (~20kD, ~40kD, and ~60kD), further supporting the idea that γ-kafirins can form oligomers with cross-linked subunits. γ-kafirin's tendency to form oligomers also can also explain, at least in part, the low digestibility of sorghum proteins. Western Blots with α-kafirins (Figure 2 E-F) also show some presence of high molecular weight proteins. However, the signals for high molecular weight proteins are weaker and the proteins are not in multiple folds of molecular weights. Therefore, the blots are not sufficient to support the possibility that α-kafirins may also form oligomers. δ-kafirin is a recently identified type of kafirin and its antibody displays that these kafirins represent a minimal portion of kafirins (Figure 2G).

Data in Figure 3 represents the time course analyses of pepsin digestion of the kafirins in P8 and T4. After running the SDS-PAGE gel of the time course protein digestions (Figure 3A), we used known molecular weights of antibodies to follow the time course pepsin digestions of kafirins and glutelins in P8 and T4. Each line

in the graphs (Figs. 3B and C) represents remaining amounts of the different kafirins during the time course of pepsin digestion as determined by densitometric quantification (described in *Materials and Methods 2.6*). From the data from this experiment, we infer that α-kafirins digest faster than γ-kafirins in each sorghum line, based on the steeper slopes in the graph in the digestion time course. This result supports claims from the literature that α-kafirins are easier to digest compared to γ-kafirins (Taylor, Bean et al. 2007). Comparing western blots for α-kafirins (anti 22kD, 19kD α-zein antibodies) and for γ-kafirins (27kD, 50kD γ-zein antibodies), it is obvious that the high molecular weight protein components observed with the γ-zein antibodies are more visible and harder to digest over time course, compared to α-kafirins. This suggest that γ-kafirin is less digestible than α-kafirin. Moreover, all types of kafirins in the T4 sorghum line digest faster than those in P8, suggesting that these two sorghum lines have varying digestibility. This result – that digestibility of T4 is faster than P8 – is also confirmed by DUMAS combustion.

Examining P8 and T4, the two tannin-containing sorghum lines in the gel analysis as well as western blots using 27kD and 50kD γ-zein antibodies, displays reasonably high levels of high molecular weight protein components

3.2 Differential digestibility of tannin and non-tannin sorghums revealed in gel-based assay

3.2.1 Comparative analysis of tannin and non-tannin sorghums

The sorghum lines selected for extraction were non-GMO, GMO-PR, GMO-JH, Seredo, and RIA 19, all of which are known to contain tannins, and Macia

and 296B, which do not contain tannins. Differential protein digestibility of these tannin and non-tannin sorghum lines was estimated by comparing their extractabilities in the gel-based system. This simple gel procedure easily reveals the types of kafirins that are left undigested following the 2-hour pepsin digestion.

The one-solvent extraction system, using 0.0125 M borate buffer pH 10 with 1% SDS and 2% 2-ME, extracts most kafirins as described (Aboubacar et al., 2001). As shown in Figure 4, all sorghum lines regardless of the presence or absence of tannins have high molecular weight protein complexes (~191 Kb) before pepsin treatment that barely enter the gel and stay in the vicinity of the bottom of the wells. Interestingly, these high-molecular weight bands disappear after pepsin digestion only in non-tannin sorghum lines, Macia and 296B. From this observation of the different degrees of disappearances of the high molecular weight proteins between tannin and non-tannin sorghum lines, we are able to predict that these proteins may be the high-molecular weight γ-kafirins, which form aggregates in the presence of tannin.

In the selected lines containing tannins high molecular weight (~191 Kb) aggregates are formed that inhibit pepsin from accessing the protein matrix. Therefore these bands may indicate that high-molecular weight γ-kafirins complex with tannins, leaving them undigested after pepsin digestion. Further study using western blot analysis with γ-zein antibodies will be permit us to examine whether our hypothesis that the high molecular weight bands are indeed γ-kafirins that form aggregates with tannin.

3.2.2 Effect of cooking on sorghum digestibility

Two identical extraction experiments with borate-SDS-ME were performed concurrently on uncooked and cooked meals in order to examine the effects of cooking on sorghum digestibility. Interestingly, there was a high molecular weight mass observed in gels both in Seredo and RIA 19, which remained at the bottom of the sample well. This might suggest that cooking further promotes formation of high molecular weight aggregates that are unable to enter the gel matrix and remain in the sample wells. We hypothesized that the increased formation of high molecular weight aggregates in cooked meal is due to the gelatination of starch during cooking that inhibit pepsin from accessing protein bodies and performing digestion, because we experimentally observed opaque substance formation due to starch gelatination from taking up of water during cooking. Furthermore, in Figure 4 cooked samples after pepsin digestion show increased intensities of high molecular weight protein bands around 38kD (Figure 4B). In comparison to the uncooked samples (Figure 4A), the gel analysis indicates that the cooked sample (Figure 4B) has more proteins after pepsin digestion at the high molecular weight location. A paper suggests that cooking reduces digestibility of protein by affecting a conformational change in proteins that could facilitate formation of disulfide-linked polymers (Oria, Hamaker et al. 1995). Hence our result further supports the idea that cooking promotes formation of oligomers that are linked by disulfide bonds, therefore leading to the low protein digestibility.

3.3 Data supporting claim that sorghum grain with tannin has lower protein

digestibility compared to non-tannin sorghum grains.

Currently, presence of tannin in sorghum grain is known to inhibit protein digestibility (Taylor, Bean et al. 2007). We performed a three-solvent, gel-based analysis in order to further investigate this claim. The three-solvent system was modified from the gel-based assay in Aboubacar et al., 2001. This system was developed in the Buchanan laboratory to better extract α-kafirins. This was needed since our previous western analyses following kafirin extraction did not show α-kafirin as the major kafirin (Figure 2 E-F), causing discrepancies with the literature that indicates α-kafirins make up 80% of total kafirins (Oria, Hamaker et al. 2000). We felt that the established one-solvent sorghum protein extraction method might not be sufficient to completely extract α-kafirins.

The new extraction system used was a three-step, sequential extraction method, designed to differentially extract different types of kafirins at each step. As shown in Figure 5, the uncooked (Fig. 5B) and the cooked samples (Figs. 5A) show differential extractability in the first solvent fraction with 60% t-butanol. Whereas in uncooked meal, this solvent extracts non crossed-linked prolamins well; in cooked meal extracted proteins are hardly visible. This suggests that cooking promotes protein cross-linking that makes the proteins insoluble in this solvent. Macia and Seredo are two African sorghum lines. While Macia does not contain tannins, Seredo does. Before pepsin treatment, the two lines show similar extractability in both cooked and uncooked samples (Figure 5A-C controls). A noticeable changes in the extractabilities occurs after the pepsin treatments (Figure 5A-C pepsins), especially when these sorghum lines are extracted with the 60% t-butanol-ME solvent (Figs. 5B). Macia shows much lighter bands after pepsin

digestion with the butanol-ME solvent, compared to the control before digestion, indicating that most proteins are α kafirins (also corroborated by western), and they are easily digested by pepsin. In contrast, bands for Seredo are not nearly as affected, suggesting that its protein is not as efficiently digested as Macia's. The hypothesis that most of kafirins extracted with the 60% butanol-ME solvent was initially drawn from the multiple performances of the western blots with the three solvent extraction method, for example, as shown in Figure 7 I-III. Whereas most of the α kafirins are shown to be extracted in the second extraction with 60% butanol-ME (Figure 7 II), most of the γ-kafirins were extracted in the third extraction step with borate-SDS-ME (Figure 7 III). Since α kafirins compose the majority of total kafirin protein, the noticeable difference in digestibility of α kafirins indicates that the digestibility of total kafirin in Macia and Seredo is also distinctly different. Moreover, bands present in extracts from treatment with the third solvent, borate-SDS-ME, also show similar patterns, with Seredo showing less differences in the bands, hence indicating lower digestibility of γ-kafirins of Seredo compared with Macia. The higher molecular weight protein mass (~191 KDa) disappears in Macia after pepsin digestion, whereas it remains visible in Seredo. These results indicate that the different digestibility between the tannin and non-tannin lines is due to formation of high molecular aggregates in the presence of tannin, making the tannin-containing sorghum, Seredo, less digestible.

3.4 Data not supporting claim that sorghum grain with tannin has lower protein digestibility – comparative study of 296B and RIA19

Our comparative studies of tannin and non-tannin lines with various other

sorghums, however, showed evidence of an exception that seem to deviate from the result drawn from our previous experiments that tannin sorghums have lower protein digestibility than non-tannin sorghums. 296B and RIA19 are two sorghum lines known to be very resistant to pepsin digestion. The standard Dumas combustion (Table 1, below) of the remaining residues after the pepsin digestions with cooked meal suggests that the percent protein digestibility of 296B and RIA19 are only 19.9% and 25.3% respectively. However, 296B does not contain tannins whereas RIA19 does. In results from the gel based-assay of the sequential 3-solvent extraction (Figure 6), the amount of extracted proteins from 296B and RIA19 before and after pepsin digestion are similar for the first two extraction steps of the uncooked (Figs. 6 A,B I, II) and cooked (Figs. 6 A,B III, IV) samples. This result indicates that these two lines have similar protein digestibility of α-kafirins, regardless of the fact that one does not contain tannin and the other does. However, proteins in the borate-SDS-ME fraction did show noticeable differences in pepsin digestion of both uncooked (Fig. 6 A,B I, II) and cooked (Fig. 6 A,B III, IV) samples. This result suggests that presence of tannin in the sorghum lines is not the only factor that determines protein digestibility. For 296B and RIA19, it is suggestive that there are other factors, such as formation of aggregates with starch, which determines protein digestibility and allows non-tannin sorghum lines, such as 296B, to be extremely resistant to pepsin digestion.

Table 1. Dumas combustion of protein residues after pepsin digestion

Sample	% Total protein	% Protein digestibility (Uncooked)	% Protein digestibility (Cooked)

Lee, Youngin
UC Berkeley

P8	11.2%± 0.83	58.8% ± 7.23	46.9% ± 6.5
T4	11.2% ± 0.35	66.5% ± 4.84	48.6% ± 5.1
296B	16.8% ± 4.11	52.7% ± 2.33	19.9% ± 15.3
RIA19	14.9% ± 0.57	44.8% ± 6.86	25.3% ± 2.5

3.5 Data not supporting claim that sorghum grain with tannin has lower protein digestibility – comparative study of P8 and T4

The following experiment illustrates another exceptional result that does not show differential protein digestibility of tannin and non-tannin sorghums. Figure 7 shows the collective study of gel-based assay and western analyses of T4 and P8 compared with 296B and RIA19. T4 is a distant transgenic offspring of the tannin-containing line, P8, and therefore both are supposed to contain tannin. However, analysis of results from the three-solvent system gel assay of uncooked meal indicates that the digestibility of both T4 and P8 in all three extractions is higher compared to that of RIA19. Comparison of control and pepsin-digested bands shows that intensity of the bands is clearly reduced in T4 and P8, whereas disappearance of the same bands was not as marked in RIA19. This result is supported by the DUMAS combustion result (Table 1), where the remaining percentages of the proteins for T4 and P8 after pepsin digestion are noticeably higher than that of RIA 19. Especially when the meal is cooked, the percentages of the residual proteins of T4 and P8 (48.6% ± 5.1, 48.6% ± 5.1, respectably) after the pepsin digestion is about two-folds higher than that of RIA 19 (25.3% ± 2.5). Western blot analyses with α- and γ- zein antibodies further supports the combustion resuts. In the second solvent fraction extracted with 60% t-butanol-ME,

hybridization with the α-zein antibody shows a clear difference between P8/T4 and RIA19 in digestibility of α-kafirin, showing that α-kafirin is more digested in P8 and T4 than in RIA19. Moreover, the western blot analysis with γ- zein antibody also clearly shows differential digestibility of γ-kafirin between T4/P8 and RIA19 in the third fraction extracted with borate-SDS-ME. This result indicates that presence of tannin does not solely determine protein digestibility of sorghum lines. Also, different concentrations of tannin in diverse sorghum varieties may also result in differential digestibility (Guiragossian, Chibber et al. 1978). Of special note, results from western blots indicate that α-kafirin is more soluble in 60% t-butanol-ME, while γ-kafirins are preferentially extracted in borate-SDS-ME buffer, indicating that γ-kafirins require harsher and more extreme condition than α-kafirins in order to be extracted.

Conclusions

In summary, our results highlight the importance of the structural and chemical properties of kafirins in determining their digestibility. The microscopy study from the previous study in our research group gave insights into the role of the protein matrix and its interaction with starch in determining sorghum protein digestibility (Wong, Lau et al. 2009). Our biochemical studies broadened these observations by investigating the function of disulfide proteins and tannins in reducing the protein digestibility as was demonstrated in our gel-based assay and western analyses. These data showed that tannins have differential effects on protein digestibility among sorghum lines, suggesting that there are more exogenous factors affecting digestibility of various sorghum lines, other than the presence of tannins.

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