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Transgenic Sorghum with Altered Kafirin Synthesis: Kafirin Solubility, Polymerization, and Protein Digestion

Laura S. da Silva,[†,‡] Janet Taylor,[†] and John R. N. Taylor[*,†]

[†]Department of Food Science, University of Pretoria, Private Bag X20, Hatfield 0028, South Africa

[‡]Department of Biotechnology and Food Technology, Tshwane University of Technology, Pretoria 0001, South Africa

ABSTRACT: Transgenic sorghum (TG) lines with altered kafirin synthesis, particularly suppression of γ-kafirin synthesis, and improved protein quality have been developed. The proportion of kafirin extracted with 60% *tert*-butyl alcohol alone was greatly increased in the TG lines. However, the total amount of kafirin remained unchanged. Further, in the TG lines, the kafirin was much less polymerized by disulfide bonding. There was also evidence of compensatory synthesis of other kafirin proteins. Cooked protein digestibility was increased in the TG form, even after removal of interfering starch. The TG protein bodies were intermediate in appearance between the normal type and the invaginated high digestibility mutants. Hence, the increased protein digestibility of these TG lines is probably related to their lower levels of disulfide-bonded kafirin polymerization, allowing better access of proteases. This work appears to confirm that disulfide bond formation in kafirin is responsible for the reduced protein digestibility of cooked sorghum.

KEYWORDS: Sorghum, transgenic, kafirin, protein digestibility, disulfide cross-linking, polymerization

INTRODUCTION

The poor protein nutritional quality of sorghum is due to the major storage proteins, the kafirins, being essentially free of lysine and to the sorghum protein having low digestibility, especially after cooking.[1] Numerous factors contribute to the low protein digestibility of sorghum. However, cross-linking of the kafirin proteins through disulfide bonding and the organization of the various kafirin subclasses within protein bodies are major factors.[2,3]

Sorghum lines with improved protein quality have been developed through reduced expression of different kafirin subclasses, using genetic engineering techniques by the Africa Biofortified Sorghum (ABS) project, under the Bill and Melinda Gates Foundation Grand Challenges in Global Health initiative.[4] We have shown that transgenic sorghum lines (TG) with cosuppression of synthesis of α-, γ-, and δ-kafirin subclasses and removal of the tannin trait have high cooked in vitro protein digestibility (IVPD) (approximately 60%) and improved lysine score (0.5) and protein digestibility corrected amino acid score (0.4).[5] The improved protein quality traits are maintained when the TG sorghum is used to produce porridge, a major African staple food,[6] and a wide range of other sorghum food products.[5]

A problem, however, with these high protein quality sorghum TG lines is that the they have a floury endosperm phenotype and a modified endosperm structure, with the protein bodies irregularly shaped and surrounded by a dense protein matrix.[7] Therefore, this study investigated the effect of reduced synthesis of the major kafirin subclasses on kafirin solubility, polymerization, and protein digestibility, with the aim of understanding of the mechanisms responsible for the TG sorghum lines having improved protein digestibility but modified endosperm structure.

MATERIALS AND METHODS

Materials. The sorghum samples used were two independent transgenic (TG) events (T1 event 5/15028; T2 event 6/15032) and their respective nontransgenic null controls (Cs) (C1 and C2, parent variety P898012, a purple plant, type II tannin type, 3.26 mg of catechin equiv/100 mg of flour). The transgenic samples were T2 self-ed seeds, 75% pure with respect to the ABS032 gene construct that suppresses synthesis of α-kafirin A1 and α-kafirin B1and B2 (which correspond to 19 and 22 kDa α-kafirin classes, respectively[3]), γ-kafirin 1 and 2, and δ-kafirin 2. The transgenics and their null controls were developed using *Agrobacterium*-mediated transformation, as described by Zhao et al.,[8] and cultivated in a confined trial under the same environmental conditions at Johnston, IA, in 2008. These were compared with a high protein digestibility line, 07HW PRGE 103 (BTx635*P850029)-CS9-CS1-CS1 (HD) (a tan plant, nontannin sorghum with P721 opaque in its pedigree, supplied by Texas A&M University, Weslaco, TX), and a normal sorghum, Macia (developed from SDS 3220, ICRISAT SMIP) (cultivated at Makoro Lands, Central District, Botswana, 2004, a popular southern African tan plant, nontannin variety).

Methods. Sorghum was received as whole grain or crushed whole grain. They were milled using a hand-held mill (IKA A11 Basic, Staufen, Germany) until all the whole-grain flour passed through a 500 μm opening screen. The flours were stored at ± 8 °C until use. The samples were previously characterized with respect to tannin content, protein, lysine, and in vitro protein digestibility of raw and wet cooked flour.[5]

Kafirin Solubilization. Extraction 1: Whole grain flour (8 g) was suspended in 40 mL of 60% (v/v) aqueous *tert*-butyl alcohol and agitated for 5 h at ambient temperature and centrifuged (2000*g* for 10 min), and the supernatant was collected. The residue was resuspended in the same solvent (40 mL), agitated overnight, and centrifuged, and the two supernatants were pooled and termed kafirin-1, as defined.[9] Extraction 2: The residue from extraction 1 was resuspended in 40 mL of 60% (v/v) aqueous *tert*-butyl alcohol plus 5% (v/v) 2-mercaptoethanol, agitated for 3 h at room temperature, and then centrifuged, and supernatant was

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collected. The residue was resuspended in the same solvent (40 mL), agitated for a further 3 h, centrifuged, and the two supernatants were pooled and termed kafirin-2.[9] The supernatants and residues were freeze-dried, the weights recorded, and the protein contents determined.

Isolation of Protein Preparations by Wet-Milling. To study the protein body proteins, protein preparations (PP) were prepared by wet-milling.[9] In brief, defatted flour was wet-milled using an Ultra Turrex (Janke & Kunkel, Staufen, Germany). The resulting slurry was passed through sieves of different opening size. The 75 μm fraction containing the protein bodies and starch was centrifuged several times to separate the protein from the more dense starch. The PP by wet-milling was then freeze-dried and the protein content determined.

Isolation of Protein Preparations by Starch Digestion. To study the pattern of protein digestion, PP were prepared by starch digestion. This was performed by a modification of the Megazyme Total Starch Assay Kit method (Megazyme International Ireland Limited, Wicklow, Ireland). α-Amylase in 3-(*N*-morpholine)propanesulfonic acid (MOPS) buffer (6 mL) was added to flour (200 mg) and the samples incubated at boiling temperature (95 °C) for 15 min. Sodium acetate (8 mL) and amyloglucosidase (0.2 mL) was added, and the samples were further incubated at 50 °C for 30 min. The samples were centrifuged and the supernatant decanted off and directly subjected to pepsin digestion.

Analyses. *Protein.* Protein (N x 6.25) was determined by a Dumas combustion method (AACC Standard Method 46−30).[10]

Pepsin Digestion. The in vitro pepsin digestibility (IVPD) method of Hamaker et al. [11] was used, modified for small-scale assay (200 mg).[5]

Transmission Electron Microscopy (TEM). TEM of the PP by wet-milling and starch digestion after pepsin digestion was performed. Specimens were fixed in 2.5% glutaraldehyde (0.075 M phosphate buffer, pH 7.4) for 2 h, dehydrated in a graded aqueous acetone series, and then infiltrated with Quetol resin. Sections were stained with aqueous uranyl acetate and further stained in Reynold's lead citrate and then examined using a JEOL JEM 2100F field emission electron microscope (Tokyo, Japan).

SDS−PAGE. This was carried out under both nonreducing and under reducing conditions using a X Cell SureLock Mini-Cell electrophoresis unit (Invitrogen Life Technologies, Carlsbad, CA) and preprepared NuPAGE 4−12% Bis-Tris gradient gels 1 mm thick, with Invitrogen Mark 12 unstained standard molecular weight markers (2.5−200 kDa). For reducing conditions 10% 2-mercaptoethanol was added to the sample buffer. Procedures were according to Gallagher,[12] and to ensure complete protein solubilization, samples were boiled for at least 15 min with vigorous vortexing every 5 min. Samples were loaded at constant protein (10 μg), unless stated otherwise. Staining was with Coomassie Brilliant Blue R250.

Statistical Analysis. Samples were analyzed in duplicate twice (four values). The data were analyzed by one-way analysis of variance (ANOVA) at a confidence level of $p < 0.05$. Means were compared by Fisher's least significant difference (LSD) test.

RESULTS AND DISCUSSION

PP by Wet-Milling. During the wet-milling process to produce the PP by wet-milling, the protein and starch fractions in the TG and HD samples separated more easily than the C and Macia samples. Additional washing and centrifugation steps were required with the latter to obtain PP by wet-milling with similar protein contents (25−30%). The TG and HD samples had all floury endosperm texture, whereas C had some peripheral corneous endosperm and Macia had intermediate endosperm texture.[7] The floury endosperm texture of TG and HD probably facilitated the wet-milling process, allowing easier separation of the protein bodies from the starch granules because of the weaker starch−protein and protein−protein matrix compared to grains of medium to hard endosperm texture.[13] The relatively low protein contents of the PP by wet-milling were due to the fact that a reducing agent, such as sodium metabisulfite, was not used during the wet-milling process, unlike in commercial wet-milling.[14] The reducing agent was not included so as study the proteins in their natural state.

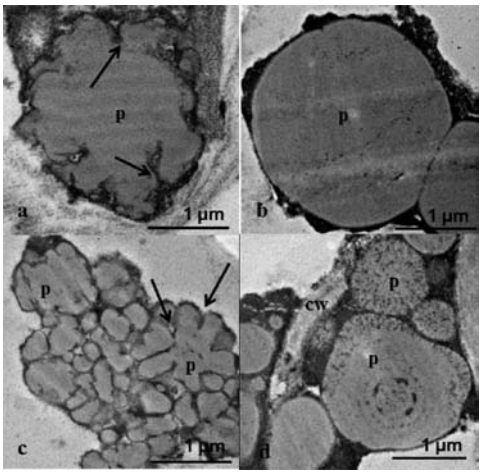

Figure 1. Representative images of TEM of sorghum protein body preparations by wet-milling: (a) T1, (b) C1, (c) HD, (d) Macia (cw = cell wall, p = protein body; arrows show invaginations).

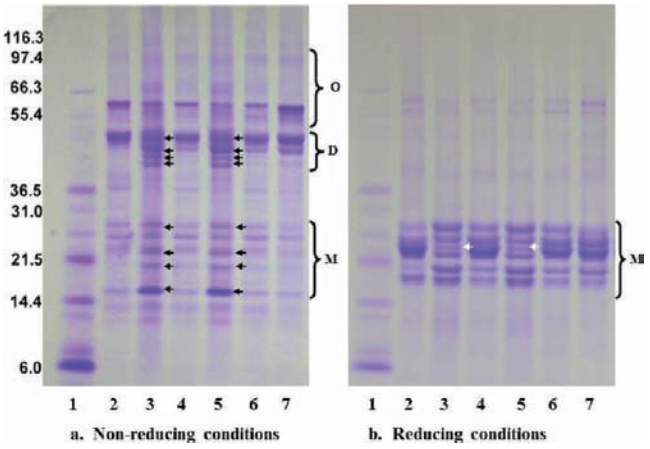

Figure 2. SDS−PAGE of sorghum protein preparations by wet-milling: (1) Molecular weight markers (kDa), (2) C1, (3) T1, (4) C2, (5) T2, (6) HD, (7) Macia. M = monomers, D = dimers, O = oligomers. Black arrows show high band intensity and white arrows show reduced band intensity. Loading was at a constant protein (10 μg) level.

The PP by wet-milling mainly comprised endosperm material made up of protein bodies surrounded by matrix protein, with some starch granules and cell wall material, as reported in similar work.[9] Significantly, the TG had somewhat more irregular shaped and invaginated protein bodies (Figure 1a), as compared to those of C (Figure 1b) and Macia (Figure 1d). The TG protein bodies were, however, less irregular and invaginated than the characteristic HD protein bodies[15] (Figure 1c). The TG protein bodies could be considered intermediate in form between those of C and Macia and HD. The protein bodies in the PP by wet-milling had the same form as those observed in endosperm sections from these types.[7]

The protein composition of the PP by wet-milling was heterogeneous (Figure 2), as they comprised all aqueous insoluble

Table 1. Kafirin Extracted with 60% (v/v) *tert*-Butyl Alcohol Followed by 60% (v/v) *tert*-Butyl Alcohol Plus 5% (v/v) Mercaptoethanol from Transgenic, Null Controls, High Protein Digestibility Mutant, and Macia Sorghums

	kafirin extraction yield (g/100 g protein)[a]		
	kafirin-1[b]	kafirin-2[c]	total
NC-P898012-2 (C1)	19.9 ± 1.5 b (42.1)	27.3 ± 2.5 c (57.9)	47.2 ± 1.0 c
TG-P898012 (ABS032)-2 (T1)	34.8 ± 1.2 d (69.9)	15.0 ± 2.3 a (30.1)	49.8 ± 1.1 c
NC-P898012-3 (C2)	19.3 ± 0.8 b (40.4)	28.5 ± 1.3 c (59.6)	47.8 ± 0.4 c
TG-P898012 (ABS032)-3 (T2)	32.9 ± 0.9 d (69.1)	14.8 ± 3.3 a (30.9)	47.7 ± 2.4 bc
HPDM8 (HD)	14.1 ± 1.0 a (42.8)	18.9 ± 1.2 b (57.2)	33.0 ± 0.2 a
Macia	22.9 ± 1.4 bc (55.6)	18.3 ± 1.9 b (44.4)	41.2 ± 0.5 b
Overall mean	24.0 (53.3)	20.5 (46.7)	44.5

[a] Means and standard deviations. Values in parentheses indicate each kafirin fraction as a percentage of the total kafirin extracted. Different letters in columns indicate significant difference ($p < 0.05$). [b] Kafirin-1 = protein extracted from whole grain flour using 60% *tert*-butyl alcohol. [c] Kafirin-2 = protein extracted from residual whole grain flour using 60% *tert*-butyl alcohol plus 5% (v/v) mercaptoethanol.

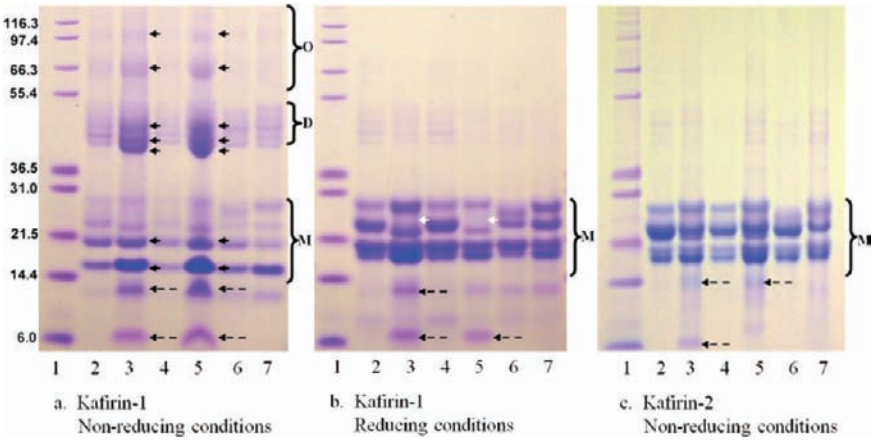

Figure 3. SDS−PAGE of extracted kafrin: (a, b) kafirin-1 extracted with 60% *tert*-butyl alcohol, (c) kafirin-2 extracted with 60% *tert*-butyl alcohol plus 5% 2-mercaptoethanol. (1) Molecular weight markers (kDa), (2) C1, (3) T1, (4) C2, (5) T2, (6) HD, (7) Macia (in all cases). M = monomers, D = dimers, O = oligomers. Black arrows show high band intensity. White arrows show reduced band intensity/or absence. Black dashed arrows show additional bands. Loading was at a constant protein (10 μg) level.

proteins in the grain. However, as would be expected, the kafirin proteins predominated due to their high concentration relative to other grain proteins (Table 1). Under both nonreducing and reducing conditions at least six monomeric kafirin bands with apparent molecular weights ranging from approximately 18 to 27 kDa were present, as was described by El Nour et al.[16] The individual kafirin monomers are not identified, as unpublished work (R. Jung, Pioneer Hi-Bred, personal communication) showed that the important 27 kDa γ-kafirin was not separated from the α-kafirins by SDS−PAGE in these sorghum lines.

Under nonreducing conditions, kafirin dimers and oligomers were also present in relatively high concentrations (Figure 2a). The latter have been identified as disulfide-bonded kafirins, pre-existing in the endosperm tissue.[9,16,17] Of significance is that the band intensities of the monomers and the number of resolving bands for the dimers were significantly higher in the TG lines (black arrows) compared to their Cs, HD, and Macia. As the protein loading was constant for all the sorghums, this suggests that a lower proportion of the highly cross-linked polymeric kafirins (>200 kDa) [17,18] was formed in the TG lines during protein body synthesis.

Under reducing conditions, essentially only kafirin monomers were present (Figure 2b). This suggests that the kafirin polymers, oligomers, and dimers were essentially all reduced by disulfide bond reduction into the various kafirin monomer subclasses. In the TG lines, a major monomeric kafirin band was absent or present, at much lower concentration, (white arrow) compared to their Cs, HD, and Macia, presumably as a result of suppressed synthesis of that particular kafirin protein.

Kafirin Solubility and Composition. Total kafirin extracted from whole grain flour of the TG lines and their Cs was in the range 47−50 g/100 g protein (Table 1), showing that they both had similar total kafirin content. The total kafirin content of the TG lines falls within normal range for whole grain flour, approximately 50−60% of the total protein in whole grain flour.[19] The high kafirin content of the TG lines is somewhat surprising, as the ABS032 gene construct causes suppressed synthesis of α-kafirin A1, B1, and B2; γ-kafirin 1 and 2; and δ-kafirin 2 through RNA interference technology.[6] Also, these TG lines were shown to contain considerably more total lysine, mean 2.65 g/100 g protein, than their Cs, mean 1.84 g/100 g protein. Also, the total protein content of the TG lines fell within the normal range for sorghum.[5] Hence, the suppression of synthesis of these kafirin subclasses also did not result in substantial reduction in total protein content. In native and mutant high lysine cereals, their increased lysine content is attributed to a decrease in total prolamin content, with a compensatory increase

Table 2. Effect of Altered Kafirin Synthesis on the in Vitro Protein Digestibility of Cooked Sorghum Flour Pretreated with Amylase (PP by starch digestion) To Digest the Starch[a]

	IVPD (%)		
	no pretreatment (%)	pretreated (%)	difference
NC-P898012-2 (C1)	37.7 ± 1.4 a	52.7 ± 2.7 a	15.0
TG-P898012 (ABS032)-2 (T1)	57.8 ± 2.3 bc	65.7 ± 3.3	7.9
NC-P898012−3 (C2)	38.8 ± 2.0 a	51.7 ± 1.9 a	12.9
TG-P898012 (ABS032)-3 (T2)	55.2 ± 2.0 b	68.0 ± 5.2 b	12.8
HPDM8 (HD)	55.0 ± 2.0 b	74.8 ± 4.6 c	19.8
Macia	60.0	69.2 ± 0.8 b	9.2
overall mean	50.7	63.7	13.0

[a] Means and standard deviations. Different letters in columns indicate significant difference ($p < 0.05$).

in the lysine-rich nonprolamin proteins,[1] as can be seen in the low percentage of total kafirin in the HD mutant, 33 g/100 protein. The fact that the TG lines had normal levels of total kafirin but increased protein lysine content suggests that compensatory synthesis of high-lysine, nonkafirin proteins occurred at the expense of other nonkafirin endosperm proteins.

With regard to the nature of the kafirins in the TG lines, it is significant that extraction with 60% *tert*-butyl alcohol alone extracted substantially more kafirin-1 [9,16,17] from the TG lines (approximately 69% of total kafirin extracted) compared to their Cs (40−42%), HD (43%), and Macia (56%) (Table 1). Since the total amount of kafirin extracted (kafirin-1 plus kafirin-2) from the TG lines was the same as their Cs and more than from HD and Macia, this indicates that the kafirin proteins in the TG lines were less cross-linked by disulfide bonding than the kafirins in normal sorghums, as was suggested by SDS−PAGE (Figure 2).

SDS−PAGE of kafirin-1 under nonreducing conditions (Figure 3a) showed that the kafirin-1 of the TG lines comprised much higher concentrations of oligomers, dimers, and certain monomers (black arrows) compared to their Cs, HD, and Macia. As the protein loading was the same for all the sorghum types, this indicates that the kafirin-1 of the TG lines contained a lower proportion of kafirin polymers (>200 kDa) than the other sorghums. With SDS−PAGE under reducing conditions (Figure 3b), all the sorghum types essentially only exhibited kafirin monomers, which were at much higher band intensity than under nonreducing conditions (Figure 3a). This was due to the kafirin polymers, oligomers, and dimers essentially all being reduced into kafirin monomers. Together, these results clearly show that the kafirin-1 in the TG lines was much less polymerized than that of their Cs, HD, and Macia (Figure 3a). This can be attributed to suppression of synthesis of the cysteine-rich γ-kafirins, which are required for polymerization through disulfide bonding.[16]

Additionally, there were significant differences between the kafirin monomer bands present in the TG lines and the other sorghums (Figure 3b), as in the PP by wet-milling (Figure 2). At least one major band was absent in T1 and T2 at approximately 25 kDa (white arrows), which was present in their Cs, HD, and Macia, showing that its synthesis was suppressed. Also, at least one new band was present in the TG lines at approximately 23 kDa (top dotted black arrow), which was absent in the normal sorghums, indicating that compensatory synthesis of other kafirin proteins or possibly nonprolamin proteins had taken place. Further, with SDS−PAGE under both nonreducing and reducing conditions additional high-intensity low molecular weight bands (<14.4 and 6.0 kDa) were also present in kafirin-1 of the TG lines (Figure 3a,b lower black dashed arrows). These low molecular weight proteins are presumably kafirins as the extraction conditions used (60% *tert*-butyl alcohol extract) is specific for solubilizing prolamin proteins such as kafirin.[16,19] The presence of these low molecular weight kafirins may be due to compensatory overexpression of additional kafirin proteins, kafirin fragments, or alcohol-soluble nonprolamin proteins,[20] as a result of modified gene expression.

The kafirin-2 proteins (Figure 3c), which were extracted under reducing conditions after extraction of kafirin-1, showed generally similar band patterns to the kafirin-1 proteins separated under reducing conditions (Figure 3b). The kafirin-2 proteins are presumably originally made up of the disulfide-bonded kafirin polymers, which were reduced into monomers. The low molecular weight kafirins were again present in the TG lines, although at a reduced intensity (Figure 3c, black dashed arrows). Of significance is that the pattern of T1 and T2 kafirin-2 proteins is more similar to that of the Cs, HD, and Macia than was the case with the kafirin-1 proteins under reducing conditions (Figure 3b). This can be attributed to the kafirin-2 proteins originally being disulfide-bonded polymers, where the cysteine-rich γ- and β-kafirins must be present in order for the kafirins to be polymerized.[16]

Pepsin Digestion of the PP by Starch Digestion. Treating the cooked flours with amylases to remove all the gelatinized starch formed during cooking, which can embed the protein bodies and matrix, improved the cooked IVPD of all the sorghum samples by 14−36% compared with no pretreatment (Table 2). The improved IVPD of the samples following starch removal is presumed to be due to the pepsin having greater access to the protein structures.[9] Importantly, however, after removal of the starch the TG lines still had substantially higher IVPD (25−32%) compared to their Cs. This shows that the protein in the TG lines was intrinsically more available to proteolysis. The fact that the IVPD of the TG lines was the same as that of Macia, a normal sorghum, and lower than that of HD can be attributed to the presence of tannins in the TG lines, which would interfere with protein digestion.[21,22]

The compositions of residual pepsin-indigestible proteins in the PP by starch digestion (Figure 4) were generally similar to those of the native proteins in the PP by wet-milling (Figure 2). However, under nonreducing conditions it appeared that the relative ratio of oligomers and dimers to monomers was higher in the pepsin indigestible residues for PP by starch digestion (Figure 4a) than in the PP by wet-milling (Figure 2a). Also, of note is that higher numbers of oligomer and dimer bands occurred in the TG lines compared to their Cs, HD, and Macia

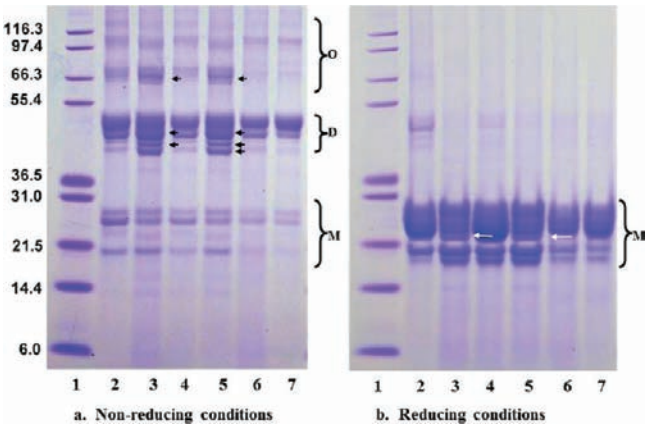

Figure 4. SDS−PAGE of pepsin-indigestible residue from cooked protein preparations by starch digestion: (1) molecular weight markers, (2) C1, (3) T1, (4) C2, (5) T2, (6) HD, (7) Macia. M = Monomers; D = Dimers; O = Oligomers. Black arrows show high band intensity. White arrows show reduced band intensity/or absence. Loading was proportional to the quantity of protein remaining after digestion.

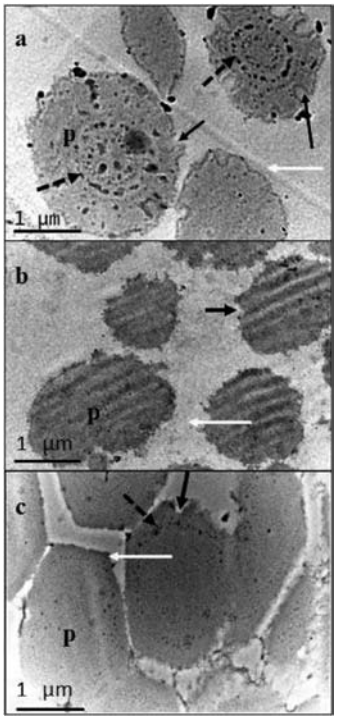

Figure 5. Representative images of TEM of pepsin indigestible residue from cooked protein preparations by starch digestion: (a) C1, (b) T1, (c) Macia; p = protein body. Solid black arrows show proteolysis of protein body periphery. Dashed black arrows show dark staining regions, presumed primarily γ- and β-kafirin.[26] White arrows show where matrix protein was.

(Figure 4a, black arrows), as in the PP by wet-milling, but the levels of monomers remaining in TG and their Cs were similar, unlike the situation in the PP by wet-milling. The fact that substantial amounts of kafirin oligomers and dimers were present in the pepsin indigestible residues implies that even these relatively small disulfide-bonded kafirins are more resistant to hydrolysis than the monomers.

Upon reduction, the polymeric proteins were resolved into the different kafirin monomer subclasses (Figure 4b). Significantly, the low molecular weight kafirins, which were present in T1 and T2 PP by wet-milling, kafirin-1 and kafirin-2 preparations (Figures 2 and 3), were absent. This indicates that they were readily hydrolyzed by pepsin. However, similar to the PP by wet-milling (Figure 2b) and the kafirin-1 (Figure 3b), particular kafirin monomers, probably γ-kafirin, which was present in high concentration in the pepsin indigestible residues of PP by starch digestion of the Cs were absent or present at very reduced concentration in the TG lines (Figure 4b, white arrows). This suggests that the kafirins in pepsin indigestible residues of PP by starch digestion of the TG lines were less polymerized than those of their Cs, due to the suppression of synthesis of γ-kafirin. This is consistent with the higher pepsin digestibility of the TG lines compared to their Cs.

TEM of the protein bodies in the PP by starch digestion of the TG lines, Cs, and Macia after pepsin digestion revealed disappearance of the matrix protein between the protein bodies and digestion of the kafirin ground material from the periphery of the protein bodies (Figure 5). This pattern of sorghum protein body digestion is normal.[23−25] In normal sorghum, the γ- and β-kafirins are concentrated at the periphery of the protein bodies and appear as dark regions[26] [dashed black arrows in null control (Figure 5a) and Macia (Figure 5c)] and are believed to inhibit access of proteases to the α-kafirin ground material,[27] thus majorly contributing to the poor protein digestibility of wet cooked sorghum. The absence of, or greatly reduced amount of, dark staining regions (cross-linked γ- and β-kafirins) in the protein bodies of the TG (Figure 5b) presumably facilitates more rapid digestion of the protein bodies but as observed does not affect the general pattern of protein body digestion.

With the HD, protein bodies could not be observed in the PP by starch digestion after pepsin digestion, presumably due to its higher IVPD (Table 2). However, in these HD mutants the protein bodies are very folded (invaginated) (Figure 1c), with the γ-kafirin located at the base of the invaginations,[15] with the effect that proteases have easy accessibility to the α-kafirin. Thus, it appears that the reason for the high digestibility of the protein bodies for the TG lines is different from that in the HD types.

Suppressed synthesis of the cysteine-rich γ-kafirin subclass in these TG sorghum lines results in a substantially lower proportion of disulfide bonded kafirin polymers. Despite the suppression of synthesis of these and other major kafirin subclasses, there is, however, compensatory synthesis of other kafirins. This results in a normal proportion of kafirins in the grain, which is probably related to their protein bodies being intermediate in appearance between that of the normal type and the characteristic invaginated HD type. The higher protein digestibility of these TG lines is most probably related to their lower levels of disulfide-bonded kafirin polymerization, allowing better access of proteases. This work seems to confirm the theory of Hamaker[28] and Rom et al.[29] that disulfide bond formation in kafirin is responsible for the reduced protein digestibility of cooked sorghum, where it was found that the disulfide bond breaking reducing agents improved sorghum protein[28,29] and protein body digestion.[29]

AUTHOR INFORMATION

Corresponding Author

*Tel.: +27 12 420 4296. Fax: +27 12 420 2839. E-mail: john.taylor@up.ac.za.

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■ ABBREVIATIONS USED

ABS, Africa biofortified sorghum; TG, transgenic sorghum; HD, high protein digestibility mutant; C, nontransgenic null control; IVPD, in vitro protein digestibility; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; PP, protein preparations; MOPS, 3-(*N*-morpholine)propanesulfonic acid

■ REFERENCES

(1) Shewry, P. R. Improving the protein content and composition of cereal grain. *J. Cereal Sci.* **2007**, *46*, 239–250.

(2) Duodu, K. G.; Taylor, J. R. N.; Belton, P. S.; Hamaker, B. R. Factors affecting sorghum protein digestibility. *J. Cereal Sci.* **2003**, *38*, 117–131.

(3) Belton, P. S.; Delgadillo, I.; Halford, N. G.; Shewry, P. R. Kafirin structure and functionality. *J. Cereal Sci.* **2006**, *44*, 272–286.

(4) African Biofortified Sorghum Project (ABS). 2009; http://biosorghum.org/ (accessed 10 March 2010).

(5) Taylor, J.; Taylor, J. R. N. Protein biofortified sorghum: Effect of processing into traditional African foods on their protein quality. *J. Agric. Food Chem.* **2011**, *59*, 2386–2392.

(6) Henley, E. C.; Taylor, J. R. N.; Obukosia, S. D. The importance of dietary protein in human health: Combating protein deficiency in sub-Saharan Africa through transgenic biofortified sorghum. *Adv. Food Nutr. Res.* **2010**, *60*, 21–52.

(7) Da Silva, L. S.; Jung, R.; Zhao, Z.; Glassman, K.; Taylor, J.; Taylor, J. R. N. Effect of suppressing the synthesis of different kafirin subclasses on grain endosperm texture, protein body structure and protein nutritional quality in improved sorghum lines. *J. Cereal Sci.* **2011**, *54*, 160–167.

(8) Zhao, Z.-Y.; Cai, T.; Tagliani, L.; Miller, M.; Wang, N.; Pang, H.; Rudert, M.; Schroeder, S.; Hondred, D.; Seltzer, J.; Pierce, D. *Agrobacterium*-mediated sorghum transformation. *Plant Mol. Biol.* **2000**, *44*, 798–798.

(9) Duodu, K. G.; Nunes, A.; Delgadillo, I.; Parker, M. L.; Mills, E. N. C.; Belton, P. S.; Taylor, J. R. N. Effect of grain structure and cooking on sorghum and maize in vitro protein digestibility. *J. Cereal Sci.* **2002**, *35*, 161–174.

(10) AACC. Crude protein-combustion, Standard Method 46−30. *Approved Methods of the AACC*, 10th ed.; American Association of Cereal Chemists: St Paul, MN, 2000.

(11) Hamaker, B. R.; Kirleis, A. W.; Mertz, E. T.; Axtell, J. D. Effect of cooking on the protein profiles and in vitro digestibility of sorghum and maize. *J. Agric. Food Chem.* **1986**, *34*, 647–649.

(12) Gallagher, S. R., Electrophoretic separation of proteins. In *Current Protocols in Molecular Biology*; Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. E., Seidman, J. G., Smith, J. A., Strule, K., Eds., John Wiley and Sons, New York, 1999; pp 10.2A1−9.

(13) Xie, X. J.; Seib, P. A. Laboratory procedure to wet-mill 100 g of grain sorghum into six fractions. *Cereal Chem.* **2000**, *77*, 392–395.

(14) Jackson, D. S.; Shandera, D. L. Corn wet milling: Separation chemistry and technology. *Adv. Food Nutr. Res.* **1995**, *38*, 271–300.

(15) Oria., M. P.; Hamaker., B. R.; Axtell, J. D.; Huang, C. P. A highly digestible sorghum mutant cultivar exhibits a unique folded structure of endosperm protein bodies. *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 5065–5070.

(16) El Nour, I. N.; Peruffo, A. D. B.; Curioni, A. Characterisation of sorghum kafirins in relation to their cross-linking behaviour. *J. Cereal Sci.* **1998**, *28*, 197–207.

(17) Emmambux, M. N.; Taylor, J. R. N. Properties of heat-treated sorghum and maize meal and there prolamin proteins. *J. Agric. Food Chem.* **2009**, *57*, 1045–1050.

(18) Ezeogu, L. I.; Duodu, K. D.; Taylor, J. R. N. Effects of endosperm texture and cooking conditions on the in vitro starch digestibility of sorghum and maize flours. *J. Cereal Sci.* **2005**, *42*, 33–44.

(19) Taylor, J. R. N.; Novellie, L.; Liebenberg, N., VdW. Sorghum protein body composition and ultrastructure. *Cereal Chem.* **1984**, *61*, 69–73.

(20) Shewry, P. R.; Pandya, M. J. The 2S albumin storage proteins. In *Seed Proteins*; Shewry, P. R.; Casey, R.; Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1999; pp 563−86.

(21) Butler, L. G.; Riedl, D. J.; Lebryk, D. G.; Blyit, H. J. Interaction of proteins with sorghum tannin: Mechanism, specificity and significance. *J. Am. Oil Chem. Soc.* **1984**, *61*, 916–920.

(22) Taylor, J.; Bean, S. R.; Ioerger, B. P.; Taylor, J. R. N. Preferential binding of sorghum tannins with gamma-kafirin and the influence of tannin binding on kafirin digestibility and biodegradation. *J. Cereal Sci.* **2007**, *46*, 22–31.

(23) Taylor, J. R. N.; Novellie, L.; Liebenberg, N.v.d.W. Protein body degradation in the starchy endosperm of germinating sorghum. *J. Exp. Bot.* **1985**, *36*, 1287–1295.

(24) Taylor, J. R. N.; Evans, D. J. Action of sorghum proteinase on the protein bodies of sorghum starchy endosperm. *J. Exp. Bot.* **1989**, *40*, 763–768.

(25) Ng'andwe, C. C.; Hall, A. N.; Taylor, J. R. N. Proteolysis of sorghum endosperm proteins when mashing with raw grain plus exogenous protease and potassium metabisulphite. *J. Inst. Brew.* **2008**, *114*, 343–348.

(26) Shull, J. M.; Watterson, J. J.; Kirleis, A. W. Purification and immunocytochemical location of kafirins in *Sorghum bicolor* (L.) Moench endosperm. *Protoplasma* **1992**, *171*, 64–74.

(27) Oria, M. P.; Hamaker, B. R.; Shull, J. M. Resistance of sorghum α-, β-, and γ-kafirins to pepsin digestion. *J. Agric. Food Chem.* **1995**, *43*, 2148–2153.

(28) Hamaker, B. R.; Kirleis, A. W.; Butler, L. G.; Axtell, J. D.; Mertz, E. T. Improving the in vitro protein digestibility of sorghum with reducing agents. *Proc. Natl. Acad. Sci. U. S. A.* **1987**, *84*, 626–628.

(29) Rom, D. L.; Shull, J. M.; Chandrashekar, A.; Kirleis, A. W. Effects of cooking and treatment with sodium disulfite on in vitro protein digestibility and microstructure of sorghum flour. *Cereal Chem.* **1992**, *69*, 178–181.