Transgenic sorghum with suppressed synthesis of kafirin subclasses: Effects on flour and dough rheological characteristics

Mohammed S.M. Elhassan[a], M. Naushad Emmambux[a], John R.N. Taylor[a]*

[a]University of Pretoria, Institute for Food, Nutrition and Well-being and Department of Food Science, Private Bag X20, Hatfield 0028, South Africa

*Corresponding Author: John R.N. Taylor
Phone: +27 12 4204296
Fax: +27 12 4202839
E-mail: john.taylor@up.ac.za

Keywords: dough rheological properties; kafirin; sorghum; transgenic

Abbreviations used: CLSM – confocal laser scanning microscopy; N – null control sorghum; TG-HD – transgenic sorghum with high protein digestibility,WAI – Water Absorption Index; WSF - Water Soluble Fraction.

Running head: Modified kafirin expression transgenic sorghum flour properties

Abstract

Arising from work showing that conventionally bred high protein digestibility sorghum types have improved flour and dough functionality, the flour and dough properties of transgenic biofortified sorghum lines with increased protein digestibility and high lysine content (TG-HD) resulting from suppressed synthesis of several kafirin subclasses, especially the cysteine-rich γ-kafirin, were studied. TG-HD sorghums had higher flour water solubility at 30°C ($p < 0.05$) and much higher paste viscosity (41% higher) than their null controls (NC). TG-HD doughs were twice as strong as their NC and dynamic rheological analysis indicated that the TG doughs were somewhat more elastic up to 90°C. CLSM of doughs and pastes indicated that TG-HD had a less compact endosperm protein matrix surround the starch compared to their NC. The improved flour and dough functional properties of the TG-HD sorghums seem to be caused by reduced endosperm compactness resulting from suppression of synthesis of several kafirin subclasses which modifies protein body and protein matrix structure, and to improved protein-starch interaction through hydrogen bonding specifically caused by reduction in the level of the hydrophobic γ-kafirin. The improved flour functionality of these transgenic biofortified sorghums can increase their commercial utility by complementing their improved nutritional quality.

1. Introduction

Sorghum's inferior protein quality with respect to the indispensible amino acid lysine and the low digestibility of its protein in wet cooked foods are major factors that limit its utilization when compared to other major cereals (Henley et al., 2010). Another protein related drawback of sorghum with respect the production of dough-based foods is that kafirin, its prolamin protein, does not form a viscoelastic, gas-holding dough in an aqueous system, unlike wheat gluten (Oom et al., 2008; Goodall et al., 2012). The Africa Biofortified Sorghum (ABS) consortium has developed transgenic sorghum lines with improved nutritional quality with respect to protein quality and availability, mineral availability and provitamin A (Biosorghum, undated). Particular ABS lines have been produced with substantially improved lysine content and protein digestibility resulting from suppression of synthesis of specific kafirin subclasses. The Protein Digestibility Corrected Amino Acid Score (PDCAAS) of these protein biofortified sorghums is double that of their null controls (Henley et al., 2010).

A wide range of food products, including porridges, couscous, flatbreads and biscuits have been made with early generation lines of the transgenic protein biofortified sorghums, which were tannin containing (Taylor and Taylor, 2011). Their quality was similar to that of their null controls (also tannin-containing), although their flour properties were not studied. However, Kruger et al. (2012) showed that these transgenic HD sorghums, which also expressed a low phytate trait, give higher extract (starch solubilisation) and free amino nitrogen in brewing, which indicated that they do have improved starch and protein functionality.

With specific respect to dough and bread quality of high protein digestibility sorghums, Goodall et al. (2012) working with conventionally bred high protein digestibility, high lysine (HD) sorghum showed that when it was composited with wheat flour, the dough had greater resistance to extension and time to dough breakage than normal sorghum-wheat composite dough; and also the HD sorghum-wheat composite had similar strain hardening to wheat dough. Furthermore, composite HD sorghum-wheat breads had higher loaf volume than the normal sorghum-wheat composite. Recently, we showed that sorghum lines with combined waxy and high protein digestibility traits, which were produced by conventional breeding, using the same high protein digestibility germplasm as Goodall et al. (2012), had improved flour quality compared to normal sorghum lines (Elhassan et al., 2015). Specifically, they exhibited higher paste viscosity and formed much softer and less sticky pastes and had much higher flour solubility. At 30^{o}C, flour solubility was similar to commercial wheat bread flours. The improvements in flour quality were attributed to the less dense (floury-type endosperm), high amylopectin content and unique endosperm protein composition of the lines.

In this present study, the flour quality of improved transgenic high protein digestibility, high lysine ABS sorghum lines was investigated. The lines were non-tannin, white tan-plant (so-called food grade) types (Da Silva et al., 2011b) and hence potentially more suitable as flour for dough-based food making.

2. Materials and Methods

2.1. Sorghum lines

Crushed whole grain of two non-tannin, white tan-plant, transgenic high protein digestibility (TG-HD) sorghum lines (TG-HD-1 and TG-HD-2) and their normal digestibility null controls (NC1 and NC2) was obtained. The lines were produced through the ABS consortium by DuPont Pioneer, Johnston, Iowa in single controlled field trial.

. The TG-HD sorghum lines have suppressed expression of certain kafirin subclasses by means of RNAi (RNA interference) technology, as described by Da Silva et al. (2011a). The lines were T2 self-ed seeds. TG-HD-1 and -2 were 75% pure with respect to the ABS032 gene construct which suppresses synthesis of α-kafirin A1 and α-kafirin B1and B2 (corresponding to the 19 and 22 kDa α-kafirin classes, respectively), γ-kafirin 1 and 2, and δ-kafirin 2.

2.2. Milling

A laboratory hammer mill fitted with a 250 μm opening screen was used to mill the sorghum samples into whole grain flour. The milled flours were then sealed in zip-lock type polyethylene bags and kept at 8-10°C until used for analysis.

2.3. Extraction of kafirins

For analysis of the kafirin composition of the sorghum lines, total kafirin was extracted essentially as described by Taylor et al. (2005). The TG and NC whole grain flours were extracted with 70% (w/w) ethanol plus 0.35% (w/w) glacial acetic acid and 0.5% (w/w) sodium metabisulphite at 70˚C with vigorous stirring for 1 hour. The supernatant was collected after centrifugation at 1000 x g at 25°C for 5 minutes. The alcohol was allowed to evaporate from the solute and the precipitated protein washed with cold distilled water (<10°C). The recovered protein was separated by filtration and air dried at 25°C.

2.4. Two-dimensional gel electrophoresis (2-DE)

Kafirin composition was analysed by 2-DE as it separates proteins by their isoelectric point in addition to molecular size. The dry kafirin preparations were dialyzed against distilled water for 36 h at 10°C, using dialysis tubing with a 12–14 kDa cut off (Visking ex. Labretoria, Pretoria, South Africa) with frequent changes of water and then freeze dried. Isoelectric focusing was carried out using 7 cm ZOOM® immobilised pH gradient strips with a range of pH 3–10 (Invitrogen, Carlsbad, CA). The strips were focused on a gradient at 200 V for 15 min, 450 V for 15 min, 750 V for 15 min and 2000 V for 70 min using a ZOOM® IPGRunner™ System according to the manufacturer's instructions. The strips were then equilibrated in the equilibration buffer, which contained dithiothreitol (as disulphide bond reducing agent) for 15 min and then in buffer containing the alkylating reagent iodoacetamide (125 mM) for 15 min. SDS-PAGE was carried out using Novex NuPAGE® 4-12% polyacrylamide gradient gels (Invitrogen).

2.5. Protein content

Dumas combustion was used to determine the protein content (N x 6.25) following AACC method 46-30 (AACC International, 2000).

2.6. Starch amylose content

The Megazyme amylose/amylopectin assay kit (Megazyme Ireland International, Bray, Ireland) was used to determine starch amylose/amylopectin ratio.

2.7. In vitro pepsin protein digestibility

In vitro protein digestibility of the flours was determined according to the pepsin digestibility method of Hamaker et al. (1986) as modified by Da Silva et al. (2011a).

2.8. Differential scanning calorimetry (DSC) of flour thermal behaviour

The method described by Beta et al. (2000) as detailed by Elhassan et al. (2015) was used.

2.9. Flour WAI and WSF

The method of by Anderson et al. (1970) was used to determine Water absorption index (WAI) and water soluble fraction (WSF) of the flours at 30°C and 60°C. These temperatures were selected to represent normal dough mixing temperature and an elevated dough mixing temperature, just below the sorghum starch gelatinization temperature (Delcour and Hoseney, 2010).

2.10. Flour pasting properties

A Physica MCR 101 Rheometer (Anton Paar, Ostfildern, Germany) using a cup and a stirrer was used to determine flour pasting properties as detailed by Elhassan et al. (2015).

2.11. Gel strength (texture)

A TA-XT2 type texture analyser (Stable Micro Systems, Godalming, UK) was similarly used as detailed by Elhassan et al. (2015) to determine flour gel texture properties.

2.12. Dough stress-relaxation behaviour

The relaxation properties of the sorghum dough were determined according to the method of Singh et al. (2006) as modified by Falade et al. (2014). A texture analyser (EZ-L, Shimadzu, Kyoto, Japan) was used. Sorghum dough was prepared with 1 g flour and 0.9 g water. Homogeneous discs of doughs of diameter 19 mm and height 7 mm were made using a syringe. To compress the dough disc, a plastic rod (43 mm diam. and 10 mm height) was used at a 25% strain to compress the sorghum dough for 5 s, then the dough was left to relax over a period of 180 s and the maximum force was recorded. Relaxation time was calculated as the time required for the maximum force to drop to 36.8% to its value (Singh et al., 2006).

2.13. Dough dynamic rheological analysis

A Physica MCR 101 Rheometer was used to perform dynamic rheological analysis on the doughs, as described by Falade et al. (2014). A parallel plate configuration (PP25) of 25 mm diameter and 2 mm gap between the top and bottom plates was used. Sorghum dough was prepared with at a 1:1 (w/w) ratio of flour to water. Dough samples were placed between the parallel plates and the edges of the dough pieces were trimmed using a thin spatula. Oscillatory measurements were performed in two steps. Amplitude sweep was measured at constant temperature (25^{o}C) and constant frequency (6.3 rad/s) to determine the linear viscoelastic range. Storage shear modulus (G') and the loss shear modulus (G''), and damping factor/loss tangent ($\tan \delta = G''/G'$) were recorded at increasing strain from 0.01 to 100%. Temperature sweep was determined as a dough baking process simulation at the same constant frequency as the amplitude sweep analysis and a constant strain of 0.1% (within the linear viscoelastic range). A temperature range from 25 to 150°C for 20 min at a heating rate of 6.25°C/min was applied. Mineral oil (paraffin) was used to cover the edges of the dough samples to prevent dehydration.

2.14. Confocal laser scanning microscopy (CLSM)

Confocal laser scanning microscopy (CLSM) was performed using a Zeiss 510 META system (Jena, Germany) with a Plan-Neofluar 10 × 0.3 objective at an excitation wavelength of 405 nm. Four g water was added to 1 g sorghum flour to prepare a batter. To determine the effects of starch gelatinization, the batters were additionally incubated in test tubes at 75°C for 25 minutes in a water bath to form pastes. They were stirred every 5 minutes using a glass rod. A layer of about 2 mm thickness of each batter or paste was placed on a glass slide and left for 3 minutes at ambient temperature. Three drops 0.02% Acid Fuchsin dye in 1% acetic acid was used to stain the protein (Autio et al., 2005). The samples were then incubated for 1 minute into an oven at 60°C to fix the dye before CLSM.

2.15. Statistical analysis

Data were analysed using IBM SPSS Statistics 22 (SPSS, Chicago, IL). One-way analysis of variance (ANOVA) was applied at a confidence level of $p = 0.05$ to determine the effect of the kafirin alteration on the applicable parameters. Means were compared by Fisher's least significant difference (LSD) test. Each experiment was repeated at least two times.

3. Results and discussion

3.1. 2-D electrophoresis

Both the transgenic sorghum lines (TG) and their null controls (NC) exhibited a major row of protein spots of apparent relative molecular weight (M_r) approx. 26 kDa and a minor row (M_r approx. 21 kDa) of kafirin polypeptide spots, which were predominantly of basic isoelectric point (pI) (Figure 1). These correspond primarily to the α-1 and α-2 kafirin subclass monomers (Bean and Ioerger, 2015). The major difference between the TG lines an NC lines, was that spots of M_r approx. 25 kDa with basic pI were largely absent in TG-HD-2 and reduced in intensity in TG-HD-1 (dashed arrow). This was due to down regulation of γ-kafirin synthesis in these TG-HD lines (Da Silva et al., 2011a). TG-HD-1, however, additionally exhibited a row of very minor polypeptide spots of approx. M_r 23 kDa (dotted arrow) between α-1 and α-2 kafirin polypeptides. This is evidence of compensatory up regulation of synthesis of other kafirins (Da Silva et al., 2011a).

3.2. Proximate composition, starch amylose content and protein digestibility

The TG lines and their null controls had similar proximate composition (Table 1S). Their protein contents were very similar, average 10.6%, and 10.8%, respectively, as were their mineral contents. The NCs had on average a slightly higher fat content, 3.1% compared to the TG lines, 2.1%. In terms of starch amylose content TG-HD-1, NC1 and NC2 had normal starch amylose contents in the range of 19-20% (Table 1S). TG-HD-2 indicated a heterowaxy trait with a starch amylose content of 14.9%. This heterowaxy trait could be due to mutation during the

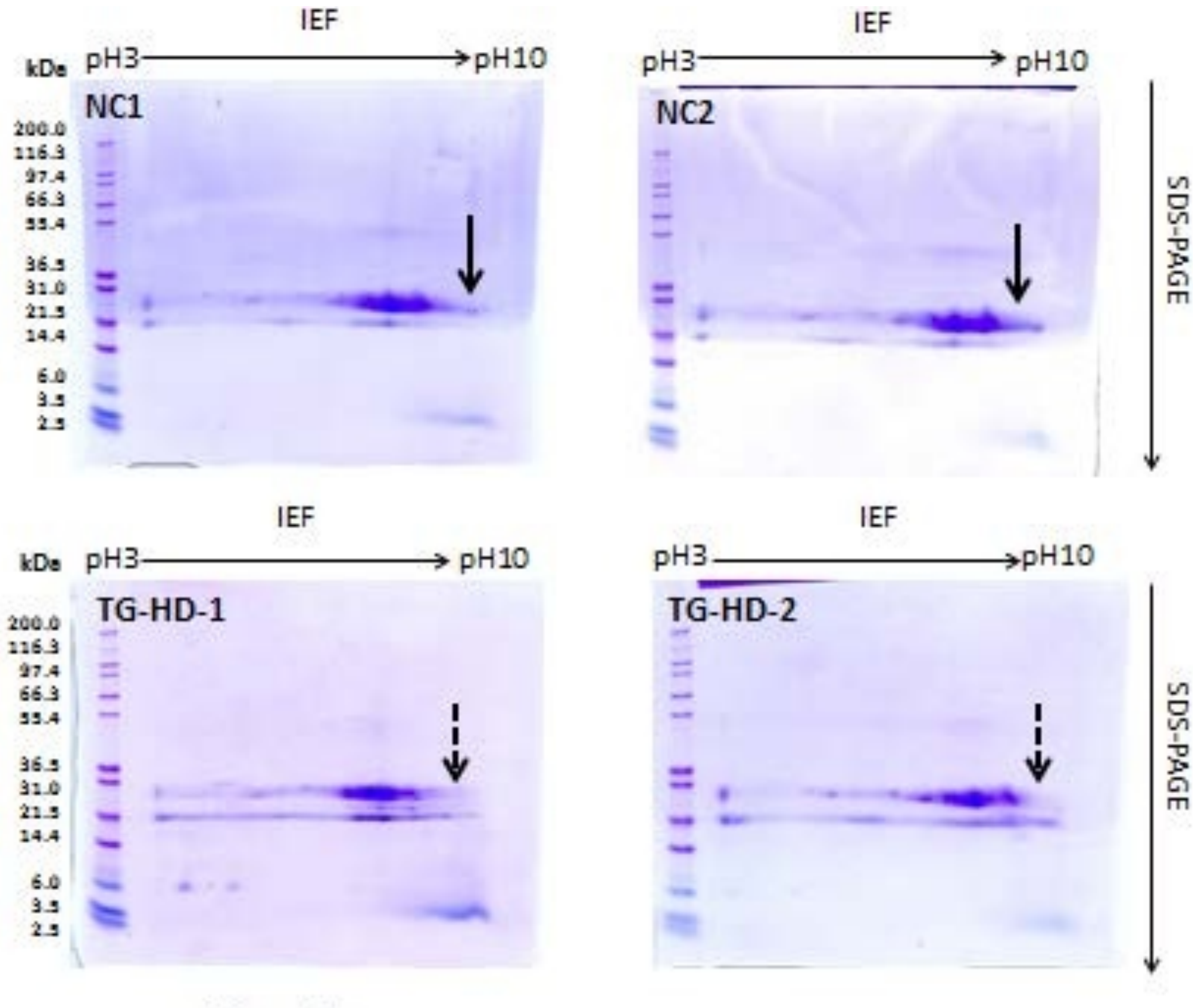


Fig. 1. 2-D electrophoresis of the kafirins extracted from the TG-HD sorghums and their null controls (NCs). Solid arrows = 25 kDa spots present in NCs, Dashed arrows 25 kDa spots largely absent in TD-HD lines.

genetic modification and back-crossing processes. Hence, these TG-HD sorghums were not waxy types, unlike the conventionally bred sorghum lines with both the HD and waxy traits investigated by Elhassan et al. (2015) but more similar to the conventionally bred HD sorghum studied by Goodall et al. (2012). Table 1S also confirms that the TG-HD sorghums had substantial higher ($p < 0.05$) in vitro protein digestibility than their N controls in both raw and wet cooked flour form. Thus, in terms of chemical composition, the TG lines only differed substantially from their NCs with respect to the high protein digestibility trait being studied.

,Da Silva et al. (2011a) attributed the high protein digestibility of these transgenic lines to the co-suppression of synthesis of several kafirin subclasses, which resulted in modified protein body and protein matrix structure. The protein bodies in these TG sorghums are irregular shaped with few to numerous invaginations and they are less densely packed than in their N controls but with a thick protein matrix around them. As a result of these changes, the endosperm is floury, whereas it is largely corneous in the N controls. Notably, the invaginated protein body structure is also a characteristic of conventionally bred HD sorghum lines (Oria et al., 2000; Da Silva et al., 2011a; Elhassan et al., 2015).

3.3. Flour thermal characteristics

The general trend of the flour thermal parameters (Onset (T_0), peak (T_p) and endset (T_c) temperatures and enthalpy (ΔH) measured by Differential scanning calorimetry (DSC) suggests that the TG-HD sorghums had slightly higher starch gelatinization temperature and larger enthalpy compared to their N controls (Table 1). Sorghum starch thermal properties are affected by the type (Sang et al., 2008), possibly as a consequence of amylopectin double helix length. Elhassan et al. (2015) reported that the waxy (high amylopectin) trait significantly ($p < 0.05$) increased the starch thermal properties of conventionally bred HD sorghum lines. This current work indicates that sorghum flour starch thermal properties are also slightly affected by suppression of synthesis of certain kafirin subclasses, particularly γ-kafirin, probably as a consequence of the effect that this has on endosperm texture, and hence flour properties.

3.4. Flour water hydration, pasting and gel properties

Table 1 shows that TG-HD sorghums had a substantially higher ($p < 0.05$) water soluble fraction (WSF), approx. 18% higher, than their N controls at 30^{o}C. The increase in the WSF of the TG-HD flours was presumably a result of the higher hydrophilicity, or strictly speaking reduced hydrophobicity, of the TG-HD kafirin as a result of suppression of synthesis of the cysteine-rich γ-kafirin (Figure 1). This leads to notably reduced disulphide bonded polymerisation of the kafirins in these transgenic lines (Da Silva et al., 2011a). Furthermore, the simple reduction in the level of cystine in the endosperm could have been involved as cystine is a hydrophobic amino acid (Nagano et al., 1999). TG-HD-2 had a higher WSF than TG-HD-1, which can be ascribed to its higher amylopectin content. In waxy type starch the amylopectin branches can bind with more water molecules through the hydrogen bonding (Wootton and Bamunuarachchi, 1978). At 60^{o}C TG-HD-2 had a significantly higher WSF (about 14%) than both N controls, which can also be attributed to its heterowaxy trait. As reported by Elhassan et al. (2015) with the conventionally bred sorghums with HD and waxy traits, there was increase in WSF in the waxy lines. There was no clear difference between TG-HD lines and their null controls in terms of flour WAI.

Table 1 Flour thermal properties, water absorption and solubility of the transgenic sorghums and their null controls

Sorghum type	**Onset temp. (°C)**	**Peak temp. (°C)**	**Endset temp. (°C)**	**Enthalpy (J/g)**	**WAI (g/g)[4] at 30°C**	**WAI (g/g) at 60°C**	**WSF (%)[5] at 30°C**	**WSF (%) at 60°C**
TG-HD-1	70.44^{b} ± 0.17	75.59^{b} ± 0.12	81.79^{b} ±0.40	3.1^{b} ±0.2	2.42^{a}[1,2] ± 0.30	2.34^{a} ± 0.05	5.66^{c} ± 0.06	5.97^{b} ± 0.16
TG-HD-2	69.74^{a} ± 0.06	75.00^{ab} ± 0.47	81.39^{ab} ±0.48	3.1^{b} ±0.0	2.32^{a} ± 0.04	2.53^{b} ± 0.03	6.21^{d} ±0.14	6.68^{d} ± 0.07
NC1	69.39^{a} ±0.01	74.92^{ab} ±0.12	81.43^{ab} ±0.13	2.7^{a} ±0.1	2.31^{a} ±0.01	2.47^{b} ±0.00	4.92^{a} ±0.04	5.53^{a} ± 0.03
NC2	69.37^{a} ± 0.20	74.59^{a} ± 0.12	80.74^{a} ±0.20	3.0^{b} ±0.1	2.33^{a} ±0.00	2.46^{b} ± 0.02	5.25^{b} ±0.01	6.34^{c} ± 0.02

[1]Means and Standard deviation. n = 2

[2]Means with different superscript letters within a column are significantly different ($p < 0.05$).

[3]Water Absorption Index

[4]Water Soluble Fraction

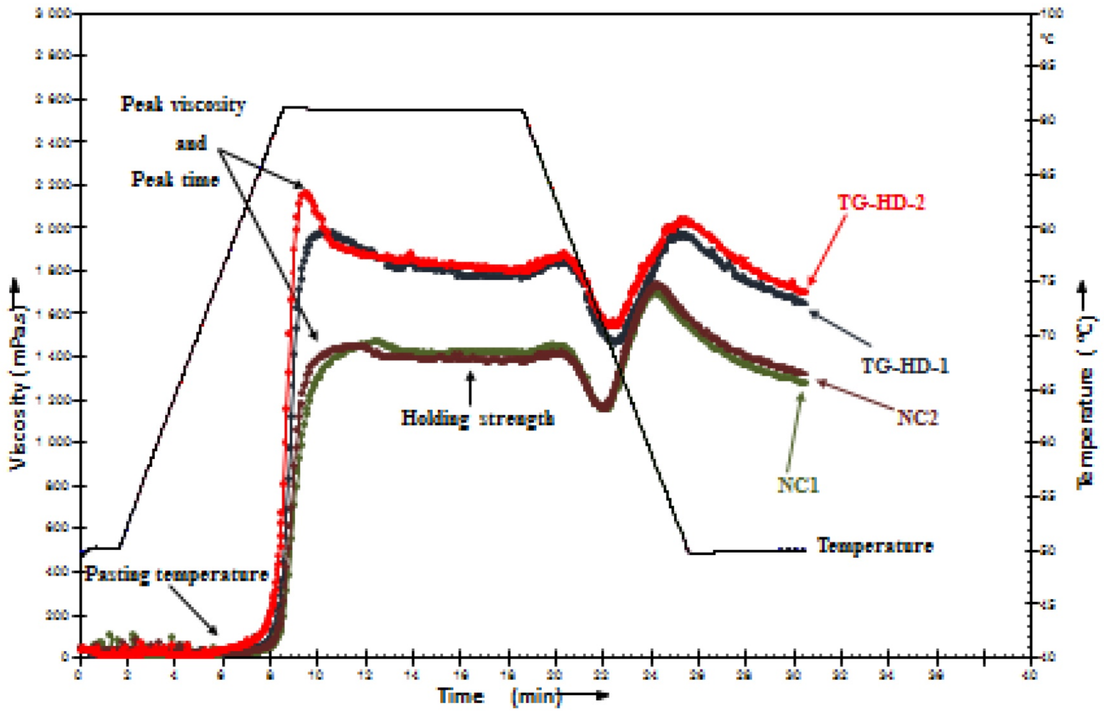


Fig. 2. Flour pasting curves of the TG-HD sorghums and their null controls.

Regarding pasting properties, TG-HD sorghums had much higher ($p < 0.05$) peak viscosity, holding strength, breakdown viscosity and final viscosity than their N controls (Table 2 and Figure 2.A). The TG-HD sorghums also had a significantly shorter peak time and lower pasting temperature and setback. Notably, the peak viscosity of TG-HD sorghums was substantially higher (about 41% higher) than their null controls. The higher peak viscosity of the TG-HD sorghums can be attributed to the less dense, non-compact endosperm protein matrix in these mutants (Da Silva et al., 2011b). The dense protein matrix in the corneous endosperm of normal sorghum can act as a barrier and retard starch granule expansion (Ezeogu et al., 2008). This protein barrier was presumably also the reason for the longer peak time and higher pasting temperature of the N controls compared to TG-HD sorghums. When the protein matrix is less dense this would provide more space for the starch granules to swell and lead to higher peak viscosity. Notably, TG-HD-2 also had significantly higher peak viscosity than its counterpart TG-HD-1 (Table 2). This was probably due to the heterowaxy trait of TG-HD-2. A similar result was obtained by Elhassan et al. (2015) where sorghum lines with the waxy (low amylose) trait displayed higher peak viscosity. The higher peak viscosity is due to the greater swelling of the more amylopectin-rich starch granules (Tester and Morrison, 1990).

There was no significant difference the flour gel strength between TG-HD lines and their null controls (Table 2). However, TG-HD-2 with heterowaxy trait showed the lowest gel strength, indicating that the gel texture of sorghum flour was affected by starch type than the endosperm protein. The low gel strength of the heterowaxy line can be attributed to the slower retrogradation of amylopectin compared to amylose. Sang et al. (2008) found that normal sorghum starch retrogrades more rapidly than heterowaxy and waxy sorghum starches.

3.5. Dough rheological properties

When subjected to dough stress-relaxation testing, the doughs of the TG-HD sorghums exhibited considerably higher maximum force during compression (approx. twice as high) as their N controls (Figure. 3A). This shows that the TG-HD sorghum doughs were much stronger. This can be attributed to the more hydrophilic nature of the kafirin proteins which would enable greater hydrogen bonding between the proteins and starch, and proteins and water. Furthermore, the TG sorghums exhibited shorter relaxation time (average 5.4 seconds) than the N1 (6.1 seconds) and N2 (6.6 seconds) lines. Thus, it can be implied that because of their modified kafirin composition resulting in greater hydrogen bonding, the TG sorghum doughs were stronger than their N controls.

With regard to the dynamic rheological properties of the doughs, the storage shear modulus (G') (Figure 3B.I) and loss shear modulus (G'') (Figure 3.B.II) of the TG-HD sorghums were higher than their N controls when subjected to amplitude sweep measurement. Higher G' relates to a more elastic dough, while higher G'' refers to higher viscosity (Dobraszczyk and Morgenstern, 2003; Oom et al., 2008). Shear stress plotted against strain amplitude showed that the N sorghums had lower shear stress (Figure 3B.III), which can be due to their softer doughs (Figure 3A). This is similar to what Falade et al. (2014) found working with maize doughs, where dough containing sourdough had a lower shear stress than untreated maize dough. In this present work,

Table 2. Flour pasting, gel texture and dough relaxation properties of the transgenic sorghums and their null controls

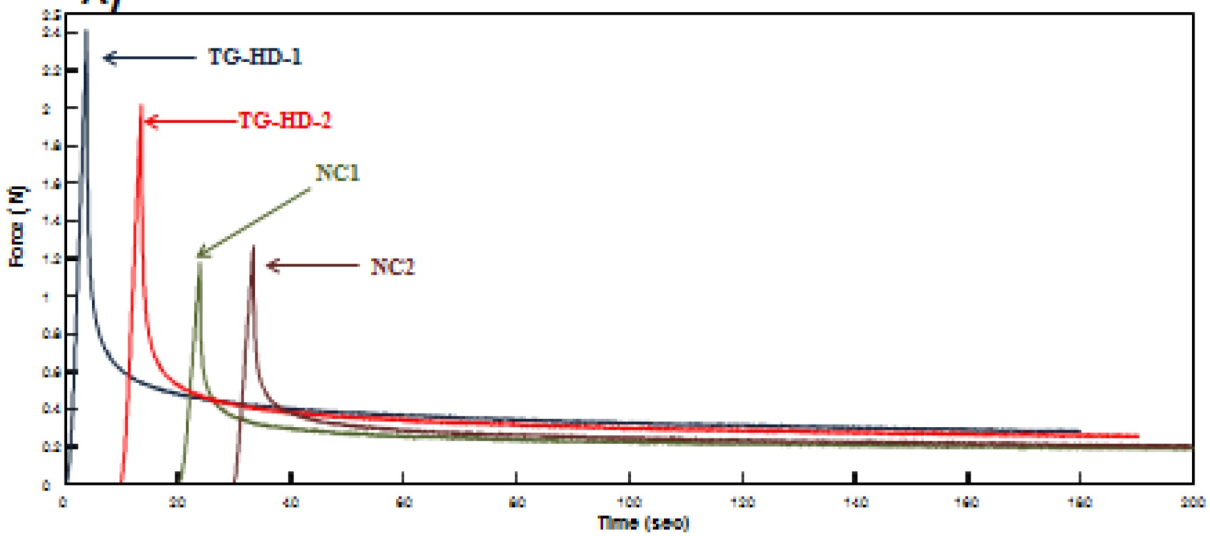
A. Flour pasting characteristics

Sorghum type	Peak time min	Pasting temp. (°C)	Peak viscosity (mPa.s)	Holding strength (mPa.s)	Breakdown (mPa.s)	Setback (mPa.s)	Final viscosity (mPa.s)
TG-HD-1	5.70[a][1,2] ±0.09	74[a] ± 1	1985[b] ± 9	1776[b] ± 5	209[b] ± 4	120[b] ± 4	1656[c] ± 9
TG-HD-2	5.50[a] ±0.07	73[a] ± 0	2149[c] ± 12	1786[b] ± 16	363[c] ± 5	127[b] ± 3	1659[c] ± 14
NC1	7.36[b] ±0.11	84[b] ±1	1472[a] ± 4	1405[a] ± 12	67[a] ± 8	148[b] ± 24	1257 ± 36
NC2	7.49[b] ±0.07	85[b] ± 0	1458[a] ± 12	1389[a] ± 8	69[a] ± 4	58[a] ± 8	1331[b] ± 0

[1]Means and Standard deviation. n = 2

[2]Means with different superscript letters within a column are significantly different ($p < 0.05$).

A)



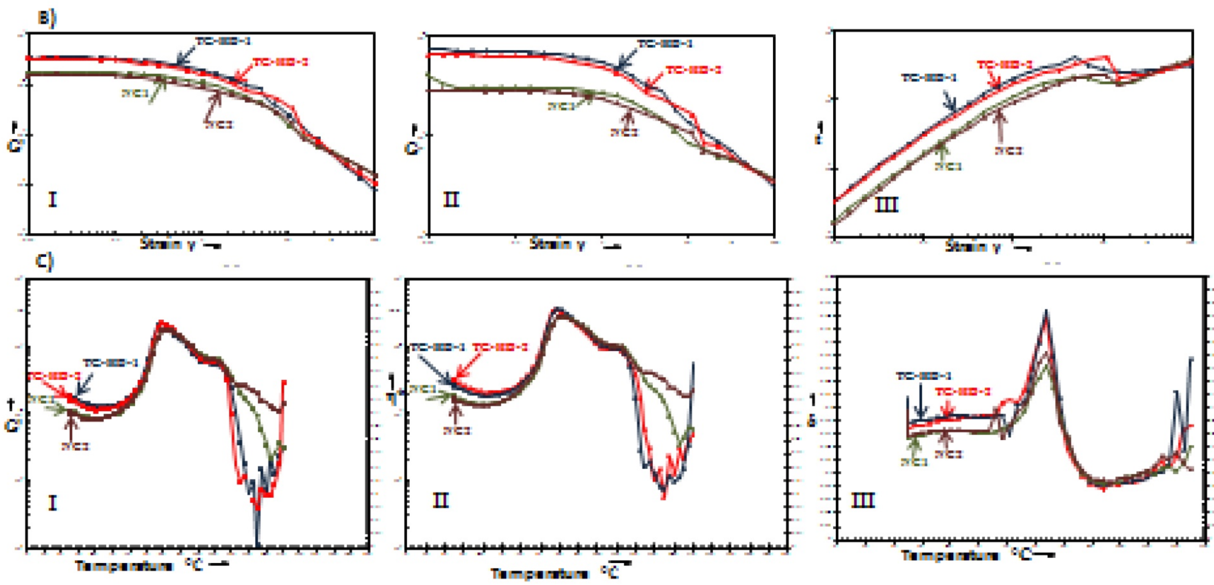


Fig. 3. Rheological properties of the TG-HD sorghum doughs and their null controls.
A) Stress-relaxation test.
B) Dynamic properties: Amplitude sweep mode over a strain of 0.01–100%. I. Storage modulus, II. Loss modulus, III. Shear stress.
C) Dynamic Properties: Temperature sweep mode over a temperature range of 25–150 °C. I. Storage modulus, II. Complex viscosity. III. Loss tangent.

the softer doughs related to a less elastic behaviour as indicated by the lower G′ of the N sorghums when compared to the TG-HD sorghums.

Storage shear modulus (Figure 3C.I), complex viscosity (η*) (Figure 3C.II) and loss tangent (tan δ) (Figure 3B.III) of the TG-HD sorghum doughs were higher than their null controls at low temperatures when subjected to temperature sweep measurement. Concerning G′ and G″, within the range of 25^{o}C to about 90^{o}C, the G′ of the TG-HD lines were higher than their null controls, which indicates higher viscoelastic behaviour at this temperature range (Dobraszczyk and Morgenstern, 2003; Oom et al., 2008) Above 90^{o}C, the G′ of both the TG-HD and NC lines declined. However, above approx. 110^{o}C the G′ and G″ of the TG-HD doughs more rapidly declined to become considerably lower than their null controls. The lower G′ of TG-HD doughs at high temperature could be due to less rapid drying compared to their null controls as the TG-HD dough bound more water. As stated, modification of endosperm protein in the TG-HD lines resulted in a floury endosperm texture as well as higher water binding capacity.

Complex viscosity (η*) (Figure 3C.III) followed a very similar pattern to G′ over the temperature range. The higher η*, higher G′ and G″ of the TG-HD doughs compared to their null controls at lower temperatures suggests that the TG-HD dough had better viscoelastic properties, as explained by Edwards et al. (2003) with reference to durum semolina dough. At the low temperature range (25 to about 80°C), the loss tangent (tan δ) of the TG-HD lines was higher than their N controls (Figure 3.B.III), a high loss tangent suggests a moist and slack dough (Lazaridou et al., 2007).

3.6. Sorghum batter and paste microstructure

Confocal laser scanning microscopy (CLSM) indicated that the TG-HD sorghum flour batters (prepared at ambient temperature) displayed a considerably less dense protein matrix (less red stain, dashed arrows) compared to their null controls (more red stain, solid arrows) (Figure 4A). The pattern of dense less protein matrix for the TG-HD sorghums compared to their null controls also occurred with the flour pastes (heated to 75^{o}C, above starch gelatinization temp.) (Figure 4B). Thus, the change in endosperm protein structure due to the suppression of synthesis of γ-kafirin in particular (Da Silva et al., 2011b) was maintained when the flours were hydrated with water into a batter and subsequently cooked into a paste. The less dense endosperm protein matrix of the TG-HD sorghum lines presumably enabled better interaction between the proteins and starch in water to form a dough of higher strength (Figure 3A) with somewhat improved viscoelastic properties (Figure 3B,C).

4. Conclusions

The transgenic sorghums with suppressed expression of several kafirin subclasses, in particular γ-kafirin, exhibit improved flour functional properties, in particular higher flour water solubility, higher pasting viscosity, considerably stronger doughs and somewhat improved dough elasticity. These effects are presumed to be due to a combination of their less dense endosperm due to suppression of several kafirin subclasses which modifies the protein body and protein matrix structure, and to improved protein-starch interaction through hydrogen bonding as a specific result of reduction in the level of the hydrophobic γ-kafirin. However, the magnitude of flour

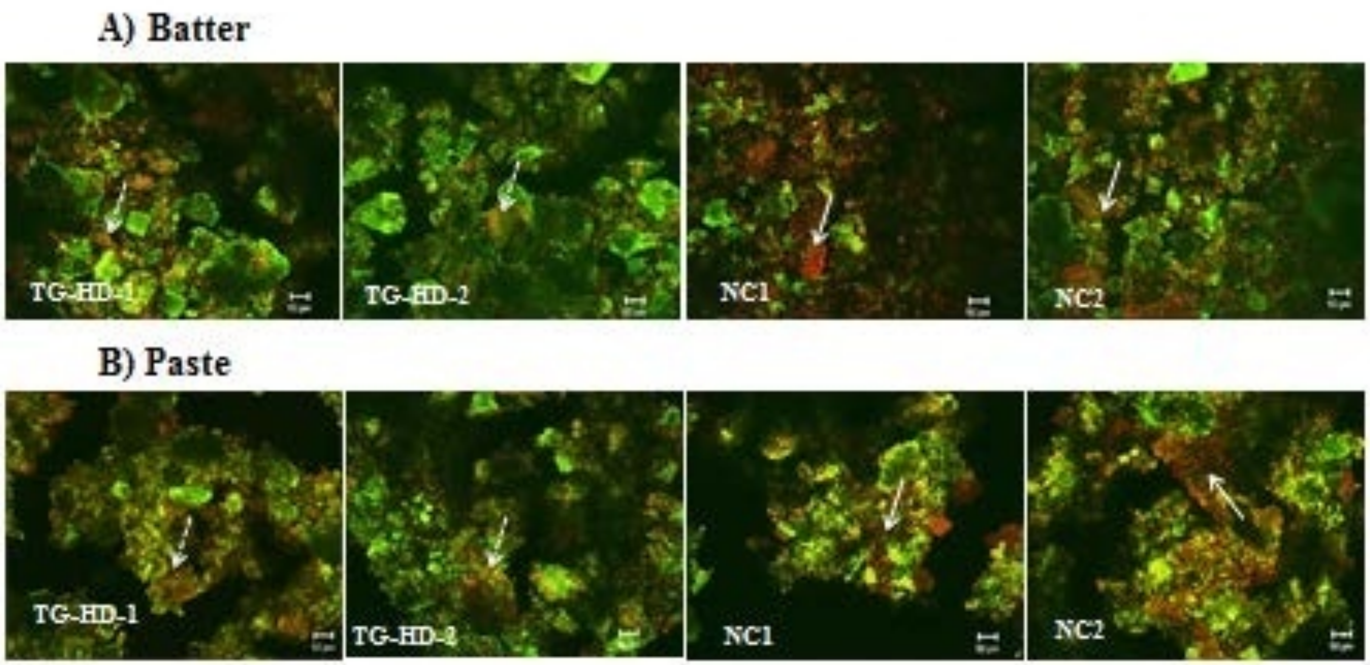


Fig. 4. CLSM of the microstructure of the batters and pastes from the TG-HD sorghums and their null controls.
A) Batters prepared at ambient temperature. TG-HD-1 and TG-HD-2, dashed arrows indicate less dense protein matrix (red colour). NC1 and NC2, solid arrows indicate more dense protein matrix (red colour).
B) Pastes prepared at 75 °C. TG-HD-1 and TG-HD-2, dashed arrows indicate less dense protein matrix (red colour). NC1 and NC2, solid arrows indicate more dense protein matrix (red colour) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

functionality improvements by suppression of kafirin subclass synthesis alone seems to be somewhat less than that obtained by combining the waxy (high-amylopectin) trait with the conventional HD type mutant (Elhassan et al., 2015). Notwithstanding this, the improvement in the flour functionality of these transgenic biofortified sorghums as a result of kafirin expression modification can potentially improve their commercial utility particularly for bread making by complementing their improved nutritional quality. The TG-HD sorghum lines have similar properties to the non-transgenic high protein digestibility-high lysine sorghum lines studied by Goodall et al. (2012) with respect to improved rheological properties compared to the normal sorghum. Thus, it is predicted that TG-HD sorghum flour will behave similarly to that of high protein digestibility-high lysine sorghum flour with regard to improved sorghum-wheat composite bread quality compared to normal sorghum flour.

Acknowledgements

The Howard G. Buffett Foundation through the Norman E. Borlaug Institute for International Agriculture of Texas A&M University. The Bill and Melinda Gates Grand Challenges 9, Africa Biofortified Sorghum Project through a sub-grant from the Africa Harvest Biotechnology Foundation International. The University of Pretoria for a Research Support Bursary for MSM Elhassan. Mr Alan Hall of the Laboratory for Microscopy and Microanalysis for assistance with the CLSM.

References

Autio, K., Kruus, K., Knaapila, A., Gerber, N., Flander, L., Buchert, J., 2005. Kinetics of transglutaminase-induced cross-linking of wheat proteins in dough. J. Agric. Food. Chem. 53, 1039-1045.

Bean, S. R., Ioeger, B.P., 2015. Sorghum and millet proteins. In: Ustunol, Z. (Ed.), Applied Food Protein Chemistry. John Wiley & Sons, Oxford, pp. 323-362.

Beta, T., Corke, H., Rooney, L.W., Taylor, J.R.N., 2000. Starch properties as affected by sorghum grain chemistry. J. Sci. Food Agric. 81, 245-251.

Biosorghum., undated. ABS Project: Technology development. www.biosorghum.org (Accessed July 2016).

Da Silva, L.S., Taylor, J., Taylor, J.R.N., 2011a. Transgenic sorghum with altered kafirin synthesis: kafirin solubility, polymerization, and protein digestion. J. Agric. Food Chem. 59, 9265-9270.

Da Silva, L.S., Jung, R., Zhao, Z., Glassman, K., Grootboom, A.W., Mehlo, L., O'Kennedy, M. M., Taylor, J., Taylor, J.R.N., 2011b. Effect of suppressing the synthesis of different kafirin sub-classes on grain endosperm texture, protein body structure and protein nutritional quality in improved sorghum lines. J. Cereal Sci. 54, 160-167.

Delcour, J.C., Hoseney R.C., 2010. Principles of Cereal Science and Technology, 3rd ed. AACC International, St. Paul, MN, Table2.1.

Dobraszczyk, B.J., Morgenstern, M.P., 2003. Rheology and the breadmaking process. J. Cereal Sci. 38, 229-245.

Edwards, N. M., Mulvaney, S. J., Scanlon, M. G., Dexter, J.E., 2003. Role of gluten and its components in determining durum semolina dough viscoelastic properties. Cereal Chem. 80, 755-763.

Elhassan, M.S.M., Emmambux, M.N., Hays, D.B., Peterson, G.C., Taylor, J.R.N., 2015. Novel biofortified sorghum lines with combined waxy (high amylopectin) starch and high protein digestibility traits: Effects on endosperm and flour properties. J. Cereal Sci. 65, 132-139.

Ezeogu, L.I., Duodu, K.G., Emmambux, M.N., Taylor, J.R.N., 2008. Influence of cooking conditions on the protein matrix of sorghum and maize endosperm flours. Cereal Chem. 85, 397-402.

Falade, A.T., Emmambux, M.N., Buys, E.M., Taylor, J.R.N., 2014. Improvement of maize bread quality through modification of dough rheological properties by lactic acid bacteria fermentation. J. Cereal Sci. 60, 471-476.

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

ICRISAT., undated. Sorghum. http://exploreit.icrisat.org (accessed July 2016).

Kruger, J., Oelofse, A., Taylor, J., Taylor, J.R.N., 2012. Potential for improvement in yeast nutrition in raw whole grain sorghum and maize lager brewing and bioethanol production through grain genetic modification and phytase treatment. J. Inst. Brew. 118, 70-75.

Lazaridou, A., Duta, D., Papageorgiou, M., Belc, N., Biliaderis, C.G., 2007. Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations. J. Food Eng. 79, 1033-1047.

Mertz, E.T., Hassen, M.M., Cairns-Whittern, C., Kirleis, A.W., Tu, L., Axtell, J.D., 1984. Pepsin digestibility of proteins in sorghum and other major cereals. Proc. Natl. Acad. Sci. USA 81, 1-2.

Nagano, N., Ota, M., Nishikawa, K., 1999. Strong hydrophobic nature of cysteine residues in proteins. Fed. Eur. Biochemical Soc. 458, 69-71.

Oom, A., Pettersson, A., Taylor, J.R.N., Stading, M., 2008. Rheological properties of kafirin and zein prolamins. J. Cereal Sci. 47, 109-116.

Oria, M.P., Hamaker, B.R., Axtell, J.D., Huang, C.P., 2000. A highly digestible sorghum mutant cultivar exhibits a unique folded structure of endosperm protein bodies. Proc. Natl. Acad. Sci. USA 97, 5065-5070.

Sang, Y., Bean, S., Seib, P.A., Pedersen, J., Shi, Y.C., 2008. Structure and functional properties of sorghum starches differing in amylose content. J. Agric. Food Chem. 56, 6680-6685.

Singh, H., Rockall, A., Martin, C. R., Chung, O. K., Lookhart, G. L., 2006. The analysis of stress relaxation data of some viscoelastic foods using a texture analyzer. J. Text. Stud. 37, 383-392.

Taylor, J., Taylor, J.R.N., 2011. Protein biofortified sorghum: effect of processing into traditional African foods on their protein quality. J. Agric. Food Chem. 59, 2386-2392.

Taylor, J., Taylor, J. R. N., Dutton, M .F., De Kock, S. 2005. Glacial acetic acid - A novel food-compatible solvent for kafirin. Cereal Chem. 82, 485-487.

Tester, R.F., Morrison, W.R., 1990. Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose, and lipids. Cereal Chem. 67, 551-557.

Wootton, M., Bamunuarachchi, A., 1978. Water binding capacity of commercial produced native and modified starches. Starch-Stärke 30, 306-309.

Appendix A. Supplementary data

Table 1S. Flour starch amylose content, in vitro pepsin protein digestibility, protein, fat and mineral contents of the transgenic sorghums and their null controls

Sorghum type	Amylose (% of starch)	Protein digestibility of raw flour (%)	Protein digestibility of cooked flour (%)	Protein (g/100 g db)	Fat (g/100 g db)	P (mg/100 g db)	Ca (mg/100 g db)	Fe (mg/100 g db)	Zn (mg/100 g db)
TG-HD-1	19.3^{b}[1,2] ±0.0	89.5^{b} ± 0.5 (31.0)[3]	73.7^{b} ± 0.7 (41.7)	10.4	2.6	403	41.3	6.4	13.1
TG-HD-2	14.9^{a} ±0.1	90.2^{b} ± 0.6 (30.0)	70.5^{b} ± 1.8 (29.8)	10.7	1.6	399	45.3	5.7	13.4
NC1	19.0^{b} ±1.3	68.4^{a} ± 1.0	52.0^{a} ± 2.0	11.1	2.6	363	38.0	6.8	11.7
NC2	20.1^{b} ±0.0	69.4^{a} ± 2.0	54.3^{a} ± 3.3	10.5	3.5	422	44.3	5.5	14.0

[1]Means and Standard deviation. For PD n= 3, for other data n = 2,

[2]Means with different superscript letters within a column are significantly different (p <0.05).

[3]Values in brackets are percentage increase relative to the null controls