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Transglutaminase Shows Better Functionality on High Digestible, High Lysine Sorghum-Wheat Composite Dough and Bread, Compared to Normal Sorghum-Wheat Composites[#]

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ABSTRACT

Highly digestible high-lysine (HDHL) sorghum-wheat composites have previously been shown to produce better composite dough and bread compared to normal sorghum-wheat composites. This study aimed to test whether improved HDHL lines can provide further enhanced functionality through the effects of transglutaminase (TG) enzyme to improve dough rheological properties. Sorghum-wheat composite doughs were made using HDHL and normal sorghum flours at substitution levels of 10, 20, and 30%, with and without 0.15% TG. Rheological properties of dough were tested using a mechanical spectrometer at 0.05% strain amplitude (within the linear viscoelastic region) over a 0.01- 50 rad/sec frequency range. A more elastic system was observed in HDHL sorghum-wheat composites above 10% substitution levels compared to normal sorghum-wheat composite dough. Addition of TG to HDHL sorghum-wheat composites resulted in a decrease in phase angle values at all substitution level, indicating that TG increased the dough elasticity. However, TG did not change viscoelastic properties of normal sorghum-wheat composites. Bread from HDHL sorghum-wheat composites had significantly higher ($P<0.05$) loaf volume, compared to those made from normal sorghum-wheat composites. These results clearly show that HDHL sorghum has ability to produce dough with improved viscoelastic properties and higher quality bread; and, due to presence of protein body kafirins, addition of TG on HDHL sorghum-wheat composite further enhances dough rheological properties.

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Introduction

Sorghum (*Sorghum bicolor* L.) is an important cereal food crop grown in the semi-arid regions of the world. Although the majority of sorghum grown is used for animal feed, ethanol production and industrial applications, there has been an increasing interest in utilization of sorghum for human consumption due to its comparably low cost and its potential health benefits, including antioxidant activity, slowly digestible starch, anti-inflammatory and anti-carcinogenic effects (Awika and Rooney, 2004; Rooney and Awika, 2005; Taylor et al., 2006).

Cakes, cookies, pasta, rice-like product, porridges, snack foods, and beverages have been successfully produced from sorghum (Rooney and Awika, 2005). However, production of sorghum bread remains the main challenge as gluten-free doughs are similar to cake batters in viscosity (Cauvain, 1998) and cannot entrap gas produced during the leavening. A major opportunity for expanding the use of sorghum in Africa, and to create larger markets for sorghum for local farmers, is in composite breads containing both wheat and sorghum. Studies on wheat-sorghum composite breads have revealed that acceptable breads can be achieved when sorghum is used at a substitution level of 15-20% (Dendy, 1970; Kim and Deruiter, 1968; Schober et al., 2005). Higher substitution levels resulted in formations of dough with impaired rheological properties and firmer breads with decreased loaf volume. These negative effects of sorghum flour on dough rheology and bread quality are mainly attributed to its storage proteins, kafirins, that lack good gluten-like properties, but also are encapsulated in rigid protein bodies that do not break apart during dough mixing due to strong cross-linking associations, thereby inhibiting their possible functional role (Goodall et al., 2012).

In addition, strong cross-linking associations of kafirins in the protein body diminishes the activities of digestive enzymes, making kafirin proteins considerably less digestible (Oria et al., 1995). For example, apparent protein digestibility of sorghum protein was found to be 46% in children, whereas that of wheat proteins was 81% (Axtell et al., 1981). Although low protein digestibility of sorghum proteins can be partially reversed by using reducing agents that reduce disulfide cross-linkages (Elkhalifa et al., 1999; Hamaker et al., 1987), this increases the overall cost of production and may negatively impact the sensory attributes. Moreover, growing consumers' demands for clean label increased the need of sorghum lines having more-digestible kafirins. For such purpose, a high digestible sorghum genotype was identified at Purdue University, named high digestible, high lysine (HDHL) sorghum (Ejeta et al., 1987; Oria et al., 2000; Weaver et al., 1998). HDHL sorghum has altered folded protein body shape, rather than normal spherical morphology, which makes kafirin proteins more accessible for enzyme attack (Oria et al., 2000).

A previous study indicated that the increase in the accessibility of kafirins also results in improvement in the functionality of sorghum proteins. Specifically, when incorporated into the wheat-sorghum composite bread formulations, HDHL lines provided better functionality by improving the viscoelastic properties of dough and final bread quality, compared to normal sorghum lines (Goodall et al., 2012). Here, we conducted a study to investigate whether the functionality of HDHL-sorghum proteins could be further enhanced by addition of a cross-linking agent to improve the viscoelastic properties of dough and final bread quality.

One of the cross-linking agents that is widely used in bakery industry is transglutaminase (TG) (Meertz et al., 2017). TG have the ability to create cross-links between the gluten chains. Specifically, TG is an enzyme that catalyzes covalent interactions between lysine and glutamine, resulting in permanent ε (γ-glutamyl) lysine isopeptic bonds between the gluten chains, and improves the properties of gluten (Meertz et al., 2017). TG has been widely used to improve the gluten quality in bakery products (Meertz et al., 2017). Moreover, TG addition has been reported to increase the level of non-wheat cereals incorporation in wheat flour breads (Basman et al., 2003). The purpose of this study was to investigate whether addition of TG on HDHL sorghum-wheat composite flour trigger improving the viscoelastic properties of dough and final bread quality.

Materials and Methods

Sorghum Flour

Sorghum flours were prepared from normal sorghum (P721N), and HDHL sorghum (4312) grains, as previously described (Goodall et al., 2012). Briefly, grains were decorticated to remove about 10% of their weight, were pin milled (Alpine American Corp., Natick, MA), followed by passing through the reduction roll of a roller mill. The obtained flour was then sieved using 54GG sieve, which has 315 µm mesh opening, to remove the large particles.

Composite Dough and Bread Preparation

Sorghum flour was combined with organic wheat flour at three different substitution levels (10, 20, 30%) before mixing. Dough samples were formed using the following ingredients: 2% yeast (Fleischmann's Active Dry Yeast, San Francisco, CA), 2% salt (Morton International, Inc., Chicago, IL), 2% soybean oil, and 54 – 62% water. Mixing properties of dough, mixing time and water needed for optimum dough formation, were determined using a 35-g Swanson-Working mixograph (National Manufacturing Co., Lincoln, NE) (Table 1). To test the effects of TG, 0.15% TG was added to the formulation. All doughs were mixed at 35 °C in a temperature-controlled room using a pin-type mixer with 100 g capacity (National Mfg. Co., Lincoln, NE). This temperature was chosen because protein body-free kafirin was shown to be mobilized and participate in viscoelastic dough formation above its glass transition temperature (T_g) (Bugusu et al., 2001; Lawton, 1992; Oom et al., 2008; Schober et al., 2008), which is believed to be around 28 °C (Goodall et al., 2012). After mixing, doughs were used for rheological testing and baking. For baking, fermentation was done at 35 °C and 85% relative humidity in a temperature and humidity controlled proofing cabinet (InterMetro Industries Co., Wilkes-Barre, PA) for 60 mins, whereupon they were baked in a rotary electric oven (National Mfg. Co., Lincoln, NE) at 218 °C for 30 mins. Organic wheat flour was used as a control. Organic wheat flour was chosen as the commercial wheat flour could contain other ingredients which could impact our findings.

Table 1 Amount of water added to composite flours and mixing time for optimum dough formation as determined by mixograph[a]

	Sorghum substitution level (%, wb)	Water added (%)	Mixing time (min)
Normal variety	10	60	5
	20	58	4.5
	30	56	4
HDHL variety	10	62	5
	20	58	5
	30	57	5
Control	0	64	5

[a] Two sets were prepared from each formulation; one with transglutaminase (TG) at a ratio of 0.15% and one without TG.

Dough Rheology

Small amplitude oscillatory tests were performed using a rheometer (ARG-2 Model, from TA instruments, Newcastle, DE) with parallel plate geometry (40 mm diameter plate) as described by Fevzioglu et al. (Fevzioglu et al., 2012). Linear viscoelastic regions for the samples were determined through strain sweep tests (at a constant frequency of 1 Hz and strain range of 0.1- 40%). Frequency sweep tests were applied at a 0.5% strain amplitude (within the linear viscoelastic region) over the 0.01 – 50 rad/s frequency range. Results were expressed in terms of phase angle (^{o}), storage modulus (G'), and loss modules (G"). In order to obtain information regarding the strength of doughs, complex modulus (G*) that account for both G' and G", were calculated using following formula:

$$G^* = \sqrt{(G')^2 + (G'')^2}$$

Bread Specific Volume

Baked loaves were allowed to cool for 1 h before measuring their mass (g) and volume (cm^3). Volume was measured by the rapeseed displacement method (AACCI, 2000). Specific volume was calculated as loaf volume/bread weight (cm^3/g).

Bread Crumb Firmness

Bread crumb firmness was determined with bread compression test using a Texture Analyzer HD Plus (TA instruments, Newcastle, DE) with a 50 kg load cell as described by Goodall et al. (Goodall et al., 2012). Results were expressed in terms of force (g).

Statistical Analyses

All analyses were performed in duplicate. Data are presented as mean ± SEM. Statistical analyses were done using GraphPad Prism version 7.0 for Mac OS X (GraphPad Software, Inc. La Jolla, CA). Differences among the samples and controls were determined using analysis of variance (ANOVA) at $\alpha = 0.05$ significance level. Tukey's multiple comparison test was applied at $\alpha = 0.05$ level to see if mean differences were statistically different.

Results and Discussion

Small Amplitude Oscillatory Tests

Rheological properties of doughs prepared from normal sorghum-wheat composite and HDHL sorghum-wheat composite flours were determined using small amplitude oscillatory tests, where the results were expressed in in terms of phase angle (^{o}), storage modulus (G'), and loss modules (G"). The phase angle value varies between 0 and 90^o and gives information regarding the viscoelastic properties of dough. The lower phase angle value corresponds to more elastic behavior, whereas the higher phase angle value refers to more viscous nature (Fevzioglu et al., 2012; Steffe, 1996). Moreover, G' and G" values obtained were used to calculate the complex modulus (G*) that is a good indicator of dough strength. Lower G* corresponds to lower dough strength, and vice versa (Fevzioglu et al., 2012; Tuncil et al., 2016).

The effects of sorghum substitution and TG addition on phase angles of dough samples with different sorghum-wheat composites are given in Figure 1 as a function of angular frequency. At 10% substitution level, using of HDHL sorghum or normal sorghum varieties displayed no differences in phase angle values (Figure 1a), suggesting that HDHL sorghum and normal sorghum lines resulted in formations of doughs with similar viscoelastic properties. This could be attributed to the fact that, at this substitution level, wheat flour dominates the system; thus, gluten proteins mainly determine viscoelastic properties of doughs. At this level of substitution, addition of TG on any type of sorghum-wheat composites decreased the phase angle values of doughs, referring to more elastic nature.

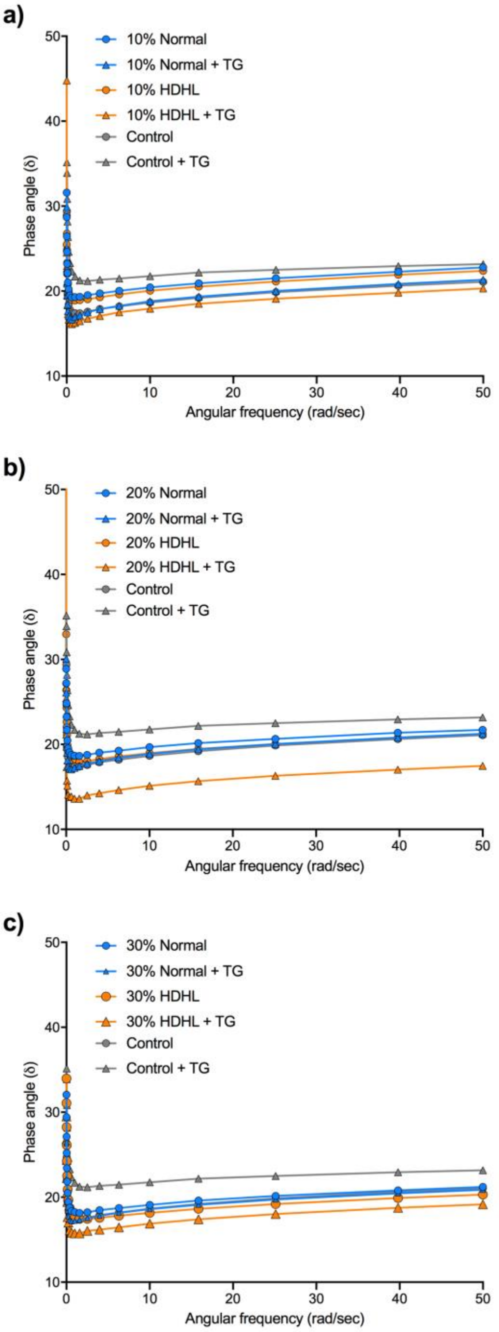

Figure 1 Phase angle values of doughs made from sorghum-wheat composite flours as a function of angular frequency. a) 10% substitution level, b) 20% substitution level, c) 30% substitution level Control doughs were made using 100% organic wheat flour.

At 20% substitution level, doughs generally had similar phase angle values except HDHL sorghum-wheat composite dough containing TG that revealed lower phase angle value, indicating more elastic nature (Figure 1b). Similarly, at 30% substitution level, addition of TG into HDHL sorghum-wheat flour composites increased the dough elasticity, while that into normal sorghum did not change dough properties (Figure 1c). These results indicate that TG shows more activity in HDHL sorghum-wheat composite dough when compared to normal sorghum-wheat composite dough. This could be attributed to the altered folded protein body shape of HDHL sorghum that makes kafirin proteins more accessible to form network-forming interactions.

Above 20% substitution level, HDHL sorghum-wheat flour composites formed slightly more elastic dough, compared to dough produced from normal sorghum composites (Figure 1c). Similar results were also observed by Goodall et al. (Goodall et al., 2012). This is attributed to the protein body free-kafirins presented in HDHL sorghum that are available for interaction, thereby possibly contributing to a protein network formed during mixing.

The effects of sorghum substitution and TG addition on G* values of dough samples with different sorghum-wheat composites are given in Figure 2 as a function of angular frequency. Compared to doughs prepared from normal sorghum-wheat composite flours, doughs prepared from HDHL sorghum-wheat composites had higher G* values, suggesting that HDHL sorghum varieties caused the formation of stronger dough matrix. This further shows that kafirins in HDHL sorghum show better functionality than those in normal sorghum. Thus, compared to normal sorghum-wheat composites, HDHL sorghum-wheat composite doughs are expected to have better ability to form matrix which could entrap CO_2 produced during fermentation, thereby resulting in a production of higher quality breads.

Addition of TG also significantly impacted the G* values of dough; however, the impact was sorghum variety- and substitution level- dependent. For example, at low substitution levels (10% and 20%), addition of TG resulted in increases in G* values of doughs prepared from HDHL sorghum-wheat composite flours, whereas this trend was reversed at 30% substitution level. Opposite to HDHL sorghum, addition of TG resulted in decreases in G* values of doughs prepared from normal sorghum-wheat composite flours. These discrepancies in the effects of TG on composite doughs can be attributed to the different natures of the kafirins found in these sorghum varieties that impact the accessibility of TG. Moreover, compared to kafirins in normal sorghum, those found in HDHL sorghum variety contains higher amount of lysine (Weaver et al., 1998; Goodall et al., 2012), which is, therefore, expected to stimulate the development of stronger dough matrix, because TG is known to catalyze covalent interactions between lysine and glutamine resulting in ε (γ-glutamyl) lysine isopeptic bonds (Basman et al., 2003).

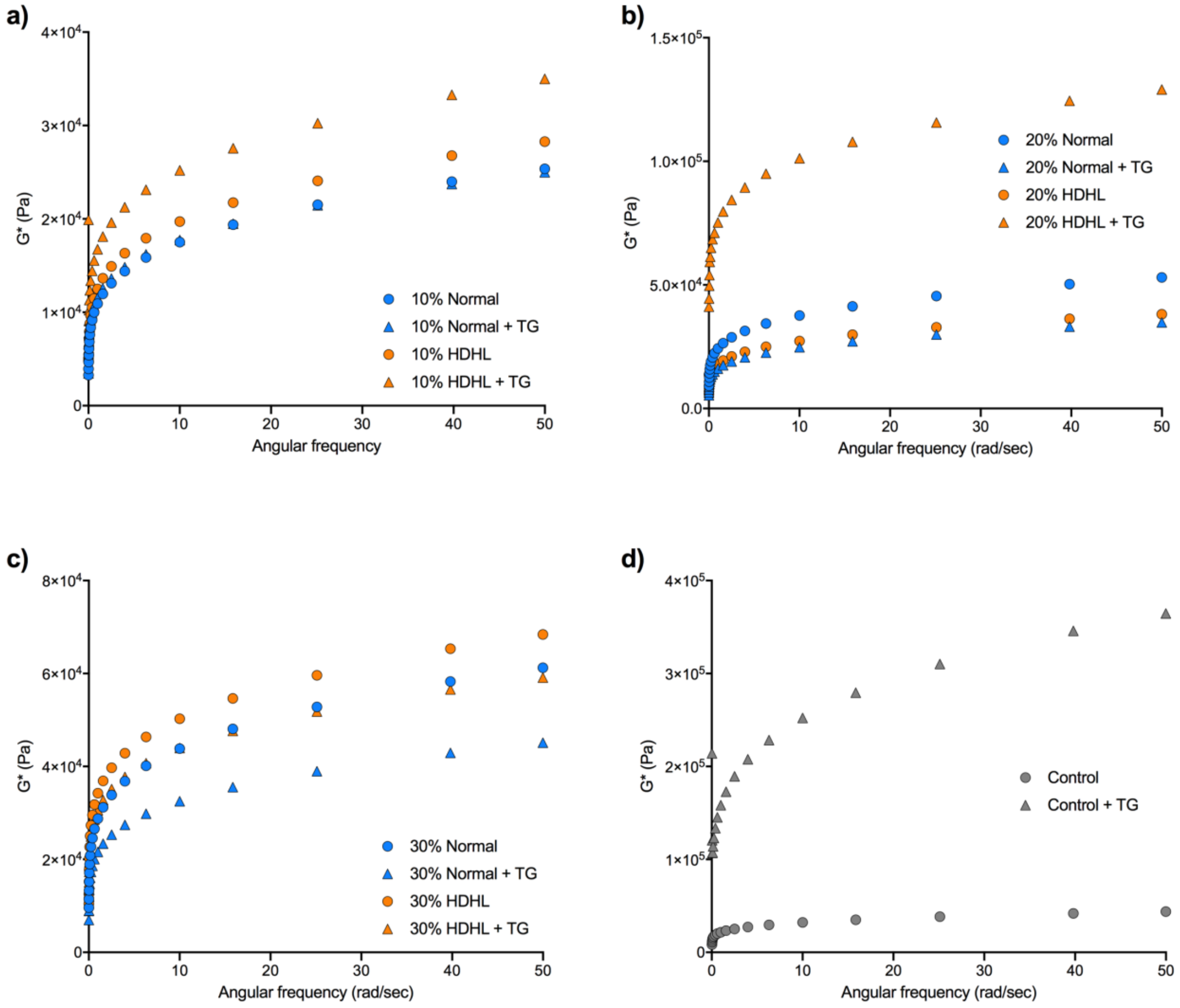

Figure 2 Complex modulus (G*) of doughs made from sorghum-wheat composite flours as a function of angular frequency. a) 10% substitution level, b) 20% substitution level, c) 30% substitution level, d) control. Control doughs were made using 100% organic wheat flour.

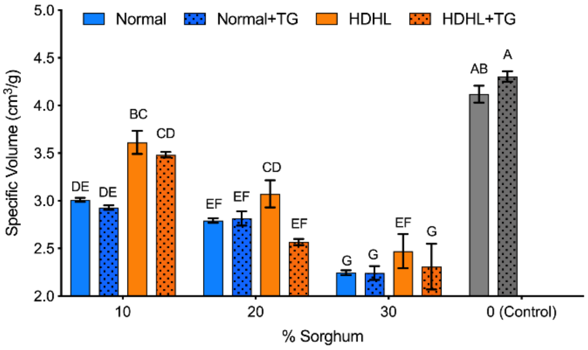

Figure 3 Specific volumes of bread made from sorghum-wheat composite doughs with- and without- TG addition. Control doughs were made using 100% organic wheat flour. Different letters on the bars represent significant differences between the samples (P<0.05)

Bread Specific Volume

Specific volume is one of the quality parameters of bread, which is calculated as loaf volume/bread weight (cm^3/g). High quality breads are desired to have high specific volume. Specific volume of breads prepared from sorghum-wheat composite doughs are given in Figures 3. As expected, substitution of any variety of sorghum flours resulted in a decrease in the bread specific volume. This is in an agreement with previous reports, where the effects of sorghum incorporation on bread quality were studied (Keregero and Mtebe, 1994; Schober et al., 2005). This negative effect of sorghum flours on bread volume is mainly attributed to its storage proteins, kafirins, which lack good gluten-like properties, and normally cannot participate in formation of strong gluten fibril matrix that entraps CO_2 produced during fermentation. Moreover, it is also noteworthy to mention here that sorghums are rich in phenolic compounds (Dykes et al., 2005; Dykes and Rooney, 2006; Dykes and Rooney, 2007), and phenolics have been shown to restrict the activity of *Saccharomyces cerevisiae* (Rauha et al., 2000); therefore, phenolics naturally presented in sorghum might cause the production of lower amount of CO_2 during leavening by reducing the activity of the yeast and lower the specific volume.

On the other hand, breads prepared from HDHL sorghum-wheat composite doughs had significantly higher specific volume, compared to those made from normal sorghum-wheat composites (Figure 3). This agrees with our previous finding (Goodall et al., 2012) and clearly indicates that the kafirins in HDHL sorghum have better functionality than those in normal sorghum. Moreover, this further suggests that, during dough development, kafirins in HDHL sorghum possibly have better ability to participate in formation of fibril matrix, which entraps CO_2 produced during leavening.

Although addition of TG into sorghum-wheat composites significantly improved the rheological properties of dough, it did not result in an increase in the specific volume of any of the composites. This suggests that TG cannot trigger the formation of fibril matrix that is strong enough to entrap CO_2 produced during the fermentation. Moreover, significant decreases in the specific volume were even observed by addition of TG into the formulation of HDHL sorghum-wheat composites prepared at a substitution level higher than 10 %. (P<0.05)

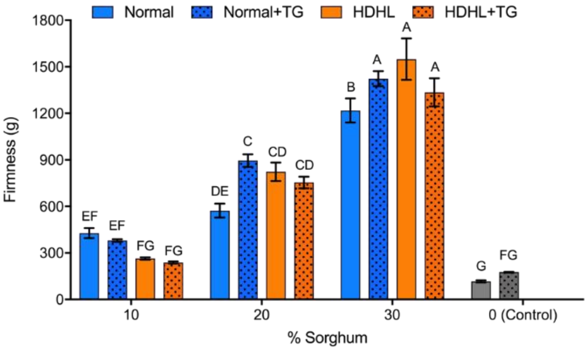

Figure 4 Firmness of bread crumbs made from sorghum-wheat composite doughs with- and without- TG addition. Control doughs were made using 100% organic wheat flour. Different letters on the bars represent significant differences between the samples (P<0.05)

Bread Firmness

Firmness is another important quality parameter of bread that is quantitatively measured by determining the force required to compress the bread. Higher force required to compress corresponds to firmer bread structure, and vice versa. Force required to compress values of the samples tested in this study are given in Figure 4. Sorghum-wheat composite doughs, except HDHL sorghum at 10% substitution level, resulted in productions of bread that require higher force to compress, compared to controls. These indicate that composite dough yielded in a production of firmer breads, compared to control. Moreover, increasing the substitution levels of any sorghum variety resulted in further increases in the bread firmness.

Above 10% substitution level, breads from HDHL sorghum-wheat composites required higher force to compress indicating that they were harder than breads made from normal sorghum-wheat composites. This could be attributed to the presence of protein body-freed kafirins in HDHL sorghum because, compared to kafirins in normal sorghum, protein body-freed kafirins are more available for interactions with gluten proteins, thus possibly contributing to the formation of stronger network in the dough and resulting in a production of more firmness bread.

The effect of TG addition on firmness of composite breads started to be observed above 10% substitution level; however, its effect was sorghum type-dependent. For example, above 10% substitution level, addition of TG into normal sorghum-wheat composites significantly increased bread firmness, while that into HDHL sorghum-wheat flour composites did not significantly changed the bread firmness (P<0.05).

Conclusions

TG was found to be more effective on HDHL sorghum-wheat composites, compared to normal sorghum-wheat composite. For example, TG addition into HDHL sorghum-wheat formulations resulted in a formation of more elastic dough. This could be due to the altered protein body shape of HDHL sorghum that makes kafirin proteins more accessible by enzymes such as TG. Although TG generally improved viscoelastic properties of dough, improvement in the bread quality with addition of TG was not observed.

These data support our previous findings that, compared to normal sorghum, kafirins presented in HDHL sorghum are more readily available for interactions and appear to participate in gluten network development in composite systems. This results in formation of dough with improved viscoelastic properties, and thus increasing the final bread quality. Overall, our results show that HDHL sorghum is more suitable for incorporation into the bread-making, compared to normal sorghum lines.

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