Novel biofortified sorghum lines with combined waxy (high amylopectin) starch and high protein digestibility traits: Effects on endosperm and flour properties

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Abbreviations used: DSC – Differential scanning calorimetry, SEM – Scanning electron microscopy; WAI – Water Absorption Index; WSF - Water Soluble Fraction.

Running head: Waxy and high protein digestibility sorghum

HIGHLIGHTS

Combined waxy-high protein digestibility traits in sorghum gave high peak viscosity

High protein digestibility overrode the waxy trait giving floury endosperm texture

Combined waxy-high protein digestibility traits gave high flour water solubility

Flour solubility of waxy-high protein digestibility traits was similar to wheat flour

Abstract

Novel biofortified sorghum lines have been developed with both waxy starch (high amylopectin) and high protein digestibility traits. Eight sorghum lines with different combinations of waxy, non-waxy, high- and normal-protein digestibility traits were studied in relation to flour properties. Lines with the high protein digestibility trait had loosely packed starch granules and floury endosperm, irrespective of whether they were waxy or non-waxy. In terms of thermal properties, waxy-high digestibility lines had the highest onset endothermic temperature as well as endothermic energy compared to non-waxy-normal protein digestibility lines. The waxy-high protein digestibility lines had higher paste viscosity and formed much softer and less sticky pastes than the non-waxy, normal protein digestibility lines. Flours of the waxy-high protein digestibility sorghum lines had much higher solubility than the non-waxy-normal protein digestibility lines. At 30^{o}C flour solubility, waxy-high protein digestibility sorghum lines flour was similar to commercial wheat bread flours. The high flour solubility, high pasting viscosity and soft paste of sorghum lines with combined waxy and high protein digestibility traits indicates that their flours are likely to produce more functional doughs and hence better quality food products than normal non-waxy, normal protein digestibility sorghums.

1. Introduction

Sorghum is the fifth most important cereal crop in terms of production, about 58 million tonnes in 2011, with Africa being the major producing region accounting for > 40% of world production (FAOSTAT, 2013). Sorghum is highly suited for cultivation in the semi-arid and sub-tropical regions of Africa as it is one of the most drought-tolerant cereal crops (Srinivas et al., 2009). Further, sorghum does not elicit an adverse reaction in coeliacs (Ciacci et al., 2007). However, despite its high production and its applicability in gluten-free foods, the commercial utilization of sorghum is limited, particularly as flour for dough-based food products, and especially in bread. This is largely due to the fact that its flour does not form gas-holding dough (reviewed by Taylor et al., 2006).

This drawback of sorghum presents a major challenge in Africa, where increasing dependence on wheat to produce bread to meet the needs of the rapid expanding urban population is negatively affecting the continent's economic situation. According to the International Food Policy Research Institute, in 2013 African countries were spending US $12 billion to import 40 million tons of wheat (IFPRI, 2013).

The major reason for the inferior bread-making properties of sorghum is that kafirin, the sorghum prolamin protein, does not exhibit the visco-elastic properties of wheat gluten in normal dough systems (reviewed by Taylor et al., 2014). Furthermore, the starch granules in the corneous endosperm of sorghum are surrounded by a hydrophobic matrix of membrane bound kafirin protein bodies (Munck, 1995). These hydrophobic matrix proteins can reduce the extent of water absorption and solubilisation of sorghum starch. In turn, this may lead to inadequate functionality of sorghum flour because in wheat flour starch water holding is related to dough functionality (Dexter et al., 1994).

Novel biofortified sorghum lines with combined waxy (high amylopectin) starch, and hence high starch digestibility (Rooney and Pfugfelder, 1986) and high protein digestibility traits have developed by Texas A&M University (Jampala et al., 2012). It has been shown that sorghums with these traits individually have improved end-use quality with regard to bioethanol production (Wu et al., 2010).

The objective of this work was to examine the effects of sorghum lines with the waxy and high protein digestibility traits individually and in combination on characteristics related to flour functionality with the aim of determining the potential of these novel biofortified sorghum lines for making good quality dough-based food products.

2. Materials and Methods

2.1 Sorghum samples

Eight sorghum lines, developed and bred through conventional plant breeding by Texas A&M University, were studied. All the lines were of the white tan-plant type. They comprised: two non-waxy-normal protein digestibility normal lines coded 199 and 200, two lines with waxy-normal protein digestibility traits (coded 175 and 179) and one line of non-waxy-high protein digestibility traits (coded 106), and three waxy-high protein digestibility lines (coded 109, 142 and 146). Sorghum lines coded 109, 142 and 146 were obtained via crossing lines Tx2907 and P850029 (Jampala et al., 2012). Tx2907 was released from the Texas AgriLife Research sorghum breeding program as a waxy and normal protein digestibility sorghum (Miller et al., 1996). P850029, a high protein digestibility line, was developed at Purdue University from a population derived from the high lysine line P721Q (Weaver et al., 1998).

The lines were increased in Halfway, Texas in 2012. The lines were blocked using rows of photosensitive lines as pollen breaks to avoid cross pollenation. The soil type at Halfway was Pullman Clay loam. The field plots were managed using agronomic practices typical for production of sorghum at each location (Rooney et al., 2005). Prior to planting the land was prepped with shredding of stalks after grain harvest, disc harrowing twice and bedding with a disc lister-bedder during the proceeding fall. In January/February, fertilizer was added at 168 kg ha^{-1} (10-34-0) and 4.5 kg ha^{-1} $ZnSO_4$. In mid-March, the seed bed was prepared with a rod-weeder, then planting occurred on March 20, prior to spraying with pre-emergent 1.42 L Atrazine and 0.6 L Brawl in 10 L ha^{-1} H_2O. After 6 weeks, plots were side-dressed with N at 110 kg ha^{-1} (32-0-0) then sprayed with 3.5 L Prowl in 10 L ha^{-1} H_2O. Plots were furrow irrigated as needed and aerial spraying at 9.3 L ha^{-1} of Asana was performed as needed for the control of sorghum midge and earworms. Grain was harvested on August 1. Plots were 5 m long after cutting alleyways with 75 cm row spacing. Tests were harvested using an MF8 plot combine.

MR Buster, a red, non-tannin, non-waxy, normal protein digestibility commercial hybrid sorghum was used as a standard. It was cultivated in Botswana and kindly supplied by the National Food Technology Research Centre in Botswana.

2.2 Grain endosperm and protein body structure

Twenty sound grains from each sorghum line were dissected longitudinally. Endosperm texture was recorded by stereo light microscopy. By reference to sorghum endosperm type illustrations (ICC, 2011), the relative proportion of corneous endosperm to floury endosperm was estimated for each line. Endosperm structure was evaluated using scanning electron microscopy (SEM). Whole sorghum grains were dissected longitudinally by scalpel after freezing in liquid nitrogen. Then samples mounted on aluminium stubs using poster gum and were sputter coated with gold and then viewed using a Zeiss Evo LS15 SEM (Carl Zeiss, Oberkochem, Germany) operated at an acceleration voltage of 8 kV. Protein body structure was investigated using Transmission Electron Microscopy (TEM) as described (Da Silva et al., 2011a,b)

2.3 Flour preparation

Twenty g sound grains of each line were decorticated using a Tangential Abrasive Dehulling Device (TADD, Venables Machine Works, Saskatoon, Canada), removing approx. 20% by weight of the grain outer layers. A laboratory hammer mill (Mikro-Feinmuhle-Culatti MFC Grinder, Janke and Kunkel, Staufen, Germany) with a 500 µm opening screen was used to mill the decorticated grain.

2.4 Flour moisture content

Moisture content was determined by NIR (DA 7200 NIR analyser, Perten Instruments Springfield, IL) using the supplier's calibration for sorghum.

2.5 Protein content

Protein content (N x 6.25), was determined by Dumas combustion according to AACC method 46-30 (AACC International, 2000).

2.6. Starch amylose content

Amylose content was determined by the Megazyme amylose/amylopectin assay kit procedure (Megazyme Ireland International, Bray, Ireland). Amylopectin in the sample is specifically precipitated by the addition of the lectin concanavalin A (Con A) and removed by

centrifugation. The concentration of amylose in the sample is determined colorimetrically using the glucose oxidase-peroxidase (GOPOD) reagent (Wong et al. 2009).

2.7 In vitro 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. (2011b). After pepsin digestion of the sample under specific conditions, the quantity protein remaining is measured as percentage of total protein in the sample and percentage digestibility calculated.

2.8 Flour thermal properties

Differential scanning calorimetry (HP DSC 827e (Mettler Toledo, Schwerzenbach, Switzerland) was used to determine the thermal properties of the sorghum flours, as described by Beta et al. (2000). Sorghum flour (9 mg dwb) was weighed into a 100 µl aluminium DSC pan and deionized distilled water was added to a total weight of 36 mg. After sealing, the sample was equilibrated at room temperature for 2 h. Each sample was scanned from 30 to 120ºC at a heating rate of 10ºC/min. Nitrogen was used at normal air pressure with flow rate of 30 ml/min. Onset (T_0), peak (T_p) and conclusion endotherm (T_c) temperatures were measured and endothermic enthalpy was calculated.

2.9 Flour pasting properties

The pasting properties of the sorghum flours were determined using a Physica MCR 301 Rheometer (Anton Paar, Ostfildern, Germany) using a cup and a stirrer. Flour and distilled water were mixed to a ratio 1: 9. Samples were stirred for 30 s at 50°C before measurement. The pasting programme was: hold for 1 min at 50°C, heat to 91°C over 7 min at a rate of 5°C/min, hold for 10 min, cool down to 50 °C over 7 min and then hold for 5 min. Pasting temperature, Peak viscosity (mPa.s), Holding strength, Breakdown, Setback and Final viscosity were measured.

2.10 Gel texture properties of flour

The gel texture properties were performed according to D'Silva et al. (2011). In brief, the pasted samples from the rheometic analysis were allowed to stand overnight at 25°C to allow gelation to take place. Gel texture was determined using a TA-XT2 texture analyser (SMS

Stable Microsystems, Godalming, UK) using a cylindrical plunger of 20 mm diam. at a test speed of 05 mm/s. Samples were compressed to a distance of 5 mm. Hardness was recorded as maximum force on the compression phase. Adhesiveness was recorded as negative force area of the curve resulted from withdrawing the probe.

2.11 Flour WAI and WSF

Water absorption index (WAI) and water soluble fraction (WSF) were measured at 30°C and 60°C essentially as described by Anderson et al. (1970). The WAI and WSF of two commercial wheat white bread flours (Golden Cloud, Tiger Brands, Bryanston, South Africa and Snowflake, Premier Foods, Isando, South Africa) were measured for comparison.

2.12 Statistical analysis

All experiments were performed at least two times. Data were analysed by one-way analysis of variance (ANOVA) in the case of amylose content and protein digestibility and two-way ANOVA for the other analyses, at a confidence level of $P < 0.05$. Means were compared by Fisher's least significant difference (LSD) test. Principal Component Analysis (PCA) for all numerical results was performed using STATISTICA verson 12 (StatSoft, Tulsa, OK, USA).

3. Results and discussion

3.1 Waxy and high protein digestibility traits

As expected, the five waxy lines (109, 142, 144, 175 and 179) had a much lower percentage of amylose then the normal lines (Table 1). However, since all the waxy lines contained some amylose it would appear from comparison with the findings of Sang et al. (2008) that all were heterowaxy types, containing at least one recessive waxy gene. Also, as expected, all the four lines that had the high protein digestibility trait (106, 109, 142 and 146) had significantly higher ($p < 0.05$) in vitro protein digestibility in raw flour and higher or generally higher (line 142) in cooked flour when compared to the four lines with the normal protein digestibility trait. Of significance is that the waxy trait did not appear to influence protein digestibility as line 142 (waxy, high protein digestibility) had lower digestibility than line 106 (non-waxy, high protein digestibility). However, the cooked protein digestibility of the high protein digestibility lines was rather lower than observed with transgenic sorghum with suppressed kafirin synthesis, but similar to their null controls (Da Silva et al., 2011b). Further, only lines 146 and 109 had

Table 1: Starch amylose content and *in vitro* pepsin protein digestibility of waxy and high protein digestibility sorghum lines and their controls (199 and 200) and normal red sorghum cultivar MR Buster

Line	Starch type	Protein digestibility trait	Amylose (%)	Protein digestibility of raw sorghum flour (%)		Protein digestibility of cooked sorghum flour (%)	
109	Waxy	High	3.9^{a}	72.8^{f}	± 1.1	46.7^{f}	± 0.2
142	Waxy	High	7.3^{c}	58.1^{c}	± 0.3	36.5^{de}	± 0.0
146	Waxy	High	5.8^{b}	68.6^{e}	± 0.2	48.1^{f}	± 1.7
175	Waxy	Normal	12.1^{d}	55.4^{ab}	± 0.3	32.3^{ab}	± 1.1
179	Waxy	Normal	7.9^{c}	54.3^{a}	± 0.3	31.9^{a}	± 1.1
106	Non-waxy	High	23.2^{e}	64.1^{d}	± 0.0	38.7^{e}	± 0.9
199	Non-waxy	Normal	25.7^{f}	55.4^{ab}	± 0.1	32.7^{abc}	±0.1
200	Non-waxy	Normal	27.8^{g}	56.3^{b}	± 0.1	35.4^{cd}	± 1.2
MRBuster	Non-waxy	Normal	30.8^{h}	57.9^{c}	± 0.2	35.0^{bcd}	± 1.2

12

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

n = 2

substantially higher protein digestibility than cultivar MR Buster, the normal sorghum standard. Also, as observed with transgenic sorghum and with this non-transgenic high protein digestibility mutant (Da Silva et al., 2011b), the in vitro protein digestibility of the cooked flours was considerably lower than the raw flours.

3.2 Grain endosperm texture and structure

All the four high protein digestibility lines, lines 109, 142 and 146 with waxy trait and line 106 (non-waxy) had a floury endosperm texture (Figure 1A). The floury endosperm texture of transgenic high protein digestibility sorghum (Da Silva et al., 2011a) and the soft endosperm character of this non-transgenic high protein digestibility mutant (Tesso et al., 2006) have been reported previously. In contrast, and as expected, the corneous endosperm of the two waxy-normal protein digestibility lines (175 and 179) had the typical "waxed floor-like" appearance of waxy sorghum described by Rooney and Miller (1982). Also, the two non-waxy-normal protein digestibility lines (199 and 200) had an intermediate (part corneous) endosperm texture. Thus, both the high protein digestibility and waxy traits modified the endosperm texture. Notably, however, the high protein digestibility trait seemed to override the waxy trait when they were in combination, resulting in a floury endosperm.

SEM showed that the peripheral starchy endosperm cells of all four high protein digestibility lines, both waxy (109, 142 and 146) and non-waxy (106), contained loosely packed starch granules (Figure 1B), hence their floury endosperm texture (Figure 1A). In contrast, the starch granules were tightly packed in all the four normal protein digestibility lines (175, 179, 199 and 200), irrespective of whether they had the waxy trait. The floury endosperm texture of the high protein digestibility mutants is a consequence of the altered kafirin synthesis, which causes the kafirin containing protein bodies to have a folded (invaginated) structure (Figure 2C), as first described by Oria et al. (2000). This in turn results in an incomplete protein matrix surrounding the starch granules in the outer floury endosperm.

3.3 Flour thermal properties

Both the waxy trait and the high protein digestibility trait affected the sorghum flour endotherms (Table 2A). The waxy trait significantly ($p<0.05$) increased the onset, peak and endset endotherm temperatures by approx. $2^{o}C$, and the enthalpy by approx.1 J/g (Table 2B). The high protein digestibility trait significantly ($p<0.05$) increased the onset temperature (by

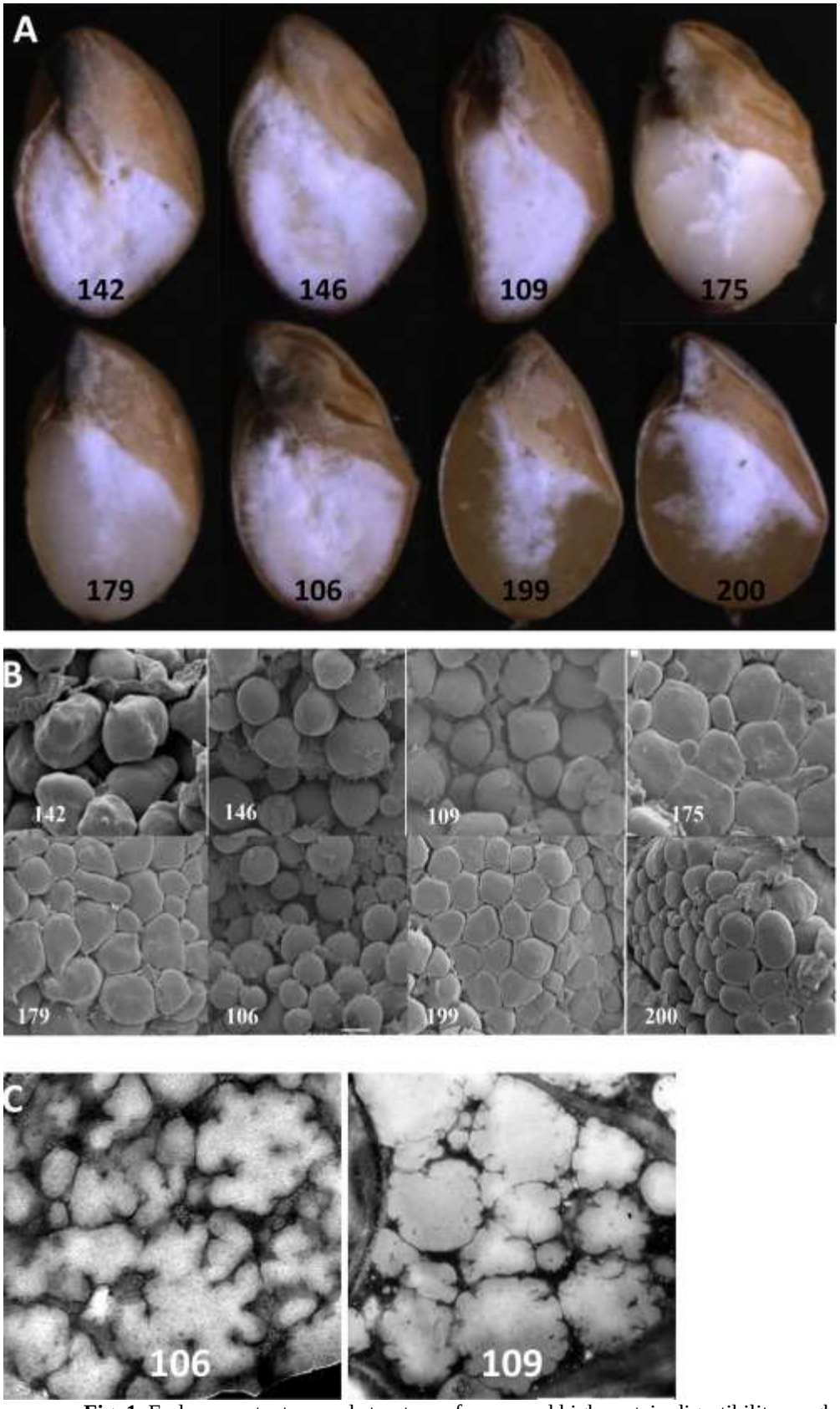

Fig. 1. Endosperm texture and structure of waxy and high protein digestibility sorghum lines
A: Longitudinal sections through the grains.
B: SEM of starch granules.
C: TEM of protein bodies.

Table 2: Thermal properties of waxy and high protein digestibility sorghum lines and their controls and normal red sorghum cultivar MR Buster

(A) Onset, peak, endset temperatures and enthalpy

Line	Starch type	Protein digestibility trait	Onset temp. (℃)	Peak temp. (℃)	Endset temp. (℃)	Enthalpy (J/g)
109	Waxy	High	71.67^{d}	76.44^{c}	84.10^{ab}	4.48^{a}
142	Waxy	High	71.81^{d}	77.50^{e}	87.95^{d}	4.35^{a}
146	Waxy	High	72.87^{e}	77.82^{e}	86.54^{c}	4.35^{a}
175	Waxy	Normal	70.64^{c}	77.10^{d}	86.44^{c}	3.73^{b}
179	Waxy	Normal	71.63^{d}	77.66^{e}	89.79^{e}	4.82^{a}
106	Non-waxy	High	70.15^{c}	76.45^{c}	86.31^{c}	3.34^{c}
199	Non-waxy	Normal	69.58^{b}	75.00^{b}	84.71^{b}	3.36^{bc}
200	Non-waxy	Normal	69.65^{b}	75.33^{b}	83.57^{a}	3.35^{c}
MR Buster	Non-waxy	Normal	67.23^{a}	74.15^{a}	86.61^{c}	3.28^{c}

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

n = 3

(B) Effects of starch type and protein digestibility over the eight lines

Parameter	Protein digestibility trait	Starch type		Means for high or normal protein digestibility sorghums	Means for waxy and non-waxy sorghums	
		Waxy	Non-waxy		Waxy	Non-waxy
Onset (°C)	High	72.12^{c}	70.15^{a}	71.63^{b}	71.72^{b}	69.79^{a}
	Normal	71.14^{b}	69.61^{a}	70.37^{a}		
Peak (°C)	High	77.25^{c}	76.45^{b}	77.05^{a}	77.03^{b}	75.59^{a}
	Normal	77.38^{c}	75.16^{a}	76.27^{a}		
Endset (°C)	High	86.20^{b}	86.31^{abc}	86.23^{a}	86.96^{b}	84.67^{a}
	Normal	88.11^{c}	84.14^{a}	86.13^{a}		
Enthalpy (J/g)	High	4.43^{a}	3.34^{b}	4.15^{a}	4.37^{a}	3.33^{b}
	Normal	4.28^{a}	3.30^{b}	3.81^{a}		

Means with different superscript letters within a cell are significantly different ($p < 0.05$).

n = 3

approx. 1^{o}C) but did not affect the peak and endset temperatures or the enthalpy. Overall, the waxy-high digestibility sorghums had the highest onset endotherm temperature and the non-waxy, normal protein digestibility sorghums had the lowest, or among the lowest, onset, peak and endset temperatures and least enthalpy. This was clearly illustrated by Principal Component Analysis (PCA) (Figure 2). The first PCA component, which accounted for 60.2% of the variation separated the waxy-high protein digestibility lines on the left side of the plot and the non-waxy-normal protein digestibility lines (including the standard hybrid MR Buster) on the right hand side of the plot

The higher endotherm temperatures and larger enthalpy of the flours from the sorghum lines with the waxy trait is in agreement with work where starches isolated from waxy and normal sorghum were examined (Sang et al., 2008). It was suggested by these authors that peak gelatinization temperature is an indicator of crystallite quality which is related to amylopectin double helix length. In support of this, they found that the low degree of polymerisation fraction (DP 6-15) of amylopectin was present in slightly higher proportion in waxy sorghum starch compared to normal sorghum starch. The higher onset temperature of the high protein digestibility lines suggests that the floury texture resulting from this trait impacted on the gelatinization of the starch. Possibly, it resulted in increased competition for available water, thus delaying the onset in change in molecular order in the starch granules.

3.5 Flour pasting and gel properties

Table 3 shows that the pasting temperatures of the flours from the sorghums with the various traits (69.4-71.2^{o}C) were not significantly different ($p \geq 0.05$). This pasting temperature range is very similar to the 67.9-70.3^{o}C found for starches isolated from Zimbabwean sorghums (Beta et al., 2000). However, the flours with the combined waxy-high protein digestibility traits (lines 109, 142 and 146) gave the highest paste peak viscosity ($p<0.05$), whereas only one of the waxy, normal protein digestibility lines (175) gave a higher peak viscosity than the non-waxy lines. All the waxy lines (normal and high protein digestibility) and the non-waxy- high protein digestibility waxy line (106) had lower paste holding strength than the two non-waxy, normal protein digestibility lines, but not compared to MR Buster, the red non-waxy-normal protein digestibility standard. These effects on peak viscosity and paste holding strength are also clearly illustrated by PCA (Figure 2). Paste holding strength is clearly associated with the two non-waxy, normal protein digestibility lines (199 and 200). The impacts of the high protein

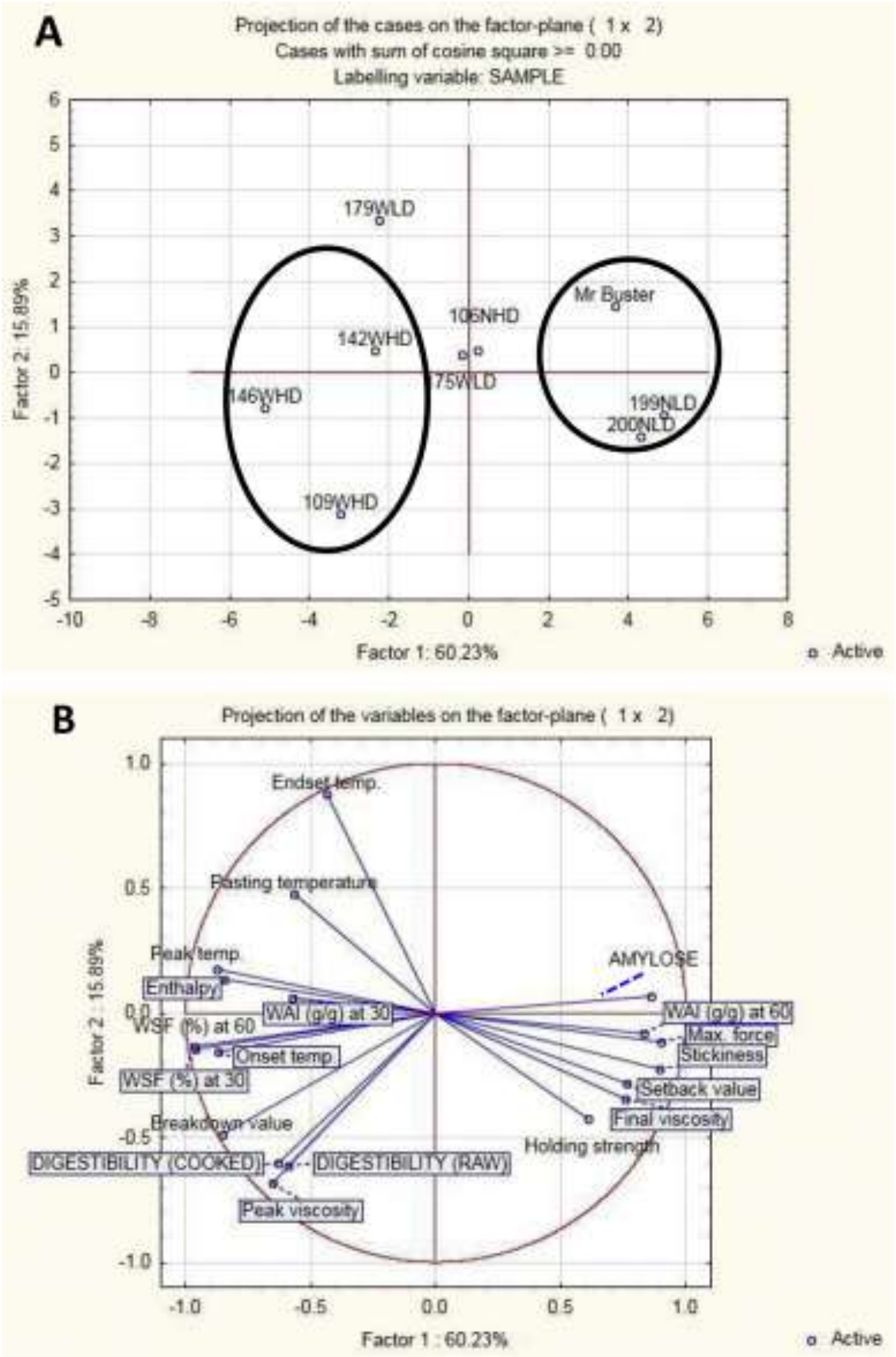

Fig. 2. PCA showing the correlations between the sorghum lines with the different traits and starch type, protein digestibility, flour thermal properties, pasting and gel properties and WAI and WSF.
A: Sample scores.
B: Loadings.

Table 3: Pasting properties and gel texture characteristics of waxy and high protein digestibility sorghum lines and their controls and normal red sorghum cultivar MR Buster

Line	Starch type	Protein digestibility trait	Pasting temperature (°C)	Peak viscosity (mPa.s)	Holding strength (mPa.s)	Breakdown value (mPa.s)	Setback value (mPa.s)	Final viscosity (mPa.s)	Max. force calc. at entire areas (N)	Stickiness (N)
109	Waxy	High	69.4^{a}	1759^{i}	909^{d}	850^{f}	478^{b}	1387^{b}	0.122^{a}	0.039^{c}
142	Waxy	High	70.2^{a}	1424^{g}	959^{e}	466^{d}	505^{b}	1463^{c}	0.125^{a}	0.045^{c}
146	Waxy	High	71.2^{a}	1557^{h}	801^{a}	756^{e}	389^{a}	1190^{a}	0.125^{a}	0.038^{c}
175	Waxy	Normal	70.9^{a}	1394^{f}	950^{e}	444^{d}	868^{e}	1818^{e}	0.215^{ab}	0.076^{c}
179	Waxy	Normal	71.1^{a}	1099^{c}	819^{b}	279^{c}	392^{a}	1212^{a}	0.123^{a}	0.045^{c}
106	Non-waxy	High	70.1^{a}	1066^{b}	807^{ab}	259^{c}	714^{d}	1521^{d}	0.238^{b}	0.081^{c}
199	Non-waxy	Normal	69.0^{a}	1147^{d}	1003^{f}	143^{b}	903^{f}	1907^{f}	2.609^{e}	0.300^{a}
200	Non-waxy	Normal	70.2^{a}	1267^{e}	1131^{g}	136^{b}	980^{g}	2110^{g}	1.754^{c}	0.232^{ab}
MR Buster	Non-waxy	Normal	69.7^{a}	879^{a}	874^{c}	5^{a}	537^{c}	1411^{b}	1.996^{d}	0.136^{bc}

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

digestibility trait on sorghum flour pasting properties have not previously been described, although it has been found that both isolated waxy and heterowaxy sorghum starches (Sang et al., 2008) and their flours (Wu et al., 2010) had much higher peak viscosities than non-waxy sorghum.

In terms of flour gel texture, all the waxy lines (high and normal protein digestibility) and the non-waxy-high protein digestibility line (106) were far softer and less sticky ($p<0.05$) than the non-waxy-normal protein digestibility lines (Table 3 and Figure 2). The findings with regard the effects of the waxy trait on gelling are similar to those of Sang et al. (2008), who observed that whereas isolated non-waxy sorghum starch formed a strong, gel, heterowaxy sorghum starch only formed a very weak gel and waxy sorghum starch remained a paste. The same effect of the high protein digestibility trait on sorghum flour gel properties has not been previously reported. The very soft, non-sticky gel texture of the all the lines with the high protein digestibility trait is probably related to their floury endosperm texture.

3.6 Flour water absorption and solubility

Both the waxy and high protein digestibility traits significantly affected flour WAI and WSF ($p<0.05$) (Table 4). Overall, at 30^{o}C flours of the high protein digestibility lines had slightly higher WAI (Table 4B, Figure 2). The higher WAI of the high protein digestibility lines is presumably related to their floury endosperm texture (Figure 1A). In contrast, at 60^{o}C, just below sorghum starch gelatinization temperature (Delcour and Hoseney (2010), the non-waxy lines had slightly higher WAI (Table 4B, Figure 2). This may be related to the lower endotherm temperature of the non-waxy lines (Table 2B).

The effects of the traits on flour WSF were much greater. Both the waxy and the high protein digestibility traits increased WSF by approx. 60% (Table 4B). Hence, at both 30 and 60^{o}C, on average the WSF of the waxy-high protein digestibility lines was more than twice that of the non-waxy-normal protein digestibility lines (Table 4A) and the combined waxy-high protein lines were strongly associated with a high WSF (Figure 2). In fact, at 30^{o}C the WSF of the flours of these sorghum lines was similar to that of the wheat flours. At 60^{o}C the WSF of the waxy-high protein digestibility lines was substantially higher than the wheat flours at 30^{o}C. However, it was lower than that of the wheat flours at 60^{o}C , but this was as a consequence of the wheat starch gelatinizing at this temperature (Delcour and Hoseney, 2010).

Table 4: Water Absorption Index (WAI) and Water Soluble Fraction (WSF) of waxy and high protein digestibility sorghum lines and their controls and normal red sorghum cultivar MR Buster

A: WAI and WSF at 30°C and 60°C

Line/name	Starch type	Protein digestibility	WAI (g/g) at 30°C	WAI (g/g) at 60°C	WSF (%) at 30°C	WSF (%) at 60°C
109	Waxy	High	2.55^{de}	2.65^{ab}	8.24^{e}	9.92^{d}
142	Waxy	High	2.59^{f}	2.72^{bc}	8.45^{ef}	9.87^{d}
146	Waxy	High	2.63^{f}	2.68^{ab}	9.88^{g}	12.02^{e}
175	Waxy	Normal	2.55^{de}	2.69^{ab}	5.96^{c}	6.89^{b}
179	Waxy	Normal	2.52^{cde}	2.58^{a}	6.71^{d}	8.13^{c}
106	Non-waxy	High	2.61^{f}	2.82^{cd}	6.21^{c}	7.15^{b}
199	Non-waxy	Normal	2.49^{c}	2.91^{de}	4.21^{b}	4.83^{a}
200	Non-waxy	Normal	2.51^{cd}	2.82^{cd}	3.95^{a}	4.31^{a}
MR Buster	Non-waxy	Normal	2.58^{ef}	2.96^{e}	4.02^{ab}	4.57^{a}
*Golden Cloud	NA	NA	2.06^{a}	3.24^{f}	6.92^{d}	18.46^{g}
*Snow Flake	NA	NA	2.13^{b}	3.59^{g}	8.52^{f}	15.50^{f}

Means with different superscript letters within a column are significantly different ($p < 0.05$).n = 2,

*Wheat bread flour, NA = not applicable

B: Effects of starch type and protein digestibility individually and in combination on WAI an WSF

Parameter	Protein digestibility trait	Starch type		Means for high and normal protein digestibility sorghums	Means for waxy and non-waxy sorghums	
		Waxy	Non-waxy		Waxy	Non-waxy
WAI at 30℃	High	2.59^{b}	2.61^{b}	2.60^{b}	2.57^{a}	2.54^{a}
	Normal	2.54^{a}	2.50^{a}	2.52^{a}		
WAI at 60℃	High	2.68^{a}	2.82^{b}	2.72^{a}	2.66^{a}	2.85^{b}
	Normal	2.63^{a}	2.86^{b}	2.75^{a}		
WSF at 30℃	High	8.86^{c}	6.21^{b}	8.20^{b}	7.85^{b}	4.79^{a}
	Normal	6.34^{b}	4.08^{a}	5.21^{a}		
WSF at 60℃	High	10.61^{c}	7.15^{b}	9.74^{b}	9.37^{b}	5.43^{a}
	Normal	7.51^{b}	4.57^{a}	6.04^{a}		

Means with different superscript letters within a cell are significantly different ($p < 0.05$).

n = 2

The high solubility of the flours of the waxy-high protein digestibility lines seems to be related their floury (less dense) endosperm texture (Figure 1A, B), high amylopectin content and also their unique endosperm protein composition. We have shown that sorghum lines with this high protein digestibility trait, although having a similar protein content to normal digestibility types, have a much lower proportion of kafirin proteins, which are water insoluble, relative to total protein (Da Silva et al., 2011a), approx.. 33% compared to 40-50% in normal sorghums.

4. Conclusions

The novel biofortified sorghum lines with combined waxy and high protein digestibility traits have much higher flour water solubility, high pasting viscosity and form much softer and less sticky pastes than regular non-waxy-normal protein digestibility sorghums. Hence, their flours are likely to give more functional doughs both below and above starch gelatinization temperature, and thus produce better quality food products.

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