Sorghum waxy and high protein digestibility traits and their relationship with malting and dough-based product making quality

By

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Submitted in the partial fulfillment of the requirements for the Degree

PhD Food Science

In the

Department of Consumer and Food Sciences

Faculty of Natural and Agricultural Sciences

University of Pretoria

South Africa

September, 2018

DECLARTION

I hereby declare that this thesis submitted at the University of Pretoria for the award of PhD degree is my work and has not been submitted by me for a degree at any other University or Institution of Higher Education.

Abadi Gebre Mezgebe
September 2018

ABSTRACT

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Abadi Gebre Mezgebe

Supervisor: Prof J.R.N. Taylor
Co-supervisors: Prof H.L. de Kock
Dr Kebede Abegaz

Sorghum is a critical cereal crop in Africa, owing to its heat- and drought- tolerance, its role in nutrition and potential in commercial food and beverage manufacture. However, sorghum has limitations for producing quality foods and beverages e.g. malt, breads and biscuits. This is due to its high starch gelatinization temperature and poor protein functionality, specifically its inability to form a viscoelastic dough like wheat.

Novel sorghum lines with combined waxy (high amylopectin) and high protein digestibility (HD) traits have been developed through conventional breeding. These lines are hypothesized to have superior functionality in food and beverage applications due to their higher digestibility and improved dough functionality.

The objective of this work was to determine the relationship between waxy and HD traits in sorghum and malting quality as well as dough-based product (injera-fermented flatbread and biscuit) making quality. Novel white tan-plant sorghum lines with differing endosperm traits: waxy, heterowaxy, waxy-HD, non-waxy-HD and non-waxy-normal digestibility traits were malted at a laboratory scale and their malting qualities were studied. Additionally, injera and biscuits were prepared using standard methods and their qualities were evaluated using a trained descriptive sensory panel (DSP) and instrumental texture analysis.

The endosperm of the waxy lines had a pale waxed floor-like appearance (typical of waxy sorghum), high starch amylopectin and intermediate to corneous texture. Transmission electron

microscopy revealed that the HD lines had protein bodies which were not densely packed in the protein matrix, of irregular shape and small size (0.48-0.56 mm diam.), and had a floury endosperm.

Malt produced from waxy and heterowaxy sorghum lines generally had improved endosperm modification and starch granule degradation. Only non-waxy-HD and one waxy line malts exhibited clear evidence of endosperm protein degradation. Malt α- and β-amylase activities were not evidently affected by the traits. Malt from waxy lines had improved hot water extract (HWE) and free amino nitrogen (FAN). Principal component analysis (PCA) showed that the waxy lines were associated with high HWE, FAN, starch and protein loss.

DSP showed that injera made from waxy sorghums were softer, more rollable, and flexible compared to normal sorghum injera and were much closer to teff injera reference. Instrumental texture analysis of fresh and stored injera revealed that waxy sorghums gave lower stress and higher strain compared to injera from non-waxy lines, indicating that the injera were softer and more flexible. There was no clear trend as to whether the HD-trait affected injera quality. The textural properties of the injera measured by texture analyser and DSP showed significant correlation. Also, the instrumental texture profile of the injera prepared using full-scale (traditional) and small-scale (microwave) methods were significantly correlated. DSP and instrumental texture analysis showed that sorghum biscuit quality was not affected by the traits.

The waxy trait either alone or in combination with the HD-trait appears to improve sorghum malting quality, probably due to the better starch granule swelling property of amylopectin, which could facilitate hydrolysis by amylases and proteases. The improved injera quality is most likely due to the slower retrogradation and better water holding of amylopectin starch.

This study shows that white tan-plant waxy sorghum can produce better quality malt and flatbreads than regular sorghums, and thus has considerable potential to partially replace barley malt and teff, respectively in these products in areas in Africa where sorghum is a major cereal crop. Hence, either white tan-plant waxy or normal sorghum can be used to partially replace wheat for biscuit making in regions that have shortage of wheat.

DEDICATION

This thesis is dedicated to:

My lovely Mother: MEBRAT LEGESSE HAILE

My lovely life partner: FILMAWIT TESFAY

My lovely daughter: DANAIT ABADI

My sweet cousins: SOLOMON ASHENAFI MEWESHA

NIGUSE GEBREYESUS HAILE

My sweet relatives and friends: LETEBRHAN HAGOS

TSIREITY DESTA

TALEF DESTA

For their love, patience and contentious support

ACKNOWLEDGEMENTS

I would like whole-heartedly to express my sincere gratitude and acknowledgement to my supervisor, Prof John R. N. Taylor, for his timely advice, sharing his wealth of knowledge and constructive criticism during the research. Also, I immensely thank him for the opportunity to work with him and supervise me, for his patience, guidance and endless help at formal and informal levels until I completed my PhD study.

I am also grateful to my co-supervisors, Prof Riette H.L. de Kock and Dr. Kebede Abegaz. I thank Prof. De Kock for her supervisory role, for the opportunity to learn the advanced sensory evaluation aspect of my work and for her nurturing spirit. A special thanks goes to Dr. Kebede for his kind hearted and unlimited support and supervision throughout my study.

I am grateful to the Texas A&M AgriLife Research for the financial support for this research. I am also greatly indebted to the University of Pretoria for additional financial support in form of postgraduate research bursary.

I wish to thank Mr Alan N Hall and Ms Erna Van Wilpe of the University of Pretoria's Laboratory for Microscopy and Microanalysis for assistance with the microscopy study.

I would like to immensely thank Mrs Marise Kinnear, for her technical support during sensory analysis and Dr Tigist Shonte for sharing her experiences sensory analysis of which I learnt a lot.

I am grateful to the academic staff, support staff and fellow postgraduate students at the Department of Consumer and Food Sciences, University of Pretoria, your assistance is unforgettable. Special thanks to Mr. Emmanuel Nekhudzhiga for his assistance with lab equipments, Ms Nandi Dersley, Ms Sharon van den Berg, Ms Mpho and Mr. Eric for their assistance in one way or the other.

Finally, special thanks to my family members, relatives and friends for their moral support and companionship during the entire period of this work.
Above all, Thanks to God.

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1 INTRODUCTION

The biggest challenge as a result of climate change in Africa and other regions of the world is food insecurity (Brown et al., 2007). Drought will be one of the major impacts of climate change in sub-Saharan Africa and alternative cereals like sorghum that are well adapted to harsh climatic conditions will be appropriate in Africa (Hattori et al., 2005). Sorghum is the world's fifth most important cereal in terms of both production and area planted (FAOSTAT, 2013). The greatest production of sorghum is concentrated in sub-Saharan Africa (Rooney et al., 2007). Ethiopia is the second largest producer of sorghum (Sorghum bicolor (L.) Moench) in Eastern and Southern Africa after Sudan (FAOSTAT, 2013). In Ethiopia production of sorghum is increasing (CSA, 2011). However, the producers are not benefitted due to limited functionality, utilization, and marketing of sorghum.

Lager beer, injera (fermented flatbread) and biscuits are important food and beverage products in most parts of Ethiopia. Barley is the preferred grain for malting in the modern brewing industry; owing to its unique characteristics of light-yellow grain color (Gupta et al., 2010), high β-amylase activity, complete degradation of its starch into fermentable sugars and complete proteolysis (Taylor et al., 2013). Due to limited barley production and its inability to use locally available resources, the brewing industry is forced to increase importation of barley grain (Bekele et al., 2012). Efforts have been done to replace imported barley with locally produced ones in Ethiopia (Diageo, 2017). However, barley (both locally produced and imported) is still relatively more expensive than sorghum (Tefera et al., 2012). On the other hand, sorghum is increasingly becoming popular for lager beer production and beverages in other African countries (Taylor et al., 2013).

The most preferred injera is prepared from teff (*Eragrostis tef (Zucc.) Trotter*), a tiny, millet type grain. This is due to its softer texture, preferred taste, and especially its color, ranging from purple to very white (Abraha et al. 2015). FAO (2012) and Minten et al. (2012) state that teff commands a higher market price than other cereals. Preparing injera from sorghum has considerable economic benefits over teff, as sorghum commands much lower price and preparing teff is time consuming and expensive (Tefera, 2012). Concerning biscuits, wheat is the most preferred grain for making biscuits due to the unique properties of its protein (gluten properties)

(Pareyt and Delcour, 2008). The flour mills in Ethiopia have a total production capacity of 3.2 million tons of flour a year and mills have been operating at half capacity due to wheat shortages (Bergh et al., 2012). Owing to shortage of wheat, the bakery industry in Ethiopia relies on importation of wheat grain and/or flour for making biscuits and other pastry products (Habtamu, 2012). Replacing wheat flour with sorghum in making biscuits would have economic advantages.

The major limiting factors of most sorghum cultivars include higher starch gelatinization temperature (Okafor and Aniche, 1987), low protein nutritional quality (Taylor and Taylor, 2011), low malt β-amylase activity (Letsididi et al., 2008) and inability to form a visco-elastic dough like wheat gluten (Dahir et al., 2015). These constraints are thought to be due to the nature of the sorghum grain structure and its chemical composition.

Sorghum lines with combined waxy and high protein digestibility traits have been developed through conventional breeding by Texas A&M University AgriLife Research (Jampala et al., 2012). These sorghum lines, owing to their high starch digestibility are hypothesized to have superior functionality in food and beverage applications (Wong et al., 2009; Peterson, 2010; Elhassan et al., 2015). Therefore, the aim of this study was to determine whether the waxy and high protein digestibility traits in sorghum either singly or in combination have a relationship with improved malting quality as well as improved dough-based food products (injera and biscuit) making quality, which are important foods in Ethiopia.

2 LITERATURE REVIEW

This chapter discusses and reviews studies related to the structure and chemistry of sorghum grain with special reference to genetic modification of sorghum starch and proteins, the science and technology of sorghum malting and making sorghum dough-based food products, genetic improvement of sorghum with respect to malting quality and dough–based product quality (with specific reference to fermented flatbreads and biscuits). The genetic improvement of sorghum varieties for food and beverage end-use quality includes both conventional breeding as well as recombinant DNA technology (genetic engineering).

2.1 Structure of sorghum grain

The structure and chemistry of the kernel play a crucial role in determining the processing properties and product qualities of a cereal grain. Details of the structure of the sorghum grain have been reviewed by many authors, most notably Serna-Saldivar and Rooney (1995). It should be noted, however, that with respect to the grain morphological characteristics and levels of chemical constituents, notable differences exist even between varieties within cereal grain species. These differences strongly affect the quality of products made from cereal grains (Koehler and Wieser, 2013).

The sorghum grain is similar in structure to the maize kernel except for its much smaller size and generally oval shape (Taylor, 2004). The sorghum grain (also known as a kernel or caryopsis) is composed of three main parts (Figure 2.1): the pericarp (outer layer), endosperm (storage tissue), and germ (embryo) (Rooney and Miller, 1982). The endosperm represents approx. 85% of the whole grain, germ (9%), and pericarp (only 6%) (Hwang et al., 2002). The shape, size, proportion and nature of the endosperm, germ, pericarp, subcoat, and colour of pericarp are genetically determined (Rooney and Miller, 1982). Beneath the pericarp, some sorghum varieties (tannin sorghum types) have a darkly pigmented layer called the testa or subcoat (Rooney and Murty, 1981). This layer contains condensed tannins, which are complex polyphenolic compounds. Some of the sorghum grain pigmentation is associated with the presence of condensed tannins.

The sorghum endosperm consists of the aleurone layer and starchy (peripheral, corneous, and floury) portions (Serna-Saldivar and Rooney, 1995). The aleurone cells do not contain starch but they have protein, phytin, minerals, water-soluble vitamins, hydrolytic enzymes and oil (Rooney and Miller, 1982). The corneous endosperm is beneath the aleurone layer and contains starch granules embedded in a dense protein matrix (Serna-Saldivar and Rooney, 1995). The endosperm matrix protein is comprised mainly of glutelins and prolamins (kafirins) (de Mesa-Stonestreet et al., 2010). The starch granules in this tissue are very angular or polyhedral in shape with depressions where protein bodies are trapped between the starch granules (Rooney and Murty, 1981). The inner, floury endosperm has loosely packed cells. The starch granules are spherical and not held together by the protein matrix (Rooney and Miller, 1982). The relative proportions of the corneous to floury endosperm are termed as endosperm texture (Ezeogu et al., 2005). Endosperm texture plays a major role in determining sorghum grain quality particularly hardness and functionality in food applications (Svihus et al., 2005). Endosperm textures in sorghum were found to affect modification during sorghum malting (Chiremba et al., 2013).

The germ consists of two parts: the embryonic axis and the scutellum (Rooney and Miller, 1982). The embryonic axis becomes the new plant and the scutellum is the germ transport reserve tissue with large amounts of oil, protein, enzymes, and minerals.

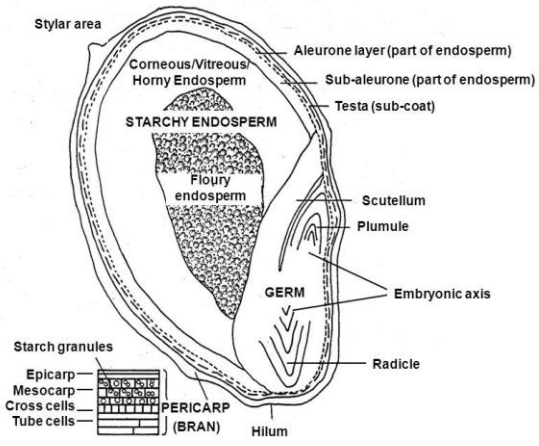


Figure 2. 1 Longitudinal section of a sorghum kernel (Taylor and Belton, 2002)

2.2 Chemistry of sorghum grain

Taylor (2004) reviewed sorghum grain chemistry as compared to that of other cereal species (maize, wheat, barley and rice) and pointed out that sorghum is well known for: its lowish

protein digestibility and reduced protein digestibility when wet cooked, that it is deficient in lysine, that it has a high starch gelatinisation temperature, that it is relatively high in fat and low in β-amylase when malted. Furthermore, it has inert endosperm protein and insoluble endosperm non-starch polysaccharides. As stated, some varieties contain condensed tannins. However, all varieties contain polyphenols (in greater or lesser amounts). The major carbohydrate in sorghum is starch (Léder, 2000), while soluble sugars are low. Sorghum is also a good source of dietary fibre, mainly insoluble fibre, some 86% of the fibre.

The general chemical composition of sorghum grain is as follows: starch (56-75%), protein (4.4-21.1%) (Ratnavathi and Patil, 2013), soluble sugars (0.7-4.2%), reducing sugars (0.05-0.53%), fat (2.1-7.6%), dietary fibre (approx. 6%) and ash (1.3-3.3%) (Table 2.1).

Table 2. 1 Chemical composition of sorghum grain (per 100 g)

Chemical constituent	Contents
Water (%)	9.2
Energy (kJ)	1.42
Protein (g)	11.3
Fat (g)	3.3
Carbohydrate (essentially starch) (g)	74.6
Dietaryfibre (g)	6.3
Ash (g)	1.6
Ca (mg)	28
Fe (mg)	4.4
P (mg)	287
Na (mg)	6
K (mg)	350
Vitamin A (I.U.)	0
Vitamin B_1 (mg)	0.24
Vitamin B_2 (mg)	0.14
Niacin (mg)	2.93

Source: USDA (2011)

2.2.1 Starch

Starch consists of two types of polymers of glucose: the almost entirely linear form amylose (linear molecule of α-1,4 linked glucose monomers) and the highly branched form amylopectin (α-1,4 glucan with α-1,6 branch points) (Zeeman et al., 2007) (Figure 2.2). There is evidence of a small degree of (α-1,6) branching in amylose, but the branching is infrequent and the branches are long, so its general physico-chemical behaviour conforms to that of linear polymers (Wrolstad, 2011). In general, sorghum starch is approx. 75% amylopectin and 25% amylose (Rooney et al., 2005). However, there is a significant inter-varietal difference in starch amylose content among sorghum varieties and waxy sorghum types exist which are high amylopectin (Beta and Corke, 2001). Many important physico-chemical, thermal, and rheological properties of starch are influenced by the ratio of amylose and amylopectin, and by the fine structure of the amylopectin. Amylose content strongly affects starch gelatinization and retrogradation (Fredriksson et al., 1998), paste viscosity (Yanagisawa et al., 2006), gelation (Biliaderis and Zawistowski, 1990), and α-amylase digestibility (Skrabanja et al., 1999). Starch granule swelling is a property of amylopectin (Tester and Morrison, 1990), which facilitate hydrolysis by amylases. Amylopectin starch also lowers pasting temperature as a result processing and hydrolysis requires less energy and time to complete (Sang et al., 2008). A sorghum starch with a high level of short chains needs to be developed to improve cold storage stability (Sang et al., 2008). In contrast, sorghum starch with a high level of long chains would result in increased retrogradation and increased resistance to enzyme digestion.

Chemical and physical modification of starch is carried out to overcome shortcomings of native starches and increase the usefulness of starch for industrial applications (Kaur et al., 2012). Starch can also be modified by plant breeding, mutant generation and crossing, genetic engineering of plants, enzymic modification, and regulatory mechanisms involving allosteric effectors (BeMiller, 1997). Genetic modification can be carried out by traditional plant-breeding techniques or through genetic engineering (Johnson et al., 1999).

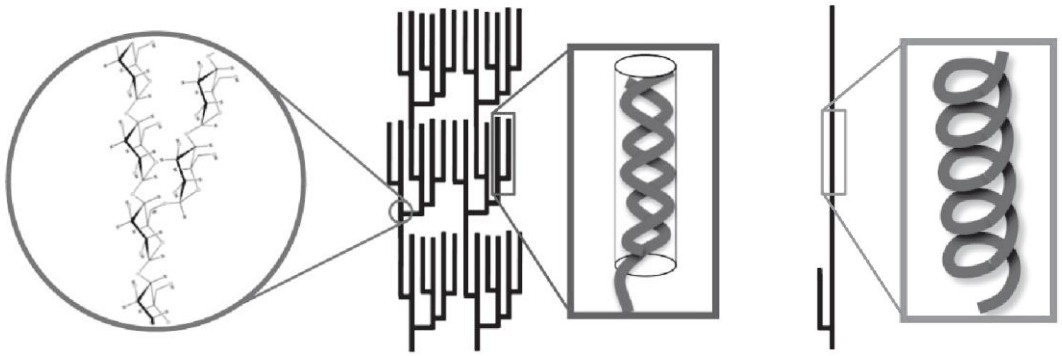


Figure 2. 2 Schematic representation of the structures of amylose and amylopectin (Zeeman et al., 2010)

Understanding starch structure and knowledge of the enzymes involved in starch biosynthesis has increased greatly in recent years and many of the genes that code for these enzymes have been cloned (Kharabian-Masouleh et al., 2012). Jeon et al. (2010) reviewed that the starch biosynthesis in the cereal endosperm requires the coordinated activities of several enzymes: including adenosine 5' diphosphate-glucose (ADP-Glc) pyrophosphorylase (AGPase), granule-bound starch synthase (GBSS), soluble starch synthase (SS), starch branching enzyme (BE), starch debranching enzyme (DBE), and plastidial starch phosphorylase (Pho1). In the endosperm, amylopectin biosynthesis requires the proper execution of a coordinated series of enzymatic reactions involving ADP glucose pyrophosphorylase (AGPase), soluble starch synthase (SS), starch branching enzyme (BE), and starched debranching enzyme (DBE), whereas amylose is synthesized by AGPase and granule-bound starch synthase (GBSS) (Jobling, 2004).

GBSS is the sole enzyme responsible for amylose biosynthesis in the cereal endosperm (Denyer et al., 2001). This 60 kD protein is entirely localized to starch granules, where it is enzymatically active both at the periphery and in the interior of the starch granule, unlike the other starch synthase isoforms. The loss of GBSS function in plants causes the waxy endosperm phenotype, named for its altered texture and appearance of the endosperm. Waxy mutants affecting either gene expression or function of GBSS have been found in several different plant species (Sattler

et al., 2009). Waxy mutants contain very low or undetectable levels of amylose, and their starch granules are nearly entirely composed of amylopectin (Denyer et al., 2001).

2.2.1.1 Waxy sorghum

In sorghum, two classes of waxy mutants have been identified based on the near absence of amylose and are classified based on absence (*waxy*^{a}; *wx*^{a}) or presence (*waxy*^{b}*; wx*^{b}) of the GBSS protein (Sattler et al., 2009). The waxy mutation alters starch composition by greatly reducing amylose content and the physico-chemical properties of waxy starch are also altered relative to normal starch (Pedersen et al., 2007). Waxy type sorghum has increased starch and protein digestibility compared to normal sorghum (Rooney and Pflugfelder 1986; Wong *et al.,* 2009). This increased digestibility could be associated with the reduced kernel density and the increased amount of space between the starch granules observed in both *wx*^{a} and *wx*^{b} (Sattler et al., 2009). Both *wx*^{a} and *wx*^{b} sorghum mutants may have potential applications, even though there are significant reductions in grain yield associated with either allele compared to non-waxy inbred lines (Sattler et al., 2009). However, there are some indications that yield drag associated with *wx* may be overcome through heterosis, different inbred backgrounds or a combination of these strategies (Rooney et al., 2005). Interestingly, in a comparison of normal, HD, waxy and waxy-high digestibility lines, some of the latter were among the best yielding, suggesting that it is possible to breed agronomically acceptable cultivars (Jampala et al., 2012)

The starch amylose content of sorghum grain depends on the dose of the recessive gene (*wx*). Waxy sorghum contains three recessive waxy genes (*wxwxwx*) and heterowaxy sorghum contains at least one recessive gene (*WxWxwx* or *Wxwxwx*) and has intermediate starch amylose content, whereas normal sorghum does not have the recessive gene (*WxWxWx*) (Sang et al., 2008). As stated, sorghum grain with waxy endosperm and a relatively weak protein matrix are more susceptible to hydrolysis by amylase and protease enzymes (Rooney and Pflugfelder, 1986; Wong et al., 2009).

2.2.2 Proteins

Sorghum proteins can be broadly classified into prolamin and non-prolamin proteins (De Mesa-Stonestreet et al., 2010). The major storage proteins in sorghum are prolamin type proteins and

are called kafirins. As such, they contain high levels of proline and glutamine, and are low in the essential amino acid lysine and are soluble in non-polar solvents such as aqueous alcohols. Kafirins account for some 77-82% of the protein in the endosperm, whereas non-prolamin proteins (namely, albumins, globulins, and glutelins) make up about 30% of the endosperm proteins (Belton et al., 2006). Sorghum grain protein is poorly digestible when wet cooked and the reduced digestibility is largely due to kafirin cross-linking by disulphide bonding (Duodu et al., 2003). Kafirins are isolated in spherical protein bodies in the starchy endosperm, which are embedded in a glutelin protein matrix, and are surrounded by starch granules (Taylor et al., 1984). The protein bodies are some 0.4-2.0 μm in diameter (Figure 2.3). The cysteine-rich, disulphide bond cross-linked β- and γ-kafirin types are generally concentrated in the out part of the protein bodies, and the interior is comprised predominantly of cysteine-poor α-kafirin (Duodu et al., 2003). The α-kafirin makes up about 80% of the total kafirin (Hamaker and Bugusu, 2003). Beta-kafirin comprises about 5% of total kafirin, and γ-kafirin about 15%.

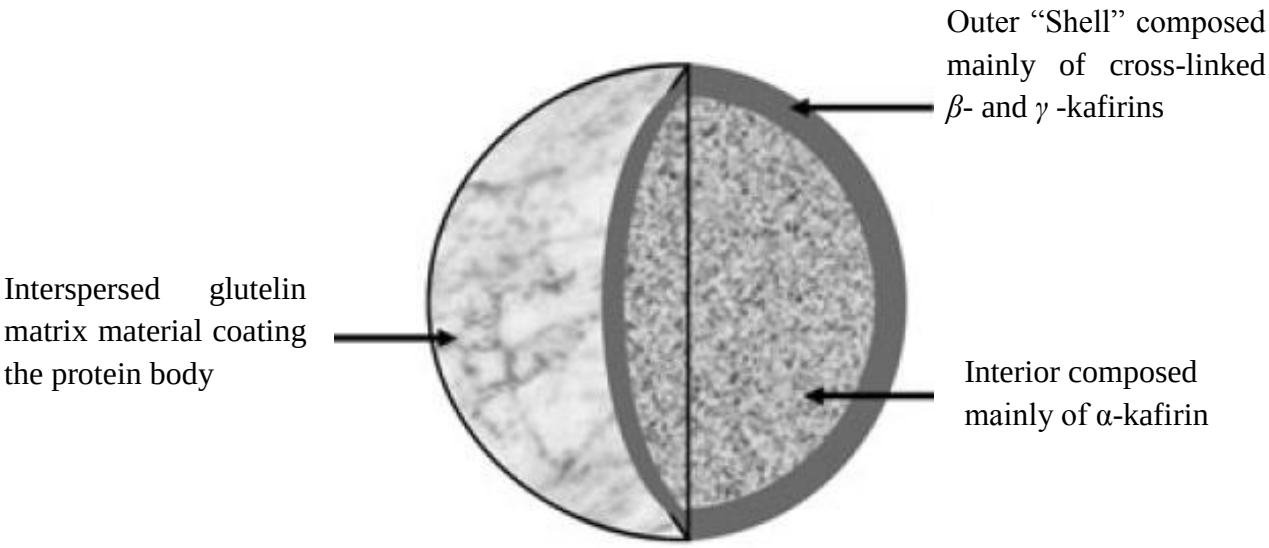


Figure 2. 3 Schematic representation of sorghum protein body (De Mesa-Stonestreet et al., 2010)

As indicated, the kafirins are classified into three main classes based on amino acid sequence, molecular weight and solubility: α-, β- and γ-kafirins (De Mesa-Stonestreet et al., 2010). Delta-kafirin has not yet been identified at the protein level (Belton et al., 2006). All classes show high homology with their zein homologues. The α-kafirin is easily digested, while the β- and γ-

kafirins are not easily digested because they form enzyme resistant structures by disulphide cross-linking (Duodu, 2003). This is due to the fact that both β- and γ-kafirins have high concentrations of the sulphur-containing amino acid, cysteine (Shull et al., 1992). Oria et al. (2000) proposed that the relatively poor digestibility of proteins in the sorghum endosperm is due to the strong disulphide bonds formed by β- and γ-kafirins that produce an enzyme-resistant structure on the periphery of the protein body. Since the highly digestible α-kafirin is predominantly located in the interior, a peripheral enzyme resistant layer of β- and γ-kafirins would negatively influence protein hydrolysis.

Additionally, the kafirin proteins seem to undergo a more severe change in secondary structure on cooking than zeins, from predominantly α-helical to β-sheet conformation, which also seems to adversely affect their digestibility (Emmambux and Taylor, 2009). Generally, the low protein digestibility of sorghum is an important aspect in terms of the limitation of starch and protein hydrolysis during malting and brewing (Mugode et al., 2011).

2.2.2.1 Improvement of sorghum protein digestibility

Research to improve sorghum protein quality has been ongoing since the 1970s, especially at Purdue University in the USA. Protein biofortification of sorghum has been achieved by both chemically induced mutation and genetic engineering (Taylor and Taylor, 2011). Through chemical mutagenesis of normal sorghum using diethyl sulphate, a high-lysine sorghum mutant called P721-opaque was developed. This has up to 60% higher lysine content due to a reduction in the relative amount of kafirins (Guiragossian et al., 1978). However, both this type of high-lysine sorghum and normal sorghums gave similarly very poor nitrogen absorption and retention performance when used in human feeding trials (MacLean et al., 1981). *In-vitro* studies carried together with the feeding trial indicated that the poor quality of sorghum protein was not only just due to its low lysine content but also due to the low digestibility of the sorghum protein in foods (Axtell et al., 1981; Mertz et al., 1984).

By crossing of P721-opaque with normal sorghums, lines were obtained that had substantially improved protein digestibility, 10-15% higher in uncooked flour and 25% higher in cooked flour (Weaver et al., 1998). These lines also have somewhat elevated levels of lysine. The improved protein digestibility appears to be due to a characteristic change in the shape of the kafirin

protein bodies in these high protein digestibility sorghum mutants from spherical to invaginated (Figure 2.4) (Oria et al., 2000). The invaginated shape of the protein bodies is probably as a result of a single point mutation, rendering the 22 kDa α-kafirin type resistant to processing (release from the protein body rough endoplasmic reticulum membrane) (Wu et al., 2013). This change in shape results in the γ-kafirin sub-class being concentrated at the bottom of invaginations where it should not interfere with the digestion of the α-kafirin. Tesso et al. (2006) identified a novel sorghum mutant with both high protein digestibility and high lysine traits, and a relatively hard endosperm. This mutant was an F6 generation of crosses between P721-opaque and hard endosperm sorghum lines. It has some 44% higher lysine than normal sorghum and 20% higher protein digestibility.

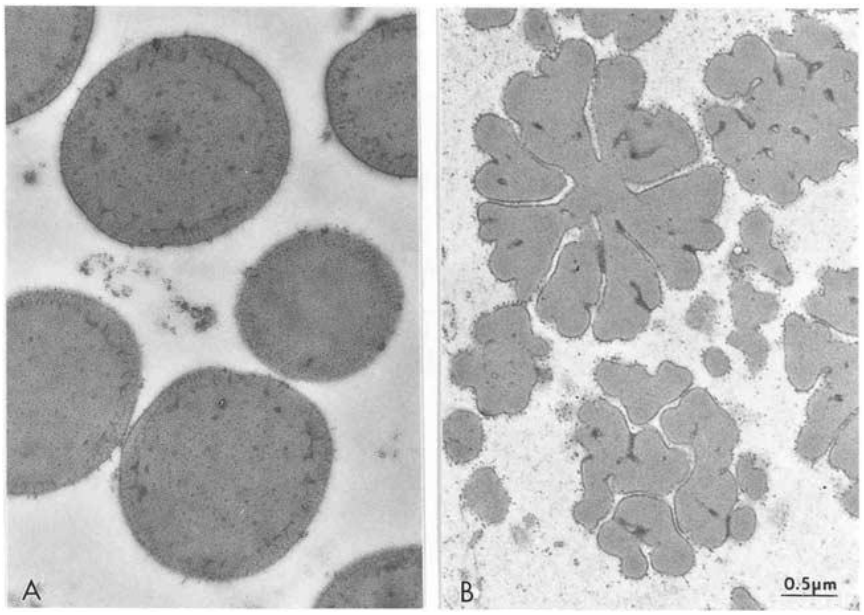


Figure 2. 4 Transmission electron micrographs of wild-type sorghum grain protein bodies (A) and mutant of high protein digestibility protein bodies (B) (Oria et al., 2000). Note the highly folded and invaginated structure of the mutant (Bar = 0.5 µm).

RNA interference (RNAi) technology has been used to suppress the synthesis of specific combinations of types of kafirins (Jung, 2010). High protein digestibility mutant lines have also been obtained through genetic modification by co-suppression of six kafirin genes (α-A1, 25 kDa; α-B1, 19 kDa; α -B2, 22 kDa; γ-kaf1, 27 kDa; γ-kaf 2, 50 kDa; and δ-kaf 2, 18 kDa) (Da Silva et al., 2011b). Grootboom et al. (2014) studied suppression of only three of these kafirin

sub-classes (27 kDa γ-kaf 1; 50 kDa γ-kaf 2; and the 25 kDa α-kaf A1) and found that suppression of their synthesis increased sorghum protein digestibility. Da Silva et al. (2011b) found that improved lines with co-suppression of α-,γ- and δ-kafirins had almost double the content of lysine, 3.7-4.1 g/100 g protein compared to their parent lines 2.1-2.4 g/100 g, and a wet cooked in vitro protein digestibility of 81%, compared to its null control with 58%. The protein bodies of these improved protein quality sorghum lines were irregular in shape and surrounded by a dense matrix of protein and the endosperm was floury (soft). Lines where the synthesis of only the γ- and δ-kafirins was suppressed had normal shaped proteins bodies and corneous (hard) endosperm structure, but the improvement in protein digestibility was less.

Sorghum flour from the high protein digestibility (HD) lines has been shown to give better dough properties (resistance to extension and time to dough breakage) and higher bread loaf volumes than normal sorghum when composited with wheat flour (Goodall et al., 2012). Furthermore, high protein digestibility sorghum flour was found to form visco-elastic dough when mixed with vital gluten in 82:18 ratios, whereas under the same conditions normal sorghum flour did not. Genetically modified sorghum (GM-HD) lines of high protein digestibility (PD) trait through γ-kafirin synthesis suppression have also shown better flour properties, stronger dough and higher viscoelasticity (Elhassan et al., 2017). The HD sorghum lines also have some better brewing attributes (Mugode et al., 2011). When malted, the high protein digestibility sorghums had substantially higher levels of free amino nitrogen (FAN), required for yeast growth and fermentation, than normal sorghums. However, their FAN production during mashing was not significantly higher.

2.2.3 Combining waxy and high protein digestibility traits in sorghum

Texas A&M University AgriLife Research has developed sorghum lines with combined waxy and high protein digestibility traits, with the aim of developing sorghum cultivars with superior functionality in food and beverage applications (Wong et al., 2009; Peterson, 2010). Combining both waxy and HD traits is expected to solve four major limitations affecting the utilization of sorghum: inhibitory kafirin protein matrices surrounding the starch granules, high temperature to starch pasting/gelatinization, poor enzymatic hydrolysis and the low lysine content of sorghum distillers dried grain (the major by-product of grain bioethanol production) (Peterson, 2010).

In fact, waxy and high protein digestibility traits in sorghum show promise for brewing, having easily pasted starch granules, yielding higher FAN, giving faster fermentation and producing more lysine-rich distillers dry grain and solubles (DDGS), compared to normal sorghum lines (Wu et al., 2010). Furthermore, transgenic HD sorghums as whole grain adjunct were found to yield higher extract and FAN (Kruger et al., 2012).

2.3 Sorghum malting science

Malting is the germination of cereal grains in moist air under-controlled conditions (Dewar et al., 1997b). The main objectives of sorghum malting are to mobilize the endogenous hydrolytic enzymes of the grain, modify the grain chemical constituents by means of these enzymes during malting so that they are readily solubilised during the mashing process of brewing to produce a fermentable medium (wort), and solubilize unmalted cereal grain (starchy adjunct) during the mashing process of brewing (Taylor, 2010). During the sorghum malting process, the starchy endosperm is modified and this is characterized by degradation of the starch granules, protein matrix and protein bodies by endogenous hydrolytic enzymes (Glennie et al., 1983; Taylor, 2010). Within the cells, the glutelin endosperm protein matrix is degraded first, while starch granules and kafirin containing protein bodies are degraded at the same time (Glennie et al., 1983). As the protein and starch are hydrolysed, a wave of grain structural changes can be observed through the endosperm starting from the germ (Figure 2.5). This is known as endosperm modification which renders the malt more soluble during mashing (Taylor, 2010).

Sorghum malt is widely used as a major functional component in traditional African beers, lagers and stouts, non-alcoholic malt beverages and porridges (Taylor and Emmambux, 2008). Beer production in general involves saccharification of starch (enzymatic hydrolysis to simple sugars) by amylase enzymes generally derived from malting, then fermentation of the resulting sugars by yeast to produce ethanol and carbon dioxide. Nutritional benefits of malting include reduction of antinutritional factors (e.g. phytate), enhancement of vitamin content (riboflavin, niacin, pyridoxine and ascorbic acid) (Malleshi and Klopfenstein, 1998), improvement of mineral availability (Ca, Mg, Zn and P) (Glennie et al., 1983) and reducing the viscosity and imparting flavour and sweetness to porridge (Taylor and Dewar, 2001). Malting has also been shown to: improve the in vitro digestibility of sorghum protein (Bhise et al., 1988) and starch (Wang and

Fields, 1978), and improve the content of essential amino acids (lysine, methionine and tryptophan) (Taylor, 1983).

Good malt is essential to produce high quality of beer as well as other malt-based food products. Quality parameters for sorghum malt include: extent of endosperm modification, as measured for example by friability (Chiremba et al., 2013); diastatic power (measure of malt amylase activity); malt extract (percentage of the malt which is made soluble during brewing) (Taylor et al., 2013), reduction in wort viscosity (Malomo et al., 2012), protein digestibility (important for improving FAN levels), and FAN (Mugode et al., 2011). As stated, FAN is important in brewing as it is used as a nitrogen source by the yeast during fermentation (Taylor et al., 2013). Adequate FAN levels are required in the wort for rapid and complete fermentation. In sorghum brewing, FAN can be of particular importance as a low ratio of malt to unmalted cereal adjunct is often used, resulting in FAN being limiting (Mugode et al., 2011).

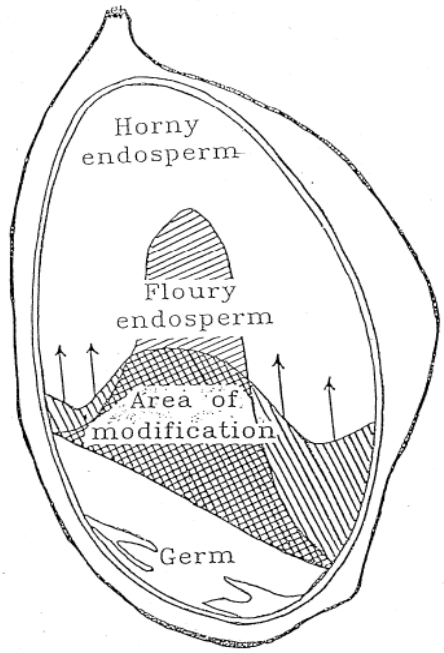


Figure 2. 5 Schematic representation of the wave of endosperm modification in sorghum during malting (Glennie et al., 1983)

2.3.1 Malting Process

Malting has three physically distinct operations: steeping, germination and drying (Dewar et al., 1997b). These process steps are necessary to ensure the proper occurrence of particular physical and biochemical changes: Steeping - to ensure good absorption of water by the grain (from 12% to at least 40% of moisture); Germination - to maintain embryo growth, enzyme synthesis and limited endosperm breakdown; and Kilning (Drying) - to ensure product stability (Gupta et al., 2010). The well-established barley malting protocols cannot be directly applied to sorghum due to its higher temperature and water requirements during germination (Taylor and Robbins, 1993). Germinating sorghum grains tend to rapidly lose water taken up during steeping. Therefore it is necessary to spray germinating grains at intervals during the germination (Morrall et al., 1986; Dewar et al., 1997a).

2.3.1.1 Steeping

Steeping is immersion of the grain in water. The grain then absorbs water and it initiates biochemical processes which lead to seed germination (Briggs, 1998). Steeping is also carried out to clean the grain. A moisture content of 33-35% (wet basis) should be achieved during steeping of sorghum grains (Daiber and Taylor, 1995). It was found that the more water that is taken up during steeping (within limits) the higher is the resulting malt quality (Dewar et al., 1997b). It was also observed by Pitz (1989) that the rate of water absorption of barley is affected by grain structure (softer grains absorb more water than hard grains), and grain size (smaller grains absorb moisture more rapidly).

Steeping temperature, time and aeration also affect the rate of water absorption by the grain and should be chosen to achieve the required level of hydration to produce quality malt (Olkku, Reinikkanen and Carregal, 1991). It was found that the optimum steeping temperature and time for sorghum grains to reach appropriate water content is 25-30°C and 16-40 h, respectively (Morrall et al., 1986; Dewar et al., 1997a). Aeration by draining the water from the grain periodically (air-resting) or sparging air through steeping water is necessary (Novellie and De Schaepdrijver, 1986). During steeping, cereal grains swell and soften, and the living tissues resume their metabolism (Briggs, 1998). It was also found that some nutrients leach out into the steep water (Pathinara et al., 1983).

2.3.1.2 Germination

Germination in sorghum malting normally takes about 6 days and it was found to occur rapidly at a green malt temperature of between 20 and 30°C, with an optimum of 25-28°C (Morrall et al., 1986; Dewar et al., 1997a). Important physiological processes associated with the germination phase are the synthesis of amylases, proteases and other endogenous hydrolytic enzymes (Palmer, 1989). The hydrolytic enzymes migrate from the germ into the endosperm where starch and protein are hydrolysed to sugars and amino acids, respectively (Glennie et al., 1983). These are then transported into the germ where they are further metabolised by the growing seedling (Taylor and Evans, 1989).

During germination, the hard (corneous) endosperm of the umalted grain is modified into more friable malt (Palmer, 1989). The conditions of germination (moisture content, temperature, germination time and oxygen availability) impact greatly on the sorghum malt quality. It was observed that the higher the level of moisture content (within the limits), the higher the resulting malt quality (Morrall et al., 1986; Dewar et al., 1997a). A germination temperature of 24-28°C was also found to result in good quality sorghum malt (Morrall et al., 1986). The germination step is completed when the whole of the starchy endosperm has been modified (partially attacked by enzymes) (Dewar et al., 1995). Optimizing germination conditions in sorghum malting is also important for ensuring that the product is safe (Lefyedi, 2007). Controlling germination conditions reduces dhurrin, a precursor of a toxigenic hydrogen cyanide (HCN) (Tokpohozin et al., 2016) and also helps prevents mycotoxin production by fungi and moulds (Taylor et al., 2005).

2.3.1.3 Kilning (Drying)

Kilning (drying) is the final stage of the malting process. The purpose of drying is to stop the growth of the green malt seedling at the end of the germination process and produce a shelf-stable product complete with active enzymes by reducing moisture content and water activity (a_w) (Novellie and De Schaepdrijver, 1986). During this phase, the germinated sorghum grains are dried at a temperature of about 50°C for 24 hours. Drying at 50°C in a forced-draft oven is specifically used in sorghum malting as it does not substantially inactivate the amylases. Drying at temperatures higher than 50°C may lower amylase activity particularly β-amylase, which is

temperature labile (Taylor and Robbins, 1993). In sorghum malting in southern Africa opaque beer brewing, the dried malt is then milled without the removal of external vegetative parts from the grain. The resultant product has a moisture content of around 10% (Daiber and Taylor, 1995).

2.3.2 Sorghum malting technologies

There are two malting germination technologies practiced to obtain sorghum malt: floor malting and pneumatic malting (Briggs et al., 2004). These technologies differ in germination phase (Taylor et al., 2005). In outdoor floor malting, steeped sorghum is germinated on a flat but slightly sloped concrete floor outside (Taylor et al., 2005). A layer of grain 10-30 cm thick is covered with shade cloth or sacking to prevent excessive loss of moisture and protect against birds and rodents. The temperature is controlled by the level of thickness (thicker beds retain more metabolic heat). The grain is watered at intervals by a hose-pipe and stopped when sufficient. The malt produced is of low quality with respect to malt quality parameters such as diastatic power (Taylor et al., 2005). In pneumatic malting, the steeped grain, which rests on a perforated steel floor, is ventilated with a stream of temperature-adjusted humidified air. Air removes excess heat and carbon dioxide and supplies oxygen (Briggs et al., 2004). Pneumatic malting of sorghum is similar to that of barley, except that the germination temperature in sorghum malting is 25-30°C, approximately 10°C higher than barley malting (Taylor et al., 2005). The grains are turned mechanically by a screw (Briggs et al., 2004). The floor malted grain can either be sun dried or dried using modern kilns in which a current of air is fan-driven from below to dry the malt to a safe moisture level (Briggs et al., 2004). Pneumatic malted sorghum is always dried through blowing warm dry air at a temperature not exceeding 50°C (Taylor et al., 2005).

2.3.3 Starch hydrolysis during sorghum malting

Studies by Glennie et al. (1983), and Chiremba et al. (2013) showed that the starch granules in modified sorghum malt as studied using scanning electron microscopy are pitted and holed where the starch has been hydrolysed. The degradation of starch during malting involves a number of hydrolytic enzymes: α-amylase (E.C. 3.2.1.1; 1, 4-α-D-Glucan, 4-Glucanohydrolase), β-amylase (E.C. 3.2.1.2; (α -1, 4)-Glucan maltohydrolase), limit dextrinase (debranching enzyme) (E.C. 3.2.1.142) and α-glucosidase (maltase) (E.C. 3.2.1.20) (Gupta et al., 2010).

However, the α-amylase and β-amylase are the most important in sorghum malting and little is known about the activity of limit dextrinase (debranching enzyme) and α-glucosidase (Taylor et al., 2013). It was found by Hibberd et al. (1982) that the starch granules of waxy (high amylopectin) sorghums showed slightly higher digestibility by amylases (amyloglucosidase and α-amylase) than those of non-waxy sorghums. Another study also found that the amount of starch hydrolysis of waxy starches incubated with α-amylase exceeded that of high amylose starch (Tester et al., 2004). This higher starch hydrolysis was reported to be as a consequence of the better starch granule swelling property of the amylopectin (Tester and Morrison, 1990), facilitating greater hydrolysis by amylases. Also, amylopectin provides larger surface area for α-amylase enzyme attack since the α-amylase can also attack native starch randomly (Tester et al., 2006).

Alpha-amylase is an endoenzyme with starch liquefying power and it attacks the α-(1->4) glucosidic bonds within starch molecules to produce dextrins (short chains of glucose molecules) and a variety of sugars including maltotriose (3 glucose molecules), maltose (2 glucose molecules) and glucose (Briggs et al., 2004). Beta-amylase is an exoenzyme which cannot attack directly the starch granules but it degrades the dextrins produced by α-amylase at the non-reducing end of the molecules, hydrolysing the penultimate α-(1->4) glucosidic bond to release maltose. The α-amylase and β-amylase work together to bring about almost complete degradation of starch into simple sugars (Taylor, 2010).

There are clear physiological differences between sorghum and barley during malting with regard to the location where synthesis of the amylase enzymes takes places (Lyumugabe et al., 2012). During germination, the hormone gibberellic acid (GA), at low concentration (0.1-0.2 ppm), induces the barley aleurone layer to produce α-amylase, but this hormone apparently plays no such role in the enzyme development in sorghum (Palmer, 1989). In sorghum, α-amylase is produced by the scutellum, while limit dextrinase develops in the starchy endosperm (Palmer, 1989). These contrasts with malting barley, where α-amylase and limit dextrinase develop in the aleurone layer, while β-amylase is produced in the starchy endosperm (Taylor et al., 2006). The β-amylase activities in sorghum malts are not nearly high as compared to the barley malt (Letsididi et al., 2008). A study by Taylor and Robbins (1993) showed that ungerminated sorghum does not exhibit β-amylase activity. The same study observed that treatment of sorghum

with reducing agents or cysteine, HCl and mercaptoethanol, or with the proteolytic enzyme papain failed to yield any β-amylase activity. This indicated that the enzyme is not in a bound form, unlike in barley. Isoelectric focusing indicated that sorghum β-amylase comprises just one major and one minor isozyme of pI approx. 4.4-4.5, unlike the many isozymes all of higher pI in barley.

The joint activity of α- and β-amylase in sorghum malt can be measured by means of the SABS Diastatic Power (DP) assay (Taylor et al., 2005) and in the sorghum beer industry it is expressed in Sorghum Diastatic Units (SDU) per gram of malt (South African Bureau of Standards, 1970). When determining the DP of malts made from high tannin (bird-proof) sorghum varieties, it is necessary to extract the amylases in the presence of peptone; otherwise they will be inactivated by the tannins, giving an artificially low value for DP (Taylor, 1989). The level of α-amylase activity in sorghum malt is similar to that in barley malt but the level of β-amylase activity is much lower (approx. one-third of the level in barley malt) (Dufour et al., 1992). As result of its low β-amylase activity, sorghum malt has a lower DP than barley malt (Dewar et al., 1995). The modification of endosperm components primarily starch during sorghum malting can also be assessed using Hot Water Extract (HWE), which as stated is a measure of soluble solids and gives an estimate of how much of the malt would be solubilised during brewing (Taylor et al., 2005). Extract content can give an indication of the modification of the malt during malting (the breakdown of endosperm reserves by amylase and protease) (Taylor, 2010). Generally, the higher the extract the better, as less material which is not solubilised is lost as spent grain.

2.3.4 Proteolysis during sorghum malting

As studied by transmission electron microscopy, both the protein bodies and the surrounding matrix protein of sorghum malt are extensively eroded where the protein has been hydrolysed (Taylor et al., 1985; Ng'andwe et al., 2008; Mugode et al., 2011). Protein degradation during malting is brought about by protease enzymes (Taylor, 2010). There are two general types of proteases (proteinases and peptidases). Hydrolysis of internal peptide bonds within protein molecule polypeptide chain to release peptides is catalysed by proteinases, while hydrolysis at the C and N terminal ends to release amino acids and dipeptides is catalysed by peptidases. It is thought that the most important peptidases in malting and brewing are the carboxypeptidases,

which hydrolyse the terminal peptide bond at the C terminal end of peptides (Evans and Taylor, 1990a). Both proteinases and peptidases are required to degrade proteins completely into amino acids (Taylor, 2010). Apparently, the proteinases (endopeptidases) and peptidases (exopeptidases) are located primarily in the sorghum endosperm and germ, respectively (Serna Saldivar and Rooney, 1995). Though it is generally accepted that unmalted cereal grains have little enzymatic activity, the work by Evans and Taylor (1990b) revealed that in resting (unmalted) sorghum grain, there was a considerable level of proteinase activity but this was accompanied by a low level of carboxypeptidase activity.

Taylor and Evans (1989) showed that the protein bodies in the sorghum starchy endosperm were degraded mainly from the periphery. Glutelin (matrix protein) was first hydrolysed, followed by the kafirin protein body. Proteinases from both the germ and endosperm of germinated sorghum were capable of degrading the protein bodies. These findings led to the conclusion that the proteinases responsible for sorghum endosperm proteolysis were synthesized in the germ and then secreted into the starchy endosperm during germination. Malted sorghum has been found to possess more endopeptidase activity than exopeptidase activity (Evans and Taylor, 1990a). The temperate cereals (e.g. wheat, barley, oats, rye and triticale) have more exopeptidase activity than endopeptidase activity (Taylor et al., 2013).

Taylor et al. (2013) explained the problems associated with hydrolysing the proline-rich cereal prolamin storage proteins into free amino acids. Barley malt contains up to 42 different endoproteases alone, which are of four different classes: metallo, serine, cysteine and aspartic (Zhang and Jones, 1995). Sorghum malt has a similar pattern of endoproteases to barley malt, but the enzymes have a low pI (Jones and Lookhart, 2005) and high levels of metallo-, cysteine- and serine-type proteases have been found (Zhang and Jones, 1995). The sorghum malt proteases are insoluble in simple aqueous solvents (Evans and Taylor, 1990a; Ogbonna et al., 2003). Hence, the enzymatic sorghum malt extracts which have been proposed for use in lager brewing (El Nour and Yagoub, 2010) could be very deficient in protease activity.

The level of endoprotease activity in sorghum does not increase substantially during malting (Evans and Taylor, 1990b). It has been suggested that the unusual prolyl-type endopeptidase is highly important with regard to hydrolysis of the proline-rich peptide products of endoprotease

cleavage of prolamins into free amino acids (Simpson, 2001). Such prolylendopeptidases have been found in germinated barley (Mikola, 1983) and there are indications that they are present in sorghum, maize and rice (Simpson, 2001). There is carboxypeptidase activity in sorghum malt at brewing type acidic pH (Ogbonna et al., 2003) and that activity releases FAN from endopeptidase hydrolysed kafirin prolamin (Evans and Taylor, 1990b). Unlike the endoprotease activity, the carboxypeptidase activity in sorghum was found to increase substantially during malting (Evans and Taylor, 1990b).

Besides the protein and starch, a number of other components of the grain are metabolised and/or solubilised during malting. They include cell wall materials (non-starch polysaccharides) such as cellulose, pentosans and β-glucans, lipids (fats), phenolic pigments and flavour compounds, vitamins and minerals (Taylor, 2010). Modifications of the non-starch polysaccharides influences wort separation. Lipids, phenolics, vitamins and minerals influence wort fermentability and the final character of the beer (Daiber and Taylor, 1995). There is no literature on effects of waxy and HD traits on hydrolysis of non-starch polysaccharides, lipids and phenolic pigments during malting. However, Gupta et al. (2010) reviewed that the presence of the waxy trait in barley does not affect the content of arabinoxylans to the same extent as that of *β*-glucans.

2.4 Research into the effect of starch and protein digestibility on malting quality

As explained, waxy sorghum grains with essentially 100% amylopectin also exist (Rooney and Miller, 1982). Research has shown that low amylose content and extractable proteins in sorghum are associated with increased malting performance (Zhao et al., 2008). Waxy starches of barley apparently facilitate amylolysis and hence have advantages for brewing (Vasanthan and Hoover, 2009). Due to the better granule swelling properties of the starch amylopectin, there has been considerable interest in using waxy sorghum in lager beer brewing (Ortega Villicaña and Serna-Saldivar, 2004) and more recently for bioethanol production (Yan et al., 2011). Waxy sorghum as adjunct in brewing trials showed more rapid starch hydrolysis and lower wort viscosity as compared to normal sorghum (Figueroa et al., 1995). A study by Osorio-Morales et al. *(*2000) indicated that waxy sorghum adjunct gave higher extract yield, filtered more rapidly and produced wort with the same level of fermentable carbohydrates as normal sorghum. Barredo-

Moguel et al. (2001a, b) also demonstrated that wort from unmalted grits of waxy sorghum was suitable for fermentation by yeast and brewing in lager beers. Similarly, a study on effect of sorghum endosperm type on bioethanol production showed that waxy and hetero-waxy lines had the highest fermentation efficiency (Wu et al., 2010). Study by Figueroa et al. (1995) found waxy and heterowaxy sorghums to produce a wort rich in complex carbohydrate and low in fermentable sugars that could be potential for low alcohol beers i.e. opaque beer.

Another constraint in the utilization of sorghum for industrial brewing is its high starch gelatinization temperature (Okafor and Aniche, 1987). Starch digestibility is higher in low-amylose, i.e. waxy (high amylopectin) sorghum, than in normal sorghum (Hibberd et al., 1982). Amylose is more compact in the granule due to its linear structure and helical chain, which in turn makes the access of enzymes difficult. In contrast, the amylopectin molecule owing to its branched chain, allows greater access of these enzymes (Denardin et al., 2012). Waxy and heterowaxy sorghums have high susceptibility to amylolysis, give shorter mashing conversion times, high wort filtration rates, with low wort glucose and fructose levels and their beer bears more resemblance to those brewed from barley (Barredo-Moguel et al., 2001; Del Pozo-Infran et al., 2004). Furthermore, Wong et al. (2009) observed that the waxy trait in sorghum enables the endosperm proteins to be exposed to proteases and result more soluble proteins.

Hydrolysable protein in sorghum is the source of FAN for the yeast during fermentation (Mugode et al., 2011). When malted, HD sorghums were found to have substantially higher levels of FAN than normal sorghums. However, their FAN production during mashing was not significantly higher. When HD lines were used as whole grain adjunct, they yielded substantially higher extract and higher FAN than their normal sorghum controls (Kruger et al., 2012).

2.5 Sorghum dough products

This section considers and reviews studies related to the science of sorghum dough products with particular reference to flatbreads and biscuits. Researches into the effects of sorghum endosperm modifications (waxy and HD traits) on quality of dough based products (with specific importance in fermented flatbreads and biscuits) are also reviewed.

2.5.1 Science of sorghum flatbreads

Most sorghum for human use is consumed as porridges and flatbreads (FAO, 1995). Flatbread is a bread made from a flattened dough or batter of flour, water, salt, yeast or mixed culture naturally fermented sourdough and other optional ingredients (Al-Dmoor, 2012). Sorghum is used for production of naturally fermented traditional flat or semi-leavened breads such as kisra of Sudan (Ejeta, 1982), kisar of Chad (Murty and Kumar, 1995), injera of Ethiopia and Eritrea (Gebrekidan and GebreHiwot, 1982; Yetneberk et al., 2004) and dosa of India (Rooney et al., 1986) and thosai of Sri Lanka (Murty and Kumar, 1995). It has been found that sourdough fermentation improves the quality and shelf life of wheat bread (Dal Bello et al., 2007). Texture and aroma profile of bread was also found to be affected as a consequence of sourdough fermentation (Corsetti and Settanni, 2007). A study by Urga et al. (1997) has also found that sourdough fermentation to make injera (teff flatbread) improved the starch and protein digestibility.

During preparation of fermented flatbreads like injera or kisra, a slurry of flour is subjected to lactic acid fermentation (Yetneberk et al., 2004). The fermentation to produce these foods involves controlled souring through naturally occurring lactic acid bacteria (Chavan and Kadam, 1989). The fermentation is originally spontaneous and dependent upon the load and flora of microorganisms naturally present in the flour, mixing water and air-borne contaminants (Yetneberk, 2004; Chavan and Chavan, 2011). However, households are generally able to carry out consistently successful fermentations through practising a system of back-slopping, whereby a portion of liquid from a successful fermentation is used to inoculate freshly prepared dough of sorghum flour. Nout et al. (1989) showed that by back-slopping each day the normally slow fermentation process (2-3 days) was accelerated by enrichment with acid producing strains of lactic acid bacteria. This simple method of carrying out predictable lactic acid fermentation is also practiced in Ethiopian households for injera production.

Teff dough fermentation for injera making involves several groups of microorganisms, viz: Gram-negative rods, lactic acid bacteria and yeasts, growing in succession (Gashe et al., 1982). The dominating yeast floras at the peak of the fermentation were *Torulopsis* and *Saccharomyces* species (Gifawesen and Bisrat, 1982). An amylase-producing bacteria (*Bacillus sp. A-001*) has

been isolated from fermenting teff dough, which might be involved in the hydrolysis of the starch (Lealem and Gashe, 1994). In teff injera fermentation, it was found that there was a reduction in pH of the dough from about pH 5.8 to pH 3.8; lactic acid and acetic acid being the major organic acids (Umeta and Faulks, 1989).

The initial step in the process of injera preparation is mixing sifted flour with water and kneading well by hand to make thick dough (Abraha et al., 2013). It generally involves two fermentation stages. The first takes 2-3 days (depending on the sourness desired) from mixing the flour with water and adding back-slopped culture. Afterwards, a portion of the fermented dough (5-10%) is cooked and added back to the fermented dough to initiate the second fermentation. The mixture is then brought to a batter consistency and allowed to ferment for about 2-3 hours. After gas bubbles have formed and subsided, the batter is then baked covered (Yetneberk et al., 2004).

Good quality injera is characterized by having a large number of evenly spaced "eyes" (honeycomb-like holes) on the top surface, non-sticky top and bottom surfaces, a slightly sour taste and it remains soft, supple and flexible after overnight storage (Gebrekidan and GebreHiwot, 1982; Yetneberk et al., 2004). The appearance, size, and distribution of gas holes on the injera surface and its taste and texture all impact the preference and acceptability of injera (Abraha et al., 2013). In addition, flavour and colour play significant roles in acceptability of sorghum injera (Zegeye, 1997).

Sorghum does not contain wheat glutenin-like proteins (Taylor et al., 1984). Thus, sorghum dough, unlike wheat dough, is poorly cohesive and not elastic. During production of sorghum breads including flatbreads, there are two possibilities for creating a cohesive dough: adding elastic and water binding substances such as gums as gluten substitutes or modification to the bread making procedure through pre-gelatinizing some of the starch, as in the "custard process", to make the dough more viscous so that it will hold gas during fermentation (Taylor and Dewar, 2001). In a traditional injera making procedure, part of the fermented dough is cooked to gelatinize the starch, then carbon dioxide produced by the fermentation is trapped and leavens the injera on baking (Yetneberk et al., 2004). Hence, starch and flour properties probably play a critical role in injera quality. On baking injera, the batter with partially gelatinized starch is poured on a very hot clay griddle and cooked covered. Covering allows steam to cook the upper

surface of the injera and prevent it from drying out (Attuquayefio, 2014). The starch granules fuse into a continuous amorphous matrix in which bubbles of gas are trapped (Parker et al., 1989). These authors reported that the protein bodies played no role in the formation of the matrix-gas bubble interface.

Staling is a major quality problem with flatbreads, which involves sensorial and physico-chemical changes like firming, declining flavour, increasing opacity, and decreasing starch solubility (Kulp and Ponte, 1981). Perhaps the most important change is firming. A review on bread staling by Fadda et al. (2014) indicates that waxy wheat flour and high amylopectin flours of other grains such as barley retards the rate of staling of bread.

2.5.2 Research into effect of starch and protein composition on fermented sorghum flatbread quality

Gebrekidan and GebreHiwot (1982) found that soft endosperm types of sorghum with white or red pericarp, regardless of sub-coat presence, produced the best injera. Yetneberk et al. (2004) found that the texture and sensory quality of injera made from soft endosperm was better and concluded that sorghum varieties have an influence on both injera making and keeping qualities. Among the soft sorghum varieties that appeared the same visually, significant differences in texture and keeping quality of injera were observed (Gebrekidan and GebreHiwot, 1982; Yetneberk et al., 2004). The major problem is that sorghum injera rapidly becomes firm and friable upon storage (Yetneberk et al., 2004). Poor texture and keeping quality are the major limiting factors of the acceptability of many sorghum cultivars for injera as compared to teff injera (Yetneberk et al., 2005). Sorghum injera quality has been found to be significantly and positively correlated with protein and linoleic acid content, and negatively correlated with tannin content, lipids, floury endosperm, stearic and oleic acid contents (Geleta et al., 2005). This study also indicated that with sorghum, starch content, amylose content, 1000 kernel weight, grain colour, palmitic acid and linolenic acid content did not show significant correlation with injera quality.

Concerning staling, high amylose rice has been found to be dry and become hard upon cooling (Ring et al., 1982). In contrast, the low amylose types are moist and sticky when cooled under optimum conditions. Guo et al. (2003) also reported that wheat flour with low amylose content

gave fresh tortillas (an unfermented flatbread) which had higher extensibility after three or more days of storage. However, tortillas made with low amylose wheat flours required more force to break the tortillas and the rupture distances became shorter. The fine structure of amylopectin is important with regard to retrogradation properties (Jane et al., 1999). It was found that staling rate is retarded in laboratory-produced breads using waxy and pregelatinized waxy barley starch at the 3% level (Purhagen et al., 2011).

There seems to be no literature on the influence of sorghum protein composition on starch retrogradation and bread staling. However, Xie et al. (2004) found that protein (gluten) retards wheat bread staling mainly by diluting starch. It was concluded that starch and protein interaction reduces the staling rate of wheat bread but is less important than starch retrogradation. A study on the effects of wheat proteins (albumins, globulins, gliadins, and glutenins) on retrogradation of wheat starch showed that only glutenins retarded retrogradation of the starch and the other three protein types promoted it within a certain concentration range (Xijun et al., 2014).

2.5.3 Science of sorghum biscuits

Biscuits (cookies) are sweet baked dough products with a low moisture content (1-5%) (Chevallier et al., 2000b). The major ingredients are flour, sugar, fat and water (Maache-Rezzoug et al., 1998) and additional ingredients may include milk, salt, flavouring agent, aerating agent and other additives. Biscuits are normally prepared from soft wheat flours, but they can also be prepared with non-wheat flours such as sorghum (Dendy, 1993). Up to 100% sorghum flour can be used in biscuit making but this results in the biscuits having a drier more sandy texture (Rooney, 2010). Biscuits made from sorghum flour (Badi and Hoseney, 1976) and pre-gelatinized sorghum flour dough (Dendy, 1993) have been found to be gritty and fragile. As reviewed by Pareyt and Delcour (2008), most authors agree that wheat biscuit quality is only influenced slightly by the flour starch but highly by the flour protein. Starch granules remain almost intact in biscuits, whereas the proteins appear aggregated in the biscuits when compared to the dough (Chevallier et al., 2000a). Kaldy et al. (1991) found that a higher amylose content was related to a large biscuit diameter. The level of damaged starch is also an important parameter for biscuit quality (Chevallier et al., 2000a). In study by Adedara (2017) the roles of

sorghum starch and sugar were observed to be critical for determining and understanding development of sorghum biscuit texture.

There seems to be no literature related to the influence of sorghum protein composition on sorghum biscuit quality. However, as stated, wheat flour protein composition has been found to have a major influence on biscuit quality and, in particular, on their diameter (Pareyt and Delcour, 2008). Souza et al. (1994) stated that the total protein content is more important for wheat sugar-snap type biscuit quality than is the protein composition. In fact, varying wheat flour protein content from 14 to 20% has been found to induce major changes in the dough rheological properties and the dimensions and texture of biscuits (Maache-Rezzoug et al., 1998). Serrem et al. (2011) found that sorghum biscuits had a dry and crispy texture as compared to wheat-soy and sorghum-soy composite biscuits. However, in study by Omoba et al. (2015) sorghum biscuits were found to be indistinguishable from whole wheat biscuits in terms of hardness, roughness and coarseness. The crispiness and dry texture of sorghum biscuits was attributed to the hydrophobic nature of kafirin proteins of the endosperm (Duodu et al., 2003), as well as probably the absence of gluten. Adedara (2017) found that the sorghum kafirins, when sorghum biscuits are studied by SEM and TEM, remained isolated in their protein bodies and were unlikely to contribute to structure and texture of sorghum biscuits. It has been found that compositing soya flour to sorghum in biscuits imparted positive sensory characteristics such as crisp texture and reduce the hard and dense texture (Serrem et al., 2011).

2.6 Conclusions

The above review on studies related to the sorghum structure and chemistry in reference to genetic modification of sorghum starch and proteins, revealed that sorghums with these modifications are desirable in improvement of sorghum for food and beverage end-use quality. The starch composition (amylose/amylopectin ratio) and protein digestibility of sorghums could impact on both processing and product quality characteristics as applied in malt and sorghum dough–based food products (injera and biscuits). It also showed that there are very limited studies concerning the relationship of waxy (high amylopectin) and high protein digestibility (HD) traits in sorghum and malting quality and sorghum dough–based products (injera and biscuit) quality. This study is therefore intended to determine whether the novel sorghum lines developed by Texas A&M Agrilife which express the waxy and high protein digestibility traits

either singly or in combination have improved malting and malt quality and improved dough-based food quality. Furthermore, the study is also ultimately aimed at using waxy and HD- traits in sorghum for malt beverages and dough–based food products.

3 HYPOTHESES AND OBJECTIVES

3.1 Hypotheses

i. The waxy (high amylopectin) and high protein digestibility (HD) traits in the novel sorghum lines either singly or in combination due to the waxy trait and protein body alteration will result in improved malting quality compared to normal sorghum.

Waxy (high amylopectin) type barley has been found to show improved starch hydrolysis as a result of improved susceptibility to amylolysis (Vasanthan and Hoover, 2009). Unmalted waxy sorghum as adjunct in brewing trials also showed more rapid starch hydrolysis (Figueroa et al., 1995). It also gave higher extract, filtered more rapidly and produced wort with the same level of fermentable carbohydrates as normal sorghum (Osorio-Morales et al., 2000). The improved starch hydrolysis was thought to be as a result of the better starch granular swelling property of amylopectin which could facilitate greater hydrolysis by amylases. Starch granule swelling is considered as a property of amylopectin (Tester and Morrison, 1990) and as a result, waxy (high amylopectin) starch is easily hydrolyzed by α-amylase (Wu et al., 2010). Mugode et al. (2011) found that malted HD lines gave substantially higher FAN than normal sorghums. The improved FAN was thought to be as a consequence of the improved protein digestibility and susceptibility to proteolytic enzymes. Furthermore it has also been found that transgenic unmalted sorghum lines with the HD trait gave increased hot water extract (HWE) and wort FAN (Kruger et al., 2012).

ii. The waxy and HD traits in the novel sorghum lines either singly or in combination will result in softer injera with reduced staling property and biscuits with improved textural properties compared to normal sorghum.

It has been observed that high amylose rice cooks dry, is less tender, and becomes hard upon cooling (Ring et al., 1982). In soft wheat, high amylose content was found to be associated with larger cookie diameter (Kaldy et al., 1991). In sorghum, amylopectin is less susceptible to re-association during retrogradation than amylose (Sang et al., 2008). Amylopectin retrogradation occurs very slowly (Lii et al., 2004), which may result in softer and slower staling baked products (Fadda et al., 2014). Adedara (2017) found sorghum starch to sugar glass structure

formation that seemed to produce a functionality that compensates for the absence of gluten which was responsible for the similar texture of sorghum to wheat biscuits.

3.2 Objectives

The general objective of this study was to determine the relationship between waxy and HD traits in sorghum and malting quality and dough-based products (Injera and biscuit) making quality. The study also in the long term intends using of the traits in making beer and malt beverages in Ethiopia, improving the nutrition of consumers of injera and biscuits because of the low cost of sorghum, promoting food security and income generation of farmers.

Specific objectives:

1. To characterize presumed waxy and HD sorghum lines in terms of endosperm texture, amylopectin content, in vitro protein digestibility, and protein body morphology.
2. To determine the relationship between the waxy and HD traits in the novel sorghum lines and malting quality in terms of endosperm modification during malting, and malt quality parameters such as malting loss, α- and β -amylase activity, hot water extract (HWE), free amino nitrogen (FAN), starch and protein losses.
3. To determine the relationship between the waxy and HD traits in the sorghum lines and dough-based products quality (softness, staling and sensory characteristics of injera and biscuits).

4 RESEARCH

4.1 GRAIN CHARACTERISATION OF SORGHUM LINES WITH PRESUMED WAXY (HIGH AMYLOPECTIN) AND HIGH PROTEIN DIGESTIBILITY TRAITS

4.1.1 Abstract

Sorghum lines with waxy starch and high protein digestibility (HD) traits have been developed through conventional breeding by Texas A&M AgriLife Research. Sorghum lines presumed to express these traits were cultivated in Texas A&M University, Texas, USA (29 lines), Nanga farm (Zambia) (8 lines) and Ukulima farm (South Africa) (8 lines) and characterised for endosperm texture, starch amylopectin, protein content and in vitro protein digestibility. Characterisation of the sorghum lines grown in Texas showed that only two lines expressed the waxy trait, four lines had high raw flour in vitro protein digestibility and floury endosperm. However, all other lines were non-waxy and heterowaxy with corneous and intermediate endosperm texture. The eight sorghum lines grown at Ukulima and Nanga farm showed differing endosperm traits: waxy, heterowaxy, waxy-HD, non-waxy-HD and non-waxy-normal protein digestibility traits. The protein content and in vitro protein digestibilities of the sorghum lines from Ukulima and Nanga farm showed no significant correlations. The growing environments had some effect on the quality of sorghum lines obtained from the different locations. TEM of sorghum lines grown at Ukulima revealed that two waxy-HD lines had protein bodies which were not densely packed in the protein matrix, of irregular shape and small size. These lines had also high cooked in vitro protein digestibility. As all the eight lines were closely related, had similar starch (73.0-78.5%) and protein (12.0-13.8%) contents, they were considered to be appropriate for the doctoral research study.

4.1.2 Introduction

Sorghum, in terms of both production and area planted, is the world's fifth most important cereal FAOSTAT (2013). The data also shows that Africa is the major sorghum producing region with more than 40% of world production. Worldwide the largest areas of sorghum cultivation are in sub-Saharan Africa and India, where sorghum is a staple crop providing food (Rooney et al., 2007). Sorghum is one of the most heat- and drought-tolerant cereal crops. Hence, it is highly suited for cultivation in the semi-arid and sub-tropical regions of Africa (Srinivas et al., 2009). Furthermore, sorghum does not bring an adverse reaction in coeliacs (Ciacci et al., 2007). However, despite its high production, drought tolerance and applicability in gluten-free foods and beverages, worldwide sorghum commercialization for foods and beverages is limited.

The major reasons for its limited use include its poor functionality in malt for brewing due to the low β-amylase activity, incomplete starch degradation into fermentable sugars and limited proteolysis (Taylor et al., 2013). Moreover, sorghum cultivars produce injera (Ethiopian fermented flatbread) with poor texture and keeping quality (Yetneberk et al., 2005) and biscuits with a dry and sandy texture (Rooney, 2010).

Sorghum lines with waxy (high amylopectin) and high protein digestibility traits have recently been developed by Texas A&M University in the USA through conventional breeding (Jampala et al., 2012). The aim is to develop sorghum cultivars with superior functionality in food and beverage applications (Wong et al., 2009, Peterson, 2010). However, work on end-use functionality of these sorghum lines for malting and dough-based products has been very limited.

The objective of this work was to characterize the presumed waxy and HD traits in the novel sorghum lines in terms of endosperm texture, amylopectin content, in vitro protein digestibility, and protein body morphology.

4.1.3 Materials and Methods

4.1.3.1 Sorghum samples

Thirty seven sorghum lines were characterized. All were derived from crosses between lines RTx2907 and P850029 by Texas A&M Agrilife Research, Texas, USA and provided by Texas

A&M Agrilife. RTx2907 is a waxy and normal protein digestibility sorghum released from the Texas Agrilife sorghum breeding program (Miller et al., 1996). P850029 is a high protein digestibility line that was developed by Purdue University from the high lysine line P721Q (Weaver et al., 1998). They comprised 29 lines which had been grown in Texas, and another eight lines which had been increased at Nanga farm, Zambia and at Ukulima research farm, Limpopo Province, South Africa. All were cultivated in controlled field trials. The lines grown in Texas were a mixture of grain and glume colours, whereas those selected for increase at Nanga and Ukulima farms were all white tan-plant types. The weight of each of the sorghum lines grown in Texas was 200 grams for each, while the lines increased at Nanga and Ukulima farm were each 1.5 kg and 20 kg, respectively.

The lines grown at each location were characterised for starch amylopectin content, protein content, raw protein digestibility and endosperm texture. Additionally, the sorghum lines increased at Ukulima farm were characterised for starch content, cooked in vitro protein digestibility and protein body morphology. Grains were milled for analyses using a laboratory hammer mill (Falling Number AB, Huddinge, Sweden) fitted with a 0.5 mm opening screen.

4.1.3.2 Sorghum grain endosperm texture

Endosperm texture, defined as the proportion of corneous endosperm relative to floury endosperm in the grain, was determined according to ICC Standard 176 (ICC, 2011) by viewing 20 longitudinally sectioned kernels (with germ) using a stereomicroscope.

4.1.3.3 Moisture

The moisture and dry matter content of the samples was determined using AACC Method 44-15A (AACC, 2000), so as to correct to dry basis the data for the various attributes measured.

4.1.3.4 Starch

The total starch content of the sorghum lines was determined using the Megazyme Total Starch assay procedure (Amyloglucosidase/α-amylase method) (Megazyme Ireland International, Bray, Ireland). The assay employs thermostable α-amylase to hydrolyze starch into soluble branched and unbranched maltodextrins and amyloglucosidase to quantitatively hydrolyze the

maltodextrins to D-glucose. Then oxidised D-Glucose is quantitatively measured colorimetrically.

4.1.3.5 Protein

The protein content (N x 6.25) of the sorghum lines was determined by a Dumas combustion assay according to AACC method 46-30 (AACC International, 2000).

4.1.3.6 Starch amylopectin

The Megazyme assay kit for Amylose/Amylopectin (Megazyme Ireland International, Bray, Ireland) was used to determine starch amylopectin content of the sorghum lines. Amylopectin is specifically precipitated by the addition of the lectin concanavalin A (Con-A) and removed by centrifugation. The amylose in the supernatant is enzymatically hydrolysed to D-glucose, and measured colorimetrically. The starch amylopectin content is then calculated by subtracting the amylose content from 100.

4.1.3.7 In vitro protein digestibility (IVPD)

IVPD of raw and wet cooked flours of the sorghum lines was determined by the pepsin digestion assay described by Da Silva et al. (2011b) , based on that of Hamaker et al. (1986). Nitrogen was then quantified by the Dumas combustion assay.

4.1.3.8 Transmission Electron Microscopy (TEM)

Protein body morphology of the sorghum lines was assessed using TEM as described (Da Silva et al., 2011b) with some modifications in fixing and dehydration. Sections of the periphery of the mid-endosperm (1-2 mm thick) were fixed in glutaraldehyde in pH 7.4 phosphate buffer (18 h) and stained with osmium tetraoxide. Samples were dehydrated sequentially in ethanol solutions. Samples were infiltrated with Quetol resin and polymerised at 60°C. Ultra-thin sections were stained with uranyl acetate, and Reynold's lead citrate, and viewed using a field emission transmission electron microscope (JEOL JEM-2100F, Japan).

4.1.3.9 Statistical analyses

All chemical analyses were repeated at least twice. The data for starch content, protein content, starch amylopectin content and IVPD were analysed using one-way ANOVA. The means were separated using Tukey's HSD test at $p<0.05$.

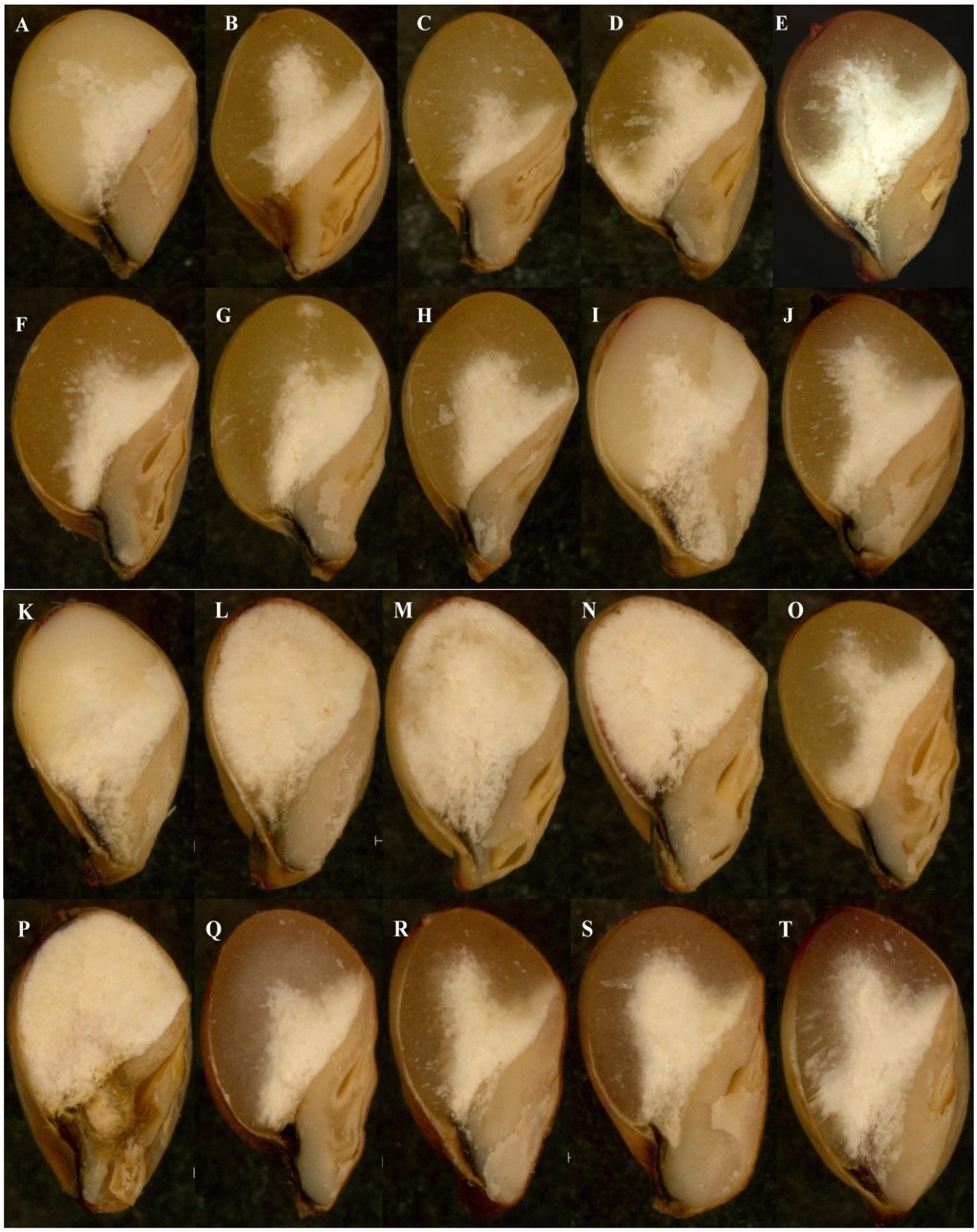
4.1.4 Results and discussion

4.1.4.1 Grain endosperm texture

The sorghum lines exhibited a range of endosperm types (Figures 4.1.1, 4.1.2 and 4.1.1.3). Out of the twenty nine lines cultivated in Texas, four lines (113-W/P, 114-W/P, 119-W/P, and 121-W/P) had a predominantly floury endosperm (Figure 4.1.1 L, M, N, P). These lines had also higher raw flour IVPD (Table 4.1.1). This is in agreement with Elhassan et al. (2015) that sorghum lines of high protein digestibility have a floury endosperm. The floury endosperm texture of transgenic HD sorghum mutants (Da Silva et al., 2011a) and the soft endosperm character of this non-transgenic HD mutant (Tesso et al., 2006) have been reported previously. Three lines (101-W/T, 102-W/P and 105-W/P) had a pale waxed floor-like appearance, typical of waxy sorghum (Rooney and Miller, 1982). These lines had intermediate endosperm (Figure 4.1.1A, I, K). All the remaining 22 sorghum lines had corneous (Figure 4.1.1B, C, F, G, H, O, Q, R, S, U, V, W, X, Y, Z, AA) and intermediate (Figure 4.1.1D, E, J, T, AA, AC) endosperm texture.

Longitudinal sections through the eight lines increased at Nanga farm (Zambia) and Ukulima farm also showed variable endosperm types (Figure 4.1.2 and Figure 4.1.3, respectively). Of the lines increased at Nanga farm, four lines (Figure 4.1.2f, h, d, g) had a pale waxed floor-like appearance, of which the first two lines had an intermediate and the latter two had a predominantly floury endosperm. Of the other four lines, two lines had a predominantly corneous endosperm (Figure 4.1.2a, c), while one line had a floury endosperm (Figure 4.1.2b) and another line with an intermediate endosperm (Figure 4.1.2e). Regarding the lines increased at Ukulima, the endosperm of five lines (Figure 4.1.3d, e, f, g, h) had a pale waxed floor-like appearance, typical of waxy sorghum (Rooney and Miller, 1982). Three of these waxy lines (Figure 4.1.3e, f, h) had an intermediate to corneous endosperm. In contrast, two of the lines (Figure 4.1.3d, g) had a predominantly floury endosperm. Of the other three lines, two lines

(Figure 4.1.3a, c) had a predominantly corneous endosperm, while one line (Figure 4.1.3b) had a floury endosperm.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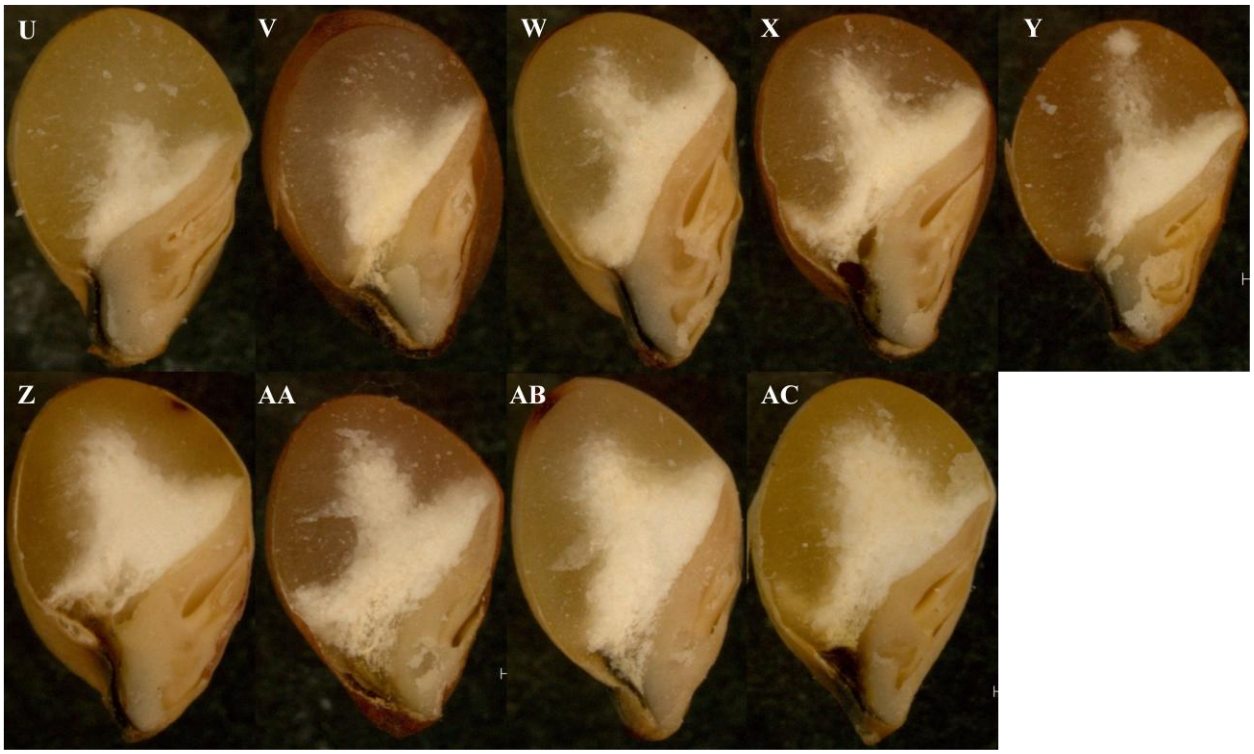

Figure 4.1. 1 Endosperm texture of the sorghum 29 lines grown in Texas.

A (line 101-W/T), B (103-W/T), C (106-W/T), D (115-W/T), E (122-W/T), F (123-W/T), G (124-W/T), H (125-W/T), I (102-W/P), J (104-W/P), K (105-W/P), L (113-W/P), M (114-W/P), N (119-W/P), O (120-W/P), P (121-W/P), Q (107-R/T), R (108-R/T), S (109-R/T), T (110-Rw/P), U (111-Rw/T BL), V (112-Rw/T BL), W (116-Rw/T BL), X(117-R/P), Y (118-R/T), Z (110-white), AA (111-white), AB (112-white), AC (116-white).

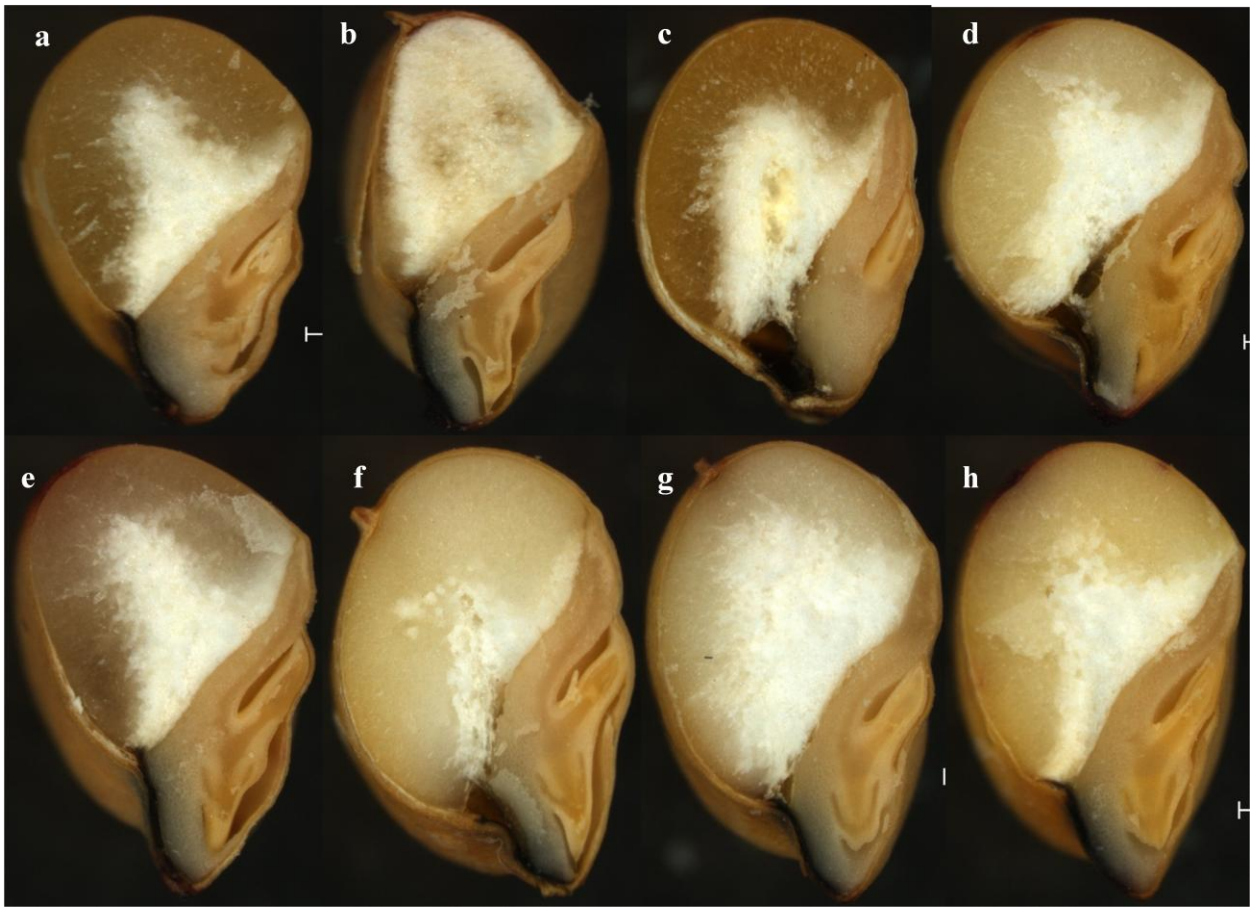

Figure 4.1. 2 Endosperm texture of the eight sorghum lines grown at Nanga farm (Zambia). a (line 98109), b (97983), c (98089), d (97999), e (98039), c (98075), g (98131), h (98045).

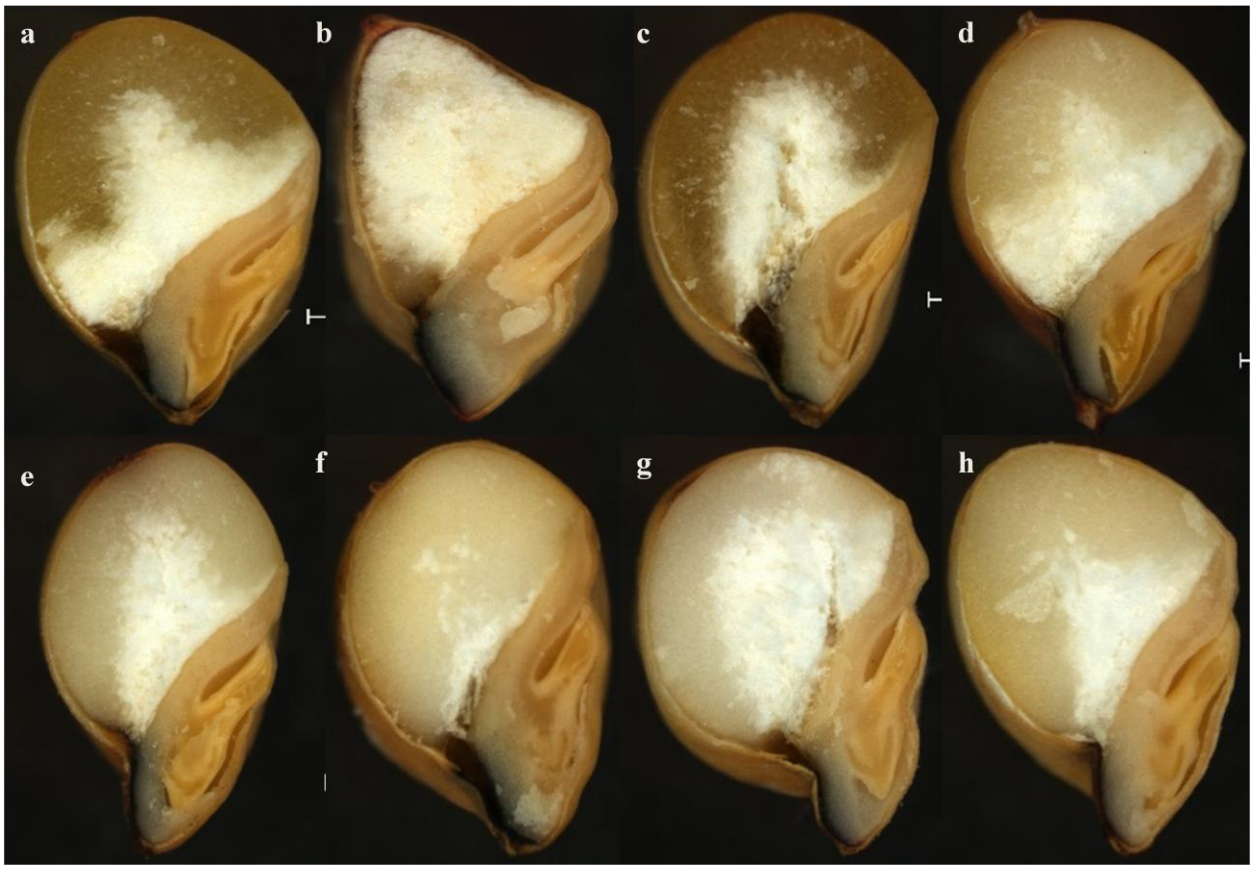

Figure 4.1. 3 Endosperm texture of the eight sorghum lines grown at Ukulima farm (South Africa). a (line 98109), b (97983), c (98089), d (97999), e (98039), f (98075), g (98131), h (98045)

4.1.4.2 Protein

Among the 29 sorghum lines grown at Texas, protein content ranged from 9 to 11.3% on dry basis (Table 4.1.1). Five of the lines (101-W/T, 123-W/T, 119-W/P, 107-R/T and 116-white) had significantly higher ($p<0.05$) protein content compared to the other lines, with no significant difference among them. Six lines (117-R/P, 118-R/T, 111-Rw/T BL, 121-W/P, 108-R/T and 110-white) had lower protein content. All the other lines had intermediate protein content. The protein contents of the eight sorghum lines grown at Nanga farm ranged from 9.7 to 13.4% (Table 4.1.2). Three of these lines (e, c and f) had higher ($p<0.05$) protein content (11.2, 12.2 and 13.4%, respectively). Sorghum lines a, b and d had an intermediate protein content, while two other lines (g and h) had lower ($p<0.05$) protein content. As shown in Table 4.1.3, the protein content of the sorghum lines grown at Ukulima farm varied between 12 and 13.8% on dry basis. Only one of the lines (e) had higher ($p<0.05$) protein content (13.8%). Sorghum lines b, c, d, f and h had intermediate protein content. The other lines (a and g) had lower ($p<0.05$) protein content. The protein content of all the sorghum lines was within the range of the waxy, heterowaxy and HD sorghums studied by Wu et al. (2010).

4.1.4.3 Starch

The starch content of the sorghum lines grown at Ukulima farm was determined and it ranged from 73.0 to 78.5% on dry basis (Table 4.1.3). There was no statistical significant difference ($p>0.05$) in the starch content between the sorghum lines. The starch content of these sorghum lines was slightly higher than the sorghum genotypes studied by Wu et al. (2007), Wang et al. (2008), and Wu et al. (2010).

4.1.4.4 Waxy (high amylopectin) and high protein digestibility (HD) traits

The starch amylopectin content of the sorghum lines grown in Texas varied considerably from 63.4 to 90.6% (Table 4.1.1). Out of the 29 lines, two lines (101-W/T, and 105-W/P) had significantly ($p<0.05$) and much higher starch amylopectin content (90.6 and 88.2%, respectively) indicating that they were waxy lines (Sang et al., 2008). All the remaining lines had lower starch amylopectin contents (63.4–85.3%). Thus, they were non-waxy and heterowaxy sorghum types. Three of the eight lines (h, d and g) grown at Nanga farm (Zambia) had significantly higher ($p<0.05$) starch amylopectin content (87-89.4%), thus they were waxy lines

(Table 4.1.2). The other five lines had lower starch amylopectin content showing that they were non-waxy and heterowaxy sorghum types. Concerning these eight lines increased at Ukulima farm, five lines (d, e, f, g and h) were typical waxy sorghum as their starch amylopectin contents were high, ranging between 87.9 and 94.1% (Table 4.1.3). The other three lines had lower starch amylopectin content (79.9–85.4%) and hence they were non-waxy and heterowaxy sorghum types. This finding is in agreement with Elhassan et al. (2015) and Sang et al. (2008) working with sorghum, as all the waxy lines in the current study contained some amylose, it would appear that all were heterowaxy types, containing at least one recessive waxy gene. Moreover, Table 4.1.4 shows that the starch amylopectin content (waxy trait) of the sorghum lines increased at Ukulima farm was significantly ($p<0.01$) correlated with that of the lines increased at Nanga farm.

As shown in Table 4.1.1, the raw flour IVPD of the sorghum lines grown in Texas varied between 67.2 and 78.5%. Only four lines (113-W/P, 114-W/P, 119-W/P and 121-W/P) had significantly higher ($p<0.05$) raw flour IVPD. All the other lines had raw flour IVPD ranging between 67.2 and 74.2%. These IVPD data correlated with the IVPD data from Texas A&M University of the same lines grown at two locations. The IVPD data of Texas A&M University was kindly provided by Prof J.M. Awika of the Soil and Crop Science Department. The correlations were significant at $p<0.05$ with Pearson's correlation value ($r = 0.409$), degrees of freedom ($df = 27$) for the data from location-1 (Figure 4.1.4) and $p<0.01$ for location 2, $r= 0.577$ and $df = 27$ (Figure 4.1.5). The raw flour IVPDs of these non-transgenic HD sorghum lines were within the range of HD sorghum raw flour IVPD studied by Da Silva et al. (2011b) and Elhassan et al. (2015) in this laboratory, but were lower than found by Weaver et al. (1998) at Purdue University. This difference was probably due to differences in IVPD assay methodology between the two laboratories.

Table 4.1. 1 Endosperm texture, starch amylopectin content, protein content and in vitro protein digestibility of the 29 sorghum lines grown in Texas

Sorghum line	Endosperm texture	Starch Amylopectin (%)	Protein content (%, db)	IVPD (raw flour) (%)
101-W/T	Intermediate, Waxy	$90.6^{q} \pm 0.6$	$11.3^{j} \pm 0.2$	$71.0^{bcdefg} \pm 1.0$
103-W/T	Corneous, Non-waxy	$74.9^{hijkl} \pm 0.9$	$9.3^{abcd} \pm 0.1$	$71.9^{defg} \pm 1.3$
106-W/T	Corneous, Non-waxy	$76.2^{jkl} \pm 1.4$	$10.2^{fg} \pm 0.0$	$69.7^{abcdef} \pm 0.4$
115-W/T	Intermediate, Non-waxy	$71.4^{defghi} \pm 1.6$	$9.7^{bcde} \pm 0.1$	$73.1^{fg} \pm 0.9$
122-W/T	Intermediate, Non-waxy	$75.3^{ijkl} \pm 1.6$	$10.8^{hi} \pm 0.2$	$72.7^{fg} \pm 1.0$
123-W/T	Corneous, Non-waxy	$78.6^{lmn} \pm 1.4$	$11.2^{ij} \pm 0.1$	$69.6^{abcdef} \pm 0.2$
124-W/T	Corneous, Non-waxy	$72.8^{ghij} \pm 0.1$	$9.8^{cdef} \pm 0.2$	$74.2^{gh} \pm 0.1$
125-W/T	Corneous, Non-waxy	$64.1^{ab} \pm 1.1$	$9.9^{ef} \pm 0.1$	$74.1^{gh} \pm 0.3$
102-W/P	Intermediate, Heterowaxy	$85.3^{op} \pm 0.3$	$10.0^{efg} \pm 0.1$	$72.3^{efg} \pm 1.4$
104-W/P	Intermediate, Non-waxy	$71.8^{fghi} \pm 0.7$	$10.2^{efg} \pm 0.2$	$71.3^{bcdefg} \pm 0.3$
105-W/P	Intermediate, Waxy	$88.2^{pq} \pm 0.7$	$9.8^{cdef} \pm 0.0$	$73.0^{fg} \pm 0.3$
113-W/P	Floury, Non-waxy	$70.2^{defg} \pm 0.4$	$9.8^{def} \pm 0.1$	$78.6^{i} \pm 0.6$
114-W/P	Floury, Heterowaxy	$82.0^{mno} \pm 0.9$	$9.3^{abcd} \pm 0.0$	$76.9^{hi} \pm 0.6$
119-W/P	Floury, Non-waxy	$73.8^{ghijk} \pm 0.0$	$11.1^{ij} \pm 0.1$	$78.4^{i} \pm 0.4$
120-W/P	Corneous, Heterowaxy	$82.7^{no} \pm 1.0$	$10.1^{efg} \pm 0.1$	$73.0^{fg} \pm 0.7$
121-W/P	Floury, Non-waxy	$76.4^{jkl} \pm 0.7$	$9.2^{ab} \pm 0.2$	$78.5^{i} \pm 0.8$
107-R/T	Corneous, Non-waxy	$68.1^{bcdef} \pm 1.3$	$11.3^{j} \pm 0.2$	$67.2^{a} \pm 0.8$
108-R/T	Corneous, Non-waxy	$64.9^{abc} \pm 1.5$	$9.2^{ab} \pm 0.0$	$69.5^{abcdef} \pm 1.2$
109-R/T	Intermediate, Non-waxy	$63.4^{a} \pm 1.0$	$9.3^{abcd} \pm 0.1$	$69.8^{abcdef} \pm 1.1$
110-Rw/P	Intermediate, Heterowaxy	$82.2^{mno} \pm 0.8$	$9.3^{abcd} \pm 0.0$	$67.8^{ab} \pm 1.5$
111-Rw/T BL	Corneous, Non-waxy	$67.7^{abcdef} \pm 1.1$	$9.0^{a} \pm 0.1$	$71.6^{cdefg} \pm 0.7$
112-Rw/T BL	Corneous, Non-waxy	$65.2^{abc} \pm 1.2$	$10.0^{efg} \pm 0.1$	$72.1^{defg} \pm 0.2$
116-Rw/T BL	Corneous, Non-waxy	$67.1^{abcd} \pm 0.7$	$10.4^{gh} \pm 0.1$	$71.2^{bcdefg} \pm 0.2$
117-R/P	Corneous, Non-waxy	$70.4^{defg} \pm 1.0$	$9.0^{a} \pm 0.2$	$73.0^{fg} \pm 0.3$
118-R/T	Corneous, Non-waxy	$71.6^{efghi} \pm 0.9$	$9.0^{a} \pm 0.2$	$69.4^{abcdef} \pm 2.8$
110-white	Corneous, Non-waxy	$68.4^{cdef} \pm 1.2$	$9.2^{ab} \pm 0.1$	$68.0^{abc} \pm 0.7$
111-white	Intermediate, Non-waxy	$70.8^{defgh} \pm 1.4$	$9.4^{bcd} \pm 0.2$	$68.6^{abcde} \pm 0.4$
112-white	Corneous, Non-waxy	$67.5^{abcde} \pm 1.1$	$10.1^{fg} \pm 0.0$	$68.6^{abcde} \pm 0.4$
116-white	Intermediate, Non-waxy	$77.9^{klm} \pm 1.5$	$11.3^{j} \pm 0.1$	$72.2^{defg} \pm 0.3$

Values are Mean ± standard deviation (n=2); Values with different letter superscripts in the same column are significantly different ($p < 0.05$).

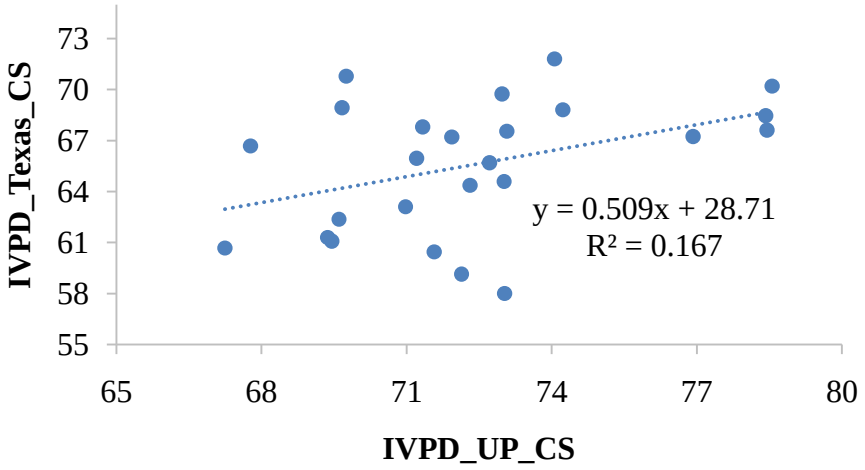

Figure 4.1. 4 Correlation of the raw grain in vitro protein digestibility (IVPD) of the 29 sorghum lines grown in Texas at location 1 (14CS) as analysed by Texas A&M University and the University of Pretoria (Two tailed significance p = 0.042; r = 0.409; df = 27)

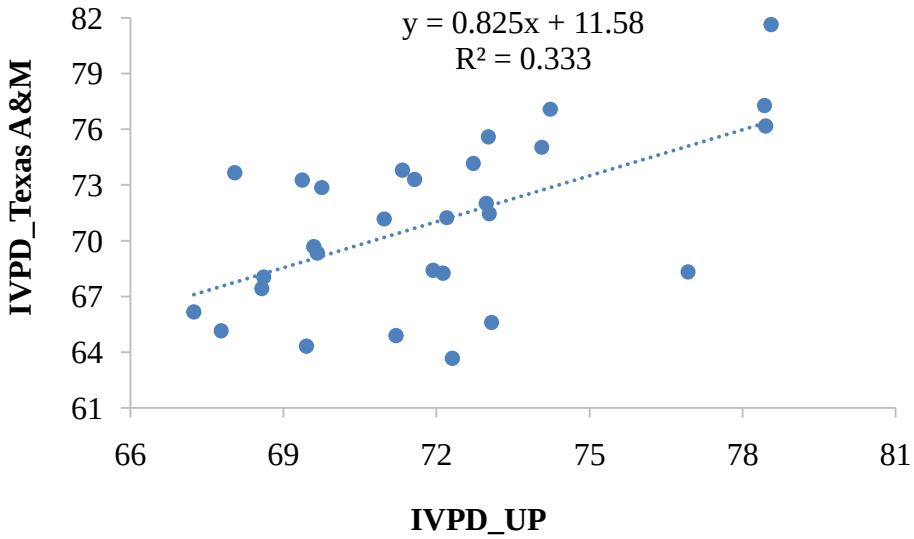

Figure 4.1. 5 Correlation of the raw grain in vitro protein digestibility (IVPD) of the 29 sorghum lines grown in Texas at location 2 (14HW) as analysed by Texas A&M University and the University of Pretoria (Two tailed significance p = 0.001; r = 0.577; df = 27).

As shown in Table 4.1.2 the IVPD of the sorghum lines grown at Nanga farm reveals that two lines (b and g) had significantly higher ($p<0.05$) raw flour IVPD (82.3 and 75.9%, respectively). These lines had also higher ($p<0.05$) cooked flour IVPD (59.6 and 54.5%, respectively). The other six lines had IVPD of raw and cooked flour IVPD ranging between 72.2 and 74.9%, and between 46.9 and 51.5, respectively. Among these sorghum lines, the IVPD of raw flours were significantly ($p<0.01$) correlated with the IVPD of cooked flours (Table 4.1.4). The cooked flour protein digestibilities of these sorghum lines were slightly higher than that of Elhassan et al. (2015). Moreover, as observed by Da Silva et al. (2011a) with transgenic HD sorghums and with these non-transgenic sorghums, the IVPD of the cooked flours were significantly lower than those of the raw flours.

The protein body morphology and protein digestibility of the sorghum lines grown at Ukulima farm are shown in Figure 4.1.6 and Table 4.1.3, respectively. Transmission electron microscopy (TEM) of the lines revealed that two waxy-HD lines (d and g) had protein bodies which were not densely packed in the protein matrix, of irregular shape and small size (0.48-0.56 µm diam.), typical of HD sorghum lines (Oria et al., 2000; Da Silva et al., 2011b). These lines had generally higher cooked flour IVPD (57.1-62.5%) than the other lines (Table 4.1.3). Furthermore, as explained in section 4.1.4.1 these lines had floury endosperms. Hence, this is in agreement with previous work that has shown that the sorghum types with the HD trait exhibit a floury character (Tesso et al., 2006; Da Silva et al., 2011a; Elhassan et al., 2015). Line b also had a relatively high cooked IVPD (65.9%), but its protein bodies were normal in shape (Figure 4.1.6b). However, the kernels were indented (as opposed to being essentially spherical) and the endosperm was relatively small and almost completely floury (Figure 4.1.3b), suggesting that other mutations had occurred. The smallish and floury nature of the endosperm may have been responsible for its relatively high protein digestibility.

Table 4.1. 2 Endosperm texture, starch amylopectin content, protein content and in vitro protein digestibility (IVPD) of sorghum lines increased at Nanga farm, Zambia

Sorghum line	Endosperm texture	Starch amylopectin content (%)	Protein content (%, db)	Protein digestibility traits	
				IVPD (raw flour) (%)	IVPD (cooked flour) (%)
a	Corneous, Non-waxy	$69.3^{a}\pm 0.7$	$10.4^{b}\pm 0.1$	$72.2^{a}\pm 0.6$	$50.8^{b}\pm 1.9$
b	Floury, Non-waxy	$75.1^{b}\pm 0.4$	$10.6^{b}\pm 0.2$	$82.3^{d}\pm 1.0$	$59.6^{d}\pm 1.2$
c	Corneous, Non-waxy	$74.0^{b}\pm 0.8$	$12.2^{d}\pm 0.1$	$74.7^{abc}\pm 0.8$	$48.9^{ab}\pm 1.0$
d	Intermediate, Waxy	$89.4^{d}\pm 0.7$	$10.7^{bc}\pm 0.1$	$72.7^{ab}\pm 1.5$	$46.9^{a}\pm 0.8$
e	Intermediate, Non-waxy	$80.5^{c}\pm 0.9$	$11.2^{c}\pm 0.1$	$75.6^{c}\pm 0.9$	$49.9^{ab}\pm 1.4$
f	Corneous, Heterowaxy	$82.1^{c}\pm 0.6$	$13.4^{e}\pm 0.3$	$74.5^{abc}\pm 1.3$	$49.8^{ab}\pm 1.6$
g	Floury, Waxy	$88.6^{d}\pm 0.6$	$9.7^{a}\pm 0.4$	$75.9^{c}\pm 1.6$	$54.5^{c}\pm 1.9$
h	Intermediate, Waxy	$87.0^{d}\pm 0.9$	$9.7^{a}\pm 0.1$	$74.9^{bc}\pm 1.0$	$51.5^{bc}\pm 1.3$

Values are Mean ± standard deviation (n = 2). Values in a column with different letter superscripts are significantly different ($p< 0.05$). a (line 98109), b (97983), c (98089), d (97999), e (98039), f (98075), g (98131), h (98045)

Table 4.1. 3 Endosperm texture, starch amylopectin content, starch and protein content, protein body size and invitro pepsin protein digestibility (IVPD) of the eight sorghum lines increased at Ukulima farm (South Africa)

Values are Mean ± standard deviation (n = 2). Values in a column with different letter superscripts are significantly different ($p < 0.05$ a (98109), b (97983), c (98089), d (97999), e (98039), f (98075), g (98131); h (98045); *lines had irregular shaped protein bodies.

Sorghum line	Endosperm texture	Starch amylopectin content (%)	Starch content (%, db)	Protein content (%, db)	Protein digestibility traits		
					IVPD (raw flour) (%)	IVPD (cooked flour) (%)	Protein body diameter(µm) from TEM
a	Corneous, Non-waxy	$79.9^{a} \pm 1.1$	$75.4^{a} \pm 3.9$	$12.2^{a} \pm 0.1$	$74.0^{ab} \pm 2.6$	$55.0^{bc} \pm 1.8$	0.84 ± 0.16
b	Floury, Non-waxy	$81.1^{ab} \pm 1.0$	$73.8^{a} \pm 2.9$	$13.0^{b} \pm 0.2$	$77.8^{b} \pm 2.7$	$65.9^{d} \pm 1.9$	0.81 ± 0.24
c	Corneous, Heterowaxy	$85.4^{bc} \pm 0.1$	$75.8^{a} \pm 5.3$	$13.0^{b} \pm 0.1$	$73.6^{ab} \pm 2.5$	$52.5^{ab} \pm 1.6$	1.18 ± 0.22
d	Floury, Waxy	$87.9^{c} \pm 0.7$	$76.1^{a} \pm 4.5$	$12.0^{a} \pm 0.2$	$72.8^{ab} \pm 2.2$	$62.5^{d} \pm 1.9$	*0.48 ± 0.10
e	Intermediate, Waxy	$88.7^{cd} \pm 0.9$	$73.8^{a} \pm 2.9$	$13.8^{c} \pm 0.1$	$71.9^{ab} \pm 2.9$	$48.9^{a} \pm 1.9$	0.89 ± 0.17
f	Corneous, Waxy	$89.1^{cde} \pm 2.5$	$73.0^{a} \pm 4.7$	$13.4^{bc} \pm 0.2$	$76.0^{ab} \pm 2.1$	$56.4^{bc} \pm 1.7$	1.25 ± 0.16
g	Floury, Waxy	$93.6^{de} \pm 2.0$	$74.7^{a} \pm 1.5$	$13.4^{bc} \pm 0.2$	$71.6^{a} \pm 2.7$	$57.1^{c} \pm 1.7$	*0.56 ± 0.06
h	Intermediate, Waxy	$94.1^{e} \pm 0.4$	$78.5^{a} \pm 4.6$	$13.0^{b} \pm 0.2$	$70.7^{a} \pm 2.8$	$49.1^{a} \pm 1.8$	1.45 ± 0.22

Table 4.1. 4 Correlation matrix of grain quality attributes of sorghum lines increased at Ukulima and Nanga farms

	Starch AMP 1	Protein 1	IVPD raw 1	IVPD cooked 1	Starch AMP 2	Protein 2	IVPD raw 2
Protein 1	0.430 ns						
IVPD raw 1	-0.695 ns	-0.039 ns					
IVPD cooked 1	-0.440 ns	-0.415 ns	0.701 ns				
Starch AMP 2	0.859**	0.107 ns	-0.531 ns	-0.004 ns			
Protein 2	-0.169 ns	0.288 ns	0.483 ns	0.003 ns	-0.254 ns		
IVPD raw 2	-0.185 ns	0.411ns	0.537 ns	0.443 ns	-0.133 ns	-0.109 ns	
IVPD cooked 2	-0.206 ns	0.266 ns	0.456 ns	0.427 ns	-0.187 ns	-0.369 ns	0.881**

Two tailed correlation significant at * $p<0.05$, ** $p<0.01$, *** $p<0.001$ and ns – not significant at $p\geq0.05$, respectively. AMP (Amylopectin content), IVPD (invitro protein digestibility), 1 (lines increased at Ukulima farm), 2 (lines increased at Nanga farm).

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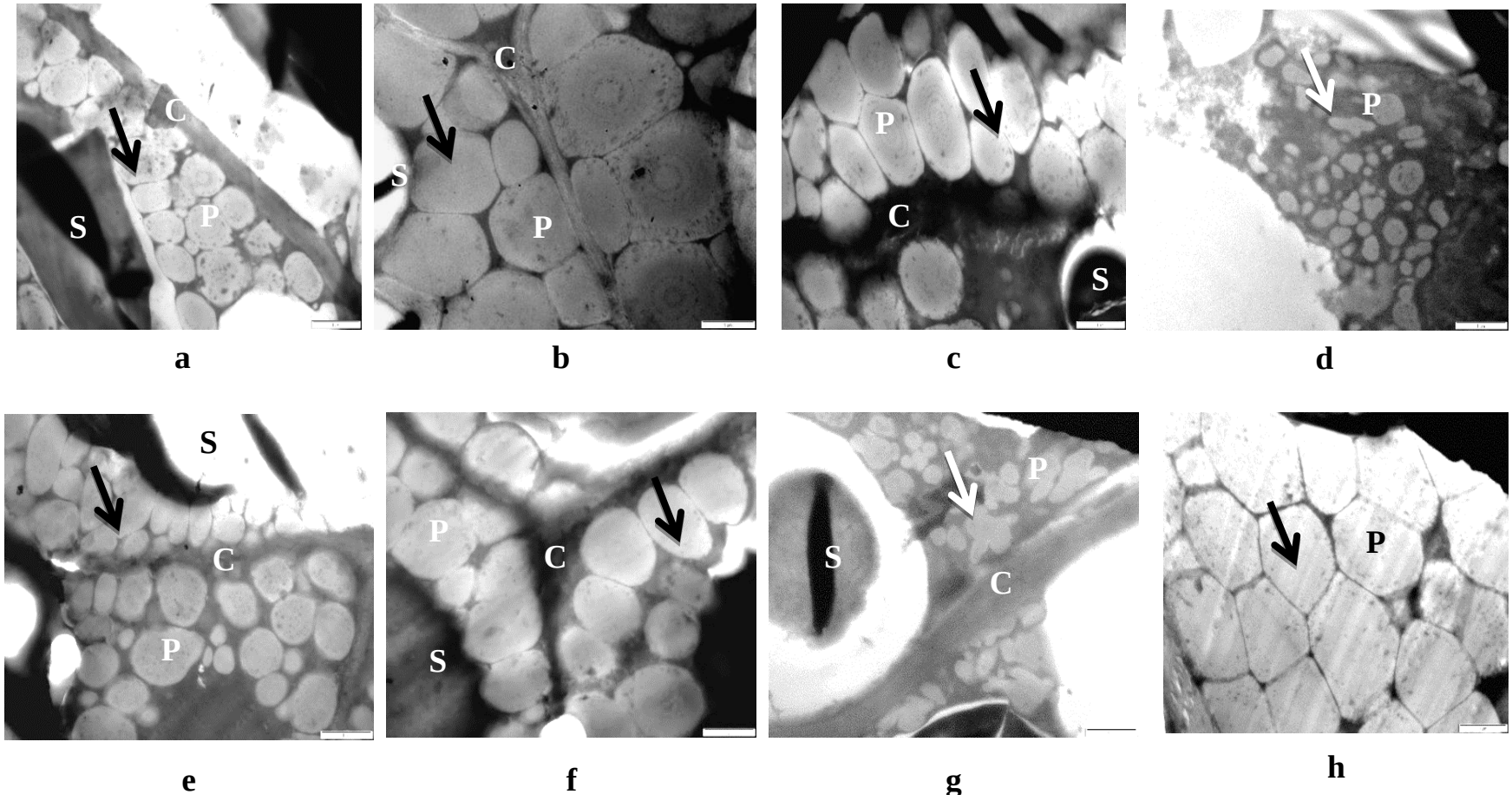

Figure 4.1. 6 TEM of protein bodies in the endosperms of the eight sorghum lines increased at Ukulima farm, South Africa.

P (protein body), S (starch granule), C (cell wall). P (protein body), S (starch granule), C (cell wall). White arrow in indicates irregular shaped protein body and black arrow indicates normal shaped and smooth surface protein body. a (Non-waxy-normal protein digestibility), b (Non-waxy-high protein digestibility), c (heterowaxy-normal protein digestibility), d and g (waxy-high protein digestibility; e , f and h (waxy-normal protein digestibilities), Bar is 1 µm.

4.1.5 Conclusions

Sorghum lines grown in Texas did not show all the presumed combinations of waxy and HD traits. However, the lines increased at Ukulima and Nanga farm showed variable endosperm modifications with the presumed waxy and HD traits. These include three waxy lines, one heterowaxy, two waxy-HD, one non-waxy-HD and one non-waxy-normal digestibility traits. These observations further show the complex nature of the physiology of cereals in general and of the sorghum studied. The eight sorghum lines increased at Ukulima farm (South Africa) are closely related in starch and protein content, demonstrated the presumed traits and had enough sample weight to conduct the doctoral study. Therefore, these sorghum lines were selected for further study on malting quality and dough-based products making quality.

4.1.6 References

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4.2 RELATIONSHIP BETWEEN WAXY (HIGH AMYLOPECTIN) AND HIGH PROTEIN DIGESTIBILITY TRAITS IN SORGHUM AND MALTING QUALITY

4.2.1 Abstract

This study determined the relationship between waxy (high amylopectin) and high protein digestibility (HD) traits in sorghum and malting quality with the aim of replacing barley malt in arid, tropical regions. Eight sorghum lines with differing endosperm traits: waxy, heterowaxy, waxy-HD, non-waxy-HD and non-waxy-normal digestibility traits were malted at laboratory scale and their malt quality and modification during malting were studied and their quality compared with commercial barley and sorghum malts. Two germination moisture levels (medium and high) were investigated. Malt from waxy and heterowaxy sorghum lines generally had improved endosperm modification and starch granule degradation. Only non-waxy-HD and one waxy line malt exhibited clear evidence of endosperm protein degradation. Malt α-amylase activity of the sorghum lines varied more than β-amylase activity. However, the α- and β-amylase activity of the sorghum malts was not evidently affected by the waxy and HD traits. Malt from waxy lines had improved hot water extract (HWE) and free amino nitrogen (FAN). Principal component analysis showed that waxy lines were associated with high HWE and FAN, and starch and protein losses. The waxy and heterowaxy novel sorghum lines germinated at high moisture also had slightly improved malting quality compared to the non-waxy lines. Medium moisture germination of the novel sorghum lines resulted in better malt quality. The improved malt quality of the waxy sorghum lines was probably due to the better starch granule swelling property of amylopectin which could have facilitated hydrolysis by amylases and proteases. This study shows that although β-amylase activity is not affected by the waxy trait, white tan-plant waxy sorghum produces higher quality malt than regular sorghum and thus has potential as a better partial barley malt replacement for brewing.

4.2.2 Introduction

Alternative cereals to barley for malting are essential because of climate change, non-viable cultivation of barley in tropical and sub-tropical regions, demand for gluten-free products, increased food product variety and to improve global food security (Phiarais and Arendt, 2008; Taylor et al., 2013; Hager et al., 2014). Sorghum malt is used as a functional component of many traditional African beers and porridges, and in modern lagers, stouts, and non-alcoholic malt beverages (Taylor and Emmambux, 2008). However, there are several factors that limit the use and functionality of sorghum malts for modern beers and non-alcoholic beverages. Among these are low β-amylase activity, incomplete degradation of its starch into fermentable sugars and limited proteolysis (Taylor et al., 2013).

Sorghum malt starch gelatinization temperature is high, 64-68°C, some 10°C higher than that of barley malt starch and this majorly adversely influences the hydrolysis of the starch by the malt α-amylase (Taylor, 1992). This problem is exacerbated by disulphide bond mediated cross-linking of the prolamins and other endosperm proteins which may also limit starch granule expansion (Chandrashekar and Kirleis, 1988; Ezeogu et al., 2008) and hence subsequent hydrolysis of the starch to fermentable sugars (Ezeogu et al., 2005). Prolamin cross-linking during wet heating also restricts enzymatic hydrolysis of the endosperm proteins into free amino nitrogen (FAN) (Duodu et al., 2003).

Waxy (high amylopectin) type barley has been found to show improved amylolysis (Vasanthan and Hoover, 2009). Furthermore, there has been considerable interest in using malted and unmalted waxy sorghum in lager beer brewing (Ortega Villicaña and Serna-Saldivar, 2004) and bioethanol (Yan et al., 2011). Unmalted waxy sorghum as adjunct in brewing trials showed more rapid starch hydrolysis (Figueroa et al., 1995) and gave higher extract, filtered more rapidly and produced wort with the same level of fermentable carbohydrates as normal sorghum (Osorio-Morales et al., 2000). Barredo-Moguel et al. (2001a, b) also demonstrated that wort from unmalted grits of waxy sorghum was suitable for fermentation by yeast and brewing in lager beers.

Sorghum lines with improved protein digestibility (HD) due to endosperm protein expression modification either by conventional breeding or genetic engineering also have improved brewing

quality. Mugode et al. (2011) found that malted HD lines were substantially higher in FAN than normal sorghums. However, the HD lines were not higher in FAN when mashed. Furthermore, it has been found that transgenic unmalted sorghum lines with the HD trait gave increased hot water extract (HWE) and wort FAN (Kruger et al., 2012).

Recently, sorghum lines that express both the waxy and HD-traits have been developed by Texas A&M University through conventional breeding (Jampala et al., 2012). Some of these lines have been found to be promising for grain bioethanol use, having more easily pasted starch granules, yielding higher FAN, giving faster fermentation and producing more lysine-rich distillers dried gain and solubles (DDGS) (Wu et al., 2010). Despite the potential advantages of waxy and HD sorghums in brewing, there has been very little research into their malting quality and the malting properties and malt quality of the combined waxy and HD sorghum types has not been investigated. Hence, the objective of this work is to evaluate the malting performance and quality of the novel sorghum lines in respect of endosperm modification during malting, and critical malt quality parameters: malting loss, α- and β-amylase activity, HWE, FAN, starch and protein losses.

4.2.3 Materials and Methods

4.2.3.1 Sorghum samples

The eight sorghum lines that were increased at the Ukulima research farm (Chapter 4.1) were investigated. All were tannin-free white, tan-plant types and comprised of three waxy-normal protein digestibility (WND), one heterowaxy-normal protein digestibility (hWND), two waxy-HD (WHD), one non-waxy–high digestibility (NWHD) and one non-waxy–normal digestibility (NWND) type. The grains of the lines were all visually indistinguishable.

Commercial barley malt (variety Cocktail) kindly provided by the Cereal and Malt Extract company (Johannesburg, South Africa) and commercial sorghum malt (white Type-II tannin sorghum, Feterita-type variety) kindly supplied by SABMiller Africa (Johannesburg) were included as references. The white Type-II tannin sorghum had a tannin content of approx. 0.5 g catechin equiv./100 g (Adetunji et al., 2013). Additionally, the general desired range of each critical malt quality parameter of barley malt for brewing is also included (Table 4.2.1). All the

malts were milled for analyses using a hammer mill (Falling Number AB, Huddinge, Sweden) fitted with a 0.5 mm opening screen.

4.2.3.2 Malting

All the sorghum lines had a Germinative Energy of ≥90%. They were malted at the laboratory scale under standard conditions following the method of Dewar et al. (1997b). Cleaned grains (100 g) were steeped at 25°C for 24 h with the steeping vessel being drained every 3 hours and the steeped grain given a 1 h air-rest. The steeped sorghum was germinated for 3 days at 25°C. A three-day germination period was chosen because scanning electron microscopy revealed significant differences between the levels of endosperm modification at the endosperm distal end between the sorghum lines (section 4.2.4.2) and also to minimise the high malting losses that occur with sorghum. Germination was conducted at medium moisture and high moisture levels following the method used by Morrall et al. (1986). For medium moisture, the moisture content of the green malt was kept constant throughout germination period, while for the high moisture level sufficient water was applied at the end of 6 h period till the grain felt wet. After germination, the malt was dried at 50°C for 24 h in a forced-draft oven to a shelf-stable moisture content of 5-7%. The roots and shoots were separated from the kernels by rubbing the dried malt against a sieve (1.4 mm opening screen).

4.2.3.3 Germinative energy

Germinative energy of the sorghum lines was measured at 72 h according to ICC Standard 174 (ICC, 2011).

4.2.3.4 Green malt moisture

The green malt moisture content was measured by weighing the germinating grains every 12 h and comparing it with the weight of the sound unmalted grains (Morrall et al., 1986).

4.2.3.5 Moisture

The moisture and dry matter content of the samples was determined using AACC Method 44-15A (AACC, 2000), so as to correct to dry basis the data for the malt quality attributes measured.

4.2.3.6 Starch

The starch content of the malted sorghum lines was determined using the Megazyme Total Starch assay procedure (Amyloglucosidase/α-amylase method) (Megazyme International, Bray, Ireland) as described in Chapter 4.1.

4.2.3.7 Protein

The protein content (N x 6.25) of the malted sorghum lines was determined by a Dumas combustion assay according to AACC method 46-30 (AACC International, 2000).

4.2.3.8 Transmission Electron Microscopy (TEM)

Protein body morphology of the malted sorghum lines was assessed using TEM as described (Da Silva et al., 2011b) with some modifications in fixing and dehydration as detailed in Chapter 4.1.

4.2.3.9 Scanning Electron Microscopy (SEM)

All novel sorghum lines malted for 3 days following steeping and four selected lines malted for 1 and 5 days following steeping were selected for the endosperm modification study. Endosperm modification during malting was evaluated using SEM according to Chiremba et al. (2013) with modification in the method of coating the kernels. Fixed kernels were sputter coated with carbon (5 times on the top, 2 times on each side) and then viewed using a Zeiss Evo LS15 field emission scanning electron microscope (Carl Zeiss, Oberkochen, Germany) operated at an acceleration voltage of 8 kV.

4.2.3.10 Alpha-amylase activity

Malt α-amylase activity was determined using the Megazyme Ceralpha kit method (Megazyme International). The assay employs non-reducing-end blocked *p*-nitrophenyl maltoheptaoside (BPNPG7) as substrate. On hydrolysis of the BPNPG7 by endo-acting α-amylase, the excess quantities of α-glucosidase present in the mixture give instantaneous and quantitative hydrolysis of the *p*-nitrophenyl maltosaccharide fragment to glucose, which is then measured colorimetrically.

4.2.3.11 Beta-amylase activity

Malt β-amylase activity was determined using the Megazyme Betamyl-3 kit method (Megazyme International). The assay employs high purity *p*-nitrophenyl-β-D-maltotrioside (PNPβ-G3) as substrate. On hydrolysis of the PNPβ-G3 to maltose and *p*-nitrophenyl-β-D-glucose by β-amylase, the *p*-nitrophenyl-β-D-glucose is immediately cleaved to D-glucose and free *p*-nitrophenol by the β-glucosidase present in the substrate mixture, and the glucose is measured colorimetrically.

4.2.3.12 Hot Water Extract (HWE)

Malt HWE was determined according to the European Brewery Convention (EBC, 1998) Method 4.5.1 Extract of Malt: Congress Mash (AM), modified to a 10 g malt sample size by reducing volumes in proportion. HWE was quantified by specific gravity in degrees Plato (^{o}P) using pycnometry and in °Brix using refractometry. The rising temperature EBC Congress barley malt mashing procedure was used in this study because when sorghum malt is used in large-scale lager-type brewing, it is invariably as a partial barley malt replacement and mashed together with barley malt using a barley type mashing process (Taylor and Emmambux, 2008).

4.2.3.13 Free amino nitrogen (FAN)

FAN content of the malts was determined using the European Brewery Convention ninhydrin assay Method 4.10 (EBC, 1998) as modified by Morall et al. (1986). Glycine was used as a standard and the results were expressed as mg FAN/100 g dry malt.

4.2.3.14 Malting loss

Malting loss was measured according to Dewar et al. (1997b) modified for 100 sound kernels. The malting loss was assessed by weighing batches of 100 sound kernels of the dried malt (after root and shoot removal) and comparing it with the the weight of 100 sound unmalted grains.

4.2.3.15 Starch and protein losses

The starch and protein losses after malting were assessed by comparing the starch and protein contents of the dried malts (after root and shoot removal) with those of the unmalted grains.

4.2.3.16 Statistical analyses

Malting was performed at least twice for sorghum lines and closely agreeing replicates were obtained. All chemical analyses on both replicates were repeated at least twice. The data for the malt quality attributes were analysed using one-way ANOVA. The means were separated using Tukey's HSD test at $p<0.05$. Principal Component Analysis (PCA) for all numerical results was performed using XLSTAT version 2016.03.30882 (Addinsoft, New York).

4.2.4 Results and discussion

4.2.4.1 Green malt moisture

Sorghum lines germinated at medium moisture had constant weight throughout the germination phase of malting (Figure 4.2.1), while the lines malted at high moisture had higher green malt moisture content. At 72 h germination, sorghum lines malted at medium moisture had an average green malt moisture content of 42.8 g/100g grain, while lines malted at high moisture had a moisture content of 62.5 g/100 g grain.

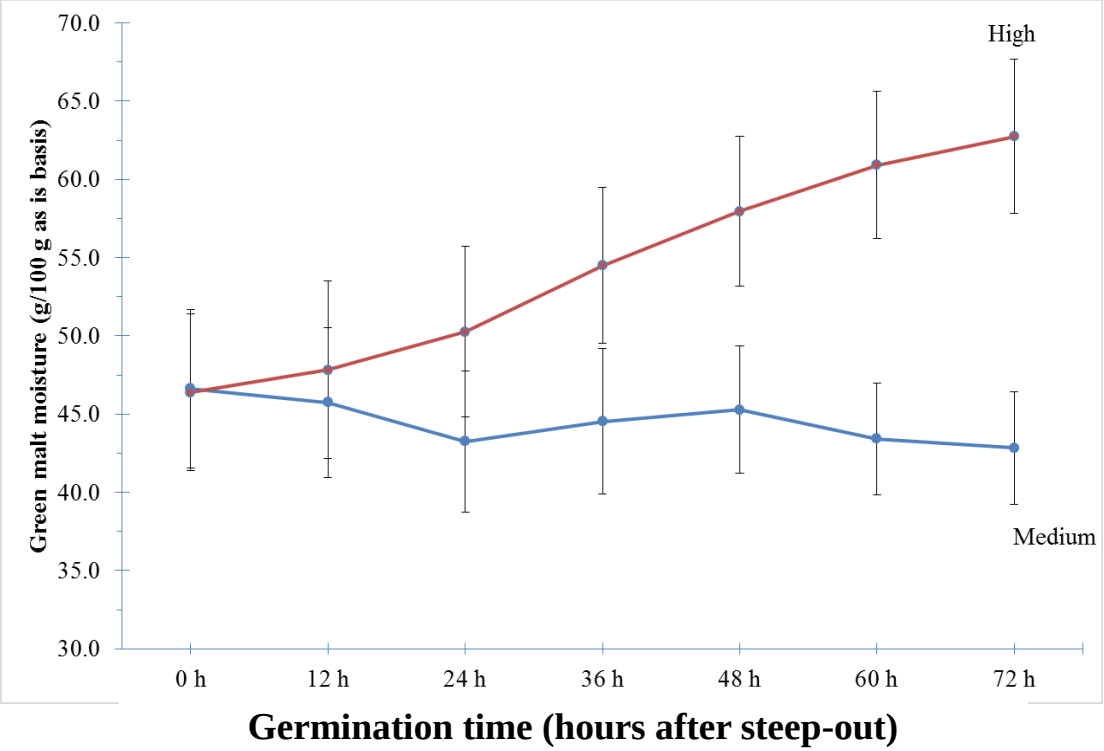

Figure 4.2. 1 Green malt moisture content of sorghum lines germination at medium and high moisture levels during germination phase of the malting.

4.2.4.2 Malt endosperm modification

SEM of the malts of all lines after 3 days germination revealed that in the floury endosperm near the endosperm–scutellum interface (proximal region), there was extensive degradation of the starch granules and the protein bodies were no longer visible (Figure 4.2.2). The extent of the degradation of starch granules and endosperm protein seemed to reduce gradually towards the distal region. The waxy-normal protein digestibility (WND1, WND2, WND3) (Figure 4.2.2M,P,V), heterowaxy-normal protein digestibility (hWND) (Figure 4.2.2G) and the waxy-high protein digestibility (WHD2) (Figure 4.2.2S) lines showed greater starch granule modification compared to the normal starch and protein digestibility line (NWND) (Figure 4.2.2A), as evidenced by greater pitting of starch granules (indicated by SGd with two arrows). However, the floury endosperm non-waxy-HD line (NWHD) (Figure 4.2.2B) did not show a higher degree of endosperm modification. Though the sorghum lines were not statistically significant different ($p > 0.05$) in their malting loss (Table 4.2.1), the higher degree of endosperm modification of the waxy lines was consistent with the indication of relatively higher malting loss than the normal sorghum lines. The greater tendency of endosperm modification with the waxy sorghum lines, combined either with high or normal protein digestibility, indicates that the amylopectin-rich starch was more readily modified.

SEM of the periphery of the mid endosperm region revealed slight modification in all the lines (Figure 4.2.2). The corneous endosperm cells of the waxy-normal digestibility lines (WND1, WND2, WND3) (Figure 4.2.2N,Q,W) and the waxy-HD line (WHD2) (Figure 4.2.2T) showed greater modification than the non-waxy, normal digestibility line (NWND) (Figure 4.2.2B). The distal regions of the waxy lines (WND2, WND3 and WHD2) showed slightly greater modification, and these lines also had the highest starch amylopectin content (Table 4.1.3) (Figure 4.2.2R,X,U).

Selected sorghum lines germinated for 1 and 5 days showed variable endosperm modification, as indicated by degradation of the starch granules and the protein bodies (Figure 4.2.3 and Figure 4.2.4, respectively). The waxy lines (WND3 and WHD2) after 1 day germination showed slight evidence of higher modification (Figure 4.2.3G, J) in the floury endosperm near the endosperm–scutellum interface (proximal region) compared to the non-waxy lines (NWND and NWHD)

(Figure 4.2.3A, D). However, all the lines showed no modification in the middle and distal sections (Figure 4.2.3B, C, E, F, H, I, K, L).

The endosperm modification of the selected sorghum lines germinated for 5 days indicated that starch and protein degradation were extensive, intermediate and minimal at proximal, middle and distal sections, respectively (Figure 4.2.4). The proximal region revealed extensive starch granule degradation (Figure. 4.2.4A, D, G, J), but with the endosperm cell wall remaining present. This was consistent with study of Glennie et al. (1983) of 4 days germinated sorghum, Correia et al. (2008) of 7 day germinated sorghum and Chiremba et al. (2013) of 5 day germinated sorghum in which the starch granules were strongly attacked and eroded. Waxy lines (WND3 and WHD2) showed a higher degree of endosperm modification at proximal (Figure 4.2.4G, J), middle (Figure 4.2.4H, K) and distal (Figure 4.2.4I, L) sections compared to the non-waxy lines (NWND and NWHD). These findings show that the waxy (amylopectin-rich starch) sorghum lines combined either with high or normal protein digestibility was more readily modified than normal sorghum lines.

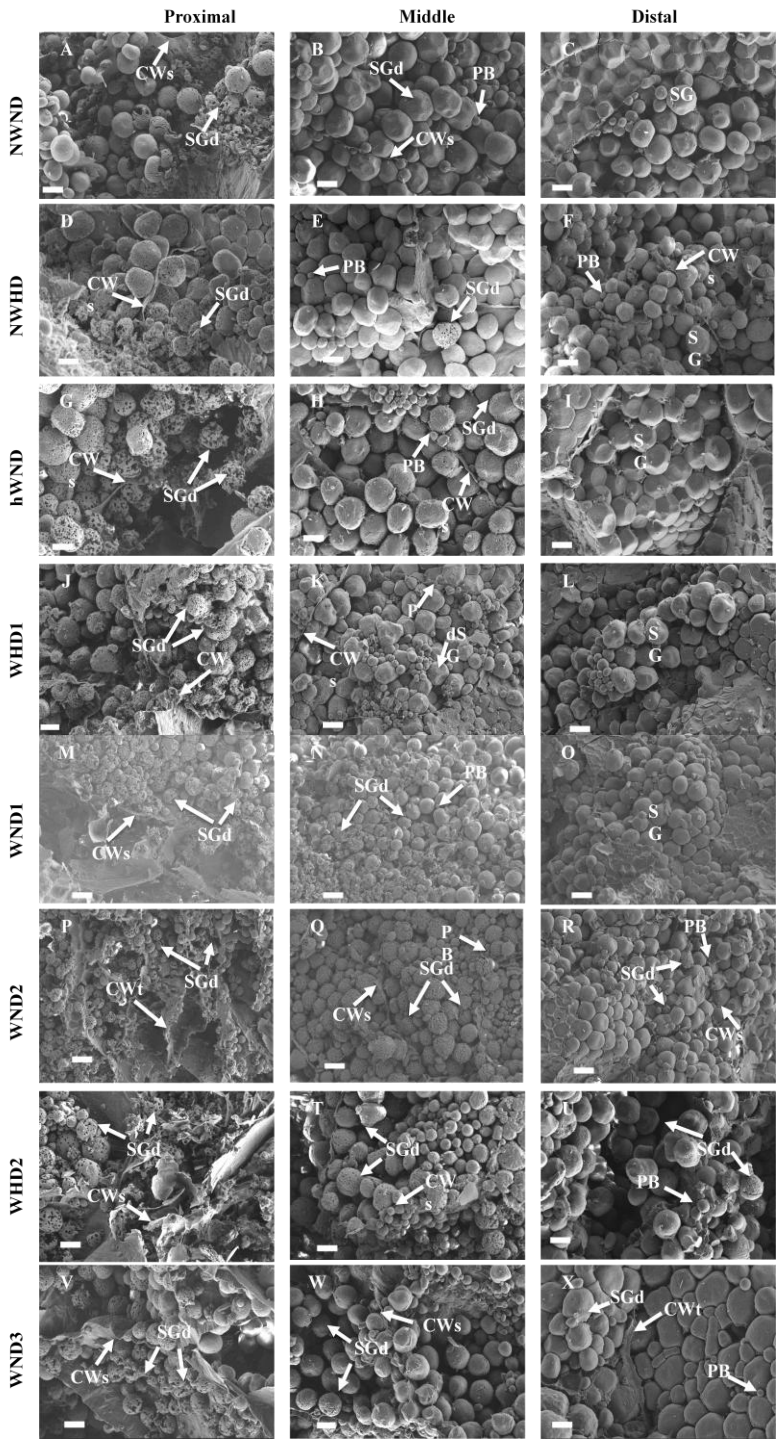

Figure 4.2. 2 SEM of proximal, mid and distal sections of sorghum lines germinated for 3 days following steeping

NWND (non-waxy-normal protein digestibility); NWHD (Non-waxy-high protein digestibility); hWND (heterowaxy-normal protein digestibility); WND (waxy-normal protein digestibility); WHD (waxy-high protein digestibility); PB= protein body, CWs = smooth cell walls,CWt = torn cell walls; SG = intact starch granules, and SGd = degraded starch granules.

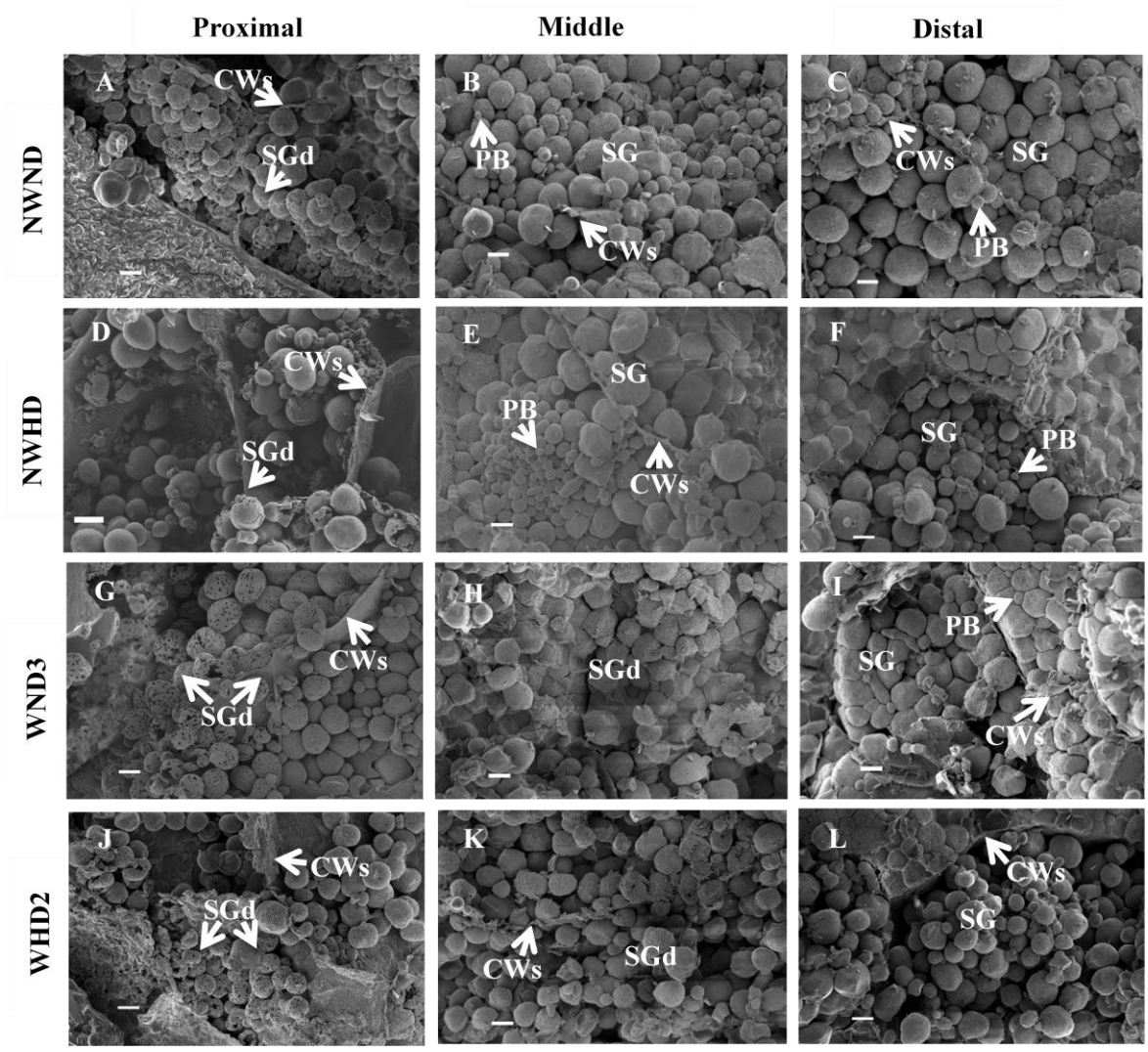

Figure 4.2. 3 SEM of proximal, mid and distal sections of selected sorghum lines germinated for 1 day following steeping

NWND (non-waxy-normal protein digestibility); NWHD (Non-waxy-high protein digestibility); WND (waxy-normal protein digestibility); WHD (waxy-high protein digestibility);PB= protein body, CWs = smooth cell walls, CWt = torn cell walls; SG = intact starch granules, and SGd = degraded starch granules.

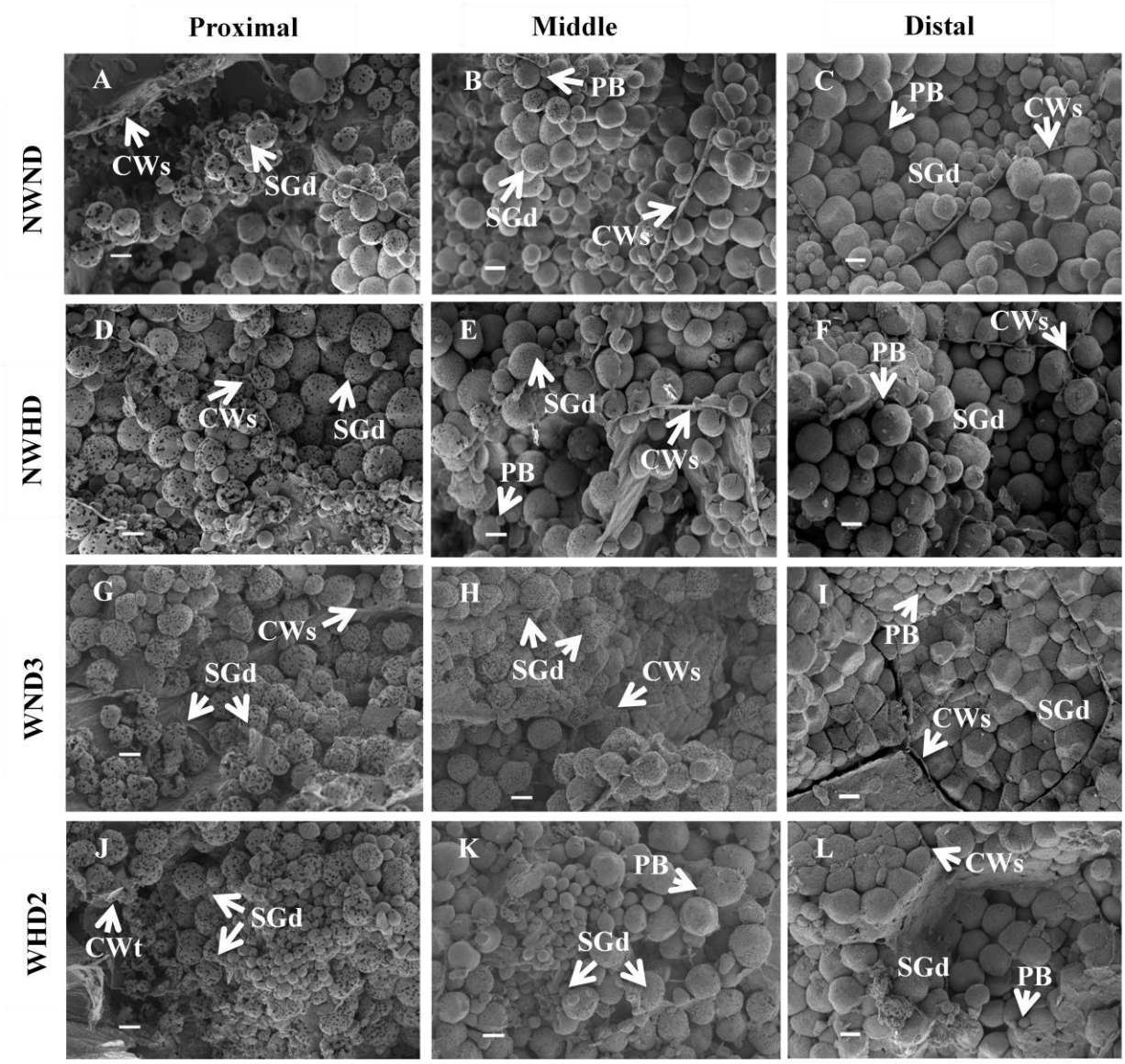

Figure 4.2. 4 SEM of proximal, mid and distal sections of selected sorghum lines germinated for 5 days following steeping

NWND (non-waxy-normal protein digestibility); NWHD (Non-waxy-high protein digestibility); WND (waxy-normal protein digestibility); WHD (waxy-high protein digestibility); PB= protein body, CWs = smooth cell walls, CWt = torn cell walls; SG = intact starch granules, and SGd = degraded starch granules.

4.2.4.3 Protein body degradation

TEM of the periphery of the mid endosperm region of the sorghum lines showed that the protein bodies and surrounded matrix were degraded to differing extents between the lines, as evidenced by the relative disappearance of margins of the protein bodies (indicated by white arrow with M) and degree of erosion (white arrow) (Figure 4.2.5). During sorghum germination the endosperm protein matrix is generally degraded before the protein bodies (Taylor et al., 1985). Lines NWHD, WND1, and WND2 showed the highest level of endosperm protein degradation. Malts of hWND and WND3 showed slight endosperm protein degradation, while lines of WHD1, WHD2 and NWND did not show any endosperm protein degradation. Hence, the study did not show any clear trend as to whether the HD trait in the lines improved the degradation of the endosperm protein, unlike the situation with starch granule degradation (Figure 4.2.2).

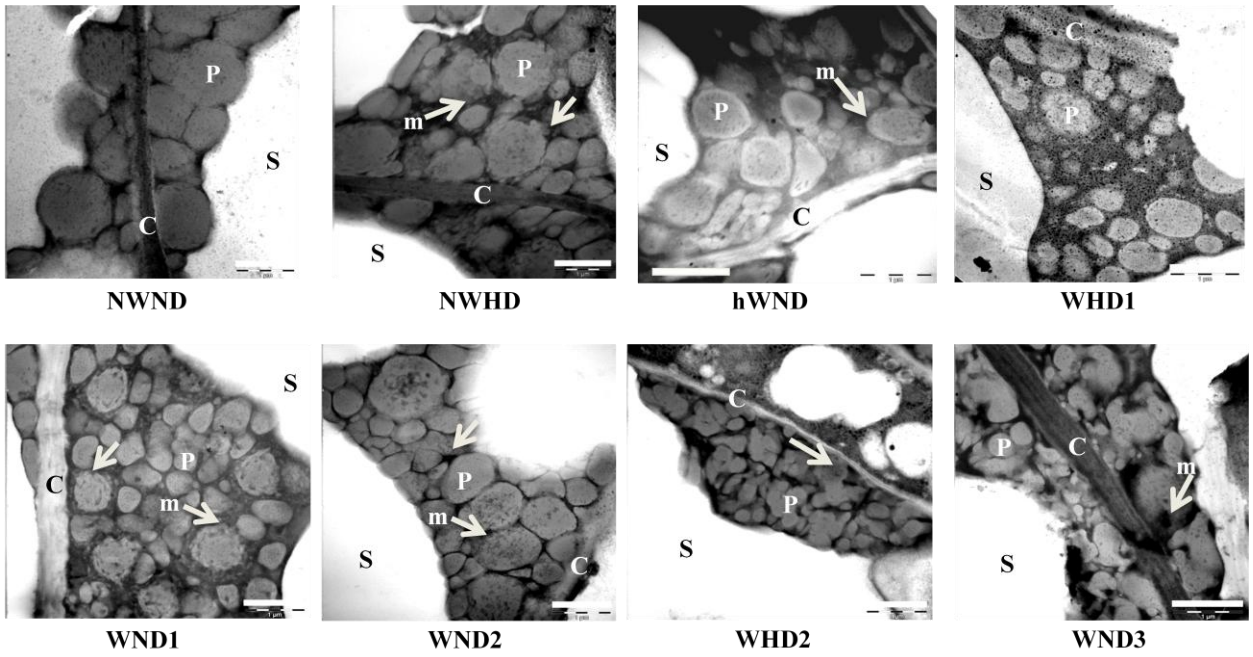

Figure 4.2. 5 TEM of protein bodies in endosperm of malted sorghum lines

P = protein body; S= starch granule, C= cell wall; White arrow in raw grain indicates irregular shaped protein body; black arrow indicates normal shaped and smooth surface protein body. In malted grain a white arrow with M indicates the disappearance of margins of the protein bodies; white arrow only indicates the eroded appearance. NWND (Non-waxy-normal protein digestibility), NWHD (Non-waxy-high protein digestibility), hWND (heterowaxy – normal protein digestibility), WHD (Waxy-High protein digestibility) and WND (Waxy-normal protein digestibility). Bar is 1 µm.

4.2.4.4 Alpha-amylase activity

The malt α–amylase activity of the sorghum lines germinated at medium moisture varied considerably, ranging from 79.3 to 168.5 CU/g (Table 4.2.1). Lines WND2 and WND3 had similar ($p \geq 0.05$) α–amylase activity to the commercial barley malt (131.2 CU/g) and line WHD2 had significantly higher activity ($p < 0.05$). Dufour et al. (1992) also found that sorghum malts exhibited similar or even higher α-amylase activities than typical lager barley malts. All the sorghum lines had lower α-amylase activity than the desired values of barley malt for brewing (200-250 CU/g) and much higher α–amylase activity (3 to 6 fold higher) than the commercial sorghum malt. However, the commercial sorghum malt was a white tannin-type II (Adetunji et al., 2013) and the tannins present could have inhibited its amylase activity.

Concerning the relationship between the waxy and HD traits and α–amylase activity, one of the waxy plus high protein digestibility lines (WHD2) had significantly ($p < 0.05$) higher α–amylase activity than the normal line (NWND), whereas the other line with same traits (WHD1) had lower α–amylase activity. A similar trend was observed for the heterowaxy and waxy sorghum malts, as WND1 and hWND had lower α–amylase activity, while WND2 and WND3 had higher compared to normal line. Only HD line (NWHD) had similar ($p \geq 0.05$) α–amylase activity to the NWND line. Thus, the malt α–amylase activity was not evidently affected by the traits.

With the sorghum lines germinated at high moisture level, malt α–amylase activity also varied considerably, ranging from 24.0 to 135.9 CU/g (Table 4.2.2). Malt α–amylase activity was lower with germination at high moisture compared to germination at medium moisture. This was consistent with Dewar et al. (1997) that showed a decline in joint α and β–amylase activity (DP) of sorghums germinated at high moisture. However, a study by Morrall et al (1986) revealed an increasing trend. One waxy-high protein digestibility line (WHD1) had similar ($p > 0.05$) α–amylase activity to the normal line (NWND) and also to the only HD line (NWHD). Among the waxy sorghum lines, the amylopectin-rich lines (WND3 and WHD2) had significantly ($p < 0.05$) lower α–amylase activity than the other two waxy lines (WND1 and WND2). Only line, WND2, had similar ($p \geq 0.05$) α–amylase activity to the commercial barley malt (131.2 CU/g). The heterowaxy line had slightly higher α–amylase activity than normal line (NWND). These results also indicate that the malt α–amylase activity varied irrespective of the waxy and HD traits.

4.2.4.5 Beta-amylase activity

The malt β-amylase activity of the lines germinated at medium moisture varied considerably, ranging from 2.1 to 4.8 Betamyl-3® U/g (Table 4.2.1). However, all the sorghum malts had much lower β-amylase activity compared to the barley malt (12 BU/g db). Studies by Dufour et al. (1992) and Letsididi et al. (2008) showed that malted sorghum β-amylase activity was much less than that of barley malt. When compared to the desired value of barley malt β-amylase activity for brewing (500 BU/g), all the sorghum lines had very much lower β-amylase activity. As with α-amylase activity, the malts of all eight lines had much higher malt β-amylase activity than the commercial sorghum malt, probably as a result of it containing tannins and of the different malting conditions used to produce the malts.

Regarding the relationship between the waxy and HD traits and β-amylase activity, the waxy-HD line (WHD1) and heterowaxy line (hWND) were significantly ($p<0.05$) lower in β-amylase activity compared to the normal line (NWND), whereas the other lines (NWHD, WND1, WND2, WND3, and WND2) all had similar ($p\geq 0.05$) β-amylase activity as of NWND. Hence, the β-amylase activity of the sorghum malts also seemed to vary irrespective of the waxy and HD traits.

With high germination moisture the malt β-amylase activity of the sorghum lines ranged between 0.2 and 1.5Betamyl-3® U/g (Table 4.2.2). The β-amylase activity of malt produced using high germination moisture was lower than that of the malt produced using medium germination moisture. The malt β-amylase activity was much lower compared to the germination at medium moisture. All sorghum malts had much lower β-amylase activity compared to the barley malt. With the exception of two lines (WND1 and WND2), all sorghum lines had similar β-amylase activity to the commercial sorghum malt. Only two waxy lines (WND1 and WND2) were significantly ($p<0.05$) higher in β-amylase activity compared to normal line (NWND). All other lines (NWHD, hWND, WND3, WHD1, WHD2 and WND2) had similar ($p\geq 0.05$) β-amylase activity as of NWND. Thus, the β-amylase activity of the sorghum malts seemed to have no relation with the waxy and HD traits.

4.2.4.6 Hot water extract (HWE)

HWE content of the malted sorghum lines measured in terms of wort density in °P varied widely, between 44.2 and 64.2%, when germinated at medium moisture (Table 4.2.1). With regard to the relationship between the waxy and HD traits and HWE, a sorghum line with both the waxy and high protein digestibility (WHD2) traits gave the highest HWE, significantly higher ($p<0.05$) HWE than the non-waxy sorghum lines (NWLD, NWHD) and comparable to the barley malt ($p\geq0.05$.). The waxy and normal digestibility (WND1, WND2 and WND3) lines also yielded higher HWE ($p\geq0.05$) than the non-waxy sorghum lines. As would be expected, HWE measured by refractometry in °Brix showed a similar trend (Table 4.2.1). The higher HWE of the waxy lines compared to the non-waxy lines is consistent with them exhibiting higher endosperm modification, as evidenced by their greater starch granule degradation (Figure 4.2.1). Line WHD1, however, gave a very low HWE (44.2%), despite it having both the waxy and high protein digestibility traits. Its low extract can be attributed to its very low α-amylase activity (Table 4.2.1). Moreover, all the sorghum lines had lower HWE compared to the generally required value for brewing (>81%).

PCA revealed that the waxy lines (WND1, WND2, WND3 and WHD2) were aligned together with HWE, together with starch and malting losses (Figure 4.2.6a). This is indicative of greater starch modification in the waxy lines resulting in more precocious germination and hence higher malting losses. In fact, with the exception of the WHD1 (the line with low α-amylase activity) all the waxy lines were in the same quadrant of PC1 (which accounted for 47.5% of total variation) as HWE. PCA with the low α-amylase WHD1 line removed revealed this more clearly (Figure. 4.2.6b). Thus, it seems that the waxy (high amylopectin) trait is highly associated with high HWE in sorghum malt. This is probably a consequence of the better starch granular swelling property of amylopectin (Tester and Morrison, 1990), which facilitate greater hydrolysis by amylases. Such greater hydrolysis by α-amylase of waxy sorghum was observed by Wu et al. (2010). The findings of the current study are in agreement with other research where waxy and heterowaxy sorghums (Osorio-Morales et al., 2000; Barredo-Moguel et al., 2001a) and waxy barley (Vasanthan and Hoover, 2009) were found to have improved starch hydrolysis. The findings are also in agreement with the work of Wong et al. (2009) who showed that in two

unmalted sorghum lines with a common pedigree, the waxy line, which also had a weak protein matrix, was more susceptible to hydrolysis by α-amylase.

When germinated at the high moisture level, HWE of the sorghum lines measured by wort density in ^{o}P varied from 35.4 to 65.1% (Table 4.2.2). The HWE was lower than that of the germination at medium moisture. Sorghum line with only waxy trait (WND1) gave the highest HWE, significantly higher HWE ($p<0.05$) than the non-waxy sorghum lines (NWLD, NWHD). The other waxy and normal digestibility lines (WND2 and WND3) and waxy-HD line (WHD2) yielded higher HWE ($p\geq0.05$) than the non-waxy sorghum lines. WHD1 gave a very low HWE (40.8%), even with it having both the waxy and high protein digestibility traits. Again, its low extract can be attributed to its very low α-amylase activity (Table 4.2.2). Again, as would be expected, HWE determined by refractometry showed similar trends. PCA showed that the waxy lines (WND1, WND2, WND3 and WHD2) were aligned together with HWE measured by wort density in ^{o}P, even when germinated at the high moisture level (Figure. 4.2.7a and b). This indicates that in sorghum malt the waxy (high amylopectin) trait was highly associated with high HWE.

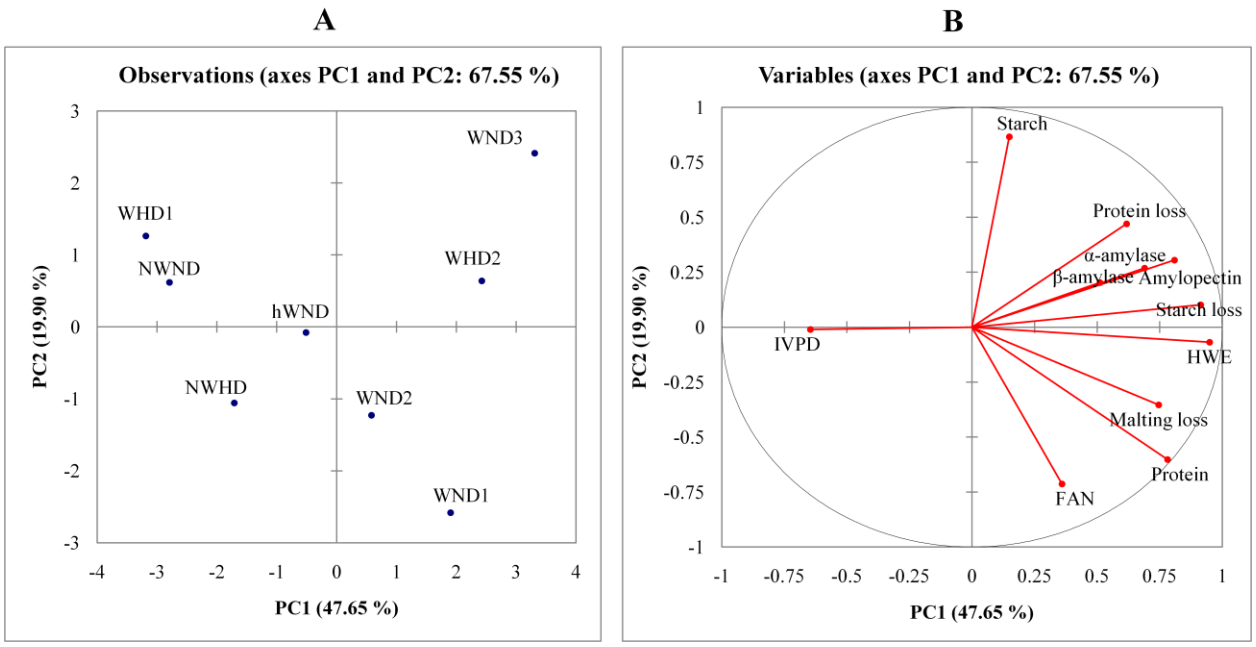

Figure 4.2.6. a Principal component analysis of sorghums with different starch and protein digestibility traits and their malting quality attributes: PCA with WHD1 (the line with low amylase activity); germination at medium moisture.

A: Sorghum lines: NWLD (Non-waxy- normal protein digestibility), NWHD (Non-waxy- high protein digestibility), hWND (heterowaxy- normal protein digestibility), WND1, WND2, WND3 (Waxy- normal protein digestibility), WHD1 and WHD2 (Waxy-high protein digestibility),

B: PCA Loadings: starch content, protein content, starch amylopectin content and cooked in-vitro protein digestibility (IVPD), α-amylase activity, β-amylase activity, hot water extract [HWE measured by wort density in ^{o}P], FAN, malting loss, starch loss and protein loss.

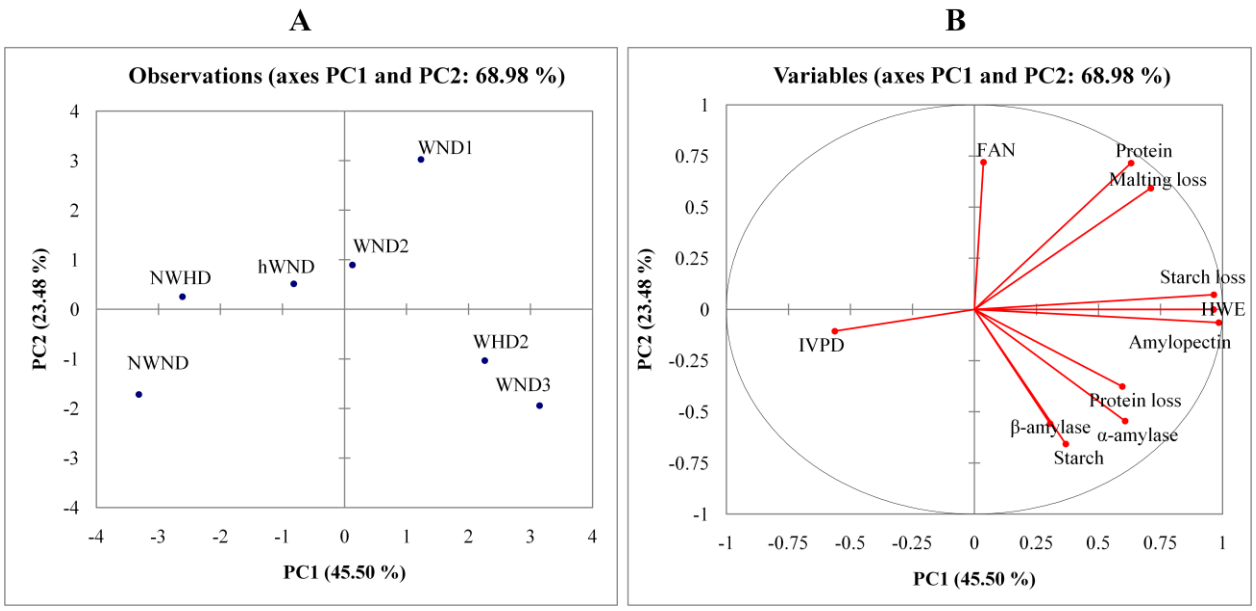

Figure 4.2.6. b Principal component analysis of sorghums with different starch and protein digestibility traits and their malting quality attributes: PCA without WHD1 (the line with low amylase activity); germination at medium moisture.

A: Sorghum lines: NWLD (Non-waxy- normal protein digestibility), NWHD (Non-waxy- high protein digestibility), hWND (heterowaxy- normal protein digestibility), WND1, WND2, WND3 (Waxy- normal protein digestibility), WHD1 and WHD2 (Waxy-high protein digestibility).

B: PCA Loadings: starch content, protein content, starch amylopectin content and cooked in-vitro protein digestibility (IVPD), α-amylase activity, β-amylase activity, hot water extract [HWE measured by wort density in ^{o}P], FAN, malting loss, starch loss and protein loss.

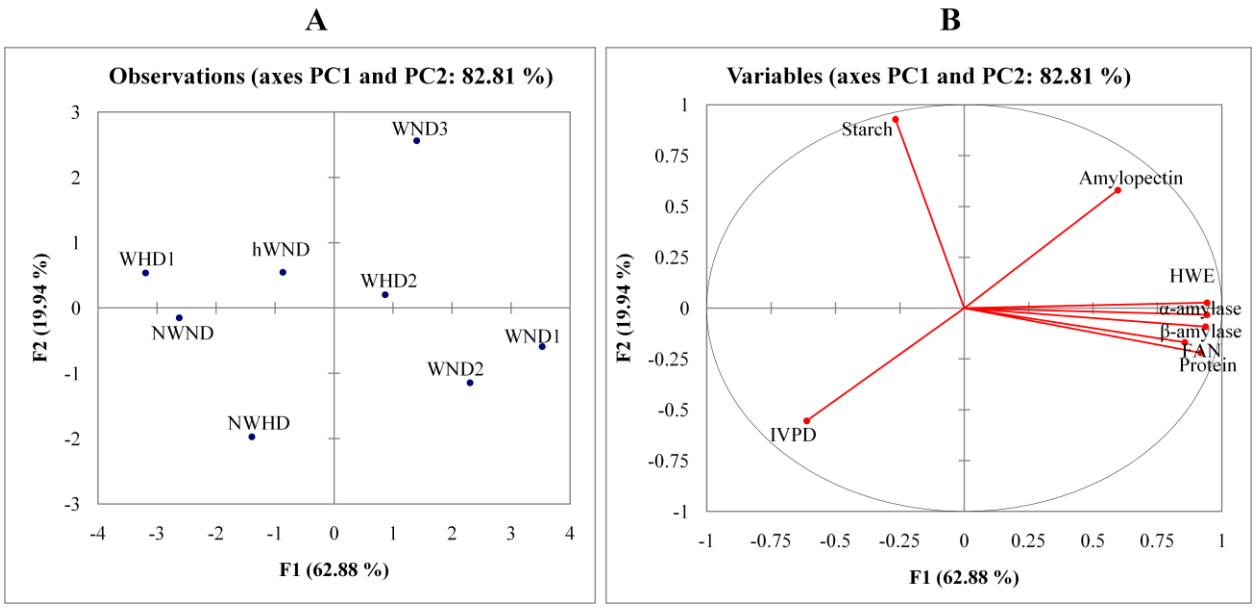

Figure 4.2.7. a Principal component analysis of sorghums with different starch and protein digestibility traits and their malting quality attributes: PCA with WHD1 (the line with low amylase activity), germination at high moisture.

A: Sorghum lines: NWLD (Non-waxy- normal protein digestibility), NWHD (Non-waxy- high protein digestibility), hWND (heterowaxy- normal protein digestibility), WND1, WND2, WND3 (Waxy- normal protein digestibility), WHD1 and WHD2 (Waxy-high protein digestibility).

B: PCA Loadings: starch content, protein content, starch amylopectin content and cooked in-vitro protein digestibility (IVPD), α-amylase activity, β-amylase activity, hot water extract [HWE measured by wort density in ^{o}P], and FAN.

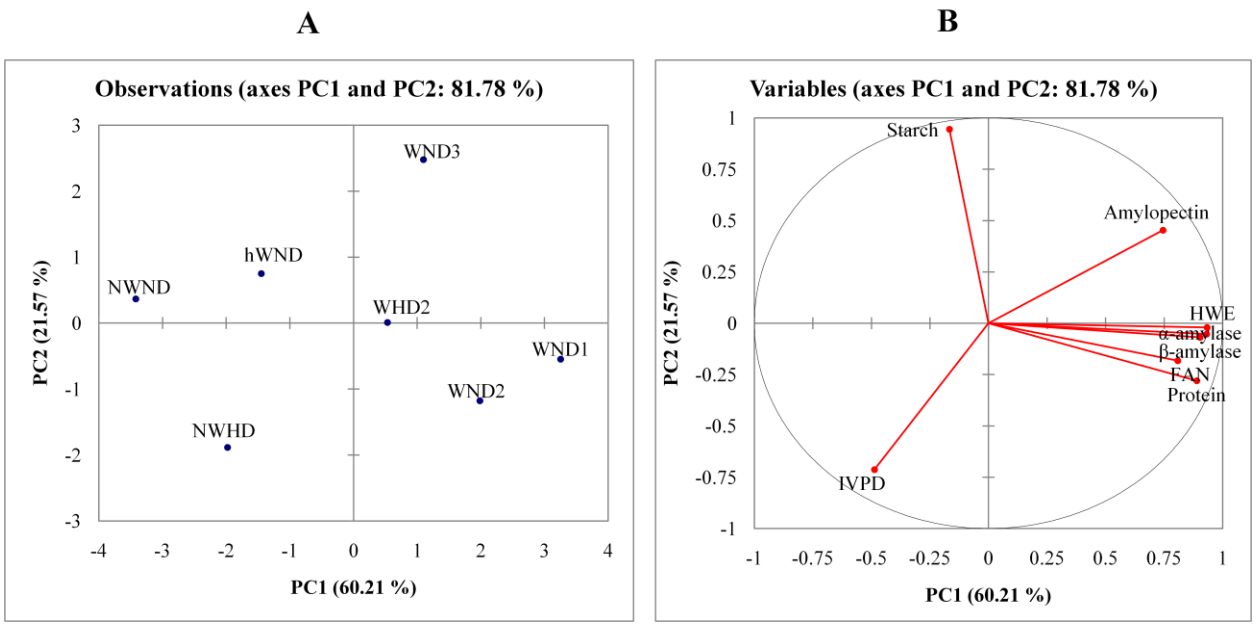

Figure 4.2.7. b Principal component analysis of sorghums with different starch and protein digestibility traits and their malting quality attributes: PCA without WHD1 (the line with low amylase activity), germination at high moisture.

A: Sorghum lines: NWLD (Non-waxy- normal protein digestibility), NWHD (Non-waxy- high protein digestibility), hWND (heterowaxy- normal protein digestibility), WND1, WND2, WND3 (Waxy- normal protein digestibility), WHD1 and WHD2 (Waxy-high protein digestibility).

B: PCA Loadings: starch content, protein content, starch amylopectin content and cooked in-vitro protein digestibility (IVPD), α-amylase activity, β-amylase activity, hot water extract [HWE measured by wort density in ^{o}P], and FAN.

4.2.4.7 Free Amino Nitrogen (FAN)

The FAN content of the malted sorghum lines varied, ranging between 71.5 and 103.2 mg/100 g, when germinated at medium moisture (Table 4.2.1). Lines WND1 and NWHD had the highest FAN content (103.2 and 99.6 mg/100 g, respectively), significantly higher ($p < 0.05$) than that of the commercial barley malt reference (83.5 mg/100 g). These lines also exhibited high endosperm protein matrix and protein body degradation (Figure 4.2.5). The waxy (WND2 and WND3) and waxy-high protein digestibility (WHD2) lines produced similar FAN ($p \geq 0.05$) to the barley malt. However, the sorghum lines were still lower in FAN compared to the desired value of barley malt FAN for brewing (130 – 160 mg/100 g). PCA (Figure 4.2.6a, b), indicating that the waxy-normal protein digestibility lines (WND1 and WND2) were associated with high malt FAN; while WND3 and WHD2 were associated with protein loss. Also, as with HWE, all the waxy lines, with exception of the low α-amylase WHD1, were in the same PC1 quadrant as high FAN. This finding is consistent with the observation by Rooney and Pflugfelder (1986) that sorghums with waxy endosperm and a relatively weak protein matrix are more susceptible to hydrolysis by amylase and protease enzymes. However, the HD trait was not associated with high malt FAN. In fact, malt FAN seemed to be more associated with grain protein content. It seemed that any effect of the moderate increase in protein digestibility in the HD lines was not significant in comparison to the effect of the waxy trait. The finding is consistent with the observation by Wong et al. (2009) that the waxy trait in sorghum could enable the endosperm proteins to be exposed to proteases.

Regarding sorghum lines germinated at high moisture level, FAN content of the lines varied from 64.7 to 100.5 mg/100 g (Table 4.2.2). The FAN content was lower compared to FAN with the germination at medium moisture. Line WND1 had the highest FAN content (100.5 mg/100 g), significantly higher ($p < 0.05$) than that of the commercial barley malt reference (83.5 mg/100 g). The waxy (WND2 and WND3) lines produced similar FAN ($p \geq 0.05$) to the barley malt. Only HD line (NWHD) also had similar FAN to the barley malt. PCA (Figure 4.2.7a, b) indicated that the waxy-normal protein digestibility lines (WND1 and WND2) were associated with high malt FAN. Also, all the waxy lines, with exception of the low α-amylase WHD1, were in the same PC1 quadrant as high FAN.

Table 4.2. 1 Malt and malting quality attributes of malt prepared from waxy and high protein digestibility novel sorghum lines germinated at medium moisture level and of commercial barley and sorghum malt references

Sorghum line	Malt Quality				Malting Quality		
	α–amylase (CU/g db)	**β-amylase (BU/g db)**	**HWE [°P] (%, db)**	**FAN (mg/100 g db)**	**Malting loss (%)**	**Starch loss (%)**	**Protein loss (%)**
NWND	$113.2^{e} \pm 1.0$	$4.0^{de} \pm 0.4$	$51.1^{bc} \pm 0.3$	$76.3^{bc} \pm 1.2$	$11.0^{a} \pm 1.8$	$8.4^{a} \pm 1.0$	$2.61^{a} \pm 0.26$
NWHD	$102.3^{e} \pm 1.2$	$4.0^{ef} \pm 0.3$	$50.5^{bc} \pm 0.7$	$99.6^{e} \pm 3.6$	$11.2^{a} \pm 0.3$	$10.5^{abc} \pm 1.6$	$4.98^{c} \pm 0.40$
hWND	$106.3^{d} \pm 1.7$	$2.1^{b} \pm 0.3$	$55.2^{cd} \pm 1.8$	$82.2^{cd} \pm 2.1$	$14.1^{a} \pm 1.3$	$10.4^{ab} \pm 1.6$	$4.84^{c} \pm 0.62$
WHD1	$79.3^{b} \pm 0.8$	$2.3^{bc} \pm 0.3$	$44.2^{b} \pm 0.9$	$71.5^{b} \pm 1.5$	$12.0^{a} \pm 0.1$	$10.8^{abc} \pm 1.4$	$3.50^{ab} \pm 0.28$
WND1	$99.2^{c} \pm 1.8$	$3.4^{cd} \pm 0.1$	$59.7^{de} \pm 2.1$	$103.2^{e} \pm 4.6$	$16.0^{a} \pm 0.5$	$13.5^{cd} \pm 0.8$	$3.54^{ab} \pm 0.34$
WND2	$126.0^{f} \pm 1.7$	$3.6^{d} \pm 0.2$	$58.6^{de} \pm 3.3$	$83.8^{d} \pm 4.8$	$14.3^{a} \pm 1.6$	$11.6^{bcd} \pm 1.5$	$3.62^{b} \pm 0.49$
WHD2	$168.5^{h} \pm 0.8$	$4.2^{de} \pm 0.1$	$64.2^{ef} \pm 1.0$	$80.6^{cd} \pm 4.6$	$13.1^{a} \pm 0.8$	$14.1^{d} \pm 1.6$	$4.85^{c} \pm 0.55$
WND3	$136.3^{g} \pm 0.2$	$4.8^{e} \pm 0.1$	$62.0^{de} \pm 0.7$	$85.0^{d} \pm 3.3$	$14.3^{a} \pm 2.1$	$14.7^{d} \pm 0.5$	$7.21^{d} \pm 0.32$
SMC	$26.0^{a} \pm 1.5$	$0.6^{a} \pm 0.1$	$36.2^{a} \pm 0.6$	$26.2^{a} \pm 1.6$	Not applicable		
BMC	$131.2^{fg} \pm 0.9$	$12.0^{f} \pm 0.4$	$70.4^{f} \pm 1.5$	$83.5^{d} \pm 3.3$			
Desired barley malt quality for brewing	200-250*	500*	>81.0**	130-160**			

Values are Mean ± standard deviation (n=2).Values in a column with different letters in superscript are significantly different ($p < 0.05$). CU (Ceralpha Unit), BU (Betamyl-3® Unit;db (dry basis);NWND (Non-waxy-normal protein digestibility); NWHD (Non-waxy - high protein digestibility), hWND (heterowaxy-normal protein digestibility), WHD (waxy-high protein digestibility), WND (waxy-normal protein digestibility), SMC = commercial sorghum malt), BMC = commercial barley malt.* (Zarnkow et al., 2007), **(Steiner et al., 2012).

Table 4.2. 2 Malt and malting quality attributes of malt prepared from waxy and high protein digestibility novel sorghum lines germinated at high moisture level and of commercial barley and sorghum malt references

Sorghum line	α–amylase (CU/g db)	β-amylase (BU/g db)	HWE[^{o}P] (%, db)	HWE[^{o}Brix] (%, db)	FAN (mg/100 g db)
NWND	$30.9^{a}\pm 1.4$	$0.7^{abc}\pm 0.1$	$35.4^{a}\pm 1.5$	$39.7^{a}\pm 1.3$	$67.7^{b}\pm 1.7$
NWHD	$31.0^{a}\pm 1.1$	$0.6^{abc}\pm 0.0$	$50.6^{c}\pm 1.7$	$55.3^{c}\pm 1.4$	$80.8^{d}\pm 2.2$
hWND	$48.9^{b}\pm 2.2$	$0.5^{ab}\pm 0.1$	$53.3^{c}\pm 0.5$	$58.3^{cd}\pm 0.7$	$69.0^{bc}\pm 2.6$
WHD1	$24.0^{a}\pm 1.9$	$0.2^{a}\pm 0.0$	$40.8^{b}\pm 0.8$	$43.9^{b}\pm 0.6$	$64.7^{b}\pm 2.4$
WND1	$116.6^{e}\pm 2.0$	$1.5^{d}\pm 0.3$	$65.1^{e}\pm 1.3$	$69.7^{ef}\pm 1.7$	$100.5^{e}\pm 2.5$
WND2	$135.9^{f}\pm 2.8$	$1.4^{d}\pm 0.1$	$62.9^{de}\pm 1.6$	$67.9^{ef}\pm 1.4$	$81.6^{d}\pm 3.4$
WHD2	$74.5^{c}\pm 1.6$	$0.9^{bcd}\pm 0.1$	$59.9^{d}\pm 0.7$	$60.8^{d}\pm 0.6$	$74.5^{c}\pm 2.7$
WND3	$86.6^{d}\pm 1.4$	$1.1^{cd}\pm 0.1$	$60.3^{de}\pm 1.9$	$66.7^{e}\pm 0.6$	$84.1^{d}\pm 2.8$
SMC	$26.0^{a}\pm 1.5$	$0.6^{abc}\pm 0.1$	$36.2^{ab}\pm 0.6$	$38.6^{a}\pm 0.4$	$26.2^{a}\pm 1.6$
BMC	$131.2^{f}\pm 0.9$	$12.0^{e}\pm 0.4$	$70.4^{f}\pm 1.5$	$71.5^{f}\pm 0.5$	$83.5^{d}\pm 3.3$

Values are Mean ± standard deviation (n=2).Values in a column with different letters in superscript are significantly different ($p< 0.05$). CU (Ceralpha Unit), BU (Betamyl-3® Unit; db (dry basis); NWND (Non-waxy-normal protein digestibility); NWHD (Non-waxy - high protein digestibility), hWND (heterowaxy- normal protein digestibility), WHD (waxy-high protein digestibility), WND (waxy-normal protein digestibility), SMC = commercial sorghum malt), BMC = commercial barley malt.

4.2.5 Conclusions

Malts from waxy sorghums exhibit greater endosperm modification during germination and generally yield higher malt hot water extract, despite the fact that β-amylase activity is not affected. The HD trait, however, does not clearly affect malt FAN, probably because its affects are obscured by those of the waxy trait. The germination at medium moisture level resulted in best malt quality. As the level of HWE from malted waxy sorghums was higher than that of the regular sorghums and more close to malted barley, white tan-plant waxy sorghum malt has considerable potential to replace part of the barley malt used in beer brewing in arid, tropical regions where barley cannot be economically cultivated.

4.2.6 References

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4.3 RELATIONSHIP BETWEEN WAXY (HIGH AMYLOPECTIN) AND HIGH PROTEIN DIGESTIBILITY TRAITS IN SORGHUM DOUGH-BASED (INJERA AND BISCUIT) PRODUCT QUALITY

4.3.1 Abstract

This study determined the relationship between waxy (high amylopectin) and high protein digestibility (HD) traits in sorghum dough-based product (injera and biscuit) quality with aim of using sorghum types expressing these traits in injera (flatbread) and biscuit making. Eight novel sorghum lines from Texas A&M Agrilife that expressed waxy and HD-traits, and three normal sorghums were studied. Injera and biscuits were prepared according to standard methods. Teff and wheat were included as references for injera and biscuits, respectively. Fresh injera, stored injera (5°C for 2 and 4 days), and biscuit quality were evaluated using a trained descriptive sensory panel and by instrumental texture analysis. Descriptive sensory profiling (DSP) showed that injera made from waxy sorghums were softer, and more rollable, and flexible compared to injera from normal sorghum lines. Instrumental texture analysis of fresh and stored injera revealed that waxy sorghums had lower stress and higher strain compared to non-waxy lines, indicating that the injera were softer. Injera of the waxy sorghum lines were softer and were much closer in softness to teff injera reference. There was no clear trend as to whether the HD trait affected injera quality. DSP showed that biscuits made from waxy and HD sorghum lines were similar in crunchiness and dryness compared to normal sorghums. Furthermore, biscuits of the waxy sorghum lines were not softer compared to normal sorghum and wheat biscuit reference. Thus waxy and HD traits did not affect biscuit quality. Principal Component Analysis showed that soft, flexible and rollable texture traits of injera were highly associated with the waxy trait. The improved injera quality is probably due to the slower retrogradation of amylopectin starch. This study clearly shows that white tan-plant waxy sorghum can produce injera of better quality than regular sorghum and thus have potential to partially replace teff for injera making in Ethiopia. However, the white tan-plant waxy sorghums do not produce biscuits of better quality than regular sorghum. Thus, either waxy or normal sorghum can be used to partially replace wheat for biscuit making.

4.3.2 Introduction

Injera (fermented flatbread) and biscuits are important food products in most parts of Ethiopia. The most preferred injera and biscuits are prepared from teff and wheat, respectively. However, teff commands a higher market price than other cereals (Minten et al., 2014). Teff grain is very small in size; 150 teff grains have comparable weight with almost one seed of wheat (Dijkstra et al., 2008). Owing to its size, cultivating and harvesting teff is time consuming and expensive (Tefera, 2012). Additionally, wheat grain and/or flour for making biscuits and other pastry products are being imported due to limitations in local production (Habtamu, 2012). The teff and wheat protein structures and compositions are different and there is no gluten-like protein in teff (Callejo et al., 2016). Sorghum is locally grown in Ethiopia and is a relatively inexpensive grain with good adaptation to harsh environments and has good production yield (Mitaru et al., 2012). Hence, making injera and biscuits from sorghum has considerable economic benefits over teff, and wheat, respectively. However, the poor texture and keeping quality of sorghum injera (Yetneberk et al., 2005) and poor sensory quality of sorghum biscuits (Rooney, 2010) are major limiting factors.

Staling is a major quality problem with flatbreads involving physico-chemical and sensory changes like firming, declining flavour, increasing opacity, and decreasing starch solubility (Kulp and Ponte, 1981). Perhaps the most important change is firming. Starch retrogradation is a major cause of staling (Hug-Iten et al., 2003). Starch retrogradation takes place when disaggregated starch components (amylose and amylopectin chains) in cooked, gelatinized starch realign themselves as the cooked starch cools (Wang et al., 2015). In sorghum, amylopectin is less susceptible to re-association during retrogradation than amylose (Sang et al., 2008). In fact, amylopectin retrogradation occurs very slowly (Lii et al., 2004), which may result in slower staling and softer baked products (Fadda et al., 2014).

Waxy (high amylopectin) barley starch has been found to retard the staling rate of laboratory-produced breads (Purhagenet al., 2011). Furthermore, Bhattacharya et al. (2002), Morita et al. (2002a, b), Baik et al. (2003), Hayakawa et al. (2004) and Mouliney et al. (2011) observed that inclusion of waxy wheat flour into normal wheat flour has an anti-staling effect and produces softer breads. Fresh tortillas (unfermented flatbread) made from high amylopectin wheat flour

has been found to have higher extensibility after three or more days of storage (Guo et al., 2003). Regarding biscuits, it has been found that higher amylose content of wheat flour was related to large biscuit diameter (Kaldy et al., 1991). Also, especially with regard to sorghum, Adedara (2017) reported that the roles of starch and sugar were critical for determining and understanding development of sorghum biscuit texture.

Sorghum lines with high protein digestibility (HD) resulting from endosperm protein expression modification by conventional breeding or genetic engineering have shown potential for improved dough-based product quality. Goodall et al. (2012) observed that HD sorghum flours produced better quality sorghum-wheat composite doughs and breads. Combining the HD-trait with waxy trait resulted in better flour properties for making dough-based food products (Elhassan et al., 2015). Taylor and Taylor (2011) found that biofortified HD sorghums had improved lysine content and protein digestibility in a range of African foods (including injera). With wheat, protein composition and content majorly influences biscuit quality in terms of diameter (Pareyt and Delcour, 2008) and texture (Maache-Rezzoug et al., 1998).

As stated, novel sorghum lines that express both the waxy and HD-traits have been developed by Texas A&M University through conventional breeding (Jampala et al., 2012). Despite the potential of these waxy and HD sorghums in improving dough-based products quality, there has been no research into their injera and biscuit making quality. Therefore, the objective of this work was to evaluate effects of the waxy and HD traits in sorghum on dough-based product (injera and biscuit) making quality in respect of descriptive sensory quality and instrumental texture analysis.

4.3.3 Materials and Methods

4.3.3.1 Materials

The eight novel sorghum lines that were increased at the Ukulima Research Farm (Chapter 4.1 and Chapter 4.2) plus three other normal types of sorghum were investigated. The novel sorghum lines comprised three waxy-normal protein digestibility (WND), one heterowaxy-normal protein digestibility (hWND), two waxy-HD (WHD), one non-waxy–high digestibility (NWHD) and one non-waxy–normal digestibility (NWND) type. The normal types of sorghum grains also grown at Ukulima Research Farm were: white non-tannin sorghum (WNTS) (PAN 606), Red non-tannin

sorghum (RNTS) (PAN 8816) and Red tannin sorghum (RTS) (PAN 8625). Moreover, Teff flour kindly supplied by Bloemfontein teff growers S.A (Pty) and wheat cake flour were included as references for injera and biscuits, respectively.

Ingredients used for biscuit making were purchased from a retail outlet in Pretoria, South Africa. These included: Snowflake wheat cake flour (Premier) (Waterfall city, South Africa), Illovo pure white sugar (Illovo sugar group, Durban, South Africa), Robertson's baking powder (Unilever, Durban, South Africa) and Sunfoil Sunflower oil (Willowton group, Kwazulu-Natal, South Africa).

4.3.3.2 Milling

The sorghum lines were milled according to the method of Kebakile et al. (2007) with modification in re-milling the second bran fractions to achieve 84-86% extraction rate. A commercial break roller mill (Maximill, Kroonstad, South Africa) with two pairs of fluted rolls was used. The top rolls (coarse break rolls) has eight flutes per 25 mm, and the bottom rolls (fine break rolls) has 22 flutes per 25 mm. This milling process that has been optimized for acceptable sorghum meal quality involves tempering clean sorghum grain (5 kg) to 16% moisture for 15 min in tightly closed plastic buckets at ambient temperature. The sorghum grain was mixed thoroughly at 5 min interval and immediately roller milled at constant feed rate using top and bottom roller gaps of 0.80 and 0.30 mm, respectively. Milled sorghum was separated on vibrating sieves of mesh sizes 1.00, 0.850, 0.710 and 0.710 mm (Tyler standard 16, 20, 26, and 26, respectively) arranged in descending order by size (top to bottom). The bran fraction was retained by the first two sieves, which was then designated as "coarse", while the meal was fractionated by the last two sieves into three streams "medium-coarse", "medium-fine", and "fine", respectively.

The extraction rate at this step was 65%; the bran fractions from sieve outlets 0.850 and 0.710 mm were mixed and re-milled to obtain the extraction rate of 84-86%. The sorghum flours were finely milled to pass through a 500 μm screen using a hammer mill (Drotsky S1, Alberton, South Africa). The milled flours were then stored in tightly closed 5 L buckets at 8°C until further analysis.

4.3.3.3 Preparation of injera (full-scale method)

Injera was prepared using a method adapted from Yetneberk et al. (2004) modified to a 1 kg sample size by increasing the volumes in proportion. The sorghum flour or teff flour (1 kg) was mixed with 900 mL tap water and kneaded well for about 5 minutes. Starter culture from pre-fermented injera dough (about 10% of the dough) was added on the top of dough. The mixture was then allowed to ferment for 3 days at room temperature (20°C). Part of the fermented dough (20%) was mixed with 130 mL and cooked in 550 mL boiling water (NB: for teff only 2/3 of these amounts was used) for 2-3 minutes, cooled to about 45°C and added back to the fermenting dough and mixed well. About 300 mL water was added and the batter was then allowed to ferment again for 2-3 h, at room temperature (20°C) until it formed foam and bubbles. Injera was baked covered for about 2 minutes by pouring 500 ml of the batter in a circular manner, on 50 cm diameter electrically heated hot clay griddle (Mitad). The injera was then removed by lifting it off the hot griddle and sliding it onto a straw mat.

4.3.3.4 Preparation of small-scale injera (microwave method)

Small-scale injera was prepared at small-scale according to Anyango et al. (2011), modified for a sample size of 250 g by adjusting the volumes in proportion and omitting the addition of baker's yeast and sugar in the 2nd phase of fermentation. In preliminary testing, different sorghum types resulted in variable size and distribution of eyes (gas cells) in the injera which was found to interfere with measurements of texture. Omitting baker's yeast and sugar was subsequently found to result in no or few eyes and improve the texture evaluation of injera made from various sorghum types. About 250 g sorghum or teff flour was mixed with 400 mL water in plastic bucket. The mixture was thoroughly kneaded by hand for about 3-5 minutes until uniformly hydrated. Starter culture from pre-fermented dough (about 10% of the dough) was added. The plastic buckets containing the dough were covered tightly and placed in an incubator set at 25ºC to ferment for 72 h. After this time, 10% of the sediment (about 75 g) was cooked with 250 ml water for 5 minutes in cooking pan. The cooked sediment was allowed to cool to 40-45ºC and added back to the un-cooked dough. Then the mixture was mixed and placed in the incubator at 25ºC for 2-3 h (2nd phase of fermentation). The batter was stirred to obtain a uniform consistency. The batter (20 g) was weighed into a 90 mm plastic Petri dish, baked for 45 seconds in a 900-Watt microwave oven and cooled to ambient temperature.

4.3.3.5 Biscuit making

Biscuits were made according to Adedara (2017), modified for a sample size of 500 g by adjusting the volumes and ingredients in proportion. Sorghum or wheat cake flour dough was prepared by mixing all dry ingredients (sorghum/wheat flour, sugar, baking powder) and wet ingredients (water and oil) in separate stainless bowls (Table 4.3.1). Both dry and wet ingredients were then combined and thoroughly mixed at medium speed for 3 minutes using an electric mixer (Kenwood Electronic Chef Excel, Mariasburg, South Africa) with an A-beater attachment. The dough was transferred to a Rollfix 300 pastry sheeter (Fritsch, Bahnofstrasse, Germany) and rolled out evenly on the surface of the sheeter using a wooden rolling pin. The dough was then sheeted to height of 5 mm and cut into circular shapes using 38 mm diameter biscuit cutter. The dough pieces were then transferred onto a lined baking tray on which its surface had been covered by silicone baking paper (Bidvest Bakery solutions, Polokwane (Pietersburg), South Africa) to prevent the dough from sticking. The baking tray containing dough pieces was then placed in a conventional oven (Miwe-Condo 4E, Müschenbach, Germany) and baked at 190^{o}C for 20±3 minutes. The typical baked aroma of baked products was used as indication that the biscuits were properly baked. Biscuits were cooled for 20 minutes at ambient temperature (20^{o}C), vacuum packed in polyethylene bags and stored at 10°C until further analysis. The moisture content of the biscuits of the different sorghum lines ranged between 3.2 and 5.1%.

Table 4.3. 1 Composition of ingredients for sorghum and wheat biscuits

Ingredients	Sorghum (g) [% based on dough weight]	Wheat (g) [% based dough weight]
Flour	500 (54.74)	500 (52.72)
Sunflower oil	145 (15.87)	145 (15.29)
Water	140 (15.32)	175 (18.45)
Sugar	125 (13.69)	125 (13.18)
Baking powder	3.4 (0.37)	3.4 (0.36)
Total dough weight	**913.4 g (100)**	**948.4 (100)**

Figures in brackets are percentages of the ingredients based on dough weight.

4.3.3.6 Moisture

The moisture and dry matter content of flours and biscuits was determined using AACC Method 44-15A (AACC, 2000), so as to correct to dry basis the proximate composition of the flours.

4.3.3.7 Starch

Starch content of the flours was determined using the Megazyme total starch assay procedure (Amyloglucosidase/α-amylase method) (Megazyme International, Bray, Ireland) as described in Chapter 4.1.

4.3.3.8 Protein

The protein content (N x 6.25) of the flours was determined by a Dumas combustion assay according to AACC method 46-30 (AACC International, 2000).

4.3.3.9 Crude fat

Crude fat (ether extraction) of the flours was determined using Soxhlet extraction according to AACC method 30-25 (AACC, 2000).

4.3.3.10 Ash

Ash content of the flours was determined using AACC approved method 08-17 (AACC, 2000).

4.3.3.11 Dietary fibre

Dietary fibre of the flours was determined by difference; subtracting all other components of the flour (moisture, protein, starch, fat and ash) from 100.

4.3.3.12 pH and Titratabile acidity (TA) of the fermenting dough

pH and TA of the doughs was measured at 0, 24, 48 and 72 hours of fermentation. TA was measured by taking 10 g dough and blending with 100 ml distilled water. The mixture was centrifuged at 3170 g and 25ºC for 10 min. Supernatant (in triplicate) (10 ml) of the centrifuged sample was then taken into beakers and 2-3 drops of 1% phenolphthalein added. This mixture was titrated against 0.1M NaOH until the end point (persistent pink colour change). The

titratable acidity (%) was then calculated by multiplying the titre value by 0.09 (Wakil and Kazeem, 2012).

4.3.3.13 Descriptive sensory analysis

Descriptive sensory analysis of fresh injera, stored injera (at 5°C for 2 and 4 days) and biscuits was conducted using a trained sensory panel of 10 people. Only panelists who voluntarily accepted and signed a consent form that informed them of the nature of the injera and biscuit samples and the activities involved in the study were included. Sensory profiling of the products was performed using the generic descriptive analysis method (Einstein, 1991). The panel was trained in two sessions of 2 hours per day during which the aroma, appearance, texture, flavour, and aftertaste sensory properties of injera and biscuits with definitions, reference standards and methodology of evaluation were developed (Table 4.3.4 and Table 4.3.11 for injera and biscuits, respectively). The attributes were evaluated on a 10-point scale (0-10) anchored with verbal descriptions.

Evaluation of the fresh injera, stored injera and biscuits (12 samples including the teff for injera reference and wheat for biscuit reference) was performed on duplicate products in replicated sessions of 2h per day. The actual product evaluation was done in a sensory laboratory with individual booths following standard good sensory practices (Lawless and Heymann, 1999). Rolled triangular pieces of injera (from $1/16^{th}$pieceas shown in Figure 4.3.1) were presented to the panelists in a small mixing glass bowl on a tray at ambient temperature (25°C). Freshly prepared injera was presented within 3h after baking. Biscuits were presented in transparent polyethylene zip-lock type bags with random three–digit codes. A glass of drinking water was provided for rinsing between samples. Responses were collected using Compusense® five release 4.6 (Compusense Guelph, ON, Canada).

Application for ethical approval of this sensory study was applied and the ethical issues were approved by the University of Pretoria Faculty of Natural and Agricultural Sciences Research Ethics Committee with a reference number EC180417-186.

4.3.3.14 Instrumental texture analysis of injera

Textural properties of injera prepared using full-scale (griddle method) and small-scale (microwave method) was measured using a 3-point bending rig with aluminium bar (5 mm thick and 90 mm long) mounted on an EZ-L SHIMADZU texture analyser (Nakagyo-ku, Japan). Injera were cut into strips (6 cm long and 3 cm width) using a sharp knife for a uniform shape and size. The injera strips were placed in a separate polythene bag and stored at 5°C for 0, 2, and 4 days. The thickness of the fresh and stored injera strips was also measured using calipers. The two adjustable supports of the rig base plates were set at 30 mm apart. The testing profile in the tensile testing machine was set; pre-test speed (1.0 mm/s), test speed (3.0 mm/s), post-test speed (10.0 mm/s), distance (15 mm), trigger type (0.049 N, Auto) (Anyango et al., 2011). The injera strips were then placed over the vertical struts (30 mm apart) clamped in place at both ends. The injera strips were compressed by a constant rate of 3.3 mm/s over a distance of 15 mm. The mean peak force (N) at break, stress (kPa) (Abang-Zaidel et al., 2008) and strain (%) of 6 injera strips were obtained and reported.

$$\sigma = \frac{3FL}{2bd^2} \quad \text{..... 1.1}$$

$$\varepsilon = \frac{\Delta L}{L} \quad \text{..... 1.2}$$

Where F (force (N)); L (support span length); b (width); d (thickness); σ (stress (kPa); ε (strain (%)); ΔL (the change in L [Calculated by subtraction of the initial L from compressed L (mm)]).

Equation 1.1 was used to calculate the true stress of the product and ε is multiplied by 100 for expressing the strain in %. The textural properties of sorghum injera were compared with the teff injera standard.

4.3.3.15 Instrumental texture analysis of biscuits

The hardness of biscuits was determined using a 3-point bending rig with aluminium bar (5 mm thick and 90 mm long) mounted on an EZ-L SHIMADZU texture analyser (Nakagyo-ku, Japan). The two adjustable supports of the rig base plates were set at 20 mm apart. A vertical force was applied using the upper blade on a biscuit placed horizontally like a bridge over the two supports at a cross-head pre-test speed of 1.0 mm/sec, test speed of 3.0 mm/sec, post-test speed of 10.0 mm/sec and distance of 10 mm. The mean maximum peak force required to break 7 biscuits was

obtained. The fracture properties of the biscuits were analyzed by determining stress using equation 1.1 (section 4.3.3.14) and strain by the following equation (Baltsavias et al., 1997).

$$\varepsilon = \frac{6h}{L^2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1.3$$

Where ε (strain (%)), L (distance between the supports (mm)), and h (biscuit thickness (mm)). The texture properties of sorghum biscuits were compared with wheat biscuit standard.

4.3.3.16 Statistical analyses

Baking of injera and biscuits was performed at least twice for all sorghum lines including references and closely agreeing replicates were obtained. All chemical and sensory analyses on both replicates were repeated at least twice. The data for the dough acidity, proximate composition, biscuit sensory quality and instrumental texture properties was analysed using one-way analysis of variance (ANOVA). Data for sensory properties of injera was analysed using both two-way ANOVA and one-way ANOVA. The means were separated using Tukey's HSD test at p<0.05. Principal Component Analysis (PCA) for all numerical results was performed using XLSTAT version 2016.03.30882 (Addinsoft, New York).

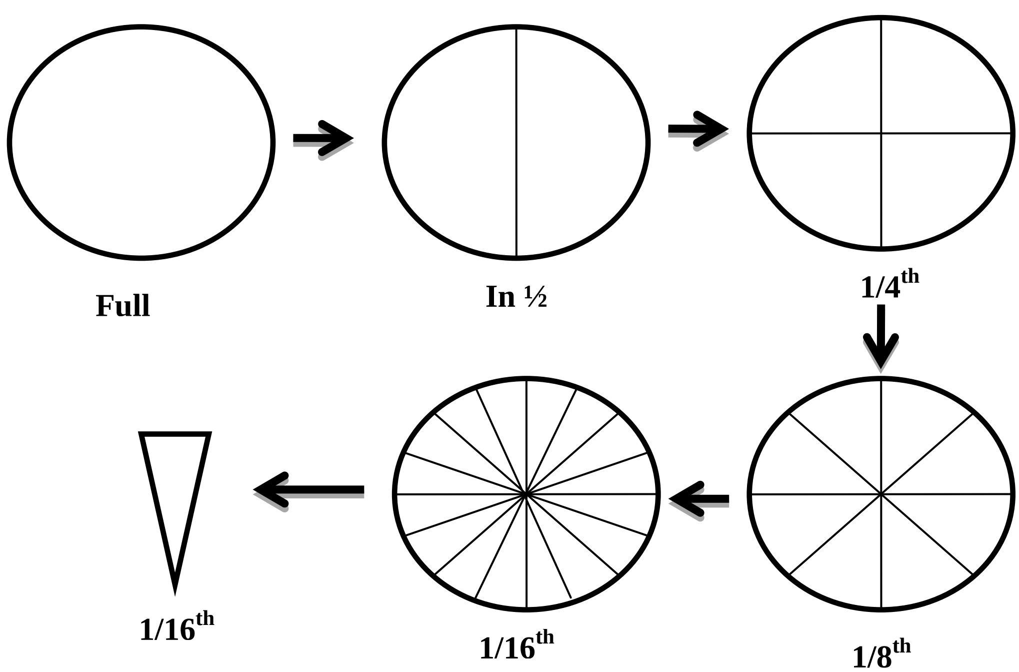

Figure 4.3. 1 Sampling and presentation of injera for descriptive sensory panels

4.3.4 Results and discussion

4.3.4.1 Proximate analyses

The starch content of the flours used for making injera and biscuit was determined and it ranged from 76.6 to 81.2% on dry basis (Table 4.3.2). All sorghum flours had similar ($p \geq 0.05$) starch content to teff flour and with no significant difference ($p \geq 0.05$) among the sorghum lines. Most of the sorghum lines also had starch content closer to the wheat flour. The starch content of the flours of the novel sorghum lines had slightly increased compared to the starch content of their grains determined in Chapter 4.1. This is because the sorghum lines were milled to an extraction rate (ER) of 84-86% and the starch is located in the starchy endosperm (Corredor et al., 2006). The starch content of these novel sorghum lines was slightly higher than the sorghum genotypes studied by Wu et al. (2007), Wang et al. (2008), and Wu et al. (2010).

The protein content of the flours ranged from 11.2 to 13.5% db (Table 4.3.2). Flours of hWND, WND1, WND2 and WHD2 had similar ($p \geq 0.05$) protein contents to NWND, NWHD, teff and had the same protein content as the wheat flour. These sorghum lines had higher ($p < 0.05$) protein content compared to waxy lines (WHD1 and WND3) and normal sorghums (WNTS, RNTS and RTS). Protein content of flours of the novel sorghum lines seemed to be within the range of the protein content of their grains (Chapter 4.1) and sorghum genotypes studied by Wu et al. (2010).

The crude fat content of the flours varied between 2.6 and 4.6% db (Table 4.3.2). WND1 had a higher crude fat content, while NWND and WHD2 had lower ($p < 0.05$) fat compared to other lines. NWND and WHD2 flours had similar ($p \geq 0.05$) crude fat to teff flour. The remaining sorghum lines (NWHD, hWND, WHD1, WND2, WND3, RNTS WNTS and RTS) were intermediate in fat. All sorghum lines had a higher fat content than the wheat flour. Fat content of all the flours was within the range of the waxy, heterowaxy and HD sorghums studied by Wu et al. (2010) and slightly higher than the decorticated normal sorghum flours studied by Corredor et al (2006).

The dietary fibre content of the flours ranged from 2.4 to 5.7% db (Table 4.3.2). hWND and RTS had higher ($p < 0.05$) dietary fibre content compared to all other sorghums. All sorghum flours had higher dietary fibre contents compared to the wheat flour. NWND, WHD1, WHD2, RNTS and WNTS were lower ($p < 0.05$) in dietary fibre. NWHD, WND1, WND2, WND3 and teff flour

were intermediate in dietary fibre. The dietary fibre content of the sorghum flours was consistent with data from Yousif et al. (2012).

The ash content of the sorghum flours varied between 1.3 and 2.2% db (Table 4.3.2). WHD2 and RTS had higher ($p<0.05$) ash compared to the other sorghums. NWND, NWHD, hWND, WND1, WND2, WND3, WHD1, WNTS, RNTS and RTS had similar ($p\geq0.05$) ash. The teff flour had the highest ash. The ash contents of all the flours were within the range of the sorghum genotypes studied by Wu et al. (2010).

As all the novel sorghum lines and normal sorghum types were similar in proximate composition they were considered suitable to study the influence of waxy and HD traits in sorghum dough-based products (injera and biscuits) quality.

Table 4.3. 2 Proximate compositions of flours (g /100 g, db) of novel sorghum lines with waxy and HD-traits, normal sorghum types and teff

Sorghum line	Starch	Protein	Crude fat	Dietary fibre*	Ash
NWND	$80.3^{a} \pm 1.2$	$12.8^{cd} \pm 0.5$	$2.7^{a} \pm 0.0$	$2.6^{a} \pm 0.4$	$1.7^{ab} \pm 0.2$
NWHD	$78.0^{a} \pm 2.8$	$12.9^{cd} \pm 0.0$	$4.1^{bcd} \pm 0.7$	$3.3^{ab} \pm 0.5$	$1.7^{ab} \pm 0.0$
hWND	$76.9^{a} \pm 3.0$	$13.1^{cd} \pm 0.0$	$3.7^{abcd} \pm 0.2$	$4.9^{c} \pm 0.9$	$1.4^{a} \pm 0.1$
WHD1	$80.0^{a} \pm 3.8$	$11.7^{a} \pm 0.1$	$4.1^{bcd} \pm 0.2$	$2.7^{a} \pm 0.4$	$1.5^{a} \pm 0.0$
WND1	$76.6^{a} \pm 2.5$	$13.5^{d} \pm 0.0$	$4.6^{d} \pm 0.4$	$3.5^{ab} \pm 0.7$	$1.8^{ab} \pm 0.1$
WND2	$79.3^{a} \pm 4.0$	$13.2^{cd} \pm 0.3$	$3.0^{ab} \pm 0.1$	$2.9^{ab} \pm 0.0$	$1.6^{ab} \pm 0.1$
WHD2	$80.0^{a} \pm 1.3$	$12.8^{cd} \pm 0.0$	$2.6^{a} \pm 0.0$	$2.4^{a} \pm 0.4$	$2.2^{b} \pm 0.2$
WND3	$80.7^{a} \pm 1.4$	$11.9^{ab} \pm 0.1$	$3.1^{ab} \pm 0.0$	$2.9^{ab} \pm 0.2$	$1.3^{a} \pm 0.0$
WNTS	$81.2^{a} \pm 1.1$	$11.4^{a} \pm 0.2$	$3.5^{abcd} \pm 0.1$	$2.7^{a} \pm 0.5$	$1.3^{a} \pm 0.0$
RNTS	$79.0^{a} \pm 1.2$	$12.7^{bc} \pm 0.1$	$4.3^{cd} \pm 0.4$	$2.4^{a} \pm 0.3$	$1.6^{ab} \pm 0.4$
RTS	$78.5^{a} \pm 3.8$	$11.2^{a} \pm 0.2$	$3.3^{abc} \pm 0.1$	$5.7^{d} \pm 0.6$	$1.3^{a} \pm 0.1$
TEFF	$76.3^{a} \pm 0.8$	$13.4^{cd} \pm 0.0$	$2.9^{a} \pm 0.1$	$4.0^{abc} \pm 0.5$	$3.3^{c} \pm 0.2$
Wheat**	75.0	13.0	1.4	0.3	n/a

Values are Mean ± standard deviation (n=2).Values in a column with different letters in superscript are significantly different ($p< 0.05$). db (dry basis); NWND (Non-waxy-normal protein digestibility); NWHD (Non-waxy - high protein digestibility), hWND (heterowaxy- normal protein digestibility), WHD (waxy-high protein digestibility), WND (waxy-normal protein digestibility), WNTS (white non-tannin sorghum), RNTS (red non-tannin sorghum), RTS (red tannin sorghum), *(dietary fibre determined by difference), n/a (not applicable), **(composition determined by the producer of the wheat flour).

4.3.4.2 pH and titratable acidity of injera doughs

The pH of the fermenting doughs was determined at 0, 24, 48 and 72 hours (Table 4.3.3). Initially all sorghum lines had higher ($p<0.05$) pH values compared to the fermenting teff. The pH of all sorghum lines and teff showed a reduction during the fermentation periods. With exception of RTS, all the sorghum lines and teff had similar ($p\geq0.05$) pH at 48 h and 72 h. This indicates that the pH was not affected by the waxy and HD traits. The higher pH value of RTS after fermentation is due to inhibitory effects of the tannin (Osman, 2004). The pH of the sorghum at the end of the fermentation (72 h) was within the pH ranges for different brands of injera batters (pH 3.65–4.02) reported in (Attuquayefio, 2014).

Titratable acidity (TA) of the fermenting doughs was measured at 0, 24, 48 and 72 h (Table 4.3.3). TA of all the sorghum lines and teff showed increased trend during the fermentation period. All sorghum lines had lower ($p<0.05$) TA compared to teff and were higher ($p<0.05$) in TA than RTS at the end of the fermentation. The lower TA of RTS is consistent with the observation that its pH was higher than all sorghum lines and because of it containing tannins (Osman, 2004). The higher titratable acidity of the teff dough can be attributed to its high buffering capacity (Urga et al., 1997; Wolter et al., 2014), resulting from its high content of protein and ash content (Table 4.3.2). There was no significant difference in TA among the novel sorghum lines and normal sorghums; hence, TA was not affected by the waxy and HD traits.

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Table 4.3. 3 pH and titratable acidity of fermenting doughs novel sorghum lines with waxy and HD-traits, normal sorghum types and teff

Sorghum line	pH at fermentation periods (h)				TA (%) at fermentation periods (h)			
	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
NWND	6.41^{c}	4.79^{ab}	4.29^{bcd}	3.81^{ab}	0.018^{c}	0.027^{bc}	0.038^{bc}	0.086^{bc}
NWHD	6.32^{bc}	5.15^{d}	4.28^{abcd}	3.89^{ab}	0.014^{abc}	0.030^{bcd}	0.040^{bc}	0.077^{b}
hWND	6.31^{bc}	4.74^{ab}	4.14^{ab}	3.88^{ab}	0.013^{abc}	0.023^{ab}	0.040^{bc}	0.086^{bc}
WHD1	6.30^{bc}	5.18^{d}	4.19^{abc}	3.92^{ab}	0.016^{bc}	0.033^{cd}	0.044^{bc}	0.090^{bc}
WND1	6.30^{bc}	4.70^{ab}	4.34^{cd}	3.89^{ab}	0.012^{abc}	0.038^{d}	0.049^{c}	0.096^{cd}
WND2	6.23^{b}	4.89^{bc}	4.22^{abcd}	3.92^{ab}	0.014^{abc}	0.032^{bcd}	0.044^{bc}	0.086^{bc}
WHD2	6.28^{bc}	5.10^{cd}	4.38^{d}	3.70^{a}	0.011^{ab}	0.029^{bc}	0.048^{c}	0.099^{cd}
WND3	6.29^{bc}	4.63^{a}	4.13^{ab}	3.85^{ab}	0.014^{abc}	0.030^{bcd}	0.048^{c}	0.109^{d}
WNTS	6.25^{b}	4.66^{ab}	4.09^{a}	3.72^{a}	0.014^{abc}	0.026^{bc}	0.045^{c}	0.090^{bc}
RNTS	6.22^{b}	5.09^{cd}	4.21^{abcd}	3.96^{b}	0.012^{abc}	0.025^{bc}	0.033^{ab}	0.077^{b}
RTS	6.16^{b}	5.92^{e}	5.87^{e}	5.89^{c}	0.010^{a}	0.016^{a}	0.023^{a}	0.032^{a}
TEFF	5.87^{a}	5.71^{e}	4.13^{ab}	3.80^{ab}	0.016^{bc}	0.029^{bc}	0.083^{d}	0.129^{e}

Values are Mean ± standard deviation (n=2). Values in a column with different letters in superscript are significantly different ($p < 0.05$). h (hours); NWND (Non-waxy-normal protein digestibility); NWHD (Non-waxy - high protein digestibility), hWND (heterowaxy- normal protein digestibility), WHD (waxy-high protein digestibility), WND (waxy-normal protein digestibility), WNTS (white non-tannin sorghum), RNTS (red non-tannin sorghum), RTS (red tannin sorghum).

4.3.4.3 Descriptive sensory analysis of injera

The sensory panel generated twenty-eight sorghum injera quality descriptors and their definitions, reference standards and anchors are given (Table 4.3.4). Aroma attributes were not affected ($p \geq 0.05$) by the sorghum line traits, storage and interaction of the trait and storage (Table 4.3.5). Appearance attributes were affected ($p<0.05$) by the sorghum line traits but not by the storage and interaction. Texture attributes evaluated by tactile handfeel were significantly affected ($p<0.001$) by the traits and storage. Only softness of the texture attributes evaluated by in-mouth sensation and sourness from flavour and aftertaste attributes were significantly affected ($p<0.001$) by the traits and by storage.

Aroma, appearance, flavour and aftertaste attributes of freshly prepared (Table 4.3.6) and stored (Table 4. 3.7 and Table 4.3.8) sorghum injera were not evidently affected by the waxy and HD

traits. However, the waxy and HD-traits greatly affected the texture profile of the freshly prepared and stored sorghum injera (Table 4.3.5). Moreover, the texture attributes evaluated by tactile handfeel were very much influenced by the waxy and HD traits; while texture attributes evaluated in-mouth sensation were not affected.

Texture qualities evaluated by tactile handfeel showed highly variable ratings (Table 4.3.6). The waxy-HD lines (WHD1 and WHD2) had a softness similar ($p \geq 0.05$) to teff and normal sorghum type (WNTS, RNTS and RTS) injera. With regard to the relationship between the waxy and HD traits and softness of fresh injera, waxy sorghums (WND1, WND2, WND3) and waxy-high protein digestibility (WHD1, WHD2) lines gave a softer ($p<0.05$) injera compared to non-waxy lines (NWND, NWHD). A two days stored (5°C) injera of the waxy lines (WND1, WND2, WND3, WHD1 and WHD2) were similar ($p \geq 0.05$) in softness to teff and RTS injera (Table 4.3.7). These lines also had softer ($p<0.05$) texture compared to non-waxy (NWND, NWHD) and normal sorghum types (WNTS and RNTS). Four days stored (5°C) injera did not show clear trend of significant differences in softness of the novel and normal sorghum lines and teff (Table 4.3.8).

PCA of freshly prepared injera revealed that waxy lines (WND1, WND2, WND3, WHD1 and WHD2) were aligned with softness, together with flexibility and rollability (Figure 4.3.2) and were much closer to teff injera reference (Figure 4.3.5). The PCA also showed that the waxy and high protein digestibility lines (WHD1 and WHD2) were associated with softness and stickiness, while the lines with only waxy trait were associated with softness (Figure 4.3.5). This indicated that the waxy trait combined with HD resulted in soft and sticky injera and only the waxy trait (higher starch amylopectin) resulted in softer and more flexible injera. In fact, all the waxy lines were in the same PC1 quadrant as softness. Furthermore, PCA of two-days stored injera revealed that all the waxy lines were in the same PC1 quadrant as softness (Figure 4.3.3). This also indicated that the waxy trait was associated with softness and flexibility of injera much better than the non-waxy sorghums (Figure 4.3.6). PCA of the four-days stored injera showed similar trend as of the two-days stored injera (Figure 4.3.4 and Figure 4.3.7). Hence, it seems that the waxy (high amylopectin) trait is highly associated with soft texture in fresh and stored sorghum injera. This is probably as consequence of the lower susceptibility of amylopectin to re-association during retrogradation (Sang et al., 2008), and better water holding property of the

amylopectin (Fadda et al., 2014), resulting very slow retrogradation (Lii et al., 2004), producing slower staling and softer baked products (Fadda et al., 2014). The findings of this current study are in agreement with other research into breads from different cereals where waxy barley (Purhagen et al., 2011) and waxy wheat (Morita et al., 2002a, b; Bhattacharya et al., 2002; Baik et al., 2003; Mouliney et al., 2011) were found to retard staling and produce softer breads. The current findings are also in agreement with the observation of Park and Baik (2007) who observed softer crumb texture in French bread when using wheat flours of low (15.4 to 16.6%) starch amylose content.

Only fresh prepared hWND injera had lower ($p<0.05$) flexibility compared to teff (Table 4.3.6). All other novel sorghum lines had similar ($p\geq0.05$) flexibility to teff injera with no significant difference among them. The flexibility of the stored injera (2 and 4 days) of all the sorghum lines (Table 4.3.7 and Table 4.3.8) showed a similar trend to the freshly prepared injera. Hence, there was no clear trend as to whether the flexibility of sorghum injera was affected by the traits.

Regarding rollability, fresh injera from all the novel sorghum lines had similar ($p\geq0.05$) rollability to teff and normal sorghum injera (Table 4.3.6). Only hWND injera was less rollable ($p<0.05$) compared to teff injera. Furthermore, there was no significant difference in rollability of the waxy and non-waxy sorghum injera. The rollability of stored injera (2 and 4 days) of all the sorghum lines followed similar trend as with the fresh injera (Table 4.3.7 and Table 4.3.8). Thus, there was no clear trend as whether the traits affected the sorghum injera rollability.

Fresh injera of hWND, WND1, WND2 and WND3 had similar ($p\geq0.05$) stickiness to injera from the non-waxy lines (NWND, NWHD), teff and normal sorghum injera (Table 4.3.6). The waxy-HD lines (WHD1 and WHD2) produce stickier ($p<0.05$) injera compared to non-waxy lines (NWND, NWHD). This was reflected in the PCA (Figure 4.3.5) which showed that the waxy trait combined with the HD-trait resulted in sticky and soft injera. However, the injera of waxy-HD lines had similar ($p\geq0.05$) stickiness to hWND, WND2 and WND3 injera. In addition, stored injera (2 and 4 days) of all sorghum lines had similar ($p\geq0.05$) stickiness (Table 4.3.7 and Table 4.3.8). Thus, there was no clear trend as whether the waxy traits affected stickiness of sorghum injera.

Freshly prepared injera of hWND line had lower ($p<0.05$) sponginess compared to teff injera (Table 4.3.6). All other injera of the novel sorghum lines had similar ($p\geq0.05$) sponginess to teff and normal sorghum type injera. Sponginess of the stored injera (2 and 4 days) of the sorghum lines followed similar trend as of the fresh injera (Table 4.3.7 and Table 4.3.8). Thus, there was no clear trend as whether sorghum injera sponginess was affected by the traits.

Table 4.3. 4 Lexicon used to describe the sensory properties of fresh and stored injera made from waxy and HD sorghum lines and teff

Sensory category	Attributes	Definition /reference	Scale anchors (0, 10)
Aroma	Fermented	Intensity of aroma associated with beer	No fermented aroma, Intense fermented aroma
	Sorghum	Intensity of aroma associated with sorghum porridge	No sorghum aroma, Intense sorghum aroma
	Musty	Intensity of aroma associated with moldy smell	No musty aroma, Intense musty aroma
	Overall aroma	Overall intensity of aroma of injera	No overall aroma intensity, Intense overall aroma
Appearance	Shininess*	The property of having a smooth shiny/ glossy/ lustrous surface	Not shiny surface, Very shiny surface
	Eye size*	Associated to the size of pores formed on surface	Very large, Very small
	Eye evenness & distribution*	Associated to the distribution and evenness of the pores	Not evenly distributed, Very evenly distributed
Texture	Softness*	Property of a product that displays slight resistance to deformation	Not soft, Very soft
	Flexibility**	The property of bending easily without breaking.	Not flexible, Very flexible
(by hand)			
	Rollability*	Property of ease to be rolled	Not rollable, Very rollable
	Stickiness*	Degree to which a product tends to give glutinous property	Not sticky, Very sticky
	Sponginess	Property of resembling a sponge; light, porous & compressible	Not spongy, Very spongy
Texture	Chewiness**	Property of remaining in the mouth without breaking or dissolving	Not Chewy, Very chewy
	Softness*	Property of a product that displays slight resistance to deformation	Not soft, Very soft
(in-mouth)			
	Breakability**	Property of a product that displays slight resistance to breaking	Not breakable, Very breakable
	Grittiness*	Degree to which small particles were noticed during mastication.	Not gritty, Very gritty
	Dry mouthfeel	Degree to which sample feels dry while chewing and absorbs saliva	No dry mouth feel, Intense dry mouth feel
Flavour	Sourness*	Fundamental taste sensation elicited by acids	No sour taste, Intense sour taste
	Bitterness*	Fundamental taste sensation elicited by caffeine	No bitter taste, Intense bitter taste
	Starchy***	Taste associated with carbohydrate-rich products (bread & pasta)	No starchy taste, Intense starchy taste
	Sweetness*	Fundamental taste sensation elicited by sugar	No sweet taste, Intense sweet taste
	Overall flavour	Overall intensity of flavour of injera	No overall flavour, Intense overall flavour
Aftertaste	Sourness*	Fundamental taste sensation elicited by acids	No sour aftertaste, Intense sour aftertaste
	Bitterness*	Fundamental taste of which caffeine is typical	No bitter aftertaste, Intense bitter aftertaste
	Dry mouth feel	Degree to which sample feels dry while chewing and absorbs saliva	No dry mouth feel, Intense dry mouth feel
	Fine particles	Degree to which small particles remained in the mouth after swallowing.	No fine particles, Many fine particles
	Astringent	Chemical sensation associated with puckering of tongue caused by substances such as tannins	No astringent taste, Intense astringent taste
	Lingering	Length of time which the taste lasts after swallowing	No lingering aftertaste, Intense lingering aftertaste

* Yetneberk et al. (2004), ** Bourne (2002), ***Lapis et al. (2016), Attributes without symbol indicator were developed by the panel.

Table 4.3. 5 Two-way ANOVA table and summary of significance for effects of waxy and HD-traits, storage and the interaction on the sensory properties of sorghum and teff injera

Sensory category	Attribute	[1]Waxy and HD traits	Storage	[1]Waxy and HD traits * Storage
Aroma	Fermented aroma	NS	NS	NS
	Sorghum aroma	NS	NS	NS
	Musty aroma	NS	NS	NS
	Overall aroma	NS	NS	NS
Appearance	Shininess of top surface	***	NS	NS
	Shininess of bottom surface	*	NS	NS
	Eye size	***	NS	NS
	Eye evenness & distribution	***	NS	NS
Texture	Softness	***	***	**
(by hand)	Flexibility	***	***	NS
	Rollability	***	***	*
	Stickiness	***	***	*
	Sponginess	***	***	NS
Texture	Chewiness	NS	NS	NS
(in-mouth)	Softness	***	***	NS
	Breakability	NS	NS	NS
	Grittiness	NS	NS	NS
	Dry mouthfeel	NS	NS	NS
Flavour	Sourness	***	*	NS
	Bitterness	NS	NS	NS
	Starchy	NS	NS	NS
	Sweetness	NS	NS	NS
	Overall flavour	NS	NS	NS
Aftertaste	Sourness	***	*	NS
	Bitterness	NS	NS	NS
	Dry mouthfeel	NS	NS	NS
	Fine particles	NS	NS	NS
	Astringent	NS	NS	NS
	Lingering	NS	NS	NS

*** p-value < 0.001; ** p-value < 0.01, *p-value < 0.05, NS (not significant); [1]ANOVA to determine the effect waxy and HD-traits in sorghum and storage on sensory quality of injera.

Table 4.3. 6 Descriptive sensory profile of freshly prepared injera from novel sorghum lines with waxy and HD traits, normal sorghum types and teff

Sensory category/ attributes		Sorghum lines											
		NWND	**NWHD**	**hWND**	**WHD1**	**WND1**	**WND2**	**WHD2**	**WND3**	**WNTS**	**RNTS**	**RTS**	**Teff**
Aroma	Fermented	4.2^{a}	3.6^{a}	3.0^{a}	4.1^{a}	3.6^{a}	4.5^{a}	3.8^{a}	4.4^{a}	3.8^{a}	3.9^{a}	4.3^{a}	4.9^{a}
	Sorghum	3.3^{a}	4.6^{a}	4.3^{a}	3.8^{a}	3.9^{a}	4.2^{a}	4.0^{a}	4.6^{a}	4.1^{a}	4.6^{a}	4.7^{a}	3.4^{a}
	Musty	3.3^{a}	2.9^{a}	3.6^{a}	2.9^{a}	2.7^{a}	2.6^{a}	3.1^{a}	2.6^{a}	3.4^{a}	3.5^{a}	4.7^{a}	3.9^{a}
	Overall aroma	5.5^{a}	5.2^{a}	4.8^{a}	5.0^{a}	5.5^{a}	5.4^{a}	5.1^{a}	5.8^{a}	5.2^{a}	5.5^{a}	6.9^{a}	5.9^{a}
Appearance	Shininess top surface	4.3^{c}	1.5^{ab}	1.1^{a}	3.3^{abc}	2.7^{abc}	1.5^{ab}	3.5^{bc}	2.3^{abc}	1.8^{ab}	2.4^{abc}	1.5^{ab}	2.3^{abc}
	Shininess bottom surface	3.2^{a}	3.5^{a}	2.4^{a}	3.5^{a}	2.9^{a}	2.1^{a}	3.4^{a}	2.8^{a}	2.4^{a}	2.1^{a}	1.5^{a}	2.4^{a}
	Eye size	7.1^{bcd}	6.4^{abcd}	5.3^{abc}	8.3^{d}	6.5^{abcd}	5.2^{abc}	7.8^{cd}	8.3^{d}	6.0^{abcd}	6.0^{abcd}	4.0^{a}	5.1^{ab}
	Eye evenness & distribution	5.3^{ab}	6.2^{ab}	3.3^{a}	5.3^{ab}	5.0^{ab}	5.0^{ab}	6.7^{b}	5.8^{ab}	5.8^{ab}	6.1^{ab}	6.1^{ab}	7.6^{b}
Texture (By hand)	Softness	4.4^{a}	4.1^{a}	4.6^{ab}	7.8^{d}	7.0^{cd}	6.9^{cd}	7.4^{cd}	7.3^{cd}	4.8^{abc}	5.1^{abcd}	6.2^{abcd}	7.4^{cd}
	Flexibility	7.6^{b}	6.3^{ab}	4.1^{a}	7.6^{b}	7.8^{b}	5.9^{ab}	6.1^{ab}	6.9^{ab}	6.1^{ab}	6.1^{ab}	5.1^{ab}	7.5^{b}
	Rollability	7.2^{bc}	6.6^{abcd}	3.7^{a}	7.5^{bc}	8.3^{c}	6.5^{abcd}	6.3^{abcd}	7.2^{bc}	5.6^{abcd}	6.8^{bc}	4.9^{ab}	8.0^{c}
	Stickiness	3.1^{ab}	2.9^{ab}	3.6^{abc}	6.4^{c}	2.2^{a}	4.9^{abc}	5.9^{bc}	4.8^{abc}	2.0^{a}	2.6^{a}	2.3^{a}	2.4^{a}
	Sponginess	5.9^{ab}	4.2^{ab}	3.9^{a}	6.5^{ab}	6.2^{ab}	5.1^{ab}	5.0^{ab}	4.3^{ab}	4.1^{ab}	5.0^{ab}	5.7^{ab}	7.0^{b}
Texture (In-mouth)	Chewiness	7.6^{a}	6.4^{a}	5.2^{a}	6.7^{a}	7.3^{a}	6.9^{a}	6.0^{a}	6.8^{a}	6.3^{a}	6.8^{a}	6.2^{a}	7.1^{a}
	Softness	7.8^{a}	5.3^{a}	5.0^{a}	7.3^{a}	7.4^{a}	7.1^{a}	6.6^{a}	7.3^{a}	5.3^{a}	5.3^{a}	6.7^{a}	7.6^{a}
	Breakability	6.6^{a}	6.6^{a}	5.8^{a}	6.3^{a}	6.6^{a}	6.7^{a}	6.5^{a}	6.5^{a}	5.9^{a}	6.4^{a}	6.9^{a}	6.7^{a}
	Grittiness	3.1^{a}	4.5^{a}	3.8^{a}	2.5^{a}	3.4^{a}	3.6^{a}	3.1^{a}	3.2^{a}	3.7^{a}	3.6^{a}	4.7^{a}	3.6^{a}
	Dry mouthfeel	3.8^{a}	3.9^{a}	4.0^{a}	3.0^{a}	3.5^{a}	4.1^{a}	3.6^{a}	3.7^{a}	4.1^{a}	3.9^{a}	3.5^{a}	3.2^{a}
Flavour	Sourness	4.6^{b}	2.6^{ab}	2.7^{ab}	3.7^{ab}	3.5^{ab}	4.4^{b}	5.3^{b}	3.9^{ab}	2.5^{ab}	3.2^{ab}	1.3^{a}	4.4^{b}
	Bitterness	3.0^{a}	2.5^{a}	3.6^{a}	3.7^{a}	3.1^{a}	4.2^{a}	3.3^{a}	3.1^{a}	2.7^{a}	2.5^{a}	1.3^{a}	3.6^{a}
	Starchy taste	2.8^{a}	2.8^{a}	3.0^{a}	2.9^{a}	1.9^{a}	2.6^{a}	2.0^{a}	2.6^{a}	3.0^{a}	2.8^{a}	3.7^{a}	2.8^{a}
	Sweetness	1.7^{a}	1.5^{a}	1.4^{a}	1.8^{a}	1.6^{a}	2.1^{a}	1.7^{a}	1.7^{a}	1.5^{a}	1.7^{a}	1.9^{a}	1.7^{a}
	Overall flavour	5.8^{a}	4.8^{a}	4.6^{a}	5.2^{a}	5.7^{a}	5.3^{a}	5.6^{a}	5.6^{a}	5.1^{a}	4.9^{a}	5.0^{a}	5.3^{a}

Aftertaste	Sourness	2.7[a]	2.0[a]	1.9[a]	2.4[a]	2.3[a]	3.1[a]	3.3[a]	2.7[a]	1.8[a]	2.0[a]	1.1[a]	2.9[a]
	Bitterness	2.6[a]	2.5[a]	2.1[a]	2.7[a]	2.6[a]	2.9[a]	2.6[a]	2.7[a]	2.0[a]	2.4[a]	1.6[a]	2.7[a]
	Dry mouthfeel	3.4[a]	3.4[a]	3.2[a]	3.5[a]	3.2[a]	3.6[a]	3.1[a]	3.5[a]	3.1[a]	3.2[a]	2.9[a]	3.2[a]
	Fine particles	3.6[a]	3.9[a]	4.9[a]	2.6[a]	3.6[a]	3.7[a]	3.1[a]	4.4[a]	4.2[a]	3.8[a]	4.5[a]	3.2[a]
	Astringent	2.5[a]	2.2[a]	2.7[a]	2.0[a]	2.3[a]	2.9[a]	2.8[a]	2.6[a]	2.2[a]	2.4[a]	1.9[a]	2.5[a]
	Lingering	3.1[a]	2.5[a]	3.4[a]	2.5[a]	2.8[a]	3.1[a]	3.6[a]	3.4[a]	2.4[a]	3.5[a]	3.3[a]	3.4[a]

Values are Mean ± standard deviation (n=2).Values in a row with different letters in superscript are significantly different ($p<0.05$). NWND (Non-waxy-normal protein digestibility); NWHD (Non-waxy -high protein digestibility), hWND (heterowaxy- normal protein digestibility), WHD (waxy-high protein digestibility), WND (waxy-normal protein digestibility), WNTS (white non-tannin sorghum), RNTS (red non-tannin sorghum), RTS (red tannin sorghum)

Table 4.3. 7 Descriptive sensory profile of stored injera (2 days at 5°C) prepared from novel sorghum lines with waxy and HD traits, normal sorghum types and teff

Sensory category/ attributes		Sorghum lines											
		NWND	**NWHD**	**hWND**	**WHD1**	**WND1**	**WND2**	**WHD2**	**WND3**	**WNTS**	**RNTS**	**RTS**	**Teff**
Aroma	Fermented	2.4^{a}	2.6^{a}	3.7^{a}	3.5^{a}	3.6^{a}	4.1^{a}	2.9^{a}	3.8^{a}	3.3^{a}	3.3^{a}	3.9^{a}	3.1^{a}
	Sorghum	3.2^{a}	3.0^{a}	3.3^{a}	2.7^{a}	3.5^{a}	3.4^{a}	3.8^{a}	4.5^{a}	4.1^{a}	3.4^{a}	4.0^{a}	3.9^{a}
	Musty	2.6^{a}	3.1^{a}	3.0^{a}	2.6^{a}	3.0^{a}	2.3^{a}	3.5^{a}	2.6^{a}	2.8^{a}	3.1^{a}	3.7^{a}	3.9^{a}
	Overall aroma	4.1^{a}	4.6^{a}	4.7^{a}	4.2^{a}	4.7^{a}	4.3^{a}	4.5^{a}	4.9^{a}	4.7^{a}	4.3^{a}	5.8^{a}	5.5^{a}
Appearance	Shininess top surface	3.3^{bc}	1.6^{abc}	0.9^{a}	3.5^{bc}	2.4^{abc}	1.7^{abc}	1.7^{abc}	2.4^{abc}	1.4^{ab}	2.4^{abc}	1.5^{abc}	3.6^{c}
	Shininess bottom surface	3.1^{a}	1.9^{a}	1.8^{a}	3.0^{a}	2.3^{a}	2.3^{a}	2.3^{a}	2.3^{a}	2.2^{a}	2.5^{a}	1.5^{a}	2.8^{a}
	Eye size	7.3^{cd}	7.5^{cd}	5.4^{abc}	6.3^{abcd}	5.8^{abcd}	6.0^{abcd}	7.1^{cd}	8.1^{d}	6.8^{bcd}	5.6^{abcc}	4.2^{a}	4.7^{ab}
	Eye evenness & distribution	7.1^{cd}	6.1^{bcde}	3.4^{a}	5.0^{abcd}	6.5^{bcde}	6.3^{bcde}	4.3^{ab}	4.5^{abc}	6.9^{bcde}	6.9^{cde}	6.8^{bcde}	8.2^{e}
Texture (By hand)	Softness	2.5^{a}	2.4^{a}	2.6^{a}	3.1^{bc}	3.2^{bc}	3.1^{bc}	3.5^{bc}	4.4^{bc}	2.7^{a}	2.6^{a}	5.9^{bc}	7.1^{c}
	Flexibility	3.5^{a}	1.5^{a}	1.2^{a}	3.7^{a}	3.3^{a}	2.2^{a}	1.3^{a}	3.6^{a}	2.0^{a}	2.5^{a}	2.9^{a}	6.8^{b}
	Rollability	3.7^{b}	1.1^{ab}	0.8^{a}	3.4^{ab}	3.0^{ab}	1.4^{ab}	1.0^{a}	3.0^{ab}	1.6^{ab}	1.6^{ab}	2.1^{ab}	6.5^{c}
	Stickiness	3.0^{a}	2.3^{a}	4.2^{a}	2.1^{a}	1.7^{a}	2.3^{a}	4.7^{a}	4.2^{a}	1.8^{a}	2.8^{a}	3.1^{a}	2.6^{a}
	Sponginess	3.2^{ab}	2.1^{ab}	1.7^{a}	2.9^{ab}	3.1^{ab}	2.1^{ab}	2.0^{ab}	3.3^{ab}	2.2^{ab}	2.2^{ab}	4.8^{ab}	6.4^{b}
Texture (In-mouth)	Chewiness	6.4^{a}	6.0^{a}	5.0^{a}	6.9^{a}	6.0^{a}	5.5^{a}	4.9^{a}	5.8^{a}	5.9^{a}	4.9^{a}	6.2^{a}	6.6^{a}
	Softness	4.4^{ab}	4.1^{ab}	3.2^{a}	4.7^{ab}	4.4^{ab}	4.9^{ab}	4.4^{ab}	5.3^{ab}	4.1^{ab}	4.2^{ab}	6.3^{ab}	7.2^{b}
	Breakability	6.9^{a}	7.6^{a}	6.7^{a}	6.7^{a}	6.6^{a}	7.2^{a}	7.0^{a}	7.1^{a}	7.3^{a}	7.5^{a}	6.8^{a}	6.3^{a}
	Grittiness	4.4^{a}	5.0^{a}	4.7^{a}	3.4^{a}	3.9^{a}	3.9	4.1^{a}	4.9^{a}	4.1^{a}	4.7^{a}	3.3^{a}	3.6^{a}
	Dry mouthfeel	3.7^{a}	3.8^{a}	3.1^{a}	2.9^{a}	3.6^{a}	4.0^{a}	3.9^{a}	4.2^{a}	3.6^{a}	4.3^{a}	3.6^{a}	3.5^{a}
Flavour	Sourness	2.8^{ab}	2.8^{ab}	2.0^{a}	4.8^{b}	3.8^{ab}	4.2^{ab}	4.0^{ab}	4.7^{b}	3.0^{ab}	2.6^{ab}	1.0^{a}	3.7^{ab}
	Bitterness	2.0^{a}	3.0^{a}	3.0^{a}	2.5^{a}	3.1^{a}	2.6^{a}	3.7^{a}	3.5^{a}	2.4^{a}	2.4^{a}	1.7^{a}	3.2^{a}
	Starchy taste	2.9^{a}	2.5^{a}	2.5^{a}	2.3^{a}	1.9^{a}	3.0^{a}	2.5^{a}	2.3^{a}	2.3^{a}	2.7^{a}	2.9^{a}	2.6^{a}
	Sweetness	1.3^{a}	1.1^{a}	1.2^{a}	1.2^{a}	1.6^{a}	1.6^{a}	1.5^{a}	1.6^{a}	1.5^{a}	1.4^{a}	1.6^{a}	1.4^{a}
	Overall flavour	4.4^{a}	4.6^{a}	4.0^{a}	5.3^{a}	5.0^{a}	4.7^{a}	5.0^{a}	5.3^{a}	4.3^{a}	4.7^{a}	4.6^{a}	5.7^{a}

Aftertaste	Sourness	1.5^{ab}	1.8^{ab}	1.6^{ab}	3.5^{b}	2.8^{ab}	2.4^{ab}	2.0^{ab}	2.6^{ab}	1.5^{ab}	1.5^{ab}	1.0^{a}	2.3^{ab}
	Bitterness	1.6^{a}	2.1^{a}	2.1^{a}	1.7^{a}	2.2^{a}	2.2^{a}	2.8^{a}	2.6^{a}	1.9^{a}	2.0^{a}	1.9^{a}	3.0^{a}
	Dry mouthfeel	2.9^{a}	3.6^{a}	3.3^{a}	2.9^{a}	3.6^{a}	3.6^{a}	3.3^{a}	3.4^{a}	3.4^{a}	3.3^{a}	2.8^{a}	3.6^{a}
	Fine particles	4.2^{a}	4.7^{a}	4.9^{a}	3.8^{a}	4.4^{a}	4.3^{a}	3.8^{a}	4.4^{a}	4.7^{a}	5.0^{a}	3.4^{a}	3.4^{a}
	Astringent	1.6^{a}	2.1^{a}	2.1^{a}	1.8^{a}	2.3^{a}	2.1^{a}	2.7^{a}	2.4^{a}	2.0^{a}	2.3^{a}	1.8^{a}	3.3^{a}
	Lingering	2.0^{a}	2.0^{a}	2.5^{a}	2.4^{a}	2.8^{a}	2.5^{a}	3.3^{a}	3.0^{a}	2.5^{a}	3.0^{a}	3.0^{a}	3.9^{a}

Values are Mean ± standard deviation (n=2). Values in a row with different letters in superscript are significantly different ($p<0.05$). NWND (Non-waxy-normal protein digestibility); NWHD (Non-waxy -high protein digestibility), hWND (heterowaxy- normal protein digestibility), WHD (waxy-high protein digestibility), WND (waxy-normal protein digestibility), WNTS (white non-tannin sorghum), RNTS (red non-tannin sorghum), RTS (red tannin sorghum)

Table 4.3. 8 Descriptive sensory profile of stored injera (4 days at 5°C) prepared from novel sorghum lines with waxy and HD traits, normal sorghum types and teff

Sensory category/ attributes		Sorghum lines											
		NWND	**NWHD**	**hWND**	**WHD1**	**WND1**	**WND2**	**WHD2**	**WND3**	**WNTS**	**RNTS**	**RTS**	**Teff**
Aroma	Fermented	3.1^{a}	3.7^{a}	3.2^{a}	3.9^{a}	3.9^{a}	3.4^{a}	3.1^{a}	3.5^{a}	2.8^{a}	2.7^{a}	3.7^{a}	3.1^{a}
	Sorghum	3.0^{a}	3.3^{a}	3.3^{a}	2.9^{a}	3.4^{a}	3.1^{a}	3.9^{a}	3.9^{a}	3.8^{a}	3.5^{a}	4.0^{a}	3.6^{a}
	Musty	3.2^{a}	2.5^{a}	3.4^{a}	2.6^{a}	3.1^{a}	2.7^{a}	3.5^{a}	3.0^{a}	3.2^{a}	3.4^{a}	3.9^{a}	3.5^{a}
	Overall aroma	4.3^{a}	4.9^{a}	4.6^{a}	4.7^{a}	4.9^{a}	4.4^{a}	4.7^{a}	4.6^{a}	4.3^{a}	4.6^{a}	5.7^{a}	5.0^{a}
Appearance	Shininess top surface	2.8^{ab}	2.1^{ab}	1.1^{a}	3.6^{b}	3.1^{ab}	2.4^{ab}	1.4^{ab}	2.3^{ab}	2.1^{ab}	2.5^{ab}	1.6^{ab}	3.2^{ab}
	Shininess bottom surface	3.3^{a}	2.6^{a}	2.3^{a}	3.3^{a}	2.7^{a}	2.3^{a}	1.5^{a}	2.4^{a}	2.6^{a}	2.8^{a}	1.6^{a}	2.3^{a}
	Eye size	7.1^{ab}	6.9^{ab}	5.4^{ab}	7.0^{ab}	6.4^{ab}	6.6^{ab}	7.8^{b}	7.7^{b}	6.8^{ab}	6.0^{ab}	5.1^{a}	5.1^{a}
	Eye evenness & distribution	6.8^{b}	7.4^{b}	3.3^{a}	6.6^{b}	7.1^{b}	6.6^{b}	3.8^{a}	5.3^{ab}	5.8^{ab}	7.3^{b}	7.0^{b}	7.5^{b}
Texture (By hand)	Softness	4.5^{a}	3.2^{a}	2.8^{a}	3.4^{a}	3.7^{a}	3.5^{a}	3.4^{a}	3.8^{a}	3.9^{a}	3.3^{a}	5.9^{a}	5.6^{a}
	Flexibility	2.3^{ab}	2.4^{ab}	1.0^{a}	3.9^{b}	2.5^{ab}	1.7^{ab}	1.3^{a}	2.5^{ab}	1.6^{ab}	1.4^{ab}	2.3^{ab}	3.3^{ab}
	Rollability	2.2^{ab}	1.6^{ab}	0.8^{a}	3.9^{b}	2.8^{ab}	1.3^{ab}	1.3^{ab}	2.5^{ab}	1.3^{ab}	1.1^{a}	2.0^{ab}	3.0^{ab}
	Stickiness	3.2^{ab}	1.9^{ab}	3.3^{ab}	1.9^{ab}	1.1^{a}	2.4^{ab}	4.2^{b}	2.3^{ab}	2.2^{ab}	2.1^{ab}	2.2^{ab}	1.5^{a}
	Sponginess	3.3^{ab}	2.2^{ab}	1.9^{a}	3.0^{ab}	3.7^{ab}	2.5^{ab}	2.2^{ab}	2.4^{ab}	3.3^{ab}	2.2^{ab}	4.6^{b}	4.4^{ab}
Texture (In-mouth)	Chewiness	6.1^{a}	5.8^{a}	5.6^{a}	6.3^{a}	6.1^{a}	5.5^{a}	5.0^{a}	6.2^{a}	5.9^{a}	6.0^{a}	6.1^{a}	7.0^{a}
	Softness	5.4^{a}	3.8^{a}	3.8^{a}	4.2^{a}	4.7^{a}	4.6^{a}	4.2^{a}	5.3^{a}	4.9^{a}	4.2^{a}	6.2^{a}	6.5^{a}
	Breakability	6.8^{a}	7.2^{a}	7.1^{a}	7.0^{a}	6.3^{a}	7.2^{a}	6.5^{a}	6.6^{a}	6.1^{a}	6.6^{a}	6.8^{a}	6.8^{a}
	Grittiness	3.6^{a}	3.6^{a}	4.6^{a}	4.0^{a}	3.4^{a}	3.8^{a}	3.7^{a}	3.8^{a}	4.0^{a}	4.0^{a}	2.9^{a}	3.6^{a}
	Dry mouthfeel	3.3^{a}	3.6^{a}	3.4^{a}	3.8^{a}	3.7^{a}	4.0^{a}	3.2^{a}	3.1^{a}	3.1^{a}	3.4^{a}	3.0^{a}	3.7^{a}
Flavour	Sourness	4.1^{bc}	3.1^{abc}	1.6^{ab}	4.2^{bc}	4.3^{bc}	4.7^{c}	4.2^{bc}	4.2^{bc}	2.3^{abc}	2.9^{abc}	0.5^{a}	3.2^{abc}
	Bitterness	1.8^{a}	1.9^{a}	2.4^{a}	3.0^{a}	2.7^{a}	2.1^{a}	2.6^{a}	3.4^{a}	2.5^{a}	2.5^{a}	1.6^{a}	2.6^{a}
	Starchy taste	2.3^{a}	2.5^{a}	2.9^{a}	2.2^{a}	2.4^{a}	2.2^{a}	2.0^{a}	2.2^{a}	2.2^{a}	2.0^{a}	2.3^{a}	1.8^{a}
	Sweetness	1.3^{a}	1.1^{a}	1.3^{a}	1.0^{a}	1.2^{a}	1.7^{a}	1.4^{a}	1.5^{a}	1.4^{a}	1.2^{a}	1.0^{a}	1.2^{a}
	Overall flavour	4.3^{a}	4.2^{a}	4.1^{a}	4.9^{a}	4.8^{a}	4.7^{a}	5.0^{a}	5.2^{a}	4.6^{a}	4.4^{a}	4.0^{a}	5.2^{a}

Aftertaste	Sourness	2.0^{ab}	1.3^{ab}	0.8^{a}	2.4^{ab}	2.6^{ab}	2.8^{b}	2.2^{ab}	2.5^{ab}	1.2^{ab}	1.5^{ab}	0.7^{a}	2.0^{ab}
	Bitterness	1.5^{a}	1.6^{a}	1.6^{a}	2.3^{a}	1.9^{a}	1.6^{a}	2.0^{a}	2.0^{a}	2.3^{a}	2.0^{a}	1.5^{a}	2.8^{a}
	Dry mouthfeel	3.0^{a}	3.1^{a}	2.9^{a}	3.4^{a}	3.0^{a}	3.3^{a}	2.9^{a}	2.9^{a}	2.8^{a}	3.4^{a}	2.7^{a}	3.0^{a}
	Fine particles	4.0^{a}	4.3^{a}	4.9^{a}	3.4^{a}	3.7^{a}	3.8^{a}	4.2^{a}	3.3^{a}	4.5^{a}	4.1^{a}	3.7^{a}	3.3^{a}
	Astringent	2.0^{a}	1.8^{a}	1.5^{a}	2.0^{a}	1.9^{a}	2.4^{a}	2.5^{a}	2.2^{a}	2.2^{a}	1.7^{a}	1.7^{a}	2.6^{a}
	Lingering	1.9^{a}	2.5^{a}	2.2^{a}	2.5^{a}	2.8^{a}	2.6^{a}	2.3^{a}	2.6^{a}	2.4^{a}	2.2^{a}	2.4^{a}	3.4^{a}

Values are Mean ± standard deviation (n=2).Values in a row with different letters in superscript are significantly different ($p<0.05$). NWND (Non-waxy-normal protein digestibility); NWHD (Non-waxy -high protein digestibility), hWND (heterowaxy- normal protein digestibility), WHD (waxy-high protein digestibility), WND (waxy-normal protein digestibility), WNTS (white non-tannin sorghum), RNTS (red non-tannin sorghum), RTS (red tannin sorghum)

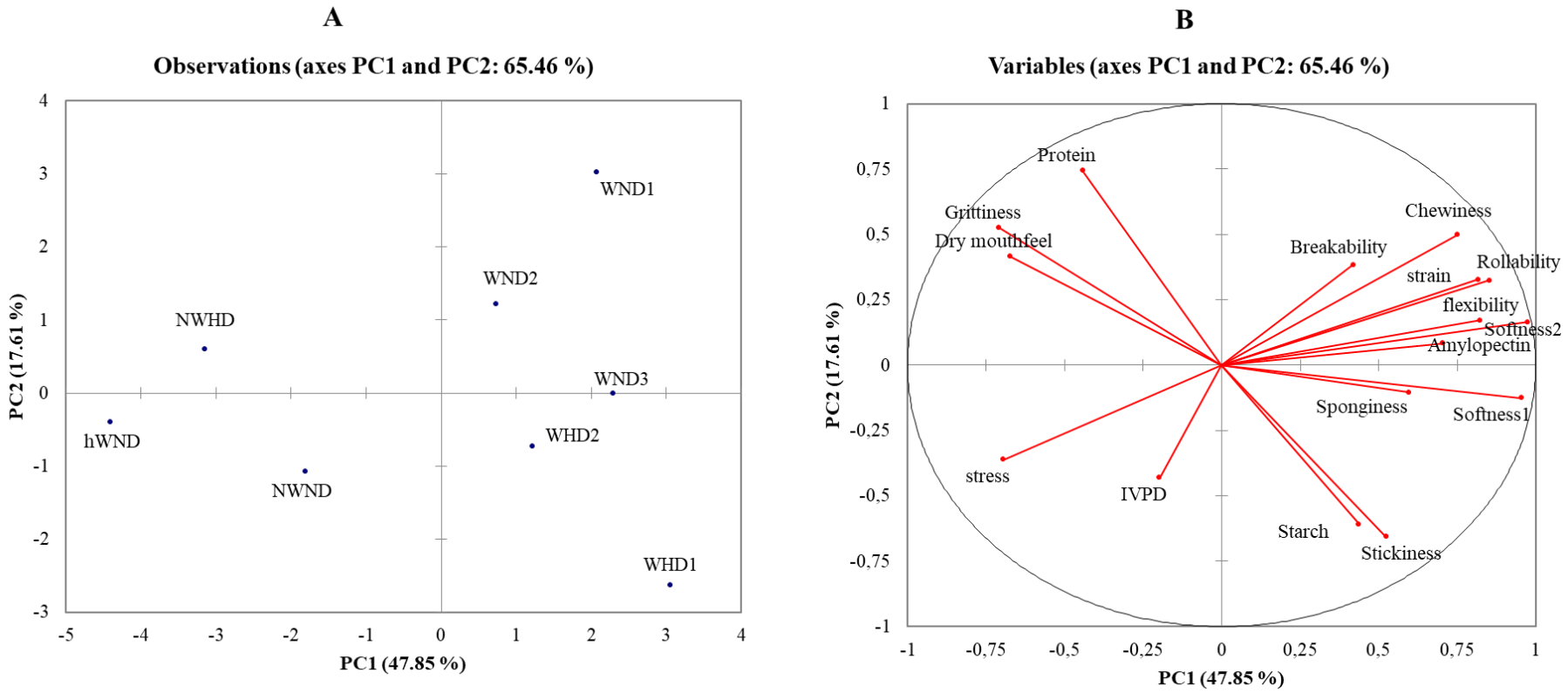

Figure 4.3. 2 Principal component analysis of sorghum lines with different starch and protein digestibility traits and their injera quality attributes: freshly prepared injera

A: Sorghum lines: NWLD (Non-waxy- normal protein digestibility), NWHD (Non-waxy- high protein digestibility), hWND (heterowaxy-normal protein digestibility), WND1, WND2, WND3 (Waxy- normal protein digestibility), WHD1 and WHD2 (Waxy-high protein digestibility),

B: PCA Loadings: starch content, protein content, starch amylopectin content and cooked in-vitro protein digestibility (IVPD), Softness-1 (as measured by touching), Softness-2 (as measured in mouth sensation), flexibility, rollability, stickiness, sponginess, chewiness, breakability, grittiness, dry mouthfeel, stress (kPa), Strain (%).

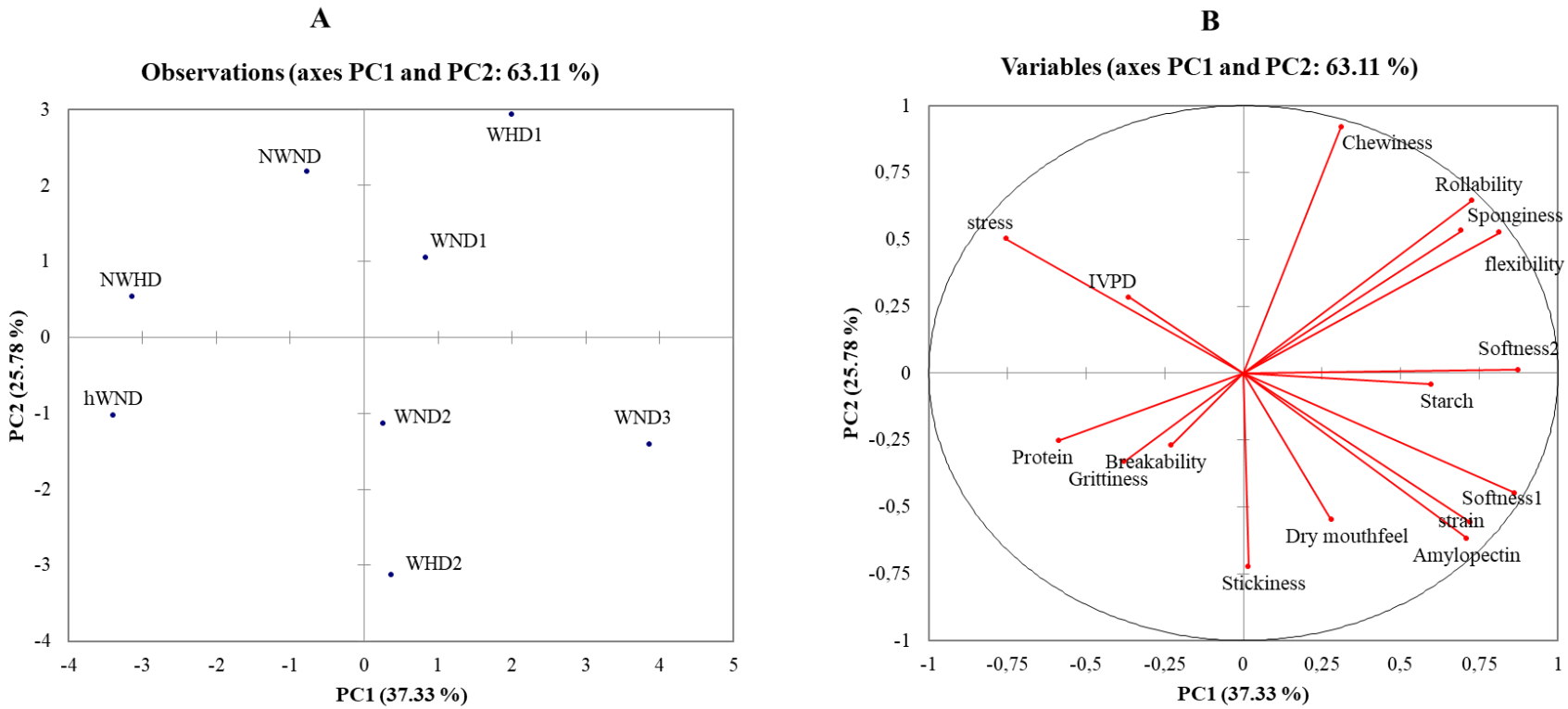

Figure 4.3. 3 Principal component analysis of sorghum lines with different starch and protein digestibility traits and their injera quality attributes: stored injera (2 days at 5°C).

A: Sorghum lines: NWLD (Non-waxy- normal protein digestibility), NWHD (Non-waxy- high protein digestibility), hWND (heterowaxy- normal protein digestibility), WND1, WND2, WND3 (Waxy- normal protein digestibility), WHD1 and WHD2 (Waxy- high protein digestibility),

B: PCA Loadings: starch content, protein content, starch amylopectin content and cooked in-vitro protein digestibility (IVPD), Softness-1 (as measured by touching), Softness-2 (as measured in mouth sensation), flexibility, rollability, stickiness, sponginess, chewiness, breakability, grittiness, dry mouthfeel, stress (kPa), Strain (%).

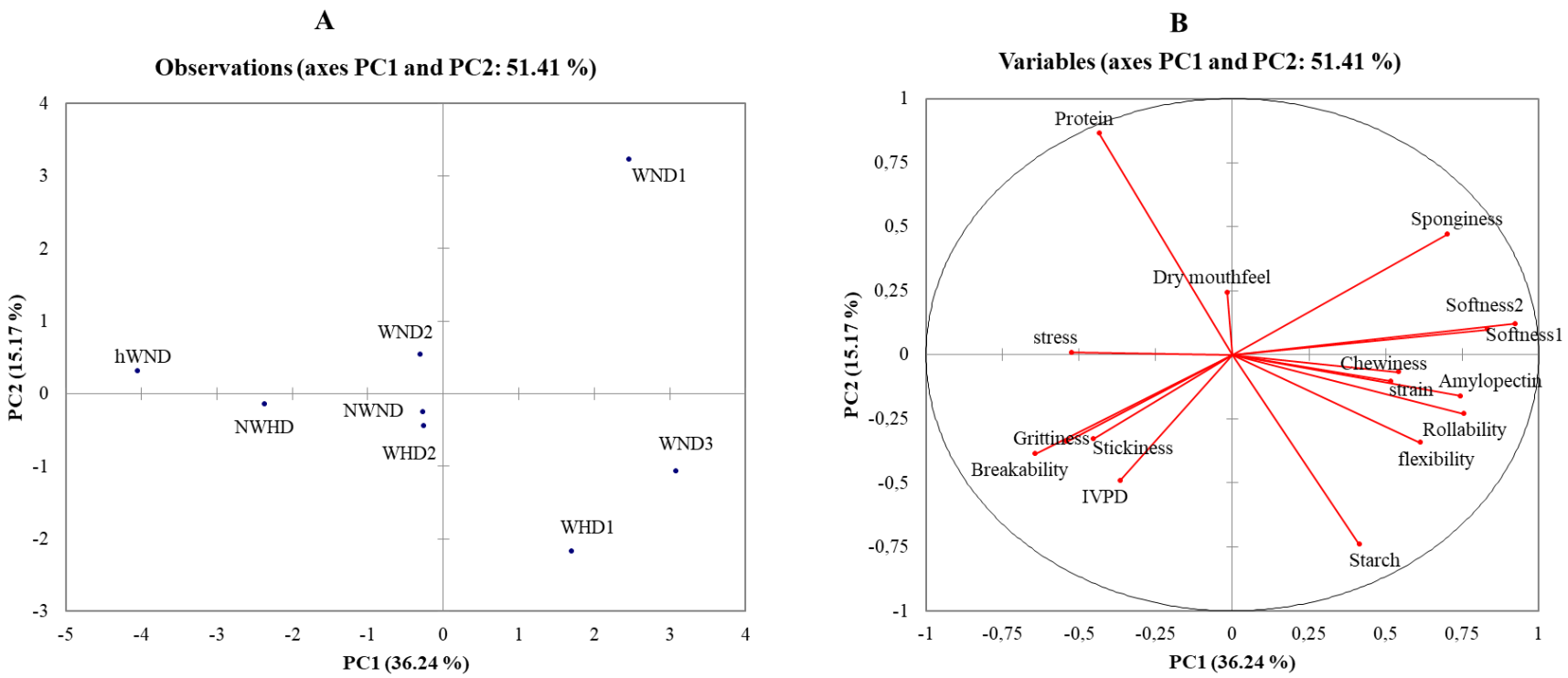

Figure 4.3. 4 Principal component analysis of sorghum lines with different starch and protein digestibility traits and their injera quality attributes: stored injera (4 days at 5°C).

A: Sorghum lines: NWLD (Non-waxy- normal protein digestibility), NWHD (Non-waxy- high protein digestibility), hWND (heterowaxy- normal protein digestibility), WND1, WND2, WND3 (Waxy- normal protein digestibility), WHD1 and WHD2 (Waxy- high protein digestibility),

B: PCA Loadings: starch content, protein content, starch amylopectin content and cooked in-vitro protein digestibility (IVPD), Softness-1 (as measured by touching), Softness-2 (as measured in mouth sensation), flexibility, rollability, stickiness, sponginess, chewiness, breakability, grittiness, dry mouthfeel, stress (kPa), Strain (%).

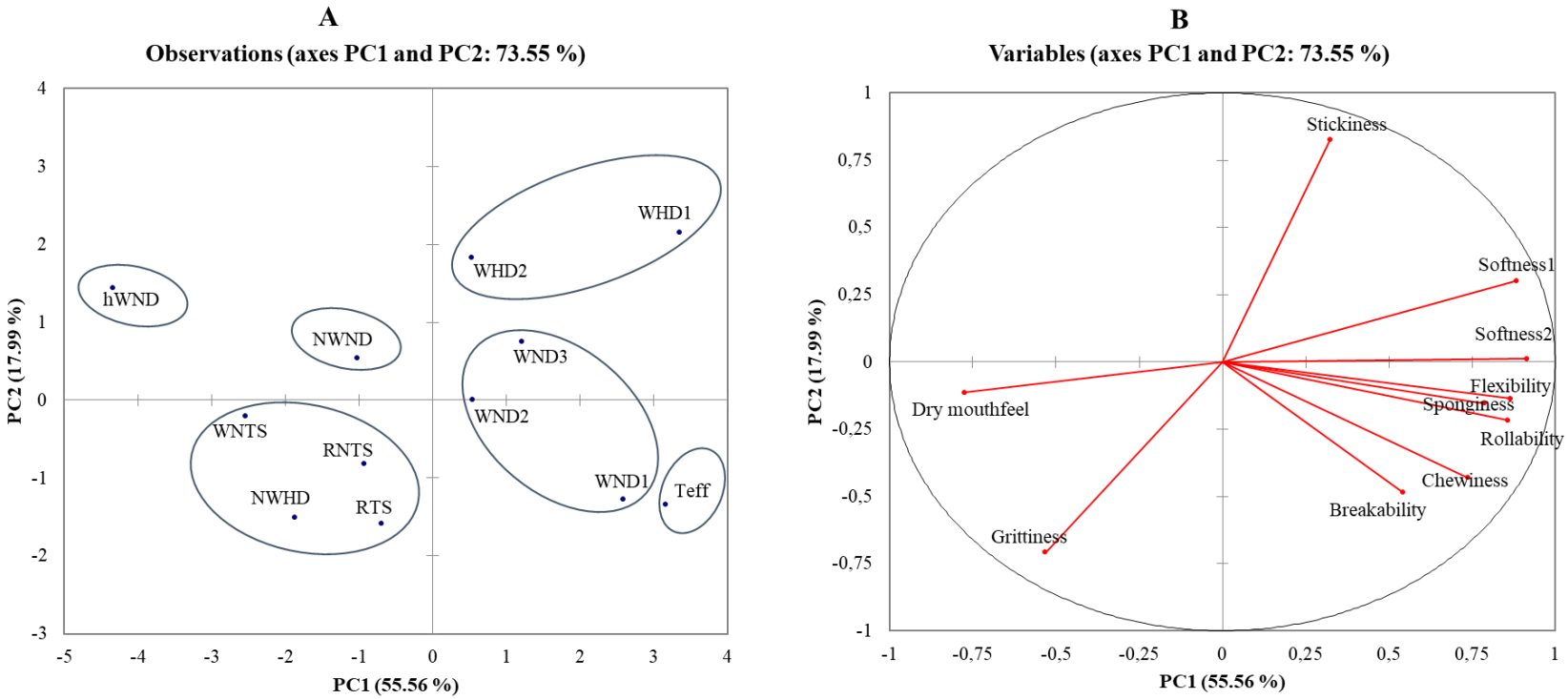

Figure 4.3. 5 Principal component analysis of novel sorghum lines, normal sorghum types and teff and their injera quality attributes: freshly prepared injera

A: Sorghum lines: NWLD (Non-waxy- normal protein digestibility), NWHD (Non-waxy- high protein digestibility), hWND (heterowaxy- normal protein digestibility), WND1, WND2, WND3 (Waxy- normal protein digestibility), WHD1 and WHD2 (Waxy- high protein digestibility), WNTS (white non-tannin sorghum), RNTS (red non-tannin sorghum), RTS (red tannin sorghum)

B: PCA Loadings: Softness-1 (as measured by touching), Softness-2 (as measured in mouth sensation), flexibility, rollability, stickiness, sponginess, chewiness, breakability, grittiness, dry mouthfeel.

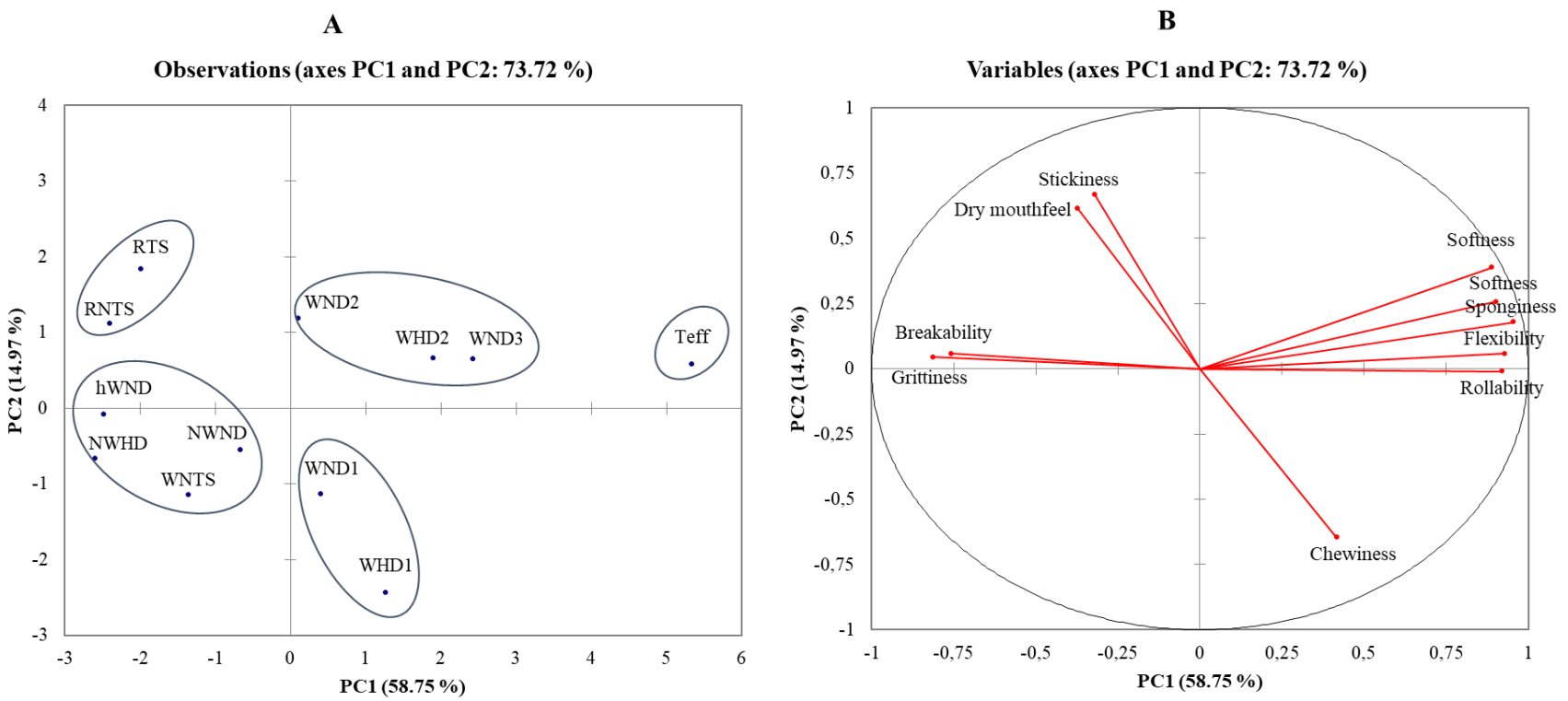

Figure 4.3. 6 Principal component analysis of novel sorghum lines, normal sorghum types and teff and their injera quality attributes: stored injera (2 days at 5°C)

A: Sorghum lines: NWLD (Non-waxy- normal protein digestibility), NWHD (Non-waxy- high protein digestibility), hWND (heterowaxy- normal protein digestibility), WND1, WND2, WND3 (Waxy- normal protein digestibility), WHD1 and WHD2 (Waxy- high protein digestibility), WNTS (white non-tannin sorghum), RNTS (red non-tannin sorghum), RTS (red tannin sorghum)

B: PCA Loadings: Softness-1 (as measured by touching), Softness-2 (as measured in mouth sensation), flexibility, rollability, stickiness, sponginess, chewiness, breakability, grittiness, dry mouthfeel.

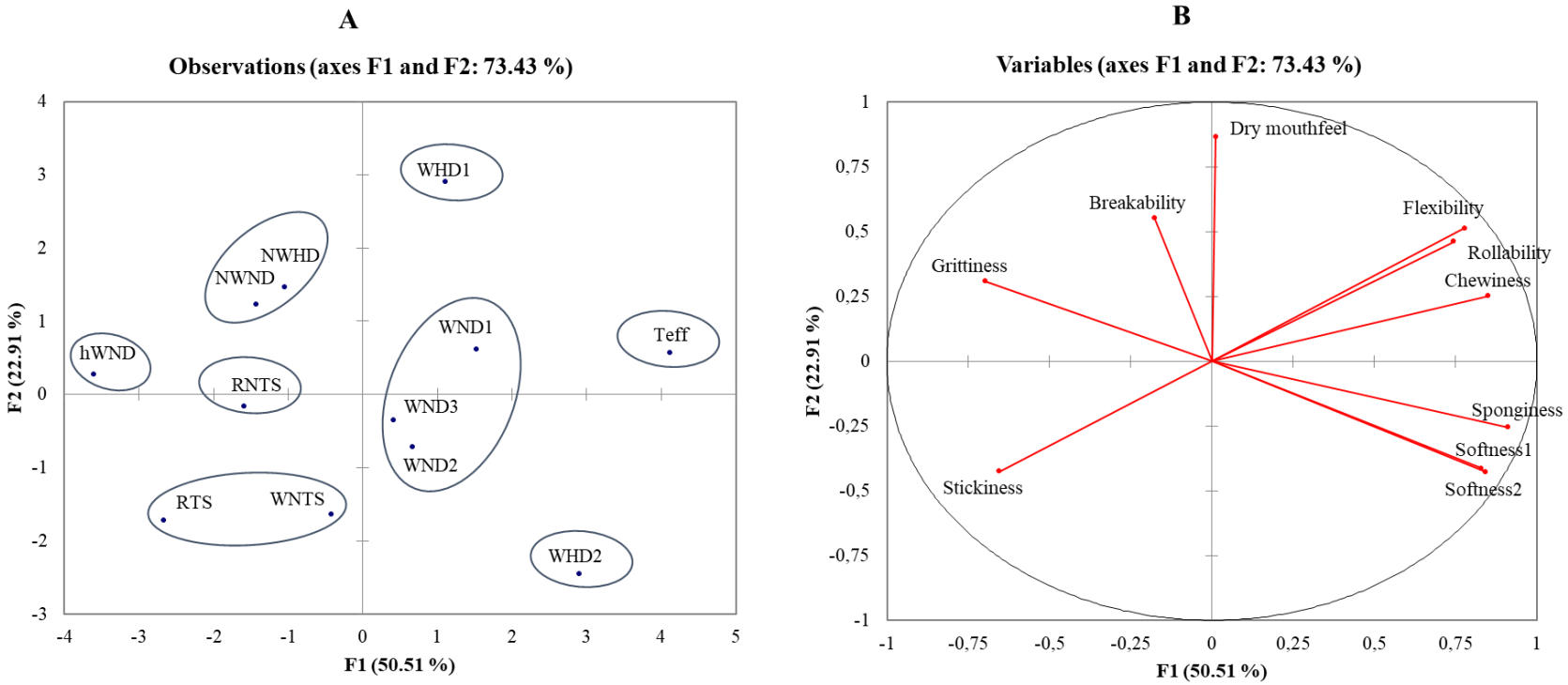

Figure 4.3. 7 Principal component analysis of novel sorghum lines, normal sorghum types and teff and their injera quality attributes: stored injera (4 days at 5°C)

A: Sorghum lines: NWLD (Non-waxy- normal protein digestibility), NWHD (Non-waxy- high protein digestibility), hWND (heterowaxy- normal protein digestibility), WND1, WND2, WND3 (Waxy- normal protein digestibility), WHD1 and WHD2 (Waxy- high protein digestibility), WNTS (white non-tannin sorghum), RNTS (red non-tannin sorghum), RTS (red tannin sorghum)

B: PCA Loadings: Softness-1 (as measured by touching), Softness-2 (as measured in mouth sensation), flexibility, rollability, stickiness, sponginess, chewiness, breakability, grittiness, dry mouthfeel.

4.3.4.4 Instrumental texture analysis of full-scale injera

Instrumental texture of the full-scale injera was determined simultaneously with DSP. Stress (strength) of the sorghum injera varied considerably, ranging from 124–159 kPa (fresh injera), 157–226 kPa (2 days stored) and 201–386 kPa (4 days stored) (Table 4.3.9). Fresh injera of WND1, WND2, WHD2 and WND3 had similar ($p \geq 0.05$) stress to teff and RTS injera. The injera of these lines also had lower ($p < 0.05$) stress compared to injera from the non-waxy lines (NWND and NWHD) and normal sorghum types (WNTS and RNTS). Stored injera of WND1, WND2, WHD2 and WND3 had higher ($p < 0.05$) stress than teff injera. The injera of waxy lines had lower ($p < 0.05$) stress compared to NWND, NWHD, WNTS, RNTS and RTS injera. However, injera from hWND and WHD1 had similar ($p \geq 0.05$) stress to injera from the non-waxy sorghums due to the fact that these lines were relatively lower in starch amylopectin compared to the other waxy lines. The lower stress of fresh and stored injera of the waxy lines compared to normal sorghums is indicative of the lines producing soft injera. As stated, this is probably a consequence of the slow retrogradation of starch amylopectin (Gudmundsson, 1994).

Strain (extensibility) of the sorghum injera varied considerably, ranging from 28–40.7% (fresh injera), 10.1–17.7% (2 days stored) and 4.3–7.8% (4 days stored) (Table 4.3.9). Fresh injera of WHD1, WND1, WND2, WHD2 and WND3 had similar ($p \geq 0.05$) strain to teff injera. These injera also had higher ($p < 0.05$) strain compared to injera from hWND, non-waxy (NWND, NWHD) and normal sorghums types (WNTS, RNTS and RTS). Stored injera (2 and 4 days) of the way lines (WHD1, WND1, WND2, WHD2 and WND3) had lower ($p < 0.05$) strain compared to teff injera. Furthermore, these injera had higher ($p < 0.05$) strain compared to injera from hWND and normal sorghums (NWND, NWHD, hWND, WNTS, RNTS and RTS). The higher strain of fresh and stored injera of waxy lines compared to normal sorghums is indicative of them producing extensible injera. As stated, this is probably a consequence of the slow retrogradation of starch amylopectin (Gudmundsson, 1994). The findings are agreement with a study by Peng et al. (2009) which found waxy wheat flour blended with normal wheat to retard staling and result in softer bread.

The correlation matrix of textural properties measured by instrument and DSP showed that stress of the fresh (Table 4.3.11) and stored injera (Table 4.3.12 and Table 4.3.13) was correlated with softness measured by the DSP. Furthermore, the strain was correlated with softness, flexibility and rollability. This indicates that the stress and strain measured by

instrumental texture analysis can be used for evaluating softness, flexibility and rollability of injera in a laboratory where there is no trained sensory panel or to minimize the cost and time of analysis.

4.3.4.5 Instrumental texture analysis of small-scale injera

The texture of sorghum injera prepared using the small-scale (microwave method) (section 4.3.3.4) was determined to calibrate it against the full-scale method (Mitad/Griddle baking) (Table 4.3.10). The stress and strain of the small-scale sorghum injera (fresh and stored) showed similar trends to the full-scale injera. A correlation plot of the stress and strain data of the injera prepared using the full-scale and small-scale methods was performed (Figure 4.3.8). The stress and strain data of fresh and stored (2 and 4 days) of the small-scale injera correlated with the data of full-scale injera. The correlations of stress were significant at $p<0.01$ for fresh ($r = 0.725$) and 2 days stored injera ($r = 0.741$) (Figure 4.3.8a and b, respectively), and at $p<0.001$ for 4 days stored injera ($r = 0.852$) (Figure 4.3.8c). Furthermore, the correlations of strain were significant at $p<0.01$ for fresh ($r = 1.000$), 2 days stored ($r = 1.000$) and 4 days stored ($r = 0.999$) injera (Figure 4.3.8d, e and f, respectively). This indicates that the small-scale microwave method has considerable potential to be used for screening sorghum cultivars for making injera, where a large number of cultivars with small sample size have to be evaluated.

Table 4.3. 9 Effect of waxy and HD traits on instrumental textural properties of fresh and stored sorghum injera prepared using large scale method

Sorghum lines	Stress (kPa)			Strain (%)		
	Storage days (5°C)			Storage days (5°C)		
	0	2	4	0	2	4
NWND	$159^{f} \pm 14$	$217^{fg} \pm 12$	$351^{h} \pm 20$	$34.2^{bc} \pm 2.2$	$10.1^{a} \pm 0.6$	$5.6^{bc} \pm 0.6$
NWHD	$153^{def} \pm 10$	$226^{g} \pm 16$	$386^{i} \pm 20$	$33.5^{bc} \pm 2.1$	$11.6^{ab} \pm 1.1$	$4.7^{ab} \pm 0.5$
hWND	$150^{def} \pm 5$	$188^{cde} \pm 8$	$296^{efg} \pm 18$	$35.8^{cd} \pm 2.2$	$11.0^{a} \pm 1.0$	$5.3^{ab} \pm 0.5$
WHD1	$152^{def} \pm 11$	$202^{ef} \pm 16$	$303^{fg} \pm 10$	$39.9^{de} \pm 2.6$	$14.9^{cd} \pm 1.2$	$7.2^{d} \pm 0.8$
WND1	$137^{bc} \pm 9$	$178^{bcd} \pm 10$	$247^{cd} \pm 19$	$42.3^{e} \pm 1.2$	$14.4^{c} \pm 1.0$	$6.8^{d} \pm 0.5$
WND2	$130^{bc} \pm 10$	$157^{b} \pm 13$	$266^{de} \pm 10$	$40.7^{e} \pm 1.7$	$16.8^{de} \pm 1.2$	$6.9^{d} \pm 0.7$
WHD2	$126^{ab} \pm 7$	$161^{b} \pm 6$	$215^{bc} \pm 15$	$39.3^{de} \pm 2.4$	$17.7^{e} \pm 0.7$	$7.8^{d} \pm 0.5$
WND3	$124^{ab} \pm 8$	$166^{bc} \pm 12$	$201^{ab} \pm 9$	$39.9^{de} \pm 3.5$	$17.1^{e} \pm 1.4$	$7.6^{d} \pm 0.8$
WNTS	$157^{ef} \pm 10$	$204^{efg} \pm 11$	$294^{ef} \pm 20$	$32.0^{abc} \pm 2.7$	$13.3^{bc} \pm 1.0$	$4.3^{a} \pm 0.5$
RNTS	$146^{cdef} \pm 6$	$207^{efg} \pm 8$	$330^{gh} \pm 26$	$30.6^{ab} \pm 2.0$	$11.4^{ab} \pm 1.0$	$5.5^{b} \pm 0.8$
RTS	$140^{bcde} \pm 8$	$197^{def} \pm 18$	$278^{def} \pm 23$	$28.4^{a} \pm 1.9$	$14.3^{c} \pm 1.2$	$5.3^{ab} \pm 0.6$
Teff	$111^{a} \pm 6$	$120^{a} \pm 8$	$169^{a} \pm 12$	$42.5^{e} \pm 2.7$	$21.5^{f} \pm 1.5$	$10.6^{e} \pm 0.5$

Values are Mean ± standard deviation (n=2).Values in a column with different letters in superscript are significantly different ($p<0.05$). NWND (Non-waxy-normal protein digestibility); NWHD (Non-waxy -high protein digestibility), hWND (heterowaxy- normal protein digestibility), WHD (waxy-high protein digestibility), WND (waxy-normal protein digestibility), WNTS (white non-tannin sorghum), RNTS (red non-tannin sorghum), RTS (red tannin sorghum).

Table 4.3. 10 Effect of waxy and HD traits on instrumental textural properties of fresh and stored sorghum injera prepared using the small scale microwave method

Sorghum line	Stress (kPa)			Strain (%)		
	Storage (days)			Storage (days)		
	0	2	4	0	2	4
NWND	$398^{d} \pm 29$	$555^{f} \pm 34$	$869^{gh} \pm 56$	$23.7^{cd} \pm 1.8$	$5.2^{a} \pm 0.4$	$2.1^{c} \pm 0.3$
NWHD	$407^{d} \pm 26$	$561^{f} \pm 30$	$829^{fgh} \pm 53$	$23.1^{bcd} \pm 1.8$	$6.4^{b} \pm 0.8$	$1.5^{ab} \pm 0.3$
hWND	$389^{d} \pm 25$	$499^{e} \pm 19$	$812^{fg} \pm 26$	$25.1^{d} \pm 1.8$	$5.9^{ab} \pm 0.7$	$1.9^{bc} \pm 0.3$
WHD1	$375^{cd} \pm 30$	$445^{cd} \pm 27$	$719^{e} \pm 54$	$28.8^{ef} \pm 2.2$	$8.9^{d} \pm 0.9$	$3.2^{def} \pm 0.5$
WND1	$313^{b} \pm 24$	$426^{bcd} \pm 27$	$672^{de} \pm 54$	$30.9^{f} \pm 1.0$	$8.5^{cd} \pm 0.8$	$2.9^{d} \pm 0.3$
WND2	$306^{ab} \pm 11$	$428^{bcd} \pm 24$	$591^{bc} \pm 49$	$29.5^{ef} \pm 1.5$	$10.5^{e} \pm 0.9$	$3.0^{de} \pm 0.4$
WHD2	$295^{ab} \pm 14$	$393^{ab} \pm 28$	$515^{a} \pm 36$	$28.3^{e} \pm 2.1$	$11.3^{e} \pm 0.5$	$3.5^{f} \pm 0.4$
WND3	$276^{a} \pm 16$	$392^{ab} \pm 30$	$566^{ab} \pm 31$	$28.8^{ef} \pm 3.0$	$10.8^{e} \pm 1.1$	$3.4^{ef} \pm 0.5$
WNTS	$402^{d} \pm 27$	$458^{d} \pm 29$	$803^{f} \pm 56$	$21.8^{bc} \pm 2.3$	$7.7^{c} \pm 0.8$	$1.3^{a} \pm 0.3$
RNTS	$406^{d} \pm 23$	$433^{cd} \pm 21$	$885^{h} \pm 73$	$20.5^{ab} \pm 1.6$	$6.2^{ab} \pm 0.7$	$2.0^{bc} \pm 0.5$
RTS	$394^{d} \pm 26$	$413^{abc} \pm 23$	$644^{cd} \pm 39$	$18.7^{a} \pm 1.6$	$8.5^{cd} \pm 0.9$	$1.9^{bc} \pm 0.4$
Teff	$356^{c} \pm 28$	$389^{a} \pm 30$	$589^{bc} \pm 36$	$31.1^{f} \pm 2.3$	$14.4^{f} \pm 1.3$	$5.6^{g} \pm 0.4$

Values are Mean ± standard deviation (n=2).Values in a column with different letters in superscript are significantly different ($p<0.05$). NWND (Non-waxy-normal protein digestibility); NWHD (Non-waxy -high protein digestibility), hWND (heterowaxy- normal protein digestibility), WHD (waxy-high protein digestibility), WND (waxy-normal protein digestibility), WNTS (white non-tannin sorghum), RNTS (red non-tannin sorghum), RTS (red tannin sorghum)

Table 4.3. 11 Correlation matrix of textural properties of the freshly prepared sorghum injera measured using sensory panel and instrumental texture analysis

	Softness-1	Flexibility	Rollability	Stickiness	Sponginess	Chewiness	Softness-2	Breakability	Grittiness	Dry mouthfeel	Stress
Flexibility	0.600*										
Rollability	0.584*	0.961**									
Stickiness	0.555 ns	0.106 ns	0.103 ns								
Sponginess	0.650*	0.579*	0.535 ns	0.001 ns							
Chewiness	0.479 ns	0.805**	0.893**	-0.070 ns	0.437 ns						
Softness-2	0.940**	0.675*	0.670*	0.347 ns	0.688*	0.640*					
Breakability	0.454 ns	0.305 ns	0.396 ns	-0.034 ns	0.562 ns	0.445 ns	0.599*				
Grittiness	-0.605*	-0.479 ns	-0.412 ns	-0.629*	-0.335 ns	-0.158 ns	-0.386 ns	0.192 ns			
Dry mouthfeel	-0.691*	-0.608*	-0.509 ns	-0.282 ns	-0.800**	-0.317 ns	-0.680*	-0.336 ns	0.365 ns		
Stress	-0.729**	-0.373 ns	-0.445 ns	-0.505 ns	-0.071 ns	-0.400 ns	-0.690*	-0.270 ns	0.411 ns	0.121 ns	
Strain	0.725**	0.618*	0.633*	0.456 ns	0.404 ns	0.460 ns	0.714**	0.141 ns	-0.590*	-0.417 ns	-0.750**

Two tailed correlation significant at * $p<0.05$, ** $p<0.01$, *** $p<0.001$ and ns – not significant at $p\geq0.05$, Softness -1 (measured by hand tactile feel), Softness-2 (measured by mouth sensation)

Table 4.3. 12 Correlation matrix of textural properties of the stored (2 days) sorghum injera measured using sensory panel and instrumental texture analysis

	Softness-1	Flexibility	Rollability	Stickiness	Sponginess	Chewiness	Softness-2	Breakability	Grittiness	Dry mouthfeel	Stress
Flexibility	0.791**										
Rollability	0.741**	0.987**									
Stickiness	-0.010 ns	-0.374 ns	-0.376 ns								
Sponginess	0.935**	0.886**	0.867**	-0.230 ns							
Chewiness	0.154 ns	0.302 ns	0.344 ns	-0.482 ns	0.362 ns						
Softness2	0.946**	0.813**	0.770**	-0.164	0.957**	0.383 ns					
Breakability	-0.613*	-0.647*	-0.690*	0.070 ns	-0.648*	-0.091 ns	-0.494 ns				
Grittiness	-0.723**	-0.639*	-0.619*	0.208 ns	-0.699*	-0.311 ns	-0.706*	0.663*			
Dry mouthfeel	-0.132 ns	-0.204 ns	-0.263 ns	0.183 ns	-0.172 ns	-0.356 ns	-0.053 ns	0.661*	0.426 ns		
Stress	-0.718**	-0.571 ns	-0.483 ns	-0.007 ns	-0.552 ns	0.188 ns	-0.616*	0.478 ns	0.744**	0.020 ns	
Strain	0.948**	0.827**	0.773**	-0.123 ns	0.870**	0.205 ns	0.900**	-0.570 ns	-0.787**	-0.168 ns	-0.779**

Two tailed correlation significant at * $p<0.05$, ** $p<0.01$, *** $p<0.001$ and ns – not significant at $p\geq0.05$, Softness -1 (measured by hand tactile feel), Softness-2 (measured by mouth sensation)

Table 4.3. 13 Correlation matrix of textural properties of the stored (4 days) sorghum injera measured using sensory panel and instrumental texture analysis

	Softness-1	Flexibility	Rollability	Stickiness	Sponginess	Chewiness	Softness-2	Breakability	Grittiness	Dry mouthfeel	Stress
Flexibility	0.402 ns										
Rollability	0.348 ns	0.961**									
Stickiness	-0.262 ns	-0.612*	-0.548 ns								
Sponginess	0.911**	0.526 ns	0.514 ns	-0.484 ns							
Chewiness	0.604*	0.748**	0.692*	-0.726**	0.660*						
Softness-2	0.950**	0.386 ns	0.367 ns	-0.282 ns	0.854**	0.669*					
Breakability	-0.146 ns	0.112 ns	-0.043 ns	0.107 ns	-0.274 ns	-0.047 ns	-0.236 ns				
Grittiness	-0.732**	-0.395 ns	-0.354 ns	0.328 ns	-0.721**	-0.283 ns	-0.604*	0.144 ns			
Dry mouthfeel	-0.289 ns	0.340 ns	0.299 ns	-0.357 ns	-0.108 ns	0.148 ns	-0.289 ns	0.531 ns	0.175 ns		
Stress	-0.733**	-0.445 ns	-0.518 ns	-0.015 ns	-0.640*	-0.392 ns	-0.742**	0.253 ns	0.633*	0.443 ns	
Strain	0.735**	0.694*	0.697*	-0.401 ns	0.694*	0.820**	0.793**	0.023 ns	-0.423 ns	0.110 ns	-0.694*

Two tailed correlation significant at * $p<0.05$, ** $p<0.01$, *** $p<0.001$ and ns – not significant at $p\geq0.05$, Softness -1 (measured by hand tactile feel), Softness-2 (measured by mouth sensation)

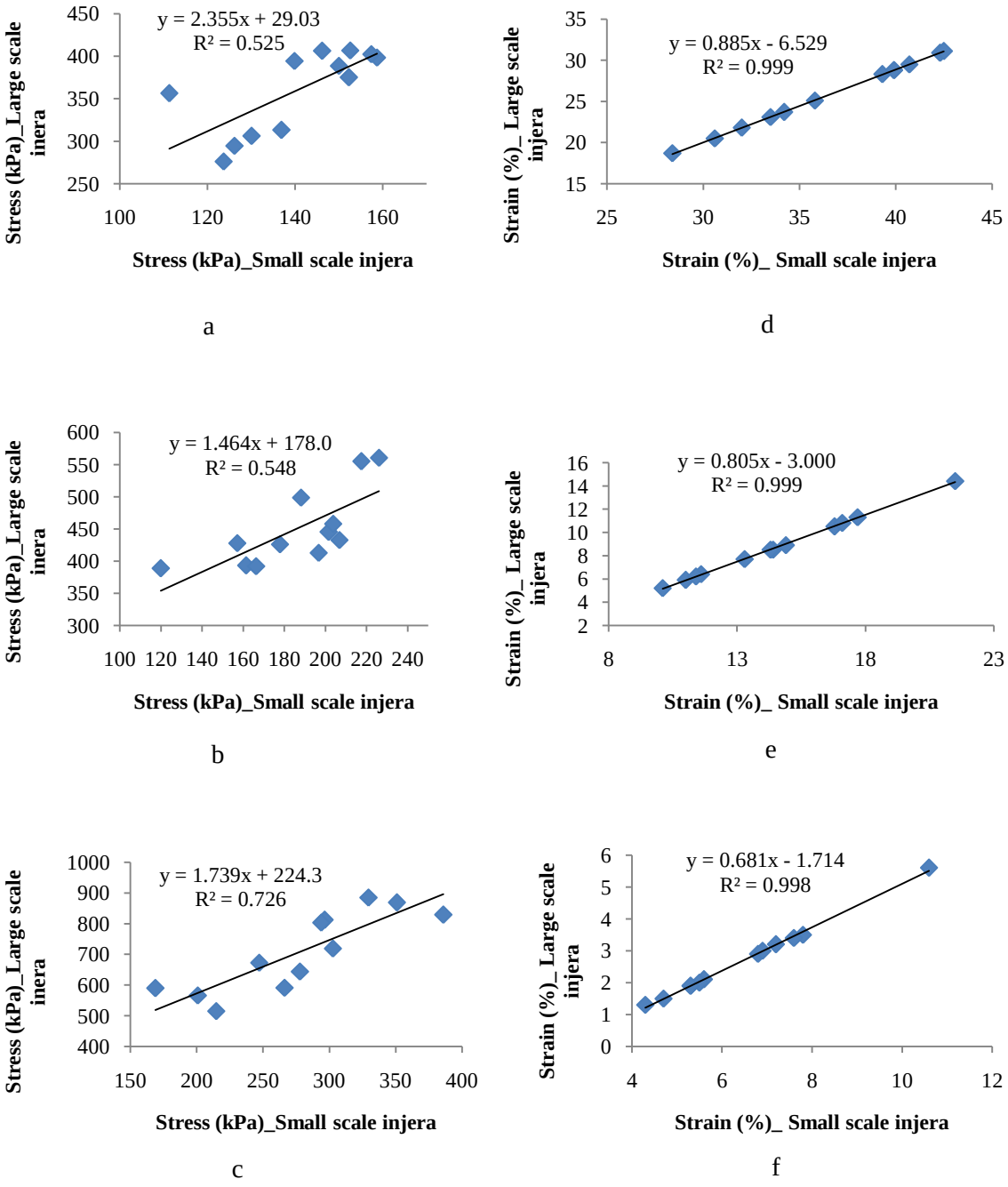

Figure 4.3. 8 Correlation of the stress (a, b and c) and strain (d, e and f) of sorghum injera prepared using full-scale and small-scale (microwave) methods: a) fresh injera (two tailed significance p = 0.01, r = 0.725, df = 10); b) stored injera (2 days) (two tailed significance p = 0.01, r = 0.741, df = 10), c) stored injera (4 days) (two tailed significance p = 0.001; r = 0.852, df = 10); (d) fresh injera (two tailed significance p = 0.01; r = 1.000, df = 10); (e) stored injera (2 days) (two tailed significance p = 0.01; r = 1.000, df = 10); f) stored injera (4 days) (two tailed significance p = 0.01; r = 0.999, df = 10).

4.3.4.6 Descriptive sensory analysis of biscuits

The DSP generated twenty-three sorghum biscuit quality descriptors and their definitions, reference standards and anchors (Table 4.3.14). There was no clear trend as to whether the waxy and HD traits affected aroma, appearance, flavour and aftertaste attributes of sorghum biscuits (Table 4.3.15). Only the texture attributes (hardness, coarseness, crunchiness and dryness) seemed to vary considerably and were significantly affected ($p<0.001$) by the traits.

Biscuits of WHD1 and WND3 were harder ($p<0.05$) compared to wheat and all other sorghum biscuits (Table 4.3.15). NWHD biscuit had similar ($p\geq0.05$) hardness to wheat and RNTS biscuits. Serrem et al. (2011), Dovi (2013), and Omoba et al. (2015) found that sorghum biscuits had similar hardness to wheat biscuits. With regard to the relationship between the waxy and HD traits and hardness of biscuits, heterowaxy (hWND) and waxy (WND1, WND2 and WHD2) biscuits had similar ($p\geq0.05$) hardness to non-waxy (NWND), and normal sorghum (WNTS and RTS) biscuits. Thus, there was no clear trend as to whether the waxy and HD traits affected the hardness of sorghum biscuits.

Regarding coarseness of the sorghum biscuits, only NWHD biscuits had similar ($p\geq0.05$) coarseness to wheat biscuit (Table 4.3.15). The study by Omoba et al. (2015) found that sorghum biscuits had similar coarseness to whole grain wheat biscuits. All other sorghum biscuits were coarser ($p<0.05$) than the wheat biscuits. Biscuits of the heterowaxy (hWND) and waxy (WHD1, WND1, WND2, WHD2 and WND3) lines had similar ($p\geq0.05$) coarseness to non-waxy (NWND) and normal sorghum (WNTS, RNTS and RTS) biscuits. Hence, there was no clear trend as to whether the traits affected coarseness of sorghum biscuits.

All the sorghum biscuits had higher ($p<0.05$) graininess rating compared to the wheat biscuits (Table 4.3.15). All biscuits of heterowaxy (hWND) and waxy (WHD1, WND1, WND2, WHD2 and WND3) lines had similar ($p\geq0.05$) graininess to normal sorghums. This shows that the waxy and HD traits did not affect graininess of sorghum biscuits.

Biscuits of heterowaxy (hWND) and waxy (WHD1, WND1, WND2, WHD2 and WND3) had similar ($p\geq0.05$) crunchiness to wheat and normal sorghum type biscuits (Table 4.3.15). The studies by Serrem et al. (2011) and Omoba et al. (2015) found that biscuits from sorghum were less crispy in texture compared to wheat biscuits. The biscuits of heterowaxy and waxy lines also had similar ($p\geq0.05$) crunchiness to the non-waxy lines (NWND and

NWHD). Hence, there was no clear trend as to whether the traits affected crunchiness of sorghum biscuits.

NWND and WND1 sorghum biscuits had similar ($p \geq 0.05$) dryness to wheat and RTS biscuits (Table 4.3.15). NWHD, hWND, WHD1, WND2 and WND3 biscuits were drier ($p < 0.05$) than wheat biscuit and were similar ($p \geq 0.05$) to WNTS and RNTS biscuits. The studies by Serrem et al. (2011) and Omoba et al. (2015) showed the sorghum biscuits were less dry than wheat biscuits. Two waxy lines (WHD2 and WND3) were drier ($p < 0.05$) than the non-waxy line (NWHD) biscuits. These waxy line biscuits were also similar ($p \geq 0.05$) in dryness to the other non-waxy lines (NWND). This shows that the waxy and HD traits did not affect the graininess of sorghum biscuits.

PCA of the biscuits of the eight novel sorghum lines showed that three waxy lines (WHD1, WHD2 and WND3) were associated with crunchiness and dryness as these lines were in the same PC1 quadrant (57.1% of the variation) as high crunchiness and dryness (Figure 4.3.9). Furthermore, all sorghum biscuits had harder and drier texture compared to the wheat biscuits. This could be attributed to the poor-water absorption and hydrophobic nature of kafirin proteins (Serrem et al., 2011; Duodu et al., 2003). Omoba et al. (2015), however, have found sorghum biscuits to be indistinguishable from wheat biscuits in terms of hardness. However, PCA of all sorghum biscuits plus the wheat biscuit standard showed that the waxy lines (WHD1, WHD2 and WND3) were not associated with crunchiness and dryness (Figure 4.3.10), as the normal sorghums were also in the same PC1 quadrant. Thus, the waxy and HD sorghum lines did not produce biscuits of better quality than regular sorghum. This is probably in part because the sorghum biscuits had low moisture content (3.2-5.1%) and products with very low moisture levels have a slow rate of starch retrogradation and staling (Ottenhof and Farhat, 2004). Also, in biscuit making, the flour constitutes only about half of the dough components and the transformation of the ingredients into biscuit comprises a complex biochemical and physical processes, which can still not be fully explained (Pareyt and Delcour, 2008).

Table 4.3. 14 Lexicon used to describe the sensory properties of biscuits made from novel sorghum lines and wheat, and summary of significance for effects of waxy and HD-traits

Sensory /Category	Attribute	Definition /reference	Scale anchors (0, 10)	[1]Waxy and HD-traits (p-value)
Aroma	Baked biscuit	Intensity of baked aroma associated with a biscuit	Not intense, Very intense	***
	Sunflower oil	Intensity associated with sunflower oil	Not intense, Very intense	**
	Sorghum[*a]	Intensity of aroma associated with sorghum porridge	Not intense, Very intense	***
	Milky	Intensity of aroma associated with milk	Not intense, Very intense	***
	Maize meal	Intensity of aroma associated with cooked maize meal	Not intense, Very intense	***
	Popcorn	Intensity of aroma associated with smell of popcorn	Not intense, Very intense	***
Appearance	Surface colour[*a]	From light cream to dark brown	Not dark, very dark	***
	Rough surface[*a]	Degree of roughness perceived on surface of a biscuit	Not rough, very rough	***
	Specks[*a]	Quantity on biscuit surface	No specks, many specks	***
Texture	Hardness[*a]	Force required to compress a biscuit between molar teeth	Not hard, very hard	***
	Coarseness[*a]	Degree to which the mass feels coarse or abrasive during mastication	Not coarse, very coarse	***
	Graininess[*a]	Amount of small particles perceived by the tongue when the mass is gently compressed between the tongue and palate	Not grainy, very grainy	***
	Crunchiness	The sensation of muffled grinding of the biscuit	Not crunchy, Very crunchy	***
	Dryness[*a]	Degree to which the sample feels dry or absorbs saliva in mouth	Not dry, very dry	***
Flavour	Sweetness[*a]	Fundamental taste sensation associated with sugars	Not sweet, intense sweet taste	NS
	Milky	Intensity of flavour associated with milk	No milky flavour, Intense milky flavour	***
	Maize meal	Intensity of flavour associated with cooked maize meal	No maize meal flavour, Intense maize meal flavour	***
	Sorghum[*a]	Intensity of flavour associated with cooked sorghum	No sorghum flavour, Intense sorghum flavour	***
	Wheat biscuit[*a]	Intensity of flavour associated with baked wheat biscuits	No wheat biscuit flavour, Intense wheat biscuit flavour	NS
Aftertaste	Bitterness[*a]	Fundamental taste of which caffeine is typical	Not bitter, Very bitter	***
	Astringent	Chemical sensation associated with puckering of tongue caused by substances such as tannins	Not astringent, Very astringent	***
	Sourness[*a]	A taste that is commonly associated with lemon	Not sour, Very sour	*
	Grittiness[*a]	Degree to which mouth contains small particles after all of the sample has been swallowed	Not gritty, Very gritty	***

*** p-value < 0.001; ** p-value < 0.01, *p-value < 0.05, NS (Not significant); [1]ANOVA to determine the effect waxy and HD-traits in sorghum and biscuit quality.*a (Omoba et al., 2015).

Table 4.3. 15 Effect of waxy and HD traits in sorghum and biscuit making quality

Sensory attributes		NWND	NWHD	hWND	WHD1	WND1	WND2	WHD2	WND3	WNTS	RNTS	RTS	Wheat
Aroma	Baked biscuit	3.9^{bc}	2.7^{ab}	3.2^{ab}	2.4^{a}	2.7^{ab}	3.2^{ab}	2.3^{a}	2.3^{a}	3.1^{ab}	2.9^{ab}	2.2^{a}	4.5^{c}
	Sunflower oil	3.8^{ab}	2.8^{a}	3.8^{ab}	3.7^{ab}	2.6^{a}	3.6^{ab}	2.9^{ab}	4.0^{ab}	4.5^{b}	3.7^{ab}	3.1^{ab}	3.3^{ab}
	Sorghum	3.4^{b}	4.6^{bc}	3.6^{b}	3.5^{b}	4.6^{bc}	4.0^{bc}	3.9^{bc}	4.0^{bc}	3.4^{b}	4.5^{bc}	5.4^{c}	1.5^{a}
	Milky	1.8^{a}	1.3^{a}	1.6^{a}	1.4^{a}	1.8^{a}	1.9^{a}	1.7^{a}	1.4^{a}	1.8^{a}	1.3^{a}	1.3^{a}	3.1^{b}
	Maize meal	2.4^{b}	2.4^{b}	2.3^{b}	2.6^{b}	2.7^{b}	2.9^{b}	2.6^{b}	2.6^{b}	2.4^{b}	2.5^{b}	2.5^{b}	0.6^{a}
	Popcorn	4.4^{d}	3.2^{abcd}	4.4^{d}	4.6^{d}	2.0^{ab}	4.2^{cd}	3.5^{bcd}	4.5^{d}	4.7^{d}	3.9^{cd}	2.3^{abc}	1.4^{a}
Appearance	Surface colour	3.3^{b}	4.5^{bcd}	3.5^{b}	5.2^{cd}	4.3^{bcd}	5.1^{cd}	4.36^{bcd}	5.1^{cd}	4.1^{bc}	5.4^{d}	9.6^{e}	0.17^{a}
	Rough surface	3.7^{bcd}	2.0^{a}	2.97^{abc}	4.0^{cd}	3.0^{abc}	2.8^{abc}	5.0^{d}	4.8^{d}	2.9^{abc}	3.4^{abcd}	2.2^{ab}	1.79^{a}
	Specks	5.1^{def}	2.9^{bc}	4.4^{cde}	5.3^{def}	3.5^{bcd}	3.1^{bc}	6.3^{f}	6.1^{ef}	4.0^{bcd}	3.9^{bcd}	2.6^{b}	0.49^{a}
Texture	Hardness	5.8^{ed}	2.9^{ab}	4.9^{cde}	8.7^{f}	4.3^{bcd}	5.1^{cde}	6.2^{e}	8.4^{f}	4.8^{cde}	4.0^{abc}	4.7^{cde}	2.7^{a}
	Coarseness	4.8^{cd}	3.2^{ab}	4.6^{bcd}	5.7^{d}	3.9^{bc}	4.17^{bc}	5.2^{cd}	5.78^{d}	4.7^{bcd}	4.3^{bcd}	3.77^{bc}	1.75^{a}
	Graininess	5.3^{b}	4.2^{b}	5.0^{b}	5.2^{b}	4.4^{b}	4.5^{b}	5.2^{b}	5.2^{b}	5.0^{b}	4.6^{b}	4.5^{b}	0.8^{a}
	Crunchiness	6.0^{ab}	5.1^{ab}	5.6^{ab}	6.0^{ab}	5.0^{a}	5.7^{ab}	6.4^{b}	6.5^{b}	6.2^{ab}	5.6^{ab}	5.1^{ab}	5.1^{ab}
	Dryness	5.3^{bcd}	3.9^{ab}	4.7^{bc}	4.6^{bc}	4.0^{ab}	4.8^{bc}	6.6^{d}	6.0^{cd}	4.7^{bc}	4.5^{bc}	4.3^{b}	2.3^{a}
Flavour	Sweetness	4.2^{a}	4.0^{a}	4.5^{a}	3.7^{a}	4.8^{a}	3.7^{a}	4.1^{a}	4.2^{a}	4.5^{a}	4.3^{a}	4.0^{a}	4.4^{a}
	Milky	3.0^{a}	2.9^{a}	2.8^{a}	2.0^{a}	3.5^{ab}	2.2^{a}	3.1^{a}	2.4^{a}	3.1^{a}	2.4^{a}	2.6^{a}	4.9^{b}
	Maize meal	3.1^{b}	2.4^{b}	2.6^{b}	3.0^{b}	2.9^{b}	3.1^{b}	3.2^{b}	2.8^{b}	2.8^{b}	2.6^{b}	2.6^{b}	0.2^{a}
	Sorghum	3.5^{b}	4.2^{b}	3.4^{b}	3.8^{b}	4.2^{b}	3.7^{b}	3.8^{b}	3.6^{b}	3.5^{b}	3.9^{b}	4.5^{b}	1.3^{a}
	Wheat biscuit	1.7^{a}	1.7^{a}	2.0^{a}	1.8^{a}	1.5^{a}	2.1^{a}	1.7^{a}	2.0^{a}	2.1^{a}	2.5^{a}	1.5^{a}	2.7^{a}
Aftertaste	Bitterness	1.9^{ab}	1.4^{ab}	1.8^{ab}	2.8^{b}	1.3^{ab}	2.6^{b}	1.5^{ab}	1.7^{ab}	1.4^{ab}	2.2^{b}	1.5^{ab}	0.2^{a}
	Astringent	2.5^{b}	2.0^{ab}	1.8^{ab}	3.3^{b}	2.7^{b}	3.3^{b}	2.9^{b}	1.8^{ab}	2.4^{b}	2.2^{b}	2.3^{b}	0.6^{a}
	Sourness	0.5^{a}	0.6^{a}	0.7^{a}	0.9^{a}	0.7^{a}	1.0^{a}	0.7^{a}	0.5^{a}	1.0^{a}	0.7^{a}	0.7^{a}	0.5^{a}
	Grittiness	4.4^{bc}	3.1^{b}	4.4^{bc}	4.5^{bc}	4.2^{bc}	4.5^{bc}	4.5^{bc}	4.9^{c}	4.1^{bc}	3.6^{bc}	3.4^{bc}	0.27^{a}

Values are Mean ± standard deviation (n=2).Values in a row with different letters in superscript are significantly different ($p<0.05$). NWND (Non-waxy-normal protein digestibility); NWHD (Non-waxy -high protein digestibility), hWND (heterowaxy- normal protein digestibility), WHD (waxy-high protein digestibility), WND (waxy-normal protein digestibility), WNTS (white non-tannin sorghum), RNTS (red non-tannin sorghum), RTS (red tannin sorghum)

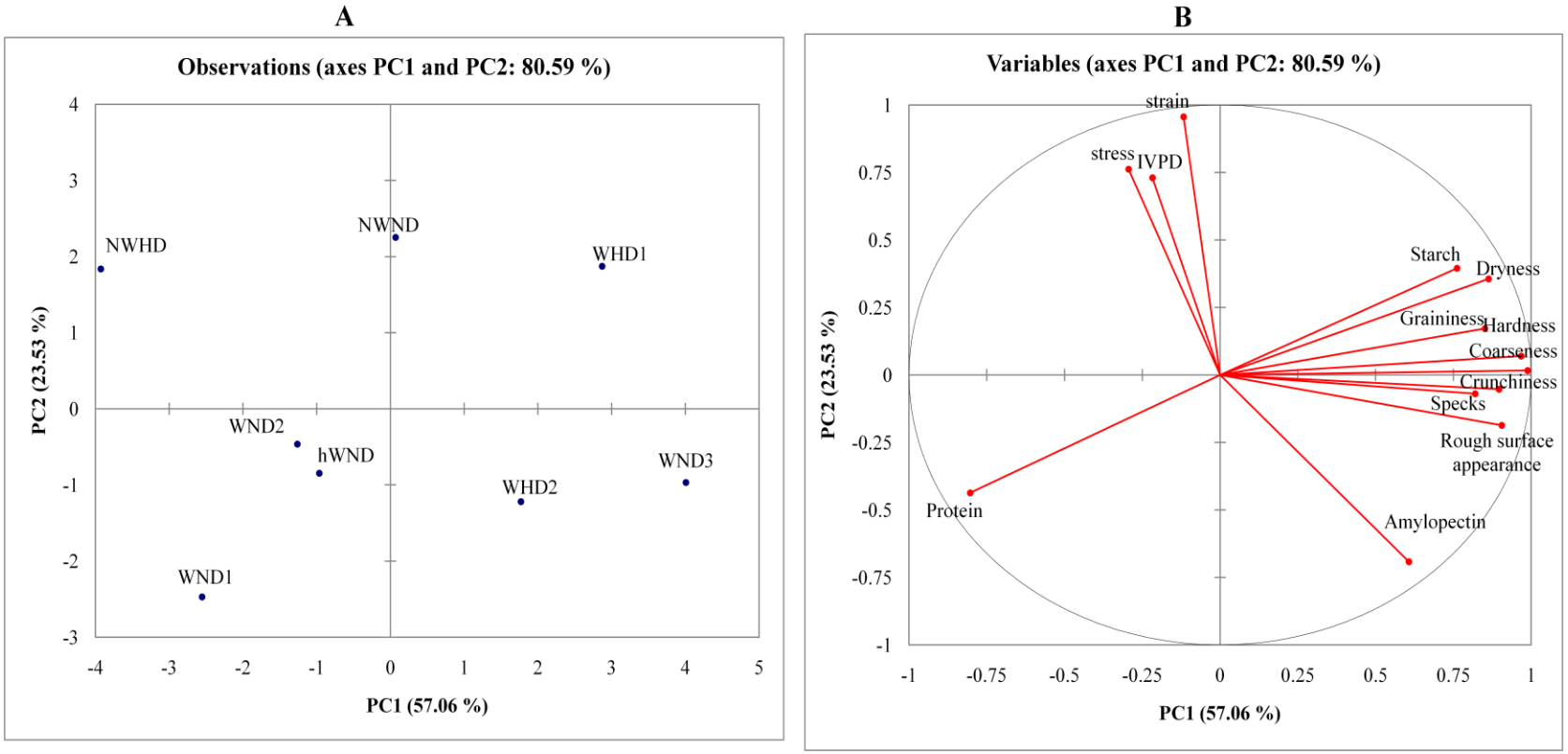

Figure 4.3. 9 Principal Component analysis of sorghums with different starch and protein digestibility traits and their biscuit quality attributes

A: Sorghum lines: NWLD (Non-waxy- normal protein digestibility), NWHD (Non-waxy- high protein digestibility), hWND (heterowaxy-normal protein digestibility), WND1, WND2, WND3 (Waxy- normal protein digestibility), WHD1 and WHD2 (Waxy-high protein digestibility),

B: PCA Loadings: starch content, protein content, starch amylopectin content and cooked in-vitro protein digestibility (IVPD), Rough surface appearance, Specks, Hardness, Coarseness, Graininess and Dryness (as measured by DSP), stress (kPa), Strain (%).

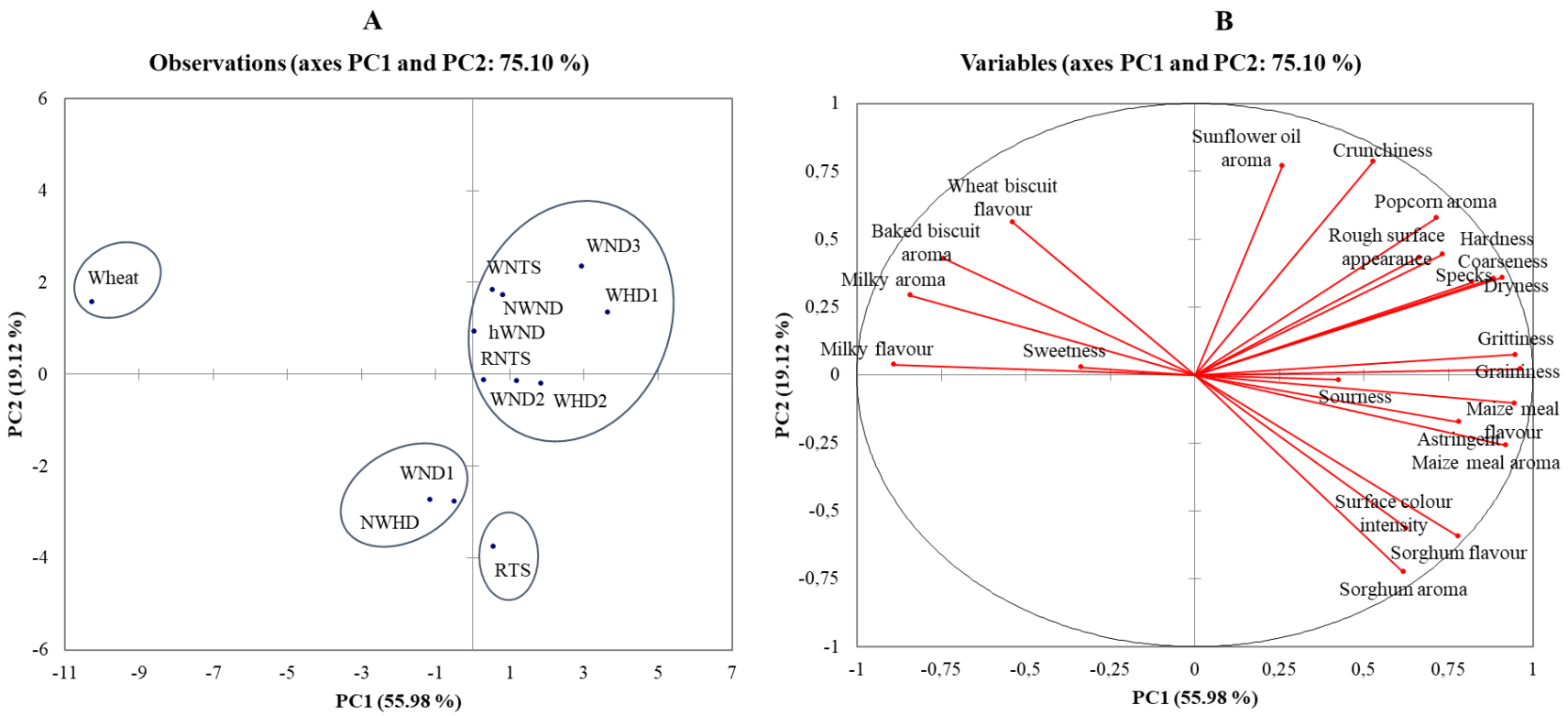

Figure 4.3. 10 Principal component analysis of novel sorghum lines, normal sorghum types and wheat and their biscuit quality attributes

A: Sorghum lines: NWLD (Non-waxy- normal protein digestibility), NWHD (Non-waxy- high protein digestibility), hWND (heterowaxy-normal protein digestibility), WND1, WND2, WND3 (Waxy- normal protein digestibility), WHD1 and WHD2 (Waxy-high protein digestibility), WNTS (white non-tannin sorghum), RNTS (red non-tannin sorghum), RTS (red tannin sorghum)

B: PCA Loadings: aroma attributes (milky, baked biscuit, sunflower oil, popcorn, maize meal, sorghum), flavour attributes (milky, wheat biscuit, maize meal, sweetness, sourness, astringent), appearance attributes (surface colour, rough surface, specks), texture attributes (hardness, coarseness, crunchiness, graininess and dryness), aftertaste attributes (bitterness, astringent, sourness, grittiness).

4.3.4.7 Instrumental texture analysis of biscuits

Stress (strength) of the sorghum biscuits varied considerably; ranging from 530-819 kPa (Table 4.3.16). All sorghum biscuits had higher ($p<0.05$) stress compared to the wheat biscuits. This indicated that the sorghum lines were stronger than the wheat biscuit reference. In a study by Adedara (2017), sorghum biscuits were found to be higher in stress compared to sugar-snap and Marie-wheat biscuits. Sorghum biscuits texture was also observed to be harder and drier than wheat biscuits by Serrem et al. (2011). Omoba et al. (2015), however, found sorghum biscuits were indistinguishable from wheat biscuits in terms of hardness. Biscuits of hWND, WHD1, WND1, WND2, WHD2 and WND3 lines had lower ($p<0.05$) stress compared to WNTS biscuits. These lines had similar ($p\geq0.05$) stress to RNTS and RTS biscuits. WHD2 and WND3 biscuits had lower ($p<0.05$) stress compared to non-waxy (NWND and NWHD) biscuits; while hWND and WHD1 had similar stress to the non-waxy line (NWHD) biscuits. Hence, there was no clear trend as to whether the waxy and HD-traits affected the stress of sorghum biscuits.

Strain (extensibility) of the sorghum biscuits varied considerably ranging from 10-37.1% (Table 4.3.16). All sorghum biscuits had higher ($p<0.05$) strain compared to the wheat biscuit standard. Higher strain of sorghum biscuits compared to Marie-wheat biscuits and lower strain of sorghum biscuits compared to sugar-snap wheat biscuit was observed by Adedara (2017). Biscuits of hWND, WHD1, WND1, WND2, WHD2 and WND3 had lower ($p<0.05$) strain compared to the WNTS and RNTS. hWND and WND1 biscuits had similar ($p\geq0.05$) strain to RTS biscuits. Regarding the relationship between waxy and HD traits and strain, biscuits of one waxy line (WND3) had similar ($p\geq0.05$) strain to the non-waxy lines (NWND and NWHD), while another waxy line (WHD1) biscuit had similar ($p\geq0.05$) strain to the NWHD biscuits. Biscuits of the heterowaxy (hWND) and other waxy lines (WND1, WND2 and WHD2) had lower ($p<0.05$) strain compared to the non-waxy sorghums. Thus, the strain of sorghum biscuits seemed to vary irrespective of waxy and HD traits.

Table 4.3. 16 Effect of waxy and HD traits on mechanical properties of sorghum biscuits

Sorghum lines	Stress (kPa)	Strain (%)
NWND	$819.2^{f} \pm 40.2$	$23.8^{ef} \pm 1.7$
NWHD	$697.7^{e} \pm 40.5$	$26.7^{f} \pm 2.5$
hWND	$648.6^{cde} \pm 30.5$	$15.2^{cd} \pm 1.5$
WHD1	$659.6^{de} \pm 31.5$	$22.5^{e} \pm 2.2$
WND1	$612.7^{cd} \pm 39.6$	$10.0^{b} \pm 1.6$
WND2	$613.6^{cd} \pm 85.7$	$18.1^{d} \pm 2.2$
WHD2	$530.0^{b} \pm 19.0$	$16.6^{d} \pm 1.7$
WND3	$583.0^{c} \pm 35.7$	$23.3^{ef} \pm 1.8$
WNTS	$778.4^{f} \pm 36.4$	$37.1^{h} \pm 1.5$
RNTS	$603.2^{bcd} \pm 43.0$	$30.8^{g} \pm 3.7$
RTS	$582.3^{bc} \pm 24.3$	$12.0^{bc} \pm 1.6$
Wheat	$358.7^{a} \pm 16.5$	$3.9^{a} \pm 0.5$

Values are Mean ± standard deviation (n=2).Values in a column with different letters in superscript are significantly different ($p<0.05$). NWND (Non-waxy-normal protein digestibility); NWHD (Non-waxy -high protein digestibility), hWND (heterowaxy- normal protein digestibility), WHD (waxy-high protein digestibility), WND (waxy-normal protein digestibility), WNTS (white non-tannin sorghum), RNTS (red non-tannin sorghum), RTS (red tannin sorghum).

4.3.5 Conclusions

Waxy sorghum produces softer, flexible and rollable injera without affecting aroma, appearance, flavour and aftertaste quality attributes. When stored, waxy sorghum also produces softer and flexible injera compared to normal sorghums. The HD trait, however, does not clearly affect injera quality. Regarding biscuits, the waxy and HD traits do not affect the quality of biscuits. This study shows that white tan-plant waxy sorghum can produce injera of better quality than regular sorghum and much closer to teff injera. Thus, these waxy sorghums have potential to partially replace teff for injera making in Ethiopia. The white tan-plant waxy sorghums do not produce biscuits of better quality than regular sorghum. Hence, either waxy or normal sorghum can be used to partially replace wheat for biscuit making in regions where wheat is not economically cultivated.

4.3.6 References

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5 GENERAL DISCUSSION

This general discussion critically reviews the methodology applied in this study, primarily the challenges faced in selecting sorghum lines with the required combination of waxy (high amylopectin) and high protein digestibility (HD) traits. This section then discusses the main findings with regard to the relationship between waxy and HD traits in sorghum and malting quality and dough-based products (injera and biscuit) making quality. Lastly, future work is suggested to answer questions related to the potential use of these novel sorghum lines with waxy and HD traits in food and beverage applications.

5.1 Methodological considerations

Sorghum lines with the waxy and HD traits were small in number (only 8 lines demonstrated the traits). HD-traits were demonstrated only with two sorghum lines (WHD1 and WHD2) and with only a relatively moderate improvement in in-vitro protein digestibility (56-62% IVPD). A higher number of novel sorghum lines (12 and more) would have been better. However, when 29 sorghum lines produced by Texas A&M Agrilife were characterized, they did not show the required combination of traits. Another limitation was the available quantity of samples and representation of the traits. Only the sorghum lines that were increased at Ukulima research farm were high enough in quantity to conduct the research. Also, for the traits there was only one sorghum line to represent lines of hWND (heterowaxy-normal protein digestibility), NWND (non-waxy starch and normal protein digestibility) and NWHD (non-waxy starch and high protein digestibility traits). However, sorghum lines with non-waxy, waxy and waxy-HD traits were represented by at least two lines.

A further challenge was also associated with the lack of stability of the waxy and HD traits. The waxy trait of the sorghum lines showed a relatively good stability as the starch amylopectin content of the sorghum lines increased at Ukulima farm in South Africa was significantly correlated ($r=0.859$, $p=0.01$) with that of the lines increased at Nanga research farm in Zambia. The waxy trait in rice has been found to show some stability over varying environments (Li et al., 2017). However, the HD-trait was unstable as the protein digestibility data of the sorghum lines increased at Ukulima and Nanga research farms were not significantly correlated. The expression of high protein digestibility trait (Tesso et al., 2008) and endosperm texture trait (Tesso et al., 2006) in certain sorghum genotypes has been found to be unstable often varying between environments under which the crops are grown. Tesso et al. (2008) stated that the expression of a high protein digestibility major gene, the endosperm

phenotype and the likely presence of modifier genes are either suppressed or activated under certain environmental conditions.

A further weakness of the study was also that it considered commercial sorghum malt (white Type-II tannin sorghum, Feterita-type variety) as a reference for studying the malting quality instead of commercial sorghum malt from white tan-plant sorghum. Also, the commercial malts were produced under industry malting conditions rather than the laboratory malting conditions used in this study. However, the general requirement of barley malt quality for commercial brewing was included in the results to evaluate the potential of the novel sorghum lines for brewing.

5.2 Research findings

5.2.1 Relationship between waxy and HD traits in sorghum and malting quality

The role of waxy and high protein digestibility in quality of sorghum malt is summarized (Table 5.1). The study indicated that the waxy (high amylopectin) trait in sorghum resulted in greater malt endosperm modification (partial hydrolysis) and starch granule degradation (section 4.2.4.2) during germination. Also, germination of selected sorghum lines for 5 days (Figure 4.2.4) showed that the waxy lines combined with either the high or normal protein digestibility traits were more readily modified than non-waxy and normal protein digestibility sorghum. This extensive starch degradation of the waxy sorghums is indicative of the amylopectin-rich starch being more readily modified. Adequate endosperm modification in sorghum malt by enzymic hydrolysis is an important factor for good fermentability in brewing (Chiremba et al., 2013). The malt modification is required so as to supply yeast with adequate nutrients, primarily fermentable sugars and free amino acids, but also micronutrients such as minerals and vitamins during brewing (Briggs, 2002; Edney et al., 2007). Large-scale beer production with sorghum malt can be adversely affected by limited yeast growth during fermentation due to poor endosperm modification resulting in low wort free amino nitrogen (FAN) (Mugode et al., 2011) and low extract yield (Aisen and Muts, 1987). Importantly, however, a study by Barredo-Moguel et al. (2001b) clearly demonstrated that worts produced from waxy sorghum grits are a suitable substrate for yeast as brewing adjuncts for production of lager beers. In summary, this current study shows that the waxy trait in sorghum is highly associated with improved malt endosperm modification (partial hydrolysis) and starch granule degradation.

Protein body degradation of malted sorghum (section 4.2.4.3) did not show any clear trend as to whether the HD trait in the lines improved the degradation of the endosperm protein. This is probably due to the fact that the level of protein digestibility was only relatively moderately increased. Protein degradation during sorghum malting is brought by various protease enzymes (Taylor, 2010). During germination both the protein bodies and the surrounding matrix protein of sorghum malt are extensively eroded when the protein has been hydrolysed (Taylor et al., 1985; Ng'andwe et al., 2008; Mugode et al., 2011).

Malt α–amylase activity of the sorghums was not evidently affected by the waxy and HD traits (section 4.2.4.4). Alpha-amylase is important as it is the starch liquefying enzyme in sorghum malting, producing dextrins (short chains of glucose molecules) and a variety of fermentable sugars including maltotriose (3 glucose units), maltose (2 glucose units) and glucose (Briggs et al., 2004). Sorghum malts have been found to exhibit similar or even higher α-amylase activities than typical lager barley malts (Dufour et al., 1992).

The β-amylase activity of the malted sorghum was also not evidently affected by the waxy and HD traits (section 4.2.4.5). Brewing with sorghum malt is associated with a problem of low β-amylase activity that results in limited saccharification and low levels of fermentable extract (Palmer, 1992; Taylor et al., 2006). Studies by Dufour et al. (1992) and Letsididi et al. (2008) showed that malted sorghum β-amylase activity was much lower than that of barley malt. Also, it has been found that sorghum malt β-amylase is highly heat labile (Taylor and Robbins, 1993). The same study also showed that ungerminated sorghum does not exhibit β-amylase activity. Efforts to optimise the limited β-amylase activity in sorghum malt have been made through selecting and breeding cultivars of high β-amylase activity, optimizing malting conditions and decantation mashing procedure whereby the enzymic mash is separated first and mixed later when the starch has been completely gelatinized (Palmer, 1992).

Waxy sorghums yielded higher malt hot water extract (HWE) (section 4.2.4.6) than the normal sorghum. The higher malt HWE of the waxy sorghums as compared to normal sorghum was reflected at both germination moisture levels (medium and high). This improved malt HWE is probably due to the better starch granule swelling property of amylopectin (Tester and Morrison, 1990). Thus, the waxy starch was more easily hydrolyzed by α-amylase (Tester et al., 2004; Wu et al., 2010). The use of unmalted sorghum in beer brewing is linked to incomplete saccharification and consequently low fermentable extract

(Taylor et al., 2006). Also, the hydrolysis of starch in sorghum malt is majorly and adversely influenced by the high gelatinization temperature and low β-amylase activity of sorghum malts (Taylor, 1992). Sorghum starch digestibility remains low even after cooking (Ezeogu et al., 2005) due to the tendency of complex disulphide-linking of kafirin polymers (Duodu et al., 2003) that restricts α-amylase access to starch granules. Hence, higher HWE is desirable for improved sorghum malt quality. Unmalted waxy and heterowaxy sorghum cultivars have higher starch digestibility (Del Pozo-Insfran et al., 2004). Improved starch hydrolysis of unmalted cereals; waxy barley (Vasanthan and Hoover, 2009) and waxy sorghums (Osorio-Morales et al., 2000; Barredo-Moguel et al., 2001a, b) has been well documented. In a study by Wong et al. (2009) it was found that unmalted waxy sorghum line, which also had a weak protein matrix as in HD sorghum lines, was more susceptible to hydrolysis by α-amylase. Also, unmalted waxy sorghum when used as adjunct in brewing trials showed more rapid starch hydrolysis (Figueroa et al., 1995). This present study reveals that sorghum waxy (high amylopectin) trait either with high or normal protein digestibility is highly associated with a higher level of malt HWE.

Waxy sorghums also had improved malt FAN (section 4.2.4.7). As with malt HWE, with the exception of low amylase sorghum line (WHD1), all the waxy sorghum lines had higher malt FAN compared to regular sorghum. This is in agreement with Rooney and Pflugfelder (1986) who observed that sorghums with waxy endosperm and relatively weak protein matrix were more susceptible to hydrolysis by protease and amylase enzymes. On the other hand, the HD trait was not associated with high malt FAN. However, study by Mugode et al. (2011) found that malted HD lines were substantially higher in FAN than normal sorghums. Nevertheless, the same study also reported that the HD lines were not higher in FAN when mashed. However, as mentioned when brewing with sorghum malt, obtaining adequate proteolysis (Palmer, 1992) and FAN (Mugode et al., 2011) for rapid and complete fermentation is a problem. Thus, high malt FAN is important in sorghum malt. Furthermore, unmalted transgenic sorghum lines with the HD trait have been found to give increased wort FAN (Kruger et al., 2012). However, in this present study FAN seemed to be more associated with grain protein content. It seemed that the moderate increase in protein digestibility in the HD lines was not significant compared to the waxy trait. A similar observation was reported by Wong et al. (2009) that the waxy trait in sorghum could enable the endosperm proteins to be exposed to proteases.

5.2.2 Relationship between waxy and HD traits in sorghum and dough-based product (injera and biscuit) quality

The aim of this part of the study was to determine the relationship between waxy and HD traits in sorghum dough-based product (injera and biscuit) quality as assessed by descriptive sensory profiling (DSP) and instrumental texture analysis. The role of waxy and high protein digestibility traits in quality of sorghum injera and biscuits is summarized (Table 5.1). The waxy trait greatly influenced the texture profile of fresh and stored sorghum injera (section 4.3.4.3 and section 4.3.4.4). Aroma, appearance, flavour and aftertaste attributes of fresh prepared and stored sorghum injera (section 4.3.4.3) and sorghum biscuits (section 4.3.4.6) were not evidently affected by the waxy and HD traits. However, there was no clear trend as to whether the HD trait affected injera and biscuit quality.

Descriptive sensory profiling and instrumental texture analysis revealed that injera of waxy (high amylopectin) sorghums were softer, more rollable, and flexible compared to injera from normal sorghums (section 4.3.4.3 and section 4.3.4.4). Also, the injera from waxy sorghums were very close in texture (softness, flexibility, sponginess, rollability, chewiness and breakability) to the standard injera produced from teff. However, injera made from combined waxy-HD lines (WHD1 and WHD2) was too sticky and that of regular sorghum lines was dry and gritty. The improved texture of waxy sorghum injera is presumably due to the slower staling and better water holding property of the starch amylopectin (Fadda et al., 2014). As stated, staling is a major problem associated with use of sorghum cultivars for injera making (Gebrekidan and GebreHiwot, 1982). Therefore, this work is significant in that the waxy trait in sorghum resulted in softer, more rollable, and flexible injera compared to regular sorghum (section 4.3.4.3). This is in agreement with other research into breads made from other cereals, where for example waxy barley (Purhagen et al., 2011) and waxy wheat (Bhattacharya et al., 2002; Mouliney et al., 2011) were found to retard staling and produce softer breads.

Comparison of the textural properties of injera measured by instrument (texture analyser) and descriptive sensory panel showed that the stress of the injera correlated with softness (section 4.3.4.4) ($p=0.01$) and the strain correlated with softness, flexibility and rollability ($p=0.01$). Hence, stress and strain measured by instrumental texture analysis can be used for evaluating softness, flexibility and rollability of injera in a laboratory where there is no trained sensory panel or to minimize cost and time of analysis. Furthermore, it was found that the

instrumental texture profile (stress and strain data) of injera prepared using full-scale (traditional) and small-scale (microwave) methods were significantly correlated ($p=0.01$). The large-scale injera making used 1 kg flour while that of the small-scale injera used 250 g flour. Also, the injera made from large-scale and small-scale had a diameter of 50 cm and 9 cm, respectively. Thus, the small-scale microwave method has considerable potential to be used for screening sorghum cultivars for making injera, where a large number of cultivars of small sample size have to be evaluated.

Descriptive sensory panel and instrumental texture analysis showed that biscuits made from waxy and HD sorghum lines were similar in crunchiness and dryness compared to biscuits from normal sorghum. This indicates that waxy and HD traits do not affect biscuit quality and these traits do not produce better biscuits compared to regular sorghum. Furthermore, sorghum biscuits from all the sorghum types studied had harder and drier texture compared to wheat biscuits. This is in agreement with studies by Adedara (2017) and Serrem et al. (2011) that observed sorghum biscuits having harder and dryer texture than wheat biscuits. This could be due to the poor water absorption and hydrophobic nature of the kafirin proteins (Serrem et al., 2011; Duodu et al., 2003). However, a study by Omoba et al. (2015) found that sorghum biscuits were indistinguishable from wheat biscuits in terms of hardness as determined by descriptive sensory panel. These inconsistent findings could be due to the fact that biscuits have low moisture content (1-5%) (Wade, 1988) and cereal products (e.g. rice pasta (Riva et al., 2000) and wheat noodles (Fukuzawa et al., 2016)) with such low moisture levels have slow starch retrogradation rate and staling (Ottenhof and Farhat, 2004). Also, in biscuit making the flour constitutes only about half of the dough components and the transformation of the ingredients into biscuit comprises complex biochemical and physical processes, which is not fully understood (Pareyt and Delcour, 2008).

Table 5. 1 Summary of the roles of waxy and HD traits in the quality of sorghum malt, injera and biscuits

Trait	Product	Role and effects	Scientific explanation
Waxy (high amylopectin)	Malt	— Improves endosperm modification (partial hydrolysis) and starch granule degradation during germination — Gives higher malt hot water extract (HWE) — Improves pre-formed malt FAN	— Starch amylopectin has better starch granule swelling property that facilitates greater hydrolysis by amylases — Endosperms with high amylopectin and a relatively weak protein matrix are more susceptible to hydrolysis by protease and amylase enzymes.
	Injera	— Improves softness of fresh and stored injera — Gives more rollable and flexible injera	— Starch amylopectin is less susceptible to re-association during retrogradation and has better water holding property, results in very slow retrogradation, producing slower staling and softer injera
	Biscuits	— Does not appear to have any effect on the biscuit quality	— Starch amylopectin is unable to contribute to the slow retrogradation and staling at the low moisture level of biscuits — The starch amylopectin is unable to contribute to biscuit quality as the flour is only about 50% of the dough components and transformation into biscuits is a complex biochemical and physical processes
HD (high protein digestibility)	Malt	— Does not appear to have any effect on the malt quality	— The moderate increase in protein digestibility in the HD lines is insufficient to improve malt quality.
	Injera	— Does not appear to have any effect on the injera quality	— The HD trait is does not contribute to injera quality due to the inadequate level protein digestibility.
	Biscuit	— Does not appear to have any effect on the biscuit quality	— Moderate increase in protein digestibility of the HD lines does not improve the biscuit quality as the increase in protein digestibility is not enough to improve the biscuit quality.
Waxy-HD (high amylopectin, high protein digestibility)	Malt	— Improves endosperm modification and starch granule degradation during germination — Gives high malt hot water extract (HWE) — Improves pre-formed malt FAN	— Endosperms with high amylopectin and a relatively weak protein matrix are more susceptible to hydrolysis by amylase and protease enzymes.
	Injera	— Gives soft and sticky injera	— Starch amylopectin has lower susceptibility to re-association during retrogradation, resulting in very slow retrogradation, producing slower staling and softer injera
	Biscuits	— Does not appear to have any effect on biscuit quality	— High starch amylopectin and protein digestibility do not contribute to the slow retrogradation and staling at low moisture level of biscuits — High starch amylopectin and protein digestibility do not contribute to biscuit quality as the flour is only about 50% of the dough components and transformation into biscuit is complex biochemical and physical processes

5.3 Future research and development of waxy sorghums

As this study has indicated, partial replacement of barley malt with waxy sorghum malt for brewing could be the option to solve problems related to the limited local production and the costly importation of barley in the semi-arid regions of Africa and Asia. Furthermore, in Ethiopia as teff is difficult to cultivate and harvest, and is also becoming very expensive, waxy sorghum partly replacing teff in injera making could be an economic alternative. Hence, based on the findings of this study it is essential to pilot trial sorghum for milling, malting and brewing, and injera production in Ethiopia.

If successful the next research would be to conduct viable hybrid seed production of the waxy sorghum lines, industrial waxy sorghum malting trial and trial partial substitution of barley malt in beer brewing in Ethiopia. This trial sorghum malting and brewing must consider hybrid seed production of certified waxy sorghum lines, it must be multiplied by farmers and farmer-unions, and the particular sorghum line cultivated for commercial malting and brewing. This needs to be done in partnership with local brewing companies.

Also, the waxy sorghum seeds production and optimization of replacement of teff with waxy sorghum for injera production should be researched. Similarly, piloting the sorghum lines for injera production should address the whole sorghum value chain, as hybrid seed of certified waxy sorghum line has first to be multiplied, farmers and farmer unions have to be contracted to multiply and grow the particular sorghum lines, for a commercial injera production in partnership with local injera producing companies.

Furthermore, there is no commercial sorghum flour milling in Ethiopia, despite the fact that a huge quantity of the crop is plate milled and consumed in villages in different forms of food. Therefore, a study on pilot industrial sorghum milling for use in various cereal based foods is required. This should also include the whole sorghum value chain: hybrid seed of certified waxy sorghum line has first to be multiplied, farmers and farmer unions be contracted to multiply and grow the particular sorghum line suitable for industrial milling e.g. Pin milling or roller milling. This needs to be done in partnership with commercial millers.

6 CONCLUSIONS AND RECOMMENDATIONS

This study to investigate sorghum waxy (high amylopectin) and high protein digestibility (HD) traits and their relationship with malting and dough-based products quality has revealed important findings about the relationship of the waxy trait with malt, injera and biscuit quality.

The waxy (amylopectin-rich starch) trait combined with either the high or normal protein digestibility traits results in kernels with an endosperm that is more susceptible to hydrolysis by amylase and protease enzymes compared to regular sorghums, which improves the malt endosperm modification (partial hydrolysis), hot water extract (HWE) and FAN. This improved hydrolysis during germination is probably due to the better starch granular swelling property of the amylopectin that facilitates greater hydrolysis by amylases.

Also, the waxy (amylopectin-rich starch) trait, which results in very slow retrogradation and staling, produces softer, more flexible and rollable sorghum injera without affecting aroma, appearance, flavour and aftertaste quality attributes. Even during storage the waxy trait improves softness and flexibility of sorghum injera. This improved injera quality due to the waxy trait is probably a result of the better water holding and slow retrogradation property of starch amylopectin. However, the waxy trait combined with either the high or normal protein digestibility traits does not affect quality of sorghum biscuits. This is probably due to the fact that the starch amylopectin is unable to contribute to the slow retrogradation and staling at the low moisture level of biscuits. Also, the starch amylopectin is unable to contribute to biscuit quality as the flour is only about half of the dough components.

Although the waxy (amylopectin-rich starch) trait in sorghum improves the malt and injera quality, these improvements could be more effective if the sorghum is used as a partial replacement of barley malt in beer brewing and teff in injera making. This is due to the fact that waxy sorghum malt quality is still below the value of barley malt quality required for beer brewing and the waxy sorghum injera is only close to teff injera in quality. Therefore, it is important to determine the optimum level of waxy sorghum for partial replacement of barley malt in brewing, and teff in injera production.

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8 PUBLICATIONS, PRESENTATIONS AND POSTERS BASED ON THIS RESEARCH

Mezgebe, A.G., Abegaz, K., Taylor, J.R.N., 2018. Relationship between waxy (high amylopectin) and high protein digestibility traits in sorghum and malting. J. Cereal Sci. 79. 319–327.

Mezgebe, A.G., Abegaz, K., Taylor, J.R.N., De Kock, H.L., 2018. Relationship between waxy (high amylopectin) and high protein digestibility traits in sorghum dough-based products (injera and biscuits) making quality. Oral presentation at the International Sorghum Conference "Sorghum in the 21st Century", Cape Town, South Africa.

Mezgebe, A.G., Abegaz, K., Taylor, J.R.N., 2017. Relationship between waxy (high amylopectin) and high protein digestibility traits in sorghum and malting quality. Oral presentation at the New Voices Symposium of the Association of Cereal Science and Technology Southern Africa (CST-SA), Pretoria, South Africa.

Mezgebe, A.G., Abegaz, K., Taylor, J.R.N., 2017. Relationship between waxy (high amylopectin) and high protein digestibility traits in sorghum and malting quality. Oral presentation at the 22nd Biennial International South African Association for Food Science and Technology (SAAFoST) Congress and Exhibition, Cape Town, South Africa.