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1 INTRODUCTION

Maize and sorghum are important cereals in semi-arid and subtropical Africa as these are the major sources of energy in the region. In Africa, maize ranks first followed by sorghum, although globally the cereals are ranked second and fifth, respectively (FAOSTAT 2009; Taylor 2004). As the contribution of maize and sorghum to food security and nutrition is critical, there is a need to continuously improve their processing and utilization.

The primary processing of sorghum and maize involves dry milling. Grain hardness is the most important parameter for assessing dry milling quality (Munck 1995) as a high yield of grits is desirable and harder grain should give higher milling yield than softer grain (Taylor and Duodu 2009). In turn, grain hardness influences product quality such as porridge stickiness and texture (Bello et al 1995, Rooney et al 1986; Taylor et al 1997). Therefore simple tests are applied by breeders, millers and traders to estimate hardness and grain milling properties. These simple tests include density (Paulsen et al 2003), endosperm texture (Rooney and Miller 1982; ICC 2008), breakage susceptibility, stress cracking and decortication (Reichert et al 1986). Also, near infrared transmittance and reflectance spectroscopy have been used to estimate grain hardness (Robutti 1995; Wehling et al 1996) but these methods require calibration against data of standard chemical and physical tests. Despite the numerous grain quality tests being applied for routine grain screening and cultivar selection, the relationship between these tests and their application to commercial sorghum and maize has not been ascertained in depth.

Besides the physical tests, the biochemical basis for grain hardness is not well understood particularly in maize although the quantity and distribution of γ-kafirins is thought to play a major role in sorghum hardness (Da Silva et al 2011a; Mazhar and Chandrashekar 1995). Therefore, there is a need to determine measurements that can be used in such a situation. Phenolic acids are also thought to play a role in grain hardness (Garcia-Lara et al 2004; Del

Pozo-Insfran et al 2006) because of their high concentration and cross linking to grain cell walls. Thus, phenolic acids may affect structural properties that affect grain hardness.

In terms of application, sorghum and maize are used for porridges, which are a staple in most parts of the continent. Grain hardness plays a major role in porridge quality and influences textural properties and consumer acceptability (Kebakile et al 2008). Sorghum malt is a widely used component of sorghum porridges used to improve sorghum digestibility, viscosity and protein profile (Belton and Taylor 2004). However, the modification of sorghum during malting as affected by grain hardness and the effects of malting on milling yield and porridge quality are not known. Hence, for economic reasons and processing quality, it is desirable to determine the extent to which malting affects sorghum grain hardness and the ideal malting conditions that would give good flour yield and desirable porridge consistency.

In summary, sorghum and maize grain quality evaluation can be improved by identifying and selecting tests that can be rapidly used to distinguish grain and malt for hardness. At the biochemical level, the effect of phenolic acids and their contents on grain hardness needs to be established as these compounds are major bioactive components in cereal grains. A relationship between phenolic acids and hardness may mean that phenolic acids could be used as markers for sorghum and maize grain hardness

2 LITERATURE REVIEW

This chapter will briefly describe the structures of sorghum and maize grain in relation to hardness. Research into several methods that are used for cereal grain hardness evaluation will be discussed in detail and with respect to their relevance to sorghum and maize quality testing. The influence of sorghum and maize hardness on porridge quality and the relationship between sorghum grain hardness and malt modification will be discussed. Lastly, the potential role of phenolic acids in sorghum and maize hardness will be reviewed.

2.1 Sorghum and maize kernel structures

The structure and chemistry of a kernel play a crucial role in determining the processing properties of a cereal grain. According to Kent and Evers (1994), the kernel characteristics of shape, size and mass are the most important in respect of cereal grain quality. Sorghum and maize kernels are similar in their structure, chemical composition and biochemical basis for hardness (reviewed by Chandrashekar and Mazhar 1999). However, the relative proportions of the pericarp, germ and floury and corneous endosperm in kernels vary among varieties.

The structure of the sorghum kernel has been reviewed in depth by Rooney and Miller (1982). Sorghum is a naked caryopsis, comprising 8% pericarp, 10% germ and 82% endosperm. Serna-Saldivar and Rooney (1995) and Watson (2003) described the structure of the maize kernel. It is also a naked caryopsis and comprising about 85% endosperm, 10-14% germ and 5-6% tip cap and pericarp. The maize kernel is the largest of cereal grains and weighs about 350 mg compared to 30 mg of sorghum. Figs 2.1a and 2.1b show the longitudinal sections of the sorghum and maize kernels, respectively.

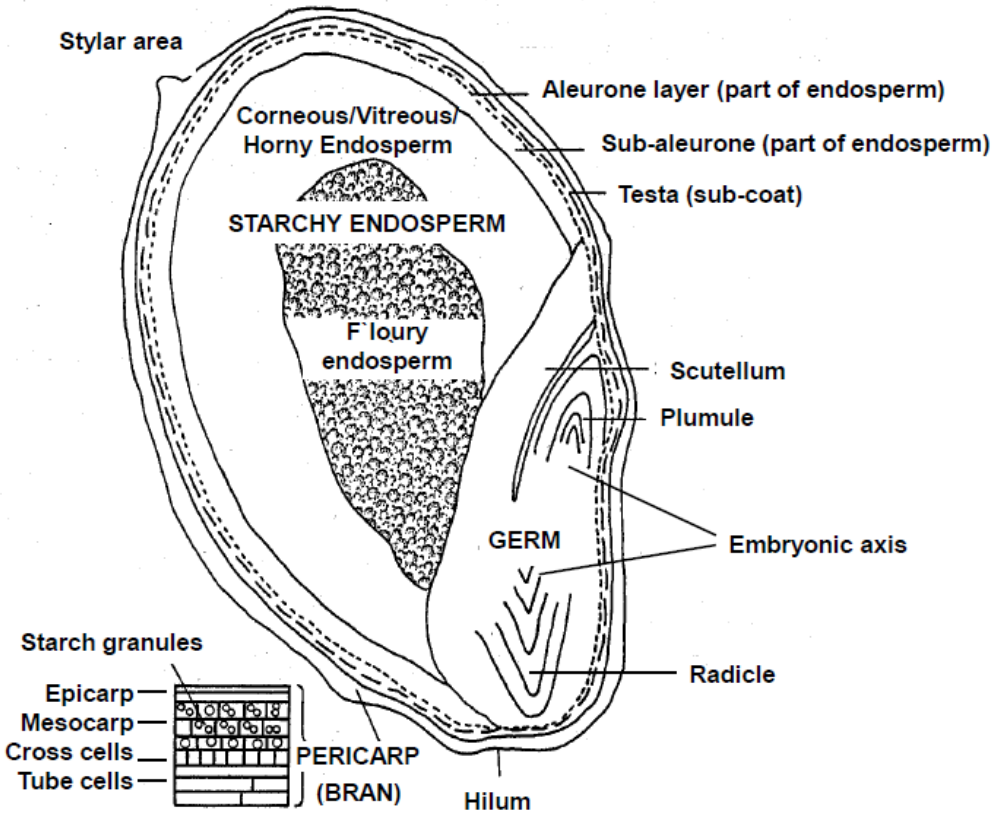


Fig 2.1a. Longitudinal section of a sorghum kernel (Taylor and Belton 2002)

2.1.1 Endosperm

As stated, the endosperm is the largest component of the maize kernels, constituting about 82-84% of the kernel of which 86-89% is starch (reviewed by Watson 2003). Likewise, in sorghum, the endosperm is the largest component ranging from 82-87% of the kernel (reviewed by Serna-Saldivar and Rooney 1995). According to Rooney and Serna-Saldivar (1993) the starchy endosperm of sorghum contains both floury and corneous (also referred to as horny or vitreous) endosperms. The endosperm is composed of starch granules, protein bodies, protein matrix and cell walls rich in cellulose, arabinoxylans and other hemicelluloses. According to Taylor et al (1984) endosperm starch granules are polygonal and round in the corneous and floury endosperm, respectively. The starch granules in the corneous endosperm

are embedded in a protein matrix that contains protein bodies, which cause dents on the granules. These protein bodies vary from 0.4 to 2.0 µm in diameter. In maize, the floury endosperm breaks along cell walls, resulting in low levels of damaged starch and floury grits, while the corneous endosperm with a thick protein matrix breaks across cells producing high levels of damaged starch (reviewed by Watson, 2003). This is also presumably true for sorghum.

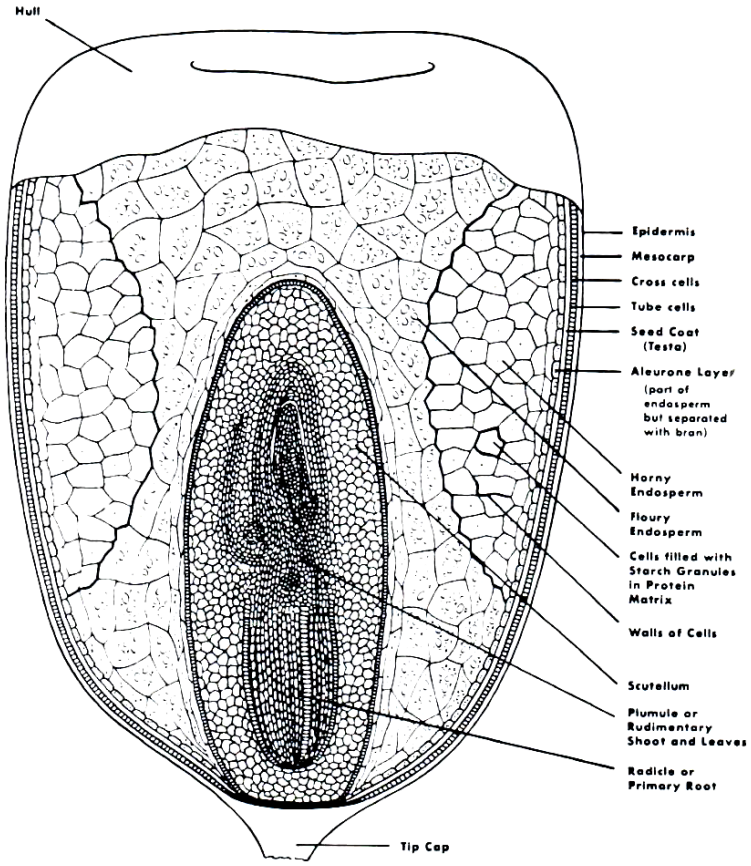


Fig 2.1b. Longitudinal section of a maize kernel (Hoseney, 1994)

In their review, Taylor et al (2006) described the composition of sorghum endosperm cell walls and compared it to that of maize and other cereals. Maize endosperm cell wall composition is similar to that of sorghum, characterised by water insoluble

glucuronoarabinoxylans compared to the soluble barley (1-3, 1-4)-β-glucans. The maize and sorghum glucuronoarabinoxylans are highly substituted and are linked to ferulic acid through ester bonds (Glennie 1984). The causes of endosperm hardness in sorghum and maize are not fully understood. Chandrashekar and Mazhar (1999) comprehensively reviewed the state of knowledge on sorghum and maize grain hardness knowledge. Grain hardness is apparently affected by a number of factors including cell wall structure and the types and concentrations of endosperm storage proteins, the prolamins. Mazhar and Chandrashekar (1995) studied the role of sorghum proteins in grains varying in hardness. The authors concluded that harder sorghum grains contained the highest levels of kafirins and that the α- and γ-kafirins were implicated in modifying endosperm texture by increasing protein body size and cross-linking, respectively. A study on maize by Lee et al (2006) showed that α- and β- zeins had an effect on grain hardness and the α-zein subclass was thought to contribute more to endosperm texture. The role of proteins in grain hardness is reviewed in detail in Section 2.3.

2.1.2 Pericarp

Rooney and Miller (1982) explained that the pericarp of sorghum grain has three sections, namely the epicarp, mesocarp and endocarp. The epicarp is the outer most layer with thick walled rectangular cells and pigments, which strongly influence kernel colour. Pericarp colour is genetically controlled by R and Y genes resulting in red (R Y), yellow (rrY) and white (R-yy or rryy) sorghum colours (Earp et al 2004). The endocarp is the innermost layer of the pericarp and consists of cross and tube cells. The sorghum mesocarp is several layers thick and seemingly determines pericarp thickness. The pericarp thickness varies among sorghum genotypes and within individual kernels with the thickest part at the crown and the thinnest area over the embryo. The study by Earp et al (2004) revealed that the pericarp thickness varied among sorghum varieties related to the quantity of starch granules in the mesocarp. Their study showed varieties with a thin pericarp had fewer starch granules than those with a thick pericarp. Fig 2.2 shows the sections through the pericarp of tannin sorghum with starch granules in the mesocarp, and the testa and aleurone layers. The testa layer is thicker in type II and type III sorghums, which are pigmented and contain condensed tannins.

Pericarp thickness is an important property in sorghum grain milling. Thin pericarps are more tightly attached to the kernel, than thick pericarps (Bassey and Schmidt 1989). According to Beta et al (2001a) thin pericarp sorghum varieties decorticate efficiently and are more suitable for mechanical decortication than those with a thick pericarp. According to Taylor and Dewar (2001), starch granules in the mesocarp contribute to pericarp friability during dry milling. A friable pericarp is undesirable as it is not separated as fines but becomes incorporated into the meal or flour, causing contamination (Perten 1984). Watson (2003) described the maize pericarp as being comprised of dead cells except the seed coat, which is amorphous and thought to be a semi-permeable membrane that affects hydration of the kernel. The maize mesocarp is devoid of starch granules unlike sorghum. Maize pericarp thickness is uneven around the kernel due to differences in compression than the number of cell layers.

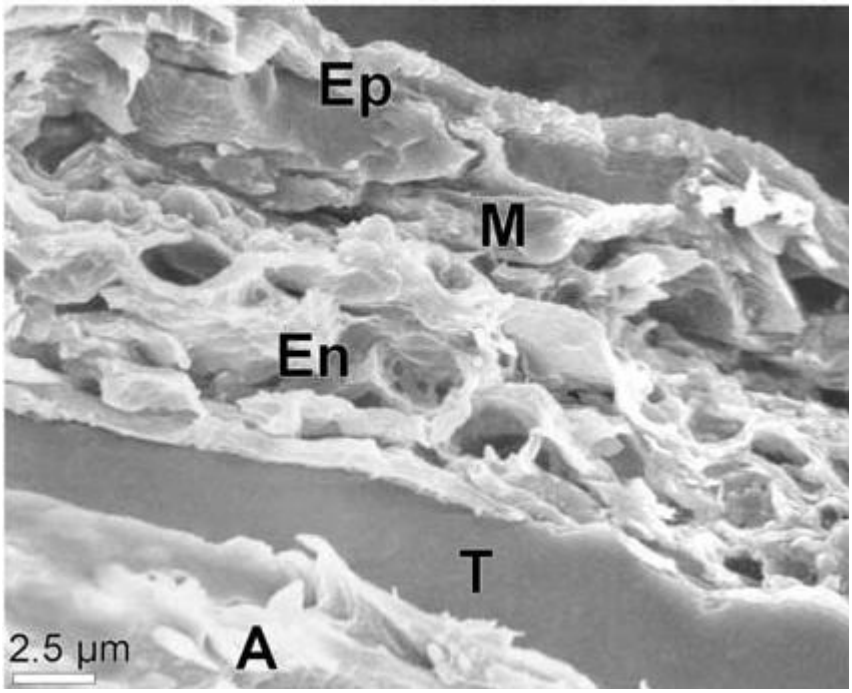


Fig 2.2. Scanning electron micrograph of a tannin sorghum pericarp containing; Ep, epicarp; M, mesocarp; En, endocarp;T, testa layer: A, aleurone layer (Earp et al 2004).

2.1.3 Germ

The germ of maize (and sorghum) is composed of two parts: the embryonic axis and the scutellum (Watson 2003). The scutellum cells contain oil bodies, protein bodies and only a few starch granules, hence the high concentration of lipid. Sorghum and maize both have a proportionally large germ relative to the size of the endosperm, resulting in high grain oil content. Degerming during milling removes the germ reducing the impact of oil rancidity. This process is widely used in commercial maize milling. However, Taylor and Dewar (2001) highlighted that degerming is incomplete in sorghum as the sorghum kernel is round shaped and its germ is embedded.

2.2 Research into methods for measuring sorghum and maize hardness

Several techniques, destructive and non-destructive, are used to measure sorghum and maize grain hardness. Taylor and Duodu (2009) described in detail, testing methods for predicting the processing quality of maize and sorghum and other non-wheat cereals. Despite the numerous methods used, it is not known which methods are more suitable for sorghum and for maize kernel hardness evaluation. The sections that follow will review hardness testing methods commonly used for sorghum and maize quality evaluation. These methods are divided into destructive and non-destructive ones.

2.2.1 Destructive methods

2.2.1.1 Abrasive milling

One of the most common methods used to measure sorghum and maize grain hardness involves decortication. A small scale laboratory decorticator such as the Tangential Abrasive Dehulling Device (TADD) is used for decortication to partially process grain for hardness

testing and porridge cooking tests. Oomah et al (1981) and Reichert et al (1986) described the TADD. The instrument comprises sample cups on a sample-cup plate. Decortication is effected by the rotation of a grinding wheel or other abrasive material below sample cups. Grains move freely in the cups and are decorticated on contact with the abrasive disk. Grain hardness is then measured by weight difference and expressed as percentage kernel removed or as Abrasive Hardness Index (AHI), which is derived by plotting retention time against percentage kernel removed during decortication. The TADD is robust and can be applied to cereals, legumes and oil seeds. The limitation of the TADD is that the abrasive disk (normally abrasive paper) may be worn out with the time giving inconsistent results. This can be monitored with the use of a standard sample of known yield.

In a study conducted in several laboratories to predict maize hardness, Lee et al (2007) found that maize TADD hardness results were highly reproducible and repeatable, an advantage of using the instrument. Using a TADD, Reichert et al (1982) decorticated 31 sorghum cultivars and found that the floury cultivars had the lowest AHI and extraction rates (percentage of kernel weight removed) and the highest AHI was in the mostly corneous varieties. Besides the TADD, other researchers have used various mills to decorticate sorghum. Kirleis and Crosby (1982) used a Strong Scott laboratory barley pearler to decorticate 15 sorghum cultivars varying in endosperm texture. Abrasive milling performance, expressed as pearling index, was related to percent vitreousness (corneousness), kernel density and particle size index. The vitreous endosperm textured cultivars had better abrasive milling performance than floury cultivars. Desikachar (1982) used a McGill laboratory rice mill to decorticate 16 sorghum cultivars and reported similar findings. Higher decortication yields and lower endosperm fragments were obtained with hard kernels. An alternative technique to abrasive decortication is the Single Kernel Characterization System (SKCS). Bean et al (2006) found that sorghum TADD hardness and SKCS-HI were correlated ($r = 0.67$, $p < 0.001$). However, the mode of action of SKCS is different from that of TADD. According to Osborne and Anderssen (2003), SKCS-HI, is determined by a response to crushing compared to the successive removal of grain outer layers using the TADD (Oomah et al 1981; Reichert et al 1986). The initial crush

response affects the aleurone layer and lastly, compression of the endosperm (Osborne and Anderssen, 2003).

2.2.1.2 Pasting

Workers have investigated whether there are relationships between sorghum and maize pasting properties and grain hardness. Almeida-Dominguez et al (1997) used a Rapid Visco Analyser (RVA) to distinguish maize kernels of varying hardness. The authors found that peak viscosity was correlated with kernel hardness values measured with a TADD, density by floatation and endosperm texture. In their study, endosperm texture and proteins were thought to affect the pasting behaviour of maize of different hardness levels. In floury kernels, hydration proceeds with ease as the starch granules are loosely packed. However, in harder grains, the starch granules are compacted by the protein matrix and may require longer hydration times, thereby exhibiting lower peak viscosities than floury cultivars. Taylor et al (1997) observed lower peak viscosity in harder sorghum. Kafirins of sorghum are also thought to play a role in lowering viscosity of hard sorghum since they surround starch granules and their hydrophobicity and disulphide bonding presumably limit water penetration to the starch granules (Chandrashekar and Mazhar 1999).

According to Chandrashekar and Kirleis (1988), higher levels of kafirin containing protein bodies in hard sorghum affects pasting by hindering starch gelatinisation (actually granule expansion). The protein bodies remain buried in the protein matrix even after cooking. Ezeogu et al (2008) showed that in hard sorghum (corneous endosperm), the protein matrix collapsed and matted extensively due to high levels of disulphide bonding between matrix proteins. However, this matting was lower in maize, due to limited disulphide bonding. Moreover, starch granules of hard sorghums appeared to be enclosed in protein matrix and cell wall and this packing also affected granule expansion (Ezeogu et al 2008). In soft sorghum, starch granules were loosely packed in the protein and expanded more on cooking than that of hard sorghum (Chandrashekar and Kirleis 1988). This was due to higher water uptake resulting in protein matrix expansion and breaking down to some extent (Ezeogu et al 2008).

Chandrashekar and Kirleis (1988) and Ezeogu et al (2008) used the reducing agent 2-mercaptoethanol to reduce the disulphide bonds in sorghum kafirins and open up the protein matrix structure of the sorghum proteins. The 2-mercaptoethanol treatment allowed expansion of the protein matrix and starch granules during cooking. Working on maize, Almeida-Dominguez et al (1997) found that coarse particles from hard grains took longer to reach peak viscosity and produced lower peak heights than in fine particles. They suggested that this was due to the relatively larger surface area of fine particles, which would increase water uptake.

Phenolic compounds have been implicated as influencing pasting properties of sorghum. Beta and Corke (2001) reported varying levels of pasting and retrogradation in condensed tannin sorghum starches. In their study, condensed tannin sorghum with a high peak viscosity apparently had lower final viscosity, while sorghum, which had low peak viscosity, had higher final viscosity. As condensed tannins are known to bind proteins, the implication is that the protein-tannin interactions may alter the functionality of sorghum starch during pasting and retrogradation.

2.2.1.3 Endosperm texture

Endosperm texture is generally determined visually by estimating the relative proportion of the corneous to floury endosperm and scoring a value against a set of standards. Rooney and Miller (1982) described endosperm texture measurements by assigning ratings on a scale of 1 (most corneous) to 5 (very floury) of longitudinal sections of cut grains. International Association for Cereal Science and Technology (ICC) has since recommended a three point rating system to denote endosperm texture against a set of standards as shown in Fig 2.3. This method is as Draft Standard Method No. 176 of the International Association for Cereal Science and Technology (ICC 2008).

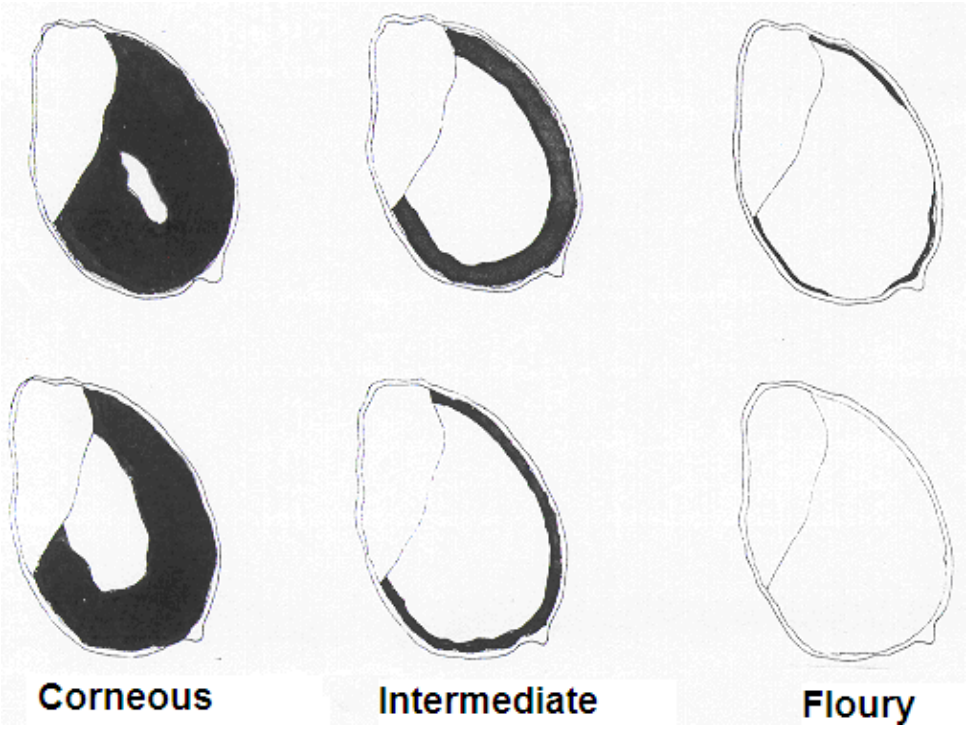


Fig 2.3. A 3-point rating system for evaluating sorghum endosperm texture (ICC 2008).

2.2.2 Non-destructive methods

Non-destructive techniques do not involve grinding or breaking down of the grain. Thus, they generally require less time and labour compared to destructive methods. Also, in an early breeder's collection, destruction of the grain can be limiting as the breeding material is only available in small quantities. Among the methods documented are Near Infrared Spectroscopy (NIRS) (Williams 1979), digital image analysis (Erasmus and Taylor 2004), test weight (Method 55-10.01, AACC International 2010,) and density tests (Paulsen et al 2003). It should be noted that NIRS can also be destructive where ground sample is used instead of whole kernels.

2.2.2.1 Near infrared spectroscopy

The principle underlying near infrared spectroscopy (NIRS) is that light of a particular wavelength in the near infrared region is absorbed by some bonds such as C-H, O-H and N-H, which vibrate in proportion to their concentration in the grain. Samples can either reflect the light in Near Infrared Reflectance (NIR) or transmit light in Near Infrared Transmittance (NIT) spectroscopy. Williams (1979) used the NIR for screening wheat for protein and hardness. The equipment was calibrated for wheat hardness using the particle size index (PSI) test. Three types of mills were used to grind samples and the burr mill was considered the most suitable for NIR hardness testing as it could clearly screen wheat cultivars of different hardness. De Alencar Figueiredo et al (2006) tested sorghum for hardness with NIR calibrated using PSI. The authors also concluded that the nature of the sample affected the calibration and that ground samples gave better calibration equations than whole grain.

Wehling et al (1996) used NIR spectroscopy to predict dry milling quality of dent maize calibrated to TADD AHI. The authors found a correlation coefficient of r = 0.87 between TADD and NIR. The authors recommended a wavelength of between 1100 and 1175 nm. The absorption band was thought to correspond to the –CH and –OH bonds due to carbohydrate, protein and lipids of the grain. Thus, interaction of the chemical bonds and the strength between them could be related to grain hardness, which is dependent on protein and starch interactions.

Robutti (1995) used NIT instead of NIR for maize quality testing. The author found a strong relationship between Near Infrared Transmittance hardness and test weight, percentage floaters and the ratio of coarse particles to fines. NIT spectra ranging 600 and 1100 nm was used to scan whole maize. Orman and Schumann (1991) compared calibrations developed for grain by NIT and NIR. Transmission data were more reliable than those of reflectance data. They recommended a wavelength of 1100 to 2500 nm for whole grain using reflectance and 680 to 1235 nm for transmittance spectroscopy. The wavelengths were within the ranges used by Wehling et al (1996) and Robutti (1995) for reflectance and transmission measurements,

respectively. Van Loggerenberg and Pretorius (2004) developed a maize hardness testing technique, commonly known as the Milling Index using Near Infrared Transmittance. The NIT Milling Index was developed by roller miller maize samples through three rollers with width gaps of 0.08, 0.3 and 0.38 mm. The NIT Milling Index was calculated from the relative proportions of meal and bran and used to develop a calibration for a whole grain NIT instrument. Hardness of whole grains was analysed at 860 nm and the results were found to be satisfactory.

Baye et al (2006) attempted to develop calibrations for maize composition using a single kernel spectroscopy. NIT and NIR spectra were collected from maize kernels of varying genotypes and environments. NIT was found unsuitable as the spectra gave high levels of noise because of NIT sensitivity to kernel density or total mass. The authors explained that this was caused by the failure of the long wavelength to penetrate the single kernels of the relatively large maize grain.

2.2.2.2 Translucency

According to Hoseney (1994), the appearance of the endosperm is as a result of the packing of the starch granules. In translucent (corneous) endosperm, starch granules are tightly packed without airspaces and allow light to diffuse through the kernel. In the floury endosperm there are air spaces, which diffract light because of the loosely packed structure. The air voids give the endosperm an opaque or chalky appearance (Serna-Saldivar and Rooney 1995).

A light box to estimate endosperm translucency in maize is commonly used to estimate grain hardness. Erasmus and Taylor (2004) refined the light box technique by developing a digital image analysis procedure to measure maize kernel translucency. This involved placing a whole kernel on top of an illuminated surface, which was smaller than the kernel to eliminate light from external sources. The light was allowed to pass through the kernel creating a contrast between the vitreous and opaque endosperms. Translucency as a percentage of the

whole kernel was correlated with the percentage vitreous endosperm as determined by hand dissection. A highly significant correlation between translucency of whole kernel and vitreous endosperm yield was obtained ($r = 0.77$, $p < 0.001$). However, the drawback of the technique was that considerable time was spent adjusting illumination and positions of the kernels in the box, which was key to obtaining accurate results. Image analysis was also used by Louis-Alexandre et al (1991). Their technique involved photography of longitudinal sections of cut kernels held by modeling clay. The computer vision generated an outline of the endosperm components and calculated endosperm area. A vitreousness index was developed as the percentage of vitreous kernel to total endosperm area. Vitreousness correlated with total endosperm area. Despite using digital image analysis, this technique involved destruction of the sample by cutting through the grain and required an extra step of using modeling clay. The method by Erasmus and Taylor (2004) eliminated these problems with the use of whole kernels and optimising illumination around the kernels.

Another technique that can be considered as a variant of maize kernel translucency is stress crack determination, also observed on an illuminated surface. Heat causes stress cracks (internal fissures), which weaken the grain structure. Stress cracks cause brittleness of the grain such that during dry milling the grain cannot handle the mechanical force of milling, resulting in poor grit yield and low grit quality. Peplinski et al (1989) found that air drying maize at 60ºC caused fissures in the grain and increased stress cracks by 25 to 30 fold. These observations were in agreement with those of Kirleis and Stroshine (1990) who also reported severe stress cracking at 60°C. The severity of stress cracking was expressed in terms of a stress crack index (SCI), which quantified stress cracks by categorizing them into single, double and multiple. According to Jackson et al (1988), the counting and quantification of stress cracks is the most reliable method to predict stress cracking. Alternatively, the use of the Fast Green colorimetric test, which stains the cracks makes it easy to identify and count the cracks (Chowdhury and Buchele 1976).

Stress crack index (SCI) can be calculated as follows:

SCI = (% single stress cracks x 1) + (% double stress cracks x 3) + (% multiple stress cracks x 5) (Paulsen et al 2003).

The US Grain Council recommends an average SCI of 140 for commercial maize with a lower SCI being preferred (Paulsen et al 2003). Kirleis and Stroshine (1990) investigated the impact of stress cracking on hard and soft maize. The authors found that hard maize types were more affected by stress cracking than soft types. However, stress cracking did not influence milling quality as the hard maize, despite severe stress cracking, still gave better milling quality than the soft maize. Therefore, grain hardness seemingly has a greater effect on milling quality than stress cracking. Similarly Jackson et al (1988) found that stress cracks alone had minimal effect on alkaline processing (nixtamalization) of maize.

2.2.2.3 Test weight

Test weight is an important criterion for grain grading. The United States Department of Agriculture (USDA) has outlined the grading requirements for sorghum and maize in the Grain Inspection, Packers and Stockyards Administration (GIPSA) Handbook (GIPSA 2007). According to Rooney (2007), high test weight is an indicator of grain plumpness, kernel filling and a higher proportion of corneous to floury endosperm, hence better milling properties. Li et al (1996) found that high maize test weight was associated with a high ratio of corneous to floury endosperm, high milling energies and resistance time to grinding using the Stenvert Hardness Test. Pomeranz et al (1986) studied the relationship between test weight and other hardness properties of yellow maize. Test weight was correlated with percentage floaters, Stenvert hardness test, breakage susceptibility (Stein hardness test) and near infrared reflectance measurements except for 100-kernel weight. In a corroborative study among different laboratories, Lee et al (2007) found that test weight was correlated with pycnometer density as did studies by Lee et al (2005).

2.2.2.4 Kernel size

Kernel size is an important factor in milling while grain uniformity is desirable for milling efficiency (Gaines et al 1997). These authors showed that in soft wheat, small kernels were softer than large kernels. Moreover, small kernel size reduced milling and baking quality of wheat. In sorghum, Lee et al (2002) found that sorghum kernel size was related to grain hardness with larger kernels giving higher milling yields. This confirmed earlier studies by Kirleis and Crosby (1982) that kernel size affected sorghum milling. They found that larger kernels decorticated better than small kernel. This observation was made among cultivars exhibiting the same endosperm texture.

2.3 Sorghum and maize proteins and their influence on grain hardness

In the sorghum and maize starchy endosperm, proteins occur in the endosperm protein matrix and protein bodies (Hoseney 1994). The sorghum prolamin is called kafirin and its amino acid composition is similar to that of maize zein. The prolamins of maize and sorghum, however, differ in their solubility and cross-linking. Kafirin is not soluble in aqueous alcohol at room temperature and is more cross-linked than zein (Chandrashekar and Mazhar 1999). Zein and kafirin comprise a number of subclasses that vary in proportions in the corneous and floury endosperm.

The causes of sorghum and maize endosperm hardness are not fully understood. Chandrashekar and Mazhar (1999) comprehensively reviewed the state of knowledge concerning sorghum and maize grain hardness. Sorghum and maize grain hardness is apparently affected by a number of factors including the types and concentrations of endosperm storage proteins, specifically the prolamins. In their study to quantify and determine the distribution of sorghum kafirins in cultivars of varying endosperm hardness, Mazhar and Chandrashekar (1995) showed that the γ-kafirin subclass was predominant in the corneous endosperm. In soft sorghum types, α-kafirin was evenly distributed throughout the

starchy endosperm, while β- and γ-kafirins were concentrated in the floury endosperm. The reasons for this distribution of the kafirin subclasses in the sorghum endosperm were not clear, although it was postulated that it could be due to variations in nutrient supply, where hard grains received nutrients more uniformly throughout seed development. Gamma- and α-kafirins were thought to modify endosperm texture through disulphide cross linkages and by increasing protein body size, respectively. The cross-linking of γ-kafirins through disulphide bonding formed a rigid structure as observed in hard cultivars due to the high proportion of this kafirin subclass.

In transgenic sorghums with reduced kafirin synthesis, Da Silva et al (2011b) found that kafirin was less polymerised in these sorghums compared to normal varieties. This was because of suppressed γ-kafirin synthesis. According to Da Silva et al (2011a) suppressing kafirin synthesis in these transgenic lines altered the endosperm texture and resulted in a floury endosperm. In maize, Mestres and Matencio (1996) showed that vitreousness (corneousness) may also be associated with the γ-zein fraction and friability with the α-zein fraction, which affects milling quality. It has been shown that in maize, protein content was not correlated with vitreousness of endosperm (Mestres et al 1991). Paiva et al (1991) studied the role of proteins in Quality Protein Maize (QPM) hardness. Gamma-zein seemed to make a major contribution to the hardness of QPM compared to floury, opaque and normal genotypes. High levels of cysteine in QPM were thought to be involved in disulphide bonding and contributed to hardness and vitreousness of QPM varieties.

2.4 The influence of grain hardness on porridge quality

Maize and sorghum porridges are staples in most parts of Africa and according to Rooney et al (1986) and Taylor et al (1997) consumers prefer non-sticky stiff porridges. Grain hardness affects porridge quality, particularly pasting properties and consumer acceptance. The effect of grain hardness on porridge quality has been studied extensively. Bello et al (1990) used a penetrometer to measure firmness of sorghum tô, a West African gel-like porridge. The

authors found that tô porridges prepared from corneous endosperm grains were firmer than those from floury grains. Cagampang and Kirleis (1985) and Akingbala and Rooney (1987) had earlier confirmed the influence of a corneous endosperm to firmer sorghum tô. Kebakile et al (2008) showed that in sorghum, hard grains produced porridges of acceptable quality. Porridges made from hard sorghum grain were acceptable because they were firm, probably as a result of the hard and less water-permeable protein-starch matrix. Aboubacar et al (1999) found that sorghum porridge texture in terms of gel consistency and porridge firmness correlated with AHI.

2.5 Changes in sorghum and maize starch as they relate to grain hardness

Most of the changes in the grain structure that occur during porridge making are related to starch gelatinisation. Generally, sorghum and maize have the same swelling behaviour but differ in that the latter exhibits higher gelatinisation temperature than the former (reviewed by Taylor and Emmambux 2010).

Chen et al (2006) investigated the microstructure and morphology of maize starch granules with different amylose to amylopectin ratios. The authors showed that granules of amylopectin-rich (waxy) maize were more regular in shape than amylose-rich (amylomaize) granules. However, the surfaces of amylose-rich granules were smoother than amylopectin-rich granules. According to Rojas-Molina et al (2007), starch granular packing in both floury and corneous endosperms is random although the corneous endosperm has a relatively higher crystallinity than floury endosperm, which was attributed to the tight packing of starch granules brought about by amylopectin.

Jackson et al (1989) compared starch gelatinisation of sorghum and maize. Aqueous leaching of maize starch granules at 85°C was characterised by slight solubilisation of amylose. Solubilisation increased with an increase in amylose content and waxy maize starch was only slightly soluble due to the absence of amylose. Sorghum starch showed similar characteristics

although the initial melting temperature of the crystalline region was almost 7°C higher than that of maize. X-ray diffraction patterns of cooked maize endosperm in water at 72°C and 92°C showed that thermal treatment caused the external layers of the endosperm to lose crystallinity and become amorphous, while internal layers remained mostly crystalline (Rojas-Molina et al 2007).

Proteins also influence starch gelatinisation. Han and Hamaker (2002) found that proteins were concentrated in the envelopes of swollen starch ghosts isolated from normal maize starches gelatinised at 70°C. However, these starch ghost-associated proteins were scarce in the internal central region of the ghost, which implied that proteins had a structural function to maintain the integrity of the starch ghosts and could influence paste viscosity and breakdown. Using Environmental Scanning Electron Microscopy (ESEM) and Scanning Electron Microscopy (SEM), McDonough et al (1997) studied microstructural changes during steam flaking of sorghum. They showed that the swollen starch granules leached amylose, which formed a starchy paste. The starchy paste together with damaged starch granules, protein matrix and other cell components formed a continuous starchy phase, as evidenced by a stringy network between starch granules. Hydration is very important for starch gelatinisation and a moisture content of 18-20% was recommended for complete gelatinisation for the production of sorghum flakes.

2.6 Grain modification during malting and the effect of hardness on malt quality

One of the reasons for interest in sorghum modification during malting is to determine how hardness influences the duration of malting that would produce acceptable malt for porridge making. However, the optimal duration for malting of cultivars varying in hardness to produce porridges is not known. It would be economical to produce desired malt within a short time such that more malt can be produced with the same equipment and labour.

Glennie et al (1983) found that sorghum endosperm modification during malting was characterised by degradation of the starch granules and protein bodies and protein matrix by endogenous hydrolytic enzymes into simple sugars and free amino nitrogen, respectively. Modification started at the endosperm-scutellum interface followed by the floury endosperm and lastly the corneous endosperm. The glutelin endosperm protein matrix degraded first, while starch granules and protein bodies degraded at the same time. Degradation of starch granules was evidenced by pitting of the granules. Only the starch granules in the pericarp remained unchanged. Aleurone layer modification was characterised by mineral loss probably as a result of phytic acid hydrolysis by the phytase enzyme during malting (Eskin and Wiebe 1983) resulting in the release of the complexed minerals. Importantly, in sorghum, endosperm cell walls remained intact after malting (Glennie et al 1983; Glennie 1984). Taylor (1983) found that malting sorghum reduced prolamins by 84% with respect to their original quantity in the grain. However, the electrophoretic pattern of the malted grain prolamin was identical to that of native grain and the author concluded that the prolamins were degraded to low molecular weight peptides or amino acids, which presumably accounted for the increased non-protein nitrogen content.

Barley malting and its modification during malting is the most researched among cereals. Osborne and Anderssen (2003) and Osborne et al (2005) studied barley malt modification. In their studies, barley malt showed a decrease in hardness by the second day of malting. The decrease was attributed to the softening of the grain outer layers during steeping and loss of cellular structure, reduced dry matter (malting loss), loss of kernel orientation and endosperm collapse. Earlier, Brennan et al (1997) studied the modification patterns of barley malt among cultivars of high and low malting quality. Generally, modification of high quality malting barley was found to be faster and more uniform than in cultivars of poor quality malting. Modification of high quality malting cultivars was characterised by protein degradation from the sub-aleurone layer towards the inner endosperm, although protein breakdown in the inner endosperm occurred after more than four days of malting. Starch granule degradation was evidenced by the pitting of the granules and partial destruction of the concentric shells after almost six days of malting. The endosperm cell walls were no longer visible after this malting

period, a very significant difference to sorghum where cell walls remained intact during malting (Glennie et al 1983, Glennie 1984).

Several studies have reported a relationship between the duration of barley malting and grain hardness (Nielsen 2003; Psota et al 2007; Vejrazka et al 2008). Brennan et al (1996), Nielsen (2003) and Psota et al (2007) were in agreement that grain hardness adversely affected accessibility of hydrolytic enzymes in barley. This was because in hard grains, the starch-protein interaction was strong and slowed amylolytic and proteolytic enzyme migration, hence slowing modification and collapse of the endosperm cell structure. Using the SKCS, Nagamine et al (2009) found a negative relationship between the SKCS-HI and malting quality of barley cultivars. Grain and malt hardness indices were negatively correlated with malt extract ($r = -0.48$ and $r = -0.70$, at $p < 0.01$, respectively). Diastatic power (DP) (amylase activity) was positively correlated with grain hardness index ($r = 0.79$, $p < 0.01$) and not with malt hardness index ($r = 0.31$).

2.7 Sorghum and maize phenolic acids and their role in grain hardness

Several phenolic acids have been reported in sorghum and maize grain in both the free and bound forms. However, the role that phenolic acids play in sorghum and maize grain hardness has not been fully ascertained. Hahn et al (1983) separated eight phenolic acids from sorghum grain. Protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, *p*-coumaric, ferulic acids were found in both free and bound forms, gallic acid was in the bound form and vanillic acid mostly in the free form. Cinnamic acid was found in some varieties in the free form. In maize, Li et al (2007) also found eight bound phenolic acids. Bound phenolic acids can be released with alkali, which hydrolyses ester bonds between phenolic acids and the grain cell walls (Mujica et al 2009). In the endosperm cell walls, ferulic acid cross-links with arabinoxylans (Glennie 1984). Proteins are also found adhering to endosperm cell walls (Glennie 1984; Piot et al 2001). This association of ferulic acid and proteins with endosperm cell walls could affect grain mechanical properties related to kernel hardness (Piot et al 2001).

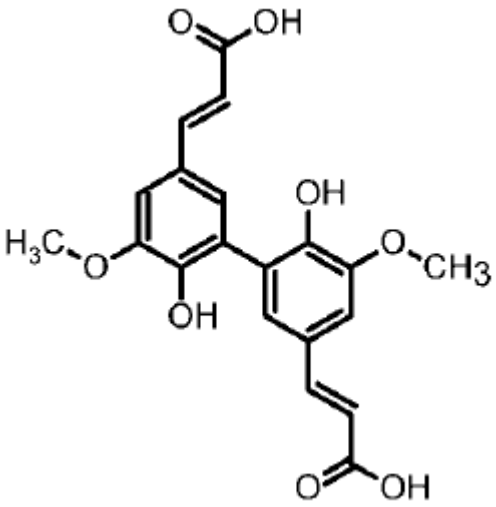
Ferulic acid occurs in highest quantities among phenolic acids in several cereals. Bily et al (2004) found high levels of cell wall bound ferulic acid in whole grain sorghum, maize, rice and wheat. The study also revealed that ferulic acid was distributed unevenly in the different grain fractions. The pericarp had the highest levels of ferulic acid followed by the embryo and lastly the starchy endosperm. Glennie (1984) found only ferulic acid and its dimers in the sorghum endosperm cell walls cross-linked with glucuronoarabinoxylan. Del Pozo-Insfran et al (2006) found almost three times more ferulic acid in white than blue maize. The high concentration of ferulic acid was thought to be responsible for the harder endosperm of white maize compared to the blue maize. In their study, Bily et al (2004) also identified diferulic acids (DFA) from whole grain sorghum and maize and their pericarp and embryonic tissues. Major diferulic acids found in sorghum and maize were 8-*O*-4′ DFA, 5-5′ DFA, 8-5′ linear form DFA, and 8-5′ benzofuran form DFA (Fig 2.4). Besides the dimers, trimers of ferulic acid have also been reported in maize bran by Rouau et al (2003). They found a triferulic acid corresponding to 4-*O*-8′, 5′-5′ dehydrotriferulic acid. However, the structural role and covalent bridging of the trimer could not be ascertained, although it was thought to form a three-fold link with glucuronoarabinoxylans, which would likely influence cell wall structure.

2.7.1 Mechanisms of cross linking of phenolic acids to cell walls and their influence on grain hardness

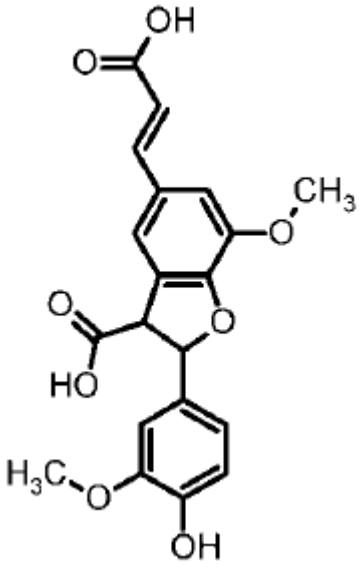
The ratio of corneous to floury endosperm affects grain hardness and the majority of the research has focused on the influence of proteins. However, there is limited fundamental research on the role of phenolic acids on grain hardness.

The mechanism by which phenolic acids influence grain hardness could be related to chemical bonding through cross linking of the compounds within the plant cell walls. Most studies have shown that ferulic acid and its oligomers are the most prevalent in forming linkages with endosperm cell walls in sorghum grain (Glennie 1984). According to Lam et al (1992a), ferulic acid simultaneously forms ester-ether linkages between the arabinoxylan and lignin.

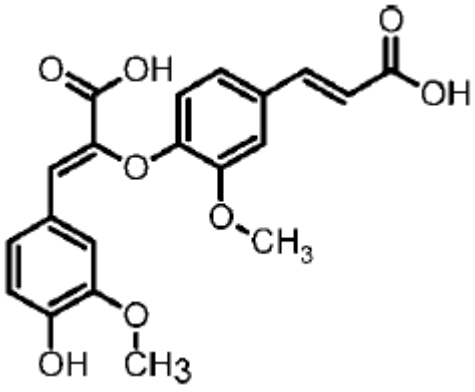
Ferulic acid ester linkages are formed during early maturation to primary cell walls of glucuronoarabinoxylans and later react with lignin quinone methide intermediates to form benzyl ether linkages in lignified cells walls at maturity. The ether linkages presumably further reinforce the cell walls. The proposed scheme for the formation ester-ether bridge between polysaccharides and lignin in cell walls is shown in Fig 2.5.



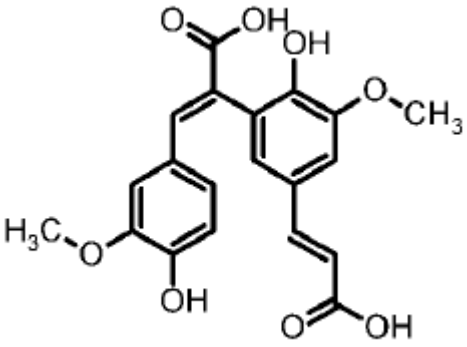
5-5′ DFA



8-5′ -benzofuran form DFA



8-*O*-4 DFA



8-5′ DFA

Fig 2.4. Chemical structures of some of the diferulic acids found in sorghum and maize (Adapted from Callipo et al 2010).

Para-coumaric acid is also likely to play a role in cell wall cross-linking. Lam et al (1992b) showed that small amounts of *p*-coumaric acid are esterified to arabinoxylan cell walls and more extensively to lignified cell walls at maturity, which was confirmed by Ralph et al (1994b) and Sun et al (2002). Thus, coupled with the ferulic acid ester linkages to arabinoxylans and etherification to lignin, *p*-coumaric acid, is also likely to form strong linkages with cell walls.

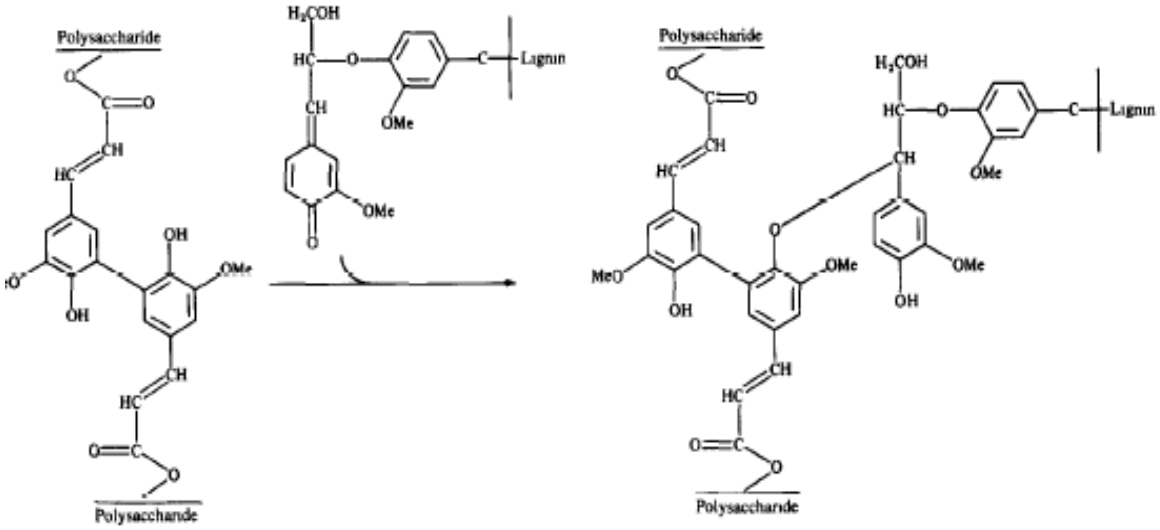


Fig 2.5. Proposed scheme for the formation of ester-ether bridges between polysaccharides and lignin in cell walls (Lam et al 1992b).

2.8 CONCLUSIONS

There are several methods used for evaluating sorghum and maize grain hardness. Differences in grain hardness cause variations in pasting properties and textural quality of sorghum and maize porridges. Although sorghum malts are important in porridge making, their modification as affected by grain hardness is not known. The knowledge of sorghum malt modification of cultivars varying in hardness will be important to determine the duration of

malting required to produce acceptable malt porridges. The review has also shown that in addition to the prolamins, phenolic acids may also influence sorghum and maize grain hardness, however their role has not been fully ascertained. Ferulic acid and its oligomers appear to likely play a role in sorghum and maize hardness through their cross-linking with grain cell walls and this will form the basis of investigation in this study.

3 HYPOTHESES AND OBJECTIVES

3.1 HYPOTHESES

1. Quality tests for evaluating sorghum grain hardness would be the same as those of maize. Sorghum and maize are similar in structure, chemical composition and the basis for hardness (Chandrashekar and Mazhar 1999).

2. Sorghum malts will be softer than sorghum grain and will produce sticky porridges. Sorghum malting will reduce grain hardness through modification of the starchy endosperm (Glennie et al 1983). Sorghum flours from hard grain will produce stiff porridges (Kebakile et al 2008) as the corneous endosperm particles will restrict starch granule swelling resulting in a high proportion of non-ruptured gelatinised starch granules that reinforce the porridge matrix (Kebakile 2008).

3. Soft sorghum cultivars should modify easily and give better quality malt than hard sorghum grain (Glennie et al 1985). Hard grains have strong starch-protein interactions, which slow amylase enzyme migration, hence slowing modification (Psota et al 2007).

4. Sorghum and maize cultivars with high levels of bound phenolic acids are likely to be harder than those cultivars with lower phenolic acid content. Phenolic acids are bound to sorghum and maize cell walls through cross linkages with arabinoxylans. The phenolic acid-arabinoxylan cross linkages will affect grain mechanical properties such as hardness by reinforcing and strengthening cell walls.

3.2 OBJECTIVES

1. To determine the relationships between simple grain quality tests and their applicability to sorghum and maize grain hardness screening and selection.

2. To determine the relationship between sorghum grain hardness, malt hardness and malt porridge quality.

3. To determine the effect of malting on sorghum hardness and the relationship between sorghum malt modification and grain hardness.

4. To determine the relationship between phenolic acid content and composition in sorghum and maize bran and flour, and sorghum and maize grain hardness.

4 RESEARCH

4.1 Relationships between Simple Grain Quality Parameters for the Estimation of Sorghum and Maize Hardness in Commercial Hybrid Cultivars

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ABSTRACT

Grain hardness affects sorghum and maize processing properties especially for dry milling. Initially, the hardness of diverse sorghum and maize cultivars were determined. A variety of simple grain quality parameters were then assessed on 17 sorghum, and 35 white maize commercial hybrid cultivars grown in six and four locations, respectively, in South Africa. The purpose was to determine tests that can be used to distinguish hardness in commercial sorghum and maize. The grains were characterised by test weight (TW), thousand kernel weight, decortication using the Tangential Abrasive Dehulling Device (TADD) and kernel size (KS). Maize was also characterised for susceptibility to breakage, stress cracking and Near Infrared Transmittance (NIT) Milling Index. In the cultivars varying widely in hardness, TADD and TW were correlated in both cereals and formed the basis for hardness testing of the commercial cultivars. Transluceny was also strongly correlated with maize TADD hardness. Among the commercial sorghum cultivars, principal component analysis showed that in non-tannin and tannin sorghums, TADD hardness and TW were closely correlated ($p < 0.001$). In maize, TADD hardness was closely correlated with NIT Milling Index and TW. Hence, TADD hardness and NIT Milling Index or TADD hardness and TW would be suitable for maize hardness evaluation. A combination of TADD hardness, TW, TKW and kernel size > 3.35 mm can be used together to select sorghum grain for hardness. Similarly, in the diverse sorghum and maize cultivars TADD and TW were correlated. It thus appears that TADD hardness and TW are an excellent way of estimating both sorghum and maize hardness that can be applied for routine batch analysis and cultivar evaluation in closely related and diverse cultivars in terms of hardness.

4.1.1 INTRODUCTION

In sorghum and maize, grain hardness is the most important parameter for assessing dry milling quality (Munck 1995). In dry milling, a high yield of pure endosperm grits is desirable. Harder grain should give higher milling yield than softer grain (Taylor and Duodu 2009). In turn, grain hardness influences product quality such as porridge stickiness and texture (Bello et al 1995; Rooney et al 1986; Taylor et al 1997). Therefore, simple tests are applied by breeders, millers and traders to estimate hardness and milling properties.

Several tests are used to estimate sorghum and maize grain hardness. These include bulk density tests such as test weight (Method 55-10.01, AACC International 2010), percentage of floaters and density by gas displacement (Paulsen et al 2003). With sorghum, grain decortication using a Tangential Abrasive Dehulling Device (TADD) is commonly used to estimate grain hardness and milling quality (Reichert et al 1986) in terms of time required to remove a certain percentage of the grain (Taylor and Duodu 2009). With maize, endosperm texture can be visually assessed using a light box to determine the relative proportion of corneous to floury endosperm, which is related to grain hardness (Rooney and Miller 1982; ICC 2008). Alternatively, digital image analysis can be used to measure maize kernel translucency (Erasmus and Taylor 2004; Louis-Alexandre et al 1991). Near infrared transmittance and reflectance spectroscopy have also been used to estimate grain hardness (Robutti 1995; Wehling et al 1996) but these methods require calibration against data of standard chemical and physical tests.

Sorghum and maize grain hardness testing methods and their relevance to end use quality are described in detail by Taylor and Duodu (2009). Several grain quality tests are applied for routine grain batch screening and cultivar selection. However, the relationships amongst these test methods are not well understood. Moreover in the real situation, the range of hardness encountered is small as commercial cultivars have been selected for specific quality attributes and tend to be closely related. Hence, screening for hardness among closely related cultivars presents a problem to the milling industry.

Hence, the objective of the work was to determine the relationships between simple grain quality tests and their value in commercial sorghum and maize hybrid grain quality selection, with respect to assessing grain hardness. Based on our experience gained in this study, we were able to summarize some of the methods commonly used for determining sorghum and maize grain hardness shown in Table 4.1.1. The Table compares the methods as to quality indicators measured, cost of equipment, speed and practical application to evaluate quality in breeding programmes and in grain marketing where millability is important.

4.1.2 MATERIALS AND METHODS

4.1.2.1 Materials

Initially, seven sorghum and five maize cultivars with diverse hardness properties were evaluated. The identity of maize cultivars could not be disclosed for confidentiality, hence only areas of origin are given. Sorghum cultivars were obtained from the Agricultural Research Council, Potchefstroom, South Africa. Maize cultivars originated from Brazil, Spain, Argentina, Australia and USA. A study was then conducted on 35 maize cultivars and 17 sorghum cultivars grown in South Africa, representing commercial hybrids of the National Cultivar Trials during the 2008/2009 growing season. Maize cultivars, all of the white dent type, were grown in four locations covering the western region (Klerksdorp and Potchefstroom localities), temperate eastern region (Petit) and the cold eastern temperate region (Bethlehem locality). Sorghum hybrids were grown in six locations, mainly of the western region where sorghum is largely grown in South Africa namely Klipdrift, Kafferskraal, Goedgedacht, Dover, Platrand, and Parys. For ease of comparison, non-tannin sorghums were evaluated separately from condensed tannin sorghums. Sorghum was grown under dryland conditions. All samples were thoroughly threshed and cleaned to remove broken and foreign material to minimize their effects on grain quality measurements. The samples were stored at 4°C until analysed.

TABLE 4.1.1 Simple Methods used for Grain Quality Evaluation, their Advantages, Disadvantages and Applicability

Method	Parameter/quality indicator	Advantages	Disadvantages	Applicability
Test weight Test weight per bushel or kg/hl apparatus	Grain density	Inexpensive device, low maintenance cost Rapid, high repeatability and reproducibility Non-destructive method	Affected by grain packing in measuring apparatus, moisture content, kernel shape, broken kernels and foreign material Not suitable for early generation breeding	Applicable to breeding programs and cultivar evaluation with limited grain sample size. Rapid test on dockage for commercial large and small-scale milling plants and grading for grain marketing
Thousand kernel weight Seed counter and balance	Grain size and grain density	High repeatability and reproducibility, non-destructive indirect measure of grain density	Time consuming if done manually (without a seed counter)	Suitable for breeding programs with limited grain sample size. Also applicable in commercial grain quality control and processing, both large and small-scale
Abrasive Decortication Tangential Abrasive Dehulling Device (TADD)	Ease of grain to be abraded- indirect measure of grain hardness and milling quality	TADD is robust and can be applied to both maize and sorghum High repeatability and reproducibility Low maintenance cost Equipment can be manufactured locally	The abrasive disk may be worn out with time and vary milling yields although this can be monitored with the use of a standard sample of known yield.	Potential use at commercial level (both small and large scale) The multi-cup sample holder allows several samples to be decorticated simultaneously within a short time (5 to 10 min)
Stress cracks Light box	Proportion of grain with cracks and number of cracks	Apparatus cheap to set up Stress cracks may be quantified using the Stress Crack Index	Stress crack counting tedious and time consuming and to a degree subjective Unsuitable for sorghum as it is opaque and does not transmit light like maize	Time consuming for routine analysis, but suitable for small sample size
Stein Breakage Susceptibility Stein Breakage Tester	Susceptibility of grain to break under stress	Allows quantification of the potential of grain to break. Rapid analysis (4 min)	Apparatus is no longer manufactured, although other mills may be used	Suitable for commercial grain evaluation. Destructive, could have limited use in breeding programs where grain sample size is limiting
Milling Index Near Infrared Transmittance (NIT) spectrometry	Grain milling quality	Automated and rapid analysis once a calibration is developed Calibration can be used by other users. None destructive method.	Requires calibration against physical or chemical data which, could be time consuming and costly Very sensitive to sample preparation affecting precision and accuracy High initial cost to purchase the instrument and operating software Regular software and service upgrade required. Requires a relatively large grain sample size (approx 500 g)- limited use in breeding programs where grain sample size is limiting	Rapid for online processing at commercial milling plants and routine analysis in breeding programs and cultivar evaluation Skilled technical maintenance required Use could be limited to well established institutions; not economically appropriate for small-scale grain quality control and processing
Kernel size Set of sieves and sieve shaker	Kernel size	Analysis is relatively cheap. Non-destructive. Direct measure of kernel size. Does not require a large grain sample size	Can be time-consuming especially if batches are very heterogeneous in terms of kernel size.	Due to lengthy analysis time, it is not applicable in commercial grain quality analysis. Applicable in research laboratories.

4.1.2.2 Methods

Tests of maize and sorghum cultivars with a wide range of physical and hardness properties included specific density using the gas pycnometer, percentage floaters, visual assessment of endosperm texture, percentage translucency, one thousand kernel weight (TKW), Single Kernel Characterization System Hardness Index (SKCS-HI) and kernel removal using the Tangential Abrasive Dehulling Device and pasting properties. For the study of South African commercial sorghum and maize cultivars, test weight, kernel size, TKW and TADD kernel removal were evaluated. NIT Milling Index was determined for maize samples.

Density

Specific density of grain was determined using a gas pycnometer (Model MUP-1 S/N 232, Quantachrome, Syosset, NY). Density was calculated after grain volume of 80 g sample was measured by helium gas displacement. For the floatation test, fifty sound kernels were immersed in a clean solution of sodium nitrate with a specific gravity of 1.275g/cm$^{3.}$ Floating kernels were counted and expressed as a percentage. Test weight was done as outlined in the United States Department of Agriculture (USDA) Grain Inspection, Packers and Stockyards Administration (GIPSA) Handbook (GIPSA, 2007) section 1.11. A quart cup (946.35 ml) was used on the Seedburo test weight apparatus (Seedburo Equipment, Chicago, IL). Test weight was converted to the metric system and reported as kg/hl.

Kernel size

Sorghum kernel size was measured by sieving the grain according to Gomez et al (1997). A clean sample of 100 g sorghum grain was placed on a 4.00 mm round hole sieve stacked on 3.35 mm, 3.15 mm and 2.36 mm sieves and a collecting tray, respectively. The samples were screened manually by performing horizontal movements for 1 min. The grain remaining on each sieve was collected, weighed and recorded as a percentage. A 500 g sample was used for maize. The sample was placed on a 8 mm round hole sieve and stacked on top of a collecting tray. Grain was screened manually as described for sorghum. The kernels remaining on top of

the 8 mm round hole sieve were collected, weighed and expressed as a percentage of the initial weight.

Abrasive hardness

Maize and sorghum hardness were determined using a prototype Tangential Abrasive Dehulling Device (TADD) at the Agricultural Research Council, South Africa. A 50 g sample was decorticated for 5 min and sorghum and maize hardness were measured in terms of percentage kernel removed (Gomez et al 1997).

Endosperm texture

Translucency was used to visually assess maize endosperm texture of the diverse cultivars using a fluorescent tube light box. A set of standards were used to rate fifty kernels on a scale of 1 (floury) to 5 (corneous) based on the proportions of corneous to floury endosperm. Translucency was reported for kernels with a rating of at least three and expressed as a percentage of fifty kernels. Translucency was not performed on sorghum due to pigmentation of the grains. Sorghum endosperm texture was done according to ICC (2008). Twenty kernels were cut longitudinally and viewed with a naked eye to assess the relative proportions of corneous to floury endosperm on a scale of 1 (corneous) to 3 (floury) endosperm.

Stress Cracks

One hundred maize kernels were placed on a light box with the germ side facing downward and individually evaluated for stress cracks. Kernels were turned up and checked on the edges for any cracks and fissures. The number of kernels with cracks was counted and expressed as a percentage. Kernels were further categorized according to the number of cracks into single, double and multiple.

Stress crack index (SCI) was calculated as follows;

SCI = (% single stress cracks x 1) + (% double stress cracks x 3) + (% multiple stress cracks x 5).

SCI was an indicator of the severity of stress cracking (Paulsen et al 2003).

One Thousand Kernel Weight

One thousand kernel (TKW) was determined by simply weighing 1000 kernels of a representative sample and recording the weight. High TKW values indicated large grains with proportionately lower surface area.

NIT Milling Index

Grain hardness was measured using near infrared transmittance (NIT), (Infratec 1241, Grain Analyzer, Foss Tecator, Eden Prairie, MN). A Milling Index (MI) was first developed from roller milled maize samples through three rollers with width gaps of 0.08, 0.3 and 0.38 mm. The MI was calculated from the relative proportions of meal and bran and used to develop a calibration for a whole grain NIT instrument (Van Loggerenberg and Pretorius 2004). Hardness of whole kernels was analysed at 860 nm.

Stein breakage test

Breakage susceptibility was determined by analysing a 100 g sample of whole maize kernels in a Stein Breakage (SB) tester (Fred Stein Laboratories, Atchison, KS) for 4 min and weighing the broken kernels passing through a 6.35 mm round hole opening sieve (Pomeranz et al 1986).

Single Kernel Characterisation System Hardness Index

Single kernel hardness of sorghum grain was measured with a Single Kernel Characterization System (SKCS) 4100 (Perten Instruments, Huddinge, Sweden). Three hundred kernels of each sample were tested. The kernel response to crushing was recorded as the Hardness Index (HI) (Bean et al 2006).

4.1.2.3 Statistical analyses

Laboratory experiments were done in triplicate. Data was analysed by multifactor analysis of variance and means compared by Fisher's least significant differences. Pearson correlation and principal component analysis (PCA) were performed on sorghum and maize data sets. Mean square values and their significance were used to determine the effects of cultivar (C), locality (L) and C x L interaction of the measured hardness parameter. Calculations were performed using Statgraphics Centurion XV (StatPoint, Herndon,Virginia, USA).

4.1.3 RESULTS AND DISCUSSION

4.1.3.1 Physical and hardness properties of sorghum and maize cultivars with a wide range of properties

Table 4.1.2 shows physical and hardness properties of sorghum cultivars of different types. TADD hardness was significantly different between cultivars. Kernel removal with the TADD was highest in condensed tannin cultivar PAN 8625 (63.2%) followed by the white non-tannin cultivar PAN 8648 with kernel removal of 40.9%. The red non-tannin sorghum types had kernel removal of 24.0% to 37.6. According to TADD hardness results, cultivar ranking from soft to hard would be in the order; PAN 8625, PAN 8648 and then the other non-tannin cultivars, which were hard types. Endosperm texture was not significantly different for the non-tannin sorghums. Percentage floaters and density differed significantly between cultivars. PAN 8625 had a high proportion of floaters and the lowest density. Floury endosperm is associated with loosely packed starch granules and air spaces (Hoseney 1994), which probably reduces density. Test weight ranged from 74.1 to 78.2 kg/hl and was highest in PAN 8247 (red non-tannin) and lowest in PAN 8625 (condensed tannin). Test weight of PAN 8648 was similar to that of the hard sorghums.

Single Kernel Characterization System Hardness Index (HI) was also significant for cultivars. According to the SKCS, the white non-tannin cultivar PAN 8648 was the hardest followed by PAN 8247 (red non-tannin). The condensed tannin cultivar PAN 8625 had the lowest HI in agreement with TADD hardness, floaters, specific density and endosperm texture. SKCS has been successful in measuring wheat hardness. The SKCS calibration is originally for wheat although the instrument settings can be adjusted for other cereals and their HIs may be used as indicators of hardness rather than exact values (Osborne and Anderssen 2003; Pedersen et al 1996). The SKCS could be useful for sorghum when settings are adjusted and with proper sample cleaning. Attempts made by Bean et al (2006) to predict sorghum hardness using SKCS showed a moderate correlation between TADD hardness and SKCS-HI ($r = 0.67$, $p < 0.001$).

Table 4.1.3 shows hardness and physical properties of the maize cultivars. Thousand kernel weight did not vary substantially between cultivars. Spain maize had the highest TKW and no significant differences were observed amongst other cultivars. Australian and Argentinian maize cultivars were the densest. Australian maize seemed to be the hardest with low rates of kernel removed (30.0%) using the TADD. Translucency of USA maize was low, 3.33% compared to other cultivars, which ranged from 45.3 to 79.3%. Test weight ranged from 76.0 to 82.7 kg/hl and varied significantly between cultivars. Translucency indicated that USA maize was soft and these results were in agreement with those of density and TADD hardness. Due to sorghum pigmentation, translucency could not be applied to sorghum except to visually assess the endosperm texture of sectioned kernels.

A comparison between the sorghum and maize data of the cultivars with a wide range of properties showed that sorghum could be harder than maize. Kernel removal by the TADD was 35.8% in sorghum (Table 4.1.2) compared to 48.9% in maize (Table 4.1.3). Mean test weights of both grains were very similar, 77.1 kg/hl for sorghum compared to 78.5 kg/hl for maize. Notwithstanding this, sorghum grain was denser than maize, 1.36 g/cm^3 compared to 1.34 g/cm^3 for maize. Similarly percentage floaters averaged only 13.8% for sorghum compared to 23.6% in maize.

TABLE 4.1.2 Physical and Hardness Properties of the Sorghum Cultivars of Different Types

Cultivar	Endosperm texture[a]	TADD (%)	TW (kg/hl)	TKW (g)	SKCS-HI	Floaters (%)	Density Pycnometer (g/cm^3)
PAN 8901(NT)	2.10 a (0.17)	31.8b (1.3)	77.8cd (0.1)	29.2 cb (1.4)	69.6 cd (1.8)	18.7 c (1.15)	1.366 bcd (0.001)
PAN 8903(NT)	2.03 a (0.03)	37.6 c (0.8)	77.3 b (0.3)	26.0 abc (2.2)	63.0 b (1.9)	20.0 c (3.46)	1.351 ab (0.002)
PAN 8564(NT)	1.97 a (0.06)	26.2 a (2.4)	78.1 cd (0.3)	25.8 ab (1.1)	71.3cde (0.7)	1.33 a (1.15)	1.366 bcd (0.001)
PAN 8609(NT)	2.05 a (0.15)	26.8 a (0.6)	77.7cd (0.4)	30.4 c (1.0)	67.4 b (0.6)	9.33 b (2.31)	1.380 d (0.017)
PAN 8625(T)	2.47 b (0.15)	63.2 d (2.4)	74.1 a (0.5)	23.9 a (1.0)	57.7 a (2.7)	34.7 d (3.06)	1.334 a (0.003)
PAN 8247(NT)	2.00 a (0.17)	24.0 a (0.9)	78.2 d (0.1)	25.8 ab (2.6)	73.6 def (0.8)	6.00 ab (2.00)	1.370 bc (0.001)
PAN8648(W)	1.90 a (0.10)	40.9 c (1.7)	77.3 c (0.2)	25.7 ab (0.8)	75.5 f (2.7)	6.67 ab (1.15)	1.356 bc (0.001)
Mean	2.07 (0.21)	35.8 (13.0)	77.1 (1.4)	26.7 (2.5)	68.3 (6.1)	13.8 (11.01)	1.360 (0.015)

[a] Endosperm texture rated on a scale 1 (hard) to 3 (soft)
TADD, Percentage kernel removed using a Tangential Abrasive Dehulling Device (TADD); TW, Test weight; TKW, Thousand kernel weight; SKCS-HI, Single Kernel Characterization System-Hardness Index; (NT), Red, non-tannin; (T), Condensed tannin sorghum; (W), White tan-plant, non-tannin sorghum
Different letters within a column denote statistically significant differences ($p < 0.05$)
Values are means and figures in parentheses are standard deviations
n = 3

TABLE 4.1.3 Physical and Hardness Properties of Maize Cultivars

Cultivar	TADD[a] (%)	TW[b] (kg/hl)	TKW[c](g)	Translucency (%)	Density	
					Floaters (%)	Pycnometer (g/cm^3)
Brazil	49.0 c (0.8)	76.0 a (0.1)	316 a (2)	45.3 b (6.0)	12.7 b (1.2)	1.354 c (0.001)
Argentina	44.0 b (0.8)	79.0 c (0.3)	310 a (6)	62.0 c (9.2)	2.67 a (1.2)	1.369 e (0.002)
Spain	52.3 d (1.9)	77.7 b (0.1)	363 b (0)	64.0 c (2.3)	12.7 b (3.1)	1.338 b (0.001)
Australia	30.0 a (0.1)	82.7 d (0.1)	312 a (14)	79.3 d (4.2)	5.33 a (2.3)	1.361 d (0.002)
USA	69.0 e (0.6)	77.3 b (2.3)	304 a (23)	3.33 a (3.1)	84.7 c (1.2)	1.273 a (0.003)
Mean	48.9 (13.2)	78.5 (2.3)	321 (23)	50.8 (27.4)	23.6 (31.9)	1.340 (0.036)

TADD, Percentage kernel removed using a Tangential Abrasive Dehulling Device (TADD)
TW, Test weight; TKW, Thousand kernel weight
Different letters within a column denote statistically significant differences ($p < 0.05$)
Values are means and figures in parentheses are standard deviations
n = 3

Among sorghum cultivars, TADD hardness (percentage kernel removed) was highly correlated ($p < 0.001$) with floaters ($r = 0.806$), TW ($r = 0.953$), density ($r = -0.835$) and endosperm texture ($r = 0.731$) (Table 4.1.4). There was no significant correlation between TADD and SKCS-HI which may be due to the differences in their mode of action.

TABLE 4.1.4 Correlation Matrix of Physical and Hardness Properties of Sorghum Cultivars

	TW	TKW	Floaters	TADD	Pycnometer	SKCS-HI	ET
TKW	0.534*						
Floaters	-0.849***	-0.332 ns					
TADD	-0.953***	-0.542 ns	0.806***				
Pycnometer	0.866***	0.625 ns	-0.678**	-0.835***			
SKCS-HI	0.162 ns	0.534*	-0.093 ns	-0.201 ns	0.342 ns		
ET	0.843***	0.359 ns	-0.869***	-0.731**	0.586 ns	-0.081 ns	

*$p < 0.05$, *** $p < 0.01$ and ***$p < 0.001$, ns- not significant at $p \geq 0.05$.
TADD; % kernel removed by Tangential Abrasive Dehulling Device, TKW; Thousand kernel weight, TW; test weight, SKCS-HI; Single Kernel Characterization System-Hardness Index, ET; endosperm texture.

Table 4.1.5 shows the correlations amongst several maize properties. TADD hardness (percentage kernel removed) was significantly correlated with the grain density, (r = -0.868), test weight (r = -0.785) and floaters (r = 0.854). Endosperm texture in terms of percentage translucency was also significantly correlated with hardness and all parameters measuring density. However, TKW did not agree with hardness, density and endosperm texture measurements, observed with sorghum. In this wide range of maize cultivars, TKW may not be a useful indicator of grain hardness.

TABLE 4.1.5 Correlation Matrix of Physical and Hardness Properties of Maize Cultivars

	Floaters	Pycnometer	TADD	TKW	TW
Pycnometer	-0.976***				
TADD	0.854**	-0.868***			
TKW	-0.248 ns	0.044 ns	0.030 ns		
TW	-0.435 ns	0.443 ns	-0.785 **	-0.170 ns	
Translucency	-0.915***	0.862***	-0.903***	0.290 ns	0.691*

*$p < 0.05$, *** $p < 0.01$ and ***$p < 0.001$, ns- not significant at $p \geq 0.05$.
TADD, % kernel removed measured by Tangential Abrasive Dehulling Device; TKW; Thousand kernel weight; TW; test weight

The study shows that sorghum is somewhat harder than maize in terms of kernel removal by the TADD, density tests and test weight. Endosperm texture, floaters, specific density, test weight, peak viscosity and TADD kernel removal seem to be promising hardness tests for sorghum. The selection of these methods is based on their significant relationships with each other. TKW and SKCS-HI did not seem suitable for sorghum hardness testing as there were not significantly related with other hardness parameters. It would seem that TW, TADD kernel removal and translucency or floaters, density, TADD hardness and translucency can be used to evaluate maize hardness among such cultivars.

4.1.3.2 Commercial sorghum physical and hardness properties

There are three sorghum types grown in South Africa, the red condensed tannin (type III), red non-tannin (type I) and white tan-plant, non-tannin sorghums (type I). For this study, red non-tannin and condensed tannin sorghums were evaluated separately for their physical properties and hardness.

Red, non-tannin sorghum cultivars had test weights ranging 72.1 to 76.9 kg/hl compared to 71.9 to 74.2 kg/hl for condensed tannin sorghums (Table 4.1.6). Test weights were not consistent for cultivars across localities wherein in some localities they fell below the minimum level of US No.1 grade. On average, cultivars PAN 8006T, PAN 8625, PAN 8902 and PAN 8904 had test weights below 73.3 kg/h. Cultivars PAN8006T and PAN 8625 were condensed tannin sorghums and the other two cultivars were entries from breeding material for screening. Cultivar PAN 8901 produced the densest kernels (76.9 kg/hl) followed by NS 5655 (76.8 kg/hl). Locality was significant for test weight as well ($p < 0.05$). The highest mean test weight for red non-tannin sorghums was in Dover. The USDA regulations recommend a minimum of 73.3 kg/hl for US No.1 grade (GIPSA 2007). Condensed tannin sorghums are normally less dense and are floury hence the lower test weights.

The mean TKW range for both sorghum types was slightly different and ranged from 21.7 to 29.0 g and 23.4 to 27.8 g for non-tannin and condensed tannin sorghums, respectively (Table 4.1.7). Cultivars of Dover had the lowest weights for both condensed tannin and non-tannin sorghums.

TABLE 4.1.6 Effects of Cultivar and Locality on Test Weight (TW) (kg/hl) of Red, Non-tannin Sorghum Cultivars

	Localities						
Cultivar	Klipdrift	Kafferskraal	Goedgedacht	Dover	Platrand	Parys	Mean
Non-tannin sorghum							
PAN 8901	76.9 (0.1)	76.1 (0.5)	77.7 (0.2)	76.7 (0.2)	78.0 (0.2)	77.5 (0.3)	77.1 (0.7)
PAN 8902	73.1 (7.3)	74.9 (0.7)	77.4 (0.1)	77.8 (0.2)	77.5 (0.3)	77.8 (0.3)	76.4 (3.1)
PAN 8903	76.3 (0.1)	75.3 (0.2)	74.3 (0.2)	76.3 (0.4)	72.2 (0.4)	76.4 (0.5)	75.1 (1.6)
PAN 8905	73.7 (0.5)	76.1 (0.2)	74.3 (0.9)	76.1 (0.3)	72.1 (0.3)	75.8 (0.2)	74.7 (1.6)
PAN 8906	73.3 (0.1)	71.5 (1.4)	74.1 (0.3)	75.9 (0.4)	73.7 (0.7)	75.3 (0.5)	74.0 (1.6)
PAN 8564	74.8 (0.5)	75.2 (0.9)	77.8 (0.1)	77.7 (0.2)	76.2 (0.3)	76.4 (0.9)	76.4 (1.3)
PAN 8657	76.2 (0.3)	76.1 (0.2)	74.3 (3.9)	76.9 (0.1)	74.4 (0.3)	76.0 (0.3)	75.7 (1.7)
PAN 8488	76.1 (0.2)	78.1 (0.0)	76.3 (0.1)	77.9 (0.2)	76.5 (0.1)	77.2 (0.3)	77.0 (0.8)
PAN 8816	76.1 (0.1)	76.9 (0.4)	76.4 (0.1)	77.6 (0.1)	77.0 (0.2)	77.6 (0.3)	76.9 (0.6)
PAN 8609	75.8 (0.2)	74.1 (0.3)	76.4 (0.1)	76.2 (0.2)	74.7(0.2)	76.9 (0.2)	75.7 (1.0)
PAN 8247	76.2 (0.5)	72.1 (0.4)	77.3 (0.7)	76.1 (0.4)	75.7 (0.3)	77.8 (0.2)	75.9 (1.9)
NS 5655	76.8 (0.1)	76.1 (0.5)	77.5 (0.1)	76.3 (0.0)	76.5 (0.1)	70.8 (0.3)	75.7 (2.3)
PAN 8904	72.1 (0.7)	72.5 (1.2)	75.0 (0.2)	75.6 (0.1)	74.8 (0.2)	74.9 (0.5)	74.1 (1.5)
Mean	75.2 c (2.3)	75.0 c (2.0)	76.1 b(1.7)	76.7 a (0.8)	75.3 c (1.8)	76.2 b (1.8)	75.7 (1.9)
Range	72.1-76.9	71.5-78.1	74.3-77.7	75.6-77.9	72.1-78.0	70.8-77.8	74.0-77.1
CV	3.05	2.67	2.23	1.04	2.39	2.36	2.54
F value	1.85 ns	3.82***	4.98***	32.3***	113.3***	59.8***	18.68***
Condensed tannin sorghum							
PAN 8006T	71.9 (0.3)	74.2 (0.2)	73.7 (0.3)	74.2 (0.1)	73.7 (0.2)	74.6 (0.4)	73.7 (0.9)
PAN 8625	72.2 (0.3)	75.5 (0.3)	73.0 (0.2)	73.4 (0.2)	71.6 (0.8)	72.7 (0.4)	73.1 (1.3)
PAN 8389	74.2 (0.1)	75.0 (0.6)	74.0 (0.3)	74.6 (0.2)	72.9 (0.3)	74.2 (0.4)	74.1 (0.7)
NS 5511	74.1 (0.2)	75.6 (0.2)	75.2 (0.4)	75.6 (0.1)	73.7 (0.4)	75.3 (0.5)	74.9 (0.8)
Mean	73.1 bc (1.1)	75.1a (0.7)	74.0abc (0.9)	74.5a (0.8)	73.0a (1.0)	74.2ab (1.1)	74.0 (1.2)
Range	71.9-74.2	74.2-75.6	73.0-75.2	73.4-75.6	71.6-73.7	72.7-75.3	73.1-74.9
CV	1.50	0.93	1.21	1.07	1.34	1.48	1.62
F value	91.6***	9.64**	25.7***	135.5***	13.6**	22.3**	11.2***

Figures in parentheses are standard deviations
Different letters in the same row denote significant differences at $p < 0.05$
*$p < 0.05$, *** $p < 0.01$ and ***$p < 0.001$, ns- not significant at $p \geq 0.05$.

TABLE 4.1.7. Effects of Cultivar and Locality on Thousand Kernel Weight (TKW) (g) of Red, Non-tannin Sorghum Cultivars

	Localities						
Cultivar	Klipdrift	Kafferskraal	Goedgedacht	Dover	Platrand	Parys	Means
Non-tannin sorghum							
PAN 8901	28.3 (2.8)	25.3 (2.1)	27.0 (1.4)	21.2 (2.0)	27.2 (2.8)	24.7 (1.7)	25.6 (3.0)
PAN 8902	31.5 (2.5)	25.5 (3.3)	29.4 (0.9)	25.5 (1.4)	28.2 (2.0)	28.2 (4.4)	28.0 (3.1)
PAN 8903	30.6 (1.6)	26.8 (0.6)	24.7 (1.5)	24.1 (0.2)	21.5 (1.7)	26.4 (0.8)	25.7 (3.1)
PAN 8905	23.4 (0.5)	27.5 (1.7)	23.8 (2.5)	23.1 (0.8)	21.5 (1.3)	26.0 (0.7)	24.2 (2.5)
PAN 8906	24.1 (2.1)	24.5 (1.8)	23.8 (0.4)	23.7 (1.3)	22.3 (2.0)	27.9 (0.6)	24.4 (2.2)
PAN 8564	23.4 (2.4)	25.1 (1.3)	25.8 (2.0)	23.4 (2.0)	24.1 (2.0)	27.4 (4.4)	24.9 (2.6)
PAN 8657	28.4 (4.5)	28.2 (2.5)	28.4 (0.5)	24.6 (0.4)	27.0 (0.7)	23.1 (1.1)	26.6 (2.8)
PAN 8488	26.5 (2.5)	28.3 (1.2)	29.4 (1.2)	24.7 (0.9)	26.4 (2.2)	26.4 (1.6)	27.0 (2.1)
PAN 8816	25.5 (2.3)	25.9 (1.4)	28.1 (1.4)	24.4 (0.9)	23.6 (1.1)	26.4 (1.2)	25.6 (1.9)
PAN 8609	28.3 (3.2)	26.2 (1.3)	27.1 (2.5)	22.5 (1.3)	24.8 (2.6)	28.3 (2.6)	26.2 (2.9)
PAN 8247	30.2 (4.9)	28.6 (2.5)	29.7 (2.8)	24.2 (3.0)	29.9 (1.5)	31.4 (3.0)	29.0 (3.5)
NS 5655	26.6 (1.4)	26.2 (1.2)	27.0 (1.8)	19.7 (0.7)	24.7 (2.3)	24.9 (0.7)	24.9 (2.8)
PAN 8904	20.5 (2.7)	20.2 (2.5)	23.7 (2.1)	19.9 (0.6)	26.1 (1.3)	19.6 (0.9)	21.7 (2.9)
Mean	26.7a (4.0)	26.2ab (2.6)	26.8a (2.6)	23.2c (2.1)	25.2b (3.0)	26.2a (3.3)	25.6 (3.2)
Range	20.5-31.5	20.2-28.6	23.7-29.4	19.7-25.5	21.5-29.9	19.6-30.7	21.7-29.0
CV	14.8	10.2	9.75	9.19	11.8	12.7	7.04
F value	3.87**	26.0**	4.68***	5.25***	5.49***	4.81***	14.20***
Condensed tannin sorghum							
PAN8006T	26.5 (2.0)	28.5 (1.9)	30.0 (1.5)	24.2 (2.0)	26.6 (0.1)	30.7 (3.4)	27.8 (2.9)
PAN 8625	20.6 (1.0)	25.3 (2.2)	31.4 (5.2)	20.4 (1.9)	22.2 (2.0)	23.9 (3.8)	24.0 (4.6)
PAN 8389	28.5 (2.4)	30.1 (0.3)	25.1 (4.5)	25.0 (1.2)	25.4 (1.7)	26.5 (4.1)	26.8 (3.1)
NS 5511	21.6 (.8)	25.7 (1.6)	24.5 (0.6)	21.0 (1.5)	21.9 (1.6)	25.9 (2.7)	23.4 (2.5)
Mean	24.3ab (3.8)	27.4a (2.5)	27.7 a (4.4)	22.7b (2.5)	24.0ab (2.5)	26.7a (4.0)	25.5 (3.8)
Range	20.6-28.5	25.3-30.1	24.5-31.4	20.4-25.0	21.9-26.6	23.9-30.7	23.4-27.8
CV	8.23	9.12	15.9	11.0	10.4	15.0	14.9
F value	12.0**	5.67*	2.88 ns	5.72*	7.20*	1.97 ns	7.08***

Figures in parentheses are standard deviations
Different letters in the same row denote significant differences at $p < 0.05$
*$p < 0.05$, *** $p < 0.01$ and ***$p < 0.001$, ns- not significant at $p \geq 0.05$.

Tables 4.1.8a to 4.1.8d show the kernel size distribution of sorghum cultivars. The influence of cultivar and location was significant for all four kernel sizes. The percentage of kernels with a size of at least 4.00 mm was very low, averaging 1.05%.

There was a high percentage of kernels retained in the 3.35 mm sieve for most cultivars. The kernel size between non-tannin and condensed tannin sorghum ranged from 23.4 to 59.5% and 29.2 to 56.3% respectively (Table 4.1.8b). PAN 8247, a non-tannin cultivar had the highest percentage of kernels with a size of 3.35 mm (59.5%) PAN 8006T and PAN 8389 had the highest percentage of kernels of size 3.35 mm for condensed tannins. The influence of location on kernel size was very high and cultivars of Dover and Platrand were the smallest compared to the same cultivars in other localities. For the 3.15 mm size, cultivars were also significantly different ($p < 0.001$) (Tables 4.1.8c). Kernel size ranged 18.0 to 31.0 and 20.4 to 31.9% for red non-tannin and red condensed tannin cultivars, respectively.

Kernels of Dover were small and were mostly retained in the 2.36 mm test sieve with a mean kernel size of 43.8% compared to the overall mean of 26.7% (Table 4.1.8d). Overall, non-tannin and condensed tannin sorghums showed similarities in grain size. A large proportion of kernels were of size 3.35 mm. Only 1.05% and 1.10% of the kernels were at least 4 mm. These sorghum cultivars could be of intermediate size. Beta et al (2001a) reported 0.7% for kernels greater than 4 mm and most kernels distributed between 2.6 and 4.0 mm sizes which were classified as intermediate size.

TABLE 4.1.8a Effects of Cultivar and Locality on Kernel Size (% kernels retained on a 4.00 mm round hole sieve) of Red, Non-tannin Sorghum Cultivars

	Localities						
Cultivar	Klipdrift	Kafferskraal	Goedgedacht	Dover	Platrand	Parys	Mean
PAN 8901	0.59 (0.25)	0.21 (0.18)	2.40 (0.92)	0.41(0.16)	0.48 (0.05)	2.40 (0.61)	1.08 (1.0)
PAN 8902	1.04 (0.46)	0.57 (0.43)	0.99 (0.22)	0.61 (0.08)	0.95 (0.13)	0.66 (0.15)	0.81 (0.3)
PAN 8903	0.81 (0.12)	0.44 (0.08)	3.57 (0.66)	0.42 (0.37)	0.25 (0.06)	1.43 (0.30)	1.15 (1.2)
PAN 8905	0.41 (0.37)	1.11 (0.55)	1.79 (0.23)	0.29 (0.07)	0.39 (0.09)	0.70 (0.25)	0.78 (0.6)
PAN 8906	1.14 (0.31)	0.63 (0.34)	4.39 (0.98)	0.59 (0.17)	0.17 (0.01)	2.00 (0.27)	1.49 (1.5)
PAN 8564	0.61 (0.12)	0.43 (0.37)	4.25 (0.88)	0.13 (0.10)	0.11 (0.02)	0.19 (0.18)	0.95 (1.6)
PAN 8657	0.91 (0.42)	0.50 (0.21)	1.05 (0.19)	0.30 (0.18)	0.37 (0.20)	0.45 (0.11)	0.60 (0.4)
PAN 8488	0.90 (0.16)	0.63 (0.46)	0.81 (0.24)	0.18 (0.10)	0.69 (0.35)	3.48 (0.29)	1.12 (1.1)
PAN 8816	0.76 (0.18)	0.40 (0.03)	0.29 (0.09)	0.15 (0.06)	0.12 (0.04)	0.39 (0.21)	0.35 (0.2)
PAN 8609	2.52 (0.58)	0.37 (0.10)	0.93 (0.42)	0.82 (0.28)	0.62 (0.15)	2.73 (0.40)	1.33 (1.0)
PAN 8247	2.74 (0.65)	1.27 (0.21)	2.95 (0.13)	1.49 (0.44)	4.05 (0.46)	4.71 (1.62)	2.87 (1.4)
NS 5655	0.16 (0.16)	0.55 (0.21)	1.21 (0.31)	0.25 (0.02)	0.15 (0.09)	0.15 (0.04)	0.41 (0.4)
PAN 8904	0.12 (0.12)	0.18 (0.16)	1.51 (0.11)	0.71 (1.23)	0.52 (0.17)	0.14 (0.09)	0.53 (0.7)
Mean	0.98c (0.83)	0.56de (0.39)	2.01a (1.40)	0.49e (0.49)	0.68d (1.03)	1.49b (1.50)	1.05 (1.16)
Range	0.12-2.74	0.21-1.27	0.28-2.95	0.13-1.49	0.11-0.94	0.14-4.71	0.41-2.87
CV	84.6	69.9	69.8	99.9	149.9	100.0	37.1
F value	15.7***	3.33**	20.8***	2.67*	89.5***	23.5***	48.4***
			Condensed tannin sorghum				
PAN 8006T	1.08 (0.40)	1.97 (0.30)	0.90 (0.20)	0.75 (0.39)	0.99 (0.14)	4.71 (0.82)	1.74 (1.5)
PAN 8625	0.33 (0.31)	0.30 (0.15)	2.68 (0.52)	0.07 (0.07)	0.16 (0.10)	0.21 (0.08)	0.62 (1.0)
PAN 8389	1.95 (0.58)	2.03 (0.37)	1.68 (0.41)	0.51 (0.25)	0.39 (0.22)	3.26 (0.71)	1.64 (1.1)
NS 5511	0.57 (0.23)	0.38 (0.48)	1.17 (0.12)	0.28 (0.21)	0.07 (0.08)	0.02 (0.02)	0.42 (0.4)
Mean	0.98ab (0.7)	1.17ab (0.9)	1.61ab (0.8)	0.41b (0.3)	0.40b (0.4)	2.05a (2.1)	1.10 (1.2)
Range	0.33-1.95	0.30-2.03	0.90-2.68	0.07-0.75	0.07-0.99	0.02-4.71	0.42-1.74
CV	71.4	76.9	49.7	73.1	100	102.4	109.1
F value	9.55**	23.4***	14.9**	3.97 ns	24.8***	54.1***	7.44***

Figures in parentheses are standard deviations
Different letters in the same row denote significant differences at $p < 0.05$
*$p < 0.05$, *** $p < 0.01$ and ***$p < 0.001$

TABLE 4.1.8b Effects of Cultivar and Locality on Kernel Size (% kernels retained on a 3.35 mm round hole sieve) of Red, Non-tannin Sorghum Cultivars

	Localities						
Cultivar	Klipdrift	Kafferskraal	Goedgedacht	Dover	Platrand	Parys	Mean
PAN 8901	35.5 (1.8)	36.7 (1.9)	53.2 (2.0)	21.6 (1.6)	36.7 (2.7)	66.9 (2.0)	41.7 (15.0)
PAN 8902	49.0 (2.4)	57.3 (1.2)	53.2 (2.8)	41.0 (1.7)	51.5 (1.0)	62.9 (0.7)	52.5 (7.2)
PAN 8903	37.2 (0.5)	37.6 (1.7)	58.9 (1.6)	24.7 (1.4)	17.1 (1.5)	56.1 (0.9)	38.6 (15.6)
PAN 8905	37.1 (5.2)	54.8 (1.1)	46.8 (3.6)	18.9 (1.7)	23.6 (0.9)	62.9 (0.2)	40.7 (16.5)
PAN 8906	24.3 (2.4)	40.3 (0.8)	58.6 (1.6)	25.8 (2.6)	18.0 (1.6)	62.3 (2.0)	38.2 (17.7)
PAN 8564	33.2 (2.3)	35.1 (0.9)	61.2 (2.4)	20.6 (1.8)	27.1 (1.5)	53.0 (1.3)	38.4 (14.7)
PAN 8657	48.3 (0.9)	43.9 (1.3)	47.6 (2.4)	24.9 (0.9)	30.5 (1.0)	61.0 (7.7)	42.7 (12.6)
PAN 8488	52.2 (0.7)	44.6 (0.5)	43.7 (3.7)	26.9 (1.2)	37.3 (2.4)	66.1 (2.5)	45.1 (12.6)
PAN 8816	58.1 (1.6)	42.7 (3.5)	35.5 (1.0)	30.9 (2.4)	19.0 (1.9)	50.6 (1.3)	39.5 (13.3)
PAN 8609	61.7 (0.3)	46.6 (2.8)	48.9 (8.6)	22.5 (2.9)	43.8 (2.4)	63.2 (2.6)	47.8 (14.3)
PAN 8247	71.9 (1.5)	59.1 (1.0)	55.5 (0.7)	46.9 (1.6)	57.9 (1.8)	65.9 (1.1)	59.5 (8.2)
NS 5655	26.5 (1.3)	49.0 (0.9)	55.1 (0.6)	13.6 (1.0)	37.1 (2.8)	57.9 (0.4)	39.9 (16.4)
PAN 8904	5.6 (1.3)	12.9 (1.5)	57.7 (0.8)	6.6 (2.4)	38.4 (1.0)	19.1 (0.7)	23.4 (19.4)
Mean	41.6d (17.3)	43.1c (11.7)	52.0b (7.5)	25.0f (10.3)	33.7e (12.5)	57.5a (12.5)	42.3 (16.3)
Range	24.3-71.9	12.9-59.1	35.5-61.2	6.6-46.9	17.1-57.9	19.1-66.9	23.4-59.5
CV	41.7	27.1	14.5	41.4	37.2	21.7	5.41
F value	219.1***	148.6***	15.6***	92.1***	145.1***	71.8***	250.8***
Condensed tannin sorghum							
PAN 8006T	54.1 (1.9)	66.8 (0.3)	43.2 (2.2)	48.3 (1.0)	57.4 (2.0)	67.7 (2.3)	56.3 (9.3)
PAN 8625	27.7 (1.3)	43.1 (1.3)	52.9 (0.9)	10.5 (3.1)	14.7 (0.8)	28.5 (1.2)	29.6 (15.3)
PAN 8389	59.2 (0.8)	66.5 (1.0)	58.9 (0.8)	43.4 (1.5)	38.2 (2.6)	70.1 (1.6)	56.0 (12.0)
NS 5511	29.8 (1.8)	36.0 (1.7)	43.6 (1.7)	19.5 (2.3)	9.5 (0.4)	36.8 (0.5)	29.2 (11.9)
Mean	42.7bc (14.8)	53.1a (14.4)	49.7a (7.0)	30.4b (16.6)	30.0b (20.1)	50.8a (19.3)	42.8 (18.1)
Minimum	27.7-59.2	36.0-66.8	43.2-58.9	10.5-48.3	9.5-57.4	28.5-70.1	29.2-56.3
CV	34.6	27.1	14.1	54.6	67.0	38.0	42.3
F value	109.2***	527.6***	74.5***	228.7***	514.1***	571.7***	28.4***

Figures in parentheses are standard deviations
Different letters in the same row denote significant differences at $p < 0.05$
***$p < 0.001$

TABLE 4.1.8c Effects of Cultivar and Locality on Kernel Size (% kernels retained on a 3.15 mm round hole sieve) of Red, Non-tannin Sorghum Cultivars

	Localities						
Cultivar	Klipdrift	Kafferskraal	Goedgedacht	Dover	Platrand	Parys	Mean
PAN 8901	33.4 (2.2)	33.0 (1.3)	21.4 (1.6)	28.8 (0.6)	27.5 (0.5)	14.2 (0.9)	26.4 (7.0)
PAN 8902	27.0 (1.2)	23.5 (1.2)	21.6 (0.3)	27.0 (0.9)	20.1 (0.5)	18.3 (0.1)	22.9 (3.4)
PAN 8903	29.5 (0.9)	29.3 (0.9)	18.6 (1.2)	27.7 (0.3)	21.9 (1.6)	13.3 (0.3)	23.4 (6.3)
PAN 8905	28.4 (0.5)	23.9 (1.1)	21.0 (1.1)	32.7 (1.3)	25.7 (1.2)	18.7 (0.3)	25.1 (4.8)
PAN 8906	34.8 (0.6)	31.8 (1.3)	18.3 (0.2)	26.9 (1.1)	30.5 (0.3)	12.4 (0.1)	25.8 (8.2)
PAN 8564	39.5 (1.5)	33.1 (0.6)	17.7 (1.6)	34.8 (1.5)	35.4 (1.9)	16.8 (0.5)	29.6 (9.2)
PAN 8657	31.0 (0.3)	29.8 (0.6)	29.3 (0.2)	34.5 (1.4)	26.5 (0.9)	16.6 (1.5)	27.9 (5.8)
PAN 8488	27.0 (0.7)	30.6 (1.0)	25.8 (1.6)	29.5 (1.9)	26.7 (0.3)	11.9 (0.3)	25.2 (6.4)
PAN 8816	23.8 (1.1)	30.3 (2.8)	35.8 (1.3)	35.8 (0.8)	42.8 (1.4)	17.5 (0.2)	31.0 (8.7)
PAN 8609	20.5 (0.3)	28.0 (1.4)	26.7 (2.8)	31.4 (1.3)	21.2 (1.3)	11.2 (0.6)	23.2 (6.9)
PAN 8247	15.3 (1.5)	22.0 (1.2)	24.2 (3.1)	25.2 (0.6)	13.4 (1.2)	7.6 (0.1)	18.0 (6.7)
NS 5655	36.1 (1.0)	25.3 (1.2)	23.5 (0.6)	21.9 (1.8)	29.1 (0.4)	14.2 (0.9)	25.0 (7.0)
PAN 8904	23.3 (2.1)	22.3 (1.6)	22.4 (0.6)	23.1 (3.0)	22.8 (0.7)	31.3 (1.6)	24.2 (3.6)
Mean	28.4b (6.6)	27.9b (4.1)	23.6d (5.0)	29.2a (4.5)	26.4c (7.2)	15.7e (5.6)	25.3 (7.2)
Range	15.3-36.1	22.0-33.1	17.7-35.8	21.9-35.8	13.4-42.8	7.6-31.3	18.0-31.0
CV	23.3	16.7	21.3	15.4	27.2	35.4	5.32
F value	90.8***	26.5***	32.0***	29.2***	140.6***	165.5***	121.6***
Condensed tannin sorghum							
PAN 8006T	25.0 (0.4)	18.9 (0.8)	26.8 (0.9)	25.1 (0.2)	18.5 (0.9)	7.8 (0.6)	20.4 (6.6)
PAN 8625	37.5 (1.5)	32.6 (0.8)	25.1 (4.7)	34.9 (4.6)	31.0 (2.8)	30.2 (0.4)	31.9 (4.7)
PAN 8389	22.0 (0.3)	17.8 (0.7)	21.3 (1.4)	27.3 (0.7)	23.2 (1.3)	14.0 (1.1)	20.9 (4.4)
NS 5511	33.2 (0.9)	33.7 (1.0)	23.9 (0.2)	31.7 (1.3)	29.9 (0.6)	28.3 (0.5)	30.1 (3.5)
Mean	29.4a (6.5)	25.8ab(7.8)	24.3ab(3.0)	29.8a(4.5)	25.7ab(5.5)	20.1b(9.9)	25.8(7.1)
Range	22.0-37.5	17.8-33.7	21.3-26.8	25.1-34.9	18.5-31.0	7.8-30.2	20.4-31.9
CV	22.1	30.2	12.3	15.1	21.4	49.2	27.5
F value	186.4***	338.0***	2.59 ns	9.95**	38.5***	741.3***	26.7***

Figures in parentheses are standard deviations
Different letters in the same row denote significant differences at $p < 0.05$
*$p < 0.05$, *** $p < 0.01$ and ***$p < 0.001$, ns- not significant at $p \geq 0.05$.

TABLE 4.1.8d Effects of Cultivar and Locality on Kernel Size (% kernels retained on a 2.36 mm round hole sieve) of Red, Non-tannin Sorghum Cultivars

	Localities						
Cultivar	Klipdrft	Kafferskraal	Goedgedacht	Dover	Platrand	Parys	Mean
PAN 8901	29.9 (0.4)	28.0 (1.2)	20.4 (1.6)	46.4 (1.8)	23.5 (1.3)	12.3 (0.6)	26.7 (10.8)
PAN 8902	22.7 (2.4)	18.0 (0.9)	21.0 (0.3)	30.2 (0.7)	16.0 (0.9)	12.8 (0.7)	20.1 (5.8)
PAN 8903	31.4 (0.6)	31.5 (0.6)	16.8 (1.2)	45.1 (1.8)	42.4 (1.1)	10.1 (0.5)	29.5 (13.0)
PAN 8905	33.4 (4.8)	19.0 (0.5)	28.5 (1.1)	46.7 (2.5)	37.5 (1.1)	12.0 (1.0)	29.5 (12.0)
PAN 8906	38.3 (1.5)	26.3 (0.7)	17.2 (0.2)	45.2 (1.4)	38.8 (1.5)	8.6 (0.5)	29.1 (13.4)
PAN 8564	25.8 (1.7)	30.2 (0.9)	15.6 (1.6)	43.7 (1.2)	27.2 (1.5)	10.2 (0.6)	25.5 (11.1)
PAN 8657	18.6 (0.5)	25.0 (0.6)	20.4 (0.2)	39.6 (1.1)	30.6 (1.4)	12.0 (1.1)	24.4 (9.2)
PAN 8488	19.3 (0.6)	23.6 (0.6)	27.4 (1.6)	41.2 (2.5)	24.3 (0.6)	13.3 (0.6)	24.8 (8.9)
PAN 8816	17.0 (0.4)	25.6 (0.5)	27.0 (1.3)	32.2 (2.4)	37.9 (2.6)	14.6 (0.5)	25.7 (8.4)
PAN 8609	14.7 (0.2)	23.9 (1.3)	20.4 (2.8)	43.9 (4.4)	20.8 (0.7)	7.8 (0.6)	21.9 (11.6)
PAN 8247	9.3 (0.6)	16.5 (1.1)	20.7 (3.1)	25.3 (0.6)	13.3 (0.5)	4.8 (0.3)	15.0 (7.1)
NS 5655	36.1 (0.6)	24.6 (0.6)	20.3 (0.6)	62.3 (2.4)	26.4 (1.5)	8.9 (0.3)	29.8 (17.1)
PAN 8904	69.8 (2.4)	61.9 (1.4)	19.7 (0.6)	68.4 (4.1)	26.0 (0.8)	40.2 (3.0)	47.7 (20.8)
Mean	28.2b (15.0)	27.2c (11.1)	21.2d (4.1)	43.8a (11.6)	28.1b (8.8)	12.9e (8.4)	26.7(13.9)
Range	9.3-69.8	16.5-61.9	15.6-30.1	25.3-68.4	13.3-42.4	4.8-40.2	15.0-47.7
CV	53.1	40.6	19.6	26.4	31.5	65.3	6.31
F value	220.9***	479.5***	20.8***	74.9***	143.3***	209.7***	421.4***
				Condensed tannin sorghum			
PAN 8006T	20.9 (0.2)	11.6 (0.5)	27.2 (0.9)	24.2 (0.7)	16.8 (1.9)	5.0 (0.3)	17.6 (7.8)
PAN 8625	32.6 (1.9)	22.5 (1.2)	24.0 (4.7)	53.9 (7.1)	40.0 (1.2)	25.9 (1.9)	33.2 (11.6)
PAN 8389	16.1 (1.1)	12.8 (0.5)	19.1 (1.4)	28.1 (2.0)	25.1 (2.7)	11.2 (2.0)	18.7 (6.5)
NS 5511	35.9 (2.2)	29.5 (1.4)	30.1 (0.2)	47.5 (3.0)	46.5 (0.9)	19.1 (1.6)	34.8 (10.4)
Mean	26.4bc (8.6)	19.1c (7.7)	25.1bc (4.3)	38.4a (13.6)	32.1ab (12.3)	15.3c (8.4)	26.1(12.1)
Range	16.1-35.9	11.6-29.5	19.1-30.1	24.2-53.9	16.8-46.5	5.0-25.9	17.6-34.8
CV	32.6	40.3	17.1	35.4	38.3	54.9	46.3
F value	109.2***	229.9***	66.5***	39.3***	168.7***	96.1***	17.4***

Figures in parentheses are standard deviations
Different letters in the same row denote significant differences at $p < 0.05$
***$p < 0.001$

The mean percentage kernel removed was higher for non-tannin sorghum than condensed tannin sorghums (Table 4.1.9). Percentage kernel removal by the TADD ranged from 29.4 to 45.2% for non-tannin sorghums and 35.9 to 40.9% for condensed tannin sorghums. The high

overall CV for TADD decortication (18.4%) suggests that these parameters could be used to resolve differences in TADD hardness between batches of commercial sorghum. The range of TADD kernel removal was from 29.4 to 40.6% and 35.9 to 45.2% for non-tannin and condensed tannin sorghums, respectively. Condensed tannin sorghums are generally softer than non-tannin sorghums (Mwasaru et al 1988), although the TADD data in this study did not indicate substantial differences in hardness between the two. Cultivars of Dover were harder than those of other localities.

Both cultivar and location and their interaction were highly significant ($p < 0.001$) with respect to all the parameters in both the non-tannin and condensed tannin sorghums. In non-tannin sorghums mean squares indicated that locality had a greater effect on TKW, KS and TADD than cultivar. Test weight was the only parameter affected by cultivar although there was a slight difference between the contribution of cultivar (45%) and location (41%). In contrast, the mean squares of condensed tannin cultivars indicated that cultivar had a greater effect on TKW, TW and KS than locality.

Table 4.1.11 shows that there were highly significant correlations between TADD hardness (inverse percentage kernel removed) and TW ($r = 0.673$, $p < 0.001$) and TADD hardness and TKW ($r = 0.757$, $p < 0.001$) for the non-tannin sorghums. TADD hardness of non-tannin sorghums was also highly significantly correlated with large kernel size > 4.00 mm ($r = 0.817$, $p < 0.001$), and kernels > 3.35 < 4.00 mm ($r = 0.560$; $p < 0.001$). However, TADD was not correlated with TKW nor with TW for condensed tannin sorghums. This could be partly attributed to the few condensed tannin samples analysed; hence, limiting variation compared to non-tannin sorghums. The significant ($p < 0.001$) correlations between, TW, TADD, TKW, and kernels retained on 3.35 mm round hole sieve implies that these parameters could be associated with grain hardness.

TABLE 4.1.9 Effects of Cultivar and Locality on Kernel Hardness as Measured by the TADD (% kernel removed) of Red, Non-tannin Sorghum Cultivars

	Localities						
Cultivar	Klipdrift	Kafferskraal	Goedgedacht	Dover	Platrand	Parys	Mean
PAN 8901	37.2 (1.4)	40.3 (2.4)	37.6 (0.1)	33.4 (2.5)	41.0 (5.1)	48.7 (6.5)	39.7 (5.8)
PAN 8902	28.2 (2.4)	39.4 (2.7)	17.5 (1.3)	35.2 (4.3)	27.0 (3.0)	33.4 (2.8)	30.1 (7.7)
PAN 8903	35.5 (0.4)	49.7 (2.2)	41.4 (0.7)	35.5 (2.7)	43.2 (0.6)	38.3 (1.9)	40.6 (5.3)
PAN 8905	30.3 (1.2)	34.8 (1.9)	31.4 (0.6)	22.1 (1.1)	36.3 (3.6)	37.6 (2.5)	32.1 (5.7)
PAN 8906	38.0 (3.7)	47.1 (1.8)	39.4 (0.6)	35.1 (4.2)	39.2 (0.9)	38.7 (1.1)	39.6 (4.3)
PAN 8564	34.2 (3.2)	37.8 (2.7)	25.0 (0.6)	17.1 (1.0)	37.3 (0.0)	36.3 (2.6)	31.3 (8.1)
PAN 8657	38.7 (1.1)	41.4 (3.0)	24.5 (0.6)	32.1 (4.4)	42.0 (3.6)	40.1 (2.3)	36.5 (6.9)
PAN 8488	35.5 (1.7)	34.7 (2.0)	22.8 (0.8)	21.3 (0.7)	34.9 (1.4)	27.4 (2.5)	29.4 (6.3)
PAN 8816	31.1 (0.8)	39.9 (1.5)	32.7 (0.4)	24.8 (1.2)	35.6 (3.5)	37.8 (2.6)	33.7 (5.4)
PAN 8609	34.3 (0.7)	42.3 (1.0)	29.5 (0.5)	37.1 (4.0)	33.9 (1.8)	33.6 (2.0)	35.1 (4.4)
PAN 8247	32.0 (1.6)	45.9 (2.1)	30.4 (0.3)	31.3 (2.7)	37.7 (1.9)	31.9 (2.4)	34.9 (5.9)
NS 5655	46.6 (3.9)	38.8 (1.1)	30.5 (0.4)	26.7 (1.1)	27.7 (1.4)	38.3 (2.3)	34.7 (7.5)
PAN 8904	34.9 (3.5)	44.8 (1.2)	35.0 (0.3)	34.1 (0.8)	36.6 (2.8)	45.5 (1.6)	38.5 (5.3)
Mean	35.1d (4.9)	41.3a (4.7)	30.6e (6.9)	29.6e (6.7)	36.3c (5.2)	37.5b (5.9)	36.4 (7.0)
Range	28.2-46.6	34.7-47.1	17.5-39.4	17.1-37.1	27.0-43.2	27.4-48.7	29.4-45.2
CV	13.9	11.5	22.7	22.7	14.3	15.6	6.83
F value	12.2***	14.8***	32.1***	16.7***	9.73***	11.4***	41.1***
				Condensed tannin sorghum			
PAN 8006T	51.2 (3.6)	50.6 (3.2)	33.7 (0.9)	36.1 (3.5)	34.9 (3.2)	38.9 (2.3)	40.9 (7.9)
PAN 8625	47.9 (3.1)	55.3 (2.3)	44.4 (0.7)	42.0 (4.7)	39.0 (0.8)	42.6 (1.1)	45.2 (5.9)
PAN 8389	42.6 (4.3)	41.7 (0.4)	43.8 (0.9)	31.0 (2.4)	34.6 (1.7)	47.3 (0.9)	40.2 (6.1)
NS 5511	34.8 (3.7)	41.6 (1.2)	40.1 (0.6)	18.5 (1.3)	33.1 (1.1)	47.2 (1.9)	35.9 (9.5)
Mean	44.1a (7.2)	47.3a (6.4)	40.5ab (5.0)	31.9c (9.5)	35.4bc (2.8)	44.0a (3.9)	40.5 (8.0)
Range	34.8-51.2	41.6-55.3	33.7-44.4	18.5-42.0	33.1-39.0	38.9-47.3	35.9-40.9
CV	16.3	13.5	12.3	29.9	7.9	8.9	19.8
F value	11.4**	32.3***	10.7**	28.9***	5.02*	17.8***	4.70**

Figures in parentheses are standard deviations
Different letters in the same row denote significant differences at $p < 0.05$
*$p < 0.05$, *** $p < 0.01$ and ***$p < 0.001$

TABLE 4.1.10 Mean Squares for Cultivar and Locality Effects on Thousand Kernel Weight, Test Weight, Kernel Size Distribution and Kernel Removal by TADD Decortication of Non-Tannin and Condensed Tannin Sorghum Cultivars Grown in Six Localities

Source	d.f	TKW	TW	>4.00	>3.35<4.00	>3.15<3.35	>2.36<3.15	TADD
Non-Tannin Sorghum[a]								
Cultivar (C)	12	60.7***	19.5***	7.7***	1288.9***	191.8***	1026.9***	251.5***
Locality (L)	5	72.5***	17.6***	14.1***	5469.9***	1000.5***	4049.3***	743.9***
(C x L)	60	10.8***	5.8***	2.1***	306.9***	77.0***	197.5***	61.3***
Condensed Tannin Sorghum[b]								
Cultivar (C)	3	80.3***	10.7***	8.3***	4299.3***	654.2***	1506.2***	262.5***
Locality (L)	5	52.5***	8.0***	5.2***	1282.5***	152.8***	851.0***	410.6***
(C x L)	15	14.4***	1.3***	2.9***	251.5***	51.2***	94.8***	94.3***
Overall for Non-Tannin and Condensed Tannin Sorghums								
Cultivar (C)	16	60.7***	27.5***	7.32***	1774.1***	267.8***	1055.0***	340.2***
Locality (L)	5	106.0***	17.7***	17.6***	6398.4***	1109.0***	4657.3***	998.3***
(C x L)	80	12.0***	5.1***	2.2***	299.3***	70.1***	181.0***	73.4***

TW, test weight (kg/hl); TKW, thousand kernel weight (g); TADD (% kernel removed); 4.00 mm, 3.35 mm, 3.15 mm and 2.36 mm; percentage kernels retained on the respective sieve sizes; C, cultivar; L, locality; C x L, cultivar x locality interactions; d.f, degrees of freedom; MS, mean square values

[a] Data of 13 cultivars cultivated in 6 locations (n=78)

[b] Data of 4 cultivars cultivated in 6 locations (n=24)

Data in parentheses are standard deviations

***$p < 0.001$

TABLE 4.1.11 Pearson Correlation Coefficients between Test Weight, Thousand Kernel Weight, Kernel Size Distribution and TADD Kernel Removal of Non-Tannin and Condensed Sorghum Cultivars Grown in Six Localities

	TW	TKW	>4.00	>3.35<4.00	>3.15<3.35	>2.36<3.15
Non-Tannin Sorghum						
TKW	0.242 ns					
>4.00	0.134 ns	0.317 ns				
>3.35<4.00	0.191 ns	0.567***	0.602***			
>3.15<3.35	0.004 ns	-0.213ns	-0.591***	-0.649***		
>2.36<3.15	-0.195 ns	-0.586***	-0.485***	-0.929***	0.497 ns	
TADD	-0.673***	-0.757***	-0.817***	-0.560***	-0.197 ns	0.101 ns
Condensed Tannin Sorghum						
TKW	0.122 ns					
> 4.00	0.101 ns	0.560***				
>3.35<4.00	0.212 ns	0.677***	0.327 ns			
>3.15<3.35	-0.124 ns	-0.561***	-0.093 ns	-0.812***		
>2.36<3.15	-0.160 ns	-0.663***	-0.028 ns	-0.926***	0.753***	
TADD	-0.327 ns	0.212 ns	-0.064 ns	-0.354 ns	-0.098 ns	-0.423 ns

TW, Test weight (kg/hl); TKW, thousand kernel weight; TADD (% kernel removed); 4.00 mm, 3.35 mm, 3.15 mm and 2.36 mm; percentage kernels retained on the respective sieve opening sizes
***$p < 0.001$, ns- not significant at $p \geq 0.05$

Principal component analysis was performed to further explain the relationships among the parameters. In non-tannin sorghum, the first two components together explained 83% of the variability in the data (Fig 4.1.1). Principal component (PC) 1 accounted for 56% of the total variation contributed. Large kernel size (> 3.35 mm < 4.00 mm) was associated with TKW, but small kernel size (> 2.36 mm < 3.15 mm) was inversely related to TKW. TADD (percentage kernel removed) was inversely related to TW. These findings are similar to those of Kirleis and Crosby (1982) who showed that sorghum pearling index, as measured by a Strong-Scott barley pearler, was correlated with kernel density. In condensed tannin sorghums (Fig 4.1.2), like non-tannin sorghums, TADD (percentage kernel removed) was inversely related to TW (PC 2).

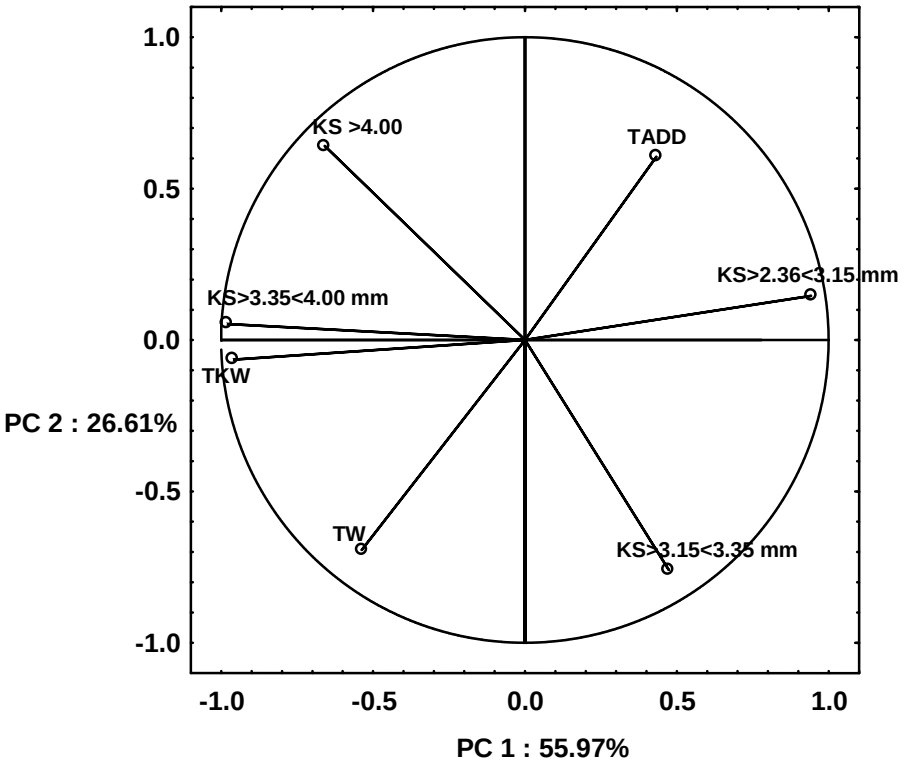

Fig 4.1.1. Factor coordinates of the first two principal components (PC) for non-tannin sorghums with respect to test weight (TW), thousand kernel weight (TKW), kernel size (KS) fractions and Tangential Abrasive Dehulling Device (TADD) (% kernel removed) properties.

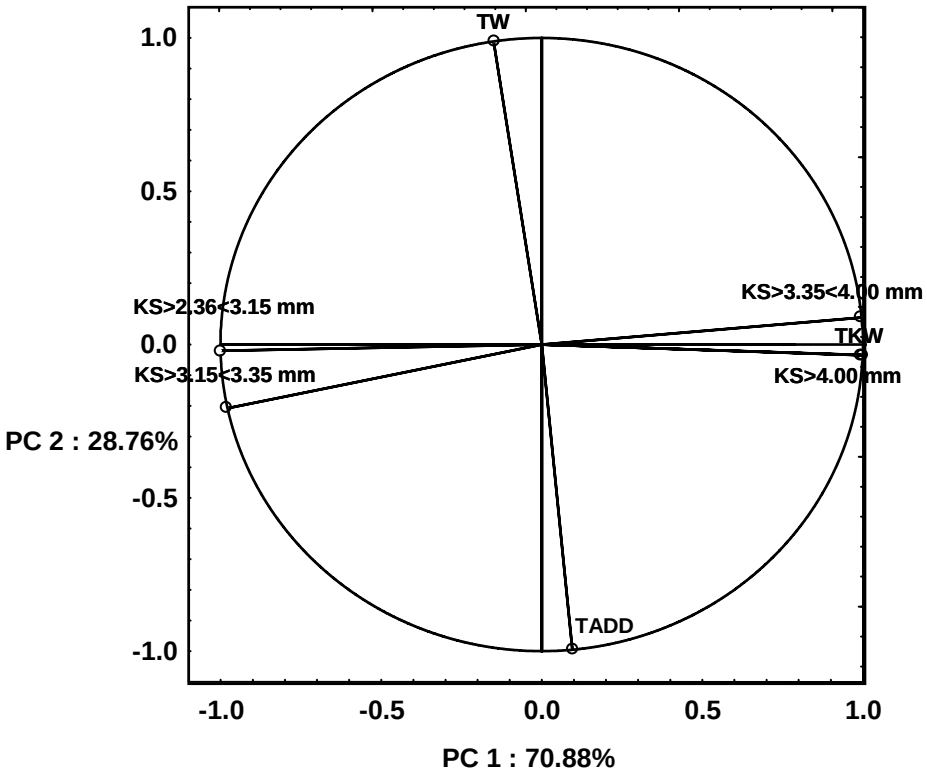

Fig 4.1.2. Factor coordinates of the first two principal components (PC) for condensed tannin sorghums with respect to test weight (TW), thousand kernel weight (TKW), kernel size (KS) fractions and Tangential Abrasive Dehulling Device (TADD) (% kernel removed) properties.

4.1.3.4 Physical and hardness properties of commercial maize cultivars

Table 4.1.12 shows TKW of cultivars within and across localities. There was little variation in TKW of cultivars. Generally, all cultivars had a TKW more than 300 g and ranged from 335 to 412 g. Within each locality, weights of cultivars were not significantly different ($p \geq 0.05$). However, locality seemed to have an effect on TKW. TKW varied with location, Potchefstroom had the highest weights and Petit the lowest (Table 4.1.12). The percentage coefficient of variation (CV) of cultivars in all localities was 1.21% showing minimal variation within cultivars despite significant differences for locality. Nago et al (1997) found

values less than 300 g amongst local ecotypes and hybrids. A corroborative study of maize quality across laboratories yielded TKW in the range of 30.6 to 45.3 g (Lee et al 2007). High TKW was associated with hard endosperm and white maize types averaging 345 and 342 g (Paulsen et al 2003). Their findings were in agreement with the results of this study. However, TKW is not a grading criterion according to the United States Department of Agriculture (USDA) Grain Inspection, Packers and Stockyards Administration (GIPSA) Handbook (GIPSA 2007).

There was no significant variation in the test weights of cultivars within localities ($p \geq 0.05$), (Table 4.1.13). The CV also confirmed the small variations of the cultivars within all localities and the mean test weights ranged from 77.0 to 79.9 kg/hl. Potchefstroom had the highest TW compared to other localities and the cultivar weights ranged from 78.1 to 82.5 kg/hl. Petit had the lowest weights. According to the USDA regulations, U.S. No. 1 grade should have a test weight of minimum of 72 kg/hl (GIPSA 2007). Maize cultivars had higher test weights than sorghum which ranged from 77.0 to 79.9 kg/hl. Sorghums seemed to have a wider range of TW values than maize. South African regulations only stipulate permissible defects and foreign matter in a sample and do not give guidelines for TW. These weights are higher than previously reported (Lee et al 2006; Lee et al. 2007). Differences may be expected due to regional and location differences and cultivar effects. Higher test weights have been associated with a high ratio of hard to soft endosperm and milling energies and resistance time to grinding using the Stenvert hardness test (Li et al 1996).

TABLE 4.1.12 Effects of Cultivar and Locality on One Thousand Kernel Weight (TKW) (g) of Maize Cultivars

	Localities				
Cultivar	Potchefstroom	Klerksdorp	Petit	Bethlehem	Mean
Phb 32A05 B	349 (31)	395 (16)	301 (66)	396 (41)	358 (55)
AFG 4321	380 (41)	317 (47)	304 (62)	340 (24)	335 (49)
PAN 6223 B	403 (41)	309 (73)	287 (38)	397 (41)	343 (65)
PhB 32B10	375 (19)	329 (31)	329 (52)	342 (50)	350 (41)
LS 8527 BR	351 (69)	380 (86)	309 (70)	391 (7)	364 (65)
PAN 4P - 313 B	418 (38)	341 (28)	323 (30)	419 (51)	375 (56)
Phb 31 M 09	403 (28)	367 (27)	293 (37)	447 (32)	374 (61)
AFG 4383	443 (17)	398 (64)	337 (34)	407 (66)	390 (57)
AFG 4445	411 (26)	360 (21)	341 (46)	391 (15)	376 (38)
AFG 4473	422 (33)	365 (7)	320 (35)	342 (56)	362 (44)
DKC 78 - 45 BR	443 (10)	354 (13)	365 (11)	363 (15)	383 (38)
IMP 52 – 11	391 (41)	385 (47)	311 (7)	370 (24)	371 (47)
DKC 77 - 61 B	438 (73)	377 (16)	343 (18)	364 (54)	377 (51)
LS 8519	386 (58)	359 (20)	341 (31)	403 (35)	380 (46)
PAN 6Q -445 B	392 (55)	362 (14)	355 (34)	379 (42)	380 (43)
CRN 3505	459 (27)	367 (28)	357 (16)	403 (21)	397 (46)
Saffier	450 (32)	374 (24)	346 (35)	421 (68)	393 (52)
AFG 4555	439 (8)	367 (4)	386(28)	414 (11)	401 (31)
Phb 30D07 B	444 (20)	379 (32)	367 (3)	391 (39)	396 (42)
DKC 78 - 15 B	408 (29)	354 (37)	342 (36)	384 (25)	368 (35)
PAN 6Q - 521 R	390 (15)	386 (15)	352 (44)	400 (51)	383 (36)
PAN 6611	402 (47)	393 (89)	324 (46)	363 (47)	375 (60)
LS 8521 B	395 (16)	401 (20)	344 (20)	365 (64)	376 (39)
DKC 78 - 35 R	407 (8)	378 (17)	354 (31)	395 (15)	382 (26)
PhB 30Y79 B	421 (38)	414 (21)	357 (18)	424 (54)	408 (4)
PAN 6723	421 (15)	390 (25)	359 (59)	391 (11)	391 (37)
AFG 4517	398 (31)	350 (37)	369 (66)	382 (42)	375 (43)
LS 8523 B	405 (26)	388 (18)	350 (52)	390 (54)	387 (43)
PhB 30Y83	411 (45)	375 (16)	351 (34)	400 (26)	380 (34)
DKC 77- 87 R	406 (14)	402 (19)	376 (56)	420 (7)	399 (31)
PAN 5Q - 433 B	404 (27)	381 (30)	376 (24)	384 (24)	383 (23)
PhB 30B95 B	435 (22)	387 (54)	392 (24)	373 (86)	404 (48)
DKC 78 - 83 R	425 (33)	391 (32)	394 (63)	406 (42)	410 (42)
LS 8511	395 (30)	360 (39)	372 (42)	411 (10)	389 (37)
CA 9001	450 (34)	405 (41)	387 (66)	424 (18)	412 (42)
Mean	410 a (3.9)	373 c (3.6)	346 d (4.4)	394 b (3.8)	381 (4.6)
Range	349-459	309-405	287-394	340-424	335-412
CV	0.95	0.97	1.27	0.96	1.21
F value	1.52 ns	1.25 ns	1.36 ns	0.97 ns	2.50***

Figures in parentheses are standard deviations
Different letters in the same row denote significant differences at $p < 0.05$
***$p < 0.001$, ns- not significant at $p \geq 0.05$

TABLE 4.1.13 Effects Cultivar and Location on Test Weight (TW) (kg/hl) of Maize Cultivars

	Localities				
Cultivar	Potchefstroom	Klerksdorp	Petit	Bethlehem	Mean
Phb 32A05 B	79.2 (1.3)	79.0 (0.7)	76.2 (2.1)	79.0 (1.4)	78.3 (1.8)
AFG 4321	81.2 (0.5)	76.6 (2.3)	74.1 (3.2)	79.8 (0.8)	77.6 (3.8)
PAN 6223 B	78.8 (2.3)	76.5 (5.0)	77.3 (3.8)	77.5 (1.4)	77.0 (3.2)
PhB 32B10	81.7 (1.2)	78.0 (2.1)	76.0 (2.1)	78.9 (0.8)	78.4 (3.0)
LS 8527 BR	78.4 (1.0)	76.4 (4.4)	76.9 (4.0)	78.3 (0.7)	77.0 (3.1)
PAN 4P – 313 B	80.0 (0.7)	77.4 (1.5)	74.9 (1.0)	79.5 (0.5)	77.9 (2.3)
Phb 31 M 09	79.8 (2.0)	78.7 (1.6)	75.2 (1.5)	78.0 (0.8)	77.8 (2.5)
AFG 4383	80.4 (1.6	77.5 (0.7)	74.3 (2.1)	78.3 (1.7)	77.4 (2.6)
AFG 4445	80.7 (0.2	78.8 (1.7)	76.0 (1.7)	80.4 (0.7)	79.0 (2.2)
AFG 4473	80.0(0.1)	77.3 (1.5)	74.4 (1.3)	78.3 (2.3)	78.6 (5.0)
DKC 78 – 45 BR	80.3(1.1)	78.8 (0.9)	73.2 (2.7)	76.9 (2.6)	77.9 (2.7)
IMP 52 – 11	81.3 (1.5)	80.6 (0.5)	74.9 (1.8)	79.0 (1.5)	79.0 (2.8)
DKC 77 – 61 B	79.3 (1.3	77.3 (1.4)	76.2 (1.4)	78.3 (1.8)	77.7 (1.8)
LS 8519	80.9 (2.1)	78.7 (2.4)	76.4 (0.9)	79.0 (2.2)	78.8 (2.3)
PAN 6Q –445 B	80.8 (1.4)	78.4 (1.3)	73.4 (0.5)	79.4 (1.6)	78.0 (3.2)
CRN 3505	80.6 (1.6)	79.9 (0.4)	77.7 (2.2)	78.7 (0.7)	78.9 (2.0)
Saffier	79.0 (0.7)	79.4 (1.6)	75.2 (2.4)	77.8 (1.7)	78.3 (2.1)
AFG 4555	82.1 (0.4)	79.6 (1.0)	76.3 (1.1)	82.1 (1.3)	79.9 (2.4)
Phb 30D07 B	78.7 (0.2)	78.3 (3.2)	74.9 (1.8)	78.3 (4.1)	77.1 (2.9)
DKC 78 – 15 B	81.0 (1.8)	77.6 (2.7)	75.1 (2.8)	78.9 (0.5)	78.2 (2.8)
PAN 6Q – 521 R	80.8 (0.5)	78.8 (0.3)	75.0 (2.2)	79.4 (0.6)	78.7 (2.3)
PAN 6611	80.7 (0.1)	78.4 (3.1)	75.9 (2.9)	78.5 (1.5)	78.2 (2.6)
LS 8521 B	79.6 (0.2)	80.3 (3.0)	76.6 (2.7)	77.0 (1.1)	78.4 (2.5)
DKC 78 – 35 R	80.8 (1.4)	78.8 (1.4)	76.6 (1.4)	78.3 (1.8)	78.6 (2.0)
PhB 30Y79 B	82.0 (1.8)	78.6 (2.0)	76.0 (1.6)	80.5 (0.7)	79.1 (2.8)
PAN 6723	80.4 (2.7)	78.9 (0.6)	75.3 (0.4)	78.8 (1.8)	78.4 (2.1)
AFG 4517	80.0 (1.8)	77.3 (1.5)	75.9 (2.8)	79.6 (1.4)	77.8 (2.9)
LS 8523 B	78.1 (2.2)	77.7 (2.1)	74.6 (0.9)	77.6 (3.0)	77.1 (2.4)
PhB 30Y83	80.6 (1.2)	79.3 (1.2)	75.5 (2.1)	78.7 (2.5)	78.8 (2.2)
DKC 77- 87 R	80.4 (2.2)	79.1 (1.0)	76.4 (2.8)	78.8 (1.9)	78.4 (2.8)
PAN 5Q – 433 B	80.1 (1.0)	79.1 (2.2)	76.1 (0.3)	79.0 (1.3)	78.6 (1.9)
PhB 30B95 B	81.4 (1.0)	79.5 (1.5)	76.0 (2.5)	78.8 (1.9)	78.6 (3.0)
DKC 78 – 83 R	81.2 (0.7)	79.2 (1.5)	76.0 (2.3)	79.5 (0.6)	78.7 (2.7)
LS 8511	80.5 (0.6)	78.0 (0.7)	75.7 (2.3)	79.4 (0.9)	78.2 (2.6)
CA 9001	82.5 (0.3)	79.4 (1.2)	75.1 (0.6)	80.4 (2.0)	79.1 (3.0)
Mean	80.5a (1.6)	78.7 b (1.9)	75.6 c (2.0)	78.8 b (1.9)	78.3 (2.7)
Range	78.1-82.5	76.4-80.6	73.2-77.3	76.9-82.1	77.0-79.9
CV	1.98	2.41	2.64	2.41	3.44
F value	1.43 ns	0.81 ns	0.61 ns	1.09 ns	1.49*

Figures in parentheses are standard deviations
Different letters in the same row denote significant differences at $p < 0.05$
*$p < 0.05$, ns- not significant at $p \geq 0.05$

Kernel size differed slightly amongst localities (Table 4.1.14). Cultivars grown in Petit were significantly smaller ($p < 0.05$) than those of Potchefstroom, Klerksdorp and Bethlehem. Kernel size did not vary significantly ($p < 0.05$) among cultivars within Potchefstroom and Bethlehem. Slight variations were noticed in Klerksdorp and Petit. The CVs of cultivars within localities were less than 10%. Kernel size is an important factor in milling for grain uniformity to reduce rejected kernels through roller sieves, which would result in reduced milling yields. In the case of sorghum, kernel size was related to grain hardness and larger kernels had higher milling yields (Lee et al 2002).

Kernel breakage susceptibility did not vary with cultivars within the same locality (Table 4.1.15). However, there were large variations within the same cultivar as evidenced by the relatively large overall standard deviations and CV. The overall mean breakage susceptibility was 2.15% over a range of 1.75 to 2.96%. Cultivars of Potchefstroom and Klerksdorp were more susceptible to breakage than those of Petit and Bethlehem. Among yellow dent corn hybrids, breakage susceptibility was 0.5 to 43.8% (Pomeranz et al 1986). The ease of kernel breakage has an impact on handling and processing of grain for dry milling and breakage should be minimized. The values obtained were too low to cause concern on grain quality.

Stress cracking in all the cultivars was minimal and did not vary significantly ($p \geq 0.05$) within each locality despite the high CV (Table 4.1.16a). High standard deviations and CV were observed for cultivars in all localities. As with breakage susceptibility, stress cracking was high in cultivars of Potchefstroom. Kernel stress cracks are not desirable in dry milling because they weaken the kernel making it more susceptible to breakage during transportation and milling. Peplinski et al (1989) recommended an upper limit of 25% stress cracked kernels and results of this study show that stress cracking was less than this limit.

TABLE 4.1.14 Effects of Cultivar and Locality on Maize Kernel Size (% kernels retained on 8mm opening sieve)

	Localities				
Cultivars	Potchefstroom	Klerksdorp	Petit	Bethlehem	Mean
Phb 32A05 B	76.2 (9.1)	59.5 (2.1)	55.3 (1.5)	78.0 (13.0)	67.9 (13.0)
AFG 4321	67.2 (5.7)	64.6 (5.9)	52.6 (0.8)	47.8 (14.4)	61.9 (9.2)
PAN 6223 B	76.5 (4.4)	69.6 (8.9)	78.7 (3.2)	83.7 (4.0)	79.2 (7.8)
PhB 32B10	66.8 (5.9)	70.9 (8.2)	56.1 (2.2)	55.2 (18.7)	68.2 (12.9)
LS 8527 BR	84.9 (4.0)	71.3 (7.4)	81.3 (2.6)	79.2 (0.8)	79.4 (6.6)
PAN 4P - 313 B	78.6 (7.1)	71.4 (12.9)	77.0 (4.4)	78.5 (9.1)	76.4 (8.2)
Phb 31 M 09	81.1 (1.5)	78.2 (8.2)	78.8 (5.8)	78.6 (4.8)	79.3 (4.9)
AFG 4383	75.1 (7.0)	81.5 (4.2)	77.3 (4.0)	75.9 (8.7)	78.7 (5.7)
AFG 4445	79.3 (7.3)	78.4 (5.0)	79.0 (5.1)	77.5 (4.2)	78.6 (4.8)
AFG 4473	80.3 (0.9)	75.0 (2.4)	78.8 (4.6)	78.7 (1.2)	78.3 (3.0)
DKC 78 - 45 BR	77.0 (6.0)	74.9 (3.0)	79.3 (2.9)	76.2 (3.6)	76.9 (3.9)
IMP 52 – 11	81.3 (1.7)	87.8 (4.8)	70.5 (5.7)	78.3 (5.4)	79.8 (7.6)
DKC 77 - 61 B	74.5 (10.1)	77.4 (2.8)	79.7 (6.6)	76.9 (6.8)	78.1 (6.1)
LS 8519	80.8 (6.0)	72.7 (13.2)	79.6 (4.5)	79.4 (7.4)	77.3 (7.8)
PAN 6Q -445 B	83.8 (3.8)	78.8 (5.9)	79.2 (4.6)	77.7 (4.5)	79.6 (4.5)
CRN 3505	75.8 (4.5)	75.3 (3.0)	77.9 (1.2)	74.9 (3.9)	76.0 (3.1)
Saffier	76.3 (4.4)	68.8 (9.4)	74.9 (1.8)	80.2 (2.7)	75.7 (6.6)
AFG 4555	79.4 (8.2)	74.0 (5.5)	75.0 (8.3)	81.6 (8.4)	76.4 (6.9)
Phb 30D07 B	78.3 (3.8)	78.1 (2.8)	77.4 (8.3)	76.3 (3.5)	77.3 (4.4)
DKC 78 - 15 B	74.9 (3.2)	73.8 (8.0)	78.2 (3.3)	75.0 (3.1)	75.2 (4.6)
PAN 6Q - 521 R	77.3 (6.3)	83.7 (2.2)	81.8 (5.6)	77.6 (5.9)	79.9 (5.4)
PAN 6611	80.1 (4.6)	70.8 (10.6)	72.7 (4.2)	80.7 (1.9)	76.1 (7.0)
LS 8521 B	75.3 (5.4)	80.3 (7.5)	74.2 (6.2)	73.8 (5.4)	75.9 (5.9)
DKC 78 - 35 R	73.7 (0.4)	76.6 (3.0)	79.1 (1.0)	73.7 (1.2)	75.8 (2.7)
PhB 30Y79 B	85.5 (8.9)	83.3 (3.5)	79.9 (2.3)	81.2 (11.0)	81.2 (6.4)
PAN 6723	78.8 (8.5)	79.2 (5.3)	79.5 (4.0)	78.8 (4.4)	78.1 (5.4)
AFG 4517	80.2 (3.5)	79.4 (3.6)	77.4 (6.1)	79.4 (2.2)	78.8 (3.6)
LS 8523 B	78.3 (4.0)	79.9 (2.6)	80.5 (5.1)	79.8 (3.7)	79.4 (3.6)
PhB 30Y83	72.7 (6.3)	78.0 (6.2)	78.4 (3.5)	77.1 (5.6)	77.3 (4.8)
DKC 77- 87 R	75.4 (5.1)	80.0 (7.5)	74.3 (5.3)	82.4 (6.4)	77.6 (5.7)
PAN 5Q - 433 B	78.5 (2.2)	76.7 (8.1)	80.0 (4.1)	78.3 (1.5)	78.4 (4.2)
PhB 30B95 B	76.2 (8.5)	76.8 (5.8)	76.1 (7.8)	80.0 (9.5)	76.6 (7.0)
DKC 78 - 83 R	73.7 (7.7)	78.8 (5.3)	75.8 (5.4)	84.8 (12.0)	76.6 (7.2)
LS 8511	81.7 (4.9)	83.4 (3.2)	77.5 (5.8)	82.8 (1.4)	81.6 (4.4)
CA 9001	68.7 (6.7)	77.9 (5.0)	76.9 (4.1)	76.2 (5.9)	75.2 (5.3)
Mean	78.1 a (7.1)	76.2 ab (7.4)	75.7 b (7.9)	77.3 ab (5.6)	76.8 (7.1)
Range	67.2-85.5	49.5-87.8	52.6-81.8	47.8-84.8	61.9-81.6
% CV	9.09	9.71	10.4	7.24	9.24
F value	1.08 ns	2.76***	6.32***	0.91 ns	4.68***

Figures in parentheses are standard deviations
Different letters in the same row denote significant differences at $p < 0.05$
***$p < 0.001$, ns- not significant at $p \geq 0.05$

Results in Table 4.1.15b show that stress cracking was not severe amongst cultivars within each locality. Overall, SCI ranged from 2.0 to 17.6%. SCI differed with locality and Potchefstroom had a higher level of stress cracking index (17.3%) than other localities with means of 3.5% to 8.1%. This was expected considering the percentage stress cracks observed. Stress crack index (SCI) is a factor of percentage stress cracks, therefore similar observations were made between the two parameters. The advantage of SCI is that it indicates the severity of stress cracking within a cultivar. Multiple stress cracks would increase breakage susceptibility and decrease yield of large flaking grits. An average of 140 SCI is recommended for commercial grain by the US Grain Council with a lower SCI being preferred (Paulsen et al 2003). Overall stress crack and SCI values were very low which may imply that stress cracking is not a major problem as the maize is field dried as opposed to artificial drying which would greatly increase stress cracking.

There were significant differences ($p < 0.05$) in TADD hardness among cultivars within localities except in cultivars of Potchefstroom as indicated by percentage kernel removed (Table 4.1.17). Potchefstroom cultivars exhibited lower kernel loss with a mean of 28% and Petit cultivars had the highest kernel removal of 41%, on average. The overall mean range of TADD hardness for all cultivars was 30.0 to 39.1% and the means were highly significantly different ($p < 0.001$). Compared with sorghum, non tannin sorghums ranged from 29.4 to 45.2%. As with TW, sorghum seems to have a wider range of values and probably more varied than maize. Thus, there was little variability of TW in the maize data set. Results of Potchefstroom seemed to contradict those of breakage susceptibility and stress cracking as high levels of breakage and cracking would be associated with reduced hardness. However, stress cracking was seen as a secondary factor in influencing milling quality in hard maize type despite being more susceptible to stress cracking (Kirleis and Stroshine 1990). Severely stressed hard maize grain still produced better milling quality than soft maize. Besides the higher values of cracking and breakage susceptibility, Potchefstroom cultivars did not reach the threshold limits that would affect grain hardness. TADD is a widely used test for sorghum grain hardness (Reichert et al 1986), which has high reproducibility and repeatability (Lee et al 2007).

TABLE 4.1.15 Effects of Cultivar and Location on Kernel Breakage Susceptibility of Maize Cultivars (%) as Measured by the Stein Breakage Test

	Localities				
Cultivar	Potchefstroom	Klerksdorp	Petit	Bethlehem	Mean
Phb 32A05 B	3.00 (2.06)	2.19 (1.02)	1.46 (0.59)	1.80 (1.87)	2.11 (1.42)
AFG 4321	3.59 (2.79)	2.80 (2.03)	2.41 (1.57)	1.92 (1.73)	2.68 (1.89)
PAN 6223 B	4.34 (0.59)	3.04 (0.78)	1.05 (0.65)	1.45 (0.24)	2.47 (1.46)
PhB 32B10	4.40 (1.55)	2.74 (0.66)	1.56 (1.20)	2.14 (1.58)	2.71 (1.57)
LS 8527 BR	4.49 (0.05)	3.18 (1.17)	1.47 (0.32)	1.36 (0.53)	2.63 (1.47)
PAN 4P - 313 B	2.05 (0.65)	3.06 (0.30)	2.04 (1.04)	1.76 (0.53)	2.23 (0.78)
Phb 31 M 09	4.87 (2.07)	2.29 (0.91)	1.32 (0.73)	1.51 (0.38)	2.50 (1.80)
AFG 4383	3.09 (2.14)	3.16 (0.75)	2.63 (1.39)	1.58 (1.05)	2.62 (1.38)
AFG 4445	1.96 (1.45)	3.51 (0.42)	1.11 (0.26)	0.92 (0.29)	1.87 (1.26)
AFG 4473	4.26 (1.37)	1.81 (0.43)	3.06 (1.45)	2.71 (0.60)	2.96 (1.29)
DKC 78 - 45 BR	1.68 (0.49)	2.49 (1.09)	1.93 (1.12)	1.23 (1.22)	1.84 (0.99)
IMP 52 – 11	3.14 (1.88)	1.89 (0.87)	1.77 (0.32)	1.53 (0.58)	2.08 (1.13)
DKC 77 - 61 B	2.70 (1.25)	2.36 (0.78)	1.11 (0.74)	1.11 (0.23)	1.82 (1.03)
LS 8519	2.31 (0.96)	3.09 (1.72)	1.46 (0.68)	1.98 (1.02)	2.21 (1.17)
PAN 6Q -445 B	2.28 (1.14)	3.72 (1.10)	2.25 (2.22)	1.69 (1.00)	2.48 (1.47)
CRN 3505	1.22 (0.17)	2.98 (1.59)	0.85 (0.71)	2.20 (2.53)	1.81 (1.57)
Saffier	2.99 (1.97)	2.28 (1.02)	2.22 (2.85)	1.27 (0.60)	2.19 (1.69)
AFG 4555	2.40 (0.63)	3.11 (0.77)	1.29 (1.12)	1.08 (0.52)	1.97 (1.10)
Phb 30D07 B	4.35 (1.99)	2.54 (0.82)	1.86 (1.96)	1.82 (0.46)	2.65 (1.65)
DKC 78 - 15 B	1.29 (0.34)	3.80 (2.58)	0.98 (0.35)	0.96 (0.28)	1.76 (1.67)
PAN 6Q - 521 R	2.52 (1.41)	2.18 (1.19)	1.23 (0.43)	1.09 (0.14)	1.75 (1.03)
PAN 6611	1.69 (0.76)	3.13 (1.76)	1.32 (0.60)	2.81 (1.33)	2.24 (1.29)
LS 8521 B	3.09 (1.09)	2.30 (0.88)	0.94 (0.35)	1.57 (0.43)	1.98 (1.05)
DKC 78 - 35 R	1.94 (1.70)	3.64 (0.44)	0.62 (0.32)	1.07 (0.58)	1.82 (1.45)
PhB 30Y79 B	2.58 (1.35)	2.88 (1.37)	0.71 (0.41)	1.47 (0.59)	1.91 (1.26)
PAN 6723	2.53 (1.48)	2.12 (0.75)	0.93 (0.55)	1.94 (0.82)	1.88 (1.03)
AFG 4517	3.80 (0.66)	2.89 (0.75)	0.99 (0.64)	1.30 (0.68)	2.25 (1.34)
LS 8523 B	4.76 (2.63)	2.32 (1.08)	0.98 (0.82)	1.51 (0.55)	2.39 (1.98)
PhB 30Y83	2.79 (1.63)	2.03 (0.51)	0.58 (0.49)	1.62 (1.09)	1.76 (1.22)
DKC 77- 87 R	2.59 (1.20)	2.69 (0.65)	1.27 (0.21)	1.47 (0.22)	2.01 (0.89)
PAN 5Q - 433 B	2.75 (0.43)	2.45 (0.18)	1.01 (0.71)	1.82 (0.61)	2.01 (0.83)
PhB 30B95 B	2.99 (2.31)	2.82 (1.06)	1.26 (0.74)	1.96 (0.52)	2.26 (1.36)
DKC 78 - 83 R	2.57 (1.84)	2.55 (1.43)	1.05 (0.43)	1.81 (0.61)	1.99 (1.23)
LS 8511	2.43 (0.44)	2.02 (1.21)	0.98 (0.81)	2.02 (0.81)	1.86 (0.92)
CA 9001	1.86 (1.27)	1.66 (1.36)	1.24 (0.54)	1.60 (0.30)	1.59 (0.87)
Mean	2.89 a (0.88)	2.68 a (1.07)	1.40 b (1.02)	1.63 b (1.57)	2.15 (1.33)
Range	1.22-4.87	1.66-3.80	0.58-3.06	0.92-2.81	1.75-2.96
CV	29.5	40.6	39.9	96.3	61.9
F value	1.33 ns	0.78 ns	0.96 ns	0.68 ns	1.06ns

Figures in parentheses are standard deviations
Different letters in the same row denote significant differences at $p < 0.05$
Ns, not significant at $p \geq 0.05$

TABLE 4.1.16a Effects of Locality and Cultivar on Stress Cracks (SC) (%) of Maize Cultivars

	Localities				
Cultivar	Potchefstroom	Klerksdorp	Petit	Bethlehem	Mean
Phb 32A05 B	6.00 (4.00)	4.00 (4.00)	2.67 (2.31)	4.67 (4.16)	4.33 (3.39)
AFG 4321	8.00 (2.00)	4.67 (4.16)	2.00 (2.00)	2.00 (2.00)	4.17 (3.66)
PAN 6223 B	4.00 (3.46)	0.67 (1.15)	0.00 (0.00)	2.67 (2.31)	1.83 (2.48)
PhB 32B10	12.67 (6.43)	5.33 (5.03)	3.33 (4.16)	4.67 (3.06)	6.50 (5.60)
LS 8527 BR	6.67 (6.43)	2.67 (2.31)	2.67 (2.31)	0.00 (0.00)	3.00 (3.95)
PAN 4P - 313 B	4.67 (1.15)	1.33 (2.31)	0.67 (1.15)	2.00 (2.00)	2.17 (2.17)
Phb 31 M 09	12. 00 (7.21)	0.67 (1.15)	2.00 (0.00)	2.00 (2.00)	4.17 (5.75)
AFG 4383	5.33 (5.03)	0.67 (1.15)	5.33 (3.06)	0.67 (1.15)	3.00 (3.57)
AFG 4445	5.33 (4.16)	2.67 (3.06)	0.00 (0.00)	0.00 (0.00)	2.00 (3.19)
AFG 4473	5.33 (5.03)	3.33 (4.16)	1.33 (1.15)	3.33 (1.15)	3.33 (3.23)
DKC 78 - 45 BR	4.67 (3.06)	1.33 (2.31)	4.00 (2.00)	1.33 (1.15	2.83 (2.48)
IMP 52 – 11	9.33 (5.03)	0.00 (0.00)	2.67 (1.15)	2.00 (2.00)	3.50 (4.36)
DKC 77 - 61 B	6.00 (5.29)	2.67 (3.06)	2.00 (3.46)	0.67 (1.15)	2.83 (3.66)
LS 8519	6.67 (4.16)	0.67 (1.15)	2.67 (2.31)	2.00 (3.46)	3.00 (3.46)
PAN 6Q -445 B	7.33 (1.15)	2.00 (3.46)	0.67 (1.15)	1.33 (2.31)	2.83 (3.35)
CRN 3505	6.67 (3.06)	2.00 (3.46)	1.33 (2.31)	2.67 (2.31)	3.17 (3.24)
Saffier	5.33 (5.03)	1.33 (2.31)	1.33 (2.31)	3.33 (3.06)	2.83 (3.35)
AFG 4555	12.00 (5.29)	1.33 (2.31)	2.67 (1.15)	0.00 (0.00)	4.00 (5.53)
Phb 30D07 B	10.00 (2.00)	0.00 (0.00)	4.67 (4.62)	0.67 (1.15)	3.83 (4.71)
DKC 78 - 15 B	2.00 (0.00)	4.00 (3.46)	4.67 (2.31)	0.00 (0.00)	2.67 (2.61)
PAN 6Q - 521 R	6.00 (6.93)	6.67 (4.16)	2.00 (3.46)	2.00 (2.00)	4.17 (4.47)
PAN 6611	8.67 (9.02)	2.00 (2.00)	2.00 (2.00)	0.67 (1.15)	3.33 (5.21)
LS 8521 B	11.33 (7.57)	1.33 (2.31)	3.33 (4.16)	4.00 (5.29)	5.00 (5.94)
DKC 78 - 35 R	2.67 (2.31)	0.00 (0.00)	0.67 (1.15)	0.67 (1.15)	1.00 (1.60)
PhB 30Y79 B	7.33 (1.15)	0.00 (0.00)	1.33 (1.15)	0.00 (0.00)	2.17 (3.24)
PAN 6723	3.33 (2.31)	4.67 (4.16)	8.00 (12.17)	2.67 (2.31)	4.67 (6.05)
AFG 4517	8.00 (2.00)	1.33 (2.31)	2.67 (3.06)	0.67 (1.15)	3.17 (3.56)
LS 8523 B	8.67 (9.87)	4.67 (3.06)	4.67 (3.06)	3.33 (4.16)	5.33 (5.35)
PhB 30Y83	4.00 (2.00)	0.67 (1.15)	3.33 (5.77)	0.67 (1.15)	2.17 (3.13)
DKC 77- 87 R	3.33 (2.31)	0.67 (1.15)	5.33 (5.77)	4.00 (2.00)	3.33 (3.34)
PAN 5Q - 433 B	4.00 (2.00)	0.00 (0.00)	3.33 (3.06)	0.00 (0.00)	1.83 (2.48)
PhB 30B95 B	8.00 (7.21)	0.67 (1.15)	0.67 (1.15)	1.33 (1.15)	2.67 (4.54)
DKC 78 - 83 R	9.33 (9.02)	0.67 (1.15)	3.33 (5.77)	1.33 (1.15)	3.67 (5.84)
LS 8511	5.55 (3.06)	2.67 (2.31)	4.67 (2.31)	0.67 (1.15)	3.67 (2.74)
CA 9001	2.67 (2.31)	0.00 (0.00)	2.00 (2.00)	0.67 (1.15)	3.33 (1.78)
Mean	6.65 a (2.23)	1.92 b (2.69)	2.69 b (3.36)	1.68 b (4.92)	3.23 (4.01)
Range	2.00-12.67	0.00-6.67	0.00-8.00	0.00-4.67	1.00-4.17
CV	33.5	140.1	124.9	292.9	124.1
F value	0.91 ns	1.39 ns	0.99 ns	1.25 ns	1.31ns

Figures in parentheses are standard deviations
Different letters in the same row denote significant differences at $p < 0.05$
Ns, not significant at $p \geq 0.05$

TABLE 4.1.16b Effects of Cultivar and Location on Stress Crack Index (SCI) of Maize Cultivars

	Localities				
Cultivar	Potchefstroom	Klerksdorp	Petit	Bethlehem	Mean
Phb 32A05 B	18.0 (18.3)	9.33 (10.07)	8.67 (8.08)	18.00 (15.88)	13.50 (12.62)
AFG 4321	22.7 (15.0)	14.00 (12.17)	4.67 (6.43)	3.33 (5.77)	11.17 (12.16)
PAN 6223 B	10.7 (9.5)	0.67 (1.15)	0.00 (0.00)	8.00 (6.93)	4.83 (6.95)
PhB 32B10	39.3 (14.5)	8.67 (8.08)	5.67 (8.14)	16.67 (5.77)	17.58 (16.05)
LS 8527 BR	10.7 (13.3)	8.00 (6.93)	5.33 (4.62)	0.00 (0.00)	6.00 (7.86)
PAN 4P - 313 B	12.7 ((1.7)	0.67 (1.15)	0.67 (1.15)	4.00 (3.46)	4.50 (7.34)
Phb 31 M 09	23.3 (15.0)	2.00 (3.46)	3.33 (2.31)	2.00 (2.00)	7.67 (11.59)
AFG 4383	12.0 (11.1)	1.33 (2.31)	8.67 (6.11)	3.33 (5.77)	6.33 (7.48)
AFG 4445	17.3 (21.6)	3.33 (4.16)	0.00 (0.00)	0.00 (0.00)	5.17 (11.98)
AFG 4473	13.3 (15.3)	0.67 (1.15)	4.00 (3.46)	11.33 (7.57)	7.33 (9.20)
DKC 78 - 45 BR	16.7 (9.5)	6.67 (11.55)	10.67 (6.43)	2.67 (3.06)	9.17 (8.88)
IMP 52 – 11	20.7 (17.0)	4.00 (6.93)	10.67 (8.08)	2.67 (3.06)	9.50 (11.41)
DKC 77 - 61 B	18.0 (16.4)	0.67 (1.15)	4.67 (8.08)	0.67 (1.15)	6.00 (10.79)
LS 8519	17.3 (12.1)	2.67 (3.06)	8.00 (8.00)	4.00 (6.93)	8.00 (9.19)
PAN 6Q -445 B	16.7 (1.2)	3.33 (5.77)	0.67 (1.15)	4.67 (4.16)	6.33 (7.13)
CRN 3505	20.0 (12.0)	3.33 (5.77)	4.00 (6.93)	8.00 (6.93)	8.83 (9.93)
Saffier	7.3 (8.1)	2.00 (3.46)	2.67 (4.62)	4.67 (4.16)	4.17 (5.08)
AFG 4555	31.3 (18.6)	0.67 (1.15)	6.67 (1.15)	0.00 (0.00)	9.67 (15.54)
Phb 30D07 B	19.3 (3.1)	0.00 (0.00)	8.67 (2.31)	0.67 (1.15)	7.17 (8.33)
DKC 78 - 15 B	6.0 (4.0)	3.33 (5.77)	10.00 (8.00)	0.00 (0.00)	4.83 (5.94)
PAN 6Q - 521 R	20.0 (27.8)	7.33 (2.31)	4.67 (8.08)	0.67 (1.15)	8.17 (14.51)
PAN 6611	6.0 (8.7)	8.00 (10.58)	6.00 (5.29)	2.67 (4.62)	5.67 (6.87)
LS 8521 B	26.0 (22.5)	3.33 (4.16)	3.33 (4.16)	10.00 (12.49)	10.67 (14.85)
DKC 78 - 35 R	6.7 (8.3)	0.00 (0.00)	0.67 (1.15)	0.67 (1.15)	2.00 (4.59)
PhB 30Y79 B	12.7 (4.1)	0.00 (0.00)	2.67 (3.06)	3.33 (5.77)	4.67 (5.99)
PAN 6723	8.7 (8.3)	6.67 (5.77)	23.33 (35.35)	8.00 (10.58)	11.67 (17.78)
AFG 4517	29.3 (12.1)	4.00 (6.93)	13.33 (15.28)	0.00 (0.00)	11.67 (14.72)
LS 8523 B	25.3 (26.9)	6.00 (6.00)	18.67 (16.04)	11.33 (17.93)	15.33 (17.36)
PhB 30Y83	9.3 (1.15)	6.00 (6.00)	12.67 (21.94)	0.67 (1.15)	7.17 (10.77)
DKC 77- 87 R	31.3 (30.3)	0.67 (1.15)	18.67 (22.30)	7.33 (8.08)	14.50 (20.43)
PAN 5Q - 433 B	10.0 (10.6)	0.00 (0.00)	10.00 (8.72)	2.00 (3.46)	5.50 (7.68)
PhB 30B95 B	24.0 (21.6)	0.67 (1.15)	0.67 (1.15)	4.00 (3.46)	7.33 (13.81)
DKC 78 - 83 R	20.7 (18.6)	0.67 (1.15)	15.33 (26.56)	6.67 (7.02)	10.83 (16.28)
LS 8511	12.0 (9.2)	3.33 (4.16)	8.67 (4.62)	0.67 (1.15)	6.17 (6.63)
CA 9001	9.3 (8.3)	0.00 (0.00)	4.67 (6.43)	3.33 (5.77)	4.33 (6.20)
Mean	17.28 a (7.01)	3.49 c (5.08)	7. 17 b (10.86)	4.40 bc (14.92)	8.10 (11.60)
Range	6.0-39.3	0.00-14.00	0.00-23.33	0.00-18.00	2.00-17.58
CV	40.6	145.6	151.6	339.1	143.2
F value	0.86 ns	1.18 ns	0.97 ns	1.55 ns	1.41 ns

Figures in parentheses are standard deviations
Different letters in the same row denote significant differences at $p < 0.05$
Ns, not significant at $p \geq 0.05$

NIT Milling Index derived from data from a pilot scale roller milling process was used to determine maize hardness. The overall Milling Index means ranged from 69.0 to 94.8, and the means were highly significantly different for cultivars ($p < 0.001$) (Table 4.1.18). Milling Index did not differ significantly among Potchefstroom and Bethlehem except with Petit and

Klerksdorp cultivars. However, Potchefstroom and Bethlehem cultivars had higher milling indices than Petit and Klerksdorp. Unlike TW, NIT Milling Index had a wider range of data as well as the TADD and would potentially screen cultivars which are closely similar.

TABLE 4.1.17 Effects of Cultivar and Locality on Kernel Hardness as Measured by the TADD (% kernel removed), of Maize Cultivars

	Localities				
Cultivar	Potchefstroom	Klerksdorp	Petit	Bethlehem	Mean
Phb 32A05 B	28.9 (5.2)	34.0 (3.7)	40.3 (3.0)	31.8 (1.4)	32.8 (5.5)
AFG 4321	29.6 (2.1)	35.6 (3.8)	38.4 (1.2)	35.6 (1.0)	33.8 (3.9)
PAN 6223 B	32.6 (3.3)	44.8 (1.7)	52.1 (3.4)	31.2 (2.7)	32.8 (9.7)
PhB 32B10	27.7 (3.8)	37.3 (9.4)	33.5 (2.2)	30.9 (3.3)	33.5 (6.2)
LS 8527 BR	29.6 (2.1)	36.5 (10.4)	32.5 (3.2)	30.2 (2.7)	34.5 (5.6)
PAN 4P – 313 B	31.3 (4.0)	40.2 (7.0)	49.4 (3.3)	27.7 (3.6)	37.1 (9.6)
Phb 31 M 09	27.0 (2.7)	32.0 (3.4)	38.1 (3.8)	32.3 (2.1)	33.3 (4.8)
AFG 4383	29.9 (4.7)	38.7 (3.7)	42.8 (2.7)	33.3 (3.3)	34.8 (6.0)
AFG 4445	29.5 (5.5)	36.7 (4.6)	45.5 (6.4)	32.7 (2.9)	33.7 (7.6)
AFG 4473	30.9 (2.5)	38.8 (2.3)	42.0 (6.2)	34.3 (3.4)	36.3 (5.6)
DKC 78 – 45 BR	28.9 (3.9)	32.6 (2.6)	44.6 (11.3)	32.4 (2.5)	32.6 (8.2)
IMP 52 – 11	25.3 (1.5)	28.0 (3.7)	41.1 (2.0)	28.9 (1.1)	33.1 (6.6)
DKC 77 – 61 B	25.1 (1.7)	31.4 (5.4)	37.0 (4.1)	30.0 (1.0)	30.9 (5.4)
LS 8519	26.2 (3.8)	35.7 (8.8)	39.7 (8.1)	32.6 (0.8)	32.9 (7.5)
PAN 6Q –445 B	28.0 (6.0)	36.6 (4.4)	46.4 (6.2)	29.6 (1.1)	33.9 (8.0)
CRN 3505	26.4 (4.6)	33.9 (2.3)	43.9 (5.9)	32.0 (2.1)	32.9 (7.5)
Saffier	27.0 (5.8)	29.8 (4.7)	36.3 (4.6)	28.6 (1.1)	32.7 (5.5)
AFG 4555	25.6 (4.2)	31.5 (3.1)	35.5 (2.1)	29.6 (4.0)	30.5 (4.7)
Phb 30D07 B	27.8 (4.8)	36.4 (5.2)	38.1 (2.5)	29.6 (0.9)	31.3 (5.8)
DKC 78 – 15 B	30.1 (1.8)	39.7 (3.5)	48.0 (1.5)	35.7 (1.7)	39.1 (7.3)
PAN 6Q – 521 R	28.4 (1.20)	41.1 (4.4)	38.7 (4.4)	31.5 (0.9)	35.9 (6.3)
PAN 6611	27.4 (4.4)	42.2 (2.7)	50.5 (2.8)	32.7 (5.4)	36.9 (10.0)
LS 8521 B	26.1 (4.9)	34.2 (2.1)	41.4 (7.1)	27.3 (2.5)	30.0 (7.5)
DKC 78 – 35 R	29.6 (4.6)	34.4 (3.0)	43.9 (3.4)	35.8 (1.3)	35.4 (6.1)
PhB 30Y79 B	29.2 (1.9)	33.7 (2.9)	36.0 (1.4)	29.8 (1.1)	31.5 (3.4)
PAN 6723	27.1 (4.6)	34.6 (4.8)	42.0 (2.5)	34.0 (1.9)	35.6 (6.3)
AFG 4517	30.4 (2.2)	33.0 (2.4)	39.1 (2.6)	29.4 (3.8)	31.5 (4.6)
LS 8523 B	26.6 (3.2)	35.6 (4.3)	33.4 (2.0)	32.4 (2.4)	35.0 (4.5)
PhB 30Y83	29.1 (0.8)	33.5 (4.8)	34.3 (1.1)	31.4 (2.6)	34.8 (3.2)
DKC 77- 87 R	30.0 (3.2)	36.1 (1.1)	43.0 (3.8)	35.4 (2.7)	34.2 (5.3)
PAN 5Q – 433 B	27.8 (2.1)	37.0 (4.0)	42.9 (2.6)	29.3 (4.6)	34.0 (7.0)
PhB 30B95 B	23.2 (4.2)	35.6 (0.4)	41.0 (5.5)	32.8 (3.7)	32.3 (7.5)
DKC 78 – 83 R	27.3 (0.6)	29.9 (0.9)	40.6 (4.5)	25.2 (1.4)	31.8 (6.5)
LS 8511	26.4 (4.6)	31.7 (1.0)	39.1 (5.2)	30.0 (2.8)	32.6 (5.8)
CA 9001	25.1 (1.4)	25.9 (0.7)	41.2 (8.0)	28.0 (2.2)	34.5 (7.7)
Mean	28.0d (3.3)	35 1b (5.3)	41.0a (5.9)	31.3c (3.6)	33.8 (6.6)
Range	23.2-32.6	25.9-44.8	32.5-52.1	25.2-35.8	30.0-39.1
CV	11.7	15.1	14.9	11.5	19.5
F value	0.94 ns	3.19 ***	3.25***	2.67***	4.47***

Figures in parentheses are standard deviations
Different letters in the same row denote significant differences at $p < 0.05$
***$p < 0.001$, ns- not significant at $p \geq 0.05$

TABLE 4.1.18 Effects of Cultivar and Locality on NIT Milling Index of Maize Cultivars

	Localities				
Cultivar	Potchefstroom	Klerksdorp	Petit	Bethlehem	Mean
Phb 32A05 B	96.4 (5.5)	86.8 (3.6)	79.6 (2.6)	90.7 (2.8)	88.4 (7.2)
AFG 4321	89.5 (7.3)	96.9 (1.3)	91.8 (2.7)	85.6 (16.3)	90.9 (8.8)
PAN 6223 B	88.4 (9.7)	54.5 (4.1)	42.8 (5.0)	90.3 (0.3)	69.0 (22.7)
PhB 32B10	94.4 (1.8)	84.6 (4.4)	77.4 (5.8)	93.7 (4.5)	87.5 (8.2)
LS 8527 BR	89.4 (2.3)	87.9 (2.2)	91.8 (3.1)	93.1 (11.9)	90.5 (5.8)
PAN 4P – 313 B	88.4 (5.6)	68.9 (4.2)	50.8 (5.2)	93.6 (8.7)	75.5 (18.5)
Phb 31 M 09	94.0 (1.8)	90.1 (0.1)	78.6 (3.7)	95.4 (2.6)	89.3 (7.1)
AFG 4383	96.7 (2.9)	78.0 (2.3)	69.6 (3.0)	83.4 (8.2)	81.6 (10.7)
AFG 4445	92.3 (1.9)	80.8 (2.0)	59.6 (13.9)	92.6 (4.5)	81.8 (15.8)
AFG 4473	93.3 (5.2)	83.4 (0.2)	75.7 (2.4)	96.5 (8.1)	87.2 (9.5)
DKC 78 – 45 BR	90.1 (1.7)	80.7 (4.1)	65.0 (10.8)	92.6 (2.3)	81.9 (12.2)
IMP 52 – 11	98.0 (5.2)	96.7 (2.7)	87.8 (3.0)	96.7 (6.1)	94.8 (5.7)
DKC 77 – 61 B	92.3 (6.2)	82.4 (0.8)	75.1 (8.3)	91.3 (2.3)	85.5 (8.8)
LS 8519	97.9 (0.3)	88.3 (2.8)	72.2 (12.0)	92.7 (3.6)	87.3 (11.0)
PAN 6Q –445 B	89.3 (3.9)	83.9 (0.4)	54.2 (13.2)	93.3 (1.4)	80.5 (17.3)
CRN 3505	96.5 (5.0)	89.3 (0.5)	71.3 (5.8)	94.4 (2.1)	87.5 (10.6)
Saffier	97.3 (5.3)	100.2 (1.9)	87.5 (4.7)	89.8 (13.9)	94.2 (8.9)
AFG 4555	93.7 (4.8)	91.5 (3.5)	85.3 (1.0)	98.9 (3.2)	92.5 (5.9)
Phb 30D07 B	93.1 (3.3)	78.5 (4.9)	75.3 (5.7)	89.2 (11.2)	83.6 (9.3)
DKC 78 – 15 B	90.2 (2.0)	69.8 (3.9)	64.8 (2.4)	89.2 (4.1)	78.6 (12.2)
PAN 6Q – 521 R	95.9 (3.5)	69.9 (7.9)	79.4 (6.9)	96.1 (5.0)	85.2 (12.7)
PAN 6611	96.8 (3.3)	56.8 (17.5)	59.4 (6.2)	96.3 (6.9)	77.0 (21.6)
LS 8521 B	103.2 (5.5)	84.5 (1.6)	73.9 (8.1)	102.2 (3.1)	90.7 (13.3)
DKC 78 – 35 R	91.4 (9.2)	77.2 (6.2)	71.4 (7.5)	90.0 (5.4)	83.2 (11.5)
PhB 30Y79 B	95.8 (3.3)	87.7 (3.9)	82.6 (0.5)	94.6 (3.3)	90.1 (6.1)
PAN 6723	93.0 (5.6)	92.4 (0.2)	75.5 (4.7)	97.9 (4.1)	90.1 (9.7)
AFG 4517	87.4 (4.6)	86.1 (4.6)	75.9 (3.3)	94.4 (3.3)	85.8 (7.7)
LS 8523 B	93.7 (4.0)	89.7 (5.5)	80.9 (19.5)	94.0 (4.2)	89.2 (10.4)
PhB 30Y83	93.5 (4.4)	84.8 (3.6)	86.2 (2.2)	93.4 (4.1)	90.0 (5.7)
DKC 77- 87 R	92.2 (2.3)	79.6 (2.2)	72.0 (4.2)	93.3 (2.8)	84.1 (9.5)
PAN 5Q – 433 B	97.7 (5.4)	84.6 (7.7)	75.1 (3.5)	94.9 (2.8)	87.8 (10.0)
PhB 30B95 B	91.1 (1.0)	79.4 (5.8)	70.9 (12.5)	89.7 (7.8)	83.2 (11.3)
DKC 78 – 83 R	97.7 (5.0)	89.9 (0.5)	75.9 (4.7)	102 4 (2.2)	91.1 (10.7)
LS 8511	95.6 (0.3)	92.3 (3.7)	81.7 (3.5)	99.9 (3.2)	92.4 (7.5)
CA 9001	97.0 (2.1)	98.0 (6.1)	68.2 (7.5)	94.9 (4.0)	89.4 (13.6)
Mean	93.7a (6.2)	83.6b (10.1)	73.9c (11.5)	93.6a (5.1)	86.2 (11.7)
Range	88.4-103.2	54.5-100.2	42.8-91.8	83.4-102.4	69.0-94.8
CV	6.61	12.0	15.6	5.45	13.6
F value	1.56 ns	13.85***	10.46***	1.14 ns	11.13***

Figures in parentheses are standard deviations
Different letters in the same row denote significant differences at $p < 0.05$
***$p < 0.001$, ns- not significant at $p \geq 0.05$

The TW of maize cultivars had a narrower range (77.0 to 79.9 kg/hl) than those reported for cultivars grown elsewhere (Duarte et al 2005; Lee et al 2007; Johnson et al 2010). South Africa has selected for hard white maize for many years, hence the closeness of the values.

TKW was, however, within the range reported by Duarte et al (2005), Lee et al (2007) and Johnson et al (2010). TADD hardness was remarkably similar for maize (33.8% ± 6.6%) and sorghum (35.1% ± 7.0%). The high CVs for TKW (12.3%) and TADD decortication (19.5%) suggest that these parameters could be used to resolve differences in quality between batches of commercial maize. The mean squares (Table 4.1.19) indicated that locality affected maize grain quality parameters more than cultivar and cultivar x locality interactions except for kernel size. The cultivar effect was not significant for breakage susceptibility and stress cracking. Location contributed to 98%, 97% and 95% variation in TW, TADD hardness and NIT Milling Index, respectively. The results of this study are in contrast to previous reports where genotype was found to have a more profound effect on grain hardness parameters than environmental conditions, growing seasons and cultural practices (Duarte et al 2005). Dent maize genotypes of the same temperate germplasm were found to have differences in their grain quality parameters and the authors suggested that genotypic evaluation may be used to identify hard kernels suitable for dry milling (Duarte et al 2005). Lee et al (2007) and (Li et al 1996) also reported the effect of hybrid on maize quality.

TABLE 4.1.19 Mean Squares for Cultivar and Location Effects on Test Weight, Breakage Susceptibility, Kernel Size, Stress Cracking, Thousand Kernel Weight, TADD Kernel Removal and NIT Milling Index of Maize Cultivars Grown in Four Localities

Source	d.f	TKW	TW	KS	SB	SC	SCI	TADD	NIT
Cultivar (C)	34	31.6***	4.71*	156.2***	1.41ns	14.1ns	139.1ns	64.3***	326.8***
Location (L)	3	725.2***	468.6***	108.6*	56.0***	570.2***	4046.0***	3093.2**	8583.5***
(C x L)	102	12.1ns	2.5ns	49.8*	1.3ns	10.3ns	84.2ns	22.7***	108.7***

TW, test weight(kg/hl); SB, % breakage susceptibility by Stein Breakage Tester; SC, % stress cracks; SCI; stress crack index; TKW; Thousand kernel weight(g); TADD (% kernel removed); KS; % kernel size ≥ 8 mm; NIT, NIT Milling Index; C, cultivar; L, locality; C x L, cultivar x locality interactions; d.f, degrees of freedom; MS, mean square values
[a]Data of 35 maize cultivars cultivated in 4 locations (n=140)
Data in parentheses are standard deviations
*$p < 0.05$, ** $p < 0.01$ and ***$p < 0.001$, ns- not significant at $p \geq 0.05$

Correlation analyses for cultivars and localities showed that the analysed parameters were mostly significantly related to each other except kernel size (Table 4.1.20). TKW was highly correlated ($p < 0.001$) with test weight and TADD hardness although the *r* values were very low ($r = 0.415$ and $r = -0.435$, respectively), indicating that only a relatively small amount of the variation is accounted for by these relationships. The relationship of TKW with TW indicated that to some extent, TKW is related to kernel density. For maize, kernel size was not related with any other hardness properties. TADD hardness (percentage kernel removed) of non tannin sorghum cultivars was highly correlated with large kernel sizes (at least 3.35mm). Sorghum TKW was correlated with kernel size and not with test weight as with maize. TKW may not be related to grain density in sorghum as observed with maize. Stress cracking and SCI were also highly correlated ($r = 0.873$, $p < 0.001$) because of their dependence on each other. NIT Milling Index was highly significantly negatively correlated with TADD hardness (percentage kernel removed) ($r = -0.659$, $p < 0.001$).

TABLE 4.1.20 Pearson Correlation Coefficients between Test Weight, Breakage Susceptibility, Kernel Size, Stress Cracking, Thousand Kernel Weight, TADD Kernel Removal and NIT Milling Index of Maize Cultivars Grown in Four Localities

	TW	SB	SC	SCI	TKW	TADD	KS
SB	0.085 ns						
SC	0.126 ns	0.285 ns					
SCI	0.128 ns	0.265 ns	0.873***				
TKW	0.415 ***	0.041 ns	0.180 ns	0.199 ns			
TADD	-0.636***	-0.155 ns	-0.194 ns	-0.172 ns	-0.435 ***		
KS	0.108 ns	0.013 ns	0.051 ns	0.030 ns	0.100 ns	-0.065 ns	
NIT	0.540***	0.112 ns	0.151 ns	0.145 ns	0.328 ns	-0.659***	0.067 ns

***$p < 0.001$, ns- not significant at $p \geq 0.05$
TW, Test weight(kg/hl); SB, % breakage susceptibility by Stein Breakage Tester; SC, % stress cracks; SCI; Stress crack index; TKW; Thousand kernel weight(g); TADD (% kernel removed); KS; % kernel size ≥ 8 mm; NIT, NIT Milling Index.

With regard to the PCA data for maize, the first two principal components explained almost 65% of the total variation (Fig 4.1.3). PC 1 was influenced by TW and TKW and by SB. The

second principal component (PC 2) was characterised strongly by TADD and NIT Milling Index, with TADD (percentage kernel removed) being inversely related to NIT Milling Index. Maize hardness was therefore clearly associated with PC 2.

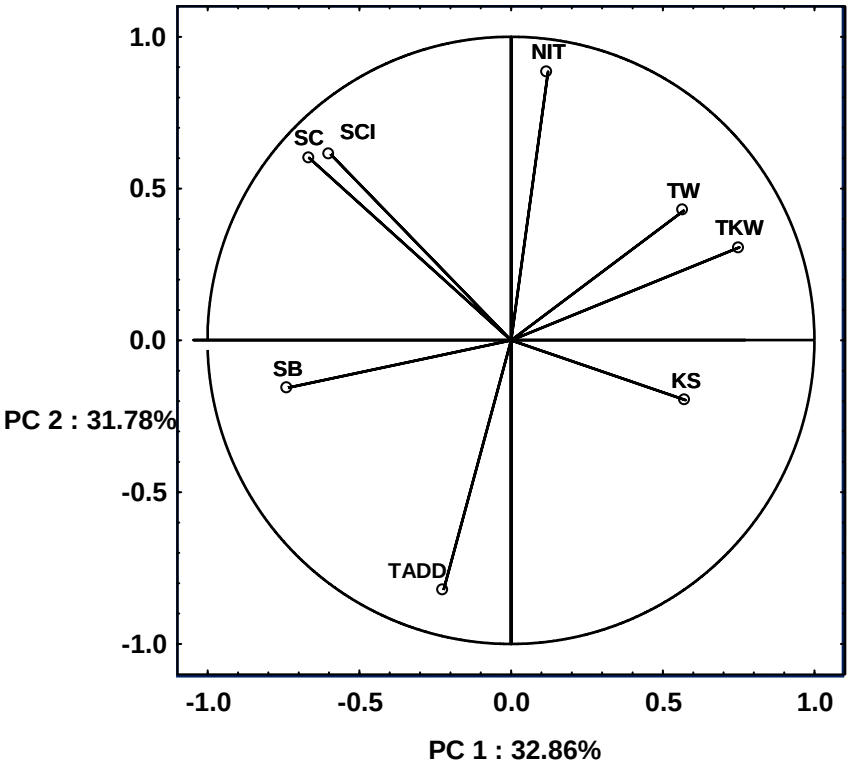

Fig 4.1.3. Factor coordinates of the first two principal components (PC) for maize with respect to test weight (TW), Stein Breakage (SB), stress cracks (SC), stress cracking index (SCI) thousand kernel weight (TKW), kernel size (KS), Tangential Abrasive Dehulling Device (TADD) (% kernel removed) and NIT Milling Index properties.

4.1.4 CONCLUSIONS

Not all simple grain quality parameters are related to each other. Grain quality tests for evaluating sorghum are different from those of maize. Locality generally affected the grain quality parameters more than cultivar or cultivar x locality interactions. TADD, TW, TKW

and kernel size > 3.35 mm can be used together to select sorghum grain for hardness. TADD and NIT Milling Index, or TADD and TW are useful for maize. TADD and TW thus seem suitable for evaluating both grain types. These methods to measure grain hardness worked the best among the ones tested. However, it is quite possible that others which were not tested would also work. The high CV for TADD for both sorghum and maize indicates that it is useful to distinguish among commercial cultivars specifically for grain hardness. The results of the widely varying maize cultivars point to the fact that if used accurately, translucency may be a quick and efficient method for screening maize cultivars without destroying sample material.

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4.2 Relationship between sorghum and maize grain hardness, porridges and sorghum malt modification.

ABSTRACT

The effect of grain hardness on pasting of flours and porridge texture was evaluated in sorghum and maize cultivars varying in hardness. Changes in the hardness of sorghum malt and its modification was also studied and related to malting quality. Maize pasting properties were clearly affected by grain hardness, harder grains in terms of TADD decortication, as found in Chapter 4.1, produced porridges with high final and setback viscosities. The viscosity of sorghum pastes varied between the condensed tannin and non-tannin cultivars. Thus, sorghum porridge quality may not only be affected by intrinsic grain hardness but also chemical composition. SEM showed that during modification the starchy endosperm was degraded from Day 1 of malting in the soft cultivar which also had the highest Diastatic Power (DP) (amylase activity). The hard sorghum with high DP was also modified fast during malting and by Day 3 of malting, both the hard and soft cultivars had similar hardness properties. The cultivar of intermediate hardness, which had the lowest DP was modified to a lesser extent with most of the starch granules in the grain remaining intact. The study showed that the amount of amylase activity (DP) affected modification more than intrinsic grain hardness

4.2.1 INTRODUCTION

Maize and sorghum porridges are staples in most parts of Africa and consumers prefer non-sticky stiff porridges (Rooney et al 1986, Taylor et al 1997). Grain hardness influences texture of porridges with hard grains producing porridges of acceptable quality (Kebakile et al 2008). In addition, the milling process also has an effect on porridge quality. Among sorghum milling techniques, Kebakile et al (2008) also recommended abrasive decortication followed by hammer milling as a technology for producing high quality porridges. In sorghum porridges, texture in terms of gel consistency and porridge firmness measured by a penetrometer were significantly correlated with grain abrasive hardness index ($r = 0{\cdot}81$, $p < 0{\cdot}01$) and ($r = 0.55$, $p < 0{\cdot}05$), respectively (Aboubacar et al 1999).

In selecting cultivars suitable for porridges, Taylor et al (1997) found a negative correlation between grain Brabender hardness and flour pasting peak viscosity (PPV) of sorghum porridges. The authors recommended that both hardness and PPV be used to select cultivars for porridge making quality. From findings in Chapter 4.1, hard and soft sorghum and maize cultivars were selected to determine the relationship between grain hardness and porridge quality.

Malt is widely used as a component of sorghum porridges, among them weaning porridges to improve sorghum digestibility, viscosity and protein profile nutritional value (Dewar 2003). Protein quality is improved through proteolysis and transamination, while quantity increases at the expense of carbohydrate loss as a result of respiration (Belton and Taylor 2004). Generally, hard grains are desirable for yields of grits (Taylor and Duodu 2009). However, grain hardness of malted sorghum and its effect on milling yield and porridge quality is not known. Several studies have reported a relationship between the duration of malting and hardness as a predictor of malting quality of barley (Psota et al 2007, Vejrazka et al 2008). Psota et al (2007) confirmed that grain hardness affected accessibility of hydrolytic enzymes to the starchy endosperm in barley. Grain hardness had a negative effect on accessibility of barley starchy endosperm by amylase enzymes thereby reducing soluble wort yield. In studies

on barley, malt showed losses in hardness by the second day of malting, which were attributed to softening of the grain outer layers during steeping and loss of cellular structure, reduced dry matter (malting loss), loss of kernel orientation and endosperm collapse (Osborne and Anderssen 2003; Osborne et al 2005). During sorghum malting the starchy endosperm is modified, which is characterised by degradation of the starch granules, protein bodies and the protein matrix by endogenous hydrolytic enzymes into simple sugars and free amino nitrogen, respectively (Glennie et al 1983). Hence, this study sought to determine the relationship between sorghum grain hardness and malt modification.

4.2.2 MATERIALS AND METHODS

4.2.2.1 Samples

Four sorghum cultivars grown in Potchefstroom South Africa and five maize cultivars from different geographical locations worldwide were used for the study. The sorghum cultivars were PAN 8901 and PAN 8247 (hard, red non-tannin), PAN 8648, (intermediate, white tan-plant non-tannin) and PAN 8625 (soft, red condensed tannin). Sorghum cultivars were commercial hybrids collected from the 2008-2009 growing season. They were all grown under dryland conditions, field dried and harvested at less than 14% moisture content. The identity of maize cultivars could not be disclosed for reasons of confidentiality but were sourced from Brazil, Argentina, Spain, Australia and USA.

4.2.2.2 Malting

Sorghum grain was malted according to Dewar et al (1995), with modifications. Sorghum samples were cleaned to remove broken kernels. Samples weighing 500 g were steeped in tap water for 24 h at 25°C before malting, with air rests every three hours. Steeped grain was weighed to determine water uptake. Malting was done for five days excluding the steeping

period by allowing the steeped grain to germinate in an incubator set at 25°C and 100% humidity. On each day of malting, a portion of malt was sampled, dried at 50°C in a force-draught oven. The dried malts were weighed to determine malting loss after five days and then evaluated for Thousand Kernel Weight (TKW), abrasive decortications using a Tangential Abrasive Dehulling Device (TADD hardness), SKCS-HI, density and floaters. The remaining malt was milled to pass through a 1.0 mm screen of the UDY Cyclotec Sample Mill (UDY Corporation, Fort Collins, Colorado) and the flour used for preparing porridges. Diastatic power was measured on 5 g malts extracted with peptone (Dewar et al 1995). PAN 8625, the condensed tannin cultivar was soaked in 0.2% (w/v) sodium hydroxide solution for four hours to deactivate condensed tannins.

4.2.2.3 Physical sorghum and maize grain characteristics

Procedures to measure sorghum and maize kernel size, thousand kernel weight (TKW), NIT Milling Index and TADD hardness are described in the Chapter 4.1, Section 4.1.2.2.

Single Kernel Hardness Test

Single kernel hardness of sorghum grain and malt was measured with a Single Kernel Characterization System (SKCS) 4100 (Perten Instruments, Huddinge, Sweden). Three hundred kernels of each sample passed through the instrument and their responses to crushing were recorded (Bean et al 2006).

4.2.2.4 Viscosity

Flour pasting properties were analysed using a Rapid Visco Analyser (RVA) Model 3C (Newport Scientific, Warriewood, Australia) according to Almeida-Dominguez et al (1997). Slurries of sorghum grain and malt, and maize flours were prepared at 18% (w/w, dry basis) solids to a total weight of 28 g in distilled water. The slurries were equilibrated at 50°C for 1

min then heated to 95°C at a speed of 6°C/min. The pastes were held at 95°C for 15 min. The hot pastes were cooled to 50°C at a rate of 6°C/min and held at this temperature for 6 min. The paddle was rotated at a constant speed of 150 rpm. Peak viscosity (viscosity at the start of the 95°C holding period), holding strength (viscosity before the start of cooling), final peak viscosity (viscosity after cooling), setback (final peak viscosity-holding strength) and breakdown (peak viscosity-holding strength) were determined.

4.2.2.4 Porridge texture measurements

Texture of porridges was determined by firmness and stickiness measured with a TX-XT2i Texture Analyser (model TA.XT2i, Texture Technology Corp., Scarsdale, NY) as described by Perdon et al (1995). The test was conducted under compression mode and the settings are shown in Table 4.2.1. Firmness was determined as the area under the force during compression and stickiness as the area of the curve during retraction. Sorghum grain and malt porridges containing 24% (w/w, dry basis) solids were prepared by directly adding flours into boiling water in 200 ml stainless steel cans. Porridges were stirred vigorously to avoid lumping and simmered on low heat for 15 min. Porridges were transferred into 50 ml stainless steel tubes and placed 10 mm from the bottom of the can. The porridges were securely covered with aluminium foil and kept for 30 min at 50°C before texture analysis. The tests were similar to those performed on maize flours.

4.2.2.5 Scanning Electron Microscopy (SEM)

Sorghum grains and malts of PAN 8247, PAN 8625 and PAN 8648 were immersed in liquid nitrogen at -196°C. The frozen samples were cut across with a sharp blade and mounted on aluminium stubs using adhesive tape. The mounted samples were sputter coated with gold and then viewed using a Zeiss Evo LS15 (Carl Zeiss, Oberkochem, Germany) scanning electron microscope operated at an acceleration voltage of 8 kV

TABLE 4.2.1 TX-XT2i Texture Analyser Settings for Determination of Firmness and Stickiness of Porridges

Settings	
Pre-test speed	2 mm/s
Test speed	1 mm/s
Post test speed	5 mm/s
Distance	8 mm
Load cell	25 kg
Trigger type	Auto (0.05 N)
Parameters	
Firmness	Area under the force-time curve during compression (Ns)
Compression	Area under the force-time curve during retraction (Ns)

4.2.2.6 Statistical analyses

Laboratory experiments were done in triplicate. Data were analysed by multifactor analysis of variance and means compared by Fisher's least significant differences. Calculations were performed using Statgraphics Centurion XV (StatPoint, Herndon,Virginia, USA).

4.2.3 RESULTS AND DISCUSSION

4.2.3.1 Pasting properties of sorghum grain flours and textural properties of their porridges

Hardness properties of the sorghum cultivars are described in detail in Chapter 4.1, Section 4.1.3.2. With regards to the pasting properties of the sorghum grain flours (Table 4.2.2 and Fig

4.2.1), peak viscosity (PV) was similar for hard cultivars PAN 8901 and PAN 8247, and PAN 8625 (soft) except for PAN 8648 (intermediate), which had low peak viscosity. It was expected that PAN 8625 being a soft cultivar, would produce highly viscous pastes, probably due to the more accessible starch in the floury endosperm by water, while lower viscosities were expected in hard cultivars with a large proportion of corneous endosperm. This was not the case probably due to the interaction of condensed tannins with other grain components, hence altering starch granular hydration.

Breakdown viscosity was high in PAN 8625 (soft) and PAN 8901 (hard), showing that the swollen starch granules were susceptible to breaking down easily (Beta et al 2001b). Final peak viscosity (FV) and setback (SB) were lowest in PAN 8625 and highest in PAN 8247. High holding strength (421 RVU) and low breakdown (348 RVU) of PAN 8247 indicated that the starch granules did not breakdown easily. FV showed that cultivar PAN 8247 (1061 RVU) a hard type sorghum formed the thickest gel compared to the soft cultivar PAN 8625 (689 RVU). The corneous endosperm particles of hard sorghum probably restricted starch granule swelling resulting in a high proportion of non-ruptured gelatinised starch granules that reinforce the gel matrix (Kebakile 2008).

Table 4.2.3 shows the texture of sorghum flours in terms of firmness and stickiness. Firmness was determined from the area (Ns) under the force-time curve during compression and stickiness as the area under the curve during retraction (Fig 4.2.2). Porridges of PAN 8901 and PAN 8625 were firmer than that of PAN 8247. Porridge stickiness varied slightly among cultivars except for porridge of PAN 8247 which was the least sticky. The differences in sorghum porridge texture were not consistent with grain hardness. Thus hardness may not be the only factor affecting porridge texture. The starch properties and their reaction kinetics during retrogradation may play a role (Perdon et al 1999) and other factors such as kernel structure and phenolic content (Beta et al 2001b).

TABLE 4.2.2 Pasting Properties of Sorghum Flours and Two Day Malted Sorghum

Cultivar	Day	Peak Viscosity	Holding Strength	Final Viscosity	Breakdown	Setback
PAN 8901(Hard)	0	773[1]a (1)	342 bc (3)	836 c (2)	431 a (5)	494 c (5)
	1	158 e (8)	81 e (2)	128 f (15)	76 e (3)	46 g (10)
	2	42 h (4)	22 g (5)	23 i (1)	17 i (2)	1 i (0.0)
PAN 8247 (Hard)	0	769 a(11)	421 a (6)	1061 a(14)	348 b (8)	640 a (7)
	1	112 f (1)	62 f (1)	94 g (3)	49 f (1)	32 h (4)
	2	38 h (1)	21 g (0)	22 i (0)	38 f (2)	2 i (0)
PAN 8648 (W)	0	665 c (14)	360 b (7)	946b (7)	304 c (6)	586 b (10)
(Intermediate)	1	154 e (6)	61.3 f (3)	158 f (15)	98 d (4)	97 f (3)
	2	49 h (1)	33 g (1)	34 i (1)	17 g (0)	2 i (0)
PAN 8625 (T)	0	745 ab (11)	318 c (6)	689 d (4)	427 a (8)	370 d (10)
(Soft)	1	531 d (15)	194 d (9)	514 e (39)	336 b (10)	320 e (19)
	2	89 g (3)	47 g (1)	59 h (2)	40 f (2)	12 i (1)

[1]Rapid Visco units (RVU)
(T), Condensed tannin sorghum; (W), White tan-plant, non-tannin sorghum
Figures in parentheses are standard deviations
Different letters in the same column denote significant differences at $p < 0.05$
n=2

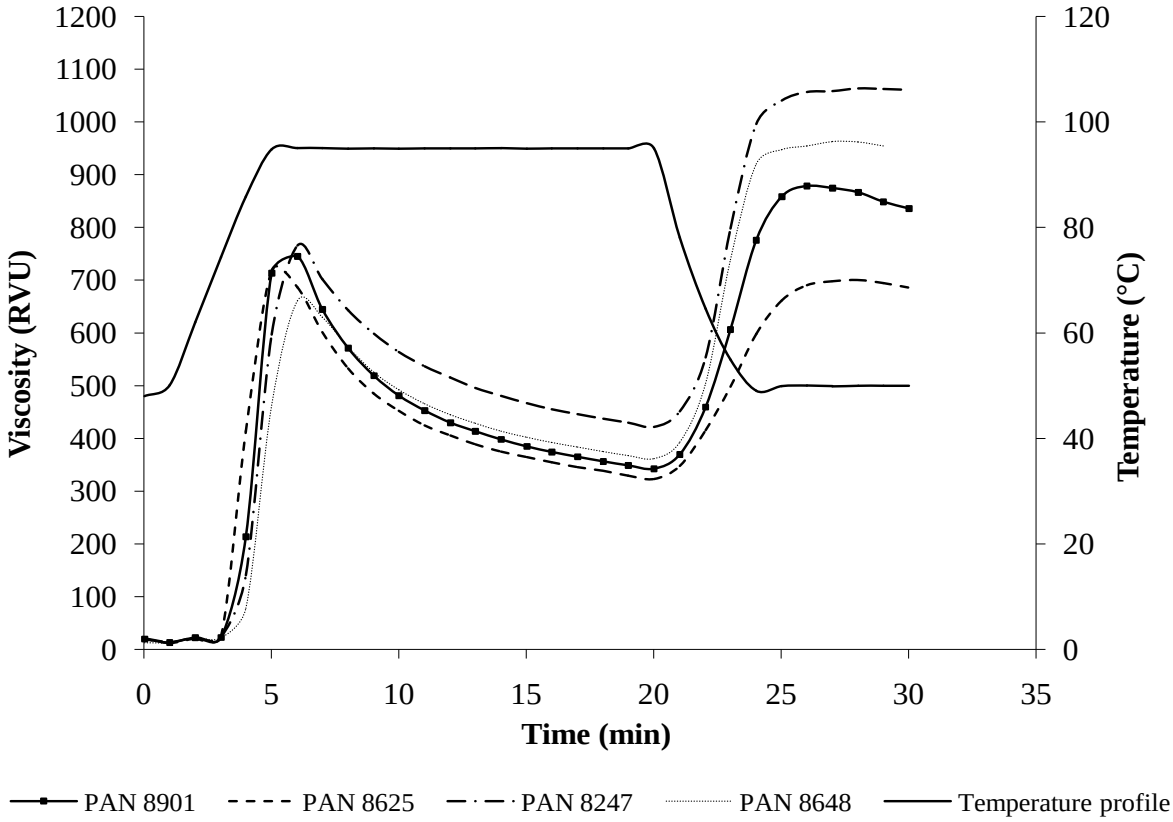


Fig 4.2.1. Pasting profiles of flours from sorghum cultivars varying in hardness (means of two separate analyses). PAN 8901 (hard), PAN 8247 (hard), PAN 8648 (white tan-plant, intermediate) and PAN 8625 (condensed tannin, soft).

TABLE 4.2.3 Firmness and Stickiness of Sorghum Grain Porridges and of One Day Malted Sorghum

Cultivar	Day	Firmness (Ns)	Stickiness (Ns)
PAN 8901 (Hard)	0	35.2 a (0.1)	5.68 b (0.15)
	1	28.7 b (2.0)	9.85 a (0.35)
PAN 8247 (Hard)	0	22.7 c (0.9)	4.10 c (0.17)
	1	22.6 c (3.1)	8.05 a (2.31)
PAN 8648 (W) (Intermediate)	0	25.3 b (2.6)	5.71 b (0.72)
	1	19.5 c (0.9)	8.47 a (0.33)
PAN 8625 (T) (Soft)	0	38.8 a (2.4)	5.61 a (1.20)
	1	13.3 c (3.0)	7.62 a (0.84)

Figures in parentheses are standard deviations
(T), Condensed tannin sorghum; (W), White tan-plant, non-tannin sorghum
Different letters in the same column denote significant differences at $p < 0.05$

n=2

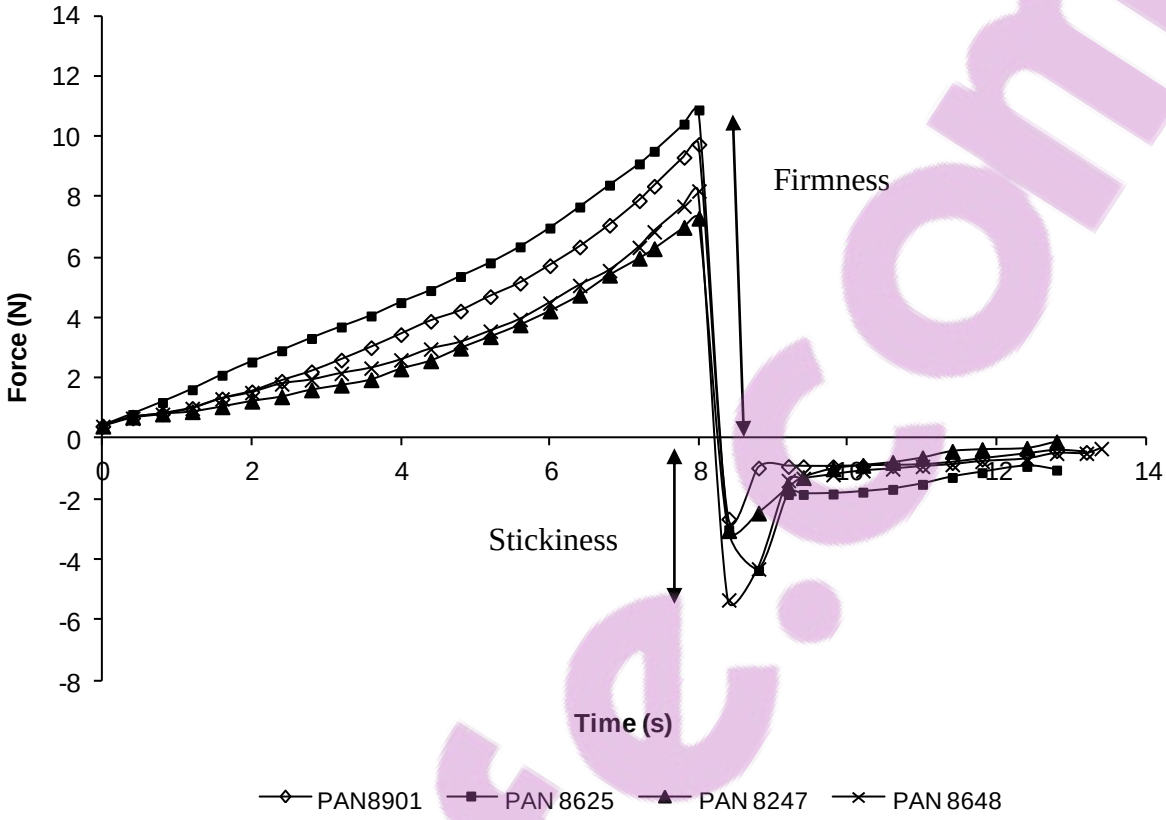


Fig 4.2.2. Firmness and stickiness of porridges prepared from flours of sorghums varying in hardness (means of two separate analyses). PAN 8901 (hard), PAN 8247 (hard), PAN 8648 (white tan-plant, intermediate) and PAN 8625 (condensed tannin, soft).

4.2.3.2 Pasting Properties of Maize Flours and Texture of their Porridges

Peak viscosity (PV) of maize samples ranged from 426 to 752 RVU (Table 4.2.4, Fig 4.2.3). Peak viscosity was low in the Australian maize, which was the hardest cultivar. The soft USA maize had the highest PV. Subsequently, USA maize had low holding strength (HS) and high breakdown (BD). The wide range in peak viscosity of the different maize samples could be due to both cultivar and cultivation environment effects. In soft maize, the loosely packed starch granules of the highly floury endosperm would be easily accessible by water and hence a large swelling capacity (Almeida-Dominguez et al 1997). The high HS and BD in USA

maize would imply that the starch granules easily disintegrated and did not have the capacity to form stable thick pastes. The low PV in Australian maize could be due to protein bodies bound to starch granules in the corneous endosperm flour forming a barrier and restricting starch hydration (Almeida-Dominguez et al 1997; Chandrashekar and Kirleis 1988). Breakdown of the hot paste was low in Australian and Argentinean maize, which meant resistance to shear thinning. Australian maize had high FV and SB showing the stability of hard cultivars to form thick gels on cooling. However the soft USA cultivar did not form a thick gel compared to other cultivars. The FV and SB of USA maize were lower than the other samples probably owing to less during retrogradation in this soft cultivar.

The sorghum and maize pasting properties were somewhat different. The mean PV of sorghum grain starch was higher, 730 RVU compared to 571 RVU for maize. Beta et al (2001b) also observed similar differences between sorghum and maize starches, which was attributed to the higher water binding capacity of the sorghum starch. Sorghum had a higher mean HS (366 RVU) than maize (220 RVU) and BD was higher in maize (61%) than sorghum (50%), with respect to PV. Probably the corneous endosperm protein matrix was stronger in sorghum than in maize. This is believed to be because of extensive matting of the collapsed protein matrix enhanced by disulphide bonding in sorghum when sorghum flour is cooked in water (Ezeogu et al 2008). HS is of significance in preparation of porridges. In practice, HS would reflect the level of thinning and determines the amount of additional flour to form a thick paste characteristic of ‘stiff’ porridges. Hence, cultivars, which resist thinning are desirable for economic reasons, to minimize quantities of flour used in porridge making.

The mean FV of sorghum was also higher, 912 RVU compared to 827 RVU in maize. However, setback pointed to the fact that retrogradation could be higher in maize, (607 RVU) than in sorghum (546 RVU). In both maize and sorghum, hard and intermediate types would produce firmer porridges on cooling than soft types.

TABLE 4.2.4 Pasting Properties of Maize Flours

Cultivar	Peak Viscosity	Holding Strength	Final Viscosity	Breakdown	Setback
Argentina (Hard)	426 d (17)	190 b (4)	820 c (10)	236 d (21)	630 a (6)
Australia (Hard)	441 d (8)	257 a (8)	899 a (5)	185 e (5)	643 a (13)
Brazil (Intermediate)	563 c (11)	201 b (7)	847 b (11)	365 c (8)	658 a (15)
Spain (Intermediate)	671 b (16)	271 a (2)	855 b (10)	400 b (14)	584 b (9)
USA (Soft)	752 a (20)	182 b (7)	719 d (7)	570 a (13)	536 c (2)
Mean	570 (134)	220(38)	828 (64)	351 (143)	610 (47)

Figures in parentheses are standard deviations
Different letters in the same column denote significant differences at $p < 0.05$

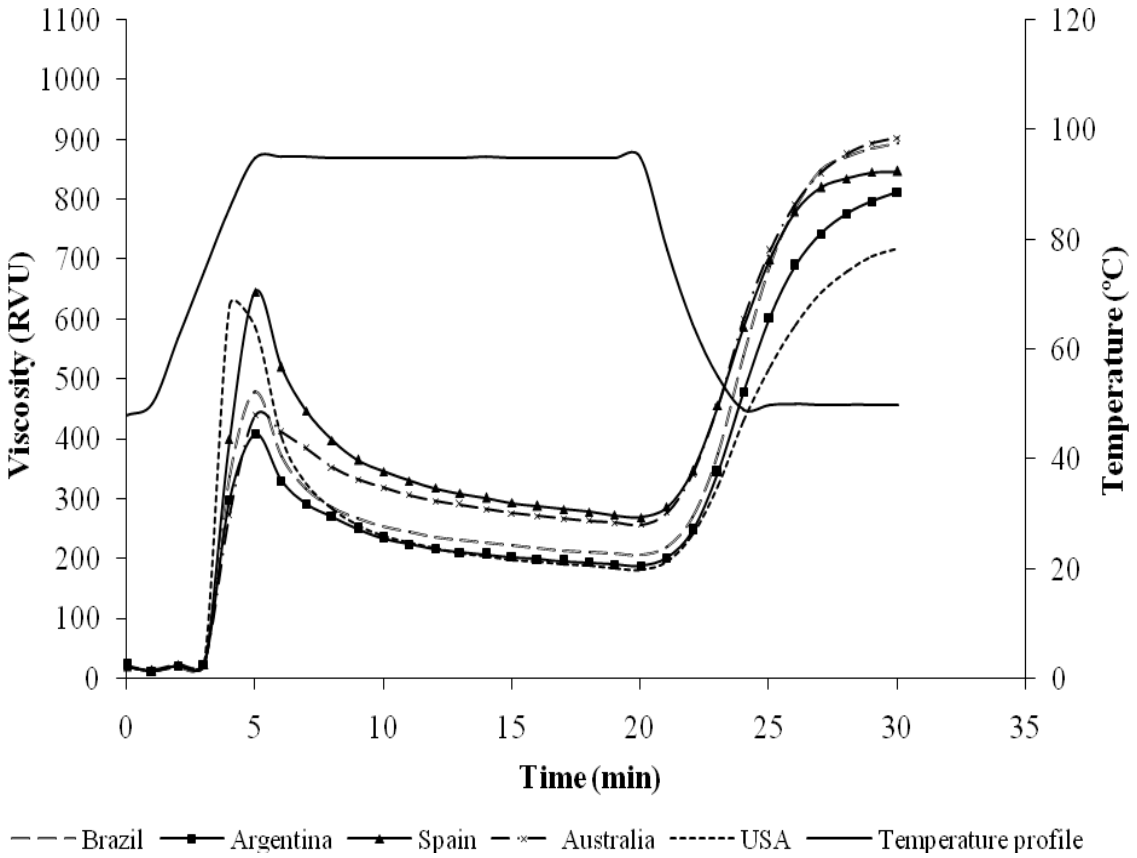


Fig 4.2.3. Pasting profiles of flours from maize cultivars varying in hardness (means of two separate analyses). Argentina (hard), Australia (hard), Brazil (intermediate), Spain (intermediate), USA (soft).

The porridge made from soft USA maize was less firm and sticky (Table 4.2.5, Fig 4.2.4) compared to those from the harder maize cultivars. The same reasons given for sorghum porridges could explain differences in the firmness and stickiness of maize porridges. Textural properties of maize were somewhat different from those of sorghum porridges. Maize porridges were firmer (28.9 to 46.3 Ns) compared to sorghum (25.3 to 35.2 Ns) despite the higher viscosities of the sorghum porridges. As described above, the setback of maize was higher than that of sorghum indicating that maize porridges retrograded more strongly on cooling than those of sorghum, hence the firmer maize porridges.

TABLE 4.2.5 Firmness and Stickiness of Porridges Prepared from Maize Flours

Cultivar	Firmness (Ns)	Stickiness (-Ns)
Argentina (Hard)	41.2 a (0.7)	5.12 bc (0.17)
Australia (Hard)	45.9 a (1.1)	6.34 b (0.22)
Brazil (Intermediate)	41.7 a (3.3)	5.41 bc (0.30)
Spain (Intermediate)	46.3 a (3.5)	7.10 a (0.10)
USA (Soft)	28.9 b (1.0)	4.13 c (1.55)
Mean	40.8 (1.9)	5.62 (0.22)

Figures in parentheses are standard deviations
Different letters in the same column denote significant differences at $p < 0.05$
n=2

4.2.3.3 Changes in grain hardness during sorghum malting

Germinative Energy was at least 90% and similar for all the cultivars (Table 4.2.6). Thus the grain germinated uniformly and was suitable for malting (Dewar et al 1995). Water uptake after steeping was substantially lower in the hard cultivars PAN 8901 and PAN 8247 than the softer cultivars. The strong starch-protein interactions in the corneous endosperm may have

limited moisture migration into the grain. PAN 8625 (soft) had the highest Diastatic Power (DP) suggesting that this cultivar had high amylase activity to degrade starch granules at a faster rate than PAN 8648 (intermediate) with the lowest DP. Malting loss was the lowest in PAN 8648, indicating that amylase activity was insufficient to breakdown much of the starchy endosperm.

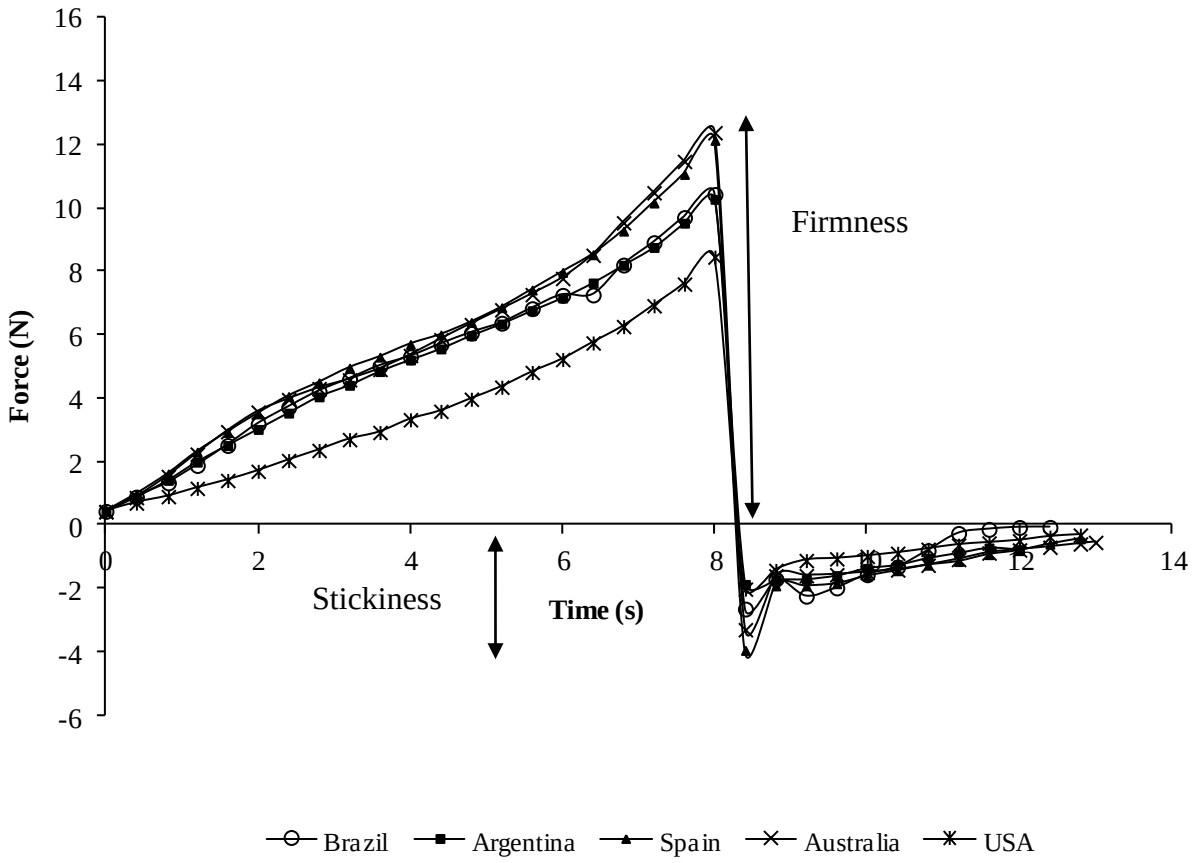


Fig 4.2.4. Firmness and stickiness of porridges prepared from flours of maize cultivars varying in hardness (means of two separate analyses). Argentina (hard), Australia (hard), Brazil (intermediate), Spain (intermediate), USA (soft).

TABLE 4.2.6 Malting Properties of Sorghum Cultivars Varying in Hardness

Cultivar	Germinative Energy (%)	Water Uptake[a] (%)	Malting Loss (%)	Diastatic Power[b] (SDU/g, db)	Free Amino Nitrogen[b] (mg/100 g, db)
PAN 8901 (Hard)	91.8 a (3.7)	33.3 b (3.0)	17.4 ab (0.2)	44.4 a (1.7)	214 ab (12)
PAN 8247 (Hard)	92.3 a (5.7)	33.8 b (1.6)	17.3 ab (0.5)	41.3 b (0.5)	206 b (4)
PAN 8648 (W) (Intermediate)	92.5 a (2.5)	41.3 a (6.9)	16.3 c (0.2)	25.2 c (0.5)	184 c (13)
PAN 8625 (T) (Soft)	93.1 a (4.5)	40.7 a (1.5)	17.9 a (0.4)	47.0 a (1.3)	236 a (16)
Mean	92.4 (4.1)	37.2 (5.0)	17.2 (0.7)	39.4 (9.3)	210 (11)

(T), Condensed tannin sorghum
(W), White tan-plant, non-tannin sorghum
[a] Water uptake during steeping, percentage of original grain weight, as is
[b] Results of whole malt including external roots and shoots
Figures in parentheses are standard deviations
Different letters in the same column denote significant differences at $p < 0.05$
n=3

TADD hardness (percentage kernel removed) was used to classify cultivars as hard, intermediate or soft (Chapter 4.1). The cultivars differed significantly in TADD hardness. Those with the lowest percentage kernel removed were hard (PAN 8901 and PAN 8247) followed by PAN 8648 (intermediate) and PAN 8625 (soft). According to TADD hardness, PAN 8648 had intermediate hardness, but SKCS indicated that the cultivar was hard. These differences can be attributed to different modes of action of the TADD and SKCS. The SKCS operates by crushing kernels. The TADD operates by abrasive removal of outer grain layers (Shepherd 1982). Light microscopy showed that the proportion of the corneous to floury endosperm of PAN 8648 was similar to that of the hard cultivar PAN 8247 (Fig 4.2.5), which confirms the SKCS HI results (Table 4.2.7). However, close examination of the PAN 8648

kernel with SEM clearly showed starch granules in the pericarp cell walls, which were not evident in PAN 8247 pericarp cell walls (Fig 4.2.5). Starch granules cause weak points in the pericarp and increase friability during decortication (Taylor and Dewar 2001), which was probably responsible for the lower hardness with the TADD.

The effect of malting on sorghum hardness was assessed using hardness techniques over a period of five days (Table 4.2.7). On Day 1, grain density was greatly reduced in all sorghums as determined by the floatation test. Floaters were 91 to 95% at Day 1 and on Day 3 all cultivars had 100% floaters, indicating considerable endosperm modification had taken place, which reduced density of the grains. The SKCS HI also decreased dramatically with malting time. On Day 1, the soft condensed tannin cultivar PAN 8625 had the lowest SKCS HI. On Day 2 all the cultivars except PAN 8648 had similar HI. The SKCS HI of PAN 8648 remained higher than that of other cultivars on Day 2 due to minimal endosperm modification in this cultivar. The SKCS rejected most of the kernels beyond Day 2. SKCS measures hardness by a response to crushing (Osborne and Anderssen 2003). The initial crush response is a factor of the pericarp and aleurone layer and finally, compression of the endosperm. With continued malting, the endosperm collapsed and kernel size and shape changed, hence the malt kernels were not evaluated. Using the SKCS, Osborne et al (2005) observed a substantial loss in hardness of barley malt on the second day of malting and attributed this to the softening of the grain outer layers during steeping and loss of cellular structure and protein in the endosperm. However, unlike barley, the sorghum endosperm cell walls persist during malting (Glennie 1984; Palmer 1991) although they undergo physical and chemical changes such as the reduction in protein and amount of cell wall (Glennie et al 1983).

Sorghum malt density measured by gas pycnometry decreased by 7% after five days of malting (Table 4.2.7). The greatest reduction was first two days of malting. As with floatation, a reduction in density is probably a result of airspaces left as a result of hydrolysis of the protein matrix and starch granules (Glennie et al 1983). Thousand kernel weight (TKW) declined by 30% in the five day malting period.

Malt hardness measured by percentage kernel removed using a TADD rapidly decreased between Days 1 and 3 (Table 4.2.7) in PAN 8648 (intermediate) and hard cultivars PAN 8247 and PAN 8901. The rate of percentage kernel removal was lower in malt of PAN 8648 than the other cultivars. PAN 8625 had the highest initial kernel removal and there was no difference in TADD hardness between Days 0 and 1, an observation similar with SKCS-HI. The high proportions of the malt kernels removed by the TADD increased dramatically because the kernels became friable and were crushed into fine particles rather than abraded.

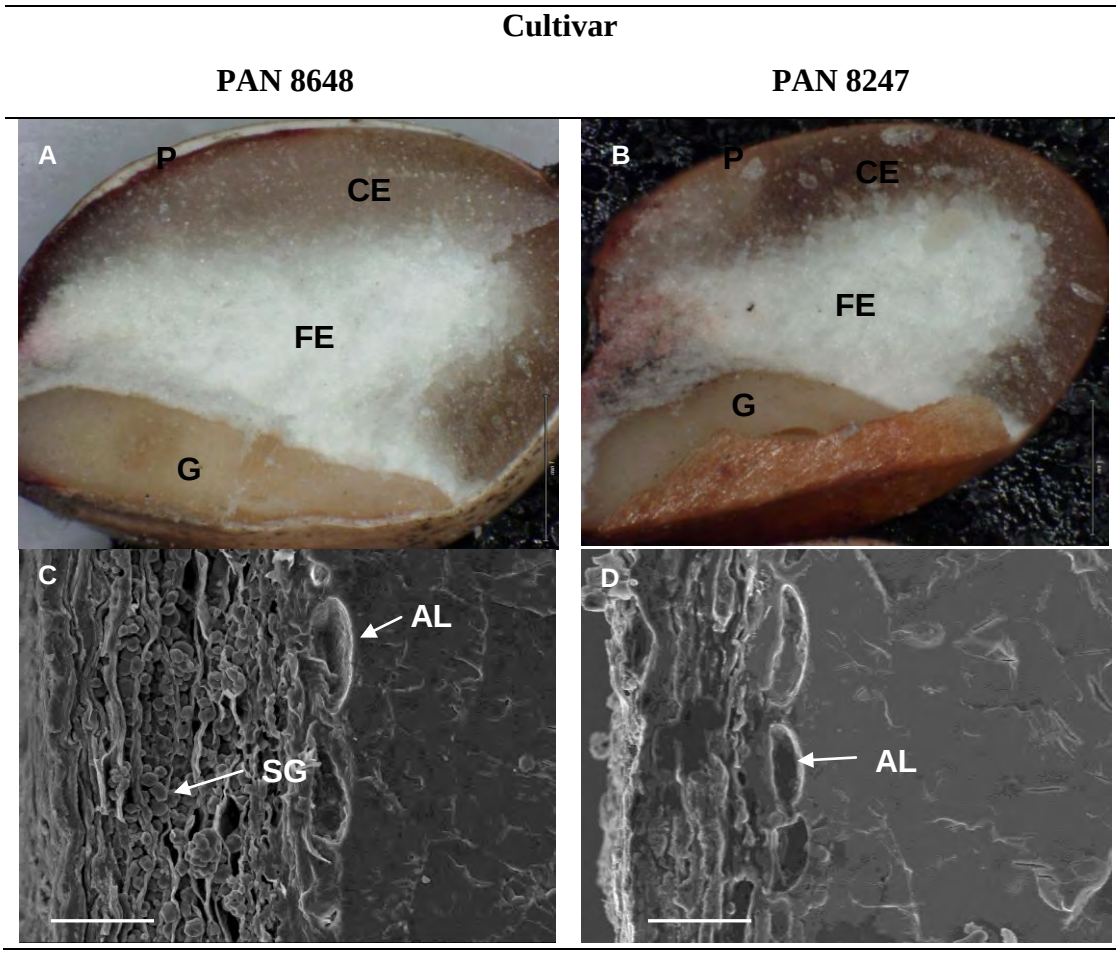


Fig 4.2.5. (A-B) Light micrographs of longitudinal sections of PAN 8648 (white tan-plant, intermediate) and PAN 8247 (hard) showing the pericarp (P), corneous endosperm (CE), floury endosperm (FE) and the germ (G). (C-D) SEM of pericarp sections of PAN 8648 (white

tan plant, intermediate) and PAN 8247 (hard) showing starch granules (SG) and the aleurone layer (AL) Bar 10 µm.

4.2.3.4 Modification of the sorghum kernel during malting

Fig 4.2.6 shows malt modification in the pericarp, corneous endosperm and floury endosperm of sorghum malted for five days. The pericarp, aleurone layer and sub-aleurone region apparently remained unchanged on Day 3 of malting. Changes in the corneous endosperm occurred on Day 5 as the cell walls were torn and the starch granules were exposed (Fig 4.2.6H). The aleurone layer was slightly compressed on Day 5 (Fig 4.2.6D). According to Glennie et al (1983) the aleurone layer modification was characterised by mineral loss. Aleurone layer modification could be a result of phytic acid hydrolysis by the phytase enzyme during malting releasing complexed minerals (Eskin and Wiebe 1983). The released minerals then migrate to the germ to sustain it during malting.

In the corneous endosperm, starch granule packing remained compact and the granules themselves remained intact and obscured by cell walls until Day 3. There were pits, which were randomly distributed on the surface of starch granules (Fig 4.2.6G). These are likely to be surface pores characteristic of native starch granules, which are thought to be sites of initial enzymatic attack (Huber and BeMiller 2000). Changes in the corneous endosperm occurred later than those in the floury endosperm, which were only observed on Day 5 malt (Fig 4.2.6H). Starch granules were partially degraded. Modification was observed in floury endosperm on Day 1 (Fig 4.2.6J). Starch granule packing was less compact in floury endosperm compared to the corneous endosperm. On Day 5, the starch granules were extensively pitted by amylases and lost their integrity (Fig 4.2.6L). Their structures were hollowed and emptied resulting in a concentric sphere structure, as observed by Glennie et al (1983).

TABLE 4.2.7 Effect of Malting Time on Hardness of Sorghum Malt

Cultivar	Malting (Days)	Floaters (%)	Gas Pycnometer (g/cm^3)	TKW (g)	SKCS (Hardness Index)	TADD (% Kernel Removed)
PAN 8901	0	18.7d(1.2)	1.37a(0.00)	29.2a(1.4)	69.6ab(1.8)	31.8l(1.3)
(Hard)	1	93.0b(4.2)	1.32bcd(0.01)	28.5a(0.4)	58.1bc(1.5)	50.3i(1.2)
	2	95.0b(7.1)	1.32bcd(0.01)	27.3abc(0.7)	44.7d(4.3)	64.4g(2.4)
	3	100.0a	1.31bcd(0.01)	23.3d-h (0.7)	ND	83.4e(1.2)
	4	100.0a	1.31bcd(0.00)	22.4g-j(1.1)	ND	92.2bcd(1.1)
	5	100.0a	1.28cde(0.00)	20.3e-i(0.8)	ND	96.9ab (0.5)
PAN 8247	0	6.00e(2.0)	1.37a(0.001)	28.0ab(1.6)	73.6a(0.8)	23.9l(0.9)
(Hard)	1	91.0b(1.4)	1.32bcd(0.00)	26.9a-d (0.9)	60.2b(0.5)	48.9j(0.4)
	2	100.0a	1.29e-i(0.01)	25.8a-d(0.9)	42.8d(0.4)	66.2g(1.8)
	3	100.0a	1.27c-g(0.00)	22.5e-i(0.7)	ND	87.3ef(0.6)
	4	100.0a	1.25g-j(0.01)	20.4g-j (1.9)	ND	96.5abc(0.3)
	5	100.0a	1.24h-k(0.01)	19.1ij (0.2)	ND	97.7a(0.3)
PAN8648 (W)	0	6.67e(1.2)	1.36ab(0.01)	26.8a-e(0.8)	75.5a(2.7)	40.9j(1.7)
(Intermediate)	1	91.0b(1.4)	1.32bcd(0.00)	25.7a-e(0.2)	63.1b(1.3)	45.4 ij(1.9)
	2	100.0 a	1.31c-f(0.01)	25.6a-d(0.4)	54.4c(0.9)	58.6h(1.6)
	3	100.0 a	1.30f-j(0.01)	22.3e-j(0.7)	ND	77.9f(0.2)
	4	100.0 a	1.29cd(0.00)	21.0f-j(1.4)	ND	82.2fg(1.5)
	5	100.0 a	1.26c-g(0.01)	18.8 ij(0.7)	ND	91.4cd(0.3)
PAN 8625 (T)	0	34.7c(3.1	1.33abc(0.00)	27.3c-g(1.0)	57.7bc(2.7)	63.2i(2.4)
(Soft)	1	95.0b(7.1)	1.30c-f(0.00)	24.5abc(1.1)	57.0bc(0.6)	63.2gh(1.7)
	2	100.0a	1.27f-i(0.01)	23.9b-f(0.7)	41.9d(0.5)	77.8f(0.5)
	3	100.0a	1.23ijk (0.00)	20.0hij(0.4)	ND	89.3e(1.0)
	4	100.0a	1.22jk (0.00)	19.8ij (0.2)	ND	95.0abc(0.0)
	5	100.0a	1.21j (0.00)	18.1j (0.7)	ND	99.5a(0.1)

ND, Not determined, most kernels rejected by the SKCS
(T), Condensed tannin sorghum; (W), White tan-plant, non-tannin sorghum
Figures in parentheses are standard deviations
Different letters in the same column denote significant differences at $p < 0.05$
Day 0; unmalted grain; Days 1-5; malting time after steeping, n=3

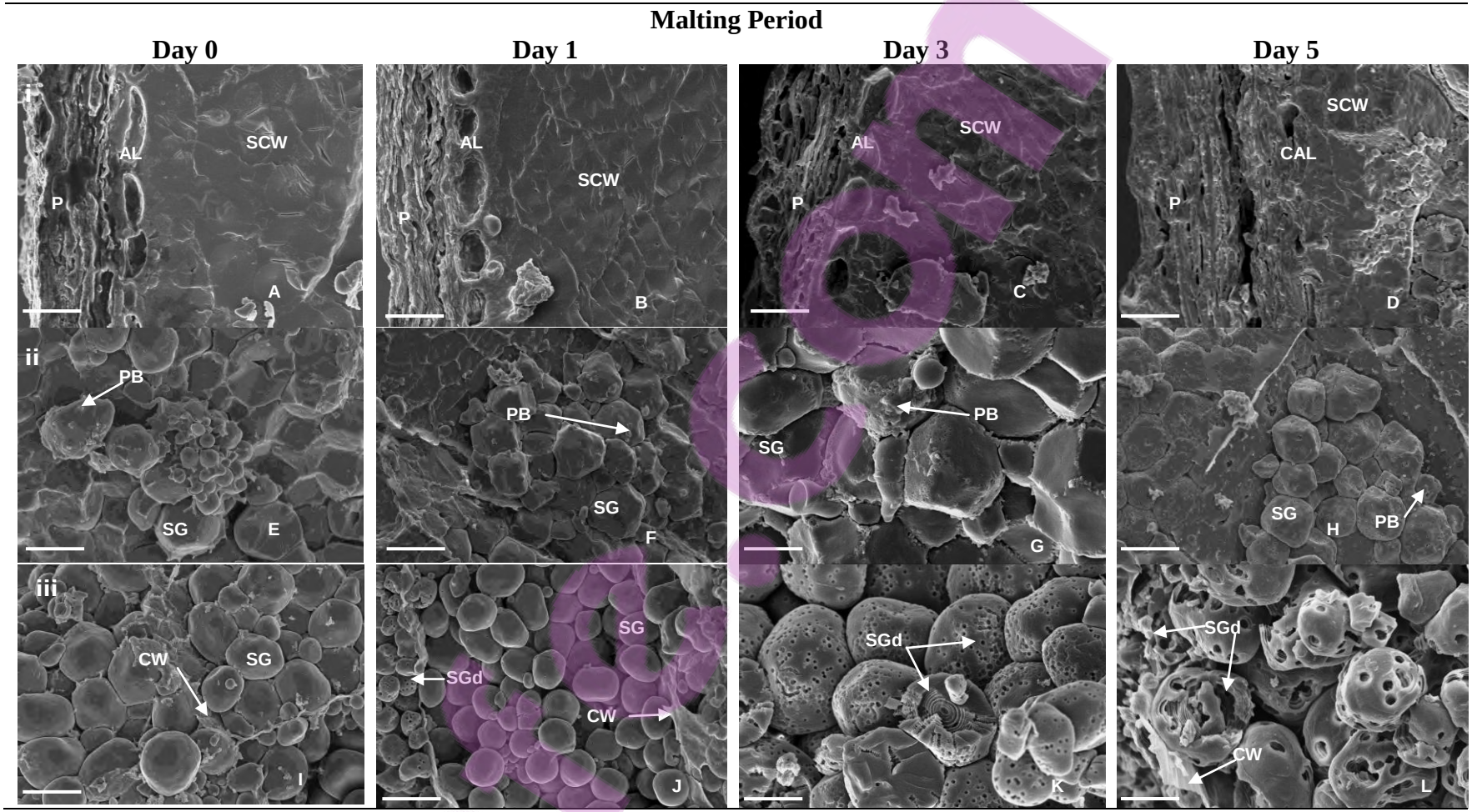


Fig 4.2.6. SEM of (i) pericarp, (ii) corneous endosperm and (iii) floury endosperm sections of sorghum that had been malted for up to 5 days following steeping. The SEM micrographs show the aleurone layer (AL), compressed aleurone layer (CAL), pericarp (P), cell wall (CW), intact starch granules (SG), starch granules obscured by cell walls (SCW), degraded starch granules (SGd) and protein bodies (PB). Bar is 10 µm.

4.2.3.5 The effect of sorghum grain hardness on malt modification

Light micrographs of sorghum cultivars varying in hardness malted up to three days show that grain of PAN 8625 (soft) had the largest area of floury endosperm and on Day 3 the malted grain had an entirely floury endosperm (Fig 4.2.7). The floury endosperm of PAN 8625 confirmed all the hardness data (Table 4.2.7). The floury endosperm area in PAN 8247 (hard) and PAN 8648 (intermediate) gradually increased as malting progressed, which agrees with the hardness data (Table 4.2.7).

Fig 4.2.8 to 4.2.11 show SEM of sorghum grain (Day 0), and malts of the three cultivars at Days 1 and 3 after steeping. Changes in malt hardness occurred mostly during this period (Table 4.2.7). The longitudinal sections of the SEM images (Fig 4.2.8) give an overview of the floury and corneous endosperm, the pericarp and the general structural changes with time. The floury endosperm area of PAN 8625 grain (soft) (Fig 4.2.8G) was larger than for PAN 8247 (hard) and PAN 8648 (intermediate). On Day 1, the cultivars showed evidence of starch degradation at the scutellum-endosperm interface (Fig 4.2.9B, E and H) confirming that modification starts in this region into the inner endosperm (Brennan et al 1997; Glennie et al 1983). The scutellum-endosperm interface showed a network of cell walls devoid of starch granules, which can be attributed to enzymatic hydrolysis of starch granules, protein bodies and protein matrix, while the cell walls remained.

The grain middle region, (Fig 4.2.10) was not modified in comparison to the proximal area. The starch granules of both the floury and corneous endosperm were intact and unchanged in all cultivars. SEM of the distal region (Fig 4.2.11) showed that there were no structural changes in malt of PAN 8247 and PAN 8648 on Day 3 but there were more loose starch granules in the floury endosperm of the soft cultivar PAN 8625 (Fig 4.2.11I). However, the starch granules were intact.

Generally modification progressed from the germ to the floury endosperm, as was also described for sorghum by Glennie et al (1983). In PAN 8247 (hard), modification was slower

than in PAN 8625 even though the malts had similarly high Diastatic Power (Table 4.2.6), indicating that the progression of amylase enzymes into the distal region was hindered by the compactness of the endosperm in PAN 8247. Endosperm modification in PAN 8625 had progressed to the distal region by Day 3 (Fig 4.2.11I), which means that the amylase enzymes migrated right through the endosperm. Modification of PAN 8625 (condensed tannin, soft) was the fastest, probably owing to its high level of amylase activity and the largely floury endosperm structure since starch granule degradation was evident as from Day 1. The open structure of the floury endosperm cells allowed faster enzyme migration than in the corneous endosperm (Nielsen 2003; Psota et al 2007).

Although malt hardness had reduced drastically on Day 3 (Table 4.2.7), modification continued as shown by SEM of Day 5 malt (Fig 4.2.12). However, there were minimal changes between Day 1 and 3 in the endosperm of PAN 8648, with low DP (Table 4.2.6). In PAN 8625, which had high DP, starch granules of the corneous endosperm and those of the pericarp were degraded (Fig 4.2.12C). Starch granules of the middle region in PAN 8625 were partially pitted (Fig 4.2.12F), while those of the proximal region were completely degraded (Fig 4.2.12I).

The endosperm cell walls were still present in Day 5 malted sorghum (Fig 4.2.12G, H and I). This finding agrees with that of Glennie et al (1984). This is in contrast to barley malt where endosperm cell walls are degraded during malting (EtokAkpan and Palmer 1990). One of the reasons for the persistence of the sorghum endosperm cell walls is that sorghum glucuronoarabinoxylans are highly substituted compared to those of barley (Verbruggen et al 1998). The pattern of substitution is thought to hinder enzyme activity of the xylanases, arabinofuranosidases and glucuronidases among hydrolases that break down the xylan backbone and the other side units of the glucuronarabinoxylan chain. Although the endosperm cell walls were persistent in the proximal region of Day 5 malt, these cell walls were torn (Fig 4.2.12G, H and I). Cell wall tearing was caused by partial degradation by enzymes (Palmer 1991). Since malt kernels were cut and fixed in preparation for SEM, it is possible that physical damage also contributed to endosperm cell wall tearing. Physical damage was also

highly likely to occur considering that the endosperm cell contents (starch granules, protein bodies and matrix), which provided support for the cell walls were removed by enzymatic hydrolysis. The emptied cell walls were weakened and probably became susceptible to physical damage.

The pattern of starchy endosperm cell wall degradation differed among the sorghum malts. PAN 8648 Day 5 had a distinct smooth surface of the endosperm cell walls showing minimal tearing, which can be attributed to limited cell wall enzymatic degradation (Fig 4.2.12H). The endosperm cell walls of PAN 8625 and PAN 8247 malts, which had higher DP, showed more cell wall tearing (Fig 4.2.12G and I). Extensive endosperm cell wall tearing was seen in PAN 8625, the cultivar with the highest DP. In view of these observations it seems that endosperm cell wall degradation is influenced by levels of enzymatic action. In turn, the extent of endosperm cell wall tearing affects kernel strength through its ability to hold cell components intact thereby contributing to malt hardness. The pattern of endosperm cell wall degradation and DP levels of the different malts (Table 4.2.6) agree with hardness data (Table 4.2.7). Thus, malt with low amylase activity could have low levels of endosperm cell wall degrading enzymes that would limit endosperm hydrolysis, hence modification. Slightly modified malt would resist collapse of the kernel, hence maintaining hardness, as was the case with PAN 8648 malt. Thus, endosperm structure organisation influences starch granular packing and malting quality in terms of enzyme migration in the endosperm (Rojas-Molina et al 2007; Holopainen et al 2005).

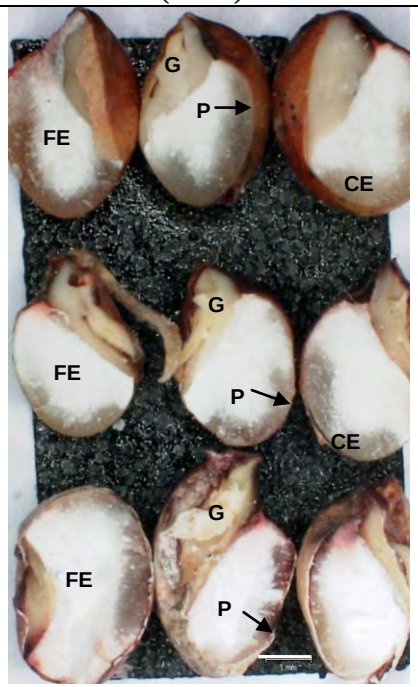
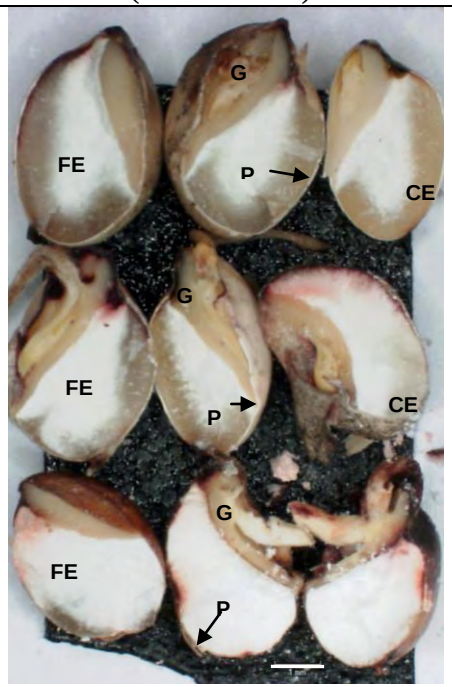
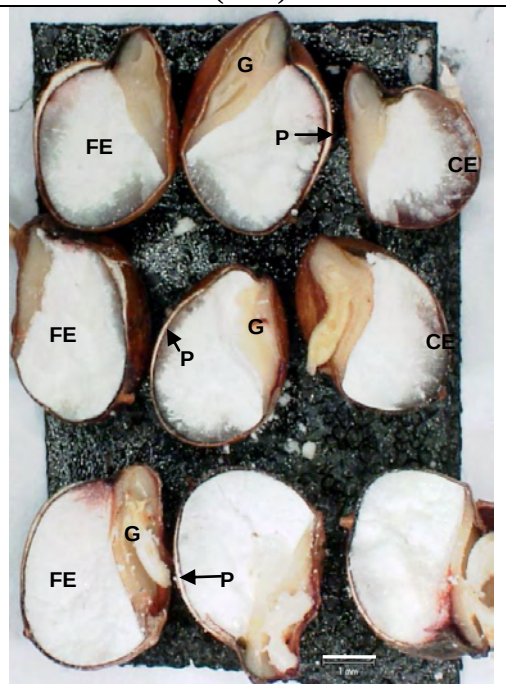
Malting	Cultivar		
	PAN 8247 (Hard)	**PAN 8648** (Intermediate)	**PAN 8625** (Soft)
Day 1			
Day 2			
Day 3			

Fig 4.2.7. Light micrographs of longitudinal sections of sorghum grain of different hardness that had been malted for up to 3 days following steeping. PAN 8247 (hard), PAN 8648 (white tan-plant, intermediate), PAN 8625 (condensed tannin, soft), pericarp (P), corneous endosperm (CE), floury endosperm (FE) and germ (G). Bar is 1 mm.

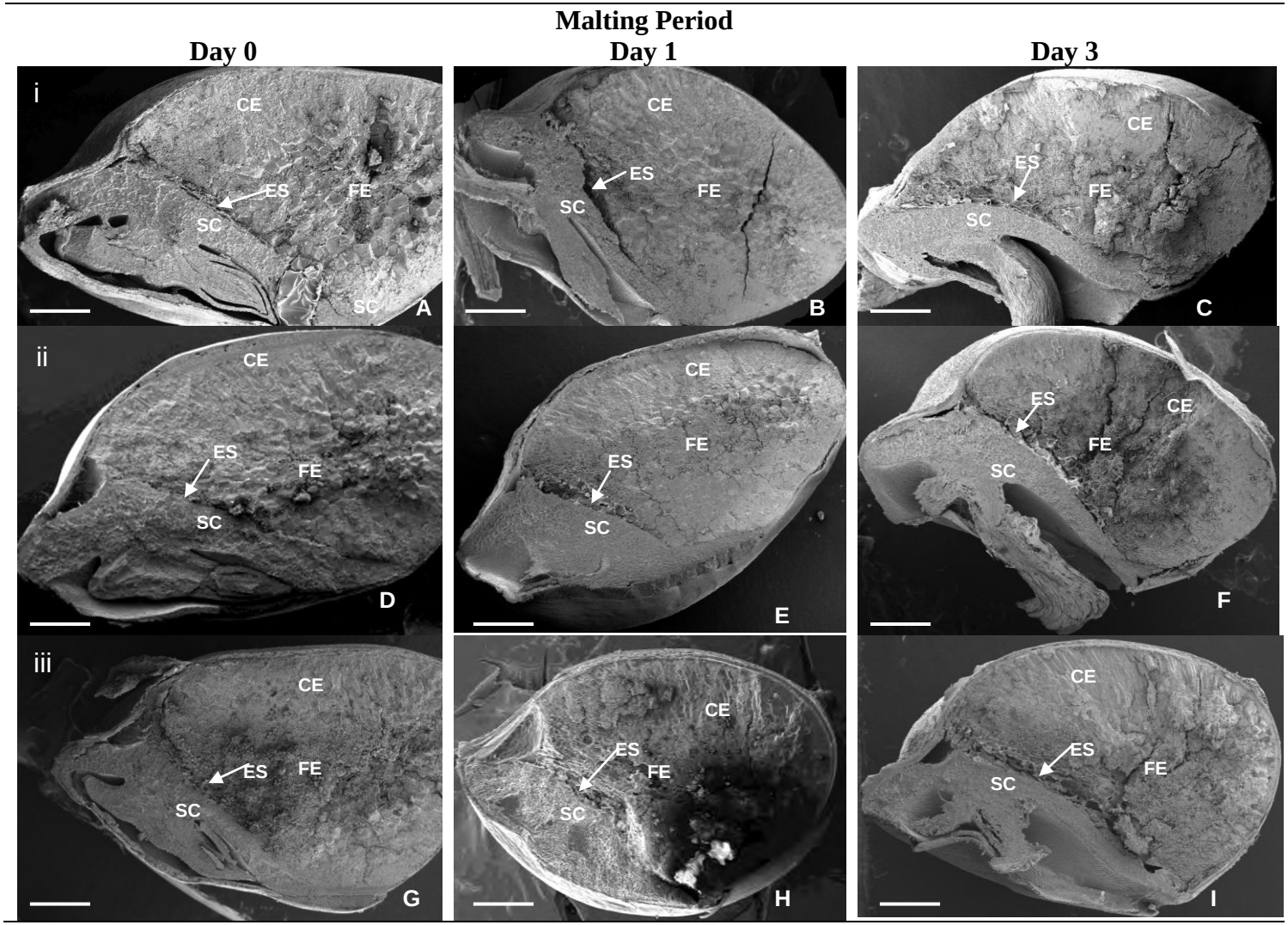


Fig 4.2.8. SEM of longitudinal sections of sorghum grain of different hardness that had been malted for up to 3 days following steeping. (i) PAN 8247 (hard), (ii) PAN 8648 (white tan-plant, intermediate), (iii) PAN 8625 (condensed tannin, soft), corneous endosperm (CE), floury endosperm (FE), scutellum (SC) and endosperm degradation at interface with scutellum. Bar is 1 mm.

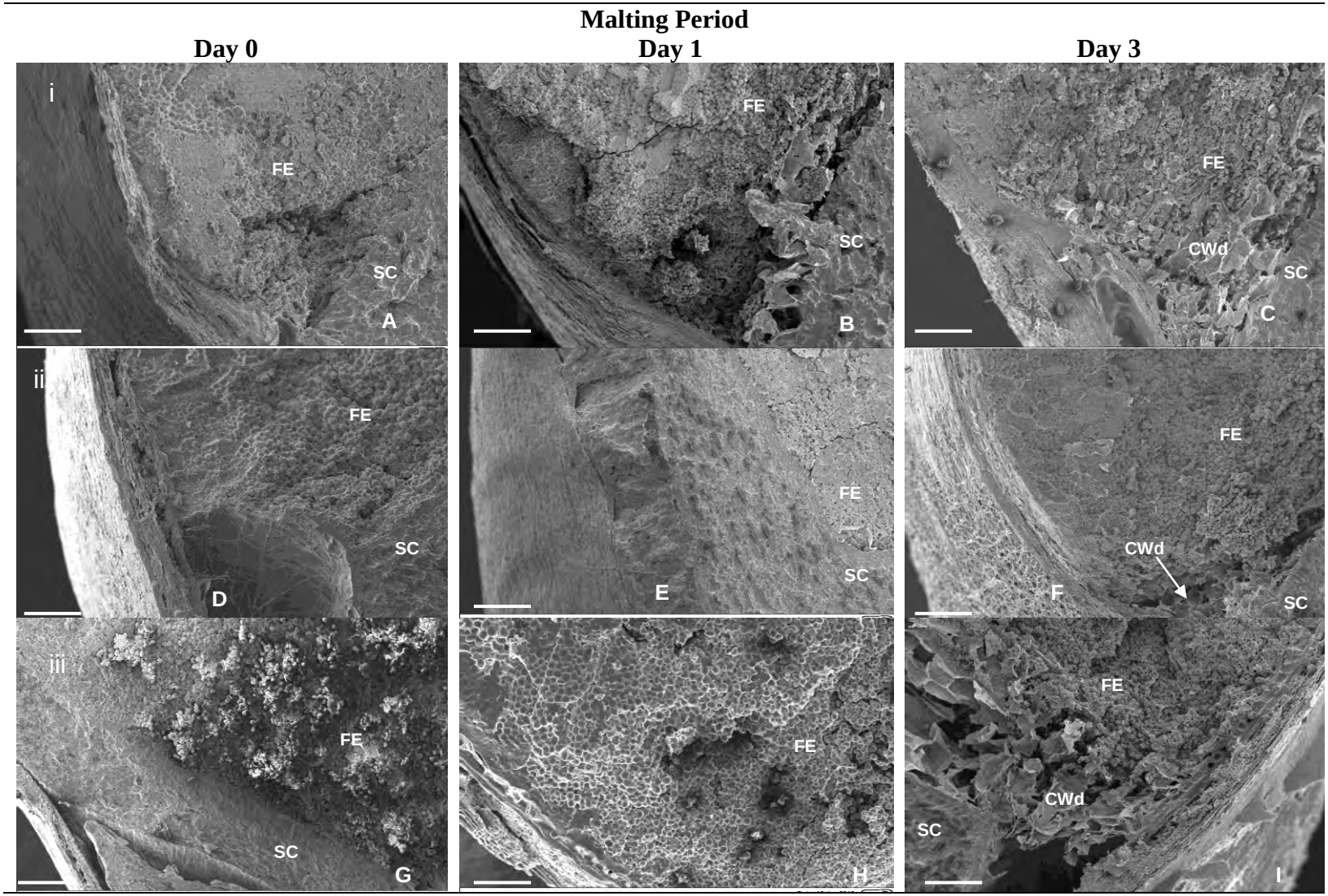


Fig 4.2.9. SEM of proximal sections of sorghum grain of different hardness that had been malted for up to 3 days following steeping. (i) PAN 8247 (hard), (ii) PAN 8648 (white tan-plant, intermediate), (iii) PAN 8625 (condensed tannin, soft), floury endosperm (FE), network of cell wall devoid of starch granules (CWd) and scutellum (SC). Bar is 200 µm.

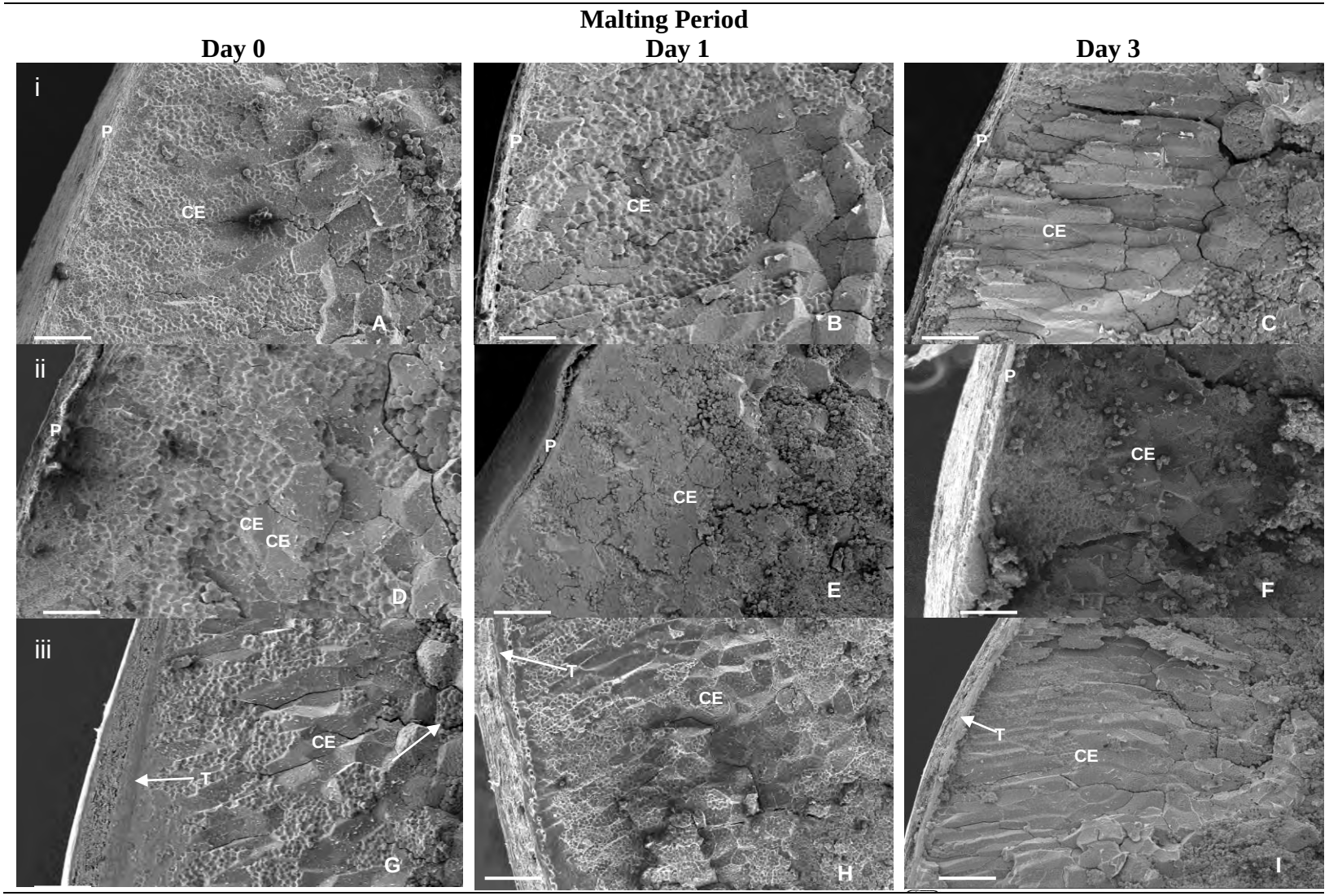


Fig 4.2.10. SEM of middle sections of sorghum grain of different hardness that had been malted for up to 3 days following steeping. (i) PAN 8247 (hard), (ii) PAN 8648 (white tan-plant, intermediate), (iii) PAN 8625 (condensed tannin, soft), the pericarp (P), corneous endosperm (CE) and testa (T). Bar is 200 µm.

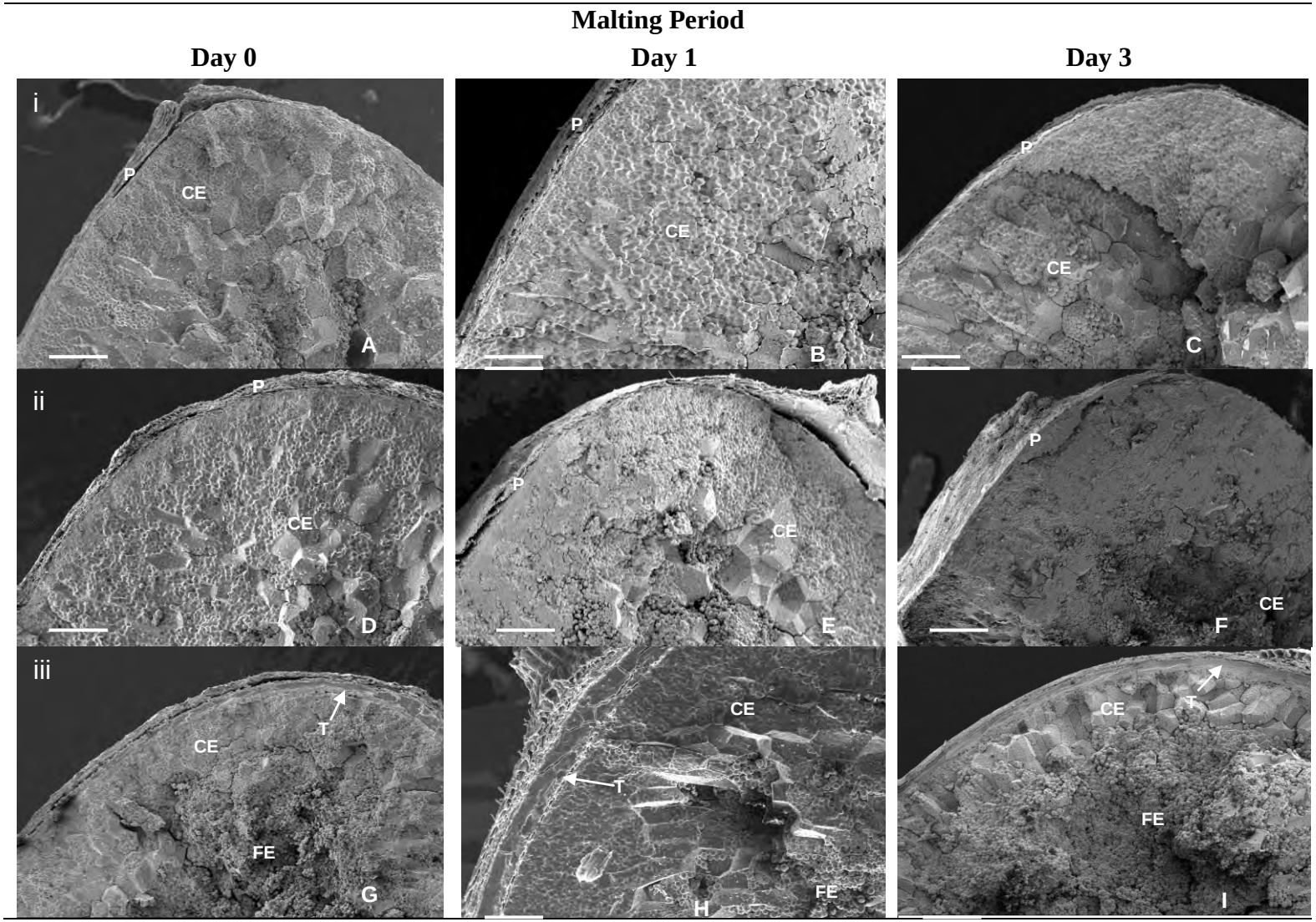


Fig 4.2.11. SEM of distal sections of sorghum grain of different hardness that had been malted for up to 3 days following steeping. (i) PAN 8247 (hard), (ii) PAN 8648 (white tan-plant, intermediate), (iii) PAN 8625 (condensed tannin, soft), pericarp (P), corneous endosperm (CE), floury endosperm (FE) and testa (T). Bar is 200 µm.

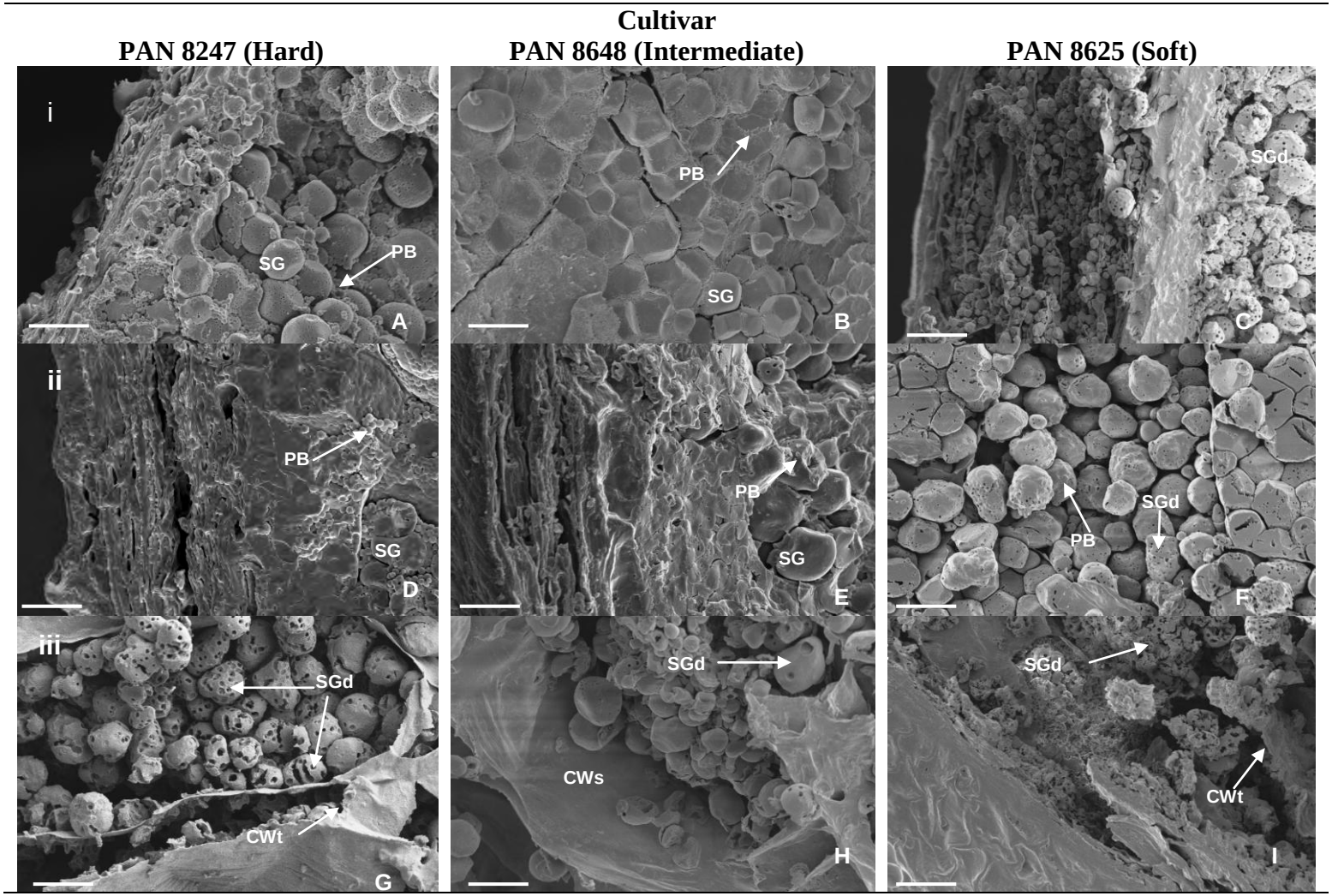


Fig 4.2.12. SEM of (i) distal, (ii) middle and (iii) proximal sections of sorghum grain of different hardness that had been malted for up to 5 days following steeping. PAN 8247 (hard), PAN 8648 (white tan-plant, intermediate), PAN 8625 (condensed tannin, soft), smooth cell wall (CWs), torn cell walls (CWt) intact starch granules (SG), degraded starch granules (SGd) and protein bodies (PB). Bar is 10 µm.

4.2.3.6 The effect of malting on pasting properties of sorghum malt flours and on the texture of malt porridges made from cultivars varying in hardness.

There was a reduction of 14 to 86% in peak viscosity during Day 1 of germination, with respect to grain peak viscosity (Table 4.2.2 and Fig 4.2.13). Malt of condensed tannin sorghum PAN 8625 (soft) had the lowest reduction in PV and PAN 8247 (hard) the highest. Likewise, FV was high in PAN 8625 and lowest in PAN 8247 malt. The results are in contrast to those of grain flours where PAN 8625 had the lowest FV (Table 4.2.2). Further reduction in viscosity occurred by Day 2. PV decreased by 88 to 95% and FV by 91 to 98% with respect to grain. Figs 4.2.13a and 4.2.13b show the pasting curves of sorghum malts malted for two days. By Day 2 the viscosity curves showed only slightly distinct peaks and by Days 3 and 4, they had flattened out almost completely. Therefore, only pasting curves up to two days of malting are shown. The general reduction in viscosities was due to high DP, which increased with malting time. However, in terms of grain hardness, there was no clear relationship between pasting properties and malt hardness.

Porridge texture was determined on sorghum malt flours produced from grain malted for one day. The texture of porridges malted longer than one day could not be assessed as they were very runny. Firmness of malt porridges was lower than that of grain except for PAN 8247 (Table 4.2.3, Fig 4.2.8) where the grain and malted porridge were similar. Firmness of PAN 8625 and PAN 8901 malt porridges decreased by almost 50% with respect to that of grain. Stickiness was not significantly different ($p \geq 0.05$) (Table 4.2.3, Fig 4.2.14) among sorghum malt porridges. Malt porridges were stickier (8.5 Ns) than those of grain (5.2 Ns) and less firm. Despite high final and setback viscosities of PAN 8625 malt, its porridge was less firm and sticky than that of other malt porridges. Firmness is due to retrogradation, which increases with time (Mohamed et al 1993; Perdon et al 1999). Firmness was affected by endosperm texture, cultivar and was higher in corneous endosperm flours than in soft sorghum floury endosperm flours (Mohamed et al 1993). This implies that the porridges of hard malts of PAN 8901 and PAN 8247 retrograded more than that of PAN 8625 during cooling. Hence the porridges became firmer since they were kept for 30 min before texture analysis.

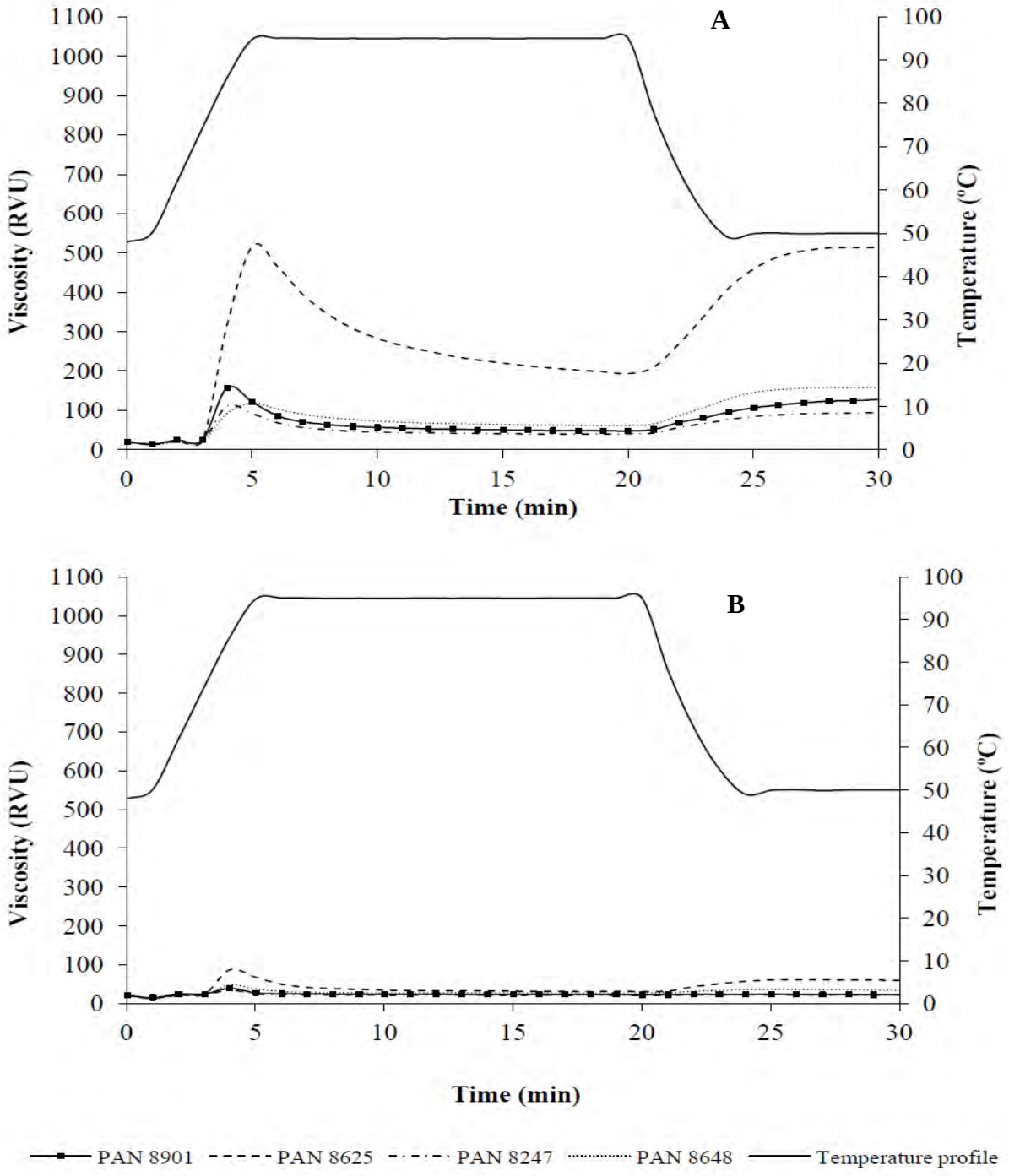


Fig 4.2.13. Effect of malting on the pasting profiles of flours obtained from sorghum grains with a wide range of hardness and physical properties, (A) malted for one day; and (B) two days (Curves based on means of duplicate runs). PAN 8247 (hard), PAN 8648 (white tan-plant, intermediate) and PAN 8625 (condensed tannin, soft).

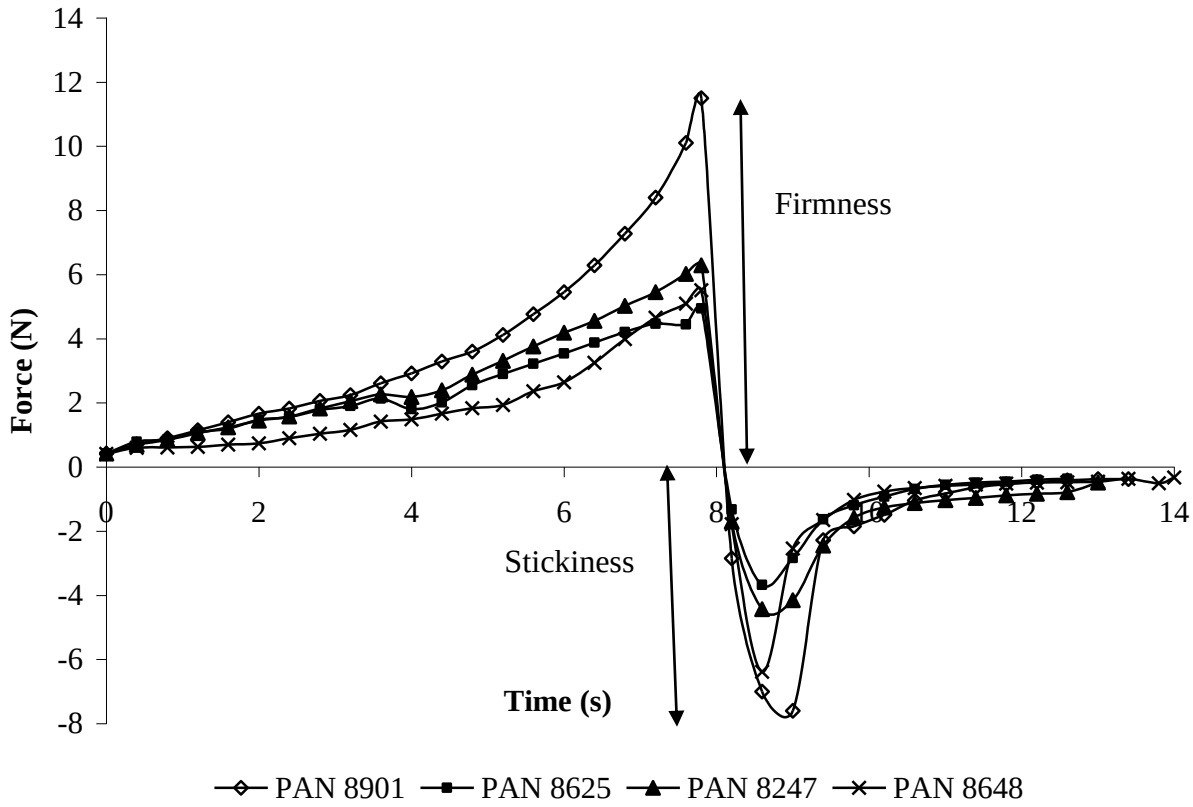


Fig 4.2.14. Firmness and stickiness of porridges prepared from sorghums flours of grain malted for one day (curves are means duplicate runs). PAN 8247 (hard), PAN 8648 (intermediate) and PAN 8625 (soft).

4.2.4 CONCLUSIONS

The pasting properties of sorghum flour of cultivars varying in hardness are not related to intrinsic grain hardness. In addition to grain hardness, the heterogeneity of sorghum in terms of condensed tannin presence may affect the pasting of flours. With maize, grain hardness affects pasting, with final viscosity high in flours of hard grains. Sorghum malting for two days is sufficient to distinguish between malts for hardness. Amylase activity and intrinsic grain hardness seem to affect sorghum modification, and hence malt hardness. However, amylase activity overrides grain hardness. Thus grain with low DP modifies slower and maintains hardness than with high DP. Sorghum with low DP has a potential for malt porridges where high DP is not sought.

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4.3 Phenolic acid content composition of sorghum and maize cultivars varying in hardness

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ABSTRACT

The role of phenolic acids on sorghum and maize hardness was evaluated among eight cultivars of each of the cereals representing hard and soft classes. Bran and flour fractions were evaluated for monomeric and diferulic phenolic acids using high performance liquid chromatographic and mass spectrometric (LC–MS/MS) techniques. Bran samples of harder grains had more phenolic acids than those of soft types. Intra-class testing showed slight differences in cultivars within the hard and soft classes. The content of phenolic acids was a useful indicator of hardness distinguishing between hard and soft maize and sorghum cultivars. Correlation coefficients between monomeric acids of maize bran, mostly ferulic acid, and grain hardness were higher than those of sorghum. Maize bran ferulic acid content was strongly correlated with Tangential Abrasive Dehulling Device (TADD) hardness ($r = -0.776$, $p < 0.001$). This study is the first to show that there is a relationship between bran phenolic acid content and sorghum and maize hardness.

4.3.1 INTRODUCTION

Dry milling quality of sorghum and maize primarily depends on grain hardness as it generally involves abrasive decortication and roller milling respectively, to obtain grits or meal. Hard grain types are desirable to obtain high extraction rates. Several physical tests have been used to estimate sorghum and maize grain hardness including density (Paulsen et al 2003), endosperm texture (Rooney and Miller 1982; ICC 2008), breakage susceptibility, stress cracking and decortication (Reichert et al 1986). Alternatively digital image analysis can be used to measure grain translucency (Erasmus and Taylor 2004; Louis-Alexandre et al 1991) and near infrared transmittance and reflectance spectroscopy to estimate grain hardness (Robutti 1995; Wehling et al 1996). These physical tests can only effectively differentiate between samples varying greatly in hardness (Duarte et al 2005; Johnson et al 2010). As mentioned in Chapter 4.1 commercial cultivars are selected for specific quality attributes and tend to be closely related, hence a need to also determine methods suitable for screening such cultivars. The findings in Chapter 4.1 have shown the appropriateness of TADD hardness and test weight for hardness determination in sorghum and maize cultivars differing slightly in hardness.

The biochemical basis for grain hardness is not well understood particularly in maize although the quantity and distribution of γ-kafirins is believed to play a major role in sorghum hardness (Da Silva et al 2011a; Mazhar and Chandrashekar 1995). Therefore, there is a need to determine measurements that can be used in such a situation. Phenolic acids are also thought to play a role in maize grain hardness (García-Lara et al 2004; Del Pozo-Insfran et al 2006). The high concentration and cross linking of phenolic acids to cell walls of the pericarp and aleurone layers are important. Thus, phenolic acids may affect structural properties that affect grain hardness.

The purpose of the study was to identify and quantify bound phenolic acids of sorghum and maize cultivars varying slightly in hardness to determine the relationship between phenolic acid types and content and grain hardness. A relationship between phenolic acids and hardness may mean that phenolic acids could be used as markers for sorghum and maize grain hardness.

4.3.2 MATERIALS AND METHODS

4.3.2.1 Samples

A study was conducted on eight sorghum and eight maize cultivars grown in South Africa representing commercial hybrids from the National Cultivar Trials harvested during the 2008/2009 growing season. Maize cultivars were white dent types grown in Potchefstroom, in the Northwest province. Sorghum cultivars were red, non-tannin and grown in Platrand, Free State province. The cultivars were all grown in one location so as to eliminate environmental effects on phenolic content. All cultivars were grown in dryland conditions, harvested at less than 14% moisture and dried slowly. Cultivars were classified as hard and soft according to the percentage of kernel removed by the Tangential Abrasive Dehulling Device (TADD). Findings of Chapter 4.1.1 showed that TADD hardness was suitable for evaluating sorghum and maize hardness. All samples were thoroughly cleaned to remove broken and foreign material threshed and cleaned samples were stored at 4°C until analyses.

4.3.2.2 Physical and hardness tests

Maize and sorghum grain physical and hardness tests are described in Chapter 4.1, Section 4.1.2.2.

4.3.2.3 Sample preparation

Maize and sorghum grains were decorticated with a TADD to 80% extraction rates to obtain bran and flour fractions. Bran was ground with a cyclone mill UDY Cyclotec Sample Mill (UDY Corporation, Fort Collins, Colorado, USA) to pass through a 0.5 mm opening screen. The ground fractions were wrapped tightly in plastic sample bags and stored at -20°C before analyses of total phenolic content and phenolic acids.

4.3.2.4 Total phenolic content (TPC)

A modified Folin-Ciocalteu method was used (Waterman and Mole 1994). Briefly, phenolic extracts were prepared in 15 ml acidified methanol (1% conc. HCl in methanol, v/v) from 1 g flour or bran samples. Centrifuged extracts were mixed with Folin Ciocalteu phenol reagent and then with sodium carbonate (20%, w/v) solution within 8 min from the addition of the phenolic reagent. The contents were left to stand for 2 h, after which absorbance was read at 734 nm. Catechin was used as a standard.

4.3.2.5 Extraction of bound phenolic acids

Soluble phenolics were extracted according to Qiu et al (2010), with modifications. Ground flour and bran samples (1 g) were extracted twice with 80% methanol (v/v) (15 ml) for 1 h by mechanical shaking. The methanolic mixture was centrifuged at 2 683 *g* for 5 min. The residue was retained for alkaline hydrolysis and washed with distilled water to remove organic solvent and filtered through Whatman No. 1 filter paper. Then 200 mg portion of the residue was hydrolysed at room temperature using NaOH under nitrogen to release insoluble ester linked phenolics. To optimize the extraction method, different extraction times and alkaline concentrations varying from 2 to 24 h and 2 to 4 M NaOH, respectively, were investigated. Hydrolysis for 2 h using 2 M NaOH was found sufficient for the release of phenolic acids. The hydrolysate was adjusted to a pH of 1.5 to 2.0 using 6 M HCl and extracted three times with 15 ml hexane to remove lipids. The organic phase was removed with a separating funnel and the aqueous phenolic phase extracted three times with ethyl acetate to obtain the alkali released phenolics. The organic phase was further dehydrated with 1 g Na_2SO_4. The combined ethyl acetate extracts were dried and concentrated under vacuum using a rotary evaporator. The dried phenolic extracts were redissolved in 2 ml of 50% (v/v) methanol and filtered through 0.45 µm and 0.22 µm PTFE filters before HPLC and MS/MS analyses, respectively.

4.3.2.6 HPLC-MS/MS analysis

HPLC analysis of phenolic acids was performed on a Waters 2695 HPLC (Waters, Milford, MA) equipped with a Waters 996 photodiode array (PDA) and a reverse phase ShimPack HRC-ODS, C18 (250 x 4.6 mm) analytical column (Shimadzu, Kyoto, Japan) and an auto sampler (717 Plus, Waters) to inject 20 µL of sample. The gradient mobile phase solvent A was 0.1% acetic acid in high purity water and solvent B was 0.1 % acetic acid in methanol. Phenolic acid separation was achieved using a 70 min linear solvent gradient at a flow rate of 0.7 ml/ min, as follows: 0 min 4% B, 18 min 18% B, 35 min 30% B, 58 min 42% B, 70 min 60% B, and 10 min to rinse and equilibrate the column. Phenolic acid quantification was based on the standard curves of the corresponding phenolic acids at a wavelength of 320 nm and peak area was used for calculations. Identification of phenolic acids was performed by comparison to the retention time and MS/MS spectra with external standards. MS/MS was conducted using a quadrupole time-of-flight mass spectrometer (Q-TOF MS) (Micromass, Milford, MA). Full mass spectra were acquired in the negative mode using cone and capillary voltages of 30 V and 1.6 kV, respectively. Desolvation and cone gases (He) were set to flow at 900 L/h and 35 L/h, respectively while the desolvation temperature and the source temperatures were 350°C and 150°C, respectively. MS/MS spectra were acquired using collision energy of 25 V in the range m/z 100 - 1500.

4.3.2.7 Statistical analyses

All extracts were analysed three times. Means were compared by Fisher's Least Significant Difference (LSD) test and significant differences were reported at $p < 0.05$. Pearson's correlation was performed to determine the relationship between phenolic acids and grain hardness.

4.3.3 RESULTS AND DISCUSSION

4.3.3.1 Physical and hardness characteristics of sorghum and maize cultivars

The physical and hardness properties of sorghum and maize cultivars are shown in Tables 4.3.1a and 4.3.1b, respectively. In general, analysis of variance could not verify significant differences among the cultivars. Thus, cultivars were simply ranked into hard and soft using TADD as a common measure of hardness for both sorghum and maize (Chapter 4.1). The hard and soft sorghum cultivars had on average 33.3 and 42.6% kernel removed by TADD decortication versus 24.1 and 30.3 % for hard and soft maize types, respectively. The average TKW was slightly higher but not significantly different for hard compared to soft cultivars of both grain types. However, there were significant differences in kernel sizes between 3.35 and 4.00 mm for hard and soft sorghums (Table 4.3.1a). The breakage susceptibility (SB) was generally high for all soft maize cultivars except for PAN 4P-313B while NIT Milling Index was generally low for all soft types except for cultivar AFG 4473 (Table 4.3.1b).

4.3.3.2 Total phenolic content of sorghum and maize bran and flour methanolic extracts

Bran TPC of hard sorghum and maize was significantly higher ($p < 0.05$) than that of soft cultivars (Table 4.3.2). The significant differences between hard and soft cultivars suggest that bran TPC may be used as an indicator of sorghum and maize hardness. However, when comparing TPC among cultivars of similar hardness or softness, TPC may not be useful to distinguish individual cultivars in the same hardness group. TPC of the flours, contributed mainly by the endosperm, seemed consistent in all cultivars and was not affected by grain hardness. Since phenolic compounds are concentrated in sorghum and maize bran (Awika et al 2005; Bily et al 2004) it was expected that TPC in the flour would not vary to a large extent among cultivars. Since most of the phenolic compounds exist in the bound form (> 85%) in maize (Adom and Liu 2002) and in other cereals, the samples were hydrolysed to release the major portion of the bound phenolic compounds and further identified and quantified with HPLC.

TABLE 4.3.1a Physical and Hardness Characteristics of Sorghum [a,b]

Cultivar	TW	TKW	>4.00 mm	>3.35<4.00 mm	>3.15<3.35 mm	>2.36<3.15 mm	TADD
Hard Cultivars							
PAN 8902	77.7aA(0.3)	25.7aA(1.2)	0.6cdB(0.05)	62.6aAB(0.3)	18.2bA(0.1)	13.2bA (0.7)	32.7bcAB(3.5)
PAN 8905	75.9bB(0.0)	26.2aA(0.9)	0.6cdB(0.30)	62.9aAB(0.3)	18.8bA(0.4)	11.5cdB(0.2)	36.2abcA(1.5)
PAN 8564	76.9aA(0.5)	25.0aA(1.0)	0.3dC(0.11)	52.5cC(1.4)	17.0bcB(0.5)	9.9efC(0.1)	37.6abcA(2.1)
PAN 8488	77.4aA(0.2)	25.5aA(0.6)	3.6aA(0.25)	65.8aA(3.4)	11.8dC(0.4)	13.0bA(0.2)	26.7cC(3.2)
Mean	77.0[a](0.7)	25.6[a](0.9)	1.3[a](1.46)	60.9[a](5.6)	16.5[a](3.0)	11.9[a](1.4)	33.3[a](4.9)
Soft Cultivars							
PAN 8901	77.7aA(0.3)	25.3aB(1.9)	0.1dC(0.0)	65.8aA(0.8)	14.6cdB(0.6)	12.6bcB(0.1)	49.2aA(9.1)
PAN 8903	76.4aA(0.6)	26.8aAB(0.7)	2.1bA(0.4)	55.6bcC(0.5)	13.4dC(0.1)	10.4deC(0.3)	42.3abcB(2.0)
PAN 8906	75.6bB(0.3)	28.0aA(0.8)	1.4cB(0.4)	61.9abB(2.7)	12.5dD(0.1)	8.8fD(0.3)	38.4abcB (1.4)
PAN 8904	75.2bB(0.3)	19.8bC(1.2)	2.2bA(0.1)	19.4dD(0.3)	31.2aA(2.3)	38.5aA(0.6)	45.1aA(2.1)
Mean	76.2[a](1.0)	25.0[a](3.5)	1.4[a](0.9)	50.7[b](19.7)	17.9[a](8.3)	17.6[a](13.0)	42.6[a](6.3)

[a] All cultivars were bred by Pannar Seed South Africa; TW, test weight (kg/hl); TKW; thousand kernel weight (g); kernels passing through > 2.36 mm > 4.00mm (%); TADD; % kernel removed by TADD decortication.
[b] Figures in parentheses are standard deviations.
Lower case letters (e.g a) in the same column denote significant differences ($p < 0.05$) among all cultivars.
Upper case letters (e.g A) in the same column denote significant differences ($p < 0.05$) within the hard and soft cultivars.
Superscript letters (e.g [a]) in the same column denote significant differences ($p < 0.05$) between hard and soft cultivars.

TABLE 4.3.1b Physical and Hardness Characteristics of Maize Cultivars [a,b]

Cultivar	TW	SB	KS	TKW	TADD	NIT
Hard cultivars						
IMP 52 – 11	81.5(1.5)	2.26(1.57)	83.0(1.0)	397(53)	25.2(2.0)	98.8(7.1)
DKC 77 – 61 B	79.9(1.5)	1.98(0.04)	74.5(10.5)	438(9)	24.2(0.9)	91.0(6.7)
AFG 4555	82.0(0.2)	2.73(0.34)	77.1(10.1)	444(3)	23.7(3.4)	93.7(6.5)
LS 8521 B	79.4(0.3)	2.57(0.89)	78.2(2.3)	404(8)	23.4(2.3)	99.6(5.2)
Mean	80.6(1.4)	2.38(0.76)	78.2(6.5)	421(48)	24.1(1.9)	95.8(6.2)
Soft cultivars						
PAN 6223 B	78.6(3.3)	4.04(0.41)	83.7(5.6)	373(54)	31.2(3.3)	85.0(11.1)
PAN 4P – 313 B	80.0(1.0)	1.70(0.30)	82.7(1.1)	403(37)	29.1(2.0)	86.3(5.5)
AFG 4473	86.1(8.1)	3.55(0.83)	80.3(0.2)	422(3)	29.6(2.0)	95.7(3.4)
AFG 4517	79.9(2.5)	4.11(0.55)	77.0(0.6)	413(23)	31.2(2.4)	84.2(1.2)
Mean	81.2(4.7)	3.35(1.13)	80.9(3.5)	403(33)	30.3(2.1)	87.8(6.9)

[a] Cultivars were bred by South African-based seed companies Agricol, Monsanto, AFGRI, Link, PANNAR, PANNAR, AFGRI, AFGRI, respectively; TW, test weight (kg/hl); SB, % breakage susceptibility by Steiner breakage tester; TKW; thousand kernel weight (g); TADD; % kernel removed by TADD decortication; KS; % kernel size ≥ 8 mm; NIT, Near Infrared Transmittance Milling Index
[b] Figures in parentheses are standard deviations. Means were not significantly different

TABLE 4.3.2 Total Phenolic Content of Sorghum and Maize Bran and Flour Fractions (g/100 g Catechin Equivalents) [a]

Sorghum			Maize		
Cultivar	Bran	Flour	Cultivar	Bran	Flour
Hard cultivars					
PAN 8902	0.89abA(0.17)	0.34aAB(0.09)	IMP 52 – 11	0.76abA(0.01)	0.29aA(0.04)
PAN 8905	0.96aA(0.02)	0.29aB(0.06)	DKC 77 – 61 B	0.78aA(0.08)	0.36aA(0.02)
PAN 8564	0.96aA(0.03)	0.48aA(0.01)	AFG 4555	0.76abcA(0.04)	0.29aA(0.09)
PAN 8488	0.71bB(0.08)	0.37aAB(0.18)	LS 8521 B	0.71abcA(0.03)	0.33aA(0.01)
Mean	0.88[a](0.13)	0.37[a](0.11)	Mean	0.75[a](0.05)	0.31[a](0.05)
Soft cultivars					
PAN 8901	0.70bcA(0.11)	0.36aB(0.03)	PAN 6223 B	0.50efA(0.00)	0.28aA(0.04)
PAN 8903	0.77bcA(0.04)	0.27aB(0.05)	PAN 4P – 313 B	0.59defA(0.06)	0.31aA(0.00)
PAN 8906	0.63cB(0.04)	0.49aA(0.06)	AFG 4473	0.45fB(0.03)	0.39aA(0.04)
PAN 8904	0.71bcA(0.05)	0.31aB(0.03)	AFG 4517	0.56defA(0.02)	0.32aA(0.04)
Mean	0.70[b](0.07)	0.36[a](0.09)	Mean	0.52[b] (0.06)	0.33[a](0.03)

[a] Figures in parentheses are standard deviations.
Lower case letters (e.g a) in the same column denote significant differences ($p < 0.05$) among all cultivars.
Upper case letters (e.g A) in the same column denote significant differences ($p < 0.05$) within the hard and soft cultivars.
Superscript letters (e.g [a]) in the same column denote significant differences ($p < 0.05$) between hard and soft cultivars.

4.3.3.3 Phenolic acid composition of sorghum and maize cultivars

Four simple phenolic acids were identified in the alkaline hydrolysates, namely caffeic acid (CA), *p*-coumaric acid (PCA), ferulic acid (FA) and sinapic acid (SA) against standards (Fig4.3.1). All of the phenolic acids were identified in sorghum bran and only PCA and FA were found in the sorghum flour. In maize, PCA, FA and SA were found in the bran fraction and only PCA and FA were detectable in the flour.

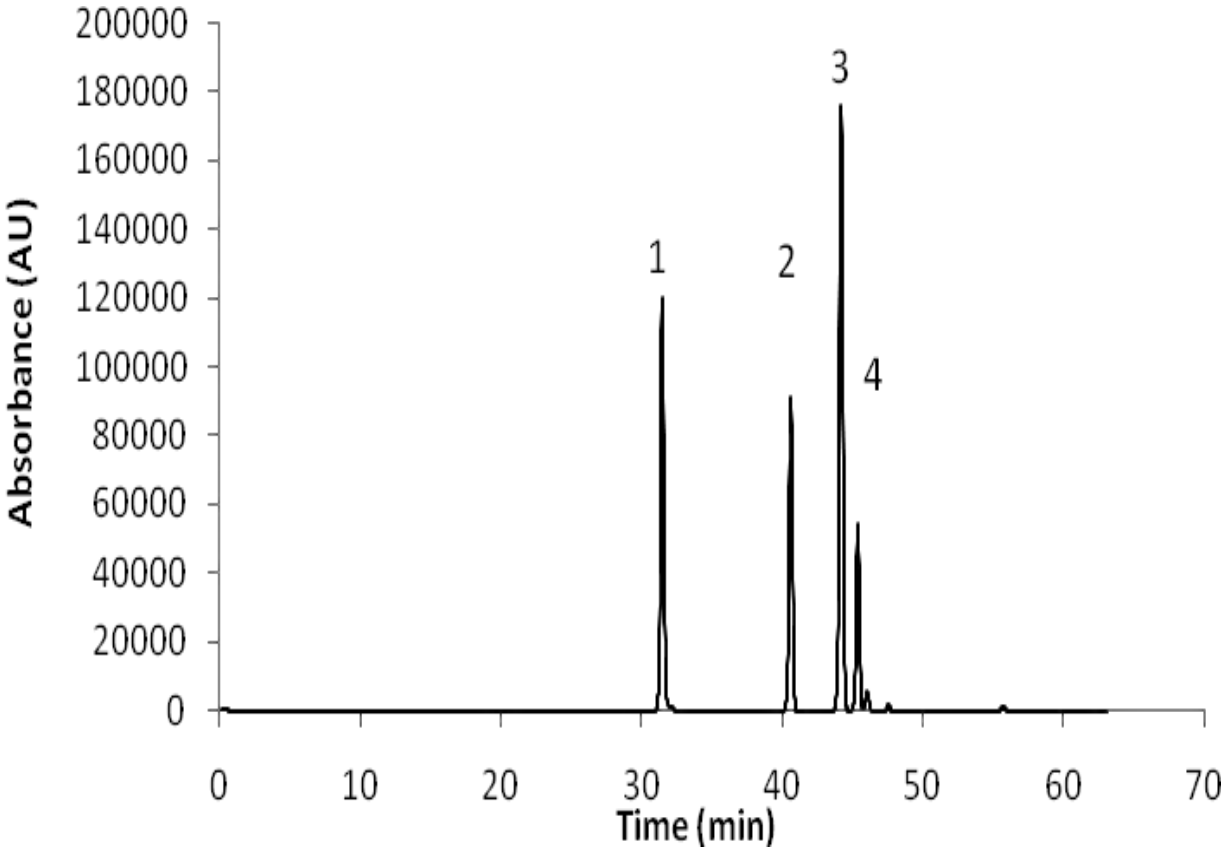

Fig 4.3.1. Chromatogram of caffeic acid (1), *p*-coumaric acid (2), ferulic acid (3) and sinapic acid (4).

4.3.3.4 Bound phenolic acids of sorghum bran and flour fractions

Ferulic acid content was significantly different ($p < 0.05$) among brans of hard and soft sorghum cultivars (Table 4.3.3a). Ferulic acid was the most abundant phenolic acid in sorghum bran (1727 to 3532 µg/g) as previously reported in several grains including maize, rice, wheat, buckwheat, sorghum, rye and barley (Bily et al 2004; Dobberstein and Bunzel, 2010; Gallardo et al 2006; Li et al 2007; Ring et al 1988; Rao and Muralikrishna, 2004). Within the hard cultivars, bran FA was similar except for PAN 8488 which had significantly lower ($p < 0.05$) content than other cultivars. The low FA content in PAN 8488 could be attributed to possible contamination of bran with flour resulting in FA dilution. Bran from hard sorghum grains had two times more PCA than soft types (Table 4.3.3a). Similar to findings with FA, the trends in the quantities of PCA between hard and soft sorghums demonstrated that hardness could be related to phenolic acid content and type.

Only PCA and FA were found in flour, almost two and seventeen times lower than in bran, respectively. The content of PCA and FA of hard sorghum flours was, respectively, hree and two times more than soft types, an indication that phenolic acid content can be used to distinguish between hard and soft cultivars even in low amounts such as those found in the flour compared to bran. PAN 8488 had bran total phenolic acid content (BTPC) that differed significantly ($p < 0.05$) with other grains within the hard cultivars. Total phenolic acid content (FTPA) of soft sorghum flour was approximately 50% that of hard type flours. The significant differences ($p < 0.05$) in phenolic acid content between hard and soft cultivars suggest that phenolic acids affect grain hardness as suggested by Garcia-Lara et al (2004) and Del Pozo-Insfran et al 2006).

TABLE 4.3.3a Bound Phenolic Acids of Sorghum Bran and Flour Fractions (µg/g) [a,b]

	Bran						Flour		
Cultivar	Caffeic	*p*-Coumaric	Ferulic	Sinapic	DFA[a]	BTPA[b]	*p*-Coumaric	Ferulic	FTPA
Hard cultivars									
PAN 8902	103bBC(16)	250cC(21)	3532aA(245)	57.3bB(3.6)	436aA(47)	4378aA(333)	166aB(12)	205aA(14)	371aA(26)
PAN 8905	136aA(6)	329bB(28)	3507aA(166)	51.5bcB(4.6)	326cC(28)	4350aA(233)	198aA(8)	185aB(13)	383aA(21)
PAN 8564	102bBC(10)	396aA(9)	3412aA(32)	78.6aA(2.8)	406aA(18)	4395aA(72)	152aBC(14)	202aA(7)	354aA(21)
PAN 8488	83cC(6)	223cC(12)	2675bB(71)	59.3bB(0.8)	397aAB(18)	3437bB(110)	140aC(9)	169bB(7)	310aB(16)
Mean	106[a](22)	300[a](79)	3282[a](408)	61.7[a](11.8)	416[a](47)	4140[a](469)	164[a](25)	190[a](17)	354[a](32)
CV						6.5			6.9
Soft cultivars									
PAN 8901	43dB(1)	103dC(9)	1886dB(42)	74.5aA(3.1)	341bcB(27)	2448dB(82)	70bB(4)	89cA(9)	160bA(13)
PAN 8903	114bA(11)	175cdA(14)	2401bcA(207)	74.5aA(3.1)	389aA(29)	3153bcA(254)	84bA(5)	79cA(10)	163bA(15)
PAN 8906	31dC(3)	139dBC(25)	2342cA(124)	75.0aA(3.8)	345bcB(24)	2939cA(180)	26dC(1)	81cA(7)	107cB(7)
PAN 8904	46dB(3)	151dAB(6)	1727dC(26)	41.4cB(3.4)	337bcB(16)	2302dB(53)	33dC(5)	79cA(4)	112cB(9)
Mean	59[b](38)	142[b](30)	2089[b](148)	66.4[a](17)	353[a](24)	2711[b](402)	53[b](28)	82[b](5)	135[b](30)
CV						7.3			8.0

[a] Figures in parentheses are standard deviations.
Lower case letters (e.g a) in the same column denote significant differences ($p < 0.05$) among all cultivars.
Upper case letters (e.g A) in the same column denote significant differences ($p < 0.05$) within the hard and soft cultivars.
Superscript letters (e.g [a]) in the same column denote significant differences ($p < 0.05$) between hard and soft cultivars.
[b] DFA, diferulic acids; BTPA, total phenolic acid content in bran; FTPA, total phenolic acids in flour; CV, average cultivar coefficient of variation

4.3.3.5 Bound phenolic acids of maize bran and flour fractions

Ferulic acid had the highest content among acids quantified in maize bran (Table 4.3.3b). Significant differences ($p < 0.05$) were observed among hard and soft maize grains. The mean FA of hard type maize bran (3214 µg/g) was substantially higher than that of soft types (2198 µg/g). The differences in FA content between soft and hard maize cultivars were also observed in sorghum. However, LS 8521 B bran had 18% less FA than other hard cultivars. Within the soft types, bran of PAN 4P – 313 B had at least 28% more FA. FA content of 2480 mg/kg was reported in white maize of intermediate to hard flour texture (Del Pozo-Insfran et al., 2006). This FA content is similar to the levels found in bran samples from soft cultivars in this present study. The present study also showed that bran PCA of hard types was higher (two times) than that of soft types. Within hard and soft cultivars, AFG 4555 and PAN 6223 B had significantly ($p < 0.05$) high and low PCA contents, respectively.

Ferulic acid and PCA occurred in lower amounts in flour compared to the bran, due to low concentrations of phenolic compounds in the endosperm (Bily et al 2004), which comprised most of the flour component. Only 6% and 4% of bran FA occurred in hard and soft grain flours, respectively. PCA was also lower in flours compared to bran, by a margin of 22 to 32%. Del Pozo-Insfran et al (2006) reported 6.6 mg/kg PCA in hard to intermediate white maize, values lower than found in this study likely due to cultivar differences and extraction methods.

TABLE 4.3.3b Bound Phenolic Acids of Maize Bran and Flour Fractions (µg/g) [a,b]

	Bran					Flour		
Cultivar	*p*-Coumaric	Ferulic	Sinapic	DFA[a]	BTPA	*p*-Coumaric	Ferulic	FTPA
Hard cultivars								
IMP 52 – 11	244bB(22)	3471aA(142)	89bB(2)	320bcB(14)	4124aA(180)	74.3aB(2)	83bB(6)	157bB(7)
DKC 77 – 61 B	242bB(18)	3273aA(137)	123aA(7)	350abB(17)	3989aA(179)	83.4aA(3)	112aA(10)	195aA(7)
AFG 4555	488aA(21)	3373aA(41)	117aA(7)	439aA(18)	4413aA(87)	47.1cdD(2)	107aA(1)	154bB(3)
LS 8521 B	232bB(15)	2740bB(186)	120aA(9)	436aA(10)	3528bA(36)	56.0bC(1)	129aA(1)	185aA(2)
Mean	302[a](124)	3214[a](326)	112[a](16)	386[a](18)	4013[a](369)	65.2[a](15)	108[a](18)	173[a](19)
CV					5.2			2.8
Soft cultivars								
PAN 6223 B	85eC(6)	2044cdB(176)	47dC(1)	259cB(16)	2435dB(199)	21.8eC(1)	113aA(8)	135bcA(7)
PAN 4P – 313 B	169cA(4)	2742bA(158)	68cA(0)	267cB(24)	3246cA(92)	43.8dB(2)	67cC(2)	110cB(4)
AFG 4473	175cA(14)	1973dB(157)	69cA(7)	331bcA(29)	2548cdB(39)	54.0bcAc(1)	82bB(4)	136bcA(4)
AFG 4517	104deB(8)	2032cdB(100)	61cdB(3)	274cB(18)	2471cdB(92)	52.7bcA(5)	63cC(1)	115cB(6)
Mean	133[b](32)	2198[b](356)	61[b](10)	283[b](27)	2675[b](386)	43.1[b](13.9)	81[a](21)	124[b](13)
CV					6.7			4.2

[a] Figures in parentheses are standard deviations.
Lower case letters (e.g a) in the same column denote significant differences ($p < 0.05$) among all cultivars.
Upper case letters (e.g A) in the same column denote significant differences ($p < 0.05$) within the hard and soft cultivars.
Superscript letters (e.g [a]) in the same column denote significant differences ($p < 0.05$) between hard and soft cultivars.
[b] DFA, diferulic acids; BTPA, total phenolic acid content in bran; FTPA, total phenolic acids in flour; CV, average cultivar coefficient of variation

4.3.3.6 Identification and quantification of sorghum and maize diferulic acids

The identification of diferulic acids (DFAs) was confirmed by their mass spectra in comparison with literature. By performing a scan at *m/z* 385, typical of diferulates, four DFAs were identified in the bran of both hard and soft sorghum and maize cultivars (Fig 4.3.2). The DFAs were assigned 8-5' (A), 5-5' (B), 8-*O*-4' (C) and 8-5'-benzofuran form (Fig 4.3.3a-d), in agreement with mass spectra data and fragmentation patterns (Bily et al 2004; Callipo et al 2010; Qiu et al 2010). The data from mass spectrometry shown in Fig 4.3.2 and Fig 4.3.3a-d is representative of both sorghum and maize cultivars. All the deprotonated diferulic acids $[M - H]^-$ produced a fragment at *m/z* 341 due to the loss CO_2 (44 Da) from the carboxylic acid group. The fragmentation pattern is characteristic of phenolic acids with the resultant $[M - H-COO]^-$ anion (Parejo et al 2004; Hossain et al 2010). The DFAs 8-*O*-4' and 8-5'-benzofuran form were the most abundant confirming previous reports by Andreasen et al (2000) and Waldron et al (1996).

Only DFAs of sorghum and maize bran were quantified as the flours contained very low amounts since most of these oligomeric compounds occur as part of dietary fibre (Bunzel et al 2001). Due to lack of DFA standards, FA was used for their quantification. DFAs of sorghum and maize were higher in bran of hard cultivars than soft ones. The presence of DFAs in bran could enhance cross linking with arabinoxylan chains (Gallardo et al 2006). The cross-linking of arabinoxylan chains probably strengthens cell walls hence affecting grain mechanical properties (Renger and Steinhart 2000), and also grain hardness. Arabinoxylans have been shown to have a greater effect in modifying grain hardness in soft wheat than in hard wheat (Bettge and Morris 2000). High levels of polymer, which was similar to water-soluble arabinoxylans is a characteristic of the peripheral endosperm of soft wheat cultivars (Barron et al 2005). Within the class of soft sorghum cultivars, PAN 8903 apparently had DFA content similar to that of hard sorghums. Within hard maize cultivars, IMP 52-11 and DKC 77-61 B could be distinguished from AFG and LS 8521 B as having lower DFA content than the latter. In the case of soft types, cultivar AFG 4473 had DFA content similar to that of IMP 52-11, a hard type.

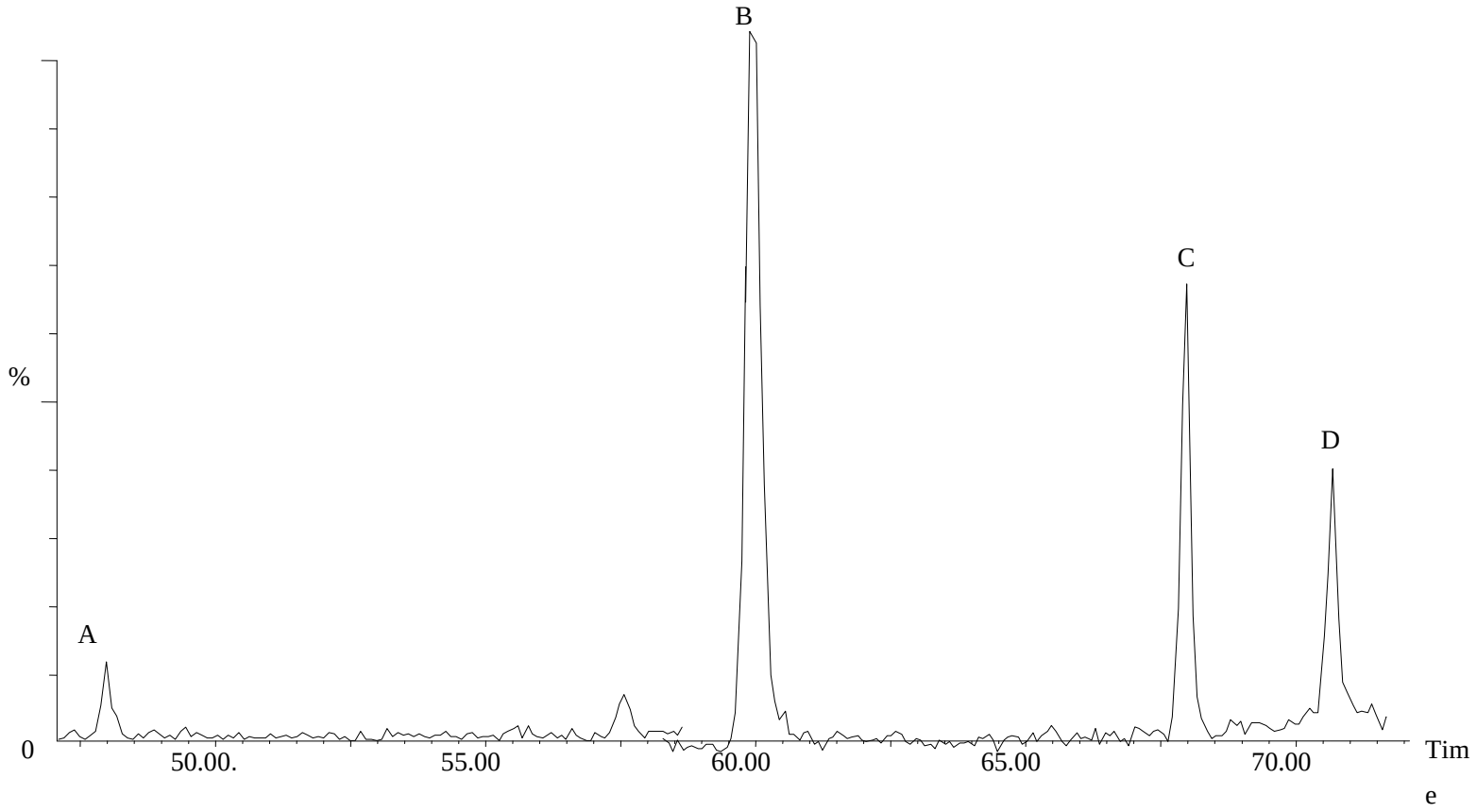

Fig 4.3.2. Selected ion chromatogram at *m/z* 385 with four of the identified diferulic acids namely 8-5' (A), 5-5' (B), 8-*O*-4' (C) and 8-5'-benzofuran form (D), respectively from the sorghum cultivar PAN 8902.

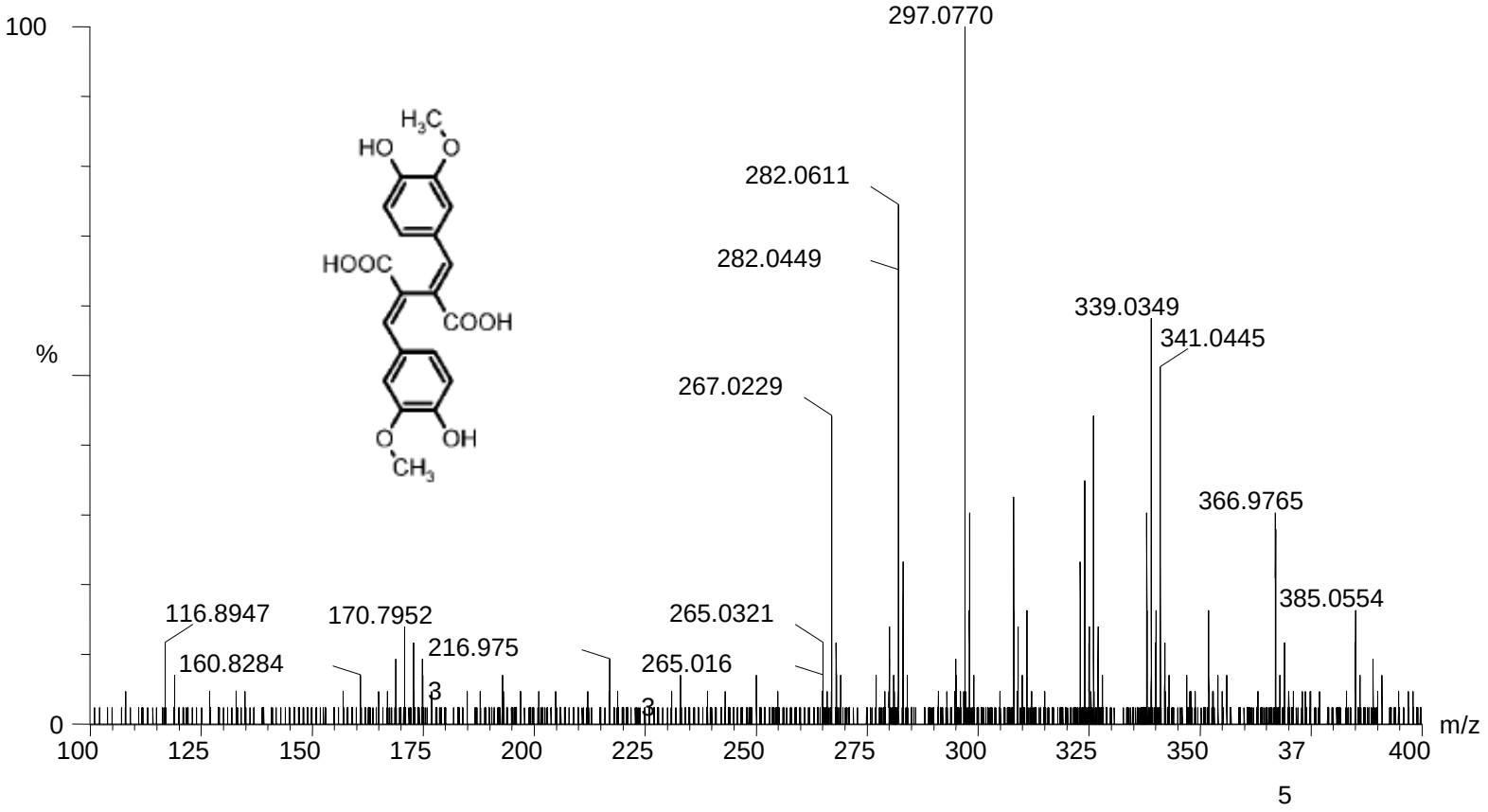

Fig 4.3.3a. MS/MS spectra of 8-5' diferulic acid from the sorghum cultivar PAN 8902.

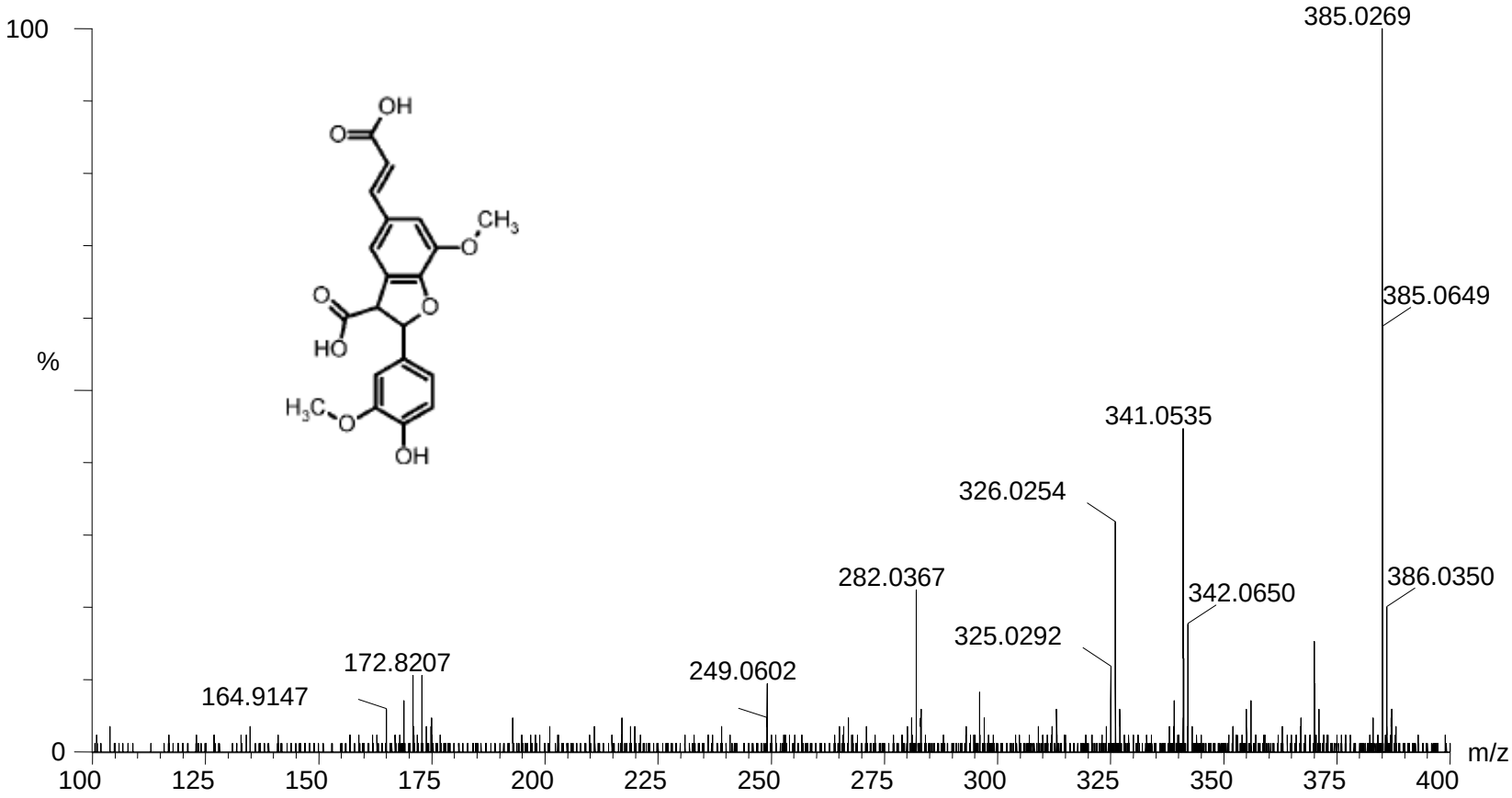

Fig 4.3.3b. MS/MS spectra of 5-5'diferulic acid from the sorghum cultivar PAN 8902.

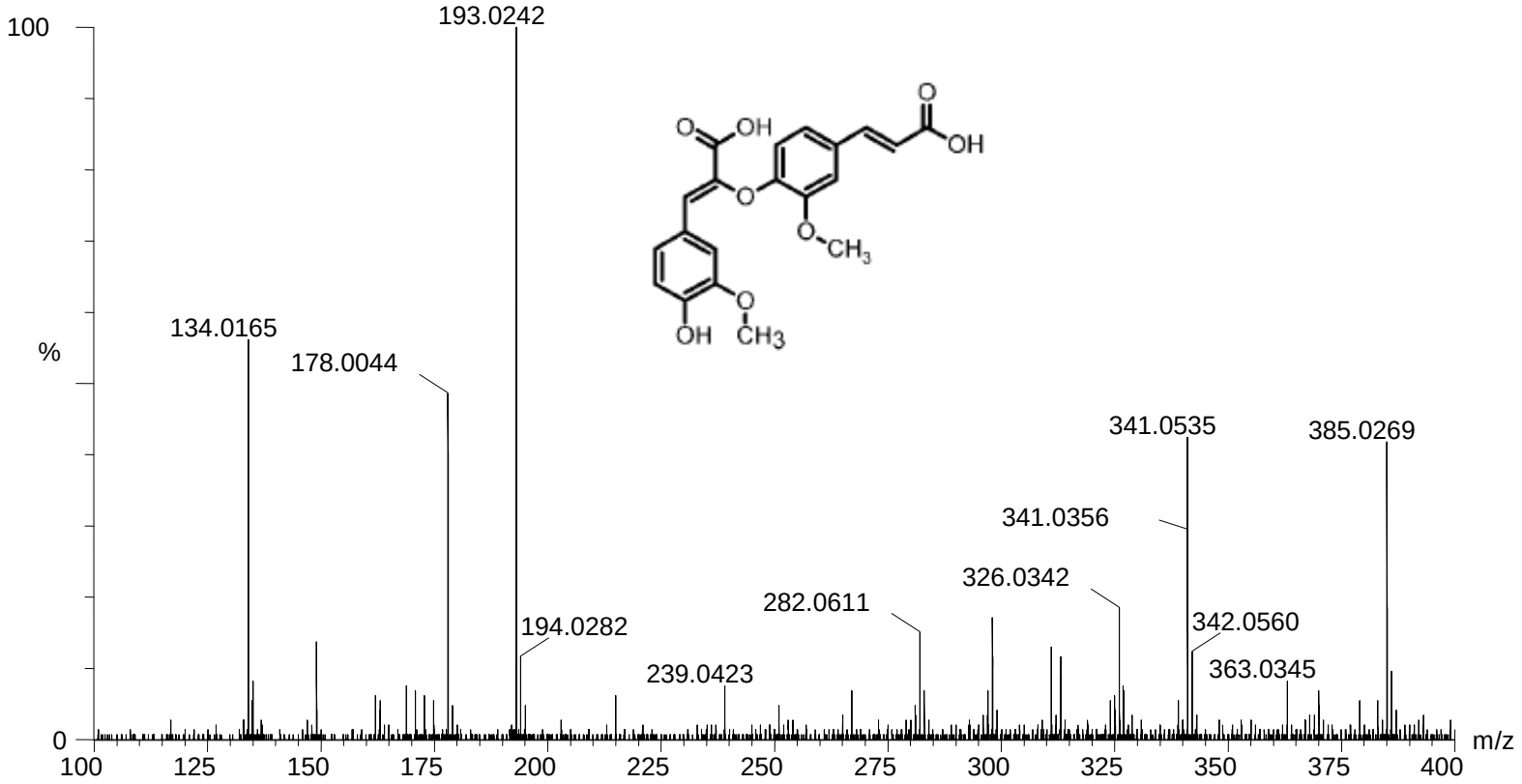

Fig 4.3.3c. MS/MS spectra of 8-*O*-4' diferulic acid from the sorghum cultivar PAN 8902.

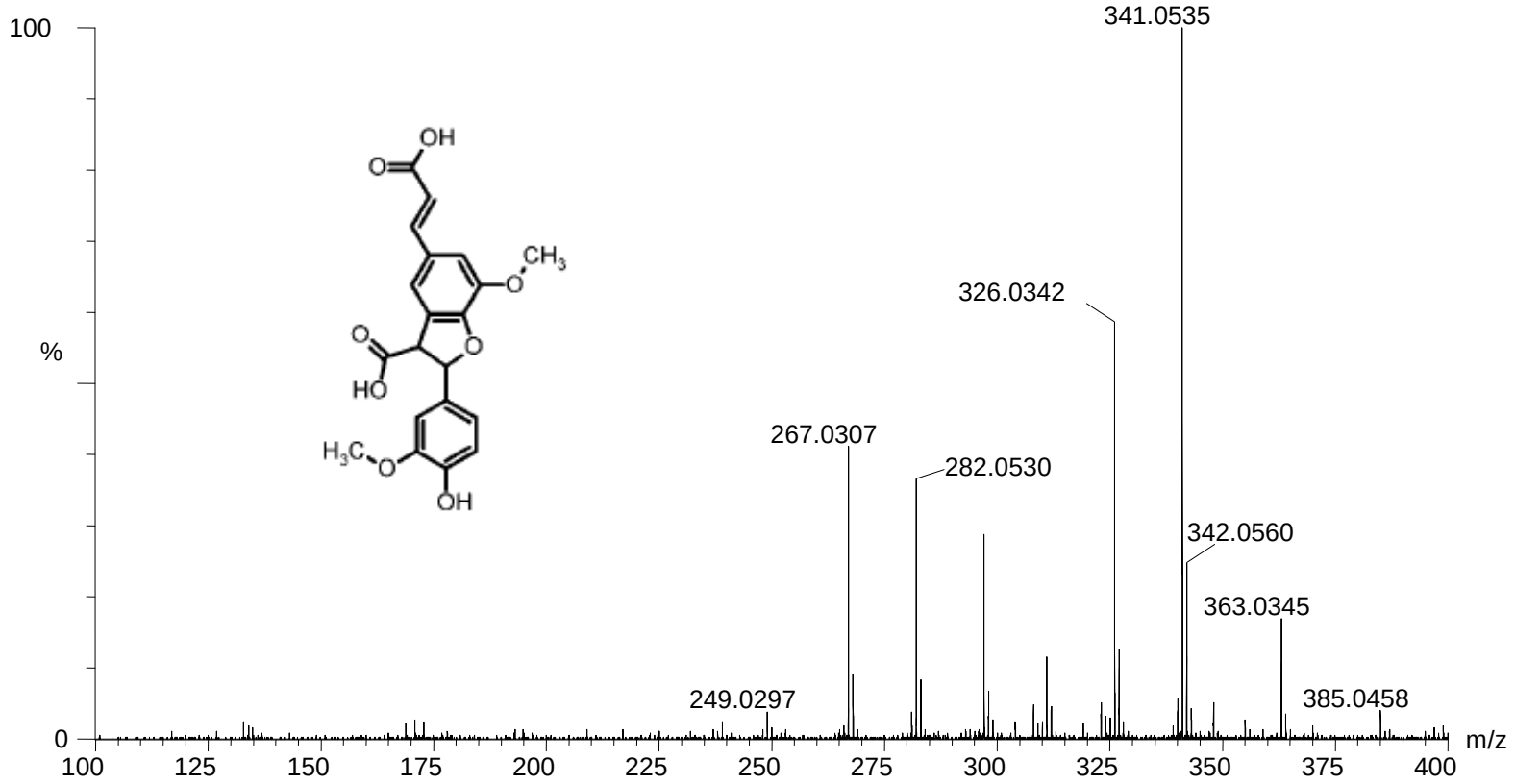

Fig 4.3.3d. MS/MS spectra 8-5'-benzofuran form diferulic acid from the sorghum cultivar PAN 8902.

4.3.3.7 Relationship between phenolic acids of sorghum and maize with grain hardness parameters

To confirm the relationships and possible role of sorghum and maize phenolic acids in grain hardness, Pearson's correlation coefficients were determined against grain physical properties as shown in Tables 4.3.4a and 4.3.4b, respectively. FA, as the major phenolic acid quantified, was significantly negatively correlated with TADD ($r = -0.447$, $p < 0.05$) of sorghum bran. Although the results indicated a significant correlation between TADD and FA, the relationship was not strong, explaining 22% of the variation. BTPA was also weakly negatively correlated with TADD ($r = -0.474$, $p < 0.05$). Correlations of BFA and BTPA with TW were slightly stronger than those for TADD ($r = 0.611$, $p < 0.05$) and ($r = 0.597$, $p < 0.05$), respectively. The significant correlation between BSA and sorghum kernel size (> 2.36 < 3.35 mm) was unexpected as it related to small kernel size. In Chapter 4.1, large sorghum kernel size (> 3.35 mm) was correlated with TADD hardness. Further investigations are needed to confirm this relationship.

In contrast, maize phenolic acids showed stronger correlations with grain physical properties than sorghum. The phenolic acids were mostly correlated with TADD hardness (Table 4.3.4b). TADD of maize bran was significantly correlated with BFA, BTPC, BSA, FPCA, FFA, BTPA and FTPA. The notable correlations at $p < 0.001$ were between TADD with BTPC ($r = -0.717$), BFA ($r = 0.-776$) and FTPA ($r = -0.730$). The correlation between FTPA and grain hardness is noteworthy given the low phenolic acid content in the flour. Since bran is a by-product of maize milling, the implication is that the retained flour could be evaluated for total phenolic acid content as an indicator of grain hardness. TW was significantly correlated with FTPC ($r = 0.503$, $p < 0.05$) and BPCA ($r = 0.579$, $p < 0.05$). Breakage susceptibility was negatively correlated with BTPC and BFA. The results clearly show that FA influences maize grain mechanical properties as the negative correlations imply that cultivars with low FA would break easily.

TABLE 4.3.4a Pearson Correlation Coefficients between Sorghum Physical and Hardness Characteristics and Phenolic Acids of Bran and Flour Fractions [a,b]

	TW	TKW	K4.00	K3.35	K3.15	K2.36	TADD	BTPC	FTPC	BCA	BPCA	BFA	BSA	FPCA	FFA	DFA	BTPA
TKW	0.260																
K400	0.372	0.170															
K335	0.582**	0.660***	0.575**														
K315	-0.449*	-0.635**	-0.677**	-0.885**													
K236	-0.454*	-0.728***	-0.372	-0.908***	0.909***												
TADD	-0.230	-0.469*	-0.252	-0.370	0.376	0.368											
BTPC	0.109	0.063	-0.538*	0.042	0.175	-0.164	-0.191										
FTPC	-0.019	0.253	0.161	0.168	-0.234	-0.243	-0.042	-0.107									
BCA	0.416	0.185	-0.314	0.236	-0.046	-0.224	-0.411*	0.441	-0.294								
BPCA	0.432	0.120	-0.328	0.149	-0.146	-0.362	-0.380	0.664**	0.218	0.486*							
BFA	0.611*	0.258	-0.107	0.381	-0.327	-0.468*	-0.447*	0.553*	-0.180	0.711**	0.787***						
BSA	0.342	0.579*	0.292	0.508*	-0.719**	-0.723**	0.052	-0.103	0.411	-0.116	0.295	0.237					
FPCA	0.095	0.109	0.198	0.083	-0.242	-0.212	-0.223	-0.063	0.774***	-0.314	0.404	-0.113	0.392				
FFA	-0.468	-0.342	0.056	-0.514*	0.267	0.412	0.049	-0.312	0.518*	-0.619**	-0.080	-0.580*	-0.084	0.712**			
DFA	0.268	0.076	0.497*	0.073	-0.233	0.058	-0.166	-0.564*	-0.154	0.229	-0.256	0.040	0.049	-0.112	-0.046		
BTPA	0.597**	0.236	-0.155	0.332	-0.300	-0.457	-0.474*	0.581*	-0.053	0.698**	0.887***	0.980***	0.269	0.061	-0.437*	0.009	
FTPA	0.078	0.014	-0.021	0.119	-0.095	-0.127	-0.389	0.142	0.398	0.044	0.388	0.134	0.012	0.492*	0.272	-0.162	0.227

[a] TW, Test weight (kg/hl); TKW; Thousand kernel weight (g); K4.00, K3.35, K3.15 and K2.36; % kernels passing through >2.36 mm > 4.00 mm; TADD; % kernel removed by a Tangential Abrasive Dehulling Device; BCA; caffeic acid in bran; BPCA, p-coumaric acid in bran; BFA, ferulic acid in bran; BSA; sinapic acid in bran; BTPC, total phenolic content in bran; FTPC; total phenolic content in flour; FPCA, p-coumaric acid in flour, FFA; ferulic acid in flour; DFA, diferulic acids; BTPA, total phenolic acid content in bran; FTPA, total phenolic acids in flour

[b] *$p < 0.05$, *** $p < 0.01$ and ***$p < 0.001$

TABLE 4.3.4b Pearson Correlation Coefficients between Maize Physical and Hardness Characteristics and Phenolic Acids of Bran and Flour Fractions [a,b]

	TW	SB	TKW	TADD	KS	NIT	BTPC	FTPC	BPCA	BFA	BSA	FPCA	FFA	DFA	BTPA
SB	0.217														
TKW	0.010	0.020													
TADD	-0.131	0.504*	-0.227												
KS	0.152	-0.045	-0.691***	0.010											
NIT	0.496	-0.165	-0.049	-0.648***	0.105										
BTPC	-0.269	-0.554*	0.318	-0.717***	-0.391	0.293									
FTPC	0.503*	0.230	0.226	-0.036	0.095	0.068	-0.320								
BPCA	0.579*	-0.131	0.148	-0.135	0.008	0.475	-0.167	0.587*							
BFA	-0.076	-0.672**	0.190	-0.776***	-0.077	0.438	0.881***	-0.266	-0.044						
BSA	-0.079	-0.320	0.344	-0.585*	-0.433	0.190	0.445	0.197	0.100	0.340					
FPCA	0.130	-0.451	0.280	-0.542*	-0.305	0.425	0.625**	0.197	0.392	0.589**	0.266				
FFA	-0.207	-0.035	0.132	-0.498*	-0.239	0.267	0.400	-0.083	-0.057	0.243	0.574*	-0.011			
DFA	0.406	-0.049	0.425	-0.372	-0.285	0.466	0.191	0.268	0.670**	0.259	0.447	0.275	0.211		
BTPA	0.096	-0.159	0.487	-0.508*	-0.454	0.503*	0.616*	-0.031	0.004	0.542*	0.383	0.361	0.279	0.272	
FTPA	-0.085	-0.306	0.277	-0.730***	-0.377	0.474	0.703	0.055	0.196	0.556*	0.620**	0.606*	0.788	0.337	0.444

[a] TW, Test weight (kg/hl); SB, % breakage susceptibility by Stein breakage tester; TKW; Thousand kernel weight (g); TADD; % kernel removed by a Tangential Abrasive Dehulling Device; KS; % kernel size ≥ 8 mm; NIT, NIT Milling Index; BTPC, total phenolic content in bran; FTPC; total phenolic content in flour; BPCA, p-coumaric acid in bran; BFA, Ferulic acid in bran; BSA; sinapic acid in bran, FPCA, coumaric acid in flour, FFA; ferulic acid in flour; DFA, diferulic acids; BTPA, total phenolic acid content in bran; FTPA, total phenolic acids in flour

[b] *$p < 0.05$, *** $p < 0.01$ and ***$p < 0.001$

These findings are not surprising, as there have been indications that phenolic acids, in particular FA, could be related to maize grain hardness. Del Pozo-Insfran et al (2006) compared FA of white and two blue maize genotypes varying in flour texture. The relatively harder white maize genotype had higher FA content (2480 mg/kg) than blue maize genotypes which contained 202 mg/kg and 927 mg/kg. This present investigation further supports the role phenolic content and phenolic acid type, mainly FA and other hydroxycinnamic acids in maize hardness. At the biochemical level, this finding will contribute to understanding the basis of maize hardness, which remains unresolved to date. Moreover, it shows that phenolic acid content and type could be used to distinguish between soft and hard maize cultivars with small variations in hardness as is the case with the cultivars in this study. Despite differences in DFA content between hard and soft cultivars, the compounds did not significantly influence sorghum and maize hardness in contrast to García-Lara et al (2004) who found the opposite probably as a result of the longer extraction period. These authors found that diferulic acids 5,5′-DiFA, 8-*O*-4′-DiFA, 8,5′-DiFA and total DiFAs extracted from maize were significantly correlated ($p < 0.001$) with whole grain hardness.

4.3.4 CONCLUSIONS

This study is the first to show a relationship between phenolic acid content and sorghum and maize grain hardness. Sorghum and maize bran of harder grains have higher phenolic acid content than those of soft types. Maize phenolic acids seem to have greater effect on grain hardness than those of sorghum. Phenolic acid content could be useful as an indicator of hardness to distinguish between hard and soft types of these two species of cereals. The study indicates the important role of FA in sorghum and maize grain hardness and its position as the most predominant phenolic acid.

4.3.5 LITERATURE CITED

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5 GENERAL DISCUSSION

This discussion will first critique the methods used in this present study and suggest ways of improvement. The second section will integrate the research findings to determine the factors affecting sorghum and maize grain hardness and also makes recommendations for further research.

5.1 METHODOLOGIES

In the research described in Chapter 4.1 sorghum and maize commercial cultivars were obtained from major growing regions in South Africa and were representative of commercial cultivars grown in South Africa. The 17 sorghum and 35 white maize cultivars were obtained from a single harvest in 2009 grown in six and four localities, respectively. The sample collection was large enough to give comprehensive data. However, it would have been ideal to have acquired samples from the following growing season to determine cultivar and environmental effects including temperature and rainfall data, soil type and treatments such as fertilizer application. This was not done because the objective was mainly to determine the relationships between hardness methods for sorghum and maize grain quality. Notwithstanding this, both cultivar and location were found to have an effect on sorghum and maize hardness. In Chapter 4.2, while sorghum cultivars were grown and harvested from the same location maize cultivars were grown in diverse environments and randomly selected for hardness. This is a weakness in the study because ideally both sorghum and maize should have been grown in the same locality to make the data comparable.

Some of the methods applied to maize could not be used for sorghum such as stress cracking. This was because of the opacity of sorghum due to pigmentation. NIT hardness was only determined for maize as the grain analyser was calibrated for whole grain maize. It could be a good idea to develop a useful calibration for sorghum but this might have limitations since sorghum is pigmented and might not transmit light as much as maize.

A few cultivars of both sorghum (n = 4) and maize (n = 6) were evaluated in Chapter 4.2 to determine the effect of grain hardness on pasting properties. Sorghum cultivars used varied in hardness but also comprised of the three sorghum types (red, non-tannin, white tan plant, non-tannin and red condensed tannin). Since only four sorghum cultivars were used, these were too few to group the results of each of the sorghum types and report them separately as in Chapter 4.1. Instead of cultivar being the only variable, sorghum type presumably affected the pasting of these cultivars. Hence, for the comparison of hardness and pasting properties of the sorghum cultivars, it was difficult to determine if the results obtained were because of an effect of cultivar or sorghum type or both. This was a weakness in experimental design. The maize cultivars were selected because of the wide variation in their hardness properties and were all of the dent type. Thus, the pasting behaviour was distinct between the hard and soft maize cultivars in contrast to sorghum.

After the comprehensive study of the assessment of grain hardness of sorghum and maize commercial cultivars, eight cultivars of each of the grains were selected for phenolic acid content analysis and profiling (Chapter 4.3). Cultivars including soft and hard sorghum and maize cultivars were selected on the basis of TADD hardness (percentage of kernel removed). TADD hardness was used as selection criteria because of its appropriateness to determine hardness of both sorghum and maize as found in Chapter 4.1. Sorghum and maize cultivars analysed for phenolic content were from a single location so as to eliminate environmental effects.

Sorghum and maize test weight was measured by passing the grain through a hopper and into a quart size cup (946.35 ml) of the test weight apparatus. Although test weight is a rapid and simple procedure, it is affected by grain packing, moisture content, broken kernels and foreign material (Rooney 2007). The grain had to be leveled off in the cup and this had to be consistent for repeatability. To maintain consistency, one person did the work. Test weight requires a relatively large sample to fill the quart cup for three independent tests. The large sample size used for test weight has an advantage in that the sample is more representative. Moreover, the test is non-destructive and the sample is retained. In this study at least 1 kg of grain was adequate for a single test. Other density tests requiring small sample portions such

as true density measured with a gas pycnometer and a floatation test can be done. The measure of density with a gas pycnometer requires grain to fill a volume of 50 cm^3 (Pomeranz et al 1984), which is approximately 80 g.

The TADD was used for both sorghum and maize hardness determination and decortication. Decortication was done for 5 min according to Gomez et al (1997). A fixed decortication time is a means of controlling the process. However, the carborundum disk, which is the abrasive material, is subject to wear with time, a limitation to the use of the apparatus. In this study, the efficiency of the TADD was monitored by decorticating a standard sample of known hardness (percentage kernel removed). The TADD results were presumed acceptable when the standard sample did not deviate by ±2% kernel removed. TADD hardness was reported as percentage kernel removed. Alternatively, the abrasive hardness index (AHI) can be used which is defined as the time in seconds required to remove 1% of the grain (Reichert et al 1982). AHI could have been more applicable as it gives the rate of grain loss with time. However, expressing TADD hardness as percentage kernel removed was the feasible option because of the large sample size (sorghum n = 306, maize n = 420, including repeats). Using AHI would have meant decorticating and weighing each sample after every min for 5 min and would have been laborious and time consuming.

Similar decortication conditions in terms of time and grain weight were used for both sorghum and maize. Although sorghum and maize kernels are similar in their structure and chemical composition (reviewed by Chandrashekar and Mazhar, 1999) they differ in size. The sorghum kernel is considerably smaller than maize. In the eight sample cup holder TADD used in the study, it can be assumed that sorghum had a larger surface area and greater exposure to the abrader than maize. Reichert et al (1982) recommended a larger sample size and a five cup (7.3 cm diameter) decorticating headplate for large kernels, presumably suitable for maize. Since the objective was to compare the relationship between hardness methods applied to each of the grains, the TADD in this case was useful for decorticating sorghum and maize.

Maize grain for NIT Milling Index was thoroughly cleaned to remove foreign material and broken kernels, which would otherwise absorb light differently from sound, whole kernels.

NIT sample preparation has the greatest influence on the accuracy and precision of analytical results (Williams 1979). In this study a highly significant inverse correlation between NIT and TADD percentage kernel removal) was obtained ($r = 0.659$, $p < 0.001$) (Chapter 4.1, Table 4.1.20) with R^2 (the coefficient of determination) accounting for 43% of the variation between the parameters in the large data set. Such a correlation is relatively weak. One of the reasons was that the maize samples used were closely related in their hardness properties. South African commercial maize cultivars are selected on the basis of hardness thereby eliminating the soft and intermediate cultivars and creating a narrow range of hardness. Thus, the NIT calibration can be improved if maize cultivars differing widely in hardness are used.

Maize breakage susceptibility was tested using a Stein Breakage Tester (SBT). According to the Watson and Herum (1986) the SBT works as both an impact and abrasive device of which most of kernel breakage is caused by abrasion against the cup wall and other kernels. The abrasive and impact breakage action of the SBT compares to that of maize roller milling. During maize milling, bran is generally removed using a degerminator to obtain grits and the highest grit yield is desirable (Taylor and Duodu 2009). The SBT is no longer manufactured. However, other mills can be used for example the Stenvert Hardness Tester (SHT) described by Pomeranz et al (1985). The SHT measures the time required to obtain a specific volume of ground grain. A 20 g sample is ground through a grooved grinding chamber and the time required to collect 17 ml of whole ground meal into a column is recorded. The time to grind a sample is presumably an index of resistance to grinding and the column height of the ground sample is an index of packing. Soft grain particles occupy more space than those of corneous endosperm from the hard grain. However, SBT was more relevant for this study because of its mode of action described above.

Stress cracking tests were done on maize samples by first determining the percentage of stress cracked kernels (SC) and then quantifying the stress cracks as an index (SCI). Fifty kernels of each of the maize samples were evaluated for the presence of stress cracking under a light box and the procedure was repeated three times. The individual assessment of the kernels was time consuming, especially when the stress cracks were counted to determine the SCI. The test becomes tiring and causes fatigue, hence results may be subjective. Therefore in practice,

stress cracking may be difficult to apply in routine grain quality testing such as in industrial milling where rapid tests are desirable. However, this test remains important where stress cracking is a problem since it can be used to directly measure and quantify stress cracks. Stress cracking also weakens the kernel and makes it susceptible to breakage (Peplinski et al 1989). Another alternative to the problem of fatigue that could be caused by SC would be to use digital image analysis to measure stress cracks similar to work done by Erasmus and Taylor (2004) to measure translucency.

Four sieve sizes were used for sorghum kernel size determination, according to Gomez et al (1997). The weighing of the different fractions was time-consuming and may be a limitation in routine analysis and cultivar evaluation where large sample numbers are handled. A 6.35 mm round hole sieve was used for maize kernel size determination. However, with this sieve more than 99% of the maize kernels passed through making it difficult to determine cultivar variability for kernel size. Therefore, an 8 mm sieve was used for this study as it is widely used by the South African maize industry (Mr C. Wootton, Milling Consultant, Johannesburg, South Africa, personal communication).

Sorghum cultivars varying in hardness (Chapter 4.1) were selected to determine the relationship between sorghum grain hardness and malt modification. Changes in malt hardness and modification in the sorghum cultivars differing in hardness were shown by SEM. With SEM, the sorghum endosperm cell walls of PAN 8625 (soft, high DP) and PAN 8247 (hard, high DP) were different from those of PAN 8648 (intermediate, low DP) (Fig 4.2.12). The endosperm cells of PAN 8625 and PAN 8247 seemed fragmented or shrunken in some areas. However, sorghum endosperm cell walls were shown to remain intact even after prolonged malting periods (Glennie 1984). Thus, the apparent shrinking of cell walls in sorghum malt could have been artifact due to physical damage of the endosperm cell walls, which surrounded empty cells caused by sample preparation. The preparation of SEM specimens in this study involved cryo freezing the grain and malt samples by immersing them in liquid nitrogen and sectioning them using a sharp blade. Since the cell walls were presumably weak due to the loss of supporting material, these are likely to have collapsed or seemingly shrunk during cutting and freezing into the voids left by starch granules. The observed endosperm cell

wall artifacts could have been minimised probably by rapidly freezing the samples in liquid nitrogen and subliming the samples on a cold stage of the SEM (Freeman et al 1991). This technique has been shown to cause minimal alterations to original specimen structure by only dehydrating the surface layer of the sample while the rest of the structure remains unaffected (Freeman et al 1991). Bozzola and Russell (1999) also recommended plunging samples into liquid nitrogen chilled fluids such as propane to rapidly freeze samples.

In the research described in Chapter 4.3 phenolic acids were extracted with 2 M NaOH for 2 h at room temperature. Alkaline hydrolysis breaks ester bonds between ferulic acid and arabinoxylans (Mujica et al 2009). This step was followed by acidification with HCl, which released Na^+ from Na ferulate. Alkaline concentrations greater than 2 M and longer extraction periods resulted in poorly resolved and asymmetric peaks with the HPLC method used in this study. Long extraction periods result in oxidation and dimerisation of phenolic compounds (Rubino et al 1996; Charlton and Lee 1997). In this study, oxidation was minimised by flushing samples with nitrogen

A higher alkali concentration (accompanied by refluxing the sample with 4 M NaOH at 170°C) is required for the release of etherified phenolic acids (Morrison and Mulder, 1994). This is because ether bonds are heat labile at 170°C (Lam et al 1992a). However, these conditions could not be employed in this study. Such a high temperature can be obtained safely by using techniques such as microwave assisted extraction. Studies show that the microwave assisted extraction technique is superior in terms of rapid heating, low solvent consumption and higher yield of phenolic acids (Beejmohun et al 2007). The results in this study presumably underestimated the amount of bound phenolic acids, particularly diferulic acids since the extraction conditions were mild.

Monomeric phenolic compounds were easily identified by comparing their retention times (t_R) with those of external standards and confirmed by their mass spectra. Diferulic acids could not be readily quantified. The limitation with diferulic acid quantification is that there are currently no commercially available standards. Hence, ferulic acid was used for their quantification. The identity of the diferulic acids was confirmed with MS/MS *m/z* and their

fragmentation patterns. The assignments were in agreement with MS/MS data and fragmentation patterns reported in literature (Bily et al 2004; Callipo et al 2010; Qiu et al 2010).

5.2 RESEARCH FINDINGS

TADD decortication and TW were correlated in both sorghum and maize. The correlation between these two tests indicates that they measure a similar property of the grain and their relationship can be explained in terms of the grain physical and chemical structures and the mechanisms of action of these methods. The relative proportions of corneous to floury endosperm affect sorghum (Reichert et al 1986) and maize grain hardness (Li et al 1996). The corneous endosperm grain has a compact structure of tightly adhered starch granules with protein bodies, embedded in a protein matrix (Rooney and Serna-Saldivar 2003). The mechanism of action of the TADD involves shearing of the grain to successively remove its outer layers. Thus, grain with a high proportion of corneous endosperm would be resistant to shear, due to the strong starch-protein interactions. Similarly, TW is a measure of the packing of the endosperm and high TW is associated with relatively high proportions of the corneous to floury endosperm (Rooney 2007). Therefore both TADD and TW measure grain hardness as it relates to endosperm structure.

Ferulic acid occurred in higher amounts in sorghum and in maize grain than other phenolic acids (Chapter 4.3). Ferulic acid cross links with arabinoxylans in the pericarp (Ralph et al 1994a) and endosperm cell walls (Glennie 1984). Thus, the assumption is that bran ferulic acid is involved in sorghum and maize hardness by cross-linking between arabinoxylan chains. In the endosperm, the cross-links would be fewer since the endosperm contains less phenolic acids than the pericarp (Bily et al 2004). During TADD decortication, ferulic acid linkages with arabinoxylans would result in greater resistance to shear since they hold the pericarp and endosperm cells together. A higher resistance of grain to shear i.e. hard grain would result in a slower rate of decortication. Test weight may be influenced by phenolic acids through their interactions with starch and proteins affecting endosperm packing. Grains with high ferulic acid would be expected to have high TW because of the increased compactness in the

corneous endosperm, resulting in higher density. Therefore the relationship between TADD hardness and TW can be further explained by role of phenolic acids in cross linking with grain cell wall components, which presumably strengthens the adhesion of grain components.

During decortication, there are two modes of action of the TADD on sorghum and maize grain, first on the pericarp and then the endosperm. TADD decortication is likely effected by breakage of the cell walls of the pericarp. In the endosperm, TADD decortication will break the cell walls and the adhering protein matrix. Fig 5.1 illustrates the action of the TADD on the pericarp of hard (Fig 5.1a-b) and soft (Fig 5.1c-d) grains. The reinforcements caused by ferulic acid cross-linkages prevent the cell walls from breaking easily. Although the purpose of decortication is to remove the pericarp and maintain an intact endosperm, variations in grain hardness affect decortication efficiency such that the endosperm gets incorporated with the decorticate (Shepherd 1982), hence the model shows potential effects of the TADD on both the pericarp and endosperm. The corneous endosperm is also subject to shearing by breaking cell walls and then the protein matrix, although to a lesser extent in compact corneous endosperm with more ferulic acid-arabinoxylan linkages (Fig 5.1e). Glennie (1984) suggested that protein in sorghum adhered to endosperm cell walls and was similar to the protein matrix. Parker et al (1999) found that in sorghum and maize protein there was matrix lining the cell walls, which may suggest that there could be ferulic acid linkages at the edge of the protein matrix and the cell walls. In the present study the assumption is that the protein matrix is linked to the cell walls through diferulic acid linkages (Fig 5.1e).

This study showed that the duration of sorghum germination affects sorghum grain hardness; hardness is dramatically reduced with germination time (Chapter 4.2). The results showed that all the measured hardness parameters including, pycnometer density, floaters, TADD hardness, TKW, SKCS-HI, reduced drastically after Day 2 of malting. The TADD hardness (percentage kernel removed) results were different from those of SKCS-HI. The reason for this could be that TADD decortication was inefficient since the grain had lost its rigid form as a result of endosperm modification (Osborne et al 2005). Grain rigidity loss was as a result of the softening of the grain outer layers during steeping, reduced dry matter content (malting loss), and endosperm cell collapse (Osborne et al 2005). Thus, the soft malt endosperm is expected to crush and be lost mainly as fines. The SKCS-HI gave different results since it

selectively picks individual kernels and records the response to crushing of each grain passing through (Osborne and Anderssen 2003).

This study also indicated that there was interplay of two factors that affect both sorghum malt hardness and endosperm modification. These are intrinsic grain hardness and amount of amylase activity (DP) in the malt. It appeared that starch granule modification by amylase had a greater effect than the intrinsic hardness of the grain. Logically grain with low amylase activity should remain harder and if the amount of amylase activity is high, the amount of undegraded starch granule will be low. Since there are apparently two factors that seem to affect sorghum malt hardness, there is a need to balance between the original (or intrinsic) hardness of grain and the rate of modification for optimal milling yield of sorghum malt.

Sorghum malt endosperm cell walls persisted during the five day malting period. This has been attributed to the highly substituted nature of glucuronoarabinoxylan hindering cell wall degrading enzymes (Verbruggen et al 1998). It is also likely that ferulic and diferulic acid cross-linkages with glucuronoarabinoxylan chains contributed to covalent bonding of the chains together. Thus, the substituted glucuronoarabinoxylan chains and the cross-linkages with ferulic acid could have hindered enzyme activity of the xylanases, arabinofuranosidases and glucuronidases among other enzymes that break down the xylan backbone and the other side units of the glucuronoarabinoxylan chain (Verbruggen et al 1998). The interaction of ferulic acid with endosperm cell walls could play a role in maintaining the integrity of the sorghum cell walls during malting ultimately contributing somewhat to malt hardness. The endosperm cell walls can also be beneficial by maintaining the integrity of the grain during sprouting facilitating the flow of nutrients from the endosperm to the germ. Perhaps this is parallel with the structural support provided by the barley husk to the grain during sprouting.

Hard grain	**Soft grain**
a. Pericarp	c. Pericarp
b. Pericarp-decorticarted	d. Pericarp-decorticated
e. Corneous endosperm	

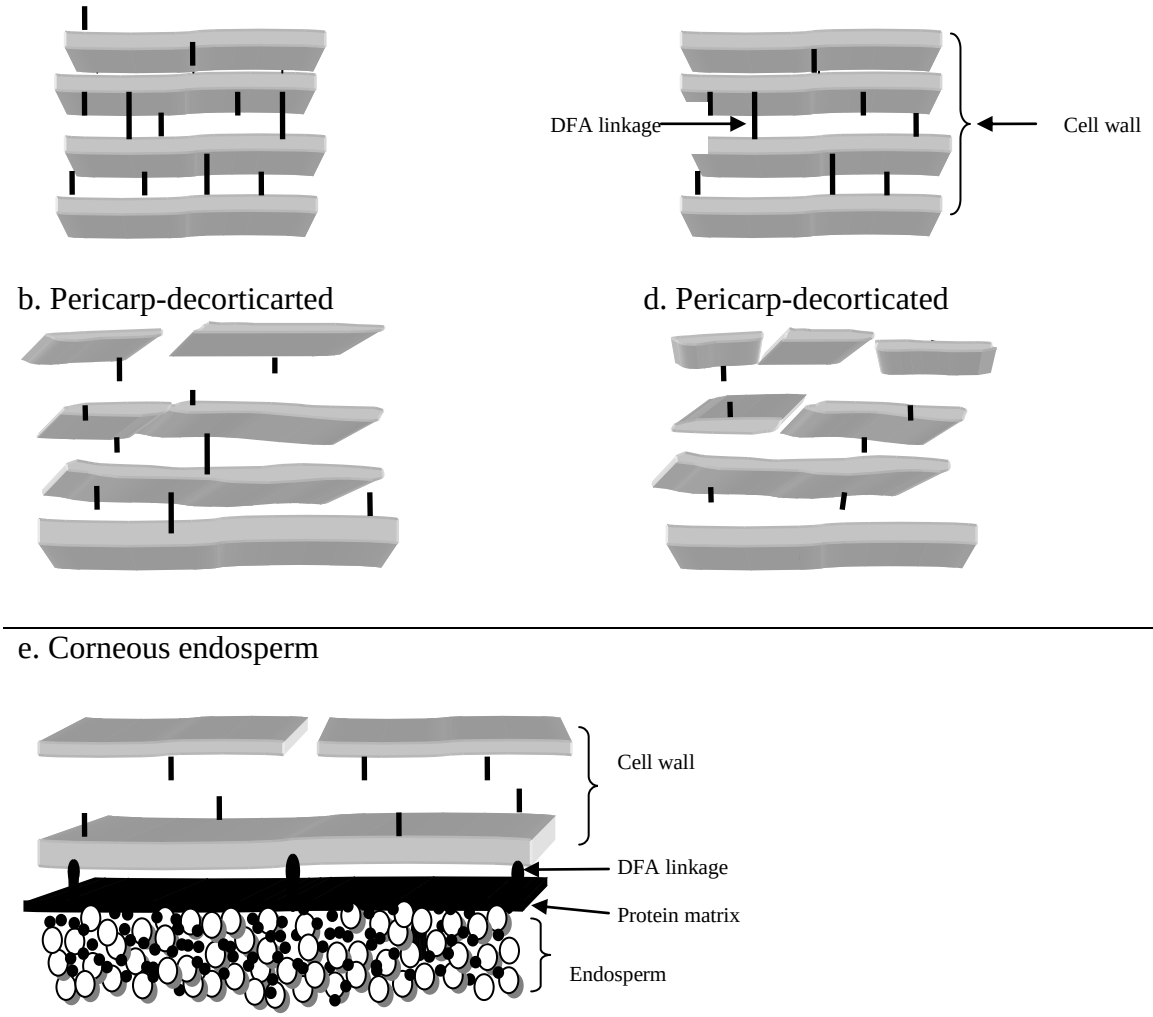

Fig 5.1a-e. Models illustrations of the shearing of pericarp and endosperm layers, and breakage of ferulic acid (FA) and diferulic acid (DFA) linkages during TADD decortication of hard and soft sorghum and maize grains. Black and white circles represent protein bodies and starch granules, respectively.

The findings of this research indicate that among the phenolic acids, ferulic acid in bran could be the most influential in the hardness of sorghum and maize. Therefore, the discussion in relation to phenolic acid content with grain hardness will be mostly with reference to ferulic acid. Bily et al (2004) showed that in rice, wheat, sorghum and maize, the pericarp had the highest levels of ferulic acid, followed by the embryo and lastly the starchy endosperm.

Fulcher (1982) using fluorescence microscopy found that ferulic acid in wheat grain was mostly distributed in the aleurone layer with lower quantities in the germ, embryo and small amounts in the starchy endosperm. Diferulic acids in wheat have been found in high concentrations in the outer pericarp (Parker et al 2005). Similar results regarding the location of ferulic acid in maize were also reported by (Sen et al 1994). This study supports these previous findings since both sorghum and maize bran had higher quantities of ferulic acid than the flour.

Ferulic acid occurs esterified to glucuronoarabinoxylans. The glucuronoarabinoxylan complex is composed of a β-(1-4)-D-xylan backbone with single α-L-arabinosyl and α-D-glucuronosyl residues linked to the *O*-3 and *O*-2 of some xylosyl residues (reviewed by Harris and Trethewey 2010). The glucuronic acid occurs in small quantities and the glucoronoarabinoxylan is largely composed of xylan and arabinosyl units. Esterification with ferulic acid occurs through its COOH group with the *O*-5 OH group of some of the arabinosyl units. Thus in the cell walls of the aleurone layer of sorghum and maize, ferulic acid is directly esterified to arabinoxylan chains through the chemical process explained above. The esterified ferulic acid residues then form ester linkages with each other through oxidative coupling reactions producing ferulic acid dimers and cross linked arabinoxylan chains. Iiyama et al (1994) postulated that the dimerisation of esterified ferulic acid residues could occur enzymatically, catalysed by peroxidases when the residues occasionally come together as the arabinoxylan chains move within the gel-like primary cell walls.

Fig 5.2 illustrates possible mechanisms of ferulic acid linkages in the pericarp and corneous endosperm cell walls of sorghum and maize. Esterification of ferulic acid is expected to occur in three ways; through direct esterification of ferulic acid with arabinoxylan chains (Fig 5.2i), esterification of the ferulic acid residues from different arabinoxylan chains to form diferulic acid bridges (Fig 5.2ii) and diferulic acid bridges between ferulic acid residues on the same arabinoxylan chain (Fig 5.2iii). In the pericarp cell walls, ferulic acid is expected to form linkages with both lignin and arabinoxylan chain, resulting in ether and ester linkages, respectively (Fig 5.3). The linkages would form simultaneously (Lam et al 1992a). Para-coumaric acid is also expected to form ester bonds with arabinoxylan units in the aleurone

layer in much the same way as ferulic acid although to a lesser extent as it occurs in smaller quantities (Lam et al 1992b). In the pericarp, *p*-coumaric acid is esterified to lignin more extensively than its esterification with arabinoxylans (Lam et al 1992a; Ralph et al 1994b; Sun et al 2002). Triferulic acids have also been reported in the pericarp of maize (Bunzel et al 2003) and wheat (Hemery et al 2009). In wheat, the triferulic acids were more concentrated in the pericarp than the aleurone layer (Hemery et al 2009). Although triferulic acids were not detected in this study, it can be infered that most of the ferulic acid and its oligomers are located in the pericarp cell walls and can possibly enhance grain hardness through their interaction with arabinoxylan chains and lignin. Thus, the proposed model between ferulic acid and *p*-coumaric acid with bran components is expected to influence mechanical properties of grain related to hardness.

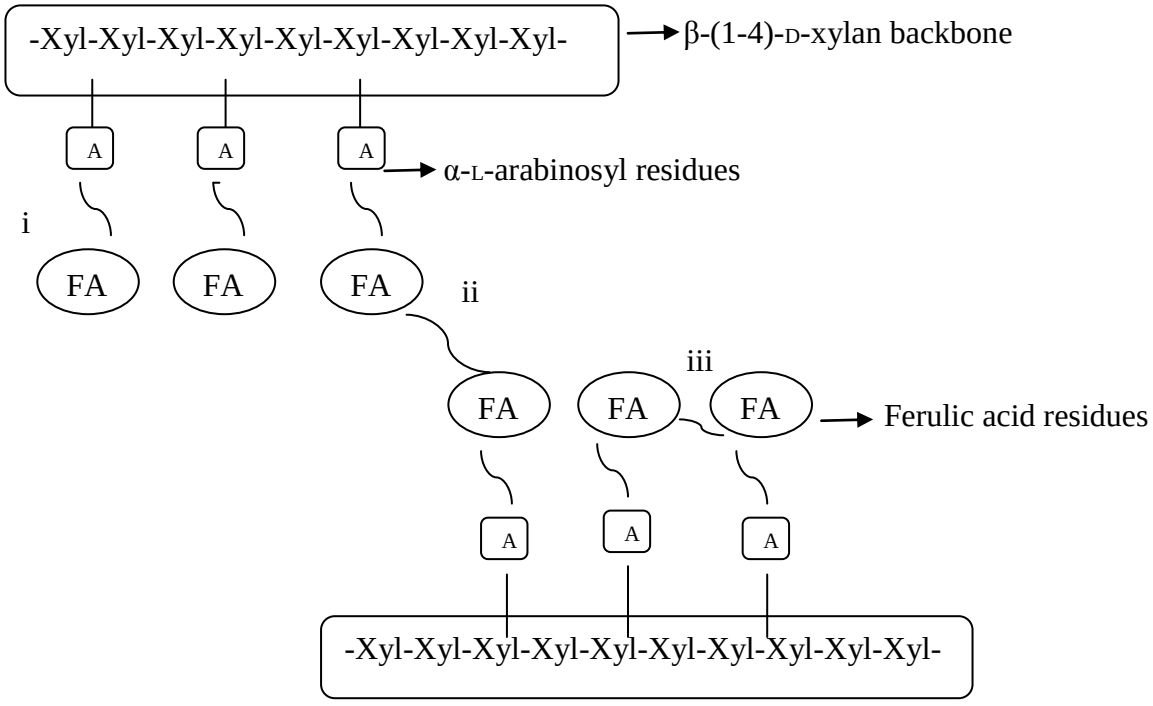

Fig 5.2. Illustration of the possible ferulic acid linkages with the arabinoxylan chains in the aleurone layer and endosperm cell walls; i. Esterification between ferulic acid and arabinoxylan; ii. Diferulic acid diester linkage between arabinoxylan chains; iii. Diferulic acid diester linkage between two ferulic acid residues on the same arabinoxylan chain.

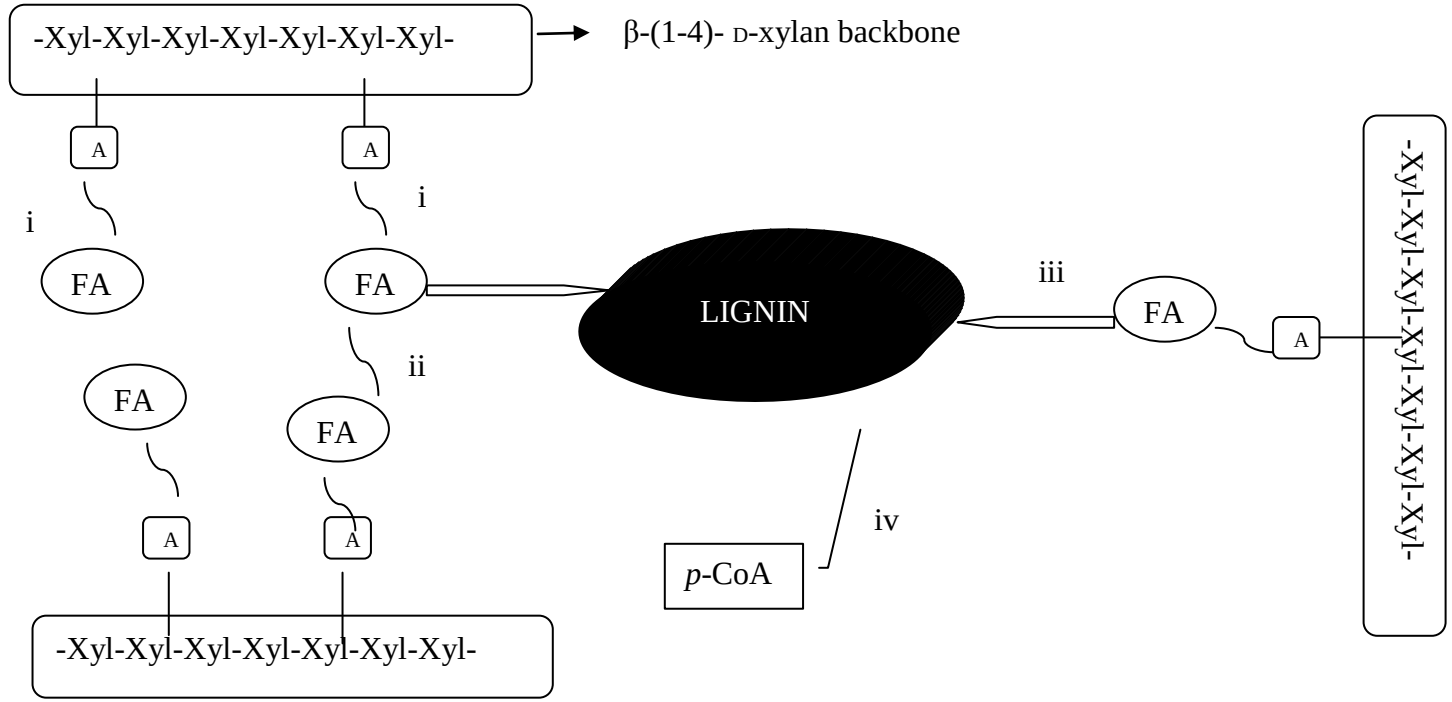

Fig 5.3. Illustration of the possible mechanisms of ferulic acid (FA) and p-coumaric acid (p-CoA) linkages with arabinoxylan chains and lignin in the pericarp cell walls; i. FA-arabinoxylan ester linkage; ii. Diferulic acid diester-ether linkage between ferulic acid residues, arabinoxylan chains and lignin; iii. Ester-ether linkage between FA, lignin and arabinoxylan chain; iv. Ester or ether linkage between lignin and p-coumaric acid.

To understand the influence of bran ferulic acid on grain hardness, ferulic acid interactions with grain outer layers and its effects on grain physical properties were evaluated. Most reports are on development of phenolics in insect and disease resistance. Serratos et al (1987) found that in maize, cultivars with a high fluorescence intensity associated with ferulic acid, were more resistant to the maize weevil *Sitophilus zeamais* than those with lower intensity. The same maize cultivars with a high fluorescence intensity and weevil resistance were also the hardest, as measured with an Instron instrument in compression mode. Although the authors acknowledged the role of the phenolic acids as chemical deterrents for the weevils, they suggested investigating the contribution of phenolics to grain hardness considering the structural organisation (esterification) of ferulic acid in the pericarp and aleurone layers.

McKeehen et al (1999) suggested that cross-linked ferulic acid and the diferulic acids strengthen cell walls to resist insect and fungi invasion. The authors showed that wheat cultivars with high concentrations of ferulic acid during grain development were resistant to *Fusarium* infection. Ferulic acid content increased during wheat grain development as the aleurone, pericarp and testa layers rapidly differentiated. Moreover, in sorghum, hard grains with high levels of phenolics are less susceptible to moulding in wet and humid environments, hence resisting deterioration (Waniska 2000). This is important to obtain high quality food grade sorghum with high milling yields. Although these studies did not address the mechanisms of mould resistance it is evident that hard grain exhibits a competitive advantage over soft grain. From the knowledge of phenolic acid cross linking in the pericarp, the assumption is that the phenolics strengthen cell walls thereby forming a physical barrier against fungal invasion and moisture migration into the endosperm. It is therefore hypothesised that high levels of ferulic acid are involved in the formation a strong network through cross-linking with, arabinoxylans in the endosperm, aleurone and pericarp tissues of mature hard sorghum and maize kernel, hence contributing to greater hardness than in grains with less ferulic acid. During milling, the aleurone and pericarp layers with a high concentration of ferulic acid would resist abrasion. For example, in red winter wheat aleurone cells with high ferulic acid have been shown to be hard and do not mill easily among red winter wheats, as evidenced by their contamination of flour streams (Pussayanawin et al 1988).

The present study showed that sorghum and maize flours had lower contents of phenolic acids than the bran. The reason for this could be that during grain development, most phenolic acids, mainly ferulic acid are produced in high concentrations in the cell walls of the pericarp and aleurone layer to protect the rapidly dividing endosperm from infection (McKeehen et al 1999). However, when the grain matures, the activity of the phenylalanine ammonia-lyase, the enzyme that catalyses the formation of cinnamic acid, a precursor of ferulic acid decreases and finally ceases (McKeehen et al 1999). The assumption is that there will be less phenolic acid production in the endosperm cell walls than in the bran layers, which differentiate rapidly and accumulate large quantities of ferulic acid during early developmental stages when the enzyme is still be highly active (McKeehen et al 1999). This could be the reason the starchy endosperm has the lowest quantities of phenolics compared to the pericarp and aleurone. In the case of condensed tannin sorghum, it can be can inferred that during grain development the plant uses extra energy in phenolic acid production and cross-linking in the testa, which is essentially absent in non-tannin sorghum, as a means of strengthening its defence mechanism. Phenolic cross-linking in condensed-tannin endosperm cell walls may not be a priority for the developing grain once the tannin defence barrier by the outer grain tissues has been formed. This could explain the largely soft, floury endosperm of condensed-tannin sorghum. However, further studies should be undertaken to determine phenolic acid formation in the grain tissues of condensed-tannin sorghum during development and its effect on grain hardness.

Chapter 4.3 showed that in maize flour, phenolic acids (*p*-coumaric and ferulic acids) and the total flour phenolic acids were correlated with TADD hardness (percentage kernel removed). While these flour phenolic acids may seem to contribute to endosperm hardness, there was a possibility of flour contamination by the bran. Contamination is likely because TADD decortication is not a precise method of grain separation and at the 80% extraction rate used in this study, there was no guarantee that bran was effectively removed from the grain. Since grain also varies in hardness some of it can easily crush before complete decortication and either be lost as fines with the bran or remain attached to the kernel and become incorporated with the flour fraction (Shepherd 1982). The effect can either be a dilution of the phenolic acids in the bran if it is contaminated by the endosperm particles or an overestimation of the phenolics in the flour because of bran contamination. The effect of bran contamination in

wheat was shown in different milling fractions by Pussayanawin et al (1988). Flour contamination was greatest at extraction rates above 65% and in this study the extraction rate was 80%, hence contamination could not be ruled out. Flours with more bran contamination had 10-20 times more ferulic acid than low bran contaminated flours. However in this present study, the degree of bran contamination in the sorghum and maize flours was not determined. Ash content could have been a possible way of detecting bran contamination although fluorescence microscopy would have been ideal to quantitatively detect ferulic acid contamination in the flour, as recommended by Pussayanawin et al (1988).

Although the physicochemical properties of sorghum and maize grains are similar, this study indicates that maize phenolics could have a greater influence on hardness. The reason for the differences could be related to kernel size. Large kernels of sorghum were shown to decorticate more efficiently and result in higher milling yields than small kernels (Lee et al 2002). Similarly, this study also showed that large sorghum kernel size is associated with grain hardness. Pussayanawin et al (1988) showed that milling fractions from large wheat kernels were less contaminated with bran and ferulic acid than of small kernels. Small wheat kernels contaminated the flour more implying that larger kernels mill more efficiently. Since maize has a larger kernel than sorghum, hence a high proportion of endosperm to bran, it can be expected to decorticate better than sorghum. Moreover, maize kernels were mostly 8 mm in size while sorghum kernels were distributed over a wide range (2.36 to 4.00 mm). The lack of homogeneity in sorghum kernel size probably adversely affected the efficacy of decortication. Considering the mechanism of TADD decortication where bran and flour particles from broken kernels are collected together as fines, there is a high probability of sorghum bran contamination by flour, hence dilution of phenolics. Moreover, sorghum is unique among cereals because it contains starch granules in its mesocarp, which contribute to pericarp friability (Taylor 2003). Friability eases kernel breakage resulting in problems already discussed. These could be some of the reasons of failing to establish a strong relationship between sorghum grain phenolic acids and hardness. Therefore, to overcome these influences, it would be recommended to separate sorghum grain tissues manually, quantify their phenolics and correlate them with hardness to offset the problem of phenolic dilution, as recommended by Greffeuille et al (2006).

6 CONCLUSIONS AND RECOMMENDATIONS

For routine analysis, there are related methods that can be used to effectively select non-tannin sorghum and maize cultivars on the basis of hardness. TADD, TW, TKW and kernel size > 3.35 mm can be used together to evaluate sorghum hardness. TADD and NIT Milling Index, or TADD and TW are useful for maize. TADD and TW thus seem suitable for evaluating both sorghum and maize for grain hardness. The association between TADD decortication and TW is probably because they both measure a similar property of the grain and their relationship can be explained in terms of the proportion of corneous to floury endosperm. Grains with a high proportion of corneous endosperm would be resistant to shear during TADD decortication due to the strong starch-protein interactions, resulting in increased hardness. Similarly, TW is a measure of the packing of the endosperm and high TW is associated with relatively high proportions of the corneous to floury endosperm. Therefore, TADD and TW seem to measure sorghum and maize grain hardness as it relates to endosperm structure but not all methods are applicable for both sorghum and maize hardness testing.

The study also indicates that malt amylase activity and intrinsic grain hardness (proportion of corneous to floury endosperm) are two factors that predominantly affect the modification of sorghum and hardness changes during malting. Amylase activity seems to have a greater impact on malt hardness. Therefore there has to be a balance between grain hardness and malt quality depending on the intended use of the sorghum malt. However, there was no clear relationship between hardness and porridge texture probably, as a result of the interaction of both amylase activity and hardness although amylase activity seemed to have a greater influence in the later stages of malting as observed with SEM. Ferulic acid cross-links with arabinoxylans and can reinforce the endosperm cell walls, thus resisting degradation by hydrolytic enzymes. Therefore, the interaction of ferulic acid with endosperm cell walls could play a role in maintaining the integrity of the sorghum cell walls during malting ultimately contributing to malt hardness.

The content of ferulic acid in bran fractions of maize can be a useful indicator for distinguishing between hard and soft cultivars. Further, the phenolic acids of maize bran may have a greater effect on grain hardness than those of sorghum. Among the phenolic acids identified and quantified, ferulic acid seems to play a major role in both sorghum and maize grain hardness. Thus high levels of ferulic acid are probably involved in the formation of ha strong network through cross-linking to arabinoxylans in the aleurone and pericarp tissues of mature hard sorghum and maize kernel, hence contributing to greater hardness than in grains with less ferulic acid.

Further studies should be conducted on sorghum cultivars to determine the relationship between phenolic acids and hardness because the correlations obtained were very low and were not conclusive. Some of the recommendations would be to manually separate sorghum tissues and correlate their phenolic acids with grain hardness. Also, breeding for high ferulic acid could reduce yield losses due to increased resistance to abiotic stresses and ultimately improve grain milling quality. The extraction of diferulic acids can also be enhanced by microwave assisted extraction, a rapid heating extraction technique that is capable of releasing etherified phenolic acids.

Some of the hardness tests such as stress crack determination are time consuming and can cause fatigue. Therefore, it is recommended that digital image analysis be used. To determine the effect of sorghum grain hardness on pasting properties, it is recommended that cultivars of the same type be tested. This is because if different types of sorghum cultivars are used (condensed tannin and non-tannin) it is difficult to determine if the pasting data obtained is as a result of cultivar or sorghum type or both. To minimize artifacts in sorghum malt specimens for SEM, rapidly freezing samples in liquid nitrogen and subliming them is recommended.

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8 APPENDIX

Publications and presentations from this work

Chiremba, C., Taylor, J. R. N., Rooney, L. W. and Beta, T 2012. Microwave assisted extraction of bound phenolic acids in bran and flour fractions of sorghum and maize cultivars varying in hardness. J. Agric. Food Chem. 60:4735-4742.

Chiremba, C., Taylor, J. R. N., Rooney, L. W. and Beta, T. 2012. Phenolic acid content of sorghum and maize cultivars varying in hardness. Food Chem. 134:81-88.

Chiremba, C., Rooney, L. W. and Taylor, J .R. N. 2011. Relationships between simple grain quality parameters for the estimation of sorghum and maize hardness in commercial hybrid cultivars. Cereal Chem. 88:570-575.

Conference posters

Chiremba, C., Rooney, L.W., Taylor J.R.N. and Beta, T., 2012. Microwave assisted extraction of bound phenolic acids from sorghum and maize bran. Institute of Food Technologists Annual Meeting and Food Expo. 25-28 June 2012. Las Vegas, USA.

Chiremba, C, Beta, T., Rooney, L. W. and Taylor J. R. N. 2011. Relationships between simple grain quality parameters of sorghum and maize hardness. American Association of Cereal Chemists International Annual Meeting, Palm Springs, CA, USA. 16-19 October 2011.