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.

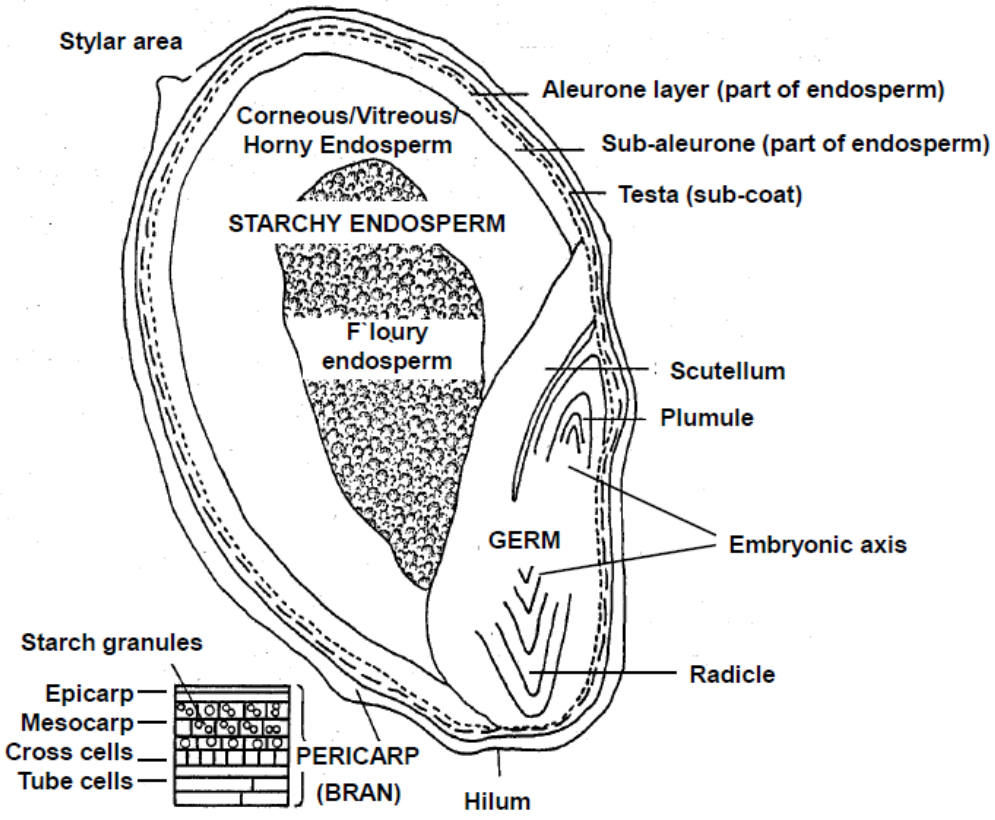


Fig 2.1a. Longitudinal section of a sorghum kernel (Taylor and Belton 2002)

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.

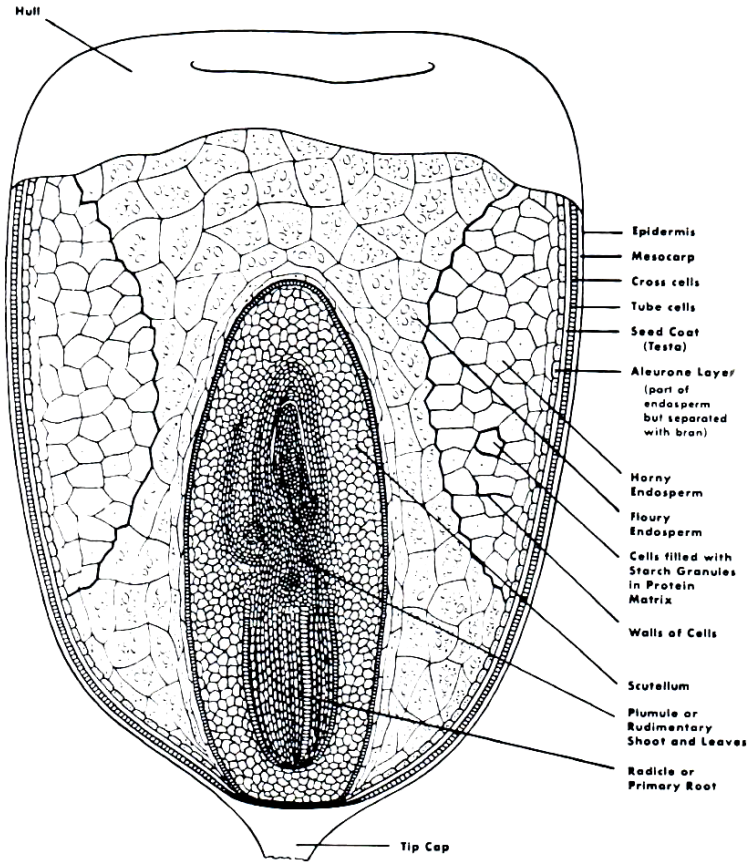


Fig 2.1b. Longitudinal section of a maize kernel (Hoseney, 1994)

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.

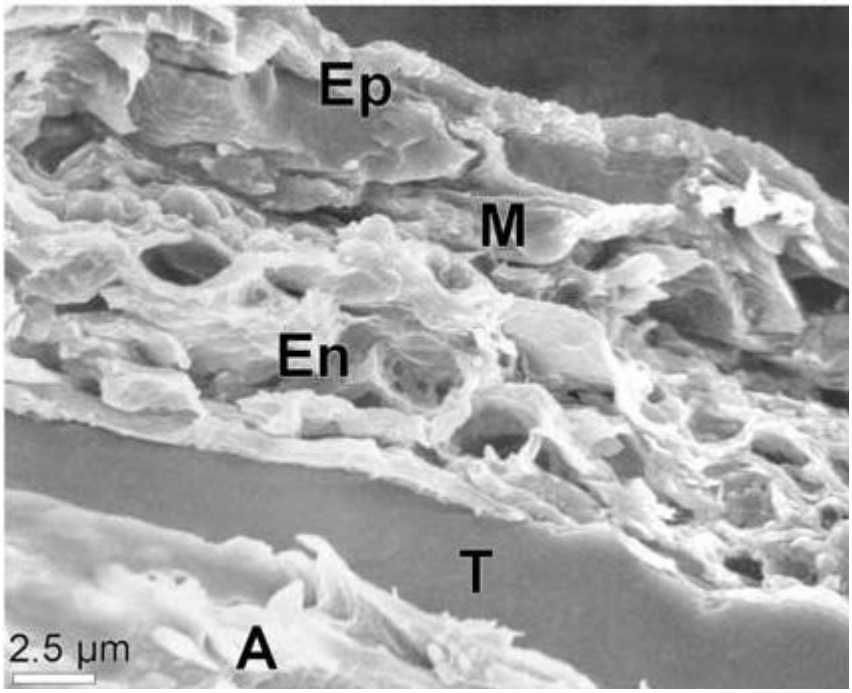


Fig 2.2. Scanning electron micrograph of a tannin sorghum pericarp containing; Ep, epicarp; M, mesocarp; En, endocarp;T, testa layer: A, aleurone layer (Earp et al 2004).

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).

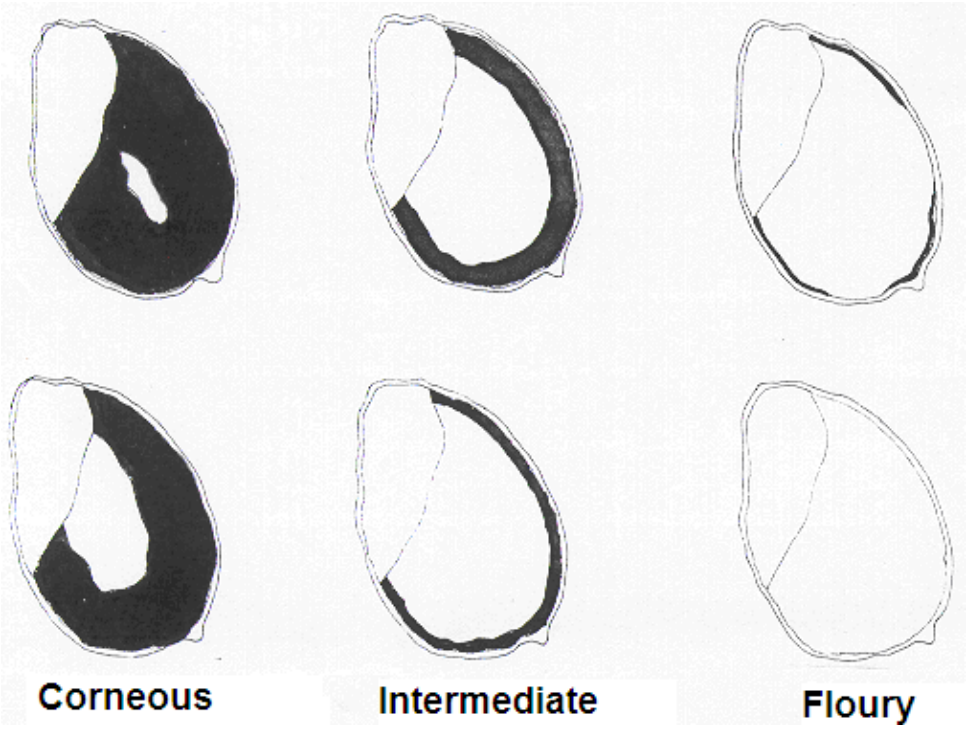


Fig 2.3. A 3-point rating system for evaluating sorghum endosperm texture (ICC 2008).

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).

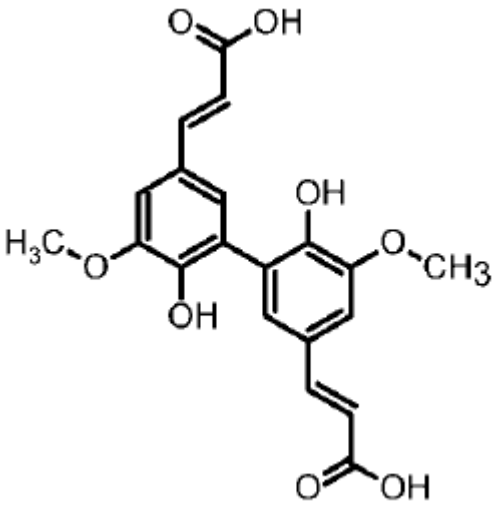
Ferulic acid occurs in highest quantities among phenolic acids in several cereals. Bily et al (2004) found high levels of cell wall bound ferulic acid in whole grain sorghum, maize, rice and wheat. The study also revealed that ferulic acid was distributed unevenly in the different grain fractions. The 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

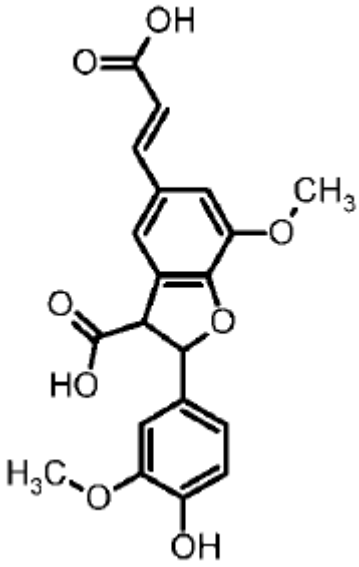
The ratio of corneous to floury endosperm affects grain hardness and the majority of the research has focused on the influence of proteins. However, there is limited fundamental research on the role of phenolic acids on grain hardness.

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.

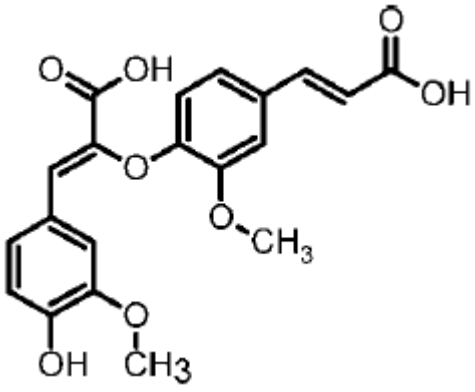
Ferulic acid ester linkages are formed during early maturation to primary cell walls of glucuronoarabinoxylans and later react with lignin quinone methide intermediates to form benzyl ether linkages in lignified cells walls at maturity. The ether linkages presumably further reinforce the cell walls.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



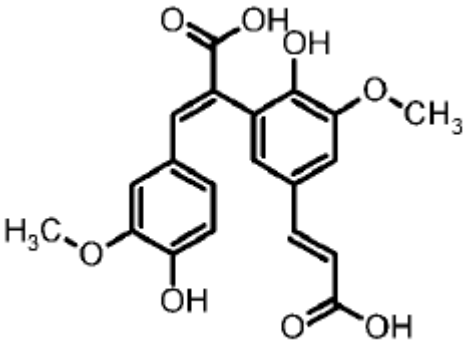
5-5′ DFA



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.

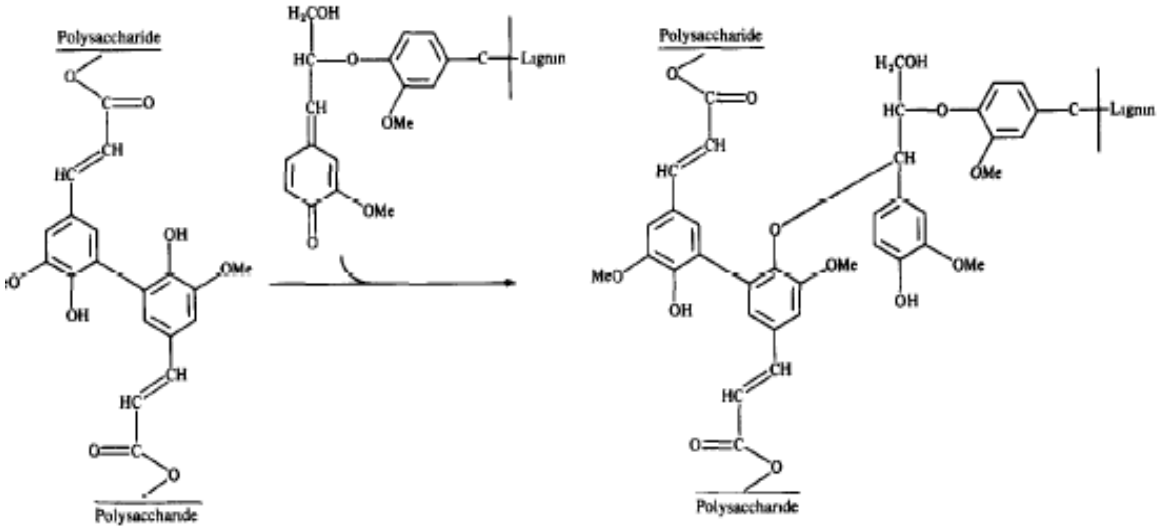


Fig 2.5. Proposed scheme for the formation of ester-ether bridges between polysaccharides and lignin in cell walls (Lam et al 1992b).

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.