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	

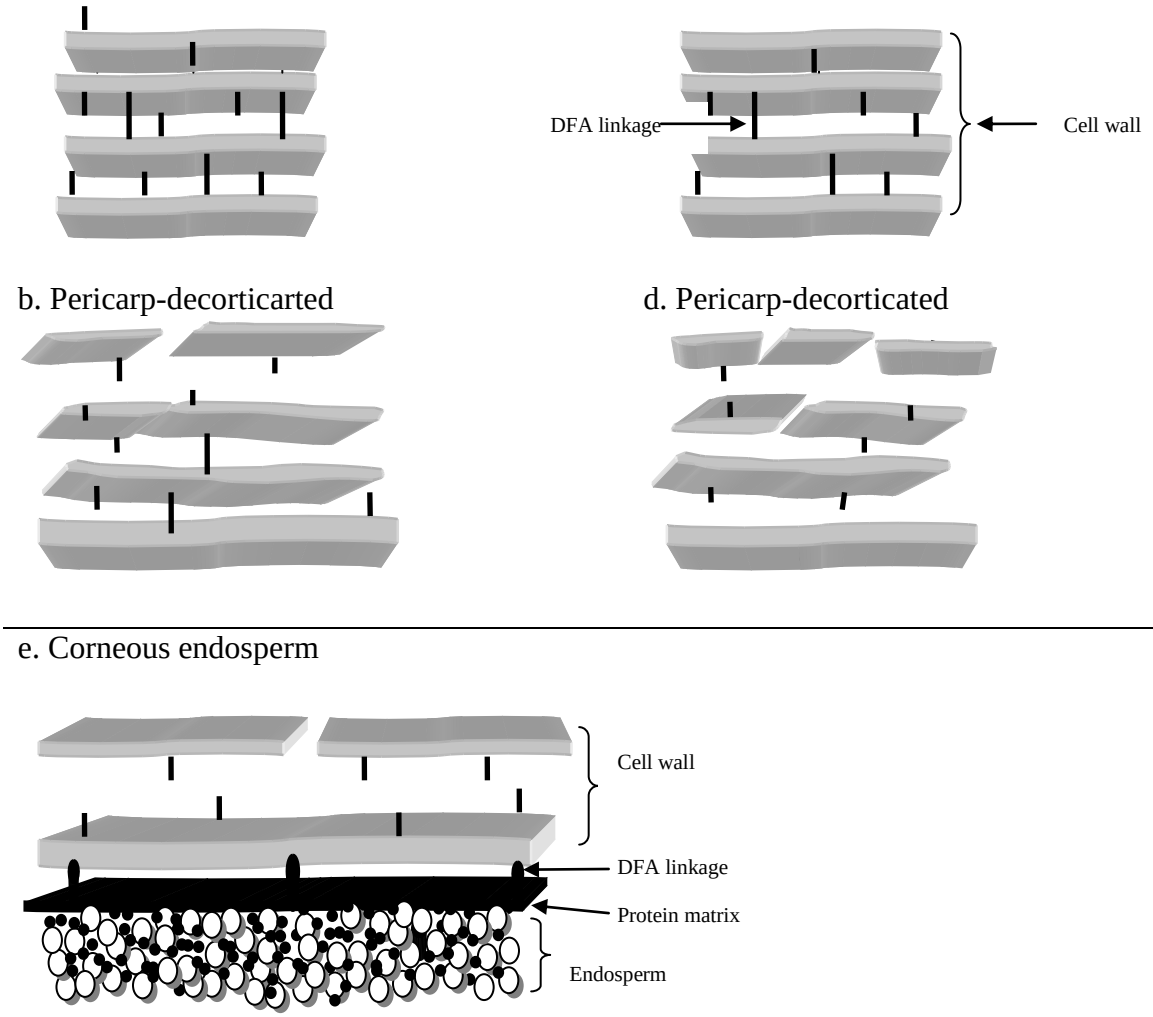

Fig 5.1a-e. Models illustrations of the shearing of pericarp and endosperm layers, and breakage of ferulic acid (FA) and diferulic acid (DFA) linkages during TADD decortication of hard and soft sorghum and maize grains. Black and white circles represent protein bodies and starch granules, respectively.

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.

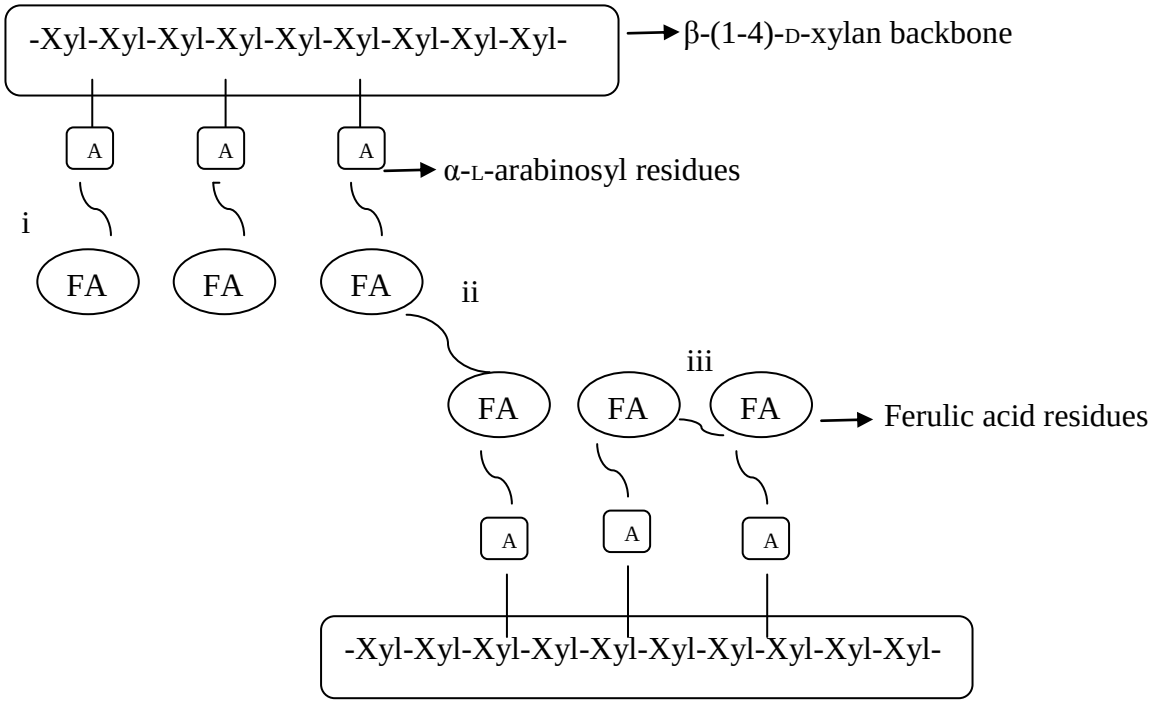

Fig 5.2. Illustration of the possible ferulic acid linkages with the arabinoxylan chains in the aleurone layer and endosperm cell walls; i. Esterification between ferulic acid and arabinoxylan; ii. Diferulic acid diester linkage between arabinoxylan chains; iii. Diferulic acid diester linkage between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

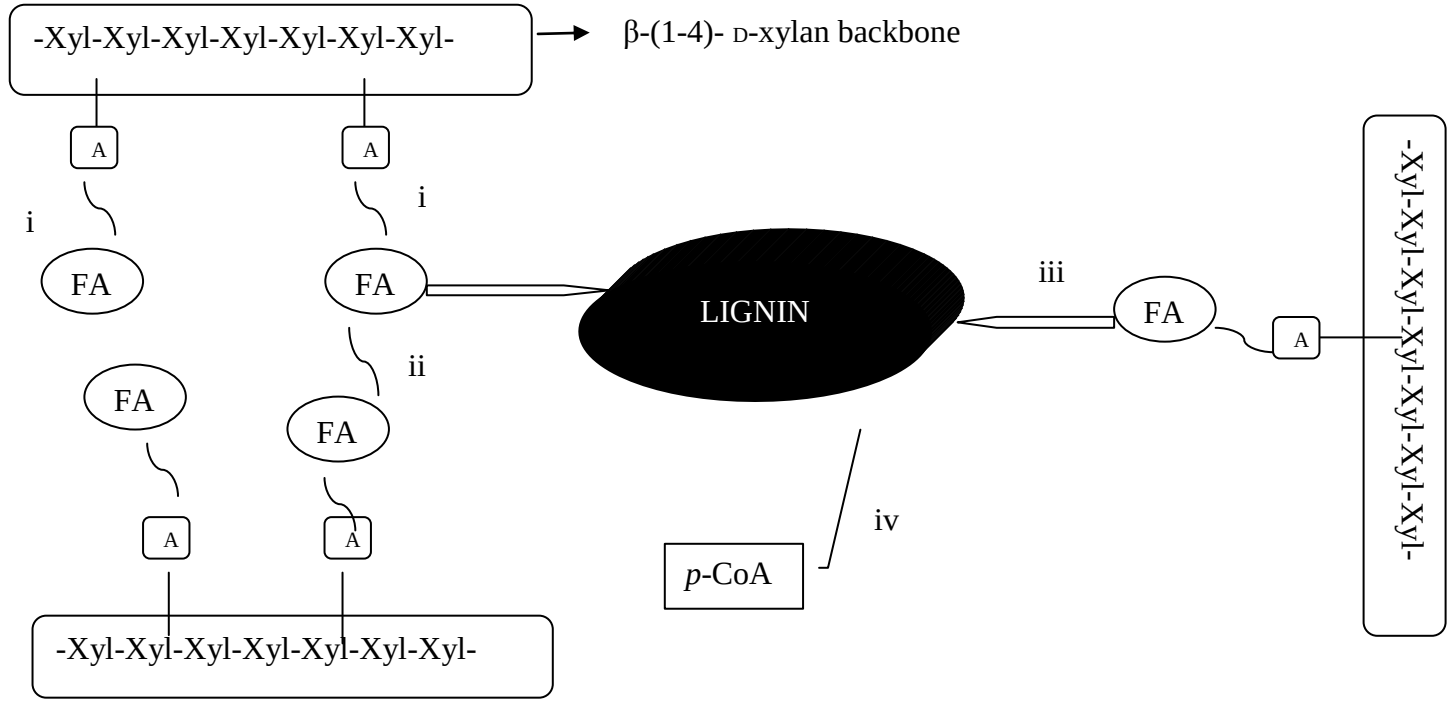

Fig 5.3. Illustration of the possible mechanisms of ferulic acid (FA) and p-coumaric acid (p-CoA) linkages with arabinoxylan chains and lignin in the pericarp cell walls; i. FA-arabinoxylan ester linkage; ii. Diferulic acid diester-ether linkage between ferulic acid residues, arabinoxylan chains and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