3. GENERAL LITERATURE REVIEW

3.1 Sorghum grain morphology

The sorghum caryopsis is composed of three anatomical parts: pericarp (outer layer), germ (embryo) and the endosperm tissue. The pericarp is divided to form the epicarp which is covered by a thin layer of wax, the mesocarp and the endocarp (Earp & Rooney 1982). Rooney and Miller (1982) described the structure of the sorghum kernel and the various roles played by the different cell layers. Each of the layers that make up the pericarp is composed of two or more cell layers. The mesocarp in sorghum is unique in that it contains starch granules. The endocarp consists of cross and tube cells which are responsible for water uptake. The pericarp may have a role in mould susceptibility as varieties with thin mesocarps are generally more resistant to mould infection (Glueck and Rooney 1980). The thickness of the pericarp is also important in traditional and mechanical milling (Scheuring et al 1983). The pericarp is thickest in areas below the stylar and near the hilum. The inner integument (testa or seed coat), derived from ovule integuments, is pigmented only in sorghums with the dominant B_1 and B_2 genes (Rooney and Miller 1982). The testa is thicker around the crown and thinner around the embryo. Phenols and tannins are located mostly in the pericarp and testa layers of the sorghum kernel (Hahn and Rooney 1986) and in some glumes (Doherty et al 1987), with only the bound phenols occurring in the endosperm (Hahn 1984). Maxson & Rooney (1972) divided sorghum grains into three groups based on polyphenol and tannin content: group I, white or cream colour, with <0.40 mg/100 mg 'tannin'; group II, red or brown colour, containing 0.3-1.0 mg/100 mg tannin content; and, group III, grain with testa and tannin content of 2.0-3.25 mg/100 mg. The classification is useful as it relates broadly to the

nutritional value and the presence or absence of the testa. Price and Butler (1977) classified sorghum into types I, II and III based on chemical analyses. Both type II and III sorghums have pigmented testa but the latter contain more tannins due to the presence of the dominant spreader gene and have the greatest bird resistance (Hahn et al 1984). In Zimbabwe, red sorghums belonging to type I and II tend to be mixed, undermining the nutritional value of the condensed tannin-free sorghums.

The endosperm tissue is made up of the aleurone layer, peripheral, corneous (waxy or horny) and floury areas (Rooney & Miller 1982). The corneous endosperm appears translucent with the protein matrix having a continuous interphase with the starch granules. The protein bodies are largely circular and embedded in the matrix (Hoseney et al 1974; Taylor et al 1984), hence imparting dents on the polygonal starch granules. The opaque, floury endosperm located around the centre of the kernel has a discontinuous protein phase and loosely packed, round starch granules. The air voids diffract incoming light giving the kernel a chalky appearance. Doherty et al (1982) indicated a relationship between resistance to moulds and insects and the degree of hardness of the sorghum grain. Waniska et al (1989) concluded that sorghums from open panicles, with thin pericarp, condensed tannins, corneous endosperm, and large tight glumes are generally more resistant to weathering. The germ is made up of the embryonic axis and the scutellum. The former contains the new plant and is divided into a radicle and plumule. The scutellum is the transport tissue and connects the endosperm and the germ. Removal of the germ during milling improves shelf-life but results in nutritionally inferior products (Eggum et al 1982) as the germ contains large amounts of oil, protein, enzymes and

minerals.

3.2 Colour of sorghum grain

Pericarp colour and thickness, presence of pigmented testa, endosperm colour and endosperm texture are the major genetic factors that influence the overall appearance of sorghum kernels (Rooney & Miller 1982). The presence of the *R* gene results in a red pericarp but only in the presence of the dominant *Y* gene. White pericarps result when the genetic composition is *R-yy* or *rryy* while *rr-Y* results in a yellow pericarp (Rooney and Miller 1980). Plant and glume colour also influence kernel appearance especially in weathered sorghums. A pigmented brown or purple testa containing condensed tannins is present if a dominant spreader gene (*S*) appears together with *B* genes (Rooney and Miller 1982). Kernels with a thick, colourless pericarp look chalky white as the thick mesocarp masks the colour of the testa and endosperm (Earp & Rooney 1982). Kernels attacked by insects secrete phenolic compounds giving discoloured spots in the affected areas, which include the endosperm (Rooney & Miller 1982). The colour of sorghum grain cannot be used as a measure of tannin content (Boren & Waniska 1992).

3.3 Chemistry of sorghum phenolic compounds

The phenolic compounds present in sorghum grain can be divided into three basic groups: phenolic acids, flavonoids, and tannins (Hahn et al 1984). Phenolic acids are derivatives of benzoic or cinnamic acids and contain hydroxyl (OH) and methoxy (OCH_3) groups substituted at various places on the benzene ring. The amount of phenolic acids differs among genotypes with white sorghum varieties having the lowest (Waniska et al 1989).

Flavonoids consist of a C6-C3 fragment (A) from cinnamic acid and a C6 fragment (B) from malonyl-CoA (Figure 1). The major groups of flavonoids are flavones, flavonols and flavans. Flavans are the major group of flavonoids in sorghum. Flavonoids have been shown to play a major role in pericarp pigmentation. For example, Nip and Burns (1969) identified two yellow and one orange anthocyanin pigments whose pigmentation is controlled by light, hence the observed colour differences in the pericarp of various sorghum grains. In red pericarp and lemon yellow pericarp, luteophorol (an anthocyanidin) and erodictyol (a chalcone) respectively are the major pigments (Kambal & Bate-Smith 1976).

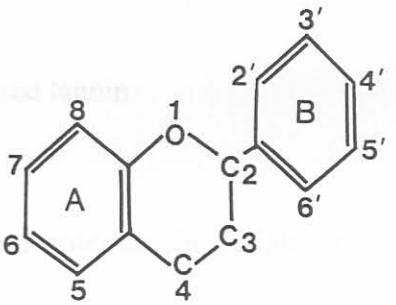

Figure 1. Basic flavonoid ring.

Tannins are divisible into two groups of polyphenols, the condensed tannins, also known as proanthocyanidins, and the hydrolysable tannins (Haslam 1981). They differ in their component subunits and the bonding between these. Only condensed tannins have been found in sorghum (Hahn et al 1984). The condensed tannins (Figure 2) are polymers derived from flavan-3-ols such as catechin and/or flavan-3,4-diols (Haslam 1981). The interflavan bonds are carbon-carbon bonds, C-4 to C-8 linkage is typical but C-4 to C-6 links have also been reported for the molliscacidin derived tannins of *Acacia mearnsii*

(Haslam 1981). Condensed tannins are termed proanthocyanidins as they yield anthocyanidins when treated with acids. The molecular weight of condensed tannins range from 560 Da upwards, given that the monomer flavanol cannot act as a tannin (Mole 1986). Molecular size may be a factor influencing the relative abilities of tannins to precipitate proteins (Oh and Hoff 1979).

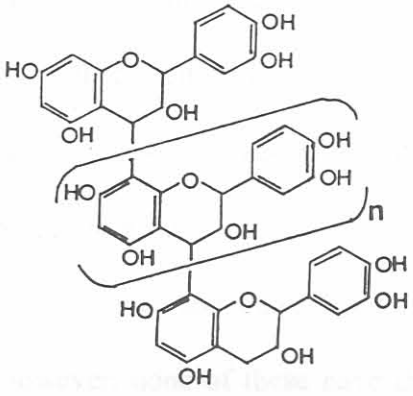

Figure 2. Structure of condensed tannin

Swain (1979) used a three-fold criterion for defining tannins: that (i) they are of high molecular weight, typically 1000 to 3000 Da, and (ii) they have sufficient phenolic hydroxyl groups to complex with proteins and other macromolecules containing –OH and $–NH_2$ groups, by forming hydrogen bonds at normal pH. These hydrogen bonds should (iii) be susceptible to auto- or enzyme-catalysed oxidation to form covalent linkages. Swain (1979) also classified tannins into four sub-types: true tannins (condensed tannins or hydrolysable tannins), prototannins (their precursors; for example catechin), β-tannins (low molecular weight substances which satisfy criteria (ii) and (iii) only) and oxytannins (oxidation products of small molecules which satisfy criteria (i), (ii), and (iii) upon their formation. The monomeric flavanol will hence act as tannin under Swain's scheme. While Swain's definition is biologically useful, it may not be practical to make the three

measurements required to define a substance as tannin. Classically, tannins are thought to bind proteins by the formation of hydrogen bonds between their phenolic hydroxyl groups and the carbonyl oxygens and amido nitrogens of the protein's peptide bonds (Loumis and Battalie 1966; Swain 1979). Mole (1986) maintained the historically important phenomenon of protein precipitation in the definition: tannins are operationally and strictly defined as being able to form precipitates with proteins in aqueous solutions. However, soluble complexes resulting from the interaction of tannins with proteins are also likely to be formed. Tannins are also able to bind to a range of other biochemicals such as starch (Davis and Hoseney 1979), cellulose, pectin, and other plant cell wall components as well as alkaloids (Swain 1965). However, none of these have the same diversity of potential binding sites as in protein (Zucker 1983).

Most of the total phenolics present in crude tannins may be composed of related substances, for example, condensed tannins of different polymer lengths (Oh and Hoff 1979). The problem of defining tannins is thus compounded by the general co-occurrence of several chemically distinct types of tannin in the same plant. Two problems often arise when attempting to relate tannins to their chemical and protein precipitation properties, that of isolating each structurally unique condensed tannin and studying it alone, and that of potential synergistic interaction between the components of crude tannins.

3.4 Qualitative and quantitative analysis of phenolic compounds

Non-quantitative methods of detecting tannin-containing sorghums include the scratch test which involves scratching away the outer layers (pericarp) to reveal the presence or

absence of a pigmented testa layer (Rooney et al 1980; Earp et al 1981), or the bleach test which causes the pericarp to dissolve, revealing the presence or absence of the testa (Rooney et al 1980; Earp et al 1981).

Table 1. Main assays used for the determination of phenolic compounds

Assay type	Determination of		
	Phenols*	HA† & PA+	PA+
Protein and alkaloid precipitation			
-Precipitation from solution (Hagerman and Butler 1978)		X	
-Diffusion in a gel (Hagerman 1987)		X	
-Precipitation on chromatography paper		X	
-Enzyme inhibition (Daiber 1975)		X	
Reaction with phenolic rings			
All phenols			
-Complexation with ferric ions (Mole and Waterman 1987)	X		
-Complexation with titanium ions (Eskin et al 1978)	X		
-Reduction of ferric ions (Prussian blue method) (Price and Butler 1977)	X		
m-Diphenols			
-Reaction with vanillin (Burns 1971; Price et al 1978)			X
Depolymerization			
-Oxidative depolymerization in BuOH/HCl (Swain and Hillis 1959)			X

*Phenols and polyphenols (including non-tannin polymers)
†Hydrolysable tannins
+Proanthocyanidins or condensed tannins

Scalbert (1992) classified the different quantitative assays used for tannin estimation in

plant tissues into three groups according to the type of reaction involved: those based on reaction with phenolic rings, depolymerization and precipitation of proteins or alkaloids. Some of these assays give a reaction with all kinds of tannins and others with a specific class of tannins, for example, the proanthocyanidins (condensed tannins) found in sorghum (Table 1).

Condensed tannins may contain phenolic rings based on phloroglucinol, resorcinol, catechol, pyrogallol or phenol (Scalbert 1992). The reagents used are chosen for their ability to react at a more or less equal stoichiometry with any type of phenolic group, whereas others will react specifically with only some of them, thus allowing the estimation of a particular group of tannins (Scalbert 1992). Several colorimetric methods are used to determine total phenols in plant extracts. They are based on the formation of coloured complexes with metals (ferric or titanium salts), the reduction of ferric to ferrous ions or on the reduction of the phosphotungstic-phosphomolybdic reagent. The coloured complexes have been formed in SDS/triethanolamine (Mole and Waterman 1987) for iron (III) and in concentrated HCl for titanium (Eskin et al 1978). Most of these complexes are unstable probably due to redox reactions (Scalbert 1992). The reduction of Fe^{3+} to Fe^{2+} and subsequent detection of ferrous ions by formation of the hexacyanoferrate (II) chelate (prussian blue) is widely used for the quantitative determination of phenols (Price and Butler 1977). Most of the phenols reduce Fe^{3+} with a yield that depends on structure and pH (Scalbert 1992). The assay is carried out on a ground grain sample, on an extract or on single seeds out in the fields (Price and Butler 1977). Another method is based on the reduction of a phosphotungstic-phosphomolybdic reagent in alkaline medium. The Folin

Ciocalteu method was recommended by Scalbert (1992) for determining absolute concentrations of complex mixtures although the assay is affected by the following: 1) sequence and timing of the addition of the reagents, 2) the linear response is observed at absorbance <0.5, 3) methanol induces precipitation when brought into the reaction medium with phenol sample, and 4) several substances such as ascorbic acid, ferrous ion, sulphur dioxide, cysteine and phenolic amino acids of proteins interfere with the assay.

The reaction between *m*-diphenols and aldehydes, in concentrated acid, has been used for assaying proanthocyanidins quantitatively as their A-rings usually contain phloroglucinol and resorcinol functionality (Scalbert 1992). Vanillin, the most widely used aldehyde, gives a red chromophore with proanthocyanidins (λ_{max}=640 nm) (Swain and Hillis 1959). HCl or H_2SO_4 can be used as catalyst. The vanillin-HCl assay, studied in detail, has been shown to be affected by several factors that include temperature (Dalby and Schuman 1978), HCl concentration (Broadhurst and Jones 1978; Price et al 1978a), vanillin concentration (Price et al 1978a) and reaction kinetics variation with proanthocyanidins or catechin (Price et al 1978a). Exposure to light may also result in a loss of absorbance (Broadhurst and Jones 1978). The vanillin reaction as described by Burns (1971) remains the most widely used assay for sorghum polyphenols. Price et al (1978a) modified the assay to include a reagent blank which corrects for the colour of the grain. The standard, catechin, produces an absorbance which is not linear with concentration when HCl is used as catalyst. Some authors prefer the use of H_2SO_4 as a higher colour intensity is obtained, and (+)-catechin gives a linear response and can be used as a standard (Scalbert et al 1989). The extinction coefficient of catechin is much less than that of tannin and the

time course of the reaction of catechin and tannin are different (Price et al 1978a). Tannin polymers are less reactive than the monomeric catechin (Goldstein and Swain 1963) and this is probably due to the C-substitution on position 6 or 8 of the *m*-diphenol A-rings involved in interflavonoid bonds. The vanillin assay will detect any monomeric or polymeric flavonol but will underestimate the weight of proanthocyanidins in an extract when catechin is used as the standard.

Proanthocyanidins give coloured anthocyanidins with absorbance maxima around 550 nm when treated in hot mineral acid solutions (Swain and Hillis 1959). The cleavage of the interflavanoid bond results in the formation of carbocations, which then undergo autoxidation to yield anthocyanidins (Porter et al 1986). Side-reactions are common however, and lead to the formation of red-brown polymers (often called phlobaphenes) absorbing around 450 nm (Swain and Hillis 1959). Methods based on depolymerization are only reliable if the depolymerization products represent the starting compound and / or depolymerization yields. The incidence of side-reactions can be reduced by replacing water with isopropanol or the more commonly used n-butanol (Swain and Hillis 1959). Light, temperature (95°C) and the duration of the reaction should be carefully controlled (Swain and Hillis 1959). Cyanidin has been used as the standard (ε_{mol}=34,700) (Scalbert et al 1989). The values obtained, however, are underestimated as the maximum yield of cyanidin from a proanthocyanidin (of average chain length 9.4 units) does not exceed 58% (Porter et al 1986). The yields should be even lower for proanthocyanidins with shorter chain length because of the increase in proportion of lower terminal units, which do not form anthocyanidins (Scalbert 1992). Using a dimer as an example, the calculated

values will represent only one half (given stoichiometric yield) to about one quarter (assuming usual yields) of the actual proanthocyanidin content (Scalbert 1992). The degree of polymerization (DP) of sorghum polyphenols is of interest as the increase in DP of sorghum tannin during maturation might be responsible for the apparent decrease in tannin content of several varieties during maturation (Davis & Hoseney 1979; Price et al 1979; Bullard & Elias 1980). Structural factors also affect the yield of anthocyanidins as the 4β→8 linkages are more easily cleaved than the 4β→6 linkages (Hemingway and McGraw 1983). Proanthocyanidins with upper units having a 2,3-cis configuration will be converted faster than their 2,3-trans analogues (Hemingway and McGraw 1983).

Methods based on protein precipitation have been used mainly for two reasons (Scalbert 1992). Firstly, the interaction of tannin with proteins is the essential property involved in economically important processes such as transformation of hide into leather or clarifying of beverages. Secondly, biological properties of tannins are often believed to be based on interactions with proteins. The use of enzymatic methods to determine the inhibiting fractions of polyphenols in sorghum grain has been proposed (Daiber 1975b). The results are affected by the ratio between sample size, enzyme concentration and concomitant substances, particularly proteins, which can all participate in complex formation (Daiber 1975b). Methods based on the precipitation of a protein out of solution differ on the choice of protein and the parameters used for evaluating the precipitation. The protein precipitating capacity using bovine serum albumin (BSA), for example, has been used to estimate the amount of tannin in sorghum (Hagerman & Butler 1978). Factors affecting protein precipitation include temperature and pH, the precipitation being maximal near pI

of the protein (Hagerman and Butler 1978). The duration and type of organic solvent will affect protein precipitation. Acetone, for example, is strongly inhibitory (Hagerman and Robbins 1987). Interferences from low molecular weight phenols (Hagerman and Butler 1978) and hydrophobic proteins, which may also co-precipitate with BSA in the absence of tannins, have been cited (Asquith et al 1985).

At equal concentrations, large variations in the relative astringency of various tannins have been observed (Haslam 1974). At high protein/tannin ratios, part of the protein is kept in solution, probably in the form of soluble tannin-protein complexes (Hagerman and Robbins 1987). When initial protein concentration is kept fixed while tannin concentration is varied, a threshold of precipitation is often observed (Hagerman and Robbins 1987). Consequently, the tannin concentration range in which linear response with the amount of protein precipitated is obtained, is often narrow (Hagerman and Robbins 1987). Protein-binding efficiency of a tannin extract rather than tannin content is better measured by precipitation assays (Scalbert 1992). Some methods have the advantage of being independent from the initial protein concentration, one of which consists of the diffusion of a tannin solution within a BSA-containing gel (Hagerman 1987). The method has the advantage of requiring no specialized equipment, is well adapted to small amounts of tannins (about 0.5 mg), small phenols do not interfere and solvent effects are limited. However, the value of precipitation assays is questionable as variations by as much as three orders of magnitude in competitive binding were shown when different proteins were compared (Asquith et al 1985). The results used with BSA, the most often used protein, may not be representative of the interactions of proteins and

tannins in the plant sample of interest (Scalbert 1992).

Tannins bind strongly to a wide variety of materials (Swain 1965; Zucker 1983). Sorghum tannins isolated using conventional methods, including chromatography, contain up to 20% protein (Strumeyer & Malin 1975; Davis & Hoseney 1979). Some additional steps to aid in the removal of protein have been introduced (Hagerman and Butler 1980). Methods using high performance liquid chromatography (HPLC) on sorghum polyphenols give complex but irreproducible patterns followed by permanent blockage of the column (Butler 1982). Free and bound phenolic acids of sorghum have been separated via reverse phase HPLC (Hahn et al 1983).

The most difficult step to control in tannin estimation is the extraction of tannins (Scalbert 1992), as the state of the sample at time of extraction may lead to large variations in tannin yields. Storage, drying and grinding of the sample together with the nature of the solvent mixture and temperature chosen for the extraction may alter tannin chemical structures and extraction efficiency (Scalbert 1992). The method thus used will determine not only the extract yield but also the composition and hence its chemical, physico-chemical, or biological properties due to large differences in solubility or extractability of the various tannin molecules present in the particular plant tissue.

Water exerts a profound effect on the extractability of sorghum tannin (Price et al 1979a). The amount of assayable tannins is diminished in wet sorghum grain that has either been germinated or allowed to imbibe water, acid, and alkali (Reichert et al 1980). The

assayable tannin content of mature high-tannin sorghums harvested at more than 16% moisture content was increased by about 190% on drying (Price et al 1979a). Addition of small amounts of water (15% of grain weight) to whole grain just before grinding or to ground grain just after grinding has no effect on assayable tannin (Butler 1982). However, large amounts of water added to ground grain results in the formation of insoluble tannin-protein complexes from which tannin cannot be extracted (Price et al 1980).

Sorghum tannins are extracted using acidic methanol or methanol alone (Maxson & Rooney 1972; Cummings & Axtell 1973). Similar amounts of tannins have been extracted in both solvents for several sorghums. Polyphenols extractable in acidic methanol and those extractable in methanol alone usually occur together (Price et al 1978a). Type II sorghums have significant amounts of tannins that are not extractable in methanol alone unlike type III sorghums. Flavan-4-ol and condensed tannins, that require acidic methanol for extraction, become soluble in methanol once extracted from the grain. Thus, in the grain, they occur together in structures not accessible to methanol in some lines of sorghum, other than covalently bound to a component of the grain (Price et al 1978a).

The absolute amounts of polyphenols present in the sorghum kernel are virtually impossible to determine because a significant proportion cannot be extracted and assayed (Hahn et al 1984; Hahn & Rooney 1986; Butler 1990) and a suitable standard for sorghum tannin is not available. Different polyphenol assays are likely to yield different

values because they respond to different chemical parts of the tannin molecule (Hagerman & Butler 1981). A simple method for the separation and identification of high molecular weight polyphenols is still to be developed; hence no specific standards are available for use in identification purposes.

3.5 Techniques to reduce or minimise the effects of phenolic compounds

Several approaches, reviewed below, have been proposed to deactivate polyphenol-rich sorghums.

Cooking results in a decrease in digestibility of tannin-free and high-tannin sorghums (Axtell et al 1981; Hamaker et al 1986). The results of cooking on the effects of tannin may thus be confounded by this reduction in digestibility. Both the role of moisture and the integrity of the grain seem to be crucial (Price et al 1980; Butler 1982). Boiling followed by drying can lower the amount of assayable tannin by up to 60% but rat weight gains and feed utilisation could not be improved (Price et al 1980). Autoclaving whole grain has been found to reduce assayable tannins by 83% without the corresponding improvement in rat weight gains (Price et al 1978b). In another study, autoclaving ground, tannin-containing sorghum could not improve chick weight gains or feed utilisation (Rostagno et al 1973). High-tannin sorghums can be ground and made into a batter, thereby reducing the tannin content by 95% (Price et al 1980). Price et al (1978b) have shown that dry heat has little effect on assayable tannin or nutritional quality of sorghum grain. The reduction in assayable tannins during some cooking processes is possibly due to formation of higher molecular weight polymers that are insoluble (Gupta

and Haslam 1978). However, cooking does not appear to alleviate the anti-nutritional effects of sorghum tannins (Price et al 1980).

Decortication removes the grain's outer layers and therefore most tannins, located in the pericarp and pigmented testa of high-tannin sorghums (Chibber et al 1978). However, most high-tannin sorghums have a soft, floury endosperm which makes decortication inefficient (Chibber et al 1980; Mwasaru et al 1988; Reichert et al 1988). The flour extraction rate is significantly correlated with tannin content, hardness, and seed shape of sorghum (Mwasaru et al 1988). Research efforts have been made to develop high tannin sorghums with good dehulling characteristics (Reichert et al 1988) and on the development of milling technologies (Reichert & Young 1977; Willis & Ali 1983; Sahay & Gandhi 1985; Ali & Wills 1986). Reichert (1982) described the Prairie Regional Laboratory (PRL) dehuller machine where abrasive action is provided by carborundum stones or resinoid discs mounted on a horizontal shaft inside a barrel. Machine decortication of tannin-containing sorghum grains, previously tempered up to 18% moisture, can reduce tannin levels (Youssef et al 1988). Parboiling prior to decortication of high-tannin sorghums has been proposed (Young et al 1990). Chemical decortication with alkali solutions followed by neutralization with acid diminishes levels of assayable tannins (Blessin et al 1971; Kock et al 1985; Butler 1990).

In milling sorghum, a high extraction rate of about 80% is recommended to avoid loss of nutritious embryo and endosperm while removing testa and pericarp (Eggum et al 1982). Low extraction rates and quality of products encountered with sorghum in the

conventional roller milling system makes the process uneconomic (Perten 1977), since clean separation of the bran from the endosperm is normally unattainable. Hahn (1969) concluded that colour and speckiness of roller-milled sorghum flour limit its usefulness and recommended lowering extraction and proper tempering. Roller milling results may, however, be improved by processing under semi-wet conditions (Cecil 1989; Gomez 1993) to improve the quality of the product. Dada and Dendy (1987) obtained relatively white flours of reduced tannin content from high-tannin sorghum using semi-wet roller milling.

A general decrease in tannin or polyphenol content in some germinating tannin-containing sorghum has been reported by several workers (Reichert et al 1980; Chavan et al 1981; Osontogun et al 1989; Banda-Nyirenda and Vohra 1990; Obizoba and Atti 1991; Bvochora et al 1999). The diminished extractability of tannin from moist grain probably results in low assayable tannin levels (Reichert et al 1980). However, other genotypes remain low in extractable tannin even after drying (Butler 1982; Okoh et al 1989; Osuntogun et al 1989). The decrease is possibly due to leaching of tannins from their location in the seed coat during the process of germination (Chavan et al 1981) and/or continued polymerisation of tannins and enhanced formation of tannin-protein complexes caused by imbibed water (Price et al 1980; Reichert et al 1980). Kruger (1976) attributed the decrease to increased polyphenol oxidase enzyme and other catabolic enzymes as observed for wheat. Germination may not always give positive results as a means of detoxifying sorghum tannins. It has been reported to reduce protein and energy utilisation in rats and support inadequate performance in growing pigs (Shem et al 1990). Recent

findings by Ahmed et al (1996) and Nwanguma and Eze (1996) have shown increases in polyphenols in some tannin-containing sorghum cultivars with germination. Thus there appears to be genotypic variation in changes of polyphenol content during germination, depending on the polymerisation reactions occurring and the solubility or extractability of the polyphenols in the malt.

Studies by various workers (Narciso and Dalmacio 1986; Khalifa and Tinay 1994; Bvochora et al 1999) have shown that lactic acid fermentation of high-tannin sorghums results in a decrease in the levels of tannins. The proanthocyanidins found in sorghum are not susceptible to acid hydrolysis. Thus tannin degradation by fermenting micro-organisms may be responsible for the improvement in nutritional value of fermented, high-tannin sorghum (Hassan and El Tinay 1994). Chavan et al (1981) attributed the improved *in vitro* protein digestibility of high-tannin sorghum to partial degradation of complex storage proteins into simple and soluble products during fermentation.

A number of chemicals have been evaluated for potential use in deactivating sorghum tannins. Soaking sorghum grains in solutions of alkalis such as NaOH (Chavan et al 1979) and sodium sesquicarbonate salt (Muindi and Thomke 1981; Muindi et al 1981) prior to incorporation into the diet have been used in reducing the amount of assayable tannins and in improving chick performance during feeding trials. Other alkalis, which include ammonium hydroxide, sodium or potassium hydroxide, potassium or sodium carbonate and to a lesser extent, calcium oxide, have been found to detoxify high tannin sorghum grain (Price et al 1979b; Babikir & Tinay 1992; Babikir & Tinay 1993).

Mohammed and Ali (1988) and Mukuru et al (1992) confirmed the effectiveness of wood ash leachate as an alkaline material, in detoxifying high-tannin sorghum during traditional processing. It has been shown that effectiveness of alkali treatment depends on the concentrations used and the duration of treatment because higher concentrations reduce crude protein digestibility (Muindi et al 1981; Schutte and Smith 1991b). According to Butler (1982), optimum detoxification is obtained by treatment with 0.5 L of 0.5 M NH_4OH (0.2% NH_3) per kilogram of whole grain for 12-24 hours at room temperature, with or without subsequent drying. Waichungo and Holt (1995) have shown reduced tannin levels of up to 88% through soaking sorghum in dilute NH_4OH (0.01-1M) for up to 20 days and drying between 25 and 50°C. Sodium bicarbonate (0.25%) has been found to improve the nutritional value of tannin-containing sorghums in chick feeding experiments (Banda-Nyirenda & Vohra 1990). Alkaline conditions are known to promote oxidative polymerization of tannins (Porter 1992). The resulting products are probably highly polymeric and nutritionally inactive.

South African high-tannin sorghums are treated with very dilute formaldehyde solution to detoxify the tannins (Daiber 1975a; Daiber & Taylor 1982). Phenolic materials such as tannins are known to react with formaldehyde thereby giving high-molecular-weight resins (Morrison and Boyd 1986). Treatment of high-tannin sorghum with HCl (1 M) has been shown to reduce the level of assayable tannins (Reichert et al 1980). The mechanism of tannin deactivation by acid probably involves formation of higher molecular weight polymers that are highly cross-linked and insoluble (Swain 1965; Gupta and Haslam 1978; Kennedy et al 1984; Porter 1992). In contrast to the beneficial effects of alkaline

treatment, Gieseman et al (1992) have reported that acid steeping of sorghum disrupts the protein matrix and gelatinises the starch component and does not improve nutrient digestibility without subsequent recovery of soluble or suspended substances from the steeping solution.

Infrared treatment of high-tannin sorghums has been reported to improve the weight gain and feed consumption of chicks (Douglas et al 1991b). However, tempering grains to 18% and infrared heating to about 150°C for 3 min did not affect total amount of assayable tannins. Thus the method probably enhances digestibility of other nutrients without deactivating tannins.

Elkin et al (1991) have shown that feeding a pelleted or crumbled tannin-containing sorghum diet to broiler chicks can improve chick weight by 57 %. Thus the form in which a diet containing high-tannin sorghum is fed may influence the extent of toxicity (Nyachoti et al 1997).

Addition of moisture to dry sorghum grain followed by storage can reduce assayable tannin content (Reichert et al 1980). In poultry feeding experiments, Mitaru et al (1983) have shown an improved feeding value of high-tannin sorghum by adding 25% additional moisture followed by storing for 10 or 20 days. Anaerobic storage and reconstitution cause a reduction in the effects of sorghum tannin possibly due to formation during incubation, of higher oligomeric polymers that are not readily soluble in water and, hence less likely to interfere with digestive enzymes and other proteins (Reichert et al 1980).

Several workers (Armstrong et al 1973, 1974a, 1974b; Featherston and Rogler 1975; Elkin et al 1978, 1991) have shown that supplementing poultry diets containing high-tannin sorghum with methionine and choline may overcome some of the deleterious effects. Methionine possibly acts as a donor for the methyl group required to hydrolyse tannins into gallic acid, which is then excreted in the urine as 4-O-methyl gallic acid (Potter and Fuller 1968). However, supplementing a tannin-containing sorghum diet with the methyl donor choline alone failed to reverse the adverse effects of sorghum tannins (Armstrong et al 1973). Thus methionine supplementation appears to overcome only certain tannin-associated effects.

The energy value of poultry diets containing high-tannin sorghum is lower than that of diets containing low-tannin sorghum or maize (Mitaru et al 1984; Douglas et al 1990) and fat supplementation has been used to reduce the effects of tannins (Douglas et al 1990). The extra fat serves as an additional energy source, which compensates for the lower metabolisable energy value of tannin-containing sorghum. Tannins are able to reduce the energy value of sorghum by interfering with starch digestibility (Flores et al 1994). Addition of orthophosphoric acid and dicalcium phosphate to chick diets containing high-tannin sorghum has been shown to alleviate the negative effects of tannins (Ibrahim et al 1988). However, excess residual phosphorus tends to interfere with calcium metabolism (Scott et al 1982).

Supplementation of a high-tannin sorghum diet with extra protein diminishes the harmful

effects of tannins (Schaffert et al 1974). Thus the extra protein serves as a binding agent rather than a source of additional amino acids. Supplementation with non-nutritive synthetic tannin-binding polymers including polyvinylpyrrolidone (Armstrong et al 1973) or polyethylene-glycol (Ford and Hewett 1977) is similarly effective.

3.6 Summary

Based upon the foregoing text, it is clear that a universally ideal sorghum cannot be defined since several desirable characters appear inversely related to others. The influence of grain structure and composition on processing characteristics and acceptability deserves much greater attention. The nature and range of phenolic substances found in sorghum poses a number of analytical questions. Various chemical assays measure different groups of phenolic compounds. In the following text, methods that are commonly used to measure total phenols, flavonoids and condensed tannins, were selected to represent the three groups of phenolics found in sorghum, that is, phenolic acids, flavonoids and condensed tannins. Phenols and their oxidation products have long been known to react with proteins possibly through hydrogen, ionic and covalent bonds. Condensed tannins in sorghum appear to produce more stable complexes than simple phenols and flavonoids. The adverse effects of sorghum polyphenols on total digestibility and nitrogen balance may be reduced by absorbants such as polyvinylpyrrolidine (PVP), or by acid or alkali hydrolysis, or by removing the high tannin seed coats by alkali or abrasive decortication. In African communities, several traditional processing methods that include a combination of alkaline treatment and germination are used to reduce the effects of polyphenols. An industrial process in Southern Africa makes use of a non-

acidic carbonyl containing-compound, formaldehyde to reduce the polyphenol content of milled products of sorghum. Very dilute solutions of formaldehyde are used to reduce the adverse effects of tannins in the malting and brewing industry in Southern Africa. However, formaldehyde has been implicated as a potential carcinogen in some studies and its continued use in foods has been questioned. The present study therefore investigates the use of other chemicals as alternatives to formaldehyde for tannin deactivation during malting. Chemical treatments using food grade chemicals, combined with simple primary processing may be beneficial in the utilisation of high-tannin sorghum for food. Alkali (NaOH) and acid (HCl) treatments have been shown to lower the amount of assayable tannins in sorghum. The concentrations are selected based on findings by Daiber (1975a) on HCHO and by Dewar et al (1997a) on NaOH. Data from preliminary experiments on steep water uptake using various concentrations of HCl will be used as the basis for selecting the concentration of the acid. Abrasive decortication followed by hammer milling is the common method of milling sorghum in Southern Africa but sorghum roller milling using simple roller mills may be advantageous. Conditioning moisture for roller milling was selected following findings by Gomez (1993). The above chemical treatments are also used in conditioning prior to abrasive milling or roller milling. Currently, maize is the source of industrial starch in Southern Africa but sorghum starch has been shown to resemble maize starch. The use of chemical treatments could also be beneficial in wet milling of sorghum. Thus chemical treatments in malting or milling are investigated as means of reducing the adverse effects of polyphenols and improving the quality of the primary product.