

# PROCESSING OF POLYPHENOL-RICH SORGHUMS FOR FOOD

by

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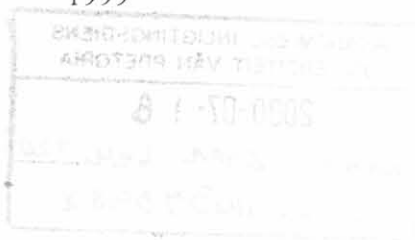
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## Dedication

This dissertation is dedicated to my three siblings who died during the course of my research:

- 1) Senitah Beta, who passed away on 17 August 1997, when I was conducting the steeping and germination experiments in Pretoria;
- 2) Sennie Beta, who passed away on 29 June 1998, when I was carrying out the milling experiments at Matopos; and
- 3) Norman Garikayi Beta, who passed away on 5 September 1998, prior to my departure for a China conference, where I intended to present a paper on sorghum phenolics.

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## ABSTRACT

### PROCESSING OF POLYPHENOL-RICH SORGHUMS FOR FOOD

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Phenolic compounds in sorghum play an important agronomic role. They are divided into three groups: phenolic acids, flavonoids, and condensed tannins. The cultivation of polyphenol-rich sorghums reduces pre-harvest losses due to bird predation and post-harvest storage losses. Condensed tannins bind proteins, thus reducing nutritional and functional value. Phenolic compounds may also impart undesirable colours in sorghum products during food processing. Sixteen sorghum varieties widely cultivated in Zimbabwe were examined for levels of phenolic compounds and kernel characteristics, to consider how to improve the acceptance and utilisation of sorghum for food in Zimbabwe and southern Africa as well as overall food security.

Three varieties, DC-75, Mutode, and Chirimaugute had high levels of condensed tannins. Chibonda had polyphenols that could not be extracted with methanol alone. A positive correlation was observed between pericarp thickness and polyphenol content ( $r > 0.64$ ).

Most varieties had an intermediate to floury (soft) endosperm texture. Zimbabwean sorghums were found to lack ideal agronomic and processing physico-chemical characteristics defined in terms of high polyphenols, plus hard endosperm and thin pericarp. Research was carried out to develop effective methods of processing the available polyphenol-rich sorghums. Chemical treatments, using HCl (0.25M), formaldehyde (0.017M), NaOH (0.075M) and water as control, were applied in steeping for malt or starch production. Conditioning moisture and chemical treatment were also used in milling. Three out of the sixteen varieties were selected for processing, each representing a tannin-free, high- and medium-tannin sorghum.

All chemical treatments reduced the polyphenol content of the grain after steeping. Treatment with NaOH or formaldehyde (HCHO) was more effective than water or HCl. Available DP was low in malt from tannin-containing varieties that had been treated with water or HCl. Malting alone, was not an effective method of reducing the enzyme inhibitory power of the sorghum tannins. Steeping in dilute NaOH was effective in deactivating high-tannin sorghums, reducing the steeping period through increased grain water uptake and enhancing malt DP. NaOH appears to be an alternative to HCHO for treatment of high-tannin sorghums in the malting industry.

Roller milling and abrasive decortication reduced the polyphenol content as the grain's outer layers were lost as bran. Chemical treatment and conditioning moisture up to 20 % did not significantly reduce the polyphenol content after abrasive milling. However,

treatment with NaOH or HCHO significantly reduced the polyphenol content of roller-milled flour. Moisture and chemical treatment appear to be useful in roller milling but not in abrasive milling.

Chemical treatments were used to improve the physico-chemical properties of starch isolated from high-tannin sorghums. The pink colour of starch from polyphenol-rich sorghums could not be removed but was further brightened by NaOH treatment. NaOH caused an intense colour for all but SV2. The tan plant colour of SV2 was important as a way to reduce colour in starch processes. NaOH steeping gave starches with higher peak viscosity (PV), cool paste viscosity (CPV) and setback than when water, HCl and HCHO were used. HCl-treated starches had higher PV temperature, and took longer to reach PV. The time to reach PV and PV temperature were markedly reduced by treatment with NaOH. Thus NaOH caused some form of partial pre-gelatinisation. Treatment with alkali could be advantageous in lowering temperatures needed to gelatinise sorghum starch. Dilute alkali steeping during wet milling could enhance the properties of starch isolated from tannin-free and tannin-containing sorghums. Polyphenol content and kernel characteristics of sorghum varieties influenced starch properties.

Dilute alkali steeping during malting or wet milling for malt or starch production could offer advantages in improving the functionalities of products made from polyphenol-rich sorghums.

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## 1. GENERAL INTRODUCTION

Zimbabwe with a total area of 390,760 km<sup>2</sup>, is divided into five agro-ecological zones based on rainfall and soil types (Nyamapfene 1991). Natural regions I and II, receiving >750 mm of rainfall per annum, are suitable for annual cropping and intensive agriculture. The rest of the regions receive less than < 750 mm of rainfall per annum. In general rainfall decreases from the eastern to the western parts of the country. Natural regions III, IV and V, where 65% of Zimbabwe's 10 million people live, have low and erratic rainfall with severe mid-season dry spells, which consistently result in maize crop failure. The lack of food security in these regions is partly caused by the use of maize, which is less suitable than more drought tolerant crops such as sorghum (SADC/ICRISAT Annual Report 1990). Shumba (1993) indicated that sorghum production is increasing in communal areas of Zimbabwe, but utilisation is not expanding. Obilana (1997) cited lack of information on the usability and nutritional qualities as one of the reasons for under-utilisation of sorghum grain. Sorghum grain has traditionally been milled for porridge, boiled like rice, or malted for sour, non-alcoholic and alcoholic beverages. The major commercial use of sorghum is in the industrial brewing of traditional opaque beer. DC-75, a high-tannin sorghum hybrid, has been the major variety promoted by the leading Zimbabwean opaque-brewing company, the latter of which engages contract farming to get its grain supplies. The move towards contract farming has left the Grain Marketing Board (GMB), a parastatal of the Zimbabwean government, with fewer markets for the polyphenol-rich sorghums. Apart from the opaque beer market, polyphenol-rich sorghums have not been processed for other major food products for the urban areas. Recently, small-scale millers have embarked on

sorghum dry milling using grain of inconsistent quality. Market penetration has not yet been realized due to quality defects of the sorghum meal in comparison with the predominant maize meal from large-scale millers. Lacking markets, it remains a crop of the small cultivator, consumed largely on the land where it is produced. Research and development aimed at producing quality primary and secondary products from both tannin-free and polyphenol-rich grains for use by those who are not farmers, will remove this particular restraint on sorghum and would do much to offset Zimbabwe's shift in demand towards imported rice, wheat and maize. However, initial emphasis should be placed on understanding the biochemical components that constitute the grain and how they affect the desired food products.

The presence of polyphenols in sorghum has, however, some negative

Phenolic compounds comprise some of the important biochemical components found in sorghum. They include phenolic acids, flavonoids and condensed tannins (Hahn et al 1984), the latter two are grouped as polyphenols. Tannin-free sorghums contain phenolic acids and some flavonoids. Phenolic acids play a role in imparting resistance to fungal attack; the degree of resistance is linked to grain that contains both a greater variety and larger amounts in the free form (Hahn et al 1983). However, resistance to fungal attack is not solely attributed to phenolic acid content but to other physical grain characteristics as well (Hahn et al 1983). The flavonoid compounds are responsible for the colour of the pericarp of sorghum grains (Kambal & Bate-Smith 1976). Polyphenols protect sorghum grain against weathering (Harris and Burns 1973) and pre-harvest germination (Harris and Burns 1970). Tannins also impart a degree of bird and mould resistance or tolerance in sorghum grain (McMillan et al 1972; Hahn et al 1984). Thus the cultivation of

polyphenol-rich sorghums reduces pre-harvest losses due to bird depredation and post-harvest storage losses. High-tannin sorghum varieties are preferred by some industrial sorghum maltsters (for example, in Zimbabwe, Botswana and South Africa) as the grain is more resistant to mould infection during the moist, warm conditions (95-100% RH, 25-30°C) used for malting. In Southern Africa, tannin deactivation using formaldehyde is achieved during malting of high-tannin sorghums (Daiber 1975a). During malting, Zimbabwean high-tannin sorghum varieties, DC-75 and Mutode can accumulate higher levels of reducing sugars and free amino nitrogen (FAN) compared to the low tannin varieties, SV2 and Chihumani (Bvochora et al 1999).

The presence of phenolic compounds in sorghum has, however, some negative implications. Phenolic acids do not affect nutritional quality of sorghum grain but may form undesirable colours during maturation as well as under some food processing conditions, for example under alkaline conditions (Hahn et al 1984). Flavonoids and phenolic acids would be undesirable in sorghum flour and meal if the products are meant to compete with their white maize and wheat counterparts in urban markets. The condensed tannins in sorghum are the only group of phenolic compounds that bind proteins reducing nutritional value (Hahn et al 1984). Tannins are able to bind more strongly to large proteins and to proteins high in proline (Butler 1982). They inhibit all enzymes in *in vitro* assays (Hagerman & Butler 1981). The non-specificity of their ability to bind protein could be the reason behind the general lack of evidence that tannins could be deactivated. Tannin-containing sorghum produces malt of lower quality (Daiber 1975a; Chavan et al 1981; Daiber and Taylor 1982) as the tannins will inhibit the amylase

enzymes required for starch hydrolysis once the milled malt is mixed with water. Tannins inhibit growth and fermentation of some microorganisms and hence could influence the balance of the fermentation medium (Strumeyer and Malin 1975). Studies using sorghum have shown that tannins diminish growth rates, protein digestibility, and feed efficiency in rats (Jambunathan & Mertz 1973), hamsters (Mehansho et al 1987), swine (Cousins et al 1981; Knabe 1990), and poultry (Muindi & Thomke 1981; Sell et al 1983). High-tannin sorghum varieties are most often very soft (Chibber et al 1980; Waniska et al 1989) probably because they have not been required to develop the hard grain trait when already equipped with the protection given by the polyphenols. Polyphenol-rich sorghum presents challenges in food processing and deactivation methods using acid or alkali (Reichert et al 1980), and formaldehyde (Daiber 1975) have been proposed.

Polyphenols play important agronomic roles but their negative implications as regards the food value of sorghum present several challenges. Firstly, there is need to retain, and even enhance, the beneficial effects of sorghum phenolic compounds in the ecosystem. Secondly, the anti-nutritional effects of condensed tannins, also known as antifeedants (Mole 1986), need to be dealt with if the full food value of sorghum is to be realised. The agronomic advantages of sorghum tannins can be maintained if post-harvest food processing is used to reduce or deactivate the anti-nutritional polyphenols. Thirdly, while the simple polyphenols in sorghum grain are not anti-nutritional, they may impart undesirable colours to food products (Hahn et al 1984) making them less attractive. Success in food processing can diversify the uses of polyphenol-rich varieties and improve the nutritive value of high-tannin sorghums. Hence there is need for simple



## 2. OBJECTIVES

The objectives of this study were to:

- Characterize sorghums that are widely grown in Zimbabwe in terms of levels of phenolic compounds and kernel properties;
- Determine the effect of various chemical treatments of polyphenol-rich sorghums on their malting and malt quality;
- Determine the effect of various chemical treatments of polyphenol-rich sorghums on their milling and flour quality;
- Determine the effect of various chemical treatments of polyphenol-rich sorghums on their starch properties; and
- Determine the physicochemical properties of starch isolated from sorghum varieties of different polyphenol content and kernel characteristics.

### 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<sub>1</sub> and B<sub>2</sub> 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.

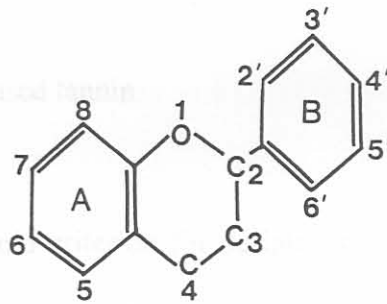
### 3.2 Colour of sorghum grain

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<sub>3</sub>) 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).

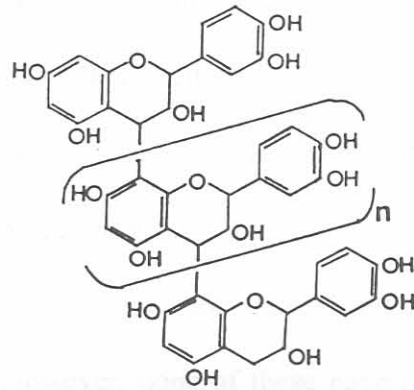
Flavonoids consist of a C<sub>6</sub>-C<sub>3</sub> fragment (A) from cinnamic acid and a C<sub>6</sub> fragment (B) from malonyl-CoA (Figure 1). The major groups of flavonoids are flavones, flavonols and flavans. Flavans are the major group of flavonoids in sorghum. Flavonoids have been shown to play 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* While 3-oxo-4-hydroxyflavan-3-ols are the most common, it may not be practical to make the

(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 protein (Zocker 1983).

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),  $\beta$ -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 Hosenev 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 <sup>*</sup>	HA <sup>†</sup> & PA <sup>†</sup>	PA <sup>†</sup>
<b>Protein and alkaloid precipitation</b>			
-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	
<b>Reaction with phenolic rings</b>			
<i>All phenols</i>			
-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		
<i>m-Diphenols</i>			
-Reaction with vanillin (Burns 1971; Price et al 1978)			X
<b>Depolymerization</b>			
-Oxidative depolymerization in BuOH/HCl (Swain and Hillis 1959)			X

<sup>\*</sup>Phenols and polyphenols (including non-tannin polymers)

<sup>†</sup>Hydrolysable tannins

<sup>†</sup>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 ( $\lambda_{\max}=640$  nm) (Swain and Hillis 1959). HCl or H<sub>2</sub>SO<sub>4</sub> 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<sub>2</sub>SO<sub>4</sub> 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 interflavonoid 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 ( $\epsilon_{\text{mol}}=34,700$ ) (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 & Hosney 1979; Price et al 1979; Bullard & Elias 1980). Structural factors also affect the yield of anthocyanidins as the  $4\beta\rightarrow 8$  linkages are more easily cleaved than the  $4\beta\rightarrow 6$  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 & Hosney 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  $\text{NH}_4\text{OH}$  (0.2%  $\text{NH}_3$ ) 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  $\text{NH}_4\text{OH}$  (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, Gieseeman 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 polyvinylpyrrolidone (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.

## 4. CHAPTER I

### Phenolic Compounds and Kernel Characteristics of Zimbabwean Sorghums

#### ABSTRACT

Sixteen sorghum varieties widely cultivated in Zimbabwe were examined for levels of phenolic compounds and kernel characteristics, to help identify those with desirable properties and develop suitable processing methods. Assays for polyphenols included the chlorox test, the vanillin-HCl, the ferric ammonium citrate and the butanol-HCl methods. Free phenolic acids were analysed using reverse-phase high performance liquid chromatography. Varieties DC-75, Mutode and Chirimaugute had the highest levels of condensed tannins. The polyphenols in Chibonda were mostly unextractable in methanol. No significant levels of polyphenols were found in 13 varieties. Phenolic acid content was related to pericarp colour. Endosperm texture and pericarp thickness were evaluated using video image analysis. Katandanzara and SV1 had relatively corneous endosperms (<30% floury). Mutode, Chibonda, and DC-75 had thick pericarps (>0.060 mm). Endosperm texture was not correlated with phenolic compounds. A positive correlation, however, was observed between pericarp thickness and polyphenol content ( $r>0.64$ ). Zimbabwean sorghums lack ideal agronomic and processing physico-chemical characteristics defined in terms of high polyphenols, plus hard endosperm and thin pericarp. Research is required to develop effective methods to process the available polyphenol-rich sorghums.

## INTRODUCTION

Sorghum remains an important crop in southern Africa and other semi-arid regions of the world due to its relatively drought-tolerant nature compared to other cereals. Thus it is critically important for food security. In Zimbabwe and elsewhere in southern Africa, sorghum is milled to produce flours for use in porridges. It is malted for the production of opaque beer and nonalcoholic beverages. Sorghum varieties vary widely in kernel characteristics such as pericarp colour and thickness, presence or absence of pigmented testa, endosperm texture and endosperm colour, all of which are genetically controlled (Rooney & Miller 1982). The structure of these grains has an important bearing on various processing and food quality parameters (Hoseney et al 1981). For example, endosperm texture is important in determining the quality of traditional sorghum foods (Rooney and Murty 1982), milling yield (Maxson et al 1971) and resistance to grain moulds (Waniska et al 1992). Similarly, pericarp thickness affects milling properties (Scheuring et al 1983) and mould susceptibility (Glueck and Rooney 1978). For commercial dry milling, white sorghums with thin pericarps and corneous endosperms have superior properties (Maxson et al 1971).

Certain sorghum varieties have a pigmented testa layer which contains condensed tannins. These tannins confer resistance to bird predation (McMillan et al 1972). Losses due to bird predation are a major problem in Africa especially in the western part of Zimbabwe. Tannin and polyphenols protect the grain against weathering (Harris and Burns 1973) and pre-harvest germination (Harris and Burns 1970). The cultivation of high-tannin sorghums reduces pre-harvest losses due to bird predation and post-harvest storage losses. Tannins bind proteins, however, reducing nutritional value (Hahn et al 1984). Hence, the sorghum food processor has the task of finding suitable processing

techniques for tannin removal or inactivation while at the same time producing quality products of high nutritional value.

The objective of this study was to classify and evaluate the physical and chemical attributes of the most important local Zimbabwean sorghums in terms of their levels of phenolic compounds and kernel properties. The information is needed to consider how to improve the acceptance and utilisation of sorghum for food in Zimbabwe and Southern Africa as well as overall rural food security.

## MATERIALS AND METHODS

### Materials

A total of 16 sorghum varieties grown during the 1995/96 season were obtained from the Crop Breeding Institute, Department of Research & Specialist Services, Zimbabwe. The selection included 13 local varieties, 2 improved cultivars (SV1 and SV2), and one international hybrid (DC-75). The sorghums were chosen to represent the most widely cultivated varieties in Zimbabwe. The grains were ground to pass through a 1 mm sieve for the quantitative determination of polyphenols.

### Chlorox test

The chlorox test was used to detect sorghums with a pigmented testa (Waniska et al 1992). Sorghum grains were placed in a glass beaker and just covered with the chlorox reagent (50g litre<sup>-1</sup> NaOH in commercial bleach, minimum 3.5% sodium hypochlorite).

The beaker and contents were placed in an oven set at 70°C for 20 min. Sorghums with a testa had dark colour after bleaching which removed the pericarp and clearly showed

the testa. The number of kernels with complete testa, partial testa and no pigmented testa were counted after draining the bleach and results expressed as a percentage.

### **Ferric Ammonium Citrate method**

A modified International Standardisation Organisation (ISO) (sorghum - determination of tannin content) was used to determine total polyphenols (ISO 1988). Milled grain (250 mg) was extracted with 5 ml of dimethyl formamide at room temperature for 1 h, with vortex mixing at 5-min intervals to stir the ground grain. Measures, 0, 10, 20, and 40 g kg<sup>-1</sup>, of tannic acid (BDH Chemicals, England) were used to prepare a calibration curve. After centrifugation for 10 min at 1200 x g, the following were pipetted into a test tube in the sequence given and mixed carefully after each addition: 5 ml distilled water, 1 ml carboxymethylcellulose/EDTA, 0.2 ml DMF extract or working standard, 0.2 ml ferric reagent and 0.2 ml alkali reagent. Sample blanks were included in which the ferric reagent was replaced by distilled water. The results were expressed as mg tannic acid per 100-mg sample.

### **Vanillin-HCl method**

The vanillin-HCl method of Burns (1971) was used to measure condensed tannins. Milled grain (1 g) was extracted with 50 ml methanol at room temperature for 20 min, with vortex mixing at 5-min intervals. The supernatant was obtained by centrifuging for 10 min at 1200 x g. Sample blanks were included. Catechin (Sigma, St Louis, USA) was used as a standard. The results were expressed as mg CE per 100-mg sample.

## **Butanol-HCl method**

A sample extract from the vanillin-HCl assay was used for the butanol-HCl assay of condensed tannins (Porter et al 1986). A measure, 6 ml, of acid butanol (50 ml HCL [32%]  $\text{dm}^{-3}$ ) was added to 1 ml of sample extract in a test tube. Iron chloride was omitted. The test tubes were placed in a forced-air oven at  $100^{\circ}\text{C}$  for 50 min. Absorbance was read at 550 nm. No standards were used in the butanol-HCl method.

## **RP-HPLC Analysis of Free Phenolic Acids**

### *Extraction*

The procedure of Hahn et al (1983) was followed with modifications. First 5 g of ground samples were extracted twice in 20 ml of 100% methanol for 30 min with vigorous shaking at 5-min intervals. The solid material was removed by centrifugation for 10 min at  $700 \times g$ . The pooled extracts were evaporated under vacuum to near dryness at  $35^{\circ}\text{C}$  using a rotary evaporator. The extract was dissolved in 8 ml of the mobile phase consisting of 25 mM potassium hydrogen phosphate in water (and adjusted to pH 2.67 using phosphoric acid) and acetonitrile in a ratio of 75:25. This extract was applied to an isolute solid phase extraction (SPE) column (International Sorbent Technology, Mid-Glamorgan, UK) to retain the high molecular weight polyphenols. The column was washed with 2 ml of the mobile phase to elute the benzoic and cinnamic acids. The final volume was measured and the extract filtered through a  $0.45\text{-}\mu\text{m}$  pore size filter (Millipore). A  $100\ \mu\text{l}$  sample was applied to the HPLC column.

### *Instrumentation*

A Waters model 501 liquid chromatograph equipped with a model U6K injector (Waters Associates, Milford, MA) was used for the determination of free phenolic acids in sorghum grain. The column was a Supelcosil ABZ+ 15cm x 4.6 mm id, 5  $\mu\text{m}$  particle cartridge with a guard column (Supelguard ABZ+Plus kit) obtained from Anatech Instruments (Johannesburg, South Africa). Detection was by ultraviolet absorption at 254 nm using a Waters Model 440 Absorbance Detector. Chromatograms were acquired and monitored from the Chromatography Signal Interface (CSI) using an Apex Chromatography Station (1988) on a computer. Retention times and peak areas were obtained using the process module to examine and integrate the chromatograms. Protocatechuic acid content in the grain was calculated using the standard. The total free phenolic acids were then calculated as protocatechuic acid equivalents.

### *Standards*

Gallic, protocatechuic, vanillic, *p*-hydroxybenzoic, caffeic, ferulic, *p*-coumaric and cinnamic acids were obtained from Sigma. Each of the standards was dissolved in the mobile phase to give 5  $\mu\text{g}$  100  $\mu\text{l}^{-1}$ . A mixture of these standards, excluding caffeic acid and cinnamic acids, containing 5  $\mu\text{g}$  100  $\mu\text{l}^{-1}$  each was also prepared. Samples of 20  $\mu\text{l}$  were chromatographed singly and as mixtures at a flow rate of 0.5 ml  $\text{min}^{-1}$  for 20 min. Optimum separation was obtained using acetonitrile and 25 mM  $\text{K}_2\text{H}_2\text{PO}_4$  adjusted to pH 2.67 in the ratio 25:75 as eluent. Cinnamic acid was omitted as preliminary runs did not detect significant concentrations in the sorghum samples and its retention time was longer than 40 min. Caffeic acid eluted at the same time as *p*-hydroxybenzoic acid and was omitted from the standard mixture.

### **Video image analysis**

Endosperm texture, endosperm colour and pericarp colour were evaluated visually as described by Rooney and Miller (1982). Endosperm texture and pericarp thickness were then determined using video image analysis of longitudinal sections of the kernels produced by hand-sectioning. The analysis was performed on an Apple Macintosh computer using the public domain NIH Image programme (developed at the US National Institute of Health and available on the Internet at <http://rsb.info.nih.gov/nih-image/>). Sections were magnified 1.18 times for endosperm texture and 6.0 times for pericarp thickness with a scanning electron microscope (Nikon SMZ-2T, Japan). The images were captured using a Panasonic CCTV camera (model WV-BL 200/C) on an Apple Macintosh computer. The image was divided into square pixels, each of which had a grey scale value ranging from 0 to 256. The image was enhanced for contrast for proper measurement. The floury portion of the endosperm was measured as a density slice and compared to the total area of the sectioned kernel using the NIH images programme. Photographs of the pericarp were taken so as to avoid the tip area and its opposite end. Pericarp thickness was measured using the conversion 1 mm = 639 square pixels, obtained by measuring an equivalent length of a cm-ruler in square pixels. A total of eight kernel sections were used.

### **Statistical analysis**

Statgraphics version 5.0 (Statistical Graphics Corporation, 1991) was used to analyse the data using multifactor analysis of variance and multiple range analysis. Pearson correlation was used to relate phenolic compounds and kernel properties of the grains.

## **RESULTS AND DISCUSSION**

The sorghum varieties containing a pigmented testa were identified using the chlorox bleach test (Table I-1). Grains of DC-75, Chibonda, Chirimaugute and Mutode contained kernels with pigmented testa which showed up using the chlorox test for the presence of pigmented testa. None of the other varieties tested had a pigmented testa.

Condensed tannins impart bird and mould tolerance and reduce the nutritional value of the grain by 10-30% (Hahn et al 1984). Chemical assays which identify sorghums with high tannin levels are useful in predicting relative bird resistance. The vanillin assay is specific for flavanols (Sakar and Howarth 1976) in which an aromatic aldehyde condenses with certain flavonoids, mainly flavan-3-ols and their oligomers, to form soluble pigments. The use of methanol as solvent quenches the reaction of vanillin with monomeric flavanols, such as catechin, so that the reaction is more specific for oligomers (tannins). The acid butanol assay is specific for proanthocyanidins (condensed tannins) if the optimized reaction conditions described by Porter et al (1986) are used. In this assay, the flavonoid subunits of the condensed tannin polymer are oxidatively cleaved to yield the anthocyanidin (Porter et al 1986). The ISO method is a redox method which is non-specific, responding not only to tannins, but also to other polyphenols and even to other reducing agents such as ascorbate. According to Daiber (1975a), this ferric ammonium citrate procedure distinguishes between tannin-containing and tannin-free sorghums.

Table I-1 shows the amount of condensed tannins (proanthocyanidins) expressed as catechin equivalents or absorbance units and total polyphenols expressed as % tannic acid. The blank subtraction in both the vanillin and ferric ammonium citrate method reduced the polyphenols levels significantly. Cummings and Axtell (1973) classified

sorghum lines into three groups: type I sorghums do not have significant amounts of tannins; type II sorghums have tannins unextractable in methanol; and type III sorghums have tannins extractable in methanol. The three polyphenol analyses indicated varieties DC-75, Chirimaugute and Mutode are probably type III although this cannot be stated categorically as acidified methanol was not used as an extractant. These sorghums differed significantly in polyphenol content, but they all contained more than 1.1% (w/w) total polyphenols, and would therefore not be suitable for malting purposes (Daiber 1975a), as the tannins inactivate the malt amylase enzymes. Treatment of the grain to inactivate the tannins, however, for example, by steeping in dilute formaldehyde (Daiber, 1975b) may render the variety suitable for malting. In fact, in Zimbabwe, DC-75 is used after steeping in dilute formaldehyde for malting.

Concerning the variety Chibonda, the chlorox test indicated the presence of a pigmented testa, but the tannins could not be extracted with methanol (Table I-1). Thus Chibonda could possibly be a type II sorghum (Cummings and Axtell 1973). The other varieties can be classified as type I sorghums since they did not have a pigmented testa and were low in polyphenols as measured by the three different assays. Mukadzidzoka, Iganu, Brown Tsweta and Ntelwane, however, apparently contained some soluble tannins as assayed by the butanol method. Although this assay is said to be specific for proanthocyanidins (Porter et al 1986), other polyphenols are presumably responsible for the positive response in these condensed tannin-free sorghums. Varieties SV1, SV2 and NL330 were virtually polyphenol-free as assayed by the vanillin and butanol assays.

Phenolic acids inhibit growth of micro-organisms and may impart resistance to moulds on sorghum grain before and after grain maturity (Hahn et al 1983). Figure I-

1 shows chromatograms obtained using RP-HPLC of the free phenolic acids in the different sorghum varieties. The chromatograms are stacked in order of decreasing tannin content as assayed by the vanillin-HCl method. The retention times of the acid standards were obtained as: gallic 5.0 min; protocatechuic 6.7 min; vanillic 8.6 min; *p*-hydroxybenzoic 9.7 min; ferulic 13.8 min and *p*-coumaric 15.7 min. Protocatechuic acid levels were higher in DC-75, Mutode and Chirimaugute, those varieties containing a pigmented testa, levels of  $>50 \mu\text{g g}^{-1}$  being measured (Table I-1). Higher levels of protocatechuic acid also occurred in Chibonda, the presumed type II sorghum. Nyamidzi and Mukadzidzoka had high levels of vanillic acid, whereas most varieties contained low concentrations of vanillic and ferulic acids. The peak due to *p*-hydroxybenzoic acid was prominent in all the varieties and could have been enhanced by caffeic acid as these were not well resolved. *p*-Coumaric could only be detected in DC-75 and Mutode. Some unidentified phenolic acids appeared in significant quantities in most varieties.

Total free phenolic acid content, calculated as protocatechuic acid equivalents, was high ( $> 400 \mu\text{g g}^{-1}$ ) in the condensed tannin-containing varieties and in type I sorghums Brown Tsweta, Iganu and Ntelwane (Table I-1). The presumed type II sorghum, Chibonda, however, was rather lower ( $291 \mu\text{g g}^{-1}$ ). According to Hahn et al (1983) sorghums which are more resistant to fungal attack contain both a greater variety and larger amounts of phenolic acids in the free form. Katandanzara, Chihumani, SV1, SV2, and Kasvikisire, varieties with a white pericarp (Table I-2) had the lowest levels of both protocatechuic acid and total free phenolic acids. Waniska et al (1989) similarly observed that white cultivars without testa contained the lowest amount of phenolic acids.

Table I-2 shows pericarp colour and thickness and endosperm colour and texture. The colour of the pericarp is genetically controlled and can be red, lemon yellow or white (Rooney and Miller 1982). Varieties DC-75, Mutode, Mukadzidzoka, Iganu, Ntelwane, and Brown Tsweta had a red pericarp. Interestingly, Chibonda and Chirimaugute had a white pericarp with a pigmented testa underneath. In the case of the red type I sorghums, Mukadzidzoka, Iganu, Ntelwane and Brown Tsveta pericarp colour appeared to be related to a highish free phenolic acid content (Table I-1). It was not, however, related to polyphenol content as determined by any of the assays performed. According to Kambal & Bate-Smith (1976), flavonoid compounds are responsible for the pericarp colour of sorghum grains. For red and lemon yellow pericarp, luteoforol (an anthocyanidin) and erodictyol (a chalcone) were the major pigments (Kambal & Bate-Smith 1976), respectively. Nip and Burns (1969) also identified two yellow and one orange anthocyanin pigments whose pigmentation was controlled by light, hence the colour differences in sorghum pericarps. Generally a lighter pericarp colour is more desirable when traditionally milled products are to be produced for porridge making. The red pericarp colour is desirable in sorghum malting for the production of traditional opaque beer.

The endosperm colour was white in all the varieties investigated. Endosperm texture is defined as the relative proportions of the corneous to floury endosperm. Figure I-2 shows SEM photographs of sectioned kernels selected to represent varieties with corneous, intermediate and floury endosperm textures (Katandanzara, Mukadziusaenda, and Chibonda, respectively). Katandanzara and SV1 had relatively corneous endosperms (<30% floury). Katandanzara (meaning that it chases away hunger) might

have been given such a name on account of superior storage properties, since hard endosperm grain is more resistant to fungal attack (Kumari et al 1992). Chihumani and DC-75 were also corneous and similar in texture. Concerning DC-75, this is contrary to the general perception that high-tannin sorghums are almost always soft (Waniska et al 1989). Chibonda and Mukadzidzoka had floury endosperms. The other varieties had intermediate to floury endosperms and differed significantly between each other. Hardness, as determined visually by scoring on a scale of 1 (floury) to 5 (corneous), was comparable to the objective image analysis measurements (Table I-2). Rooney and Murty (1982) concluded that sorghum grains with 60-100% corneous endosperm (or <40% floury endosperm) are preferred for the preparation of stiff porridges and rice-like products. Varieties Katandanzara, SV2, SV1, and NL330 met this criterion. Although DC-75, a high-tannin variety, met the criterion, the reddish colour of the meal might not be desirable.

## CONCLUSIONS

Pericarp thickness plays a role in mould susceptibility (Glueck and Rooney 1978), rendering sorghums with a very thick pericarp more susceptible to grain weathering. Three of the varieties (Mutode, Chibonda, and DC-75) had thick pericarps ( $>0.060$  mm, Table I-2). The classification into thin, intermediate and thick pericarp, as illustrated in Figure 3, was based on visual determination. The thickness of the pericarp differed significantly among varieties with most ranging from thin to intermediate. Abrasive milling studies by Maxson et al (1971) led to the general conclusion that white sorghums with thin pericarps and corneous endosperms had superior dry-milling properties. On this basis, varieties Katandanzara, Chihumani, SV1, SV2, and NL330 are likely to have better milling properties, although none of these varieties is rich in polyphenols.

The three polyphenol assays (total polyphenols, butanol-HCl and vanillin-HCl), despite the fact that they supposedly are specific for different types of polyphenols (Porter et al 1986, Sakar and Howarth 1976), were all significantly correlated ( $p < 0.05$ ) with each other ( $r \geq 0.95$ ) (Table 3). Earp et al (1981) also observed high correlations for different polyphenol assays (vanillin-HCl, Prussian blue and alpha-amylase inhibition) when a range of sorghums was assayed. There was no correlation between the polyphenol content of the Zimbabwean sorghums and endosperm texture (Table I-3). A significant correlation, however, occurred between polyphenol content and pericarp thickness ( $r > 0.64$  at  $p < 0.05$ ). Protocatechuic acid content was also significantly correlated with pericarp thickness, although total free phenolic acids were not correlated.

## CONCLUSIONS

A few of the widely grown sorghum varieties in Zimbabwe contain significant levels of polyphenols. There was a significant correlation between polyphenol content and pericarp thickness. No correlation existed between phenolic compounds and endosperm texture. None of the 16 sorghums met the criterion of high polyphenols, hard endosperm, and thin pericarp. Therefore, if polyphenol-rich Zimbabwean sorghum varieties are to be used for milling, there is a great need for suitable processing methods that will inactivate the tannins in high-tannin varieties and/or improve meal yields from varieties with a soft endosperm and thick pericarp, while giving products of high quality and acceptance.

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**Table I-1.** Polyphenol and phenolic acid content of 16 Zimbabwean sorghum varieties as measured by different methods

Variety*	Chlorox†			Van-HCl‡		Ferric ammonium§		Butanol¶	PTA⊖	TFPA**
	CT%	PT%	NT%	WB††	BS††	WB††	BS††	A/g††	ug/g††	ug/g††
1. DC-75	91	7	2	6.29k	5.48f	1.43k	0.79h	45.88h	79.3j	635.7n
2. Chirimaugute	10	14	76	3.78j	3.07e	1.18l	0.63f	29.3lg	57.0j	563.3m
3. Mutode	13	37	50	3.23i	2.79d	1.30j	0.73g	28.30f	56.0l	454.0k
4. Mukadzidzoka	0	0	100	0.66h	0.08c	0.32h	0.14e	1.62e	21.7f	277.7h
5. Chibonda	19	63	18	0.52g	0.03ab	0.20f	0.08cd	1.04cde	41.0h	291.3i
6. Iganu	1	1	98	0.36f	0.09c	0.19ef	0.05ab	1.21de	25.0g	490.7l
7. Ntelwane	0	0	100	0.36f	0.07c	0.29h	0.14e	1.05cde	21.3f	412.0j
8. Tsveta	1	1	98	0.32e	0.09c	0.25g	0.07bc	1.67e	23.0f	566.7m
9. Katandanzara	0	0	100	0.19d	0.03b	0.11b	0.05ab	0.34ab	7.0a	117.3a
10. Mukadziusaenda	0	0	100	0.18d	0.01ab	0.14bcd	0.07bc	0.42a	13.0d	257.3g
11. Nyamidzi	0	2	98	0.12c	0.00a	0.11bc	0.03a	0.87bcd	16.3e	223.0e
12. Kasvikisire	0	0	100	0.05b	0.01ab	0.14bcd	0.05ab	0.25ab	10.0c	205.7d
13. Chihumani	1	0	99	0.03b	0.00a	0.06a	0.02a	0.19a	8.0ab	136.7b
14. SV2	0	0	100	0.00a	0.00a	0.16de	0.14e	0.20a	9.0ab	192.7c
15. SV1	0	0	100	0.00a	0.00a	0.13bc	0.07bc	0.20a	7.7ab	210.3d
16. NL330	0	0	100	0.00a	0.00a	0.14cd	0.11de	0.14a	12.3d	241.0f
Mean	8	8	84	1.01	0.73	0.38	0.19	7.04	25.5	329.7

\* Listed in order of decreasing catechins equivalents (CE) as measured by the vanillin-HCL assay.

† Grains with complete testa (CT), partial testa (PT), no testa (NT).

‡ mg CE per 100-mg sample without blank subtraction (WB) and with blank subtraction (BS).

§ mg tannic acid per 100-mg sample without blank subtraction (WB) and with blank subtraction (BS).

¶ Absorbance units at 550 nm per g sample.

⊖ Protocatechuic acid in µg per g.

\*\* Total free phenolic acids expressed as protocatechuic acid equivalents in µg per g.

†† Mean of three measurements. Values with different letters within the same column are significantly different at  $p < 0.05$ .

**Table I-2.** Pericarp colour, endosperm colour, endosperm texture and pericarp thickness of Zimbabwean sorghum varieties

Variety	Testa	Pericarp*	Endosperm		Hard‡	Pericarp thickness§	
			Colourψ	Texture†			
1. DC-75	yes	red	white	31.9b¶	3.5	6.6j¶	t
2. Chirimaugute	yes	white	white	63.0i	1.4	5.3h	m
3. Mutode	yes	red	white	45.1e	3.2	8.2k	t
4. Mukadzidzoka	no	red	white	71.9k	1.3	4.1g	m
5. Chibonda	yes	white	white	69.7j	1.1	7.0j	t
6. Iganu	no	red	white	56.0h	2.1	3.0cd	tn
7. Ntelwane	no	red	white	56.1h	1.7	3.4e	m
8. Brown Tsveta	no	red	white	51.4g	2.1	3.2de	tn
9. Katandanzara	no	white	white	28.4a	4.3	2.4b	tn
10. Mukadziusaenda	no	white	white	48.1f	3.3	2.8c	tn
11. Nyamidzi	no	white	white	37.8c	3.9	5.2h	m
12. Kasvikisire	no	white	white	38.1cd	3.5	3.7f	m
13. Chihumani	no	white	white	32.9b	3.7	2.3b	tn
14. SV2	no	white	white	39.9d	3.3	3.4e	m
15. SV1	no	white	white	29.8a	3.8	2.0a	tn
16. Ntelwane	no	white	white	39.4cd	2.4	3.9fg	m
Mean value				46.2		4.1	

\*Genetics of pericarp colour.

ψEndosperm colour determined subjectively on longitudinal sections of the grain.

†Measured as % floury endosperm.

‡ Visual hardness determined on a scale of 1 to 5: 1, completely floury; 5, completely corneous. Values are an average of 10 scores.

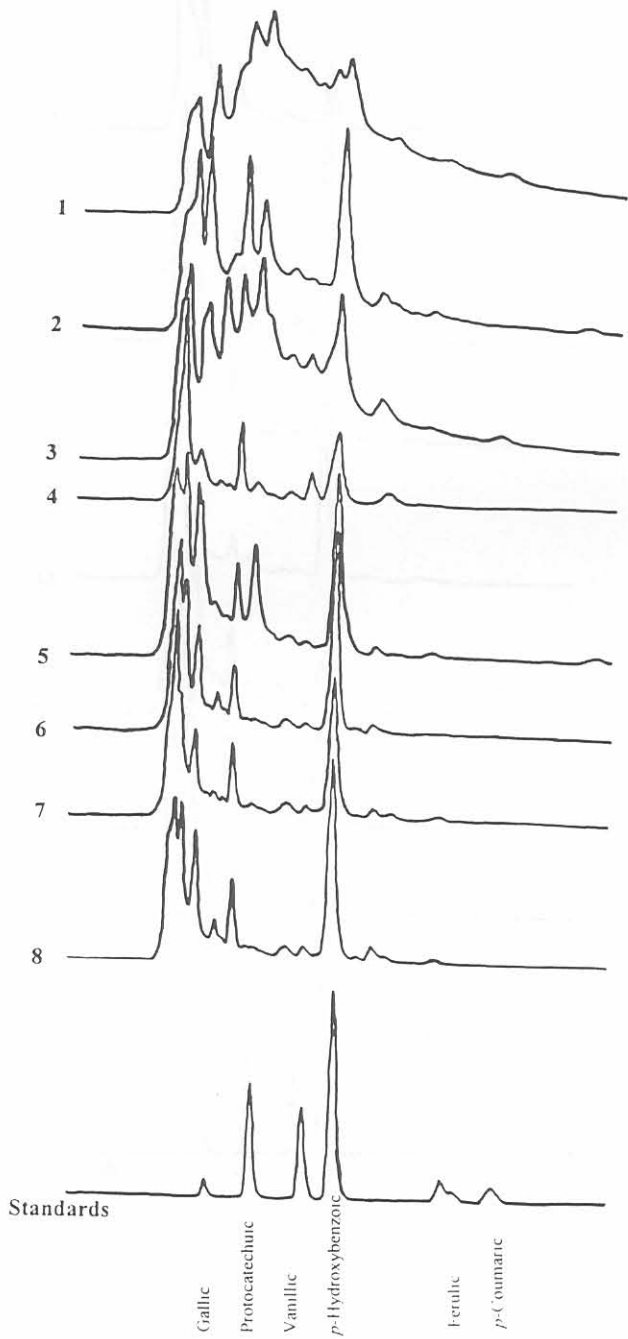
§ Pericarp thickness ( $m \times 10^{-5}$ )classification: t = thick, m = medium, tn = thin. Mean of eight measurements.

¶ Values within same column with different letters are significantly different at  $p < 0.05$ .

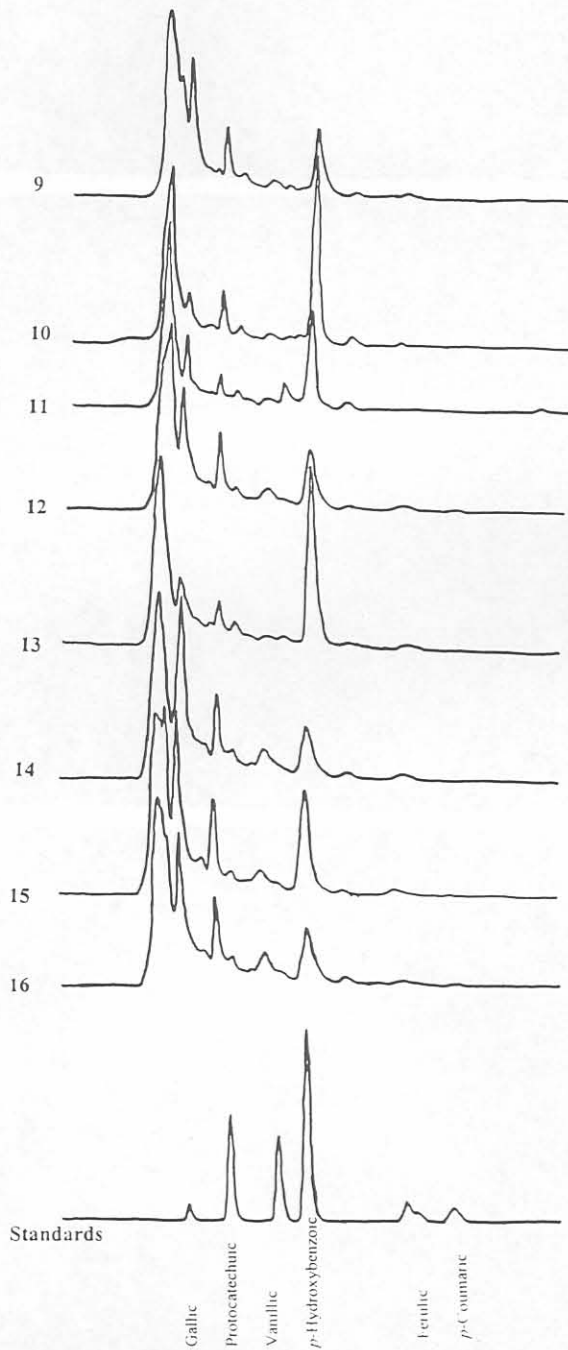
**Table I-3.** Correlation analysis between polyphenol content and kernel characteristics<sup>a</sup>

	Peri	Text	TP	TPB	BH	VH	PTA	TFPA
Text	0.29							
TP	0.72*	0.09						
TPB	0.71*	0.03	0.99*					
BH	0.67*	-0.04	0.98*	0.97*				
VH	0.66*	0.01	0.96*	0.95*	0.99*			
VHB	0.64*	-0.08	0.96*	0.96*	1.00*	1.00*		
PTA	0.80*	0.26	0.93*	0.90*	0.93*	0.94*	0.92*	
TFPA	0.46	0.36	0.73*	0.68*	0.70*	0.72*	0.69*	0.80*

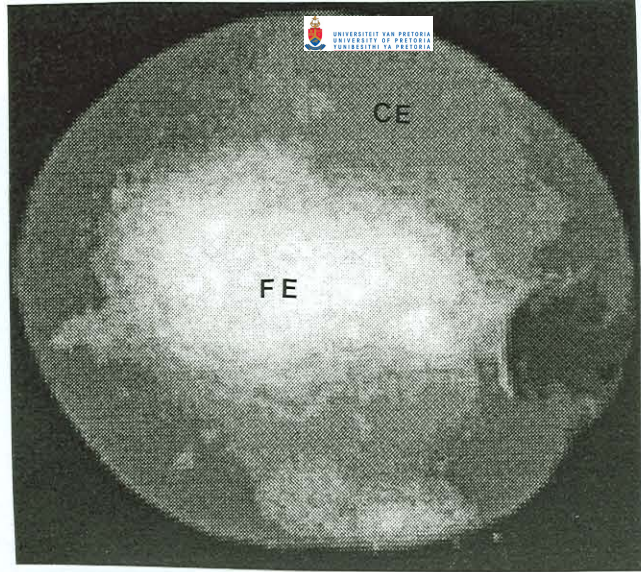
<sup>a</sup>Pearson correlation coefficients. All values marked with \* are significant at  $p < 0.05$ . Text, endosperm texture; peri, pericarp thickness; TP, total polyphenols; TPB, total polyphenols with blank subtraction; BH, butanol-HCL; VH, vanillin-HCL; VHB: vanillin-HCL with blank subtraction; PTA, protocatechuic acid; TFPA, total free phenolic acids.



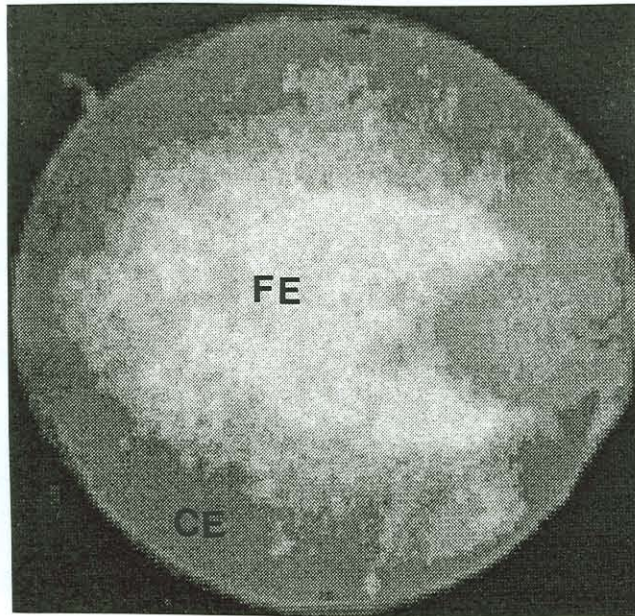
**Figure I-1.** (a) RP-HPLC chromatographic patterns of varieties: 1, DC-75; 2, Chirimaugute; 3, Mutode; 4, Mukadzidzoka; 5, Chibonda; 6, Iganu; 7, Ntelwane; 8, Brown Tsweta.



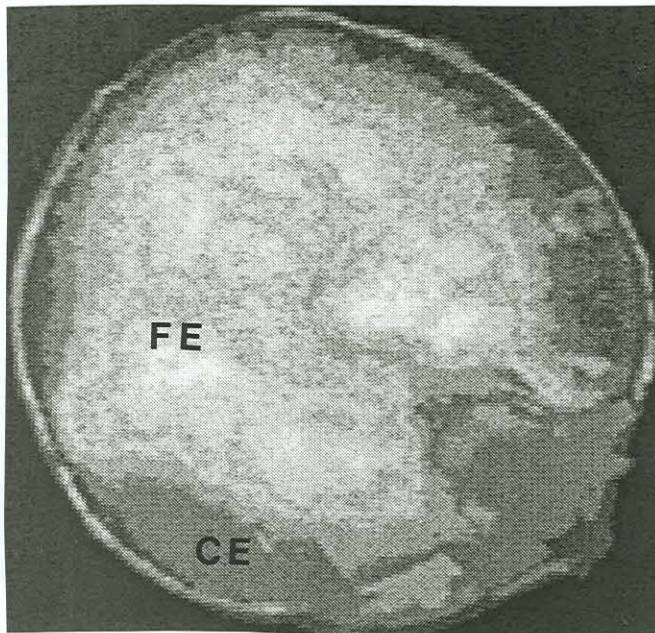
**Figure I-1.** (b) RP-HPLC chromatographic patterns of varieties: 9, Katandanzara; 10, Mukadziusaenda; 11, Nyamidzi; 12, Kasvikisire; 13, Chihumani; 14, SV1; 15, SV2; 16, NL330.



a

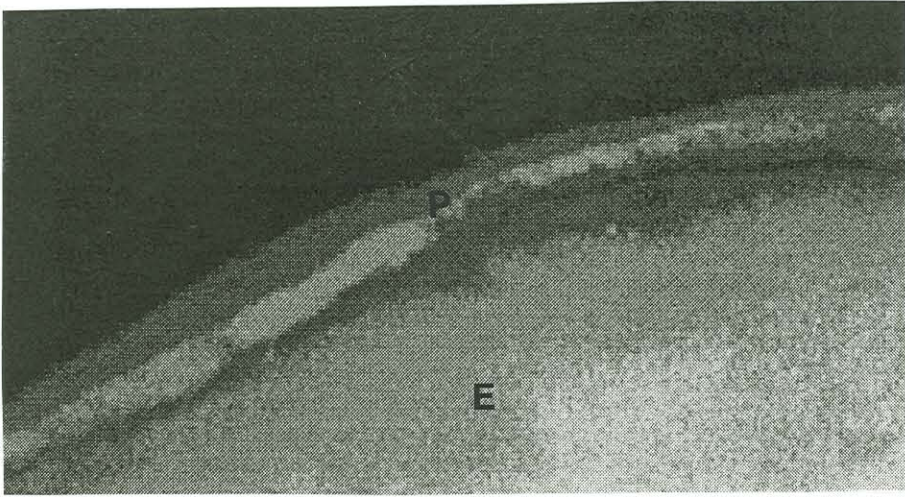


b

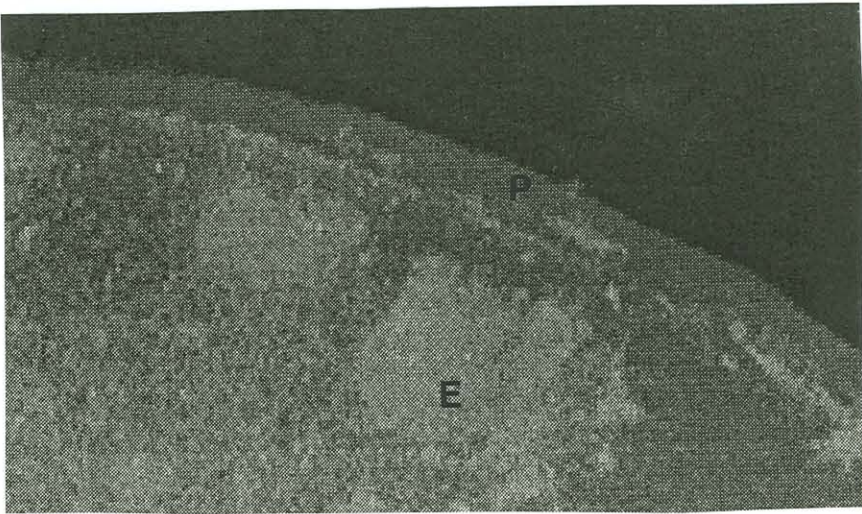


c

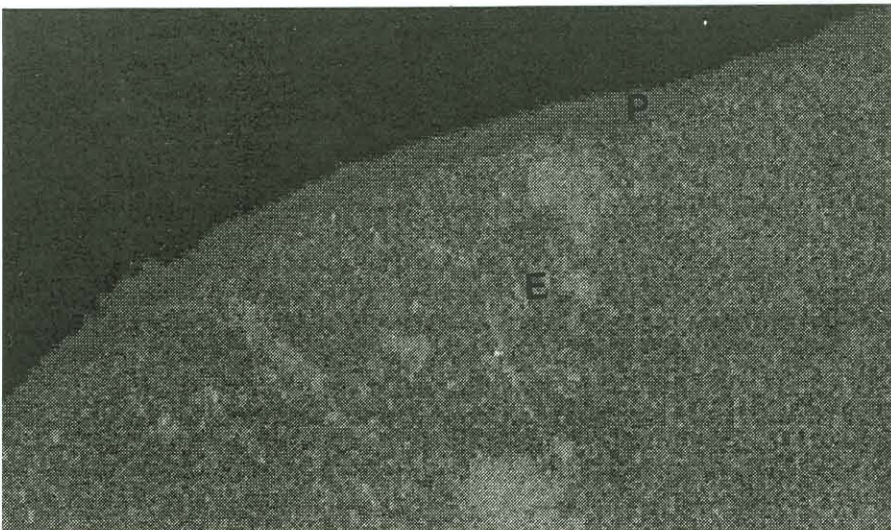
**Figure I-2.** SEM photographs of varieties Katandanzara, Mukadziusaenda, and Chibonda depicting: (a) corneous endosperm; (b) intermediate endosperm; and (c) floury endosperm, respectively. [FE, floury portion of the endosperm, CE, corneous portion of the endosperm, bar = 1 mm (22 x magnification)]



a



b



c

**Figure I-3.** SEM photographs of varieties Chibonda, SV2, and SV1 depicting: (a) thick pericarp, (b) intermediate pericarp, and (c) thin pericarp. [P, pericarp; E, endosperm; bar = 0.1 mm (140 x magnification)]

## 4. CHAPTER II

### Effect of Chemical Treatments on Polyphenols and Malt Quality in Sorghum

#### ABSTRACT

Tannin-containing sorghums, Chirimaugute and DC-75, and a tannin-free sorghum, SV2, were steeped in water, HCl (0.25M), formaldehyde (0.017M) and NaOH (0.075M) for 8 and 24 h. Germination was carried out for 2 and 5 d. Steeping in NaOH enhanced water uptake of the grains compared to the other treatments. All treatments reduced the polyphenol content of the raw grain. Treatment with NaOH or formaldehyde (HCHO) was more effective than water or HCl. Malt quality was measured in terms of diastatic power (DP). Potential DP, determined after the peptone extraction, indicated higher amylase in DC-75 malt than in Chirimaugute and SV2 malt. Available DP, determined after water extraction, was low in malt from the tannin-containing varieties that had been treated with water or HCl. Malting alone, was not an effective method of reducing the enzyme inhibitory power of the sorghum tannins. Available DP was markedly improved by the NaOH and HCHO treatments of the tannin-containing varieties. It is concluded that steeping in dilute NaOH is effective in detoxifying high-tannin sorghums, reducing the steeping period and enhancing malt quality. Steeping in NaOH appears to be an alternative to HCHO for treatment of high-tannin sorghums in the malting industry and other food uses.

## INTRODUCTION

The phenolic compounds in sorghum grain play an important agronomic role by reducing grain damage and bird predation (Hahn et al 1984). They can be divided into phenolic acids, flavonoids and condensed tannins. However, the condensed tannins in sorghum grain bind and precipitate proteins causing a reduction in nutritional value (Butler 1982). Tannins also bind with amylase enzymes in the malt (Daiber 1975a), making them unavailable for starch degradation. It is therefore important that tannin-containing sorghum grains are properly treated before they can be used for food. Three major approaches have been reported in the literature: decortication, malting and chemical detoxification. Decortication by abrasive action can remove the outer pericarp and testa layers of the grain where most of the tannins are located (Rooney et al 1980). This technique can result in low milling yield and high protein loss due to softness of the endosperm, which is characteristic of some of the high tannin sorghums (Reichert et al 1988). Malting is a specially controlled form of germination, which mobilises enzymes and modifies the original grain structure and components. In Africa, sorghum grain is malted to produce opaque beers, weaning foods and other traditional dishes. Malting has been reported to result in a decrease in assayable tannins in some genotypes of sorghum (Butler 1982, Reichert et al 1980). Chemical detoxification has also been used to treat polyphenol-rich sorghums. Several alkalis, including ammonium hydroxide, sodium or potassium hydroxide, potassium or sodium carbonate, sodium bicarbonate and calcium oxide, have been used to detoxify high-tannin sorghum grains (Price et al 1979, Banda-Nyirenda and Vohra 1990). In southern Africa, high-tannin sorghums are treated with very dilute formaldehyde solution for malting (Daiber 1975b). However, there is concern

about the toxicity of formaldehyde in food applications (Cheftel et al 1985). High tannin sorghums have also been subjected to treatment with hydrochloric acid (Reichert 1980).

In most of these studies, detoxification treatments were carried out independently of each other. The purpose of this investigation was to compare the effect of various chemical treatments on the polyphenol content and final malt quality of tannin-containing sorghum varieties.

## MATERIALS AND METHODS

### Grains

Sorghum (*Sorghum bicolor* (L) Moench) grain of varieties, SV2 and Chirimaugute (Chiri), and a hybrid, DC-75, were used. Chiri and DC-75 are tannin-containing types. SV2 is a tannin-free improved variety. The grains were grown in the 1996/97 season at Matopos, Zimbabwe in field conditions under normal agronomic practices.

### Steeping

Samples of grain (100 g) were weighed in perforated nylon mesh bags (400 x 400 mm) and steeped for periods of 8 h and 24 h at 25°C. Initially, all grains were subjected to a static steep of 8 h during which individual bags were immersed in solutions of water (control), NaOH (0.3%, w/v), HCl (0.9%, v/v) and formaldehyde (HCHO) (0.05%, v/v) contained in plastic beakers. The solutions were discarded afterwards. The grains in nylon bags were rinsed five times with tap water. Excess water was removed using paper towels after the 8-h steep period. The steeped grains were dried in a forced air oven at

50°C for 48 h inside the nylon bags. The moisture content of the steeped grains was then determined.

After rinsing, steeping was continued for 16 h for those grains that were steeped for a total period of 24 h. The 16-h steep was carried out in running tap water at 25°C by use of two steeping tanks, one of which was thermostatically controlled so that the water temperature was 25°C before entering the other tank where the grains were immersed. Grains were steeped for 4 h followed by a 1-h air rest period outside the steeping tank. This cycle was repeated over 16 h. Excess water was removed and the grains dried as above.

### **Germination**

The grains were steeped for 24 h following the procedure described above. The steeped grains were centrifuged in a domestic spin-drier for 30 sec at 300 x g to remove the excess surface-held water. The grains were then weighed. Germination was carried out at 25°C and 100% relative humidity for 2 and 5 d inside a water-jacketed incubator (Forma Scientific, Marietta, U.S.A.). The grains were placed on racks with moist cloths placed on the surface and bottom of bags to ensure that the grains remained moist throughout the germination period. Twice a day, the germinating grains were weighed and steeped in tap water for 10 min, spin-dried for 30 sec, reweighed and returned to the incubator. After germination for 2 and 5 d, the grains were dried at 50°C for 48 h in a forced-air oven.

## Milling

The steeped, germinated and dried grains were ground to pass through an 800- $\mu$ m sieve, prior to analysis.

## Chemical analyses

*Polyphenols.* The polyphenols in sorghum were measured using a method based on anthocyanidin production (Swain and Hillis 1959) and the vanillin reaction (Burns 1971) assay. By using a blank subtraction with the latter, flavonoid components of the grain were eliminated and the reaction became more specific for condensed tannins (Price et al 1978). Sample extracts for the assays were obtained by shaking 0.2 g ground grain in 10 ml methanol at 5 min intervals for 20 min on a vortex mixer at room temperature. The supernatant was obtained by centrifuging for 10 min at 1200 x g. For the anthocyanidin production assay, 6 ml of 5 % HCl in n-butanol was added to 1 ml of sample extract in a test tube. Iron chloride was omitted. The test tubes were placed in a forced-air oven at 100°C for 50 min. Absorbance was read at 550 nm against a reagent blank and no standard was used. The results were reported as absorbance units (A/g, dry basis). Catechin (Sigma Chemicals) was used as a standard in the vanillin assay and sample blanks were included. The results were expressed in catechin equivalents (g/100 g, dry basis).

*Diastatic Power (DP).* Sorghum diastatic power was determined following the South African Bureau of Standards method 235 (SABC 1970) with modifications. Water (to measure available DP) and peptone solution (to measure potential DP) were used as extractants. Malt (5 g) was weighed and the extraction volume was reduced proportionately.

The results were expressed as sorghum diastatic units (SDU/g, dry basis).

### **Statistical analyses**

The general linear model procedure of SAS version 6.12 (SAS Institute, Cary, NC) was used. Analysis of variance was used to determine the effect of variety, treatment, steeping, and germination period on polyphenol content and malt quality. Means were separated using the least significant difference at  $P < 0.05$ .

## **RESULTS AND DISCUSSION**

### **Effects of chemical treatment on steep out moisture**

Table II-1 shows the moisture content of the grains after steeping in water, HCl, HCHO and NaOH solutions for 8 and 24 h. The moisture content was significantly affected by variety, treatment, and steeping time ( $P < 0.001$ ). Chiri had higher moisture uptake than the other varieties. The variations in water uptake by the grains could be attributed to their differences in endosperm texture. Video image analysis showed that Chiri grains had a relatively soft endosperm texture compared to those of the other varieties (Chapter I). Grains that were steeped in NaOH solution had higher moisture contents compared to the other treatments, hence the alkali enhanced water uptake of the grain. As sorghum grains are required to have a moisture content of 33-35% (wet weight basis) after steeping (Hofmeyr 1970), the use of low concentrations of NaOH in steeping solutions could reduce the period required to reach such moisture levels. The treatment could also be useful in enhancing malt quality which has been shown to be related to steep-out moisture (Dewar et al 1997a). A shorter steep may also be advantageous, as longer steeping

periods may result in microbial proliferation (Dewar et al 1997b).

### **Effects of chemical treatment on polyphenols**

Table II-2 shows the condensed tannin content measured by the acid butanol assay. Variety, treatment, steeping and germination period significantly affected the tannin levels ( $P < 0.001$ ). The tannin content of the raw grains was higher in DC-75 than Chiri. All treatments resulted in a reduction in condensed tannins. Most of the reduction took place during the first 8 h steeping period. HCHO and NaOH were more effective than water and HCl. Further steeping in water for 16 h was done to bring the total steeping period to 24 h. With water and HCl treatments tannins decreased at 2 and 5 d germination. A marked increase at 5 d germination was observed with HCHO and NaOH. In the butanol assay, the anthocyanidin pigments that were measured resulted from the autoxidation of carbocations initially formed by cleavage of the interflavan bond of the condensed tannin polymer (Porter et al 1986). Given the problems with specificity of tannin assays (Mole 1986), the extracts were also assayed by the vanillin reaction. Its advantage is that vanillin reacts with the flavonoid A-ring at the C-6 position, forming a chromophore that is not influenced by the B-ring hydroxylation (Figure II-1a). The vanillin assay will thus detect any monomeric or polymeric flavanol (Sakar and Howarth 1976). A reduction in the condensed tannin content of the raw grain was observed with all treatments (Table II-3) when the vanillin method (without blank subtraction) was employed. Results closely resembled those obtained with the acid butanol assay with HCHO and NaOH being more effective than water and HCl. Polyphenols generally increased at 5 d germination with the increase being pronounced with HCHO and NaOH.

Reduction in tannins as germination proceeded did not appear to be the general trend, as had been anticipated. McGrath et al (1982) observed that during malting the properties of tannins change as a large complement of the non-inhibitory or non-tannin polyphenols are produced by roots and shoots. Stafford (1965) also reported formation of intensely red coloured cyanidins in the developing root, which cause an increase in the flavonoid content and colour of the malt (Glennie 1983). Thus the apparent increase in tannin content as measured by the butanol and vanillin (without blank subtraction) methods could be attributed to an increase in non-tannin polyphenols as germination proceeded. Modification of the vanillin assay to include a reagent blank that corrected for the colour of the seed extract was done to improve the specificity of condensed tannin measurement (Price et al 1978). The tannin levels with blank extraction (Table II-4) were lower than those without blank extraction (Table II-3). The contribution of the flavonoid components in the grain was thus eliminated. The HCHO and NaOH treatments reduced the level of condensed tannins essentially to zero. Additionally, when true condensed tannins were estimated (by the vanillin with blank extraction), and not polyphenols in general, malting did in fact substantially reduce their level, as has been stated (Butler 1982, Reichert et al 1980). The results confirmed the above observation that non-tannin polyphenols contributed to the tannin readings as the malting period increased.

Notwithstanding the limitations of the assays used, a significant decrease in polyphenols was found when the varieties were steeped in water, HCl, HCHO and NaOH solutions. All treated grains had to be dried prior to polyphenol analysis to eliminate the problem of diminished extractability of tannin from moist grain (Reichert et al 1980, Price et al

1979). Prior to the study, experiments were conducted to determine the lowest HCl concentration at which water uptake of the grain was enhanced with minimal changes in grain appearance. It was observed, however, that grains treated in HCl (0.25 M) were slow in germinating during malting. HCHO concentration chosen for this study was based on levels currently being used for tannin deactivation (Daiber 1975b) during brewing in southern Africa. The concentration of NaOH was chosen based on the finding that it improved the water uptake and apparently improved malt quality in condensed tannin-free sorghums (Dewar et al 1997a). Both the HCHO and NaOH treatments were found to be effective for the purpose of tannin reduction. Similar findings on the effectiveness of NaOH in tannin reduction were reported by Reichert et al (1980) when sorghum grain was allowed to imbibe 25% by weight of distilled water, 0.8 M HCl or 0.8 M NaOH prior to storage at 25 or 35°C for 2 d. However, the tannin values obtained were on freshly ground samples raising the question of diminished tannin extractability at higher moisture levels (Butler 1982). Our results confirm their findings, as the latter problem was eliminated by drying the samples prior to tannin analysis by the vanillin method with blank extraction.

Several authors have surmised that the mechanism of tannin deactivation involves formation of higher molecular weight polymers (Swain 1965, Gupta and Haslam 1978, Kennedy et al 1984, Porter 1992) that are highly cross-linked and insoluble. In the case of tannin deactivation by water or HCl, the mechanism may be similar to the reactions which takes place in the grain as it approaches maturity (Gupta and Haslam 1978). The acid-treated grains may form reactive carbocations from soluble polymers (n=5-6) during

storage (Reichert et al 1980). These carbocations then react to form higher oligomeric polymers which are not readily soluble in water and hence less likely to interfere with enzymes or proteins. Reichert et al (1980) postulated that, either storage of grains that had imbibed water (25% by weight) for 2 days allowed a continuation of the natural polymerization process that was taking place as the seed was drying out, or storage of the acid- or water-treated grains insolubilized the tannins by formation of covalent bonding between condensed tannins and both protein- and polysaccharide constituents of the grain.

The action of NaOH on tannins possibly involves oxidation of phenolic groups under the moist, alkaline conditions. Alkaline conditions promote oxidative polymerization of condensed tannins (Porter 1992), resulting in the formation of highly polymeric and probably nutritionally inactive compounds. In an alkaline solution, flavan-3-ols or condensed tannins can undergo C-ring opening and rearrangement via radical reactions involving traces of oxygen (Kennedy et al 1984). It has been shown that the radical anion intermediate formed in mild, alkaline solutions reacts further by a series of alternative annulation and ring migration reactions leading to a host of products (Figure II-1b). Such processes give phlobatannins, which are more rigid and less polar than the parent proanthocyanidins or condensed tannins (Porter 1992). Swain (1965) suggested additionally, a possible alteration in tannin structure, resulting in formation of insoluble phlobaphenes or the binding of tannin to a nearby component in the grain, rendering both substances insoluble and inert.

The general reaction between formaldehyde and phenols could be used to explain the action of HCHO on tannins. Formaldehyde reacts with phenols to form phenol-formaldehyde resins or Bakelite and related polymers (Morrison and Boyd 1984). The resulting substances are of high molecular weight in which phenol rings are held together by  $-CH_2-$  groups. McGrath et al (1982) postulated that either the formaldehyde simply inactivates the phenolic group in the tannin molecules or that it cross-links the tannin to form large polymers.

### **Effects of chemical treatment on diastatic power of sorghum malt**

Potential DP was assayed after extracting the enzymes from the malt using peptone solution (Table II-5). The reason for peptone use in DP determination is that the high concentration of peptone swamps out the reaction between tannins and the diastatic enzymes, as the peptone reacts preferentially with the tannins (Taylor 1989). Variety, chemical treatment and germination period significantly affected ( $P < 0.001$ ) the DP of sorghum malt and hence the brewing quality of the malt. Malt from DC-75 had the highest potential DP. The HCl treatment gave the lowest DP in all malts. Germination was reduced in HCl-treated grains raising the question of whether the treatment adversely affected the synthesis of the  $\alpha$ - and  $\beta$ -amylases which the DP assay measures. The HCHO treatment gave the highest DP in malts from tannin-containing varieties. The potential DP of malts from water and NaOH-treated grain was in general slightly lower than in malts from HCHO-treated grains.

Available DP was measured after extracting the enzymes with water (Table II-6). SV2, the

tannin-free variety, gave similar DP when water or peptone was used as the extractant. High DP was also found using both assays when the grain from tannin-containing types (DC-75 and Chiri) was treated with HCHO and NaOH. However, malts from DC-75 and Chiri grain that had been treated with HCl and water gave very low water extract DP, as the tannins inhibited the malt amylases (Daiber 1975). Table II-4 shows that malting alone reduced the levels of assayable tannins in Chiri to virtually zero and almost halved the levels in DC-75. However, if one examines the situation with regard to available DP (Table II-6) then DP in Chiri in the 5 day malt control is half that in the peptone (potential) DP and in the case of DC-75 the water DP is only about 15 % of that of peptone DP. The bottom line is that although malting only very substantially reduces assayable tannins (Table II-4), it does not actually greatly reduce the inhibitory power of the tannins in the malt. Malting was not an effective method of reducing the enzyme inhibitory power of the sorghum tannins. This can only be done in combination with treatment with NaOH or HCHO. Thus the NaOH and HCHO treatments were effective in tannin detoxification, such that tannins did not inhibit the malt amylases, whereas the water and HCl treatments were not effective.

## CONCLUSIONS

Steeping high tannin sorghum grains in water, HCl, NaOH and HCHO solutions results in lower tannin content, with the latter two being most effective in reducing the tannins. Malting is not an effective method of reducing the enzyme inhibitory power of the sorghum tannins. This can only be done in combination with treatment with NaOH and HCHO. NaOH also enhances the water uptake of the grain. Of importance is the similar

effectiveness of NaOH in tannin inactivation compared to formaldehyde, since the latter, despite its associated health risk, is currently being used to deactivate the tannins by the sorghum malting industry in southern Africa. Optimum conditions still need to be established, however, it appears that NaOH treatment is potentially a simple, relatively safe alternative for use in sorghum malting and other sorghum food applications. There is need for additional chemical work on sorghum tannins.

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**Table II-1.** Effect of steeping sorghum varieties, Chiri and DC-75, for 8 h in HCl (0.9%, v/v), HCHO (0.05%, v/v), and NaOH (0.3%, w/v), on grain moisture content (% wet weight basis).

Variety	SV2		Chiri		DC-75	
	8 h	24 h	8 h	24 h	8 h	24 h
<b>Treatment</b>						
Control (water)	28.0 <sup>1,2</sup>	33.1 <sup>a</sup>	33.8	38.8 <sup>a</sup>	32.0	37.0 <sup>a</sup>
	(0.3)	(0.6)	(0.3)	(0.2)	(0.4)	(0.1)
HCl	27.3	32.9 <sup>a</sup>	32.1	37.1 <sup>a</sup>	30.3	36.7 <sup>a</sup>
	(0.2)	(0.3)	(0.3)	(0.5)	(0.2)	(0.6)
HCHO	28.0	33.1 <sup>a</sup>	33.0	38.0 <sup>a</sup>	31.1	36.3 <sup>a</sup>
	(0)	(0.3)	(0)	(0)	(0.7)	(0.1)
NaOH	34.0	36.2 <sup>b</sup>	39.4	41.5 <sup>b</sup>	38.4	39.6 <sup>b</sup>
	(0.5)	(0.6)	(0.7)	(0.4)	(0.4)	(0.5)

<sup>1</sup>Mean and standard deviation

<sup>2</sup>Treatments with different letters in the same column are statistically different ( $P < 0.05$ )

**Table II-2.** Effect of steeping sorghum varieties, Chiri and DC-75, for 8 h in HCl (0.9%, v/v), HCHO (0.05%, v/v), and NaOH (0.3%, w/v), followed by germination, on the condensed tannin content as measured by the butanol-HCl assay (A/g, dry basis).

Variety	Chiri					DC-75				
	Malting period		2 d		5 d	Malting period		2 d		5 d
	8 h	24 h				8 h	24 h			
Raw grain			29.31					45.88		
<b>Treatment</b>										
Control (water)	13.28 <sup>a1,2</sup>	10.53 <sup>b</sup>	7.34 <sup>b</sup>	8.45 <sup>a</sup>		33.00 <sup>b</sup>	36.64 <sup>b</sup>	25.70 <sup>a</sup>	27.89 <sup>a</sup>	
	(0.55)	(0.13)	(0.30)	(0.14)		(0.40)	(1.13)	(1.31)	(1.89)	
HCl	13.08 <sup>a</sup>	16.11 <sup>a</sup>	8.64 <sup>a</sup>	6.82 <sup>b</sup>		34.35 <sup>a</sup>	38.69 <sup>a</sup>	23.77 <sup>b</sup>	20.16 <sup>b</sup>	
	(0.13)	(0.72)	(0.21)	(0.05)		(0.48)	(0.97)	(1.26)	(0.51)	
HCHO	1.59 <sup>b</sup>	1.68 <sup>c</sup>	2.30 <sup>c</sup>	5.28 <sup>c</sup>		3.24 <sup>c</sup>	3.20 <sup>c</sup>	2.46 <sup>d</sup>	5.60 <sup>c</sup>	
	(0.03)	(0.14)	(0.08)	(0.06)		(0.23)	(0.08)	(0.09)	(0.22)	
NaOH	1.16 <sup>b</sup>	1.48 <sup>c</sup>	2.02 <sup>c</sup>	6.64 <sup>b</sup>		1.66 <sup>d</sup>	1.63 <sup>d</sup>	1.26 <sup>d</sup>	6.12 <sup>c</sup>	
	(0)	(0.06)	(0.05)	(0.16)		(0.08)	(0.13)	(0.08)	(0.06)	

<sup>1</sup>Mean and standard deviation

<sup>2</sup>Values with different letters in the same column are statistically different ( $P < 0.05$ )

**Table II-3.** Effect of steeping sorghum varieties, Chiri and DC-75, for 8 h in HCl (0.9%, v/v), HCHO (0.05%, v/v), and NaOH (0.3%, w/v), followed by germination, on the condensed tannin content as measured by the vanillin-HCl assay in catechin equivalents (g/100 g, dry basis).

Variety	Chiri				DC-75				
	8 h	24 h	2 d	5 d	8 h	24 h	2 d	5 d	
Raw grain			3.78				6.29		
<b>Treatment</b>									
Control (water)	2.45 <sup>a 1,2</sup> (0.02)	2.06 <sup>a</sup> (0.02)	2.01 <sup>a</sup> (0.01)	3.74 <sup>a</sup> (0.04)	5.49 <sup>a</sup> (0.14)	5.62 <sup>a</sup> (0.02)	4.12 <sup>a</sup> (0.09)	5.90 <sup>a</sup> (0.14)	
HCl	2.24 <sup>b</sup> (0.03)	1.93 <sup>b</sup> (0.04)	1.44 <sup>b</sup> (0.03)	2.15 <sup>a</sup> (0.02)	5.32 <sup>b</sup> (0.02)	4.93 <sup>b</sup> (0.04)	3.57 <sup>b</sup> (0.07)	4.63 <sup>b</sup> (0.06)	
HCHO	0.45 <sup>c</sup> (0.03)	0.31 <sup>c</sup> (0)	0.92 <sup>c</sup> (0.01)	2.72 <sup>d</sup> (0.01)	0.16 <sup>c</sup> (0.01)	0.18 <sup>c</sup> (0)	0.15 <sup>c</sup> (0.01)	2.12 <sup>d</sup> (0.03)	
NaOH	0.31 <sup>d</sup> (0.01)	0.33 <sup>c</sup> (0)	0.83 <sup>d</sup> (0)	3.56 <sup>b</sup> (0.06)	0.01 <sup>d</sup> (0.01)	0.00 <sup>d</sup> (0)	0.13 <sup>c</sup> (0.01)	2.90 <sup>c</sup> (0.04)	

<sup>1</sup>Mean and standard deviation

<sup>2</sup>Values with different letters in the same column are statistically different ( $P < 0.05$ )

**Table II-4.** Effect of steeping sorghum varieties, Chiri and DC-75, for 8 h in HCl (0.9%, v/v), HCHO (0.05%, v/v), and NaOH (0.3%, w/v), followed by germination, on the condensed tannin levels as measured by the vanillin-HCl assay with blank subtraction in catechin equivalents (g/100 g, dry basis).

Variety	Chiri				DC-75			
	8 h	24 h	2 d	5 d	8 h	24 h	2 d	5 d
Raw grain			3.07				5.48	
<b>Treatment</b>								
Control (water)	1.77 <sup>a 1,2</sup> (0.02)	1.31 <sup>a</sup> (0.02)	0.51 <sup>a</sup> (0.01)	0.11 <sup>b</sup> (0.04)	4.77 <sup>a</sup> (0.14)	5.36 <sup>a</sup> (0.02)	3.59 <sup>a</sup> (0.09)	2.88 <sup>a</sup> (0.15)
HCl	1.09 <sup>b</sup> (0.03)	1.04 <sup>b</sup> (0.04)	0.57 <sup>a</sup> (0.03)	0.27 <sup>a</sup> (0.02)	4.78 <sup>a</sup> (0.02)	4.02 <sup>b</sup> (0.04)	3.07 <sup>b</sup> (0.07)	2.10 <sup>b</sup> (0.06)
HCHO	0.02 <sup>c</sup> (0.03)	0.00 <sup>c</sup> (0)	0.01 <sup>b</sup> (0.01)	0.07 <sup>b</sup> (0.01)	0.15 <sup>b</sup> (0.01)	0.00 <sup>c</sup> (0)	0.08 <sup>c</sup> (0.01)	0.18 <sup>c</sup> (0.03)
NaOH	0.31 <sup>c</sup> (0.01)	0.33 <sup>c</sup> (0)	0.02 <sup>b</sup> (0)	0 <sup>b</sup> (0.06)	0.01 <sup>c</sup> (0.01)	0.00 <sup>c</sup> (0)	0.02 <sup>c</sup> (0.01)	0.09 <sup>d</sup> (0.04)

<sup>1</sup>Mean and standard deviation

<sup>2</sup>Values with different letters in the same column are statistically different ( $P < 0.05$ )

**Table II-5.** Effect of steeping sorghum varieties, Chiri and DC-75, for 8 h in HCl (0.9%, v/v), HCHO (0.05%, v/v), and NaOH (0.3%, w/v), followed by germination, on the diastatic power (peptone extraction) of sorghum malt (SDU/g, dry basis).

Variety	SV2		Chiri		DC-75	
	2 d	5 d	2 d	5 d	2 d	5 d
<b>Treatment</b>						
Control (water)	19.3 <sup>a 1,2</sup> (0.7)	30.8 <sup>a</sup> (0.6)	17.8 <sup>c</sup> (0.3)	30.6 <sup>c</sup> (0.7)	28.3 <sup>b</sup> (0.2)	47.7 <sup>b</sup> (0.3)
HCl	7.3 <sup>c</sup> (0.4)	18.4 <sup>c</sup> (0.4)	6.8 <sup>d</sup> (0.4)	12.4 <sup>d</sup> (0.4)	15.2 <sup>c</sup> (0.4)	33.9 <sup>c</sup> (0.2)
HCHO	16.4 <sup>b</sup> (0.7)	30.0 <sup>a</sup> (0.6)	20.6 <sup>a</sup> (1.4)	38.5 <sup>a</sup> (0.7)	32.5 <sup>a</sup> (0.7)	49.3 <sup>a</sup> (0.4)
NaOH	17.1 <sup>b</sup> (0.4)	26.6 <sup>b</sup> (0.7)	19.6 <sup>b</sup> (0.4)	32.2 <sup>b</sup> (0.5)	32.1 <sup>a</sup> (0)	46.9 <sup>b</sup> (0.3)

<sup>1</sup>Mean and standard deviation

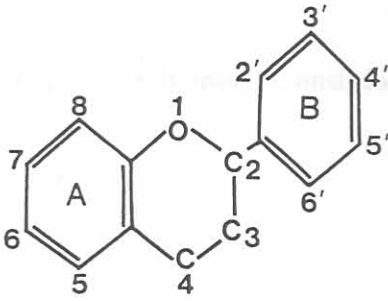
<sup>2</sup>Values with different letters in the same column are statistically different ( $P < 0.05$ )

**Table II-6.** Effect of steeping sorghum varieties, Chiri and DC-75, for 8 h in HCl (0.9%, v/v), HCHO (0.05%, v/v), and NaOH (0.3%, w/v), followed by germination, on the diastatic power (water extraction) of sorghum malt (SDU/g, dry basis).

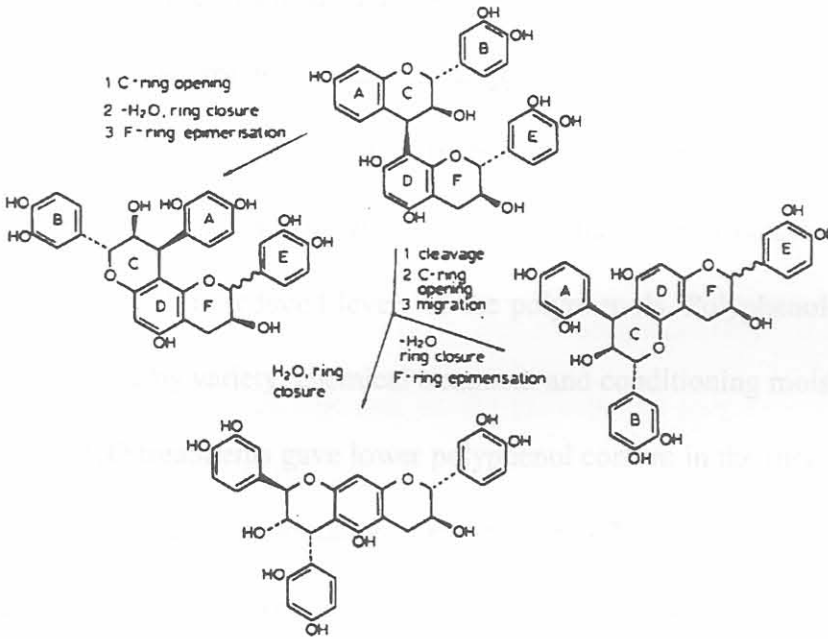
Variety	SV2		Chiri		DC-75	
	2 d	5 d	2 d	5 d	2 d	5 d
<b>Treatment</b>						
Control (water)	17.7 <sup>a1,2</sup> (0.8)	29.4 <sup>b</sup> (0.6)	1.9 <sup>c</sup> (0)	13.3 <sup>c</sup> (0.4)	0.3 <sup>c</sup> (0.2)	6.0 <sup>c</sup> (0.4)
HCl	7.4 <sup>b</sup> (0.6)	17.7 <sup>c</sup> (0.5)	0.38 <sup>d</sup> (0.1)	3.7 <sup>d</sup> (0.2)	0.0 <sup>c</sup> (0)	0.5 <sup>d</sup> (0.4)
HCHO	17.3 <sup>a</sup> (0.2)	33.0 <sup>a</sup> (0.5)	17.5 <sup>a</sup> (0.5)	32.1 <sup>a</sup> (0.9)	25.9 <sup>b</sup> (0.2)	43.5 <sup>a</sup> (0.3)
NaOH	17.4 <sup>b</sup> (1.0)	29.5 <sup>b</sup> (0.9)	15.6 <sup>b</sup> (0.4)	25.2 <sup>b</sup> (0.9)	27.6 <sup>a</sup> (0.4)	38.5 <sup>b</sup> (0.3)

<sup>1</sup>Mean and standard deviation

<sup>2</sup>Values with different letters in the same column are statistically different ( $P < 0.05$ )



(a)



(b)

**Figure II-1.** (a) Basic structure of a flavonoid ring; and (b) rearrangement reactions for proanthocyanidins to form 'phlobatannins' in mildly basic solutions. From Porter (1992).

#### 4. CHAPTER III

##### Effect of Chemical Conditioning on the Milling of High-Tannin Sorghum

###### ABSTRACT

Suitable methods are required for milling tannin-containing sorghums. Sorghum varieties, SV2 (tannin-free), Chirimaugute (medium-tannin) and DC-75 (high-tannin) were milled using a simple roller mill and a tangential abrasive decortication device. Grain was conditioned up to 20 % moisture prior to milling using HCl (0.9 %, v/v), formaldehyde (HCHO) (0.05 %, v/v), NaOH (0.3 %, v/v) and water as control. Abrasive decortication and roller milling reduced levels of the polyphenols. Polyphenol reduction was affected significantly by variety, chemical treatment and conditioning moisture ( $P < 0.001$ ). NaOH and HCHO treatments gave lower polyphenol content in the meal after roller milling, but not after abrasive decortication. Enzyme inhibition (EI) by polyphenols was reduced by 52 % after decortication or roller milling. Chemical treatment did not significantly affect EI levels after decortication. EI was lowest at 12 % and 20% moisture conditioning prior to decortication and roller milling, respectively. NaOH and HCHO treatments gave the lowest EI when Chirimaugute was roller milled at 20 % moisture. Product yield was higher at 12 % moisture for SV2 and Chirimaugute, and at 16 % for DC-75, after decortication. Conditioning to 20 % moisture prior to milling did not improve product yield. Roller milling and decortication resulted in products that were lighter in colour than the grain. Conditioning using NaOH could be advantageous in roller milling but not abrasive decortication of tannin-containing sorghums.

## INTRODUCTION

Sorghum industrial milling technology is still evolving, unlike those for wheat, rice and maize, where specialized milling technologies have been developed to give finished products of high acceptance. Roller mills with two pairs of rolls are gaining wide acceptance for small-scale maize milling in southern Africa. Conditioning sorghum using the same moisture levels applied to wheat results in a less clean fractionation as the sorghum bran is much more brittle (Perten 1977). Other researchers have revealed that roller milling results may be improved by steeping at high moisture contents under semi-wet conditions (Gomez 1993). An alternative approach to that of roller milling, is decortication. The decortication principle applied to rice and barley dehullers, decorticators and polishers has been used for sorghum (Hulse et al 1980; Reichert 1982). Abrasive decortication operates on the principle of progressively rubbing off the outer layers of the kernel (Oomah et al 1981). Traditionally, this is a dry technique requiring no conditioning so as to avoid clogging of the mill by use of high moisture grains. Decortication, followed by hammer milling, is the most common industrial sorghum milling process in southern Africa.

High-tannin sorghum challenges the miller to remove the testa layer and extract or transform the polyphenols. Mwasaru et al (1988) advocated development of harder, rounder grain with minimum polyphenol content before commercial milling of high-tannin sorghum can become economically feasible. However, it was concluded that widely cultivated Zimbabwean high-tannin sorghums lack ideal characteristics for processing (Chapter I), that is, a thin, white pericarp and corneous endosperm (Rooney

and Walker 1978). Chemical treatments have been used to detoxify tannin-containing sorghums (Daiber 1975; Reichert et al 1980; Chapter II). The objective of this study was to investigate the effect of chemical conditioning on the milling of high-tannin sorghums.

## MATERIALS AND METHODS

### Grains

Sorghum (*Sorghum bicolor* (L.) Moench) grain of varieties, SV2 and Chirimaugute, and a hybrid, DC-75, were used. DC-75 (high-tannin) and SV2 (tannin-free) have relatively corneous endosperms while Chirimaugute (medium-tannin) has a floury endosperm (Chapter I). Pericarp thickness is thick in DC-75, medium in Chirimaugute and thin in SV2 kernels (Chapter I). The sorghums were grown in the 1996/97 season at Matopos, Zimbabwe in field conditions under normal agronomic practices.

### Abrasive Decortication

A multi-sample tangential abrasive dehulling device (TADD Model 4E-230, Venables Machine Work Ltd., Saskatoon, Canada), consisting of 8-cup dehulling plate, was used for removing successive layers from sorghum grains abrasively at 1,750 rpm, following the technique developed by Oomah et al (1981) and Reichert et al (1986). Grain (20 g) was conditioned, in plastic containers, to 12, 16 or 20 % moisture using solutions of HCl (0.9 %, v/v), HCHO (0.05 %, v/v), NaOH (0.3 %, w/v) and water as control. Grains and the solution were thoroughly mixed twice. The samples were left to equilibrate overnight in the cold store at 4°C. The conditioned grains were milled for 2 min in the TADD and

the yield (calculated at 16 % moisture basis) of decorticated grain was recorded.

### **Roller Milling**

A double roll, roller mill with 1.5-mm vibrating screen, single pass (Maximill Model, Kroonstad, South Africa) was used to mill conditioned grain. The roll gaps on the mill were set at 0.15 mm for the top and 0.10 mm for the bottom roll. The top and bottom rolls were corrugated to 7 and 22 flutes/inch, respectively. Roll speeds were set at a differential of 1.25:1. Conditioning was carried out prior to milling using solutions of HCl (0.9 %, v/v), formaldehyde (HCHO) (0.05 %, v/v), NaOH (0.3 %, w/v) and water as the control. Grain (5 kg) was weighed into plastic bags. The moisture content (MC) of the grain was determined. The grain was conditioned by adding the solution to 16 or 20% moisture, the amount of which was determined by using the formula:  $\{[(100-MC)/(100-\text{desired moisture content})] - 1\} \times \text{sample weight in g}$  (AACC Method 26-95 1983). The grain was mixed thoroughly with the solution and the bag was sealed. The sample was left to equilibrate at 4°C overnight. The grain was thoroughly mixed twice during the conditioning period. The yield (calculated at 16 % moisture basis) of meal and offals obtained after roller milling was recorded.

### **Chemical analyses**

The products obtained after roller and abrasive milling were ground to pass through an 800-µm sieve prior to analyses.

*Polyphenol Analysis.* The polyphenols in milled sorghum products were measured using

the butanol-HCl (Porter et al 1986) and the vanillin-HCl (Burns 1971) methods. By using a blank subtraction with the vanillin-HCl assay, flavonoid components of the grain were eliminated and the reaction became more specific for condensed tannins (Price et al 1978). Sample extracts for the assays were obtained by shaking ground grain (0.2 g) in methanol (10 ml) for 20 min at 5 min intervals on a vortex mixer at room temperature. The supernatant was obtained by centrifuging for 10 min at 1200 x g. For the anthocyanidin production assay, 5 % HCl in n-butanol (6 ml) was added to the sample extract (1 ml) in a test tube. Iron chloride was omitted. The test tubes were placed in a forced-air oven at 100°C for 50 min. Absorbance was read at 550 nm against a reagent blank and no standard was used. The results were reported as absorbance units (A/g, dry basis). Catechin (Sigma Chemicals) was used as a standard in the vanillin assay and sample blanks were included. The results were expressed in catechin equivalents (g/100 g, dry basis).

*Enzyme Inhibition.* Enzyme inhibition in sorghum flour was determined following the method of Daiber (1975b). An enzyme extract was obtained by milling malt (5 g) in distilled water (100 ml) in an Ultra Turrax model T25 (Janke and Kunkel IKA-Labortechnik, Staufen, Br., Germany) for 5 min and centrifuging the sample for 5 min. The extract (12 ml) was added to sorghum flour (120 mg) and the sample incubated for 30 min at 30°C. A blank sample containing no sorghum flour was included. After incubation, samples were centrifuged for 5 min and the supernatant was used for diastasis. Diastatic power (DP) was determined and percentage enzyme inhibition calculated as  $100 \times (\text{original} - \text{residual activity}) / (\text{original activity})$ .

*Colour Values.* Hunter L, a, and b values were obtained using a Hunter Colour Quest 45/0 LAV (Hunter Associates Laboratory, Inc. Reston, Virginia). An average of three readings per sample was taken.

### **Statistical analyses**

The general linear model procedure of SAS version 6.12 (SAS Institute, Cary, NC) was used. Analysis of variance was used to determine the effect of variety, treatment, and moisture level prior to milling on polyphenol content, yield and quality of product. Means were separated using the least squares difference (LSD) at  $P < 0.05$ .

## **RESULTS AND DISCUSSION**

The choice of chemical treatments for milling was based on previous reports using alkali (Chavan et al 1979, Reichert et al 1980), HCl (Reichert et al 1980), HCHO (Daiber 1975a), high-moisture reconstitution (Reichert et al 1980; Mitaru et al 1983) to improve the nutritional value of high tannin-sorghums. The selected concentrations were based on findings by Daiber (1975a) on HCHO and by Dewar et al (1997) on NaOH. The information obtained from preliminary experiments on steep water uptake using various concentrations of HCl was used as the basis for selecting the concentration of the acid. Conditioning moisture for roller milling was selected following findings by Gomez (1993). Conditioning during decortication is practised in the traditional mortar and pestle method up to about 20 % (Eggum et al 1982) but machine decortication in an abrasive mill is a relatively dry milling technique.

## Product Yield

Yield of decorticated grain (87 to 96 %) was highest in SV2, a tannin-free variety (Table III-1). Oomah et al (1983) found extraction rates ranging from 71 to 98 % for 23 white sorghum varieties. DC-75 gave slightly higher yields than Chirimaugute (84 and 81 %, respectively) at 12 % moisture. Video image analysis of the endosperm texture of these grains indicated an increasing floury endosperm from SV2, DC-75 and Chirimaugute (Chapter I). Thus yield was more related to the floury nature of the grains as previously reported by Chibber et al (1978) and Reichert (1982) than their polyphenol content. The importance of a relatively corneous endosperm was emphasized by Munck et al (1982) when he noted that the introduced Tanzanian sorghum with 31 % soft endosperm was giving lower yields of 50 % compared to the local hard cultivars (3.9 to 13.6 % soft endosperm) that gave recoveries of 72 to 86 %.

Conditioning to 20 % resulted in lower yields for all varieties. Highest yields were achieved at 12 % with SV2 and Chirimaugute. Thus conditioning prior to decortication was not an advantage for the purpose of increasing product yield. However, yields were slightly improved at 16 % for DC-75. Meal yield was decreased by up to 8, 6, or 7 % on average when SV2, DC-75 and Chiri, respectively, were conditioned to 20 % moisture. High-tannin sorghum lines that could be abrasively decorticated and yield at least 70 % of product were also identified elsewhere (Reichert et al 1988). Subramanian et al (1988) found a correlation coefficient of +0.89 ( $P < 0.01$ ) when the traditional mortar and pestle method and the TADD abrasion equipment were used for sorghum milling. However, the

reported yields could be slightly lowered under commercial operations as in most laboratory decorticators such as the modified Udy Cyclone mill by Shepherd (1979) and the TADD abrasion mill, the frictional forces between the grains are minimized, and the grains are decorticated in a gentler way compared to commercial decorticators (Munck 1995).

Treatment did not significantly affect yield of roller-milled product (Table III-1). SV2 and DC-75 gave higher product yield than Chirimaugute. The latter has a floury endosperm (Chapter I) and loss of endosperm fractions into the offal (bran) resulted in lower yield. However, yield of product was relatively higher than values previously reported for brown sorghums (Chibber et al 1978). The findings on product yield confirm the report by Gomez (1993) that roller milling of sorghum under semi-wet conditions (16 % moisture) is superior to dry milling (12 % moisture). Eggum et al (1982) demonstrated that only 59 % (compared to 79 % in wheat) of lysine remain in hand-dissected sorghum endosperm, and hence, in milling sorghum, a high extraction rate of 85 % is desirable. Extraction rates were reasonably high at the 16 % conditioning moisture with all the three varieties. There was no advantage in conditioning to 20 % moisture as yield of offal increased (Table III-1). Yield of offal, a by-product of sorghum milling, should be minimal as offal has less economic value as an animal feed than meal.

### **Polyphenol Content (Butanol-HCl Assay)**

The polyphenol content of the meal was significantly affected ( $P < 0.001$ ) by variety, chemical treatment and conditioning moisture prior to abrasive decortication or roller

milling (Table III-2). Reduction in polyphenols, after milling, was due to loss of grain pericarp and testa layers as offal. Polyphenol content was markedly lower when abrasive decortication was used. Roller milled flour apparently had more offal contamination. Conditioning to 12 % moisture followed by decortication, a technique equivalent to the traditional dry abrasion practice, reduced polyphenol levels by 71 to 81 % in DC-75 and Chirimaugute, respectively. Polyphenol reduction was greatest at 12 % moisture and no further advantage was obtained by conditioning to higher moisture prior to decortication. It was presumed that polyphenol reduction was largely due to the abrading action. At 16 and 20 %, DC-75 flour had higher polyphenol content than at 12 % (Table III-2). Polyphenol content was 29 to 36 % less when DC-75 grain was conditioned to 16 % moisture and roller milled. Higher conditioning treatment resulted in better separation of offal from endosperm as polyphenol content was lower when Chirimaugute was conditioned to 20 than 16 % moisture. NaOH and HCHO generally gave lower polyphenols than water and HCl for both varieties as previously observed in steeping for malting (Chapter II). NaOH and HCHO treatments of high-tannin sorghums for feed have also been found to improve the weight gain of birds (Schutte and Smith 1991).

### **Polyphenol Content (Vanillin-HCl Assay)**

Polyphenol reduction (range 12 - 53 %) was less pronounced in decorticated samples with the vanillin than with the butanol-HCl assay (Table III-3). The vanillin method tends to underestimate the weight of condensed tannins due to their reduced reactivity (Goldstein and Swain 1963). Polyphenol content was reduced by 41 % for DC-75 and 49 % for Chirimaugute after decortication at 12 % moisture. Reduction in polyphenol content was

due to loss of the grain outer layers by abrasion. Chibber et al (1978) and Mwasaru et al (1988) reduced polyphenol content by up to 91 % using a similar dry abrasion technique, but at lower extraction rates. Polyphenol reduction exceeding 55 % was not achieved as yield of product was kept relatively high. Higher conditioning moisture during decortication resulted in less polyphenol extraction for both varieties presumably due to reduced abrasion of the outer layers of moist grain. Roller milling DC-75 after conditioning to 16 % moisture gave flour products that had higher polyphenol content than dry abrasive decortication at 12 %, giving further evidence that polyphenols were largely removed by the abrading action. However, polyphenol content was markedly reduced by 56 to 71 % after roller milling Chirimaugute at 20 % moisture. Polyphenol content was lower at 20 than 16 % in Chirimaugute roller milled meal. At 20 % moisture, HCHO and NaOH gave lower polyphenol content in Chirimaugute roller meal. Polyphenol reduction possibly involved formation of high molecular weight polymers (Porter 1992) that were cross-linked and insoluble.

### **Polyphenol Content (Vanillin-HCl with Blank Subtraction)**

Tannin values differed significantly ( $P < 0.001$ ) among treatments. Decortication resulted in lowest tannin levels at 12 % than at 16 and 20 % (Table III-4). The abrasive action of the TADD was mostly responsible for tannin removal in Chirimaugute and DC-75 grains. The tannin content of roller milled meal was reduced by 15 to 20 % and 18 to 44 % when DC-75 and Chirimaugute respectively, were conditioned to 16 % (Table III-4). Tannin reduction was higher for Chirimaugute than DC-75 when the vanillin method with blank extraction was used. The findings confirm the work of Reichert et al (1980) where

tannins were reduced by 97, 83, 39 % when sorghum grains imbibed 0.8 M NaOH, 0.8 M HCl and water, respectively at 25 % by weight at 25°C with an equilibration period of 2 d. The differences in tannin reduction could be due to higher solution concentrations used in their experiments. Tannin reduction levels of 24-52 % were also reported when 10 % by weight of sorghum grain of 0.5 or 0.2 M NaOH was used for treatment of high-tannin sorghum that were then stored for 3 and 11 days (Price et al 1979). At 16 and 20 % moisture HCHO and NaOH generally gave lower tannin content in Chirimaugute meal.

### **Tannin Content of Offal (Modified Vanillin Assay)**

Tannin levels differed significantly ( $P < 0.001$ ) among treatments (Table III-4). The tannin content of the offal from the roller mill was higher than that of the whole grain, as the offal contained most of the pericarp and testa layers. Tannin content of offal was 72 - 126 % greater than in DC-75 grain at 16 % conditioning moisture. However, Chirimaugute had 0 - 30 % more tannin in its offal at the same moisture presumably due to higher endosperm contamination. Tannin content in the offal increased by 49 to 133 % when Chirimaugute was conditioned to 20 % moisture prior to roller milling. Thus conditioning Chirimaugute grains to higher moisture resulted in more tannins being extracted into the offal or less endosperm contamination of the offal. Tannin content of the offal was lower with NaOH and HCHO treatment at 16 % moisture. The findings are very important if offal is used for feeding purposes. At 20 % moisture, the tannin levels of Chirimaugute offal were markedly high with HCl. This confirms the findings in Chapter II that NaOH and HCHO are more effective than HCl in tannin deactivation.

## Enzyme Inhibition by Polyphenols in the Meal

Enzyme inhibition (EI) assays indicated significant differences between the two varieties (Table III-5). The mean EI for Chirimaugute and DC-75 flour was 16 and 41 %, respectively. This was presumed to be due to the higher tannin content in DC-75 grains (Chapter I) that could not be completely removed. Chemical treatment had no effect on EI after abrasive decortication confirming the findings that abrasion was mostly responsible for polyphenol reduction. EI was similar at 16 and 20 % (mean 30 % EI) but differed significantly from the 12 % (mean 25 % EI) conditioning moisture. Thus conditioning to higher moisture was not important in abrasive decortication for the purpose of reducing the enzyme inhibitory power of polyphenols.

The control (water) gave higher inhibition that differed significantly from HCl, HCHO and NaOH treatment in roller milling. EI in Chirimaugute meal was higher at 16 than 20 % moisture (31 and 24 %, respectively). NaOH and HCHO gave the lowest enzyme inhibition when Chirimaugute was conditioned to 20 % moisture prior to roller milling. Thus roller milling at higher moisture showed the effectiveness of NaOH and HCHO on polyphenol reduction as previously reported in steeping experiments (Chapter II).

## Product Colour

In sorghum milling, colour improvement is of primary importance for consumer acceptance. Variety, conditioning treatment and moisture significantly affected ( $P < 0.001$ ) product colour (Table III-6). There was no advantage in conditioning up to 20 % moisture prior to abrasive decortication for the purpose of improving flour colour as L

values were highest at 12 % (Table III-6). Chirimaugute produced the darkest flour although its polyphenol content was significantly less than that of DC-75. Factors other than polyphenol content alone, are involved in determining the colour of sorghum products (Rooney and Miller 1982). Hunter L values differed significantly among treatments with HCl giving a slight improvement in flour colour.

SV2 had higher L values than DC-75 and Chirimaugute after roller milling. SV2 is tannin-free variety, unlike the latter (Chapter I). Flour colour was improved more at 20 % than 16 % moisture for SV2 and Chirimaugute. HCl gave slightly higher L values than other treatments after roller milling SV2 and DC-75 as found in abrasive milling. The mean increase in L values was 7, 8 and 15 % after roller milling SV2, Chirimaugute and DC-75, respectively.

## CONCLUSIONS

Conditioning treatment and moisture prior to roller milling of high-tannin sorghums improved product yield and reduced polyphenol content of the meal. Conditioning grains prior to abrasive decortication neither gave significant reduction in polyphenol content nor improved colour and yield of decorticated flour. Enzyme inhibition was higher in products obtained from the high-tannin than the medium-tannin variety. Polyphenol content was not related to yield and colour of the product after milling. The findings that abrasive and roller milling plus HCHO and NaOH reduced tannin content of offal could be important if offal is used for animal feed. Additional chemical work is needed on sorghum tannin so as to understand its chemical fate during processing.

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**Table III-1.** Effect of conditioning sorghum varieties, DC-75 and Chiri, to 16 and 20%, using water, HCl (0.9%, v/v), HCHO (0.05%, v/v), and NaOH (0.3%, w/v), prior to abrasive decortication and roller milling, on the meal and offal yield (% extraction at 16 % moisture).

Variety	SV2			DC-75			
% Conditioning moisture content	12 AD <sup>1</sup>	16 AD	20 AD	16 RM	20 RM	16 RM-offal	20 RM-offal
Water (control)	95.0b <sup>2</sup>	94.7ab	87.8a	90.9	79.8	6.9	13.1
HCl	95.3ab	94.9a	87.8a	90.5	85.2	6.4	9.1
HCHO	95.3ab	94.6bc	86.7b	91.8	84.7	6.6	9.2
NaOH	95.6a	94.1c	87.8a	89.5	83.9	7.4	9.8
Mean	95.3	94.6	87.5	90.7	83.4	6.8	10.3

Variety	DC-75			Chiri			
% Conditioning moisture content	12 AD <sup>1</sup>	16 AD	20 AD	16 RM	20 RM	16 RM-offal	20 RM-offal
Water (control)	83.6c <sup>2</sup>	86.8ab	84.6ab	88.5	82.2	7.4	10.6
HCl	84.1bc	87.0a	84.4ab	87.5	82.0	8.0	11.3
HCHO	84.4ab	86.9ab	84.8a	88.1	81.8	7.0	10.4
NaOH	85.0a	86.3b	84.3b	89.9	82.9	6.7	10.9
Mean	84.3	86.8	84.5	88.5	82.2	7.3	10.8

<sup>1</sup>AD = abrasive decortication, RM = roller milling.

<sup>2</sup>Values with similar letters in the same column are not statistically different (P < 0.05).

**Table III-2.** Effect of conditioning sorghum varieties, DC-75 and Chiri, to 16 and 20%, using water, HCl (0.9%, v/v), HCHO (0.05%, v/v), and NaOH (0.3%, w/v), prior to abrasive decortication or roller milling, on the tannin content of the meal as measured by the butanol-HCl assay (A/g, dry basis).

Variety	DC-75				Chiri				
	12 AD <sup>1</sup>	16 AD	20 AD	16 RM	12 AD	16 AD	20 AD	16 RM	20 RM
% Conditioning moisture content	45.9	45.9	45.9	45.9	29.3	29.3	29.3	29.3	29.3
Tannin content of raw grain	45.9	45.9	45.9	45.9	29.3	29.3	29.3	29.3	29.3
<b>Treatment</b>									
Water (control)	10.6b <sup>2</sup>	18.3b	20.8a	32.3a	6.5a	8.2a	8.0a	22.1a	6.3b
HCl	13.2a	15.7c	17.5bc	32.6b	6.2a	7.9a	7.9a	17.6b	9.3a
HCHO	13.1a	18.0b	15.2c	30.2c	5.4b	6.8b	5.3b	14.2c	5.0c
NaOH	13.4a	22.9a	17.9b	29.6c	6.3a	7.7a	4.8b	15.3c	5.8bc

<sup>1</sup>AD = abrasive decortication, RM = roller milling.

<sup>2</sup>Values with similar letters in the same column are not statistically different ( $P < 0.05$ ).

**Table III-3.** Effect of conditioning sorghum varieties, DC-75 and Chiri, to 16 and 20%, using water, HCl (0.9%, v/v), HCHO (0.05%, v/v), and NaOH (0.3%, w/v), prior to abrasive decortication or roller milling, on the polyphenol content of the meal or offal as measured by the vanillin-HCl assay in catechin equivalents (g/100 g, dry basis).

Variety	DC-75					Chiri						
	12 AD <sup>1</sup>	16 AD	20 AD	16 RM	16 RM	12 AD	16 AD	20 AD	16 RM	20 RM	16 RM	20 RM
% Conditioning moisture content	6.3	6.3	6.3	6.3	(Offal)	3.8	3.8	3.8	3.8	3.8	(Offal)	(Offal)
Polyphenol content of raw grain	6.3	6.3	6.3	6.3	(Offal)	3.8	3.8	3.8	3.8	3.8	(Offal)	(Offal)
<b>Treatment</b>												
Water (control)	3.8a <sup>2</sup>	4.7b	4.8c	5.0a	12.9b	1.8c	2.3b	2.3b	2.4d	1.3b	5.1b	7.4c
HCl	3.6b	4.6c	5.6a	4.6c	13.7a	1.9b	2.4a	2.6a	3.0a	1.7a	5.7a	9.6a
HCHO	3.6b	4.5d	4.8c	4.7b	11.0c	1.9b	2.1c	2.0d	2.5c	1.1d	4.8c	6.1d
NaOH	3.8a	4.9a	5.0b	4.7b	10.7d	2.1a	2.3b	2.1c	2.6b	1.2c	4.6d	8.0b

<sup>1</sup>AD = abrasive decortication, RM = roller milling.

<sup>2</sup>Values with similar letters in the same column are not statistically different ( $P < 0.05$ )

**Table III-4.** Effect of conditioning sorghum varieties, DC-75 and Chiri, to 16 and 20%, using water, HCl (0.9%, v/v), HCHO (0.05%, v/v), and NaOH (0.3%, w/v), prior to abrasive decortication or roller milling, on the tannin content of the meal as measured by the vanillin-HCl assay with blank subtraction in catechin equivalents (g/100 g, dry basis).

Variety	DC-75					Chiri						
	12 AD <sup>1</sup>	16 AD	20 AD	16 RM	16 RM	12 AD	16 AD	20 AD	16 RM	20 RM	16 RM	20 RM
% Conditioning moisture content												
Tannin content of raw grain	5.5	5.5	5.5	5.5	(Offal)	3.1	3.1	3.1	3.1	3.1	(Offal)	(Offal)
<b>Treatment</b>												
Water (control)	3.4b <sup>2</sup>	4.3b	4.3c	4.7a	11.6b	1.2c	1.6b	1.6b	1.7d	0.8b	3.4b	5.6c
HCl	3.2d	4.3b	5.1a	4.4b	12.4a	1.3b	1.7a	1.8a	2.5a	1.2a	4.0a	7.2a
HCHO	3.3c	4.1c	4.3c	4.4b	9.8c	1.2c	1.4c	1.3d	2.1b	0.7c	3.0d	4.6d
NaOH	3.5a	4.5a	4.5b	4.4b	9.4d	1.4a	1.6b	1.5c	2.0c	0.7c	3.1c	6.2b

<sup>1</sup>AD = abrasive decortication, RM = roller milling.

<sup>2</sup>Values with similar letters in the same column are not statistically different (P < 0.05).

**Table III-5.** Effect of conditioning sorghum varieties, DC-75 and Chiri, to 16 and 20%, using water, HCl (0.9%, v/v), HCHO (0.05%, v/v), and NaOH (0.3%, w/v), prior to abrasive decortication or roller milling, on enzyme inhibition (%) by the polyphenols in the meal.

Variety	DC-75				Chiri				
	12 AD <sup>1</sup>	16 AD	20 AD	16 RM	12 AD	16 AD	20 AD	16 RM	20 RM
% Conditioning moisture content	80	80	80	80	33	33	33	33	33
% Enzyme inhibition of raw grain									
<b>Treatment</b>									
Water (control)	36b <sup>2</sup>	46a	42c	54a	17a	12c	23a	19a	13a
HCl	24c	40b	51a	41b	12c	14bc	18b	17b	13a
HCHO	38b	41b	38d	38c	14b	17a	19b	14c	9b
NaOH	40a	46a	45b	43b	17a	16ab	11c	19a	9b
Mean (% reduction in inhibition)	58	46	45	46	55	56	46	48	67

<sup>1</sup>AD = abrasive decortication, RM = roller milling.

<sup>2</sup>Values with similar letters in the same column are not statistically different ( $P < 0.05$ ).

**Table III-6.** Effect of conditioning sorghum varieties, DC-75 and Chiri, to 16 and 20%, using water, HCl (0.9%, v/v), HCHO (0.05%, v/v), and NaOH (0.3%, w/v), prior to abrasive decortication and roller milling, on meal lightness as measured by the Hunter Lab (L values).

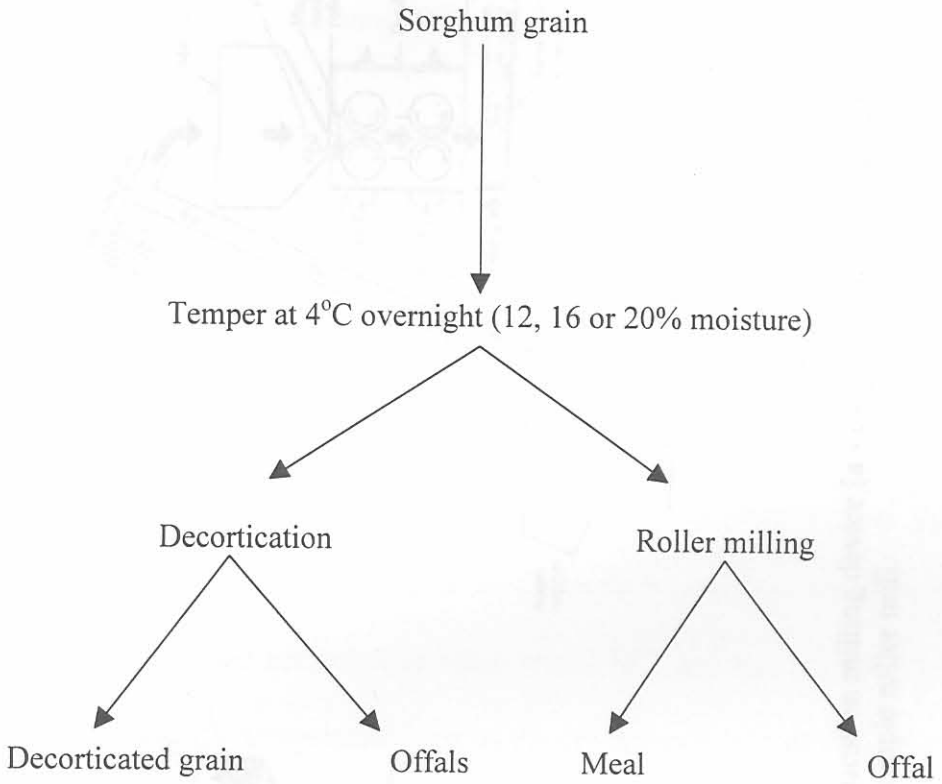
Variety	SV2			DC-75			Chiri		
Conditioning moisture content	12% AD <sup>1</sup>	16% AD	20% AD	12% AD	16% AD	20% AD	12% AD	16% AD	20% AD
L value of whole grain flour	74.35	74.35	74.35	60.68	60.68	60.68	59.52	59.52	59.52
Water (control)	76.7c <sup>2</sup>	76.1b	76.0b	68.5d	66.5c	66.8b	66.3a	65.6a	65.1d
HCl	75.0a	76.3a	76.5a	68.8b	67.6a	67.2a	66.2ab	65.4b	65.6a
HCHO	76.9b	75.9c	75.8c	69.0a	67.0b	66.2c	66.2b	65.6a	65.4b
NaOH	76.5d	76.2a	75.7c	68.7c	66.3c	66.1c	66.2ab	65.3b	65.2c
Mean (% increase in L value)	3	2	2	10	10	10	11	10	10

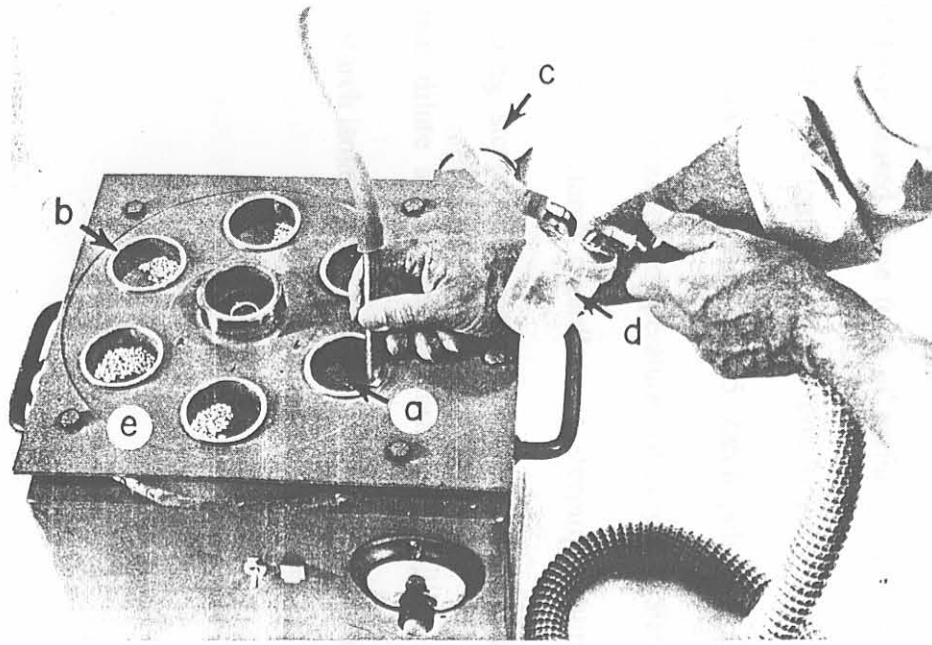
Variety	SV2		DC-75	Chiri	
Conditioning moisture content	16% RM	20% RM	16% RM	16% RM	20% RM
L value of whole grain flour	74.35	74.35	60.68	59.52	59.52
Water (control)	79.7a <sup>2</sup>	80.4b	67.1d	65.6a	68.2b
HCl	79.8a	80.5a	69.0a	63.8b	67.7c
HCHO	78.8b	80.4b	68.7b	64.4d	68.5a
NaOH	79.7a	80.1c	67.7c	63.7c	67.6d
Mean (% increase in L value)	7	8	11	8	14

<sup>1</sup>AD = abrasive decortication, RM = roller milling.

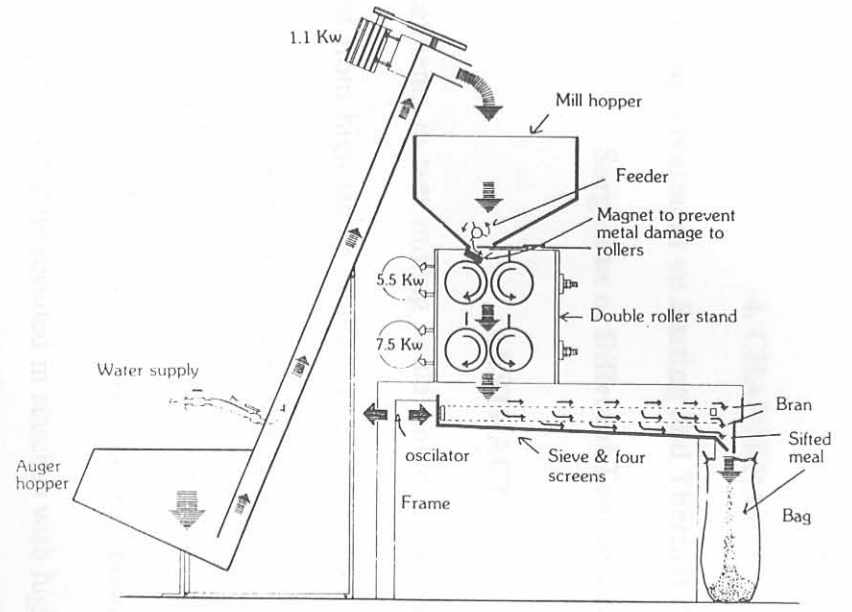
<sup>2</sup>Values with similar letters in the same column are not statistically different (P < 0.05).



**Figure III-1.** Procedures used for abrasive decortication and roller milling of sorghum.



(a)



(b)

**Figure III-2.** Diagrams illustrating the (a) abrasive decortication milling device [a - carborundum stone, b - sample cups, c - internal fan, d - vacuum device, e - dehulling headplate]; and (b) simple roller mill.

#### 4. CHAPTER IV

### Effect of Steeping Treatment on Pasting and Thermal Properties of Starches from Sorghums of Different Tannin Content

#### ABSTRACT

Chemical treatments in wet milling could improve the physico-chemical properties of starch isolated from high-tannin sorghums. Sorghum varieties, Chirimaugute (medium-tannin), DC-75 (high-tannin) and SV2 (tannin-free) were steeped in water, dilute HCl (0.9%, v/v), formaldehyde (0.05%, v/v) and NaOH (0.3%, w/v) solutions prior to wet milling and starch separation. Pasting, textural and thermal properties of starch were determined. Steeping in NaOH resulted in starches with higher peak viscosity (PV), cool paste viscosity (CPV) and setback than when water, HCl and formaldehyde were used. The time to PV and PV temperature were markedly reduced by treatment with NaOH. NaOH could have caused some form of partial pre-gelatinisation. HCl treatment gave starches with higher PV temperature and took longer to reach PV presumably due to acid hydrolysis. The low hardness of DC-75 starch gels was slightly improved in NaOH-treated grains. Gelatinization temperatures of sorghum starches were generally low in comparison to maize starch regardless of steeping treatment. Surprisingly, chemical treatments during steeping of sorghum grains greatly affect starch properties. It is concluded that dilute alkali steeping during wet milling could be used to improve properties of starch isolated from tannin-containing sorghums.

## INTRODUCTION

Tannin-containing sorghum grain poses problems to the food processor due to appearance and the anti-nutritional effects of condensed tannins (Butler 1982). Milling sorghum grains by decortication, as practised in southern Africa, removes most of the tannins located in the outer layers of the grain (Chibber et al 1978). However, most high-tannin sorghums have a soft, floury endosperm that makes decortication inefficient (Chibber et al 1980; Mwasaru et al 1988). Chemical treatments using water, NaOH, or HCl (Reichert et al 1980) and formaldehyde (Daiber 1975) have been shown to reduce or deactivate tannins in sorghum grain. NaOH also improves water uptake of sorghum grains (Dewar et al 1997). Yang and Seib (1995) used NaOH to improve the brightness of sorghum starch. However, there is no information on the effect of chemical treatment on starch properties of high-tannin sorghums.

The possible adsorption and retention of tannins by starch if extracted from tannin-containing sorghum varieties has been indicated (Davis and Hosney 1979). The pink colour of sorghum starch has been associated with pigments in the pericarp and endosperm of the grain (Freeman and Watson 1971, Norris 1971). Subramanian et al (1994) implicated alcohol-soluble components as causing the dullness of sorghum starch. Sorghum starches also exhibit high gelatinisation temperature ranges (71-81°C) (Akingbala et al 1982). The objective of this investigation was to determine the effect of chemical treatment during steeping on pasting, textural, and thermal properties of starch extracted from a tannin-free, medium-tannin and tannin-rich sorghum.

## MATERIALS AND METHODS

### Samples

Three sorghum varieties grown under uniform field conditions in the 1996/97 season at Matopos, Zimbabwe, were used: Chirimaugute (landrace, medium-tannin) and DC-75 (hybrid, high-tannin) and SV2 (cultivar, tannin-free).

### Starch Extraction

Starch was extracted following a combination of the methods used by Watson et al (1955), Perez et al (1993) and Zhao and Whistler (1994). Sorghum grain (100 g) was steeped in 200 ml water, HCl (0.9 %, v/v), formaldehyde (HCHO) (0.05 %, v/v) or NaOH (0.3 %, w/v) at 5°C for 24 hr. The steeped grain was washed and ground with an equal volume of water using a Waring blender. The slurry was filtered through a 200-mesh sieve (75 µm opening screen). The material remaining on the sieve was rinsed with water. Grinding and filtering was repeated on this material. The material still remaining on the sieve was discarded. The filtrate was centrifuged at 760 x g for 10 min. The grey-coloured top protein layer was removed. Excess water was added to resuspend the sample and centrifugation was carried out for 3 min. Washing and re-centrifugation was repeated until the top starch layer was white. The starch was dried for 24 hr at 40°C.

### Pasting Properties

Pasting properties of sorghum starches were determined using the Rapid Visco Analyser model 3D (RVA) (Newport Scientific, Narrabeen, Australia). Sorghum starch (3 g, 14 % moisture basis) was mixed with 25 g of accurately weighed water (10.32 % dry solid

content) in the aluminium canister. Peak viscosity (PV), temperature at PV, time to PV, holding strength (hot paste viscosity) (HPV), cool paste viscosity (CPV), and setback (CPV-PV) were recorded. Two replicates per sample were analysed.

### **Textural Properties**

The sample from RVA testing was allowed to stand for 24 hr at room temperature for gelation to take place (Bhattacharya et al 1997). Hardness or firmness of the starch gel was measured using an SMS Model TA-TX2 texture analyser (Stable Micro Systems, Godalming, England). A standard two-cycle programme was used to compress the gels for a distance of 10 mm at a crosshead speed of 30 mm/min using a 7-mm cylindrical probe with a flat end. Hardness was recorded as maximum force on cycle one in grams (g). Four repeat measurements were taken of each of the two gel replicates per sample.

### **Amylose / Amylopectin Ratio**

An iodine-binding spectrophotometric method using 0.2 % iodine in 2.0 % potassium iodide was used (Juliano et al 1981). Samples of starch (100 mg) were weighed in volumetric flasks (100 ml). One millilitre of ethanol (95 %) was used to wash the sample down the flask. Nine millilitres of NaOH (1 M) was added to the starch sample before heating the flasks in a boiling water bath for 10 min. The samples were cooled and the volume made up to 100 ml with distilled water. The contents were mixed vigorously to disperse the starch. Amylose/amylopectin standard mixtures were prepared to represent from 0 to 60 % amylose content. For the iodine colour development, an aliquot (5 ml) of each solution was taken to which 1 M acetic acid (1 ml) was added. The contents were

mixed. The solutions were allowed to stand for 20 min after mixing with iodine solution (2 ml). Absorbance was read at 620 nm.

### **Thermal Properties**

A Mettler DSC-20 differential scanning calorimeter (Mettler-Toledo AG Instruments, Naenikon-Uster, Switzerland) was used to measure thermal properties of the starch. Starch (2 mg, dwb) was weighed directly into a 40- $\mu$ L aluminium standard pan and water added to give a final weight of 6.5 mg. The pan was covered with the lid and hermetically sealed. After equilibration at room temperature, the sample was heated from 30°C to 120°C at the rate of 10°C per min. The gelatinisation temperature ( $^{\circ}$ C) parameters of onset temperature ( $T_o$ ), peak ( $T_p$ ), and conclusion ( $T_c$ ) were determined. Gelatinisation enthalpy ( $\Delta H$ ) in J/g was also recorded. Two replicates per sample were analysed.

### **Statistical Analysis**

Data was analysed using ANOVA procedures of the Statistical Analysis System version 6.10 (SAS Institute, Cary, NC). Means were compared at the 5 % significance level using Duncan's Multiple Range Tests.

## **RESULTS AND DISCUSSION**

### **Starch colour**

The colour of starch from NaOH-treated grain, was brighter than when water, HCl or HCHO were used during steeping. The effect of NaOH on brightness of sorghum starch

colour has been reported before (Yang and Seib 1995). SV2 gave a white starch with all treatments. Concerning SV2, it is a tannin-free variety with white pericarp and tan glumes. However, DC-75 and Chirimaugute gave pink-coloured starches presumably due to the polyphenols present in the pigmented testa layer.

### **Pasting and Swelling Properties**

The pasting properties of sorghum starches are arranged in Table IV-1 according to treatment and variety. Starch pasting and swelling properties were affected by treatment and variety at  $P < 0.05$  (Table IV-1). The peak viscosity (PV) was increased by the NaOH treatment. PV is an indication of the water binding capacity of the starch (Newport Scientific 1998). Treatment with NaOH apparently enhanced starch swelling causing an increase in viscosity. Molecules containing ionic groups have intensified water-binding ability at the ionic location, and, in addition, their repulsive charges cause the starch molecules to repel each other (Whistler and BeMiller 1997). Thus NaOH presumably hydrated the starch molecules and also caused some partial pre-gelatinisation. HCl and HCHO treatment gave relatively lower PV. HCl-treated sorghum could have undergone mild, acid-catalysed hydrolysis resulting in starch depolymerisation and hence lower-viscosity products (Whistler and BeMiller 1997). HCHO presumably cross-linked the polymers, particularly protein and polyphenols, present as minor constituents in the starch causing a reduction in swelling power. Starch from DC-75 had the highest PV regardless of treatment probably due to its relatively higher amylopectin content than the other starches (Table IV-2).

The ability of starch to withstand heating and shear stress was recorded as hot paste viscosity (HPV). Subjecting a starch sample to a constant high temperature (95°C) and mechanical shear causes more granules to disintegrate, and amylose molecules will generally leach out into the solution and undergo alignment, resulting in a greater decrease in viscosity (Whistler and BeMiller 1997). NaOH and HCl treatments gave markedly higher HPV than water and HCHO for SV2 and Chirimaugute starches. Starch from NaOH-treated grain, apparently underwent excessive shear thinning because the highly, swollen granules were broken up easily. Acid-modified starches also break up easily and dissolve when heated in water (Whistler and BeMiller 1997). The tannin-rich variety, DC-75 gave starch with the lowest HPV presumably due to the low starch amylose content. Researchers have surmised that the degree of shear thinning may also be related to the morphology and rigidity of the swollen granules (Williams and Bowler 1982, Steeneken 1987). Cool paste viscosity (CPV) and setback were generally higher in starch from NaOH-treated grain. Mistry and Eckhoff (1992) reported that an alkali extraction process results in maize starch with higher viscosity and higher hydration capacity than the commercial maize starch.

Starch from NaOH-treated grain took less time to reach PV presumably due to partial gelatinisation (Table IV-1). However, HCl treatment gave starches that took longer to reach PV probably due to acid hydrolysis. The time to reach PV was lowest for DC-75 due to its higher amylopectin content that accelerated granule swelling than in other starches. PV temperature was markedly lowered in starches from NaOH-treated grain as the partially gelatinised starch granules easily produced high viscosity pastes. Treatment

with HCl gave starches with higher PV temperature as more energy was required to produce viscous pastes from some of the relatively shortened starch chains. The PV temperature range (80.8-82.3°C) was lower for DC-75 starch compared to other starches that had higher amylose content.

### **Textural Properties**

Hardness or firmness of the starch gels was significantly affected by both treatment and variety at  $P < 0.05$  (Table IV-2). All treatments resulted in DC-75 starches with very soft gels. This was due to low amylose content of DC-75 as retrogradation of cooked starch involves both starch polymers, with amylose undergoing retrogradation at a much more rapid rate than does amylopectin (Whistler and BeMiller 1997). A firmer gel can also be prepared with starch from corneous rather than the floursy endosperm of sorghum (Cagampang and Kirleis 1984). However, a firm starch gel was still obtained from Chirimaugute, a variety with a floursy endosperm texture largely due to its relatively high amylose content. With the exception of Chirimaugute, NaOH generally gave starches with firmer gels than other treatments. Thus treatment with NaOH enhanced retrogradation as evidenced by the high setback values of the starch pastes (Table IV-1). The control (water) resulted in a gel that was less firm and significantly different from all the other treatments for SV2 starch. Gel firmness was largely determined by the amount of amylose present in the starches.

### **Thermal properties**

Starch from NaOH-treated grain generally had slightly higher gelatinisation temperatures

than the water, HCl and HCHO treatments (Table IV-3). The effect of NaOH on lowering PV temperature during pasting could not be compared with starch thermal properties. Gelatinisation refers to the disruption of molecular order within starch granules while PV is reached when some of the highly swollen granules have ruptured and fragmented due to shearing forces (Whistler and BeMiller 1997). Steeping SV2 and DC-75 in HCHO or NaOH gave starches with similar  $T_p$  values.  $T_p$  values for starches isolated from Zimbabwean varieties were lower, regardless of treatment, than values reported by Akingbala et al (1982) that ranged from 74 to 76°C for normal sorghum starches.  $T_p$  values for sorghum starches from NaOH-treated grain were also lower than those reported for eight maize starches (72-75 °C) extracted in alkali (Mistry and Eckhoff 1992).  $T_c$  was lowest when water was used for steeping. Means of  $T_o$ ,  $T_p$  and  $T_c$  for starches isolated from 35 tropical maize germplasm accessions were 63.0, 71.0, 77.8°C, respectively (Li et al 1994), values similar or slightly higher than the sorghum starches studied. These findings confirm theories by Taylor et al (1997) that southern African sorghums may have a relatively low gelatinisation temperature range. The enthalpy for gelatinisation ( $\Delta H$ ) was quite narrow and ranged from 9.0 to 10.8 J/g.  $\Delta H$  was significantly higher in HCHO-treated DC-75 starch than when other steeping solutions were used. HCHO could have largely reacted with tannins (Daiber 1975) or proteins and had little effect on the molecular order of the starch granule. However, starches of SV2 and Chirimaugute, from water- and HCl-treated grain respectively, had lower  $\Delta H$  than other treatments, an indication of less ordered granules.

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### CONCLUSIONS

Sorghum starch pastes had higher peak viscosity, cool paste viscosity and setback when grain was steeped in NaOH than water, HCl and formaldehyde. NaOH also reduced the time to reach peak viscosity and peak viscosity temperature. NaOH appears to improve sorghum starch properties for certain applications by causing partial pre-gelatinisation. The low hardness of DC-75 starch gels was slightly improved by the NaOH treatment of grain. Gelatinisation temperatures of Zimbabwean sorghum starches were generally low regardless of treatment. It is surprising how much soaking treatments effected sorghum starch properties. Dilute alkali solution, if used during steeping of sorghum grains, could enhance sorghum starch properties.

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**Table IV-1.** Effect of steeping sorghum varieties, SV2, Chirimaugute, and DC-75, in HCl (0.9%, v/v), HCHO (0.05%, v/v), NaOH (0.3%, w/v), on the pasting properties of starch<sup>1</sup>.

Variety	SV2						Chirimaugute						DC-75					
	PV	HPV	CPV	STB	Time	PVT	PV	HPV	CPV	STB	Time	PVT	PV	HPV	CPV	STB	Time	PVT
<b>Treatment</b>	(RVU)	(RVU)	(RVU)	(RVU)	(min)	(°C)	(RVU)	(RVU)	(RVU)	(RVU)	(min)	(°C)	(RVU)	(RVU)	(RVU)	(RVU)	(min)	(°C)
Control	373 <sup>b,2</sup>	112 <sup>c</sup>	257 <sup>d</sup>	145 <sup>bc</sup>	7.27 <sup>b</sup>	87.6 <sup>b</sup>	373 <sup>b</sup>	124 <sup>c</sup>	270 <sup>d</sup>	146 <sup>c</sup>	7.10 <sup>b</sup>	86.5 <sup>c</sup>	463 <sup>b</sup>	116 <sup>bc</sup>	239 <sup>b</sup>	124 <sup>b</sup>	6.27 <sup>ab</sup>	81.5 <sup>b</sup>
HCl	360 <sup>d</sup>	151 <sup>a</sup>	299 <sup>b</sup>	149 <sup>b</sup>	7.93 <sup>a</sup>	91.6 <sup>a</sup>	368 <sup>bc</sup>	162 <sup>b</sup>	306 <sup>b</sup>	145 <sup>c</sup>	7.90 <sup>a</sup>	91.3 <sup>a</sup>	443 <sup>c</sup>	126 <sup>bc</sup>	245 <sup>b</sup>	119 <sup>b</sup>	6.37 <sup>a</sup>	82.1 <sup>a</sup>
HCHO	368 <sup>bc</sup>	125 <sup>b</sup>	272 <sup>c</sup>	148 <sup>b</sup>	7.34 <sup>b</sup>	87.9 <sup>b</sup>	361 <sup>cd</sup>	127 <sup>c</sup>	281 <sup>c</sup>	153 <sup>b</sup>	7.18 <sup>b</sup>	87.1 <sup>b</sup>	424 <sup>d</sup>	116 <sup>a</sup>	239 <sup>b</sup>	123 <sup>b</sup>	6.40 <sup>a</sup>	82.3 <sup>a</sup>
NaOH	396 <sup>a</sup>	151 <sup>a</sup>	319 <sup>a</sup>	168 <sup>a</sup>	6.44 <sup>c</sup>	82.6 <sup>c</sup>	410 <sup>a</sup>	177 <sup>a</sup>	356 <sup>a</sup>	179 <sup>a</sup>	6.27 <sup>c</sup>	81.5 <sup>d</sup>	495 <sup>a</sup>	118 <sup>b</sup>	255 <sup>a</sup>	136 <sup>a</sup>	6.14 <sup>b</sup>	80.7 <sup>c</sup>

<sup>1</sup>PV = peak viscosity, HPV = hot peak viscosity, CPV = cool paste viscosity, STB = setback (CPV-HPV), Time = time to PV, PVT = temperature at PV, RVU = Rapid Visco Analyser Units.

<sup>2</sup>Means with the same letter in each column are not significantly different ( $\alpha = 0.05$ ) according to Duncan's Multiple Range test.

F-values from analysis of variance of starch pasting and textural properties<sup>1</sup>.

Source	Degrees of Freedom	Time	PVT	PV	HPV	CPV
Variety	2	622.8 <sup>2</sup>	682.0	1833.0	235.2	409.2
Treatment	3	373.0	401.1	309.5	223.6	195.2
Variety*Treatment	6	58.3	62.4	20.3	44.0	29.6

<sup>1</sup>Time = time to peak viscosity (PV), PVT = temperature at peak viscosity (PV), PV = peak viscosity, HPV = hot peak viscosity, CPV = cool paste viscosity, STB = setback (CPV-HPV).

<sup>2</sup>All means significant at  $P < 0.05$ .

**Table IV-2.** Effect of steeping sorghum varieties, SV2, Chirimaugute, and DC-75, in HCl (0.9%, v/v), HCHO (0.05%, v/v), NaOH (0.3%, w/v), on the hardness of starch gels.

Variety	SV2	Chirimaugute	DC-75
Amylose content (%) <sup>*</sup>	27.4	28.7	21.5
	Hardness	Hardness	Hardness
<b>Treatment</b>	(g)	(g)	(g)
Control	73.6 <sup>b,1</sup>	82.9 <sup>a</sup>	31.0 <sup>c</sup>
HCl	81.5 <sup>a</sup>	82.9 <sup>a</sup>	31.5 <sup>c</sup>
HCHO	81.5 <sup>a</sup>	79.2 <sup>b</sup>	33.9 <sup>b</sup>
NaOH	80.6 <sup>a</sup>	77.4 <sup>c</sup>	36.8 <sup>a</sup>

<sup>\*</sup>Determined on starch from NaOH-treated grain.

<sup>1</sup>Means with the same letter in each column are not significantly different ( $\alpha = 0.05$ ) according to Duncan's Multiple Range test.

F-values from analysis of variance of starch gel hardness.

Source	Degrees of Freedom	Hardness
Variety	2	2942.6 <sup>1</sup>
Treatment	3	5.0
Variety*Treatment	6	12.4

<sup>1</sup>All means significant at  $P < 0.05$ .

**Table IV-3.** Effect of steeping sorghum varieties, SV2, Chirimaugute, and DC-75, in HCl (0.9%, v/v), HCHO (0.05%, v/v), NaOH (0.3%, w/v), on the thermal properties of starch<sup>1</sup>.

Variety	SV2				Chirimaugute				DC-75			
	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)
Control	59.7 <sup>c,2</sup>	67.0 <sup>c</sup>	75.9 <sup>b</sup>	9.4 <sup>c</sup>	60.6 <sup>a</sup>	66.8 <sup>b</sup>	75.8 <sup>b</sup>	9.1 <sup>ab</sup>	61.2 <sup>a</sup>	67.5 <sup>a</sup>	77.9 <sup>b</sup>	9.7 <sup>b</sup>
HCl	60.6 <sup>b</sup>	68.3 <sup>b</sup>	78.5 <sup>a</sup>	10.8 <sup>a</sup>	60.3 <sup>a</sup>	66.5 <sup>b</sup>	76.1 <sup>ab</sup>	9.0 <sup>b</sup>	60.6 <sup>b</sup>	67.0 <sup>b</sup>	78.3 <sup>b</sup>	9.7 <sup>b</sup>
HCHO	62.7 <sup>a</sup>	69.0 <sup>a</sup>	78.1 <sup>a</sup>	9.8 <sup>bc</sup>	60.6 <sup>a</sup>	66.8 <sup>b</sup>	76.2 <sup>ab</sup>	9.1 <sup>ab</sup>	60.7 <sup>b</sup>	67.5 <sup>a</sup>	78.6 <sup>ab</sup>	10.6 <sup>a</sup>
NaOH	62.9 <sup>a</sup>	69.3 <sup>a</sup>	78.3 <sup>a</sup>	10.1 <sup>b</sup>	60.7 <sup>a</sup>	67.5 <sup>a</sup>	76.9 <sup>a</sup>	9.6 <sup>a</sup>	61.2 <sup>a</sup>	67.5 <sup>a</sup>	79.4 <sup>a</sup>	9.7 <sup>b</sup>

<sup>1</sup>To = onset temperature, Tp = peak temperature, Tc = conclusion temperature, ΔH = gelatinization enthalpy.

<sup>2</sup>Means with the same letter in each column are not significantly different ( $\alpha = 0.05$ ) according to Duncan's Multiple Range test.

F-values from analysis of variance of starch thermal properties<sup>1</sup>.

Source	Degrees of Freedom	To	Tp	Tc	ΔH
Variety	2	34.24 <sup>2</sup>	112.00	77.18	20.66
Treatment	3	36.59	30.33	20.53	3.23 <sup>†</sup>
Variety*Treatment	6	33.87	15.33	4.35	6.46

<sup>1</sup>To = onset temperature, Tc = conclusion temperature, Tp = peak temperature, ΔH = gelatinization enthalpy.

<sup>2</sup>All means significant at  $P < 0.05$  except for value marked with †.

#### 4. CHAPTER V

### **Physico-chemical Properties of Starch Isolated from Sorghum Varieties of Different Polyphenol Content and Kernel Structure**

#### **ABSTRACT**

To determine the relationship between sorghum grain polyphenol content, grain structure and starch properties, starch was isolated from ten sorghum varieties using an alkali steep and wet milling procedure. SV2, a tannin-free variety with white pericarp gave a white starch. Varieties having red or white pericarp and different polyphenol levels gave pink starches. The bright pink colour of these starches could not be removed. Hunter L, a and b values of starches were not correlated with grain polyphenol content. Grain appearance in terms of pericarp colour, presence or absence of pigmented testa, did not relate to the intense pink colouration of sorghum starches. Amylose content was significantly correlated ( $r = -0.88$  at  $P < 0.001$ ) to grain floury endosperm texture. Sorghum starches had higher peak viscosities than commercial maize starch. The time taken to reach peak viscosity (PV) from the initial viscosity rise was less for sorghum starches than maize starch. However, sorghum starches had higher rates of viscosity decrease, from PV to hot paste viscosity, than maize starch. There was a significant positive correlation ( $P < 0.01$ ) between grain polyphenol content and starch pasting peak viscosity. Starch gel hardness was negatively correlated to pasting properties of rate of viscosity decrease and paste breakdown ( $r = -0.78$  and  $-0.77$ , respectively) at  $P < 0.01$ . Peak gelatinization temperature ( $T_p$ ) occurred over a narrow range of 66 to 69°C.  $T_p$  was negatively correlated to the floury endosperm portion of the grain ( $r = -0.77$ ) at  $P < 0.01$ . It is concluded that sorghum

grain polyphenol content and grain characteristics influence its starch properties.

Starch properties are less consistent than those of kernel structure and other grain quality traits (Miller 1982). The chemical composition of starch has been shown to be heterogeneous in the pericarp and endosperm of maize (Graham and Vadas 1971; Davitt and Hoseney 1971). Davitt and Hoseney (1971) have shown that starch content in maize varies widely among whole grain and endosperm samples. This variation is due to differences in the amount of starch in the pericarp and endosperm.

The starch content of maize varies widely among whole grain and endosperm samples. This variation is due to differences in the amount of starch in the pericarp and endosperm.

This variation has been applied in isolating *Amaranthus* starch (Perez et al 1993, Chiu et al 1994; Wu et al 1995) and the maize starch (Perez et al 1993).

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## INTRODUCTION

Sorghum grain tends to be less consistent than maize in terms of kernel structure and colour (Rooney and Miller 1982). The observed pink colour of sorghum starch has been associated with pigments in the pericarp and endosperm of the grain (Freeman and Watson 1971; Norris 1971). Davis and Hosenev (1979) showed that starch could adsorb and retain condensed tannins when extracted from tannin-containing sorghum varieties. Studies by Subramanian et al (1994a) implicate alcohol-soluble components as the source of the dull colour of sorghum starch. The brightness of sorghum starch can be improved by washing with dilute NaOH solution (Yang and Seib 1995) or steeping the grain in alkali prior to wet milling (Chapter IV).

Alkali extraction has been applied in isolating *Amaranthus* starch (Perez et al 1993; Zhao and Whistler 1994; Wu et al 1995) and the method has also been proposed for starch extraction from cereals (Mistry 1991). Alkali extraction results in maize starch with high viscosity, high hydration capacity, and more heat and shear stability than the commercial maize starch (Mistry and Eckhoff 1992). NaOH-treated starches had higher peak viscosity and setback than when water, HCl and formaldehyde were used (Chapter IV). The advantages of using alkali solution for steeping sorghum grain include rapid water uptake (Dewar 1997; Chapter II) and polyphenol reduction (Reichert et al 1980; Chapter II). Enhanced water uptake is presumed to be due to the alkali opening up the pericarp cell walls (Dewar et al 1997). Alkaline conditions also promote oxidative polymerization of condensed tannins (Porter 1992), resulting in the formation of highly polymeric and probably nutritionally inactive compounds.

The objectives of this study were: 1) to isolate starch from sorghum varieties of different polyphenol content and kernel structure, using an alkali steep and wet milling; 2) to measure important physico-chemical properties, including pasting, textural, and thermal properties of sorghum starch; and 3) to determine the relationship between starch properties, kernel structure and polyphenol content of sorghum.

## MATERIALS AND METHODS

### Samples

Ten sorghum varieties grown under uniform field conditions in the 1996/97 season at Matopos, Zimbabwe, were used. The selection represented sorghum varieties differing in polyphenol content, endosperm texture and pericarp thickness. Chirimaugute, Chibonda, Mutode and DC-75 (a hybrid), are tannin-containing varieties. Katandanzara, Kasvikisire, Mukadziusaenda, Mukadzidzoka, Tsveta and SV2 (an improved cultivar) are tannin-free varieties. Katandanzara, DC-75 and SV2 have relatively corneous endosperms. Other varieties have an intermediate to floury endosperm texture. Mutode, Chibonda and DC-75 have thick pericarps. Pericarp thickness ranges from thin to medium in other varieties.

### Starch Extraction

Starch was extracted following a combination of the methods described by Watson et al (1955), Perez et al (1993) and Zhao and Whistler (1994). This method has been used to isolate and characterise *Amaranthus* starch (Wu et al 1995). Sorghum grain (100 g) was steeped in 200 ml NaOH (0.25 %, w/v) at 5°C for 24 hr. The steeped grains were washed

and ground with an equal volume of water using a Waring blender for 5 min at full speed. The slurry was filtered through a 200-mesh sieve (75  $\mu\text{m}$  opening screen). The material remaining on the sieve was rinsed with water. Grinding and filtering was repeated on this material. After rinsing, the material still remaining on the sieve was discarded. The collected filtrate was allowed to stand for 1 hr. The filtrate was centrifuged at 760 x  $g$  for 10 min. The grey-coloured, top protein-rich layer was removed using a spatula. Excess water was added to resuspend the sample and centrifugation was done for 3 min. The washing and recentrifugation was repeated several times until the top starch layer was white. The starch was dried for 24 hr at 40°C.

### **Amylose/Amylopectin Ratio**

An iodine-binding spectrophotometric method using 0.2 % iodine in 2.0 % potassium iodide was used (Juliano et al 1981). Samples of starch (100 mg) were weighed in volumetric flasks (100 ml). One millilitre of ethanol (95 %) was used to wash the sample down the flask. Nine millilitres of NaOH (1 M) was added to the starch sample before heating the flasks in a boiling water bath for 10 min. The samples were cooled and the volume made up to 100 ml with distilled water. The contents were mixed vigorously to disperse the starch. Amylose/amylopectin standard mixtures were prepared to represent from 0 to 60 % amylose content. For the iodine colour development, an aliquot (5 ml) of each solution was taken to which 1 M acetic acid (1 ml) was added. The contents were mixed. The solutions were allowed to stand for 20 min after mixing with iodine solution (2 ml). Absorbance was read at 620 nm.

### **Pasting Profile Determination**

Pasting properties of sorghum starches were determined using a Rapid Visco Analyser model 3D (RVA) (Newport Scientific, Narrabeen, Australia). A commercial maize starch (Sigma, St Louis, U.S.A.) was also analysed for comparison with sorghum starch. Sorghum starch (3 g, 14 % moisture basis) was mixed with 25 g of accurately weighed water in the aluminium canister. A programmed heating and cooling cycle was used, in which the mixture was held at 50°C for 1 min, heated to 95°C in 7.5 min at the rate of 6°C/min, held at 95°C for 5 min before cooling to 50°C in 7.5 min and holding at 50°C for 1 min (Bhattacharya et al 1997). Peak viscosity (PV), PV temperature, temperature at initial viscosity increase (Ti), time to reach PV (time at PV - time at initial viscosity increase), holding strength (hot paste viscosity) (HPV), cool paste viscosity (CPV), rate of viscosity decrease calculated as  $(PV - HPV)/(13.5 \text{ min} - \text{PV time})$ , breakdown calculated as  $(PV - HPV)$ , and setback calculated as  $(CPV - PV)$ , were recorded. Two replicates per sample were analysed.

### **Texture Analysis**

The stirring paddle was removed from the aluminium canister after RVA testing. The sample was allowed to stand for 24 hr at room temperature (about 20°C) for gelation to take place (Bhattacharya et al 1997). An SMS Model TA-TX2 texture analyser (Stable Micro Systems, Godalming, England) was then used to measure the textural properties of the sorghum starch gels. A standard two-cycle programme was used to compress the gels for a distance of 10 mm at a crosshead speed of 30 mm/min using a 7-mm cylindrical probe with a flat end. Textural parameters of hardness (maximum force on first cycle in

g) and cohesiveness (total positive work done on second cycle divided by total positive work done on first cycle, no units), and gumminess (cohesiveness x hardness in g) were automatically computed, from the force-time curve obtained, using the data processing software supplied with the instrument. Four repeat measurements were taken of each of the two gel replicates per sample.

### **Differential Scanning Calorimetry**

Thermal properties of sorghum starches were determined using a Mettler DSC-20 differential scanning calorimeter (Mettler-Toledo AG Instruments, Naenikon-Uster, Switzerland) equipped with a ceramic sensor and a Mettler TC II data analysis station. Starch (2 mg, dwb) was weighed directly into a 40- $\mu$ L aluminium standard pan and water added to give a final weight of 6.5 mg. The pan was covered with the lid and hermetically sealed. The sample was allowed to equilibrate at room temperature for at least 1 hr before running it on the differential scanning calorimeter. The sample was heated from 30°C to 120°C at the rate of 10°C per min. Gelatinisation parameters of onset ( $T_o$ ), peak ( $T_p$ ), and conclusion ( $T_c$ ) temperature were determined. Gelatinization temperature range ( $T_c - T_o$ ) was calculated. Gelatinization enthalpy ( $\Delta H$ ) in J/g was also recorded. Two replicates per sample were analysed.

### **Colour Determination**

The colour of sorghum starches was determined using the Hunter Lab Colour Quest 45/0 LAV (Hunter Associates Laboratory, Inc. Reston, U.S.A.) equipped with Universal Software Version 3.1. The mean of three readings per sample was taken at different

angles.

### Statistical Analysis

The general linear model procedure of the Statistical Analysis System version 6.10 (SAS Institute, Cary, U.S.A.) was used for data analysis. Means were compared at the 5 % significance level using Fisher's least significant difference (LSD). Pearson correlation coefficients ( $r$ ) were calculated among starch pasting, textural, and gelatinisation properties and grain polyphenol content and kernel characteristics using SAS Proc Corr.

## RESULTS AND DISCUSSION

### Swelling and Pasting Properties

Swelling and pasting properties of sorghum starches are shown in Table V-1. The mean initial swelling temperature ( $T_i$ ) of sorghum starches was lower ( $69.4^\circ\text{C}$ ) than that of maize starch ( $73.6^\circ\text{C}$ ). Subramanian et al (1994b) also reported higher  $T_i$  values of 69 to  $77^\circ\text{C}$  for 7 sorghum starches. The differences in swelling temperatures possibly resulted from genetic variation in kernel structure of the varieties as most Zimbabwean sorghums have intermediate to floursy endosperm (Chapter I). Peak viscosity (PV) temperature for sorghum starch ranged from  $78.8$  to  $88.1^\circ\text{C}$ . Maize starch had a higher PV temperature of  $93.5^\circ\text{C}$ . The results confirm the findings of by Abd Allah et al (1987) that sorghum starches had lower PV temperatures than maize starch. The lower swelling temperature of sorghum starch could have been due to the alkali treatment that caused some partial pre-gelatinisation (Chapter IV). Sorghum starches took less time (2.4 min) to reach PV than maize starch (3.5 min). Sorghum starch granules were thus easily swollen. The time to

reach PV and PV temperature were positively correlated ( $P < 0.001$ ) and both were negatively correlated ( $P < 0.01$ ) to grain polyphenol content (Table V-2). During alkali steeping, polyphenols were presumably oxidatively polymerised to highly polymeric compounds that formed minor constituents of sorghum starches, thereby influencing the swelling properties. The higher PV temperature of starch from tannin-free varieties, SV2 and Mukadziusaenda, was further evidence that polyphenolic products were involved in granule swelling. The mean rate of viscosity decrease was 31.6 and 21.0 RVU/min for sorghum and maize starch, respectively. Presumably, the highly, swollen sorghum starch granules broke up easily. Subramaniam et al (1994b) also found a higher rate of viscosity decrease when they compared sorghum starches with maize starches. Starch from the tannin-rich variety, DC-75 had the highest rate of viscosity decrease whereas that from Kasvikisire, a tannin-free variety, had the lowest.

Peak viscosity (PV) of starches is related to the swelling power and indicates the water binding capacity of the starch as it occurs at the equilibrium point between swelling and polymer leaching which causes a viscosity increase, while rupture and polymer alignment cause a viscosity decrease (Newport Scientific 1998). The mean PV of sorghum starches (332 RVU) was markedly higher compared to that of maize starch (239 RVU). Thus sorghum starches had greater water binding capacity than maize starch. The pasting profiles shown in Figure V-1 depicts the greater swelling power of sorghum starches of DC-75, Chirimaugute, and SV2 compared to maize starch. However, the alkali extraction method could have enhanced PV of sorghum starch (Chapter IV). An alkali extraction process also gives maize starch with high viscosity and high hydration capacity than

commercial maize starch (Mistry and Eckhoff 1992). PV was positively correlated ( $P < 0.05$ ) to rate of viscosity decrease as excessively swollen granules break down easily. There was a significant positive correlation ( $P < 0.05$ ) between PV and grain polyphenol content (Table V-2). The oxidatively polymerized products of polyphenols presumably appeared as minor starch constituents that enhanced granule swelling. Hence, DC-75, the tannin-rich variety, gave starch with highest PV (398 RVU) (Table V-1). The higher amylopectin content of DC-75 starch caused greater swelling. SV2 and Kasvikisire (both tannin-free) and Chirimaugute (medium-tannin) gave starch pastes with lowest PV (300 RVU) as their amylose content were relatively high (Table V-3).

Hot paste viscosity (HPV) is a shear thinning property of the starch (Newport Scientific 1998). Sorghum and maize starch had mean HPV of 117 and 133 RVU, respectively (Table V-1). There was a significant negative correlation ( $P < 0.001$ ) between HPV and rate of viscosity decrease (Table V-2). In other words the higher the rate of viscosity decrease the lower the HPV. HPV was highest (132 RVU) in starches from Chibonda and Kasvikisire. Chibonda has polyphenols largely unextractable in methanol whereas Kasvikisire is tannin-free (Chapter I). There was no significant correlation between HPV and grain polyphenol content or endosperm texture. The relatively higher amylose content of both starches could have contributed to higher shear thinning. Starches from Mukadziusaenda, DC-75 and Mutode gave the lowest HPV. Starches with low shear thinning suggest that they are stable under hot conditions. Subramanian et al (1994b) concluded that hot pastes sorghum starches generally undergo more shear thinning than maize starches. My observation is similar to that of Yang and Seib (1996) who found low

shear thinning from starches isolated from two commercial yellow grain sorghums. The viscosity of a starch paste is not entirely caused by swelling (Miller et al 1973) but the fine structure and molecular weights of the amylose and amylopectin fractions could be important in controlling shear thinning (Takeda et al 1988; Jane and Chen 1992). Several other authors (Steeneken 1987; Williams and Bowler 1982) have surmised that the degree of shear thinning may be related to the morphology and rigidity of the swollen granules.

On cooling a hot starch paste, a firm, viscoelastic gel is generally produced due to re-association of starch molecules (Newport Scientific 1998). Cool paste viscosity (CPV) is important in some food processing operations such as canning (Wu et al 1995). The mean CPV (244 RVU) of sorghum starches was similar to that of maize starch, as has been previously noted by Yang and Seib (1996). There was a significant positive correlation ( $P < 0.001$ ) between CPV and HPV. Thus the same factors that influence shear thinning of sorghum pastes are also involved in final viscosity. Starches from tannin-free variety, Kasvikisire and tannin-rich variety, DC-75 had the highest (277 RVU) and lowest (208 RVU) CPV, respectively. A significant negative correlation ( $P < 0.01$ ) was found between CPV and starch paste breakdown. Varieties, Kasvikisire and Chirimaugute having similar hardness scores but differing widely in polyphenol content (Chapter I), gave starches with the lowest breakdown values.

The mean setback values for maize and sorghum starch pastes were 111 and 128 RVU, respectively (Table V-1). High setback is associated with syneresis, or weeping, during freeze / thaw cycles for example, and substituted starches are commonly used where this

presents a quality defect (Newport Scientific 1998). Starch from the tannin-rich variety, DC-75 had the lowest setback (109 RVU). Chirimaugute, Kasvikisire and Katandanzara starches had the highest setback (>140 RVU).

### **Amylose Content and Textural Properties**

The amylose content of sorghum starches ranged from 21.5 to 29.9 % (Table V-3). Thus varieties had normal, nonwaxy endosperm. There was a significant negative correlation ( $P < 0.01$ ) between starch amylose and grain polyphenol content, flourey endosperm and pericarp thickness (Table V-2). Hence, the higher the amylose content the more corneous the endosperm. Cagampang and Kirleis (1984) also reported a positive correlation between grain vitreousness and amylose content of 15 sorghum hybrids. Katandanzara, a tannin-free variety with corneous endosperm, had the highest amylose content. DC-75, the tannin-rich variety with relatively corneous endosperm, had the lowest amylose / amylopectin ratio. There was not a significant correlation between endosperm texture and polyphenol content (Chapter I).

The RVA was used as a cooker for the preparation of starch gels for textural measurements. Textural properties of sorghum starch gels are shown in Table V-3. The hardness of the starch gels ranged from 36.1 to 71.3 g. The tannin-rich variety, DC-75 gave starch with the softest gel. Chirimaugute, a variety of intermediate tannin content and flourey endosperm, gave starch with the hardest gel. Gel firmness was presumably due to the amylose content of the starches. Grain flourey endosperm and polyphenol content were both negatively correlated to amylose content (Table V-2). Cagampang and Kirleis

(1984) also prepared a firmer gel with starch from corneous rather than the floury endosperm of sorghum. Amylopectin starch polymers contribute more to solubilization (Jackson et al 1989) and retrogradation of starch (Cagampang and Kirleis 1984). Bello et al (1995) also reported increased soluble starch and a firmer, thick porridge texture as associated with a more corneous endosperm texture of sorghum. However, starch gel hardness was not significantly correlated to grain endosperm texture among the varieties studied (Table V-2). There was a significant negative correlation ( $P < 0.05$ ) between gel hardness and pasting PV. Hence grain polyphenol content influenced gel hardness as PV was high in tannin-containing varieties. DC-75 gave the most cohesive starch gel while SV2 and Chirimaugute gave the least. Apparently, factors involved in gel hardness produce the opposite effect in gel cohesiveness. However, gel gumminess was presumably controlled by factors that control gel hardness. Gel gumminess was high in Chirimaugute and SV2 starches and low in DC-75 starch.

### **Thermal Properties**

Table V-4 expresses the gelatinization temperatures of sorghum starches from the onset ( $T_o$ ), peak ( $T_p$ ) and conclusion ( $T_c$ ) of gelatinisation. Gelatinization temperatures (GT) ranged from 60.1 ( $T_o$ ) to 76.2°C ( $T_c$ ). Taylor et al (1997) reported  $T_o$  and  $T_c$  values of 63.6 and 69.5°C respectively, for South African sorghums. However, GT values ranging from 71 to 80°C were reported by Sweat et al (1984) and Akingbala et al (1982) for sorghum starches.  $T_p$  values (72 to 75°C) reported for eight maize starches extracted in alkali (Mistry and Eckhoff 1992) were higher than those of sorghum starches. Thus GT of starches from Zimbabwean sorghums were lower than the literature values, as found by

Taylor et al (1997). The differences in GT can be explained largely by variation in genetic and environmental factors. Thermal properties could not be correlated to shear thinning properties as previously reported (Subramanian et al 1994). A significant correlation ( $P < 0.05$ ) was found between  $T_p$  and starch amylose content. Thus starches with higher amylopectin content required lower temperatures to disrupt the molecular order of the granules. There was a significant correlation ( $P < 0.01$ ) between endosperm texture and  $T_p$  and  $T_o$ . In other words, the more corneous the grain the higher the  $T_p$  or  $T_o$ . Similar observations had been reported on the latter when starches from the corneous endosperm gave higher  $T_p$  than those isolated from the floury types of three sorghum cultivars (Cagampang and Kirleis 1984). A significant negative correlation ( $P < 0.05$ ) was found between  $T_o$  and grain polyphenol content (Table V-2). The oxidation products of polyphenols possibly contributed to molecular order disruption of granules resulting in lower  $T_o$  values of the starch. The significant negative correlation between  $T_o$  and grain pericarp thickness could not be explained. The enthalpy for gelatinization ( $\Delta H$ ) was highest for SV2 (8.3 J/g) and lowest for Tsveta (6.9 J/g). The two varieties differ in that SV2 is relatively more corneous and contains lower levels of phenolic acids than Tsveta (Chapter II). Thus the high phenolic acids of Tsveta grain could have undergone polymerisation resulting in products that lowered the energy needed to gelatinise starch. A significant negative correlation ( $P < 0.05$ ) between  $\Delta H$  and grain polyphenol content was also found. Thus starch from polyphenol-rich varieties required less enthalpy to completely gelatinise them.

## Starch Colour and Kernel Properties

The starches obtained generally were of different shades of pink (Figure V-2 a and b). Only SV2, a tannin free variety with white pericarp and no testa (Chapter I), gave a white starch. Apparently, the tan plant colour of SV2 was important in reducing the colour of the starch product. The other sorghum varieties have either red or white pericarp and different levels of polyphenols. Starch colour is probably due to the presence of pigments in the pericarp that were leached into the endosperm during weathering in the field or during steeping for wet milling (Norris 1971). Hunter L (lightness), a (red to green) and b (blue to yellow) values of sorghum starches are shown in Table V-4. Grain pericarp colour, presence or absence of pigmented testa and polyphenol content did not correlate to the pink colour of sorghum starches. The starch colouration obtained even from grains that apparently looked white with a few red or purple spots, was remarkably intense. The explanation by Freeman and Watson (1971) that starch made from certain white-seeded cultivars could still be off-white due to carotenoid pigment in the endosperm was not a satisfactory explanation in this case. The colour changes of phenolics which occur when pH is increased generally result from ionization of the phenolic; the phenolate ion is generally more brightly coloured than the neutral phenol. In addition, the phenolate ion can complex with metals (especially iron) to form coloured complexes. Kennedy et al (1984) also found that in alkaline solution, flavan-3-ols or condensed tannins could undergo C-ring opening and rearrangement via radical reactions involving traces of oxygen. The radical anion intermediate formed in mild, alkaline solutions will then react further by a series of alternative annulation and ring migration reactions giving a host of products. These reactions could probably have caused the intense pink pigmentation of

the starch. SV2 starch had the highest L value (90.5). However, Chibonda, a variety with white pericarp but containing pigmented testa underneath, gave the darkest starch. Varieties, Chirimaugute, DC-75, and Mutode also have pigmented testa beneath white, red and red pericarp, respectively (Chapter I). However, the polyphenolic compounds of Chibonda, which are unextractable in methanol (Chapter I), could have remained adsorbed to the starch during wet milling.

## CONCLUSIONS

A tan plant colour is critically important to lower colour in tannin-free sorghum meant for production of white starch. Starch pasting properties of peak viscosity, peak viscosity temperature, and time to reach peak viscosity were significantly correlated to grain polyphenol content. Starch amylose content and gelatinization peak temperature were significantly correlated to grain endosperm texture. Hence the polyphenol content and kernel structure of sorghum varieties influence starch properties.

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**Table V-1.** Pasting and swelling properties of starches isolated from Zimbabwean sorghum varieties<sup>1</sup>

Variety	Ti (°C)	Rate (RVU/min)	TPV (°C)	Time (min)	PV (RVU)	HPV (RVU)	CPV (RVU)	BKD (RVU)	STB (RVU)
DC-75	69.5 <sup>b,2</sup>	40.4 <sup>a</sup>	79.9 <sup>f</sup>	1.73 <sup>f</sup>	398 <sup>a</sup>	99 <sup>f</sup>	208 <sup>h</sup>	299 <sup>a</sup>	109 <sup>f</sup>
Chirimaugute	70.3 <sup>a</sup>	26.2 <sup>g</sup>	85.4 <sup>c</sup>	2.53 <sup>c</sup>	301 <sup>g</sup>	125 <sup>b</sup>	268 <sup>b</sup>	176 <sup>h</sup>	144 <sup>a</sup>
Mutode	69.3 <sup>b</sup>	34.1 <sup>c</sup>	82.8 <sup>e</sup>	2.24 <sup>d</sup>	338 <sup>d</sup>	102 <sup>c</sup>	218 <sup>g</sup>	236 <sup>c</sup>	117 <sup>c</sup>
Mukadzidzoka	67.9 <sup>d</sup>	30.1 <sup>f</sup>	82.0 <sup>e</sup>	2.39 <sup>c</sup>	326 <sup>c</sup>	114 <sup>c</sup>	232 <sup>f</sup>	212 <sup>f</sup>	118 <sup>de</sup>
Chibonda	68.6 <sup>c</sup>	29.7 <sup>f</sup>	80.0 <sup>f</sup>	1.92 <sup>c</sup>	353 <sup>c</sup>	132 <sup>a</sup>	263 <sup>c</sup>	222 <sup>e</sup>	131 <sup>b</sup>
Tsveta	68.1 <sup>d</sup>	31.2 <sup>c</sup>	78.8 <sup>h</sup>	1.80 <sup>ef</sup>	363 <sup>b</sup>	124 <sup>b</sup>	250 <sup>d</sup>	240 <sup>b</sup>	127 <sup>c</sup>
Katandanzara	70.2 <sup>a</sup>	30.3 <sup>ef</sup>	86.8 <sup>b</sup>	2.71 <sup>b</sup>	315 <sup>f</sup>	126 <sup>b</sup>	263 <sup>bc</sup>	189 <sup>g</sup>	140 <sup>a</sup>
Mukadziusaenda	70.3 <sup>a</sup>	37.8 <sup>b</sup>	87.8 <sup>a</sup>	2.97 <sup>a</sup>	324 <sup>c</sup>	96 <sup>g</sup>	218 <sup>g</sup>	228 <sup>d</sup>	122 <sup>d</sup>
Kasvikisire	70.1 <sup>a</sup>	24.4 <sup>h</sup>	84.4 <sup>d</sup>	2.40 <sup>c</sup>	300 <sup>g</sup>	132 <sup>a</sup>	277 <sup>a</sup>	168 <sup>i</sup>	144 <sup>a</sup>
SV2	70.0 <sup>a</sup>	32.2 <sup>d</sup>	88.1 <sup>a</sup>	3.03 <sup>a</sup>	304 <sup>g</sup>	111 <sup>d</sup>	245 <sup>e</sup>	193 <sup>g</sup>	134 <sup>b</sup>
<b>Maize starch</b>	<b>73.6</b>	<b>21.0</b>	<b>93.5</b>	<b>3.5</b>	<b>239</b>	<b>133</b>	<b>244</b>	<b>106</b>	<b>111</b>
Mean (sorghum starch)	69.4	31.6	83.5	2.37	332	116	244	216	128
LSD (sorghum starch)	0.45	0.86	0.65	0.14	4.25	2.32	5.40	3.41	4.00

<sup>1</sup>Ti = temperature at initial viscosity rise, Rate = rate of viscosity breakdown, Time = time from initial to PV, TPV = temperature at peak viscosity, PV = peak viscosity, HPV = hot paste viscosity, HPV = holding strength at 13.5 min, CPV = cool paste viscosity, BKD = breakdown (PV-HPV), STB = setback (CPV-HPV).

<sup>2</sup>Values within the same column with different letters are significantly different at  $p < 0.05$ .

**Table V-2.** Pearson correlation coefficients (r values) of the sorghum starch properties, grain structural characteristics and polyphenol content<sup>1,2</sup>

	Tp	To	Tc	Tr	$\Delta H$	Ti	Rate	Time	TPV	PV	HPV	CPV	BKD	STB	L	Hard	Amy	Van	Et
To	0.94***																		
Tc	0.82**	0.70*																	
Tr	-0.41	-0.62	0.12																
$\Delta H$	0.38	0.33	0.36	-0.06															
Ti	0.86**	0.82**	0.65*	-0.44	0.18														
Rate	0.33	0.08	0.44	0.37	0.54	-0.004													
Time	0.54	0.62	0.15	-0.69*	0.41	0.63	-0.12												
TPV	0.67*	0.73*	0.31	-0.66*	0.39	0.79**	-0.09	0.97***											
PV	-0.27	-0.49	0.10	0.79**	-0.01	-0.50	0.66*	0.81**	-0.78**										
HPV	-0.49	-0.36	-0.41	0.04	-0.72*	-0.12	-0.88***	-0.18	-0.18	-0.32									
CPV	-0.26	-0.11	-0.30	-0.17	-0.63	0.17	-0.90***	0.11	0.13	-0.56	0.95***								
BKD	-0.05	-0.28	0.23	0.64*	0.24	-0.37	0.85**	-0.61	-0.59	0.94***	-0.61	-0.80**							
STB	0.04	0.18	-0.12	-0.38	-0.45	0.47	-0.83**	0.41	0.46	-0.75*	0.77**	0.93***	-0.89***						
L	-0.25	-0.24	-0.08	0.23	0.36	-0.20	0.06	0.18	0.09	-0.07	-0.12	-0.11	-0.01	-0.07					
Hard	-0.36	-0.14	-0.58	-0.43	-0.24	0.04	-0.78**	0.32	0.27	-0.69*	0.57	0.67*	-0.77**	0.69*	0.18				
Amy	0.73*	0.82**	0.40	-0.69*	0.43	0.62	-0.01	0.67*	0.72*	-0.58	-0.35	-0.14	-0.35	0.13	0.03	0.02			
Van	-0.60	-0.73*	-0.32	0.67*	-0.46	-0.52	0.14	-0.86**	-0.83**	0.75*	0.21	-0.01	0.55	-0.26	-0.21	-0.12	-0.85**		
Et	-0.77**	-0.80**	-0.53	0.53	-0.60	-0.61	-0.13	-0.53	-0.60	0.39	0.42	0.25	0.17	0.03	0.21	0.14	-0.88***	0.71*	
Pt	-0.66*	-0.76*	-0.43	0.58	-0.64*	-0.47	-0.17	-0.48	0.52	0.35	0.55	0.38	0.10	0.14	-0.14	0.09	-0.87***	0.71*	0.83**

<sup>1</sup>Tp = peak gelatinization temperature, To = onset temperature of gelatinization, Tc = conclusion temperature of gelatinization, Tr = Tc-To,  $\Delta H$  – gelatinization enthalpy, Ti = temperature at which viscosity begins to increase, Rate =rate of viscosity breakdown, Time =time from initial increase to PV, TPV =temperature at PV, PV =peak viscosity, HPV =holding paste viscosity, CPV =final or cool paste viscosity, BKD =breakdown (PV-HPV), STB =setback (CPV-HPV), L =Hunter L value, Hard = hardness, Amy = amylose, Van = polyphenol content, Et = % floury endosperm, Pt = pericarp thickness.

<sup>2</sup>\*\*\*, \*\*, \* = P < 0.001, 0.01, and 0.05, respectively; n=10.

**Table V-3.** Endosperm texture, starch amylose content and gel properties of Zimbabwean sorghum varieties

Variety	Floury endosperm* (%)	Amylose (%)	Hardness (g)	Cohesiveness	Gumminess (g)
DC-75	31.9	21.5 <sup>g,1</sup>	36.1 <sup>g</sup>	0.63 <sup>a</sup>	22.8 <sup>g</sup>
Chirimaugute	63.0	28.7 <sup>b</sup>	71.3 <sup>a</sup>	0.48 <sup>e</sup>	33.9 <sup>a</sup>
Mutode	45.1	28.1 <sup>bcd</sup>	58.2 <sup>cd</sup>	0.48 <sup>dc</sup>	28.1 <sup>e</sup>
Mukadzidzoka	71.9	27.1 <sup>de</sup>	56.5 <sup>de</sup>	0.52 <sup>c</sup>	29.1 <sup>de</sup>
Chibonda	69.7	28.3 <sup>bc</sup>	59.2 <sup>cd</sup>	0.56 <sup>b</sup>	32.9 <sup>ab</sup>
Tsveta	51.4	28.2 <sup>bc</sup>	59.6 <sup>c</sup>	0.51 <sup>cd</sup>	30.1 <sup>cd</sup>
Katandanzara	28.4	29.9 <sup>a,2</sup>	53.8 <sup>e</sup>	0.56 <sup>b</sup>	29.9 <sup>cde</sup>
Mukadziusaenda	48.1	26.0 <sup>f</sup>	47.5 <sup>f</sup>	0.53 <sup>c</sup>	25.0 <sup>f</sup>
Kasvikisire	38.1	27.1 <sup>e</sup>	59.8 <sup>c</sup>	0.48 <sup>dc</sup>	28.9 <sup>de</sup>
SV2	39.9	27.4 <sup>cde</sup>	66.9 <sup>b</sup>	0.47 <sup>e</sup>	31.5 <sup>bc</sup>
Mean	48.8	27.2	56.9	0.52	29.2
LSD		0.99	2.78	0.03	1.93

<sup>1</sup>Hard = hardness, cohes = cohesiveness, gumm =gumminess, chew = chewiness.

<sup>2</sup>Values within the same column with different letters are significantly different at p<0.05.

\*From Chapter 1.

**Table V-4.** Thermal properties of starches isolated from Zimbabwean sorghum varieties<sup>1</sup>

Variety	To (°C)	Tp (°C)	Tc (°C)	Tr (°C)	$\Delta H$ (J/g)
DC-75	61.9 <sup>cd,2</sup>	68.0 <sup>b</sup>	76.2 <sup>a</sup>	14.3 <sup>a</sup>	7.85 <sup>ab</sup>
Chirimaugute	62.3 <sup>c</sup>	67.5 <sup>bc</sup>	74.3 <sup>c</sup>	12.0 <sup>d</sup>	7.15 <sup>bcde</sup>
Mutode	62.3 <sup>bc</sup>	67.7 <sup>bc</sup>	75.3 <sup>b</sup>	13.0 <sup>bcd</sup>	7.50 <sup>bcde</sup>
Mukadzidzoka	61.2 <sup>d</sup>	66.5 <sup>d</sup>	73.9 <sup>c</sup>	12.7 <sup>cd</sup>	7.70 <sup>abcd</sup>
Chibonda	60.4 <sup>e</sup>	66.3 <sup>de</sup>	74.4 <sup>c</sup>	14.0 <sup>ab</sup>	7.10 <sup>cde</sup>
Tsveta	60.1 <sup>e</sup>	65.8 <sup>e</sup>	74.2 <sup>c</sup>	14.1 <sup>ab</sup>	6.90 <sup>e</sup>
Katandanzara	62.2 <sup>c</sup>	68.0 <sup>b</sup>	75.4 <sup>b</sup>	13.2 <sup>abcd</sup>	7.00 <sup>de</sup>
Mukadziusaenda	63.2 <sup>a</sup>	68.7 <sup>a</sup>	75.7 <sup>ab</sup>	12.5 <sup>cd</sup>	7.80 <sup>abc</sup>
Kasvikisire	63.0 <sup>ab</sup>	68.0 <sup>bc</sup>	75.9 <sup>ab</sup>	12.9 <sup>bcd</sup>	7.25 <sup>bcde</sup>
SV2	61.9 <sup>cd</sup>	67.5 <sup>c</sup>	75.2 <sup>b</sup>	13.3 <sup>abc</sup>	8.25 <sup>a</sup>
Mean	61.8	67.4	75.0	13.2	7.45
LSD	0.72	0.51	0.79	1.22	0.70

<sup>1</sup>Tp = peak gelatinization temperature, To = onset temperature of gelatinization, Tc = conclusion temperature of gelatinization, Tr = (Tc-To),  $\Delta H$  = gelatinization enthalpy

<sup>2</sup>Values within the same column with different letters are significantly different at p<0.05.

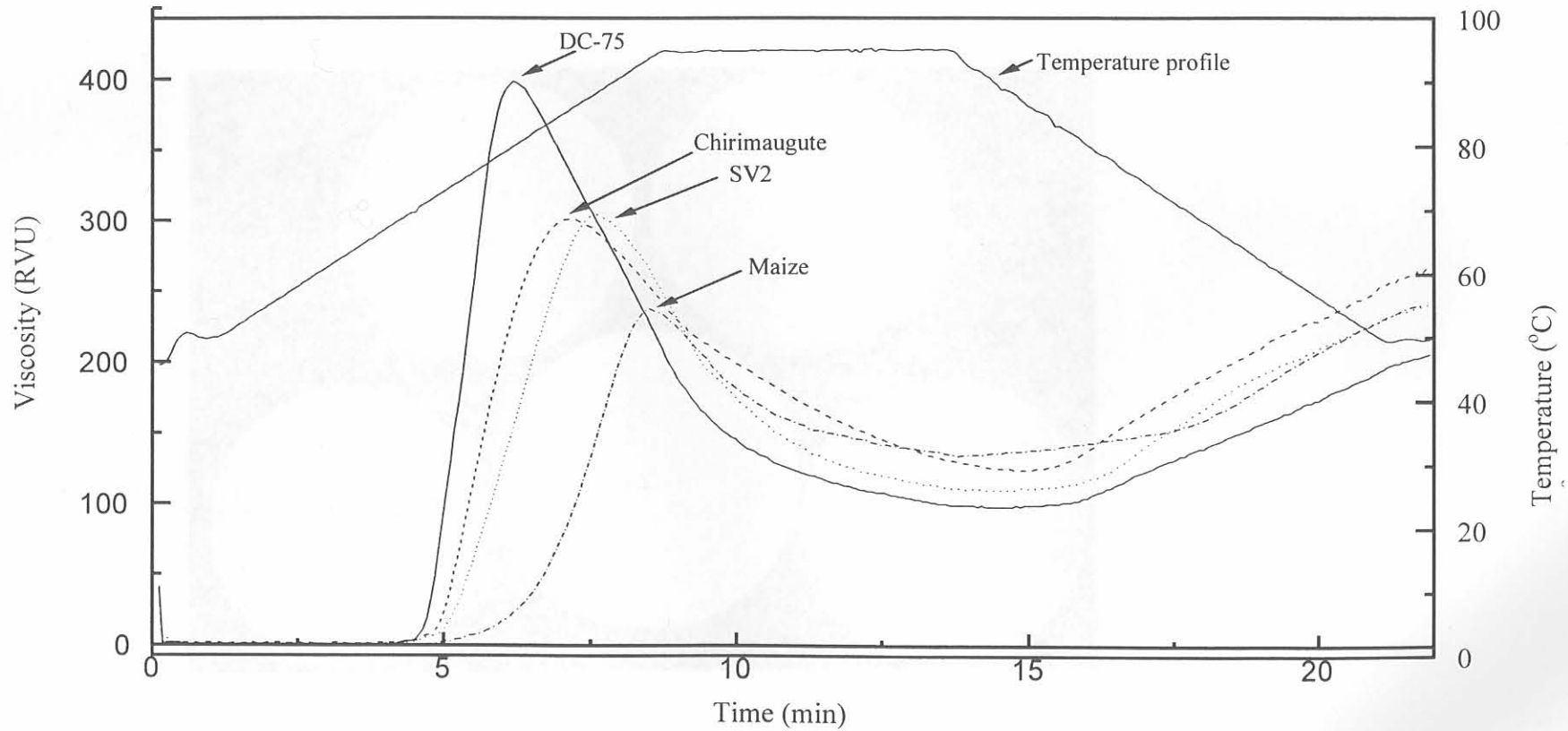
**Table V-5.** Hunter L, a, and b colour values of starches isolated from Zimbabwean sorghum varieties of different kernel properties and polyphenol content<sup>1</sup>

Variety	Testa*	Pericarp colour*	Total polyphenol**	L	a	b
DC-75	Yes	Red	6.29	81.1 <sup>2</sup> (0.01)	3.90 (0.02)	4.11 (0.02)
Chirimaugute	Yes	White	3.78	77.9 (0.01)	4.51 (0.02)	4.36 (0.02)
Mutode	Yes	Red	3.25	81.5 (0.01)	5.81 (0.02)	5.34 (0.01)
Mukadzidzoka	No	Red	0.66	84.1 (0.03)	4.30 (0.03)	4.66 (0.02)
Chibonda	Yes	White	0.52	75.9 (0.01)	4.30 (0.01)	4.74 (0.02)
Tsveta	No	Red	0.32	87.9 (0.01)	2.20 (0.01)	4.66 (0.02)
Katandanzara	No	White	0.19	81.7 (0.01)	4.95 (0.02)	4.95 (0.02)
Mukadziusaenda	No	White	0.18	79.2 (0.01)	3.47 (0.03)	4.58 (0.03)
Kasvikisire	No	White	0.05	81.4 (0.02)	4.95 (0.02)	4.95 (0.02)
SV2	No	White	0.00	90.5 (0.01)	-0.34 (0.00)	5.47 (0.01)
Mean			1.52	82.1	3.81	4.76

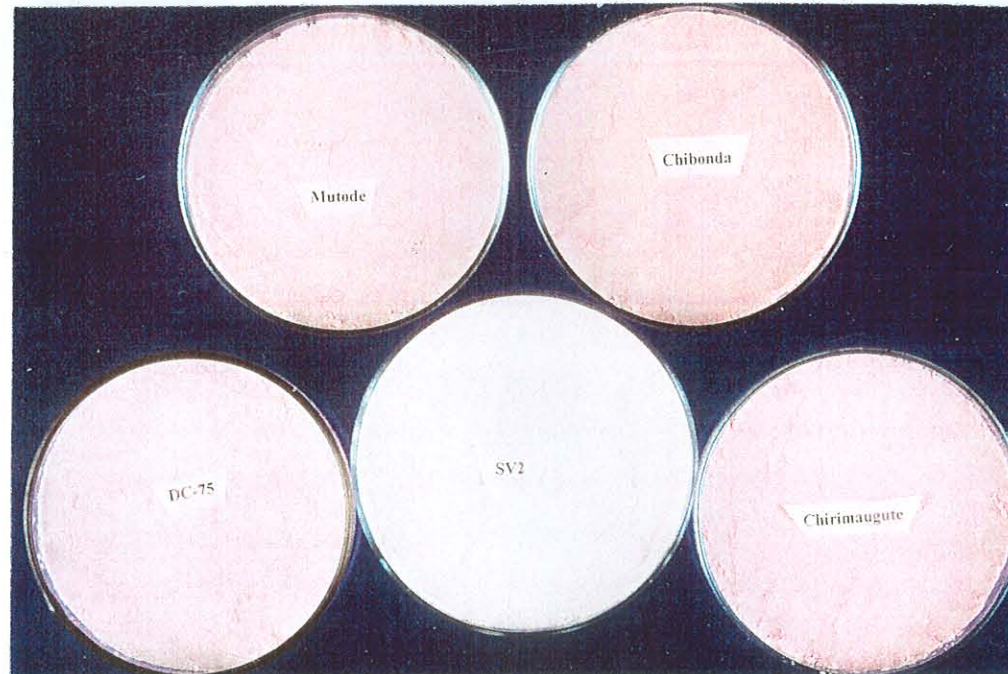
<sup>1</sup>Hunter L, a, and b values.

<sup>2</sup>Mean and standard deviation.

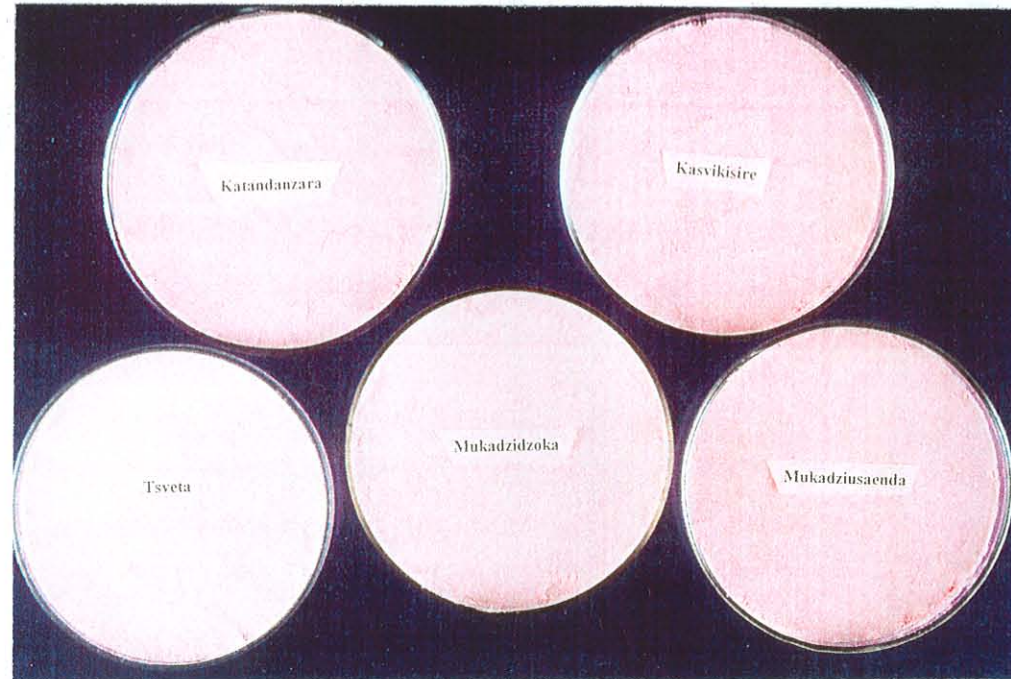
\*From Chapter 1; +mg/100g.



**Figure V-1.** RVA pasting profiles of sorghum starches compared to maize starch. DC-75 and Chirimaugute are tannin-containing varieties and SV2 is a tannin-free variety.



**Figure V-2.** (a) Photographs illustrating different colours of sorghum starches from tannin-containing (DC-75, Mutode, Chirimaugute and Chibonda) and tannin-free (SV2) varieties.



**Figure V-2.** (b) Photographs illustrating different colours of sorghum starches from tannin-free varieties (Katandanzara, Kasvikisire, Tsveta, Mukadzidzoka, Mukadziusaenda).

## 5. GENERAL DISCUSSION

Concerning the levels of polyphenols, in the 16 widely cultivated Zimbabwean sorghums, three had high polyphenol content and one had polyphenols that were not extractable in methanol solvent. The same varieties had a pigmented testa, that is the layer in which polyphenols are concentrated, as shown by the bleach test. Varieties identified as high in polyphenols represent a fourth of the sorghums widely grown in Zimbabwe. The figure represents a significant fraction of sorghum genotypes that normally have an agronomic advantage (McMillan et al 1972) but presents problems in processing. Condensed tannins will bind and precipitate proteins making them nutritionally unavailable (Hahn et al 1984). Sorghum tannins are inhibitory to the malt enzymes required for brewing (Daiber 1975b; Daiber and Taylor 1982). Therefore, the condensed tannins that form most of the phenolic compounds in high-tannin sorghum have to be removed or deactivated to prevent them from binding the grain proteins during food processing. The challenge is to maintain the agronomic benefits of the tannins while providing effective post-harvest methods of processing tannin-containing sorghums.

In milling operations, a relatively hard endosperm is a requirement for sorghum varieties (Maxson et al 1971) so as to minimise loss of endosperm material into the bran. While some of the Zimbabwean varieties had an intermediate to corneous endosperm, they lack the high polyphenol content that protects them against mould (Harris and Burns 1973), insect and bird attack (McMillan et al 1972). DC-75 was identified as a high-tannin variety with a relatively corneous endosperm, contrary to

the general perception that high-tannin sorghums are almost always soft (Waniska et al 1989). DC-75 apparently has most of the ideal agronomic and processing physico-chemical characteristics. However, DC-75 grain will still pose problems given its red, thick pericarp and pigmented testa layer, both of which need to be removed, for production of lighter-coloured food products in dry milling. Chirimaugute and Chibonda, both displaying pigmented testa, had soft endosperms. Hence grain endosperm texture and polyphenol content could not be correlated among the varieties studied.

The significant positive correlation ( $P < 0.05$ ) found between grain polyphenol content and pericarp thickness was presumably due to the additional pigmented testa layer under the pericarp. In traditional decortication, using hand pounding, varieties with a thick pericarp are preferred as they take less time to decorticate (Scheuring et al 1982). The surveys conducted in communal areas of Zimbabwe indicated that red sorghums without testa were being used for malting. On the other hand, sorghums containing high polyphenols were hardly used for food in rural Zimbabwe. Villagers highlighted the lack of suitable methods for removing the testa as hand pounding was inefficient in removing the testa. The use of variety DC-75 is thus strictly confined to commercial malting for opaque beer brewing as tannins are deactivated through use of formaldehyde (Daiber 1975a), a treatment that is not available at village level. Hence varieties with thick pericarp and high polyphenols were grown for marketing purposes, as simple treatments were not available for rural food processing. The thick pericarp of DC-75, Chibonda and Mutode is desirable in traditional decortication but

would render these varieties susceptible to grain weathering (Glueck and Rooney 1980). However, their high polyphenol content would protect them against mould (Harris and Burns 1973) and pre-harvest germination (Harris and Burns 1970). These findings confirm the observation by Scheuring et al (1982) that desired agronomic properties may not be compatible with good milling quality. The ideal characteristics for machine decortication are a thin, white pericarp and corneous endosperm (Rooney and Walker 1978). None of the 16 widely cultivated Zimbabwean sorghum varieties met the agronomic and processing criteria of high polyphenol, hard endosperm and thin pericarp.

In view of this, simple chemical treatments of the grain were examined in order to produce food products of low polyphenol content and therefore better nutritional and functional value. The three varieties selected represented tannin-free (SV2), medium-tannin (Chirimaugute) and high-tannin (DC-75) sorghum types. The use of water, HCl (0.25 M), HCHO (0.017 M) and NaOH (0.075 M) during steeping of tannin-containing varieties reduced polyphenol content of the grain. Several authors have postulated that polyphenol reduction by water, HCl, HCHO and NaOH probably involves formation of cross-linked and insoluble, high molecular weight polymers (Swain 1965; Gupta and Haslam 1978; Kennedy et al 1984; Morrison and Boyd 1984; Porter 1992). The fact that NaOH and HCHO were more effective than water and HCl was evidence that the polymerised reaction products of polyphenols were different. Gupta and Haslam (1978) surmised that water or acid-treated grain may form reactive carbocations from soluble polymers ( $n=5-6$ ) which in turn react to form higher

oligomeric polymers which are not readily soluble. The degree of cross-linking and polymerisation into insoluble polyphenolic compounds would probably serve to differentiate the effectiveness of these treatments. McGrath et al (1980) postulated that either HCHO simply inactivates the phenolic group or that it cross-links the tannin to form large polymers. The latter is supported by the general reaction between HCHO and phenols that results in phenol-formaldehyde resins and related polymers (Morrison and Boyd 1984). Alkaline conditions are known to promote oxidative polymerisation of polyphenols (Porter 1992). Thus the NaOH treatment presumably caused formation of highly polymeric and probably inactive compounds. Flavan-3-ols or condensed tannins are known to undergo C-ring opening and rearrangement via radical reactions involving traces of oxygen (Kennedy et al 1984). The resultant products formed by a series of alternative annulation and ring migration reactions are less polar and more rigid than the parent polyphenols (Kennedy et al 1984). The formation by polyphenols of higher molecular weight and less reactive polymers can be postulated for all treatments. However, it may seem that NaOH and HCHO were more effective in formation of highly cross-linked, rigid and inactive oligomeric polymers. HCHO, despite its associated health risk, is currently being used in southern Africa to deactivate tannins in high-tannin sorghum used for malting. In this respect, the similar effect of NaOH on assayable tannins is of great potential economic importance.

NaOH also enhanced water uptake of both tannin-free and tannin-containing grains. Dewar et al (1997a) reported increased water uptake by NaOH in tannin-free, but not

tannin-containing sorghum. The effects of NaOH are presumed to be due to the alkali opening up the pericarp cell wall structure of the grain, allowing rapid water uptake (Dewar et al 1997a). The same authors argued that water uptake was not increased to the same extent in tannin-containing sorghum as the alkali preferentially reacted with tannins. My observation differed from theirs in that both the tannin-containing varieties had increased water uptake in addition to polyphenol reduction. Thus there might be differences in varietal response. The water uptake after an 8-hr steep in NaOH was similar to that achieved only after 24 hr of steeping in other solutions. Longer steeping periods may result in microbial proliferation (Dewar et al 1997b) and hence NaOH could be used to shorten the steeping time. The cost implications to the malting industry are obvious if malt production time could be cut by several hours.

The tannin-rich variety, DC-75 had highest potential DP, a measure of malt quality. Chirimaugute, a medium-tannin variety with a soft endosperm could not reach the same DP level even though its water uptake was better than that of the relatively hard DC-75 grain. Thus enhanced water uptake (Dewar et al 1997b), if used alone, may not be a reliable measure of malt quality among different sorghum varieties. DP was incompletely available when water or HCl was used for steeping DC-75 or Chirimaugute grain prior to germination. In other words, polyphenols remained inhibitory to the enzymes even after malting when water or HCl were used. Hence malting alone could not be used to reduce the enzyme inhibitory power of the tannins although it decreased the polyphenol content. These results contradict the recommendations in the literature that malting alone can be used as a way of reducing

tannins in sorghum (Osontugun et al 1989; Reichert et al 1980). These findings support the above postulations that oligomeric polymers, formed when water or HCl were used for grain treatment (Gupta and Haslam 1978; Reichert et al 1980), were less rigid, more polar and still reactive. Thus treatment with NaOH and HCHO was effective for purposes of increasing available DP in sorghum malt from tannin-containing varieties.

Roller milling or abrasive decortication resulted in flour products that had lower polyphenol content than the grain, due to removal of some of the pericarp and testa, the layers where tannins are located (Hahn and Rooney 1986). In the case of decortication, conditioning moisture up to 20 % using water, HCl (0.25 M), HCHO (0.017 M) and NaOH (0.075 M) as chemical treatments, did not seem to be directly responsible for polyphenol reduction as evidenced by enzyme inhibition assays of decorticated flour. This is also supported by reports that small amounts of water (15 % of grain weight) added to whole grain just before milling has no effect on assayable tannin (Butler 1981). In traditional decortication, the washing of grain, followed directly by pounding and wetting, does not allow time for significant amounts of water to move into the endosperm of the grain (Scheuring et al 1982). Hence, conditioning overnight allowed moisture equilibration throughout the grain that adversely affected abrasion of outer layers. With decortication, chemical treatment did not significantly affect polyphenol reduction presumably due to the abrading action that was largely responsible for removal of the outer layers. In contrast, conditioning aided in bran removal during roller milling. NaOH and HCHO further lowered the levels of

polyphenols, as observed in steeping for malt production. This evidence further supports the above hypothesis that polymerisation reaction products (Kennedy et al 1984; Morrison and Boyd 1984; Porter 1992) formed between polyphenols and NaOH or HCHO are more cross-linked, rigid and less reactive than when water or HCl is used. However, the effects of NaOH and HCHO were not apparent in abrasive decortication presumably due to the pearling action that removed most of the polyphenols.

Flour colour was not related to polyphenol content of the grain as DC-75 gave lighter-coloured products than Chirimaugute, presumably due to the fact that polyphenols that were measured in the raw grain were not the only compounds responsible for the colour of sorghum products. Rooney and Miller (1982) indicated that grain appearance is affected by pericarp colour and thickness, presence of pigmented testa, endosperm colour and texture. Thus substances other than the methanol-soluble polyphenols could be important in colour of sorghum grain and products. Swain (1965) also suggested a possible alteration in tannin structure as in formation of insoluble phlobaphenes or binding of tannin to a nearby grain component under different conditions. Thus there was also a possibility that polyphenols present in grain were converted to different reaction products during conditioning and milling for flour production. It remains difficult to accurately predict product colour from grain appearance if polyphenol assays are not targeted at specific phenolic compounds. However, roller milling and abrasive decortication still offered advantages over whole grain grinding in flour colour improvement as the dark outer

layers of tannin-containing grains were largely removed. Dada and Dendy (1987) also obtained relatively white flour of low tannin and high-tannin sorghum using semi-wet roller milling.

Grain was tempered up to a maximum of 20 % moisture since conditioning to 30 % prior to roller milling (Cecil 1986) is way too high to be practical in terms of water use, equilibration and then drying. The increase in flour yields in the order SV2 > DC-75 > Chirimaugute, can be attributable to differences in the floury endosperm portion of the cultivars. There was no advantage in conditioning to 16 or 20 % moisture prior to abrasive decortication for purposes of improving product yield and quality. Addition of water just prior to milling could have changed the above results as the water would still be concentrated in the outer layers of the grain. Higher yields were obtained at 12 % moisture, which equates to the traditional dry abrasion technique. The extent of enzyme inhibition in milled products was assayed, as tannins are known to inhibit most enzymes in *in vitro* assays (Hagerman and Butler 1981). Enzyme inhibition was also lowest in products obtained after decortication at 12 % moisture. Enzyme inhibition assays supported the effectiveness of NaOH and HCHO in polyphenol reduction compared to water and HCl. Conditioning Chirimaugute grain to 20 % moisture and roller milling resulted in flour products with lower enzyme inhibition than at 16 % moisture. Thus grain meant for food or feed could be conditioned to higher moisture with NaOH for the purposes of improving nutritional value where roller mills are already available. However, the practical implications of having to dry products to a relatively safer moisture for storage will still need to be

addressed. The tannin-rich variety, DC-75 gave products with higher enzyme inhibitory power than the medium-tannin variety, Chirimaugute, an indication that there were still more inhibitory polyphenols in DC-75 products.

Concerning steeping treatments using very dilute solutions in sorghum wet milling, differences in physico-chemical properties of sorghum starch were encountered. The importance of tan plant colour was evident with variety SV2. SV2 gave a white starch presumably due to lack of pigments in the pericarp and endosperm (Freeman and Watson 1971; Norris 1971). SV2 has a white pericarp, lacks pigmented testa and is devoid of polyphenols. Tannin-containing varieties, Chirimaugute and DC-75 gave pink coloured starches probably due to adsorption and retention of tannins by the starch (Davis and Hosney 1979). None of the treatments could prevent the starch from being coloured pink in tannin-containing varieties, presumably due to migration of phenols from the pericarp into the endosperm and the starch during isolation. Polymerisation reactions involving grain polyphenols and water, HCl, HCHO and NaOH (Gupta and Haslam 1978; Kennedy et al 1984; Morrison and Boyd 1984) could also have played a limited role. The colour which was further brightened by NaOH treatment (Yang and Seib 1995) did not disappear when starch was cooked but resulted in attractive, coloured starch pastes. In work not included in this thesis, sorghum noodles from white- and pink-coloured starch were rated higher in acceptability than those produced from sweet potato starch and wheat noodles (Hong Kong University). In selecting a particular steeping solution, it would be more

appropriate to choose reagents that enhance starch properties as desired by the end user.

Starches from NaOH-treated grain had higher PV and CPV. NaOH apparently caused some form of partial pre-gelatinisation, resulting in starches with greater swelling capacity. Molecules containing ionic groups have intensified water-binding ability at the ionic location, and, in addition, their repulsive charges cause starch molecules to repel each other (Whistler and BeMiller 1997). Treatment with NaOH was advantageous in cutting down on the time and temperature needed to reach PV as starches had enhanced swelling power. Thus there could be cost-saving implications to the NaOH-treatment on starch pasting properties. DC-75 starch had highest PV, probably due to its relatively high amylopectin content. Starches from HCl-treated grain had higher PV temperature presumably due to mild, acid-catalysed hydrolysis and would need more energy input to obtain the high viscosity from relatively short-chain polymers. Treatment of sorghum grain with HCHO gave starches with relatively higher PV temperature than when water or NaOH was used possibly due to the cross-linking effect of formaldehyde on starch and polyphenol polymers. Cool paste viscosity (CPV) and setback were high in NaOH-treated starches as the hydrating effect of NaOH enhanced the ability of starch to form viscous pastes after cooking and cooling. Alkali extraction also results in maize starch with high viscosity and high hydration capacity (Mistry and Eckhoff 1992). Starches from the tannin-rich variety, DC-75 had the softest gel regardless of treatment presumably due to the low amylose content of DC-75. A related sorghum variety, DC-333, also contains a low amylose

content (Taylor et al 1997) and both could presumably have been bred for higher amylopectin amounts. The medium-tannin Chirimaugute produced starches with the hardest gels. The unique gelling properties of starches from these two varieties could be taken advantage of in different food products. Variety and growth site would both affect the desired pasting, thermal and textural properties and it is important that varietal and environmental variations in starch properties be assessed in breeding programmes. In work not included in this thesis, environmental influence was observed on starch properties of sorghums that were grown in several locations in Zimbabwe. Sorghums identified to have unique starch properties could then be treated and modified to give the desired properties for end use.

Grain structure and polyphenol content affected the properties of starch isolated using the NaOH-steep. However, grain colour, polyphenol content and presence or absence of pigmented testa did not relate to the colour of isolated starch. Possible hypotheses for the lack of relationship, are threefold. Firstly, it could be presumed that the methanol-extractable phenolic components (Burns 1971) that were measured in the grain, were not directly responsible for starch colour. Secondly, the bound phenolic compounds that could not be extracted with methanol alone, should also be contributing to starch colour as significant amounts of tannins are extracted in acidic methanol than in methanol alone (Maxson and Rooney 1972; Cummings and Axtell 1973). Price et al (1980) surmised the formation of insoluble tannin-protein complexes, when large amounts of water were added to ground grain, from which tannin could not be extracted. The darker colour of Chibonda starch, compared to the

others, was evidence that polyphenols that were unextractable in methanol alone were important in starch colour. Bound phenolic compounds would presumably be less reactive than their unbound counterparts during the starch extraction procedure. When NaOH reacts with polyphenols to form highly polymeric and relatively inactive compounds (Kennedy et al 1984; Porter 1992), it would seem likely that the free phenolic compounds readily undergo the reactions in the presence of oxygen than their bound counterparts. Thirdly, it could be postulated that the phenolic compounds of the grain underwent numerous reactions during wet milling and surfaced as completely different entities in the isolated starch. The latter is supported by reports by Kennedy et al (1984) and Porter (1992) that flavan-3-ols and tannins give rise to a host of products including the less polar and rigid phlobatannins when exposed to mild alkaline conditions in the presence of oxygen.

In addition to steeping treatments, grain chemistry will also influence starch properties. For example, grain endosperm texture was correlated to starch amylose content, as previously reported (Cagampang and Kirleis 1984). Amylose, being essentially linear, could probably be closely packed within the sorghum grain than the more branched, amylopectin fraction of starch. Thus grains with relatively corneous endosperms have slightly more amylose, the starch polymer that readily undergoes retrogradation. Grain polyphenol content was positively correlated to starch pasting PV. Gelatinization temperatures previously reported for sorghum starches (Akingbala et al 1982) are higher than values obtained for Zimbabwean varieties. It could be that sorghum starches from southern Africa gelatinize at relatively lower temperatures than

varieties found elsewhere. This is supported by Taylor et al (1997) who also observed lower gelatinization temperatures for South African sorghums. Peak gelatinization temperature ( $T_p$ ) was positively correlated to both amylose content ( $P < 0.05$ ) and corneous endosperm fraction of the grain ( $P < 0.01$ ). Hence lower gelatinization temperatures were also attributed to kernel characteristics as most widely cultivated Zimbabwean sorghums have intermediate to floury endosperm. Sorghum grains with relatively corneous endosperm are known to have high gelatinization temperature (Cagampang and Kirleis 1984). The observation that starch from polyphenol-rich varieties required less enthalpy to gelatinize them could also be an indication of the role of polyphenols in disrupting the molecular order of starch granules. Starch gelatinization temperatures are influenced by both genetic and environmental factors (Akingbala et al 1984). The influence of endosperm texture on starch gelatinization temperatures of Zimbabwean sorghums was also important.

## 6. CONCLUSIONS AND RECOMMENDATIONS

Zimbabwean sorghums lack ideal agronomic and physico-chemical characteristics defined in terms of high polyphenols, plus hard endosperm and thin pericarp. Simple and effective treatments are recommended for processing the available polyphenol-rich sorghums.

Malting, alone, reduces polyphenol content but does not reduce the enzyme inhibitory power of sorghum tannins. Concerning chemical treatments during steeping of tannin-containing grain, malting should be done in combination with NaOH or HCHO treatment in order to reduce the enzyme inhibitory power of sorghum tannins. NaOH will also reduce the length of malting as it enhances water uptake of the grain. It appears that NaOH treatment is potentially a simple, relatively safe alternative to HCHO for use in sorghum malting.

In abrasive milling, reduction in polyphenol content is largely due to the abrasion of grain outer layers. The traditional, dry abrasive decortication technique is, therefore, recommended for milling tannin-containing grains since conditioning moisture and chemical treatments using water, HCl, HCHO and NaOH neither give significant reduction in polyphenol content nor improve the yield of decorticated flour. However, chemical treatments using HCHO and NaOH and conditioning moisture appear to be advantageous in roller milling, as the enzyme inhibitory power of tannins is significantly reduced in roller-milled flour. Thus enzyme inhibitory assays are recommended as a way of assessing treatment effects where animal-feeding

experiments cannot be conducted. Conditioning just prior to roller milling or abrasive decortication is recommended for future work.

Concerning chemical treatments in wet milling, steeping sorghum grain in dilute NaOH will give starch pastes with higher PV, CPV and setback than when water, HCl or HCHO is used. NaOH treatment will also give starches with reduced PV temperature and brighter colours due to polymerisation reactions between polyphenols and alkali. Thus dilute alkali steeping during wet milling is recommended to enhance the physico-chemical characteristics of sorghum starch. The polyphenol content of sorghum will influence starch properties, for example, grain polyphenol content is positively correlated to starch pasting PV. Similarly, kernel characteristics will also affect sorghum starch properties as grain floury endosperm portion is negatively correlated to starch amylose content and peak gelatinisation temperature.

Developing quality products using highly-coloured starch, isolated from polyphenol-rich sorghums, is an alternative towards improving the overall image of sorghum but the challenge will lie in obtaining a uniform pink colour from different batches of the same sorghum varieties during commercial operations. Future work should consider assessing the exact composition of starch and the yield of prime grade starch obtained under alkaline processing conditions.

The chemistry of sorghum phenolic compounds should be further investigated to understand the reactions that take place during food processing of high-tannin sorghums.

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## 8. PUBLICATIONS

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- Beta, T., Rooney, L. W., Marovatsanga, L. T., and Taylor, J. R. N. 1999. Phenolic compounds and kernel characteristics of Zimbabwean sorghums. *J. Sci. Food Agric.* 79:1003-1010.
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- Beta, T., Rooney, L. W., and Taylor, J. R. N. Effect of chemical conditioning on the milling of high-tannin sorghum. *J. Sci. Food Agric.*

### Presentations

- Beta, T., Rooney, L. W., Marovatsanga, L. T., and Taylor, J. R. N. 1997. Grain quality evaluation of sorghum varieties in Zimbabwe. Poster presented at the 14<sup>th</sup> biennial South African Association of Food Science and Technology (SAAFOST) international congress and exhibition, 1-4 September 1997, Pretoria,

South Africa.

Beta, T., Corke, H., and Taylor, J. R. N. 1999. Starch properties of Southern African sorghums. Poster presented at the 15<sup>th</sup> biennial South African Association of Food Science and Technology (SAAFOST) international congress and exhibition, 27-29 September 1999, Cape Town, South Africa.

Beta, T., Rooney, L. W., Marovatsanga, L. T., and Taylor, J. R. N. 1999. Physicochemical properties of Zimbabwean sorghums. Paper presented at the 10<sup>th</sup> World Congress of Food Science and Technology, 3-8 October 1999, Sydney, Australia.