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 pericap 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 Hoseney 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 theses 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 (Tp) 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.