Lowry, O. H.; Rosenbrough, N. J.; Farr, A. L.; Randal, R. J. *J. Biol. Chem.* **1951**, *193*, 265.
Meredith, P. *Cereal Chem.* **1976**, *47*, 492.
Novellie, L. *J. Sci. Food Agric.* **1962**, *13*, 115.
Saric, R. M. *Proc. U. N. Int. Conf. Peaceful Uses At. Energy, 2nd, 1958* **1958**, *27*, 233.
Tipples, K. H.; Norris, F. W. *J. Sci. Food Agric.* **1963**, *14*, 646.

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Polyphenol Changes in Sorghum Grain during Malting

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Bird-resistant and non-brid-resistant sorghum cultivars were malted to study the effects of tannin on malting as well as the change in polyphenols during malting. No difference could be found in the percent germination nor in the root and shoot production of the malts of the two cultivars. There was an increase in the anthocyanidin content of the roots and shoots during malting. Since there was also an increase in the anthocyanidin content of the non-bird-resistant kernels, it would appear that there is no migration of anthocyanidins from the kernels to the roots and shoots. A transmission electron micrograph of the testa after malting showed a testa very similar to that before malting. The tannin in the bird-resistant sorghum picked up extra protein or peptides during malting. Although it was difficult to establish the source of this extra material, amino acid analysis suggested that it was not from the prolamin storage protein in the endosperm.

Although sorghum beer has been traditionally brewed only in Africa, studies involving the malting of sorghum grain have been carried out in several countries having hot climates. In Sri Lanka it was hoped to replace imported barley malt with locally produced sorghum malt (Jayatissa et al., 1980). In Nigeria, studies involved the brewing of the lager beer (Okafor and Aniche, 1980), as well as the local Otika (Ogundiwin and Tehinse, 1981), from malted sorghum grains. In South Africa, sorghum is still malted for the brewing of traditional sorghum beer although some of the beer is now brewed by modern industrial methods (Novellie, 1968).

Besides geographical diversity, these sorghum malting studies covered a wide variety of modification changes. Unlike barley, gibberellic acid has little effect on amylase production in sorghum and it is probable that this production is a function of the scutellum rather than the aleurone layer (Daiber and Novellie, 1968; Aisien, 1980). The carbohydrate products of enzyme modification were established as glucose, maltose, and maltotriose (Aisien, 1982). Since malted sorghum is now used for brewing other types of beer as well as the traditional one, enzyme composition and activity are of considerable importance. The development of enzyme activity in germinating sorghums of different varieties has been reported by Nout and Davies (1982). They concluded that most sorghum malts were not desirable for conventional lager but some varieties were acceptable for production of a "tropical lager".

Because of their high diastatic power and good agronomic properties, bird-resistant sorghums are being produced extensively in South Africa. The large amounts of tannin found in the testae of these bird-resistant grains can inhibit enzymes essential to the brewing process (Daiber, 1975). This inhibition is caused by the tannins reacting with the enzymes to form insoluble complexes. Besides reducing enzyme activities, these tannins can also reduce the nutritional quality of the grain. Hence, there are numerous reports in the literature regarding the problems of tannin in sorghum grain. Such aspects as tannin structure, biosynthesis, location in the kernel, biochemical effects, and bird resistance are ably discussed by experts in proceedings edited by Hulse (1979).

The research literature on malting is very extensive and much has been reported on tannin, but very little has been reported on the effect of malting on tannin or on the effect of tannin on malt quality. Chavan et al. (1981) reported that approximately 73% of the tannins of a high-tannin sorghum were lost during germination. Also, they found that seedling growth was suppressed in these high-tannin grains and that the rate of starch and protein degradation was reduced. In another study it was found that after 6 days of malting some of the sorghum tannins required more solvent to elute them from a Sepharose CL-6B column than was necessary before malting (McGrath et al., 1982). The fate of the tannins during malting have now been more extensively studied, especially in respect to their location in the testa. Besides the tannins, the fate of other phenolic compounds such as anthocyanidins, phenolic acids and the cyanogenic compound, dhurrin, have been studied during malting and these studies are now reported.

MATERIALS AND METHODS

Malting. Three cultivars of sorghum grain, Barnard Red, NK283 and SSK30, were harvested from the 1981/1982 crop. The first two are not bird resistant while the third one is. The grain was surface sterilized with 0.2% Adcodyne (an idophore) and steeped for 16 h (water changed after every 3 h with a 1-h air rest). Malting was carried out in nylon mesh bags loaded so that the layer of grain was three to five kernels thick. A Forma Scientific incubator was used to keep the temperature at 28 °C and the relative humidity at 99%. The grain was watered twice a day by steeping (1 × 5 min, 1 × 10 min) in water containing 0.05% hypochlorite. Excess moisture was removed

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by centrifuging at 1500 rpm for 3 min. Samples were taken every second day up to 6 days and dried overnight in an oven at 50 °C.

For much of this work the roots and shoots were first separated from the malt kernels. This involved rubbing the dried seedlings in a nylon bag with a mesh size that allowed the roots and shoots to escape while retaining the kernels.

Effect of Tannins on Malting. Samples of Barnard Red (low tannin) and SSK52 (high tannin) were germinated on moist filter paper in 9 cm diameter Petri dishes (50 grains each) as above. Each day one dish of seedlings was taken and root and shoot length as well as percentage germination determined.

Extraction and Separation of Polyphenols. The polyphenols were extracted from 50 g of grain, malted kernels, or roots and shoots and separated on a Sepharose CL-6B column as previously described (Kaluza et al., 1980; McGrath et al., 1982). Some tannins from the malted kernels required an extra 1500 mL of 50% v/v aqueous acetone to elute them from the column.

Determination of Polyphenols and Tannins. Grain, malted kernels, and roots and shoots (0.5 g) were exhaustively extracted with 70% v/v aqueous acetone. The extract was taken to dryness on the rotary evaporator and then dissolved in 2 mL of 70% v/v aqueous acetone and an appropriate aliquot taken for analysis. The polyphenols were determined by a modified Folin-Ciocalteu method (McGrath et al., 1982).

Samples (250 mg) of grain, malted kernels, and roots and shoots were extracted with 5 mL of 75% v/v aqueous dimethylformamide for tannin determinations. An aliquot of this was analyzed according to the method of Jerumanis (1972) using tannic acid (Merck, Dormstadt) as a standard.

Nitrogen Determination. Samples (25 mg) of tannin were analyzed for protein ($N \times 6.25$) by the method of Thomas et al. (1967). Determinations were carried out in duplicate.

Reducing Sugar Determination. Sugars were obtained by hydrolyzing the tannin with 2.0 M HCl at 96 °C for 1.5 h. The ratio of HCl to polyphenol was 2.5 mL to 1 mg. The hydrolyzed samples were cleaned up according to McGrath et al. (1982). Duplicate aliquots of the samples were analyzed manually by using the reduction of potassium ferricyanide, $K_3[Fe(CN)_6]$, as described by Hoffman (1937).

Electron Microscopy. Samples of grain, hybrid SSK52, were malted and prepared for transmission electron microscopy as previously described (Glennie et al., 1983). A Phillips EM301 transmission electron microscope was operated at 60 kV.

Amino Acid Analysis. Tannin fractions were isolated from the Sepharose CL-6B column as already described (Kaluza et al., 1980). Aliquots of these fractions were hydrolyzed in 6 M HCl at 110 °C for 22 h in evacuated tubes. After being cooled, the tubes were opened and the samples were dried in a vacuum desiccator over P_2O_5 powder and NaOH pellets. The dried samples were purified by using procedures described by Higgins and Payne (1981). Appropriate aliquots were then analyzed for amino acids on a Technicon TSM amino acid analyzer.

Measurement of Color in Malted Sorghum. Malted kernels or roots and shoots (2 g) were extracted with 100 mL of 70% v/v aqueous acetone, evaporated to dryness, and taken up in 5 mL of 70% acetone. The extract (1 mL) was hydrolyzed with 1 mL of 2 M HCl at 96 °C for 30 min. The sample was extracted with 5 mL of 1-pentanol and diluted to 10 mL with 1% v/v HCl in methanol. Measurements were carried out in duplicate and absorbance was read at 520 nm on a Pye Unicam SP1800 ultraviolet spectrophotometer.

Table I. Polyphenol Content of Sorghum Grain before and after Malting[a]

cultivar	polyphenol content, % grain	malted kernels[b]	roots and shoots
Barnard Red	0.10	0.20	0.46
NK 283	0.08	0.14	0.42
SSK 30	0.89	0.68	0.43

[a] Polyphenol content was determined by the Folin-Ciocalteu method. [b] These values have been corrected for malting loss, where malting loss is the loss in dry weight which occurred during malting—mainly through respiration.

Cyanogenic Glycoside Analysis. Grain, malted kernels, and roots and shoots (mixed) as well as freeze-dried wort and beer were examined separately for cyanogenic glycosides. Small, milled samples (500 mg) of each of the above were put into 100-mL flasks and 5 mL of H_2O was added. Approximately 5 mg of β-glucosidase (BDH; from sweet almond meal) was added, and the released hydrogen cyanide was detected by picrate papers as described by Harborne (1973).

RESULTS AND DISCUSSION

Effect of Tannin on Malting. The tannin contents of the Barnard Red (low tannin) and the SSK30 (high tannin) were 0.10% and 1.20% respectively. The percentage germination was 93% for both cultivars, and no difference could be found in root and shoot length of the malts of the two cultivars. To check these results, the results from several hundred malt samples analyzed over several years were compared. No statistical difference could be found between bird-resistant (12.8 ± 1.8%) and non-bird-resistant (12.0 ± 1.8%) seedling yield on a mass basis (Daiber, 1983). Statistical analyses were carried out as correlation analyses with dummy variables for both bird-resistant and non-bird-resistant samples.

In contrast, Chavan et al. (1981) reported that in a similar study the root and shoot growth of their high tannin cultivar was suppressed. They suggested that this slower growth could be attributed to the higher tannin content. This is unlikely as the tannin is compartmentalized in the testa where it remains during germination (see below). It is only when the grain is milled and the testa is disrupted, releasing the tannin, that it becomes a problem as in the brewing process or in enzyme assays (Daiber, 1975). Is it possible that the results presented by Chavan et al. (1981) are cultivar dependent rather than related to tannin content?

When a solution of tannic acid was used to treat low-tannin sorghum during germination, it suppressed root growth and starch degradation (Chukwura and Muller, 1982). Tannic acid (hydrolyzable tannin) is not found in sorghum that contains condensed tannin. However, the inhibitory effect for both types of tannin would probably be similar. As mentioned above, the condensed tannin in sorghum is confined to the testa where it apparently does not interfere with the germination process. The tannic acid was not confined to the testa but rather was in solution so it could penetrate into the grain and thus inhibit enzyme activity during germination. Hence, the inhibition displayed by the soluble tannin was due to its mobility while the testa-bound tannin could not reach the site of enzyme activity and was, therefore, not inhibitory.

Changes in Polyphenols during Malting. After

Table II. Protein Content of Tannin Fractions Isolated from Bird-Resistant Sorghum Malt (SSK30)

malting period	protein content, %				
	F_1	F_2	F_3	F_{red}	F_{yellow}
grain	5.6	1.1	1.3	1.5	–[a]
day 2	8.5	1.4	0.8	1.5	5.4
day 4	8.8	2.1	1.3	2.9	4.8
day 6	8.2	3.4	1.4	3.2	5.0
day 8	8.9	2.9	1.6	3.2	7.5
day 10	10.6	2.9	1.6	1.8	6.0
day 12	10.8	2.8	1.1	4.1	–[a]

[a] Did not elute off the column. It is probable that in the ungerminated grain such a fraction did not exist and in the grain germinated for 12 days it is possible that the fraction had picked up sufficient protein so that it would not elute from the column with the solvents used.

Table III. Reducing Sugar Content of Tannin Fractions Isolated from Bird-Resistant Sorghum before and after a Six-Day Malt

fraction	reducing sugar content, %	
	grain	6-day malt
F_1	0.92	0.69
F_2	1.30	1.33
F_3	0.67	1.47

malting for 6 days, there was a loss in polyphenols in the kernels of the bird-resistant sorghum while the two non-bird-resistant cultivars showed an increase (Table I). The polyphenols discussed in this paper are polyphenols that react with Folin-Ciocalteu reagent rather than tannins that are determined by the Jerumanis method. Polyphenols determined this way probably included the tannins. The loss in polyphenols in the kernels of the bird-resistant cultivars during malting could probably be accounted for by the binding of the tannins by protein, thus reducing their solubility. The increase in polyphenols in the non-bird-resistant kernels could come about by solubilization that occurred as the grain took up water or by synthesis.

The polyphenol contents of the roots and shoots of all three samples were similar and were not related to the original polyphenol content of the grain. This, along with an increase in the polyphenol content in the kernels of the non-bird-resistant grain, would suggest that the polyphenols in the roots and shoots were synthesized de novo and did not migrate from the kernels during malting. Since the shoots are sites of active biosynthesis, Stafford (1965) used the first internodes (2–3 cm in length) of 4-day-germinated sorghum kernels to study various factors affecting formation of phenolic compounds. In the present study the polyphenols were extracted from the roots and shoots and chromatographed on Sepharose CL-6B. With this method, which distinguishes between the low molecular weight polyphenols and the tannins, no tannins could be detected in the roots and shoots. This would suggest that the tannins remained in the kernel and were probably still restricted to the testa.

It has already been established that the elution patterns of sorghum polyphenols from a Sepharose CL-6B column changed after malting for 6 days (McGrath et al., 1982). After malting, extra solvent was required to elute the red and yellow peaks from fraction III. It was suggested that either sugars or polypeptides could be removed from the tannin during malting, allowing it to bind more strongly to the column. Actually, as Table II shows, the tannin fractions increased in protein content during malting. It is probably this increased protein content that caused the change in elution patterns of the tannins.

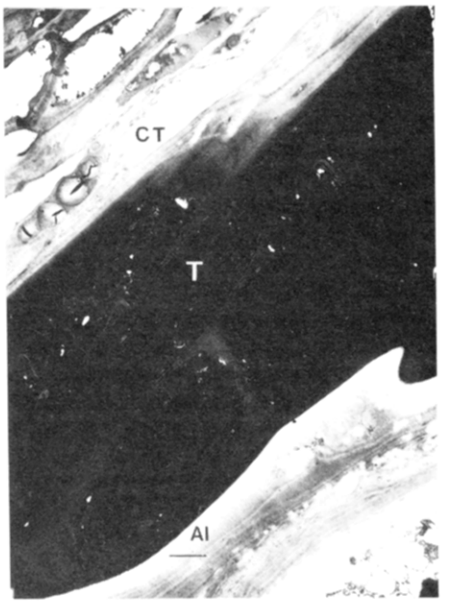

Figure 1. Electron micrograph of the testa of a malted bird-resistant sorghum (Al = aleurone cells; T = testa; CT = cross and tube cells). Bar = 3.5 μm.

Table III shows the change in reducing sugar content of the tannin fractions after malting. F_I decreased in sugar content but these sugars were probably not bound to the low molecular weight polyphenols found in this fraction. Rather, the sugars and polyphenols probably eluted from the column in the same volume. F_{II} did not change its sugar content during malting while F_{III} doubled in sugar. However, it is doubtful whether this small amount of sugar would change the elution patterns of the tannins.

Although there was a slight change in the chemical composition of the isolated tannin fractions, there was no discernible difference in the tannin in situ after 6 days of malting. Figure 1 shows an electron micrograph of the testa of a malted bird-resistant grain, and it appears very similar to that of an unmalted grain (Morrall et al., 1981). Sections of bird-resistant grain were prepared (unstained) for the light microscope, and the testa appeared as described by Blakely et al. (1979). That is, the testa was often composed of two distinct layers: one layer was a reddish brown and the other a yellowish brown. It could be that these two layers contribute to the red and yellow fractions isolated from the Sepharose CL-6B column. Microscopic examination suggests that the reddish brown layer is the larger, and when the fractions are isolated from the column, there is usually more of the red fraction than of the yellow.

Amino Acid Composition of Tannin Associated Protein. The tannin fractions isolated from the Sepharose CL-6B column had protein associated with them. Because sorghum is used as a malting grain it is important to establish the source of such protein. During malting the proteinases degrade the storage protein in the endosperm, thus creating nutrients that the yeast utilizes during the brewing process. But, if the tannins are tying up this storage protein, it then becomes unavailable for the yeast, resulting in poor fermentation. Table IV shows the amino acid composition of the protein associated with F_{III} tannin as well as the composition of prolamin storage proteins isolated from whole sorghum grain (Taylor, 1983). This comparison shows that the protein being complexed by the tannin did not have the same amino acid composition as the prolamin that constituted the major storage protein in the sorghum endosperm.

Table IV. A Comparison of the Amino Acid Composition of Proteins Associated with Tannin with the Composition of Prolamin Storage Protein (g/100 g of Protein)

amino acid	F_{III} tannin protein	prolamin
lysine	2.5	0
histidine	0	1.0
ammonia	excess	2.5
arginine	0	2.3
aspartic acid	0.7	4.8
threonine	1.9	2.1
serine	3.2	3.1
glutamic acid	7.4	25.0
proline	12.5	7.2
half-cystine	0	1.9
glycine	5.0	1.1
alanine	10.1	12.3
valine	8.4	5.8
methionine	0	1.2
isoleucine	11.6	5.2
leucine	25.2	15.9
tyrosine	2.5	3.7
phenylalanine	5.1	5.1
γ-aminobutyric acid	3.9	0

It is not known what exactly is associated with the tannin in the intact testa, but it is clear that, after milling, the tannins have peptides or proteins associated with them that increase during malting (Table II). Since the grain and malt samples were prepared similarly, the increase was probably a result of the malting process. It is not known where this extra protein or peptide comes from, but since it is unlikely to come from the prolamins, it could come from the pericarp or germ. The roots and shoots were removed before the malted kernels were milled so they could not act as a source for this extra material.

Finding γ-aminobutyric acid associated with the tannin implies that free amino acids could also be associated with the tannins. γ-Aminobutyric acid is a non-protein amino acid found in germinating grains, and in bird-resistant sorghums it became complexed with the tannin. When milled grain was extracted and the polyphenols separated on a Sepharose CL-6B column, then the first fraction was composed mainly of low molecular weight phenolic compounds. However, when the extract was prepared from malted kernels, this fraction contained a large amount of γ-aminobutyric acid (≃12% of the protein fraction). Thus, it is not clear what the tannins in the malted kernel were complexing with, but the results in Table IV suggest that it was not the storage proteins.

Color in Malted Sorghum. Visual inspection of the germinating grain each day showed that the roots became colored with anthocyanins especially in the region near wounds while the shoots did not. There was no indication that color was being translocated from the kernels to the roots. The changes in color, expressed as anthocyanidins, followed the same patterns as found for the polyphenol content in general. The color in the kernel as well as the roots and shoots increased during malting in both the bird-resistant and non-bird-resistant samples (Figure 2). As Figure 2 shows, the bird-resistant sorghum contained more color in the kernels than the non-bird-resistant one while the non-bird-resistant grain produced considerably more color in its roots and shoots.

Cyanogenic Glycosides. The distribution of the cyanogenic glycoside dhurrin in germinating sorghum seedlings has been established (Akazawa et al., 1960). Upon disruption of the seedlings, the dhurrin is enzymatically hydrolyzed to form equimolar amounts of HCN and *p*-hydroxybenzaldehyde. Etiolated seedlings and green seedlings contained similar amounts of dhurrin while ungerminated grain did not. Because of its dhurrin production, sorghum has been used to study the intermediate compounds in dhurrin biosynthesis from L-tyrosine (Conn, 1979).

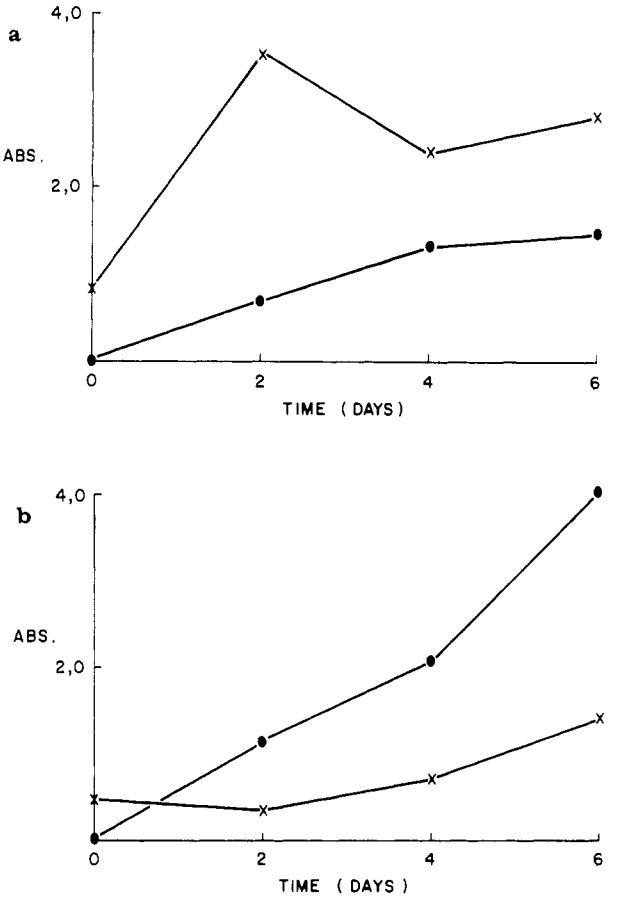

Figure 2. Changes in anthocyanidin concentration (expressed as absorbance at 530 nm) of roots and shoots and kernels of sorghum during malting: (a) bird-resistant sorghum (SSK30); (b) non-bird-resistant sorghum (Barnard Red). (×) Kernels; (●) roots and shoots.

The present study confirms the above results. When various tissues were tested for the presence of the cyanogenic glycoside, the roots and shoots were positive while the grain and the germinated kernels were negative. Since the roots and shoots are not removed before sorghum malt is used in the brewing of sorghum beer, the various stages of brewing were analyzed for HCN production. All stages were weakly positive, and in the final beer the concentration was extremely low and detection became very difficult.

In conclusion, the results of the investigation presented in this paper suggest that the tannins remain in the testa during malting and do not affect the malting process. The roots and shoots of the malt develop a red color that is carried over to contribute to the characteristically pink color of sorghum beer. Also, dhurrin is found in the roots and shoots but not in the grain and could contribute to the flavor of the final beer.

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LITERATURE CITED

Aisien, A. O. Ph.D. Thesis, Heriot-Watt University, Edinburgh, 1980.

Aisien, A. O. *J. Inst. Brew.* **1982**, *88*, 164.

Akazawa, T.; Miljanick, P.; Conn, E. E. *Plant Physiol.* **1960**, *35*, 535.
Blakely, M. E.; Rooney, L. W.; Sullins, R. D.; Miller, F. R. *Crop Sci.* **1979**, *19*, 837.
Chavan, J. K.; Kadam, S. S.; Salunkhe, D. K. *J. Food Sci.* **1981**, *46*, 638.
Chukwura, E. N.; Muller, H. G. *J. Food Sci.* **1982**, *47*, 1380.
Conn, E. E. *Naturwissenschaften* **1979**, *66*, 28.
Daiber, K. H. *J. Sci. Food Agric.* **1975**, *26*, 1399.
Daiber, K. H., Sorghum Beer Unit, CSIR, Pretoria, unpublished results, 1983.
Daiber, K. H.; Novellie, L. *J. Sci. Food Agric.* **1968**, *19*, 87.
Glennie, C. W.; Harris, J.; Liebenberg, N. v. d. W. *Cereal Chem.* **1983**, *60*, 27.
Harborne, J. B. "Phytochemical Methods"; Chapman and Hall: London, 1973; p 194.
Higgins, C. F.; Payne, J. W. *Plant Physiol.* **1981**, *67*, 785.
Hoffman, W. S. *J. Biol. Chem.* **1937**, *120*, 51.
Hulse, J. H., Ed. "Polyphenols in Cereals and Legumes"; International Development Research Centre: Ottawa, Canada, 1979.
Jayatissa, P. M.; Pathirana, R. A.; Sivayogasunderam, K. *J. Inst. Brew.* **1980**, *86*, 18.
Jerumanis, J. *Brauwissenschaft* **1972**, *25*, 313.
Kaluza, W. Z.; McGrath, R. M.; Robberts, T. C.; Schröder, H. H. *J. Agric. Food Chem.* **1980**, *28*, 1191.
McGrath, R. M.; Kaluza, W. Z.; Daiber, K. H.; van der Riet, W. B.; Glennie, C. W. *J. Agric. Food Chem.* **1982**, *30*, 450.
Morrall, P.; Liebenberg, N. v. d. W.; Glennie, C. W. *Scanning Electron Microsc.* **1981**, *3*, 571.
Nout, M. J. R.; Davies, B. J. *J. Inst. Brew.* **1982**, *88*, 157.
Novellie, L. *Wallerstein Lab. Commun.* **1968**, *31*, 17.
Ogundiwin, J. O.; Tehinse, J. F. *Brew. Distilling Int.* **1981**, *11*, 42.
Okafor, N.; Aniche, G. N. *Brew. Distilling Int.* **1980**, *10*, 32.
Stafford, H. A. *Plant Physiol.* **1965**, *40*, 130.
Taylor, J. R. N., Sorghum Beer Unit, CSIR, Pretoria, unpublished data, 1983.
Thomas, R. L.; Sheard, R. W.; Moyer, J. R. *Agron. J.* **1967**, *59*, 240.

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Characterization of the Condensed Tannin (Proanthocyanidin) from a Group II Sorghum

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Most of the tannin in seeds of high-tannin bird-resistant group III sorghums [*Sorghum bicolor* (L.) Moench] extracts readily into methanol or aqueous acetone. In contrast, the tannin of group II sorghums is not extracted by these solvents but is extracted by methanol containing 1% (v/v) HCl. Group II sorghums have been reported to be nutritionally less harmful than group III types, presumably due to differences in their tannins. We have purified tannin from a group II sorghum (IS 8768) and compared it to tannin purified from a group III sorghum (DeKalb BR 64). We do not find significant differences in the properties of the purified tannins. Tannin extractable only in acidic methanol is present along with methanol-extractable tannin in most group III sorghums, especially in immature grain. The differentially extractable tannins do not appear to have a precursor/product relationship.

One of the constraints on utilization of sorghum grain as food or feed is the occurrence, in some cultivars, of condensed tannins (proanthocyanidins, oligomers of flavan-3-ols). The presence of tannins is associated with decreased bird preference (Bullard and Elias, 1980) and lower digestibility (Jambunathan and Mertz, 1973; Hartigan, 1979) of the grain. Nutritionally superior low-tannin and tannin-free cultivars and hybrids are available but are more difficult to produce than high-tannin "bird-resistant" sorghums in areas where bird depredation is a severe problem (Tipton et al., 1970; Hoshino and Duncan, 1980). In addition to resistance to bird depredation, tannins may also confer resistance to preharvest germination (Harris and Burns, 1970) and molding (Harris and Burns, 1973).

Sorghum cultivars have been classified into three groups according to the tannin content of the grain and the genes which control it (Cummings, 1973; Price and Butler, 1977; Hartigan, 1979; Rooney and Miller, 1982). Group I sorghums do not have a pigmented testa layer and have no tannin, although other polyphenols may be present. Group II sorghums have a pigmented testa layer that contains condensed tannins. Most of these tannins are unusual in that they cannot be extracted from the seed with methanol or aqueous acetone. However, the tannins are readily extracted by methanol to which 1% concentrated HCl has been added (Maxson and Rooney, 1972). Group II sorghums, of which Hegari is an example, have dominant B_1 and B_2 genes with a recessive *s* gene resulting in the occurrence of polyphenols, including tannin, in the pigmented testa layer (Rooney and Miller, 1982). Group III sorghums have a pigmented testa layer (B_1–B_2–) and a dominant *S* gene resulting in polyphenols occurring in both the pigmented testa layer and the pericarp (Rooney and Miller, 1982). Group III sorghums, which include the well-known "bird-resistant" hybrids such as DeKalb BR 64, contain tannin readily extractable into methanol without HCl; many of them also contain tannin extractable only in acidic methanol (see Results).

Oswalt (1975), Hartigan (1979), and Bullard and Elias (1980) have suggested that tannins from group II sorghums may have less severe antinutritional effects than tannins from group III sorghums. The chemical characterization of tannins from group II sorghums is less complete than that of tannins from group III sorghums. Bullard et al. (1981) have analyzed polyphenols extracted by acetone, methanol, and aqueous methanol from three group II sorghums. We report here the purification and characterization from a group II sorghum (IS 8768) of those condensed tannins that do not extract into acetone, methanol, or their aqueous mixtures but that do extract

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