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Polyphenols of Sorghum Grain, Their Changes during Malting, and Their Inhibitory Nature

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An automated system for the detection of tannins is described. It uses the precipitation of bovine serum albumin (BSA), and this, in tandem with an automated detection of phenolic groups, has been used for the rapid investigation of four sorghum varieties. Two of the varieties were bird resistant and were distinguishable from the non-bird-resistant grains by the presence of a tannin fraction that precipitates protein. The noninhibiting fraction (F_1) of all four varieties contained a large number of varied phenolics. During malting the roots and shoots developed a large complement of F_1 polyphenols and the properties of the tannins changed. The ability of the grain to resist mold growth is related to polyphenol content; resistance was ascribed to the physical barrier set up by the tannin-containing testa. Formaldehyde treatment of whole grain reduced both the number of available phenolic hydroxyl groups in the tannin and its ability to precipitate BSA.

Polyphenols are a large and important group of secondary metabolites found in higher plants. One group of polyphenols comprises the tannins, and besides giving the usual phenolic reactions, they also have the ability to precipitate proteins. It is these compounds that are found in the testae of certain varieties of grain sorghum, thus making them bird-resistant.

These tannins can inactivate enzymes required during the brewing of sorghum beer (Daiber, 1975) and also reduce the nutritional quality of the grain. Because of this it is important to develop a better understanding of tannins. The monomeric building blocks of tannins have been established (Gupta and Haslam, 1978), and a sequence for their biosynthesis has been proposed (Gupta and Haslam, 1979).

Work has recently been reported on the extraction of the polyphenols from bird-resistant sorghums and their separation into several fractions (Kaluza et al., 1980). Aqueous acetone (70%) was found to be a good solvent for sorghum polyphenols and it did not extract proteins. For separation of the polyphenols Sepharose CL-6B was used. It separated the polyphenols into three fractions; the first fraction contained low molecular weight polyphenols which did not precipitate protein while the other two fractions contained tannins of different molecular weights and they did precipitate protein.

The present study uses these techniques to compare the polyphenols of two bird-resistant sorghum varieties with those of two non-bird-resistant varieties. While working with the bird-resistant grains we observed that their polyphenolic patterns changed during malting. One purpose of this study was to observe this phenomenon more closely. Additionally, we have tried to overcome the inhibitory nature of the tannins by treating a bird-resistant grain with formaldehyde.

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EXPERIMENTAL PROCEDURES

Four varieties of sorghum grain, SSK2, NK300, DC36, and Breytenbach Red, were collected as described before (Kaluza et al., 1980). The first two were bird-resistant varieties, with SSK2 having the higher tannin content. The last two are not bird resistant but represent marketing classes known in South Africa as KM and KR, respectively. Additional SSK2 grain was (a) treated with formaldehyde and (b) malted and the phenolic pattern examined.

Extraction and Separation of Polyphenols. The polyphenols were extracted with aqueous acetone and separated into the major fractions (F_1, F_2, and F_3) by using an acetone gradient on Sepharose CL-6B columns (Kaluza et al., 1980). Usually, 50 g of ground grain was extracted for each column run. Two gradients were applied sequentially: (1) H_2O–H_2O–80% v/v aqueous MeOH (250:250:300 mL); (2) 80% aqueous MeOH–25% v/v aqueous Me_2CO–50% Me_2CO (280:250:264 mL). This solvent was found to be the best of many tried, and it separated the polyphenols of sorghum into a fraction containing the noninhibitory polyphenols and two fractions containing the inhibitory tannins.

A dimethylformamide (DMF) elution was also used during the study of the effect of formaldehyde on polyphenols. It consisted of two parts: (1) H_2O–H_2O–80% aqueous MeOH (250:250:300 mL); (2) 80% aqueous MeOH–20% v/v aqueous DMF–40% aqueous DMF (250:209:211 mL). DMF was sometimes used in preference to acetone because acetone interferes slightly with the automated phenolic detection system (Kaluza et al., 1980).

Automated Detection of Tannins by BSA Precipitates. We have been able to show in this study that if the pH is constant at pH 4.8 and ionic strength is 0.1, then the precipitation of BSA is proportional to the amount of

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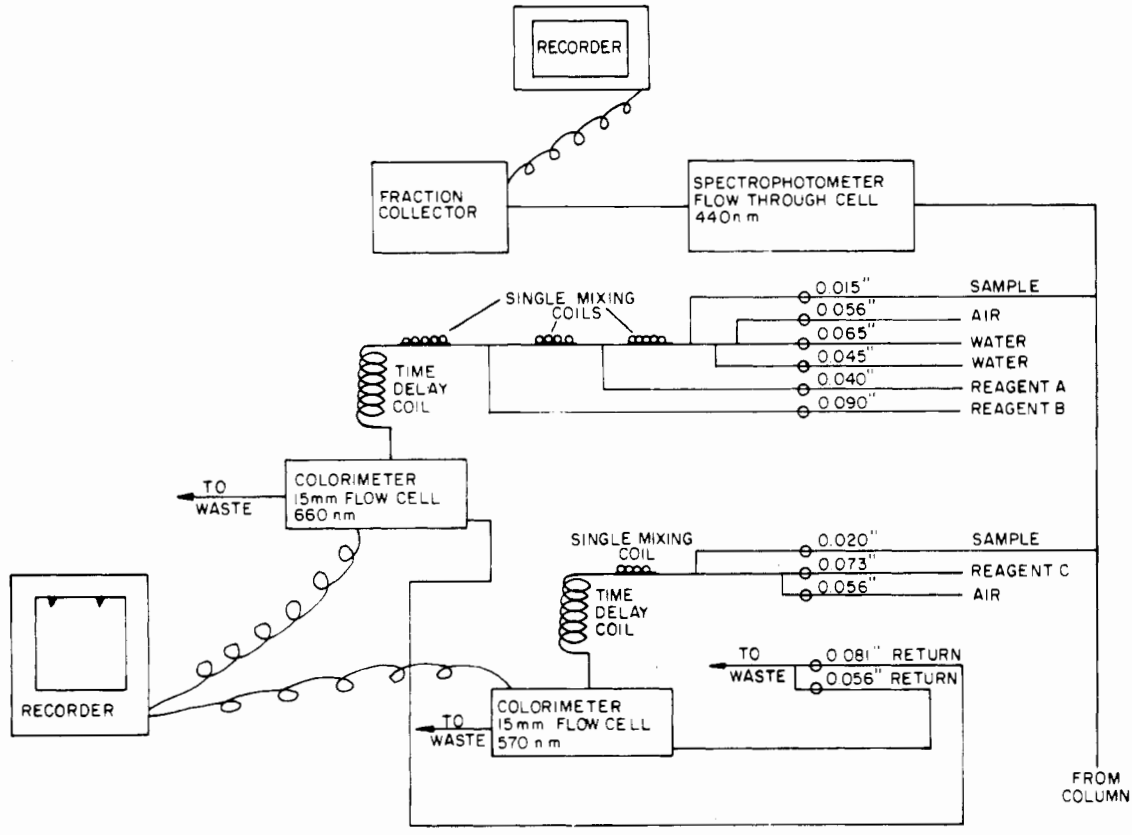

Figure 1. Scheme showing the Technicon Automatic Analyzer system used in the continuous automatic detection of total sorghum polyphenols and inhibitory (tanning) phenolics. Reagent A: 1:4 dilution of Folin–Ciocalteu reagent (Merck, West Germany). Reagent B: 0.5 M ethanolamine. Reagent C: 2 mg/mL BSA in 0.05 M sodium citrate buffer, pH 4.8.

tannin (F_3). However, this proportionately does not hold at either high pH or high ionic strength. Using this fact, we designed the fully automated and continuous system described diagrammatically in Figure 1. It should be noted that since this method depends on the continued suspension of a protein–tannin complex, then no rapid slow down in movement should be allowed to occur. If this happens, the suspension subsides, which is disastrous if it happens in the flow cell. So that this could be prevented from happening, all joints should be made by using the same inner diameter rubber tubing. This is particularly true for the inlet and outlet from the flow cell. Light dispersion was measured at 570 nm.

Automated Detection of Total Polyphenols. This method has been described previously (Kaluza et al., 1980) but is shown in tandem with the detection of tannins. The modified Folin–Ciocalteu detection of phenolic hydroxyls was used rather than the modified Jerumanis simply because of its greater sensitivity.

Detection of Colored Compounds. This was done by monitoring the effluent at 440 nm with a flow cell and a simple spectrophotometer (Figure 1).

Manual Estimation of Polyphenols. Either the method of Jerumanis (1972) or a modified Folin–Ciocalteu was used. A suitable aliquot of the polyphenol solution (5–100 μL) was added to 5 mL of H_2O, followed by 0.5 mL of reagent. After 1 min, 10 mL of 1.0 M ethanolamine was added to give an immediate phosphomolybdate blue color, the OD of which was read after 10 min at 750 nm.

The usual Na_2CO_3 solution cannot be used in place of ethanolamine; otherwise a white precipitate results. The blue color developed is directly related to the concentration of phenolics in the range 0–150 μg at least. Proteins have a very low color production (8%) compared to phenols on a weight basis under the conditions described here. It should be remembered that the grain was extracted with 70% aqueous acetone to specifically exclude proteins from the extract.

Protein Estimation. The method of McGrath (1972) with half the reported NaCN concentration was used because phenols do not interfere.

Sugar Identification and Estimation. Sugars were obtained by hydrolyzing F_1, F_2, and F_3 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. Because the polyphenols interfered with the ion-exchange columns used to separate the sugars, they were removed. This was done by chromatography on a small column (1 × 15 cm) of defined polycaprolactam (MN-Polyamide SC6, Macherey Nagel and Co., Düren, West Germany). The column was eluted with H_2O and this allowed the polyphenols to bind to the column while the sugars were eluted. The sugar-containing effluent was dried under vacuum on a rotary evaporator and the residue dissolved in 5 mL of H_2O. The sugars of an aliquot were separated on an ion-exchange column (Technicon Type S Chromobeads) (Khym and Zill, 1956) and detected by an orcinol reaction described by Brown (1946).

Formaldehyde Treatment of SSK2 Grain. SSK2 grain was steeped for different times at various concentrations of formaldehyde in H_2O in the ratio of 1:1 (w/v). After the grain was steeped, the liquor was drained off, and the grain was washed 3 times with H_2O and then dried at 50 °C in a forced draught of air. The time of contact and formaldehyde concentration are specified in the relevant text.

Malting of Bird-Resistant Grain. The grain was steeped and malted for 6 days at 28 °C in the laboratory as described by Daiber et al. (1973). The malt was polished to remove the roots and shoots, and these as well as the berries were extracted with 70% aqueous acetone. The roots and shoots were combined to form one sample. A portion of this extract was fractionated on a column of

Sepharose CL-6B as already described, while other aliquots were hydrolyzed. Acid hydrolysis yielded anthocyanidins, and their UV spectra and R_f values were determined according to Harborne (1973a). Alkaline hydrolyses as described by Harborne (1973b) were used to determine phenolic acid patterns in berries, roots, and shoots. After examination of the TLC plates under UV light, the phenolic acids were detected by spraying the plates with Pauly's reagent (diazotized sulfanilic acid).

Thin-Layer Chromatography (TLC). The three fractions (F_1, F_2, and F_3) from four varieties of grain were examined by TLC on commercially prepared Cellulose-F plates (Merck, West Germany). They were visualized by spraying with 2-aminoethyl diphenylboric acid ester (Somaroo et al., 1973). The abbreviations used in the description of colors developed when viewed under UV (254 nm) are as follows: bl, blue; br, brown; g, gray; v, violet; y, yellow; r, red; or, orange; fl, fluorescent; l, light; w, weak.

High-Performance Liquid Chromatography (HPLC). The three varieties examined by HPLC were SSK32, NK300, and DC90. The ground grain (2 g) was exhaustively extracted with 70% aqueous acetone and the extract concentrated to 5 mL. Thirty microliters of this was injected into the HPLC. A Varian 5000 HPLC was used and the detector was a Micrometrics Chromoniter 785 set at 280 nm. The column (25 × 0.4 cm) was packed by the balanced density slurry method of Asshauer and Halász (1974) with Nucleosil 5 C_{18} (Macherey Nagel & Co.). Various solvents and programs were tried and the conditions reported here were found to be optimal. The solvents used were (A) 5% HOAc in H_2O and (B) 5% HOAc in MeOH. The solvent program was 10% B (90% A) at time 0 changing to 60% B (40% A) at 100 min and 100% B at 114 min which was maintained for 6 min. The flow rate was 1.0 mL/min.

Fungal Growth in the Presence of F_2 and F_3 from SSK2. A chemical medium was prepared which consisted of (A) $NaNO_3$ (3 g), K_2HPO_4 (1 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), KCl (0.5 g), and $FeSO_4 \cdot 7H_2O$ (0.01 g) in 500 mL of H_2O and (B) starch for diastase determination (5 g) free from low molecular weight carbohydrates (Merck) in 500 mL of H_2O. The solutions were sterilized separately by filtration and were then asceptically mixed. To 5 × 100 mL of the medium (in 500-mL Erlenmeyer flasks) were added 0.1, 1, 5, 10, and 50 mg of F_2 and F_3, respectively. The flasks were then inoculated with 0.5 mL of an aqueous spore suspension of *Aspergillus flavus*. Two sets of flask were thus prepared: one set underwent stationary incubation and the other was shaken at 180 rpm, both at 25 °C for a period of 1 week. Duplicates were prepared in either case.

The bird-resistant and non-bird-resistant varieties were steamed for 5 min, to prevent germination, before placing in moist Petri dishes and incoulation with *A. flavus* and *Penicillium brevi-compactum* spores. Incubation was carried out for 6 days at 30 °C.

RESULTS AND DISCUSSION

Column Chromatography Using Sepharose CL-6B. Figure 2 shows the phenolic patterns when SSK2, NK300, Breytenbach Red, and DC36 extracts were chromatographed on Sepharose CL-6B by using the acetone gradient system. Three fractions of polyphenols could be separated; namely, F_1 which contained low molecular weight phenolic monomers while F_2 and F_3 contained almost exclusively condensed tannins. Tannin was found in the bird-resistant varieties and it was the tannin which caused enzyme inhibition. F_3 tannin contained relatively little color in comparison to F_1 when they were measured at 440 nm.

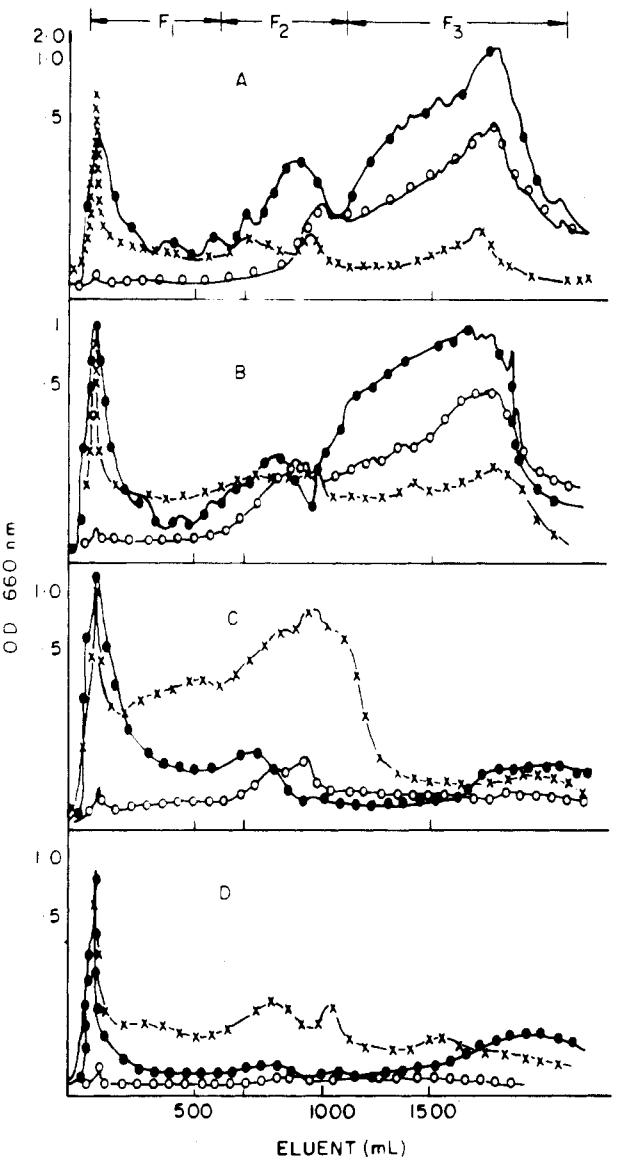

Figure 2. Separation of aqueous acetone extracts of sorghum grains on Sepharose CL-6B by using an acetone gradient. Folin-Ciocalteu phenolics (●), BSA precipitation (○), and light adsorption at 440 nm (×) were continuously monitored as described under Experimental Procedures. (A) SSK2, 25 g extracted; (B) NK300, 50 g; (C) Breytenbach Red, 75 g; (D) DC36, 50 g.

The testa of grain taken immediately from the plant contained no detectable color. The brown color, normally associated with tannin, only developed on aging, either on the plant or at 5 °C in the dark. This correlates well with a normally reliable field test for bird-resistant sorghum varieties, which is the presence of a dark brown testa beneath the pericarp.

The polyphenol content of the different sorghum grains varied widely. SSK2 grain contained twice as much polyphenols as the other bird-resistant variety examined (NK300). Breytenbach Red contained much less polyphenols than the two bird-resistant varieties, but it did contain a small amount of F_2 tannins. DC36 has the lowest polyphenol content of all four varieties with F_2 being lower than that in Breytenbach Red.

TLC and HPLC of Sorghum Grain Polyphenols. There were substantial differences in the patterns of polyphenols when the extracts of the different varieties were examined by TLC. In all four varieties F_1 was the most varied in its content (Figure 3) and so it was examined for its potential in a chemotaxonomic study of sorghum grains.

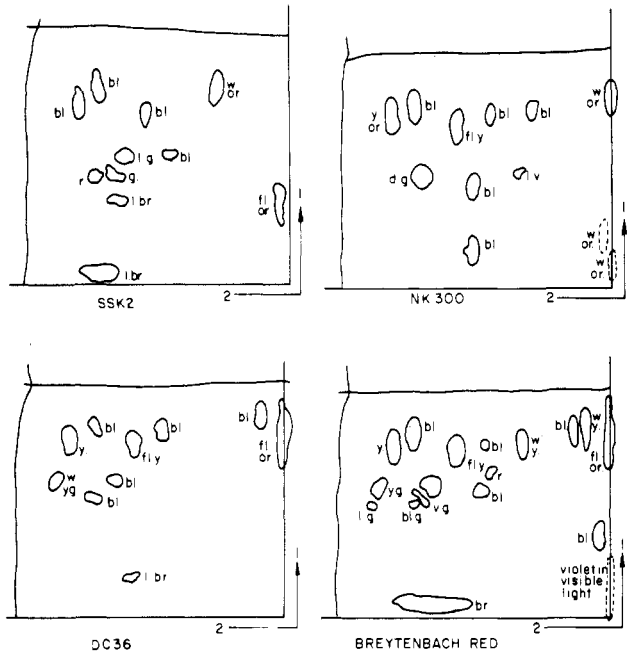

Figure 3. Thin-layer chromatography of F_1 on Cellulose-F from the four varieties shown, using in the first direction 1-BuOH–HOAc–H_2O (6:1:2 v/v/v) and in the second 15% aqueous HOAc as solvents. See Experimental Procedures for visualization and abbreviations.

Table I. Mold Index[a] of Growth during Malting

variety	Breytenbach Red	DC36	NK300	SSK2
fungal growth	1.8	4.4	1.3	1.3

[a] The mold index is a subjective assessment of visible mold infection of a batch of grain on the scale 0–5, where 0 indicates no growth and 5 complete coverage of the grain.

This chemotaxonomic study was carried out by HPLC.

Three varieties were chosen for HPLC analysis, and they represent the most popular varieties in South Africa; i.e., they are the most widely planted. The three varieties, between them, yielded 40 different peaks by HPLC. It is probable that some of the peaks are cis and trans isomers of the same phenolic acid compounds as these isomers are readily separated in aqueous solvents as used in this study. Thirty of the peaks separated by HPLC were common to the three varieties studied and shows their similarity. However, there were sufficient differences to distinguish the varieties one from the other. Attempts are being made to identify the peaks, and until this is done, the validity of the differences between the three varieties remains uncertain.

Taxonomy of man-created cereal varieties has always been a problem and grain sorghum is no exception. If the HPLC technique shows real differences between sorghum varieties, then the results could be applied to a chemotaxonomic classification of sorghum grain.

Figure 4 shows the TLC separation of the F_2 polyphenols. All four varieties are similar with the exception of Breytenbach Red which showed three extra spots. The TLC patterns of F_3 polyphenols are shown in Figure 5. It would appear that there are more compounds than previously described by Kaluza et al. (1980), who showed only two major compounds and a minor one using Sephacryl S-200 as the absorbent.

Mold Resistance of Sorghum Grain. The ability of grain to resist fungal growth is related to polyphenol content (Table I). The exact relationship is vague in that Breytenbach Red grain inhibited fungal growth almost as well as NK300 and SSK2, but it contained a smaller amount of F_2 and no F_3 (Figure 2). However, DC36, which contained only a trace of F_2, did not resist fungal growth.

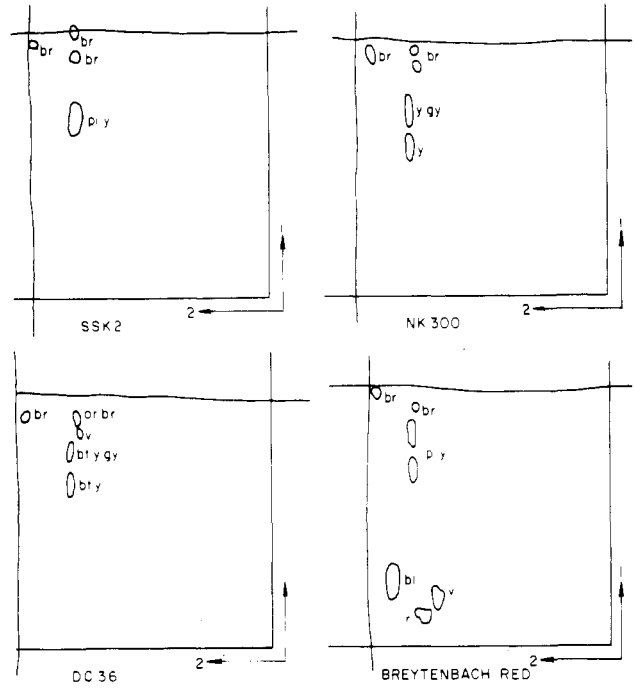

Figure 4. Thin-layer chromatography of F_2 on Cellulose-F from the four varieties shown, using in the first direction MeOH–HOAc–H_2O (8:1:8 v/v/v) and in the second 1-BuOH–HOAc–H_2O (4:2:5 v/v/v). Visualization and color abbreviations are found under Experimental Procedures.

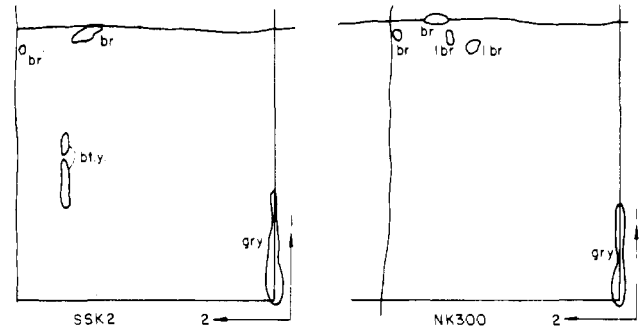

Figure 5. Thin-layer chromatography of F_3 from two varieties on Cellulose-F, using in the first direction 15% aqueous HOAc and in the second 1-BuOH–HOAc–H_2O (4:2:5 v/v/v). See Experimental Procedures for visualization and color abbreviations.

For further investigation of this antifungal activity, an experiment was set up where *A. flavus* was offered starch as a C source in a tannin-containing, and otherwise complete, medium. Growth was luxuriant in all the flasks even in the presence of 0.5 mg/mL F_2 and F_3. *A. flavus* produces an extracellular amylase to obtain sugars as a C source from starch (Le Mense et al., 1947). Strikingly, this enzyme was not inhibited whereas β-amylase from sweet potato was completely inhibited.

Bird-resistant grains fared better than non-bird-resistant ones when left intact but killed by steaming and inoculated with *A. flavus* and *P. brevi-compactum* spores. SSK2 grain supported *A. flavus* growth but at a lower level than did DC36, while neither supported *P. brevi-compactum* well. In whole grain F_2 and F_3 are found localized in the testa, and it is probably this physical barrier of tannins which is important in protecting the grain.

Effect of Malting on SSK2 Polyphenols. After the grains were malted for 6 days, the elution patterns of the two major tannins of F_3 changed. Figure 6 shows the

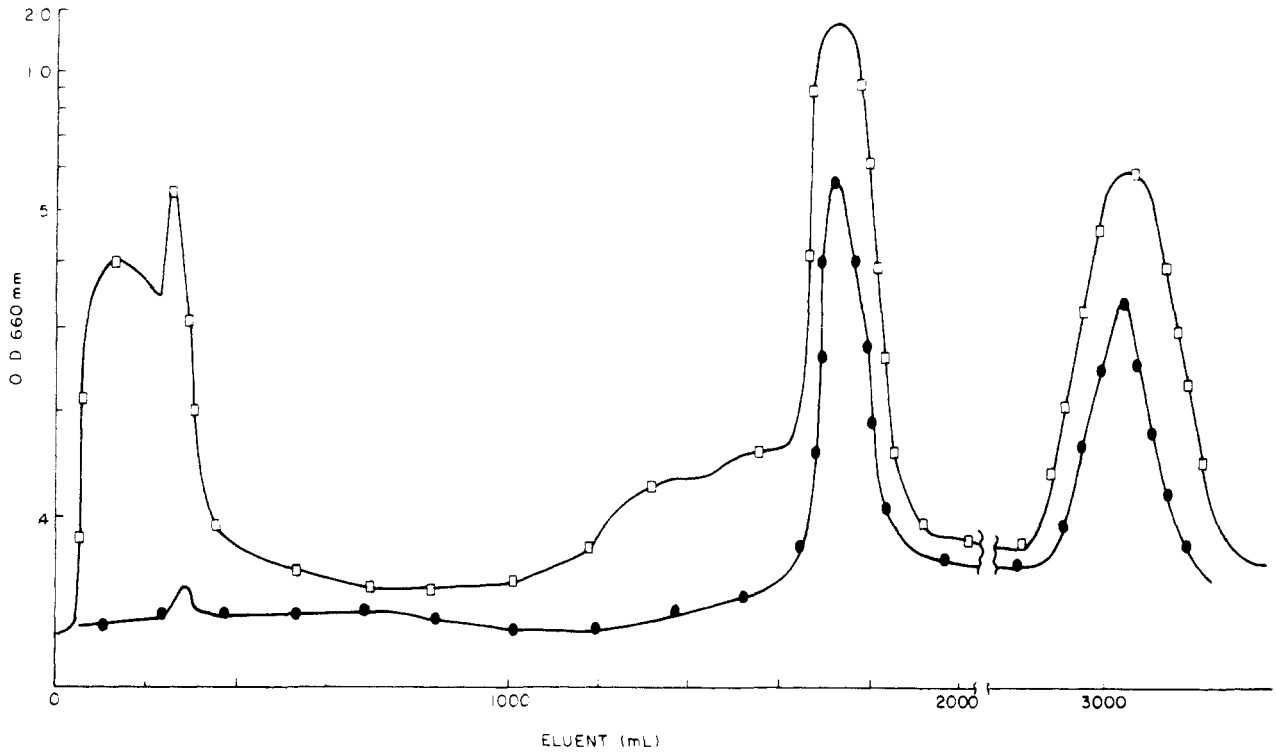

Figure 6. Separation of the phenolics from a 6-day malted SSK2 variety (20 g). The elution was carried out by aqueous acetone as described under Experimental Procedures but required an extra 1500 mL of 50% v/v aqueous acetone. Total phenolics (□) and tannins (●) are shown.

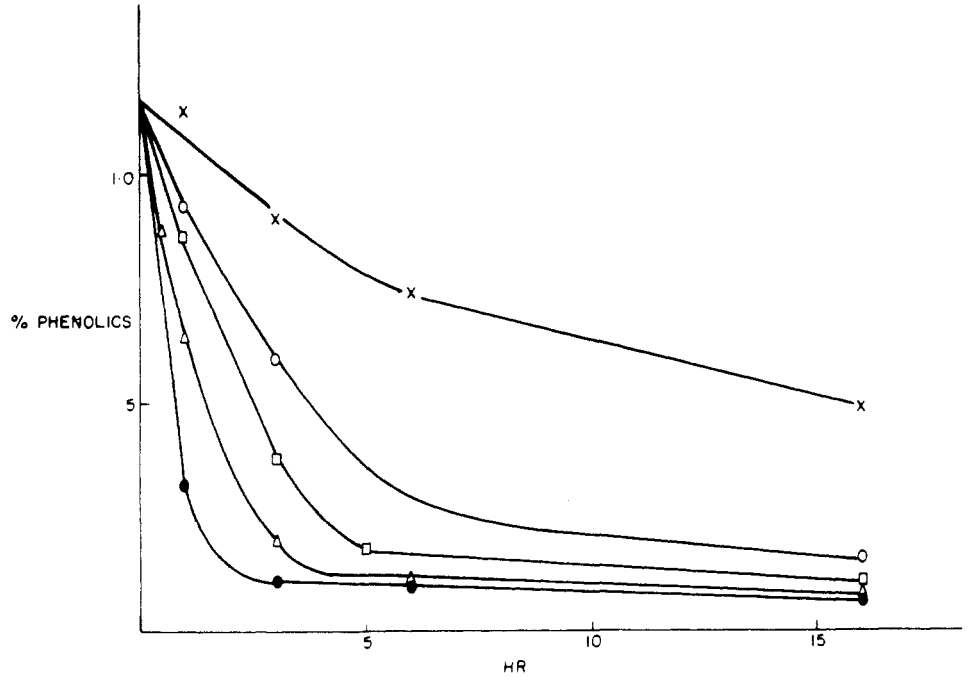

Figure 7. SSK2 variety treated as described under Experimental Procedures with 0.02% (×), 0.04% (○), 0.06% (□), 0.08% (△), and 0.16% (●) aqueous v/v formaldehyde solutions for various times. After treatment the grains were milled, extracted, and assayed for polymeric phenolics by the Jerumanis (1972) method.

elution pattern of the tannins after malting. From unmalted grain the two tannins in F_3 eluted almost together with the advent of 50% aqueous acetone, though the yellow-colored large molecular weight tannin had always eluted first. After the grains were malted, an extra 500 mL of aqueous acetone was needed to elute the smaller red compound first, and a further liter of eluant was needed to remove the larger yellow fraction.

We suggest the existence of either sugars or polypeptides bound to these tannins in unmalted grain, which would reduce the tannins capacity to bind to the Sepharose CL-6B. When the grains were malted, with all the concomitant biological activity of proteinases and carbohydrases, it seemed reasonable to suppose that the nonphenolic moiety would be removed, thus exposing more hydroxyl groups on the tannin. Table II shows F_3 of unmalted grain to contain very little glucose but 1.5% polypeptide, which is enough to mask some phenolic hydroxyls. Further work is necessary to prove or disprove this hypothesis.

The column fractionation of polyphenols from roots and shoots yielded only F_1. This fraction contains only low

Table II. Sugar and Polypeptide Content[a] of Fractions F_1, F_2, and F_3

	% sugar or protein		
	F_1	F_2	F_3
arabinose	0.39	0	0
galactose	1.82	0	0
xylose	0.28	0	0
glucose	9.31	1.92	0.19
polypeptide	3.0	2.0	1.5

[a] These figures represent the differences between the values found for the actual fractions and for a blank run from an unloaded polygalactan column.

Table III. Extraction of Formaldehyde[a]-Treated Grain

	acetone extract		
	dry wt, g/100 g of seed	Folin estimate,[c] %	H_2O suspension,[b] Folin estimate,[c] %
sorghum, untreated	2.99	2.99	2.4
sorghum, treated	1.27	0.68	0.2

[a] See Experimental Procedures. Formaldehyde concentration was 0.12% v/v and steep time 6 h. [b] 10 mg of ground grain suspended in 5 mL of H_2O. [c] See Experimental Procedures.

molecular weight polyphenols and no tannins could be detected. The roots and shoots were highly colored, and acid hydrolysis yielded cyanidin as well as the two rarer anthocyanidins luteolinidin and apigenidin. These compounds were reported by Stafford (1965) as present in the hydrolysates from the first internodes of sorghum seedlings.

Alkaline hydrolysis released simple phenolic acids from the roots and shoots as well as the berries after malting. The pattern of these compounds was more complex in the roots and shoots than in the berries or in the unmalted grain.

Effect of Formaldehyde on Bird-Resistant Grain SSK2. Figure 7 shows the effect of various concentrations of formaldehyde on the polyphenols of whole SSK grain over various times. The kinetics show that the reaction rate was dependent on the concentration of the formaldehyde and on the time of contact.

A sample of grain was treated with 0.12% formaldehyde for 6 h. Table III shows that the aqueous acetone extractable material was reduced and that the material which was extracted possessed 50% fewer phenolic hydroxyls. This is so because the modified Folin–Ciocalteu method used here depends on the reducing power of the phenolic hydroxyls. The overall reduction of hydroxyls was a drastic 92%.

Phenol–formaldehyde resins consist of two types of polymers: the base catalyzed and the acid catalyzed. The former leads to polymers with the –OH groups intact and the latter to phenol resins cross-linked via etheric bonds; i.e., the –OH groups disappear (Gilman, 1945).

The formaldehyde treatment of sorghum grain resembles acid catalyses in that etheric linkages occur. This should cause a loss in enzyme inhibitory power and BSA precipitation. This could have two explanations. The first is that the tannin molecules remain a reasonable size and the formaldehyde simply inactivates the phenolic group. The second is that the formaldehyde cross-links the tannin to form large polymers and the tannin is, therefore, no long extractable. Figure 8 supports either of these explanations where it can be seen that after formaldehyde treatment

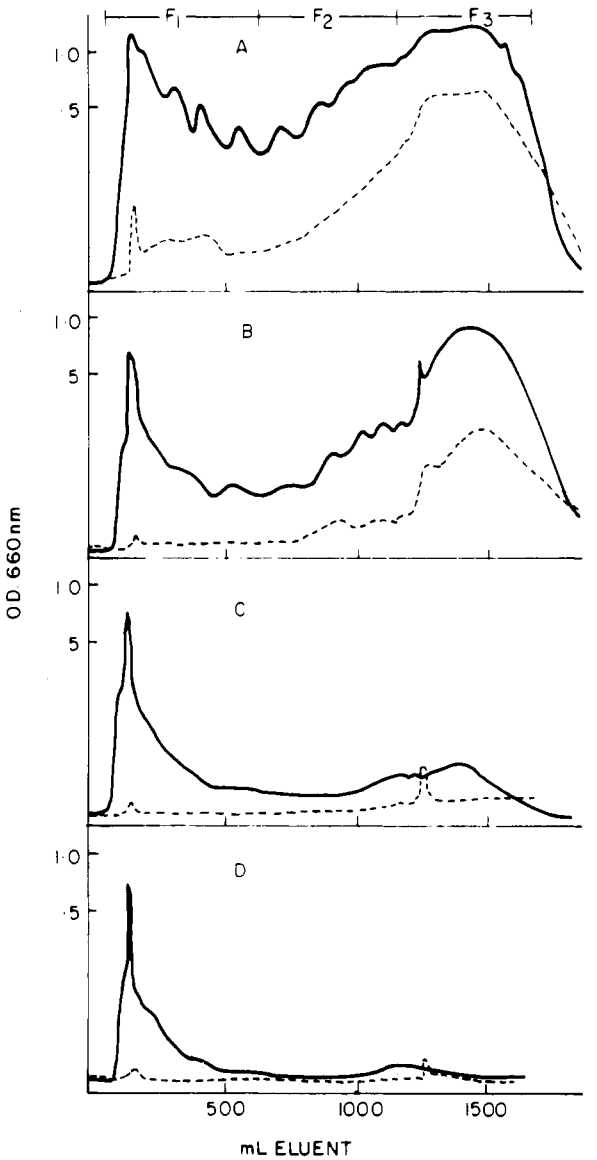

Figure 8. Chromatography on Sepharose CL-6B of extracts of bird-resistant sorghum, the grains of which had been pretreated with H_2O (A) and 0.025% (B), 0.05% (C), and 0.075% (D) v/v aqueous formaldehyde. DMF solutions were used in the gradient elution system. Total polyphenol (Folin–Ciocalteu) is shown as a solid line, and BSA precipitation is shown as the dashed line.

F_2 and F_3 respond less as regards both phenolic hydroxyl content and BSA precipitate. Furthermore, F_2 and F_3 were reduced at a faster rate than F_1. This is probably due to the high concentration of F_2 and F_3 polyphenols in the testa which was readily exposed to the formaldehyde. Table III supports the second explanation as it shows a considerable reduction in extractable material.

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Cyclitols in Soybean

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The free cyclitols present in soybean plants were pinitol (1D-3-*O*-methyl-*chiro*-inositol), D-*chiro*-inositol, *myo*-inositol, and sequoyitol (5-*O*-methyl-*myo*-inositol). Pinitol was a major component of all plant parts and was found in large quantities in all soybean cultivars examined. D-*chiro*-Inositol, *myo*-inositol, and sequoyitol were minor components. Root nodules contained the same cyclitols found in other plant parts but the relative abundance differed considerably. In soybean seed pinitol was the only monosaccharide of significance. The use of a simple procedure to separate sugars from cyclitols is discussed.

Previously Phillips and Smith (1974) reported that an *O*-methylinositol, now identified as pinitol, was a major ethanol-soluble component in the vegetative portion of soybean plants and in some cases was more prevalent than glucose, fructose, and sucrose combined. They also found *myo*-inositol and an unidentified sugar alcohol which was subsequently found to be a cyclitol.

Delente and Ladenburg (1972) identified the one major monosaccharide in soybean seeds as galactose, but Hymowitz and Collins (1974) identified it as fructose. Pinitol has been identified in soybean flakes by Honig et al. (1971) and Nielsen (1960) and in "milk" made from seeds by Schweizer et al. (1978). Schweizer et al. (1978) also reported a new disaccharide, galactopinitol, but found only traces of fructose or galactose. Streeter and Bosler (1976) and Streeter (1980) reported on cyclitols in root nodules of soybean. Ruis and Hoffmann-Ostenhof (1969) reported that pinitol was synthesized from *myo*-inositol via sequoyitol in crimson clover. Previously Phillips and Smith (1974) were not able to confirm the presence of sequoyitol in soybean plants.

Biologists frequently attempt to relate the soluble or nonstructural carbohydrate levels to various biological functions. Since cyclitols are abundant in soybean plants (Phillips and Smith, 1974) and other legumes (Smith and Phillips, 1980) and some analytical methods fail to distinguish between cyclitols and other carbohydrates or do not detect cyclitols, it was important to identify the cyclitols in soybean plants. This paper reports the identification of the cyclitols in soyben plants, including seeds and root nodules.

MATERIALS AND METHODS

Soybean (*Glycine max* L. Merr.) plants from the growth chamber, greenhouse, or field were uprooted, the soil was washed from the roots, and the plants were divided into various parts depending on the analysis desired. The plant parts were frozen as soon as possible (always within 0.25 h) by placing in a freezer at −30 °C or by shaking with finely divided dry ice. The frozen parts were lyophilized and ground in a Wiley mill to pass a 40-mesh screen and then extracted with 80% ethanol. In some cases fresh or frozen plant parts were placed directly in a blender with sufficient 95% ethanol to give a final concentration of about 80% ethanol after blending.

Clearing, ion-exchange separations, and ^{13}C NMR spectroscopy methods were as previously described (Phillips and Smith, 1974). Gas chromatography was done as described by Loewus and Shah (1972), Phillips and Smith (1973), and Lee and Ballou (1965). Mass spectrometery methods were as described previously (Phillips and Smith, 1974; Smith and Phillips, 1981). Some samples were silylated with deuterated reagents (Sherman et al., 1970) prior to obtaining mass spectra.

When necessary, sugars were removed from cyclitol solutions by a modification of the method described by Roseman et al. (1952). A column of Dowex 21-K was regenerated with 1.0 N NaOH saturated with BaOH, and a column of Dowex 50W was regenerated with 2.0 N HCl. After the columns were rinsed with CO_2-free water, they were connected with the Dowex 21-K on top. The sample was added to the Dowex 21-K column and eluted with CO_2-free water. The cyclitols and the acyclic polyols passed rapidly through the columns, and the sugars were retained.

Solutions of cyclitols deionized by the above procedure were lyophilized and recrystallized from ethanol or methanol. Inositol methyl ethers were demethylated with boiling hydroiodic acid. Infrared spectra were obtained by the KBr wafer method on a Beckman spectrophotometer. Optical rotations were measured on a Perkin-Elmer 141 polarimeter.

RESULTS AND DISCUSSION

The major cyclitol in soybean plants was previously identified as an *O*-methylinositol (Phillips and Smith,

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