Fractions A and A' were extremely deficient in lysine, and fractions B and B', in which the lysine accumulated, have a much improved amino acid balance over the original meal.

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Partial Characterization of Tannin–Protein Complexes in Five Varieties of Grain Sorghum by Automated Gel Filtration Chromatography

First-moment apparent molecular weights (M_{A_1}) for the glutelin fraction of five varieties of grain sorghum were determined by gel chromatography with detection by UV absorbance at 254 and 206 nm. For the low-tannin varieties, M_{A_1} based on absorbance at either wavelength was about 1.7×10^3. For an intermediate-tannin variety, M_{A_1} was 1.82×10^3 when based on detection at 206 nm and 2.21×10^3 when based on 254 nm, whereas the high-tannin variety had values of 0.90×10^3 and 2.36×10^3 at 206 and 254 nm, respectively. The higher values of M_{A_1} at 254 nm for intermediate- and high-tannin varieties compared to low-tannin ones were attributed to higher levels of protein–tannin complexes which have higher extinction coefficient at 254 than at 206 nm for molecular weights in excess of 2500. This conclusion was supported by measurement of tannin and protein content in the glutelin fraction.

Sorghum is an important food and feed grain in the United States and the world. High-tannin (polyphenol) varieties give higher acre yields of processed grain than low-tannin varieties. Unfortunately, however, tannin content and nutritional value in sorghum are inversely related (Jambunathan and Mertz, 1973).

Recently, Chibber et al. (1978) have postulated that tannin–prolamin interactions have caused many of these proteins to appear in the glutelin fraction when high-tannin sorghum grain was subjected to a classical fractionation scheme such as that devised by Landry and Moreaux (1970). Since protein–tannin interactions may adversely affect the nutritional value of grain sorghum through diminished protein availability, it was our purpose in this communication to examine the glutelin fraction from high-, intermediate-, and low-tannin varieties of sorghum for protein–tannin complexes.

MATERIAL AND METHODS

Seeds. Five varieties of whole grain sorghum were tested, and had been produced in 1970 at College Station, TX, and stored under refrigeration. Three of the varieties, SC 301, CK 60, and TX 615 have been designated low in tannin; one variety, NSA 740 as intermediate; and one variety, GA 615, as high (see Table I). Morphological descriptions of these varieties have been reported (Sullins, 1972; Sullins and Rooney, 1974).

Milling. The whole grain was ground in a Wiley Mill to pass a 40-mesh screen. The ground meal was defatted by suspension in stirred hexane for 1.5 h (1 L/250 g of meal) and then removal of the solvent by filtration. The particle size was further reduced by grinding in a ball mill for three 10-min intervals with periods of air cooling in between.

Protein Extraction. The glutelin fraction of each variety was obtained by a modified procedure of earlier methods (Landry and Moureax, 1970; Jambunathan and Mertz, 1973) according to Neucere and Sumrell (1979). Defatted meal was extracted with deionized water. Albumins were separated as supernatant from the insoluble residue by centrifugation. The residue was extracted with 0.5 M NaCl and centrifuged again, and globulins were removed with the supernatant. Residue from the salt extraction was extracted with aqueous *tert*-butyl alcohol (60% butyl alcohol by volume) that contained 0.1 M guanidine hydrochloride, and centrifuged to yield a supernatant containing prolamins. The residue of the previous step was extracted with 0.16 M borax–0.048 M NaOH buffer, buffer, pH 10, which was also 0.5% in sodium dodecylsulfate. After centrifugation, the supernatant glutelin fraction was separated from the residue, dialyzed against deionized water, and freeze-dried.

Protein Content. Crude protein (N × 6.25) in each variety was determined for the whole seed and the glutelin fraction by the microKjeldahl nitrogen method (Horowitz, 1975). Protein in the glutelin fraction was dissolved in 0.8 N NaCl and determined by the Lowry method (Lowry et al., 1951).

Table I. Protein and Tannin Contents of Whole Sorghum and Glutelin Fractions

	whole seed			glutelin fraction							
							206 nm		254 nm		206 nm/254 nm ratio
variety	tannin level	crude[a] protein, % N × 6.25	catechin[b] equivalents	% crude protein, % N × 6.25	% protein Lowry	catechin equivalents	$M_{A_1} \times 10^{-3}$	A^c	$M_{A_1} \times 10^{-3}$	A^c	M_{A_1} A
SC301	low	13.5[d]	0.18 ± 0.01	31.0[d]	40[d]	0.32 ± 0.01	1.62 ± 0.20	190 ± 22	1.52 ± 0.15	46.8 ± 2.9	1.06 ± 0.03 4.04 ± 0.21
CK60	low	11.9	0.15 ± 0.01	30.0	40	0.12 ± 0.03	1.62 ± 0.04	320 ± 4	1.64 ± 0.06	77.7 ± 0.1	0.99 ± 0.01 4.12 ± 0.05
TX615	low	14.0	0.05 ± 0.01	51.0	52	0.36 ± 0.01	1.69 ± 0.06	729 ± 44	1.84 ± 0.06	161 ± 9	0.92 ± 0.01 4.51 ± 0.01
NSA740	medium	17.1	0.50 ± 0.04	31.0	78	1.38 ± 0.11	1.82 ± 0.01	1204 ± 83	2.21 ± 0.00	260 ± 15	0.82 ± 0.01 4.65 ± 0.58
GA615	high	11.5	2.85 ± 0.11	36.0	200	12.0 ± 0.64	0.90 ± 0.00	237 ± 12	2.36 ± 0.02	117 ± 2	0.38 ± 0.00 2.02 ± 0.06

[a] Defatted seed (i.e., less fat and moisture). [b] Milligrams of catechin/100 mg dry weight. [c] Area [mL/mg (N × 6.25)]. [d] All columns with superscript *d* are based on single analysis, remaining on duplicate analysis.

Tannin Content. The tannin contents of the whole seed and the glutelin fraction were determined for each variety by the modified method of Burns (1971) according to Maxon and Rooney (1972). Table I contains results in milligrams of catechin/100 mg dry weight of sample (i.e., catechin equivalents).

Gel Chromatography. Freeze-dried glutelin fractions were dissolved in 0.8 N NaCl and filtered through a 0.40-μm Nucleopore membrane filter. Analytical gel chromatography was performed on an automated gel filtration apparatus (Fishman and Burdick, 1977) with an operating range of 1×10^6 to 0.43×10^3 daltons for globular proteins and peptides. The chromatograph was equipped with four columns connected in series. Each column was 6 mm in diameter. The first column was packed with 191 mm of G-10, G-25 Sephadex mixed to give equal bed volumes, the second with 179 mm of G-50, G-75 Sephadex mixed to give equal bed volumes, the third with 173 mm of Bio-Gel A-0.5 m, and the fourth with 209 mm of Bio-Gel A 5m. Up to five 0.2-mL samples were injected automatically at intervals of 228 min. Protein concentration in the sample ranged from 0.5 to 1.2 mg/mL. Flow rates were between 0.107 and 0.119 mL/min. Head pressure for the first column was between 13 and 15 psi. Absorbance of column effluent was measured with a Spectro Flow 770 UV monitor (Schoeffel Inst. Corp., Westwood, NJ) at 206 nm and a Model 1200 UV monitor (LDC, Riviera Beach, FL) at 254 nm. Data reduction and molecular weight calculations are described elsewhere (Fishman, 1976).

RESULTS AND DISCUSSION

Previously, automated gel chromatography with detection at 206 and 254 nm was used to characterize the complex mixture of proteins, polypeptides, and amino acids in Coastal Bermuda grass extracts (Fishman, 1976; Fishman and Burdick, 1977). Absorbance at 206 nm was chosen to measure amide linkages in the proteins, whereas that at 254 nm was chosen to measure aromatic groups. The researchers found that the area under the gel curve and the molecular weight distribution (MWD) varied with wavelength of detection, i.e., 206 or 254 nm. They suggested that the proteins formed complexes with aromatic groups, possibly of polyphenols endogenous to the plant, and that these complexes caused a decrease in the ratio of areas measured at 206 and 254 nm. Hence, we chose gel chromatography as a means to investigate the glutelin fractions of sorghum for protein–tannin complexes. Molecular weights obtained from the gel chromatograms of these glutelin fractions correlated well with tannin content of the fractions and of the whole seeds. The low tannin varieties had chromatograms typical of those for CK 60 shown in Figure 1. The first-moment apparent molecular weights M_{A_1} (Fishman, 1976) for these low-tannin varieties (Table I) were independent of wavelength and variety. The medium- and high-tannin varieties NSA 740 and GA 615, respectively (Figures 2 and 3), had chromatograms which were different from each other and from the low-tannin varieties. Moreover, the chromatograms of NSA 740 and GA 615 varied with the wavelength of detection. Furthermore, the values of M_{A_1} at 254 nm and the 206 to 254 nm ratios of these two varieties (Table I) also confirmed that the molecular weight distributions were different for variety and wavelength of determination. Apparently, tannins complexed more readily with proteins and polypeptides whose apparent molecular weights were above 2500 than below (cf. Figures 1–3). Since tannin–protein complexes should have a lower ratio of 206–254-nm absorbance than uncomplexed proteins or peptides (Fishman and Burdick, 1977), the average molecular weight

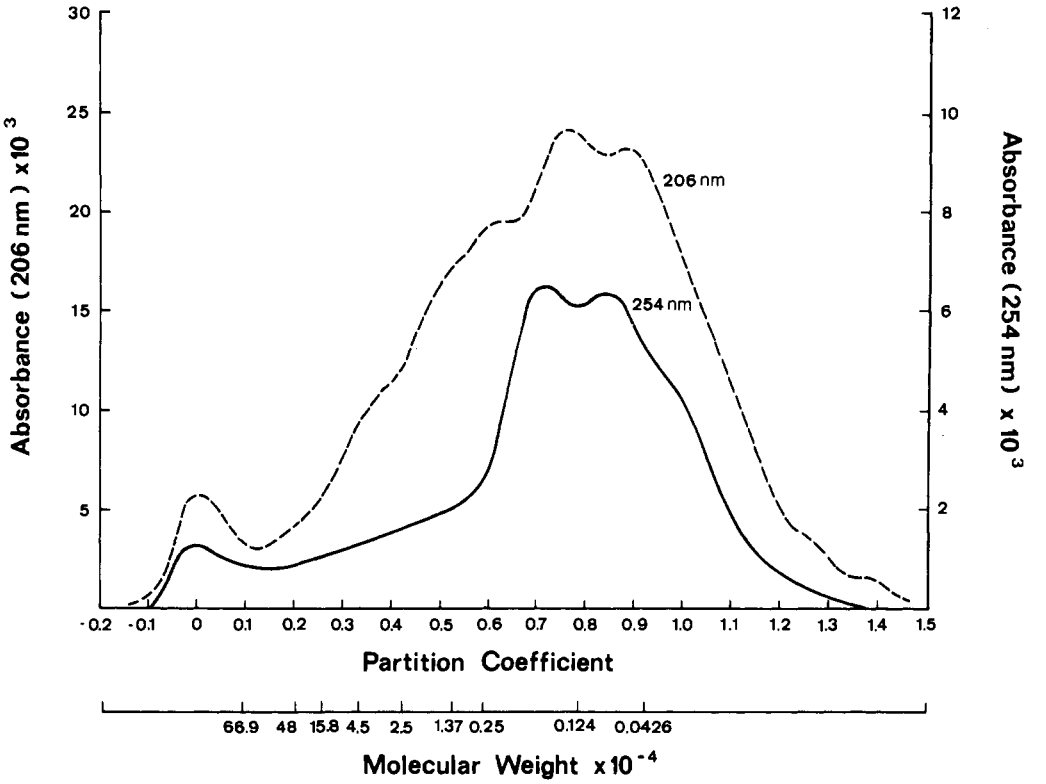

Figure 1. Gel chromatograms of glutelin fraction from CK-60 (low tannin) grain sorghum. Wavelengths of detection were 254 and 206 nm. Sensitivity was 0.08 absorbance units full scale (aufs) at 254 nm and 0.2 aufs at 206 nm. Concentration was 1.11 mg/mL.

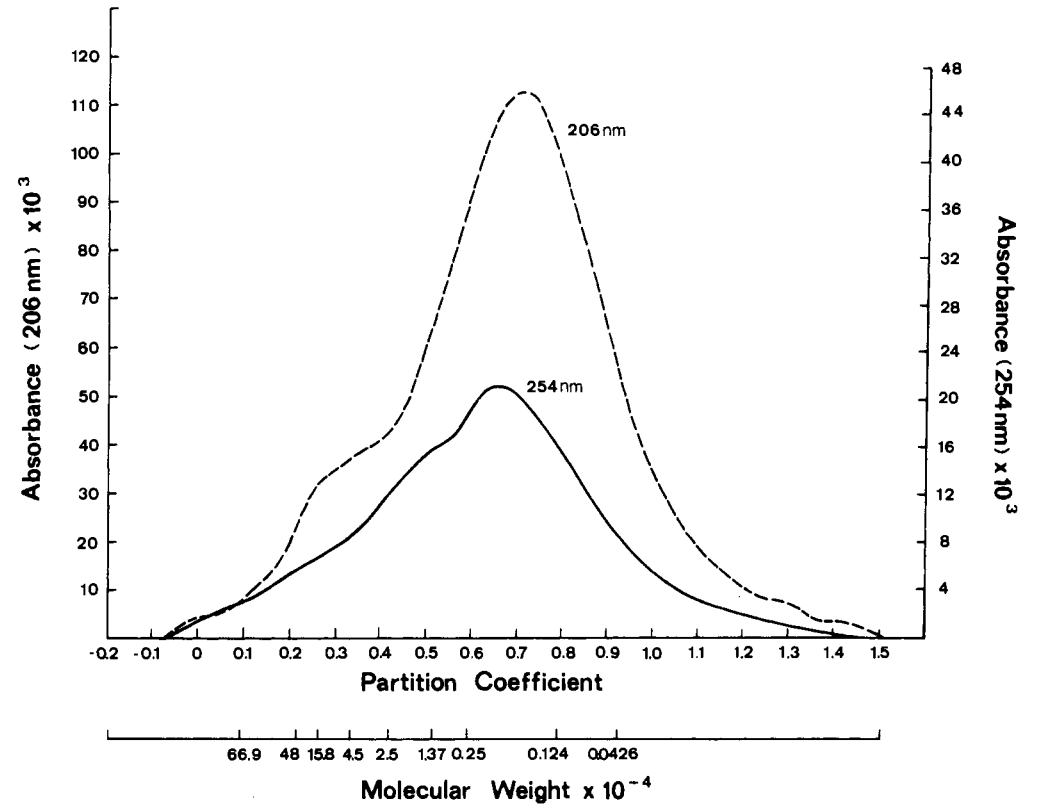

Figure 2. Gel chromatograms of glutelin fraction from NSA 740 (medium tannin) grain sorghum. Wavelengths of detection were 254 and 206 nm. Sensitivity was 0.08 aufs at 254 nm and 0.2 aufs at 206 nm. Concentration was 0.628 mg/mL.

of the complexes should be higher when based on 254 nm than on 206 nm absorbance. In general, the glutelin fractions from all the sorghum varieties had a lower than "normal" 206 to 254 nm ratio for areas under the gel curve. The ratios for these areas were between 4.51 and 2.02 (Table I), whereas those for several standard proteins were found to be between 60 and 30 (Fishman and Burdick, 1977).

A comparison of protein content based on the Kjeldahl nitrogen and Lowry methodologies (Table I) also tended to confirm the hypothesis that glutelin fractions from the medium- and high-tannin varieties had relatively higher

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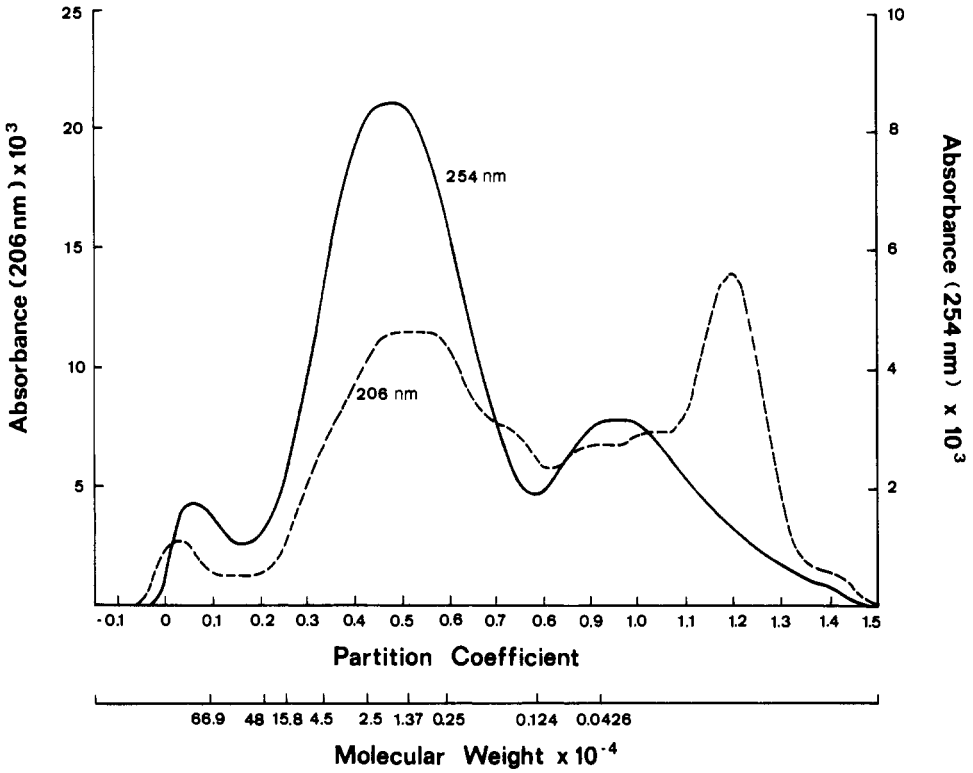

Figure 3. Gel chromatograms of glutelin fraction from GA 615 (high tannin) grain sorghum. Wavelengths of detection were 254 and 206 nm. Sensitivity was 0.08 aufs at 254 nm and 0.2 aufs at 206 nm. Concentration was 1.10 mg/mL.

levels of tannin–protein complexes than the low-tannin varieties. The Lowry protein value for NSA 740 was more than twice the Kjeldahl value, whereas the Lowry value for GA 615 was more than five times the Kjeldahl value. Sarkar et al. (1975) reported that in the presence of aromatic compounds (e.g., tannins) the Lowry method will overestimate protein.

We have shown that analysis for tannins in the glutelin fraction by the HCl–vanillin method gave values which correlated well with the whole seed values (Table I). Extraction of the tannins from the glutelin fraction by methanol is strong evidence that methanol disrupted tannin–protein complexes, and, hence, these were not covalently bound. Several polar organic solvents including ethanol–water mixtures have reversed, at least partially, reactions between tannins and proteins (Van Sumere, 1975). Chibber et al. (1978) suggested that the tannin–protein complexes appearing in the glutelin fraction were in reality prolamin–tannin complexes, which were not soluble in their solvent for prolamins, namely 70% 2-propanol. If prolamin–tannin complexes are present in the glutelin fraction, a portion of the methanol-extracted glutelin fraction, might be soluble in 70% 2-propanol because removal of tannins would leave uncomplexed prolamins.

The conclusion that there are salt (0.8 N NaCl)-soluble protein–tannin complexes, regardless of their mechanism of formation, in the glutelin fraction of grain sorghum is consistent with our gel chromatographic data, estimates of tannin, Lowry protein measurements, and crude protein assays based on Kjeldahl nitrogen. Furthermore, with the possible exception of TX615, our data indicate that the concentration of tannin–protein complexes in the glutelin fraction increased proportionately with the tannin content of the whole seed (Table I).

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