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Effects of Formaldehyde on Protein Extraction and Quality of High- and Low-Tannin Sorghum

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Dilute solutions of formaldehyde were used to inactivate the tannins in high-tannin (bird-resistant) sorghum. By means of this treatment it was shown that high- and low-tannin sorghums contain similar proportions of the different classes of protein. The differences observed in protein yield, when high-tannin sorghums are extracted (without pretreatment) by using the Landry and Moureaux fractionation, are due to interactions between tannins and albumin, globulin, and prolamin proteins, the majority of these proteins being rendered insoluble in their usual solvents. In addition, electrophoresis indicated that those proteins which were extractable from high-tannin sorghum were bound to tannins. As formaldehyde facilitated the quantitative extraction of proteins from high-tannin sorghum, it is suggested that it may have a more general application in the study of proteins from plant material rich in polyphenols.

The proteins of high-tannin (bird-resistant) and low-tannin cultivars of sorghum have been examined by a number of workers (Jambunathan and Mertz, 1973; Chibber et al., 1978; Guiragossian et al., 1978) using the fractionation procedure of Landry and Moureaux (1970). These workers found that high-tannin cultivars contain a lower proportion of salt-soluble, albumin plus globulin, and alcohol-soluble, prolamin, proteins than low-tannin types. Conversely, the proportion of glutelins which are the proteins soluble in pH 10 borate buffer containing 2-mercaptoethanol and sodium dodecyl sulfate (NaDodSO$_4$) is greater in the high-tannin varieties.

Chibber et al. (1978) postulated that the lower proportion of albumins, globulins, and prolamins found in high-tannin sorghum is due to interactions between these proteins and the tannins, rendering them insoluble in their normal solvents but extractable by the glutelin solvent. Evidence to support this theory has been obtained by Fishman and Neucere (1980), who demonstrated the presence of salt-soluble protein–tannin complexes in the glutelin fraction obtained from a high-tannin sorghum cultivar.

Daiber (1976) patented a process in which high-tannin sorghum grain for use as malt in the brewing of sorghum beer is treated with dilute formaldehyde solution. The formaldehyde reacts with the tannins, thus preventing their subsequent complexing with and inactivation of enzymes formed during malting. The reaction between formaldehyde and tannins is probably similar to that which occurs between it and phenol in the formation of "Bakelite", a phenol–formaldehyde resin (Morrison and Boyd, 1966).

In this report which forms part of a larger investigation into sorghum polyphenols (Kaluza et al., 1980; Glennie, 1981), the effect of soaking grain in formaldehyde on the extraction and quality of salt- and alcohol-soluble proteins from high- and low-tannin varieties is described.

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Table I. Albumin plus Globulin and Prolamin Proteins Extracted from Sorghum Grain Treated with Different Concentrations of Formaldehyde

		% of total protein in grain	
	% formaldehyde	albumins + globulins	prolamins
SSK 52:[a]	0	0.6	10.4
	0.04	10.4	14.5
	0.08	10.4	38.5
	0.16	10.3	36.6
G 766 W:[b]	0	15.9	37.1
	0.04	17.2	43.3
	0.08	15.9	43.3
	0.16	14.8	41.9

[a] High tannin. [b] Low tannin.

EXPERIMENTAL SECTION

Grain (30 g) of sorghum cultivars SSK 52 (high tannin) and G 766 W (low tannin) was steeped for 6 h at room temperature in 30 mL of the following solutions: distilled water and 0.04, 0.08, and 0.16% formaldehyde. It was then blotted dry and dried further at 50 °C for 16 h.

The dried grain (20 g) was ground for 2 min in a Janke and Kunkel beater-type mill. Five grams of the resulting flour was extracted sequentially by stirring for three 1-h periods with 25-mL aliquots of 0.8 M NaCl at 4 °C and then with 55% (v/v) isopropyl alcohol plus 2% (v/v) 2-mercaptoethanol at 60 °C to extract the albumin plus globulin and prolamin proteins, respectively. The supernatants were dialyzed overnight against distilled water and freeze-dried.

Protein ($N \times 6.25$) was determined by the method of Thomas et al. (1967). So that nitrogen recovery could be checked, acetanilide was included with each batch of di-

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Table II. Tannin Content of Grain and Protein Fractions Extracted from Grain Treated with Different Concentrations of Formaldehyde[a]

	% formaldehyde	mg of catechin equiv/ g of whole grain	mg of catechin equiv/ g of protein	
			albumin + globulin fraction	prolamin fraction
SSK 52:[b]	0	48	499	244
	0.04	2	10	0
	0.08	0	1	0
	0.16	0	1	0

[a] No tannins were detected in the protein fractions extracted from G 766 W (low-tannin) grain or in the grain itself. [b] High tannin.

gestions. Tannin was measured by using the vanillin assay of Price et al. (1978) with catechin as the standard. Polyacrylamide gel electrophoresis in 7% gels containing 6 M urea and $NaDodSO_4$–polyacrylamide gel electrophoresis in 12.4% gels were carried out as described by Shewry et al. (1977).

RESULTS AND DISCUSSION

The results in Table I show that the proportions of albumin plus globulin and prolamin proteins extracted from the high-tannin sorghum treated with formaldehyde approach the percentages of these proteins in the low-tannin grain. The low percentages of these proteins extracted from untreated high-tannin grain cannot be attributed to their loss from the grain during steeping in water because the residual steepwater contained less than 0.4% of the protein originally in the grain. These results indicate that during steeping formaldehyde reacted with the tannins, preventing any subsequent protein–tannin interactions during protein extraction.

The fact that less proteins were extracted from high-tannin sorghum treated with 0.16% formaldehyde compared to that treated with 0.08% and from low-tannin sorghum treated with 0.08 and 0.16% compared to 0.04% suggests that the grain had been overtreated. It is probable that the formaldehyde reacted with the proteins, rendering them less soluble (Fraenkel-Conrat and Olcott, 1948). The preferential reaction of formaldehyde with the tannins rather than with proteins is most likely due to the location of the tannins in the outer layers, pericarp, and testa of the grain (Reichert et al., 1980). The absence of a "protective" tannin-rich testa in the low-tannin grain probably accounts for why it was more adversely affected by the formaldehyde treatment.

Table II shows that the fractions containing albumin plus globulin and prolamin proteins extracted from the high-tannin cultivar were heavily contaminated with tannins. It was reported by Fishman and Neucere (1980) that the glutelin fraction extracted from a high-tannin variety was similarly contaminated. Reaction of formaldehyde with the tannins resulted in the protein fractions being free of tannins.

The patterns of protein bands obtained from SSK 52 (high-tannin) grain steeped in water are considerably different from those obtained from the grain treated with various concentrations of formaldehyde (Figures 1a and 2a). It is most unlikely that these differences were caused by formaldehyde reacting with the proteins, since no such differences are observable between the comparable fractions obtained from G 766 W (low-tannin) grain (Figures 1b and 2b). Therefore, Figures 1a and 2a indicate that tannins are actually bound to those albumin, globulin, and prolamin proteins which are extractable from high-tannin sorghum and not merely extracted with them as could be concluded from the results in Table II.

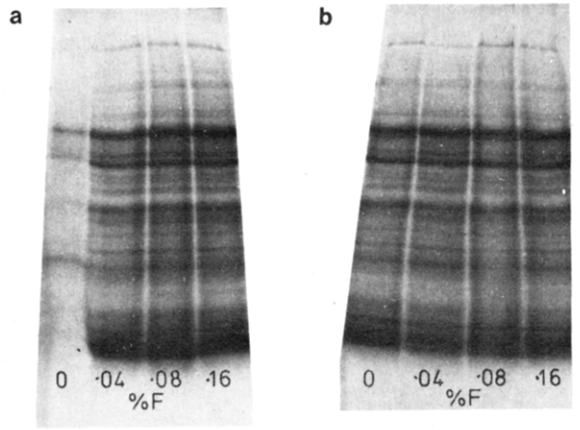

Figure 1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of albumins plus globulins extracted from sorghum grain treated with different concentrations of formaldehyde (F). (a) High-tannin sorghum. (b) Low-tannin sorghum.

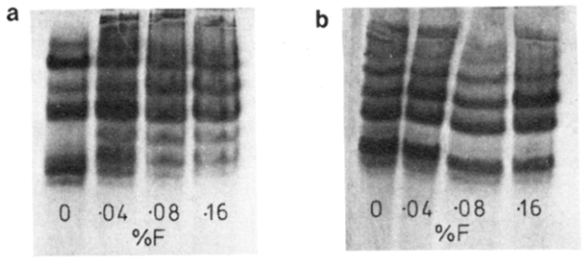

Figure 2. Polyacrylamide gel electrophoresis in 6 M urea of prolamins extracted from sorghum grain treated with different concentrations of formaldehyde (F). (a) High-tannin sorghum. (b) Low-tannin sorghum.

CONCLUSION

The use of formaldehyde to inactivate tannins confirms the hypothesis of Chibber et al. (1978) that tannin–protein interactions are responsible for the apparently low proportion of albumins, globulins, and prolamins in high-tannin sorghum cultivars. It is possible that formaldehyde may be of more general use in the inactivation "phenolics", as these secondary products create special problems in the isolation of enzymes and organelles from a wide range of plant materials (Loomis, 1974).

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Dimethylolurea as a Tyrosine Reagent and Protein Protectant against Ruminal Degradation

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Amino acid analysis of hydrolyzed proteins by ion-exchange chromatography was used to show apparent selective reaction of tyrosine side chains in bovine serum albumin (BSA), lysozyme, casein, soy protein, and wheat gluten with dimethylolurea (DMU). A plot of pH of the reaction medium vs. extent of tyrosine modification for BSA is biphasic, with maxima below pH 3.5 and above pH 10 and a minimum near pH 5. The tyrosine modification increased with the ratio of DMU to protein but not with BSA or casein concentration when the ratio of DMU was constant. These observations are the basis for a proposed mechanism of acid and base catalysis of tyrosine modification by DMU to form modified proteins. In vitro evaluation of degradation of DMU-treated casein and other DMU-treated proteins by ruminal microorganisms suggests that DMU is a potentially useful compound for protecting feed proteins against degradation by microorganisms in the rumen of sheep and cattle.

As part of a program to develop new treatments for feed proteins to increase their nutritional quality by decreasing their microbial degradation in the rumen (Friedman and Broderick, 1977), we evaluated the utility of dimethylolurea-treated casein. Amino acid analysis of DMU-treated casein revealed that only tyrosine residues gave derivatives stable to acid hydrolysis. To establish the generality of this reaction, we investigated several variables expected to govern the interaction of DMU with aromatic (phenolic) groups on tyrosine side chains. Conditions were devised to define the reactivity of tyrosine side chains in several proteins. In addition, we evaluated the degradability of DMU-treated casein by ruminal microorganisms. The results demonstrate the usefulness of dimethylolurea as a reagent for modifying tyrosine residues in proteins and its potential as a protein protectant against ruminal degradation.

MATERIALS AND METHODS

Dimethylolurea was synthesized by the method of Dixon (1918), but most of the experiments were carried out with a commercial sample obtained from Brochem, West Germany. Casein was obtained from International Casein Corp., San Francisco, CA, wheat gluten was obtained from Nutritional Biochemical Corp., Cleveland, OH, bovin serum albumin and lysozyme were obtained from Sigma Chemical Co., St. Louis, MO, and soy protein (Promine-D) was a gift from Central Soya, Chicago, IL.

Chemical Modification. The following is a typical experiment. Dimethylolurea (0.25 g) and bovine serum albumin (0.5 g) were dissolved in 10 cm^3 of buffer of appropriate pH (citrate buffer for pH 1.5–3.5; acetate buffer for pH 5; phosphate buffer for pH 7–8; borate buffer for pH 9–11). the reaction mixture was left standing for 24 h at room temperature and the final pH was measured. The mixture was then dialyzed against water for 3 days and lyophilized. In some cases, the reaction mixutre became gellike, so its pH was measured less accurately. Control experiments without DMU were done in all instances.

Amino Acid Analyses. A weighed sample of protein (about 5 mg) was dissolved in 15 cm^3 of 6 N HCl in a commercial hydrolysis tube. The tube was evacuated, placed in an acetone-dry ice bath, evacuated, and refilled with oxygen-free nitrogen twice before being placed in an oven at 100 °C for 24 h. The cooled hydrolysate was filtered through a sintered disk funnel and evaporated to dryness at 40 °C with the aid of an aspirator, and the residue was twice resuspended in water and evaporated to dryness. Amino acid analysis of an aliquot of the residue was carried out on a Durrum amino acid analyzer, Model D-500, under the following conditions: single-column Moore and Stein ion-exchange chromatography; resin, Durrum DC-4A; buffer pH, 3.25, 4.25, 7.90; photometer, 440 and 590 nm; column, 1.75 mm × 48 cm; analysis time, 105 min. Norleucine was used as an added internal standard. The reproductibility is estimated to be ±3% or better (Friedman et al., 1979).

In Vitro Incubations. Samples containing 180 mg of casein or isonitrogenous amounts of other proteins were weighed exactly into 50 mL of polyethylene centrifuge tubes. For Michaelis–Menten incubations, 25, 50, 100, 150, and 250 mg of casein were added to separate tubes. Five milliliters of McDougall's (1948) buffer was added to each tube. Protein sources plus buffer were allowed to soak overnight at 4 °C. The next morning the tubes were put in a water bath to warm to 39 °C, and 10 mL of incubation mixture was added to each tube; tubes were rapidly capped with stoppers with Bunsen valves and incubated for 2 h at 39 °C in a shaker water bath. Digestion was stopped

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