Kinsella, J. E., Shetty, K. J., in "Nutritional Improvements of Food and Feed Proteins", Friedman, M., Ed., Plenum Press, New York, 1978, p 797.
Koshiyama, I., *Cereal Chem.* **45**, 394 (1968).
Laemmli, U. K., *Nature (London)* **227**, 680 (1970).
Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., *J. Biol. Chem.* **193**, 265 (1951).
Melnychyn, P., Stapley, R. (to Carnation Co.), Acylated Protein for Coffee Whitener Formulations, U.S. Patent 3 764 711, Oct. 9, 1973.
Mitsuda, H., Yasumoto, K., Yamamoto, A., *Arch. Biochem. Biophys.* **118**, 664 (1967).
Nakagawa, Y., Capetillo, S., Jirgensons, B., *J. Biol. Chem.* **247**, 5703 (1972).
Nakagawa, Y., Perlmann, G. E., *Arch. Biochem. Biophys.* **149**, 476 (1972).
Provansal, M. M. P., Cuq, J.-L. A., Cheftel, J.-C., *J. Agric. Food Chem.* **23**, 938 (1975).
Puski, G., Melnychyn, P., *Cereal Chem.* **45**, 192 (1968).
Rackis, J. J., Sessa, D. J., Honig, D. H., *J. Am. Oil Chem. Soc.* **56**, 262 (1979).
Shetty, K. J., Kinsella, J. E., *J. Food Sci.* **44**, 633 (1979).
Singhal, R. P., Atassi, M. Z., *Biochemistry* **10**, 1756 (1971).
Thanh, V. H., Shibasaki, K., *J. Agric. Food Chem.* **24**, 1117 (1976a).
Thanh, V. H., Shibasaki, K., *Biochim. Biophys. Acta* **439**, 326 (1976b).
Thanh, V. H., Shibasaki, K., *Biochim. Biophys. Acta* **490**, 371 (1977).
Thanh, V. H., Shibasaki, K., *J. Agric. Food Chem.* **26**, 692 (1978).
Thanh, V. H., Okubo, K., Shibasaki, K., *Agric. Biol. Chem.* **39**, 1501 (1975).
Wolf, W. J., Cowan, J. C., "Soybean as a Food Source", CRC Press, Cleveland, OH, 1975, pp 58–60.

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Tannin Deactivation and Nutritional Improvement of Sorghum by Anaerobic Storage of H_2O-, HCl-, or NaOH-Treated Grain

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Extractable tannin content of sorghum grain was markedly reduced by imbibing H_2O, HCl, or NaOH solutions into whole seeds and storing them under CO_2 atmosphere. The extent of the reduction was dependent on storage time and temperature, quantity of liquid imbibed, and concentration of acid or base. Typically, tannin content was reduced from 3.63% to 2.2, 0.6, and 0.1% by imbibing 25% by weight of water, 0.8 N HCl, or 0.8 N NaOH, respectively, and storing for 2 days at 25 °C. Increasing the storage time of the water-treated sample to 9 days decreased the tannin content to 0.3%. Extractable tannin was also markedly reduced by germination. Rat feeding studies showed that these treatments produced weight gains and feed/gain ratios which were equivalent to those obtained on a low tannin sorghum (LTS) diet. The apparent digestibility of protein and total dry matter and the PER were improved but not rendered equal to the values obtained for the LTS diet in most cases. It appears that the vanillin–HCl assay for tannin content is an adequate predictor of the nutritional quality of high tannin sorghum treated by these methods.

High tannin sorghums (HTS) with an open panicle structure appear to prevent or at least decrease bird depredation (McMillian et al., 1972; Tipton et al., 1970; Niehaus and Schmidt, 1970). Another desirable characteristic associated with tannins in sorghum is weather resistance, in particular retardation of preharvest seed germination and seed molding (Harris and Burns, 1973; Harris and Burns, 1970). Sorghum tannins, however, also have deleterious effects due to their strong interaction with proteins. The resulting complexes are not readily digested by monogastrics and this leads to lower protein digestibility, lower PER's, and weight gains (Featherston and Rogler, 1975; Maxson et al., 1973; Chang and Fuller, 1964). Sorghum tannins also inhibit enzymic reactions and microbial activity which are required during the brewing of beer (Watson, 1975).

A number of methods have been used to try to overcome problems associated with HTS. Mechanical abrasion of the seed coat layers has been shown to reduce tannin content (Chibber et al., 1978). However, this method results in low yields and large protein losses. Supplementation with methionine or the addition of polyvinylpyrrolidone to the diet improved performance of chicks (Armstrong et al., 1973; Fuller et al., 1967). Extraction of tannins with aqueous alkali resulted in marked improvements in weight gain and feed efficiency of rats (Armstrong et al., 1974) and improved in vitro protein digestibility (Chavan et al., 1979). Soaking seeds in dilute formaldehyde solution has been shown to decrease tannin content, presumably by cross-linking with some constituent in the seed (Daiber, 1975a). Imbibing dilute NH_4OH into whole seeds or mixing dilute K_2CO_3 with ground grain reduced tannin content and increased chick weight gain and feed efficiency (Price et al., 1979; Price and Butler, 1978).

Except for mechanical abrasion these treatments involve chemicals and therefore there is some question regarding the economic feasibility of the processes. In addition, a certain degree of expertise, which may or may not be available in a developing country, is required in the handling of chemicals. In this investigation we report the marked reduction in tannin content caused by anaerobic

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storage of sorghum treated only with water. Treatments with HCl and NaOH, which also decrease tannin content, were compared to the water treatment. Rat growth studies were used to evaluate the improvement in nutritional quality of the grain.

EXPERIMENTAL SECTION

High tannin sorghum (HTS) grain (*Sorghum bicolor* var. sorgho X3055) and low tannin sorghum (LTS) grain (*Sorghum bicolor* var. sorgho CE90) were grown in Senegal in 1976. Moisture content was 9.7% for HTS and 10.0% for LTS. Distilled water was used throughout the investigation.

Sorghum Dissection. Sorghum grain was soaked in distilled H_2O for 10 min and then blotted dry on filter paper. The hull and testa were carefully scraped from the seed. The sorghum components were dried under vacuum at room temperature.

Grain Treatments. Imbibition medium was added to 8 g of sorghum grain in a 15-mL vial and then immediately purged with CO_2 to remove air. The vial was sealed and shaken until all of the liquid was imbibed. Although time for imbibition varied with concentration, total imbibition was usually achieved within 30 min; acidic solutions required less time than basic solutions.

To prepare water- and base-treated sorghum for the feeding trial, we added sorghum grain (2200 g) to a 4.5-L bottle and purged with CO_2. Inbibition medium was added (550 mL) and the bottle was again purged with CO_2. After shaking the bottle to imbibe all of the liquid, the bottle was stored at 25 or 35 °C ± 1 °C. Both the imbibition medium and the sorghum were preheated to the storage temperature prior to the reaction. The water- and base-treated grain received a CO_2 treatment to prevent mold growth; this was not necessary for the acid-treated grain. Acid-treated grain was similarily prepared; however, following storage, excess HCl was leached from the seeds by soaking in 28 L of distilled H_2O for 45 min. The H_2O was decanted from the grain and this process was repeated five times with 8 L of H_2O. Grains were then leached for 2 min, four times with 8 L of H_2O. Washing removed sufficient acid to effect a pH change of a 25% slurry of ground sorghum from 2.6 to 3.6. Formalin-treated sorghum was prepared by soaking grain (2200 g) in 0.03% formalin (8800 mL) for 4 h. Excess formalin solution was decanted and the grain was washed four times with 8800 mL of H_2O. The treated grain was dried 4 h at 35 °C in a forced air oven. All other treated grains were similarily dried at 25 °C.

Grains were germinated up to 5 days at 25 °C in excess water. Grains were not sterilized prior to germination because of the possible effect on the tannins. Moldy grains were removed from the petri dishes daily.

Analytical Methods. Grains were ground in a Udy cyclone mill containing a 1-mm screen. For the tannin assays, grains were not dried prior to grinding.

The vanillin–HCl assay (Burns, 1971) using reagent blanks as described by Price et al. (1978) was used to measure tannin content of freshly ground samples. Catechin was used as a standard for quantitative comparisons.

The α-amylase inhibition assay was similar to the procedure described by Daiber (1975b). Flour samples (20 mg) were weighed into centrifuge tubes and 10 mL of 0.06% α-amylase (Wallerstein Dex-Lo, from *Bacillus subtilis*) in 0.05 M phosphate buffer (pH 7.0) was added. After 30 min at 30 °C the tube was centrifuged and the activity of the enzyme was determined by the 3,5-dinitrosalicylic acid determination (Bruner, 1964) of reducing substances produced. Inhibition was calculated as

$$\alpha\text{-amylase inhibition} = [(\text{original} - \text{residual activity}) \times 100]/\text{original activity}$$

Protein content (N × 6.25) was determined by using a Hewlett-Packard 185B CHN analyzer or the Kjeldahl procedure.

All results are presented on a dry weight basis.

Feeding Trial. Male, Wistar strain rats (Canadian Breeding Farm and Laboratories, Ltd., St. Constant, Quebec) were obtained at 25 days of age and were conditioned for 3 days. Rats were housed individually in wire-bottomed stainless steel cages which were maintained in a controlled environment. Feed and water were provided ad libitum for 4 weeks (trial 1, Table IV) or 3 weeks (trial 2, Table V) duration. The rats were randomly divided into groups of six rats each. The average of their initial weights was 66.1 g ± 5.3 (standard deviation) for trial 1 and 68.7 g ± 5.6 for trial 2.

All diets were formulated to contain 1.25% vitamins (total vitamin supplement, Nutritional Biochemicals Corp., Cleveland, OH), 4.0% minerals (Bernhart and Tomarelli, 1966), 0.3% DL-methionine, 0.63% lysine, 0.5% chromic oxide, 8.0% fat with the addition of corn oil, and 5% crude fiber with the addition of cellulose (Alphacel, Nutritional Biochemicals Corp.). In addition, HTS and LTS diets contained 72.76% (dry matter) sorghum flour, whereas the casein control diet contained 10.6% ANRC casein (Humko Sheffield, Memphis, TN). The HTS diets were supplemented with 1.06% casein so that the protein content of all diets was 10.0%. The balance of each diet was wheat starch.

Feed intakes and weight gains were determined at 7-day intervals. Feces were collected during the last 14 days and analyzed for chromic oxide (Bolin and Lockhart, 1960), nitrogen, and dry matter in order to determine apparent digestibilities.

All results were statistically analyzed by a one-way analysis of variance. Differences were determined by Duncan's multiple range test (Duncan, 1955).

RESULTS

Table I. Distribution of Tannin and Protein within Sorghum Grain

component	% by wt	% tannin	% protein (N × 6.25)
testa	7.6	22.4 (81.6)[a]	14.7 (9.8)
pericarp	7.5	4.2 (15.1)	4.1 (2.7)
endosperm + germ	84.8	0.08 (3.3)	11.8 (87.5)
whole grain	100	3.63	11.5

[a] Proportion of constituent in parentheses.

Distribution of Tannin within Sorghum Grain. Tannin analysis of sorghum grain components showed that 81.6% of the condensed tannin was located in the testa layer, whereas 15.1% was located in the pericarp (Table I). Only 57% of the tannin extractable from untreated grain was accounted for by summing the tannin present in all components. This demonstrates the reactivity of the tannins in the presence of water, since simply soaking the seeds and dissecting and air-drying the components destroyed or insolubilized 43% of the tannin. The testa layer comprised 7.6% of the seed by weight and contained 14.7% protein. These figures explain, in part, the lower yields and higher protein losses which occurred when HTS was mechanically dehulled to remove the tannins (Chibber et al., 1978).

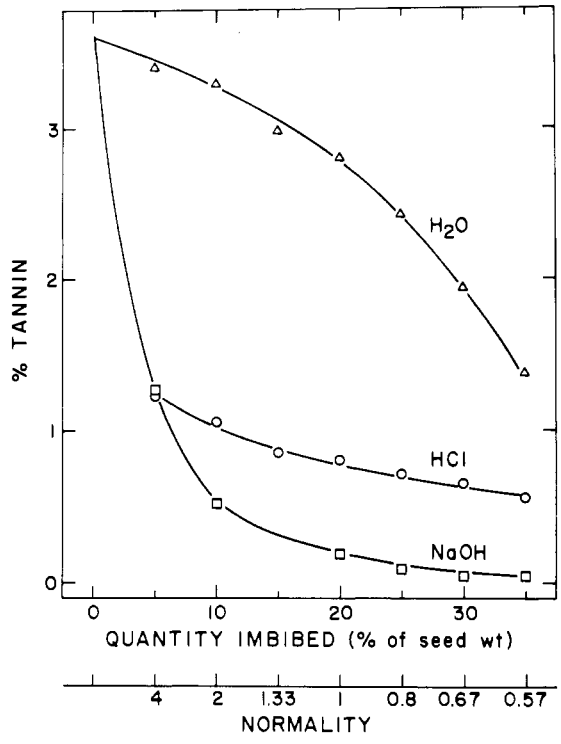

Figure 1. Tannin content of H_2O-, HCl-, and NaOH-treated sorghum stored (25 °C) as whole grains under CO_2 atmosphere for 2 days. The quantity of acid or base imbibed was kept constant at 0.02 mol/100 g of sorghum by adjusting the quantity imbibed and the normality.

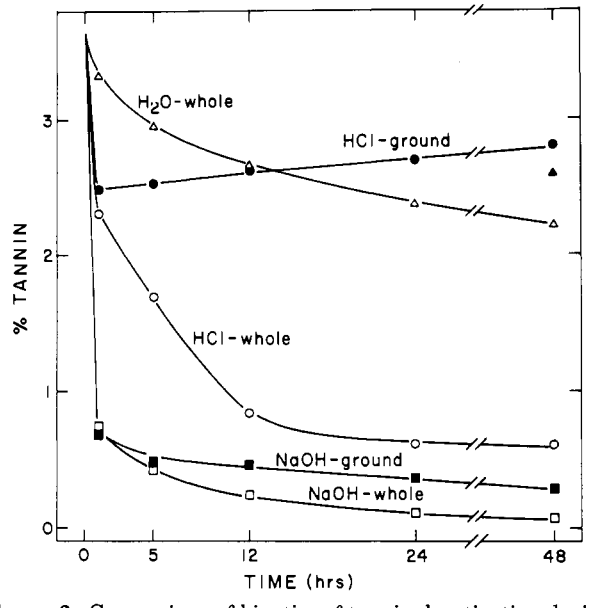

Figure 2. Comparison of kinetics of tannin deactivation during storage (25 °C) of whole seeds or ground flour treated with H_2O, 1 N HCl, or 1 N NaOH. Whole seeds were treated with 25% by weight of these solutions and immediately after imbibition one portion was ground (▲, H_2O treatment, ground).

Tannin Deactivation by H_2O, HCl, and NaOH. Extractable tannin content of HTS grain was reduced by imbibing H_2O, HCl, or NaOH solutions into whole grains and storing (25 °C) the treated grains under CO_2 atmosphere for 2 days (Figure 1). The number of moles of acid or base imbibed was kept constant (0.02 mol/100 g of sorghum) and the effect of the volume of imbibition medium was investigated by changing the concentration. Imbibing larger volumes of low normality HCl or NaOH was more effective in reducing tannin than imbibing small volumes of more concentrated solutions. The reduction of tannin by the H_2O treatment was markedly affected by the imbibition volume. The NaOH treatment most effectively reduced tannin, followed by the HCl and H_2O treatments.

Table II. Effect of Storage Temperature on Tannin Deactivation by H_2O, HCl, and NaOH Treatment

	tannin content, %	
	storage temp	
medium imbibed[a]	25 °C	35 °C
0.2 N HCl	2.34	1.14
0.8 N HCl	0.64	0.25
0.2 N NaOH	0.90	0.20
0.8 N NaOH	0.10	0.10
H_2O	2.20	1.10

[a] 25% by weight of grain of each medium was imbibed and the treated grain was stored for 2 days under CO_2 atmosphere.

Table III. Tannin Content of H_2O-, HCl-, and NaOH-Treated Grain Stored in Air or CO_2 Atmosphere

		tannin content, %	
		storage condition	
medium imbibed[a]	storage time, h	CO_2	air
H_2O	5	3.18	3.31
	48	2.36	2.51[b]
0.9 N HCl	5	1.74	1.55
	48	0.41	0.53
0.3 N NaOH	5	1.23	1.20
	48	0.31	0.36[b]

[a] 25% by weight of grain of each medium was imbibed into HTS. [b] Grain was moldy.

Tannin deactivation was more effective when the treated grains were stored whole rather than stored in the ground state (Figure 2). The tannin content of HCl-treated grains did not decrease further after the grains were ground and stored. In fact, a slight increase was observed. The figure illustrates that the rate of tannin deactivation in whole grains was highest for the NaOH-treated grains, followed by the HCl- and H_2O-treated grains.

In developing countries ambient temperature is often greater than 25 °C and therefore the effect of temperature on tannin deactivation by these treatments was investigated (Table II). For all treatments, tannin content was consistently lower for storage conditions at 35 °C than 25 °C, suggesting that shorter storage times can be used at higher temperatures.

Tannin deactivation proceeded to approximately the same extent regardless of whether treated samples were stored in air or under CO_2 atmosphere (Table III). Therefore, tannin deactivation would not be affected if only part of the air was replaced with CO_2 during the storage of the treated grains. The function of CO_2 is simply to prevent aerobic mold and bacterial growth.

Tannin content was also reduced by germinating the grain (Figure 3). The rate of tannin deactivation occurring during this process was very similar to the rate of tannin decline during anaerobic storage of water-treated sorghum (25% by weight). This indicates that the mechanism of tannin deactivation may be the same in both cases, although only one is occurring in the presence of O_2. Prolonged storage of water-treated grain nearly completely eliminated the tannin.

Nutritional Quality of Treated Grains. HTS was treated in a variety of ways to effect a range of tannin levels (Table IV). Measuring tannin by the vanillin–HCl method

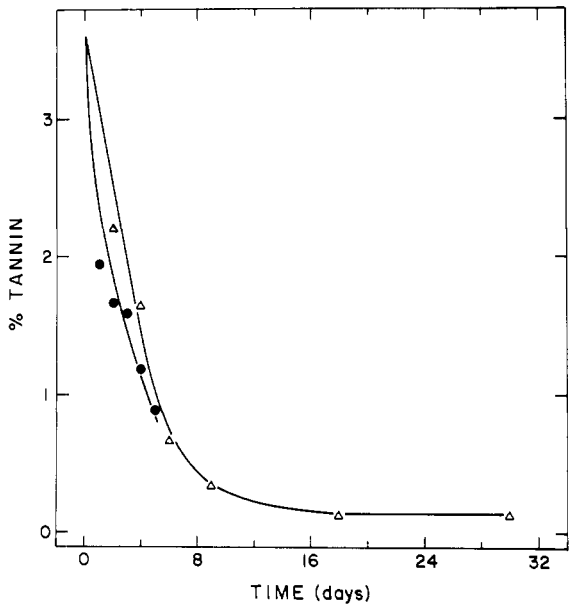

Figure 3. Comparison of the rate of tannin deactivation by anaerobic storage of whole sorghum treated (25% by weight) with water (△) and by germination of sorghum (●).

showed a reduction from 3.63% in the untreated seed to 0.92–2.21% for H_2O-treated seeds and to ≤0.5% for HCl, NaOH, and formalin treatments. The effect of the water wash on the tannin content of HCl-treated grain was negligible; tannin content was 0.39% before the wash and 0.37% after. Estimating the tannin content by quantitating the influence these treated and ground grains had on α-amylase activity appeared to give values which were generally comparable to the vanillin–HCl estimation. The correlation coefficient between α-amylase inhibition and the vanillin–HCl assay was 0.98.

The HCl- and formalin-treated grains showed the highest weight gains, lowest feed/gain ratios, and highest protein digestibility and PER values. The water treatments also markedly improved nutritional quality; however, storage at 35 °C showed no improvement over storage at 25 °C. This was unexpected since the 35 °C treatment had much lower values for tannin content and α-amylase inhibition. The H_2O-treated sorghum stored for 10 h at 35 °C showed the highest values for tannin content and showed the poorest growth and feed efficiency. The NaOH-, NH_4OH-, formalin-, HCl-, and H_2O- (25 °C, 48 h and 35 °C, 48 h) treated grains showed feed intakes, weight gains, and feed/gain ratios which were equivalent to those of the LTS. In addition, PER measurements were also equivalent for the HCl, NH_4OH, and formalin treatments. The protein digestibility of HTS was markedly improved by every treatment; however, none were equivalent to the protein digestibility of LTS.

LTS was treated with water and stored to determine whether the beneficial nutritional effect of the water treatment on HTS was due to factors other than tannin deactivation (Table V). Sullins and Rooney (1971) have shown that storage of water-treated grain caused a disruption of the protein–starch matrix and perhaps some enzymatic hydrolysis of constituents. These effects were primarily responsible for the observed improvement in nutritional quality when ruminants were fed water-treated corn (Beeson and Perry, 1958) and sorghum (Riggs, 1969). Table V shows, however, that imbibition of 25% by weight of water and storage of the moist grain for 2 days at 25 °C did not significantly improve rat growth performance. The effect of the water treatment on HTS in this investigation

Table IV. Rat Growth Performance (4-Week Trial) on Water-, Acid-, Base-, and Formalin-Treated Sorghum

treatment				tannin estimation		nutritional parameters[c]					
										digestibility	
feed source	medium imbibed[a]	storage time, h	storage temp, °C	vanillin–HCl, %	α-amylase inhibition, %	feed intake, g	wt gain, g	feed/gain	PER	protein, %	dry matter, %
casein				0	0	483.7[a]	169.7[a]	2.85[a]	3.10[a]	93.5[a]	92.4[a]
LTS				0.04	0	319.2[c]	65.2[c]	5.00[b]	1.92[b]	81.5[b]	87.5[b]
HTS				3.63	93.6	231.9[d]	19.7[d]	12.52[d]	0.79[f]	43.7[h]	79.3[ef]
HTS	H_2O[a]	48	25	2.04	32.9	317.5[c]	57.7[c]	5.57[b]	1.68[cd]	61.3[fg]	81.1[d]
HTS	H_2O[a]	48	35	0.92	6.2	311.2[c]	56.8[c]	5.56[b]	1.69[cd]	64.2[ef]	80.4[de]
HTS	H_2O[a]	10	35	2.21	49.5	251.2[d]	34.7[d]	7.73[c]	1.27[e]	58.0[g]	81.3[cd]
HTS	0.9 N HCl[a]	48	25	0.37	2.8	385.2[b]	83.5[b]	4.64[b]	1.99[b]	75.7[c]	82.8[c]
HTS	0.25 N NaOH[a]	48	25	0.50	6.7	320.0[c]	56.8[c]	5.93[b]	1.64[d]	61.2[fg]	78.9[f]
HTS	0.25 N NH_4OH[a]	48	25	0.67	3.8	353.6[bc]	71.4[bc]	5.02[b]	1.86[bcd]	65.2[e]	81.3[cd]
HTS	0.03% formalin[b]			0.40	2.8	352.9[bc]	74.7[bc]	4.73[b]	1.94[b]	70.8[d]	81.4[cd]

[a] 25% by weight of grain of each medium was imbibed into HTS. [b] Soaked in 0.03% formalin for 4 h and then water-washed and dried. [c] Values not connected by a common letter are significantly different at the 95% level.

Table V. Rat Growth Performance (3-Week Trial) on Untreated and Water-Treated Low Tannin Sorghum

treatment				nutritional parameters[b]					
								digestibility	
feed source	medium imbibed[a]	storage time, h	storage temp, °C	feed intake, g	wt gain, g	feed/ gain	PER	protein, %	dry matter, %
casein				280.4[a]	107.0[a]	2.62[a]	3.36[a]	90.6[a]	91.1[a]
LTS				214.6[b]	38.1[b]	6.22[b]	1.81[b]	80.1[b]	85.9[b]
LTS	H_2O[a]	48	25	211.4[b]	40.9[b]	5.29[b]	1.62[b]	79.0[b]	85.5[b]

[a] 25% by weight of H_2O was imbibed into LTS. [b] Values not connected by a common letter are significantly different at the 95% level.

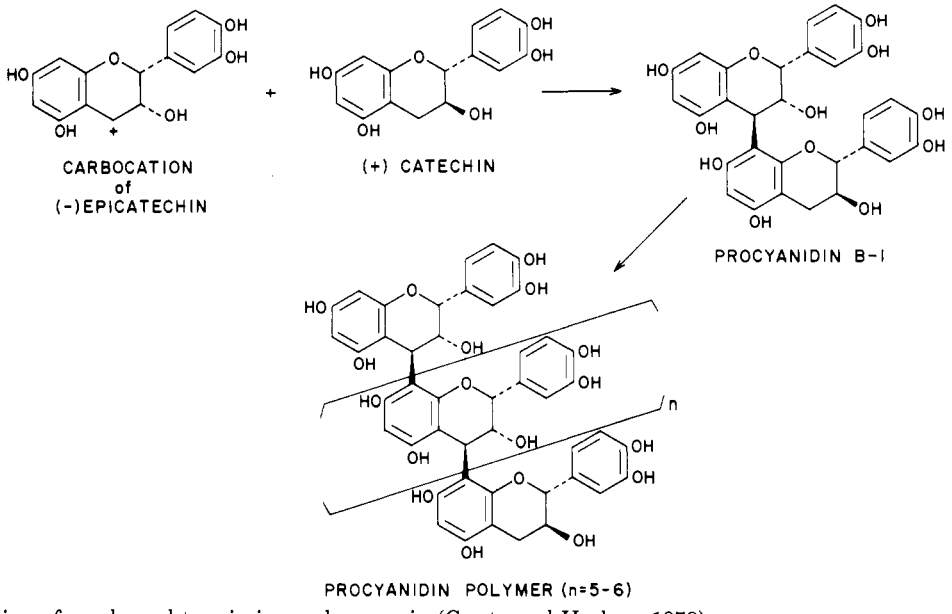

Figure 4. Formation of condensed tannin in sorghum grain (Gupta and Haslam, 1978).

is therefore likely only due to tannin deactivation.

DISCUSSION

Extractable tannin content in sorghum can be reduced by imbibing H_2O, HCl, or NaOH solutions into the whole seeds and storing the treated grain under CO_2 atmosphere. The nutritional quality was markedly improved by these treatments; however, further gains could likely be made by extending the storage period, especially for the water-treated grains.

The mechanism of tannin deactivation by the H_2O or HCl treatments may be similar to the reactions which proceed in the grain as it approaches maturity. The grain is initially formed in a sheath and at the etiolated stage no procyanidins can be detected (Gupta and Haslam, 1978). However, as chlorophyll develops in the seed coat there is apparently a rapid synthesis of procyanidin B-1 and (+)-catechin (Figure 4). As the seed ripens and approaches maturity, polymeric procyanidins are formed from the reaction of the carbocation from (−)-epicatechin and (+)-catechin. A concomitant increase in the concentrations of soluble and insoluble polymers is observed. An acid-catalyzed mechanism, generating the carbocation, is favored by many investigators for the conversion of the flavan progenitors to the condensed tannins, even though the concentration of acid in the plant is very low (Halsam, 1966). The reaction in Figure 4 is under thermodynamic control and recently it was shown that a similar reaction is possible in vitro in the presence of HCl (Fletcher et al., 1977). It is therefore possible that during the storage of acid-treated sorghum, reactive carbocations are formed from the soluble polymers (n = 5–6). These would react in a fashion similar to that shown in Figure 4 to yield higher oligomeric polymers which are not readily soluble in water and hence much less likely to interfere with enzymes or other proteins. Storage of water-treated sorghum may simply allow a continuation of the natural polymerization process (Figure 4) that was occurring as the seed was drying out.

Alternatively, storage of acid- or water-treated grains may insolubilize the tannins by protein–tannin interaction. This interaction would prevent complexation of the tannins with digestive enzymes but the resulting complexes would not likely be digestible.

The effect of water on sorghum tannins has implications for methods of tannin analysis, especially by the widely used vanillin–HCl procedure. The reproducibility of this assay has recently been shown to be affected by reaction temperature, sample preparation method, and light (Dalby and Shuman, 1978; Price et al., 1978; Broadhurst and Jones, 1978). However, we feel that the most important parameter affecting the meaningfulness of this assay is the postharvest treatment of the sample, in particular, storage time, temperature, and moisture content. Samples should be dried to less than 10% moisture and stored near 0 °C to eliminate changes in tannin content with storage time. Obviously, the postharvest treatment of HTS, particularly storage conditions, also has an effect on the nutritional quality of the grain.

Deactivation of tannin by storage of water-treated grain appears to be a simple solution to the problem of overcoming this antinutritional factor in sorghum. The advantage of the treatment for developing countries is that no chemical other than water is required. Supplementary carbon dioxide is not likely required since aerobic mold and bacterial growth can be prevented or at least greatly

diminished by tightly packing imbibed grain in a sealed container. Future research will need to determine whether HCl or H_2O treatments are applicable to other high tannin varieties. Nutritional studies will also have to be conducted to determine whether further improvements in protein digestibility and PER may be possible with prolonged storage (10–20 days) of water- or acid-treated sorghum.

LITERATURE CITED

Armstrong, W. D., Featherston, W. R., Rogler, J. C., *Poultry Sci.* **52**, 1592 (1973).
Armstrong, W. D., Rogler, J. C., Featherston, W. R., *Poultry Sci.* **53**, 714 (1974).
Beeson, W. M., Perry, T. W., *J. Anim. Sci.* **17**, 368 (1958).
Bernhart, F. W., Tomarelli, R. M., *J. Nutr.* **89**, 495 (1966).
Bolin, D. W., Lockhart, W., *Proc. N.D. Acad. Sci.* 14, 102 (1960).
Broadhurst, R. B., Jones, W. T., *J. Sci. Food Agric.* **29**, 788 (1978).
Bruner, R. L., in "Methods in Carbohydrate Chemistry", Volume IV, Academic Press, New York, 1964, p 67.
Burns, R. E., *Agron. J.* **63**, 511 (1971).
Chang, S. I., Fuller, H. L., *Poultry Sci.* **43**, 30 (1964).
Chavan, J. K., Kadam, S. S., Ghonsikar, C. P., Salunkhe, D. K., *J. Food Sci.* **44**, 1319 (1979).
Chibber, B. A. K., Mertz, E. T., Axtell, J. D., *J. Agric Food Chem.* **26**, 679 (1978).
Daiber, K. H., South Africa Patent 75/4957, 1975a.
Daiber, K. H., *J. Sci. Food Agric.* **26**, 1399 (1975b).
Dalby, A., Shuman, A. C., *Anal. Biochem.* **85**, 325 (1978).
Duncan, B. D., *Biometrics* 11, 1 (1955).
Featherston, W. R., Rogler, J. C., *Nutr. Rep. Int.* 11, 491 (1975).
Fletcher, A. C., Porter, L. J., Haslam, E., Gupta, R. K., *J. Chem. Soc., Perkin Trans. 1*, 1628 (1977).
Fuller, H. L., Chang, S. I., Potter, D. K., *J. Nutr.* **91**, 477 (1967).
Gupta, R. K., Haslam, E., *J. Chem. Soc., Perkin Trans 1*, 892 (1978).
Halsam, E., "Chemistry of the Vegetable Tannins", Academic Press, New York, 1966, p 69.
Harris, H. B., Burns, R. E., *Agron. J.* **62**, 835 (1970).
Harris, H. B., Burns, R. E., *Agron. J.* **65**, 957 (1973).
Maxson, E. D., Rooney, L. W., Lewis, R. W., Clark, L. E., Johnson, J. W., *Nutr. Rep. Int.* **8**, 146 (1973).
McMillian, W. W., Wiseman, B. R., Burns, R. E., Harris, H. B., Greene, G. L., *Agron. J.* **64**, 821 (1972).
Niehaus, M. H., Schmidt, W. H., *Agron. J.* **62**, 677 (1970).
Price, M. L., Butler, L. G., Rogler, J. C., Featherston, W. R., *J. Agric. Food Chem.* **27**, 441 (1979).
Price, M. L., Butler, L. G., *Nutr. Rep. Int.* **17**, 229 (1978).
Price, M. L., Van Scoyoc, S., Butler, L. G., *J. Agric. Food Chem.* **26**, 1214 (1978).
Riggs, J. K., *Am. Soc. Agric. Eng.* **12**, 782 (1969).
Sullins, R. D., Rooney, L. W., *Cereal Chem.* **48**, 567 (1971).
Tipton, K. W., Floyd, E. H., Marshall, J. G., McDevitt, J. B., *La. Agron. J.* **62**, 211 (1970).
Watson, T. G., *J. Appl. Bacteriol.* **38**, 133 (1975).

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Oleoresins of Pinyons

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Monoterpenoid hydrocarbons from wood and gum turpentines of 11 pinyon species and varieties are composed of α-pinene, camphene, β-pinene, 3-carene, sabinene, myrcene, limonene, β-phellandrene, γ-terpinene, terpinolene, and *p*-cymene with occasional occurrence of *cis*-ocimene, tricyclene, α-phellandrene, thujene, and α-terpinene, generally in trace amounts. Higher boiling constitutents include at least 27 sesquiterpenoid hydrocarbons and 14 oxygenated monoterpenoids and volatile nonterpenoids. Pimaric acids, mainly Δ-8(9)-isopimaric acid, account for about 70% of the resin acids, with the rest composed mainly of abietic-type acids. Paraquat treatment of the singleleaf pinyon produces negligible resinification within 1 year.

Pinyons are pines of semiarid regions of the southwestern United States and central and north central Mexico (Critchfield and Little, 1966). They are relatively small in size compared to western pines such as ponderosa pine, lodgepole pine, or western white pine, or to southeastern pines such as loblolly pine or slash pine, and often grow in association with other small size tree species, such as junipers. They are slow-growing species (Barger and Ffolliott, 1972), commonly bushy in appearance with branches down to the ground, with the result that about half of the weight of a tree represents leaves and branches and with wood including a large number of knots and other defects. Pinyons comprise 11 species and varieties, with *P. cembroides*, *P. edulis*, and *P. monophylla* responsible for most pinyon stands in existence and with the rest covering either considerably more restricted ranges or known from one or a few localities only (Critchfield and Little, 1966).

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Pinyon forests comprise about 61 million acres in the United States (Barger and Ffolliott, 1972) and about 45 million acres in Mexico. Forests of Arizona and New Mexico contain about 393 ft^3 of wood per acre in the north of the states and only 23 ft^3 per acre for the woodland zone of the south. These figures change to 744 and 595 ft^3 per acre, respectively, if one includes junipers which commonly occur together with pinyons (Howell, 1940). While this is well below the volume of softwood available on an acre of commercial timberland in the Pacific U.S. region (over 3000 ft^3/acre), it compares favorably with that of southeastern states (from 266 ft^3/acre for Virginia to 513 ft^3/acre for South Carolina) (McGuire, 1973). At the same time pinyons and junipers are lagging well behind the southern pines in terms of annual growth—6.0 and 3.6 ft^3/acre for northern Arizona and New Mexico, respectively, vs. about 28 ft^3/acre for southeastern U.S.

Commercial utilization of pinyons is not extensive. The defective nature of wood limits its use as lumber and results in a low quality veneer for plywood. The pulp of *P. edulis* and *P. monophylla* is short fibered, which is undesirable and limits its use in pulp and paper (Barger and

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