Use of Ammonium Hydroxide To Reduce the Level of Assayable Tannin in High-Tannin Sorghum Grain[†]

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Soaking in dilute alkali, followed by drying at elevated temperatures, was used to reduce the amount of assayable tannin in high-tannin sorghum. Sorghum tannin levels were reduced by up to 88% by treatment with ammonium hydroxide (0.01–1 M) for periods of up to 20 days. Soaking and drying temperatures between 25 and 50 °C were evaluated. Increased drying temperatures decreased ammonia levels in the flour.

Keywords: *Tannin; sorghum*

INTRODUCTION

High-tannin sorghums have various agronomic benefits. These advantages include resistance to preharvest seed germination (Harris and Burns, 1970), decreased bird depredation (Voigt, 1966a,b; Harris, 1969; Tipton *et al.*, 1970), and resistance to seed molding (Friend, 1977). Unfortunately, tannins cause various processing and nutritional problems. These problems include decreased protein digestibility (Chavan *et al.*, 1979, 1980; Muindi and Thomke, 1981) and inhibition of enzymatic reactions and essential microbial processes such as those necessary for beer brewing (Watson, 1975). Tannins of high molecular weight can be both bitter and astringent (Arnold *et al.*, 1980). Bitterness and astringency reduce consumer acceptability and market value of products produced from high-tannin sorghum grain (Sosulski, 1979). In addition, dietary tannins have been implicated in the development of some forms of cancer (Korpassy, 1961; Morton, 1970). Previous studies (Price *et al.*, 1978, 1979) reported that soaking sorghum grain in alkali solutions significantly reduces the level of assayable tannin and improves the feed efficiency of the grain.

The goal of this research was to reduce the level of assayable tannin using relatively low concentrations of ammonium hydroxide and to determine the changes that occur to pH and residual ammonia levels in the sorghum flour following treatment. In addition, the effect of treatment on sensory preference of a product produced from sorghum flour was evaluated.

Table 1. Treatment Combinations Derived by Face-Centered Cubic Design (Murray, 1992)

NH_4OH concn (M)	soak temp (°C)	storage time (days)	dry temp (°C)
0.1[a]	25	1	25
0.1	25	1	50
0.1	50	1	25
0.1	50	1	50
0.5	37.5	1	37.5
1	25	1	25
1	25	1	50
1	50	1	25
1	50	1	50
0.1	37.5	10	37.5
0.5	25	10	37.5
0.5	37.5	10	25
0.5	37.5	10	37.5
0.5	37.5	10	37.5
0.5	37.5	10	37.5
0.5	37.5	10	50
0.5	50	10	37.5
1	37.5	10	37.5
0.1	25	20	25
0.1	25	20	50
0.1	50	20	25
0.1	50	20	50
0.5	37.5	20	37.5
1	25	20	25
1	25	20	50
1	50	20	25
1	50	20	50

[a] 0.01 M concentration replaced by 0.1 M for replicates II and III.

MATERIALS AND METHODS

Sorghum grain (W-744DR bird-resistant hybrid sorghum) was obtained from George Warner Seed Co., Inc. (Hereford, TX). The grain was hand cleaned to remove all dirt, glumes, and broken grain.

Alkali Treatment of Grain Sorghum. Treatment combinations were chosen to conform to a face-centered cubic statistical design (Murray, 1992; Table 1). Ten grams of sorghum grain was mixed with 25 mL of aqueous NH_4OH (0.01, 0.1, 0.5, or 1 M) and 20 ppm of $NaHClO_3$ (added to prevent microbial spoilage), in 125 mL glass Erlenmeyer flasks. Flasks were sealed with Parafilm. Samples were incubated for 1, 10, or 20 days at 25, 37.5, or 50 °C. After storage, samples were rinsed with distilled water and dried for 24 h at 25, 37.5, or 50 °C in a forced air oven. Dried samples were ground in a Janke and Kunkel mill (Tekmar, Cincinnati, OH) to pass through a 0.5 mm sieve and analyzed for tannin content, pH, and residual ammonia.

Tannin Analysis. The vanillin assay (Burns, 1971) was used as modified by Price *et al.* (1978). Extractions for the modified vanillin–HCl assay were prepared by combining between 0.4 and 2 g of ground grain and 10 mL of 1% HCl in methanol in 10 × 110 mm test tubes. Sample size was adjusted so that the measured tannin values would fall within the linear portion of the standard curve as recommended by Price *et al.* (1978). The samples were mixed prior to centrifuging at 3000*g* for 20 min at 25 °C. One milliliter of supernatant from each sample was pipetted into each of two test tubes. To

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Table 2. Effect of Soaking Time, Ammonium Hydroxide Concentration, and Soaking and Drying Temperatures on Tannin, Residual Ammonia, and pH As Determined by General Linear Model Procedures

source	DF	sum square	mean square	F value	Pr >F[a]
pH					
time	2	0.15873611	0.07936637	1.42	0.2486ns
alkali concn	3	15.37030660	5.12343553	91.68	0.0001***
soak temp	2	0.30303909	0.15156955	2.71	0.0735ns
dry temp	2	1.11739518	0.55869759	10.00	0.0002***
ammonia					
time	2	56.96698097	28.48390434	13.21	0.0001***
alkali concn	3	66.96698097	22.32232699	10.35	0.0001***
soak temp	2	4.04107534	2.20253767	0.94	0.3968ns
dry temp	2	13.91491052	6.95745526	3.23	0.0458*
tannin					
time	2	1.65783645	0.82891823	12.26	0.0001***
alkali concn	3	23.46561824	7.82187275	115.71	0.0001***
soak temp	2	005395045	0.02697523	0.40	0.6725ns
dry temp	2	0.01761210	0.00880605	0.13	0.8781ns

[a] ns, not significant; *, significant at $p < 0.05$; ***, significant at $p < 0.001$.

the first aliquot was added 5 mL of vanillin−HCl reagent. Five milliliters of 4% HCl in methanol was added to the second aliquot. This second sample was used as a corresponding sample blank. After incubation for 20 min at 25 °C, the transmittance at 500 nm for the samples and sample blanks was determined using a Beckman Instruments DU 64 spectrophotometer (Arlington Heights, IL).

The machine was standardized to 100% transmittance with vanillin−HCl reagent. Corrected sample transmittance was calculated as sample transmittance minus sample blank transmittance. Tannin concentration was determined by comparison of the corrected sample transmittance to the standard curve.

Standard Curve. A stock solution was prepared by adding 100 mg of catechin (Sigma Chemical Co., St. Louis, MO) to 50 mL of methanol. Dilutions from 0.1 to 2 mg/mL were prepared from the stock solution and placed in separate test tubes. Two 1 mL aliquots of each dilution were pipetted into test tubes (duplicate samples). Five milliliters of vanillin−HCl was added to each aliquot. Transmittance at 500 nm was measured after incubation for 20 min at 25 °C. The linear portion (assessed by visual inspection) of the standard curve was between 0.25 and 1.5 mg/mL. The equation of linear portion (determined by linear regression) was $y = 0.038x + 4.22$ ($r^2 = 0.95$).

Sample Preparation for pH and Residual Ammonia Determination. Two grams of ground sample was mixed with 10 mL of distilled water in 16 mL disposable plastic centrifuge tubes. The samples were homogenized using a Janke and Kunkel Tissumizer (Tekmar). Next, samples were centrifuged at 3000g for 20 min. The supernatant was decanted into 30 mL glass beakers. The pH in the supernatant was measured and adjusted to between 11 and 14 (for minimum ammonia ionization), and ammonia was determined with an ammonia selective electrode (Model 95-12, Orion Research, Boston, MA).

Statistical Analysis. Response variables of assayable tannin, pH, and residual ammonia were subjected to statistical calculations using general linear model (GLM) procedures (SAS, 1989). Mean separations of main effects found to be significant by GLM were calculated using Fisher's protected least significant difference (lsd) (Snedecor and Cochran, 1989). Linear regression coefficients were calculated by the response surface (RS) regression procedure (SAS, 1989). Parameter estimates from linear regression were used to plot response surfaces for significant variables using the SAS-Graph program. Both 0.01 and 0.1 M concentrations were included in the statistical analysis.

Large Scale Alkali Treatment of Grain Sorghum. Three kilograms of hand-cleaned grain was mixed with 7.5 L of 1.0 M NH_4OH and 20 ppm $NaHClO_3$. The grain was stored for 1 day at 37.5 °C, rinsed with distilled water, and dried for 24 h at 37.5 °C. Treated and untreated grains were ground separately with a Wiley mill to pass through a 0.5 mm sieve. Tannin, pH, and residual ammonia levels were then determined for both samples as previously described.

Sensory Evaluation. Sensory preference of muffins made from treated and untreated sorghum flour was evaluated in a paired comparison test (Stone and Sidel, 1985). The muffin formulation (% w/w) was 20.6% wheat flour, 22% sorghum flour (treated or untreated), 2.6% baking powder, 6.8% sugar, 0.3% salt, 31% milk, 9% egg, and 7.7% butter.

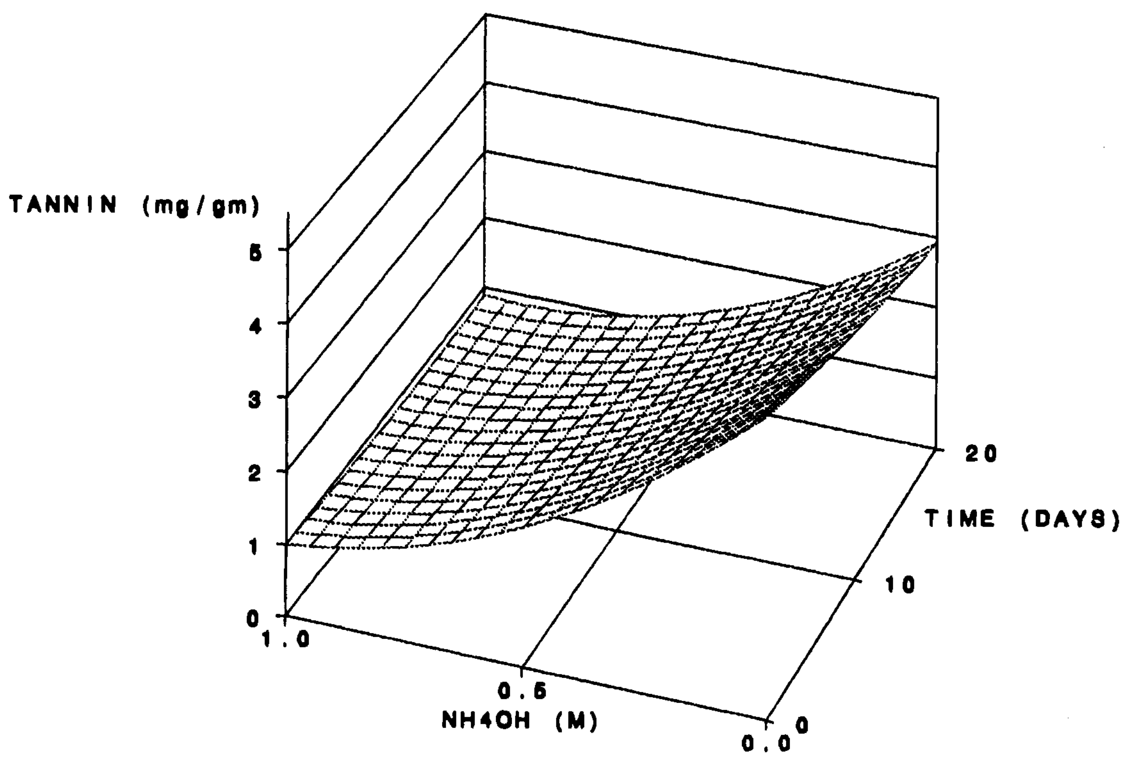

Figure 1. Tannin reduction in grain sorghum as a function of soaking time and ammonium hydroxide concentration. Soaking and drying temperatures were 37.5 °C.

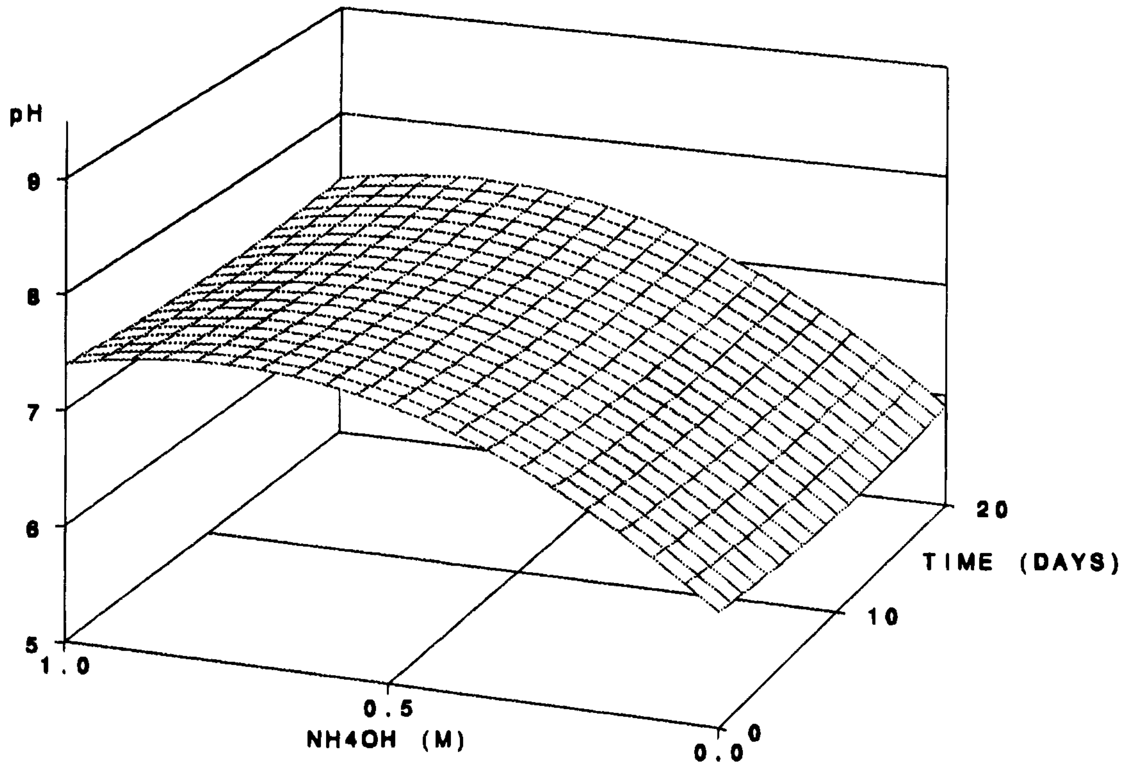

Figure 2. Change in pH in grain sorghum as a function of soak time and ammonium hydroxide concentration. Soaking and drying temperatures were 37.5 °C.

RESULTS AND DISCUSSION

As expected, aqueous ammonium hydroxide treatments successfully reduced the assayable tannin level in grain sorghum.

Ammonium hydroxide concentration and soaking time had a significant effect ($p < 0.001$) on the assayable tannin in the grain (Table 2). Soaking temperatures had no significant effect on tannin reduction. This is in contrast to the work of Price *et al.* (1979). These researchers suggested that the decrease in assayable tannin level may be more rapid when grain is soaked at 50 °C than at 4 °C. Linear regression coefficient estimates were used to generate three-dimensional plots ($r^2 = 0.86$) of the variables that were found to significantly affect tannin content, as determined by general linear model procedures (Figure 1). Increasing alkali

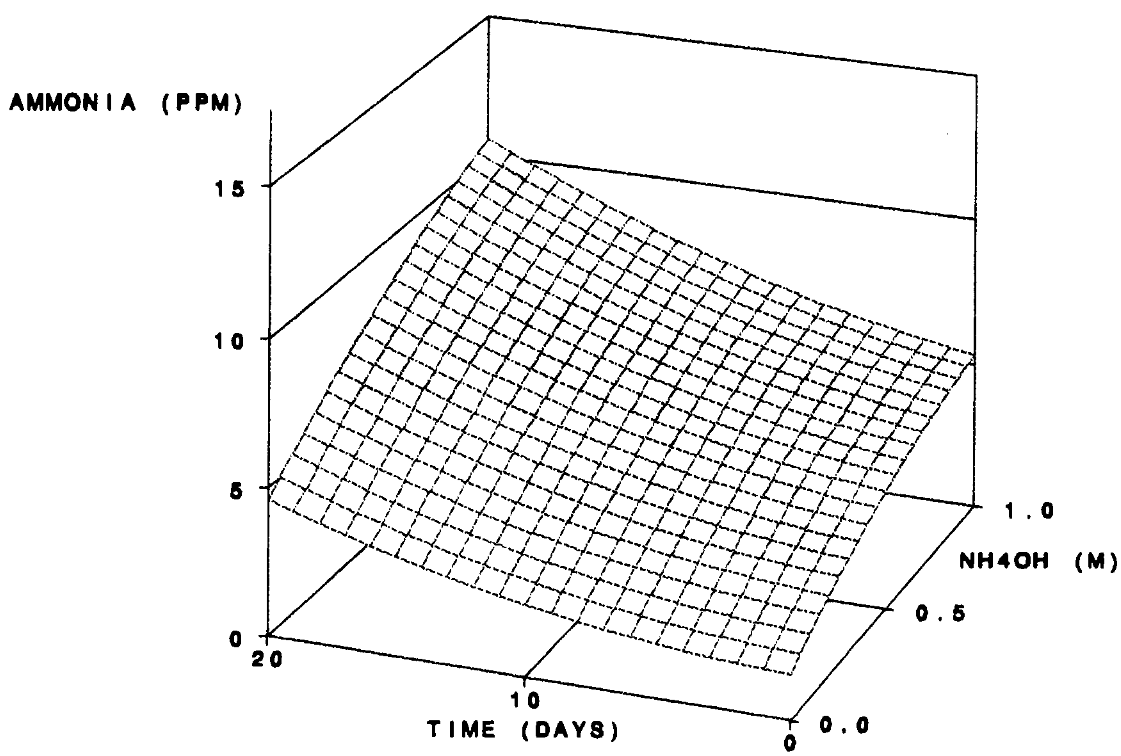

Figure 3. Residual ammonia levels in grain sorghum as a function of soak time and ammonium hydroxide concentration. Soaking and drying temperatures were 37.5 °C.

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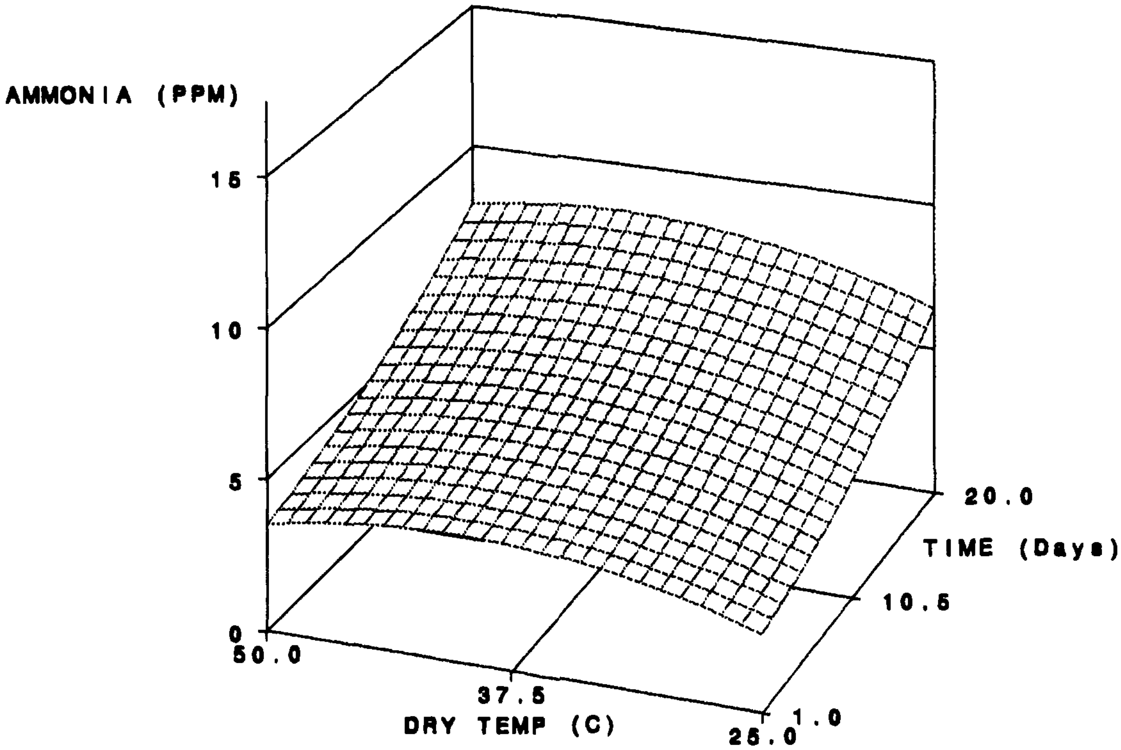

Figure 4. Residual ammonia levels in grain sorghum as a function of drying temperature and ammonium hydroxide concentration. Soaking time was 10 days, and soaking temperature was 37.5 °C.

concentration was more effective at reducing tannin levels than increasing soaking times. However, a longer soaking time in more dilute alkali could decrease tannin concentration to levels comparable to those obtained by soaking at high alkali concentrations. The most concentrated alkali solutions resulted in the lowest level of assayable tannin.

The pH of grain was significantly affected by alkali concentration ($p < 0.001$) and drying temperature ($p < 0.05$) (Table 2). Soaking time and temperature did not significantly affect pH. As expected, sorghum flour pH increased with alkali concentration (Figure 2). Drying temperatures above 37.5 °C decreased the pH of the flour.

Residual ammonia levels were significantly affected by soaking time, ammonium hydroxide concentration, and drying temperature (Table 2). Soaking temperature had no significant effect on residual ammonia levels. Response surface regression ($r^2 = 0.65$) showed a strong correlation between soaking time and NH_4OH concentration (Figure 3). Longer soaking times and higher alkali concentrations resulted in an increase in residual ammonia levels. Drying temperatures above 37.5 °C appeared to reduce the level of residual ammonia (Figure 4). As suggested by Price *et al.* (1979), it appears that residual ammonia could be reduced by volatilization. Mean values for measured parameters are compiled in Table 3.

Very low alkali concentrations (0.01 M) were found to be inadequate to prevent microbial spoilage. A simple Gram stain of the supernatant from obviously spoiled grain revealed Gram-negative rods and Gram-positive cocci. In addition to microbial growth, seed germination occurred during drying after 1 day of soaking when using 0.01 M NH_4OH. This NH_4OH concentration was therefore discontinued after the first replication and replaced by 0.1 M.

Measuring the tannin content in the supernatant using the vanillin assay (Price *et al.*, 1978) was unsuccessful. The mechanism by which alkaline conditions reduce the amount of assayable tannin in high-tannin sorghum grain remains obscure. It is speculated that the tannins may become bound to some component in the grain or are altered in some manner so as to become unreactive (Price *et al.*, 1979). Feeding studies indicate that alkali treatment of sorghum grain increases feed utilization (Price *et al.*, 1979; Muindi and Thomke, 1981).

The large scale alkali treatment of high-tannin sorghum grain resulted in a reduction in the assayable tannin level from 4.6 mg/g in the control down to 0.5 mg/g in the treatments. This accounts for an 88% reduction in the assayable tannin level. Residual ammonia level in the alkali-treated grain was 4.7 ppm.

Table 3. Effects of Soaking Time, Ammonium Hydroxide Concentration, and Drying Temperatures on Tannin, Residual Ammonia, and pH As Determined by Fisher's Protected Least Significant Difference[a]

	soak time		
	1 day	10 days	20 days
ammonia[b] (ppm)	1.69[b]	2.10[b]	3.69[a]
tannin[c] (mg/g)	1.35[a]	1.09[b]	1.00[c]

	alkali concn			
	0.01 M	0.1 M	0.5 M	1 M
ammonia[b] (ppm)	0.24[d]	2.88[c]	2.16[b]	2.68[a]
tannin[c] (mg/g)	2.53[a]	0.93[b]	0.59[c]	0.52[c]
pH[d]	6.56[c]	7.21[b]	7.97[a]	7.92[a]

	dry temp		
	25 °C	37.5 °C	50 °C
ammonia[b] (ppm)	1.83[c]	3.21[a]	2.44[b]
pH[d]	7.57[a]	7.38[b]	7.29[c]

[a] Superscripts indicate numbers in the same row that are significantly different. [b] Ammonia level in control, 0.0255 ppm. [c] Tannin level in control, 4.568 mg/g. [d] pH level in control, 6.43.

Table 4. Tannin, pH, and Residual Ammonia Levels in Large Scale Alkali-Treated Grain and Untreated Grain[a]

	treated grain		untreated grain	
	mean	SD	mean	SD
tannin (mg/g)	0.52	±0.086	4.57	±0.144
pH	7.97	±0.085	6.43	±0.036
ammonia (ppm)	4.71	±0.162	0.03	±0.005

[a] Measurement done in triplicate.

Large scale results were similar to those results achieved in laboratory bench scale experiments (Table 4).

Visual inspection of the muffins revealed differences between the treated and untreated muffins. These differences included batter viscosity, color, texture, and leavening ability. The muffins made from alkali-treated grain were considered to be more moist (by an untrained sensory panel) but had a lower loaf height than the muffins made from untreated grain (3.1 cm as compared to 3.8 cm). Sensory evaluation revealed that the muffins prepared from treated grain were significantly more preferred than the untreated grain muffins ($p < 0.0358$).

Further research is needed to determine the mechanism by which alkaline conditions reduce the amount of assayable tannins. The role of high temperatures in reducing residual ammonia should be further investigated. The quality of the products produced from alkali-treated high-tannin sorghum grain should also be further evaluated. Additional sensory evaluation is needed to study the differences between the products of the treated and untreated sorghum grains. Descriptive analysis may be a useful tool in describing the bitter and/or astringent quality of high-tannin sorghum grain.

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