EFFECT OF DIFFERENT TREATMENTS ON TANNIN CONTENTS OF SORGHUM GRAIN CULTIVARS.

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ABSTRACT: The effect of different chemicals on tannin content of two varieties of sorghum, namely sorghum white and sorghum red were determined. Triplicate samples of white and red sorghum were steeped with sodium hydroxide (0.05, 0.1 and 0.2%) and formaldehyde (0.05, 0.1 and 0.2%) at two incubation periods of 8 and 16 hours. At each incubation period alkali treatment was found to be more effective. However, results showed a significant ($p<0.05$) reduction in tannin content, when the sorghum was steeped in 0.2% NaOH for 16 hrs (0.05±0.005) compared with control (0.34±0.01). Steeping with water had significant impact on the total content of tannin (0.29±0.005) compared with untreated sorghum (0.48±0.03). The incubation time showed significant impact, however varietal difference was found to be nonsignificant. All the reagents including water showed significant ($p<0.005$) effect on tannin reduction. Reduction is influenced by incubation time, but not with the variety of sorghum.

Key words: Tannin, steeping, sodium hydroxide, incubation time, sorghum.

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INTRODUCTION

Tannins are complex polyphenolic compounds with the varying range of molecular weight and complexity. Tannins are components of the feed and food having potential to form complexes with polysaccharides and other macromolecules limiting the digestibility of the feed. Tannin containing diets resulted in depressed growth and poor feed conversion ration (FCR) in chicken (Mahmood. *et al.,* 2006).

Sorghum (sorghum bicolor) which is an old cereal grass is an important component of poultry feed (Black, 2005). Sorghum is a tough, drought resistant crop, hence can be cultivated in different geographical areas of the world (Boren *et al.,* 1992). The feeding value of the sorghum has been affected by its tannin content present in the pericarp of the grain (Selle *et al.,* 2010). Tannin reduces feed intake (Ebadi *et al.,* 2011) ,metabolizable energy and amino acid digestibility in broilers. The color of the sorghum varies in relation to tannin contents, although precise estimation require further studies (Sedghi *et al.,* 2012).

Several treatments have been employed to reduce tannin contents. Mechanical abrasion of the seed coat can be performed, but it resulted in the loss of protein content (Eggum *et al.,* 1983), ammoniation and addition of binding agent (Marquadt, 1989). Frequently used physical methods include grinding and removal of testa or soaking in water (Chibber *et al.,* 1978). Sorghum grain soaking in acid and alkali is also practiced.

The present study was designed to evaluate the effect of formaldehyde and sodium hydroxide on tannin content of sorghum grain. Three different concentrations and two different incubation times on local varieties of red and white sorghum were used.

MATERIAL AND METHODS

Two sorghum cultivars, red and white were obtained from local market of Lahore. The samples were carefully cleaned from husk, damaged grains and foreign matter.

Proximate Analysis: Moisture, ash, fat, fiber and crude protein contents were determined. For the moisture content the two varieties of sorghum samples were dried in a hot air oven at 40° C with subsequent cooling until weight become constant. The ash contents were determined using 2 gram of each variety by a muffle furnace at 550 °C according to AOAC (1985) methods. Total nitrogen was determined by the macro-kjeldhal method. The crude fiber was estimated by methods (Goering and Van Soest).

Tannin Estimation: Quantitative estimation of tannin was carried out using the Lowenthal's volumetric permanganate method AOAC (1970). The reagent was prepared daily.The infusion of sample as dissolved in indigo- carmine solution was titrated against N/25 $KMnO_4$ solution. The tannin contents were estimated by the amount of $KMnO_4$ converted into gram of tannin present (D. N. Barua and E. A. Houghton Roberts, 1940).

Steeping: Samples of sorghum grain (100 gram) were weighed after the removal of dust and husk by using a mesh. The grains were placed in perforated nylon bags.

All the bags were subjected to static steep in water (control), formaldehyde (0.05, 0.1, 0.2%) and NaOH (0.05, 0.1, 0.2%) initially for 8 hours and then for 16 hours, at 25 ℃. Post 8 hours steeping grains were rinsed with tap water. Excessive water was removed and grains were dried in hot air oven at 40 ℃ for 72 hours. The same procedure was adopted for post 16 hours steeping.

The grains were analyzed for tannin contents and proximate analysis was carried out.

Statistical analysis: Data was means of triplicate samples subjected to analysis of variance. Significance between means were calculated according to Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

The experiment conducted by steeping white and red sorghum varieties in water, NaOH and formaldehyde. The concentration chosen for this study was based upon currently used concentration range of alkaline treatments. The dry matter contents were affected significantly by all the treatments and steeping time ($P<0.05$). The sorghum red showed the highest moisture intake than sorghum white (table 1). However the effect of steeping time was different in both the varieties. Sorghum white uptakes the moisture contents after 8 hours, while sorghum red attained the same moisture contents after 16 hours steeping These findings are in line with previous studies conducted by Owuama (1997).

The difference may be attributed to the difference in the endosperm texture in two varieties. As sorghum grains requires 33-35% moisture for germination, so the treatment can be helpful in reducing the germination time.

The proximate analysis (Table 2) showed little increase in the protein contents, which could be attributed to the fact that steeping could affect the total dry matter contents, hence increased protein content may be projected. These results are in line with previous studies carried out by (Nawal *et al.,* 2009 and Makokha *et al.,* 2002).

Table 1: Effect of NaOH and formaldehyde treatments within each incubation time on dry matter contents of sorghum grains.

Treatments		Sorghum red				Sorghum white		
		8h		16 h		8 h		16 h
Control (water)		91.7± 0.02		91.32± 0.008		91.31±0.005		91.83±0.011
Formaldehyde	0.05	91.06 ±0.29[a]	0.05	91.46 ± 0.38[b]	0.05	91.2 ±0.17[c]	0.05	91.18 ± 0.14[d]
	0.1	91.1 ±0.36[a]	0.1	91.43 ±0.33[b]	0.1	91.4 ± 0.05[b]	0.1	91.22±0.21[d]
	0.2	91.4 ± 0.14[a]	0.2	91.24 ±0.23[a]	0.2	91.32 ± 0.27[b]	0.2	91.31±0.21[b]
Sodium hydroxide	0.05	90.7 ±0.14[a]	0.05	91.50 ±0.11[a]	0.05	91.3 ± 0.06[b]	0.05	91.2 ± 0.12[b]
	0.1	91.6 ± 0.02[a]	0.1	91.60 ±0.10[a]	0.1	91.07 ±0.17[b]	0.1	91.15±0.09[b]
	0.2	91.3 ±0.40[a]	0.2	91.44 ±0.10[a]	0.2	91.3± 0.06[a]	0.2	91.17 ±0.30[b]

*The mean with different subscript differ significantly.

Table 2: Effect of NaOH and formaldehyde treatments within each incubation time on crude protein contents of sorghum grains.

Treatments		Sorghum red				Sorghum white		
		8h		16 h		8 h		16 h
Control (water)		9.03±0.011		9.2±0.08		8.80±0.008		8.80±0.008
Formaldehyde	0.05	9.01 ±0.008[a]	0.05	9.07±0.20[a]	0.05	8.50 ± 0.17[b]	0.05	8.94 ±0.03
	0.1	9.05 ±0.02[a]	0.1	8.76 ±0.04[b]	0.1	8.47 ± 0.08[c]	0.1	8.72± 0.06[b]
	0.2	9.03 ±0.02[a]	0.2	9.03±0.01[a]	0.2	8.46 ± 0.20[b]	0.2	8.85± 0.05[c]
Sodium hydroxide	0.05	9.06 ± 0.02[a]	0.05	9.23 ±0.08[b]	0.05	8.49 ± 0.14[c]	0.05	8.38 ± 0.05[c]
	0.1	9.16± 0.02[a]	0.1	9.13 ±0.11[a]	0.1	8.52±0.15[b]	0.1	8.82 ±0.04[c]
	0.2	9.07±0.02[a]	0.2	9.29 ± 0.03[b]	0.2	8.43 ±0.12[c]	0.2	8.92± 0.04[b]

*The mean with different subscript differ significantly.

The crude fiber contents showed same pattern as that of crude protein, however, with 0.2% NaOH the crude fiber showed a significant ($P<0.05$) decrease (Table-3). That could be due to the fact that alkali can

dissolute the fiber content (Nawal *et al.,* 2009). Howevr a non significant difference in Ash content (Table-4) was observed as reported by (Beta *et al.,* 2000).

The tannin contents of soaked sorghum grain were measured by Lowenthal's volumetric permanganate method AOAC (1970). All treatments resulted in the reduction of tannin significantly ($P<0.05$). The variety, treatment and the steeping time all effected the tannin content significantly ($P<0.001$). The tannin contents of raw sorghum white and red were 0.34± 0.05 and 0.48±0.03, respectively (table-5).

The reduction of tannin contents with water steeping were 0.22±0.01 and 0.30±0.05 in sorghum white and red respectively. This reduction mechanism is thought to be similar to that of seed maturation mechanism (Gupta *et al.,* 1978).

The action of NaOH on tannins may involve the phenolics groups oxidation under the influence of alkali. The alkaline conditions were thought to produce polymeric and nutritionally inactive compounds as already described by (Kennedy *et al.,* 1984).

It was found that there was a significant reduction ($P<0.05$) in two varieties of sorghum in relation to tannin contents post 0.2% NaOH treatment. These results were in line with previous study carried out by (Ochanda *et al.,*2010).

Table-3: Effect of NaOH and formaldehyde treatments within each incubation time on crude fiber contents of sorghum grains.

Treatments		Sorghum red				Sorghum white		
		8h		16 h		8 h		16 h
Control (water)		2.47±0.008		2.4±0.008		2.45±0.005		2.50±0.008
Formaldehyde	0.05	2.45 ±0.03[a]	0.05	2.47±0.005[a]	0.05	2.46 ±0.02[a]	0.05	2.45 ±0.03[a]
	0.1	2.48±0.01[a]	0.1	2.56 ±0.03[b]	0.1	2.44±0.02[a]	0.1	2.59±0.03[b]
	0.2	2.47±0.01[a]	0.2	2.26±0.03[c]	0.2	1.7±0.05[d]	0.2	2.58±0.05[b]
Sodium hydroxide	0.05	2.43±0.03[a]	0.05	2.51±0.06[b]	0.05	2.43 ±0.03[a]	0.05	2.45±0.03[a]
	0.1	2.48±0.02[a]	0.1	2.52±0.03[b]	0.1	2.51 ±0.02[b]	0.1	2.42±0.03[a]
	0.2	2.49 ±0.02[a]	0.2	2.54±0.02[b]	0.2	2.44 ±0.01[a]	0.2	2.47±0.008[a]

*The mean with different subscript differ significantly.

Table- 4: Effect of NaOH and formaldehyde treatments within each incubation time on ash contents of sorghum grains.

Treatments		Sorghum red				Sorghum white		
		8h		16 h		8 h		16 h
Control (water)		1.23±0.011		1.24±0.005		1.25±0.003		1.25±0.001
Formaldehyde	0.05	1.26 ±0.01[a]	0.05	1.28±0.01[a]	0.05	1.26±0.01[a]	0.05	1.24±0.00[a]
	0.1	1.25±0.02[a]	0.1	1.25 ±0.02[a]	0.1	1.25±0.02[a]	0.1	1.24±0.01[a]
	0.2	1.26 ±0.01[a]	0.2	1.30 ±0.01[b]	0.2	1.26±0.01[a]	0.2	1.25±0.02[a]
Sodium	0.05	1.26 ±0.02[a]	0.05	1.28±0.01[a]	0.05	1.29 ±0.01[a]	0.05	1.31 ±0.02[b]
hydroxide	0.1	1.24 ±0.01[a]	0.1	1.23 ±0.01[a]	0.1	1.28±0.01[a]	0.1	1.28±0.01[a]
	0.2	1.26 ±0.01[a]	0.2	1.25±0.02[a]	0.2	1.25 ±0.00[a]	0.2	1.28±0.02[a]

Table-5: Effect of NaOH and formaldehyde treatments within each incubation time on Tannin contents of sorghum grains.

Treatments		Sorghum red				Sorghum white		
		8h		16 h		8 h		16 h
Control (water)		0.40±0.02		0.30±0.005		0.26±0.008		0.22±0.011
		P=0.016		P=0.001		P=0.001		P=0.003
Formaldehyde	0.05	0.25±0.005[a]	0.05	0.24±0.005[a]	0.05	0.16±0.005[a]	0.05	0.17±0.005[a]
	0.1	0.22±0.005[a]	0.1	0.20±0.005[a]	0.1	0.13±0.005[b]	0.1	0.13±0.26[b]
	0.2	0.19±0.005[a]	0.2	0.16±0.005[b]	0.2	0.12±0.005[c]	0.2	0.09±0.005[d]
Sodium	0.05	0.25±0.005[a]	0.05	0.23±0.005[a]	0.05	0.15±0.005[b]	0.05	0.16±0.005[b]
hydroxide	0.1	0.23±0.005[a]	0.1	0.236±0.008[a]	0.1	0.11±0.008[b]	0.1	0.11±0.008[b]
	0.2	0.15±0.005[a]	0.2	0.14±0.005[a]	0.2	0.11±0.008[b]	0.2	0.05±0.005[c]

*The mean with different subscript differ significantly.

The results showed significant (P>0.05) difference when treated with NaOH, Formaldehyde or water (Table 6). And after NaOH and formaldehyde treatment a little increase in the ether content was observed and the results were similar to the study conducted by (Asma *et al.*, 2008).

Table 6: Effect of NaOH and formaldehyde treatments within each incubation time on ether extract contents of sorghum grains.

Treatments	Sorghum red				Sorghum white			
	8h		16 h		8 h		16 h	
Control (water)	2.81±0.008		2.86±0.005		2.76±0.005		2.83±0.004	
Formaldehyde	0.05	2.92 ±0.03^{a}	0.05	2.89±0.02^{b}	0.05	2.83±0.02^{b}	0.05	2.96±0.08^{a}
	0.1	2.83 ±0.02^{b}	0.1	2.87±0.02^{b}	0.1	2.83±0.03^{b}	0.1	2.70±0.05^{c}
	0.2	2.93 ±0.02^{a}	0.2	2.90±0.02^{a}	0.2	3.00 ±0.11^{d}	0.2	2.76±0.08^{c}
Sodium	0.05	3.06 ±0.46^{d}	0.05	2.92±0.03^{a}	0.05	3.03±0.13^{d}	0.05	2.83±0.02^{b}
hydroxide	0.1	2.72 ±0.21^{c}	0.1	2.82±0.01^{b}	0.1	2.93±0.02^{a}	0.1	2.92±0.03^{a}
	0.2	2.87 ±0.02^{b}	0.2	2.93±0.26^{a}	0.2	2.91±0.02^{a}	0.2	2.92 ±0.04^{a}

Conclusion: Steeping sorghum grain in water, NaOH and formaldehyde resulted in lower tannin contents. The effectiveness of NaOH was comparable to formaldehyde.

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