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Effect of the Concentration of Wood Ash Extract and Duration of Soaking on the Chemical Composition of High Tannin Sorghum (*Sorghum bicolor*) Cultivars

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Abstract: A study was conducted to investigate the effect of the concentration of wood ash extract and duration of soaking on the chemical composition of high tannin sorghum cultivars. Ash extract solutions were prepared by mixing hard wood ash and distilled water in plastic buckets at the rate of 1:20, 1:10 and 1:5 (w/v). Two red coloured sorghum cultivars (Kabusiba and Elodir) plus one brown sorghum variety (Sekedo), were soaked in the ash extract solutions at each concentration for 6, 12, 18, and 24 hours. The treated grains were analysed for tannin content, major minerals and amino acid composition. At higher concentrations of ash extract, the reduction in sorghum tannins was correspondingly higher. However, the relationship was not linear and the various sorghum cultivars did not exhibit a similar degree of response to ash extract treatment. Kabusiba sorghum was more responsive to ash treatment than Sekedo and Elodir. Prolonged soaking did not necessarily result into corresponding reductions in tannin content of the grain. The levels of potassium and sodium in the grain were slightly increased as a result of ash extract treatment. At the highest concentration of ash extract, treatment of the grain resulted in slight decreases of some amino acids.

Key words: ash extract, sorghum, tannins, minerals, amino acids

Introduction

One major characteristic that is of nutritional interest in the utilization of sorghum grain is its content of tannins. Several studies have demonstrated the ability of aqueous alkalis to reduce the assayable tannin content of sorghum grain (Armstrong *et al.*, 1974; Muindi *et al.*, 1981; Muindi and Thomke, 1981; Mohammed and Ali, 1988; Banda-Nyirenda, 1990; Mukuru *et al.*, 1992; Mukuru, 1992; Nyachoti *et al.*, 1998). The rate of tannin extraction and the effectiveness of the alkali treatment are influenced by a number of factors. Soaking sorghum in hot alkali solutions reduces the time required to remove the same amount of assayable tannins compared to a low temperature medium (Armstrong *et al.*, 1974; Monyo *et al.*, 1992). Muindi *et al.* (1981) found that high concentrations of sodium sesquicarbonate salt (Magadi soda) were more effective than low concentrations in reducing the tannin content of sorghum grain. Variations in varietal responses to alkali treatment have been shown by Muindi and Thomke (1981) and Mukuru (1992) for sorghum grain. Wood ash is an alkaline material that is used by local communities living in south-western Uganda in the post-harvest treatment of high tannin sorghum. This local technology involves soaking sorghum grain in wood ash slurry, then allowing the grain to germinate for a period of four days. The resulting material is almost tannin free but contains ash residues and hydrocyanic acid. The treatment also results in a 20% loss of dry matter (Mukuru *et al.*, 1992). This local technology might also affect the quality of protein in the grain because treatment of proteins with high concentrations of alkalis is reported to have negative effects on protein quality and availability (De Groot and Sump, 1969; Liardon and Hurrell, 1983; Serna-Saldivar *et al.*, 1987). Apart from tannin content, there is scanty information on the effect of wood ash treatment on the chemical composition of sorghum grain. The present study was therefore conducted to investigate the effect of the concentration of ash extract and duration of soaking on the chemical composition of high tannin sorghum cultivars.

Materials and Methods

The first set of laboratory experiments were conducted in the Animal Science laboratory at Makerere University, and the second set was carried out in the Department of Animal Science at the Agricultural University of Norway. The hard wood ashes used in these studies were collected from eight institutional kitchens in Kampala. Ash extract solutions were prepared by mixing ash and distilled water in plastic buckets at the rate of 1:20, 1:10 and 1:5 (w/v). The mixtures were left to stand for 15 hours to allow maximum diffusion to take place (Etiegni and Campbell, 1991). The supernatant was carefully removed and filtered to obtain the ash extract. Samples of the extract were taken for mineral analysis and measurement of pH. The rest of the extract was used as soaking medium for sorghum grain.

Samples (100 g each) of two red coloured sorghum cultivars (Kabusiba and Elodir) plus one brown sorghum variety (Sekedo) were soaked in the ash extract solutions at room temperature (25°C), at the rate of one gram of grain to 2 mL of ash extract. After periods of 6, 12, 18 and 24 hours the grain was removed from the soaking medium and dried in aluminium trays for 24 hours in a force-air oven at 50°C. Each treatment was replicated three times.

For the determination of minerals, 2 g of each sorghum type, replicated three times were first weighed into crucibles and dried in a force-air oven at 105°C for 12 hours then ashed in a furnace at 550°C overnight. The ash obtained was digested in 10 ml 1N HCl at 30°C for one hour. The resulting solution was centrifuged at 3000 rpm for 10 minutes to obtain a clear supernatant for the analysis of minerals. Cations were measured using a GBC Atomic Absorption Spectrophotometer (GBC Scientific Equipment PTY. LTD., Melbourne, Australia). The mineral contents of ash extract samples were also determined. Phosphorus was determined using an automatic spectrophotometer (F. Hoffmann-La

Roche and Co. Ltd, CH-4002 Basel, Switzerland),
The amino acid composition of the sorghum grain was analysed by a chromatographic method. Amino acid analysis was carried out with a Biochrom 20 Amino acid Analyzer (Pharmacia Biotec Scientific Software, Inc., USA), following the Official European Communities method for amino acid analysis (Official Journal of the European Communities, 1998). Tannins in the sorghum grain were determined using the Vanillin assay (Price *et al.*, 1978).

Results

Treated sorghum grains became darker in colour with increasing concentration of ash extract. At concentrations of 1:10 and 1:5 Kabusiba grains turned black but Sekedo and Elodir grains were not as dark. Fig. 1 shows the effect of the concentration of ash extract and soaking time on the tannin content of the 3 sorghum cultivars. Tannin content of the grain decreased with increasing concentration of ash extract, but the reduction in tannin content did not exhibit a linear relationship to concentration of ash extract. For the three cultivars, prolonged soaking did not result into similar responses. In some cases, there were slight increases in assayable tannins with prolonged soaking. However, much of the reduction in tannins occurred within the first six hours of soaking.
The content of the main mineral species and pH of the leachates extracted from wood ashes, at three different concentrations, is shown in Table 1. The pH of the ash extract solutions was over 11.50 for all the ashes. An increase in the concentration of the extract solutions resulted in a corresponding increase in pH but it did not rise beyond 12.70. On average, when the concentration of ash extract was doubled, there was an increase of 70-83% in the contents of potassium and sodium. At higher concentrations, variation in potassium and sodium contents of ash extracts was quite wide. Calcium and magnesium contents of the extracts were not much affected by changes in concentration. The levels of potassium and sodium in the sorghum grain were slightly increased as a result of ash extract treatment.

Table 1:Levels of main mineral species in extracts obtained from hard wood ashes

Extract (w/v)[1]	pH	mg/litre			
		Potassium	Sodium	Calcium	Magnesium
1:20	11.68±0.120	2955±957	127±65	68±33	1.51±0.156
1:10	12.08±0.181	5128±1474	223±122	66±27	1.56±0.422
1:5	12.38±0.304	9387±2838	380±223	73±4	1.42±0.170

[1]Ash: water (w/v)

Table 2: Effect of ash treatment on the amino acid composition of Sekedo and Kabusiba sorghum cultivars (expressed as g/16 gN)

	SEKEDO					KABUSIBA				
			Concentration of ash extract (ash:water)					Concentration of ash extract (ash:water)		
	UT	water	1:20	1:10	1:5	UT	water	1:20	1:10	1:5
Cysteine	1.60	1.62	1.68	1.66	1.41	1.56	1.42	1.45	1.44	1.48
Methionine	1.72	1.84	1.89	1.90	1.60	1.67	1.58	1.68	1.63	1.65
Aspartic acid	7.08	7.09	6.70	6.49	7.07	7.02	7.04	7.14	6.86	6.97
Threonine	3.04	3.02	3.14	2.98	3.02	3.12	3.04	3.12	2.97	3.14
Serine	4.51	4.55	4.85	4.51	4.75	4.60	4.72	4.76	4.61	4.80
Glutamic acid	21.15	20.87	21.98	22.40	22.71	21.57	21.69	22.45	21.36	21.44
Proline	7.58	8.41	8.07	8.24	7.49	7.75	7.39	7.68	7.22	7.39
Glycine	3.16	3.13	3.11	3.16	3.00	3.13	3.01	2.96	3.02	2.97
Alanine	9.28	9.27	9.19	9.23	9.12	9.23	9.37	8.81	9.56	9.40
Valine	5.20	5.32	5.25	4.97	5.06	5.15	5.09	5.05	5.37	5.07
Isoleucine	4.53	4.55	4.66	4.29	4.30	4.30	4.36	4.41	4.86	4.56
Leucine	13.83	13.99	13.09	13.50	13.77	13.67	13.93	13.84	14.12	14.20
Tyrosine	3.77	3.95	3.75	3.62	3.75	3.82	3.88	3.65	3.86	3.85
Phenylalanine	5.31	5.74	5.18	5.28	5.40	5.37	5.49	5.35	5.46	5.52
Histidine	1.97	1.91	1.86	1.95	1.81	1.97	1.87	1.81	1.84	1.80
Lysine	2.44	2.38	2.38	2.30	2.24	2.16	2.22	2.12	2.16	2.06
Arginine	3.81	3.59	3.52	3.73	3.51	3.91	3.90	3.72	3.66	3.70
Protein (% grain)	9.9	10.0	9.9	10.0	9.8	9.3	9.4	9.3	9.3	9.2

The effect of ash extract concentration on the amino acid profiles of Sekedo and Kabusiba is presented in Table 2. In some cases, especially at the highest concentration of ash extract, treatment of the grain resulted in slight decreases of the amino acids. For Sekedo there were increases in the sulphur-containing amino acids at lower concentrations of

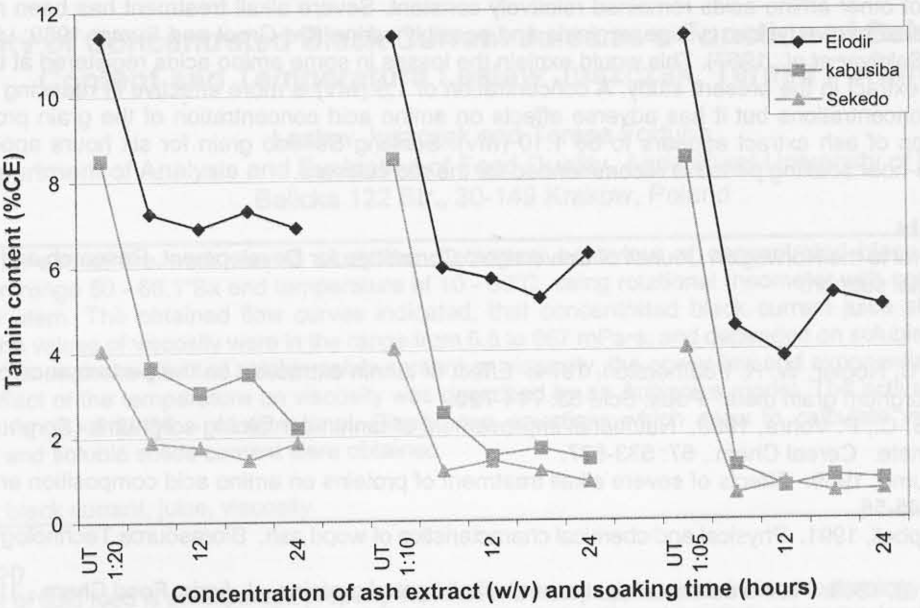

Fig. 1: Effect of concentration of ash extract and soaking time on the tannin content of three sorghum cultivars

ash extract but at the highest concentration, there were reductions of 12% and 6% for cysteine and methionine, respectively. For Kabusiba these amino acids followed a different trend. Cysteine registered a 5% reduction at the highest concentration of ash extract, while methionine suffered no losses. Reductions of up to 8% occurred in histidine, lysine and arginine for both cultivars, at the highest concentration of ash extract.

Discussion

Mukuru, (1992) similarly reported the darkening of sorghum grain following ash treatment. This phenomenon may be attributed to alkali treatment. There is likely to be corrosion of the testa when the grain is soaked in alkali solution, but also, phenolic acids may be oxidised to quinones followed by polymerisation to darker colour when exposed to high pH. The increasing reduction of assayable tannins at higher concentrations of ash extract is in agreement with the findings of other researchers (Muindi *et al.*, 1981 and Mukuru, 1992).

Muindi and Thomke (1981) and Mukuru (1992) similarly reported variations in varietal responses to alkali treatment. Mukuru (1992) found that while other varieties responded to low concentrations of ash-water mixtures, IS 8182 required the highest wood ash concentration treatment to reduce their tannin content to below 0.1% Catechin Equivalents (CE). Mukuru (1992) also reported a steady reduction in tannins after six hours of soaking after which prolonged soaking resulted in an increase in assayable tannins. The level of tannins in one of the varieties (IS 8182) appeared to increase after six hours of soaking. This is an interesting phenomenon, which suggests that different sorghum cultivars might require specific ash treatments. When sorghum grain is soaked, a considerable amount of the grain components leach into the soaking medium (Armstrong *et al.*, 1974). This causes chemical changes in the medium, including a reduction in pH. Numerous chemical reactions could take place, some of which are likely to be reversible. Tannins possibly leach into the soaking medium and when soaking is prolonged, some could migrate back into the grain due to changes in the concentration gradient.

Nolte *et al.* (1987) reported no significant differences in pH values at various concentrations of wood ash extract. When wood ash is mixed with water large amounts of potassium and sodium are rapidly leached out (Nolte *et al.*, 1987; Etiegni and Campbell, 1991). Etiegni and Campbell (1991) showed that the levels of these cations in solution increased linearly with concentration of the wood ash leachate. Calcium in wood ash occurs in both the very soluble forms, such as calcium oxide and calcium hydroxide, as well as the less soluble forms of calcite, calcium silicates and calcium-aluminium silicates (Steenari *et al.*, 1999). Filtering of the ash extract therefore could have removed the insoluble forms of calcium. This explains the relatively low levels of calcium ions in the extracts despite the fact that calcium was the most abundant mineral in the wood ashes. In an alkaline medium, phenols may dissociate to form phenoxide ions, which subsequently form salts with cations, especially potassium, sodium and calcium ions (Ribereau-Gayon, 1972). An increase in the potassium and sodium contents of the sorghum grain would imply that the salts thus formed diffuse into the grain.

The findings of the present study showed that treatment of the grain with ash extract was not harsh at low concentrations. Treatment of the grain with high concentration of ash extract resulted in slight increases in the propotions of serine and phenylalanine for both cultivars, and isoleucine and leucine for Kabusiba. When Muindi and Thomke (1981) treated sorghum grain with Magadi soda (sodium sesquicarbonate) at a concentration of 37.5 g/L, the alkali treatment resulted in slight increases of alanine, proline, serine, phenylalanine and the sulphur-containing amino acids. Arginine suffered losses. The content of other amino acids remained relatively constant. Severe alkali treatment has been reported to cause modifications that involve lysine, cystine, arginine and possibly serine (De Groot and Sump, 1969; Liardon and Hurrell, 1983; Serna-Saldivar *et al.*, 1987). This would explain the losses in some amino acids registered at the highest concentration of ash extract in the present study. A concentration of 1:5 (w/v) is more effective in reducing assayable tannins than lower concentrations but it has adverse effects on amino acid concentration of the grain protein. The optimum concentration of ash extract appears to be 1:10 (w/v). Soaking Sekedo grain for six hours appears to be sufficient but a twelve-hour soaking period is recommended for the red cultivars.

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