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Changes in chemical composition, grain malting, starch and tannin contents and protein digestibility during germination of sorghum cultivars

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Abstract

Malting loss and changes in chemical composition, starch and tannin contents and *in vitro* protein digestibility (IVPD) were determined during germination of low (0.32%) and high (1.44%) tannin sorghum cultivars. For both cultivars, crude protein, fibre, fat and ash contents were slightly decreased after soaking and germination of the seeds. Malting loss was slightly increased for both cultivars and for all germination periods. Starch degradation was high for both cultivars. Depending on the soaking time about 56–66% and 98–99% tannin were lost during 72 h germination in low and high tannin cultivars, respectively. IVPD was markedly decreased in the low tannin cultivar and markedly increased in the high tannin one with the germination time. Soaking, on the other hand, was found to have a minor effect on IVPD. It is suggested that tannins are responsible for retarding the protein digestibility and starch degradation, especially in the high tannin cultivar.

1. Introduction

Sorghum is a staple food in many African countries and it contains a reasonable amount of protein, ash, oil and fibre (Drich and Pran, 1987). One of the constraints on utilization of sorghum grain as food or feed is the occurrence, in some cultivars, of condensed tannin (Butler et al., 1983). In different parts of the world, vigorous efforts are directed towards coupling the beneficial effects of tannins in sorghum as a field crop with methods for overcoming the anti-nutritional effects of tannins in seeds by direct removal of seed testa, inactivation, or by extraction. Extractable tannin content was markedly reduced when grains were soaked in water and stored under a carbon dioxide atmosphere (Reichert et al., 1980). In feeding trials with rats (Reichert et al., 1980; Yasaman et al., 1990) and chicks (Teeter et al., 1986), tannins reduced weight gain and feed conversion. Chavan et al. (1979) reported that soaking of sorghum seeds at high temperature for different time intervals reduced tannin content and improved IVPD. They also reported that sodium carbonate-treated high tannin grains showed significantly lower tannin contents and significantly higher IVPD than untreated grains. Tannin content of low-tannin cultivars was slightly increased when seeds were germinated for different periods (Glennie, 1983). Although germination of sorghum seeds reduced the tannin content and improved IVPD, it significantly increased cyanide content which is potentially hazardous (Panasiuk and Bills, 1984). In areas where sorghum is a staple food, germinated grains are fermented and then traditionally processed. This traditional processing was found to reduce cyanide contents of germinated seeds (Ahmed et al., 1996). The study described here aims to investigate the changes in chemical composition, grain malting, tannin and starch contents and protein digestibility of sorghum cultivars.

2. Materials and methods

Seeds of two sorghum cultivars, Gadamelhamam and Cross 35:18, obtained from the Agricultural Research Station, Wad Medani, were carefully cleaned. Germinated seeds were dried at room temperature (25–30°C) for 2–3 days. For determination of protein digestibility, both treated and untreated seeds were ground to pass a 0.16 mm screen. For tannin analysis, seeds were ground to pass a 0.4 mm screen.

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2.1. Soaking and germination

Seeds were soaked in distilled water for different time intervals (10, 20 and 30 h) with water changing every 6 h. The wet seeds were soaked in 1–2 vols of 0.2% formaldehyde solution for 40 min to retard mold growth during germination. Thereafter, the seeds were washed with water several times to remove residual formaldehyde; then the treated seeds were placed on a double layer of filter paper in Petri dishes, and incubated at 30°C. The filter papers were moistened at regular intervals of 12 h. Germination was allowed to proceed for different time intervals (24, 48 and 72 h). The germinated seeds were then dried to a constant weight.

2.2. Fibre, ash, and fat determination

Fibre, ash, and fat were determined by the method of AOAC (1965).

2.3. Malting loss determination

Malting loss was indicated by the ratio of the weight difference before soaking and after drying of the germinated seeds to that of the original seed weight.

2.4. Starch determination

Starch was determined spectrophotometrically by the method of McCready et al. (1950). A standard curve was prepared expressing the results as mg ml^{-1} starch at 610 nm.

2.5. Protein and tannin determination

Protein (N×6.25) was determined by the method of AOAC (1965). Tannins were estimated by the modified procedure of Maxon and Rooney, as described by Price et al. (1978). A 200 mg sample was extracted with 10 ml 1% (v/v) conc. HCl in methanol for 20 min in capped rotating test tubes. Vanillin reagent (0.5%, 5 ml) was added to the extract (1 ml) and the absorbance of the colour developed after 20 min at 30°C was read at 500 nm. A standard curve was prepared expressing the results as catechin equivalents, i.e. amount of catechin (mg ml^{-1}) which gives a colour intensity equivalent to that given by tannins after correcting for blank.

2.6. In vitro *protein digestibility (IVPD)*

IVPD was determined by the method of Saunder et al. (1973). A sample (0.2 g) was placed in a 50 ml centrifuge tube, 15 ml of 0.1 M HCl containing 1.5 mg pepsin were added, and the tube was incubated at 37°C for 3 h. The suspension was then neutralized with 0.5 M NaOH and treated with pancreatin (4.0 mg) in 7.5 ml of 0.2 M phosphate bufffer, pH 8.0, containing 0.05% sodium azide; the mixture was then gently shaken and incubated at 37°C for 24 h. After incubation, the sample was treated with 10% trichloroacetic acid (10 ml) and centrifuged at 5000 *g* for 20 min at room temperature. Nitrogen in the supernatant was determined by Kjeldahl method (AOAC, 1965). Digestibility was calculated using the formula:

$$\text{Protein digestibility}\% = \frac{\text{N in supernatant} - \text{enzyme N}}{\text{N in sample}}$$

2.7. Statistical analysis

Samples were analyzed in triplicate and the figures were then averaged. Data was assessed by analysis of variance (ANOVA) (Snedecor and Cochran, 1987) and by Duncan's Multiple-range test with a probability $p \leq 0.05$ (Duncan, 1955).

3. Results and discussion

3.1. Compositional changes during soaking and germination

Fig. 1 shows changes in protein and fibre contents during soaking and germination of sorghum seeds for different time intervals. For both cultivars, when the seeds were soaked for 10 h, and germinated up to 72 h, no significant change in protein content was observed (Fig. 1(a)) and when they were soaked for 30 h and germinated for 72 h, protein content changed from 11.5 to 8.69% and from 11.0 to 10.1% for Gadamelhamam and Cross 35:18 cultivars, respectively. The slight change in protein content may attributed to the fact that water-soluble nitrogen was lost during soaking of seeds and also, during seed germination, part of the protein was utilized for the growth and development of the embryo (Wu and Wall, 1980). Fibre content (Fig. 1(b)) decreased significantly ($p \leq 0.05$) during 72 h germination in low tannin cultivar. Soaking of seeds for 30 h, followed by germination (72 h), was found to reduce fibre content from 3.26 to 1% for the Gadamelhamam cultivar, while that of the Cross 35:18 was slightly affected by soaking and germination. Changes in fibre content may attributed to the fact that part of the seed fibre may be solubilized enzymatically during seed germination. Fig. 2 shows changes in ash and fat contents during soaking and germination of sorghum seeds for different time intervals.

For both cultivars, and for different soaking periods and germination intervals, ash content (Fig. 2(a)) was significantly decreased only when seeds were germinated for 24 h and thereafter remained constant. Generally it

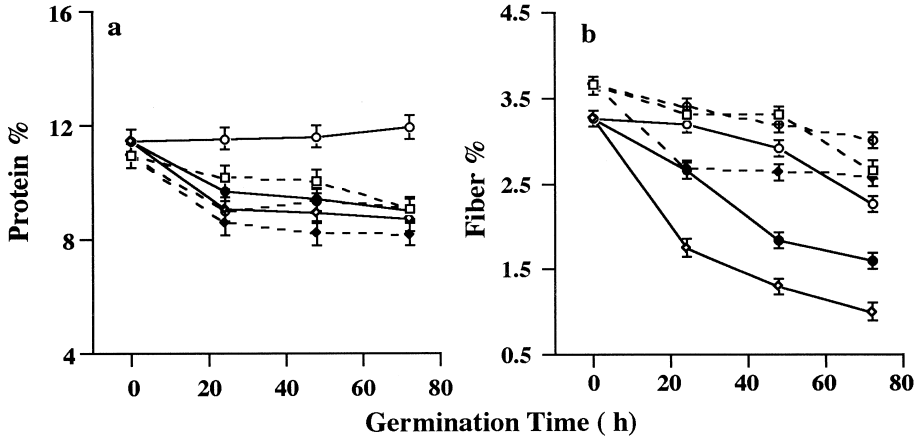

Fig. 1. Changes in (**a**) crude protein and (**b**) fibre contents during soaking and germination of Gadamelhamam (—) and Cross 35:18 (- - - - -) cultivars. Symbols: ○; ◆ 10 h soaking, ●; ⊖ 20 h soaking, ◇; □ 30 h soaking. Values are means (± SD), $n = 3$.

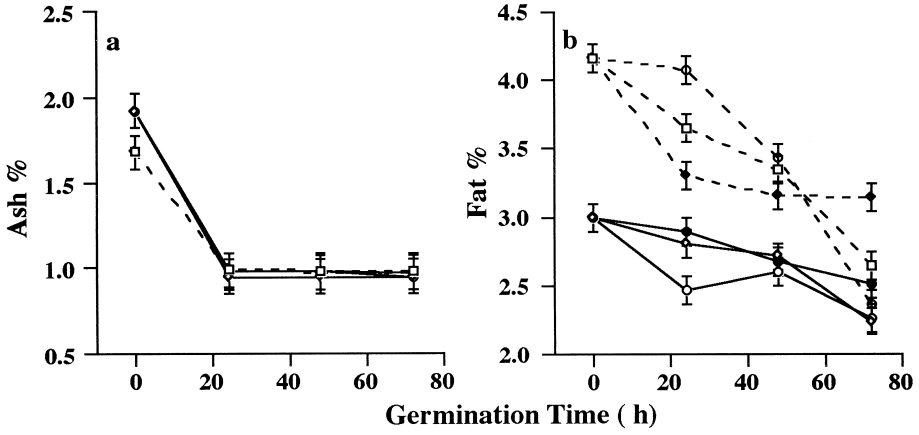

Fig. 2. Changes in (**a**) ash and (**b**) fat contents during soaking and germination of Gadamelhamam (—) and Cross 35:18 (- - - - -) cultivars. Symbols: ○; ◆ 10 h soaking, ●; ⊖ 20 h soaking, ◇; □ 30 h soaking. Values are means (± SD), $n = 3$.

decreased from 1.92 to 0.95% and from 1.68 to 0.97% for Gadamelhamam and Cross 35:18 cultivars, respectively. Results indicated that germinating grains initially require nutrients (mainly minerals) for sprouting. Fat content (Fig. 2(b)) was slightly decreased from 2.99 to 2.51% for Gadamelhamam cultivar during all soaking and germinating periods. However, for Cross 35:18 cultivar it was significantly decreased from 4.16 to 2.64 when the seeds were soaked for 30 h and germinated 72 h. The reduction in fat content may be due to the fact that biochemical and physiological changes occurred during germination; such changes require an energy to proceed, and therefore part of the seed fat was utilized for the production of this energy.

3.2. Malting loss during soaking and germination of seeds

For both cultivars, soaking of seeds in water for different time intervals followed by germination (Table 1) was found to increase malting loss of the grains with the germination time and it was found to be in the range of 11.45–15.55, 11.92–17.67 and 18.03–18.55% when the seeds were soaked for 10, 20, and 30 h, respectively, for the Gadamelhamam cultivar. That of the cultivar Cross 35:18 was found to be in the range of 14.04–14.75, 15.94–17.28 and 16.51–17.48% for the soaking periods, respectively. Results revealed that malting loss was directly proportional to the number of days allowed for soaking and germination of seeds (Pathirana and Jayatissa, 1983). The loss due to germination can be attributed to respiratory activity of the grains (Chavan et al., 1981), while soaking for a long time led to a faster rate of germination (Pathirana and Jayatissa, 1983).

3.3. Changes in tannin and starch contents and protein digestibility

Table 2 shows the effect of soaking in water and germination for different time intervals on tannin content. For both cultivars, soaking of seeds for different time intervals was found to cause slight changes in tannin content. The tannin content decreased significantly ($p \leq 0.05$) during 72 h germination in the low-tannin

Table 1
Malting loss during soaking and germination of seeds of sorghum cultivars

Cultivar	Germination time (h)	Soaking time (h)		
		10	20	30
		Malting loss %		
Gadamelhamam	24	11.45 (±0.10)[a]	11.92 (±0.02)[a]	18.03 (±0.03)[a]
	48	14.45 (±0.04)[b]	16.54 (±0.04)[b]	18.25 (±0.02)[a]
	72	15.55 (±0.03)[b]	17.67 (±0.08)[b]	18.55 (±0.05)[a]
Cross 35:18	24	14.04 (±0.05)[a]	15.94 (±0.04)[a]	16.51 (±0.02)[a]
	48	14.11 (±0.08)[a]	16.33 (±0.02)[a]	17.34 (±0.05)[a]
	72	14.75 (±0.04)[a]	17.28 (±0.02)[a]	17.48 (±0.07)[a]

Values are means ± SD.
Values not sharing a common superscript letter in a column are significantly different at $p \leq 0.05$ as assessed by Duncan's Multiple-Range Test.

cultivar (Gadamelhamam). Soaking of seeds for 30 h, followed by germination, was found to be effective in reducing tannin content (Table 2). At 24 h germination, 38% of the total tannins disappeared and the losses increased up to 66% at 72 h germination in the low-tannin cultivar. In the high-tannin cultivar, soaking in water alone was found to have a minor effect on tannin content. However, when it was accompanied by germination, tannin was significantly ($p \leq 0.05$) decreased with the germination time. Soaking of seeds for 10 h, followed by germination, significantly ($p \leq 0.05$) reduced tannin content from 1.44 to 0.03%. At 24 h germination, 74% of the total tannins disappeared and the losses significantly increased up to 98% at 72 h. Similar trends were observed when the seeds were soaked for 20 or 30 h (Table 2). Tannins are located in the seed coat (Jambunathan and Mertz, 1973). The loss of tannins, therefore, can be attributed to leaching of tannins into the growth medium as indicated by the significant browning of supporting filter paper during germination. It is also likely that part of the tannins may enter into the endosperm with imbibed water during germination. Table 3 shows the effect of soaking in water, and germination for different time intervals, on the starch content. For both cultivars, soaking of seeds in water was found to have no significant effect on starch content. However, when it was accompanied by germination, starch content was reduced from 68 to 33.5% in the low-tannin cultivar, whereas in the high-tannin cultivar, the starch was reduced from 75 to 44% depending on the germination time. Table 4 shows the effect of soaking in water and germination for different time intervals on *in vitro* protein digestibility (IVPD). For both cultivars, soaking of seeds in water, especially when the seeds were soaked for a long time (30 h), caused significant decrease in IVPD and when accompanied by germination, IVPD was significantly decreased with germination time from 91.05 to 70.62% in the low-tannin cultivar, whereas, in the high-tannin cultivar, it significantly increased from 80.75 to 95.02, 89.02 and 85.01% when the seeds were soaked for 10, 20 and 30 h and germinated for 72 h. The results indicate that the starch and protein degradations are apparently lowered in high-tannin seeds during germination (Chavan et al., 1981) as shown in Tables 3 and 4. During germination, the tannins located in the seed coat are

Table 2
Changes in tannin content during soaking in water and germination of seeds of sorghum cultivars

Cultivar	Germination time (h)	Soaking time (h)					
		10		20		30	
		Tannin					
		Total %	Loss %	Total %	Loss %	Total %	Loss %
Gadamelhamam	0	0.32 (±0.03)[a]	—	0.32 (±0.03)[a]	—	0.32 (±0.03)[a]	—
	24	0.28 (±0.04)[ab]	13	0.25 (±0.01)[ab]	22	0.20 (±0.01)[b]	38
	48	0.20 (±0.01)[bc]	38	0.17 (±0.02)[bc]	47	0.15 (±0.02[b]	53
	72	0.14 (±0.02)[c]	56	0.13 (±0.07)[c]	59	0.11 (±0.01)[b]	66
Cross 35:18	0	1.44 (±0.17)[a]	—	1.44 (±0.17)[a]	—	1.44 (±0.17)[a]	—
	24	0.38 (±0.02)[b]	74	0.35 (±0.01)[b]	76	0.34 (±0.01)[b]	76
	48	0.31 (±0.03)[b]	79	0.23 (±0.01)[c]	84	0.21 (±0.06)[c]	85
	72	0.03 (±0.01)[c]	98	0.02 (±0.00)[d]	99	0.02 (±0.00)[d]	99

Table 3
Changes in starch content during soaking in water and germination of seeds of sorghum cultivars

Cultivar	Germination time (h)	Soaking time (h)		
		10	20	30
		Starch %		
Gadamelhamam	0	68.00 (±0.10)[a]	68.00 (±0.10)[a]	68.00 (±0.10)[a]
	24	64.00 (±0.14)[b]	54.00 (±0.14)[b]	52.50 (±0.12)[b]
	48	55.00 (±0.13)[c]	48.16 (±0.28)[c]	44.00 (±0.15)[c]
	72	48.50 (±0.16)[d]	36.00 (±0.31)[d]	33.50 (±0.21)[d]
Cross 35:18	0	75.00 (±0.22)[a]	75.00 (±0.02)[a]	75.00 (±0.22)[a]
	24	70.00 (±0.11)[b]	67.30 (±0.29)[b]	59.00 (±0.15)[b]
	48	64.00 (±0.17)[c]	55.00 (±0.31)[c]	55.00 (±0.17)[c]
	72	58.50 (±0.29)[d]	55.00 (±0.23)[d]	44.00 (±0.18)[d]

Table 4
Changes in *in vitro* prtein digestibility (IVPD) during soaking in water and germination of seeds of sorghum cultivars

Cultivar	Germination time (h)	Soaking time (h)		
		10	20	30
		IVPD %		
Gadamelhamam	0	91.05 (±0.22)[a]	91.05 (±0.22)[a]	91.05 (±0.22)[a]
	24	92.66 (±0.27)[a]	84.37 (±0.32)[b]	73.58 (±0.22)[b]
	48	86.00 (±0.14)[b]	83.13 (±0.25)[b]	70.81 (±0.15)[c]
	72	83.48 (±0.46)[c]	72.39 (±0.09)[c]	70.62 (±0.21)[c]
Cross 35:18	0	80.75 (±0.12)[a]	80.75 (±0.12)[a]	80.75 (±0.12)[a]
	24	90.31 (±0.11)[b]	90.08 (±0.10)[b]	80.91 (±0.05)[ab]
	48	91.14 (±0.11)[b]	89.01 (±0.31)[b]	82.18 (±0.27)[b]
	72	95.02 (±0.21)[c]	89.02 (±0.21)[c]	85.01 (±0.12)[c]

Values are means ± SD.
Values not sharing a common superscript letter in a column are significantly different at $p \leq 0.05$ as assessed by Duncan's Multiple-Range Test.

leached out. Part of the tannins may enter into the endosperm along with the imbibed water. Such tannins are likely to form complexes with reserve seed protein and enzymes and to inactivate them (Price et al., 1978). During germination, the reserves of nutrients like starch and protein are degraded to soluble sugars and amino acids, respectively, to meet the seedling requirements (Dalvi, 1974). Depression of starch and protein degradations indicate the interference with metabolic systems operating on reserve starch and protein, mainly enzymes such as amylases and proteases (Dalvi, 1974). Tannins are reported to form complexes with hydrolytic enzymes and to inactivate them (Milic et al., 1972). In the low-tannin cultivar, both tannin content and IVPD were significantly decreased (Tables 2 and 4). This observation is a departure from an otherwise good correlation between tannin content and IVPD, whereas in the high-tannin one, tannin content significantly decreased and IVPD was significantly increased. The explanation for this observation is not clear, but may lie in chemical (as well as quantitative) differences between the tannins of the two cultivars.

In conclusion, soaking of sorghum seeds in water and germination was found to be effective in reducing tannin content of high-tannin cultivars and caused an appreciable improvement in IVPD. Also it was found to reduce fibre, fat and starch contents and it slightly increased malting loss. Soaking of sorghum seeds in water and germination is a traditional process for preparation of beverages and food. Fortunately these processes are followed by fermentation which was found to reduce cyanide content, because germination alone caused an increase in cyanide content which is reported to be potentially hazardous (Panasiuk and Bills, 1984).

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