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Effect of alkali on tannin content and *in-vitro* protein digestibility of sorghum cultivars

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Two sorghum cultivars obtained from different locations in Sudan—Mugud, obtained from Gadarif local market and Karamaka, obtained from Kadogli Research Station—were used for this study. Investigation showed that tannin contents of untreated seeds were 3·10% and 0·60% for Karamaka and Mugud cultivars, respectively. In-vitro protein digestibilities were 89·10% and 90·41% for the two cultivars, respectively. Extractable tannin content of sorghum cultivars was markedly reduced by imbibing NaOH or KOH solutions into whole seeds and by incubating them at 30°C for 1, 3, 6, 12 or 24 h or at 100°C for 5, 10 or 20 min. The extent of reduction depended on time of incubation, temperature and alkali concentration. Per cent tannin extracted and per cent in vitro protein digestibility increased with increase in time or alkali concentration, while application of high temperature reduced the time of incubation required.

INTRODUCTION

In different parts of the world, vigorous efforts are directed towards coupling the beneficial effects of tannins in sorghum and faba bean as field crops, with methods for overcoming the anti-nutritional quality of tannins in seeds. Tannins could be reduced, by direct removal of seed testa and pericarp (Chibber *et al.*, 1978) or by inactivation of the seed tannin or by extraction (Armstrong *et al.*, 1974). Extractable tannin content of sorghum grains was markedly reduced by imbibing alkaline solutions into the whole seeds. The reduction of tannin was accompanied by a significant improvement in *in-vitro* protein digestibility (Tamir & Alumot, 1969; Chavan *et al.*, 1979). In feeding trials with rats (Marquardt *et al.*, 1976; Reichert *et al.*, 1980) and chicks (Armstrong *et al.*, 1974) extraction of tannins produced weight gains and feed conversion. Further, Price *et al.* (1979) reported that most alkaline conditions in general are responsible for improved nutritional quality and reduced level of chemically assayable tannin in high tannin grains, but not to levels found in low tannin grain sorghum.

This study was conducted to determine whether the nutritional value of high tannin sorghum cultivars could be improved by imbibing NaOH or KOH solutions of different concentrations, at different temperatures and for different times.

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MATERIALS AND METHODS

Seeds of two sorghum cultivars, Mugud, obtained from local market and Karamaka, obtained from Kadogli Research Station, were used in this study. All the samples were carefully cleaned and freed from dirt, stones, chips, and other extraneous grains or grits. For tannin analysis and moisture determination, cultivars were ground to pass a 0·4 mm screen. For protein digestibility both treated and untreated seeds were ground to pass a 0·16 mm screen.

Moisture, protein and tannin analysis

To express results on a 105°C dry matter basis, moisture was determined according to the AOAC (1965). 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% conc. HCl in methanol for 10 min in capped rotating test tubes. Five millimetres of vanillin reagent (0·5%) was added to 1 ml aliquots and the absorbance of the colour developed after 20 min (30°C) was read at 500 nm. A standard curve was prepared using catechin equivalents (CE) after correcting for blank.

Determination of tannin extracted from treated seeds

Four grams of sorghum seeds were soaked in 100 ml NaOH or KOH solutions of different concentrations at either 30°C for 1, 3, 6, 12 or 24 h or at 100°C for 5, 10 or 20 min. Treated seeds were washed thoroughly with distilled water several times. All washings were collected and the volume was made to 150 ml. Tannin extracted was determined according to the modified vanillin-HCl method as described by Price *et al.* (1978).

In-vitro protein digestibility (IVPD)

IVPD was determined according to the Saunder *et al.* (1973) method. A sample (0·2 g) was placed into a 50 ml centrifuge tube, 15 ml of 0·1M HCl containing 1·5 mg pepsin was added, and the tube was incubated at 37°C for 3 h. The suspension was then neutralized with 0·5 M NaOH (*c.* 3·3 ml), then treated with 4 mg of pancreatin in 7·5 ml of 0·2 M phosphate buffer (pH = 8·0) containing 0·005 M sodium azide and incubated at 37°C for 24 h. After incubation, the sample was treated with 10 ml 10% trichloroacetic acid and centrifuged at 5000*g* for 20 min at room temperature. Nitrogen in the supernatant was estimated using the Kjedahl method. Digestibility was calculated using the formula:

$$\text{Protein digestibility\%} = \frac{\text{N in supernatant} - \text{enzyme N}}{\text{N in sample}} \times 100$$

Statistical analysis

Each sample was analyzed in triplicate and the figures were then averaged. Data was assessed by analysis of variance (ANOVA) (Snedecor & Cochran, 1987) and by the Duncan multiple range test with a probability $P \leq 0{\cdot}05$ (Duncan, 1955).

RESULTS

Per cent tannin extracted in relation to time, temperature and alkali concentration.

Per cent tannin extracted in relation to time, temperature and alkali concentration is shown in Figs 1–4 for the two sorghum cultivars. For Mugud cultivar, soaking in 0·05 M NaOH at 30°C for 1 h was found to extract 45% of the tannin. Soaking for 24 h was found to extract 84·4%. Under similar conditions, 0·05 M KOH was found to extract 39·8% after soaking for 1 h and 81·2% after soaking for 24 h. When NaOH or KOH concentration was lowered to 0·005 M, NaOH was found to extract 16·3% after 1 h and 35·3% after 24 h, while KOH extracted 12·2% after 1 h and 42·8% after 24 h (Fig. 1). When soaking was carried out at 100°C (Fig. 2), 0·05 M NaOH extracted 48·5% after 5 min and

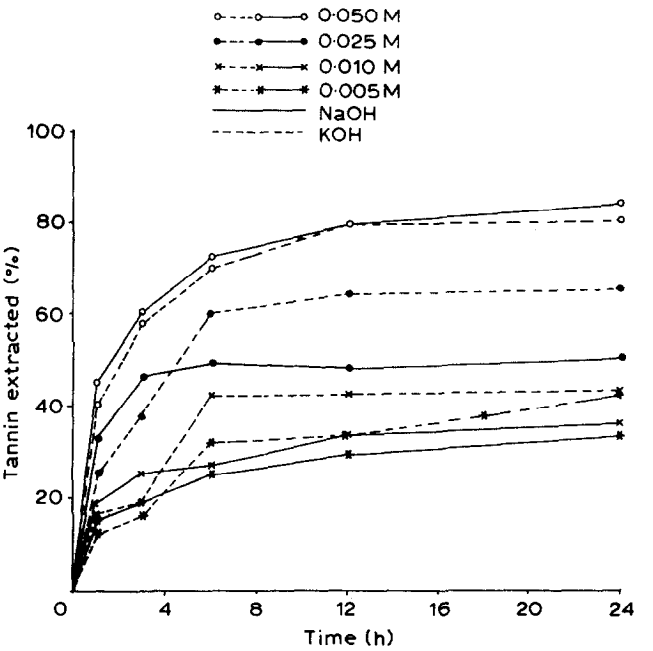

Fig. 1. % Tannin extracted at 30°C in relation to alkali concentration and extraction time for sorghum cultivar Mugud.

84·2% after 20 min, while 0·05 M KOH was found to extract 36·1% after 5 min and 86·2% after 20 min. When 0·005 M NaOH or KOH was used, NaOH extracted 16·8% after 5 min and 40·3% after 20 min, while KOH extracted 16·8% after 5 min and 40·4% after 20 min. For Karmaka cultivar (Figs. 3 and 4) soaking in 0·05 M NaOH at 30°C for 1 h was found to extract 46·8% and 83·9% when soaking was carried out for 24 h, while 0·05 M KOH under similar conditions was found to extract 42% after 1 h and 82% after 24 h (Fig. 3). When soaking was carried out at a lower con-

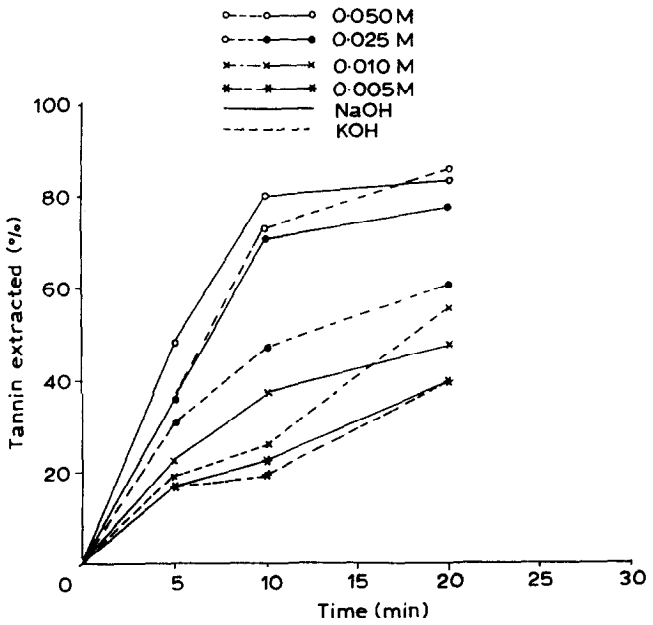

Fig. 2. % Tannin extracted at 100°C in relation to concentration and extraction time for sorghum cultivar Mugud.

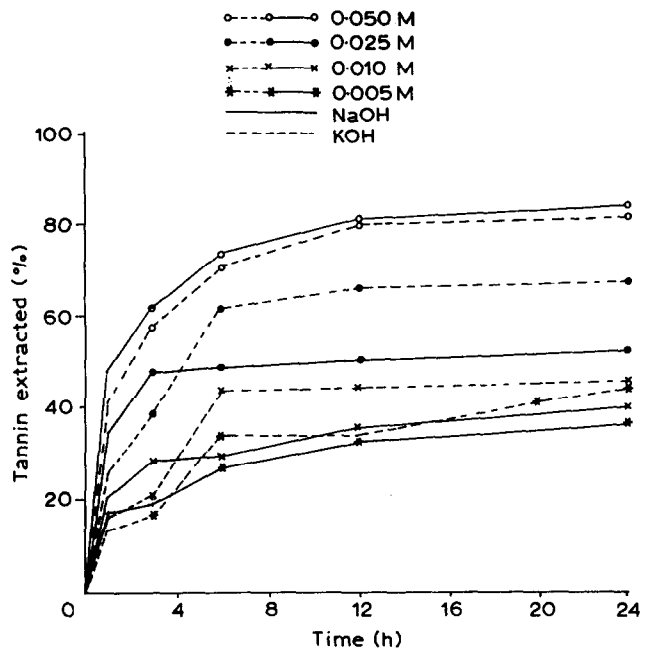

Fig. 3. % Tannin extracted at 30°C in relation to alkali concentration and extraction time for sorghum cultivar Karamaka.

centration (0·005 M; Fig. 3), NaOH extracted 16·6% after 1 h and 35·8% after 24 h, while KOH was found to extract 12·7% after 1 h and 43·8% after 24 h. Soaking at 100°C (Fig. 4) with 0·05 M NaOH extracted 49·7% after 5 min and 85·7% after 20 min, while KOH extracted 36·5% after 5 min and 87·1% after 20 min. Soaking at a lower concentration (0·005 M) NaOH extracted 17·2% after 5 min and 40·7% after 20 min, while KOH extracted 17·2% after 5 min and 41·1% after 20 min.

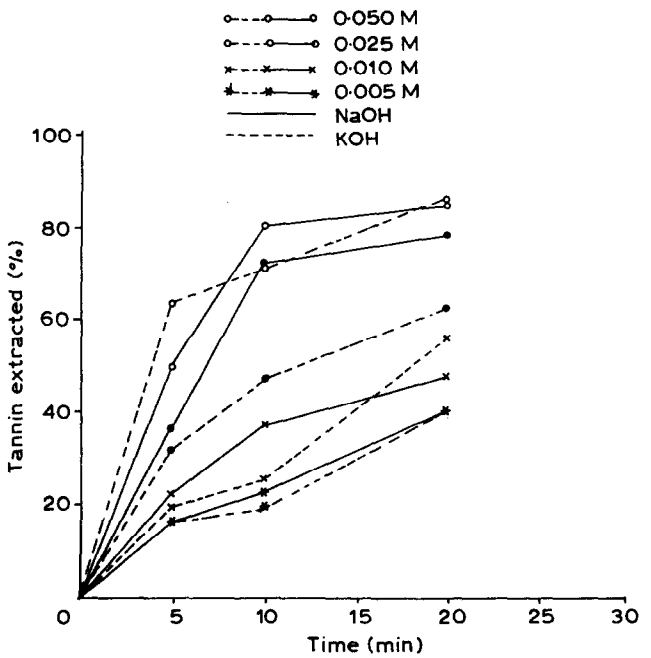

Fig. 4. % Tannin extracted at 100°C in relation to alkali concentration and extraction time for sorghum cultivar Karamaka.

Effect of alkali on tannin content and *in-vitro* protein digestibility

Per cent tannin retained and *in-vitro* protein digestibility at different soaking temperature, time and alkali concentration are shown in Tables 1–4. For Mugud cultivar, tannin content and *in-vitro* protein digestibility of untreated seeds were 0·60% and 90·41%, respectively (Table 1). Soaking in 0·05 M NaOH at 30°C significantly reduced tannin content to 0·33% and improved IVPD (97·48%) after 1 h. Soaking for 24 h resulted in a reduction in tannin content and improvement in IVPD significantly ($P \leq 0.05$) (0·09 and 98·48%, respectively; Table 1). Soaking in 0·05 M NaOH at higher temperature (100°C; Table 1) reduced tannin content to 0·31% after 5 min and 0·09 after 20 min, causing a significant ($P \leq 0.05$) improvement in IVPD: 97·56% after 5 min and 98·70% after 20 min.

NaOH, 0·005 M (Table 2), at 30°C was found to cause a significant reduction in tannin content when soaking was carried out for a relatively long period of time. IVPD was significantly ($P \leq 0.05$) improved and was independent of soaking time; soaking at 100°C gave similar results. For Karamaka cultivar, tannin content and IVPD of untreated seeds were 3·10% and 89·10%, respectively (Table 1). NaOH, 0·05 M (Table 1), at 30°C significantly ($P \leq 0.05$) reduced tannin content to 1·65% and improved IVPD to 96·58% after 1 h soaking. Soaking for 24 h resulted in reduced tannin content (0·50%) and improved IVPD (98·47%). Soaking at 100°C was found to cause a significant ($P \leq 0.05$) reduction in tannin content and improvement in IVPD for a shorter period of time (Table 1). NaOH, 0·005 M (Table 2), at 30°C was found to cause a significant reduction in tannin content and improvement in IVPD when soaking was carried out for a longer period. Soaking at 100°C gave similar results, but in a shorter period (Table 2). KOH, 0·05 M (Table 3), and KOH, 0·005 M (Table 4) were found to give results similar to those obtained by NaOH for the two sorghum cultivars.

DISCUSSION

Per cent tannin content in relation to time, temperature and alkali concentration

Results revealed that extractable tannin content in sorghum cultivars can be reduced by imbibing alkaline solutions into whole seeds; the rate of reduction was found to depend on time, temperature and alkali concentration. For a given time and temperature, it was found that, as alkali concentration was increased, per cent tannin extracted was also increased up to 80%. This indicated that higher concentrations enhanced the rate of reaction between alkali and polyphenols to form

Table 1. Effect of 0·05 M NaOH on tannin content and *in-vitro* protein digestibility of sorghum cultivars

Extraction time	Mugud				Karamaka			
	% Tannin after treatment		IVPD		% Tannin after treatment		IVPD	
Hours at 30°C								
0	0·60	(±0·15)	90·41	(±1·88)	3·10	(±0·32)	89·10	(±2·38)
1	$0·33^{a}$	(±0·13)	$97·48^{abcd}$	(±1·25)	1·65	(±0·31)	$96·58^{abcd}$	(±2·29)
3	$0·24^{ab}$	(±0·20)	$97·53^{abcd}$	(±1·47)	1·22	(±0·25)	$96·67^{abcd}$	(±2.46)
6	$0·17^{bc}$	(±0·05)	$97·65^{abc}$	(±1·52)	0·83	(±0·10)	$96·99^{abc}$	(±1·79)
12	$0·12^{cd}$	(±0·04)	$97·81^{ab}$	(±1·78)	$0·59^{a}$	(±0·21)	$97·06^{ab}$	(±1·58)
24	$0·09^{cd}$	(±0·06)	$98·48^{a}$	(±0·60)	$0·50^{a}$	(±0·14)	$98·47^{a}$	(±0·74)
Minutes at 100°C								
0	0·60	(±0·15)	90·41	(±1·88)	3·10	(±0·32)	89·10	(±2·38)
5	$0·31^{a}$	(±0·06)	$97·56^{ab}$	(±1·16)	1·56	(±0·21)	$96·17^{ab}$	(±2·07)
10	$0·12^{ab}$	(±0·07)	$97·82^{ab}$	(±1·82)	0·59	(±0·18)	$97·00^{ab}$	(±1·59)
20	$0·095^{b}$	(±0·01)	98.70^{a}	(±0·29)	0·44	(±0·21)	$98·43^{a}$	(±0·89)

Values are means (± SD).
Means 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.
IVPD, *in-vitro* protein digestibility.

soluble complexes. At a given temperature and alkali concentration, increase in time caused an increase in per cent tannin extracted, up to 80%, while high temperature was found to reduce the time needed. Several workers substantiate the present study; Price *et al.* (1979) reported that, when high tannin grains of sorghum were treated with different inexpensive chemicals to remove tannins, alkaline treatment caused 90% or greater reduction in assayable tannins. Further, Tamir *et al.* (1969) reported that, when high tannin seeds were extracted in alkaline solutions, the inhibitory effects of tannins were markedly reduced. The reduction in assayable tannin may be due to the fact that polyphenols react with alkali to form the corresponding phenates which have different properties compared to polyphenols.

Effect of alkali on tannin content and IVPD

Results revealed that tannin content of treated seeds was markedly decreased with increase in time or alkali concentration or both. The low tannin cultivar showed a higher IVPD value than the high tannin cultivar. In sorghum, most of the tannins are present in the pericarp layer; the alkali-treated seeds exhibited significant ($P \leq 0·05$) increase in IVPD as compared to untreated seeds (Chavan *et al.*, 1979). Alkaline treatment improved the nutritional quality of high-tannin sorghum.

Table 2. Effect of 0·005 M NaOH on tannin content and *in-vitro* protein digestibility of sorghum cultivars

Extraction time	Mugud				Karamaka			
	% Tannin after treatment		IVPD		% Tannin after treatment		IVPD	
Hours at 30°C								
0	$0·60^{a}$	(±0·15)	90·41	(±1·88)	3·10	(±0·32)	$89·10^{d}$	(±2·33)
1	$0·50^{ab}$	(±0·01)	$93·00^{bc}$	(±0·33)	$2·58^{a}$	(±0·21)	$90·51^{bcd}$	(±0·59)
3	$0·49^{abc}$	(±0·12)	$93·58^{bc}$	(±1·11)	$2·50^{ab}$	(±0·19)	$90·66^{bc}$	(±0·47)
6	$0·45^{bcd}$	(±0·21)	$93·67^{b}$	(±0·10)	$2·30^{abc}$	(±0·20)	$90·74^{b}$	(±0·73)
12	$0·42^{bcde}$	(±0·13)	$96·71^{a}$	(±0·59)	$2·12^{cd}$	(±0·17)	$96·05^{a}$	(±0·46)
24	$0·39^{bcde}$	(±0·10)	$97·13^{a}$	(±0·11)	$1·99^{d}$	(±0·24)	$96·25^{a}$	(±0·56)
Minutes at 100°C								
0	$0·60^{a}$	(±0·15)	90·4	(±1·88)	3·10	(±0·32)	$89·10^{b}$	(±2·33)
5	$0·50^{ab}$	(±0·10)	$92·99^{a}$	(±0·77)	$2·57^{a}$	(±0·21)	$90·52^{ab}$	(±0·63)
10	$0·47^{bc}$	(±0·08)	$93·67^{a}$	(±0·16)	$2·38^{a}$	(±0·23)	$90·72^{a}$	(±0·64)
20	$0·36^{bc}$	(±0·10)	97·46	(±0·06)	1·84	(±0·19)	96·37	(±0·78)

Values are means (± SD).
Means 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.
IVPD, *in-vitro* protein digestibility.

Table 3. Effect of 0·05 M KOH on tannin content and *in-vitro* protein digestibility of sorghum cultivars

Extraction time	Mugud				Karamaka			
	% Tannin after treatment		IVPD		% Tannin after treatment		IVPD	
Hours at 30°C								
0	0·60	(±0·15)	90·41	(±1·88)	3·10	(±0·32)	89·10	(±2·38)
1	0·36^{a}	(±0·05)	97·48^{abcd}	(±0·22)	1·83	(±0·19)	96·37^{abcd}	(±0·66)
3	0·26^{ab}	(±0·10)	97·69^{abcd}	(±1·45)	1·33	(±0·21)	96·47^{abcd}	(±2·56)
6	0·18^{bc}	(±0·01)	97·78^{abc}	(±1·53)	0·92	(±0·22)	96·65^{abc}	(±1·85)
12	0·12^{cd}	(±0·08)	97·82^{ab}	(±1·89)	0·58^{a}	(±0·24)	97·07^{ab}	(±1·59)
24	0·11^{cd}	(±0·09)	97·98^{a}	(±1·79)	0·56^{a}	(±0·25)	97·54^{a}	(±1·25)
Minutes at 100°C								
0	0·60	(±0·15)	90·41	(±1·88)	3·10	(±0·32)	89·10	(±2·38)
5	0·38	(±0·12)	97·13^{ab}	(±0·04)	1·97	(±0·26)	96·26^{ab}	(±0·57)
10	0·16^{a}	(±0·07)	97·68^{ab}	(±0·74)	0·83	(±0·19)	96·94^{ab}	(±0·33)
20	0·08^{a}	(±0·02)	98·74^{a}	(±0·93)	0·40	(±0·14)	98·57^{a}	(±0·30)

Values are means (± SD).
Means 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.
IVPD, *in-vitro* protein digestibility.

Some workers (Sternberg *et al.*, 1975; Aymard *et al.*, 1978) reported that, alkali treatment at higher temperature (100°C) or higher temperature treatment alone may lower the protein quality of treated grain due to the fact that treatments may form lysinoalanine (LAL) and lanthionine (LAT) cross-links which render proteins insoluble. Further, Sternberg *et al.* (1975) reported that LAL, an unusual amino acid implicated as a renal toxic factor in rats, has been found in proteins of home-cooked and commercial foods and ingredients; it has been reported to occur in both edible and non-food proteins only after alkali treatment. It has now been identified in food proteins that had not been subjected to alkali. Also, in experiments done to compare between dehulled and alkali-treated seed protein, Schaffert *et al.* (1974) found that there were no differences in the distribution patterns of proteins among the treatments; they exhibited similarities. The authors suggest that lower values of IVPD of treated seeds obtained in this study may be due to the fact that very little tannin goes to the endosperm due to alkali treatment at 100°C which inhibits the activities of proteolytic enzymes like pepsin and trypsin, as indicated by significantly lower values of IVPD of untreated seeds which are therefore not completely due to LAL and LAT cross-links. Alkali treatment is associated with significant ($P \leq 0·05$) improvement in sorghum nutritional value by causing a significant reduction in tan-

Table 4. Effect of 0·005 M KOH on tannin content and *in-vitro* protein digestibility of sorghum cultivar

Extraction time	Mugud				Karamaka			
	% Tannin after treatment		IVPD		% Tannin after treatment		IVPD	
Hours at 30°C								
0	0·60^{a}	(±0·15)	90·41^{c}	(±1·88)	3·10	(±0·32)	89·10^{cd}	(±2·38)
1	0·55^{ab}	(±0·20)	90·77^{c}	(±0·33)	2·71^{a}	(±0·23)	89·39^{cd}	(±1·33)
3	0·50^{abc}	(±0·12)	92·97	(±0·42)	2·60^{a}	(±0·17)	90·52^{c}	(±0·55)
6	0·41^{cd}	(±0·13)	96·78^{ab}	(±0·60)	2·08^{b}	(±0·15)	96·10^{ab}	(±0·39)
12	0·40^{cde}	(±0·05)	97·12^{ab}	(±0·16)	2·03^{bc}	(±0·20)	96·21^{ab}	(±0·64)
24	0·34de	(±0·09)	97·81^{a}	(±0·10)	1·74^{c}	(±0·18)	96·69^{a}	(±0·60)
Minute at 100°C								
0	0·60^{a}	(±0·15)	90·41	(±1·88)	3·10	(±0·32)	89·10	(±2·38)
5	0·50^{ab}	(±0·14)	93·01^{a}	(±0·33)	2·57^{a}	(±0·21)	90·66^{a}	(±0·66)
10	0·43^{abc}	(±0·04)	93·65^{a}	(±0·20)	2·49^{a}	(±0·19)	90·77^{a}	(±0·41)
20	0·36^{c}	(±0·08)	97·47	(±0·11)	1·83	(±0·22)	96·40	(±0·76)

Values are means (± SD).
Means 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.
IVPD, *in-vitro* protein digestibility.

nin content. Although this method was found to be effective, removal or lowering the content of tannins through genetic means is an important goal in both cereals and legumes (FAO, 1981).

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