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Effect of fermentation on protein fractions and tannin content of low- and high-tannin cultivars of sorghum

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Two sorghum cultivars—safra (low in tannin, 0·612%) and cross 35 : 18 (high in tannin, 1·104%)—obtained from Wad Medani Research Station were used in this study. The percentage of the protein fractions albumin, globulin, prolamin, glutelin and insoluble fraction for the safra cultivar were 11·5%, 8·2%, 60·2%, 10·2% and 10·1%, respectively, and for the cross 35 : 18 cultivar 10·0%, 4·7%, 67·9%, 9·4% and 8·1%, respectively. The tannin contents of fractions for the safra cultivar were 0·228%, 0·052%, 0·028% and 0·304% for the first four fractions, respectively, and for the cross 35 : 18 cultivar 0·376%, 0·056%, 0·136%, 0·536%, respectively, for the first four fractions. The two varieties were fermented for 14 h and the protein fractions and the tannin content of the fractions were determined at 2-h intervals. Results indicated that there was a decrease in the prolamin fraction, an increase in the glutelin and a slight increase in the albumin and globulin fractions for the safra cultivar. In the second cultivar, the prolamin content fluctuated during fermentation while the glutelin fraction increased towards the end of fermentation and the albumin fraction increased at the beginning of fermentation but decreased at the end. Most of the tannin was associated with the glutelin and albumin fractions. Fermentation decreased the tannin content for both cultivars, and the decrease of tannin reached 92% in the high-tannin variety. The tannin content of the protein fractions decreased during fermentation, especially in the albumin and glutelin fractions.

INTRODUCTION

Grain sorghum ranks third among cereals for human consumption, and is a staple food in Africa, China, and India, superseded only by rice and wheat. It is the most important cereal crop in Sudan. Sorghum flour is utilized in Sudan in the form of a fermented product known as Kisra bread. Kisra is a fermented sorghum flour which id baked on a hot plate to form thin sheets of bread (El Tinay *et al.*, 1979). According to El Hidai (1978), Kisra fermentation was mainly due to lactic acid bacteria *Lactobacillus* spp. and to a lesser degree a yeast, acetic acid and butyric acid fermentations. El Tinay *et al.* (1985) described the manufacture of Kisra bread.

The important factor that affects the nutritional quality of sorghum is the presence of tannins (Rooney & Sullins, 1973). Biological effects of tannins in human and animals vary considerably. Singleton and Kratzer (1969) and Van Busen and Robinson (1969) reported that tannins affect the growth of animals in three main ways: they have an astringent taste, which effects palatability and decreases feed consumption; they form complexes with proteins of reduced digestibility; and they act as enzyme inactivators. All these factors result from the interaction of tannins and proteins to form soluble and insoluble complexes, an interaction that depends primarily on the relative proportions of phenol and protein.

Kazanas and Fields (1981) reported that the nutritional value of sorghum could be increased by fermentation, which increases the content of lysine and methionine.

Virupaksha and Sastry (1968) and Skoch *et al.* (1970) fractionated sorghum proteins as albumins, globulins, prolamins and glutelins using the Mendel–Osborne (1914) technique to produce a water-soluble fraction, a 5% NaCl-soluble fraction, an 80% ethanol-soluble fraction and a 0·2% NaOH-soluble fraction.

The objective of this investigation was to study the effect of fermentation on protein fractions and the association of tannin in these fractions for low- and high-tannin cultivars of sorghum.

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Table 1. Protein fractions and tannin content of the fractions befors treatment

Fractions	Safra		Cross 35 : 18	
	Protein %	Tannin %	Protein %	Tannin
Albumin I	11·5(± 0·141)	0·228(± 0·001)	10·0(± 0·081)	0·376(± 0·009)
Globulin II	8·2(± 0·249)	0·052(± 0.004)	4·7(± 0·047)	0·056(± 0·004)
Prolamin III	60·2(± 0.249)	0·028(± 0·001)	67·9(± 0·081)	0·136(± 0·004)
Glutelin IV	10·2(± 0·249)	0·304(± 0·001)	9·4(± 0·163)	0·536(± 0·009)
Residue V	10·1(± 0·169)	—	8·1(± 0·141)	—
Actual Protien	13·3(± 0·124)	—	10·05(± 0·04)	—
Actual tannin	—	0·652(± 0·004)	—	1·162(± 0·081)
Total tannin	—	0·612	—	1·104
Extractable protein	90·1	—	92·0	—

Values are means (± SD).

MATERIALS AND METHODS

Materials

Two sorghum cultivars, safra and cross 35 : 18, obtained from the Wad Medani Research Station were used in this study. The two samples were carefully cleaned and freed from foreign materials; the grains were ground to pass a 0·4-mm screen.

Preparation of Kisra dough

Fermented dough was prepared in the traditional way as used by the housewife. Sorghum flour (1 kg) was mixed with 2 litres of water in a round earthenware container. Previously fermented dough (300 g) was then added to the mixture of flour and water to act as starter. After a thorough mixing, samples were taken at 2-h intervals until the end of fermentation, which was terminated after 14 h (pH 3·8–3·9) at ambient temperature (30 ± 2°C). These samples were dried in an oven at 70°C and finely ground. Samples were taken to fractionate the protein on the basis of solubility.

Protein fractionation of fermented samples

The Mendel–Osborne (1914) technique for protein fractionation was used in this study. Duplicate 2·5 g samples were taken in plastic bottles provided with screw caps. The sample was extracted twice with 50 ml of distilled water. Extraction was carried out for 30 min with continuous shaking on a shaker. The extract was separated from residue by centrifugation at 2000g for 30 min. The clear supernatant liquids were collected. The residues were then extracted successively in a similar manner with 1·0M NaCl solution, 70% ethanol and 0·2% NaOH solution and the extracts collected in the same way as described above. The residues remaining after these successive extractions with the four solvents were the insoluble residues. The protein contents of the four extracts and the residues were determined by the micro-Kjeldahl method.

Determination of tannin

Tannin was determined according to the modified vanillin–HCl method, as described by Price *et al.* (1978).

Statistical analysis

Each sample was analysed in triplicate and the figures were then averaged. Data were 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 AND DISCUSSION

The per cent of protein fractions and the tannin content of the fractions of the two sorghum cultivars before

Table 2. Effect of fermentation on protein fractions of sorghum cultivar safra

Fermentation time (h)	pH	Albumin %	Globulin %	Prolamin %	Glutelin %	Residue %
0	6·78	11·5(± 0·141)^a	8·2(± 0.249)^a	60·2(± 0·249)^a	10·2(± 0·249)^a	10·1(± 0.169)
2	5·68	10·9(± 0·216)^b	4·4(± 0·169)^b	62·3(± 0·244)^b	12·0(± 0·262)^b	10·5(± 0·163)
4	4·90	12·0(± 0·216)^c	7·6(± 0.216)^c	51·9(± 0·094)^c	19·0(± 0·084)^c	9·5(± 0·205)
6	4·70	13·3(± 0·124)^d	10·7(± 0·169)^d	48·2(± 0·249)^d	17·1(± 0·294)^d	10·8(± 0·124)
8	4·30	13·8(± 0·081)^e	13·2(± 0·244)^e	46·6(± 0·216)^e	18·5(± 0·081)^e	8·1(± 0·235)
10	4·15	13·3(± 0·124)^d	13·8(± 0·169)^f	47·2(± 0·249)^f	16·3(± 0·326)^f	9·5(± 0·094)
12	3·95	13·7(± 0·081)^f	13·3(± 0·124)^g	47·5(± 0·081)^g	14·7(± 0·169)^g	10·7(± 0·124)
14	3·90	13·7(± 0·124)^f	13·1(± 0·094)^h	51·5(± 0·169)^h	10·3(± 0·355)^h	10·9(± 0·081)

Values are means (± SD)
Means not sharing a common superscript letter in a column are significantly different at $p < 0{\cdot}05$, as assessed by Duncan's multiple-range test.

Table 3. Effect of fermentation on protein fractions of sorghum cultivar cross 35 : 18

Fermentation time (h)	pH	Albumin %	Globulin %	Prolamin %	Glutelin %	Residue %
0	6·53	10·0(± 0·081)^a	4·7(± 0·047)^a	67·9(± 0·081)^a	9·4(± 0·163)^a	8·1(± 0·141)
2	5·32	12·5(± 0·081)^b	5·6(± 0·081)^b	61·5(± 0·094)^b	11·8(± 0·094)^b	8·8(± 0·047)
4	5·15	12·5(± 0·081)^b	9·4(± 0·081)^c	59·4(± 0·081)^c	13·2(± 0·169)^c	5·6(± 0·081)
6	5·02	10·1(± 0·081)^c	5·4(± 0·309)^d	71·1(± 0.169)^d	8·1(± 0·169)^d	5·3(± 0·047)
8	4·81	10·0(± 0·081)^a	5·3(± 0·081)^e	66·5(± 0·081)^e	12·0(± 0·145)^e	6·1(± 0·047)
10	4·52	9·7(± 0·169)^d	4·5(± 0·094)^f	68·2(± 0·163)^f	12·3(± 0·081)^f	5·5(± 0·141)
12	4·39	9·3(± 0·047)^e	4·3(± 0·047)^g	68·7(± 0·092)^g	12·4(± 0·169)^g	5·3(± 0·081)
14	3·83	8·9(± 0·047)^f	4·1(± 0·169)^h	69·4(± 0·081)^h	12·4(± 0·169)^g	5·2(± 0·047)

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.

fermentation are shown in Table 1. The percentages of soluble nitrogen in the first two fractions and the fourth fraction of the low-tannin variety (11·5%, 8·2% and 10·2% respectively) were higher than in the high-tannin variety (10·0%, 4·7% and 9·4%, respectively), whereas the reverse was observed with the prolamin fraction, which was 60·2% for the low-tannin cultivar and 67·9% for the high-tannin cultivar. The amount of protein extracted by the four solvents was higher in the case of the high-tannin cultivar than in the case of the low-tannin cultivar.

Since the distribution of proteins in various fractions was distinctly different between the high- and low-tannin varieties, it was tentatively assumed that the pigments and tannins may be the cause of this uneven distribution. (Jambunathan & Mertz, 1973). This assumption was further strengthened by the observation that the first two fractions in the pigmented sample were low in protein, although one would expect them to be reasonably high because those fractions represent albumin and globulin, which are indispensable for the germination and sustenance of the plant (Jambunathan & Mertz, 1973).

As shown in Table 1, high tannin content was associated with the glutelin and albumin fractions in the two sorghum cultivars; the globulin fraction in the two cultivars has approximately identical tannin content (0·05%), whereas the prolamin fraction of the high-tannin cultivar contained four times more tannin than the corresponding fraction of the low-tannin cultivar.

Fermentation and the protein fractions

Table 2 shows the variations in protein content during Kisra fermentation of the low-tannin sorghum variety (safra). The albumin fraction decreased during the first 2 h, but started to increase significantly ($p \leq 0.05$) during the following 10 h of fermentation, reaching its maximum after 8 h of fermentation. The globulin fraction decreased in the first 2 h, but increased significantly ($p \leq 0.05$) during the following 10 h of fermentation, reaching its maximum after 10 h of fermentation. This rise in the protein content during fermentation might be due to synthesis of protein by micro-organisms (El Hidai, 1978; Romo-Parada *et al.*, 1985). Rose (1961) in his study of microbial foods reported that microbial cell matter contains appreciable amounts of protein. The albumin and globulin fractions have higher levels of the amino acid lysine (Wu & Wall, 1980). Thus, the nutritional value of sorghum would be expected to increase due to the increase in the albumin and globulin fractions as a result of the fermentation process. The prolamin fraction increased during the first 2 h and then decreased significantly ($p \leq 0.05$) during the following 10 h of fermentation, reaching its minimum after 8 h of fermentation. The glutelin fraction increased during the first 4 h, and then decreased significantly ($p \leq 0.05$) until the end of fermentation. For the insoluble fraction there was a slight increase as a result of Kisra fermentation.

For sorghum cultivar cross 35 : 18 (Table 3) the albumin, globulin and glutelin fractions increased significantly ($p \leq 0.05$) during the first 4 h of fermentation, reaching their maximum; then they began to level off. The prolamin fraction fluctuated during the fermentation process. The insoluble fraction (residue)

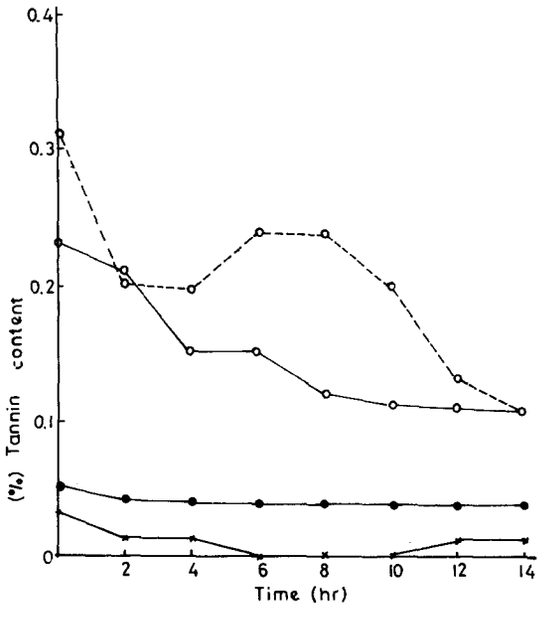

Fig. 1. Effect of fermentation on tannin content of protein fractions of sorghum cultivar safra. —○— Albumin, —●— globulin, —×— prolamin and – –○– – glutelin.

increased slightly during the first 2 h of fermentation, and then fluctuated thereafter. For the high-tannin cultivar, therefore, it appears that the nutritional quality was not improved, as the most nutritionally important fractions comparatively decreased after 4 h of fermentation and then levelled off.

Fermentation and the tannin content of the fractions

In the low-tannin sorghum cultivar (safra; Fig. 1), the tannin content of the albumin fraction started to decrease at the beginning of the fermentation process, and had decreased 52% by the end of fermentation. In the globulin fraction, tannin decreased during the first 2 h of fermentation, from 0·052% to 0·04%, and then levelled off during the remaining period of fermentation. In the prolamin fraction, tannin decreased from 0·028% to 0·012% in the first 4 h but there was no detectable tannin in this fraction after 10 h of fermentation. The tannin content of the glutelin fraction decreased from 0·304% to 0·188% during the first 4 h, increased after 6 h to 0·24%, and then decreased as the fermentation process progressed, reaching 0·108% at the end of the process. The results indicated that Kisra fermentation has a marked effect on the tannin content of the protein fractions, especially the albumin and glutelin fractions, which were reduced by 52% and 64%, respectively.

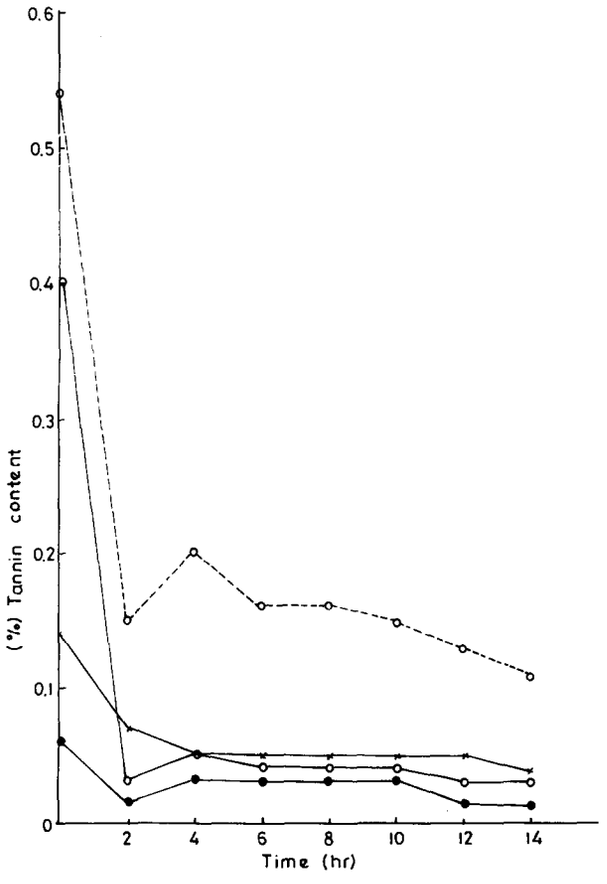

Fig. 2. Effect of fermentation on tannin content of protein fractions of sorghum cultivar cross 35 : 18. -○- Albumin, -●- globulin, -×- prolamin and - -○- - glutelin.

Figure 2 shows the tannin content of the protein fractions of the fermented sorghum cultivar cross 35 : 18. In this cultivar, the fermentation process caused significant ($p \leq 0{\cdot}05$) decrease in the tannin content for all protein fractions; the percent decrease was 92%, 78%, 70% and 80% for albumin, globulin, prolamin and glutelin, respectively. Kisra fermentation caused a significant decrease in the tannin of protein fractions. Romo-Parada *et al.* (1985) reported that fermentation decreases the tannin content of a high-tannin sorghum by 92%. Lewis and Starkey (1968) indicated that the decreasing tannin content was due to the effect of micro-organisms.

Fermentation had a significant ($p \leq 0{\cdot}05$) effect on the protein fractions, especially in the case of the low-tannin sorghum cultivar. It increased the nutritional value of this cultivar by increasing the albumin and globulin fractions; it also decreased the tannin content for both cultivars of sorghum, especially for the high-tannin cultivar, where the decrease in tannin of the protein fractions reached 92%.

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