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Effect of preincubation of sorghum flour with enzymes on the digestibility of sorghum gruel

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Abstract

A low-tannin sorghum cultivar M-35-1 was used in this study. Sorghum was germinated for 6 days and protease and amylase activities were measured every 24 h. Results showed that the 5th day germinated sorghum had a higher protease activity and a lower amylase activity. Sorghum flour was incubated for 30 min with the extract from germinated sorghum or with 0.01, 0.05 or 0.1 mg ml^{-1} papain or trypsin prior to cooking in water. Results showed increase in in vitro protein digestibility (IVPD) with the 5th day germination extract. Pretreatment of sorghum flour with small amounts of papain or trypsin (0.01 mg ml^{-1}) improved the IVPD without affecting the paste viscosity, whereas the germinated sorghum extract led to very low paste viscosity.

1. Introduction

Grain sorghum ranks third among cereals for human consumption and is a staple food in Africa, China and India. It is the most important cereal crop in Sudan. Various Sudanese foods are made from sorghum. The rural Sudanese traditionally divide these foods into two major groups. One group encompasses the foods and beverages involving the use of germinated grain and the other group is composed of the foods and beverages prepared from ungerminated grain (Dirar, 1993).

Nitrogen balance studies conducted on children and young adults using cooked sorghum showed that sorghum diets resulted in progressively lower apparent digestibility values (Kurien, Narasinga Rao, Swaminathan, & Subramanyam, 1960; MacLean, Deromana, Plocko, & Graham, 1981). It is generally accepted that cooking of sorghum reduces its digestibility, presumably through formation of disulphide linkages.

Germination induces or significantly increases various enzyme activities, plant seeds are a good starting material for enzyme production. This paper attempts to study the possibilities of using enzymes from germinated sorghum as well as papain or trypsin to inhibit the formation of aggregates during cooking of sorghum and thus increase protein digestibility of cooked sorghum.

2. Materials and methods

Sorghum cultivar M-35-1 was used in this study. The material was cleaned and kept at 4°C. Sorghum flour was prepared by milling sorghum in a hammer mill to pass through a 0.4 mm (60 BSS mesh) screen and the flour stored at 4°C.

2.1. In vitro protein digestibility

The in vitro protein digestibility procedure described by Mertz et al. (1984) was used with slight modification. Porcine pepsin (EC 3, 4, 23.1,3000 u mg^{-1} protein, SRL Chemical Ltd, Bombay, India) was used to digest the protein.

Uncooked sorghum flour (200 mg) was used either directly or soaked for 30 min at room temperature (27 ± 2°C) in 5 ml of geminated sorghum extract, and 0.01, 0.05 or 0.1 mg ml^{-1} papain or trypsin enzyme solutions. Then samples were centrifuged (4800 ***g***, 10 min) the supernatant was discarded and the residue was

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cooked in 5 ml water with stirring in a boiling water bath for 20 min. Samples were suspended in 35 ml of 0.1 M phosphate buffer containing 1.5 g pepsin per litre (pH 2) and incubated at 37°C for 2 h. Pepsin digestion was stopped by adding 2 ml of 2 M NaOH solution. After centrifugation (4800 ***g***, 20 min), the supernatant was discarded and the residue was washed with 15 ml of the buffer and recentrifuged. The residue was analyzed for nitrogen by microKjeldahl digestion (American Association of Cereal Chemists, 1986). The percentage of soluble nitrogen was reported as in vitro digestibility.

2.2. Grain sorghum germination

Sorghum seeds were germinated according to the method of Bhise, Chavan, and Kadam (1988).

2.3. Estimation of protease activity

The protease activity was assayed by the method of Chandrasekhara and Swaminathan (1953) with some modifications.

2.3.1. Preparation of enzyme extract

Two grams of germinated sorghum flour were suspended in 10 ml of double distilled water for 1 h at room temperature (27 ± 2°C) with occasional shaking, then centrifuged (10 000 ***g***, 10 min). The supernatant containing protease was taken for the assay.

2.3.2. Enzyme assay

One millilitre of the above extract was added to 1 ml of 1% casein and incubated for 1 h at 40°C in a shaking water bath. Then 2 ml of 10% trichloroacetic acid (TCA) were added to the mixture and allowed to stand for 30 min at room temperature (27 ± 2°C) and filtered through a Whatman No. 1 filter paper and the absorbance of the filtrate was read at 280 nm in a UV spectrophotometer.

2.4. Estimation of amylase activity

Amylase activity of germinated sorghum was measured using the method of Bernfeld (1955).

2.5. Pasting profile

The pasting profile of sorghum was obtained by using the procedure of Deffenbaught and Walker (1989) using a rapid visco-analyzer (RVA) (Newport Scientific, Model 3D) with software programme (RVACOM) and the rate of heating and cooling of the slurry was 3°C min^{-1}. Gelatinisation temperature, peak viscosity, peak temperature, breakdown, setback and area values were determined by using RVACOM.

2.6. Scanning electron microscopy (SEM)

After pepsin digestion of samples, the residue obtained after centrifugation was incubated with α-amylase (Bacillus species, EC 3.2.1.1, 240 u mg^{-1} protein, Sigma. St. Louis, MO, USA) in phosphate buffer (240 mg^{-1} litre, pH 6.9, 37°C, 2 h) for removal of starch. After discarding the supernatant, the pellet containing the protein was fixed in 2% (v/v) glutaraldehyde in 0.1 M phosphate buffer (pH 6.8) for 16 h at 4°C.

2.7. SEM

The fixed samples were rinsed in 0.1 M phosphate buffer (pH 6.8), and dehydrated in a graded ethanol series of 10, 20, 40, 60, 80, 95, 100, 100 and 100 for 10 min each time at room temperature (27 ± 2°C). Alcohol was removed after centrifugation for 10 min at 4000 ***g*** each time. Samples were then air-dried and fixed to copper stabs with a double adhesive tape, coated with gold and viewed in a Jeol scanning electron microscope.

2.8. Statistical analysis

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

3. Results and discussion

The protease and amylase activities in extracts from germinated sorghum were measured (Fig. 1). Results indicated that the proteolytic activity of the extract increased with increasing time of germination, reaching a maximum on the fifth day (Fig. 1). While amylase activity was highest on the third day of germination, it decreased steadily thereafter (Fig. 1). These results agree with the results obtained by Arisen (1982) and Garg and Virupaksha (1970) but are in contrast to the results obtained by Evans and Taylor (1990) who reported that, in germinated sorghum, protease activity reached a maximum on the 3rd–4th day of germination and on the 6th day of germination it declined to the base level.

The residual protein of sorghum flour treated with extract from germinated sorghum was little affected by incubation (> 2%). Incubation with the extracts significantly increased pepsin digestibility of sorghum flour (Table 1). Fifth day sorghum extract gave the highest IVPD.

Generally, sorghum proteases have narrow specificities and specifically cleave the peptide linkages between the α-carboxyl group of aspartate or glutamate and the amino group of the adjacent amino acid (Garg

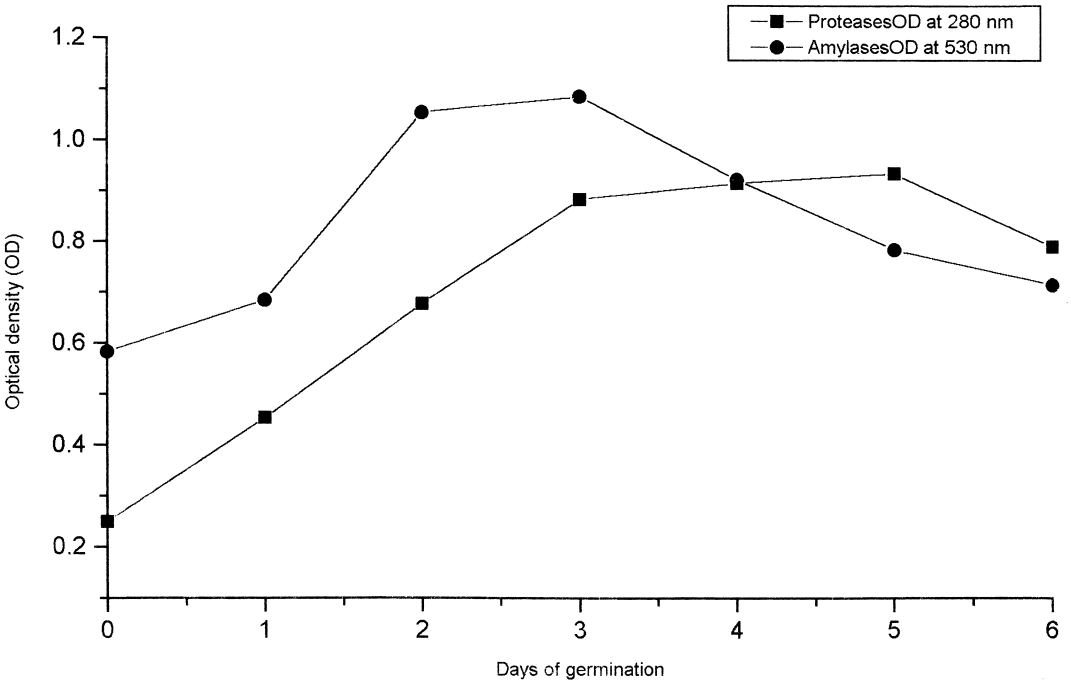

Fig. 1. Proteolytic and amylase activity during germination of sorghum.

Table 1
Effect of pretreatment of sorghum flour with enzymes on the in vitro protein digestibility of sorghum gruel

Treatment	IVPD%[ab]
Control (sorghum gruel untreated)	10.7 (±0.28)g
Extract from ungerminated sorghum	23.3 (±0.21)f
Extract from 1st day germinated sorghum	40.2 (±0.35)e
Extract from 2nd day germinated sorghum	42.3 (±0.07)d
Extract from 3rd day germinated sorghum	44.5 (±0.21)c
Extract from 4th day germinated sorghum	50.9 (±0.14)b
Extract from 5th day germinated sorghum	57.3 (±0.21)a
Extract from 6th day germinated sorghum	42.3 (±0.28)d
Papain (0.01 mg ml^{-1})	42.4 (±0.28)c
Papain (0.05 mg ml^{-1})	45.6 (±0.57)b
Papain (0.1 mg ml^{-1})	49.5 (±0.71)a
Trypsin (0.01 mg ml^{-1})	45.4 (±0.14)b
Trypsin (0.05 mg ml^{-1})	49.7 (±0.07)a
Trypsin (0.1 mg ml^{-1})	51.4 (±0.57)a

[a] Values are means (±SD).
[b] Means not sharing a common superscript letter in a column are significantly different at $p \leqslant 0.05$ as assessed by Duncan's multiple range test.

& Virupaksha, 1970). As prolamins contain large amounts of glutamic acid and aspartic acid (Virupaksha & Sastry, 1968), cleavage of the protease would make prolamins more accessible to digestive enzymes and, therefore, increase protein digestibility.

Sorghum flour was incubated with different concentrations of papain and trypsin prior to cooking. The in vitro protein digestibility of sorghum gruel was increased significantly with all the papain- and trypsin-treated samples (Table 1). The increases in the IVPD

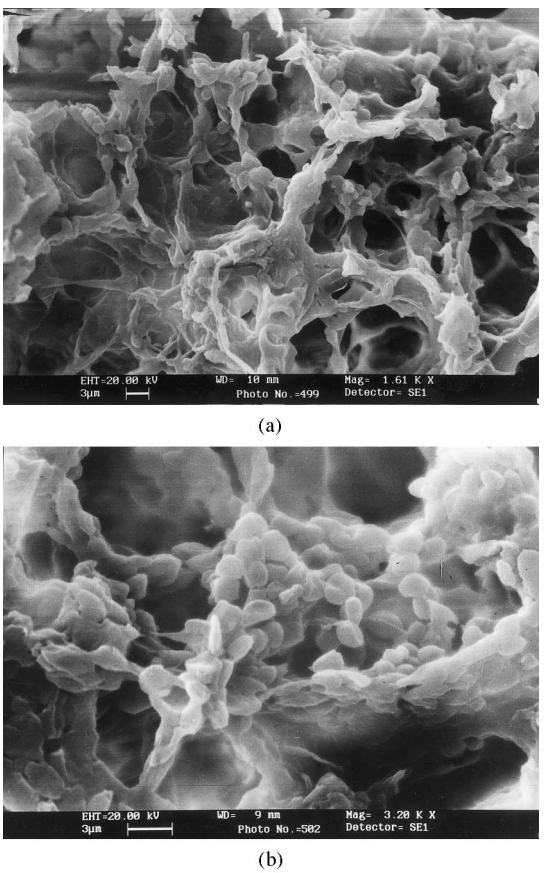

Fig. 2. SEM micrograph of sorghum gruel (A) treated with pepsin and α-amylase (control) (B) pretreated with papain then digested with pepsin and α-amylase.

Table 2
Effect preincubation of sorghum flour with enzymes on the paste viscosity of sorghum

Treatment	GT[a] (°C)	PV[b] (SNU)	PT[c] (°C)	BD[d] (SNU)	SB[e] (SNU)	Area (SNU[f]. min)
Control	70.5 (±0.42)[b]	93 (±2.83)[b]	92.95 (±0.07)[b]	33 (±2.82)[a]	73 (±4.24)[a]	2499.66 (±5.15)[b]
Papain (0.01 mg ml^{-1})	73.35 (±0.92)[a]	115 (±2.12)[a]	95.18 (±0.04)[a]	31 (±2.12)[a]	53 (±5.66)[a]	2941.77 (±86.65)[a]
Papain (0.05 mg ml^{-1})	76.15 (±0.21)[a]	121 (±3.54)[a]	95.1 (±0.07)[a]	33 (±0.71)[a]	62 (±3.52)[a]	3122.47 (±133.2)[a]
Trypsin (0.01 mg ml^{-1})	72.55 (±0.49)[a]	102 (±3.54)[a]	94.05 (±0.81)[a]	32 (±3.54)[a]	49 (±4.24)[a]	2615.74 (±74.8)[a]
Trypsin (0.05 mg ml^{-1})	75.05 (±0.41)[a]	101 (±2.12)[a]	95.15 (±0.07)[a]	25 (±0.71)[a]	48 (±0.71)[a]	2691.09 (±70.9)[a]
Extract from 5th day germinated sorghum	–	3 (±0.01)[c]	–[g]	0	0	1.25.27 (±1.25)[c]

[a] GT: gelatinization temperature.
[b] PV: peak viscosity.
[c] PT: peak temperature.
[d] BD: breakdown (difference between peak viscosity and the viscosity at the end of 95°C).
[e] SB: setback (difference between the viscosity at the end of the experiment and peak viscosity).
[f] SNU: Stirring number unit.
[g] – Not determined.

were 9, 18 and 28% when sorghum flour was incubated with 0.01, 0.05 and 0.1 mg ml^{-1} of papain, respectively, and were 17, 28 and 33% when sorghum flour was incubated with 0.01, 0.05 and 0.1 mg ml^{-1} of trypsin, respectively.

The SEM micrograph of sorghum gruel (control) digested with pepsin, then with α-amylase, showed an expanded protein matrix and the protein bodies were clearly seen attached to the protein matrix. In the case of sorghum flour pretreated with papain, then digested with pepsin and α-amylase, the SEM micrograph showed digestion of the protein matrix (Fig. 2) and this explains the high IVPD in the treated samples.

The paste viscosity of sorghum flour pretreated with enzymes is shown in Table 2. Pretreatment of sorghum with papain or trypsin at different concentrations led to increase in gelatinization temperature, peak viscosity and peak temperature; it also led to a decrease in the breakdown and setback compared with the untreated control. Treatment of sorghum flour with extracts from germinated sorghum had a negative effect on the rheological properties of sorghum starch (Table 2). This may be due to the presence of α-amylase in the germinated extract. This amylase degrades the starch granules, reducing their water-binding capacity and consequently the viscosity of the slurry (Hansen, Pedersen, Munck, & Eggum, 1989).

4. Conclusion

Pretreatment, prior to cooking, of sorghum flour, with either papain, trypsin or germinated sorghum grain extracts, significantly improved protein digestibility compared to untreated sorghum. This could be attributed to low levels of disulphide linkages in the treated samples compared to the control.

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