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Effect of reducing agents on the in vitro protein and starch digestibilities of cooked sorghum

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Abstract

A low-tannin sorghum cultivar M-35-1 was used in this study. Investigation showed that the in vitro protein digestibility (IVPD) decreased considerably when sorghum flour was cooked in water, while it increased when cysteine, sodium metabisulphite, or ascorbic acid were added to the cooking medium. The increase in the IVPD was significantly higher with increasing concentrations of cysteine up to 0.25 M and it continued to increase to 0.5 M for sodium metabisulphite; with ascorbic acid it increased up to 0.1 M then decreased. The in vitro starch digestibility (IVSD) of the treated gruel initially increased in the presence of either cysteine, sodium metabisulphite or ascorbic acid. The increase was parallel to that shown by IVPD; however, at high levels of cysteine or sodium metabisulphite the IVSD was low. Removal of cysteine from the gruel by alcohol gave higher IVSD. Altered viscosity patterns for all the treatments led to increase in the gelatinization temperature, peak viscosity and breakdown. However, the setback decreased in all treatments. Cysteine and ascorbic acid gave a negative setback but when the pH was adjusted to 4.5 or 7.0 a normal setback was obtained.

1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is a crop that is widely grown over the world for food and feed. It is the main staple food for the world's poorest people. Changes occur in protein quality during cooking and their effects on protein digestibility have been investigated by many workers. Eggum, Monowar, Bach Kundesn, Munck, and Axtell (1983) and Mitaru and Blair (1984), reported that sorghum protein digestibility decreased significantly after cooking; Mitaru, Reichert, and Blair (1985) reported a 31% drop in protein digestibility after cooking. Using an in vitro pepsin digestion assay, Hamaker, Kirleis, Butler, Axtell, and Mertz (1987), compared the protein digestibility of cooked and uncooked samples of sorghum, maize, barley, rice and wheat. Sorghum was 24.5% lower in digestibility after cooking, which was a significantly greater decrease than that of the other cereals. This result indicated that sorghum protein had higher levels of disulphide bonding than did other cereal grains.

Hamaker et al. (1987) reported that cooking sorghum flour in the presence of reducing agents improved the in vitro protein digestibility up to 25% compared with untreated cooked flour. Rom, Shull, Chandrashekar, and Kirleis (1992) reported that treatment with sodium bisulphite increased the digestibilities of both cooked and uncooked sorghum flour. Arbab and El Tinay (1997), using sodium bisulphite and ascorbic acid, improved the in vitro protein digestibility of cooked sorghum. No data have been published on the effect of reducing agents on the starch digestibility of cooked sorghum.

Since sorghum is the main staple food in Sudan, the decrease in protein digestibility upon cooking is of major concern in the country. Efforts to overcome the poor digestibility would have a significant impact on the nutritional status of the population. The present study was, therefore, directed to address this problem.

2. Materials and methods

Sorghum cultivar M-35-1 was used in this study. The material was carefully cleaned and freed from foreign materials and the grain was ground to pass through a 0.4 mm screen.

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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.

Sorghum flour (200 mg) was suspended in 5 ml of water or 5 ml of 0.05, 0.1., 0.25 or 0.5 M L-cysteine hydrochloride, sodium metabisulphite or ascorbic acid and stirred 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 Na OH 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 the microKjeldahl method (American Association of Cereal Chemists, 1986). The percentage of soluble nitrogen was reported as in vitro digestibility.

2.2. Starch digestibility

In vitro starch digestibility was determined according to the method of Dahlqvist (1964) using α-amylase to digest the starch and the reducing sugar was estimated by the dinitrosalicylic acid (DNS) method.

2.3. Removal of residual cysteine from sorghum gruel

After cooking sorghum flour in the presence of cysteine for 20 min, the samples were cooled and three volumes of 100% ethanol were added so as to precipitate the starch and then it was centrifuged (4000 ***g***, 15 min). The supernatant containing cysteine was discarded and the residue was washed with three volumes of 70% ethanol, then filtered through a Whatman No. 1 filter paper. The residue was air-dried and its in vitro starch digestibility was estimated.

2.4. 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.

2.5. Statistical analysis

Three separate batches for a particular treatment were taken and analyzed separately and the figures were then averaged. Data was 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

3.1. Effect of different reducing agents on the in vitro protein digestibility of cooked sorghum

The crude protein and starch contents of untreated sorghum were 10.3 and 74.4%, respectively. The in vitro protein digestibility (IVPD) decreased from 38.8 to 10.7 g 100 g^{-1} after cooking in water. Cooking in the presence of varying concentrations of cysteine resulted in higher levels of in vitro protein digestibility. The increase was significant ($p \leqslant 0.05$) with increasing cysteine concentration up to 0.25 M but decreased at 0.5 M (Table 1). It was noticed that the pH of cysteine-treated flour was 1.65 which is extremely acidic in nature. Therefore, the in vitro protein digestibility of cysteine-treated flour was carried out after adjustment of pH to 4.5 and 7. Results showed that there was still considerable increase in the IVPD at pH 4.5 compared to the untreated control (Table 2).

The effect of cooking, in the presence of sodium metabisulphite, on the IVPD is shown in Table 3. The IVPD increased by 272, 299, 345 and 372% for 0.05, 0.1, 0.25 and 0.5 M concentrations of sodium metabisulphite, respectively (Table 3). The IVPD of sorghum gruel cooked in ascorbic acid was higher than the control (Table 3).

3.2. Effect of different reducing agents on the in vitro starch digestibility of cooked sorghum

The in vitro starch digestibility (IVSD) of sorghum gruel treated with cysteine increased by 70 and 16% for the first two concentrations of cysteine, respectively, and decreased by 27% for the other two concentrations (Table 1). Removal of cysteine by alcohol resulted in

Table 1
Effect of cooking, in the presence of cysteine, on the in vitro protein and starch digestibilities of sorghum[a]

Treatment	IVPD (g 100 g^{-1})[b]	IVSD (g 100 g^{-1})[b]	IVSD (g 100 g^{-1}) after extraction with EtOH[b]
Control	10.7 (±0.28)c	34.3 (±0.01)d	–
0.05 M	44.7 (±0.07)d	58.2 (±0.07)a	59.5 (±0.14)c
0.1 M	50.5 (±0.21)c	39.9 (±0.14)b	62.2 (±0.07)b
0.25 M	61.2 (±0.01)a	25.1 (±0.21)c	68.8 (±0.07)a
0.5 M	58.3 (±0.35)b	25.0 (±0.14)c	68.7 (±0.01)a

[a] Values are means (±SD).

[b] Means not sharing a common following letter in a column are significantly different at $p \leqslant 0.05$ as assessed by Duncan's multiple range test.

increased starch digestibility values compared to that of the control (Table 1).

The IVSD of sorghum gruel, in the presence of sodium metabisulphite, increased by 9% for the first concentration (0.05 M) but decreased by 11, 14 and 53% with increasing sodium metabisulphite concentrations of 0.1, 0.25 and 0.5 M, respectively (Table 3). The IVSD of sorghum cooked in the presence of ascorbic acid increased significantly ($p \leqslant 0.05$) by 62, 85, 112 and 120%, for 0.05, 0.1, 0.25 and 0.5 M concentrations, respectively (Table 3).

Table 2
Effect of cooking, in the presence of 0.1 M cysteine at different pHs, on the in vitro protein and starch digestibilities of sorghum

Treatment	pH	IVPD (g 100 g^{-1})[a]	IVSD (g 100 g^{-1})[a]
0.1 M cysteine	1.65	50.5 (±0.71)a	39.9 (±0.92)b
0.1 M cysteine	4.5	43.7 (±0.99)b	49.3 (±1.13)a
0.1 M cysteine	7.0	39.9 (±0.42)b	43.8 (±0.35)ab

[a] Means not sharing a common following letter in a column are significantly different at $p \leqslant 0.05$ as assessed by Duncan's multiple range test.

Table 3
Effect of cooking, in the presence of sodium metabisulphite or ascorbic acid, on the in vitro protein and starch digestibilities of sorghum

Treatment	Concentrations	IVPD (g 100 g^{-1})[a]	IVSD (g 100 g^{-1})[a]
Control	–	10.7 (±0.28)i	34.3 (±0.01)d
Sodium metabisulphite	0.05 M	39.8 (±0.07)d	37.3 (±0.07)c
	0.1 M	42.7 (±0.14)c	30.6 (±0.14)e
	0.25 M	47.6 (±0.24)b	29.5 (±0.07)e
	0.5 M	50.5 (±0.09)a	16.3 (±0.21)f
Ascorbic acid	0.05 M	16.5 (±0.28)e	55.7 (±2.12)b
	0.1 M	26.2 (±0.21)f	63.5 (±0.71)b
	0.25 M	19.4 (±0.14)g	72.8 (±0.28)a
	0.5 M	16.5 (±0.21)e	75.3 (±0.42)a

[a] Means not sharing a common following letter in a column are significantly different at $p \leqslant 0.05$ as assessed by Duncan's multiple range test.

3.3. Effect of reducing agents on the paste viscosity of sorghum

The pasting behaviour of sorghum flour under the effect of different reducing agents is shown in Table 4. The addition of cysteine to sorghum flour at 0.1 or 0.25 M concentrations resulted in increased gelatinization temperature, peak viscosity and negative setback compared to the control (Table 4). In the presence of cysteine, and pH adjustment to 4.5 or 7, there was a slight and positive setback at pH 4.5 and very high setback at pH 7. Similarly, the gelatinization temperature and peak viscosity increased with the increased concentration of the sodium metabisulphite in the pasting medium (Table 4). The pattern with ascorbic acid was similar to that noted with cysteine. The peak viscosity and breakdown increased with increasing ascorbic acid concentration.

4. Discussion

In vitro pepsin digestion showed that sorghum had a low protein digestibility when cooked in water. Eggum et al. (1983), Hamaker, Kirleis, Mertz, and Axtell (1986), Mitaru and Blair (1984) and Rom et al. (1992) have also reported that sorghum protein digestibility decreased significantly after cooking in water. They attributed this decrease to the formation of disulphide bonds during cooking which resulted in toughening at the surface and interior of protein bodies.

Treatment with reducing agents increased the digestibility of sorghum gruel. Rom et al. (1992) reported that the IVPD of cooked sorghum increased by 58% when sodium bisulphite (100 mM) was used for cooking. Arbab and El Tinay (1997) showed that cooking sorghum in the presence of sodium bisulphite or ascorbic acid resulted in improvement in IVPD. They attributed this increase in the IVPD to the fact that, during cooking, proteins were disulphide-linked, thus decreasing their accessibility to digestive enzymes and that reducing agents minimize the formation of these linkages.

Table 4
Effect of reducing agents on the paste viscosity of sorghum

Treatment	Gelatinization temperature (°C)[a]	Peak viscosity (SNU[b])[a]	Peak temperature (°C)[a]	Breakdown (SNU)[a]	Setback (SNU)[a]	Area (SNU min)[a]
Control	69.2 (±1.13)c	102 (±5.65)c	92.8 (±0.28)c	42 (±8.49)c	75 (±7.07)b	2624.05 (±0.35)a
0.1 M cysteine	70.9 (±1.34)b	113 (±2.83)c	84.93 (±0.11)f	109 (±2.12)a	−107 (±2.12)f	629.12 (±53.12)f
0.25 M cysteine	72.98 (±0.25)a	109 (±1.41)c	82.93 (±0.04)g	105 (±2.83)	−104 (±1.41)f	536.18 (±49.25)f
0.1 M cysteine (pH 4.5)	72.03 (±0.67)	110 (±1.41)c	90.73 (±0.88)cde	48 (±0.71)b	32 (±1.41)c	2574.75 (±34.15)e
0.1 M cysteine (pH 7.0)	72.68 (±1.02)a	115 (±2.12)c	93.38 (±0.11)a	41 (±1.41)c	106 (±0.71)a	3353.35 (±6.84)a
0.1 M SMS	73.3 (±0.49)a	113 (±5.66)c	91.65 (±0.43)b	44 (±2.12)c	31 (±2.12)c	2774.80 (±103.8)b
0.25 M SMS	71.95 (±2.59)a	131 (±2.12)ab	91.08 (±0.04)b	61 (±0.71)e	17 (±0.01)d	2966.53 (±61.42)b
0.1 M ascorbic acid	70.3 (±0.42)b	130 (±1.41)ab	90.6 (±0.92)cdf	88 (±1.41)d	−51 (±0.01)d	1979.57 (±21.45)d
0.25 M ascorbic acid	68.95 (±1.20)c	143 (±2.83)a	87.85 (±0.42)ef	114 (±9.19)a	−103 (±2.83)f	1605.63 (±20.65)e

[a] Means not sharing a common following letter in a column are significantly different at $p \leqslant 0.05$ as assessed by Duncan's multiple range test.
[b] SNU: Stirring number unit.

Improvement of IVSD increased with increasing concentrations of sodium metabisulphite. In the case of cysteine the reverse was true. This decrease may be attributed to inhibition of α-amylase at these concentrations and this was substantiated by the increase in the IVSD when cysteine was removed by alcohol. The increase in the IVSD in the case of ascorbic acid may be due to hydrolysis of starch by ascorbic acid during cooking.

The effect of protein modification on starch gelatinization is very clearly related to the altered viscosity patterns in treated flour. Cooking of sorghum in water retards starch gelatinization and, conversely, starch gelatinization is increased when reducing agents are used for cooking.

Paste viscosity of the dough is an important quality attribute because, in the manufacture of *Kisra*, the dough is spread thinly on a hot plate. The increase in viscosity due to the effect of reducing agents could be of commercial importance in *Kisra* manufacture.

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