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Effect of cooking and treatment with sodium bisulphite or ascorbic acid on the *in vitro* protein digestibility of two sorghum cultivars

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Two sorghum cultivars, Karamaka (high in tannin, 3.1%) and Gadam Elhamam (low in tannin, 0.35%), were used in this study. The effect of sodium bisulphite and ascorbic acid on *in vitro* protein digestibility of the two sorghum cultivars was investigated. Varying concentrations of reducing agents, 0.05, 0.1, 0.25 and 0.5 M, and pepsin digestion times, 30, 60, 90, 120, 150 and 180 minutes, were considered. Reducing agents improved digestibility for cooked and uncooked samples. 0.1 M concentration provided the highest level of digestibility for both reducing agents. Cooking reduced *in vitro* protein digestibility for both cultivars. Formation of disulphide bonds in cooked samples is probably responsible for the reduced protein digestibility. Reducing agents prevent formation of disulphide bonds and improve the *in vitro* protein digestibility. For all treatments, Karamaka holds a lower level of *in vitro* protein digestibility than Gadam Elhamam cultivar. This could be attributed to the higher tannin content in Karamaka cultivar.

INTRODUCTION

Sorghum is a major food crop in Africa and Asia. It is grown in semiarid areas, usually as a dry land crop. Most of the grain produced in these areas is consumed by humans as food. Sorghum is the staple food in many areas in Sudan. It acts as the major source of protein. Sorghum, like other cereals, is deficient in lysine (Adrian & Sayerse, 1957). Two other limiting amino acids are threonine (Harden *et al.*, 1976), and methionine (Ilori & Conrad, 1976). Changes occur in protein quality during cooking and its effect on protein digestibility has been investigated by many scientists. Eggum *et al.* (1983) and Mitaru and Blair (1984), reported that sorghum protcin digestibility decreased significantly after cooking. Mitaru *et al.* (1985) reported a 31% drop in protein digestibility after sorghum was cooked. Using an *in vitro* pepsin digestion assay, Hamaker *et al.* (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 proteins 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* pepsin digestibility up to 25% compared with untreated cooked flour.

Sorghum grain contains tannins, which are polyphenolics, in the outer layers and in the endosperm, that interact and precipitate proteins. High tannin content causes several problems in the utilization of sorghum. These problems include lower protein digestibility (Maxson *et al.*, 1973).

MATERIALS AND METHODS

Seeds of two sorghum cultivars, Gadam Elhamam obtained from Shambat (University of Sudan for Science and Technology), and Karamaka, obtained from Kadogli Research Station, were used in this study. The seeds were cleaned and milled to a fine flour to pass through a 0.4 mm mesh screen. Milled flour was stored at 20°C until used.

PROTEIN DIGESTIBILITY

The *in vitro* pepsin digestibility procedure described by Mertz *et al.* (1984) was used with slight modification. Porcine pepsin (EC3, 4, 23.1, 1.200 units per mg protein, Sigma Chemical CO., St Louis, MO) was used to digest protein.

Uncooked sorghum flour (0.2 g) was used either directly or soaked for 18h at 4°C in 2 ml of 0.05, 0.1, 0.25 or 0.5 M sodium bisulphite or ascorbic acid

solution. Cooked samples were prepared by suspending 0.2 g of flour in 2 ml of water or 2 ml of 0.05, 0.1, 0.25 or 0.5 M sodium bisulphite or ascorbic acid solution and stirring in boiling water bath for 20 min. For protein digestion, samples were suspended in 35 ml of 0.1 M phosphate buffer containing 1.5 g of pepsin per litre (pH 2.0) and incubated at 37°C for 30, 60, 90, 120, 150 or 180 min. 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 buffer and recentrifuged. The residue was analyzed for nitrogen by micro-Kjeldahl digestion (AOAC, 1984). The percentage of soluble nitrogen was reported as *in vitro* digestibility. All treatments were carried out in triplicate.

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 Duncan's multiple range test with probability $P < 0.05$ (Duncan, 1955).

RESULTS

Effect of cooking on the *in vitro* protein digestibility (IVPD) of untreated sorghum

Percent *in vitro* protein digestion of uncooked Gadam Elhamam cultivar was 33% after 60 min of digestion time, increasing to 46% after 180 min (Fig. 1). In contrast, the cooked sample from the same cultivar was only 11% digested after 60 min which increased to 24% after 180 min. For uncooked Karamaka cultivar, IVPD was 25% after 60 min digestion and was 38% after 180 min (Fig. 1). These values are lower than those obtained for Gadam Elhamam cultivar. Cooking of Karamaka cultivar resulted in reduced IVPD: 8% after 60 min digestion and 19% after 180 min.

Effect of sodium bisulphite on IVPD

Treatment of Gadam Elhamam cultivar with 0.05 M sodium bisulphite resulted in increased protein digestion to 39% after 60 min digestion and to 77% after 180 min for the uncooked sample (Fig. 2). Cooked samples treated with 0.05 M sodium bisulphite were higher in IVPD compared to the untreated Gadam Elhamam samples. Uncooked Karamaka treated with 0.05 M sodium bisulphite increased protein digestion to 30% after 60 min, which increased to 65% after 180 min. Cooked and treated samples of Karamaka cultivar, though higher in IVPD compared to untreated samples (Fig. 1), were lower than their counterpart uncooked samples. After the flour was soaked in 0.1 M sodium bisulphite, protein digestion increased to 56% after 60 min and 81% after 180 min for uncooked Gadam Elhamam cultivar (Fig. 3). For the uncooked Karamaka cultivar, protein digestion increased to 39% after 60 min and to 72% after 180 min. In reduced, cooked flour, 22% of the protein was digested after 60 min for Gadam Elhamam cultivar. Cooked Karamaka cultivar

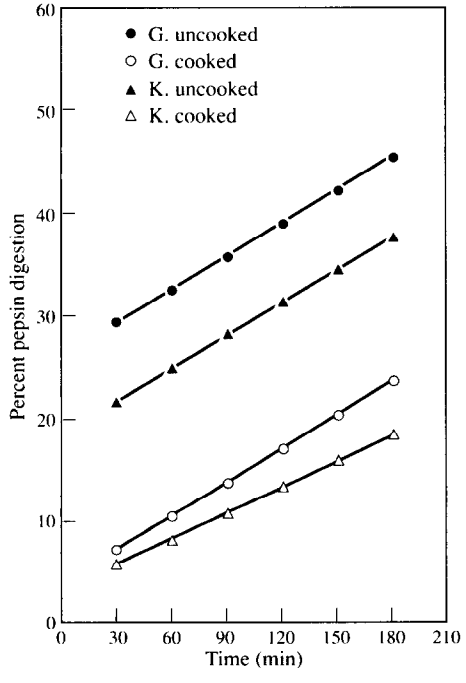

Fig. 1. Percent pepsin digestion of uncooked and cooked Gadam Elhamam and Karamaka sorghum cultivars (untreated, controls) versus time in minutes.

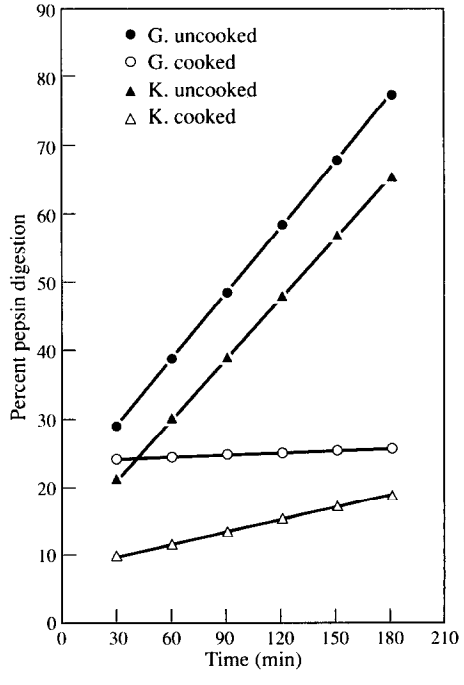

Fig. 2. Percent pepsin digestion of uncooked and cooked Gadam Elhamam and Karamaka sorghum cultivars treated with 0.05 M sodium bisulphite versus time in minutes.

gave lower values for IVPD than cooked Gadam Elhamam cultivar. Increasing the concentration of sodium bisulphite to values higher than 0.1 M (0.25 and 0.5 M), gave lower percent protein digestion compared to those obtained with 0.1 M (Figs 4 and 5); however, they were higher than those of untreated samples.

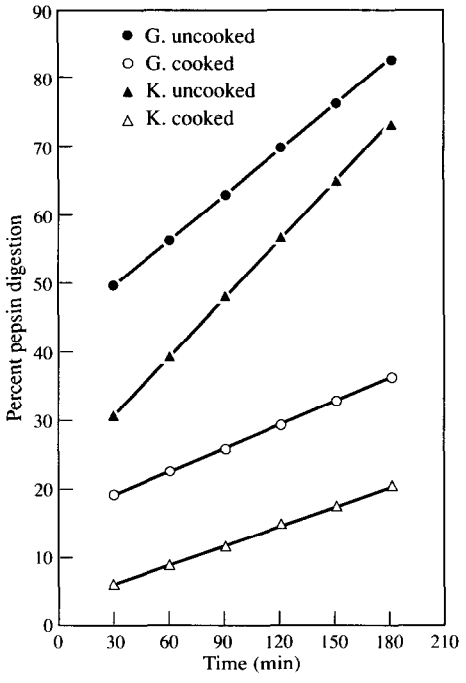

Fig. 3. Percent pepsin digestion of uncooked and cooked Gadam Elhamam and Karamaka sorghum cultivars treated with 0.10 M sodium bisulphite versus time in minutes.

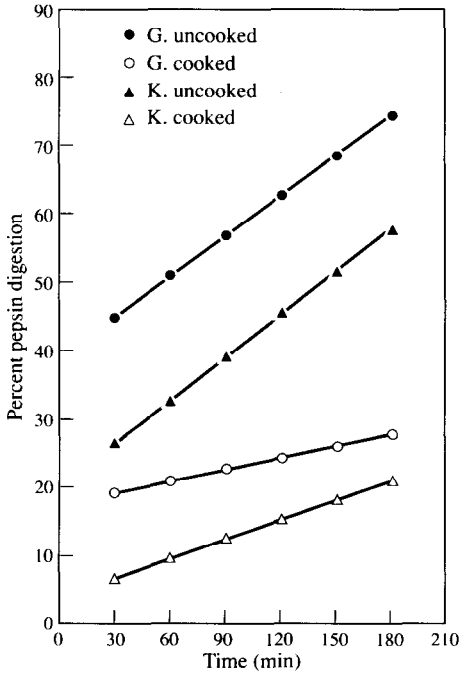

Fig. 4. Percent pepsin digestion of uncooked and cooked Gadam Elhamam and Karamaka sorghum cultivars treated with 0.25 M sodium bisulphite versus time in minutes.

Effect of ascorbic acid on IVPD

Treated samples of Gadam Elhamam cultivar with 0.05 M ascorbic acid resulted in increased protein digestion to 31% after 60 min digestion and to 51% after 180 min for the uncooked sample (Fig. 6). Cooked

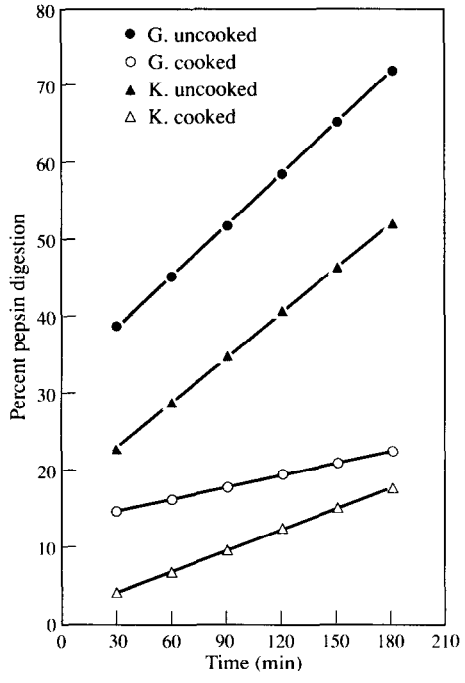

Fig. 5. Percent pepsin digestion of uncooked and cooked Gadam Elhamam and Karamaka sorghum cultivars treated with 0.5 M sodium bisulphite versus time in minutes.

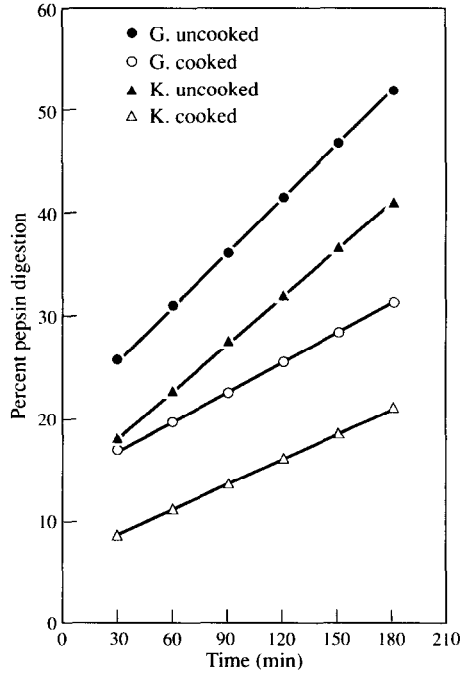

Fig. 6. Percent pepsin digestion of uncooked and cooked Gadam Elhamam and Karamaka sorghum cultivars treated with 0.05 M ascorbic acid versus time in minutes.

samples treated with 0.05 M ascorbic acid were higher in IVPD than cooked untreated samples of the same cultivar. Uncooked Karamaka cultivar treated with 0.05 M ascorbic acid increased protein digestion to 23% after 60 min, which increased to 40% after 180 min. Cooked Karamaka samples treated with 0.05 M ascorbic acid showed slight improvement in protein digestion compared to untreated cooked samples of the same cultivar. After soaking the flour in 0.1 M ascorbic acid, protein digestion increased to 35% after 60 min and 61% after 180 min for uncooked Gadam Elhamam cultivar (Fig. 7). For uncooked Karamaka cultivar, protein digestion increased to 27% after 60 min, which increased to 45% after 180 min. In reduced cooked flour of Karamaka cultivar, 14% of the protein was digested after 60 min, which increased to 27% after 180 min. Increasing the concentration of ascorbic acid beyond 0.1 M (0.25 and 0.5 M) did not bring about an improvement in protein digestion for either uncooked or cooked sorghum cultivar (Figs 8 and 9).

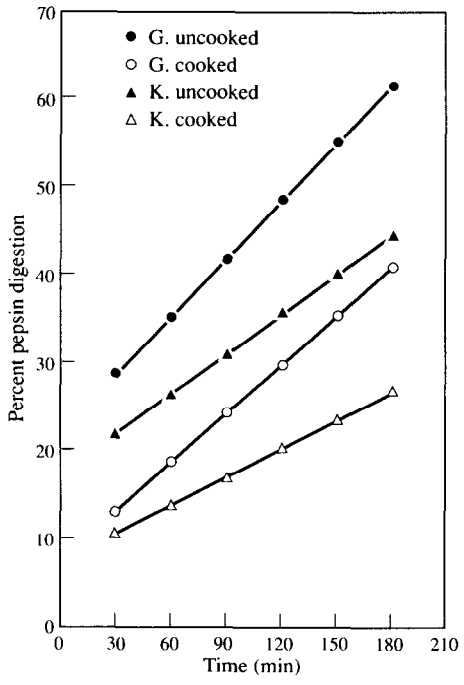

Fig. 7. Percent pepsin digestion of uncooked and cooked Gadam Elhamam and Karamaka sorghum cultivars treated with 0.10 M ascorbic acid versus time in minutes.

DISCUSSION

In vitro pepsin digestion showed that sorghum has low protein digestibility. Tannin content and high levels of disulphide bonds in sorghum protein are mainly responsible for the reduction in digestibility of sorghum protein. Gadam Elhamam (low in tannin) gave higher values for the IVPD than Karamaka (high in tannin) for all treatments. Tannins interact with proteins causing their precipitation. Treatment with reducing agents improved protein digestibility significantly. The effect of reducing agents depends on concentration, digestion time and cooking treatment. Cooking in the presence of reducing agent improved the IVPD significantly compared with untreated samples. Hamaker *et al.* (1987) attributed this to the formation of disulphide

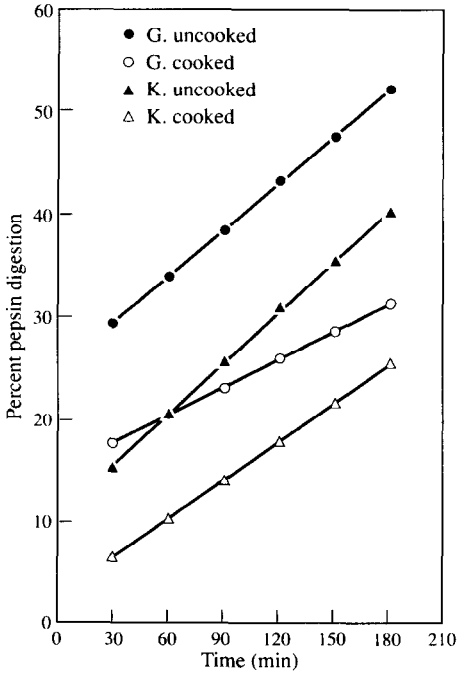

Fig. 8. Percent pepsin digestion of uncooked and cooked Gadam Elhamam and Karamaka sorghum cultivars treated with 0.25 M ascorbic acid versus time in minutes.

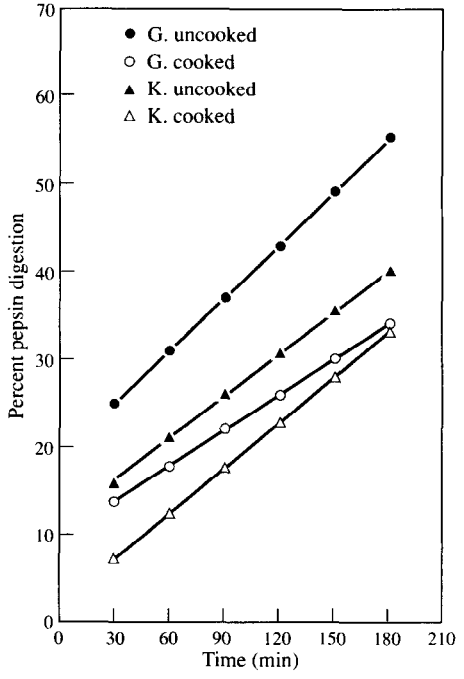

Fig. 9. Percent pepsin digestion of uncooked and cooked Gadam Elhamam and Karamaka sorghum cultivars treated with 0.5 M ascorbic acid versus time in minutes.

bonds which results in toughening at the surface and interior of the protein bodies. Since the reducing agent prevents the formation of disulphide bonds during cooking and makes the sorghum more pepsin-digestible, it is probable that disulphide bonding is responsible for reduced protein digestibility in cooked sorghum.

REFERENCES

Adrian, J. & Sayerse, C. (1957). Composition of Senegal millets and sorghums. *Br. J. Nutr.*, **11**, 99–105.

AOAC (1984). *Official Methods of Analysis*, 10th edn. Washington, DC: Association of Official Agricultural Chemists.

Duncan, B. O. (1955). Multiple range and multiple F test. *Biometrics*, **11**, 1–42.

Eggum, B. O., Monouar, L., Bach Knudsen, K. E., Munck, L. & Axtell, J. (1983). Nutritional quality of sorghum and sorghum foods from Sudan. *J. Cereal Sci.*, **1**, 127–130.

Hamaker, B. R., Kirlies, A. W., Butler, L. G., Axtell, J. D. & Mertz, E. T. (1987). Improving the *in vitro* protein digestibility of sorghum with reducing agents. *Proc. Natl. Acad. Sci.*, **84**, 626–627.

Harden, M. L., Stanaland, R., Briley, M. & Yong, S. P. (1976). The nutritional quality of protein in sorghum. *J. Food Sci.*, **41**, 1082–1085.

Ilori, J. O. & Conrad, J. H. (1976). The nutritive value of protein in selected sorghum lines as measured by rat performance. *Nutr. Rep. Int.*, **13**, 307–314.

Maxson, E. D., Roony, L. W., Lewis, R. W., Clark, L. E. & Johnson, J. W. (1973). The relationship between tannin content, enzyme inhibition, rat performance and characteristics of sorghum grain. *Nutr. Rep. Int.*, **8**, 145–152.

Mertz, E. T., Hassen, M. M., Cairns Whittern, C., Kirlies, A. W., Tu, L. & Axtell, J. D. (1984). Pepsin digestibility of protein in sorghum and other major cereals. *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 1–2.

Mitaru, B. N. & Blair, R. (1984). Comparative effect of cooking and high moisture storage of sorghum in protein digestibility in rats. *Nutr. Rep. Int.*, **30**, 397–399.

Mitaru, B. N., Reichert, R. D. & Blair, R. (1985). Protein and amino acid digestibilities for chickens of reconstituted and boiled sorghum grain varying in tannin contents. *Poult. Sci.*, **50**, 455–456.

Snedecor, G. W. & Cochran, W. G. (1987). *Statistical Methods* (7th edn). The Iowa State University Press, Ames, IA, pp. 221–222.