Effect of cooking, pH and polyphenol level on carbohydrate composition and nutritional quality of a sorghum (*Sorghum bicolor* (L.) Moench) food, ugali

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1. The present work was undertaken to study the effects of cooking, pH and polyphenol level on carbohydrate composition and nutritional quality of sorghum (*Sorghum bicolor* (L.) Moench). Three different sorghum varieties; Dabar, Feterita and Argentine containing zero, intermediate to low and high levels of polyphenols respectively were used in the study. From these varieties uncooked, uncooked acidified, cooked, and cooked acidified diets were prepared. Diets were characterized with regard to resistant starch (RS), dietary fibre (DF), acid-detergent fibre (ADF) and amino acid content. Raw materials were further analysed for content and composition of non-starch polysaccharides and Klason lignin. The nutritional properties were studied in balance trials with rats. True protein digestibility (TD), biological value (BV), net protein utilization, digestible amino acids, digestible energy (DE) and digestible DF were used as criteria in the nutritional study.

2. Cooking at neutral and acid pH resulted in significantly higher assayed values for DF. Increase in DF could be accounted for by formation of RS. Approximately 50% of RS was recovered in the faeces.

3. In vitro values for protein associated with ADF and in vivo balance values using rats suggest that an endosperm protein fraction, kafirins, was made unavailable during cooking. This resulted in reduced TD and increased BV. It is assumed that unavailable kafirins serve as a nitrogen source for microflora in the hind-gut.

4. Dietary polyphenols changed the excretory route for N from urine to faeces. This resulted in lower TD and higher BV in Argentine (high in polyphenols) than in Dabar and Feterita (low in polyphenols), although dietary lysine (first limiting amino acid) was the same in the three varieties.

5. Variation in DE of the diets was attributed to DF, RS and the amount of faecal protein, which in turn were influenced by undigested kafirins and polyphenols.

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the most important food constituents in arid parts of Africa and Asia. In these areas sorghum serves as the principal form of protein and energy for several hundred million people (Hulse *et al.* 1980). Based on nutrient composition, energy of low-polyphenol sorghum varieties are expected to be highly digestible. The starch content is higher and dietary fibre (DF) lower than in most other cereal staples (Bach Knudsen & Munck, 1985); the former fraction is almost completely hydrolysed and absorbed in the small intestine, while the latter fraction escapes digestion in the small bowel (Keys & DeBarthe, 1974; Englyst & Cummings, 1985; Sandberg *et al.* 1986). In agreement with dietary composition, balance trials in which rats were given unprocessed low-polyphenol sorghum varieties, revealed digestibility of energy (DE) to be in the upper range of DE for cereals (Eggum *et al.* 1983; Pedersen & Eggum, 1983*a, b, c, d*). However, a study by MacLean *et al.* (1981) of preschool children caused doubt about the nutritive value of sorghum. The loss of available energy in faeces was 776 kJ/d when consuming diets based entirely on cooked sorghum ugali compared with 243–477 kJ/d

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when consuming wheat, rice, potato or maize processed in a similar way. Moreover, apparent protein digestibility was only 0·46 for sorghum compared with 0·66–0·81 in reference diets.

Recent studies show that methods used to process sorghum into food have significant implications on availability of nutrients in vivo (Eggum et al. 1983; Bach Knudsen & Munck, 1985). A starch fraction which resists α-amylase (EC 3.2.1.1) digestion in vitro and in vivo was identified in cooked sorghum. This was seen as increased DF content (Bach Knudsen & Munck, 1985) as well as reduced energy digestibility (Eggum et al. 1983) in cooked compared with uncooked sorghum. Furthermore, amino acid analysis of protein associated with acid-detergent fibre (ADF) suggests that an endosperm protein fraction, kafirins, was turned into an unavailable stage during cooking (Bach Knudsen & Munck, 1985). This hypothesis was supported by investigations with rats, as true protein digestibility (TD) was reduced while biological value (BV) increased in products made from cooked compared with uncooked sorghum (Eggum et al. 1983). The overall effect of cooking sorghum was a reduction in digestibilities of protein and energy and enlargement of faecal volume.

Besides these dietary factors, polyphenols present in several sorghum varieties are known to alter digestion and absorption processes in simple-stomach animals (Eggum & Christensen, 1973; Eggum et al. 1983). Since binding between polyphenols and protein is optimal at the isoelectric point of the protein (Butler, 1982), pH of the food might be a factor to consider.

The present investigation was undertaken to study the effects of cooking, pH and polyphenols on carbohydrate composition and nutritional quality of three sorghum varieties. Criteria used included carbohydrate characteristics and balance trials with growing rats.

EXPERIMENTAL

Raw materials

Whole-grain sorghum of varieties Dabar, Feterita and Argentine (unnamed variety from Argentina) were used in the present investigation. Varieties were selected to belong to groups I, II and III (Asquith et al. 1983) respectively according to their polyphenol concentration in the bran part of the seed (pericarp + testa). Dabar did not contain detectable amounts of polyphenols. Feterita, having polyphenols in the testa layer, was intermediate to low, while Argentine had a high level of vanillin-reactive polyphenols and condensed tannins in both pericarp and testa (see Table 2, p. 36).

Pretreatment experiment

To determine whether freezing at $-20°$ or freeze-drying influences the analytical value of DF or enzyme-resistant starch (RS) the following experiment was undertaken. A sample of variety Dabar (not the same batch as used in the nutritional experiment) was cooked for 1 h as described by Eggum et al. (1983), cooled to 30° and thereafter frozen in solid carbon dioxide–methanol or at $-20°$. Samples of raw materials, solid CO_2–methanol frozen and $-20°$ frozen were thereafter freeze-dried (lyophilized). Various treatments are shown in Table 3 (p. 36).

Diets for nutritional experiments with rats

From raw materials of the three sorghum varieties the following dishes were prepared: uncooked acidified, cooked, and cooked acidified. Cooked sorghum porridge was prepared according to Eggum et al. (1983) with a cooking time of 1 h. To obtain a pH effect similar to that of fermentation during food preparation in Africa, the pH was adjusted to 3·9 with

mineral acid. Cooked and acidified materials were kept at 20–25° for 20 min, frozen at −20° and thereafter freeze-dried. Samples were labelled as: uncooked (U), uncooked acidified (UA), cooked (C), and cooked acidified (CA). Diets for rat experiments were prepared from raw and cooked materials of all three varieties (Table 1). Diets were kept constant in dietary nitrogen by adding an N-free mixture to obtain 15 g N/kg dry matter (DM). Vitamins and minerals were added as described by Eggum (1973).

Nutritional experiments with rats

The experimental procedures for N balance and digestibility trials have been described by Eggum (1973). Groups of five male Wistar rats, each animal weighing approximately 70 g, were used in the experiments with preliminary feeding periods of 4 d and balance periods of 5 d. Each animal received approximately 150 mg N and 10 g DM daily in the diet throughout preliminary and balance periods. TD, BV, net protein utilization (NPU), digestible amino acids, DE and digestible DF were used as criteria for nutritional quality. DE and digestible DF were calculated by difference after correction for digestibility of the N-free mixture of 0·917 (SE 0·013) and digestibility of cellulose powder in the N-free mixture of 0·29 (SE 0·11). Individual faeces and urine samples were analysed for N and energy and used to estimate TD, BV, NPU and DE, while calculation of digestible amino acids and digestible DF were based on analysis of pooled faecal samples.

Analytical methods

Proximate analyses were performed in accordance with standard methods of the Association of Official Analytical Chemists (AOAC) (1975), and amino acids as described by Mason *et al.* (1980). Tannin analyses were done according to Eggum & Christensen (1973). Determination of methanol-soluble phenols was based on their reactions with vanillin. Acid extractions according to methods of Maxson & Rooney (1972) and Butler (1982) were applied. Free glucose, fructose, sucrose and fructans were extracted with acetate buffer (0·1 M, 65°, pH 5·0) and glucose and fructose residues quantified with specific enzymes (Larsson & Bengtsson, 1983). Starch was gelatinized and hydrolysed to oligosaccharides with a thermostable α-amylase (Termamyl®; Novo A/S, Denmark), further degraded to glucose monomers with amyloglucosidase (*EC* 3.2.1.3; catalogue no. A9268; Sigma Chemical Co., Poole, Dorset), quantified with a glucose oxidase (*EC* 1.1.3.4; catalogue no. 124036; Boehringer Mannheim) reagent and converted to polysaccharides with the factor 0·9 in accordance with Bach Knudsen *et al.* (1987).

Total mixed linked $(1 \rightarrow 3, 1 \rightarrow 4)$-$\beta$-D-glucans ($\beta$-glucans) were measured fluorometrically using the method of Jørgensen & Aastrup (1987). Duplicate samples (50 mg) of sorghum flour were weighed into 50 ml centrifuge tubes with screw caps. Deionized water (10 ml) was added and the sample boiled in a water-bath for 1 h. After cooling to room temperature, 10 ml perchloric acid (0·1 M) was added and the sample boiled for a further 10 min to solubilize the β-glucans. Finally, the suspensions were centrifuged (3000 *g*, 10 min). The β-glucan levels in the supernatant fraction were quantified by complexing with Calcofluor (Calcofluor®; Polysciences Ltd, Northampton) and measuring the β-glucan–Calcofluor complex fluorometrically. Excitation was at 350 nm and emission at 425 nm.

Total DF (TDF) content was assayed by a gravimetric method based on enzymic digestion of starch and proteins as described by Asp *et al.* (1983). According to this method, DF can be classified into insoluble (IDF) and soluble (SDF) components. The pepsin (catalogue no. 7190; Merck) incubation step was carried out at pH 1·5 for 1 h, and the pancreatin (catalogue no. P1750; Sigma Chemical Co.) step at pH 6·8 for another hour. Starch was digested by a thermostable α-amylase during a 30 min gelatinization step at

Table 1. *Composition of experimental diets (g/kg dry matter)*

Variety...	Dabar				Feterita				Argentine			
Diet no...	1	2	3	4	5	6	7	8	9	10	11	12
Treatment...	U	UA	C	CA	U	UA	C	CA	U	UA	C	CA
Whole-grain sorghum (*Sorghum bicolor* (L.) Moench)	717·4	715·7	715·7	710·0	696·5	713·6	696·7	699·8	822·1	800·8	814·5	819·3
Nitrogen-free mixture*	226·6	228·3	228·3	234·0	247·5	230·4	247·3	244·2	121·9	143·2	129·5	124·7
Mineral mixture	40·0	40·0	40·0	40·0	40·0	40·0	40·0	40·0	40·0	40·0	40·0	40·0
Vitamin mixture	16·0	16·0	16·0	16·0	16·0	16·0	16·0	16·0	16·0	16·0	16·0	16·0

U, uncooked; UA, uncooked acidified; C, cooked; CA, cooked acidified; for details of treatments, see pp. 32–33.
* Providing (g/kg): sucrose 90·0, cellulose powder 520, soya-bean oil 52·0, potato starch (autoclaved) 806·0.

100°. Ash and protein contents of IDF and SDF fractions were determined using standard AOAC methods (AOAC, 1975).

ADF was determined gravimetrically after extraction of starch, proteins and hemi-celluloses with an acid detergent solution according to Van Soest (1963). Protein and ash were determined in the fibre residues.

Non-starch polysaccharides (NSP) of DF were determined as alditol acetates by gas–liquid chromatography (GLC) for neutral sugars and by a decarboxylation method for uronic acids as described by Theander & Åman (1979) and Theander & Westerlund (1986). Polysaccharides, after quantitative removal of starch, were swelled with sulphuric acid (12 M, 30°, 60 min), hydrolysed with 0·41 M-H_2SO_4 (125°, 60 min), reduced with potassium borohydride to alcohols and acetylated using 1-methylimidazole to catalyse the reaction; myo-inositol was used as internal standard. The NSP constituent sugar values were converted to polysaccharides by the factor 0·9.

The content of cellulose in NSP was calculated as $NSP_{glucose}$ minus β-glucan, and non-cellulosic polysaccharides (NCP) as arabinose + xylose + mannose + galactose + uronic acid + β-glucan. Lignin was defined as the residue resistant against sulphuric acid and measured gravimetrically as Klason lignin (Theander & Westerlund, 1986).

RS, i.e. starch not hydrolysed by incubation with α-amylase in the fibre determination, was solubilized from fibre residues by 2 M-potassium hydroxide, following the description of Englyst *et al.* (1982). The procedure is identical with the assay of TDF residues. Recovered fibres were quantitatively transferred to a 50 ml centrifuge tube and 5 ml 2 M-KOH added and stirred for 0·5 h, at room temperature (Whirlimixer, then magnetic stirrer). Saturated benzoic acid (25 ml) was added and 3 ml of this suspension, while still mixing, was transferred to a small centrifuge tube. Then followed the addition of 1 ml 2 M-acetic acid and 1 ml amyloglucosidase solution (20 U amyloglucosidase (catalogue no. 39114; BDH, Poole, Dorset) per ml 0·1 M-acetic acid buffer, pH 4·5, 20 mM in calcium chloride) and incubation for 1 h at 65°. Released glucose monomers were quantified with a glucose oxidase (catalogue no. 124036; Boehringer Mannheim) reagent and converted to polysaccharides by the factor 0·9.

Statistical methods

Rat nutritional values were examined by a three-way analysis of variance model, as outlined by Snedecor & Cochran (1973):

$$X_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \epsilon_{ijkl},$$

where X_{ijkl} is the dependent variable (i.e. TD, BV, NPU, DE); μ is the overall mean; α_i is the effect of sorghum variety; β_j is the effect of cooking; and γ_k is the effect of acidification. ϵ_{ijkl} is a normal distributed random variable. The 95% confidence interval was calculated as $\overline{X} \pm t_{0.975df} \cdot (s/\sqrt{n})$.

Digestibility of DF was examined by a one-way analysis of variance model according to Snedecor & Cochran (1973):

$$X_{ij} = \mu + \alpha_i + \epsilon_{ij},$$

where X_{ij} is the dependent variable (i.e. digestible DF); μ is the overall mean; α_i is the effect of sorghum variety (i.e. Dabar, Feterita or Argentine) and ϵ_{ij} is a normal distributed random variable.

Table 2. *Carbohydrate composition of raw materials of sorghum* (Sorghum bicolor (L.) *Moench) flours of varieties Dabar, Feterita and Argentine* (g/kg dry matter)

	Dabar	Feterita	Argentine
Low-molecular-weight sugars:			
Glucose	2·6	2·4	2·4
Fructose	1·2	1·0	1·0
Sucrose	8·7	6·8	6·3
Fructan	1·0	1·2	0·8
Total sugars	13·5	11·4	10·5
Starch	701·1	713·7	719·6
NSP:			
Cellulose	35·4	28·5	38·2
NCP	34·4	30·6	34·3
Arabinose	11·4	10·2	11·4
Xylose	10·8	9·7	10·7
Mannose	1·0	1·0	1·4
Galactose	2·5	2·4	2·5
Glucose	2·8	2·6	2·5
Uronic acid	5·9	4·7	5·8
Total NSP	69·8	59·1	72·5
Klason lignin	10·8	17·4	39·0
Dietary fibre	80·5	76·5	114·3
TDF	85·4	83·5	108·8

NSP, non-starch polysaccharides; NCP, non-cellulosic polysaccharides; TDF, total dietary fibre determined gravimetrically.

Table 3. *Chemical composition of uncooked* (U), *uncooked acidified* (UA), *cooked* (C) *and cooked acidified* (CA)* *whole-grain sorghum* (Sorghum bicolor (L.) *Moench) flours for diets of varieties Dabar, Feterita and Argentine* (g/kg dry matter)

Variety	Treatment	Crude protein (nitrogen × 6·25)	Ash	Fat	Starch	Tannin	Catechin equivalents
Dabar	U	122·5	14·7	32·0	701·1	4·2	0·0
	UA	122·5	14·5	32·5	693·3	5·1	0·0
	C	124·0	15·4	21·0	700·3	5·8	0·0
	CA	127·5	14·7	17·5	694·7	6·2	0·0
Feterita	U	128·0	18·9	35·0	713·7	4·3	2·6
	UA	125·5	18·3	35·5	704·9	4·6	2·3
	C	128·0	18·5	28·5	698·9	3·2	1·1
	CA	125·5	18·1	27·5	693·5	3·7	1·6
Argentine	U	110·0	15·1	33·0	719·6	23·0	31·9
	UA	110·0	14·4	32·0	721·5	12·3	22·1
	C	113·5	14·2	20·0	705·8	12·0	2·2
	CA	113·5	14·9	23·0	706·7	10·7	4·4

* For details of treatments, see pp. 32–33.

RESULTS

Chemical composition of raw materials

Chemical composition (DM basis) of raw materials (U) used to prepare diets for the nutritional experiment with rats are shown in Tables 2–3. DF and starch were higher in Argentine (108·8 and 719·6 g/kg respectively) than in Dabar and Feterita where these

Table 4. *Effect of pretreatment of sorghum* (Sorghum bicolor (*L.*) *Moench*) *samples on neutral sugars of non-starch polysaccharides and resistant starch* (*RS*) (*g/kg dry matter* (*DM*))

Treatment	Constituent neutral sugars (g/kg DM)					
	Arabinose	Xylose	Mannose	Galactose	Glucose*	RS
RM	11·5	10·9	1·1	2·0	35·4	1·5
RM, CO_2, −20°	12·4	11·8	1·2	3·0	35·0	2·4
RM, CO_2, −20°, FD	12·4	11·8	1·0	2·3	34·0	2·5
C, 30°, CO_2, −20°	11·8	12·2	1·0	2·8	44·7	10·4
C, 30°, CO_2, −20°, FD	12·2	12·8	1·0	2·4	40·0	8·4
C, 30°, −20°	11·8	11·3	1·0	2·5	46·0	11·4
C, 30°, −20°, FD	11·3	10·9	0·9	2·0	40·0	8·3

RM, raw material; C, cooked for 60 min; 30°, cooled to 30° thereafter frozen; CO_2, frozen in solid carbon dioxide–methanol; −20°, kept for 120 h at −20°; FD, freeze-dried (lyophilized).
* Glucose values include RS.

dietary constituents ranged from 83·5 to 85·4 and 701·1 to 713·7 g/kg respectively. The higher DF was accounted for by higher Klason lignin and slightly higher cellulose content. Low-molecular-weight sugars made up 10·5–13·5 g/kg, with sucrose accounting for more than 60 % of the sugar fraction. Protein displayed a variation from 110·0 g/kg in Argentine to 128·0 g/kg in Feterita. Amino acid composition was approximately the same in all sorghums with levels of lysine ranging from 2·0 to 2·1 g/16 g N.* However, in contrast to other cereals a huge amount of protein was associated with TDF and ADF. As a percentage of total protein 44, 61 and 95 % was associated with TDF, and 21, 34 and 20 % with ADF for Dabar, Feterita and Argentine respectively.

Amino acid composition of protein associated with ADF showed a high degree of similarity to endosperm protein kafirins. Characteristic for this protein is a high level of glutamic acid, proline, alanine and leucine but a low level of lysine. Contents (g/kg total amino acid) of glutamic acid, proline, alanine, leucine and lysine in kafirins were: 227, 106, 117, 174 and 2 respectively compared with 248, 91, 103, 174 and 3 respectively of protein associated with ADF (means of Dabar, Feterita and Argentine). Amino acid composition of protein associated with TDF was very close to that of whole grain except for lysine, which was intermediate to whole-grain protein and the kafirins.

Analyses of soluble polyphenols (catechin equivalents) revealed that Dabar did not contain polyphenols, Feterita having low levels of 2·6 g/kg, while Argentine had high levels of 31·9 g/kg.

Effect of cooking and pH on chemical composition

Effects of pretreatment on polysaccharide composition are shown in Table 4. RS in raw materials was low (< 3 g/kg). As a result of cooking, RS increased to 8–11 g/kg and $NSP_{glucose}$ by approximately the same amount. However, no differences were found when diets were frozen at −20° compared with those frozen in solid CO_2–methanol or between freeze-dried and non-freeze-dried samples.

The assayed content of DF in cooked materials used in the nutritional experiment was 10·7–25·4 g/kg higher than that of corresponding uncooked materials (Fig. 1). Increases in DF in the cooked materials were due to the presence of RS (Fig. 1). Moreover, partition of DF into IDF and SDF components and ADF showed that the increase in DF was

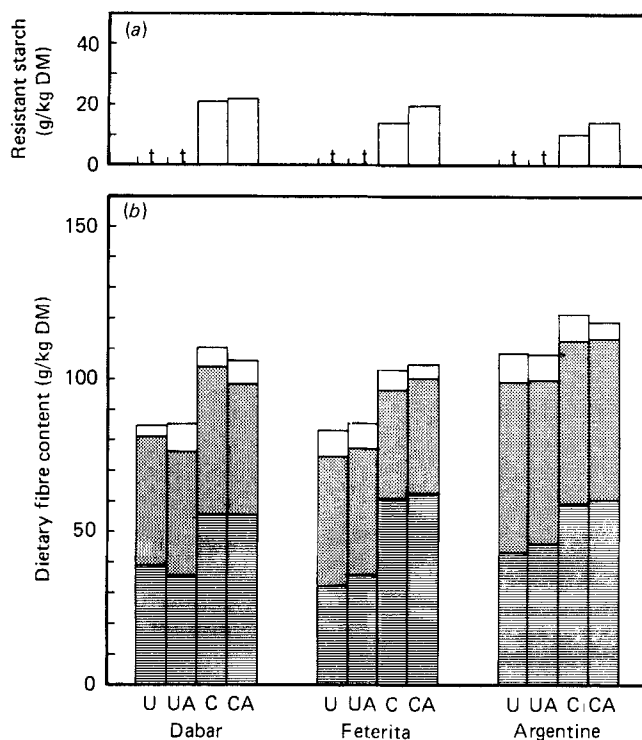* The amino acid data are available from the authors if required.

Fig. 1. (*a*) Resistant starch and (*b*) dietary fibre composition of uncooked (U), uncooked acidified (UA), cooked (C) and cooked acidified (CA) whole-grain sorghum (*Sorghum bicolor* (L.) Moench) flours of varieties Dabar, Feterita and Argentine. (▤), Acid-detergent fibre, ADF; (▤ + ▨), insoluble dietary fibre, IDF; (☐), soluble dietary fibre, SDF; (▤ + ▨ + ☐), total dietary fibre, TDF; t, traces; DM, dry matter.

assigned to IDF and ADF components. In agreement with more starch polysaccharides present as RS, analysed values for starch in cooked products were reduced by 13–14 g/kg (Table 3).

As expected, cooking and acid treatment (C, UA, CA) had no significant influence on either protein level or amino acid composition (Table 3). However, significantly more kafirin-like protein was associated with ADF. This was the case irrespective of whether the material was cooked at neutral or acid pH (C, CA) (Fig. 2). Acidification and cooking liberates polyphenols and condensed tannins from the matrix. Hence, in the variety Argentine, soluble polyphenols were reduced from 31·9 g/kg in uncooked (U) to 22·1 g/kg in acidified (UA) and further to 2·2–4·4 g/kg in cooked and cooked acidified (C, CA). Tannin values followed the same tendency, although absolute levels were different.

DE and digestibility of DF

DE of uncooked (U) Dabar and Feterita were approximately the same (0·906–0·902), whereas DE of Argentine was significantly lower (0·848) (Fig. 3, Table 5). Acidification without cooking (UA) reduced DE of Argentine to 0·829. Cooking had a clear impact on DE of all diets. Hence DE was reduced in response to cooking at neutral pH (C v. U) by 0·024, 0·023 and 0·063 and at acid pH (CA v. UA) by 0·028, 0·020 and 0·016 in diets of Dabar, Feterita and Argentine respectively. These effects are displayed in the analysis of
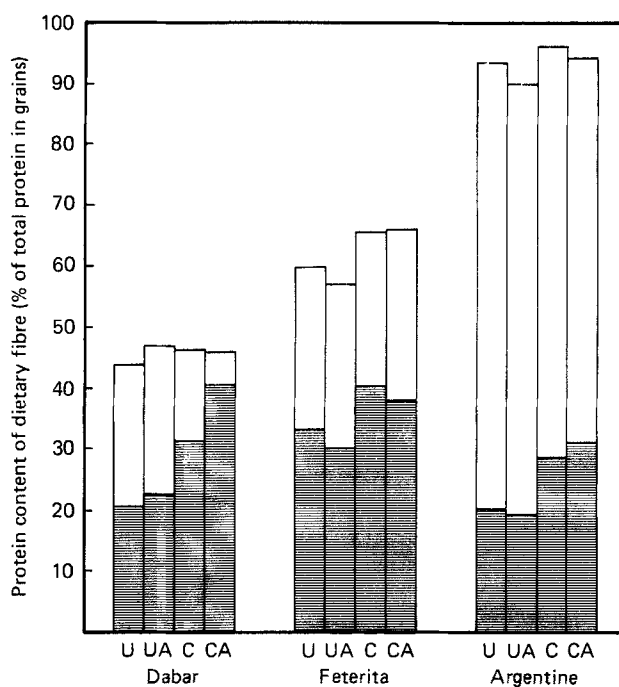
Fig. 2. Protein associated with total dietary fibre (▦ + ☐) and acid-detergent fibre (▦) of uncooked (U), uncooked acidified (UA), cooked (C) and cooked acidified (CA) whole-grain sorghum (*Sorghum bicolor* (L.) Moench) flours of varieties Dabar, Feterita and Argentine.

variance table, showing main effects of variety (polyphenols) and cooking as well as interactions between all three factors (variety, cooking, acidification) (Table 5).

DF and protein were the two bulk substances of faecal DM, making up 63 and 21 % from Dabar and Feterita and 53 and 27 % in faeces of Argentine (means of U and UA). As a result of cooking the quantitative excretion of DF + RS and protein in faeces increased. Digestibility of DF was different in the three varieties (Fig. 4). Digestibility of DF was 0·34 in Dabar, 0·22 in Feterita, and 0·20 in Argentine.

TD, BV, NPU and digestibility of amino acids

TD was much lower in uncooked (U) Argentine (0·752) compared with 0·950 and 0·957 in Dabar and Feterita respectively (Fig. 5, Table 5). Moreover, acidification (UA) reduced TD further to 0·678 in Argentine. This is in contrast to the situation for Dabar and Feterita where TD remained high at 0·965 and 0·963 respectively. Cooking at neutral (C) and acid pH (CA) had a significant negative impact on TD, the response, however, being significantly different between Dabar and Feterita compared with Argentine. Reduction in TD was 0·04–0·058 for Dabar and Feterita while it was 0·312 when Argentine was cooked at neutral pH and 0·111 when cooked at acid pH. These effects explain the two- and three-way interactions shown in the analysis of variance in Table 5.

BV did not correlate with lysine level (Fig. 5). For all three varieties the same lysine concentration was found, but BV was 0·10 higher in Argentine (0·65) than in Dabar and Feterita (0·55). On the other hand NPU was approximately the same in all three varieties in the range 0·49–0·52. As a result of cooking at neutral pH (C), BV increased by 0·05 in Dabar and Feterita which thus compensated for the decrease in TD, leaving NPU virtually
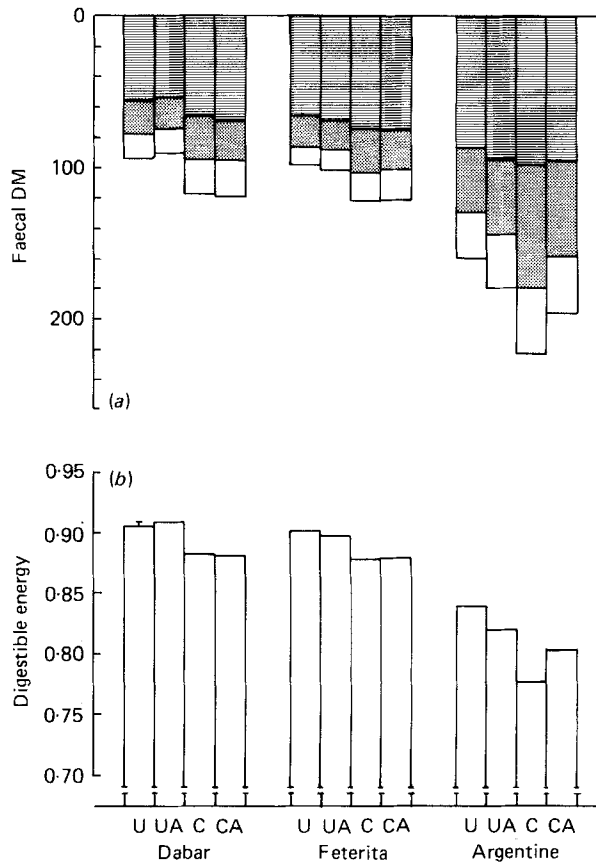
Fig. 3. (a) Partition of faecal dry matter (DM) in dietary fibre + resistant starch (▦), protein (▨) and residues (☐) and (b) digestible energy of uncooked (U), uncooked acidified (UA), cooked (C) and cooked acidified (CA) whole-grain sorghum (*Sorghum bicolor* (L.) Moench) flour of varieties Dabar, Feterita and Argentine. 95% confidence interval represented by vertical bar.

Table 5. *Analysis of variance for true protein digestibility (TD), biological value (BV), net protein utilization (NPU), and digestible energy (DE) of values expressed in Figs. 3 and 5*

	TD	BV	NPU	DE
Pooled SE	0.021	0.038	0.034	0.007
CV (%)	2.5	6.4	7.1	0.9
Variety (V)	1628.6***	55.0***	81.0***	661.8***
Cooking (C)	383.4***	12.6***	2.8NS	158.4***
Acid (A)	11.1***	17.2***	18.1***	0.0
V × C	100.1***	0.9NS	0.4NS	7.8**
V × A	0.7NS	3.8*	11.0***	0.6NS
C × A	44.5***	0.7NS	3.8*	12.0***
V × C × A	37.2***	1.5NS	14.4***	11.7***

CV, coefficient of variation; NS, not significant.
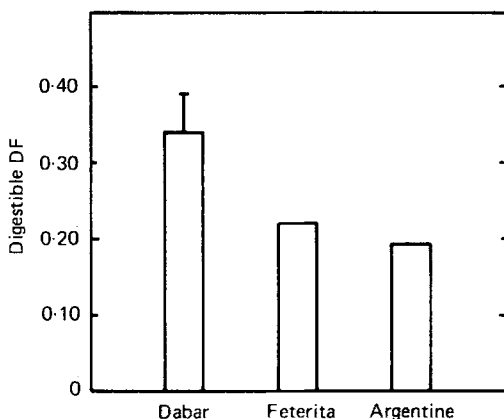* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Fig. 4. Digestibility of dietary fibre (DF) of Dabar, Feterita and Argentine varieties of whole-grain sorghum (*Sorghum bicolor* (L.) Moench) flour. 95% confidence interval represented by vertical bar.
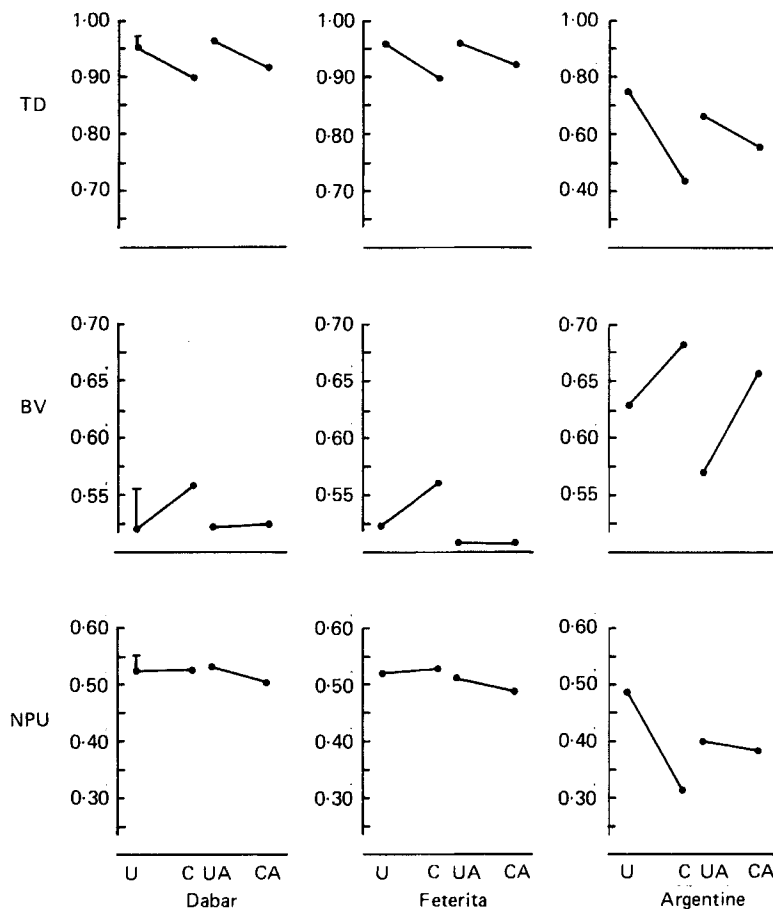

Fig. 5. True protein digestibility (TD), biological value (BV) and net protein utilization (NPU) of uncooked (U), uncooked acidified (UA), cooked (C) and cooked acidified (CA) whole-grain sorghum (*Sorghum bicolor* (L.) Moench) flour of varieties Dabar, Feterita and Argentine. 95% confidence interval represented by vertical bars.
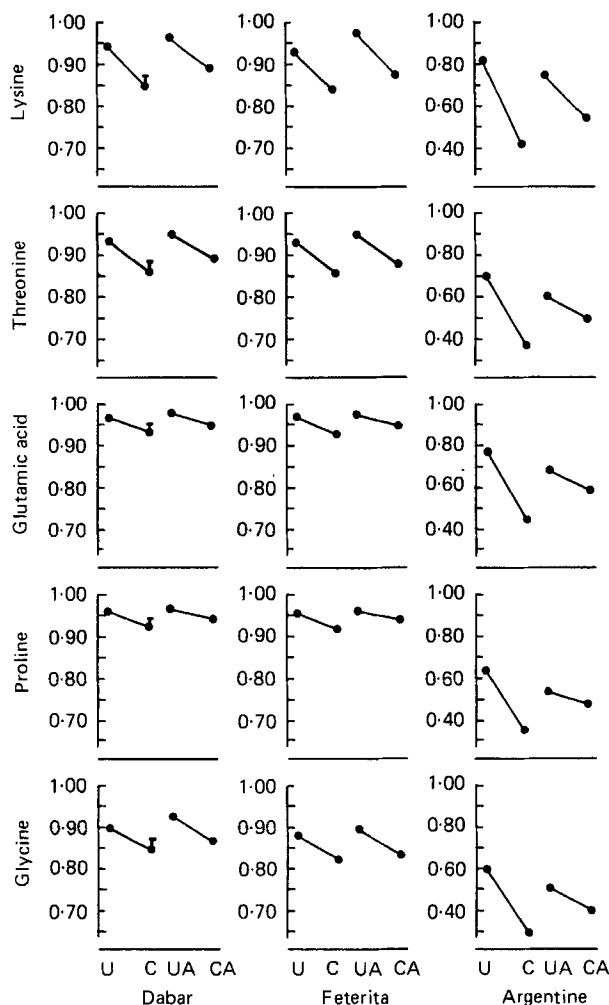
Fig. 6. Digestibilities of lysine, threonine, glutamic acid, proline and glycine of uncooked (U), uncooked acidified (UA), cooked (C) and cooked acidified (CA) whole-grain sorghum (*Sorghum bicolor* (L.) Moench) flour of varieties Dabar, Feterita and Argentine. 95% confidence interval represented by vertical bars. (The full amino acid data set are available from the authors if required.)

constant at 0·52–0·53. In Argentine, cooking caused a slightly stronger increase in BV (0·062) which, however, could not compensate for the strong negative influence of cooking on TD. NPU was therefore reduced from 0·492 in uncooked diets to 0·315 in cooked diets. Cooking at acid pH (CA) did not affect BV of Dabar and Feterita, while BV in Argentine was 0·094 higher resulting in an NPU of Argentine UA and CA of 0·40 and 0·388 respectively.

Amino acids listed in Fig. 6 were selected as they represent different types of proteins, located in various cell tissues. Thus, proteins in germ and bran are rich in lysine and threonine, while glutamic acid, proline and glycine primarily are found in high concentrations in endosperm proteins (i.e. kafirins). Generally digestibility of these amino acids varied in accordance with TD except in the following cases: first, digestibilities of proline and glycine were significantly lower while the digestibility of lysine was higher than

that of total protein in Argentine uncooked (U); second, in Dabar and Feterita, digestibilities of lysine and threonine were reduced progressively more than TD in response to cooking. Decrease in digestibility of lysine was 0·075 to 0·100 and of threonine 0·060 to 0·075, which was appreciably higher than of the non-essential amino acids glutamic acid, proline and glycine, which were reduced by 0·03–0·06.

DISCUSSION

RS

As a result of cooling cooked porridge ugali, a starch fraction undergoes recrystallization and hence resists hydrolysis with α-amylase. In the cooked products from the pretreatment and the nutritional experiment, RS was identified at levels of 8–23 g/kg. This amount approximates what was earlier found in sorghum ugali (Bach Knudsen & Munck, 1985) and in other heat-processed products such as bread, cornflakes, porridges and boiled potato (Englyst *et al.* 1982; Englyst & Cummings, 1984; Johansson *et al.* 1984; Bjørck *et al.* 1986). Neither the freezing technique nor freeze-drying itself resulted in any further increase in RS or $NSP_{glucose}$. This was also found by Johansson *et al.* (1984) and by Gøranzon & Forsum (1987). However, in contrast to these findings Englyst *et al.* (1982) found an increase in $NSP_{glucose}$ of 24·6 g/kg when boiled potato was frozen at $-25°$ compared with freezing in solid CO_2–methanol. When freeze-drying the latter sample, $NSP_{glucose}$ increased by 41·0 g/kg. The reason for differing results in the two experiments with regard to the effect of freezing technique and freeze-drying is most likely due to the procedure for starch removal before NSP determination. In our study and in the Swedish studies (Johansson *et al.* 1984; Gøranzon & Forsum, 1987), starch was removed with thermostable α-amylase in accordance with Theander & Åman (1979) and Asp *et al.* (1983), while mammalian α-amylase was used in the method of Englyst *et al.* (1982, 1983). It is known that the hydrolytic capacity of the former system is much higher than that of the latter (Åman & Hesselman, 1984). Hence, RS values reported in the literature will differ in accordance with the method used. RS defined by the method used in the present study will most likely consist of irreversible retrograded starch in the form of amylose.

Results from the pretreatment experiment further confirm the relevance of working with frozen and freeze-dried samples when studying nutritional properties of sorghum in relation to man. Although sorghum is not processed in this way before consumption by man, there is no doubt that the effect of treatment revealed in the present study will be consistent with that found in fresh samples.

Increases in DF levels of cooked diets when assayed by gravimetric methods are accounted for by formation of RS. In vivo this results in a larger stool due to higher faecal excretion of DF + RS and protein. Calculations based on dietary and faecal increases in assayed DF through cooking (diet C + CA *v.* U + UA), indicate that approximately 50 % of RS was recovered in the faeces. Current information indicates that not only DF but most RS resists breakdown in the small intestine (Englyst & Cummings, 1985; Bjørck *et al.* 1986). RS will therefore serve as an energy substrate for micro-organisms in the lower gut, this being one of the reasons for higher excretion of protein in faeces when consuming cooked diets. However, more detailed studies are needed to clarify the site and extent of RS degradations in different segments of the digestive tract.

DF

DF content of uncooked sorghum grains (83·5–108·8 g/kg) was in accordance with DF levels recently reported (Nyman *et al.* 1984; Bach Knudsen & Munck, 1985). However these values were lower than those of most other cereal grains such as wheat, rye, maize,

rice and barley whose DF content is in the range 94·0–188·0 g/kg (Nyman *et al.* 1984; Bach Knudsen & Munck, 1985). In spite of the lower DF level in sorghum, digestibility of this fraction in Dabar diets (0·34) is in the same range as found in previously mentioned cereals of 0·31–0·44 (Nyman *et al.* 1985). On the other hand, DF digestibility of Feterita and Argentine whole-grain flour was significantly lower. This is explained by the higher Klason lignin content in Feterita and Argentine than in Dabar. Lignin resists bacterial breakdown in the hind-gut (Dintzis *et al.* 1979; Nyman & Asp, 1982; Nyman *et al.* 1985) and reduces degradation of polysaccharides in secondary lignified cell walls, e.g. cellulose and NCP (Cummings, 1981). In Argentine, however, it cannot be excluded that high polyphenol levels are of importance. Experiments with ruminants indicate that polyphenols can associate with endogenous and exogenous proteins in the gut and thus reduce the amount of N available for microbial growth. These observations are based primarily on lower in vitro DM digestibility and second on lower gas production per g DM of high-tannin sorghum than of low-tannin sorghum grain (Donnelly & Anthony, 1969; Saba *et al.* 1972).

Endosperm proteins, kafirins

The suggested effect of cooking on the availability of endosperm protein, kafirins (Bach Knudsen & Munck, 1985) is also identified in the present study. TD was reduced while BV increased in cooked materials derived from Dabar, indicating a changed essential:non-essential amino acids ratio in absorbed protein. However, unavailable kafirins serve as a N source for microflora in the lower gut. This can be seen by significantly stronger negative effects of cooking on digestibility of essential amino acids (0·07–0·09) than of total protein (0·05) and of non-essential amino acids (0·03–0·05). Since the concentrations of lysine and threonine in bacterial protein are high (Mason & Palmer, 1973), small changes in the ratio of bacterial: total faecal protein will have strong negative implications on digestibility values of these amino acids.

Polyphenols

Dietary polyphenols influence digestibility of DF and protein as well as the ratio, essential: non-essential amino acids in absorbed protein. In in vitro measurements, significantly higher association of protein with TDF in Argentine than in the two other varieties was an effect of polyphenols. In vivo, significantly lower digestibility of proline and glycine compared with lysine in Argentine than in Dabar was due to the selective effect of polyphenols. A similar selective effect of polyphenols was identified in a study where purified tannins were added to a diet based on soya-bean meals (Eggum & Christensen, 1973).

The net effect of polyphenols is a change in the route of excretion for N from urine to faeces. This is illustrated by a BV which was 0·10 higher for Argentine than for Dabar and Feterita, although dietary lysine was at the same level in the three varieties. NPU values of 0·49–0·53 in the three sorghum varieties further confirmed this conclusion. Moreover acidification and cooking resulted in a further reduction in digestibility of protein and amino acids of Argentine. The reason for this is undoubtedly that these treatments liberate condensed polyphenols from their matrix, which thus react with glycine and proline residues in dietary proteins. Evidence for this are significantly lower values for catechin equivalents and tannins in acidified and cooked diets than in uncooked diets of Argentine. A similar conclusion was drawn by Price *et al.* (1980) when cooking batter of a high-tannin variety. The analysed values for tannin decreased from 19 to 1 g/kg DM and the ability to bind exogenous bovine serum albumin (BSA) decreased from 0·93 of added BSA in uncooked to 0·07 in cooked samples. However, reduction in digestibility of protein and amino acids following acidification and cooking of the variety Argentine was not compensated by a higher BV.

In contrast to the situation in Argentine, polyphenols present in Feterita did not influence TD and BV either in uncooked or in cooked materials. Although polyphenols are present in Feterita, which in vitro influences the amount of protein associated with TDF, the effect of cooking on TD, BV, NPU and amino acid digestibility was exactly the same in Feterita as in Dabar.

Effect of acidification on nutritive value

Fermentation, which lowers pH in gruel, is a common procedure for preparing sorghum diets (Vogel & Graham, 1978). A protective effect of acid during cooking, which is independent of fermentation, was suggested by Eggum *et al.* (1983) as TD and BV of cooked acidified ugali of Dabar were unchanged when compared with uncooked diets. A similar protective effect of acid could not be identified in the present experiment in diets based on varieties Dabar and Feterita. On the contrary, a beneficial effect of acidification was demonstrated in Argentine diets. TD and BV were 0·57 and 0·68 for the cooked acidified diet compared with 0·44 and 0·72 for the cooked diet. However, for uncooked Argentine based diets TD and BV were 0·75 and 0·66 respectively. Therefore to obtain fully the beneficial effect of acidification, liberated polyphenols should be washed out before cooking. This technique is already applied when preparing sorghum foods from high-tannin varieties in East Africa (Mukuru *et al.* 1982). According to this procedure, the condensed polyphenols are liberated by soaking the flour in acids (tamarind) or alkali (pot ash) and liberated polyphenols are removed by washing before cooking. On the other hand it is very unlikely that acidification (not to compare with fermentation) will have any beneficial effect on nutritional value of sorghum varieties not containing polyphenols (e.g. Dabar).

Nutritional value of sorghum compared with other cereals

The TD and DE of 0·95 and 0·90 respectively of the varieties Dabar and Feterita are high and in the upper range of values among cereals of 0·84–0·96 and 0·84–0·95 respectively (Pedersen & Eggum, 1983 *a, b, c, d*). On the other hand BV of Dabar and Feterita (0·55) was inferior to that of most other cereals (0·61–0·81) (Pedersen & Eggum, 1983 *a, b, c, d*) due to a lower concentration of dietary lysine in sorghum than in other cereals. Decreases in protein and energy digestibility as a consequence of cooking ugali are in accordance with decreases found in recent studies with rats (Eggum *et al.* 1982, 1983). These decreases, however, were of much lower magnitude than those reported from experiments with preschool children (MacLean *et al.* 1981). MacLean *et al.* (1981) found an apparent protein digestibility of 0·46, and a loss of available energy in faeces of 766 kJ/d when consuming porridge ugali. These values were significantly lower and higher respectively than of cooked wheat, rice, potato and maize processed in a similar way. Apparent protein digestibility was 0·66–0·81, and loss of available energy in faeces was 243–477 kJ/d. The higher energy loss in faeces which averages 0·21 of intake (DE 0·79) was attributed to undigested protein and carbohydrates (MacLean *et al.* 1981). Based on our results with rats it is reasonable to assume that undigested protein is accounted for by unavailable kafirins and undigested carbohydrates by RS. It is obvious that low digestibility of protein and DE is an effect of processing because a later study (MacLean *et al.* 1983) showed an apparent protein digestibility of pearled extruded sorghum of 0·81, similar to that of a casein control of 0·83 and a DE of 0·914.

Four dietary factors: RS, DF, unavailable endosperm protein kafirins and polyphenols explain the variation in nutritive value of raw uncooked and cooked sorghum products. The RS and nitrogenous substances formed in response to cooking serve as energy and N substrates for hind-gut microflora resulting in a slight reduction in DE and protein digestibility. These factors probably explain the low digestibility of sorghum dishes reported for man.

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