Effect of Cooking on the Protein Profiles and in Vitro Digestibility of Sorghum and Maize

Bruce R. Hamaker, Allen W. Kirleis,* Edwin T. Mertz, and John D. Axtell

Uncooked sorghum was almost as digestible as uncooked maize, when assayed in vitro with pepsin and/or a trypsin–chymotrypsin mixture. However, after cooking, sorghum protein digestibility was significantly decreased. In contrast, cooking maize had no effect on pepsin digestibility and increased trypsin–chymotrypsin and pepsin–trypsin–chymotrypsin digestibility. After cooking, sorghum prolamins, measured by the Landry–Moureaux procedure and SDS-PAGE, became much less soluble and less pepsin digestible than maize prolamins. These findings demonstrate that sorghum and maize proteins behave differently when cooked and may explain why cooked sorghum has a lower digestibility than maize, wheat, and rice in children.

INTRODUCTION

Sorghum grain supplies a large portion of the protein and calories for many people living in the semiarid tropics. In 1981 MacLean et al. (1981) reported results from nitrogen balance studies on Peruvian children that showed that the protein digestibility of cooked sorghum gruel was significantly lower than that of cooked wheat, maize, or rice gruels (46% vs. 81, 73, and 66%, respectively). As tannin has been shown to reduce protein digestibility (Armstrong et al., 1974; Chibber et al., 1980), the sorghum feeding studies were conducted with four low-tannin varieties (two normal and two high lysine). An in vitro assay developed by Axtell et al. (1981), based on the solubilization of proteins following pepsin digestion, also showed that sorghum protein was less digestible than other cereals. Furthermore, the in vitro study showed that the cooking process was responsible for the decreased protein digestibility in sorghum, as the uncooked flour was more digestible than the cooked gruel. Unpublished data (Mertz et al., 1983) from our laboratory show that there is only a slight or no decrease in pepsin digestibility of wheat, maize, and rice following cooking.

Two recent animal studies support the finding that cooking of sorghum has an adverse effect on protein digestibility. Mosha et al. (1983) found that rats had slightly less ability to digest cooked, decorticated, low-tannin sorghum protein than the corresponding uncooked flour. Mitaru et al. (1985), using chickens, found cooked, whole-grain, low-tannin sorghum protein was 31.5% less digestible than the uncooked flour protein. Recent work in our laboratory has shown that both fermentation (Axtell et al., 1981) and heat extrusion (Mertz et al., 1984) prior to cooking improves the in vitro digestibility of sorghum flour. These findings have been confirmed in children by Graham et al. (1985) and MacLean et al. (1983), respectively. In this paper we present data on the changes in the protein profiles of uncooked and cooked sorghum and maize flours and their pepsin-indigestible protein residues.

MATERIALS AND METHODS

Cereal samples consisted of yellow dent maize (1983 crop) and low-tannin sorghum P721N (1982 crop). Whole-grain samples were ground in a Udy Mill (Boulder, CO) to pass through a 0.4-mm screen. Flour was then defatted with petroleum ether on a Soxhlet apparatus. Samples to be cooked were suspended in water (1:10) and put in a boiling water bath for 20 min, producing a gelatinous gruel (Mertz et al., 1984). The cooked gruel was then lyophilized and reground through a 0.4-mm screen.

In Vitro Protein Digestibility Procedures. Enzymes consisted of pepsin (Sigma P-7000; activity 120 units/mg of protein) and trypsin–chymotrypsin (Sigma T-8128; trypsin activity 1045 BAEE units; chymotrypsin activity 739 ATEE units). Flour samples (200 mg) were assayed for protein digestibility by using modifications of the digestibility method as described by Mertz et al. (1984). Thirty-five milliliters of enzyme solution [1.5 g of enzyme/L of 0.1 M KH_2PO_4 buffer; pH 2.0 (pepsin), pH 7.6 (trypsin–chymotrypsin)] was added to the sample, and the resultant mixture was incubated for 2 h at 37 °C in a shaking water bath. Following centrifugation, the residue was resuspended in buffer, centrifuged, dried at 80 °C, digested, and colorimetrically assayed for nitrogen content (AACC, 1983). Digestibility was calculated by subtracting residue nitrogen from total nitrogen, dividing by total nitrogen, and multiplying by 100. For multiple enzyme digestion, the sorghum or maize flour was incubated with pepsin, followed by washing in a neutral buffer and incubation for 1 h with trypsin–chymotrypsin.

Fractionation Procedure for Uncooked and Cooked Sorghum and Maize. Whole-grain proteins were sequentially extracted into five fractions by the Landry–Moureaux method (1970) as described by Guiragossian et al. (1978). One-gram samples were suspended in a 0.5 M NaCl solution at 4 °C to yield albumins and globulins (fraction 1) and low molecular weight nitrogen fragments. The prolamins, kafirin (sorghum) and zein (maize), were then extracted first with 70% 2-propanol (fraction 2) followed by the same alcohol solution plus 2-mercaptoethanol (2-ME) to reduce disulfide bonds (fraction 3). The fourth fraction, glutelin-like, contains proteins soluble in an alkali borate buffer plus 2-ME. The true glutelin fraction (fraction 5), which contains a complex heterogeneous mixture of proteins, was extracted in an alkali borate buffer, containing 2-ME and sodium dodecyl sulfate (SDS). Nitrogen was determined by the micro Kjeldahl method. Because of the drastic changes in solubility following cooking, the classical protein nomenclature may no longer apply to these preparations; therefore, protein fractions

Table I. In Vitro Digestibility of Sorghum and Maize

	% protein digestibility[a]					
	P[b]		TC[b]		P–TC	
	U[c]	C[c]	U	C	U	C
sorghum	80.7	64.8	72.7	57.1	87.6	70.5
maize	81.5	81.9	79.4	87.7	88.3	90.7

[a] Values represent duplicate determinations. [b] P = pepsin, TC = trypsin–chymotrypsin. [c] U = uncooked, C = cooked.

Food Science (B.R.H., A.W.K., E.T.M.) and Agronomy (J.D.A.) Departments, Purdue University, West Lafayette, Indiana 47907.

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Table II. Landry-Moureaux Fractionation of Raw and Cooked Sorghum and Maize

fraction	extractn solvent	% of total N in grain[a] sorghum uncooked	sorghum cooked	maize uncooked	maize cooked
1	0.5 M NaCl	16.6	7.1	19.6	11.1
2	70% 2-propanol	17.3	0.0	34.0	8.1
3	70% 2-propanol, 2-ME	24.5	6.5	10.1	19.5
4	pH 10 buffer, 2-ME	4.8	4.6	10.3	8.3
5	pH 10 buffer, 2-ME, SDS	27.2	53.3	15.9	33.9
6	nonextractable	11.5	25.8	6.6	14.2
% rec		101.9	97.3	96.5	95.1

[a] Mean of four determinations.

in Tables I and II have been listed strictly on the basis of solvent extractability and are referred to in the text as fractions 1–6. Fraction 6 contains the nonextractable nitrogen.

Landry–Moureaux Fractionation of Pepsin-Indigestible Residue. Both uncooked and cooked sorghum and maize flours were digested with pepsin by using the method described above. A sufficient number of pepsin-indigestible residues were pooled, lyophilized, and ground through a 0.4-mm screen. Two-gram samples were then subjected to the Landry–Moureaux fractionation procedure described above.

Gel Electrophoresis. Pooled protein fractions 2–3 and 4–5 extracted by the Landry–Moureaux procedure were prepared for electrophoretic separation by extensive dialysis against at least four changes of purified H_2O at 4 °C and then lyophilized. Samples were completely redissolved in 2% SDS sample buffer. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on a vertical gel electrophoresis system (Pharmacia, Uppsala, Sweden) by using the method of Laemmli (1975). Samples were subjected to electrophoresis under reducing conditions at constant voltage, 60 V for 2 h followed by 120 V for 5 h.

RESULTS AND DISCUSSION

In vitro protein digestibility values for uncooked and cooked sorghum and maize using pepsin, trypsin–chymotrypsin, or pepsin followed by trypsin–chymotrypsin are given in Table I. In all three methods, sorghum digestibility decreased following cooking by approximately 15%. Maize showed a different pattern. The cooked maize gruel was equal to the uncooked meal after pepsin digestion, and greater than the uncooked after trypsin–chymotrypsin (8%) and multiple enzyme digestion (2%). For sorghum, pepsin alone gave differences between the uncooked and cooked flours similar to that obtained by using the multiple enzyme method.

The levels of protein extracted by the various solvents from uncooked sorghum and maize meal (Table II) agree with values obtained by Guiragossian et al. (1978) for sorghum and Misra et al. (1975) for maize. In both sorghum and maize, total alcohol-soluble proteins (fractions 2 and 3) account for approximately 40% of the total protein. However, sorghum contains more than twice the level of fraction 3 proteins compared to maize (Table II). In contrast, maize has twice the level of alcohol-soluble (fraction 2) proteins (34% in maize compared with 17% in sorghum). The fraction 4 proteins in the uncooked maize are twice the level of the same proteins in sorghum, whereas the fraction 5 proteins in the uncooked maize account for half of the quantity in sorghum.

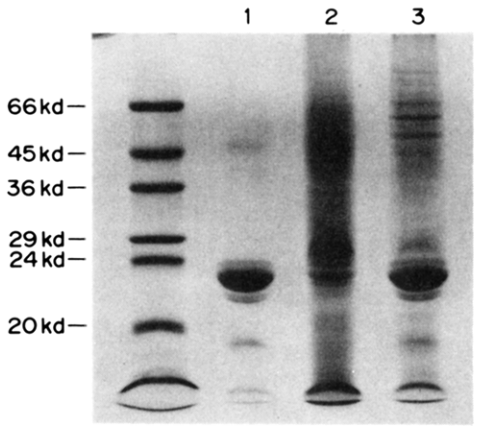

Figure 1. SDS-PAGE patterns of sorghum fractions: lane 1, uncooked sorghum prolamins (fractions 2 and 3); lane 2, uncooked sorghum glutelins (fractions 4 and 5); lane 3, cooked sorghum fractions 4 and 5; left lane, proteins of known molecular weight (kd = kilodaltons).

Table III. Landry-Moureaux Protein Profile of Pepsin-Indigestible Residue

fraction	extractn solvent	% of total N in grain[a] sorghum uncooked	sorghum cooked	maize uncooked	maize cooked
1	0.5 M NaCl	1.3	2.5	2.8	2.9
2	70% 2-propanol	1.3	3.2	1.5	1.8
3	70% 2-propanol, 2-ME	0.0	0.0	0.0	0.0
4	pH 10 buffer, 2-ME	5.4	5.8	5.0	4.9
5	pH 10 buffer, 2-ME, SDS	6.6	10.7	4.4	4.2
6	nonextractable	4.7	13.0	4.8	4.3
% indigestible protein		19.3	35.2	18.5	18.1

[a] Mean of four determinations.

When sorghum or maize are cooked (1:10 ratio of flour to water in a boiling water bath for 20 min) the solubility of the proteins is altered, in particular the prolamins (fractions 2 and 3) (Table II). Nonextractable proteins significantly increase following cooking to 25.8% for sorghum vs. 14.2% for maize. Cooking sorghum and maize meals reduced the proportion of fractions 1 and 2, reduced fraction 3 in sorghum, and increased it in maize. Fractions 5 and 6 were doubled following cooking in both sorghum and maize, accounting for approximately 80% of the protein in sorghum and 48% in maize. Thus, the shift in alcohol-soluble proteins is more pronounced in sorghum than in maize. By SDS-PAGE it has been shown (Paulis and Wall, 1979) that alcohol-soluble proteins (fraction 2 and 3) of uncooked sorghum have molecular weights in the 20 000–24 000-Da range. Examination of these proteins (fractions 2 and 3) in sorghum by SDS-PAGE (Figure 1) shows that prolamin-type proteins do in fact appear in pooled fractions 4 and 5 after cooking as evidenced by the characteristic 20 000–24 000-Da proteins in lane 3. Fractions 2 and 3 are therefore partially (or completely) rendered alcohol insoluble by cooking.

Table III shows the protein profiles of the pepsin-indigestible residues of the uncooked and cooked sorghum and maize. The amount of indigestible protein is significantly larger in the cooked sorghum (35.2%) compared to the uncooked sorghum (19.3%), while there is essentially no difference in cooked and uncooked maize. This indicates that the indigestible sorghum proteins are increased

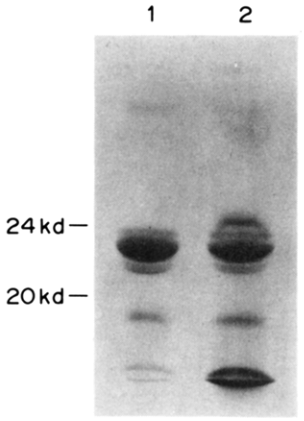

Figure 2. SDS-PAGE patterns of sorghum fractions: lane 1, uncooked sorghum prolamins (fractions 2 and 3); lane 2, proteins found in fractions 4 and 5 of the pepsin-indigestible residue of cooked sorghum.

during the cooking process while the maize proteins are not. The overall profiles are similar except for fractions 5 and 6. Here, the amount in fractions 5 plus 6 in the cooked sorghum is 2.5–3 times higher than in maize. This largely accounts for the pepsin digestibility difference between sorghum and maize seen in Table III. Fractions 2 and 3 appear to be highly digestible as they make up a smaller proportion of the pepsin-indigestible residue in both uncooked and cooked sorghum (Table III). However, electrophoretic examination of the proteins present in the pooled fractions 4 and 5 shows that the 20 000–24 000-Da prolamin bands predominate in these fractions (Figure 2). The glutelin proteins from uncooked flour (Figure 1, lane 2) have very little of this molecular weight protein. The band appearing in the 10 000–15 000-Da range may come from the glutelin class of proteins since it appears in lanes 2 and 3 of Figure 1; however, it could also be low molecular weight peptides present at the electrophoretic front. However, it is clear that the major portion of the pepsin-indigestible protein is prolamin. These data support the hypothesis of Bach Knudsen et al. (1985) that the low-quality kafirins of cooked sorghum are poorly digested in the rat. This suggestion was made because, after cooking, the biological value increased even though protein digestibility decreased. It appears that in maize the prolamins are also pepsin indigestible (not shown), but the effect is greater in sorghum due to the larger amount of proteins that becomes indigestible after cooking.

On the basis of the data presented above, we conclude that uncooked sorghum proteins are almost as digestible as uncooked maize proteins. On cooking, however, the sorghum prolamins become much less soluble and much less pepsin digestible than the prolamins in maize. This could explain the lower digestibility of cooked sorghum proteins MacLean et al. (1981) found in young children.

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Registry No. Pepsin, 9001-75-6; trypsin, 9002-07-7; chymotrypsin, 9004-07-3.

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