Effects of Processing on Sorghum Protein Digestibility

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Resumen: Estudios fueron conducidos con el objeto de determinar el porqué las proteínas del sorgo son menos digestibles que las proteínas de otros cereales. Ha sido demostrado que las proteínas indigestibles a la pepsina o tripsina/quimotripsina de sorgo cocido o sin cocer son principalmente proláminas o kafirinas. Usando cualquiera de los dos sistemas enzimáticos, el cocimiento reduce la digestibilidad de las kafirinas del sorgo pero no tiene efecto en la digestibilidad de las zeínas del maíz. La solubilidad de las kafirinas en alcohol acuoso es substancialmente menor después del cocimiento. La soludibilidad de las zeínas no se ve tan afectada como la de las kafirinas. Usando electroféresis en gel de poliacrilamida (dodecil sulfato de sodio) y cromatografía de filtración en gel, se demostró que las proteínas del sorgo forman enlaces intermoleculares de disulfuro durante el cocimiento. Estos polímeros proteicos enlazados ocurrieron en mayor proporción en la fracción proteica de las glutelinas, las cuales forman el matrix proteico. Esto también ocurrió pero en menor escala en las kafirinas. Por lo tanto, nosotros propusimos que la disminución en la digestibilidad de las kafirinas durante el cocimiento pudo haber sido debido a dos factores: 1) la formación de una capa proteica alrededor de los cuerpos proteicos, enlazada con enlaces disulfuro y 2) la polimerización de las mismas kafirinas. Una serie de agentes reductores mejoraron significativamente la digestibilidad in vitro de las proteínas del sorgo cocido y sin cocer. La adición de agentes reductores al sorgo mejoró la digestibilidad de la proteína al mismo nivel que las proteínas de maíz, cebada, arroz y trigo. Cuando la harina de sorgo fue remojada en una solución de 100 nM de mercaptoetanol por 12 h, la pepsina solubilizó más del 90% de las proteínas del sorgo en 2 h comparado con el 80% de la harina sin tratar.

Microfotografías del microscopio de barrido mostraron que los cuerpos proteicos del endospermo del sorgo (ricos en kafirinas) retuvieron su integridad estructural durante el cocimiento. También, usando esta técnica, los cuerpos proteicos parecen ser menos susceptibles a la digestión con pepsina que la proteína del matrix proteico. Los efectos de la textura del endospermo, decorticado, extrusión, fermentación y pH en la digestibilidad de la proteína de sorgo serán también discutidos.

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ABSTRACT

Studies were conducted with the goal of determining why sorghum proteins are considerably less digestible than other cereal proteins. It was shown that the pepsin or trypsin/chymotrypsin indigestible proteins of either uncooked or cooked sorghum are primarily kafirin proteins. Using either enzyme system, cooking reduced the digestibility of sorghum kafirins but had no adverse effect on the digestibility of maize zeins. The solubility of kafirins in aqueous alcohol was substantially decreased after cooking and more than for zeins.

Using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and gel filtration chromatography, sorghum proteins were shown to form intermolecular disulfide bonds during the cooking process. These disulfide linked protein polymers occurred to a greater extent among the glutelin protein fraction, which make up the protein matrix, but also occurred to some extent with the kafirin proteins. Accordingly, we propose that the decrease in kafirin digestibility after cooking may be due to two factors: 1) the formation of a disulfide-bound protein coat surrounding the protein bodies, and 2) polymerization of the kafirins themselves.

A variety of reducing agents were shown to significantly improve the *in vitro* digestibility of uncooked or cooked sorghum proteins. The addition of a reducing agent brought sorghum protein digestibility to the level of maize, barley, rice, and wheat. When sorghum flour was soaked in a 100 mM solution of 2-mercaptoethanol for 12 h, pepsin solubilized over 90% of the sorghum proteins in 2 h compared to about 80% in untreated flour.

Scanning electron micrographs showed that the kafirin-containing protein bodies of sorghum endosperm retained their structural integrity during cooking. Also, using this technique, the protein bodies appear to be less susceptible to pepsin digestion than the matrix protein. The effects of endosperm texture, decortication, heat extrusion, fermentation, and pH on sorghum protein digestibility are also discussed.

ABSTRACT

Sorghum grain supplies a large portion of the protein and calories for many people living in the semi-arid tropics. Many reports indicate that the proteins of low tannin sorghum gruel and bread are poorly digested compared to the proteins of other cooked cereals (Kurien et al., 1960; Axtell et al., 1981; MacLean et al., 1981; Mertz et al., 1984). The chemical and/or physical basis for this observation is not understood.

A number of studies have indicated that sorghum proteins are altered by cooking and thereby become less digestible (Axtell et al., 1981; Eggum et al., 1983; Mitaru et al., 1985). In two studies, where protein digestibility was measured, *in vitro* (Axtell et al., 1981) or with chickens (Mitaru et al., 1985), it was found that uncooked low tannin sorghum was far more digestible than the cooked sorghum (decreasing on cooking by 40% and 31.5%, respectively). With rats, Eggum et al., (1983) showed a smaller but significant reduction in digestibility of 7% after cooking. This phenomenon somewhat unique as it is generally thought that cooking denatures proteins and makes the raw materials' protein more digestible (Silano, 1977). The only other report of decreased digestibility in cereals after cooking was by Eggum et al., (1977) who found that rats were less able to digest the proteins of cooked rice than of the uncooked rice flour. In humans, however, MacLean et al., (1981) found that rice gruels were substantially more digestible than those made from sorghum (66% versus 46%, respectively). In 1981 MacLean et al. reported results from nitrogen balance studies on Peruvian children which showed that the protein digestibility of cooked sorghum gruel was significantly lower than that of cooked wheat, maize, or rice gruels (46% versus 81, 73, and 66%, respectively). An *in vitro* assay developed by Axtell et al., (1981) based on the solubilization of proteins following pepsin digestion also showed that sorghum proteins were less digestible than proteins from 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.

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 1.

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 and multiple enzyme digestion. For sorghum, pepsin alone gave differences between the uncooked and cooked flours similar to that obtained using the multiple enzyme method, indicating that pepsin alone can be used to determine digestibility among processed sorghum products.

Table 1. *In vitro* digestibility of sorghum and maize proteins.

	% PROTEIN DIGESTIBILITY					
	Pepsin		TC†		Pepsin/TC	
	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
Sorghum	80.7	64.8	72.7	57.1	87.6	70.5
Maize	85.5	81.9	79.4	87.7	88.3	90.7

†trypsin/chymotrypsin

When sorghum or maize are cooked the solubility of the proteins is altered, in particular the alcohol-soluble proteins called prolamins (kafirins in sorghum and zeins in maize). The prolamin proteins constitute approximately 50% of the total protein in the kernel. These storage proteins are synthesized by membrane-bound polyribosomes and transported into the lumen of the endoplasmic reticulum where they assemble into protein bodies (Larkins and Hurkman, 1978). The amount of alcohol-soluble proteins in sorghum and maize flour was reduced by cooking from 42 to 6% and 44 to 28%, respectively. Accordingly, the shift in alcohol-soluble protein fraction is more pronounced in sorghum than in maize.

An examination of the pepsin-indigestible proteins from uncooked and cooked sorghum and maize by sodium dodecyl sulfate--polyacrylamide gel electrophoresis (SDS-PAGE) showed that the prolamins (the alcohol-soluble storage proteins located in protein bodies) were the predominant indigestible proteins. This was the case irrespective of whether the sorghum

and maize was uncooked or cooked. We have also found that the sorghum prolamins (kafirins) become much less digestible than do maize prolamins (zeins) after cooking. Thus, explaining how sorghum protein digestibility is lowered by cooking.

In contrast to the findings that cooking sorghum to a gruel lowers its protein digestibility, decorticated sorghum that was heat-extruded on a Brady extruder at 180°C produced a material that gave no decrease in protein digestibility after cooking. *In vitro* protein digestibility using pepsin was 79% for cooked decorticated heat-extruded sorghum compared to 57% for gruels prepared from the same decorticated sorghum. Apparent protein digestibility of the identical extruded material fed to children was 81% digestible compared to 46% for gruels made with whole sorghum (MacLean et al., 1983).

Prolamin proteins, when extracted using the Landry-Moureaux (1970) procedure, are separated into two groups, prolamin-I which is extracted in aqueous alcohol and prolamin-II which is extracted in aqueous alcohol plus a strong reducing agent, 2-mercaptoethanol (2-ME). As mentioned above these two alcohol soluble protein fractions account for about 50% of the total protein in sorghum and maize (Table 2). The exact

Table 2. Nitrogen distribution in Landry-Moureaux fractions of whole kernel sorghum and maize.

Fraction		Solvent	% of Total N	
			Sorghum	Maize
I	albumin/globulin	NaCl 0.5 M	10.0	16.6
II	prolamin-I	isopropanol 70%	15.7	38.6
III	prolamin-II	isopropanol 70% + 2-ME 0.6%	31.3	10.1
IV	glutelin-like	borate buffer pH 10 +2-ME 0.6%	4.5	10.0
V	true glutelin	borate buffer pH 10 + 2-ME 0.6% + SDS 0.5%	29.3	20.2
	Total	% N extracted	90.8	95.5
		% protein in seed	13.5	10.5

nature of the differences between prolamin-I and -II is unknown. It is, however, known that in sorghum these protein fractions are identical in many respects, such as amino acid composition (Guiragossian et al., 1978) and electrophoretic mobility (Taylor et al., 1984). It has been assumed that the major difference between the two prolamin fractions is that fraction-II proteins have a higher degree of disulfide crosslinking than fraction-I. Hence fraction-II proteins are sometimes called the "crosslinked prolamins".

As sorghum proteins contain higher levels of crosslinked prolamins compared to maize it was suggested that this might be responsible for the low digestibility of sorghum following cooking. This led to testing the affect of adding reducing agents to sorghum and maize for improving protein digestibility. Accordingly, it was found that treating sorghum with 2-ME resulted in large increases in protein digestibility (Table 3). The reducing agent affected both uncooked (soaked) and cooked sorghum and resulted in increasing pepsin digestibility by 11 and 25%, respectively, when compared with sorghum which was soaked or cooked in water. It was found that pepsin digestibility was maximally increased by treating samples with 10 mM 2-ME solutions for uncooked sorghum and 100 mM 2-ME solutions for cooked sorghum. Addition of a reducing agent also increased the digestibility of uncooked and cooked maize proteins, but to a lesser extent then in sorghum (Table 3).

Other reducing agents also enhanced protein digestibility of sorghum. When sorghum was cooked in 100 mM solutions of dithiothreitol, sodium bisulfite, or L-cysteine pepsin digestibility

Table 3. Effect of 2-mercaptoethanol on the pepsin digestibility of sorghum and maize.

	% PROTEIN DIGESTIBILITY			
	Soaked†		Cooked	
	Water	+2-ME	Water	+2-ME
Sorghum	83.2	94.3	50.7	81.8
Maize	86.4	94.8	78.0	83.1

†Flours were soaked for 12 hr prior to digestion.

increased by 27, 25, and 23%, respectively, over sorghum cooked in water alone. Dithiothreitol was the most effective reducing agent, followed by bisulfite, 2-ME, and L-cysteine, in increasing pepsin digestibility at low concentrations. However, at 100 mM the improving effect of all the reducing agents tested was nearly equal.

Using SDS-PAGE and gel filtration chromatography, sorghum proteins were shown to form intermolecular disulfide bonds during the cooking process. These disulfide-linked protein polymers occurred to a greater extent among the glutelin protein fraction, but also occurred to some extent with the kafirin proteins. Following cooking, an extremely large molecular weight complex was found in the void volume fraction from a Sepharose CL-2B column. When separated by SDS-PAGE, these proteins when reduced were found primarily to be non-kafirin proteins with a range in apparent molecular weight (Mr) from 50 to 70 kD. These proteins, however, were well digested by pepsin. Three glutelin polypeptides with Mr of 100 to 120 kD were found to be less digestible and were solubilized by pepsin just prior to the start of kafirin digestion. These may be proteins associated with the protein bodies and may be involved with limiting kafirin digestibility. Accordingly, we propose that the decrease in kafirin digestibility after cooking is due to two factors: 1) the formation of a disulfide-bound protein coat surrounding the protein bodies, and 2) polymerization of the sorghum kafirin proteins themselves.

We have found that sorghum cooked at low pH was better digested than that cooked at neutral pH. This finding is in accordance with the theory that rearrangement of disulfides by sulfhydryl-disulfide interchange during cooking is responsible for decreasing protein digestibility. At low pH, free sulfhydryls are not likely to react in sulhydryl--disulfide interchange reactions as the mercaptide ion ($-S^-$), which is more likely to be present at neutral or alkali pH, is the reaction initiator.

A traditional fermentation process used in Sudan to prepare a sorghum food called *nasha* has been shown to increase *in vitro* protein digestibility (Mertz et al., 1984). Studies in Peru with children indicated that the apparent protein digestibility of *nasha* was 79% compared to 46% for a cooked sorghum gruel (Graham et al., 1986). On the basis of these results, Graham et al.,

(1986) concluded that *nasha* is a satisfactory food when supplemented with relatively small amounts of lysine-rich food such as milk, fish, or legumes.

CONCLUSIONS

Sorghum is used as the staple food crop by millions of people, many of whom have nutritionally inadequate diets. Two processes, fermentation and heat-extrusion, have been shown to increase the protein digestibility of sorghum in children. The fact that the decrease in *in vitro* digestibility of sorghum following cooking can be reversed by the addition of a disulfide cleaving agent should make it possible to develop other methods of processing that would lead to highly digestible sorghum foods.

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