Effects of Cooking and Treatment with Sodium Bisulfite on In Vitro Protein Digestibility and Microstructure of Sorghum Flour[1]

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ABSTRACT

The predominant indigestible proteins in cooked sorghum are kafirins, which are stored in protein bodies. In vitro pepsin digestion assay and scanning electron microscopy were used to examine the effects of cooking and treatment with sodium bisulfite on protein digestibility and protein body microstructure. In vitro pepsin digestion assay showed that sorghum decreases in protein digestibility after cooking. Treatment with sodium bisulfite increased the digestibilities of both cooked and uncooked flour. Scanning electron micrographs revealed that in all treatments the protein matrix is digested before the protein bodies. Protein bodies in uncooked samples were digested by pitting from the outer surface. In contrast, the protein bodies from the cooked sorghum did not exhibit any pitting. They did, however, become ellipsoidal. Cooking changed the protein bodies so that they could not be digested as they had before cooking. Protein bodies in cooked samples that had been soaked in sodium bisulfite did exhibit shallow pits, suggesting a reversal in the reactions that took place during cooking. Since sodium bisulfite prevents the formation of disulfide bonds during cooking and makes the sorghum more pepsin-digestible, formation of disulfide bonds is probably responsible for reduced protein digestibility in cooked sorghum.

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Sorghum (*Sorghum bicolor* (L.) Moench) is a major food crop in the semiarid tropics of Africa and Asia. In these areas where the people are typically poor and food resources are limited, sorghum is a major source of protein. Sorghum food products of high protein quantity and quality are essential. Traditionally in these developing countries, sorghum is consumed as a porridge or flat bread. The changes in protein quality that occur during cooking have interested many scientists (Maclean et al 1981, Hamaker et al 1987).

Both feeding studies and in vitro digestibility assays have shown that sorghum protein digestibility decreases significantly after cooking. In studies with rats, Eggum et al (1983) and Mitaru and Blair (1984) reported decreases in protein digestibility of cooked sorghum compared with that of uncooked sorghum. Mitaru et al (1985), using chickens, 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 decreased 24.5% in digestibility after cooking, which was a significantly greater decrease than that of the other grains.

Hamaker et al (1987) reported that cooking sorghum flour in water containing a reducing agent increased in vitro pepsin digestibility up to 25% compared with that of untreated, cooked flour. These results indicated that sorghum proteins contained higher levels of disulfide bonding than did other cereal grains. Work in our laboratory (J. J. Watterson, *unpublished*) has shown that disulfide bonding does increase in sorghum flour during cooking and is temperature-dependent.

Kafirins and glutelins are the major proteins in sorghum endosperm. Kafirins are stored in membrane-bounded protein bodies, while the glutelin proteins are present in the protein matrix (Seckinger and Wolf 1973). Using gel electrophoresis, Hamaker et al (1986) showed that kafirins are the predominant indigestible proteins in cooked sorghum. For this reason, changes that occur in the kafirin during digestion are of great interest. In this study we used electron microscopy to examine how cooking and treatment with sodium bisulfite affect protein digestibility and protein body microstructure.

MATERIALS AND METHODS

Flour Preparation

Sorghum cultivar P721N, grown at the Purdue University Agronomy Research Center, West Lafayette, IN, during the 1988 crop year, was used throughout the study. Whole grain was milled to a flour in a Cyclotec 1093 sample mill (Tecator, Hoganas, Sweden) to pass through a 0.4-mm mesh screen. Milled flour was stored at −20°C until used for the protein digestibility studies.

Protein Digestibility

The in vitro pepsin protein digestibility procedure described by Mertz et al (1984) was modified. Porcine pepsin (EC 3.4.23.1, 1,200 units per milligram of protein, Sigma Chemical Co., St. Louis, MO) was used to digest protein. Uncooked sorghum flour (200 mg) either was used directly or was soaked for 12 hr at 4°C in 2 ml of a 100 m*M* sodium bisulfite solution. Cooked samples were prepared by suspending 200 mg of flour in 2 ml of water or in 2 ml of 100 m*M* sodium bisulfite solution and stirring in a 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 liter (pH 2.0) and then incubated in a shaking water bath at 37°C for 5, 15, 30, 60, or 120 min. Pepsin digestion was stopped by adding 2 ml of 2*M* NaOH. After centrifugation (4,800 × *g*, 4°C, 20 min) the supernatant was discarded, and the residue was washed in 15 ml of buffer and recentrifuged. The residue was analyzed for nitrogen by micro-Kjeldahl digestion and colorimetric nitrogen analysis (AACC 1983). Indigestible nitrogen was subtracted from total nitrogen, and the percentage of soluble nitrogen was reported as in vitro digestibility.

In vitro pepsin protein digestibility determinations were done in triplicate. Control samples used for microscopy were treated by the same method as described above, except the pepsin solution was replaced with 0.1*M* phosphate buffer (pH 2.0).

Preparation of Microscopy Samples

After pepsin digestion, samples were sequentially digested with amyloglucosidase (Rhizopus, EC 3.2.1.3, 12,100 units per gram of solid, Sigma) and α-amylase (porcine pancreatic, EC 3.2.1.1, 1,240 units per milligram of protein, Sigma) to remove starch and, thereby, to make the protein bodies and matrix more visible. After incubation with amyloglucosidase (35 ml, 340 mg of enzyme per liter of phosphate buffer, pH 4.5, 55°C, 2 hr) the samples were centrifuged (4,800 × *g*, 4°C, 20 min). The supernatants were removed, and the samples were incubated with α-amylase (35 ml, 240 mg of enzyme per liter of phosphate buffer, pH 6.9, 37°C, 2 hr) and centrifuged (4,800 × *g*, 4°C, 20 min).

The supernatant was discarded, and the pellet containing the

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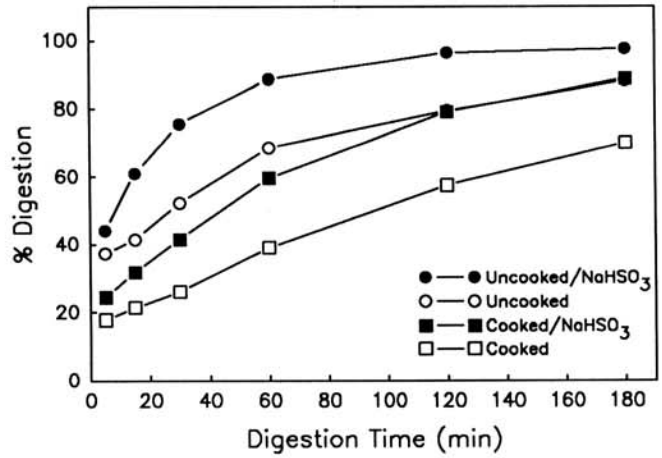

Fig. 1. Percent pepsin digestion of uncooked sorghum flour, cooked sorghum flour, sodium bisulfite-treated, uncooked sorghum flour, and sodium bisulfite-treated, cooked sorghum flour versus time in minutes.

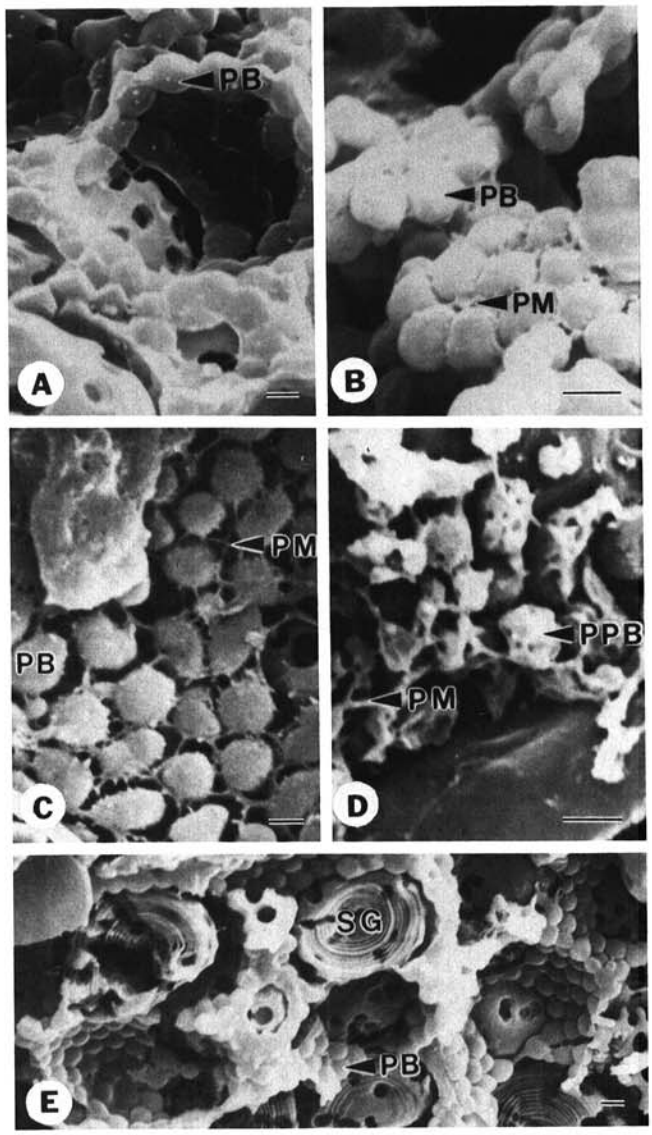

Fig. 2. Scanning electron micrographs of uncooked sorghum. **A,** Without pepsin digestion; **B,** after 5 min of pepsin digestion; **C,** after 15 min of pepsin digestion; **D,** after 30 min of pepsin digestion; **E,** 120 min control. PB = Protein body, PM = protein matrix, PPB = pitted protein body, SG = starch granule. Bars = 1.0 μm.

undigested protein was fixed in 2% glutaraldehyde (v/v) in 0.1*M* phosphate buffer (pH 6.8) for 16 hr at 4°C for scanning electron microscopy (SEM).

SEM

Fixed samples were rinsed in 0.1*M* phosphate buffer, pH 6.8. Samples were dehydrated in a graded ethanol series of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 100, and 100% for 20 min each at room temperature. Samples were then critical point-dried from CO_2 in a Polaron critical point dryer (Waterford, England). The dried samples were affixed to an aluminum stub with double-stick tape, coated with gold palladium in a Technics Hummer 1 sputter coater (Alexandria, VA), and viewed in a JEOL JSM-840 scanning microscope (Tokyo, Japan).

RESULTS

In Vitro Pepsin Digestion Assay

Percent in vitro protein digestion of uncooked sorghum flour was 37% after 5 min of digestion time, increasing to 79% after 120 min (Fig. 1). In contrast, cooked sorghum was only 18% digested after 5 min and increased to 58% after 120 min. After the flour was soaked in reducing agent, protein digestion increased to 44% after 5 min and 96% after 120 min. In reduced, cooked flour, 24% of the protein was digested after 5 min, which increased to 79% after 120 min.

SEM

Uncooked sorghum flour. In sorghum flour, protein bodies were embedded in a protein matrix that surrounded the starch granules. Amyloglucosidase and α-amylase only partially digested the starch granules, but many granules fell away from the flour particle surface so that the protein bodies were exposed (Fig. 2). Large cavities were observed in the sample where starch

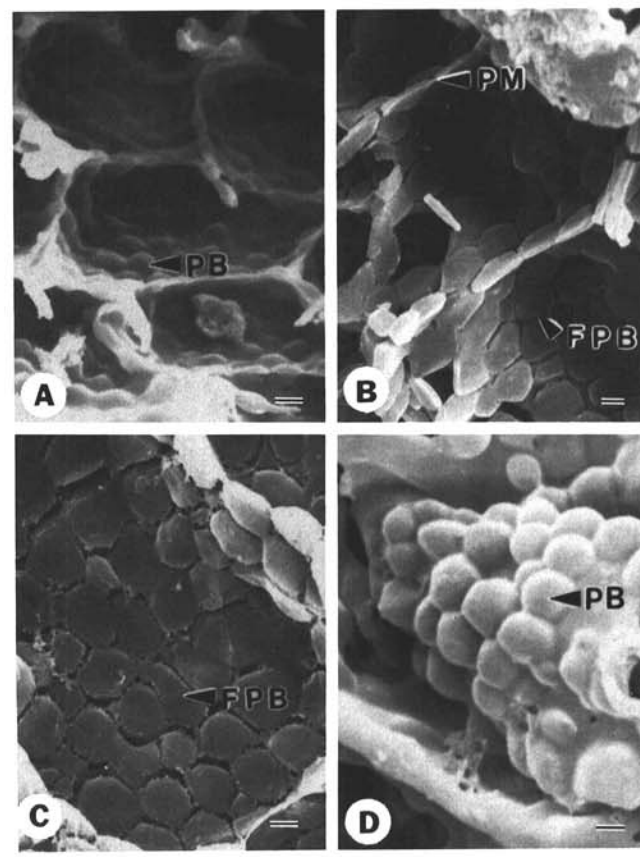

Fig. 3. Scanning electron micrographs of cooked sorghum. **A,** Without pepsin digestion; **B,** after 30 min of pepsin digestion; **C,** after 120 min of pepsin digestion; **D,** 120 min control. FPB = Flattened protein body, PB = protein body, PM = protein matrix. Bars = 1.0 μm.

granules were once located (Fig. 2A). After 5 min of pepsin digestion some protein matrix was digested, but undigested protein matrix strands remained. The protein bodies remained intact and unchanged (Fig. 2B). After 15 min, undigested protein matrix strands were observed and protein bodies showed signs of digestion in the form of small surface pits (Fig. 2C). The same pattern of matrix digestion and pitting of protein bodies continued through 30 and 60 min of pepsin digestion. By 120 min of digestion, most of the protein bodies were extensively pitted and had lost their spherical shape (Fig. 2D). The control sample (120 min) showed no protein body or protein matrix digestion (Fig. 2E).

Cooked sorghum flour. In cooked sorghum flour, the protein bodies and protein matrix were less distinguishable from each other because the protein matrix evenly coated the protein bodies (Fig. 3A). Most of the starch granules were gelatinized during cooking, making them more susceptible to enzyme hydrolysis. Thus, intact starch granules were rarely observed. Cavities were observed where the starch granules were once located. Between 5 and 15 min of pepsin digestion, the protein bodies remained unchanged. By 30 min of pepsin digestion, the digested matrix was observed and many of the protein bodies were ellipsoidal or flattened (Fig. 3B). After 120 min, increased amounts of digested matrix and flattened protein bodies were observed (Fig. 3C). However, the protein bodies showed no signs of pitting. The control sample (120 min) showed no protein body or protein matrix digestion (Fig. 3D).

Sodium bisulfite-soaked sorghum flour. Sorghum flour soaked in sodium bisulfite showed some matrix degradation even before pepsin digestion (Fig. 4A). Protein bodies, however, were unchanged by the sodium bisulfite treatment. Samples digested with pepsin for 5 min showed matrix digestion, and the majority showed some protein body pitting (Fig. 4B). By 15 min of pepsin digestion, protein bodies were severely pitted and became less spherical as their outer edges were digested away (Fig. 4C). A few protein matrix strands could be seen after 15 min, but most of the matrix was completely digested. Protein bodies and protein matrix digestion continued through 30 and 60 min of pepsin digestion. After 120 min, most protein bodies were completely digested and the remaining protein bodies showed extensive pitting (Fig. 4D). The control sample (120 min) showed no protein body or protein matrix digestion (Fig. 4E).

Sodium bisulfite-soaked, cooked sorghum flour. Reduced, cooked flour exhibited the typical appearance of cooked sorghum except that some protein matrix degradation occurred before pepsin digestion (Fig. 5A). After 30 min of digestion, many protein bodies exhibited flattened surfaces, and some of the protein bodies showed shallow pitting (Fig. 5B). Undigested matrix strands also could be seen. After 120 min, some protein bodies were irregularly shaped and many showed extensive pitting and degradation (Fig. 5C). The control sample (120 min) showed no protein body or protein matrix digestion (Fig. 5D).

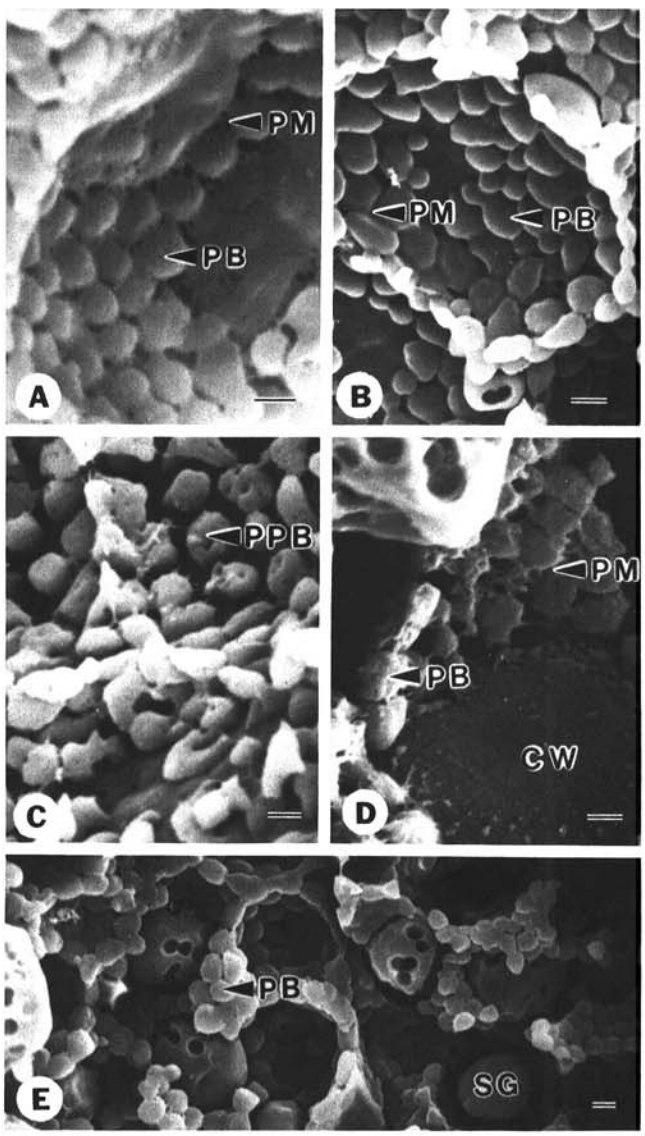

Fig. 4. Scanning electron micrographs of uncooked sorghum soaked in sodium bisulfite. **A,** Without pepsin digestion; **B,** after 5 min of pepsin digestion; **C,** after 15 min of pepsin digestion; **D,** after 30 min of pepsin digestion; **E,** 120 min control. CW = Cell wall, PB = protein body, PM = protein matrix, PPB = pitted protein body, SG = starch granule. Bars = 1.0 μm.

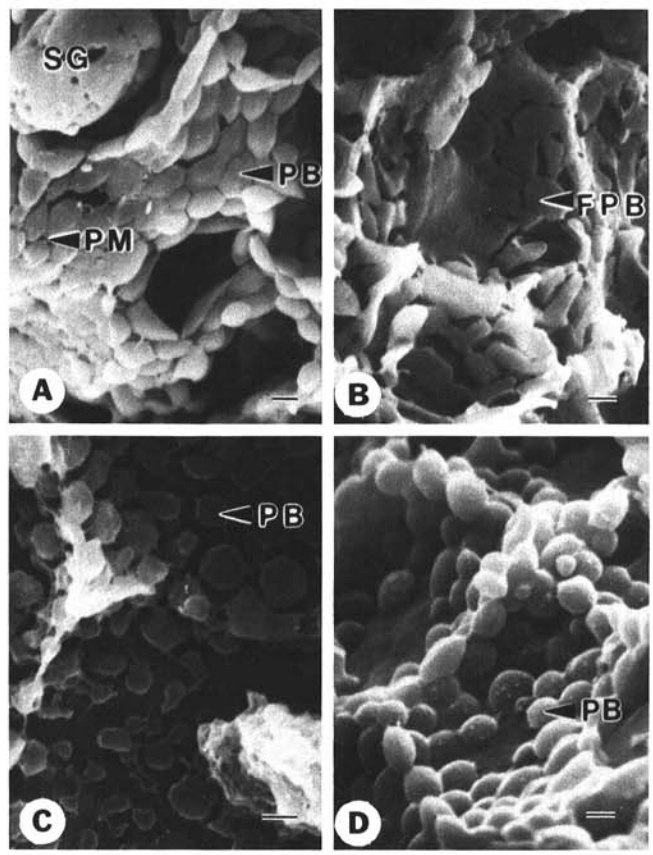

Fig. 5. Scanning electron micrographs of cooked sorghum soaked in sodium bisulfite. **A,** Without pepsin digestion; **B,** after 30 min of pepsin digestion; **C,** after 120 min of pepsin digestion; **D,** 120 min control. FPB = Flattened protein bodies, PB = protein body, PM = protein matrix, SG = starch granule. Bars = 1.0 μm.

DISCUSSION

In vitro pepsin digestion assay showed that sorghum decreases in protein digestibility after cooking. Treatment with the reducing agent increased the digestibilities of both cooked and uncooked flour. These results are in agreement with the values reported by Hamaker et al (1987). Previous research has shown that cooking sorghum increases the number of disulfide bonds (J. J. Watterson, *unpublished*). Since the reducing agent prevents the formation of disulfide bonds during cooking and makes the sorghum more pepsin-digestible, it is probable that disulfide bonding is responsible for reduced protein digestibility in cooked sorghum.

SEM micrographs revealed that in all treatments the protein matrix is digested before the protein bodies. The samples that were soaked in sodium bisulfite showed some protein matrix degradation before digestion and then took less time to digest than the unreduced samples (flour). Most matrix was digested after 30 min in sodium bisulfite-soaked flour versus 180 min in unreduced flour. The matrix is made up primarily of glutelin proteins and is presumed to exist in the form of polymers bound by intermolecular disulfide linkages (Wall 1971). If this is true, the sodium bisulfite could disrupt the protein matrix through cleavage of disulfide bonds and allow the water and salt-soluble proteins to be extracted. This would explain the partial degradation of the protein matrix observed in reduced, undigested samples.

In all treatments, protein bodies were more resistant to digestion than was the protein matrix. Since kafirins are found only in protein bodies and are the major indigestible proteins (Hamaker et al 1986), these results were expected.

Protein bodies in uncooked sorghum flour were digested by pitting from the outer surface. In contrast, protein bodies from the cooked sorghum were not pitted on exposure to digestive enzymes, suggesting that a structural change occurred during cooking. Hamaker et al (1987) proposed that the formation of disulfide bonds during cooking results in a toughening at the surface and interior of the protein body. After cooked samples were soaked in reducing agent, shallow pits again were observed in the digested protein bodies. This suggests that a reversal in the reactions that took place during cooking had occurred.

Enzymatic pitting in protein bodies similar to that observed in our study has been observed in sorghum during germination (Taylor et al 1985). Taylor et al (1984) found that in the transmission electron microscope, the protein bodies contained dark-staining inclusions that are in the form of invaginations of the protein matrix. These dark-staining regions on the protein bodies were preferentially degraded during germination (Taylor et al 1985). If the dark-staining inclusions in sorghum protein bodies were digested first, they would leave pits in the protein body. Whether the proteins of the dark-staining regions differ in composition and are involved in disulfide bonding during cooking is of interest.

Both cooked and uncooked protein bodies that had been soaked in sodium bisulfite were digested more rapidly than their unreduced counterparts. This treatment may have caused a reduction of the disulfide linkages that had formed in the protein body during cooking in addition to weakening the protein matrix so that the protein bodies were more accessible to the pepsin.

Research is in progress to determine the protein composition of the dark-staining regions in sorghum protein bodies and their involvement in the toughening of the protein body during cooking that prevents pitting and pepsin digestion.

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