Influence of Protein on Starch Gelatinization in Sorghum¹

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ABSTRACT

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Nine sorghums selected on the basis of density (as a measure of grain hardness) were decorticated and ground into flour. Kafirin content decreased with density. Alkali and acid gel consistency tests and an enzymatic measure of the degree of starch gelatinization (DG) of the cooked samples were made. Grains containing more kafirin gave higher gel consistency values and lower values for DG. The rate of gel firming during storage was greater for the hard than for the intermediate hardness grain samples. Gels from the soft sorghums firmed to a maximum value within 30 min. Addition of a reducing agent (0.05% 2-mercaptoethanol [2-ME]) before cooking decreased gel consistency values, decreased the rate of gel firming during storage, and increased the DG particularly in the hard sorghums. The effect of 2-ME on gel firming and DG was not as pronounced for the intermediate and soft samples. Scanning electron microscopy of flour particles from a hard and soft grain revealed that starch from the hard grain particles was enclosed in protein and cell wall. Flour

particles from the softer sorghum contained some particles that resembled those of the hard sorghum, yet many particles were seen where the cell wall had sloughed off and the starch granules were not tightly enclosed in protein. On cooking, the protein matrix of the hard samples appeared as convoluted sheets with protein bodies buried in the matrix. The matrix of the softer sorghum expanded to a greater degree and showed a more open structure. In the hard grain sample, 2-ME opened up the matrix structure, and the sheets of protein were broken into smaller fragments. We concluded that proteins influence starch gelatinization in sorghum. More kafirin-containing protein bodies were seen in samples with lower capacities for starch gelatinization, and the way in which these protein bodies were organized around the starch granule appeared to act as a barrier to starch gelatinization. Alteration of the protein with a reducing agent increased starch gelatinization.

Sorghum (Sorghum bicolor (L.) Moench) is the staple food crop in many regions of Asia, Africa, and Central America. Considerable information on traditional sorghum foods and on grain properties required to produce acceptable quality food products has been obtained (Rooney et al 1982, 1986). The structure of the grain has an important bearing on the food quality related properties of sorghum. Endosperm texture, the relative proportion of vitreous or transluscent endosperm to floury or opaque endosperm, has been identified as the factor that most consistently affects the processing and food-making properties of sorghum (Rooney et al 1986). Fewer kernels are broken when decorticating hard grain than grain with a floury endosperm texture (Rooney et al 1982). It has been shown that, during grinding, the vitreous endosperm portion of the grain gives rise to larger flour particles, whereas the floury endosperm forms smaller flour particles (Kirleis and Crosby 1982). With regard to cooking quality, grains with a relatively high proportion of vitreous endosperm are preferred for making thick porridges and for popping (Chandrashekar and Desikachar 1986, Murty et al 1982), and sorghums that have a high proportion of floury endosperm are preferred for making fermented and unfermented bread (Rooney et al 1986). Cagampang et al (1982) showed that hard grains take up less water during cooking and give a less sticky porridge than soft grains.

Grain hardness may be due to morphological compactness or to chemical adhesion between the various cellular components in the endosperm. Examination of the vitreous and floury endosperm parts of sorghum with the scanning electron microscope revealed that the vitreous endosperm contains numerous protein bodies that surround the starch granule, whereas the floury endosperm is relatively free of protein bodies and contains a higher concentration of starch (Sullins and Rooney 1975, Sekinger and Wolf 1973, Hoseney et al 1974). The chemical determinants of grain hardness in corn and sorghum have not been fully identified, although the involvement of prolamin proteins (zein in corn and kafirin in sorghum), located in protein bodies, has been suggested by a number of workers (Moshenin 1970, Kirleis and Crosby 1982,

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Ortega and Bates 1983, Abdelrahaman and Hoseney 1984). These findings alone, however, do not explain the functional differences between vitreous and floury endosperm sorghums during cooking. When made into a porridge, hard grains form products that are less sticky than grains with a larger proportion of floury endosperm (Cagampang et al 1982). Stickiness of cooked sorghum flour is a function of starch gelatinization (Hoseney 1986). Starch in the vitreous endosperm portion of sorghum grain is embedded in protein. Thus, it may be possible for the protein in hard grains to influence starch gelatinization.

Previous studies have shown that adding papain to sorghum semolina greatly increases the water absorption of the semolina during cooking (Chandrashekar and Desikachar 1981). This indicates that starch gelatinization in sorghum might be affected by protein. In order to understand differences in sorghum cooking quality, grains with varying endosperm texture were examined for kafirin content and indices of starch gelatinization.

MATERIALS AND METHODS

Seven sorghum varieties (Mshimbha, P721N, TX623B, P-954130, K-1597, CS-3541, and P721Q) and two hybrids (Hageen Dura-1 and IS10428AxP1362) with a range of endosperm textures (visually assessed) were selected for this study. Densities of the whole grains were determined by xylene displacement. The sorghums were grown at West Lafayette, IN, except for Mshimbha which was grown at Lubbock, TX, during the 1985 crop year (Table I).

All sorghums were decorticated in a Strong Scott barley pearler as described by Cagampang et al (1982), to yield 74–83% pearled grain. Some degerming occurred in the decortication process. Subsamples of the pearled grain were ground into flour in a Udy cyclone sample mill (Udy Corp., Ft. Collins, CO) using a 0.4-mm round-hole screen.

Water Uptake

Water uptake was determined on decorticated sorghum flour and starch isolated from the decorticated grain. Crude starch was isolated from sorghum grain by soaking in 0.005% NaOH for 16 hr, wet grinding in a Waring Blendor, and sieving through a nylon sieve. Protein was then removed from the crude starch using NaCl/toluene. The isolated starch was dried with methanol and ethyl ether (Badenhuizen 1964). The starch content of the isolated material, estimated by the glucoamylase procedure of Chiang and Johnson (1977), was 98%.

Water uptake of flour and starch was determined at 50, 60, 70, and 80° C by placing 1.0 g of flour in weighed 50-ml tubes, adding

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30 ml of water, and incubating the tubes for 3 hr. After incubation the tubes were centrifuged at $2,000 \times g$ for 15 min. The supernatant was decanted and the sides of the tube were wiped dry prior to weighing. The tubes were placed in an oven at 105° C for 16 hr and the weight of water held by 1.0 g of dry residue was determined (Chandrashekar and Desikachar 1981).

Protein and Kafirin Content Determination

A modification of the method described by Esen (1980) was used to determine kafirin protein content of sorghum flour. Decorticated flour (1 g) was suspended in 10 ml of 60% tert-butanol containing 0.6% 2-mercaptoethanol (2-ME) for 1 hr with continual shaking. After centrifugation $(1,500 \times g \text{ for } 15 \text{ min})$ and removal of the supernatant, the residue was shaken with 10 ml of fresh solvent for 30 min and again for 15 min. The supernatant from the three extractions was combined to yield the total kafirin extract. Extractions were performed in triplicate. Nitrogen was determined in flour and in the dried extracts by the method of Apostolatus (1984).

Degree of Starch Gelatinization

An enzymatic method described by Kainuma et al (1981) was used to measure the degree of starch gelatinization in cooked sorghum. Decorticated sorghum flour (1 g) was mixed with 20 ml of water and cooked in a boiling water bath for 30 min. Then 2.4 IU of β -amylase (Type 1B, sweet potato, Sigma Chemical Co., St. Louis, MO) and 10 IU of pullulanase (Sigma) were dissolved in acetate buffer (0.3M, pH 6.0) and added to the gruel. The activities of the enzymes were assayed using soluble starch and pullulan, respectively. Values for the degree of gelatinization are expressed as a ratio of the flour that is susceptible to enzyme after cooking in water to that which is susceptible after cooking the flour in 2Nalkali. Reducing sugars were assayed by the dinitrosalycilic acid (Fisher Scientific Co., Fair Lawn, NJ) method described by Bernfeld (1955), and total sugars were determined by the phenol sulfuric acid procedure of Dubois et al (1956).

Gel Consistency

Alkali and acid gel consistency measurements were performed by modifying a procedure described by Cagampang et al (1973). The modified procedure consisted of combining 200 mg of flour with 0.2 ml of 95% ethanol and 3 ml of either 0.2N KOH or 0.1N tartaric acid in 16×150 mm culture tubes. The mixture was cooked in a vigorously boiling water bath for 8 min. After cooking, the tubes were allowed to cool in an upright position at room temperature for 0.5 hr. In order to simulate the way porridges are stored overnight in parts of Africa, some tubes were held in the upright position as long as 18 hr. After cooling, the tubes were placed horizontally over ruled paper for 1 hr at room temperature and the gel front migration was read to the nearest millimeter. A high value indicated a thinner gel.

Cooking with a Reducing Agent

The β -amylase/pullulanase degree of starch gelatinization and the alkali acid gel consistency measurements were carried out as described above and on samples that were cooked in the presence of 0.05% 2-ME.

Microscopy

Uncooked flours before and after digestion with pepsin (Sigma P-7000; activity 1,200 units per milligram of protein) were examined by scanning electron microscopy. Sorghum flour (200 mg) was suspended in 35 ml of a buffer containing pepsin (1.5 g of pepsin per liter of 0.1 M K H₂ PO₄ buffer, pH 2.0) and incubated in a shaking water bath at 37° C. Pepsin digestion was stopped after 30 min with the addition of 2 ml of 2M NaOH. After centrifugation at $4,800 \times g$ at 4° C for 20 min, the supernatant was discarded, and the residue was washed in 15 ml of water and recentrifuged. The water wash was repeated two more times. The residue was then dried and coated with gold palladium, as described below, before viewing.

Scanning electron micrographs of gruels cooked in water and in 0.05% 2-ME were also made. The wet material was fixed in a 2% (v/v) solution of glutaraldehyde in 0.05M phosphate buffer (pH 7.0) for 3 hr and then dried by solvent exchange, using an ethanol series from 10 to 100% and by critical-point drying (Newbury 1986). The dried flours were placed in an ion sputtering unit, coated with gold palladium (13.3 Pa vacuum, 10 mA voltage) and viewed on a Jeol ASM22 scanning electron microscrope.

Statistics

Correlation coefficients between grain density, kafirin content, and indices of starch gelatinization were calculated using standard statistical procedures (Lapin 1980).

RESULTS

Water Uptake

The water uptake of sorghum flour and starch at temperatures from 50 to 80°C is presented in Figure 1. Prior to starch gelatinization (70-73° C for sorghum) a transition in starch and flour water uptake occurred. That is, at temperatures below 70° C the water uptake of the flour was greater than that of the starch, whereas at temperatures above 70°C the starch held more water than flour. Between temperatures of 70 and 80°C, where starch should be responsible for most of the water taken up by the flour,

TABLE I Whole Grain Hardness and Protein and Kafirin Protein Content of Decorticated Sorghums^a

Sorghum	Density (g/cm ³)	Percent Vitreousness ^b	% Protein ^c	% Kafirin of Total Protein
Mshimbha	1.376	58	11.5	70
Hageen Dura-1	1.375	57	8.4	60
IS10428AxP1362	1.360	54	10.0	54
P721N	1.344	50	9.3	49
TX623B	1.333	48	8.2	60
P-954130	1.309	43	9.9	52
K-1597	1.302	41	9.2	57
CS-3541	1.285	38	11.8	46
P721Q	1.143	8	9.7	46
CV,d %	2.83	1.07	•••	4.14

Values are means of duplicate determinations.

dCoefficient of variation.

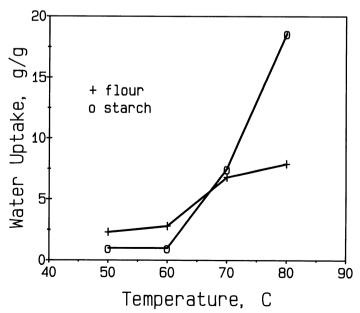


Fig. 1. Water uptake of sorghum flour and starch at temperatures from 50

^bPredicted from data presented by Kirleis and Crosby (1982).

Values reported on dry weight basis (N \times 6.25).

flour water uptake increased by only 1% but starch water uptake increased by 11%.

Grain Hardness

Grain hardness was estimated by measuring grain density. Density of the nine sorghums used in this study varied from 1.143 to 1.367 g/cm³ (Table I). According to data presented by Kirleis and Crosby (1982), percent vitreousness (percent vitreous to total endosperm area of cross-sectioned kernels) of sorghum was significantly correlated with grain density. Using their data, grain density values from the present study were converted to percent vitreousness. Accordingly, the sorghums used in the present work ranged in percent vitreousness from 0 to 58% (Table I).

Protein Composition

The protein content of the nine decorticated sorghums varied considerably and showed little relationship to grain density (Table I). The kafirin protein content expressed as the percent of total protein for the nine sorghums ranged from 46 to 70% (Table I). As previously reported by Kirleis and Crosby (1982), we found that the harder, high-density sorghums had a greater amount of kafirin protein than did the low-density grains. This finding supports the work of Abdelrahman and Hoseney (1984), who showed that removing the prolamin (kafirin) proteins from sorghum grits decreased hardness of the grit particle.

Indices of Starch Gelatinization

Alkali gel consistency of sorghum flours was measured at pH 8.2; values ranged from 40 to 110 mm (Table II). The sorghums that contained more kafirin proteins produced the thinnest gels (gels with the highest values). In most cases the alkali gel consistency decreased (gels became stiffer) when 2-ME was added to the cooking media (Table II). The extent of gel thickening caused by the addition of 2-ME was greater for harder sorghums with more kafirin protein than for the softer sorghums with less kafirin protein.

A similar trend as that found with alkali gels was observed with acid gels at pH 2.4 (Table II). The hard grain sample gave acid gel consistency values between 92 and 118 mm, whereas the values for the softer grain were near 50 mm. As with the alkali gels, the acid gels made with harder high-kafirin grains stiffened to a greater degree when 2-ME was added to the cooking media than did the softer low-kafirin grain.

Gel firmness is an important determinant of food quality as it influences textural properties (Hoseney 1986). The change in alkali gel firmness of hard (Mshimbha), intermediate (P-954130), and soft (CS-3541) grain, cooked with and without 2-ME and held up to 18 hr is shown in Figure 2. The firmness of the hard and intermediate grain gels, with and without 2-ME, decreased as holding time increased and finally reached a maximum firmness value of 40 mm after 18 hr of storage (Fig. 2). However, the soft grain gels prepared with and without 2-ME reached their

TABLE II
Alkali and Acid Gel Consistency
With and Without Addition of 2-Mercaptoethanol (2-ME)

Sorghum	Gel Consistency ^a (mm)				
	Alkali	Alkali + 2-ME	Acid	Acid + 2-ME	
Mshimbha	100	70	118	100	
Hageen Dura-1	100	60	92	52	
TX623B	110	70	118	100	
K-1597	60	50	80	40	
IS10428AxP1362	52	48	68	48	
P-954130	60	50	45	40	
P721N	40	40	83	50	
CS-3541	40	40	50	40	
P721Q	40	40	50	40	
CV, b %	1.64	1.38	1.32	1.54	

^aValues are means of duplicate determinations after 30 min holding time and 1 hr running time.

maximum firmness (40 mm) after only 0.5 hr of holding time and were unchanged thereafter (Fig. 2). The relative degree of change in gel firmness with time decreased in the following order: hard grain gels > hard grain gels with 2-ME > intermediate grain gels > intermediate grain gels with 2-ME > soft grain gels = soft grain gels with 2-ME (Fig. 2).

Degree of Starch Gelatinization

Enzymatic measurements of the degree of starch gelatinization for sorghum gels prepared with water ranged from 63 to 94% (Table III). The harder grains that contained more kafirin protein and produced the thinnest alkali and acid gels had the lowest degree of starch gelatinization. With the addition of 2-ME to the cooking media the degree of starch gelatinization increased for the harder high-kafirin grains and had little effect on the softer lower kafirin protein grain (Table III). 2-ME is a reducing agent that disrupts disulfide linkages in proteins (Freidman 1973). This indicates that proteins are possibly involved in limiting starch gelatinization and that gelatinization is limited to a greater extent

TABLE III
Enzymatically Measured Degree of Gelatinization
With and Without Addition of a Reducing Agent

	Degree of Gelatinization ^a (%)			
Sorghum	Cooked in Water	Cooked in Water + 2-ME ^b		
Mshimbha	63	100		
Hageen Dura-1	61	92		
TX623B	69	89		
K-1597	89	100		
IS10428AxP1362	85	94		
P-954130	82	97		
P721N	87	92		
CS-3541	94	100		
P721Q	82	93		
CV,° %	2.54	2.76		

[&]quot;Values are means of duplicate determinations.

^cCoefficient of variation.

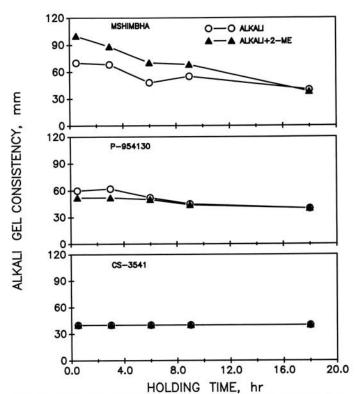


Fig. 2. Alkali gel consistency of hard (Mshimbha), intermediate (P-954130), and soft (CS-3541) sorghums cooked in the absence and presence of 2-mercaptoethanol and stored for various times.

^bCoefficient of variation.

b2-Mercaptoethanol.

in hard grains containing more kafirin than in soft grains with lower levels of kafirin.

Correlation Coefficients

Correlation coefficients between the parameters examined are presented in Table IV. Grain density was significantly related only to kafirin content (r = 0.65). Grain kafirin content was significantly related to alkali gel consistency (r = 0.85), acid gel consistency (r = 0.83), and to the degree of starch gelatinization (r = -0.71). Acid and alkali gel consistency measurements and the degree of starch gelatinization values were highly correlated with each other.

Scanning Electron Microscopy

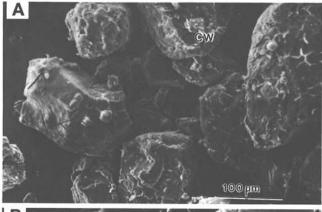
Flours. Scanning electron micrographs of flour particles from a hard high-kafirin content sorghum (Mshimbha) are presented in Figure 3. Almost all flour particles are covered with cell wall (Fig. 3A). This indicates that these particles probably originate from the vitreous endosperm portion of the grain (Chandrashekar and Kirleis, unpublished). A closer view of a typical Mshimbha flour particle shows where the cell wall has been torn away during the grinding process, and both intact and damaged starch granules surrounded by protein matrix are visible (Fig. 3B). A low magnification view of flour particles from a softer low-kafirin

TABLE IV

Correlation Coefficients Between Kafirin, Density,
and Indices of Starch Gelatinization^a

Parameter	Degree of Gelatinization	Acid Gel Consistency	Alkali Gel Consistency	Kafirin Content	
Density	0.41	0.59	0.54	0.65*	
Kafirin	-0.71*	0.83**	0.85**		
Alkali gel consistency	-0.89**	0.76*			
Acid gel consistency	-0.76*				

^{***} and * indicate significance at P = 0.01 and 0.05, respectively.



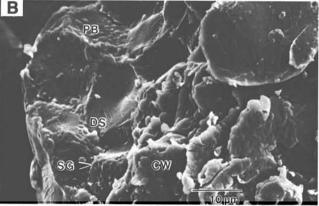
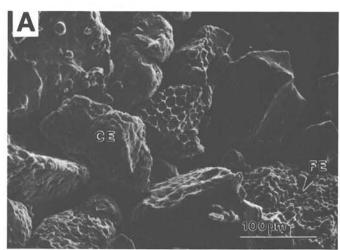
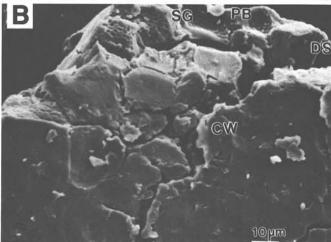


Fig. 3. Scanning electron micrographs of flour particles from hard sorghum (Mshimbha). A, Low magnification view showing corneous endosperm covered with cell wall (CW); and B, close-up of a corneous flour particle showing exposed intact starch granule (SG), damaged starch granule (DS), and protein bodies (PB) where the cell wall was removed during grinding.

content sorghum, CS-3541, shows a few particles covered by cell wall and many particles without attached cell wall (Fig. 4A). The CS-3541 flour particles covered by cell wall resemble those from Mshimbha (Figs. 4B and 3B). Unlike the Mshimbha particle, a close up view of a CS-3541 particle without a cell wall covering shows mostly undamaged starch granules and very few protein bodies (Fig. 4C). Starch granules in these particles seem to be linked together by protein but not enclosed in it. These flour particles are typically derived from the floury endosperm (Chandrashekar and Kirleis, unpublished).





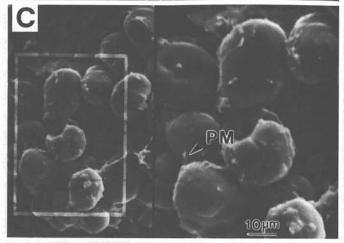


Fig. 4. Scanning electron micrographs of flour particles from soft sorghum (CS-3541). A, Low magnification view showing corneous endosperm (CE) particles covered with cell wall and a floury endosperm particle without cell wall (FE at arrow); B, close-up of corneous endosperm particle, showing intact starch granule (SG), damaged starch granule (DS), protein bodies (PB), and cell wall (CW); and C, close-up of floury endosperm particle, showing starch granules linked together by protein matrix (PM at arrow).

The effect of pepsin on uncooked flour particles from the soft sorghum (CS-3541) is shown in Figure 5. After treatment with pepsin, the flour particles lose their structural integrity, and only free starch granules with some adhering protein bodies remain. This indicates that the integrity of the flour particle is maintained by protein.

Gruels. Scanning electron micrographs of gruels made from the hard sorghum (Mshimbha) show that the protein matrix containing embedded protein bodies forms convoluted sheets after cooking (Fig. 6A). Partially gelatinized starch granules still held by the protein matrix can also be seen on the surface of the particle (Fig. 6A). In gruels prepared from the soft sorghum CS-3541, the protein matrix has a more expanded structure after cooking (Fig. 6B), the protein bodies are more loosely held by the protein matrix, and no partially gelatinized starch granules are present on the surface of the CS-3541 particle. When Mshimbha flour was cooked in the presence of 2-ME, instead of forming convoluted sheets of matrix protein, the matrix protein broke up into small fragments. Thus the protein structure is more open and the protein bodies are more exposed than in the gruels prepared without 2-ME (Figs. 7 and 6A, respectively). In addition, partially gelatinized starch granules are not visible on the surface of the Mshimbha particle cooked with 2-ME (Fig. 7).

DISCUSSION

It has been shown that endosperm texture, the relative proportion of vitreous to floury endosperm, is significantly correlated with sorghum grain hardness (Maxson et al 1971, Kirleis and Crosby 1982). Rooney et al (1986) states that endosperm texture is the factor that most consistently affects the food-making properties of sorghum. Sorghums with a large proportion of hard endosperm are preferred. Statistically significant correlations of endosperm texture and percent vitreousness with grain hardness were reported by Maxon et al (1971) and Kirleis and Crosby (1982). Hard sorghums are preferred for making traditional porridge-type foods (Chandrashekar and Desikachar 1986, Rooney et al 1986). Various gel tests have been used as a measure of sorghum food quality (Murthy et al 1982 and Cagampang and Kirleis 1984). In general these workers have shown that hard grains produce thinner, less sticky gels than soft grains. These findings were confirmed by our work using both acid and alkali gel consistency measurements (Table II). We found that the acid gels were thinner than the alkali gels; however, the ranking of the sorghums was about the same at both pHs.

Starch gelatinization is an important determinant of cereal food texture (Hoseney 1986). Degree of starch gelatinization measurements in the present work showed that acid and alkali gel consistency of sorghum flour were negatively correlated with starch gelatinization (Table IV). Furthermore, significant correlations were found between the kafirin protein content of decorticated sorghum flour and gel consistency (acid and alkali)

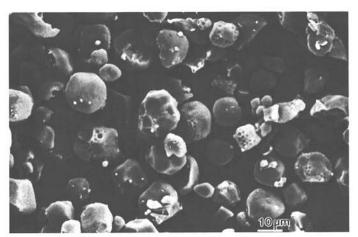


Fig. 5. Scanning electron micrographs of flour particles from soft sorghum CS-3541 after treatment with pepsin for 30 min.

and degree of starch gelatinization (Table IV). This indicates the involvement of protein in limiting starch gelatinization in hard sorghums and thereby producing thinner gels.

Further evidence for the effect of protein on limiting starch gelatinization in sorghum was obtained when 2-ME was added to the cooking media. 2-ME is a strong reducing agent that breaks disulfide linkages in proteins (Friedman 1973) and has been used to improve the digestibility of sorghum protein (Hamaker et al 1987). In the present work, addition of 2-ME to the cooking media increased the degree of starch gelatinization and decreased the gel consistency to a greater extent in the hard sorghum with more

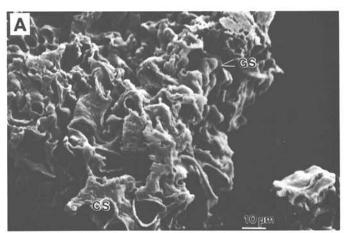




Fig. 6. Scanning electron micrographs of sorghum flour cooked in water. A, Mshimbha showing convoluted sheets (CS) made up of matrix proteins with embedded protein bodies and some attached partially gelatinized starch granules (GS); and B, CS-3541 showing exposed protein bodies (PB at arrow).

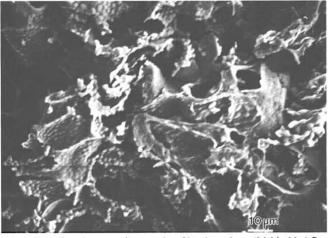


Fig. 7. Scanning electron micrographs of hard sorghum (Mshimbha) flour cooked in the presence of 2-mercaptoethanol.

kafirin protein than in the soft grain with less kafirin (Tables II and III).

The involvement of protein in limiting starch gelatinization is also supported by the large differences in water uptake of sorghum starch and flour at temperatures of 70 and 80°C (Fig. 1). Other evidence for the involvement of protein in limiting starch gelatinization was obtained by Chandrashekar and Desikachar. (1981), who showed that the addition of papain to sorghum flour prior to heating resulted in an increased water uptake.

Many studies using the scanning electron microscope have shown that two distinct morphological types of proteins are present in sorghum endosperm (Seckinger and Wolf 1973, Hoseney et al 1974, Sullins and Rooney 1975). The alcohol-soluble kafirins are found in spherical protein bodies, whereas the nonkafirin proteins (proteins not soluble in aqueous alcohol) comprise the protein matrix. More protein bodies are found in the vitreous endosperm than in the soft regions of the grain. Our work with the scanning electron microscope showed that flour particles from the hard grains were most often covered with cell wall, and, when exposed, the starch granules seemed to be surrounded by numerous protein bodies. In contrast, in the softer grains, the cell wall often appeared sloughed off the particle, and far fewer protein bodies surrounded the starch granule. The starch granule, protein bodies, and cell wall appear to be linked together by strands of protein. This observation was supported by the fact that when flour particles were treated with pepsin (Fig. 5) the organized structure in both hard and soft grains was lost. We believe that the linking proteins that hold the particle together are strands of glutelin. Thus, the protein matrix of the hard grain contains both the protein bodies and the matrix strands, whereas the soft grains contain a larger amount of the strand protein.

On cooking, the protein bodies from the hard grain remained intact, and the structure comprising the protein body and matrix remained rigid, with many partially gelatinized starch granules still surrounded by protein (Fig. 6). The structure of the protein (mainly matrix) in the softer grain, however, showed a greater alteration and expansion during cooking with liberation of starch granules. The addition of 2-ME to the cooking media caused disruption of the matrix of harder grains, resulting in a loose or more open structure.

It may be concluded that the presence of protein bodies around the starch granule provides a rigid cover and that full gelatinization of the starch granule occurs only when this barrier is removed. Softer grains gelatinize more easily as the protein covering the starch granule expands during cooking. Wall and Paulis (1984) suggested that disulfide bonds are very important in holding the glutelin proteins together and that 2-ME causes a loosening of the matrix protein by breaking the bonds.

It may be assumed that modification of protein by either varietal selection or by addition of chemicals will enable one to change the processing character of sorghum for various food uses. The results presented here provide a better understanding of the cooking characteristics of sorghum and the effect of protein on limiting starch gelatinization.

LITERATURE CITED

- APOSTOLATUS, G. 1984. Technical note: A rapid inexpensive procedure for determination of nitrogen in plant materials. J. Food Technol. 19:639.
- ABDELRAHMAN, A. A., and HOSENEY, R. C. 1984. Basis for hardness in pearl millet, grain sorghum, and corn. Cereal Chem. 61:232.
- BADENHUIZEN, N. P. 1964. General method for starch isolation. Page 14 in: Methods in Carbohydrate Chemistry. Vol. 4. R. L. Whistler, ed. Academic Press: New York.
- BERNFELD, P. 1955. Amylases, α and β. Page 149. in: Methods in Enzymology. Vol. 1. P. S. Colowick and N. O. Kaplan, eds. Academic Press: New York.
- CAGAMPANG, G. B., and KIRLEIS, A. W. 1984. Relationship of sorghum grain hardness to selected physical and chemical measurements of grain quality. Cereal Chem. 61:100.
- CAGAMPANG, G. B., PEREZ, C. M., and JULIANO, B. O. 1973. A gel consistency test for eating quality of rice. J. Sci. Food Agric. 24:1589.

- CAGAMPANG, G. B., GRIFFITH, J. E., and KIRLEIS, A. W. 1982.
 Modified adhesion test for measuring stickiness of sorghum porridges.
 Cereal Chem. 59:234.
- CHANDRASHEKAR, A., and DESIKACHAR, H. S. R. 1981. Studies on the hydration of starches, flour and semolina from different cereal grains. J. Food Sci. Technol. 18:12.
- CHANDRASHEKAR, A., and DESIKACHAR, H. S. R. 1982. Quality of sorghum for use in Indian foods. Pages 263-268 in: Proc. Int. Symp. Sorghum Grain Quality. L. W. Rooney, D. S. Murty, and J. V. Mertin, eds. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT): Patancheru, India, October 28-31, 1981.
- CHANDRASHEKAR, A., and DESIKACHAR, H. S. R. 1986. Studies in sorghum quality part II. Suitability for making dumpling (mudde). J. Food Sci. Technol. 23:7.
- CHIANG, Y. B., and JOHNSON, J. A. 1977. Measurement of total and gelatinized starch by glucoamylase and o-toluidine reagent. Cereal Chem. 54:429.
- DUBOIS, M., GILLES, K.A., HAMILTON, J.K., REBERS, P.A., and SMITH, F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350.
- ESEN, A. 1980. A simple colorimetric method for zein determination in corn and its potential in screening for protein quality. Cereal Chem. 57:129.
- FRIEDMAN, M. 1973. The Chemistry and Biochemistry of the Sulfhydryl Group in Amino Acids, Peptides, and Proteins. Pergamon Press: Oxford. 204 p.
- HAMAKER, B. R., KIRLEIS, A. W., BUTLER, L. G., AXTELL, J. D., and MERTZ, E. T. 1987. Improving the in vitro digestibility of sorghum with reducing agents. Proc. Natl. Acad. Sci. USA. 84:626.
- HOSENEY, R. C., DAVIS, A. B., and HARBERS, L. H. 1974. Pericarp and endosperm structure of sorghum grain shown by scanning electron microscopy. Cereal Chem. 51:553.
- HOSENEY, R. C. 1986. Pages 47-54 in: Principles of Cereal Science and Technology. Am. Assoc. of Cereal Chem: St. Paul, MN.
- KAINUMA, K., MATSUNAGA, A., ITAGAWA, M., and KOBAYASHI,
 S. 1981. New enzyme system β-amylase-pullulanase to determine the degree of gelatinization and retrogradation of starch or starch products.
 J. Jpn. Soc. Starch Sci. 28:235.
- KIRLEIS, A. W., and CROSBY, K. O. 1982. Sorghum hardness: Comparison of methods for its evaluation. Page 231-241 in: Proc. Int. Symp. Sorghum Grain Quality. L. W. Rooney, D. S. Murty, and J. V. Mertin, eds. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT): Patancheru, India, October 28-31, 1981.
- LAPIN, L. L. 1980. Statistics: Meaning and Method. Harcourt Brace Jovanovich: New York. 308 p.
- MAXSON, E. D., FRYAR, W. B., ROONEY, L. W., and KRISHNAPASAD, M. N. 1971. Milling properties of sorghum grain with different proportions of corneous to floury endosperm. Cereal Chem: 48:478.
- MOSHENIN, N. N. 1970. Physical Properties of Plant and Animal Materials. Gordon and Breach Science Publisher: New York. 341 p.
- MURTY, D. S., PATIL, H. D., and HOUSE, L. R. 1982. Cultivar differences for gel consistency in sorghum. Page 289-293 in: Proc. Int. Symp. Sorghum Grain Quality. L. W. Rooney, D. S. Murty, and J. V. Mertin, eds. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT): Patancheru, India, October 28-31, 1981.
- NEWBURY, D. E., JOY, D. C., ECHLIN, P., FIORI, C. E., and GOLDSTEIN, J. I. 1986. Pages 325-363 in: Advanced Scanning Electron Microscopy and X-ray Microanalysis. Plenum Press: New York
- ORTEGA, E. I., and BATES, L. S. 1983. Biochemical and agronomic studies of two modified hard-endosperm *Opaque-2* maize (*Zea mays L.*) populations. Cereal Chem. 60:107.
- ROONEY, L. W., MURTY, D. S., and MERTIN, J. V., eds. 1982. Pages 11-200. Proc. Int. Symp. Sorghum Grain Quality. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT): Patancheru, India, October 28-31, 1981.
- ROONEY, L. W., KIRLEIS, A. W., and MURTY, D. S. 1986. Traditional foods from sorghum: Their production evaluation and nutritional value. Pages 317-353 in: Advances in Cereal Science and Technology. Vol. 8. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.
- SECKINGER, H. L., and WOLF, M. J. 1973. Sorghum protein ultrastructure as it relates to composition. Cereal Chem. 50:455.
- SULLINS, R. D., and ROONEY, L. W. 1975. Light and scanning electron microscopic studies of waxy and nonwaxy endosperm sorghum varieties. Cereal Chem. 52:361.
- WALL, S. J., and PAULIS, J. W. 1978. Corn and sorghum grain proteins. Pages 135-219 in: Advances in Cereal Science and Technology. Vol. 2. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.