

# Low $\alpha$ -Amylase Starch Digestibility of Cooked Sorghum Flours and the Effect of Protein<sup>1</sup>

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## ABSTRACT

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The comparably low starch digestibility of cooked sorghum flours was studied with reference to normal maize. Four sorghum cultivars that represent different types of endosperm were used. Starch digestibilities of 4% cooked sorghum flour suspensions, measured as reducing sugars liberated following  $\alpha$ -amylase digestion, were 15–25% lower than for cooked maize flour, but there were no differences among the cooked pure starches. After the flours were predigested with pepsin to remove some proteins, the starch digestibility of cooked sorghum flours increased 7–14%, while there was only 2% increase in normal maize; however, there was no effect of pepsin treatment on starch digestibility if the flours were first cooked and

then digested. After cooking with reducing agent, 100 mM sodium metabisulfite, starch digestibility of sorghum flours increased significantly while no significant effect was observed for maize. Also, starch solubility of sorghum flours at 85 and 100°C was lower than in maize, and sodium metabisulfite increased solubility much more in sorghum than in maize. Differential scanning calorimetry results of the flour residue after  $\alpha$ -amylase digestion did not show any peaks over a temperature range of 20–120°C, indicating that sorghum starches had all undergone gelatinization. These findings indicate that the protein in cooked sorghum flour pastes plays an important role in making a slowly digesting starch.

Grain sorghum is an important human food in the semiarid tropics. The efficiency of starch utilization for cooked sorghum porridge was lower in children as compared to other major cereals (MacLean et al 1981). Of calories consumed,  $\approx$ 21% were recovered in the feces of children on a sorghum-based diet compared to our estimates (from calculations made on the presented data) of 13% for maize and 7% for wheat. In rats fed whole and decorticated flours, a 4% reduction in sorghum starch digestibility due to cooking was observed, which was attributed to a higher excretion of undegraded resistant starch and protein (Bach Knudsen et al 1988). Endosperm and starch granule structures of sorghum and maize are similar, and additionally they have very similar protein composition. However, important differences do exist in the properties of the proteins. Cooking significantly decreases protein digestibility of normal sorghum, but not that of maize, because of the formation of disulfide crosslinks within, and possibly between, protein bodies (Hamaker et al 1987, Oria et al 1995).

Among the cereals, sorghum grain generally has the lowest raw starch digestibility due to restrictions in accessibility to starch caused by endosperm proteins (Rooney and Pflugfelder 1986, Waniska et al 1990). The somewhat lower digestibility of the raw grain affects the feeding value for livestock. However, the underlying reasons for the low starch digestibility of cooked sorghum flours has not been well investigated. There has been a recent health interest in slow digesting starches in simple cooked or processed foods related to the popular desire to reduce digestible calories, increase the fiber component, and provide energy over extended periods. This was part of the impetus for the present study.

The basis for the poor digestibility of cooked sorghum starch is not clear. Bach Knudsen et al (1987, 1988) considered the effect of dietary fiber, polyphenol compounds, resistant starch, and protein kafirins on starch digestibility in African foods prepared from sorghum flours. They concluded that a high content of dietary fiber in sorghum flour may decrease starch digestibility. There was a highly negative correlation between starch digestibility and resistant starch formation. However, the mechanism resulting in resistant starch formation after cooking was not investigated. Chandrashekar and Kirleis (1988) found that degree of gelatinization, using the  $\beta$ -amylase and pullulanase method, was lower in hard sorghum cultivars with

high kernel density than in soft cultivars. The reducing agent, 2-mercaptoethanol, markedly increased degree of gelatinization. They concluded that endosperm proteins create a barrier to gelatinization in dense, vitreous sorghum kernels. However, in terms of digestibility, this did not explain the high energy excretion seen when a soft cultivar was fed to children (MacLean et al 1981). The purpose of this study was to better understand the basis of low starch digestibility of cooked sorghum flours.

## MATERIALS AND METHODS

One normal maize inbred (B73) and four genetically related sorghum cultivars that represent different types of endosperm (P721N, vitreous type; P721Q, floury type; and P851171 and P850043, dense floury types) were harvested from the Purdue University Agronomy Farm in 1995 and were conditioned (27°C, 67% rh) for two weeks to  $\approx$ 13% moisture content. Using the Stenvert hardness test (Pomeranz et al 1985), kernel hardness values for the cultivars were 28.3, 17.7, 21.3, and 17.2 sec, respectively. Flours and isolated starches were used to study the starch digestibility of sorghum. Maize flour and starch were used as a reference.

### Sample Preparation and Starch Digestibility Determination

Flour samples from whole sorghum and maize grains as well as decorticated sorghum (P721N) grains were prepared by milling through a 0.5-mm screen using a sample mill (Cyclotec 1093, Tecator, Sweden). Sorghum grains from P721N were decorticated by using a tangential abrasive dehulling device (model 4E-110, Venables Machine Work, Ltd., Saskatoon, Canada) to 10, 20, and 30%, and the removed materials were also collected. Total starch content in the flour samples was measured by the enzymatic method of McCleary et al (1994). Protein content of whole grain samples was determined by Approved Method 46-13 (AACC 1995).

Starch was isolated from whole sorghum and maize grains according to a general toluene procedure for starch isolation (Badenhuizen 1964). After the protein was thoroughly removed by 10% toluene, 85% methanol was applied for 16 hr with stirring at room temperature to extract the lipids. The starch samples were dehydrated with 70, 90, and 100% ethanol, and dried at 50°C for one day.

Starch digestibility was measured based on enzymatic hydrolysis using porcine pancreatic  $\alpha$ -amylase (Type VI-B, Sigma Chemical Co. St. Louis, MO). Flour equivalent to 200 mg of starch was cooked in 5 mL of distilled water for 20 min and cooled to 40°C. Enzyme solution (25 mL, 10 units/mL in buffer [1 mM sodium glycerophosphate-HCl, pH 6.9, 25 mM NaCl, 5 mM CaCl<sub>2</sub>]) was added to the cooked flour, and the suspension was incubated at

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37°C. Samples were obtained by taking 1 mL of reaction liquid at different times (up to 120 min), and treating with 0.1 mL of 2N H<sub>2</sub>SO<sub>4</sub> to stop the reaction. Reducing sugar concentration was measured by the Nelson-Somogyi method (Somogyi 1952). For every experiment, samples equivalent to same amount of dry weight starch were used. The reducing sugar concentrations after hydrolysis under the same conditions were used as relative starch digestibility values.

The residue of cooked flour after  $\alpha$ -amylase digestion was prepared for analysis by differential scanning calorimetry (DSC). After a 2-hr hydrolysis, samples were centrifuged at 1,100  $\times$  g for 10 min. The supernatant was discarded, and the pellet was dehydrated with 70, 90, and 100% ethanol. The dehydrated sample was dried in an oven at 50°C for one day and was subject to DSC analysis.

### Pepsin Treatment

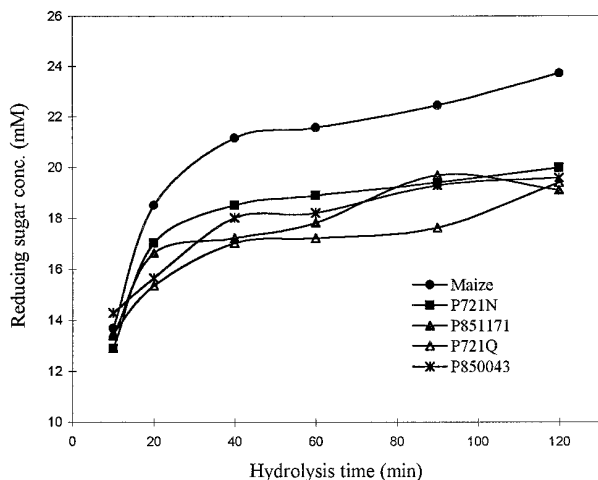
Flour samples (equivalent to 200 mg of starch) were incubated in pepsin solution (35 mL of 1.5-g enzyme [type A, Sigma]/L in 0.1M potassium phosphate buffer, pH 2.0) for 2 hr at 37°C in a shaking water bath (Mertz et al 1984). After centrifugation (4,900  $\times$  g, 20 min), the supernatant was discarded, and the residue was used for starch digestibility determination. Samples were also treated with pepsin after the flours were cooked. After pepsin digestion, the pH was adjusted to 7.0, and  $\alpha$ -amylase reaction buffer (35 mL, 20 U/mL) was added to hydrolyze the samples.

### Water Retention and Starch Solubility

Flour slurries (equivalent to 5% starch content in water or 100 mM Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>) were incubated at 85 and 100°C for 30 min with shaking every 5 min. After the samples were cooled at room temperature for 20 min, they were centrifuged at 4,900  $\times$  g for 10 min. Supernatants were collected to measure total soluble starch, and the precipitate was used to determine the water retention. Total soluble starch was precipitated by adding 80% ethanol and dried at 40°C for two days. The water retention was the ratio of sample weight after incubation to original sample weight.

### DSC

A differential scanning calorimeter (model DSC 30 with a TA11 processor, Mettler-Toledo, Highstown, NJ) was used to measure thermal parameters ( $T_o$ ,  $T_p$ ,  $T_c$ ,  $\Delta H$ ) of flour residues after digestion. Samples and distilled water (1:2, 1:3, and 1:5, w/w) with a total weight of 15–20 mg were sealed hermetically in aluminum pans. The temperature range of the scan was 20–120°C with a 10°C/min heating speed.



**Fig. 1.**  $\alpha$ -Amylolytic hydrolysis of cooked sorghum (P721N, P721Q, P851171, P850043) and maize flours as a function of time (flour used equivalent to 400 mg of starch).

### Statistical Analyses

Each experiment included three replicates. The difference of means between treatment and control within each cultivar were determined using Student's *t*-test. Values of  $P < 0.05$  were considered statistically significant. Differences among cultivars were evaluated for significance by analysis of variance (one-way ANOVA) with  $\alpha = 0.05$ . Data was analyzed using Excel software (Microsoft).

## RESULTS AND DISCUSSION

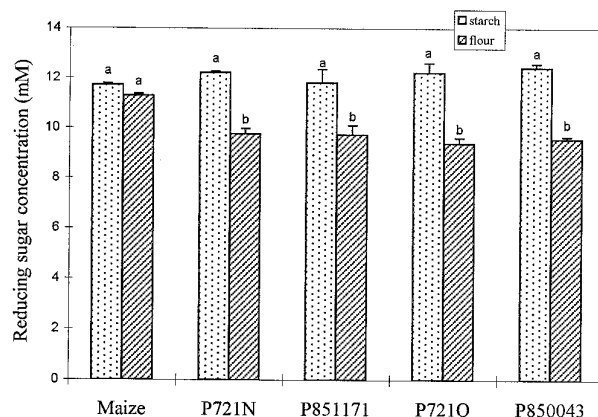
### Starch Digestibility of Sorghum

Flours of sorghum cultivars were 15–25% lower in starch digestibility, measured by reducing sugar concentration, as compared to normal maize and were similar to each other, regardless of endosperm type (floury, dense floury, or vitreous) (Fig. 1). Protein contents were not related to digestibility. Values were 9.5, 11.3, 9.4, 11.9, and 11.3% for corn, P721N, P851171, P721Q, and P850043, respectively. These results were obtained under the condition of 4% starch concentration (equivalent flour samples were used) to supply an excess of water for gelatinization. There were no significant differences in starch hydrolysis of samples cooked at 5, 20, or 60 min (data not shown).

Sorghum starch itself was not the reason for lower starch digestibilities of cooked sorghum flours when compared to cooked maize flour (Fig. 2). Cooked isolated sorghum starches had markedly higher digestibilities than starch from cooked sorghum flours, and were equal to isolated maize starch. Digestibility of isolated maize starch did not differ significantly from that of maize flour.

We initially hypothesized that materials in the sorghum pericarp (dietary fiber, phenolic compounds) could negatively affect cooked starch digestibility, and investigated this possibility with a decortication experiment. Cooked starch digestibility of sorghum cultivar P721N did not show noticeable differences due to decortication (Fig. 3), even though samples had markedly different amounts of pericarp (whole flour, 80% starch; 10% and 20% decortication, 86% starch; 30% decortication, 93% starch). The lower starch digestibility of the pooled materials removed during decortication (containing 55% starch) indicates that, in a concentrated form, pericarp substances do reduce starch digestibility. However, the surface materials of sorghum seeds (pericarp and outer endosperm) were not responsible for the low starch digestibility of cooked whole sorghum flours.

Potential digestibility lowering effect of  $\alpha$ -amylase inhibitors in sorghum grain would appear to be negligible and not a factor in the present study. An  $\alpha$ -amylase inhibitor was identified in sorghum grain (Kutty and Pattabiraman 1986). However, it became completely inactive when heat-treated for 10 min at 80°C, a less rigorous heat treatment than was used in this study. In another study,

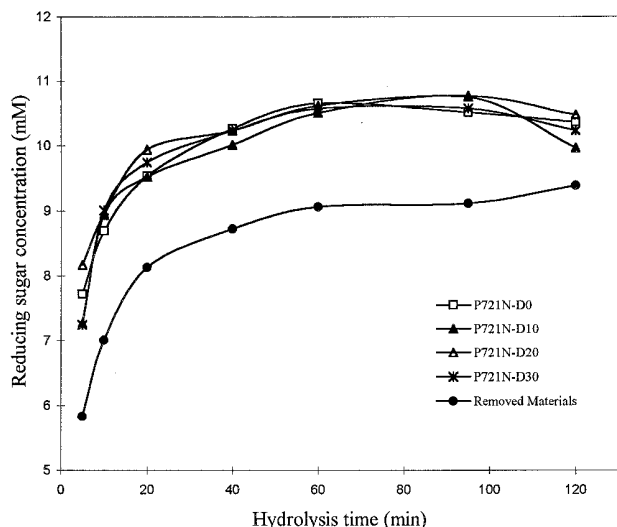


**Fig. 2.** Comparison of starch digestibilities between cooked isolated starch and flour samples after hydrolysis for 2 hr with  $\alpha$ -amylase. Means with different letters differ significantly ( $P < 0.05$ ).

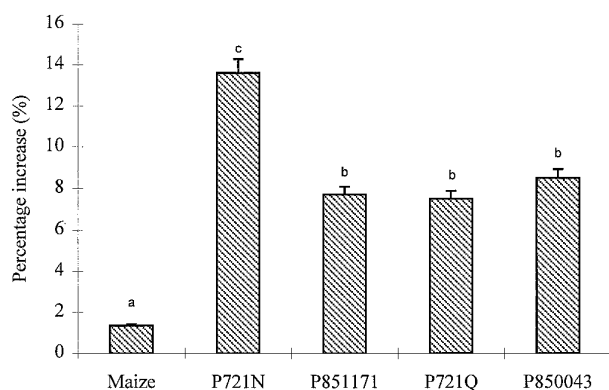
Weselake et al (1985) found no  $\alpha$ -amylase inhibitor activity in sorghum compared to significant levels of activity in wheat, barley, rye, and triticale.

### Effect of Pepsin Treatment and Reducing Agent on Digestibility

Pepsin pretreatment before cooking increased the starch digestibility of sorghum by 7–14%, while it only had a negligible effect on maize (Fig. 4). However, when pepsin treatment was performed



**Fig. 3.** Effect of decortication on cooked starch digestibility of sorghum cultivar P721N. D0, D10, D20, and D30 represents 0, 10, 20, and 30% decortication. Removed materials are pooled fractions removed during decortication.



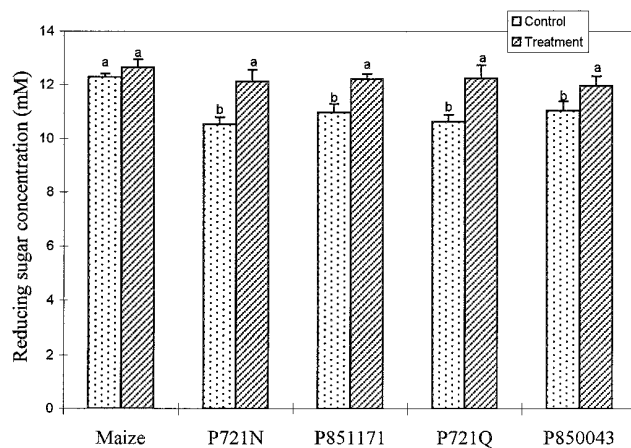
**Fig. 4.** Increased amount of cooked starch digestibility after pepsin pre-digestion of flours for 2 hr before cooking. Means with different letters differ significantly ( $P < 0.05$ ).

after cooking, no significant increase on starch digestibility was seen (data not shown). Although pepsin digestibility is known to decrease after cooking (Hamaker et al 1987), two of the cultivars used in this study have high uncooked and cooked protein digestibilities (P851171 and P850043, unpublished data). Yet, they showed no difference in  $\alpha$ -amylosis, after pepsin pretreatment and cooking, compared to the other sorghum cultivars.

A reducing agent, sodium metabisulfite, which cleaves disulfide bonds, increased the starch digestibility of sorghum flour to the same level as maize flour (Fig. 5), but it had no effect on starch digestibility of the maize flour itself. Bisulfite also increases the protein digestibility of sorghum (Hamaker et al 1987, Rom et al 1992), presumably by preventing crosslinking within protein bodies during cooking. This data supports the previous finding of Chandrashekar and Kirleis (1988), who showed that a reducing agent, 2-mercaptoethanol, increased the degree of starch gelatinization of cooked sorghum flour as measured using the  $\beta$ -amylase and pullulanase method. In the present study, hydrolysis by  $\alpha$ -amylase, a better indicator of mammalian starch digestion, was equally affected. Overall, these results strongly indicate that sorghum proteins affect starch digestibility in cooked sorghum flour, and that this effect occurs during the cooking or cooling processes.

### Starch Gelatinization

Complete gelatinization is important in obtaining highly digestible starch. DSC results on the residues after  $\alpha$ -amylase digestion (not shown) showed no peaks from 20 to 120°C within a range of flour-to-water ratios where endotherms are detected. This indicates that native crystallites had all melted in the undigested starch residues, suggesting that incomplete gelatinization



**Fig. 5.** Effect of cooking with sodium metabisulfite (100 mM) on the starch digestibility of flours. Means with different letters differ significantly ( $P < 0.05$ ).

**TABLE I**  
Swelling Power and Starch Solubility of Maize and Sorghum Flours

Cultivar	Temperature (°C)	Swelling Power (g/g)			Solubility <sup>a</sup>		
		Control	Treated <sup>b</sup>	Increase (%)	Control	Treated <sup>b</sup>	Increase (%)
Maize	85	7.0	9.1	29.7	50.9	76.2	49.7
	100	9.3	12.1	29.8	78.7	88.0	11.8
P721N	85	5.6	6.64	17.1	12.8	35.3	175.7
	100	9.3	12.1	29.3	55.1	65.8	19.4
P851171	85	6.0	7.18	18.7	10.9	28.8	164.2
	100	11.7	14.1	20.5	29.9	38.0	27.1
P721Q	85	5.8	6.8	16.9	10.0	28.6	186.0
	100	11.0	12.5	13.6	26.3	40.9	55.5
P850043	85	6.2	7.23	15.1	11.3	29.5	161.1
	100	11.2	12.6	12.5	21.6	40.1	85.6

<sup>a</sup> Measured as mg of soluble starch/500 mg of starch.

<sup>b</sup> Reducing agent 100 mM sodium metabisulfite.

was not the reason for lower digestibility of sorghum starches. This appears to be contrary to the findings of Chandrashekar and Kirleis (1988), who found that protein in flour from vitreous sorghum kernels inhibits gelatinization, resulting in decreased values for degree of gelatinization. Starch gelatinization, however, is a process, proceeding from ungelatinized granules all the way to molecularly dispersed starch. While thermal readings by DSC show melting of crystallites, this measurement may not be a good indication of whether sorghum and maize starches have reached the same level in the gelatinization process, i.e., swelling and dispersion of starch.

### Water Retention and Starch Solubility

Normal maize flour had markedly higher starch solubility at 85 and 100°C than sorghum flours (Table I). The reducing agent, sodium metabisulfite, increased water retention and starch solubility, producing a larger effect on sorghum starch solubility than on maize. Swelling power of starch reflects the ability of starch molecules to interact with water molecules. Sorghum samples had lower swelling power than maize at 85°C, while at 100°C they were, in some cases, higher than maize. This indicates that sorghum starch requires higher temperatures to reach full granule swelling. Starch solubility is an indication of the degree of starch molecule dispersion after cooking. The reducing agent, sodium metabisulfite, had a larger effect on sorghum starch solubility than on maize. Therefore, it seems that protein in sorghum, possibly in the form of a disulfide-bound network, lowers its starch solubility. Perhaps the lower solubility of sorghum starch is related to a decreased accessibility to  $\alpha$ -amylase.

### CONCLUSIONS

Cooked sorghum flours had lower starch digestibility (15–25%) than normal maize flour, regardless of whether the endosperm type was floury, dense floury, or vitreous. Neither the starch itself nor the outer layer materials of sorghum seeds appeared to be related to poor starch digestibility. The increase in starch digestibility when sorghum flour was pepsin-pretreated before cooking, or by cooking with a reducing agent, suggests that protein plays a large role in its low starch digestibility. Two possible scenarios might explain the role of sorghum protein in creating less digestible starch. First, as previously suggested by Chandrashekar and Kirleis (1988), endosperm protein may restrict the starch granules from fully gelatinizing, thereby resulting in lower digestibility. We found no trace of unmelted crystallites in undigested residues by DSC. However, there was less soluble starch in gelatinized sorghum, indicating that, after cooking, sorghum and maize starches may be found in different stages of the gelatinization process. Second, a starch-protein interaction may occur during cooking or cooling that causes gelatinized sorghum starch to be in a less digestible state. Studies are being conducted to determine the mechanism involved causing proteins to lower starch digestibility of sorghum.

### ACKNOWLEDGMENT

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