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Pepsin digestibility of proteins in sorghum and other major cereals

(improved pepsin assay/processing effects/temperature effects)

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ABSTRACT We have shown previously that sorghum is highly digestible in the rat. However, other workers have shown that sorghum is much less digestible than wheat, maize, and rice in young children. Because the rat does not show these digestibility differences, we developed an empirical pepsin digestion method, first reported in 1981, which simulates the digestion values found in children. In this report the method has been improved and used to analyze wheat, maize, rice, millet, and sorghum and certain processed samples of millet and sorghum. The pepsin digestion values parallel those found in children for wheat, maize, rice, and sorghum. In addition, a processed sorghum product that gave a high digestion value in children also gave a high value with the *in vitro* pepsin method.

Sorghum [*Sorghum bicolor* (L.) Moench] is an important source of calories and protein for a large segment of the human population in the semiarid tropics.

In a previous publication (1), the apparent digestibility of the proteins in four (cooked) sorghum gruels varied from 76.5% to 83.0% (average, 80.4%) in young rats. The same four sorghum flours, when cooked and fed to children of age 6–27 months, gave an average apparent digestibility value of only 46%, compared with values of 81%, 73%, and 66% for wheat, maize, and rice, respectively (2). Because the rat gave values for cooked sorghum that were the same as those MacLean *et al.* (2) found for cooked wheat in the child, we developed an empirical *in vitro* pepsin method (1) that more closely simulates MacLean's values in children. In this report we present improvements in our original method and data on the pepsin digestibility of the major cereals. We also include data on processed sorghum and millet products [sorghum and millet nasha (fermented baby foods) and extruded decorticated sorghum].

MATERIALS AND METHODS

The grain samples used in these studies were Eagle hard red winter wheat (1980 crop), yellow maize B73 × H60 (1981 crop), milled white rice (commercial), normal sorghum P721N (1980 crop), high-lysine sorghum P721-opaque (1980 crop), and pearl millet (Sudan variety of *Penicetum Americana*). The sorghum used to prepare nasha was a Sudan variety (Dabar), and the sorghum used for heat extrusion was normal sorghum 954062 (1977 and 1978 crops).

Processed Grains. Decorticated normal sorghum 954062 flour was extruded at low moisture content in a Brady extruder at 350°C with a throughput of 345 kg/hr at Colorado State University, Fort Collins. Dabar whole-kernel sorghum and pearl millet (Sudan variety) were ground in a Udy grinder with a 0.4-mm screen. Three hundred fifty grams of flour were suspended in 1 liter of water. Starter, 150 grams from Sudan (natural mixed culture of lactobacillus and yeast found on the grain in Sudan), was added, and the mixture was incubated at 30°C for 12 hr. The slurry was fed to a laboratory drum dryer or was freeze-dried, and the dry residue was used for digestibility studies.

Pepsin. The pepsin used was porcine pepsin 1:10,000 (Sigma). It had an activity of 1,200–2,000 units/mg of protein ($A_{280}^{1\%}$) (3).

Modified Pepsin Method (Residue Method). Ground cereal samples (200 mg) prepared with a Udy grinder and 0.4-mm screen were suspended in 35 ml of a solution of pepsin (1.5 mg/ml) in 0.1 M phosphate buffer (pH 2.0); the mixture was incubated with gentle shaking at 37°C for 2 hr. Incubation for 3 hr raises all values about 10% above the 2-hr values; incubation for 1 hr lowers all values about 10% below the 2-hr values. Therefore, time of incubation is critical in the assay. After incubation the suspension was centrifuged (12,000 × *g* for 15 min at 4°C), and the residue was suspended in 10 ml of 0.1 M phosphate buffer (pH 2.0) and centrifuged as before. The low-pepsin moist residue was freed from the walls of the centrifuge tube with a spatula and dumped in the center of Whatman filter paper no. 3 on a 43-mm Buchner funnel. Suction (aspirator) was applied, and the residue in the centrifuge tube was washed into the funnel with 5 ml of phosphate buffer (pH 2.0). The filter paper was rolled and inserted in a Technicon digestion tube. In the sulfuric acid digestion process, the temperature was started at 100°C in the digestion block and raised in increments to 375°C over a time span of 2.5 hr. H_2O_2 was used two or three times to clear carbonaceous material. Ammonia nitrogen was determined in a Technicon analyzer.

Cooking Procedure. Ground samples (200 mg) were suspended in 2 ml of water and held in a boiling water bath for 20 min. The cooked gruel was then added to 35 ml of the previously described pepsin solution, and the digestibility was determined.

RESULTS

It can be seen from Table 1 that wheat, maize, and rice have digestion values about 25 percentage points higher than that of normal sorghum. This agrees with MacLean's findings in children. High-lysine sorghum (P721O) is slightly more digestible than normal sorghum (Table 1). Pearl millet appears to be superior to normal sorghum (74.8% versus 59.0%) but inferior to wheat, maize, and rice. However, conversion of millet to the fermented baby food nasha raises its pepsin digestibility to that of wheat, maize, and rice. These findings on millet need to be confirmed in children. In contrast, conversion of sorghum to nasha appears to improve the flour by a smaller amount (from 59.8% to 65.5%).

The most pronounced effect was observed with decorticated sorghum. When the flour was extruded, the digestibility increased from 56.8% to 79%. MacLean *et al.* (4), feeding the same extruded cooked flour used in our tests, obtained an average value of 81%, which agrees very well with the *in vitro* value in Table 1 for extruded sorghum.

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Table 1. Pepsin digestibility of cooked major cereals

Cereal	Protein digestibility,* %	SD
Wheat	85.5	1.73
Maize	85.3	1.26
Rice	83.8	0.96
Sorghum P721N	59.0	2.45
Sorghum P721O	63.2	1.30
Millet, pearl	74.8	2.06
Millet nasha	85.5	0.58
Sorghum, Dabar	59.8	1.50
Sorghum nasha	65.5	1.29
Sorghum, decorticated 954062	56.8	2.22
Sorghum, decorticated/ extruded 954062	79.0	0.00

*Mean of four analyses.

DISCUSSION

In a recent paper, Eggum *et al.* (5) question the large differences in digestibilities of sorghum protein (and energy) between preschool children and growing rats. Eggum *et al.* (5) do not believe the digestibility values obtained by MacLean *et al.* (2) in children can be explained by interspecies differences alone. They state that "It is worth emphasizing that the subjects studied by MacLean *et al.* were children who had been malnourished, and that this might have affected their results." That a species difference does exist is supported by the data of MacLean *et al.* and also by the data of previous workers. Kurien *et al.* (6) found that the proteins in rice fed to seven normal boys age 10–11 yr were 75% digestible; replacement of the rice with sorghum lowered the apparent digestibility to 55%. Similar results were obtained in young girls by Daniel *et al.* (7).

MacLean *et al.* (2, 4) used children who were clinically recovered from acute malnutrition and were gaining weight. It would be necessary for us to assume that their digestive tracts had been temporarily (or permanently) damaged by their earlier bout with malnutrition in order for them to react differently to sorghum proteins. However, these same children were fed casein and gave digestion and absorption values similar to those observed in normal well-nourished children. In addition, the same type of child had been used for previous studies on casein, wheat (70% extraction), maize, and rice and gave average values of 81%, 81%, 73%, and 66%, respectively, compared with 46% for sorghum. Finally, decorticated extruded sorghum gave values in previously malnourished children equal to the values obtained with wheat (2, 4). This indicates that when the sorghum is more digestible [as indicated also by our pepsin method (Table 1)], previously malnourished children digest sorghum proteins as readily as they do wheat proteins.

The large difference in digestibility between sorghum and other major cereals observed with children cannot be detected with the rat. However, our pepsin method shows this difference and should be useful in research on improving the digestibility of sorghum for humans.

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