

Discovery of Grain Sorghum Germ Plasm with High Uncooked and Cooked In Vitro Protein Digestibilities¹

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ABSTRACT

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Grain sorghum has been documented to have low protein digestibility relative to other cereal grains. Low protein digestibility of sorghum is most pronounced in cooked foods and is ranked slightly lower than corn as a feed grain. In this article, sorghum germ plasm is identified that has substantially higher uncooked and cooked flour in vitro protein digestibility than normal cultivars. Sorghum lines were found within a high-lysine population derived from the mutant P721Q that have $\approx 10\text{--}15\%$ higher uncooked and $\approx 25\%$ higher cooked protein digestibilities using a pepsin assay. Highly digestible sorghum grain showed little reduction in digestibility after cooking, compared to the large reduction that is typical of normal sorghum cultivars. Using the three-enzyme pH-stat method, we showed that the highly digestible lines had the same degree of peptide bond hydrolysis in ≈ 5 min, as was found in 60 min in the normal cultivar, P721N. Differences in protein digestibility were related to enzyme susceptibility of the

major storage prolamins, α -kafirin, that comprises $\approx 50\text{--}60\%$ of the total sorghum grain protein. Using the enzyme-linked immunosorbent assay (ELISA) technique to track the pepsin digestion of α -kafirin, the highly digestible lines had $\approx 90\text{--}95\%$ α -kafirin digested in 60 min compared to 45–60% for two normal cultivars. γ -Kafirin, a minor structural prolamins found mainly at the periphery of protein bodies, was also somewhat more digestible in the highly digestible sorghums. Highly digestible grain was of a floury kernel type, though recently this trait has been found in a modified background. More digestible protein from sorghum grain, that additionally is high in lysine content and has a fairly hard endosperm, could be of important benefit to populations who lack adequate protein in their diets, and may, pending further studies, prove to increase the value of sorghum as a feed grain.

Sorghum (*Sorghum bicolor* (L.) Moench) grain is the principle staple food for millions of people living in the semiarid regions of Africa and Asia. It is also a major feed grain, used both in developed and developing countries. Tannin-free or low tannin sorghum cultivars, which are predominately grown around the world, have been shown to have low protein digestibility compared to other major cereals (MacLean et al 1981, Hamaker et al 1986). This low digestibility characteristic is most pronounced in cooked sorghum products, such as porridge, though is still notable in the raw grain. Additionally, this trait is independent of tannin content, which itself is known to reduce the digestibility of sorghum proteins. Elkin et al (1996) showed that protein digestibility measured in chickens varied markedly among sorghum cultivars of similar condensed tannin content and was low in some cultivars essentially free of tannin.

Sorghum protein is somewhat unique among the plant food proteins in that it becomes markedly less digestible after cooking (Axtell et al 1981, Eggum et al 1983, Mitaru et al 1985, Oria et al 1995b). In recovered malnourished children fed cooked porridges made from four sorghum cultivars, apparent protein digestibility was only 46% (mean value), compared to 81% for wheat, 73% for maize, and 66% for rice (MacLean et al 1981). Compared to the control casein diet, sorghum protein was only 57% as digestible. Another human study showed 55% apparent protein digestibility for cooked sorghum (Kurien et al 1960). Two further metabolic studies done by the same group showed that processing sorghum either by fermentation or decortication-extrusion markedly increased protein and energy digestibilities (MacLean et al 1983, Graham et al 1986). In nonruminant livestock animals, the protein digestibility of sorghum is slightly less than for other major feed cereals. For example, in swine, protein digestibility of sorghum is reported to be $\approx 95\%$ that of yellow dent maize (Rooney 1990). Low starch and protein digestibilities, which may be related, affect the overall feed efficiency and pricing of grain sorghum.

Mertz et al (1984) found that an in vitro pepsin digestion method produced protein digestibility values for sorghum, and other cereals, similar to those reported by MacLean et al (1981) for children. In vitro protein digestibility of cooked sorghum flour was $\approx 20\%$ lower than it was for other cereal flours. Using this procedure Hamaker et al (1987) showed that cooking sorghum in the presence of a number of different reducing agents prevented the digestibility decrease usually seen, resulting in digestibility values comparable to uncooked sorghum flour, and nearly equal to the levels of barley, rice, maize, and wheat. The action of the disulfide bond-cleaving agents suggested that sorghum proteins develop enzyme-resistant disulfide-bound complexes upon cooking that reduce digestibility.

The main proteins in sorghum grain are the prolamins storage proteins, kafirins, comprising in normal cultivars $\approx 70\%$ of total grain protein and $\approx 80\%$ of endosperm protein (Hamaker et al 1995). Kafirins are found exclusively in the kernel endosperm in enclosed organelles called protein bodies. They are extracted using an aqueous alcoholic solution containing a reducing agent, such as 60% t-butanol plus 2-mercaptoethanol. Sorghum prolamins are subclassified as α -, β -, and γ -kafirins based on a nomenclature system developed for maize zeins (Shull et al 1991). α -Kafirin is the true storage protein making up $\approx 80\%$ of total kafirin and is found in the central light-staining region of protein bodies (Shull et al 1992). β - and γ -Kafirins are found in dark-staining regions at the protein body periphery and inclusions within the body. They contain significant amounts of cysteine (β 5 mol% and γ 7 mol%) and γ , in particular, has been shown to be highly disulfide-bound in mature grain (Oria et al 1995a). Hamaker et al (1986) showed that, in general, kafirins are much less digestible than nonkafirins and are the last proteins to be digested whether in the uncooked or cooked state.

Our theory regarding the basis of the low protein digestibility of sorghum grain is that γ - and possibly β -kafirins form a disulfide-bound enzyme-resistant layer at the periphery of protein bodies, thus restricting access of proteases to the interior-located and more easily digested α -kafirin protein (Oria et al 1995a,b). Cooking likely promotes sulfhydryl-disulfide interchange reactions at the protein body periphery making them less digestible.

Although kafirin proteins are nutritionally incomplete with little lysine, in mixed human diets that are deficient in protein content the increase in digestible protein could be quite valuable. In the case of sorghum grain for animal feed, increased availability of protein could reduce the level of higher priced protein supplements needed in feed formulations. Furthermore, more efficiently digested protein

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may make starch more available for digestion, as the dense protein matrix in sorghum endosperm is believed to cause the comparatively low starch digestibility of sorghum for livestock (Rooney and Pflugfelder 1986).

In 1975, Axtell and colleagues (Mohan, 1975) developed a chemically induced mutant P721 Opaque (P721Q) with 60% more lysine than normal sorghum. The enhanced lysine content of that line resulted primarily from an increase in the lysine-rich nonkafirin and a small decrease in the amount of the lysine-poor kafirin proteins (Guiragossian et al 1978, Hamaker et al 1995). We report here the discovery of sorghum lines within a population derived from crosses of P721Q and hard endosperm, food-grade sorghums that have substantially higher uncooked and cooked protein digestibilities compared to normal sorghum cultivars. This is followed by findings showing the change in digestibility of α -kafirin in the highly digestible lines.

MATERIALS AND METHODS

Plant Materials

Five sorghum cultivars (P721Q, high-lysine mutant), P850029 (contains the parents P721Q and an agronomically adapted elite cultivar), P721N (normal, Mexico 1993-94 crop); P851171 (contains the parents P721Q and an agronomically adapted elite cultivar, West Lafayette, IN 1994 crop); and SAFRA (normal, Puerto Rico 1993-94 crop) were used to determine protein digestibility (in vitro pepsin and in vitro pH-stat), and for in vitro pepsin time-course digestions (0, 10, 30, and 60 min) followed by visualization of undigested proteins by electrophoresis and quantitation of the individual undigested kafirins by enzyme-linked immunosorbent assay (ELISA). Protein contents for these samples as determined by micro-Kjeldahl content (Approved Method 46-13, AACC 1995) were 14.7, 12.7, 11.8, 11.8, and 12.6% for P721Q, P850029, P721N, P851171, and SAFRA, respectively. Samples (P850029, P851171, P721N, P721Q [West Lafayette 1992 crop], and SAFRA [Puerto Rico 1992-1993 crop]) from a different crop year were analyzed for pepsin digestibility for comparative purposes. Twenty sorghum cultivars containing P721Q in their pedigree (Mexico 1993-93 crop) were also tested by the pH-stat digestibility method. In all cases, whole grain was harvested after field drydown, \approx 20% decorticated using a tangential abrasive dehulling device (model 4E, Venables Machine Work, Ltd., Saskatoon, Canada), and milled using a ball mill to pass through a 125-mesh screen.

Pepsin Assay for in vitro Protein Digestibility

Flour samples (200 mg) were subjected, uncooked and cooked, to in vitro protein digestion using porcine pepsin (Sigma P-7000, activity: 890 U/mg of protein, Sigma Chemical Co., St. Louis, MO) as described by Mertz et al (1984). For cooking, flour samples were suspended in 2 mL of water and placed in a boiling water bath for 20 min. Samples were incubated in 35 mL of enzyme solution (1.5 g of enzyme/L in 0.1M KH_2PO_4 , pH 2.0) for 2 hr at 37°C in a shaking water bath. Digestion was stopped by adding 2 mL of 2N NaOH. After centrifugation (4,900 \times g, 4°C, 20 min) the supernatant was discarded, the residue was twice washed and centrifuged in 20 mL of buffer (0.1M, K_2HPO_4 , pH 7.0), and the residue and original flour were analyzed by microKjeldahl for nitrogen. The percentage of soluble nitrogen, calculated by difference, was reported as in vitro protein digestibility.

For five sorghum cultivars (P850029, P851171, P721Q, SAFRA, and P721N), in vitro pepsin digestion was followed by ELISA analysis to quantitate undigested α -, β -, and γ -kafirins for uncooked and cooked samples. Flour samples to be cooked (200 mg) were suspended in 5 mL of 0.8M Na_2HPO_4 (pH 6.0) containing 50 μL of heat-stable bacterial α -amylase (Sigma A-3306) and cooked as above. Both uncooked and cooked samples were digested for 0, 10, 30, and 60 min. The undigested proteins of two sorghum cultivars (P850029 and P721N) were also visualized by SDS-PAGE after pepsin digested for 0, 0.5, 1, 3, 5, 10, 20, 30, and 60 min.

pH-Stat Assay for in vitro Protein Digestibility

Flour samples (equivalent to 10 mg of N) were subjected to a multienzyme in vitro pH-stat protein digestion according to a modified Pedersen and Eggum (1983) procedure. Enzyme solution was prepared to equal 1.72 mg of trypsin (Sigma T-0134 from porcine pancreas, activity 13,700 U/mg of protein), 4.43 mg of α -chymotrypsin (Sigma C-4129 from bovine pancreas, activity 42 U/mg of solid), and 0.51 mg of peptidase (Sigma P7500 from porcine intestinal mucosa, activity 120 U/g of solid) for each 1 mL of purified water. The enzyme solution was adjusted to pH 8.0 with 0.1N NaOH and placed on ice. For uncooked flours, purified water (10 mL at 37°C) was added to each 10 mg of N flour sample, and titrant (0.1N NaOH) added to bring to pH 8.0. Enzyme solution was added (1 mL) and amount of titrant (0.1N NaOH) required to hold the digestion solution at pH 8.0 was monitored for the designated digestion time or times. For cooked samples, flour equal to 5 mg of N was weighed into test tubes, 2.5 mL of purified water was added and cooked while stirring for 20 min. Samples were transferred with 5 mL of purified water, enzyme solution added (0.75 mL), and titrated NaOH was monitored for the designated digestion times.

SDS-PAGE

SDS-PAGE was performed using a vertical gel electrophoresis system (model V15-17, GIBCO BRL Life Technologies, Gaithersburg, MD) on gel slabs (160 \times 200 \times 1.5 mm). The running gel was a 10–15% (w/v) polyacrylamide gradient, with the dense solution consisting of 15% (w/v) total acrylamide, 0.2M Tris, 5% (v/v) glycerol, 6M urea, 0.1% (w/v) SDS (pH 8.5), and the light solution consisting of 10% (w/v) total acrylamide, 0.2M Tris, 6M urea, and 0.1% (w/v) SDS (pH 8.5). The stacking gel was 4% (w/v) total acrylamide, 0.1M Tris, and 0.1% (w/v) SDS (pH 6.8). Gels were polymerized with tetramethylene diamine and ammonium persulfate.

After pepsin digestion, proteins were extracted from the undigested residues for 2 hr on a shaker at room temperature with 2 mL of a solvent containing 0.125M sodium tetraborate buffer (pH 10), 2% SDS (w/v), and 1% 2-ME (v/v). Following centrifugation (4,900 \times g, 22°C, 20 min), protein extract (200 μL) and SDS sample solvent (100 μL ; 0.06M Tris, 2.0% [w/v] SDS, 2.0% [v/v] 2-ME, 10% glycerol, and 0.3% [w/v] bromophenol blue) were combined, boiled 3 min, and loaded (80 μL) into the sample wells of the gel. The tank buffer was 0.025M Tris, 0.19M glycine, and 0.1% (w/v) SDS. Electrophoresis was performed at 60V for 16 hr. Gels were stained using 0.25% (w/v) Coomassie Brilliant Blue R250 stain in 46% (v/v) methanol and 8% (v/v) acetic acid. Gels were destained in 20% (v/v) ethanol and 10% (v/v) acetic acid (Wilson 1986).

ELISA

Primary antibodies against α -, β -, and γ -kafirins were previously obtained in our laboratory by Shull et al (1992) and the ELISA assay developed by Oria et al (1995a). A check sample (P721Q) was used in each microtiter plate to monitor assay precision. Proteins were extracted (30 mg/mL) in a 0.0125M borate buffer (pH 10) containing 2% SDS and 1% 2-ME by shaking for 14 hr at 22°C. Protein extracts (100 μL) were diluted 10-fold in the same extraction solvent. For γ -kafirin analysis, the standard, control, and zero digest extracts were diluted again 250-fold in 40% (v/v) ethanol and 10% (v/v) acetic acid. Extracts from the 10-, 30-, and 60-min digests were diluted 125-fold. For β -kafirin analysis, diluted samples were further diluted twofold. For α -kafirin analysis, they were further diluted 10-fold. Diluted extracts (100 μL) were added to the second row of an ELISA microtiter plate (Corning high-binding ELISA plate 25801-96, Corning, New York) to 50 μL of 40% ethanol and 10% acetic acid. The samples were mixed immediately. Six three-fold dilutions were made into adjacent wells of consecutive rows that contained 40% ethanol and 10% acetic acid. The plates were incubated for 2 hr at 32°C to allow for binding of the antigens. After incubation, solutions were removed from the wells by aspiration, and the wells were rinsed three times with 25 mM Tris-HCl,

0.9% (w/v) NaCl, and 0.05% (v/v) Tween 20 (TBS-T), pH 7.5. Then, 200 μ L of bovine serum albumin (2.5%, w/v, in TBS-T) was added to each well. Plates were covered and incubated for 2 hr at 32°C. Solutions were aspirated, the wells rinsed with TBS-T, and 100 μ L of primary antibody solution (anti- α -kafirin, anti- β -kafirin, or anti- γ -kafirin serum [1:1,000 in TBS-T]) were added to each well. The plates were again covered and incubated for 2 hr at 32°C. After the solutions were aspirated and the wells rinsed with TBS-T, 100 μ L of goat antirabbit antibodies labeled with alkaline phosphatase (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA), diluted 1:5,000 in TBS-T, were added to each well and incubated overnight at 4°C. Aspiration and rinsing steps were repeated. Enzyme substrate (Sigma 104 phosphatase substrate tablets [paranitrophenyl phosphate]) was dissolved in diethanolamine (9.7%, v/v, pH 9.8) as described by Clark et al (1986) and added to each well. Color was developed for 45 min for α -kafirins, 90 min for γ -kafirins, and 2 hr for β -kafirins, and absorbance was read at A_{405} on a V_{max} ELISA platereader (Molecular Devices, Menlo Park, CA). Absolute amounts of the different proteins were computed by dividing the slope of their linear regression lines by the slope of the line of a standard (P721N), and then multiplied by the previously determined milligram amount of α -, β -, or γ -kafirin per 100 mg of P721N flour.

TABLE I
In Vitro Protein Digestibilities (Pepsin, %) Using Pepsin Assay of Two Normal Sorghum Cultivars (P721N and SAFRA) and Three Sorghum Cultivars from a High-Lysine Population (P721Q, P851171, and P850029)

Cultivar	1992		1994	
	Uncooked	Cooked	Uncooked	Cooked
P721N	80.2b ^a	56.8b	70.9b	48.5c
SAFRA	65.8c	57.8b	73.5b	47.3c
P721Q	79.8a,b	57.4b	86.7a	71.9b
P851171	87.0a	80.8a	88.3a	72.5a,b
P850029	87.8a	78.7a	89.4a	75.1a

^a Values followed by the same letter are not significantly different ($P < 0.05$).

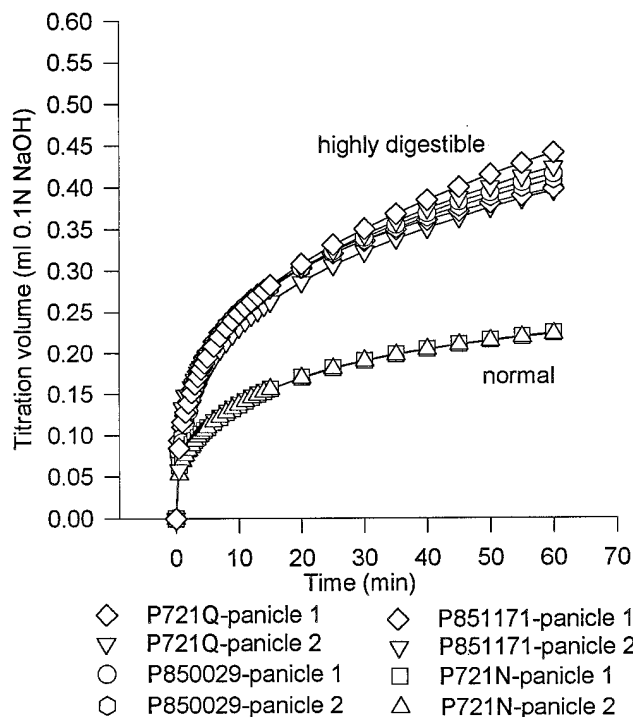


Fig. 1. Titration curves by the pH-stat method over 60 min comparing two uncooked panicles of one normal (P721N) and three highly digestible (P721Q, P850029, P851171) sorghum lines.

Statistical Analysis

Statistical analysis was performed using the one-way analysis of variance (ANOVA) test, and significant differences ($P < 0.05$) between means were determined by the Student-Newman-Keul's test for multiple comparisons (Steel and Torrie 1980).

RESULTS

In Vitro Digestibility

Sorghum lines P851171 and P850029 from two crop years, both derived from crosses containing the high-lysine mutant P721Q, showed markedly higher uncooked and cooked in vitro (pepsin) protein digestibilities than did the normal sorghum cultivars P721N and SAFRA (Table I). Cooking generally resulted in less decrease in digestibilities in P851171 and P850029 (6 and 9% decrease in 1992, and 16 and 14% decrease in 1994) than in the normal cultivars P721N and SAFRA (23 and 8% decrease in 1992, and 22 and 26% decrease in 1994). Pepsin digestibilities of 22 normal sorghum cultivars of the 1992 crop (data not shown) averaged 74.8% uncooked and 59.5% cooked, for an average decrease due to cooking of 15.3%.

Cooked digestibilities for P851171 and P850029 were somewhat different in the two crop years with the 1994 samples showing a larger decrease in digestibility following cooking. However, decreases due to cooking were also greater for the normal cultivars in 1994 compared to 1992, averaging a 24% drop. Cooked digestibilities for the two highly digestible lines were $\approx 25\%$ higher than for the normal cultivars. P721Q had lower uncooked and cooked digestibilities when compared to P851171 or P850029 in 1992, but were nearly equal to the two highly digestible lines in 1994. The differences in values found due to year have been corroborated in a recent study in our laboratory that revealed sig-

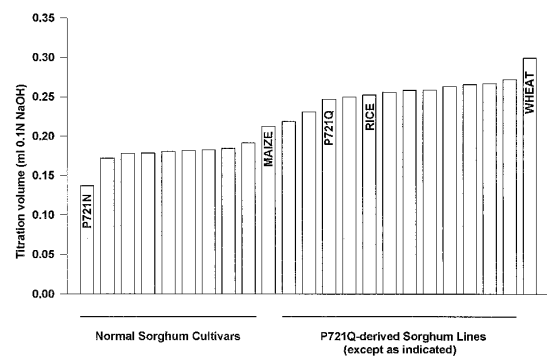


Fig. 2. Comparison of titration volumes at 10 min using the pH-stat digestibility method for 20 sorghum cultivars or lines grown in the same location and year, plus wheat, rice, and maize.

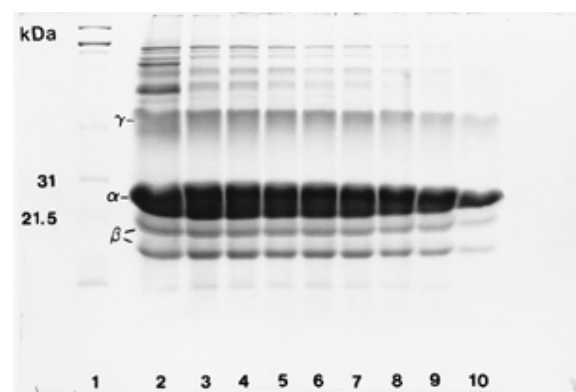


Fig. 3. SDS-PAGE gel of a time-course digestion of a normal sorghum cultivar (P721N); undigested proteins are shown. Lane 1: molecular weight standard; lanes 2–10: digestion at 0, 0.5, 1, 3, 5, 10, 20, 30, and 60 min, respectively.

nificant year-to-year variations in in vitro protein digestibility for one cultivar over seven years (Buckner and Hamaker, unpublished results).

In vitro pH-stat titration curves for uncooked flours (1994 crop) showed remarkable differences in protein hydrolysis rates between P851171, P850029, and P721Q, and the normal cultivar, P721N (Fig. 1). This technique monitors the amount of peptide bonds hydrolyzed by digestive enzymes. Lines P851171, P850029, and P721Q showed much higher initial rates of digestion, as well as later higher titration volumes when compared to P721N. Approximately the same number of peptide bonds were hydrolyzed in ≈ 5 min in highly digestible cultivars as were in 60 min in the normal cultivar. Tita-

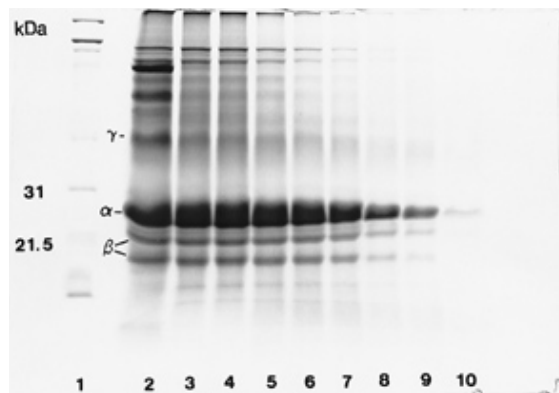


Fig. 4. SDS-PAGE gel of a time-course digestion of a highly digestible sorghum line (P850029); undigested proteins are shown. Lane 1: molecular weight standard; lanes 2–10: digestion at 0, 0.5, 1, 3, 5, 10, 20, 30, and 60 min, respectively.

tion curves of two panicles from each of the cultivars were the same, indicating genetic homogeneity.

In vitro pH-stat titrations at 10 min for 20 sorghum genotypes grown at the same location, plus wheat, rice, and maize are shown in Fig. 2. Sorghum cultivars with P721Q in their pedigree and P721Q were consistently more digestible than normal sorghum cultivars and normal maize.

Digestion of the Individual Kafirins

Analysis of pepsin undigested proteins using SDS-PAGE revealed that differences in digestibilities between normal and highly digestible cultivars are related to differences in disappearance during proteolysis of the α -, β -, and γ -kafirin proteins (Figs. 3 and 4). Nonkafirin proteins were rapidly digested in both cultivars. In the time-course digestion of the normal cultivar P721N, the α -kafirin protein was not noticeably digested until 60 min of pepsin incubation. γ -Kafirin in P721N was resistant to digestion up to 30 min. For the highly digestible cultivar P850029, α -kafirin was digested as early as 3 min and had almost disappeared by 60 min of digestion. The γ -kafirin band disappeared by 5 min. β -Kafirin was less digestible than γ -kafirin in P850029 but was still more rapidly digested than in P721N.

ELISA was used to show quantitative differences in digestibility of the individual kafirins among normal and highly digestible sorghum cultivars. Digestion of uncooked highly digestible lines (P850029, P851171, and P721Q) with pepsin showed that α -kafirin was 75–90% digested in 30 min as compared to 35–45% in the normal cultivars (P721N, SAFRA) (Fig. 5). By 60 min, α -kafirin was virtually all digested in the highly digestible cultivars compared to 50–60% digested for the normal cultivars.

The percentages of α -kafirin digested from cooked sorghum flours are shown in Fig. 6. Cooking caused some reduction in digesti-

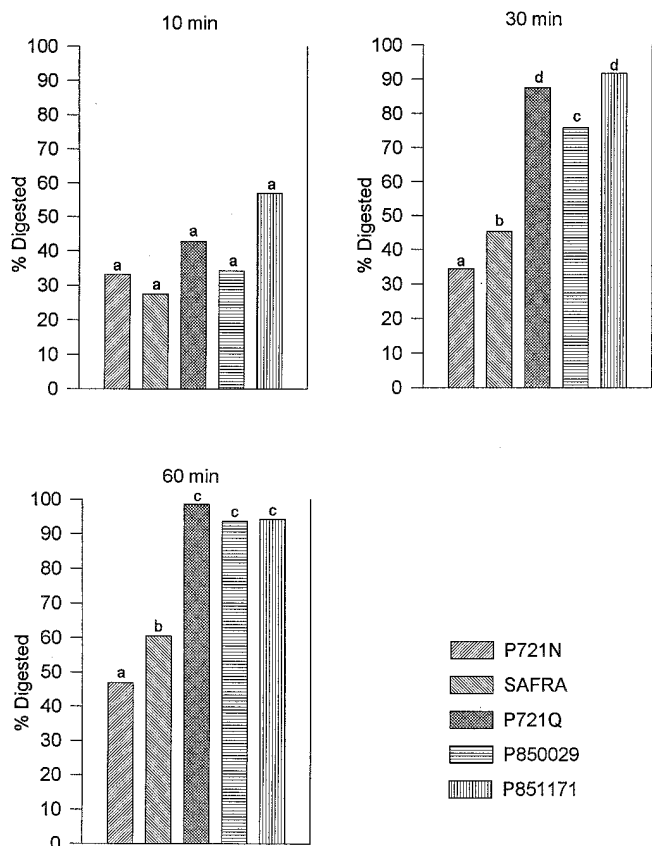


Fig. 5. Percentage of α -kafirin digested in uncooked flour of two normal (P721N and SAFRA) and three highly digestible (P721Q, P850029, and P851171) sorghum cultivars determined using enzyme-linked immunosorbent assay (ELISA) after 10, 30, and 60 min of in vitro pepsin digestion. Mean values with different letters are significantly different at $P < 0.05$.

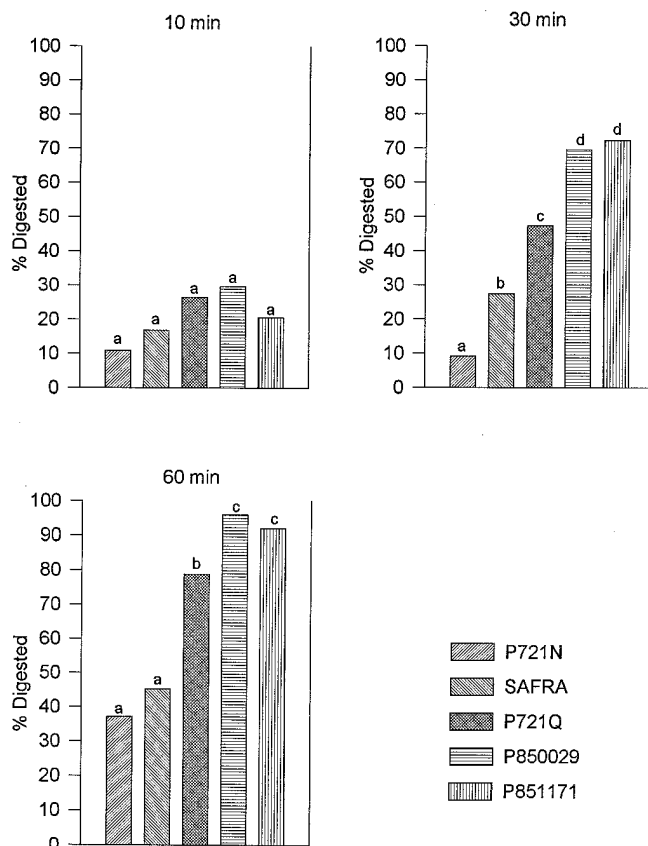


Fig. 6. Percentage of α -kafirin digested in cooked flour of two normal (P721N and SAFRA) and three highly digestible (P721Q, P850029, and P851171) sorghum cultivars determined using enzyme-linked immunosorbent assay (ELISA) after 10, 30, and 60 min of in vitro pepsin digestion. Mean values with different letters are significantly different at $P < 0.05$.

bility of α -kafirin in all cultivars, though again the highly digestible lines had much more rapid α -kafirin digestion. P721Q after cooking showed some loss in digestibility of α -kafirin. At 60 min of pepsin digestion, α -kafirin in P850029 and P851171 was nearly all digested, and in the two normal sorghum cultivars was <50% digested.

β - and γ -Kafirins in uncooked sorghum flours were more digestible in P721Q and P850029 than in the normal cultivars (Figs. 7 and 8), although the differences were not as great as with α -kafirin. Similar trends were seen in the cooked samples (not shown). In the case of γ -kafirin, there was less difference between the normal and highly digestible cultivars, although again the highly digestible group was, for the most part, more digestible at 30 and 60 min.

DISCUSSION

In this study, substantially higher *in vitro* protein digestibilities were found in sorghum lines carrying the P721Q mutant in their background, as well as P721Q itself, than in normal sorghum cultivars. Higher protein digestibilities of these improved lines were shown to be a result of more rapid digestion of the main sorghum storage protein, α -kafirin. This protein alone comprises \approx 50–60% of total grain protein, ranging from the high-lysine P721Q to normal cultivars (calculations based on values for total kafirin in Hamaker et al 1995). Highly digestible grain was of a floury kernel type, though recently this trait has been found in a modified kernel type with partial vitreous endosperm (Hamaker and Mamadou, *unpublished data*). There is a continuing effort to improve kernel type along with the high protein digestibility trait.

In addition to the importance of finding sorghum cultivars with high protein digestibility, this discovery also clarifies why normal sorghum has poor protein digestibility when compared to other cereals. We have theorized that sorghum protein in normal cultivars is less digestible because the protein bodies are more resistant to proteolysis than the protein bodies of other similar cereal grains such as maize (Hamaker et al 1987; Oria et al 1995a,b). Various experimental evidence (Oria et al 1995a,b) implicates the enzyme-resistant nature of the peripherally located γ - and possibly β -kafirins that prevents ready access of proteases to α -kafirin. Additionally, γ - and β -kafirins have been shown to form extensive intermolecular disulfide-bound complexes during seed development and during cooking (Oria et al 1995a,b). In a companion study on these same highly digestible lines (Oria et al *in press*), γ -kafirin was found to be located not at the protein body periphery, but at the base of folds of a highly invaginated protein body structure. Thus, α -kafirin in protein bodies of the highly digestible cultivars is more exposed to digestive enzymes than in normal protein bodies, and accordingly, as shown in this study, is quickly digested.

Notably, this is the first time sorghum lines have been identified that retain relatively high *in vitro* protein digestibilities after cooking. Cooking normally decreases digestibility of sorghum proteins, specifically the kafirins (Axtell et al 1981, Hamaker et al 1986, Oria et al 1995b). In previous work in our laboratory (Hamaker 1986), we showed that isolated, native (unreduced) α -kafirin is highly digestible and retains its high digestibility after cooking, unlike the γ - and β -kafirins that become more resistant to digestion (Oria et al 1995b). Thus, in the highly digestible lines, the improved accessibility of proteases to α -kafirin, even after cooking, must account for their overall higher protein digestibility.

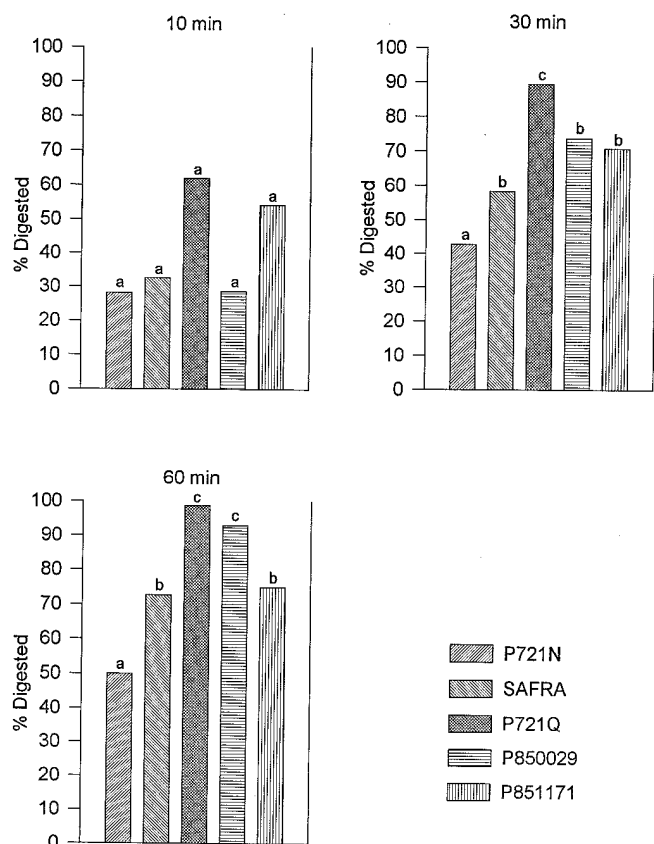


Fig. 7. Percentage of β -kafirin digested in uncooked flour of two normal (P721N and SAFRA) and three highly digestible (P721Q, P850029, and P851171) sorghum cultivars determined using enzyme-linked immunosorbent assay (ELISA) after 10, 30, and 60 min of *in vitro* pepsin digestion. Mean values with different letters are significantly different at $P < 0.05$.

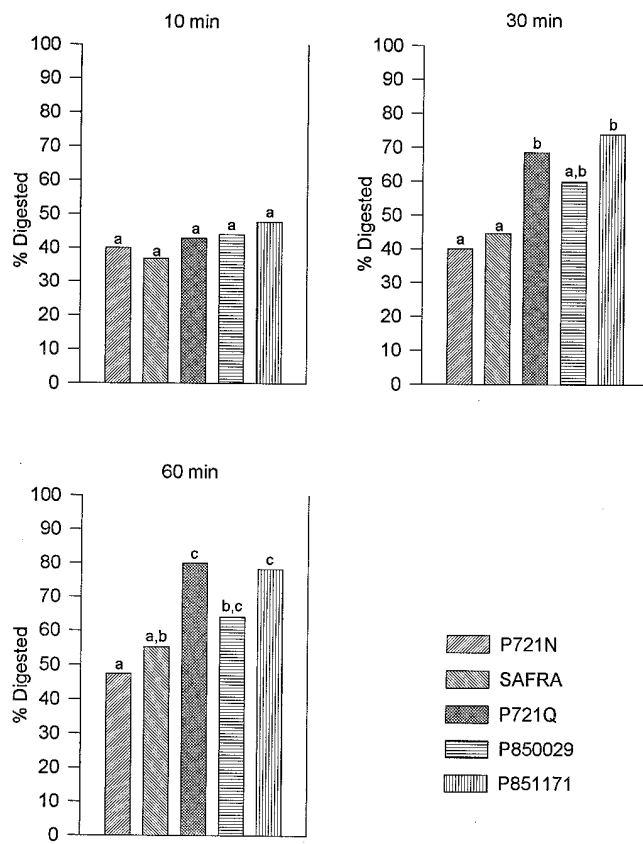


Fig. 8. Percentage of γ -kafirin digested in uncooked flour of two normal (P721N and SAFRA) and three highly digestible (P721Q, P850029, and P851171) sorghum cultivars determined using enzyme-linked immunosorbent assay (ELISA) after 10, 30, and 60 min of *in vitro* pepsin digestion. Mean values with different letters are significantly different at $P < 0.05$.

CONCLUSIONS

Sorghum germ plasma was identified with substantially higher uncooked in vitro protein digestibility when compared to normal cultivars. Sorghum grain with improved protein digestibility could be an important nutritional contribution to the diets of people and livestock depending on sorghum as a main protein source. For humans consuming a mixed diet with marginal to inadequate protein levels, the addition of more digestible protein, with an accompanying boost in lysine content (as is true in these lines), would increase the utilizable protein in the diet. In animals, in addition to increasing available protein, a highly digestible sorghum protein may increase starch availability, as the protein component is known to restrict starch digestion (Rooney and Pflugfelder 1986). This needs to be further studied. In this study we have revealed the reason for the digestibility differences between normal and highly digestible sorghum cultivars, that being increased α -kafirin digestibility. This knowledge should facilitate the development of a screening assay for breeders to use to select for highly digestible lines. The assay presently being worked on is based on relative differences in disappearance of α -kafirin between the highly digestible and normal sorghum cultivars.

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