

A Rapid Protein Digestibility Assay for Identifying Highly Digestible Sorghum Lines¹

Adam Aboubacar,² John D. Axtell,³ Chia-Ping Huang,² and Bruce R. Hamaker^{2,4}

ABSTRACT

Cereal Chem. 78(2):160–165

Protein digestibility in sorghum (*Sorghum bicolor* (L.) Moench) lines was determined using two standard procedures (pepsin digestibility and pH-stat) and compared with a newly developed, rapid electrophoresis-based screening assay. The new assay was based on the rate of α -kafirin disappearance after pepsin digestion. α -Kafirin, the major sorghum storage protein, makes up ≈ 60 –70% of the total protein in the grain. In the new assay, samples were first digested with pepsin for 1 hr, and undigested proteins were then analyzed by SDS-PAGE. The intensities of the undigested α -kafirin bands were measured. Higher band intensity indicated lower protein digestibility. The new assay was significantly correlated with the standard pepsin digestibility assay ($r = -0.96$, $n = 16$) after which it was patterned. The same was true of the pH-stat procedure ($r = -0.85$, n

$= 16$). This implies that the new assay is comparable to existing procedures and can be used for screening sorghum lines for protein digestibility. Two groups consisting of high-protein digestibility and wild-type sorghum lines were identified when the new assay was tested on 48 sorghum lines derived from crosses of wild-type and mutant high protein digestibility lines, indicating that the new assay was efficient in differentiating between the two groups. Advantages of the new assay over the standard procedures include considerable reduction in analysis time and sample size required for the analysis. For example, analysis time was reduced by 20% and sample size by 10% when the new assay was used as compared with the pH-stat procedure. We estimate that ≈ 60 sorghum lines can be screened in a day by a single operator using the new assay.

Sorghum (*Sorghum bicolor* (L.) Moench) grain ranks fifth among the world cereal grains. It is a major staple food in many parts of Africa and India. In the United States, it is mainly used as an animal feed grain. A significant problem with sorghum is its comparably poor nutritional quality. Protein digestibility in cooked sorghum was considerably lower when compared with other cereals (MacLean et al 1981; Hamaker et al 1986). The cooking process as used for human consumption reduces protein digestibility of sorghum due to rearrangement and formation of disulfide bonds (Hamaker et al 1987; Oria et al 1995b). This was demonstrated by the significant increase in protein digestibility observed when sorghum was cooked in the presence of 2-mercaptoethanol and other reducing agents that cleave disulfide bonds. Increasing protein digestibility in sorghum would benefit the undernourished poor, who rely heavily on sorghum as their main source of protein and energy. Proteins in sorghum grain used for animal feed are also somewhat less available than other grain proteins, and may further inhibit starch digestibility (Bramel-Cox et al 1995).

The main proteins in sorghum are the aqueous alcohol-soluble storage prolamins known as kafirins. Sorghum prolamins are classified as α -, β -, and γ -kafirins based on molecular weight, solubility, and structure (Shull et al 1991). α -Kafirin is the major storage protein making up ≈ 80 % of the kafirins and ≈ 60 –70% of the total protein in the sorghum grain (Hamaker et al 1995). α -Kafirin is enclosed in protein bodies surrounded by γ -kafirin, and to a lesser extent β -kafirin. β - and γ -Kafirins contain significant amounts of cysteine (Shull et al 1992), and γ -kafirin is highly disulfide-bound in mature grain. In another study, Hamaker (unpublished data) showed that isolated, native (unreduced) α -kafirin is highly digestible both before and after cooking. Oria et al (1995b) found that γ -kafirin is more resistant to digestion, especially after cooking, leading them to hypothesize that γ -, and to a lesser extent β -kafirins, form a disulfide-bound enzyme-resistant layer at the periphery of protein bodies that restricts access by proteases to easily digestible α -kafirin.

Recently, in our laboratory, we discovered sorghum lines that have remarkably higher uncooked and cooked in vitro protein digestibility than wild-type sorghum lines (Weaver et al 1998). These high-protein digestibility lines were found within a high-lysine sorghum population developed at Purdue University. Increased digestibility in these lines was due to increased rate of α -kafirin digestion. Transmission electron microscopy (TEM) examination of the endosperm of high-protein digestibility sorghum lines revealed irregularly shaped protein bodies with numerous invaginations or folds (Fig. 1B). These protein bodies were distinctly different from the spherical protein bodies of wild-type sorghum lines (Fig. 1A). Immunocytochemistry investigation (Oria et al 2000) showed similarities in localization of α - and β -kafirins within the protein bodies of high-protein digestibility and wild-type lines. However, in the high-protein digestibility lines, γ -kafirin was located at the base of the folds of the protein bodies instead of at the periphery, as is characteristic of wild-type lines. Consequently, α -kafirin in the high-protein digestibility lines is more exposed and more easily accessible to digestive enzymes. This, along with the large surface area exhibited by the invaginated protein bodies of high-protein digestibility lines, may explain the high in vitro protein digestibility. The discovery of these high-protein digestibility sorghum lines has generated interest in incorporating them into breeding programs. This, in turn, has prompted the need to develop a quick, inexpensive, and simple assay to screen breeders' lines for protein digestibility.

There are currently two in vitro procedures used to predict sorghum protein digestibility. Mertz et al (1984) developed an in vitro pepsin digestion method specifically for sorghum proteins that approximated data obtained from human feeding trials. Though very effective in differentiating for digestibility among sorghum lines, this method is lengthy and cumbersome and allows only a few samples to be analyzed at a time. Alternatively, the pH-stat procedure of Pedersen and Eggum (1983), provisionally recommended by FAO/WHO (1989), has been used by some investigators as a more rapid method to measure sorghum protein digestibility. In spite of the relatively short analysis time used, the pH-stat procedure too has limited practical use when screening hundreds of breeders' lines. Only ≈ 16 samples can be analyzed in one day using this procedure, and the operator must be present at all times. This article describes a rapid, simple, and efficient electrophoresis-based protein digestibility assay. The new assay, based on differences in the rate of α -kafirin digestion, was highly correlated with standard procedures of sorghum protein digestibility determination. Advantages of the new assay over existing procedures include considerable reduction in analysis time and sample size required for

¹ Paper 16120 of the Purdue University Agricultural Research Programs.

² Department of Food Science and the Whistler Center for Carbohydrate Research, Purdue University, West Lafayette, IN 47907-1160.

³ Department of Agronomy, Purdue University.

⁴ Corresponding author. Phone (765) 494-5886. Fax: (765) 494-7953. E-mail: hamakerb@foodsci.purdue.edu

the analysis, making it possible to screen a large number of breeders' lines for digestibility in one day.

MATERIALS AND METHODS

Materials

Sorghum lines used in this study were from a high-lysine population recently developed by researchers at Purdue University. The lines were derived from two populations of sorghum grown in Mexico during the 1996-97 crop season. Two high-protein digestibility and high-lysine (P850115 and P851171) and two wild-type (MR732 and SRN39) sorghum lines were crossed to generate the two populations (Table I). Both populations were of the F_{10} generation. A wild-type sorghum line (P721N) from which the high-protein digestibility mutants (P850115 and P851171) were derived also was

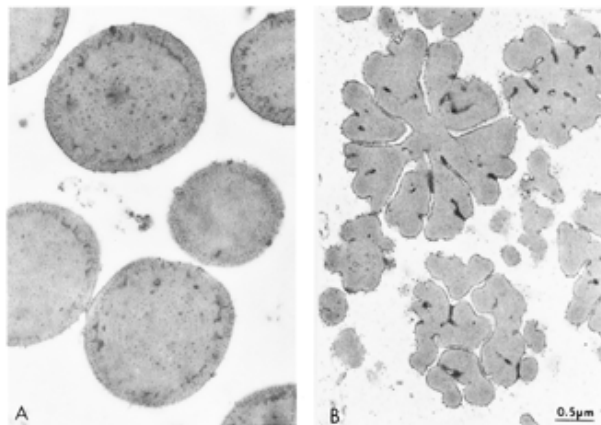


Fig. 1. Protein bodies structure of wild-type (A) and high-protein digestibility (B) sorghum lines (Oria et al 2000).

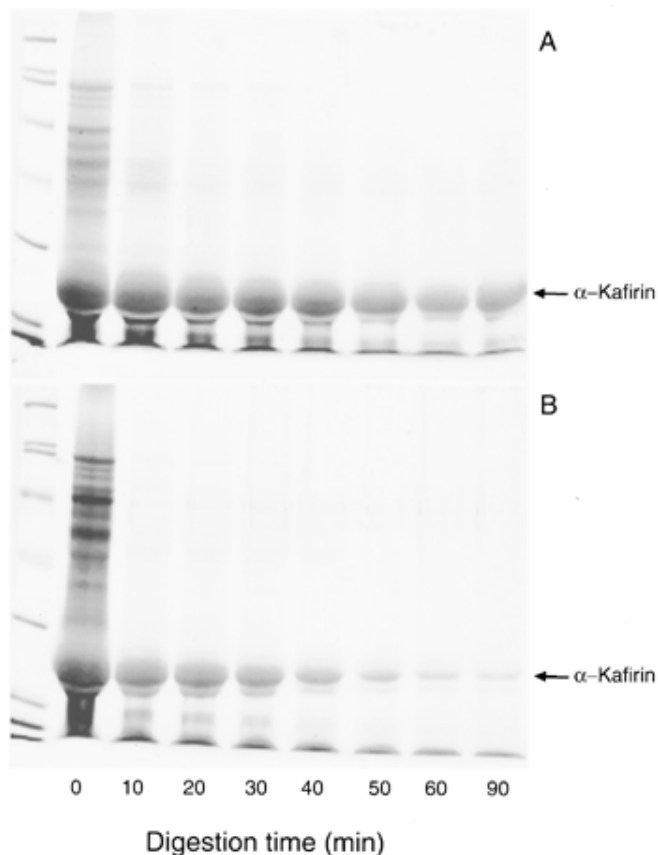


Fig. 2. SDS-PAGE gel of time-course digestion of wild-type (A) and high-protein digestibility (B) sorghum lines.

included in the study. All grain samples were ground into flour for 1 min using a ball mill (Brinkmann Instruments, Des Plaines, IL) before performing analyses.

Protein Determination

Nitrogen concentration ($N \times 5.7$) of the flour samples was determined by the micro-Kjeldahl procedure (Approved Method 46-13, AACC 2000).

Standard In Vitro Pepsin Digestibility

Pepsin digestibility (%) in sorghum flour was determined using the procedure described by Mertz et al (1984). Flour samples (200 mg) were weighed into Erlenmeyer flasks and mixed with 35 mL of porcine pepsin (Sigma P-7000, activity: 890 U/mg of protein, Sigma Chemical Co., St. Louis, MO) solution (1.5 g of pepsin/L in 0.1M KH_2PO_4 , pH 2.0). Samples were digested for 2 hr at 37°C in a shaking water bath. Digestion was stopped by addition of 2 mL of 2N NaOH. Samples were centrifuged ($4,900 \times g$, 4°C) for 20 min, and the supernatants discarded. The residues were washed and centrifuged twice with 20 mL of buffer (0.1M KH_2PO_4 , pH 7.0). Undigested nitrogen (N) was determined with a Technicon nitrogen analyzer. Digestibility was calculated as % digestibility = $(N \text{ in sample} - \text{undigested N})/N \text{ in sample} \times 100$.

Standard pH-Stat Procedure

The in vitro enzymatic pH-stat procedure described by Pedersen and Eggum (1983) was used to determine protein digestibility in the sorghum samples. Flour samples (equivalent to 10 mg of N) were weighed into pH-stat cups and digested in 1 mL of enzyme solution consisting of trypsin (Sigma T-0134 from porcine pancreas, 22,704 U), α-chymotrypsin (Sigma C-4129 from bovine pancreas, 186 U), and peptidase (Sigma P7500 from porcine intestinal mucosa, 0.052 U). The amount of 0.1M NaOH titrated to maintain pH 8.0 was used as an estimate of in vitro protein digestibility.

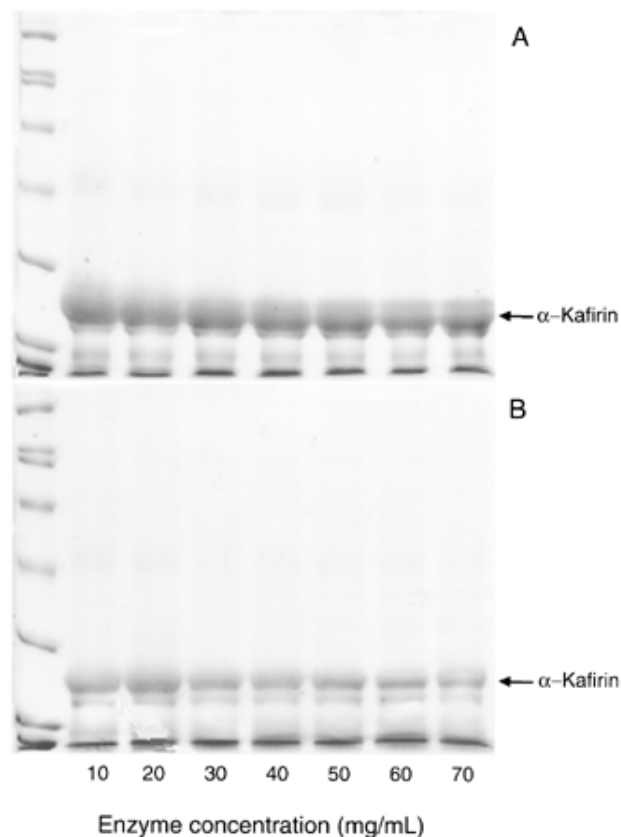


Fig. 3. SDS-PAGE gel of wild-type (A) and high-protein digestibility (B) sorghum lines after 1 hr of digestion at different pepsin concentrations.

Preliminary Experiments

Experiments were performed to determine pepsin concentration and digestion time necessary to attain optimum digestion with reduced sample size. Pepsin digestion was performed using a modification of the method described above (Mertz et al 1984). The modification consisted of reduction of sample size from 200 to 50–70 mg (depending on N content) and digestion time from 120 to 60 min. Reducing sample size also made it possible to conduct the assay using microcentrifuge tubes (Eppendorf), which was less cumbersome than using glassware. Results (Figs. 2 and 3) indicated that a pepsin concentration of 20 mg/sample and digestion time of 60 min were necessary to distinguish clearly between wild-type and high-protein digestibility lines.

Digestion

Sorghum flour was weighed into 1.5-mL Eppendorf tubes. Pepsin solution (Sigma P-7000, activity: 890 units/mg of protein) contained 20 mg of pepsin/mL of 0.1M KH₂PO₄, pH 2.0. A sample of the solution (1 mL) was then added to each tube. The suspensions were mixed on a vortex mixer, placed on a rotating shaker, and incubated at 37°C in a forced-air oven. After 1 hr of digestion, tubes were removed from the shaker and the reaction

was stopped by addition of 100 µL of 2N NaOH. The tubes were centrifuged in a microcentrifuge for 10 min at 14,000 rpm and supernatants were discarded. The pellets were resuspended in 1 mL of 0.1M potassium phosphate buffer (pH 7.0), mixed, and centrifuged. Supernatants were discarded and pellets were washed with purified water and centrifuged at 14,000 rpm for 10 min. Pellets were saved for protein extraction.

Protein Extraction

Protein was extracted from digested flour samples using the method described by Wallace et al (1990), as modified by Hamaker et al (1995). To pellets were added 0.5 mL of 0.0125M sodium tetraborate buffer (pH 10.0) containing 1% SDS (w/v) and 2% 2-mercaptoethanol (2-ME) (v/v). Protein was extracted for 1 hr at room temperature on a rotating shaker. The suspensions were centrifuged for 10 min at 14,000 rpm and supernatants analyzed by SDS-PAGE. For comparison, extraction of protein followed by SDS-PAGE also was conducted on undigested flour samples.

SDS-PAGE and Quantification of Undigested Protein

SDS-PAGE initially was performed using a vertical mini gel system (Mini-Protean II cell, Bio-Rad, Hercules, CA) with 10

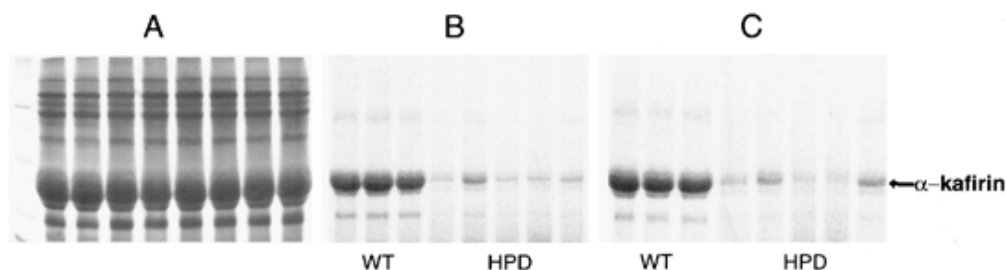


Fig. 4. Representative SDS-PAGE gels of sorghum proteins before (A) and after (B, same nitrogen basis; C, same flour basis) pepsin digestion. WT = wild-type, HPD = high protein digestibility.

TABLE I
Protein Content and Standard Pepsin Digestibility of 48 Sorghum Lines

Population I			Population II		
Sample	Protein (%)	Standard Pepsin Digestibility (%)	Sample	Protein (%)	Standard Pepsin Digestibility (%)
Parents			Parents		
SRN39	12.8de ^a	72.0f	MR732	8.3qr	63.6h
P851171	9.8mn	91.0a	P850115	9.3o	90.1a
F ₁₀ lines			F ₁₀ lines		
1	12.2fg	87.2b	1	8.4q	88.0ab
2	12.8de	89.2ab	2	10.2lm	88.9ab
3	9.4o	86.6b	3	10.6k	87.6ab
4	9.9m	88.1ab	4	11.4i	87.8ab
5	12.0gh	88.1ab	5	9.2op	86.7bc
6	12.1g	88.3ab	6	9.7n	90.9a
7	11.2i	86.9b	7	7.8s	85.4c
8	14.0b	89.9a	8	8.1r	84.8d
9	12.7ef	86.9b	9	9.0p	79.3e
10	9.9m	63.3h	10	13.5c	86.2c
11	12.5e	57.8j	11	15.7a	86.6bc
12	11.8hi	56.2j	12	10.8jk	64.0h
13	10.9j	60.7i	13	14.3b	84.6d
14	12.2fg	56.1j	14	13.2cd	82.7de
15	12.8de	65.6g	15	14.4b	83.8d
16	10.1m	71.2f	16	12.5ef	83.7d
17	10.2lm	67.3g	17	14.3b	85.1cd
18	10.8jk	66.3g	18	14.2b	87.1b
19	12.0gh	66.2g			
20	10.4l	84.1d	P721N	8.5q	76.7e
21	11.5i	83.1d			
22	7.8s	85.4c			
23	10.7k	88.2ab			
24	9.9m	88.7ab			
25	11.3ij	88.1ab			

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

wells. The resolving gel consisted of 15% polyacrylamide in 0.3M Tris buffer (pH 8.8) and 0.1% SDS (w/v). The stacking gel contained 4% polyacrylamide, 0.125M Tris buffer (pH 6.8), and 0.1% SDS (w/v). Tetramethylethylenediamine (5 μ L) and 5% ammonium persulfate (10 μ L) were used to polymerize the gels. A 15- μ L mixture (1:1) of protein extract and sample buffer (0.0132M Tris-HCl, 2% SDS, pH 6.8, 10% glycerol, 0.05% bromophenol blue and 2% 2-ME) was placed in a boiling water bath for 3 min, cooled to room temperature, and loaded into each well. Electrophoresis was done at 200 V for 45 min in tank buffer consisting of 0.2M Tris, 0.19M glycine, and 0.1% SDS (w/v). Gels were washed three times with purified water (100 mL) for 5 min each time and stained with 20 mL of Gelcode blue stain reagent (Pierce, Rockford, IL) for 60 min. Destaining was achieved by replacing the staining solution with 100 mL of purified water. Gels were sealed in plastic bags, placed under a light source, and photographed with a digital camera. Images were transferred to a Kodak 1 D Image Analyzer (Eastman Kodak Co., Rochester, NY 14650) and the intensity of the undigested α -kafirin bands was measured. Higher band intensity indicated lower protein digestibility. All experiments were conducted on same-flour and same-nitrogen concentration bases.

Statistics

All experiments were conducted in duplicate. Differences among lines were determined using Duncan's multiple comparison procedure. Simple regression and correlation analyses were performed to determine relationships between the newly developed assay and standard digestibility assays. Precision of the new assay was measured in 10 replicate samples of wild-type and high-protein digestibility lines as coefficient of variation = standard deviation/

mean \times 100 (Steel and Torrie 1980). Day-to-day variation in the assay was determined over four days using three sorghum lines. All statistical procedures were performed using SigmaPlot and SigmaStat software (Jandel Scientific Co. 1992).

RESULTS

Protein Content and Digestibility of Sorghum Samples

Table I lists the lines along with protein concentrations and standard pepsin digestibility values. Protein concentration in the samples had a range of 7.8–15.7%. In vitro digestibility values for the high-protein digestibility/high-lysine parents were 90.1% for P850115 and 91.0% for P851171, whereas the wild-type parents had digestibility values of 63.6% for MR732 and 72.0% for SRN39. Digestibility of the derived lines was 56.1–89.9% in population I and 64.0–90.9% in population II. Of the lines in population I, 40% had digestibility lower than the wild-type parent and the other 60% had digestibility higher than the wild-type parent. All lines in this population had digestibility at least slightly lower than the high-protein digestibility parent. Except for two entries, the lines in population II had digestibility values higher than the wild-type parent but lower than the high-protein digestibility parent. One line was similar in digestibility (64.0%) to the wild-type parent (63.6%) and the other had digestibility similar (90.9%) to the high-protein digestibility parent (90.1%).

Effect of Digestion Time and Enzyme Concentration on Digestion Rate

As shown in Fig. 2, most of the nonkafirin proteins in both wild-type and high-protein digestibility lines disappeared after 10 min of digestion, leaving mostly remnants of α -kafirin. The rate of digestion of α -kafirin was much faster in the high-protein digestibility than in the wild-type lines. The largest difference in digestibility of α -kafirin between wild-type and high-protein digestibility lines was observed after 60 min of digestion. The effect of enzyme concentration on α -kafirin digestion is shown in Fig. 3. As enzyme concentration increased from 10 to 30 mg of pepsin/mL, more α -kafirin was digested in both wild-type and high-protein digestibility lines. However, enzyme concentration >20–30 mg of pepsin/mL had no noticeable effect on degree of digestion of either line.

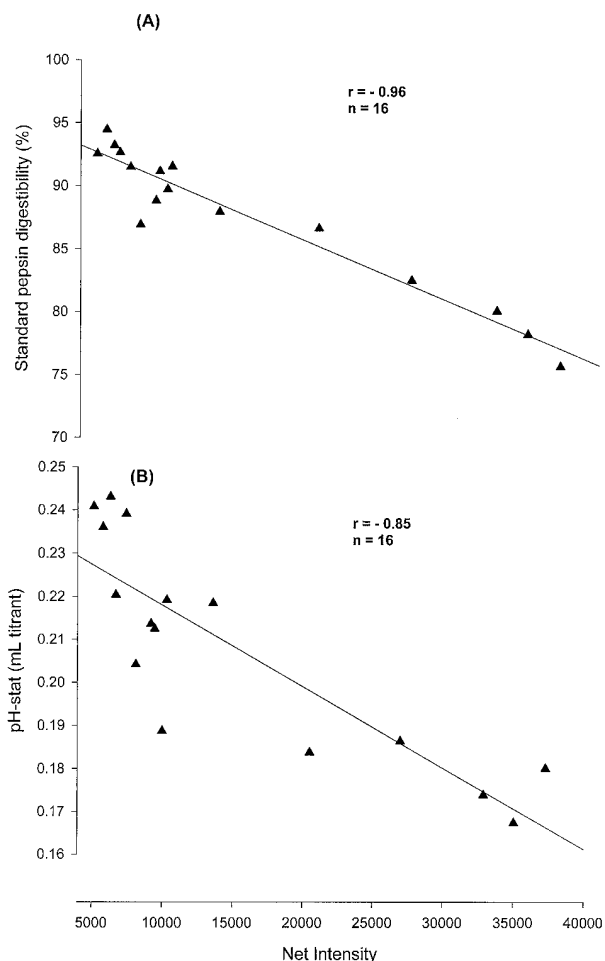


Fig. 5. Correlation of the new assay with standard pepsin digestibility (A) and pH-stat (B) procedures.

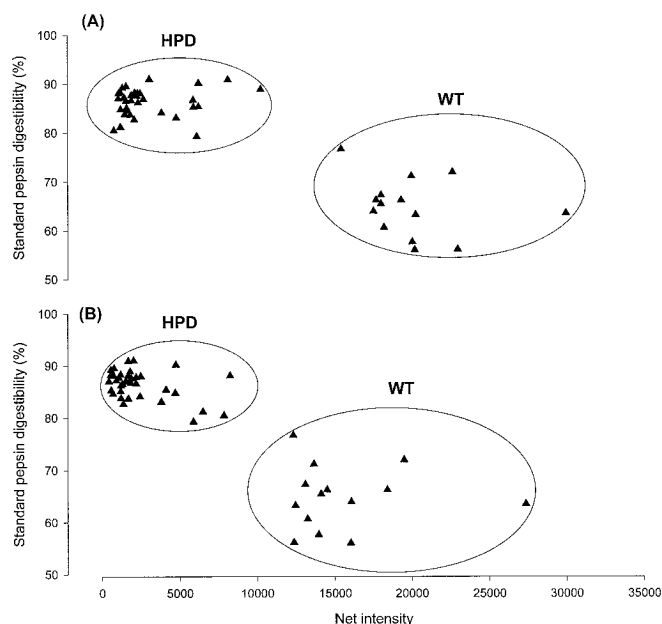


Fig. 6. New assay tested on a larger sorghum population: A, same nitrogen basis; B, same flour basis. WT = wild-type, HPD = high-protein digestibility.

SDS-PAGE of Native and Digested Sorghum Proteins

SDS-PAGE was conducted on sorghum samples to compare native (undigested) protein band patterns. Figure 4A is a representative SDS-PAGE gel of eight sorghum lines before pepsin digestion. Samples were analyzed on a same-protein basis. As expected, the SDS-PAGE band patterns of the undigested samples were similar among the lines. When the samples (same-protein basis) were digested for 1 hr followed by SDS-PAGE of undigested proteins, nonkafirin protein bands disappeared, leaving mostly α -kafirin (Fig. 4B). The amount of α -kafirin remaining after 1 hr of pepsin digestion was substantially lower in high-protein digestibility than in wild-type sorghum lines. When digestion followed by SDS-PAGE was conducted on the same lines considered on the same-flour basis, the results (Fig. 4C) were very similar to results obtained when using the same-protein basis (Fig. 4B). This implies that the higher rate of digestion in high-protein digestibility compared with wild-type lines is not necessarily protein-dependent. Therefore, protein concentration determination is not a prerequisite for the assay. The highly different band intensities of α -kafirin in the gels indicates that selecting for the high-protein digestibility lines can be accomplished simply by visual observation.

Comparing Standard Digestibility Procedures and the New Assay

In vitro protein digestibility in 16 sorghum lines was determined using standard pepsin digestibility and pH-stat procedures. Results were compared with those obtained using the new assay. Figure 5A and B shows correlation between digestibility determined using the new assay and using the standard pepsin digestibility and pH-stat procedures, respectively. Highly significant ($P < 0.01$) negative correlation coefficients were obtained. Higher correlation with the pepsin assay was expected because the SDS-PAGE assay uses pepsin as the digestion enzyme. These results indicate that the new assay is comparable to the existing standard procedures and can be used for screening sorghum lines for protein digestibility. A correlation coefficient of $r = 0.86$ ($P < 0.01$) was obtained between the pH-stat procedure and the standard pepsin digestibility assay.

New Assay Tested on a Larger Sorghum Population

The new assay was tested on 48 sorghum lines using a 15-well vertical mini gel system instead of the 10-well system. Using two electrophoresis units with two gels each, 60 samples could be analyzed at a time using the 15-well system as compared with 40 samples using the 10-well system. The results obtained were compared with digestibility values determined using the standard pepsin digestibility assay. Figure 6A and B shows plots of electrophoresis values of the mixed population of wild-type and mutant lines considered on same-nitrogen and same-flour bases, respectively. In both cases, two distinct groups of high-protein digestibility and wild-type sorghum lines were obtained, indicating that the new assay is highly efficient in distinguishing between them. Samples analyzed on the same-nitrogen basis showed somewhat better discrimination.

DISCUSSION

This study indicates that differences in digestibility between wild-type and high-protein digestibility sorghum lines were easily detected by a new assay. This assay is based on the extent of α -kafirin disappearance after 1 hr of pepsin digestion. α -Kafirin is the major storage protein and makes up ≈ 60 –70% of the total sorghum proteins. The rate of α -kafirin digestion has been shown (Weaver et al 1998) to be faster in high-protein digestibility than in wild-type sorghum lines. Results from our investigation confirm those findings. Differences in digestibility of α -kafirin between high-protein digestibility and wild-type sorghum lines have been explained by differences in protein body structure and in localization of γ -kafirin (Oria et al 2000). The new assay efficiently distinguishes between the two sorghum types based on these differences.

Advantages of the new assay over existing procedures of protein digestibility determination include considerable reduction in sample size and analysis time making it possible to screen at least 60 samples per day (four SDS-PAGE mini-gels). Both the pepsin digestibility and the pH-stat procedures require measurement of nitrogen (protein) concentration in the samples before determination of digestibility. In the pepsin procedure, nitrogen values are used to calculate % digestibility, whereas in the pH-stat procedure prior knowledge of nitrogen concentration is necessary because samples must be analyzed on the same-protein basis. In the new assay, however, prior nitrogen determination is not necessary because the assay easily distinguishes between high-protein digestibility and wild-type lines when compared on the same-flour basis. The assay is highly reliable and repeatable within and between analysis days, provided that all SDS-PAGE procedures, including staining and destaining conditions, are constant. Using 10 sorghum samples, within-day coefficients of variation (CV) of 6 and 4% were obtained for wild-type and high-protein digestibility lines, respectively. A 12% CV was obtained when the assay was repeated over four days with three sorghum lines. When using this assay, it is recommended that a wild-type or a high-protein digestibility sorghum line of known digestibility be used as a check.

When examining the SDS-PAGE pattern of native (undigested) proteins of some sorghum lines, we also observed that some proteolytic activity occurred, likely due to fungal contamination. Most of the digested proteins were nonkafirin proteins. α -Kafirin did not appear to be affected by this proteolytic activity, at least in the samples used. The proteolytic activity was not observed when freshly prepared flours from grains that were stored at refrigeration temperature (4°C) were used, indicating that samples to be analyzed using this assay should be kept refrigerated or frozen until analysis. Sorghum grain subjected to weathering before harvest may contain a protease, probably of fungal origin, that resulted in a loss of protein bands on SDS-PAGE (Huang et al 2000) in a number of lines. Proteolysis occurred during the protein extraction step. If weathering is suspected, samples should be checked for proteolysis before the digestibility assay is performed. This can be prevented by adding 20 mM phenylmethylsulfonyl fluoride (PMSF) to the extraction solvent.

The lack of rapid and efficient assays to measure protein digestibility is a major constraint in breeding programs concerned with improvement in this trait. This study has shown that the new protein digestibility assay is significantly more rapid than existing assays. In addition, the assay is very reliable and efficiently distinguishes sorghum lines on the basis of differences in protein digestibility. It is, therefore, a valuable tool for breeders in the selection and development of nutritionally superior sorghum lines.

ACKNOWLEDGMENTS

This research was supported in part by a grant from the Texas Grain Sorghum Board.

LITERATURE CITED

- American Association of Cereal Chemists. 2000. Approved Methods of the AACCC, 10th ed. Method 46-13. The Association: St. Paul, MN.
- Bramel-Cox, P. J., Anand Kumar, K., Hancock, J. H., and Andrews, D. J. 1995. Sorghum and millets for forage and feed. Pages 325-364 in: Sorghum and Millets: Chemistry and Technology. D. A. V. Dendy, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.
- FAO. 1989. Pages 1-66 in: Report of a joint FAO/WHO expert consultation on protein quality evaluation. FAO/WHO: Rome.
- Hamaker, B. R., Mohamed, A. A., Habben, J. E., Huang, C. P., and Larkins, B. A. 1995. Efficient procedure for extracting maize and sorghum kernel proteins reveals higher prolamin contents than the conventional method. Cereal Chem. 76:583-588.
- Huang, C. P., Hejlse-Kohsel, E., Han, X. Z., and Hamaker, B. R. 2000. Proteolytic activity in sorghum flour and its interference in protein analysis. Cereal Chem. 77:343-344.

- MacLean, W. C., de Romana, G. L., Placko, R. P., and Graham, G. G. 1981. Protein quality and digestibility of sorghum in preschool children: Balance studies and plasma free amino acids. *J. Nutr.* 111:1928-1936.
- Mertz, E. T., Hassen, M. M., Cairns-Whitern, C., Kirleis, A. W., Tu, L., and Axtell, J. D. 1984. Pepsin digestibility of proteins in sorghum and other major cereals. *Proc. Natl. Acad. Sci.* 81:1-2.
- Oria, M. P., Hamaker, B. R., and Shull, J. M. 1995a. In vitro protein digestibility of developing and mature sorghum grain in relation to α -, β - and γ -kafirin disulfide crosslinking. *J. Cereal Sci.* 22:85-93.
- Oria, M. P., Hamaker, B. R., and Shull, J. M. 1995b. Resistance of sorghum α -, β - and γ -kafirins to pepsin digestion. *J. Agric. Food Chem.* 43:2148-2152.
- Oria, M. P., Hamaker, B. R., Axtell, J. D., and Huang, C. P. 2000. A highly digestible sorghum mutant cultivar exhibits a unique folded structure of endosperm protein bodies. *Proc. Natl. Acad. Sci.* 97:5065-5070.
- Pedersen, B., and Eggum, B. O. 1983. Prediction of protein digestibility by an in vitro enzymatic pH-stat procedure. *Tierphysiol. Tierernaehr. Futtermittelk* 49:265-277.
- Shull, J. M., Watterson, J. J., and Kirleis, A. W. 1991. Proposed nomenclature for the alcohol soluble proteins (kafirins) of *sorghum bicolor* (L. Moench) based on molecular weight, solubility, and structure. *J. Agric. Food Chem.* 39:83-87.
- Shull, J. M., Watterson, J. J., and Kirleis, A. W. 1992. Purification and immunocytochemical localization of kafirins in *sorghum bicolor* (L. Moench) endosperm. *Protoplasma* 171:64-74.
- Steel, R. G. D., and Torrie, J. H. 1980. Principles and Procedures of Statistics. A Biometrical Approach. McGraw-Hill: New York.
- Weaver, C. A., Hamaker, B. R., and Axtell, J. D. 1998. Discovery of grain sorghum germplasm with high uncooked and cooked in vitro protein digestibilities. *Cereal Chem.* 75:665-670.

[Received April 17, 2000. Accepted November 21, 2000.]