In Vitro Digestibility of High-Tannin Sorghum at Different Stages of Dehulling

High-tannin sorghum grain was subjected to sequential dehulling to remove tannins. Stepwise removal of tannins gradually increased the percent of nitrogen solubilized by pepsin from 22 to 71% and by trypsin–chymotrypsin mixture from 3 to 35%. These studies suggest that a simple pepsin digestion could be used to predict the biological value of high-tannin sorghums.

The low nutritional quality of high-tannin sorghum varieties (Axtell et al., 1975; Jambunathan and Mertz, 1973) has hampered their use as a primary food source for animals and humans. Schaffert et al. (1974) studied the effect of tannin on in vitro dry matter and protein disappearance in sorghum grain. They concluded that the major factor limiting the utilization of high-tannin sorghum is the availability of protein. Undigestible protein and tannin complex were believed to have accounted for the differences in in vitro dry matter disappearance following 96 h of fermentation with rumen fluid. Armstrong et al. (1974) incubated ground sorghum samples with pepsin for 3 h at low pH, followed by incubation with a pancreatin solution at high pH for an additional 24 h. When the high-tannin, bird-resistant variety BR 64 was digested under these conditions, 4.8% of the total amino acids was recovered as free amino acids compared with 13% in the low tannin sample RS 610. These authors were concerned with the low levels of free amino acids released under the conditions of their tests. They state that "the low values observed raise questions concerning the validity of the test. Certainly, the chick utilized more of the grain than is indicated here and the bird resistant grains do not appear to be as low in value for chick growth in relation to the nonresistant grain as is indicated in this study." They also state that "the need for a rapid screening procedure for determining the biological value for sorghum grain varieties is indicated by the wide differences noted in the performance of chicks and other animals fed different grain sorghums."

We have reexamined the use of pepsin and a combination of trypsin and chymotrypsin in place of pancreatin for evaluation of high tannin sorghums. The effect of these enzymes was studied on a high tannin sorghum which was milled in steps to remove most of the tannins. Both pepsin and the trypsin–chymotrypsin mixture are more effective in splitting sorghum proteins when tannins have been removed. The data also suggest that our pepsin digestion method can predict the biological value of high-tannin sorghums.

MATERIALS AND METHODS

A high-tannin, bird-resistant sorghum variety (BR 64), one of the varieties studied by Armstrong et al. (1974), was used in this work. This variety was grown at the Purdue Agronomy Farm in 1974. It was subjected to sequential dehulling operations in an experimental thresher developed at the Prairie Regional Laboratory, Saskatoon, Canada, as described earlier (Chibber et al., 1978). Protein (N × 6.25) was determined by the microKjeldahl procedure and tannin content was determined by the vanillin–hydrochloric acid method of Burns (1971) and expressed as catechin equivalents (CE) per gram.

The proteolytic enzymes used were obtained from Sigma Chemicals. The proteolytic digestion of ground, defatted sorghum samples was conducted as follows.

Acid Proteolysis. Sorghum samples (100 mg) were suspended in a solution of pepsin (0.5 mg/mL, 50 mL, 0.1 M phosphate, pH 2.0), and the mixture was incubated with gentle stirring at 37 °C for 2 h. Appropriate blanks excluding the enzyme in the sample were run simultaneously. Following 2 h of incubation, the suspension was centrifuged (10000*g*, 15 min, 4 °C) and the supernatant analyzed for solubilized nitrogen.

Table I. Protein Digestion at Various Stages of Dehulling

	percent dehulled			
	0	12.3	24.2	37.0
protein, %	9.4	8.6	8.2	8.2
tannin, CE/g	4.5	4.0	1.6	0.2
digestion by pepsin, %	22.2	27.7	55.0	70.8
chymotrypsin mixture, %	3.0	8.6	23.6	35.7

Alkaline Proteolysis. Sorghum samples were incubated at pH 2.0 as described above in the absence of pepsin, and following centrifugation, the residue was suspended in phosphate buffer (50 mL, 0.1 M, pH 7.8) containing trypsin and chymotrypsin (each at 0.5 mg/mL). The mixture was incubated for 1 h at 37 °C as described above and then centrifuged (10000*g*, 15 min, 4 °C), and the supernatant was analyzed for nitrogen. Appropriate blanks were run in the absence of enzymes, sample, and sample plus enzymes.

RESULTS AND DISCUSSION

The data in Table I show that sequential dehulling reduced the protein content of the residual grain from 9.4% protein with no dehulling to 8.2% protein after 37% of the seed had been removed. A complete discussion of the dehulling procedure and the composition of the residual seed at each stage has been published (Chibber et al., 1978). The dehulling process reduced the tannin content per gram from 4.5 CE (catechin equivalents) in the original grain to 0.2 CE in the residual kernel from which 37% of the original weight had been removed. It can be seen from Table I that the stepwise removal of tannin from the high tannin sorghum grain caused a stepwise increase in the amount of protein that could be solubilized by pepsin. Thus, with the reduction of the tannin content from 4.5 to 4.0 CE/g, a slight increase in solubilization by pepsin occurred, approximately 5%. When the tannin content was reduced from 4.0 to 1.6 CE/g, the digestion by pepsin doubled. Finally, with the reduction of the tannin to a level of 0.2 CE/g, digestibility increased to 71%. In contrast, the trypsin–chymotrypsin mixture solubilized only 3% of the nitrogen in the high-tannin sorghum before dehulling and, even with the nearly complete removal of tannin, solubilized only one-third of the total nitrogen.

The solubilization of the nitrogen in sorghum is achieved much more effectively by the action of pepsin than by the action of the trypsin–chymotrypsin combination. This suggests that, in vivo, pepsin plays a key role in solubilization of sorghum proteins and that the enzymes of the intestinal tract are more involved with the breaking up of the soluble polypeptides into free amino acids. Previous studies by Armstrong et al. (1974) have shown that the high-tannin sorghum used in these studies (Br 64) does not support comparable chick growth and feed efficiency

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to that observed with low-tannin sorghum grains. The results obtained here with pepsin digestion support this in vivo observation and suggest that the simple pepsin test described could be useful as a rapid screening procedure for determining the biological value of sorghum grain varieties. Studies are underway to determine the usefulness of this method.

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Shorter High-Performance Liquid Chromatographic Method for the Determination of 25-Hydroxycholecalciferol in Cow Serum

A short high-performance liquid chromatographic procedure is reported for the analysis of 25-hydroxycholecalciferol (25-OH-D_3) in cow serum. This procedure is faster and requires only 2 mL of serum or plasma as against the 25 g that was required by the procedure that we had reported earlier. In addition, the procedure may be used for the determination of total 25-OH-D (25-OH-D_3 plus 25-OH-D_2) or specifically for 25-OH-D_3.

The primary metabolite of vitamin D_3, 25-OH-D_3, was found useful in reducing the incidence of parturient paresis in cows (Bringe et al., 1971; Olsen et al., 1973a,b). We have reported high-performance liquid chromatographic (LC) procedures for the determination of 25-OH-D_3 in cow blood (Koshy and VanDerSlik, 1976) and in cow liver, kidney, and muscle (Koshy and VanDerSlik, 1977a). Our procedure for cow blood required a large sample size of 25 g of plasma or serum and was lengthy. Besides it was not specific for 25-OH-D_3, as later, when 25-hydroxyergocalciferol (25-OH-D_2) became available, we found that the two compounds had the same retention time on the LC. This problem was resolved by the use of a partition column prior to LC (Koshy and VanDerSlik, 1978). Subsequent to our work on the cow serum, we reported an LC method for 25-OH-D_3 for human serum using 2.5 mL of serum (Koshy and VanDerSlik, 1977b) which could be used either for the determination of total 25-OH-D (25-OH-D_2 plus 25-OH-D_3) or specifically for 25-OH-D_3. This communication is an adaptation of the above method to cow serum.

METHOD

Briefly, the procedure was as follows. (1) Two milliliters of the serum was extracted with 5 mL and 4 mL each of 95% ethanol in a screw-capped centrifuge tube, diluted with 2 mL of water and extracted with 2 × 5 mL of CH_2Cl_2. (2) The extract was evaporated under vacuum and subjected to chromatography on 2 g of silica column (230–400 mesh) using hexane–ether (1:1) and ether–ethyl acetate (9:1) solvent systems. The fraction containing the 25-OH-D was evaporated under N_2. (3) The residue was subjected to partition column chromatography on 0.2 g of Celite 545 using 80:20 methanol–water as the stationary phase and pentane as the mobile phase. (4) For the determination of total 25-OH-D, the extract was subjected to LC on a C_{18} bonded microparticulate silica column. For the specific determination of 25-OH-D_3, the extract was subjected to LC on a nonbonded microparticulate silica column. The LC conditions for the two columns are shown in Table I. For more detailed information of the procedure, the reader is referred to our procedure for human serum (Koshy and VanDerSlik, 1977b).

Table I. LC Conditions

	for total 25-OH-D	for 25-OH-D_3
instrument used	Varian 8500[a]	Varian 8500[a]
column	Zorbax ODS[b] 2.1 mm × 25 cm	Zorbax Sil[b] 2.1 mm × 25 cm
mobile phase	CH_3CN, CH_3OH, H_2O 94:3:3	3% 2-propanol in hexane
flow rate	25 mL/h	45 mL/h
pressure	≈105 atm	≈190 atm
detection	UV-254 nm[c]	UV-254 nm[c]
sample size	5–7 μL	10 μL
sensitivity	0.005 aufs	0.005 aufs

[a] Varian Instrument Division, Palo Alto, CA. [b] DuPont DeNemours, Inc., Wilmington, DE. [c] Waters Model 440, Waters Associates, Inc., Framingham, MA.

RESULTS AND DISCUSSION

The method is simpler than the one we reported before and therefore at least six samples can be analyzed by one analyst in a day. One major difference between our human serum procedure and this one is that a 0.2-g Celite partition column was adequate for both the reversed phase and the adsorption mode of LC. With the human serum, it was necessary to use a 1-g Celite column for the latter

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