Rapid Methods for Estimating Protein and Lysine in Sorghum (*Sorghum bicolor* (L.) Moench)[1]

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ABSTRACT

Sorghum (*Sorghum bicolor* (L.) Moench) samples were analyzed for protein by the Technicon auto analyzer (TAA) method, and the results were compared with the micro-Kjeldahl method. The TAA method was highly and significantly correlated (r = 0.997**) with the micro-Kjeldahl method, and the results were found to be highly reliable for routine screening of samples. Lysine was estimated by the rapid dye-binding capacity (DBC) procedure and was determined using an amino-acid analyzer. The results of the DBC procedure, when expressed as a ratio with respect to the protein content, were highly and positively correlated (r = 0.933**) with the amino-acid analyzer values. The rapid procedures of TAA and DBC were observed to give reliable estimates of protein and lysine content, respectively, and are recommended when large numbers of sorghum samples are to be analyzed.

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Sorghum grain ranks fifth in acreage and in total production of cereal crops of the world. Sorghum grain is an important source of food energy for several million people in Africa and Asia. However, it is well known that the protein quality of sorghum grain is lowest among cereals, mainly because of its low levels of lysine. One of the objectives of our institute is to improve the genetic potential for grain yield and nutritional quality of sorghum. In our institute the two high-lysine (hl) Ethiopian sorghum cultivars (Singh and Axtell 1973) and a chemically induced mutant P-721 (Mohan 1975) were used as parents to study the inheritance of the high-lysine gene (Riley 1980). This necessitated identifying rapid, simple, and reasonably accurate methods for the estimation of protein and lysine in large numbers of sorghum samples. Mossberg (1969) and Finkner et al (1979) have used the dye-binding capacity (DBC) procedure for the evaluation of protein quality and quantity in barley, wheat, oats, rye, ryewheat, and corn. In this article, the progress that has been made in using such methodologies for the estimation of protein and lysine is reported. A brief account of the improvement of the nutritional quality of sorghum using these methods was reported earlier (Jambunathan 1980).

MATERIALS AND METHODS

Sorghum (*Sorghum bicolor* (L.) Moench) grain samples were obtained from the ICRISAT breeding program. These included high-lysine and normal sorghum grains and their progenies obtained from crosses. No attempt was made to study the influence of environment and agronomic practices on protein and lysine contents. The grain samples were ground in a Udy cyclone mill to pass through a 0.4-mm screen.

Protein

A slightly modified automated colorimetric procedure using the Technicon auto analyzer (TAA) was used for the estimation of nitrogen as described by Singh and Jambunathan (1980). A Technicon block digestor with a provision to digest 40 samples at a time was used in conjunction with the TAA. The standard micro-Kjeldahl (MKJ) procedure (AOAC 1970) was used for the determination of nitrogen, and the results obtained by the TAA method were compared with MKJ values. The crude protein was calculated (N × 6.25).

When comparing the TAA method with the MKJ procedure, a regression equation was first obtained using 101 samples. An additional 146 samples were used as experimental samples, and MKJ values were predicted from the regression equation using TAA values. Later MKJ values were determined on these samples. The results obtained by both the procedures were compared.

Lysine

Lysine content was estimated by the DBC procedure according to Udy (1971). The acid orange-12 dye and reference dye solutions were obtained from the Udy Analyzer Company, Boulder, CO. Dye solution (40 ml) was added to 1 g of ground sample and mixed in a reciprocating shaker for 1 hr. The percent transmittance (percent T) of the filtrate was determined in a flow-through colorimeter and was referred to as Udy Instrument Reading (UIR). The ratio of the UIR (percent T) to the percent of protein (P) in the sample (UIR/P), was calculated and was compared with the lysine values determined as described below.

Lysine content in sorghum samples was determined, using a Beckman model 120C automatic amino-acid analyzer (Spackman et al 1958). Each sorghum sample was hydrolyzed with 6*N* HCl. The excess HCl was evaporated in vacuum in a flash evaporator, and the residue was dissolved in citrate buffer (pH 2.2). An aliquot was used for the determination of lysine content of 58 samples. The results were compared with UIR/P values for the same samples, and a regression equation was calculated. An additional 42 samples were treated as unknown experimental samples, and their UIR/P values were determined. Their lysine values were estimated using the regression equation obtained with the original 58 samples. Thereafter, lysine determinations were also performed on these 42 samples using the amino-acid analyzer, and the results obtained by both these procedures were compared.

RESULTS AND DISCUSSION

Protein Estimation

A highly significant correlation coefficient (r = 0.997**) was obtained between the MKJ and TAA values. Protein content in these samples ranged from 7.1 to 19.1%. A regression equation relating the MKJ with the TAA values (Y = 0.985x + 0.159) was calculated that had a standard error of estimate of 0.25. A comparison of results obtained on 146 samples by the TAA and MKJ procedures representing various protein groups is shown in Table I. Mean error percentage was relatively low over a range of protein values (Table I), indicating the suitability of the TAA method for the estimation of sorghum grain protein content.

Lysine Estimation

Although the DBC procedure method measures all three basic amino acids, attempts were made to relate the DBC values with the lysine values determined using the amino-acid analyzer. UIR/P ratios on 58 sorghum samples were determined, and varied between 2.32 and 4.57. When the results of the determined lysine values and the UIR/P ratios of the combined low-, medium-, and high-protein samples were compared, a highly significant correlation (r = 0.927**) was obtained (Table II). However, a lower but significant correlation was obtained for the low-protein sorghum samples alone (Table II). A regression equation relating the UIR/P values and lysine content for these 58 samples (Y = 0.662×−0.144)

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was obtained that had 0.17 as standard error of estimate. This equation was used to predict the lysine values in the 42 unknown experimental samples. The results of observed and estimated lysine values were compared (Table III). The mean error percentage for the various groups ranged between −2.51 and +4.27%, indicating that the DBC method in combination with protein content in samples could give a reliable estimate of lysine content in a sorghum sample. A paired *t* test did not show any significant difference between the two means. Preliminary results obtained with nine samples that varied in their tannin content (catechin equivalents) from 1.0 to 2.4% showed that tannin content might not influence the estimated lysine values.

When the results of the 58 samples and the 42 samples were combined, the correlation coefficient between UIR/P and lysine values was observed to be 0.933**. The relationship between these two parameters is given in Fig. 1. A regression equation Y = 0.644 × −0.082 with a standard error of estimate of 0.16 was obtained for the 100 samples. This equation has been used in our laboratory for predicting the lysine values in routine screening of several thousand sorghum samples every year.

The biuret procedure of Johnson and Craney (1971) was checked carefully for the estimation of protein in sorghum in our laboratory. We modified the procedure, using 50 mg of sample and reducing the quantities of all other chemicals proportionately so that the reaction could be performed in screw-capped test tubes for the analyses of large numbers of samples. A highly significant correlation was obtained between the biuret procedure and MKJ values. However, the errors associated with the biuret procedure when used for routine screening were large and unpredictable and, hence, we could not rely on this procedure in our laboratory (Jambunathan 1977). When the UIR values obtained for the 100 sorghum samples used in the present study were compared with the micro-Kjeldahl protein values, a positive and significant correlation (r = 0.782**) was obtained, indicating the possibility of using the DBC procedure for the estimation of protein in sorghum samples. However, DBC values would overestimate the protein content of high-lysine samples. Therefore, DBC might be more accurate for the estimation of protein for each class.

Our earlier dye-binding analyses were performed on samples containing 80 mg of protein, and this procedure was found to be satisfactory in predicting the lysine content (unpublished data). Unfortunately, however, DBC analysis could not be performed unless the results of protein analyses were available for these samples, as constant protein levels were necessary. As we could analyze about 160 samples per day by the DBC procedure and only about 100–120 sample by the TAA method, we could not proceed with the DBC analysis. Therefore, we decided to use the procedure described in this article. However, our experiments showed that the UIR reading obtained at constant protein could be related to the UIR reading obtained on 1 g of sample if the latter values are calculated and expressed on a constant protein basis (unpublished data). Also, a negative and significant correlation (r = −0.743**) was obtained between the UIR values obtained at one protein level and the MKJ protein value, indicating the possible existence of a negative relationship between protein and lysine content in

TABLE III
A Comparison of Determined and Estimated Lysine Values (g/100 g P)

Lysine Level	No. of Samples	Mean Determined Lysine[a] (%)	Mean Estimated Lysine[b] (%)	Error (%) of AAA[a] Values
1.0–1.39	2	1.34	1.35	0.75
1.4–1.79	6	1.64	1.71	4.27
1.8–2.19	15	1.99	1.94	−2.51
2.2–2.59	12	2.39	2.39	...
2.6–2.99	7	2.69	2.68	−0.37

[a] Amino-acid analyzer values.
[b] Estimated as percent lysine = 0.662 (UIR/P) − 0.144.

TABLE I
Deviation of Technicon Auto Analyzer (TAA) Protein from Micro-Kjeldahl (MKJ) Protein Values

Protein Class (MKJ)	No. of Samples	Determined MKJ Protein (%) (mean)	Estimated MKJ Protein (%) from TAA Values (mean)[a]	Error (%)
6.0–6.9	3	6.40	6.43	0.5
7.0–7.9	3	7.40	7.30	−1.4
8.0–8.9	7	8.57	8.70	1.5
9.0–9.9	23	9.45	9.53	0.8
10.0–10.9	23	10.42	10.59	1.6
11.0–11.9	34	11.39	11.47	0.7
12.0–12.9	20	12.40	12.36	−0.3
13.0–13.9	15	13.56	13.63	0.5
14.0–14.9	9	14.57	14.53	−0.3
15.0–15.9	5	15.34	15.30	−0.3
16.0–16.9	4	16.33	15.78	−3.4

[a] Estimated MKJ protein (%) = 0.985 (TAA) + 0.159.

TABLE II
Correlation Between Dye-Binding Capacity (DBC) and Lysine Content

Protein Group	Protein (%)	No. of Samples	UIR[a] (% T)	UIR/P[a]	Lysine (g/100 g P)	r Between UIR/P[a] and Lysine
Low	7.3–10.7	16	22–36	2.66–3.50	1.59–2.38	0.768[b]
Medium	10.8–14.7	17	31–63	2.46–4.36	1.34–2.76	0.939[b]
High	15.0–18.2	25	35–72	2.32–4.57	1.44–2.98	0.951[b]
All[c]	7.3–18.2	58	22–72	2.32–4.57	1.34–2.98	0.927[b]

[a] UIR = Udy Instrument reading; P = percent of protein.
[b] Significant at $P = 0.01$.
[c] y = 0.662 × −0.144.

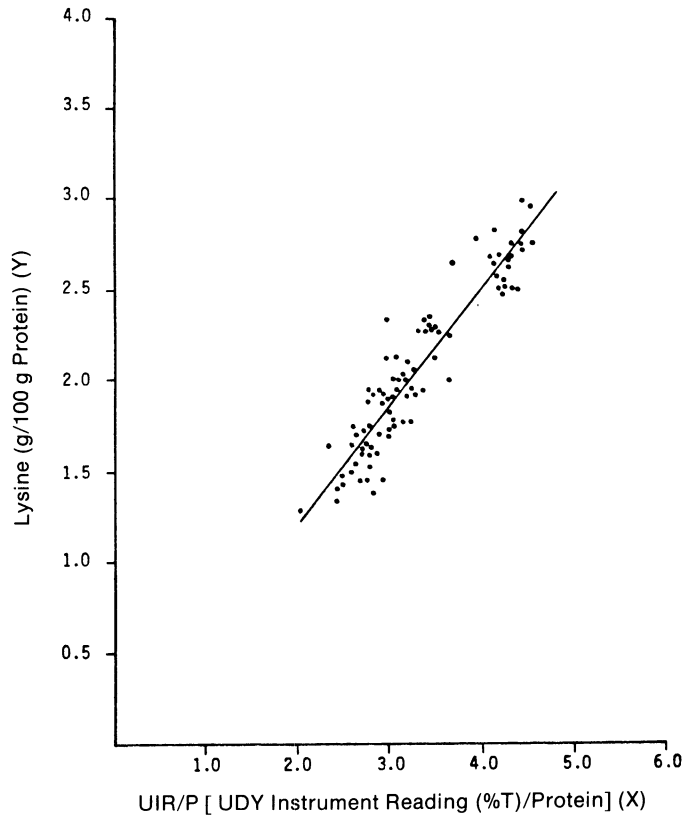

Fig. 1. Relationship between UIR/P and lysine content for sorghum. Y = 0.644x −0.082 (n = 100, r = 0.933**).

sorghum. However, conclusions cannot be drawn from this observation as this was not the objective of the study and also because of contrasting reports made about this relationship (Hulse et al 1980).

We observed that the results of protein and lysine analyses based on TAA and DBC methods agree fairly well with the MKJ and amino acid analyzer values, respectively. Considering the time and effort required to determine protein and lysine values, we recommend these two rapid procedures in any large breeding program in which attempts are being made to improve the protein content and protein quality of sorghum. However, it is important to test and standardize these two procedures independently in any laboratory, using sorghums containing a wide range of protein contents, before general use in a breeding program. Also, a bulk check sample should be used to monitor the day-to-day variations, if any, in the analytical results. We have analyzed over 30,000 sorghum samples using these two rapid procedures. This procedure was also successfully used to monitor the progenies arising out of crosses between high-lysine and normal sorghums (Riley 1980).

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