Studies on the Protein Content and Amino Acid Composition of Some Varieties of Grain Sorghum

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Determination of the protein content and lysine levels of a number of nonhybrid varieties of grain sorghum indicates large variations in the protein content. Statistical analysis of data on amounts of lysine shows that a negative correlation exists between per cent lysine in the protein and per cent protein in the seed. The proportion of various protein fractions in endosperm of five varieties of grain sorghum of both low- and high-protein type has been determined. Results show that prolamine and glutelin are the principal protein fractions, and increased protein levels in sorghum varieties are correlated with an increase mainly in the prolamine fraction. Nine high- and low-protein varieties of grain sorghum have been analyzed for their amino acid composition by ion exchange procedures. One of the high-protein genetic varieties of sorghum has a high concentration of lysine in the seed. Amino acid composition of the protein fractions of two varieties is also reported. These data permit an evaluation of the nutritional quality of sorghum protein and factors that influence the quality of the protein.

In recent years, considerable interest has been shown in breeding cereal grains with high-protein content, provided good nutritional quality can be maintained (Nelson, 1966). Earlier attempts to breed corn of high-protein content were unsuccessful because the increase in protein was largely due to an increase in the prolamine fraction of corn, which is already deficient in lysine (Hansen *et al.*, 1946). Recently, two mutant corn varieties, opaque-2 and floury-2 were reported to have a higher concentration of lysine in their endosperm proteins than the normal maize (Mertz *et al.*, 1964; Nelson *et al.*, 1965). This alteration of amino acid pattern has been shown to enhance greatly the nutritive value of corn (Nelson, 1966).

Lysine is the most limiting amino acid in diets containing sorghum as the only source of protein. The amino acid composition of grain sorghum protein is perhaps genetically controlled as in corn (Doty *et al.*, 1946; Nelson, 1966). Analysis of a large number of genetic varieties of sorghum may reveal a variety which has high amounts of good quality protein in the grain. Ion exchange chromatographic procedures for studying effects of various factors on protein quality of cereals have only been applied recently to the study of amino acid composition of a number of hybrid varieties of sorghum (Deyoe and Shellenberger, 1965). There is a need to investigate the amino acid composition of nonhybrid varieties of sorghum and the individual protein fractions of grain sorghum by ion exchange procedures, since this information is not available. These data are useful in correlating the variation in the amino acid composition of the samples with the changes in the amount of individual protein fractions in the grain.

This report deals with the authors' studies on the protein content and amino acid composition of a large number of genetic varieties and a few hybrid varieties of sorghum. The results of investigations on the solubility fractionation of a few samples of sorghum with low- and high-protein content and the amino acid composition of the individual protein fractions are also presented.

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MATERIALS AND METHODS

Forty-four genetic varieties of grain sorghum were obtained from the World Germ Plasma Collection of Sorghum at Hyderabad (A.P.). Five hybrid varieties of local origin were supplied by the Agricultural Research Stations, Mysore State, India.

Estimation of Crude Protein. All samples were analyzed for protein and moisture by standard methods (Assoc. Offic. Agr. Chemists, 1960). Nitrogen determined by the Kjeldahl method was converted to crude protein by multiplying by the factor 6.25. Correction for moisture was applied, and the results were expressed on a dry weight basis (Table I).

Solubility Fractionation of Endosperm Protein. Dehulled, defatted endosperm flour was prepared as described earlier (Sastry and Virupaksha, 1967). Solubility fractionation of the endosperm proteins was done using a modification of the classical procedure of Osborne and Mendel (1914). The solvents used for the various protein fractions were distilled water to extract the albumin, 1% NaCl solution for globulin, 60% (v./v.) aqueous ethanol for prolamine, and 0.4% NaOH solution for glutelin. The relative proportion of these protein fractions in five varieties of sorghum was determined by the following procedure. One-hundred-milligram samples of defatted 100-mesh flour were shaken for 2 hours with 2.0 ml. of each solvent in a "wrist action" shaker. Extraction was done at room temperature for albumin, globulin, and glutelin and at 60° C. for prolamine. The samples were then centrifuged, and the residues were washed with the solvents used for extraction. The extracts and washings were combined, and nitrogen was determined by the Kjeldahl method.

The procedure for the large scale preparation of albumin, globulin, and prolamine fractions from sorghum endosperm flour has been described before (Sastry and Virupaksha, 1967). Glutelin fraction was prepared by shaking 10 grams of the flour with 100 ml. of 0.4% NaOH for 2 hours. The suspension was centrifuged, and the clear supernatant was dialyzed against several changes of distilled water and lyophilized.

Table I. Protein and Lysine Content of Some Genetic Varieties of Grain Sorghum

	Variety	Nitrogen, %,[a] N (1)	Protein, %,[a] N × 6.25 (2)	Lysine, % of Sample (3)	Lysine, % of Protein (4)
1533	Conspituum	1.38	8.61	0.117	1.36
219	Dur./Nig.	1.62	10.12	0.232	2.29
326	Dur./Nig.	1.67	10.47	0.197	1.89
106	Kaura	1.68	10.52	0.182	1.73
1040	A Zera Zera	1.70	10.60	0.200	1.89
2064	Kaf. Rox	1.70	10.64	0.207	2.04
918	Caud./Caff.	1.71	10.69	0.220	2.06
1029	Hegaru	1.74	10.86	0.189	1.74
61	Nig./Durra	1.75	10.94	0.179	1.63
5205	Caff./Durra	1.76	10.98	0.226	2.06
663	Durra	1.86	11.64	0.220	1.89
1236	Nig./Durra	1.87	11.65	0.193	1.66
4503	Caud/Nig.	1.91	11.91	0.226	1.90
955	Fet. Kaf.	1.93	12.06	0.170	1.41
5285	Nig./Ft.	1.93	12.06	0.232	1.93
126	Caudatum	1.95	12.19	0.246	2.02
3687	V. C. Durra	1.95	12.19	0.186	1.53
1237	Bird Proof	1.98	12.39	0.240	1.94
269	Dobbs	1.99	12.41	0.192	1.55
149	Nigricans	2.01	12.57	0.235	1.87
1094	Tall White Kafir	2.02	12.60	0.164	1.29
1205	Milo	2.05	12.83	0.221	1.72
1122	Red Kafir	2.09	13.07	0.182	1.39
2747	Durra	2.10	13.13	0.200	1.52
304	Darso	2.10	13.13	0.199	1.51
3587	G. C. Durra	2.15	13.45	0.201	1.53
192	PJ 8K Durra	2.17	13.56	0.241	1.78
1387	Amber	2.17	13.56	0.230	1.70
1861	Shallu	2.19	13.68	0.190	1.39
4296	Nandyal	2.21	13.79	0.201	1.46
1102	Margarttiferum	2.24	14.01	0.262	1.87
3588	G. R. C. Durra	2.25	14.03	0.230	1.64
2965	Guin	2.26	14.10	0.248	1.76
1349	Tall Rox	2.29	14.29	0.203	1.42
795	Feterita	2.32	14.50	0.209	1.44
164	Subglab	2.34	14.65	0.269	1.68
5611	Kaura	2.36	14.71	0.218	1.48
3228	Red Durra	2.41	15.05	0.179	1.50
5508	Conspituum	2.43	15.21	...	...
29	Cernum	2.47	15.50	0.251	1.63
202	Subglab	2.48	15.50	0.245	1.58
981	Sudan	2.61	16.29	0.256	1.57
160	Cernum	2.83	17.68	0.413	2.13
361	Dochna	2.91	18.21	0.275	1.51
M-35-1	Raichur	1.37	8.55	0.186	2.17
D-340	Siruguppa	1.80	11.25	0.236	2.10
M-35-1	Siruguppa	1.88	11.76	0.249	2.12
BS-81-3	Annigeri	2.01	12.56	0.261	2.08
CSH-1	Bijapur	2.64	16.48	0.300	1.82

[a] On dry weight basis.

Estimation of Lysine. Lysine content of whole seeds was determined by the procedure of Selim (1965). Seeds were powdered, and weighed amounts were hydrolyzed with constant boiling HCl in sealed evacuated tubes at 110° C. for 24 hours. The hydrolyzates were freed of HCl by evaporation in vacuo, and the residue was made up to 5.0 ml. with borate buffer, pH 9.0. Amino acids in aliquots of the hydrolyzates were quantitatively converted to their copper complexes, followed by treatment with 1-fluoro-2,4-dinitrobenzene (FDNB). Absorbance of the ε-DNP-lysine in the copper-free and ether-extracted mixtures was read at 390 mμ. The concentration of lysine was calculated by matching observed absorbance against those of a series of standard solutions of authentic ε-DNP-lysine.

Concentration of lysine in the samples was expressed both as per cent of sample and as grams of lysine per 100 grams of protein (Table I). The data on protein content of the samples and the lysine content were analyzed statistically (Snedecor, 1956).

Amino Acid Analysis. Whole seed powder or the protein fractions were weighed and hydrolyzed with constant-boiling HCl in sealed evacuated tubes at 110° C. for 30 hours. Hydrochloric acid was removed from the hydrolyzates by evaporation in vacuo, and the residues were made up to a known volume in 0.2*M* citrate buffer, pH 3.26. Aliquots were taken for the estimation of nitrogen by Kjeldahl method. Hydrolyzed samples prepared for analyses were stored at −20° C. until used.

Amino acid analyses were conducted by ion exchange chromatography on a Beckman model amino acid analyzer, using the methods of Spackman *et al.* (1958). Results were expressed as grams of amino acid per 100 grams of protein. Corrections for the possible destruction of amino acids like cystine, serine, and threonine during acid hydrolysis have not been applied.

RESULTS

Protein Content of Samples. Nitrogen and protein content of 44 genetic varieties of grain sorghum and five hybrid varieties are shown in Table I (columns 1 and 2). These data indicate a wide variation in the protein content of the genetic varieties, the lowest value being 8.61% and the highest 18.21%. The hybrid varieties also show a wide range of variation from 8.55 to 16.48% (mean of all samples = 12.87% and standard deviation = 2.093). The nonhybrid varieties are arbitrarily grouped into low-, medium-, and high-protein samples with a protein content of 12.5 and 15.0% as the dividing line. The hybrid varieties are grouped together as a separate class.

Solubility Fractionation of Endosperm Protein. The proportions of albumin, globulin, prolamine, and glutelin in five samples of sorghum are shown in Table II, with the protein content in the endosperm fraction of these varieties. The results indicate that prolamine and glutelin are the principal proteins of the sorghum endosperm. Albumin and globulin together account for less than about 12% of the total endosperm protein. The distribution of various protein fractions in sorghum endosperm is similar to that of corn endosperm proteins (Bressani *et al.*, 1958).

The efficiency of extraction varies from 80.7 to 103.4% in five samples of sorghum. The high extraction efficiency obtained with BS-81-3 Annigeri is perhaps due to the breakdown of some protein on storage of the grain. This might have resulted in a higher value for the prolamine fraction of this variety. This sample responded poorly in germination tests showing that some physiological changes have occurred during storage.

All three high protein samples show an increase in prolamine content, and the variety 160-cernum shows a significantly higher proportion of glutelin than the others. Therefore, the increased protein content in sorghum varieties may be attributed mainly to an increase in the prolamine fraction of the grain. Similar changes in the propor-

Table II. Solubility Fractionation of Protein of Grain Sorghum

100-mesh; defatted endosperm protein

Variety	Protein Contents, % Endosperm	Protein Fraction, %				Total, %	Extraction Efficiency, %
		Albumin	Globulin	Prolamin	Glutelin		
M-35-1 Siruguppa	9.94	0.56	0.73	3.24	3.72	8.25	83.0
BS-81-3 Annigeri	10.56	0.57	0.77	5.93	3.65	10.92	103.4
CSH-1 Bijapur	18.13	1.40	1.16	7.88	4.86	15.30	84.4
160 Cernum	17.06	0.89	1.59	7.06	5.93	15.47	90.7
361 Dochna	19.00	0.25	0.29	11.18	3.61	15.33	80.7

tion of prolamine in the high-protein varieties of other cereals have been reported (Bressani and Mertz, 1958; Cagampang *et al.*, 1966; McDermott and Pace, 1960).

Relationship between Lysine Content and Protein Levels. Lysine content of samples determined by the colorimetric procedure is expressed both as per cent of sample and as per cent of protein (columns 3 and 4 of Table I). These data indicate a considerable range of variation for the lysine concentration of samples. When lysine values are expressed as per cent of sample, the values range from extremes of 0.117 and 0.413% (mean = 0.219%; standard deviation = 0.044). Lysine as per cent of protein varies between 1.29 and 2.17% (mean = 1.69%; standard deviation ± 0.253). The values for the lysine content determined by the colorimetric procedure are lower than those obtained for a few varieties by the ion exchange chromatography (Table III). Although the absolute concentration of lysine in the seed samples may be slightly higher than those in Table I, the relative values for lysine content remain unaffected, and the comparisons between the varieties are valid.

In Figure 1, per cent lysine in the protein is plotted against per cent protein in the seed. The regression line indicates a decrease of 0.041% lysine for each 1% increase in protein. The coefficient of correlation, r, between per cent lysine in the protein and per cent protein in the seed is −0.34. The χ^2 value is 0.014, which indicates a good fit

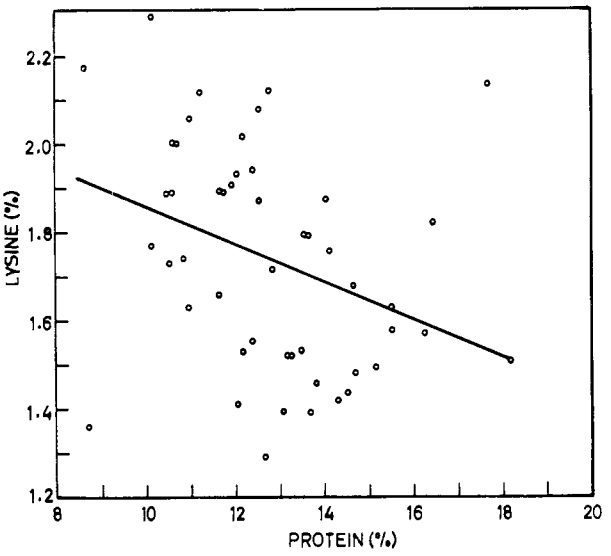

Figure 1. Relationship between lysine in the protein and protein levels in 48 samples of grain sorghum

Table III. Amino Acid Composition of Genetic and Hybrid Varieties of Grain Sorghum, Per Cent of Protein

Amino Acid	29 Cernum	160 Cernum	164 Subglab	202 Subglab	361 Dochna	1533 Conspituum	BS-81-3 Annigeri	M-35-1 Siruguppa	CSH-1 Bijapur
Lysine	2.15	3.14	2.15	1.95	1.42	1.92	2.07	1.81	1.65
Histidine	1.91	2.31	1.89	2.52	1.25	2.44	2.18	1.01	2.01
Arginine	3.90	4.87	2.72	4.05	3.68	3.78	2.94	2.54	2.85
Aspartic acid	8.94	9.09	8.30	9.95	6.89	9.26	7.86	6.72	6.18
Threonine	3.25	3.55	3.27	3.47	3.13	3.44	3.31	3.58	3.14
Serine	4.28	4.65	4.48	4.91	3.53	4.86	4.21	4.63	4.65
Glutamic acid	28.72	23.37	23.11	32.10	25.51	31.10	27.20	23.24	23.60
Proline	13.44	12.66	13.88	7.70	12.34	14.60	13.12	9.97	8.62
Glycine	2.89	3.21	3.50	3.09	2.27	3.11	2.82	3.89	2.78
Alanine	12.46	10.28	11.27	14.02	11.02	13.03	11.60	10.16	13.52
Half cystine	0.95	...	0.66	0.53	1.33	1.14	0.71	1.37	...
Valine	5.135	4.20	5.83	6.59	4.77	5.57	5.05	5.66	5.36
Methionine	1.53	1.13	1.61	1.83	0.73	1.64	1.34	1.92	0.81
Isoleucine	4.78	3.82	4.96	6.20	4.80	5.22	4.95	4.07	3.68
Leucine	17.77	12.70	16.49	20.90	17.90	19.40	17.70	12.94	13.12
Tyrosine	2.05	2.19	1.91	2.84	1.23	2.61	1.99	2.14	1.91
Phenylalanine	5.47	4.56	5.32	6.62	4.91	6.02	5.33	4.97	5.09
Protein %	15.50	17.68	14.65	15.50	18.21	8.61	12.56	11.25	16.48

between the observed and calculated values. Hence, a negative correlation probably exists between per cent lysine in the protein and per cent protein in the seed.

Amino Acid Composition of Sorghum Varieties. Data from the amino acid determinations of six genetic varieties and three hybrid varieties of sorghum are shown in Table III. In these analyses, whole seed hydrolyzates were used. Both low-protein and high-protein samples were included in the analyses. These values, expressed as grams of amino acid per 100 grams of protein, are in general agreement with those reported by Deyoe and Shellenberger (1965), for hybrid sorghum varieties. However, the values reported here for arginine, aspartic acid, glutamic acid, proline, alanine, isoleucine, and leucine are higher than those reported by other workers (Deyoe and Shellenberger, 1965; Waggle *et al.*, 1966) and may reflect the tendency for a prolamine increase with an increase in protein content.

Results of analyses (Table III) show that amino acid content of grain sorghums varied considerably among the varieties. The basic amino acids, lysine and histidine and the sulfur-containing amino acids, appear to be the most deficient amino acids in all the samples. However, one of the nonhybrid varieties, 160-cernum, has the highest concentration of lysine (3.1 grams per 100 grams of protein) of all the varieties that have been reported so far. Glutamic acid, proline, alanine, and leucine are present in high concentrations in all the varieties, and the ratio of isoleucine to leucine is nearly 1 to 4. Thus, the amino acid composition of sorghum protein is grossly similar to that of corn protein (Mossé *et al.*, 1966; Nelson, 1966).

Amino Acid Composition of Protein Fractions. Amino acid compositions of the defatted endosperm meal, globulin, prolamine, and glutelin fractions of CSH-1-Bijapur variety of sorghum and data on amino acid analyses of globulin and prolamine fractions of 160-cernum variety of sorghum are given in Table IV. Globulin fraction has a better distribution of all the essential and related amino acids compared with endosperm meal and prolamine fractions. Concentrations of lysine, arginine, glycine, cystine, and methionine in the globulin fraction are nearly twice that of the endosperm meal and several times higher than those present in the prolamine fraction. On the other hand, the levels of glutamic acid, proline, alanine, and leucine in the globulin fraction are about half that of the concentration of these amino acids in the endosperm meal and the prolamine fractions.

In comparing the amino acid composition of the prolamine and glutelin fractions, the amounts of tyrosine, histidine, arginine, glycine, and cystine present in the glutelin fraction are several times higher than the levels of these amino acids in the prolamine fraction. Serine and aspartic acid values are about 62 and 35% higher, respectively, in the glutelin fraction, whereas tyrosine and alanine are approximately 40 and 33% lower. Amounts of glutamic acid, proline, valine, isoleucine, leucine, and phenylalanine are about the same in both fractions.

Concentrations of glutamic acid, valine, leucine, and phenylalanine in the endosperm meal are higher than those in the individual protein fractions. This may be because some residual protein fraction, which cannot be extracted from the endosperm meal, has higher levels of these amino acids.

These preliminary results indicate that lysine, which is most deficient in sorghum protein, is present in much higher quantities in the glutelin fraction than in the prolamine fraction. Any change in protein composition which would increase the prolamine fraction will result in a decrease in the lysine content, and an increase in glutelin fraction will result in an increase in the lysine levels of the seed.

Additional studies to determine the effect of variation in the proportion of protein fractions and other factors on the protein content and amino acid composition of the grain sorghums are necessary.

DISCUSSION

Recent work on mutant corn has aroused renewed interest in the possibility of breeding cereals for higher content of good quality protein (Nelson, 1966). Analyses of a large number of genetic and hybrid varieties of grain sor-

Table IV. Amino Acid Composition of the Protein Fractions, Per Cent of Protein

Amino Acid	CSH-1-Bijapur				160 Cernum	
	Endosperm meal	Globulin	Prolamine	Glutelin	Globulin	Prolamine
Lysine	1.70	3.36	0.14	3.12	5.12	0.37
Histidine	2.16	1.45	0.67	3.12	1.28	0.74
Arginine	3.25	6.14	0.66	5.91	7.79	1.45
Aspartic acid	6.25	8.68	6.72	9.07	9.66	6.97
Threonine	3.81	4.87	...	4.88	4.00	2.15
Serine	4.50	5.55	3.32	5.38	4.51	3.06
Glutamic acid	29.75	15.80	25.07	24.08	10.68	28.30
Proline	10.31	5.33	11.63	14.86	4.81	12.56
Glycine	3.27	6.25	1.28	5.33	4.35	0.62
Alanine	12.58	6.74	13.96	9.40	6.36	12.67
Half cystine	1.08	1.99	Trace	1.21	1.68	Trace
Valine	7.25	6.46	5.88	5.50	4.04	3.93
Methionine	1.51	2.24	1.33	...	1.05	0.97
Isoleucine	4.91	3.45	5.04	4.07	2.80	3.80
Leucine	16.58	6.72	15.33	12.49	5.09	18.13
Tyrosine	4.64	4.01	5.17	3.23	2.13	3.64
Phenylalanine	6.40	4.77	5.84	4.90	2.84	4.75

ghum show that the protein content varies widely (Table I). Information on the amino acid composition makes it possible to evaluate the nutritive quality of the protein. Lysine is the most limiting amino acid in sorghum protein. When lysine content of the different varieties of sorghum was determined, lysine as per cent of protein was negatively correlated with the protein content of the seed. This finding is in agreement with the observed decline in the nutritive quality of sorghum as the protein in the grain increases (Vivach *et al.*, 1959; Waggle *et al.*, 1966).

Analyses by ion exchange chromatography of nine samples of grain sorghums representing both low- and high-protein varieties indicate that amino acid content of the varieties differs considerably (Table III). Variation of seed amino acid composition could be due to changes in the various protein fractions without any changes in their chemical composition, or it may be due to a change in the chemical composition of one or more protein fractions. The variations may also arise from both of these causes (Mossé, 1966). The negative correlation between lysine and protein in corn is attributed to the observed increase in the quantity of prolamine as the total protein increases (Bressani and Mertz, 1958). Mertz *et al.* (1964) have shown that changes in the amino acid make-up of opaque-2 mutant maize compared with normal seeds is due to a reduction in the amount of zein relative to other proteins. Later experiments have shown that in opaque-2 and floury-2 mutant lines of maize, synthesis of some components of normal zein is blocked accompanied by a greater synthesis of salt-soluble proteins (Mossé, 1966; Mossé *et al.*, 1966). Dalby and Davies (1967) have shown that in opaque-2 maize, there is an increase of ribonuclease activity during endosperm maturation which may account for the observed reduction in zein synthesis and the modification of the amino acid composition of the endosperm.

Solubility fractionation studies on five samples of sorghum of both low- and high-protein type reveal that increased protein content in sorghum varieties results from an increase mainly in the prolamine fraction of the grain (Table II). However, the high-protein genetic variety of sorghum, 160-cernum, has a significantly higher proportion of glutelin than the others. The proportion of prolamine to glutelin in this variety is 7.06 to 5.93, whereas 361-Dochna, another high-protein genetic variety of sorghum, has a prolamine to glutelin ratio of 11.18 to 3.61. Concentration of lysine in the glutelin fraction of sorghum is several times higher than that of the prolamine fraction (Table IV). Mention has been made that 160-cernum has the highest concentration of lysine of all the varieties which have been examined so far. This is perhaps to be attributed to the high proportion of glutelin in the endosperm protein of this variety. These results indicate that the protein of 160-cernum variety of sorghum would be superior to other varieties because of the high content of protein and high concentration of lysine in the protein. It remains to be seen whether confirmation of these tentative conclusions can be obtained in nutritional experiments. It would also be of interest to determine whether the alterations in the relative proportions of the protein fractions and amino acid pattern of this sorghum variety are under genetic control or are due to physiological factors.

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