Protein Fractionation of Sorghum Grain[1]

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ABSTRACT

Protein solubility of five samples of sorghum grain of both low- and high-protein type were compared to that of high-lysine (opaque-2) corn. A modified Mendel-Osborne technique was used. Results indicate that twice the amount of protein is extracted from opaque-2 corn when compared to that of sorghum grain. The glutelin fraction represented the major soluble-protein fraction of sorghum grain and opaque-2 corn. Amino acids were determined by ion-exchange chromatography. Comparisons of amino acid distributions were made between fractionated proteins, with the assumption that those proteins extracted were characteristic of their class. The percentages of proline and leucine increased and decreased respectively in the albumin protein as the protein content of whole sorghum grain increased. Concentrations of lysine and glycine in the nonsolubilized protein of sorghum grain decreased as protein content of the grain increased. Concentrations of lysine, arginine, and glycine in both the albumin and globulin fractions of sorghum grain were nearly double that in protein of whole sorghum grain. The glutelin fraction had an amino acid composition midway between that of the globulin and prolamine. Concentrations of lysine, histidine, arginine, threonine, serine, and glycine were higher in glutelin than in the prolamine fraction.

Published literature on protein solubility and amino acid composition of the fractionated proteins for sorghum grain is limited compared with that for corn and other cereal grains. The classic work of Osborne and Mendel (1) still forms the basis for most solubility studies. Basically, the method utilized a dilute salt solution to remove albumins and globulins, an aqueous ethyl alcohol solution to extract prolamines, and a dilute alkali solution to dissolve glutelins. Numerous workers, including Nagy et al. (2) and Jimenez (3), have used modifications of the Osborne-Mendel method in fractionating the proteins of corn.

Virupaksha and Sastry (4) fractionated protein of the endosperm portion of sorghum grain. They used solvents similar to those used in the Osborne-Mendel method, but did not specify consecutive fractionation. Lack of consecutive solvent extraction would allow an overlap of the proteins present in a given solubility class.

The objective of this study was to compare the solubilities of the proteins of corn and sorghum grain by means of the consecutive extraction technique of Osborne-Mendel (1), Nagy et al. (2), and Jimenez (3). Amino acid compositions of the fractionated proteins of sorghum grain and corn were compared under the assumption that those proteins extracted were characteristic of their class.

MATERIALS AND METHODS

Samples

Five samples of the 1966 crop of sorghum grain were obtained from test plots of the Kansas State University Agricultural Experiment Station. Samples were

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grown in Riley and Colby counties under similar conditions of soil and fertility. Hybrids grown in Riley county included Asgrow's Rica, RS-625, and TE-66, referred to as MO-6, MO-81, and MO-88 respectively.

Colby county hybrids included Asgrow's Rica and TE-66, referred to as CO-6 and CO-88, respectively. The five sorghum samples ranged in crude protein content from 8.3 to 14.8%, where CO-6 was 8.3% protein; CO-88, 8.4%; MO-81, 13.5%; and MO-88, 14.8%. The protein content of opaque-2 corn used was 9.8%. The high-lysine corn (opaque-2) was grown in Northeast Kansas during 1967. The sorghum grain and opaque-2 corn were cleaned and ground on a Micro-Sampl mill to pass through a screen opening of 0.02 in.

Protein Fractionation Procedures

Duplicate samples of sorghum grain and opaque-2 corn were subjected to successive extractions with each of the following solvents: distilled water to extract albumin, 5% sodium chloride solution for globulin, 80% ethanol plus 0.2% sodium acetate solution for prolamines, and 0.2% sodium hydroxide solution for glutelins. The residual proteins (nonsolubilized) were retained for analysis.

Water Extraction. Distilled water (100 ml.) was added to a 3-g. sample in a 250-ml. centrifuge tube. The stoppered tubes were rotated longitudinally for 24 hr. at room temperature. Suspensions were then centrifuged at 2,000 r.p.m. for 15 min., and the supernatant was decanted. Additional solvent (30 ml.) was added to the centrifuge tube and stirred with a glass rod to resuspend the insoluble material. The insolubles were washed twice with the respective solvents. The supernatant liquid was stored at 4°C. until lyophilized.

Salt Extraction. Insoluble residue from the water extraction was combined with a 5% (w./v.) solution of sodium chloride (100 ml.) by the same extraction procedures. Prior to lyophilization, salt was removed from the supernatant by dialysis against several changes of distilled water.

Alcohol Extraction. Similarly, the remaining insoluble residue was extracted with a mixture of 18.8% water, 80% ethanol, and 0.2% sodium acetate (v./v./w.) (100 ml.). The lyophilized material was redissolved in a smaller volume of the ethanol-acetate mixture to facilitate quantitative removal from the lyophilizing vessel. Aliquots of the sample were used in estimating crude protein content and amino acid composition.

Alkali Extraction. After successive extraction with distilled water, sodium chloride solution, and ethanol-acetate solution, the insoluble residue was extracted with 0.2% (w./v.) sodium hydroxide solution (100 ml.). Aliquots of the supernatant liquid or of the lyophilized product from the alkali extraction were used for analyses.

Insoluble Residue. Material remaining after extraction with the four solvents was dried for 5 hr. in an air oven at 110°C. The nonsolubilized residue was quantitatively recovered and analyzed for crude protein and amino acid composition.

Amino Acid Analyses. Amino acid analyses were performed on the whole grain and on aliquots of the liquid fractions or of the lyophilized fractions. Proteins were hydrolyzed with an excess of 6N HCl at 110°C. for 22 hr. in an anaerobic atmosphere according to the procedures of Waggle et al. (5). Amino acid analyses of

TABLE I. RESULTS OF THE FRACTIONATION OF PROTEIN FROM SORGHUM GRAIN AND OPAQUE-2 CORN BY THE MODIFIED MENDEL-OSBORNE METHOD

Fraction	Opaque-2 Corn	CO-6	CO-88	MO-6	MO-81	MO-88
Albumin[a]	9.0	4.9	4.5	4.5	2.2	2.7
Globulin[a]	11.2	5.8	3.5	4.3	3.6	2.5
Prolamine[a]	9.5	8.5	5.0	7.5	4.3	3.9
Glutelin[a]	56.4	20.8	17.9	14.9	17.4	17.3
Total soluble protein[a]	86.1	40.0	30.9	31.2	27.5	26.4
Nonsolubilized residue[a]	10.6	58.6	62.6	52.8	62.3	59.3
Total protein recovered, %	96.7	98.6	93.5	84.0	89.8	85.7
Initial protein of grain, % (d.b.)	9.8	8.3	8.4	12.8	13.5	14.8

[a]Data reported as percent of total protein.

the hydrolysates were made according to the procedures of Spackman et al. (6) and Moore et al. (7) with a Beckman 120 automatic amino acid analyzer. Cystine and methionine were determined as cysteic acid and methionine sulfone by the procedure of Moore et al. (7).

RESULTS AND DISCUSSION

Protein Fractionation

Percentages of total nitrogen extracted with each of the solvents from the five sorghum grain samples and opaque-2 corn are shown in Table I. The percent of soluble protein extracted from the sorghum samples varied from 26.4 to 40.0%; 86.1% of the opaque-2 corn protein was solubilized. These data demonstrate the insoluble characteristic of sorghum grain protein as compared to opaque-2 corn when the classic Osborne-Mendel solvent systems and techniques are used. The insolubility of the protein may be a physical phenomenon caused by the protein-starch matrix configuration of the seed. The samples, however, were sufficiently ground so that only a reasonable part of the insolubility should be attributed to this factor. It would appear that a large portion of sorghum grain protein was not solubilized by the Osborne-Mendel solvent systems.

Pushpamma (8) fractionated sorghum grain proteins by a conventional shaking technique with a modified Osborne-Mendel solvent system. Solvents were similar to those used in the present study, with the exception that 0.4% NaOH was used rather than 0.2% NaOH. Percentages of total protein extracted from commercial samples from both the United States and India were 38.2 and 41.5%, respectively. Virupaksha and Sastry[2] (4) reported an extraction of 83.0 to 103.4% of the protein

[2]Virupaksha, T. K., and Sastry, L. V. S. A personal communication given in the Final Technical Report by P. S.Sarma; U.S.D.A. Grant No. FG-In-159, Dept. of Biochemistry, Indian Inst. of Science, Bangalore-12, India.

from the endosperm of sorghum grain, but did not report protein recoveries based on successive extraction. They reported intermixing of the various protein fractions during extraction and substantiated this fact by electrophoretic studies. This suggests that some components characteristic of one protein group were also present in another group.

The glutelin fraction represented the major soluble-protein fraction in sorghum grain as shown in Table I. That agrees with reports utilizing opaque-2 endosperm (3). Though glutelin was found to be the major soluble-protein fraction of both grains, only small differences were observed between the quantities of globulin and prolamine.

The percent protein remaining in the nonsolubilized material was used to calculate the percent of total protein recovered. Total protein recovered ranged from 84 to 98.6% in the sorghum samples, whereas 96.7% of the total protein was recovered from opaque-2 corn.

Amino Acid Composition of Whole Grain

Amino acid compositions of whole-grain samples used for protein fractionation are given in Table II. The composition of the five sorghum grain samples was in

TABLE II. AMINO ACID COMPOSITION OF SORGHUM GRAIN AND OPAQUE-2 CORN USED IN PROTEIN FRACTIONATION (g. AMINO ACID PER 100 g. PROTEIN)

Amino Acid[a]	CO-6	CO-88	MO-6	MO-81	MO-88	Sorghum Average	Opaque-2[b] Corn
Lysine	2.32	2.08	1.81	1.61	1.59	1.88	4.14
Histidine	2.32	1.99	1.97	1.90	2.17	2.07	3.52
Ammonia	2.83	3.04	3.05	2.85	3.17	2.98	2.12
Arginine	3.76	3.36	3.30	3.12	3.10	3.32	6.56
Aspartic acid	6.48	6.37	6.41	6.04	6.60	6.38	9.00
Threonine	3.26	3.11	3.11	2.92	2.93	3.06	3.97
Serine	4.49	4.34	4.47	4.21	4.03	4.31	4.71
Glutamic acid	20.87	21.70	22.74	23.94	22.20	22.29	17.38
Proline	7.82	7.58	7.97	7.95	7.91	7.84	8.44
Glycine	3.14	3.04	2.76	2.57	2.53	2.81	4.93
Alanine	9.19	9.60	9.70	9.79	9.96	9.65	6.50
Cystine[c]	1.82	1.62	1.60	1.58	1.53	1.63	2.50
Valine	4.61	4.54	2.80	4.08	4.70	4.14	4.77
Methionine[c]	1.61	1.40	1.53	1.56	1.61	1.54	1.85
Isoleucine	3.90	3.94	3.99	3.74	3.92	3.90	3.32
Leucine	13.12	13.25	14.04	13.44	14.22	13.62	9.28
Tyrosine	3.98	3.90	4.10	3.89	4.03	3.98	3.82
Phenylalanine	5.06	5.05	5.09	4.95	5.18	5.07	4.15
Recovery of amino acids, %	103.10	106.79	103.75	108.88	111.08	106.72	99.55
Crude protein in grain, % (d.b.)	8.3	8.4	12.8	13.5	14.8	11.6	9.8

[a]Amino acid values were corrected to 100% recovery of Kjeldahl protein.

[b]Average of duplicate amino acid analyses.

[c]Cystine and methionine values estimated by performic acid oxidation.

close agreement with that reported by Deyoe and Shellenberger (9) and Waggle and Deyoe (10), and the amino acid composition for opaque-2 corn was similar to values reported by Cromwell et al. (11).

Amino Acid Composition of Protein Fractions

The albumins (Table III), globulins (Table IV), prolamines (Table V), glutelins (Table VI), and residual proteins (Table VII) had differing amino acid compositions. Some major trends were observed in amino acid composition of the fractions, attributable to differences in protein content of the whole grain. The proline content of the albumin fraction increased as the protein content of sorghum grain increased, whereas the leucine content of the albumin fraction decreased with increased protein content. Concentrations of lysine and glycine in the nonsolubilized protein of sorghum grain decreased as protein content of the grain increased, whereas the amounts of leucine and glutamic acid increased with increased protein content. Although some trends were observed in the amino acid content of the albumin and nonsolubilized protein fractions of the five sorghum grains, none were sufficient to indicate a major deviation in protein type owing to

TABLE III. AMINO ACID COMPOSITION OF THE WATER-SOLUBLE FRACTION (ALBUMIN) OF SORGHUM GRAIN AND OPAQUE-2 CORN (g. AMINO ACID PER 100 g. PROTEIN)

Amino Acid[a]	CO-6	CO-88	MO-6	MO-81[b]	MO-88[b]	Sorghum Average	Opaque-2[b] Corn
Lysine	5.28	4.54	5.58	3.36	3.58	4.47	5.41
Histidine	1.66	2.01	2.09	1.76	1.79	1.86	2.55
Ammonia	1.10	2.74	1.68	3.06	3.80	2.48	2.23
Arginine	6.09	6.78	4.33	4.91	4.33	5.29	6.38
Aspartic acid	12.92	11.68	10.53	11.12	11.02	11.45	9.79
Threonine	6.04	5.30	5.21	6.26	5.91	5.74	4.95
Serine	5.26	5.80	4.61	5.23	5.23	5.23	4.59
Glutamic acid	10.90	15.06	14.81	15.81	16.39	14.59	14.79
Proline	4.88	5.27	7.02	6.44	8.80	6.48	10.70
Glycine	7.40	6.94	7.33	8.25	8.47	7.68	7.74
Alanine	9.28	8.20	9.01	9.07	9.64	9.04	8.14
Cystine[c]	0.61	1.26	1.28	0.34	1.00	0.90	1.64
Valine	7.64	2.74	7.21	3.48	3.44	4.90	4.95
Methionine[c]	0.63	1.19	1.24	0.20	0.55	0.76	1.12
Isoleucine	4.66	4.37	3.57	4.32	3.26	4.04	3.51
Leucine	7.85	7.35	6.93	6.49	5.55	6.83	5.30
Tyrosine	3.30	3.54	3.96	3.48	3.12	3.48	2.77
Phenylalanine	3.85	4.31	3.63	3.30	2.99	3.62	2.60
Recovery of amino acids, %	66.31	61.57	63.69	55.04	49.52	59.23	81.44
Crude protein in grain, % (d.b.)	8.3	8.4	12.8	13.5	14.8	11.6	9.8

[a]Amino acid values were corrected to 100% recovery of Kjeldahl protein.

[b]Average of duplicate amino acid analyses.

[c]Cystine and methionine values estimated by performic acid oxidation.

TABLE IV. AMINO ACID COMPOSITION OF THE SODIUM CHLORIDE FRACTION (GLOBULIN) OF SORGHUM GRAIN AND OPAQUE-2 CORN (g. AMINO ACID PER 100 g. PROTEIN)

Amino Acid[a]	CO-6	CO-88	MO-6	MO-81	MO-88	Sorghum Average	Opaque-2[b] Corn
Lysine	5.35	4.52	4.59	4.33	4.25	4.61	5.98
Histidine	2.10	1.97	2.21	2.13	2.14	2.11	3.12
Ammonia	1.98	1.81	2.06	2.11	1.73	1.94	1.76
Arginine	8.23	9.32	9.41	9.13	9.75	9.17	9.05
Aspartic acid	9.29	10.54	9.62	9.88	9.55	9.78	9.16
Threonine	4.99	5.09	5.03	4.85	5.11	5.01	4.37
Serine	5.17	5.14	5.45	5.56	5.21	5.30	5.10
Glutamic acid	13.51	13.07	13.80	14.48	13.72	13.72	14.60
Proline	4.94	5.30	5.20	5.00	7.17	5.52	4.31
Glycine	6.06	5.91	6.02	6.16	4.25	5.68	5.89
Alanine	6.81	7.23	6.67	6.98	4.51	6.44	6.81
Cystine[c]	2.09	1.38	2.63	2.11	2.34	2.11	8.64
Valine	6.11	5.90	4.93	5.30	4.82	5.41	5.80
Methionine[c]	1.46	0.89	1.53	1.36	1.47	1.34	6.42
Isoleucine	4.34	4.54	4.21	3.84	4.46	4.28	4.03
Leucine	7.66	8.09	7.56	7.32	7.89	7.71	7.37
Tyrosine	3.67	3.35	3.39	3.32	3.60	3.47	3.62
Phenylalanine	4.23	4.11	4.28	4.15	4.42	4.24	4.60
Recovery of amino acids, %	78.63	77.12	86.81	79.37	81.48	80.68	87.79
Crude protein in grain, % (d.b.)	8.3	8.4	12.8	13.5	14.8	11.6	9.8

[a]Amino acid values were corrected to 100% recovery of Kjeldahl protein.

[b]Average of duplicate amino acid analyses.

[c]Cystine and methionine estimated by performic acid oxidation.

the wide range of total protein content. Similar effects were reported on different genotypes of corn by Jimenez (3) and Boundy et al. (12).

Concentrations of lysine, arginine, and glycine in the albumin and globulin fractions of sorghum grain were nearly double the levels found in the protein of whole sorghum grain, whereas the globulin fraction of opaque-2 corn had approximately one-third more lysine and arginine than the protein of whole opaque-2 corn. The prolamine fraction of both opaque-2 corn and sorghum grain contained less lysine than that of respective whole-grain protein.

Amounts of lysine, histidine, arginine, threonine, serine, and glycine were several times higher in the glutelin fraction of sorghum grain than the levels of those amino acids in the prolamine fraction. Amino acid compositions of the glutelin fractions of opaque-2 corn and sorghum grain were quite similar and were midway between those of the globulins and prolamines, except for the higher content of histidine and proline in the glutelin fraction.

Differences in amino acid composition of the nonsolubilized protein of both opaque-2 corn and sorghum grain followed differences observed in the respective whole-grain protein, regardless of the percent of protein solubilized.

TABLE V. AMINO ACID COMPOSITION OF THE ETHANOL-ACETATE MIXTURE FRACTION (PROLAMINE) OF SORGHUM GRAIN AND OPAQUE-2 CORN (g. AMINO ACID PER 100 g. PROTEIN)

Amino Acid[a]	CO-6	CO-88	MO-6	MO-81	MO-88	Sorghum Average	Opaque-2 Corn
Lysine	0.40	0.75	0.32	0.48	0.30	0.45	0.53
Histidine	0.83	0.80	0.89	0.81	0.79	0.82	1.18
Ammonia	3.39	2.95	3.07	2.63	2.85	2.98	2.47
Arginine	1.91	2.21	1.61	1.82	1.62	1.83	1.95
Aspartic acid	7.05	7.45	6.87	6.20	6.72	6.86	5.64
Threonine	2.54	2.68	2.68	2.50	2.52	2.58	2.81
Serine	4.08	3.95	4.13	3.72	3.75	3.93	4.98
Glutamic acid	25.04	24.08	27.62	23.99	24.72	25.09	22.81
Proline	9.93	8.45	5.75	9.25	8.88	8.45	9.67
Glycine	1.80	2.14	1.61	1.77	1.62	1.79	1.82
Alanine	10.09	9.73	10.87	9.74	9.94	10.07	8.34
Half-Cystine[b]	...[c]	...	0.33	...	...	...	1.13
Valine	4.60	4.66	1.94	4.57	4.59	4.07	3.62
Methionine[b]	0.52	1.10	0.86	2.09	1.39	1.19	0.61
Isoleucine	3.07	3.61	4.11	3.79	4.19	3.75	3.92
Leucine	15.32	15.76	16.36	16.06	15.94	15.89	17.21
Tyrosine	4.28	4.36	4.85	4.75	4.52	4.55	4.77
Phenylalanine	5.16	5.32	6.12	5.83	5.66	5.62	6.54
Recovery of amino acids, %	68.21	70.20	92.31	92.64	103.91	85.45	122.34
Crude protein in grain, % (d.b.)	8.3	8.4	12.8	13.5	14.8	11.6	9.8

[a]Amino acid values were corrected to 100% recovery of Kjeldahl protein.

[b]Cystine and methionine values were estimated by acid hydrolysis.

[c]Amino acids with missing values were not determined.

TABLE VI. AMINO ACID COMPOSITION OF SODIUM HYDROXIDE FRACTION (GLUTELIN) OF SORGHUM GRAIN AND OPAGUE-2 CORN (g. AMINO ACID PER 100 g. PROTEIN)

Amino Acid[a]	CO-6[b]	CO-88	MO-6	MO-81	MO-88	Sorghum Average	Opaque-2 Corn
Lysine	2.31	3.03	2.51	2.84	2.98	2.73	2.93
Histidine	2.80	3.02	3.24	3.41	3.25	3.14	3.28
Ammonia	2.64	2.41	2.89	2.11	2.17	2.44	1.84
Arginine	4.21	5.52	5.06	6.10	5.57	5.29	6.54
Aspartic acid	7.63	8.29	7.65	7.86	7.96	7.88	6.99
Threonine	3.89	4.23	4.02	4.20	4.35	4.14	3.78
Serine	4.38	5.17	4.68	4.98	4.67	4.78	4.00
Glutamic acid	18.52	18.28	19.16	18.60	19.07	18.73	19.39
Proline	9.56	9.28	9.46	9.47	8.29	9.21	10.13
Glycine	5.02	5.38	5.20	5.21	5.05	5.17	5.45
Alanine	8.02	7.52	7.96	7.21	7.20	7.58	6.68
Cystine[c]	0.69	...[d]	1.14	...	...	...	0.72
Valine	4.83	5.14	3.93	5.37	6.08	5.07	4.59
Methionine[c]	1.20	...	1.52	...	...	...	1.51
Isoleucine	4.76	3.69	4.52	4.31	4.82	4.42	3.88
Leucine	11.39	10.01	11.39	9.36	9.46	10.32	10.56
Tyrosine	4.23	4.08	4.44	4.10	4.08	4.20	4.67
Phenylalanine	5.05	4.96	5.22	4.70	4.87	4.96	5.09
Recovery of amino acids, %	60.38	81.25	87.81	95.00	96.09	84.11	67.26
Crude protein in grain, % (d.b.)	8.3	8.4	12.8	13.5	14.8	11.6	9.8

[a]Amino acid values were corrected to 100% recovery of Kjeldahl protein.

[b]Average of duplicate amino acid analyses.

[c]Cystine and methionine values estimated by performic acid oxidation.

[d]Amino acids with missing values were not determined.

TABLE VIII. RECOVERY OF EACH AMINO ACID FROM THE FRACTIONATED PROTEINS OF OPAQUE-2 CORN AND SORGHUM GRAIN[a]

Amino Acid[a]	Sorghum Average %	Opaque-2 Corn %
Lysine	63.0	76.3
Histidine	84.4	81.2
Ammonia	89.8	86.3
Arginine	78.1	89.5
Aspartic acid	99.3	78.0
Threonine	95.9	96.0
Serine	94.8	87.0
Glutamic acid	91.8	99.4
Proline	95.3	104.6
Glycine	102.2	103.2
Alanine	92.9	101.8
Valine	89.7	96.4
Isoleucine	99.4	110.2
Leucine	95.0	105.1
Tyrosine	94.8	103.9
Phenylalanine	97.6	112.5

[a]Recovery = the ratio (in %) of the summation of g. of an amino acid from the five protein fractions to g. of amino acid in the entire protein of the grain.

TABLE VII. AMINO ACID COMPOSITION OF NONSOLUBILIZED RESIDUE OF SORGHUM GRAIN AND OPAQUE-2 CORN (g. AMINO ACID PER 100 g. PROTEIN)

Amino Acid[a]	CO-6	CO-88	MO-6[b]	MO-81	MO-88	Sorghum Average	Opaque-2[b] Corn
Lysine	0.76	0.69	0.46	0.33	0.31	0.50	3.58
Histidine	1.74	1.62	1.64	1.54	1.40	1.60	3.50
Ammonia	2.96	3.44	3.10	3.18	3.02	3.13	2.01
Arginine	1.78	1.88	1.80	1.49	1.38	1.69	4.97
Aspartic acid	6.19	6.62	5.98	5.87	5.72	6.06	7.82
Threonine	3.17	3.03	2.60	2.64	2.44	2.75	5.37
Serine	4.58	4.78	4.40	4.30	4.16	4.44	4.70
Glutamic acid	22.72	24.47	24.22	26.92	27.33	24.98	15.04
Proline	8.10	8.43	8.38	8.53	8.01	8.31	9.25
Glycine	2.74	2.68	2.08	1.99	1.68	2.21	5.87
Alanine	9.70	10.83	11.15	11.12	11.23	10.86	6.99
Cystine[c]	0.41	0.43	0.32	0.32	0.26	0.35	0.80
Valine	4.66	2.02	3.00	4.55	2.66	3.18	6.57
Methionine[c]	1.24	1.43	1.28	1.43	1.28	1.33	1.23
Isoleucine	4.36	4.56	4.18	4.10	3.98	4.23	3.99
Leucine	15.12	15.43	16.29	16.76	16.32	16.04	10.45
Tyrosine	4.37	4.16	4.00	4.25	4.06	4.14	2.95
Phenylalanine	5.80	5.84	5.71	5.74	5.51	5.72	5.00
Recovery of amino acids, %	69.17	105.72	133.68	115.88	136.78	112.25	62.42
Crude protein in grain, % (d.b.)	8.3	8.4	12.8	13.5	14.8	11.6	9.8

[a]Amino acid values were corrected to 100% recovery of Kjeldahl protein.

[b]Average of duplicate amino acid analyses.

[c]Cystine and methionine values estimated by performic acid oxidation.

Recovery of Amino Acids

Recovery of amino acids was calculated by summing the amount (g.) of each amino acid recovered in each protein fraction and comparing the summation with the level of that amino acid present in the entire protein of the respective grain (Tables II to VII). Table VIII shows the recovery of each amino acid of the sorghum grain samples and opaque-2 corn. Lysine had the lowest recovery of any amino acid determined. The basic amino acids in general had lower recoveries than either the acidic or neutral amino acids. With the exception of lysine, recoveries are similar to those reported by Jimenez (3).

Literature Cited

1. OSBORNE, T. B., and MENDEL, L. B. Nutritional properties of proteins of maize kernel. J. Biol. Chem. 18: 1 (1914).
2. NAGY, D., WEIDLEIN, WILMA, and HIXON, R. M. Factors affecting the solubility of corn proteins. Cereal Chem. 18: 514 (1941).
3. JIMENEZ, J. R. Protein fractionation studies of high lysine corn. Proc. High-Lysine Corn Conf., p. 74 (1966).
4. VIRUPAKSHA, T. K., and SASTRY, L. V. S. Studies on the protein content and amino acid composition of some varieties of sorghum grain. J. Agr. Food Chem. 16: 199 (1968).
5. WAGGLE, D. H., PARRISH, D. B., and DEYOE, C. W. Nutritive value of protein in high and low protein content sorghum grain as measured by rat performance and amino acid assays. J. Nutr. 88: 370 (1966).
6. SPACKMAN, D. H., STEIN, W. H., and MOORE, S. Automatic recording apparatus for use with chromatography of amino acids. Anal. Chem. 30: 1190 (1958).
7. MOORE, S., SPACKMAN, D. H., and STEIN, W. H. Chromatography of amino acids on sulfonated polystyrene resins (an improved system). Anal. Chem. 30: 1185 (1958).
8. PUSHPAMMA, S. Protein quality and nutritive value of three Indian millets. A dissertation. Kansas State University, Manhattan (1968).
9. DEYOE, C. W., and SHELLENBERGER, J. A. Studies on the amino acids and proteins in sorghum grain. J. Agr. Food Chem. 13: 446 (1965).
10. WAGGLE, D. H., and DEYOE, C. W. Relationship between protein level and amino acid composition of sorghum grain. Feedstuffs 38(51): 18 (1966).
11. CROMWELL, G. L., PICKETT, R. A., and BEESON, W. M. Nutritional value of *opaque*-2 corn for swine. J. Animal Sci. 26: 1325 (1967).
12. BOUNDY, JOYCE A., WOYCHIK, J. H., DIMLER, R. J., and WALL, J. S. Protein composition of dent, waxy, and high-amylose corns. Cereal Chem. 44: 160 (1967).

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