

# Amino Acid Composition and Nutritional Value of Milled Sorghum Grain Products<sup>1</sup>

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## ABSTRACT

Nutritive values of milled products from sorghum grain were compared on the basis of amino acid composition and growth of rats. Endosperm products ranged from a soft white flour with 6.5% protein to a vitreous speckled product having 20.6% protein. Calculated on an equal nitrogen basis, nutritional value of protein in a bran-germ fraction was superior to both the original grain and the various endosperm fractions. The soft low-protein flours were higher in nutritive value than vitreous high-protein products. When whole grain and the various endosperm-containing diets were supplemented with lysine, an increase in growth resulted and protein efficiency of all diets was similar.

Sorghum grain is a high-volume cereal grain in the United States, but no information is available on protein quality of dry-milled fractions. Studies on whole sorghum grain by Waggle *et al.* (1,2) found that the nutritive value of low-protein sorghum grain, when compared on an equal protein basis, was superior to that of a high-protein sorghum grain. Dibasic amino acids, especially lysine, sulfur-containing amino acids, and threonine levels of high-protein sorghum grain were lower than those of low-protein grain.

Studies on sorghum grain by Normand *et al.* (3) indicated highest concentration of protein in outer layers of the endosperm. Peplinski *et al.* (4) and Stringfellow and Peplinski (5) reported the horny endosperm to contain more protein than the soft floury endosperm. The separation of horny and floury fractions was made by dry-milling and air classification.

This study was to determine protein quality of high- and low-protein milled fractions of sorghum grain. Protein quality was determined from amino acid composition of fractions and confirmed by nutritional studies.

## MATERIALS AND METHODS

### Milling

Grain sorghum (Paymaster-Kiowa), tempered to 14% moisture for 16 hr. and then to 20% moisture for 15 min. immediately before processing, was milled with typical dry-milling equipment. Processing was in two principal parts. The bran and germ was obtained by breaking the grain with corrugated rolls and separating with aspiration and a gravity table. The grits were then reduced by a series of rolls and sieves. The milling flow from which the various fractions were collected is shown in Fig. 1. The experimental flow as shown is complex, but for commercial milling could be simplified by combining similar streams; e.g., the streams of fine and

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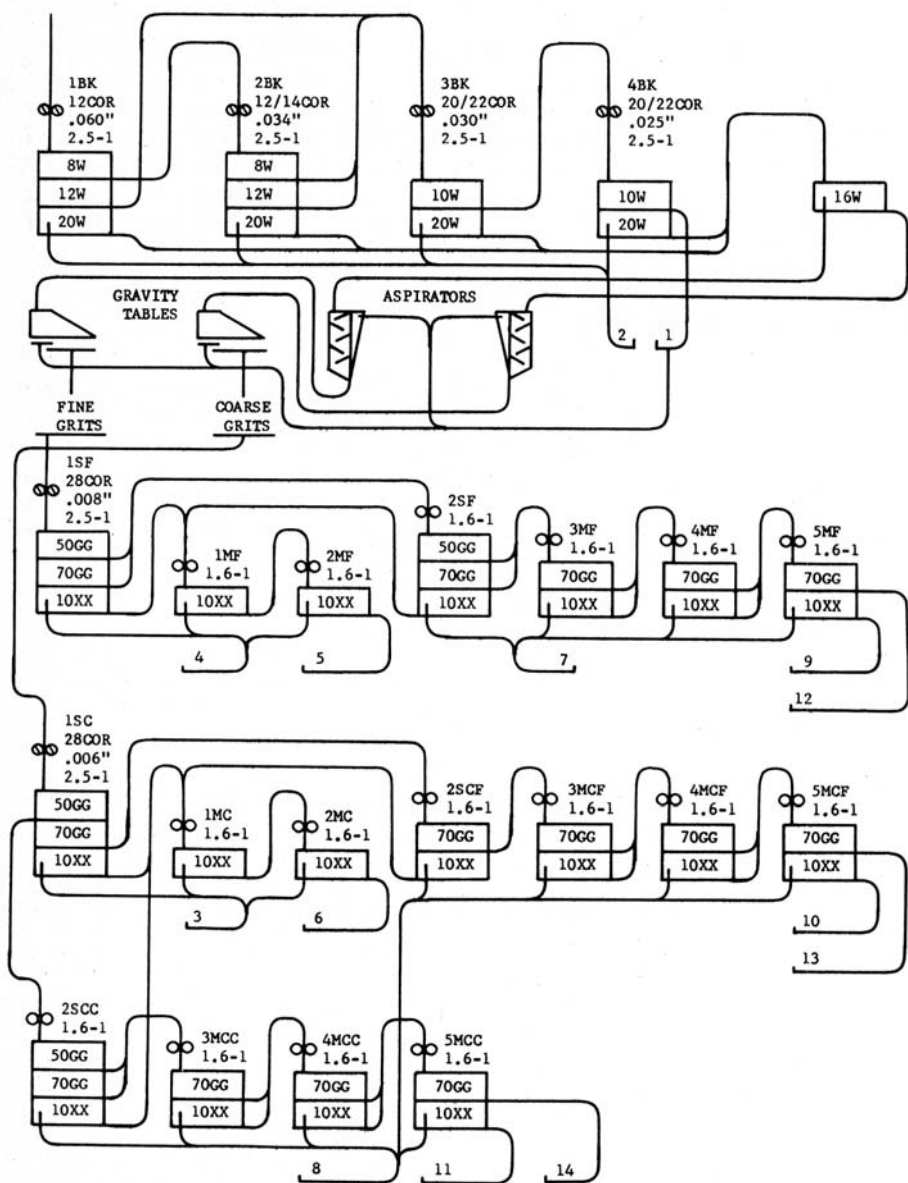


Fig. 1. Milling flow.

coarse grits could be combined and milled by one reduction system. For the nutritional study some of the milled sorghum grain fractions were composited and used in the diets as follows: diet 1, a portion of the bran and germ mixture, fraction 1, 10.3% protein; diet 2, sorghum grain, 10.1% protein; diet 3, flour fraction 3, 5.7% protein; diet 4, flour fraction 8, 9.8% protein; diet 5, endosperm fractions 10 and 11, 15.1% protein; diet 6, endosperm fractions 12, 13, and 14, 18.1% protein.

### Analytical

Crude protein, crude fat, and moisture of all samples were determined by AOAC Methods (6). Protein and fat values were adjusted to a moisture-free basis.

Amino acids and ammonia were determined on the composited dry-milled sorghum grain fractions and several commercial products by ion-exchange chromatography according to procedures of Spackman *et al.* (7) and Moore *et al.* (8) with an amino acid analyzer. Samples were hydrolyzed by the procedure of Waggle *et al.* (1). Cystine and methionine were determined as cysteic acid and methionine sulfone by the procedure of Moore (9). Quantities of those amino acids then were determined with the Autoanalyzer. Tryptophan was determined by the procedure of Lombard and Delang (10).

### Nutritional

A growth study was made with female albino rats of the Spague-Dowley strain, 21 days old. A completely randomized experimental design was used, with eight replications per diet. The rats were housed in individual wire cages in a controlled-environment laboratory. The only source of protein was sorghum grain fractions which were composites of milled fractions as described previously. Diets were calculated to be isonitrogenous (approximately 9.3% protein on 12% moisture basis) except for diet 3 which, owing to the fraction used, contained only 5.6% protein. High-protein fractions were diluted with starch to adjust protein levels. The basal diet is shown in Table I.

The study was of 6 weeks' duration. At the end of the 4th week lysine was added to the diets to meet adjusted nutritional requirements (Table II). Because the National Research Council's requirements (11) are based on a 20% protein diet, adjusted requirements for the 9.3% and 5.6% dietary protein levels used in this study were based on 9.3/20 of the NRC requirement for diets 1, 2, 4, 5, and 6 and 5.6/20 for diet 3. Although exact ratios have not been firmly established, amino acid requirements are proportional to total dietary protein.

TABLE I. BASAL DIET

Ingredient	1,000-g. Diet	Ingredient	1,000-g. Diet
	<i>g.</i>	Vitamin premix	<i>mg.</i>
Sorghum grain or fraction	variable	Vitamin A (11.6 IU/mg.)	190.00
Starch	variable	Vitamin D (15.0 IU/mg.)	133.00
Corn oil	35.00	Alpha-tocopherol	240.00
Mineral premix		Menadione	0.10
Dicalcium phosphate	27.00	Thiamine HCl	1.25
Salt	3.00	Riboflavin	2.50
Trace minerals	0.25	Pyridoxine HCl	1.20
Potassium chloride	2.75	Niacin	15.00
Magnesium sulfate	1.50	Calcium pantothenate	8.00
		Vitamin B-12 (crystalline)	5.00
		Choline chloride	750.00
		Carrier (grain fraction)	9,000.00

<sup>a</sup>Sargents Calcium Company, Des Moines, Iowa: P, 18.5%; Ca, 19.0 to 22.5%.

<sup>b</sup>CCC trace mineral mix contained: p.p.m. Mn, 10; Fe, 10; Ca, 14; Cu, 1; Zn, 5; I, 0.3; and Co, 0.1; Calcium Carbonate Company, Quincy, Ill.

## RESULTS AND DISCUSSION

### Milling

The yield of milled grits over a 20-mesh wire sieve by the process shown in Fig.

TABLE II. SUPPLEMENT LEVELS OF LYSINE<sup>a</sup>

Diet	Amount of Sorghum Fraction in Diet %	Lysine in Milled Fraction %	Lysine in Diet 0 to 4 Weeks %	Lysine Supplemented %	Lysine in Diet 5 to 6 Weeks %	Adjusted <sup>b</sup> NRC Requirement %
1	92.2	0.47	0.43		0.43	0.43
2	88.1	0.21	0.19	0.24	0.43	0.43
3	94.5	0.10	0.10	0.16	0.26	0.26
4	94.5	0.12	0.12	0.31	0.43	0.43
5	62.0	0.14	0.09	0.34	0.43	0.43
6	52.0	0.17	0.09	0.34	0.43	0.43

<sup>a</sup>Calculations made on an "as-is" moisture basis.

<sup>b</sup>National Research Council requirements based on 20% dietary protein; adjusted requirements based on 9.3/20 of NRC requirement for diets 1,2,4,5, and 6 and 5.6/20 of NRC requirement for diet 3.

1 was 68%. Grits were milled further to obtain the products listed in Table III. As milling proceeded, softer endosperm materials were reduced and removed, and protein content of the remaining fractions increased. The percentage protein in endosperm fractions obtained ranged from 6.5% in a soft flourey fraction to 20.6% in a hard vitreous product. The color gradient followed a similar trend, low-protein material being white, high-protein fractions tan and speckled.

TABLE III. YIELD AND PROTEIN AND FAT CONTENT OF FRACTIONS OBTAINED FROM DRY-MILLING SORGHUM GRAIN

Fraction	Description	Fraction of Whole Grain %	Protein in Fraction %	Fat in Fraction %
1	Bran and germ	19.3	12.8	10.4
2	Fines 1,2,3,4BK	12.0	8.0	1.9
3	Flour, 1SC; 1,2MC	12.3	6.5	0.5
4	Flour, 1SF; 1,2MF	3.0	7.2	1.1
5	Endo. over 10xx; 2MF	4.7	8.9	0.4
6	Endo. over 10xx; 2MC	14.8	12.1	1.0
7	Flour, 2SF; 3,4,5MF	7.2	8.6	1.2
8	Flour, 2SC; 3,4,5MC	6.8	11.1	1.3
9	Endo. over 10xx; 5MF	8.9	14.2	0.6
10	Endo. over 10xx; 5MCF	2.1	15.7	1.1
11	Endo. over 10xx; 5MCC	3.7	17.3	0.8
12	Endo. over 70GG; 5MF	2.2	19.7	1.1
13	Endo. over 70GG; 5MCF	1.0	18.8	1.5
14	Endo. over 70GG; 5MCC	1.9	20.6	1.9

Data expressed on moisture-free basis.

### Amino Acids

Percentages of amino acids in the various samples (moisture-free basis) are given in Table IV. The bran-germ material was relatively high in lysine, arginine, and glycine, but lower in other essential amino acids (isoleucine, leucine, and phenylalanine) than whole grain. Endosperm fractions represent a wide range in total protein content. All amino acids in the fractions increased as total protein in the fractions increased. However, relative distribution of amino acids in the protein varied as protein content of the sample changed (Table V); consequently, protein efficiencies should differ from one fraction to another. Percentages of lysine,

TABLE IV. AMINO ACID COMPOSITION OF SORGHUM GRAIN AND MILLED PRODUCTS

	Sorghum Grain %	Bran and Germ %	Endosperm Fractions			
			3 %	8 %	10,11 %	12,13,14 %
Protein <sup>a</sup>	10.10	10.30	5.70	9.80	15.10	18.10
Lysine <sup>b</sup>	0.24	0.52	0.12	0.14	0.17	0.19
Histidine	0.26	0.28	0.14	0.24	0.35	0.41
Arginine	0.44	0.82	0.21	0.32	0.41	0.51
Aspartic acid	0.81	0.95	0.45	0.72	1.11	1.31
Threonine	0.38	0.42	0.25	0.35	0.52	0.60
Serine	0.55	0.52	0.31	0.50	0.76	0.92
Glutamic acid	2.57	1.57	1.47	2.80	4.47	5.17
Proline	0.96	0.60	0.60	0.99	1.58	1.86
Glycine	0.35	0.60	0.20	0.28	0.38	0.47
Alanine	1.11	0.78	0.67	1.21	1.93	2.36
Cystine	0.24	0.24	0.14	0.21	0.30	0.36
Valine	0.57	0.58	0.26	0.58	0.88	1.09
Methionine	0.20	0.21	0.11	0.19	0.27	0.32
Isoleucine	0.47	0.39	0.27	0.51	0.78	0.91
Leucine	1.54	0.85	0.90	1.75	2.80	3.31
Tyrosine	0.50	0.32	0.27	0.49	0.72	0.86
Phenylalanine	0.62	0.45	0.35	0.64	1.01	1.20
Tryptophan <sup>c</sup>	0.16	0.25	0.21	0.20	0.20	0.27
Ammonia	0.36	0.25	0.25	0.40	0.62	0.70
Nitrogen recovery	89.50	85.14	95.92	99.68	97.91	96.94

<sup>a</sup>Crude protein, N X 6.25, 12% moisture basis.<sup>b</sup>Amino acid, g. per 100 g. sample, single analysis, moisture-free basis.<sup>c</sup>Average of duplicate analyses.

TABLE V. AMINO ACID DISTRIBUTION IN PROTEIN OF SORGHUM GRAIN AND MILLED PRODUCTS

	Sorghum Grain %	Bran and Germ %	Endosperm Fractions			
			3 %	8 %	10,11 %	12,13,14 %
Protein <sup>a</sup>	10.10	10.30	5.70	9.80	15.10	18.10
Lysine <sup>b</sup>	2.10	4.44	1.82	1.29	0.96	0.93
Histidine	2.24	2.35	2.18	2.16	2.01	1.99
Arginine	3.89	6.96	3.14	2.90	2.38	2.49
Aspartic acid	7.00	8.03	6.73	6.47	6.41	6.35
Threonine	3.33	3.54	3.76	3.18	3.04	2.92
Serine	4.77	4.45	4.65	4.56	4.42	4.47
Glutamic acid	22.32	13.31	22.20	25.32	25.95	25.14
Proline	8.39	5.10	8.97	8.96	9.18	9.06
Glycine	3.01	5.11	3.07	2.52	2.21	2.30
Alanine	9.68	6.60	10.16	10.95	11.21	11.45
Cystine	2.04	2.01	2.16	1.90	1.72	1.73
Valine	4.96	4.93	4.56	5.28	5.13	5.28
Methionine	1.75	1.74	1.82	1.75	1.57	1.54
Isoleucine	4.07	3.29	4.10	4.59	4.50	4.41
Leucine	13.31	7.24	13.64	15.84	16.27	16.08
Tyrosine	4.37	2.69	4.03	4.39	4.19	4.18
Phenylalanine	5.41	3.83	5.25	5.79	5.85	5.82
Tryptophan	1.35	2.10	3.16	1.88	1.13	1.33
Ammonia	3.56	2.11	3.82	3.65	3.61	3.86

<sup>a</sup>Crude protein, N X 6.25, 12% moisture basis.<sup>b</sup>Amino acid, g. per 100 g. protein.

cystine, methionine, threonine, and tryptophan of the protein decreased as protein content of the endosperm fractions increased. In fraction 3 the percentages of valine, isoleucine, leucine, and phenylalanine in the protein were less than those found in the higher-protein fractions. Protein of the bran-germ fraction contained approximately four times as much lysine and two times as much arginine and glycine as protein of endosperm fractions. In contrast, percentages of glutamic acid, proline, alanine, leucine, and tyrosine in the protein of the bran-germ fraction were approximately half those of the endosperm protein. As expected, amino acid values of the whole grain fell between those of the endosperm fractions and the bran-germ material.

TABLE VI. AMINO ACID COMPOSITION OF COMMERCIAL PRODUCTS FROM SORGHUM GRAIN<sup>a</sup>

	Flour %	Flour %	Flour %	Bran %	Germ %	Millfeed %
Protein <sup>b</sup>	7.30	5.10	8.00	7.60	12.10	9.00
Lysine <sup>c</sup>	0.13	0.14	0.16	0.32	0.75	0.36
Histidine	0.17	0.13	0.20	0.19	0.38	0.22
Ammonia	0.27	0.17	0.30	0.20	0.28	0.23
Arginine	0.26	0.21	0.28	0.36	1.08	0.52
Aspartic acid			0.68	0.69	1.31	0.82
Threonine			0.32	0.33	0.56	0.36
Serine			0.47	0.40	0.74	0.47
Glutamic acid			2.42	1.22	2.28	1.64
Proline			0.92	0.49	0.81	0.63
Glycine			0.29	0.48	0.82	0.45
Alanine			1.09	0.63	1.12	0.80
Valine			0.42	0.40	0.77	0.22
Isoleucine			0.42	0.33	0.51	0.37
Leucine			1.53	0.77	1.22	1.00
Tyrosine			0.42	0.22	0.47	0.31
Phenylalanine			0.56	0.36	0.64	0.46
Nitrogen recovery			104.73	83.02	97.92	84.53

<sup>a</sup>No analyses were made for cystine, methionine, and tryptophan.  
<sup>b</sup>Crude protein, N X 6.25, 12% moisture basis.  
<sup>c</sup>Amino acid, g. per 100 g. sample, single analysis, moisture-free basis.  
<sup>d</sup>Blank spaces indicate no determination made.

In Tables VI and VII are data on samples of sorghum grain products from a commercial mill. Flour samples ranging from 5.1 to 8.0% protein showed the same shift in lysine concentration of the protein as noted in the experimentally milled products; i.e., concentration of lysine in the protein of low-protein flour was highest (Table VI). The protein of the germ was notably higher in lysine, arginine, aspartic acid, and glutamic acid than was protein of the bran. However, those amino acids, except glutamic acid, were more concentrated in the protein of both germ and bran than in the protein of whole grain. Millfeed analysis was similar to that of the bran; it contained more lysine and arginine than did whole grain.

#### Nutrition

Rat growth and percentages met of amino acid requirements of the three most limiting amino acids are shown in Table VIII. Significant differences were found among weight gains of rats tested at the end of the 4th week. Those fed the

TABLE VII. AMINO ACID DISTRIBUTION IN PROTEIN OF COMMERCIAL PRODUCTS FROM SORGHUM GRAIN<sup>a</sup>

	Flour %	Flour %	Flour %	Bran %	Germ %	Millfeed %
Protein <sup>b</sup>	7.30	5.10	8.00	7.60	12.10	9.00
Lysine <sup>c</sup>	1.62	2.45	1.75	3.68	5.42	3.56
Histidine	2.09	2.26	2.20	2.20	2.76	2.16
Ammonia	3.22	3.03	3.30	2.34	2.06	2.29
Arginine	3.09	3.57	3.15	4.16	7.80	5.12
Aspartic acid	d		7.56	7.93	9.43	8.01
Threonine			3.52	3.78	4.04	3.56
Serine			5.23	4.55	5.32	4.65
Glutamic acid			26.72	14.07	16.46	16.13
Proline			10.18	5.66	5.85	6.16
Glycine			3.24	5.58	5.95	4.46
Alanine			11.97	7.26	8.11	7.80
Valine			4.69	4.65	5.58	2.13
Isoleucine			4.68	3.75	3.72	3.66
Leucine			16.88	8.89	8.79	9.86
Tyrosine			4.68	2.59	3.38	3.09
Phenylalanine			6.20	4.15	4.66	4.54

<sup>a</sup>No analyses were made for cystine, methionine, and tryptophan.

<sup>b</sup>Crude protein, N X 6.25, 12% moisture basis.

<sup>c</sup>Amino acid, g. per 100 g. protein, single analysis.

<sup>d</sup>Blank spaces indicate no determination made.

TABLE VIII. RAT GROWTH DATA

Diet	Description	Protein <sup>a</sup>		Without Lysine Supplementation, 0 to 4 Weeks		Percent of NRC Requirement, Adjusted, 0 to 4 Weeks			With Lysine Supplementation, 5 to 6 Weeks	
		Fraction %	Diet %	Av. Gain <sup>c</sup>	PER <sup>d</sup>	Lysine %	Methionine %	Valine %	Av. Gain <sup>c</sup>	PER <sup>d</sup>
1	Bran and germ	10.3	9.3	20.4 <sup>a</sup>	2.08	100.0	89.3	145.4		
2	Sorghum grain	10.1	9.3	5.2 <sup>b</sup>	1.04	44.0	88.1	135.0	24.4 <sup>e</sup>	2.60
	Endosperm fractions									
3	3	5.7	5.6	1.8 <sup>c</sup>	0.58	34.6	88.2	107.0	11.8 <sup>f</sup>	2.61
4	8	9.8	9.3	1.8 <sup>c</sup>	0.39	25.5	89.3	145.4	24.0	2.77
5	10, 11	15.1	9.3	0.6 <sup>d</sup>	0.12	20.2	78.6	145.4	21.4 <sup>g</sup>	2.57
6	12, 13, 14	18.1	9.3	0.6 <sup>d</sup>	0.16	21.0	78.6	148.4	23.4 <sup>e</sup>	2.92

<sup>a</sup>Percent protein (N X 6.25), 12% moisture basis.

<sup>b</sup>National Research Council requirements based on 20% dietary protein; adjusted requirements based on 9.3/20 of NRC requirement for diets 1, 2, 4, 5, and 6 and 5.6/20 of NRC requirement for diet 3.

<sup>c</sup>Grams per rat per week; values with the different letters are significantly different at the 10% level with LSD 1.1 for 0 to 4 weeks and 2.1 for 5 to 6 weeks.

<sup>d</sup>Protein efficiency ratio, g. of gain per g. of protein consumed.

bran-germ fraction gained fastest; those fed whole grain were second. Rats fed diets containing flouy endosperm fractions (5.7 and 9.8% protein) or horny endosperm fractions (15.1 and 18.1% protein) gained little. The flouy endosperm diets, including diet 3 with the low-protein level, supported a significantly higher gain than



diets containing horny endosperm. Dietary amino acid requirements were met except as shown in Table VIII. A positive relation existed between weight gain and percentage of lysine supplied by the diet. The protein efficiency ratio (PER) indicates that protein of diet 3 had higher nutritive value than protein of diet 4, although the difference was not indicated by weight gains. Protein efficiency ratio is defined as the ratio of gain in body weight to amount of protein ingested by the animal during the test period. The PER values reported are valid for comparison of only these experimental diets, as no control diets were fed.

Since lysine apparently was the most limiting nutrient (Table VIII) during the first 4 weeks, diets 2 to 6 were supplemented with sufficient lysine to meet the adjusted NRC requirement for 2 weeks additional. The weight gain and PER show positive responses in the 5th and 6th weeks compared with the first 4 weeks. Differences in weight gains during weeks 5 and 6 were small except for diet 3, which contained less protein than other diets. PER's during weeks 5 and 6 were similar in diets 2 to 6 when lysine deficiencies were corrected. These results tend to indicate the validity of the adjustment applied to the NRC requirements. It is recognized the good PER's of diets 2 to 6 during the last 2 weeks could have resulted in part from the condition of the rats having been fed poor-quality protein the first 4 weeks.

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#### Literature Cited

1. 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.* 88:370-374 (1966).
2. WAGGLE, D. H., DEYOE, C. W., and SMITH, F. W. Effect of nitrogen fertilization on the amino acid composition and distribution in sorghum grain. *Crop Sci.* 7:367-368 (1967).
3. NORMAND, F. L., HOGAN, J. T., and DEOBALD, H. J. Protein content of successive peripheral layers milled from wheat, barley, grain sorghum, and glutinous rice by tangential abrasion. *Cereal Chem.* 42:359-367 (1965).
4. PEPLINSKI, A. J., STRINGFELLOW, A. C., and BURBRIDGE, L. H. Fractionating commercial flour and grits from grain sorghum. *Am. Miller Processor* (October 1963).
5. STRINGFELLOW, A. C., and PEPLINSKI, A. J. Air classification of sorghum flours from varieties representing different hardnesses. *Cereal Sci. Today* 11:438-440, 455 (1966).
6. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official methods of analysis (9th ed.). The Association: Washington, D.C. (1960).
7. SPACKMAN, D. H., STEIN, W. H., and MOORE, S. Automatic recording apparatus for use in the chromatography of amino acids. *Anal. Chem.* 30:1190-1206 (1958).
8. MOORE, S., SPACKMAN, D. H., and STEIN, W. H. Chromatography of amino acids on sulfonated polystyrene resins (an improved system). *Anal. Chem.* 30:1185-1190 (1958).
9. MOORE, S. On the determination of cystine as cysteic acid. *J. Biol. Chem.* 238:235-237 (1963).
10. LOMBARD, J. H., and DELANG, J. Chemical determination of tryptophan in food and mutual diets. *Anal. Biochem.* 10:260 (1965).
11. NATIONAL ACADEMY OF SCIENCES-NATIONAL RESEARCH COUNCIL, Committee on Animal Nutrition. 1963 Nutrient requirements of laboratory animals, publ. 990. Washington, D.C.

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