strength.

The monomers (subunits) are dissociated reversibly from the protomer or the dimer at pH 11.5–12.0. They may undergo conformational changes before extensively unfolding at pH 12.5. At extreme alkaline pH (pH 13) alkaline degradation may account for the irreversible dissociation. At ionic strength less than 0.01, reversible dissociation into subunits occurs even at mild alkaline or mild acidic pH. In the alkaline region, the dissociation of B_3 to B_6 conglycinins gives α subunit. In the acidic region both α and β subunits are dissociated from β-conglycinin.

Dissociation of β-conglycinin with urea has been found to accompany simultaneously the destruction of internal structure of the protein, which led to the suggestion that the subunits were very compactly and complicatedly folded on the formation of the gross structure (Koshiyama, 1971). However, in the present study the ability of the protein to undergo dissociation at low ionic strength and at physiological pH suggests that the subunits can exist as organized monomers in equilibrium with the trimer and hexamer within soybean seeds. Thus, the dissociation may not require an extensive destruction of the secondary and tertiary structures of the protein.

Contrary to a report of Koshiyama (1968) we found that the dissociation at alkaline pH was reversible. The dissociation coincided with the ionization of tyrosine residues in β-conglycinin. Tyrosine and the electrostatic interactions that are disrupted at alkaline pH due to unionization of ϵ-amino group of lysine (pK = 10.53) and guanidine group of arginine (pK = 12.48) are considered to contribute to the interaction between the subunits. Tyrosine residues are likely to be present in the subunit contact region—therefore, exposed to the media during dissociation—rather than being buried in the interior of the subunits, because their ionization at alkaline pH was not time-dependent (Koshiyama, 1971). In a similar manner, most of the tyrosine residues of conarachin (78%), which ionized with apparent pK of 11.2 at low ionic strength, were proposed to be located at the interfaces of the subunits, stabilizing the quaternary structure of the protein (Yotsuhashi, 1973).

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Protein Fractions from Five Varieties of Grain Sorghum: Amino Acid Composition and Solubility Properties

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Classic protein fractions 1, 2, 3, and 4 (albumins, globulins, prolamins, and glutelins, respectively) were obtained from five varieties of sorghum that differed in endosperm/pericarp structure and in tannin content. Amino acid profiles and protein distribution of isolated fractions showed some differences among varieties. The chemical scores varied from 9 to 91. Methionine, cysteine, isoleucine, and leucine were most limiting in fractions 1 and 2. Fractions 3 and 4 were most deficient in lysine in all five varieties. Leucine/lysine ratio was above 20 in all of the fractions 3 except for that in a high-tannin variety; the ratio of all of the other fractions was below 4. Fractions 1 and 2 extracted from the high-tannin variety contained less protein than those from the other varieties. The comparable extent of essential amino acid deficiencies and excesses in these fractions is given.

Grain sorghum, *Sorghum bicolor* (L.) Moench, is an important food crop in many parts of the world and is a major feed grain produced in the United States. Cultivated sorghums include five races: bicolor, caudatum, durra, guinea, and kafir (Schecter and Dewet, 1975). The physical and chemical characteristics of the grain which vary among varieties, account for differences in biological values and availability of nutrients. Recent studies on several varieties suggested that the efficiency of grain fed to ruminants is related, in part, to both peripheral and internal structures of the seed (Sullins, 1972; Sullins and Rooney, 1974) and to the presence of polyphenolic compounds (Axtell, 1976). Only a small amount of nutritional data of sorghum fed to humans, however, has been reported.

The amino acid composition of the total protein in sorghum is similar to that of corn and other cereals, where lysine is the most limiting amino acid (Wall and Blessin, 1970). Low content of lysine is attributed to the high content of prolamin (fraction 3) in most normal varieties. The biological value of sorghum is further reduced by the presence of metabolic inhibitors and by chemical inter-

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Table I. Protein Analysis of Whole Seed Meals and Protein Fractions of Sorghum

	percent protein (N × 6.25)				
	variety				
sample	CK 60	SC 301	Tx 615	NSA 740	GA 615
whole seed	9.9	11.5	11.9	14.3	9.8
defatted seed	11.9	13.5	14.0	17.1	11.5
residual meal	4.7 (39)[a]	5.1 (38)	5.4 (39)	7.0 (41)	3.6 (31)
fraction 1[b]	21.5	30.4	35.4	25.5	4.3
fraction 2	9.9	17.9	14.1	15.1	1.0
fraction 3	57.6	40.6	42.6	54.8	60.3
fraction 4	10.9	11.0	7.9	4.5	34.3

[a] Percent of total protein in defatted seed in parentheses. [b] Distribution among fractions based on freeze-dried material.

actions that occur during processing (Wall and Blessin, 1970).

We compared the solubility of protein in commercial varieties that vary in pericarp/endosperm structures and in tannin content. In addition, the amino acid composition and chemical scores of isolated albumins, globulins, prolamins, and glutelins from these varieties were compared.

This research is part of a broader study of five cultivars. Though there are data in the literature on the amino acid composition of sorghum proteins (Virupaksha and Sastry, 1968; Jones and Beckwith, 1970; Skoch et al., 1970; Wall and Paulis, 1978), such information on grain sorghum is small in comparison with other major cereals, and it is deemed worthwhile to present the data reported here on five commercial cultivars.

MATERIALS AND METHODS

Seed Samples. The five varieties used in this study are characterized as follows: SC 301, all corneous endosperm with thin pericarp; NSA 740, floury endosperm with thick pericarp; CK 60, intermediate floury/corneous endosperm with thick pericarp; Tx 615, waxy endosperm with intermediate pericarp; GA 615, high tannin content with intermediate floury/corneous endosperm. The varieities were grown in 1970 under identical agronomic conditions at College Station, Texas, and stored under refrigeration after harvesting. The cultivars are further described elsewhere (Sullins, 1972; Sullins and Rooney, 1974).

Milling. Samples of whole grain (250 g each) were milled on a standard Wiley Mill with a 40-mesh screen. The meals of each variety were then extracted with hexane (1 L/250 g of meal) to remove the lipid. Particle size was further reduced by pulverizing the meals with a ball mill for 30 min at 10-min intervals.

Protein Extraction. Lipid-free meals (200-g samples) were extracted yielding four protein fractions according to Landry and Moureaux (1970) and Jambunathan and Mertz (1973) with some modifications. The exact sequential procedure follows: Fraction 1 (albumins); 200 g of each flour in two 1-L portions of deionized water, pH 6.0, was sequentially mixed with a magnetic stirrer for 60 min at 5 °C. The homogenates were centrifuged at 40000*g* for 20 min at 10 °C, yielding supernatants containing albumins. Fraction 2 (globulins); residues from the previous step, were extracted sequentially into two 1-L portions of 0.5 M NaCl for 60 min at 5 °C. Globulin fractions in supernatants were obtained by centrifugation as described above. Fraction 3 (prolamins); residues from fraction 2, were mixed with two 1-L portions of tertiary butanol/water (v/v, 60:40) that contained 0.1 M guanidine hydrochloride. Supernatants containing prolamins were collected after centrifugation as described above. Fraction 4 (glutelins); the final residues were extracted with two 1-L volumes as described above with Borax–NaOH buffer, pH 10, that contained 0.5% sodium dodecyl sulfate ($NaDodSO_4$). This buffer was prepared by mixing 6.1 g of sodium borate, 1.9 g of NaOH, and 0.5 g of $NaDodSO_4$ per liter of water.

All of the fractions except the albumins were dialyzed against deionized water for 48 h at 5 °C followed by freeze-drying.

Analytical Methods. Protein contents were determined from Kjeldahl nitrogen values (conversion factor, 6.25). Amino acid analyses were made by Analytical Bio Chemistry Laboratories, Inc., Columbia, No. 65201, in duplicate according to the method of Kaiser et al. (1974). Tryptophan was not determined.

Chemical scores were calculated from essential amino acid data based on the requirement pattern for humans established by the World Health Organization (1973): score = milligrams of amino acid/gram of test protein × 100/milligrams of amino acid/gram of reference protein; score = value of most limiting amino acid. To determine differences between means, the least significant difference (LSD) was used (Snedecor and Cochran, 1973).

RESULTS AND DISCUSSION

The protein contents of meals and fractions are given in Table I. From the data on the residual meals, about 60% of the total protein in defatted seed was extracted. The distribution of extracted protein, however, indicated a wide range of variation among the fractions. The highest proportion of fraction 1 was obtained from Tx 615, the waxy endosperm variety; the lowest proportion was extracted from GA 615, the high-tannin variety. Low extractability of fractions 1 and 2 from high-tannin sorghums has been reported (Wambunathan and Mertz, 1973). These results suggest the possible formation of insoluble complexes between tannins and proteins that could be carried over to fractions 3 and 4 in subsequent extractions. For all varieties, the usual high percent of fraction 3 was observed. The highest proportion of fraction 4 was extracted from GA 615 and the lowest from NSA 740.

The amino acid profiles of the isolated protein fractions and residual meals are given in Table II. These values are in general agreement with previous investigations (Wall and Blessin, 1970; Wall and Paulis, 1978), but, some anomalies were observed. Fraction 4 from CK 60 and fraction 2 from GA 615, for example, contain unusually high amounts of cysteine relative to comparable fractions of the other varieties. Fractions 1 and 2 from all the varieties have the expected high contents of lysine, but the highest value is in fraction 1 of NSA 740. The lowest contents of lysine were found in fraction 3 from all the varieties, NSA 740 having the least amount. The profiles of the residual meals resemble fraction 3 and are comparable to those of the whole seed reported by Sullins (1972). It appears that some protein from the four classic fractions remained in the final residue. Nitrogen recovery

Table II. Amino Acid Content of Protein Fractions of Sorghum

	variety/fraction																						
	CK 60					SC 301					Tx 615					NSA 740					GA 615				
amino acid	1	2	3	4	RM[b]	1	2	3	4	RM	1	2	3	4	RM	1	2	3	4	RM	1	2	3	4	RM
alanine	7.5	7.5	11.2	5.6	9.2	7.6	8.0	11.4	7.3	9.4	6.7	6.1	11.5	6.9	8.8	8.7	7.0	11.4	6.7	8.9	7.2	4.6	9.8	9.1	9.9
valine	6.2	4.8	4.4	4.6	4.8	5.5	4.4	4.0	5.2	4.9	5.5	5.6	5.2	6.0	4.7	6.9	6.0	4.8	6.0	4.4	4.6	3.1	5.2	5.0	5.3
glycine	6.4	6.8	1.6	5.8	2.7	6.6	6.9	1.9	6.2	2.5	6.5	6.3	1.9	5.7	2.6	6.5	6.7	1.9	6.2	2.3	6.6	4.6	3.6	3.3	2.9
isoleucine	3.4	2.9	3.7	2.8	3.9	3.1	2.6	3.7	3.6	4.0	3.0	4.2	4.1	3.9	3.7	4.3	3.6	4.1	4.0	3.8	3.0	2.1	3.8	3.6	4.4
leucine	6.8	6.0	16.1	7.6	13.7	6.8	6.5	15.5	8.6	14.3	6.6	6.4	16.7	9.5	12.9	7.7	6.1	15.5	7.8	13.1	7.0	6.7	12.7	12.6	14.7
proline	4.7	4.7	7.8	9.3	8.2	4.7	4.9	7.9	8.2	8.6	4.8	4.7	7.8	7.1	8.3	5.5	4.4	7.9	6.2	7.4	4.2	4.1	7.3	6.8	8.8
threonine	4.9	4.6	2.6	3.8	2.9	5.1	4.7	2.9	4.4	3.0	4.6	4.3	2.7	4.3	2.9	4.3	4.0	2.8	4.2	2.6	4.2	4.1	3.6	3.3	3.2
serine	5.6	7.7	4.1	5.4	4.1	5.9	7.8	4.6	6.0	4.3	7.3	6.6	4.1	5.7	4.1	5.8	6.7	4.7	5.6	4.0	6.1	6.2	5.2	4.9	4.6
methionine	1.4	1.6	0.9	1.0	1.7	1.8	1.3	1.4	1.6	1.2	1.5	1.4	1.2	2.0	1.2		1.2	1.2	1.4	1.0	1.1	1.0	1.2	2.0	1.4
hydroxyproline	0.2	0.2			0.1	0.1				0.1					0.1					0.1		1.0			0.1
phenylalanine	4.5	4.3	5.4	4.2	5.2	4.7	4.2	5.3	4.8	5.3	5.1	5.1	5.5	5.4	4.2	4.3	4.7	5.0	5.9	4.4	6.1	7.7	5.4	5.0	5.1
aspartic acid	9.9	9.5	6.3	6.8	5.5	10.8	9.9	6.3	8.0	6.0	9.0	8.5	6.5	8.0	5.5	12.6	9.3	6.5	9.4	5.5	11.4	11.3	8.0	7.3	6.6
glutamic acid	15.3	13.4	24.2	18.6	19.5	15.3	13.5	22.7	18.0	21.0	15.4	15.1	24.7	16.8	19.6	18.7	15.7	24.3	18.2	19.0	16.9	17.5	22.1	20.3	21.7
tyrosine	3.3	3.9	4.8	3.5	3.3	3.4	3.8	4.9	3.8	3.4	3.5	3.9	5.0	3.9	2.9		3.4	4.5	3.6	2.8	2.5	2.6	3.6	3.9	3.3
lysine	6.6	4.9	0.8	3.5	1.4	6.3	4.6	0.7	3.8	1.3	5.4	5.3	0.8	3.9	1.4	8.0	1.9	0.5	4.1	1.3	6.3	5.8	1.7	1.6	1.5
histidine	3.5	3.0	2.5	4.4	3.0	3.8	3.4	1.4	3.5	2.2	2.8	4.1	0.5	2.4	2.6	4.7	3.3	1.3	2.9	2.0	4.2	5.7	1.5	2.6	2.2
arginine	8.9	10.6	2.9	5.4	1.9	7.4	10.5	2.7	6.1	2.4	10.0	9.6	0.7	6.7	1.5	1.5	13.2	1.8	5.8	2.4	7.4	4.1	4.1	7.3	2.7
cyst/2	1.1	3.5	0.4	7.8	1.0	1.0	3.4	2.6	1.1	0.9	2.5	2.6	0.8	1.6	1.0	0.7	3.0	1.0	1.5	0.7	1.3	7.7	1.0	1.3	0.9
recov., %	82	84	100	100	91	83	80	97	100	94	91	80	97	59	89	81	93	62	80	86	46	48	36	80	98

[a] Grams of amino acid/100 g of protein (average of duplicate analyses). [b] RM = residual meal.

Table III. Chemical Scores of Five Varieties[a] of Grain Sorghum

	fraction																			
	1 variety					2 variety					3 variety					4 variety				
amino acid	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
lysine	120	115	98 (118)[b]	145	115	89	84	96 (82)	*35*	105	*15*	*13*	*15* (17)	*9*	*31*	*64*	*69*	*71* (62)	*75*	*29*
threonine	123	120	115 (113)	100	105	115	118	108 (109)	100	103	65	73	68 (73)	70	90	95	110	108 (100)	105	83
methionine + cyst/2	*71*	80	114 (71)	*20*	*69*	145	134	114 (152)	120	248	37	114	50 (65)	63	62	251	77	100 (121)	83	94
leucine	97	97	94 (100)	110	100	86	91	91 (90)	87	95	230	221	239 (218)	221	181	109	123	136 (132)	111	180
isoleucine	85	*78*	*75* (84)	108	75	*73*	*65*	105 (77)	90	*53*	93	93	103 (97)	103	95	70	80	98 (88)	100	90
valine	156	110	110 (121)	138	92	96	88	112 (96)	120	62	88	80	104 (94)	96	104	92	104	120 (107)	120	100
phenylalanine + tyrosine	180	135	143 (134)	72	143	137	133	150 (145)	135	172	170	170	175 (168)	158	167	128	147	155 (147)	158	148

[a] A, CK 60; B, SC 301; C, Tx 615; D, NSA 740; E, GA 615. Italic values refer to first limiting amino acids. [b] Mean values of chemical scores are in parentheses.

Table IV. Statistical Analysis of Chemical Scores[a]

	LSD between varieties in each fraction								LSD between fractions[b]	
	fraction 1		fraction 2		fraction 3		fraction 4			
amino acid	5%	1%	5%	1%	5%	1%	5%	1%	5%	1%
lysine	22.4	30.3	10.8	14.6	3.4	4.5	7.4	10.0	16.4	22.2
threonine	7.5	10.2	3.1	4.1	5.0	6.7	4.5	6.0	7.0	9.5
methionine + cyst/2	13.5	18.2	20.8	28.1	11.7	15.7	29.2	39.3	15.7	21.2
leucine	2.6	3.4	1.4	1.9	8.8	11.9	11.6	15.5	22.7	30.7
isoleucine	5.9	8.0	8.2	11.0	1.9	2.5	5.0	6.8	5.2	7.0
valine	10.0	13.5	8.4	11.3	4.2	5.6	5.0	6.8	11.3	15.3
phenylalanine + tyrosine	14.7	20.9	6.7	9.1	3.3	4.5	4.7	6.3	14.9	20.1

[a] Values show least significant differences (LSD) at 5% and 1% levels. [b] Determined from mean values shown in parentheses in Table III.

was low for some of the fractions. For GA 615, recovery was exceptionally low for fractions 1, 2, and 3. Upon hydrolysis, possible reactions between tannins and individual amino acids could account, in part, for such low recovery. Also, the presence of nonprotein nitrogen in these fractions could overestimate the initial quantity of protein analyzed for.

An excess or deficiency of certain amino acids in protein can have deleterious effects on nutrition. The disproportionately high amounts of leucine in some cereals, including sorghum, have been suggested as a possible cause of pellagra. This observation has been made in some parts of India, where sorghum is a staple food (Deosthale et al., 1970; Kakade, 1974). Inspection of Table II reveals that all of the proteins in fractions 3 had very high leucine/lysine ratios; the other fractions had ratios below 4.0 with the exception of fraction 4 of GA 615. According to Deosthale et al. (1970), any protein fraction with a leucine/lysine ratio below 4.6 could be considered nutritionally safe as a major source of protein.

Estimated biological values based on chemical scores from essential amino acid data (without tryptophan) are presented in Table III. These data indicate both the limiting amino acids and the extent of their deficiency. Compared with the provisional standard for humans established by the World Health Organization (1973), the percentage deficits are greatest for methionine, cysteine, isoleucine, and lysine. For fractions 1 and 2, the sulfur-containing amino acids (except for lysine in fraction 2 of NSA 740) are most deficient. Lysine is most limiting in the other two fractions, which agrees with the findings of other investigations (Wall and Paulis, 1978, and references cited therein). The fact that leucine is the first limiting amino acid in fraction 2 from Tx 615 is noteworthy, even though that fraction has substantial lysine and isoleucine.

Statistical analysis of the data in Table III is presented in Table IV. The least significant difference is a criterion used to establish confidence limits for the population difference between any pair of means. Hence, the significant differences in chemical scores of individual essential amino acids between varieties in each fraction and between the fractions are given here. In fraction 1, for example, only NSA 740 (D in Table III) is significantly different from the other four varieties with respect to the score of lysine at the 5% level (LSD = 22.4). Similar reasoning can be applied to determine significant differences between other means in Table III. Mean comparisons between fractions can be illustrated for the score of lysine as another example. At the 5% level (LSD = 16.4), the largest mean difference is between fractions 1 and 3 and the smallest between fractions 2 and 4 (Table III). And for all practical purposes, the score for lysine is not different in fractions 2 and 4 (82 vs. 62 ± 16.4).

These data suggest that differences in physical and chemical characteristics of the grain from different varieties of sorghum do influence solubilities and chemical scores of the classic protein fractions. Because chemical scores reported here do not take into account the availability of pertinent limiting amino acids, the values represent only a rough estimation of protein quality—results from in vivo digestibility studies might not correlate with these data. Work is now in progress to further characterize and compare proteins from these fractions by serological, electrophoretic, and chromatographic methods.

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