Evaluation of Protein Quality of Sorghum [*Sorghum bicolor* (L.) Moench]†

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Grain protein content varied from 6.8% to 19.6% in eight sorghum cultivars comprising land races, hybrids, and local cultivars. Amino acid contents and distribution of Landry and Moreaux protein fractions in grains were studied. Two Ethiopian land races had higher concentrations of lysine and threonine, as well as cystine, isoleucine, and tyrosine. Fraction I, comprising albumin–globulin including non-protein nitrogen, and fraction V (glutelin) together constituted about 41–55% of the protein in the eight sorghum cultivars. Variation in fraction II (prolamin) and fraction III (cross-linked prolamin) contents was observed among the cultivars. To elucidate the pattern of synthesis of protein fractions in grain, studies were made at different grain maturity periods, using one cultivar. Fraction I synthesis was initiated at 7 days after anthesis. Prolamin increased from 14 to 28 days and declined toward maturity. Glutelin did not change beyond 14 days after anthesis until maturity.

Sorghum [*Sorghum bicolor* (L.) Moench] grains supply energy, proteins, minerals, and vitamins to several million people in the tropics, who depend on it as their staple food. Protein content of sorghum germplasm accessions varied from 4.4% to 21.1% with a mean value of 11.4% (Subramanian and Jambunathan, 1984). The genotypes, growing environment, and nitrogen fertilization influenced the protein content in sorghum (Deosthale et al., 1972). Sorghum proteins varied in their properties and amino acid composition (Virupaksha and Sastry, 1968). This variability can be effectively utilized for incorporating desirable traits in the development of cultivars through techniques such as biotechnology. The quality of protein depends on the amino acid composition and the proportion of various classes of proteins (Gupta and Gupta, 1974). Variation in amino acid composition is a function of nitrogen content in normal sorghum grains (Mosse et al., 1988). The genes affecting protein quality do so by changing the relative quantities of protein fractions and not by changing the quality of proteins within these fractions in sorghum (Guiragossian et al., 1978). Distribution of nitrogen in different fractions of protein from the grains of IS 11167 and IS 11758 has been previously reported (Jambunathan et al., 1975; Guiragossian et al., 1978). Physical and chemical characteristics of the grain from different sorghum varieties influenced solubilities and chemical scores of protein fractions (Neucere and Sumrell, 1979). Certain lines are superior to other sorghum cultivars due to higher percent of germ, higher absolute protein content, and production of lysine-rich proteins (Ejeta and Axtell, 1987).

The aim of this paper is to compare the variation in amino acid composition, distribution of nitrogen in Landry–Moreaux protein fractions of whole grains in sorghum cultivars comprising land races, hybrids, and local varieties. The changes that occur in protein fractions at different stages of grain maturation were also studied to determine the time of accumulation of the different classes of proteins.

EXPERIMENTAL PROCEDURES

Sorghum Cultivars. Eight sorghum cultivars, M 35-1, CSV 3, CSH 6, CSH 8, P 721, RY 49, IS 11167, and IS 11758, were used in the study; the details of the cultivars and growing conditions of the crop and grain sampling method for analysis have been described earlier (Subramanian et al., 1983). Grains were ground in a Udy cyclone mill, to pass through a 0.4-mm screen. The meal was defatted in a Soxhlet apparatus, using *n*-hexane, and used for further analysis. All values reported are means of duplicate analysis.

Protein Content. Total nitrogen in sorghum meal was determined by the micro-Kjeldahl method (AOAC, 1975), and the crude protein content was calculated by using the factor (N% × 6.25).

Amino Acid Composition. The sample was hydrolyzed by refluxing in 6 N HCl for 24 h, and excess acid was removed by using a flash evaporator. Residue was dissolved in citrate buffer (pH 2.2). The amino acids were analyzed in an amino acid analyzer (Beckman Model 120C). The values for cystine and methionine were not reported.

Protein Fractionation. Solubility fractionation of protein fractions I–V was obtained by the method of Landry and Moreaux (1970), with minor modifications. The resulting residue after fraction V was treated with 0.1 N sodium hydroxide solution, and protein soluble in the alkali was referred to as fraction VI or residual protein.

For determining protein fractions during maturation, cultivar CSH 6, a popular hybrid in India, was selected. Panicles were harvested at weekly intervals, starting from day 7 after anthesis, until 49 days. The grains were removed, freeze-dried, and, after being ground in a Udy mill, processed for protein fractionation.

RESULTS AND DISCUSSION

Grain protein content of the eight cultivars varied from 6.8% to 19.6%. The grains of M 35-1, CSH 6, CSH 8, and CSV 3 had less than 10.0% protein, while protein content was more than 12.0% for the grains of P 721, RY 49, IS 11167, and IS 11758. The variation in amino

† Submitted as Journal Article No. 828 by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh 502 324, India.

0021-8561/90/1438-1344$02.50/0

Table I. Amino Acid Composition of Sorghum Cultivars [g (100 g)$^{-1}$ of Protein]

amino acid	cultivars								mean	SE
	M 35-1	CSH 6	CSH 8	CSV 3	RY 49	P 721	IS 11167	IS 11758		
lysine	2.58	2.01	2.21	2.34	1.95	2.69	2.73	3.00	2.44	±0.13
histidine	1.98	1.90	1.91	2.27	1.97	2.17	2.16	2.16	2.07	±0.05
arginine	4.24	3.65	3.78	3.90	4.00	4.37	4.41	4.93	4.16	±0.05
aspartic acid	7.30	6.61	6.60	6.93	6.57	7.90	8.61	9.17	7.46	±0.35
threonine	3.25	2.88	2.95	3.80	2.61	3.46	3.70	3.94	3.32	±0.17
serine	3.94	3.98	3.96	5.14	3.94	4.38	4.65	5.33	4.42	±0.20
glutamic acid	24.20	25.39	23.69	24.99	25.61	26.88	27.78	25.22	25.47	±0.47
proline	8.00	8.68	7.47	8.21	7.89	8.16	8.83	9.00	8.28	±0.18
glycine	4.20	3.16	3.75	4.03	3.04	4.27	4.06	4.95	3.93	±0.22
alanine	10.11	10.64	10.02	10.46	10.13	11.22	11.68	13.04	10.91	±0.37
cystine	0.73	0.78	0.75	0.86	0.57	0.75	0.90	1.41	0.84	±0.09
valine	5.59	5.16	5.39	5.56	4.92	6.07	6.48	6.93	5.77	±0.24
methionine	0.63	0.55	–	1.33	1.22	0.21	0.53	1.55	0.86	±0.19
isoleucine	3.89	3.68	3.82	4.98	3.69	4.45	4.64	5.05	4.28	±0.20
leucine	13.50	14.29	13.65	14.89	14.43	15.62	16.09	14.43	14.61	±0.32
tyrosine	3.69	3.54	3.82	3.51	3.58	4.10	4.17	4.50	3.86	±0.13
phenylalanine	4.22	4.30	4.90	4.89	4.88	4.10	4.17	4.50	4.50	±0.12
% protein in meal ($N \times 6.25$)	6.8	9.5	7.2	7.9	14.7	12.0	19.6	19.1	12.10	±1.83

[a] Values are based on moisture-free basis.

Table II. Nitrogen Distribution in Sorghum Grains[a]

protein fraction	cultivars								mean	SE
	M 35-1	CSH 6	CSH 8	CSV 3	RY 49	P 721	IS 11167	IS 11758		
I (albumin and globulin, including non-protein nitrogen)	17.1	15.6	17.8	17.3	18.6	19.4	22.4	24.4	19.1	±1.04
II (prolamin)	5.2	14.4	5.6	8.4	15.8	8.2	9.7	7.4	9.3	±1.37
III (cross-linked prolamin)	18.2	18.1	18.7	19.5	19.8	16.2	17.9	14.6	17.9	±0.61
IV (glutelin-like)	4.2	3.5	3.4	4.4	3.4	3.4	2.9	3.5	3.6	±0.17
V (glutelin)	38.3	33.3	35.0	33.7	30.4	35.4	18.9	23.1	31.0	±1.45
VI (residual protein)	10.4	6.6	10.7	10.7	9.5	9.3	17.3	18.5	11.6	±1.45

[a] Percent of total nitrogen.

acid composition of the eight cultivars is shown in Table I. Protein content has been reported to be negatively related to lysine content (Virupaksha and Sastry, 1968). The high-lysine Ethiopian land races IS 11167 and IS 11758 also had high protein content, which may be due to the high levels of lysine-rich fractions I and V as reported earlier (Guiragossian et al., 1978). These two land races had comparatively high concentrations of lysine, aspartic acid, threonine, alanine, isoleucine, tyrosine, and phenylalanine (Table I). This may be due to high levels of albumin–globulin fraction in these lines. The lysine content was low in RY 49 and CSH 6. Variation in other amino acids was also observed among the cultivars. The leucine/isoleucine ratio was lower in IS 11758 and CSV 3 grains than in other cultivars.

The lysine-rich albumin and globulin fractions are generally low in sorghum (Sastry et al., 1986; Mohammad et al., 1980). Alcohol-soluble, lysine-deficient prolamin forms the major component of sorghum proteins (Jones and Beckwith, 1970). Glutelin also constitutes a major proportion in sorghum proteins (Mohammad et al., 1980). Solubility fractionation of protein in sorghum revealed that fraction I was high in the lines having a high grain protein content (Table II). These values include both protein and non-protein nitrogen. This fraction contained more lysine (Neucere and Sumrell, 1979). The high-lysine Ethiopian land races, IS 11167 and IS 11758, had the highest quantities of the above fraction (22.4% and 24.4%, respectively). Haikerwal and Mathieson (1971) reported 28% soluble nitrogen in the saline-soluble fraction of a Nigerian sorghum. It has been reported that albumin, globulin, and glutelin offer the best source of protein nutrition in sorghum (Guiragossian et al., 1978). Prolamin content (Table II) showed variation among the genotypes, ranging from 5.2% to 15.8%. The cultivars CSH 6 and RY 49 had higher prolamin content than others. Hamaker et al. (1986) observed that after cooking sorghum flour, kafirin (prolamin) had low in vitro protein digestibility. Kafirin proteins form complexes during cooking, and complexed kafirins are less accessible for enzyme attack. The low lysine content in these cultivars may be due to high prolamin content, which is deficient in lysine. However, their protein contents varied significantly (Table I). Prolamin protein had very high leucine/lysine ratios (Neucere and Sumrell, 1979). The leucine content in grain did not show appreciable variation except that it was high in IS 11167 (Table I). In general, cross-linked prolamin is high in sorghum, as compared to pearl millet and corn (Nwasike et al., 1979). All the cultivars, except IS 11758, had more than 16% cross-linked prolamin. Cross-linked prolamin may be advantageous as it confers hardness to the grain (Abdelrahman and Hoseney, 1984). Grain hardness is beneficial to withstand breakage of grain during dehulling process. It has been observed that mutation in P 721 sorghum decreased the quantity of kafirin (Guiragossian et al., 1978) with an increase in the albumin–globulin fraction. In barley, high-lysine content in mutant endosperm was caused by a reduction in lysine-poor hordeins, with a concomitant increase in glutelins (Brandt, 1976). Sorghum cultivars such as P 721, IS 11167, and IS 11758, which had high grain-protein content, were low in prolamin content, compared to the other cultivars studied.

The distribution of fraction V (reduced glutelin) is nearly one-third of the proteins in the sorghum cultivars, except in IS 11167 and IS 11758, which had only 18.9% and 23.1%, respectively (Table III). Fraction I and V proteins together comprised more than 41–55% of protein in the sorghum cultivars. The residual protein (fraction VI) content was very high for the Ethiopian lines, IS 11167

Table III. Lysine Content in Protein Fractions of Sorghum Grains [g (100 g)$^{-1}$ of Protein]

protein fraction	cultivars							mean	SE
	M 35-1	CSH 8	CSV 3	RY 49	P 721	IS 11167	IS 11758		
I	3.47	4.37	4.38	4.70	4.93	4.99	5.03	4.55	±0.21
II	0.17	0.08	0.01	ND[a]	0.10	0.05	0.10	0.09	±0.02
III	0.10	ND[a]	ND[a]	ND[a]	ND[a]	0.05	0.07	0.07	±0.01
IV	1.07	1.04	1.21	0.91	1.14	1.08	1.46	1.13	±0.06
V	2.48	2.15	2.36	2.22	2.73	2.70	2.58	2.46	±0.09
VI	1.65	2.15	1.69	1.82	2.09	2.19	1.84	1.91	±0.08

[a] ND, not detected.

Table IV. Nitrogen Distribution in Sorghum Grain at Different Stages of Development[a]

protein fraction	days after 50% flowering						
	7	14	21	28	35	42	49
I	53.9	15.6	20.8	17.5	18.1	18.4	15.6
II	5.7	20.4	21.1	25.9	13.4	12.4	14.3
III	2.0	8.5	9.2	9.0	14.3	17.2	18.1
IV	1.1	3.4	5.1	5.0	4.2	3.5	3.5
V	15.5	27.1	30.8	25.7	34.1	33.4	33.3
VI	13.5	8.0	6.6	5.3	7.2	6.2	6.6
total	91.7	93.0	93.6	91.4	91.3	91.1	91.5
protein in grain, g (100 g)$^{-1}$	11.0	10.4	8.0	9.2	7.7	8.7	9.5

[a] Percent of total nitrogen.

and IS 11758, and low in CSH 6. The variations in distribution of different protein fractions and in total protein content in the cultivars indicated that protein synthesizing activity may be different among the cultivars. The proteins of sorghum endosperm were heterogeneous and varied in properties and amino acid composition (Virupaksha and Sastry, 1968).

It has been well established that lysine is the most limiting amino acid in sorghum (Deyoe and Shellenberger 1965; Virupaksha and Sastry, 1968). The cultivars were compared for distribution of lysine in the different protein classes. The lysine content in fraction I was high in cultivars IS 11758, IS 11167, RY 49, and P 721, which had high grain protein content (Table III), and low in M 35-1 grains. Though other cultivars had nearly similar quantities of albumin–globulin per unit quantity of protein, the lysine levels were high in IS 11167, IS 11758, P 721, and RY 49, as their protein content and albumin–globulin contents were also high. Prolamin and cross-linked prolamin had very low lysine levels, as reported earlier by Virupaksha and Sastry (1968), and may even be absent (Table III). The lysine content in glutelin, glutelin-like, and residual protein fractions did not vary among the cultivars, although the levels were high.

The sequential changes in content of protein fractions during grain development of the cultivar CSH 6, a popular sorghum hybrid in India, are given in Table IV. It had been observed earlier that protein content of developing grain was high 7 and 14 days after anthesis, and a decline was observed toward maturity (Subramanian et al., 1983). Fraction I synthesis was maximum during the first 7 days after anthesis. This fraction includes both protein and non-protein nitrogen. In barley, too, accumulation of salt-soluble proteins was observed to be maximum during the early stage of grain development (Brandt, 1976). Van Scoyoc et al. (1988) reported that the percentage contribution of fraction I, expressed as percent of total nitrogen, declined rapidly and steadily in developing sorghum grains. However, when expressed as N content/endosperm, the patterns of nitrogen per endosperm for fraction I were very different from those expressed as a percentage of total protein. Prolamin synthesis was low during the first 7 days after anthesis, tended to increase from 14 to 28 days, and declined thereafter. Synthesis of cross-linked prolamin steadily increased until maturity. This suggests that prolamin was synthesized in different proportions during different stages of grain development. Onset of active synthesis of glutelin was observed from 21 days after anthesis, with little change until maturity. However, Gupta and Gupta (1974) observed that in sorghum the levels of prolamin and glutelin progressively increased from early milky stage to maturity stage. There was a progressive increase in the synthesis of fraction IV (glutelin-like) from 7 to 35 days of maturity period, which then stabilized until maturity. Insoluble protein contents in the residue were high during the first 7 days and decreased until 21 days, with very little change thereafter.

CONCLUSIONS

The present study shows variation in the amino acid composition and distribution of protein fractions in grains of cultivated varieties/hybrids and land races. The two land races, IS 11167 and IS 11758, had higher protein content and higher concentration of lysine and threonine. The cultivars M 35-1 and P 721 also had higher concentration of lysine. These data would be useful in a breeding program where the protein quality of sorghum is considered important.

ACKNOWLEDGMENT

We thank T. Hanumantha Rao and K. A. R. Zafar for their assistance.

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Received for review November 14, 1988. Revised manuscript received January 26, 1990. Accepted February 5, 1990.

Registry No. Lys, 56-87-1; Thr, 72-19-5.

Chromatographic Profile of Carbohydrates in Commercial Coffees. 2. Identification of Mannitol

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Mannitol, a polyhydric sugar alcohol, has been identified for the first time in coffee products. Its presence in some commercial soluble coffees at levels above 0.30% indicates adulteration.

Recent studies of the carbohydrate composition of soluble coffee powders (Blanc et al., 1989) have provided evidence that coffee husk extracts are present in some commercial soluble coffees at concentrations as high as 25%. At these levels the quantities of undeclared materials in the product after industrial processing cannot be considered a nondeliberate "defect" in the product (Jobin, 1982).

Inositol is the only polyhydric alcohol reported earlier in roasted coffee beans and instant coffee powder (Mishkin et al., 1970). Zuluaga Vasco and Tabacchi (1980) found 1.2% inositol in the carbohydrate fraction of fresh wet processed coffee pulp.

Mannitol, also a polyhydric alcohol, is frequently found in exudates of plants such as the flowering ash, olive, and plane trees and in marine algae in concentrations in excess of 20% (Lohmar, 1974). It has now been identified in the carbohydrate fraction of pelletized coffee husks and also in certain commercial soluble coffees, where its presence confirms adulteration: its behavior in the conditions of soluble coffee manufacture will be discussed here.

EXPERIMENTAL PROCEDURES

Standards and Reagents. Pure sugars and polyhydric alcohol standards were obtained from local supply houses. STOX, oxime internal standard reagent, and *N*-(trimethylsilyl)imidazole were obtained from Pierce Chemical Co. (Rockford, IL). ABH, the postcolumn derivatization agent, was obtained from Aldrich Chemical Co. (Milwaukee, WI).

Free Carbohydrate and Polyhydric Alcohol Analyses. *Apparatus.* A Pierce derivatization system, consisting of a Reacti-Therm heating module, a Reacti-Block, and 3-mL Reacti-Vials, was used for preparation of volatile derivatives. Samples were analyzed by GC on a Vista 6000 instrument (Varian, Palo Alto, CA) equipped with a 30-m DB-17 fused silica megabore column, 1.0-μm film thickness (J&W Scientific, Folsom, CA). Helium was used as the carrier gas (2 mL/min) and detector make-up gas (20 mL/min). The column oven temperature program was 165–185 °C at 1 °C/min, 185–260 °C at 5 °C/min, and 260 °C isothermal for 10 min. GC peaks were integrated

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0021-8561/90/1438-1347$02.50/0