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Fractionation of Proteins from Low-Tannin Sorghum Grain

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The effects of various parameters on the extraction of the Osborne protein fractions from low-tannin sorghum grain were investigated with the objective of obtaining a protein fractionation procedure specifically optimized for sorghum. Methods were obtained for the quantitiative extraction of low molecular weight nitrogen (amino acids and peptides), albumins plus globulins, and prolamins. The protein fractionation procedure was applied to 16 locally (South African) grown sorghums and an International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) collection of 25 economically important sorghums. It was found that nearly 50% of the protein is in the form of prolamins and that the albumins plus globulins plus low molecular weight nitrogen make up nearly 25%. The figure of less than 30% for the residual glutelin fraction is much less than that found in previous investigations.

The fractionation of cereal seed proteins by the use of different solvents, based on the classical work of Osborne (1924), is still a most useful technique.

In sorghum it has been used for many purposes: Jones and Beckworth (1970) fractionated the proteins to determine their chemical composition. More recently this type of work has been extended to compare the composition of the proteins from different varieties of sorghum (Guiragossian et al., 1978; Neucere and Sumrell, 1979; Paulis and Wall, 1979). Fractionation has been used to explain the different responses of rats fed high- and low-tannin sorghum (Jambunathan and Mertz, 1973) and to determine which proteins are increased in high-lysine varieties (Jambunathan et al., 1975). It has shown which proteins are affected when sorghum grain is dehulled (Chibber et al., 1978) and micronized (Shiau and Yang, 1982) and which were rendered insoluble in high-tannin cultivars (Daiber and Taylor, 1982). The technique has also been used to investigate the changes in sorghum protein composition during seed development (Johari et al., 1981) and germination (Wu and Wall, 1980; Taylor, 1983).

Despite the importance of protein fractionation to sorghum research there has been no recent systematic investigation into the efficiency of the procedures used. This is in contrast to the situation with other cereals such as barley (Shewry et al., 1978) and maize (Landry and Moureaux, 1981). Workers have in general used adaptations of the method of Landry and Moureaux (1970) developed for maize. These authors introduce two important changes compared to previous methods of grain protein fractionation: the use of aqueous alcohol plus reducing agent after the aqueous alcohol extraction and a final extraction with basic buffer containing sodium dodecyl sulfate ($NaDodSO_4$) plus reducing agent. These changes resulted in much improved protein extraction. For example, Skotch et al., (1970), using a traditional-type procedure, only extracted an average of 31% of the protein in sorghum grain, whereas Jambunathan and Mertz (1973), using a Landry and Moureaux procedure, extracted an average of 89%.

However, the fact that procedures for fractionating sorghum proteins have not been more thoroughly investigated is surprising especially as it is known that kafirin (sorghum prolamin) is less soluble than zein, maize prolamin (Wall and Paulis, 1978). The difference is probably due to the more hydrophobic nature of kafirin.

In this study the effect of different parameters on the extraction of each protein group was investigated with the objective of obtaining a protein fractionation procedure specifically optimized for sorghum grain. The resulting procedure was then applied to a number of locally (South African) grown sorghums and to some from other parts of the world so that a general picture of sorghum seed protein

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composition could be obtained.

EXPERIMENTAL SECTION

Sorghum Sources. A collection of 25 economically important sorghum cultivars originating from eight countries, ISFQT—1981, was kindly supplied by the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India. Commercial sorghum cultivars were obtained from the Summer Grain Centre of the South African Department of Agriculture, Potchefstroom. Local examples of the cultivated races of sorghum were given by T. Arnold of the Botanical Research Institute, Pretoria.

For the investigations into a procedure for fractionating sorghum proteins, grain from two genetically similar commercial cultivars was used: Barnard Red (LP), 8.3% protein and 0.05% total polyphenols; Breytenbach Red (HP), 12.9% protein and 0.04% total polyphenols. These sorghums were chosen because they represent extremes of protein content and they were virtually polyphenol free. The presence of polyphenolic compounds in sorghum can adversely affect the quantitative extraction of proteins (Wall and Paulis, 1978; Daiber and Taylor, 1982).

Extraction of Albumins, Globulins, and Low Molecular Weight Nitrogen. Twenty-gram samples of whole grain were ground for two 30-s periods in a Janke and Kunkel beater-type mill. The resulting flour was used directly (without lipid extraction) in the protein fractionation procedure. Extraction was carried out in 50-mL screw-top centrifuge tubes, their contents mixed by magnetic stirring.

Five-gram samples of LP flour were extracted by stirring for 1-h periods with three successive 25- or 50-mL aliquots of 0.5, 0.75, 1.0, 1.25, or 1.50 M NaCl at 4 °C and then for 15 min with 25 or 50 mL of distilled water at 4 °C. After each extraction a clear supernatant was obtained by centrifugation at 16000*g* for 10 min. The supernatants from each method were combined.

Extraction of Prolamins. Residues obtained after extraction of flour for three 1-h periods with 25-mL aliquots of 1.25 M NaCl and for 15 min with 25 mL of distilled water of 4 °C were used in the prolamin extraction experiments.

Experiment 1. LP residues were extracted by stirring at room temperature (approximately 25 °C), 40 °C, or 60 °C with 25-mL aliquots of 60% (v/v) *tert*-butyl alcohol (*t*-BuOH) for three 30-min periods and thereafter with 60% *t*-BuOH plus 0.05% (w/v) dithiothreitol (DTT) for two 30-min periods, after the method of Wu and Wall (1980). In a variation on this method, residues were simply extracted with 60% *t*-BuOH plus 0.05% DTT for two 1-h periods and one 30-min period at these temperatures. The residues remaining after prolamin extraction were subjected to $NaDodSO_4$ electrophoresis to determine whether they were contaminated with prolamins.

Experiment 2. HP residues were extracted by stirring at room temperature, 40 °C, and 60 °C with 25-mL aliquots of 70% (v/v) isopropyl alcohol (*i*-PrOH) for three 30-min periods and then with 70% *i*-PrOH plus 0.6% (v/v) mercaptoethanol (ME) for two 30-min periods, after the method of Landry and Moureaux (1970) and Jambunathan and Mertz (1973). Residues were also extracted at these temperatures with 25-mL aliquots of 60% *t*-BuOH plus 0.05% DTT for two 1-h periods and then overnight. The residues resulting from these prolamin extractions were subjected to $NaDodSO_4$ electrophoresis to check for prolamin contamination.

Extraction of Glutelins. Residues obtained after extraction of LP flour for three 1-h periods with 25-mL aliquots of 1.25 M NaCl and then for 15 min with 25 mL of distilled water at 4 °C followed by extraction with 25-mL aliquots of 60% *t*-BuOH plus 0.05% DTT for two 1-h periods and then overnight at room temperature, were used in the glutelin extraction experiments. The residues were extracted at room temperature or at 90 °C for three 1-h periods with 25- or 50-mL aliquots of pH 10.0, borate buffer plus NaCl (Landry and Moureaux, 1970) containing various amounts of $NaDodSO_4$ and ME.

General Sorghum Protein Fractionation Procedure. Five grams of whole grain flour was stirred for three 1-h periods with 25-mL aliquots of 1.25 M NaCl at 4 °C and then for 15 min with distilled water to extract the albumins, globulins, and low molecular weight nitrogen (LMWN). The clear supernatants, obtained by centrifugation at 16000*g* for 10 min, were combined. The protein content of the resulting liquid was determined before the solution was dialyzed overnight against distilled water at 4 °C and then freeze-dried. The protein content of the freeze-dried powder, which contained the ablumins and globulins, was determined and the LMWN content of the flour calculated by subtraction.

The residue from the NaCl extractions was stirred for two 1-h periods and then overnight with 25-mL aliquots of 60% (v/v) *t*-BuOH plus 0.05% (w/v) DTT at room temperature to extract the prolamins. The combined prolamin supernatants were freeze-dried directly.

The residue from the NaCl and aqueous alcohol extractions was designated glutelins (Osborne, 1924; Wilson, 1981). To facilitate subsequent characterization of this fraction by electrophoresis, it was purified by using a modification of the method of Beckworth (1970). The glutelins were suspended in 100 mL of distilled water and the pH was adjusted to 5.5 with HCl. Twenty milligrams of α-amylase (*Bacillus subtilis*, Boehringer Mannheim) was added and incubation was carried out overnight at 35 °C. At the end of this period the glutelins were starch free as determined by iodine solution. The suspension was then centrifuged at 16000*g* for 10 min to remove the soluble α-amylase and sugars and the glutelin pellet was freeze-dried.

Analyses. Protein ($N \times 6.25$) was determined by the method of Thomas et al., (1967) after digestion with concentrated H_2SO_4 containing 20% (w/v) K_2SO_4 and 0.1% (w/v) SeO_2. Total polyphenols were measured by a modified Jerumanis method (Daiber, 1975) using tannic acid as the standard. $NaDodSO_4$ electrophoresis in gels containing 12.4% acrylamide was carried out as described by Shewry et al. (1977). Protein loading was 200 μg/track in 2.6 mm thick gels.

Statistical Methods. The protein compositions of the groups of sorghums were compared by means of a Kruskal-Wallis one-way analysis of variance procedure. Whenever this test established an overall difference at the 5% level of significance, pairwise comparisons of groups were conducted at a simultaneous 5% level by means of Mann–Whitney *U* tests (Siegel, 1956). Within groups, relationships between the various protein components were examined by means of classical (simple and multiple) linear regression procedures and partial correlation analyses.

RESULTS AND DISCUSSION

Derivation of Sorghum Protein Fractionation Procedure. When sequentially extracting sorghum proteins, most recent workers (Guiragossian et al., 1978; Paulis and Wall, 1979; Neucere and Sumrell, 1979) have first defatted the flour. However, there is considerable evidence from work with other cereals that this is not necessarily

Table I. Effect of Salt Concentration on Albumin plus Globulin and Low Molecular Weight Nitrogen Extraction (Grams of Protein Extracted per 100 Grams of Grain)

NaCl concn, M	salt to flour ratio 5:1	10:1
0.50	1.53	1.67
0.75	1.60	1.92
1.00	1.83	1.94
1.25	1.94	1.90
1.50	1.83	1.92

Table II. Effect of Temperature and a Reducing Agent on Prolamin Extraction from Low-Protein Sorghum (Grams of Protein Extracted per 100 Grams of Grain)

extraction temp, °C	solvent treatments: 60% t-BuOH	then 60% t-BuOH + DTT	total	60% t-BuOH + DTT
rt[a]	1.41	1.09	2.50	3.04
40	1.51	1.20	2.71	2.79
60	1.53	1.30	2.83	2.62

[a] rt = room temperature.

beneficial (Soave et al., 1977) and can even be harmful as protein solubility is reduced (Chung and Pomeranz, 1978; Miflin et al., 1980). For this reason it was decided to omit defatting in this study.

A decision was also made to employ NaCl as the first extractant to solubilize the albumin and globulin proteins and the LMWN (amino acids and peptides). LMWN was then removed from the extract by dialysis so that the albumin plus globulin and LMWN content of the grain could be calculated. Following dialysis the albumin and globulin proteins were not separated because in cereals, unlike legumes, both groups are mainly metabolic proteins (Wilson et al., 1981a). Therefore, although the separation of these two groups may be of academic interest, it has little physiological significance. Some workers with sorghum (Neucere and Sumrell, 1979; Wu and Wall, 1980) have first extracted with water and then with NaCl to solubilize what they have termed albumins and globulins, respectively. These descriptions are inaccurate since water also extracts the LMWN as well as the albumins. In addition, because of endogenous salt in the grain, some globulins are also extracted (Wilson et al., 1981a).

Table I shows the effect of salt concentration and solvent to flour ratio on the extraction of albumins plus globulins plus LMWN from LP sorghum. It can be seen that the amount of protein extracted increases with salt concentration up to an optimum of 1.25 M for a solvent to flour ratio of 5:1 and 1.00 M for a ratio of 10:1. A salt concentration of 0.5 M as employed by Landry and Moureaux (1970) with maize and subsequently by many workers with sorghum is suboptimal for sorghum. At the optimum salt concentrations it can be seen that there is no different in the amount of protein extracted at the two different solvent to flour ratios. Because it was more convenient to handle smaller volumes, 1.25 M NaCl at a solvent to flour ratio of 5:1 was chosen as the extractant for albumins plus globulins plus LMWN.

In the case of barley it has been stated that quantitative prolamin extraction required high temperature or exhaustive mixing and the presence of reducing agent in the alcoholic solvent (Shewry et al., 1977). Table II shows the effect of temperature and the addition of a reducing agent on prolamin extraction from sorghum using 60% t-BuOH (Jones and Beckworth, 1970). It can be seen that in the absence of a reducing agent only about 50% of the maximum amount of protein was extracted. The use of the reducing agent DTT plus 60% t-BuOH after extraction with 60% t-BuOH resulted in a 77–85% increase in protein extraction. Similarly, 60% t-BuOH plus DTT extracted 70–115% more protein than 60% t-BuOH on its own. Temperature did not greatly influence protein extraction. There was a slight increase with temperature when the sequential procedure was used and a small decrease with the single solvent 60% t-BuOH plus DTT. This lack of temperature dependance implies that the method of mixing used in this study was sufficiently exhaustive. $NaDodSO_4$ electrophoresis of the residues remaining after extraction with 60% t-BuOH followed by 60% t-BuOH plus DTT or with 60% t-BuOH plus DTT did not reveal any contamination by prolamins.

Table III. Effect of Different Solvents and Temperatures on Prolamin Extraction from High-Protein Sorghum (Grams of Protein Extracted per 100 Grams of Grain)

extraction temp, °C	solvent treatments: 70% i-PrOH	then 70% i-PrOH + ME	total	60% t-BuOH + DTT
rt[a]	1.30	3.61	4.91	6.89
40	4.28	1.75	6.03	5.60
60	5.41	0.22	5.63	5.36

[a] rt = room temperature.

A further observation from Table II is that at room temperature 60% t-BuOH plus 0.05% DTT extracted slightly more protein than was extracted by the Landry and Moureaux type sequential procedure of first aqueous alcohol and then aqueous alcohol plus reducing agent. This phenomenon was also noted by Shewery et al. (1978) working with barley. In sorghum the proteins extracted with the second solvent, aqueous alcohol plus reducing agent, have variously been termed alcohol soluble glutelins (Jambunathan et al., 1975), cross-linked kafirins (Guiragossian et al., 1978), and alcohol-soluble reduced glutelins (Paulis and Wall, 1979). However, amino acid analysis and electrophoresis have indicated that these proteins in sorghum, unlike those in maize, are identical with the prolamins (Paulis and Wall, 1979). Thus, separation of the alcohol-soluble proteins into two groups by the use of two solvents does not appear to be justified, especially in view of the fact that as much if not more protein was extracted by the single solvent, aqueous alcohol plus reducing agent.

The relative efficiency of prolamin extraction from HP sorghum at different temperatures of 60% t-BuOH plus 0.05% DTT and the solvent used by Jambunathan and Mertz (1973) and a number of subsequent workers, 70% i-PrOH and then 70% i-PrOH plus 0.6 ME, is compared in Table III. The inverse relationship between temperature and the amount of protein extracted by 60% t-BuOH plus DTT was again found. It is possible that this was due to the elevated temperatures oxidizing the DTT. Protein extraction by 70% i-PrOH was very temperature dependent. However, this was balanced by a corresponding decrease in the amount of protein extracted by 70% i-PrOH plus ME, the maximum total quantity of protein being extracted at 40 °C. This, however, was somewhat less than that extracted by 60% t-BuOH plus DTT at room temperature. $NaDodSO_4$ electrophoresis of the residue from this extraction showed that it was virtually free of contaminating prolamins. Therefore, 60% t-BuOH plus 0.05% DTT at room temperature was chosen for the general protein fractionation procedure. A further advantage of t-BuOH is that the prolamin extracts can be

Table IV. Effect of Different Temperatures and $NaDodSO_4$ plus ME Concentrations on Glutelin Extraction from Low Protein Sorghum (Grams of Protein per 100 Grams of Grain)

% $NaDodSO_4$ and ME	extraction temp, °C	solvent to flour ratio	glutelins extracted	residue
0.1% $NaDodSO_4$	rt[a]	10:1	0.47	1.76
0.6% ME	90	10:1	0.56	1.67
0.5% $NaDodSO_4$	rt	5:1	0.75	1.48
0.6% ME	rt	10:1	0.77	1.46
	90	10:1	1.13	1.10
1.2% $NaDodSO_4$	rt	5:1	0.59	1.64
2.0% ME	90	5:1	1.19	1.04

[a] rt = room temperature.

freeze-dried without prior dialysis due to its high melting point.

Glutelins are defined as including those proteins that are either soluble in dilute aqueous alkali or insoluble in neutral aqueous solutions, saline solutions, or alcohol (Osborne, 1924; Wilson, 1981).

The glutelins were first treated as alkali-soluble proteins. Preliminary work (data not presented revealed that as in the case of the prolamins a single solvent, pH 10, borate buffer plus $NaDodSO_4$ plus ME, extracted as much proteins as the Landry and Moureaux (1970) procedure of an initial pH 10 buffer extraction followed by buffer plus $NaDodSO_4$ plus ME. Table IV shows that there was no significant difference in the amount of protein extracted by the two solvent to flour ratios, 10:1 or 5:1. Further, increased concentrations of $NaDodSO_4$ at room temperature did not greatly affect the amount of protein extracted. However, increased concentrations of $NaDodSO_4$ and ME in conjunction with an elevated temperature resulted in considerably more protein being extracted. Nevertheless, even with the best procedure, pH 10, borate buffer containing 1.2% $NaDodSO_4$ plus 2% ME at 90 °C, only 1.19 g of protein was extracted and 1.04 g or some 12.5% of the protein in the grain remained unsolubilized. It is possible that this protein was not extracted because it was linked to cell walls (Wall and Paulis, 1978). Glennie (1984) isolated cell walls from sorghum endosperm. These were rich in protein. Electron microscopy indicated that the protein was what is termed matrix protein, which in sorghum has been found to be mostly glutelin (Seckinger and Wolf, 1973).

Consideration of the alternative definition of glutelins that they are saline and alcohol-insoluble proteins (Osborne, 1924; Wilson, 1981) revealed that the extraction of 1.19 g only represented some 53% of the total glutelin fraction. This low percentage was not deemed adequately representative, especially if the glutelins are to be characterized by amino acid composition or electrophoresis. It was, therefore, decided to treat all the protein remaining after saline and aqueous *t*-BuOH extraction as glutelins. A problem resulting from this decision is that the glutelin fraction contained on average only 3.1% protein, which was too low to enable it to be characterized by electrophoresis. The fraction is, however, rich in starch and Beckworth (1970) purified glutelin from sorghum flour by the use of α-amylase. The starch was first dispersed by the use of dimethyl sulfoxide. At the concentration of α-amylase used by Beckworth (1970) neither this method, gelatinization at 75 °C, nor no pretreatment of the starch resulted in a starch-free preparation. With all treatments it was necessary to raise the amount of α-amylase to 20 mg to free the prepartion of starch. As dispersion with dimethyl sulfoxide did not have any advantage over no pretreatment, the latter procedure was adopted. Removal of starch increased the protein concentration of the glutelin fraction from LP sorghum to 20%. The mean protein concentration of all the glutelins prepared during this study was 26.5%. This value is rather lower than that obtained by Paulis et al. (1969) and Beckworth (1970) and more αmylase was required. The probable reason for these differences is that these workers used endosperm flour and not milled whole grain. Nevertheless, the method described in this study permits satisfactory electrophoresis of the sorghum glutelin fraction (Taylor, 1983).

General Protein Composition of Sorghum Grain. A total of 41 sorghums originating from eight countries and representing all the five cultivated races of *Sorghum bicolor* ssp. *bicolor* [bicolor, candatum, durra, guinea, and kafir (Harlan and de Wet, 1972)] were subjected to the protein fractionation procedure. Of these, three were high-tannin cultivars that were either extracted in the presence of polyvinylpolypyrrolidone (Loomis, 1974) or pretreated with dilute formaldehyde solution (Daiber and Taylor, 1982) to prevent tannin-protein interactions. As these treatments are probably not totally effective, the results from these cultivars were not subjected to statistical analysis.

Table V (a) shows that the average protein content of the sorghums was 11.1 ± 1.7% (SD). There was no significant difference in protein content ($\rho < 0.3$) between the three groups examined: ICRISAT sorghums [Table V b)], South African commercial sorghums [Table V (c)], and South African noncommercial sorghums [Table V (d), Deosthale et al. (1970) examined 332 varieties of sorghum from the World Genetic Stock and found that 62% fell in the range of 11–13% protein with a mean of 11.73%.

LMWN accounts for an average of 6.6 ± 2.5% (SD) of the total protein in the 38 sorghum cultivars analyzed. This value may be a slight overestimate because some amino acids and peptides could be formed as a result of limited proteolytic activity during the 4 °C salt extractions. LMWN has not previously been quantified in sorghum, but figures of 4.6 and 6.3% of total protein have been reported for maize (Wall and Paulis, 1978). The ICRISAT sorghums contained significantly lower levels of LMWN and significantly higher levels of albumins and globulins than either the commercial or noncommercial South African sorghums ($\rho < 0.005$ in both cases). In fact, at a simultaneous 5% level the two groups of South African soghums did not differ significantly with respect to any of the protein components and for statistical analysis were considered as a single group. It is possible that the higher albumin plus globulin and lower LMWN contents of the ICRISAT group compared to the South African sorghums were a result of genetic differences. It is, however, more likely that these differences were due to environmental factors. The ICRISAT sorghums were produced together under similar conditions, whereas the South African Sorghums were produced under widely differing conditions.

If LMWN and albumin plus globulin proteins are considered together, this salt-soluble protein fraction accounted for an average of 23.0 ± 2.7% (SD) of the total protein in 38 cultivars analyzed. This figure is similar to that obtained by Neucere and Sumrell (1979), who used a water extraction followed by a salt extraction. Jambunathan and Mertz (1973) obtained a much lower value of 14.3%. However, these authors employed 0.5 M NaCl,

Table V. Protein Composition of Sorghums

cultivar or sample and race	protein content, g/100 g	LMWN, g/100 g	% of total protein	albumins + globulins, g/100 g	% of total protein	prolamins, g/100 g	% of total protein	glutelins, g/100 g	% of total protein	% recovery
(a) Average Protein Composition of Sorghums										
	11.1	0.73	6.6	1.82	16.4	5.40	48.0	2.71	27.7	95.6
SD	1.7	0.29	2.5	0.54	4.1	1.19	5.0	0.50	4.3	2.1
(b) Protein Composition of ICRISAT Sorghums										
M 35-1	9.9	0.65	6.6	2.02	20.4	4.43	44.8	2.74	27.7	99
CSH-5	9.8	0.65	6.6	1.81	18.5	4.42	45.1	2.28	23.3	94
M 50009	10.4	0.54	5.2	1.83	17.6	5.01	48.2	2.32	22.3	94
M 50013	11.0	0.55	5.0	1.93	17.6	5.54	50.4	2.31	21.0	94
M 35052	10.9	0.52	4.8	2.35	21.6	4.53	41.6	2.87	26.3	94
M 50297	11.6	0.55	4.7	2.20	19.0	6.16	53.1	2.56	22.1	99
P 721	13.9	1.06	7.6	2.57	18.5	5.21	37.5	4.48	32.2	96
CO-4	9.0	0.68	7.6	1.81	20.1	3.73	41.4	2.47	27.4	97
Patcha-Jonna	12.8	0.69	5.4	2.56	20.0	6.06	47.3	3.21	25.1	98
Mothi	11.3	0.50	4.4	2.15	19.0	5.32	47.1	3.16	28.0	98
E 35-1	13.0	0.73	5.6	2.30	17.7	6.57	50.5	2.85	21.9	96
IS-158	9.4	0.38	4.0	1.93	20.5	4.12	43.8	2.58	27.5	96
WS 1297	10.1	0.51	5.0	1.95	19.3	4.41	43.7	2.72	26.9	95
Swarna	10.0	0.47	4.7	2.21	22.1	4.44	44.4	2.54	25.4	97
S-29	13.0	0.59	4.5	3.02	23.2	6.61	50.9	1.99	15.3	94
S-13	13.2	0.55	4.2	3.29	24.9	6.31	47.8	2.50	18.9	96
IS-2317	10.3	0.33	3.2	1.81	17.6	5.31	51.6	2.64	25.6	98
IS-7035	10.6	0.42	4.0	2.08	19.6	5.28	49.3	2.30	21.7	95
IS-7055[a]	13.4	1.04	7.8	2.74	20.5	7.06	52.7	2.24	16.7	98
IS-9985	11.5	0.70	8.8	2.03	17.7	6.06	53.2	2.13	18.5	95
IS-8743	11.9	0.74	6.2	1.68	14.1	5.82	48.9	3.28	27.6	97
Dobbs[a]	12.1	1.07	8.8	2.70	22.3	4.94	40.8	2.91	24.1	96
CS 3541	12.6	1.05	8.3	1.75	13.9	5.48	43.5	3.88	30.8	97
Segaolane	13.0	0.69	5.3	1.82	14.0	7.54	58.0	2.36	18.2	95
Market-1	13.9	0.68	4.9	2.28	16.4	7.62	54.8	2.89	20.8	97
mean	11.4	0.62	5.4	2.15	18.8	5.48	47.7	2.74	24.1	96
SD	1.5	0.18	1.3	0.40	2.8	1.04	4.8	0.57	4.3	2
(c) Protein Composition of South African Commercial Sorghums										
PNR 8311	12.0	1.86	15.5	1.52	12.7	5.98	50.0	3.23	27.0	97
NK 283	9.3	0.72	7.7	1.17	12.6	4.02	43.3	2.73	29.5	93
G 766 W	9.0	0.64	7.1	1.28	14.2	3.79	42.1	2.69	29.9	93
Barnard Red	8.3	0.64	7.7	1.30	15.7	3.39	41.0	2.20	26.6	91
Breytenbach Red	12.9	0.72	5.6	1.68	13.0	6.89	53.4	2.85	22.1	94
SSK 52[b]	12.2	0.56	4.6	1.28	10.5	5.58	45.9	4.28	35.2	96
mean	10.3	0.72	7.1	1.39	13.7	4.81	45.9	2.74	27.0	94
SD	2.0	0.09	0.9	0.21	1.3	1.53	5.4	0.37	3.1	2
(d) Protein Composition of South African Noncommercial Sorghums										
56 Kafir	10.9	0.81	7.4	1.43	14.4	5.19	47.7	2.73	25.1	93
94 Kafir	12.3	1.80	14.6	0.66	5.4	7.01	57.0	2.36	19.2	96
135 Kafir	7.1	0.41	5.8	1.19	16.8	3.49	49.1	1.78	25.1	97
161 Guinea	11.9	0.88	7.4	1.73	14.8	7.05	59.2	2.41	20.2	102
213 Kafir	10.8	1.46	13.5	1.04	9.6	5.14	47.6	2.48	23.0	93
249 Caudatum	9.6	1.22	12.7	1.10	13.9	4.09	42.6	3.13	32.6	99
1552 Guinea	13.8	0.92	6.7	1.57	11.4	7.60	55.1	3.14	22.7	96
1706 Durra	8.8	0.86	9.8	1.10	13.2	3.72	42.2	2.90	32.9	97
1733 Kafir	10.9	0.84	7.7	1.50	13.8	5.02	46.1	2.32	21.3	89
1758 Bicolor	11.6	0.77	6.6	1.45	12.5	5.61	48.4	2.81	24.2	92
mean	10.8	1.00	9.3	1.29	12.2	5.40	49.6	2.62	24.7	96
SD	1.9	0.39	3.2	0.32	3.1	1.38	5.1	0.42	4.7	2

[a] High-tannin sorghums, protein extracted in the presence of polyvinylpolypyrrolidone. [b] High-tannin sorghum grain pretreated with formaldehyde.

which is suboptimal for sorghum (table I). In sorghum the salt-soluble protein fraction is richest in lysine, the first limiting amino acid of cereals (Guiragossian et al., 1978). Therefore, sorghums that are rich in this fraction are probably nutritionally superior to other types. The ICRISAT sorghum S-13 [Table V (b)], a zerazera derivative, had the highest content of salt-soluble protein and among the highest percentage with respect to total protein.

Prolamins, the major storage proteins of sorghum (Taylor, 1983), were the most abundant protein fraction, being 48.0 ± 5.0% (SD) of the grain protein [Table V (a)]. This figure is of the same order as that obtained by Paulis and Wall (1979), who used 60% *t*-BuOH followed by 60% *t*-BuOH plus ME as the extractant. It is, however, much higher than that found by Jambunathan and Mertz (1973) using the less efficient procedure of extraction with 70% *i*-PrOH followed by 70% *i*-PrOH plus ME at room temperature (Table III). It is also much higher than the value found by Neucere and Sumrell (1979), who employed the single solvent, 60% *t*-BuOH plus 0.1 M guanidine hydrochloride.

In both the ICRISAT and the South African sorghums partial correlation analysis showed that at a constant protein content increased prolamin was associated with

decreased glutelin ($\rho < 0.001$) and vice versa. Also, in the South African group, regression analysis showed that the percentage contribution of prolamin to protein content increased significantly with total protein content ($\rho < 0.001$). This was at the cost of a decreased percentage of mainly glutelin ($\rho < 0.02$) and to a lesser extent albumin plus globulin ($\rho = 0.052$). In fact, the increase in prolamin per unit total protein content (2.16) was virtually fully balanced by the corresponding decrease in albumins plus globulins plus glutelins per unit of total protein, 0.74 + 1.42 = 2.16. In the case of the ICRISAT sorghums increased total protein content resulted in the absolute amounts of all the protein fractions being significantly increased. Increases in the amounts of the four fractions were also observed with the South African sorghums, although only the prolamins were increased significantly.

The glutelin fraction accounted on average for 24.7 ± 4.3% (SD) of the total grain protein (Table V (a)]. This figure for these salt- and alcohol-insoluble proteins is much lower than that obtained by other workers with sorghum (Jambunathan and Mertz, 1973; Neucere and Sumrell, 1979; Paulis and Wall, 1979). These authors obtained values of the order of 40% or more of the total grain protein. It is probable that such high values for cereal glutelins are a reflection of incomplete extraction of the other protein fractions (Wilson et al., 1981b).

The ICRISAT and South African sorghums did not differ significantly with respect to the recovery of the total protein content in terms of the four fractions ($\rho > 0.05$). In both groups the average recovery was about 96%. Regression and partial correlation analyses showed that the errors in recovery were most consistently correlated with prolamin and glutelin contents. It is likely that most of the protein loss was from the glutelin fraction as its preparation involved several steps, where losses could occur. In contrast, the prolamins were freeze-dried directly.

The use of the protein fractionation procedure described in this paper has resulted in a much more accurate picture of sorghum grain protein composition, in terms of LMWN, albumins plus globulins, prolamins, and glutelins, than was previously available. Work is now being carried out to characterize these fractions from a range of different sorghums.

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