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Extractability, fractionation and nutritional value of low and high tannin sorghum proteins

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Kafirins and glutelins were found to be the major, while albumins and globulins were the minor components of sorghum proteins. Marked differences were noticed in both amino acid composition and electrophoretic properties among the sorghum protein fractions. Albumins and globulins were more rich in most of essential amino acids than the other sorghum protein fractions. Dehulling of sorghum grain led to a marked reduction in tannins, and slight reduction in total protein. Low tannin sorghum, Giza 15 variety, had relatively high levels of protein extraction efficiency, albumins, globulins and kafirins, slightly low levels of glutelins and the same amino acids as the high tannin sorghum SX 121 variety.

INTRODUCTION

Sorghum proteins like other cereal proteins, can be classified according to solubility, into albumins (water-soluble proteins), globulins (salt-soluble proteins), gliadins 'Kafirins' (alcohol-soluble proteins), and glutelins (dilute alkali-soluble proteins) (Osborne, 1924; Landry and Moureaux, 1970). Osborne's method extracted only 26% to 40% with an average of 31% of sorghum total proteins (Roch *et al.*, 1970). This low solubilization was as a result of the insoluble complex formed by proteins binding with polyphenols (Schröder, 1973). Therefore, Landry and Moureaux (1970) suggested the use of aqueous alcohol plus reducing agent after an aqueous alcohol extraction and a final extraction with basic buffer containing reducing and detergent agents. According to Jambunathan and Mertz (1973) and Taylor *et al.* (1984), aqueous butanol was more effective than ethanol in extraction of kafirins. Also, addition of reducing agents, such as mercaptoethanol (ME), dithiothreitol (DTT), and sodium dodecyl sulphate (SDS) as detergent increased the solubilizing of kafirins, glutelins and the yield of total protein extraction to 89–96.5%.

In this work, differences in the solubility, PAGE electrophoretic pattern, and amino acids of the different protein fractions, of low and high tannin sorghum varieties, were estimated. Also, the effects of dehulling on the tannin content, protein content, protein extractability and IVPD were studied.

MATERIALS AND METHODS

Materials

Two sorghum varieties (*Sorghum vulgare*), one high in tannins ('SX 121') and the other low in tannins ('Giza 15') were used in this study. Some of the whole sorghum grains were dehulled using a vertical shelling machine type '270' F.H. Schüle GMBH (Youssef *et al.*, 1988). Whole and dehulled grains were ground to pass through a 60 mesh sieve using a Junkel Kunkel electric blender type A.10. The ground samples were defatted by shaking with petroleum ether (40–60°C) at a ratio of 1:10 w/v for 30 min at room temp. The defatted flours were desolventized in an air oven at 60°C.

Methods

Tannins, total proteins and IVPD

Tannin content was determined in the ground defatted samples of whole and dehulled sorghum in methanol extracts according to the method of Price *et al.* (1978). Moisture and total crude protein (N×6.25) were determined in the same above samples as described in AOAC (1990). The method of Akeson and Stahmann (1964) was followed to estimate the *in vitro* protein digestibility (IVPD) in the same previous samples.

Protein fractionation

The method of Landry and Moureaux (1970), with little modification, was followed to fractionate sorghum

proteins as illustrated in Fig. 1. The extraction conditions for each of the sorghum protein fractions were: one hour stirring followed by centrifugation at 16 000 g for 10 min. The defatted sorghum flour and its centrifuged residues were subjected to successive extraction with the following extractants:

1. Distilled water to extract the albumins.
2. 5% NaCl to extract the globulins.
3. 60% tertiary butanol (60 t-BuOH) containing 0.1% guanidine hydrochloride (GH) to extract the kafirins.
4. 60% t-BuOH containing 1% GH and 0.6% merceptoethanol (ME) to extract the cross-linked kafirins 'alcohol-soluble reduced glutelins' (ASRG).
5. Borate buffer (pH 10) containing 0.6 M ME and 0.5 M sodium dodecyl sulphate 'SDS' to extract the alcohol-insoluble reduced glutelins 'AIRG').

Except for the albumin factions, the supernatants containing the other protein fractions were dialyzed against distilled water before lyophilization. The obtained fractions were weighed to estimate the efficiency of extraction.

Electrophoretic properties

SDS polyacrylamide gel (10%) electrophoresis of the obtained protein fractions was carried out according to Stegemann *et al.* (1987) using the PANTA-PHOR apparatus (Labour Muller, D-53 10 Hann Munden. W. Germany) at 30 V for 20 h running, cooling at 32°C and ~80–100 μl extract. Staining and destaining were carried out according to Stegemann *et al.* (1987) using Coomassie brilliant blue R250 for staining and a solution of methanol:acetic acid:water (2:3:5, v/v/v) for destaining. Electrophoretic relative mobility (ERM) for each band was estimated and the intensity of the bands was evaluated visually.

Amino acid analysis

Amino acids were determined in total sorghum protein and each protein fraction after hydrolysing for 24 h with 6 N HCl at 110°C using a Kontron Anocomp 500 amino acid analyzer (Moore, 1958). The obtained data were computed automatically and expressed as g amino acid per 100 g protein.

RESULTS AND DISCUSSION

Tannins

Table 1 shows the level of tannins in the ground defatted whole and dehulled (low and high tannins) sorghum varieties. The results indicate that the mechanical dehulling caused ~96% and 88% reduction in tannin content of high (SX 121) and low (Giza 15) tannin sorghum varieties, respectively. This was an indication that most of the polyphenols (tannins) in sorghum grain were located in outer layers of the grain. According to Chibber *et al.* (1978), up to 98% of sorghum tannins were removed at 64% dehulling yield.

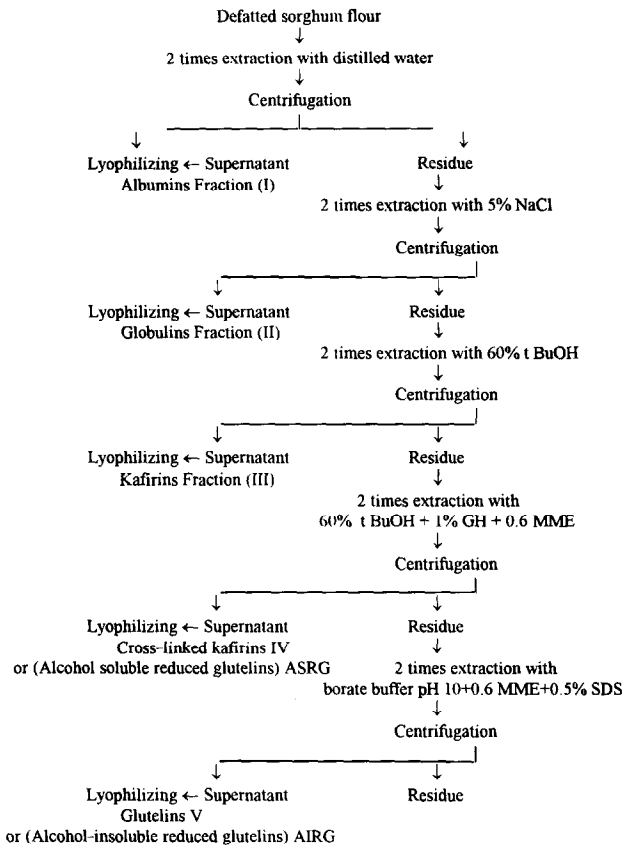

Fig. 1. Diagram for extracting proteins from defatted sorghum grain flour.

Proteins

Extractability

Total protein content and it's fractions in low and high in tannins sorghum varieties are shown in Table 1. The data in this table indicate that:

1. Dehulling reduced 7.5 and 8.3% of the total proteins of both SX 121 and Giza 15 sorghum varieties, respectively. This means that the end outer layers of sorghum grain contained protein. According to Hubbard *et al.* (1950), the protein contents in bran and germ were 5–8 and 18–19%, respectively.
2. Sorghum protein fractions can be arranged according to their concentrations in the following decreasing order, in both low and high tannin varieties, kafirins 'Fraction III' (36.2–55.1%), ASRG 'Fraction IV' (18.8–24.5%), globulins 'Fraction II' (10.9–13.7%), albumins 'Fraction I' (4.2–7%) and AIRG 'Fraction V' (3.18–4.2%), respectively. Heikerwal and Mathieson (1971) fractionated sorghum proteins into 30% kafirins,

Table 1. Tannins, total protein content and protein extracts of whole and dehulled grain of sorghum[a]

Components	Sorghum Grain Variety			
	SX 121		Giza 15	
	Whole	Dehulled[b]	Whole	Dehulled[c]
Tannins[d]	2.90	0.10	0.018	0.002
Total proteins (N×6.25)	11.9	11.0	13.30	12.2
Protein extract[e]				
Albumins	4.20	6.36	6.99	5.82
Globulins	10.9	13.3	13.7	12.3
Kafirins	36.2	57.5	55.1	57.0
ASRG[f]	24.5	19.1	18.8	19.8
AIRG[g]	4.20	1.90	3.18	3.21
Protein extraction efficiency	80.1	98.0	97.7	98.1
Protein digestibility[h]	42.6	73.2	70.2	86.4

[a]On dry weight basis.
[b]Dehulling yield was 75%.
[c]Dehulling yield was 84.4%.
[d]As catechin equivalent (g 100 g^{-1}) sample.
[e]% from total protein.
[f]ASRG Alcohol-soluble reduced glutelins.
[g]AIRG Alcohol-insoluble reduced glutelins.
[h]*In vitro* protein digestibility.

20% globulins, 8% albumins, 12% glutelins and 30% bound gelled glutelins. Meanwhile, Ivanceko *et al.* (1972) found that sorghum protein consisted of 42.5% kafirins, 23.2% glutelins, 2.95% albumins and 2.7% globulins.

3. Proteins of low tannin sorghum variety, Giza 15 are composed of relatively higher levels of albumins, globulins, kafirins and slightly lower levels of ASRG and AIRC fractions than those of high tannins ones ('SX 121'). These results confirmed the formation of complexes between albumins, globulins and kafirins with tannins (Taylor *et al.*, 1984). According to Chibber *et al.* (1973), the complex compounds between tannins and albumins on globulins were responsible for the low protein extractability of proteins from high tannin sorghum varieties.
4. Dehulling of low tannin sorghum, Giza 15, caused a slight reduction in albumins and globulins and a slight increase in other protein fractions. In contrast, dehulling of high tannin sorghum variety 'SX 121' caused an increase in albumin, globulin and kafirin fractions and a decrease in the other two fractions, ASRG and AIRG. This is as indication that the last two protein fractions, ASRG and AIRG were located in the outer parts of endosperm of the high tannin sorghum variety, SX 121. The results of Jambunathan and Mertz (1973) indicated that the dehulling of high tannin sorghum varieties increased the globulin extraction. According to Chibber *et al.* (1978), albumins and globulins were largely associated with the external portion of the sorghum, while glutelins were uniformly distributed and kafirins were more concentrated in an interior part.
5. The protein extraction efficiency was about 80% from whole SX 121 variety and 97.7% from Giza 15 type. This amount increased to nearly 98% in both varieties after dehulling. These results agree well with those reported by Wall and Paulis (1978) and Daiber and Taylor (1982). Also, it confirmed that most of the tannins in sorghum were in the outer layers of the grain. According to Chibber *et al.* (1980), the amounts of extracted proteins from low and high tannin sorghum varieties were quite similar after dehulling.

Fractionation

Using SDS-PAGE technique to fractionate the extracted sorghum proteins gave the following results (Fig. 2):

1. Albumins of protein of Giza 15 and SX 121 varieties were fractionated into 13 to 15 bands, respectively. Eleven of these fractionated bands were identical. Two of these bands with 0.7 and 0.73 ERM were found in high intensity. According to Zubaidov (1968*a*), the albumin fraction of eight sorghum varieties were fractionated by the SDS-PAGE technique into five similar fractions. Two of these fractions migrated to the positive pole and were found as major components. The others migrated to the negative pole and were found as minor components.
2. SX 121 and Giza 15 globulins were fractionated by the SDS-PAGE technique into 10 and 12 bands, respectively. Most of these bands were identical.

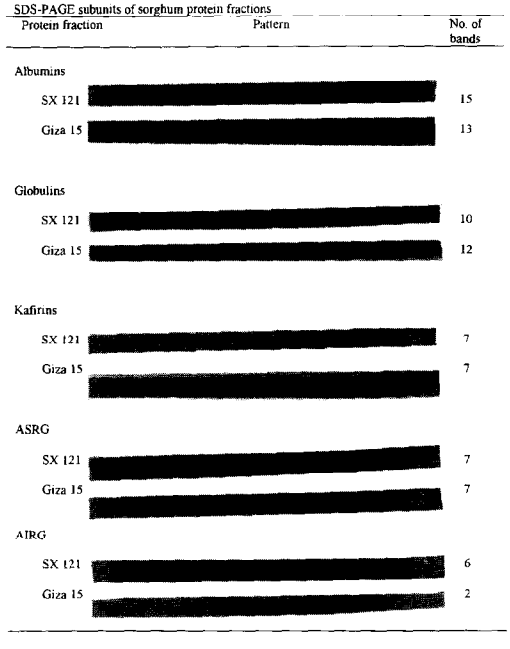

Fig. 2. SDS-PAGE subunits of sorghum protein fractions.

Results of Zubaidov (1968*b*) showed differences in the SDS-PAGE globulin fractions among sorghum varieties.

3. Both kafirins and ASRG or cross-linked kafirins of two sorghum varieties were fractionated by SDS-PAGE into seven identical bands with high concentration.
4. In contrast, AIRG was fractionated into six bands in the SX 121 sorghum variety and two bands in the Giza 15 variety.

Kilmenko and Zubaidov (1971) showed that glutelin proteins of grain sorghum consisted of smaller protein units joined together through disulfide linkages. Mazhar and Chandrashekar (1995) concluded that content and distribution of kafirins within sorghum kernels varies in grains with different endosperm hardness.

Nutritional value

IVPD

As shown in Table 1, IVPD of SX 121 and Giza 15 sorghum varieties was increased by 71.8% and 23% after the dehulling process, respectively. Schaffert *et al.* (1974) and Hulse *et al.* (1980) observed a high significant negative correlation between tannin content and decrease *in vitro* protein digestibility. Chibber *et al.* (1980) observed that stepwise removal of tannins by sequential dehulling increased the percentage of IVPD of high-tannin sorghum. Results of Oria *et al.* (1995) revealed that when untreated sorghum flour was incubated with pepsin, 31.0% of α kafirin, 15.3% of β kafirin and 13.5% of γ kafirin remained undigested.

Amino acids

The data in Tables 2 and 3 indicated that:

1. Total sorghum protein of both SX 121 and Giza 15 sorghum varieties was poor in most essential amino acids 'EAA', especially sulphur-containing amino acids, lysine and threonine, in addition to histidine and arginine. Glutamic acid, phenyl alanine, proline and leucine were found in highest amounts among the amino acids of total sorghum protein. Generally, the EAA of total protein can be arranged according to their concentration in the following decreasing order: leucine, phenylalanine, valine, isoleucine, arginine, tyrosine, threonine, lysine, histidine and methionine. These results agree with those reported by Chakravorty (1967) and Neucere and Surmell (1979).
2. Leucine/isoleucine ratio was 3.6 in total protein of both varieties. According to Hulse *et al.* (1980) and Johari *et al.* (1977), a leucine/isoleucine ratio higher than 3/1 should be regarded as potentially deleterious. The high level of leucine in sorghum was responsible for niacin deficiency. It alters the activity of some enzymes involved in the conversion of tryptophan to nicotinic acid (Gopalon and Kamalos, 1975).
3. Marked differences were noticed in amino acid content among the protein fractions. Generally, albumin was rich in lysine, threonine and glycine compared with other protein fractions. Globulins contained the highest level of valine and arginine. Among the other protein fractions, kafirin had the largest amount of leucine, glutamic acid, proline and alanine, and lowest value of sulphur-containing amino acids, lysine, arginine, glycine and histidine. The other two protein fractions, ASRG and AIRG, were more balanced in their levels of amino acids compared with the other two frac-

Table 2. Amino acid composition of high tannin sorghum protein and its fractions

Amino acid	Total protein	Albumins	Globulins	Kafirins	ASRG	AIRG
Essential						
Lysine	1.90	5.83	4.92	0.39	2.95	3.60
Methionine	1.40	1.97	1.62	1.20	1.40	1.70
Threonine	2.71	4.99	3.12	2.10	4.35	4.10
Tyrosine	3.22	3.25	3.22	4.40	7.80	6.50
Valine	4.49	5.28	6.96	4.20	5.10	5.11
Ph. alanine	4.33	4.74	4.81	5.10	2.90	4.50
Leucine	13.0	4.55	4.63	15.3	13.4	10.4
Isoleucine	3.56	3.75	3.19	3.80	3.10	3.80
Histidine	1.75	3.92	4.12	0.82	5.00	3.40
Arginine	3.10	7.37	10.53	1.03	4.20	5.80
Non-essential						
Aspartic acid	4.82	10.99	9.33	6.20	6.10	8.20
Glutamic acid	23.6	14.4	13.2	30.8	19.5	13.8
Serine	4.63	4.11	5.62	2.90	4.10	4.60
Proline	7.05	4.50	3.92	10.21	9.10	8.30
Glycine	3.94	5.31	4.95	1.30	3.90	5.20
Alanine	9.80	6.84	5.32	8.50	6.50	7.60
Ammonia	1.80	2.20	2.31	2.10	1.80	2.10

Table 3. Amino acid composition of low tannin sorghum protein and its fractions

Amino acid	Total protein	Albumins	Globulins	Kafirins	ASRG	AIRG
Essential						
Lysine	2.11	5.23	4.55	0.32	3.10	4.60
Methionine	1.36	2.05	1.89	1.30	0.92	1.70
Threonine	3.00	5.70	4.12	2.42	4.75	4.20
Tyrosine	3.43	4.20	3.55	4.21	3.11	5.80
Valine	4.55	5.32	6.55	3.50	5.11	6.40
Ph. alanine	5.62	5.10	4.95	4.00	4.61	4.40
Leucine	13.2	5.72	5.08	16.0	12.5	9.70
Isoleucine	3.65	3.90	3.47	3.80	4.07	3.70
Histidine	1.82	3.32	4.10	0.72	3.32	3.50
Arginine	3.55	6.77	9.32	1.13	4.51	5.10
Non-essential						
Aspartic acid	5.60	12.22	10.31	6.80	8.21	8.50
Glutamic acid	22.0	15.1	13.00	28.6	18.2	13.9
Serine	4.46	4.50	5.08	3.70	4.32	4.50
Proline	7.12	4.10	4.95	10.50	8.72	7.81
Glycine	2.80	6.70	4.92	1.60	3.81	4.86
Alanine	9.62	8.50	6.31	9.10	6.50	7.70
Ammonia	1.86	2.40	2.53	1.90	1.50	2.30

tions. No great variations were observed in amino acid composition between the protein fractions of the two sorghum varieties. Skoch *et al.* (1970) studied the amino acid compositions of protein fractions of sorghum and found smaller differences in the amino acid composition between protein fractions.

4. The leucine/isoleucine ratio was 1.2–1.46, 1.45–1.46, 4.0–4.2, 3.07–4.3 and 2.4–7.0 in albumins, globulins, kafirins, ASRG and AIRG sorghum protein fractions, respectively.

These results indicated that the amino acids of sorghum protein were not evenly distributed among the protein fractions. Generally, albumins and globulins were rich in the limited amino acids of sorghum, lysine, sulphur-containing amino acids and threonine, and had a low ratio of leucine/isoleucine. Kafirin, the major protein of sorghum, was poor in most of the EAA and had the highest ratio of leucine/isoleucine. ASRG protein, the second major protein in sorghum protein had the same problems as kafirin but with less intensity.

In conclusion, these results will help researchers in breeding or genetic engineering to develop new varieties rich in albumins and globulin protein fractions to overcome the nutritional problem of sorghum utilization. Also, these results confirm the importance of dehulling of sorghum before using for an edible purpose. This process increases its protein extractability and improves its IVPD.

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