

Characterization of seed storage proteins of urdbean (*Vigna mungo*)

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Abstract. Dehulled and defatted flour of urdbean (*Vigna mungo*), Var T-9, contained 25% protein with maximum contribution by globulins (63%). Albumins and glutelins contributed 12% and 21% respectively, whereas prolamins were present only in traces (1%). Globulins were further fractionated into legumin and vicilin type proteins which were present in the ratio of 4:1. All the protein fractions were heterogenous in nature as revealed by high performance liquid chromatography. SDS-polyacrylamide gel electrophoresis revealed the total protein sample to contain 21 different components with molecular weights ranging from 8.92 to 117.49 kd. Albumins, globulins, prolamins and glutelins resolved into 4, 8, 6 and 13 different sized components of molecular weights ranging from 10.23 to 25.53, 10.84 to 112.72, 10.33 to 51.52 and 8.91 to 112.72 kd, respectively. Amino acid analysis of all fractions revealed that glutamic acid was present in maximum concentration followed by aspartic acid and lysine. Just like other pulse proteins, the urdbean proteins were also deficient in sulphur containing amino acids.

Introduction

Storage proteins of seeds are an important group of proteins, which together with the reserves of carbohydrates and oils, are synthesized during seed development. These proteins being an integral component of human nutrition have gained added significance, particularly for vegetarian populations where pulses constitute the main source of dietary proteins. Since these proteins are tissue specific and present only in cotyledons, epicotyles and hypocotyles; they offer an attractive system for the study of differential gene expression. The pioneer work of Osborne and Campbell [1] extended by Dainelsson [2] showed that salt soluble globulins which account for about 70% of the total grain N in pulses could be further fractionated into 7 S vicilin type and 11 S legumin type proteins. Since then a large number of pulse crops have been studied for the characterization of seed storage

proteins [3, 4]. Here we report some of the characteristics of urdbean (*Vigna mungo*, commonly known as black gram) proteins, as little information is available on the storage proteins of this crop.

Material and methods

Seed material

Soaked seeds of T-9 variety of urdbean, after hand removal of testa, were dried at 70 °C for 24 h and milled to pass through a 40-mesh sieve. The flour was defatted with n-hexane, dried at 70 °C for 78 h and stored at room temperature in airtight polythene bottles.

Total protein and protein fractions

The crude protein in the defatted flour was estimated by the conventional microkjeldhal method as described in AOAC [5] ($N \times 6.25$). Total proteins were fractionated by the method of Naik [6] as modified by Singh et al. [7]. The defatted flour was dissolved in 0.5 M NaCl, shaken for about 2 h and then centrifuged. The procedure was repeated three times and the pooled supernatant then dialysed against water for 72 h. Albumins were present in the supernatant and globulins were obtained in the precipitate. Prolamins and glutelins were extracted according to Naik [6].

Globulins were further fractionated into legumin and vicilin by employing the method of Osborne [8]. Globulins were dissolved in 0.5 M NaCl and dialysed against 0.2 M NaCl for 72 h. Precipitates and supernatant represented the legumin and vicilin fractions, respectively.

High performance liquid chromatography (HPLC)

A Shimadzu HPLC model LC-4A was used for the HPLC of protein fractions. The separation was obtained using HSG-30W gel permeation column. The proteins were eluted at a flow rate of 1 ml/min using 0.05 M phosphate buffer (pH 6.5) containing 0.5 M NaCl and 0.02% sodium azide. The elution profile was recorded using a SPD-2 ASW detector and computed by a CR2AX data system.

SDS polyacrylamide gel electrophoresis (SDS-PAGE)

Electrophoresis in the presence of SDS was performed by the method described in Sigma Chemical Bulletin No. 8777-L [9].

Table 1. Total protein and distribution of protein in different protein fractions of urdbean

S. no.	Fraction	Protein* (g/100 g flour)	Protein (g/100 g total protein)
1.	Total Protein	25.38 $\pm$ 0.44	
2.	Albumin	3.06 $\pm$ 0.38	12.06
3.	Globulin (Total)	15.94 $\pm$ 0.44	62.81
	(i) Legumin	12.75 $\pm$ 0.25	50.24
	(ii) Vicilin	3.13 $\pm$ 0.31	12.33
4.	Prolamin	0.25 $\pm$ 0.44	1.00
5.	Glutelin	5.31 $\pm$ 0.56	20.92
6.	Residue	0.44 $\pm$ 0.19	1.73

* Mean $\pm$ S.E.

Mean represents the average of six observations. % recovery of protein was 96.79%.

Amino acid analysis

Ten mg protein was hydrolysed under nitrogen in sealed tubes using 10 ml of 6 N HCl at 110 °C for 24 h. The hydrolysate was made acid free in vacuum at 40 °C. The final sample (10 ml), in 0.2 M citrate buffer (pH 2.2), was decolourized by activated charcoal and filtered. Analysis was carried out on a Beckman multichrom B liquid column chromatograph 4255, using three buffers (pH 3.49, 4.12 and 6.4) in the elution system. Column temperature was maintained at 54 °C with a flow rate of 70 ml/h.

Results and discussion

Table 1 includes data on total and different protein fractions of urdbean. Globulins were the major storage proteins in urdbean accounting for about 63% of the total proteins. Albumins and glutelins contributed about 12% and 21%, respectively, whereas only 1% of the total protein was contributed by the prolamin fraction. The protein recovery during the fractionation was 97%. These results are in good agreement with those obtained for chickpea [7, 10].

Legumin content was four times higher (80% of the total globulins) than the vicilin content (20% of the total globulins). This compares very well with species like *larthyrus* and *Vicia* [2, 12] where legumin and vicilin are present in the ratio of 4:1. On the contrary, 7S vicilin was greater as compared to 11S legumin in *Phaseolus* [13, 14].

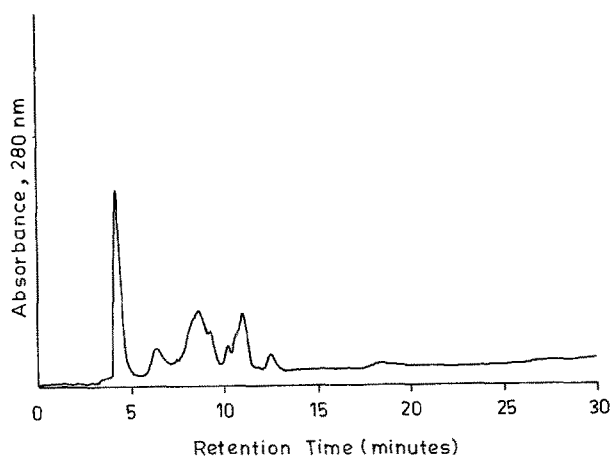


Fig. 1. HPLC profile of albumins of urdbean.

High performance liquid chromatography of various protein fractions

Albumin, globulin, prolamin and glutelin on HPLC resolved into seven, five, fifteen and four different peaks respectively (Figs. 1–4) suggesting that all of the fractions were heterogenous in nature. Prolamin fraction showed the maximum heterogeneity. Bietz [15] used this technique to characterize the heterogeneity of different protein classes of wheat and suggested that this offers an effective tool in identifying different cultivars.

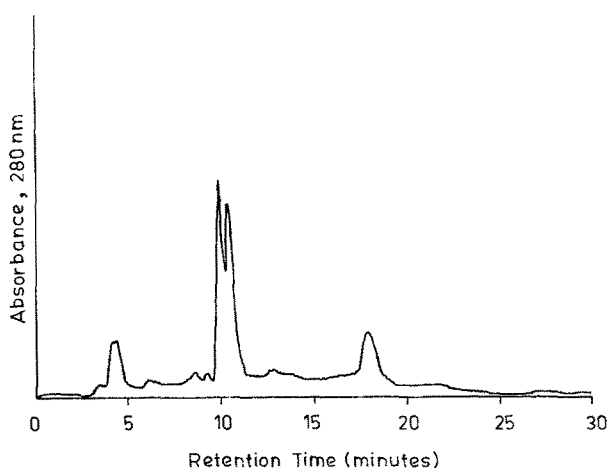


Fig. 2. HPLC profile of globulins of urdbean.

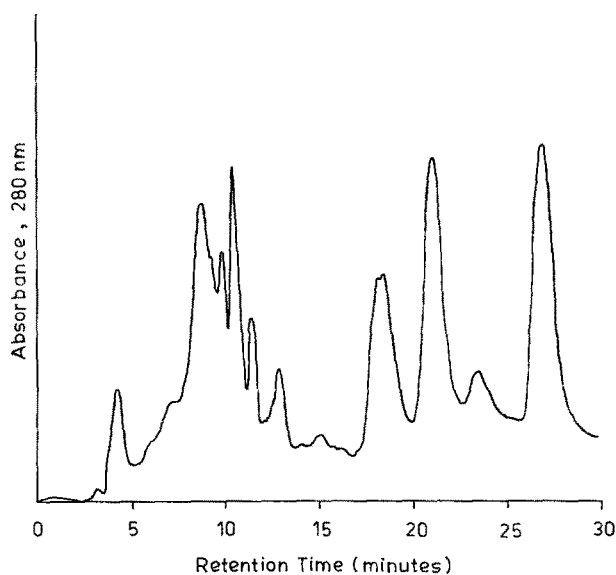


Fig. 3. HPLC profile of prolamins of urdbean.

SDS polyacrylamide gel electrophoresis

Total proteins of urdbean resolved into 21 different bands of varying molecular weight ranging from 8.92 to 117.49 kd (Plate 1), indicating that both low and high molecular weight components were present. Analyses of the

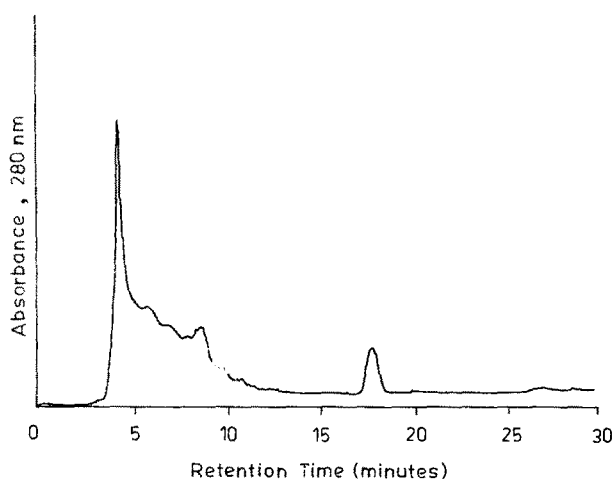


Fig. 4. HPLC profile of glutelins of urdbean.

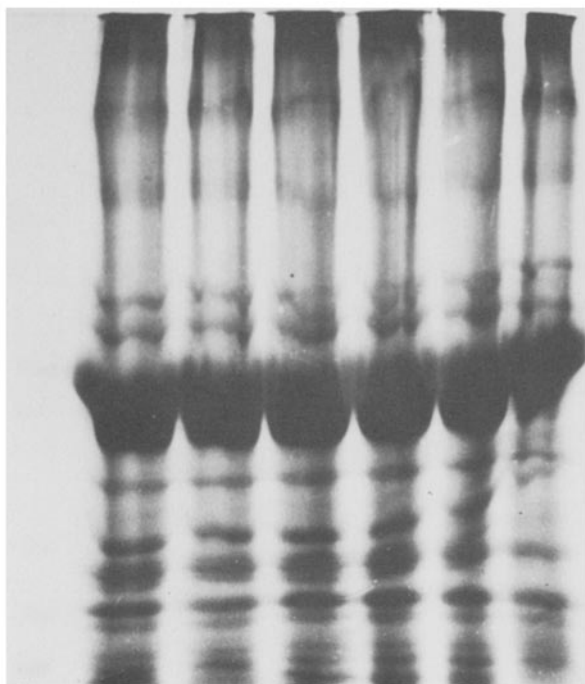


Plate 1. SDS-polyacrylamide gel electrophoregram of urdbean total proteins. The sample was run in six replicates.

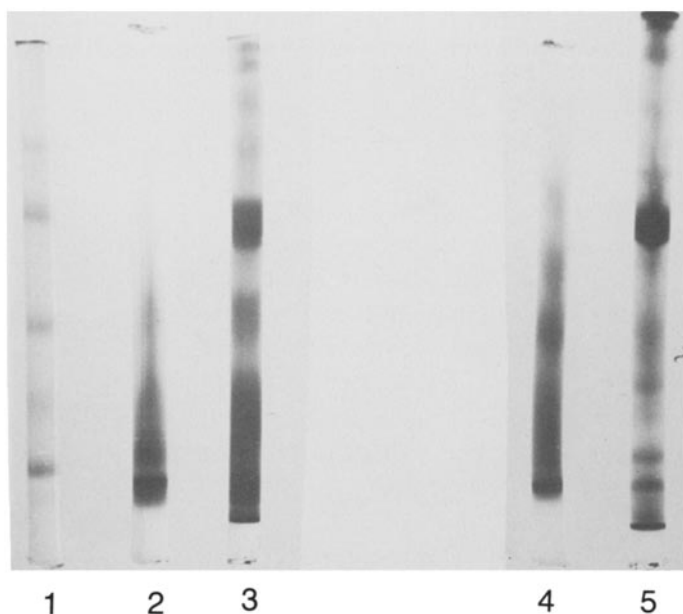


Plate 2. SDS-polyacrylamide gel electrophoregram of various fractions of urdbean. 1, standard marker proteins; 2, albumins; 3, globulins; 4, prolamins; 5, glutelins.

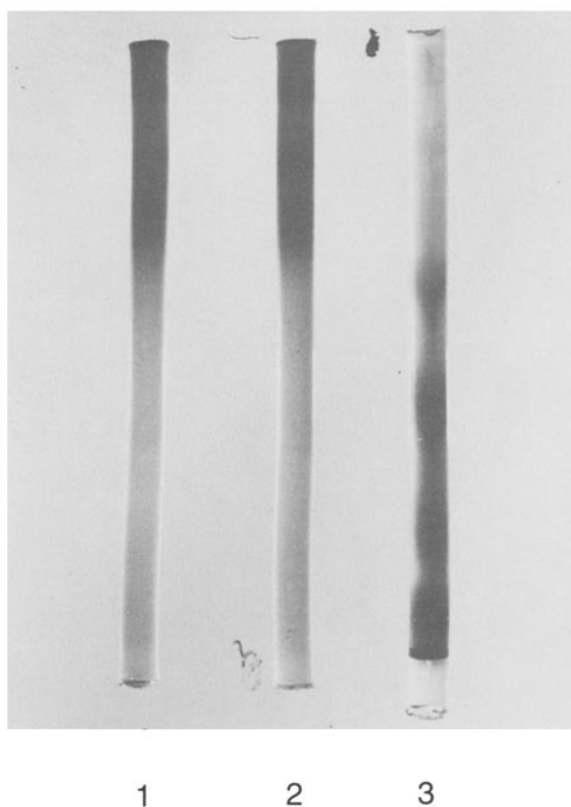


Plate 3. Polyacrylamide gel electrophoregram of legumin subfraction of urdbean. 1 and 2 (in duplicate), without SDS; 3, with SDS.

total protein sample without SDS treatment did not give satisfactory separation, suggesting that these subunits were cross linked.

The electrophoretic patterns of different protein classes viz. albumin, globulin, prolamin and glutelin are presented in Plate 2. Albumins could be separated into four different subunits of molecular weight 10.23, 12.82, 16.03 and 25.53 kd showing that this fraction of urdbean is comprised of low molecular weight components. Castorbean, cotton, lupin, soybean, mungbean, broad bean and pea have also been reported to contain low molecular weight subunits in their albumin fraction [16–18]. Globulins, prolamins and glutelins separated into eight, six and thirteen different size components of molecular weight ranging from 10.84 to 112.72 kd, 10.33 to 51.52 kd and 8.91 to 112.72 kd, respectively.

The legumin subfraction of globulin resolved into three different bands when it was not treated with SDS but after treatment with SDS, the legumin fraction was resolved into seven different bands of molecular weights ranging from 10.91 to 108.14 kd (Plate 3). The vicilin subfraction, similarly gave

Table 2. Amino acid composition (g/100 g flour) of different protein classes of urdbean

Amino acid	Total Protein	Albumin	Globulin	Glutelin
Aspartic acid	2.79 (11.00)	0.28 (9.04)	1.87 (11.75)	0.50 (9.45)
Threonine	0.42 (1.65)	0.07 (2.35)	0.10 (0.61)	0.04 (0.95)
Glutamic acid	5.64 (22.21)	0.47 (15.31)	2.85 (17.86)	0.90 (18.96)
Serine	0.38 (1.49)	0.04 (1.34)	0.27 (1.72)	0.02 (0.45)
Proline	2.09 (8.23)	0.40 (12.89)	1.24 (7.78)	0.34 (6.41)
Glycine	1.38 (5.43)	0.53 (17.32)	0.51 (3.19)	0.12 (2.22)
Alanine	0.88 (3.45)	0.58 (18.95)	0.33 (2.05)	0.30 (5.63)
Valine	0.48 (1.90)	0.05 (1.64)	0.27 (1.67)	0.10 (1.87)
Cysteine	Traces (-)	0.19 (6.26)	N.D. (-)	Traces (-)
Methionine	0.44 (1.74)	0.01 (0.18)	ND (.)	0.01 (0.10)
Isoleucine	1.03 (4.05)	0.03 (0.81)	0.30 (1.87)	0.17 (3.23)
Leucine	2.33 (9.16)	0.13 (4.07)	0.70 (4.37)	0.41 (7.69)
Tyrosine	0.69 (2.72)	0.08 (2.43)	0.51 (3.19)	0.09 (1.59)
Phenylalanine	0.38 (1.51)	0.01 (0.18)	0.02 (0.20)	0.22 (4.20)
Lysine	2.04 (8.03)	0.17 (5.55)	1.72 (10.80)	0.25 (4.90)
Histidine	1.61 (6.33)	0.17 (5.55)	1.05 (6.58)	0.34 (6.30)
Arginine	0.62 (2.42)	0.02 (0.71)	0.20 (1.26)	0.42 (7.95)
Acidic	8.43 (33.21)	0.75 (24.35)	4.72 (29.61)	1.40 (28.41)
Basic	4.27 (16.78)	0.36 (11.81)	2.97 (18.64)	1.01 (19.15)

Table 2. (Continued)

Amino acid	Total Protein	Albumin	Globulin	Glutelin
Hydrophobic	7.63 (30.04)	1.21 (38.72)	2.86 (17.94)	1.55 (29.13)
Polar (uncharged)	2.87 (11.29)	0.91 (29.43)	1.39 (8.71)	0.27 (5.21)

N.D. = Not detectable.

Values in parenthesis are g/100 g protein in the individual fraction.

only a single band when it was electrophoresed without being treated with SDS, and after its dissociation with SDS, it was resolved into five different bands of molecular weights ranging from 10.84 to 41.98 kd (Plate 4). Legumin contained components of high molecular weights, whereas low molecular weight subunits were the major components of the vicilin subfraction.



Plate 4. Polyacrylamide gel electrophoregram of vicilin subfraction of urdbean. 1, without SDS; 2 with SDS.

SDS-PAGE has been used to study the molecular weights of different subunits of protein classes in pea [19], soybean [20] and *Phaseolus vulgaris* [21].

Amino acid analysis

Data on amino acid content in total as well as in different protein fractions of urdbean have been included in Table 2. The prolamin fraction, being low in amount, could not be analysed for its amino acid composition. Glutamic acid was present in maximum concentration (22.21% of the total protein) followed by aspartic acid and lysine. Each of the fractions was distinct with respect to its amino acid composition. Meaningful comparisons between the fractions can, however, be made in terms of the proportions of different classes of amino acids. Amongst fractions, albumins contained the highest amount of hydrophobic (38.72%) and polar uncharged (29.43%) amino acids, whereas the contents of basic (11.81%) and acidic (24.35%) amino acids were the lowest. The higher proportion of glutamic acid and aspartic acid characterized the black gram globulins. Black gram glutelins were rich in basic amino acids. Padhye and Salunkhe [22], however, have observed that black gram glutelins are poor in basic amino acids.

There were three compounds of unknown identities in the aminograms for total proteins. The same proportions of these unidentified compounds were present in glutelin hydrolysate while they were absent in albumin and globulin fractions.

Urdbean proteins, just like other pulse proteins, were found to be deficient in sulphur containing amino acids and rich in lysine. This deficiency in sulphur amino acids is due to the higher amounts of globulins and glutelins which constitute the major components of urdbean protein. Cysteine was not detectable, as this amino acid is mostly destroyed by acid hydrolysis.

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