EXTRACTION, FRACTIONATION AND CHARACTERIZATION OF COWPEA SEEDS (VIGNA UNGUICULATA) ALBUMINS

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Summary

The cowpea total albumins were separated from salt extractable proteins by prolonged dialysis against distilled water. The total albumins were separated into four components by chromatography on Sephadex G-100 column. The molecular weight was determined to be 153 000, 100 000, 56 000 and 18 000 for components A, B, C and D respectively. Spectrophotometry was used for characterizing the albumin components. The amino acid composition indicated the presence of higher levels of histidine, proline and some of the essential amino acids, in the albumin component (A) than that usually found in the other albumin components.

Introduction

The seed proteins were classified by Osborne (1924) into albumins, globulins, glutelins and prolamines, on the basis of solubility criteria.

Globulins usually constitute the major portion of legume seed proteins with different species showing various amounts. However, the albumins have more favourable amino acid profile (Bajaj et al., 1971; Boulter and Derbyshire, 1971; Hirch et al., 1977) than the globulins and are quantitatively significant, contributing 20–40% of the extractable cotyledonary proteins. It has been suggested that strains of pea with higher protein efficiency ratios have a higher albumin content (Bajaj et al., 1971).

The total extractable proteins of the seeds of several cowpea varieties were analysed by Tella and Ojehomon (1980). They reported the presence of equal amounts of albumins and globulins. These two proteins were present in greater amounts than glutelins and prolamines which also occurred in almost equal amounts.

The globulins of a number of legume species have been investigated (Baily and Boulter, 1972; Derbyshire et al., 1976; Krishna et al., 1977; Casey, 1979;

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Chavan and Djurtof, 1982). In contrast, only few studies have been performed on the albumins of legume seeds (Sefa-Dedeh and Stanley, 1979; Khan et al., 1980; Bhatty, 1982).

The object of the work reported in this paper was the fractionation and characterization of the albumin components of cowpea seeds cultivated in Egypt.

Materials and methods

Cowpea seeds (*Vigna unguiculata* variety Blackeye) were obtained from the experimental Farm of Faculty of Agriculture, El-Minya University, El-Minya, Egypt. The defatted meal was prepared by treating the ground seeds with ice cold acetone at 4 °C.

Extraction and separation of total albumins

The proteins were extracted from the defatted meal by stirring with 50 mM phosphate buffer, pH 7.8 containing 0.5 *M* NaCl and 10 mM mercaptoethanol, for 8 h at 4 °C. After centrifugation at 15 000 r.p.m. for 20 min, the clear supernatant was dialyzed against several changes of distilled water for 72 h at 4 °C. The protein which precipitated (globulin) was collected by centrifugation and the clear supernatant was freeze-dehydrated to obtain the total albumins.

Gel filtration chromatography

Chromatography on Sephadex G-100 column (2.5 × 86 cm) equilibrated with 50 mM phosphate buffer pH 7.0, was used for separating the total albumin components. The column was eluted with the same buffer and fractions of 5 cm^3 were collected at a flow rate of 12 cm^3/h. Absorbance at 280 nm and the protein content using the method of Lowry et al. (1951) were determined for each fraction.

Molecular weight determination

The method of Andrews (1965) was used for estimating the molecular weight of the albumin components. The void volume (Vo) of the Sephadex G-100 column was determined by Blue Dextran 2000 and the column was calibrated using literature values for reference proteins (cytochrome C M.Wt. 12 600, trypsin M.Wt. 23 400, ovalbumin M.Wt. 45 000, bovine serum albumin M.Wt. 68 000 and aldolase M.Wt. 158 000).

Polyacrylamide gel electrophoresis

The method of Davis (1964) was employed to examine the number of albumin components.

Ultraviolet absorption spectrum

The spectrum for each protein component was recorded on Sp 18000 double beam recording spectrophotometer in the eluting buffer.

Amino acid analyses

Amino acid determinations were carried out on acid hydrolyzates by paper chromatography using three different solvents:

1. Phenol:2-butanol:0.067 *M* phosphate buffer, pH 12 (95:5:100 V/V) (Leavy and Chung, 1953), for the separation of aspartic and glutamic acid, serine, glycine, threonine and alanine.
2. n-Butanol: acetic acid: water (4:1:5 V/V) Block et al. 1955), for the separation of cysteine, lysine, histidine and arginine.
3. n-Butanol: acetic acid: water (4:1:1 V/V) (Roland and Gross, 1954), for the separation of tyrosine, methionine, valine, phenylalanine, leucine and isoleucine.

The tryptophan content was determined in the alkaline hydrolysate by the method of Blauth et al. (1963).

Results and discussion

Gel filtration chromatography of cowpea total albumins

The chromatography of cowpea albumins on Sephadex G-100 column had been conducted as a means of separating the albumin components. The elution profile is shown in Fig. 1. It is evident that the total albumins contained at least four components coded A–D in the order of their elution from the Sephadex G-100 column.

The results of protein determination using the method of Lowry et al. (1951) indicated that the albumin component (B) contained more protein than component (A). Also, component (D) contained a smaller amount of protein than component (C) (Fig. 1).

Determination of the molecular weight of the cowpea albumin components was performed by gel filtration chromatography on calibrated Sephadex

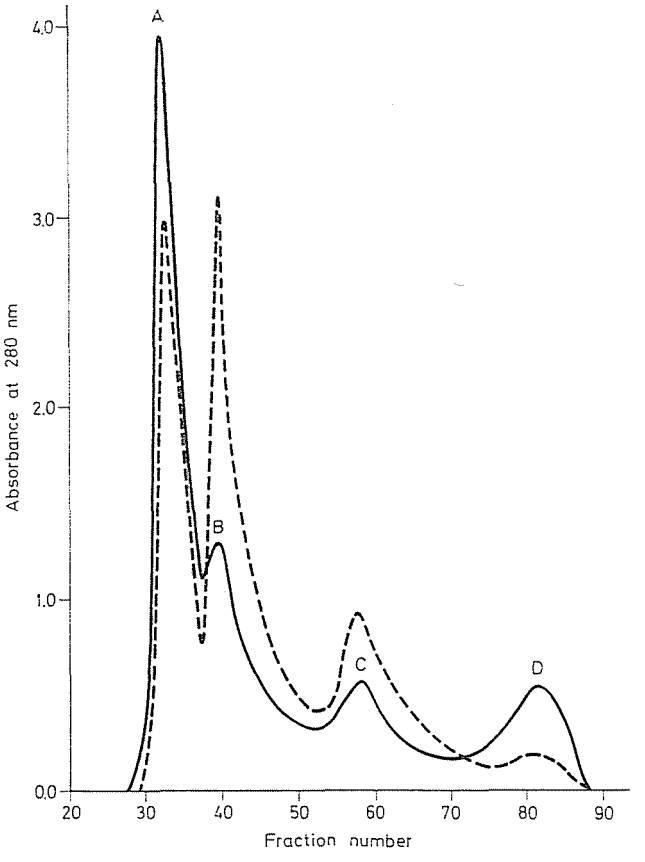

Fig. 1. Fractionation of total albumins on Sephadex G-100 column

G-100 column. The values obtained were: 153 000; 100 000; 56 000 and 18 000 for components A, B, C and D respectively. Two albumin components were separated by Khan et al. (1980) when the cowpea total proteins extractable by phosphate buffer (Salina) were chromatographed on a column of Sephadex G-200. They reported that the major albumin was a monomer of a molecular weight of 105 000, other albumins were present in significant amounts with subunits of molecular size of 32 000 and 22 500. Further lower molecular weight albumin components were found in lesser amounts (Khan et al 1980).

The separation of albumins into different molecular weight components prompted us to consider the properties of the albumin components separately. The ultraviolet absorption spectra of the components separated from the Sephadex G-100 column are shown in Fig. 2. Components B and C showed typical protein spectra with a maximum at 279 nm and a minimum at 250 nm. The ratio of absorbance at 280 nm to that at 260 nm was 1.71 for component

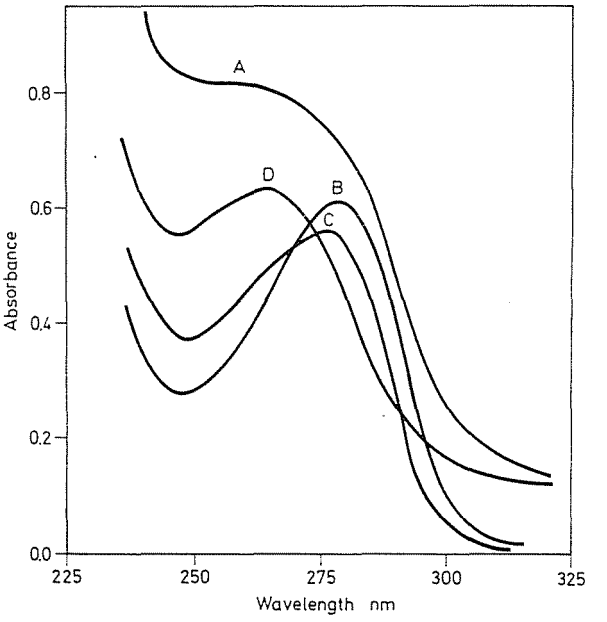

Fig. 2. Ultraviolet absorption spectra of albumin component obtained from Sephadex G-100 column

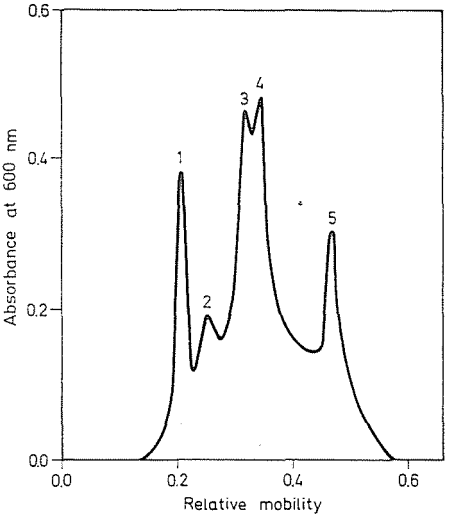

Fig. 3. Densitometric scanning of polyacrylamide gel electrophoretic patterns of total albumins

(B), indicating that this component was free of nucleic acids. On the other hand, component (C) with ratio (A_{280}/A_{260}) of 1.26 indicated the contamination with nucleic acids. The ultraviolet spectrophotometric analysis (Fig. 2), demonstrated that the maximum absorption of the albumin component (A) was between 275 and 260 nm which pointed to the contamination with nucleic acids, furthermore, as found for field bean water extractable proteins (El Morsi 1982), the eluent containing the albumin component (A) was turbid and it has been suggested (Obara and Kimura, 1967; Morita and Yoshida, 1968) that this peak (A) (Fig. 1) could be a denaturated product of some other protein components.

The cowpea total albumins were examined by polyacrylamide gel electrophoresis and the results of densitometric scanning of the polyacrylamide gel are presented in Fig. 3. Five protein bands, designated 1, 2, 3, 4 and 5, with relative mobilites of 0.21, 0.25, 0.30, 0.35, 0.47 respectively, were detected. Sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE) examination of cowpea total albumins revealed the presence of several subunits with molecular weights of 105 000, 32 000, 22 500 and lower molecular weights (Khan et al., 1980).

Amino acid analyses

The amino acid profiles of some of the albumin components (component A, B and C), separated by the Sephadex G-100 column, and the total albumins are summarized in Table 1. The predominant amino acids, in all the protein components, were glutamic and aspartic acids. In addition, substantial amounts of leucine + isoleucine were recorded.

Results in Table 1 indicate that the total albumins was characterized by the highest proportions of tyrosine and threonine.

Among the three albumin components, higher levels of histidine, proline and the essential amino acids methionine, valine, threonine and leucine plus isoleucine, were present in the albumin component (B) which endow this component with better nutritive quality.

It is evident from Table (1) that the highest amount of cystine was recorded in the albumin component (A), whereas the albumin component (B) contained more methionine. In addition, the total amount of the sulphur containing amino acids was relatively higher in the albumin component (B) and it has been suggested by Johnson and Lay (1974) that, the protein components richer in sulphur amino acids may be used to improve the level of methionine and cystine in edible seed legumes, but Boulter et al. (1978) did not support this view.

The albumin component (C) contained higher proportions of arginine, serine, alanine and aspartic acid than the other proteins (Table 1). The highest

Table 1

The amino acid composition of cowpea total albumins and the components separated from Sephadex G-100 column (g amino acid/100 g protein)

Amino acid	Total albumins	Albumin component		
		A	B	C
Cystine	1,17	1,33	1,03	1,08
Lysine	5,69	6,31	5.11	5.53
Histidine	7.14	5.97	8.07	7.69
Arginine	5.47	7.58	5.62	8.17
Proline	1.32	1.21	1.59	0.48
Tyrosine	9.60	6.31	5.28	5.14
Methionine	1.34	0.77	1.65	1.20
Valine	2.96	3.37	4.94	3.61
Phenylalanine	7.31	8.51	5.94	5.53
Leucine + Isoleucine	8.07	9.07	9.37	8.83
Aspartic acid	13.96	12.76	14.02	14.33
Glutamic acid	12.23	14.21	13.51	11.32
Serine	4.79	4.75	5.68	6.01
Glycine	2.25	2.87	2.66	2.21
Threonine	6.25	4.53	4.88	4.45
Alanine	6.14	5.42	6.53	6.97
Tryptophan	0.13	0.38	N. D.	N. D.
Total amino acids	95.82	95.25	97.86	96.55

level of lysine was found in the albumin component (A) which is the first and sometimes the second limiting amino acid in cereals. It could be concluded from the amino acid analysis (Table 1) that our data were in some aspects similar to those found by other researchers (Evans and Boulter, 1974; Molina et al. 1976; Bhatty, 1982; Ologhobo and Fetuga, 1982).

The main differences were the lower contents of tryptophan, lysine and glycine and contents of histidine and tyrosine in the total albumins studied here compared with those reported by other workers.

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