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CHROMATOGRAPHIC SEPARATION AND PHYSICO-CHEMICAL STUDY OF LEGUMIN FROM CHICKPEA (*CICER ARIETINUM* L.)

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Total salt-soluble protein, extracted from chickpea cotyledon, was precipitated with ammonium sulphate into fractions 0.60 and 60-100%. Albumin was removed from fraction 60-100 by dialysis at 3-5° against H_2O, pH 4.1 and the globulin of fraction 60-100 was subjected to chromatographic isolation of legumin in homogeneous state. The physico-chemical properties of isolated legumin were studied by gradient extraction on celite column, chromatography on hydroxylapatite and DEAE-cellulose columns, gel electrophoresis, and ultracentrifugation. The chromato-electrophoretic behaviour and sedimentation curve showed its elution constants at 76% ammonium sulphate concentration, 0.26 μ NaCl and 0.40 M phosphate buffer on celite, DEAE-cellulose and hydroxylapatite columns respectively. The sedimentation coefficient was 11.72.S. Legumin was rich in arginine and tyrosin and poor in histidine and threonine.

INTRODUCTION

Chickpea seed is a valuable source of plant protein. It is 2-3 times richer in protein content than grain and due to rising prices and shortage of meat it has considerably replaced proteins of animal origin [1]. It contains between 12-31% protein, composed of a highly heterogeneous system of individual proteins consisting of globulin components of the primary and secondary type and albumin components distributed in various morphological parts of the seed [2]. The globulins form about 80% of total protein and enrich the cotyledon, whereas the testa is poor in protein content and the embryo contains albumins and low molecular-weight globulins associated with nucleic acids and carbohydrates [3].

The chromato-electrophoretic analysis of proteins of the chickpea seed has indicated that vicilin and legumin are the major components of the total cotyledon proteins, which could not be separated by gradient extractionon celite and chromatography on hydroxylapatite columns. It has also been observed that vicilin and legumin had their elution constants at 0.26 μ and 0.33 μ NaCl concentration of phosphate buffer on DEAE cellulose columns, but were contaminated with albumin [6].

The present investigation was, therefore, carried out to devise a simple procedure for the isolation of homogenous legumin from chickpea seed and to study its physico-chemical properties including the amino acid composition.

MATERIAL AND METHODS

Chickpea *(Cicer arietinum,* L.) seeds (variety Sowkhoz-14) were procured for experiments from Biological Station, Kishinev State University, USSR in 1975. The testa and embryo were removed. The cotyledons were ground into flour, defatted with ether and the salt soluble protein was extracted with 1 M NaCl, pH 7.0 phosphate buffer. The total salt-soluble protein extract was precipitated by adding the desired amount of crystalline ammonium sulphate [4, 8] into fractions 0.60 and 60-100%. Albumin was removed from fraction 60-100 by dialysis at 3-5° against distilled water (pH 4-1) [5, 9]. The globulin thus left in the fraction 60-100 was subjected to chromatographic separation. The experimente were conducted in a cold room.

The globulin of fraction 60-100 was chromatographed on DEAE-cellulose, hydroxylapatite and celite-545 analytical and preparative columns [7, 10, 11]. The salt concentration in the eluate was determined graphically [5], while the concentration of ammonium sulphate in each tube was determined refractrometrically [12]. The concentration of protein was determined spectrophotometrically in each tube based on absorption at 278 nm and was plotted on the chromatogram, whereas the nature of chromatographic fraction was determined on the basis of extinction correlation E-260/E-278 (nucleic acid: protein concentration ratio) at 260 nm and 278 nm respectively. The proteins of chromatographic fractions were analysed electro-phoretically [13, 14] using 7.5% polyacrylamide gel and *tris*-buffer, pH 8.3 [15].

Protein of 0.7% concentration in phosphate buffer, pH 7.2 and 0.3 μ NaCl concentration, was ultracentrifuged (Ultracentrifuge, MOM-G 120, Hungary) at 20° and 45640 r.p.m., while the sedimentation coefficient was determined as reported by Shpikiter [16]. For amino acid analysis, 10 mg protein was hydrolysed with 6 N HCl at 132° for 4 hr. and the amino acid values were determined as reported earlier [17].

RESULTS AND DISCUSSION

The results of chromatographic separation of legumin from protein fraction 60-100 of the chickpea seed are presented in Fig. 1 and for homogeneity test in Fig. 2. The amino acid composition of the isolated legumin is presented in Table 1.

Table 1. Amino acid composition of the legumin of chickpea seed (g/100 g of protein)

Amino acid	%	Amino acid	%
Lysine	3.85	Glycine	3.44
Histidine	1.77	Alanine	3.97
Arginine	7.90	Valine	4.68
Aspartic acid	16.34	Isoleucine	3.86
Threonine	2.12	Leucine	5.74
Serine	4.10	Tyrosine	6.59
Glutamic acid	18.87	Phenylalanine	7.02
Proline	4.68		

It was demonstrated in an earlier work that the fraction, precipitated between 60-100% concentration of ammonium sulphate from total salt-soluble protein extract of chickpea cotyledon, is rich in vicilin and legumin, and also contains a considerable amount of secondary globulin and albumin components [6].

Therefore the globulins of fraction 60-100 were separated on DEAE cellulose column into five chromatographic fractions labelled as 1,2, 0.26, 0.33 and 0.58 (Fig. 1A). Peaks 0.26 and 0.33 were dominated by vicilin and legumin respectively while peaks 1 and 2 were enriched with secondary globulins and peak 0.58 with nucleic acids. A similar chromatographic behaviour of vicilin and legumin of chickpea seed has also been observed by Kllmenko [3]. Subfraction 0.33, enriched with legumin, was pooled together for further analysis. Electrophoresis in polyacrylamide gel revealed that subfraction 0.33 was highly heterogeneous and possessed four electrophoretic components. Consequently this fraction was rechromatographed on DEAE collulose two more times with single band was contained in the polyacrylamide gel electrophoresis (Fig. 1). The major component was highly stained.

The protein of subfraction 0.33 after purification was tested for contaminants. The isolated protein was chromatographically pure on celite (Fig. 2/1), hydroxylapatite (Fig. 2/3) and DEAE-cellulose (Fig. 2/3) columns. Electrophoretically the protein was pure and the sedimentation diagram also attests to its homogeneity (Fig. 2/4). The chromato-electrophoretically pure protein had maximum elutions with 72% ammonium sulphate 0.40 M phosphate buffer on hydroxylapatite and 0.33 μ NaCl concentration

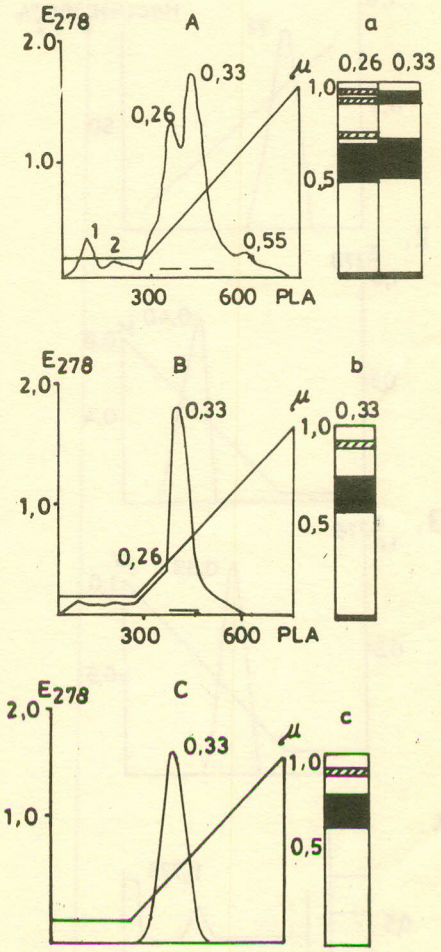

Fig. 1. Chromatographic separation and electrophoretic analysis of globulins of fraction 60-100 of chickpea seed.
A, a) Chromatography of fraction 60-100 on DEAE cellulose column and electrophoresis of subfractions 0.26 and 0.33 in polyacrylamide gel.
B, b) Rechromatography of subfraction 0.33 on DEAE cellulose and analysis of subfraction 0.33, pooled together after rechrasua tography, by gel electrophoresis.

C, c) Rechromatography of subfraction 0.33 and electrophoresis of subfraction 0.33.

of phosphate buffer on DEAE-collulose columns. The sedimentation coefficient was 11.72 S and in general was in agreement with the sedimentation coefficients of the legumins isolated by Grigorcha [4] and Lapteva [5] from chickpea and lentil seeds respectively. The isolated protein was, therefore, the legumin or 12 S component of chickpea seed. The procedure developed in this investigation for the isolation of legumin from chickpea seed is simple (Table 1) as compared to the more complicated purification schemes of the former.

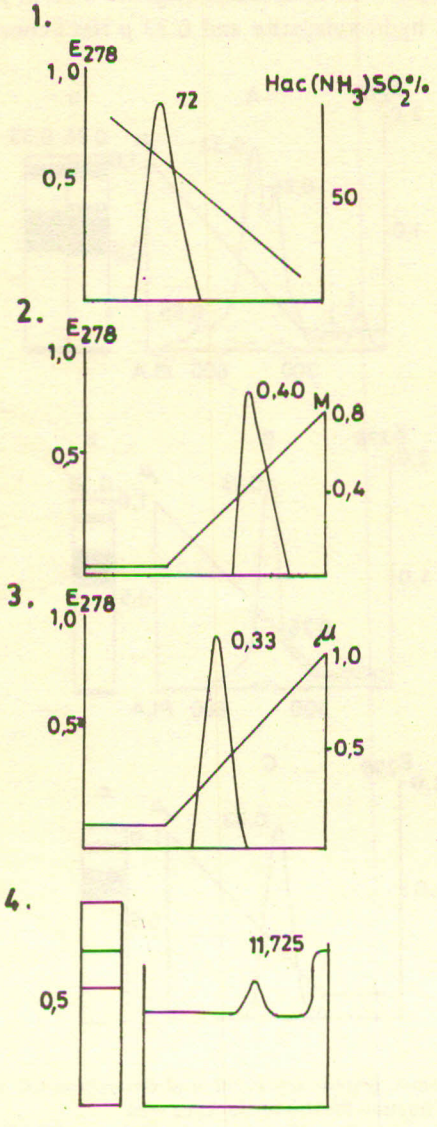

Fig. 2. Homogeneity evaluation of the legumin isolated from chickpea seed.
(1) Gradient extraction on celite column.
(2) Chromatography on hydroxylapatile column.
(3)Chromatography on DEAE cellulose column.
(4) Electrophoresis in polyacrylamide gel and ultracentrifugation.

The amino acid composition revealed that the isolated legumin is rich in glutamic acid, aspartic acid, arginine and tyrosine, while poor in histidine and threonine as compared to the value of legumin of checkpea reported elsewhere [7]. The values for glycine, lysine, *iso*leucine, alanine, serine, proline, valine and leucine were found between 3.44-5.74% (Table 1). The distribution of amino acids in the legumin isolated in this investigation, in general, is in conformity with the work conducted earlier [7]. Nevertheless the values obtained in this study for arginine, leucine, lysine and threonine were lower than those of the former. The variation observed may be due to difference in varieties and analytical methods and also because of the difference in the homogeneity status of the isolated legumins.

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