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A COMPARATIVE STUDY ON ALBUMINS OF CHICKPEA AND LENTIL COTYLEDONS BY CHROMATOGRAPHY ON HYDROXYLAPATITE AND ELECTROPHORESIS IN POLYACRYLAMIDE GEL

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The albumins were separated from total salt-soluble protein extracts of chick pea and lentil cotyledons and were subjected to chromatography on hydroxylapatite column. Chromatographic fractions were analysed by electrophoresis in polyacrylamide gel.

The albumins of investigated cotyledons were represented by a multicomponent system, which were further characterised by a complex electrophoretic composition. A noticeable difference was observed between albumins of chick pea and lentil cotyledons in the number of chromatographic fractions eluted and their electrophoretic behaviour.

INTRODUCTION

The chick pea and lentil seeds are valuable sources of plant protein and contribute substantially to qualitative improvement of human diet [1]. These seeds contain between 12-13 per cent protein, which is composed of albumin and globulins of primary and secondary type; about 20% of the total protein is mainly the albumin fraction [2].

The composition of globulins and albumins is important in the systematic classification of the plants and in the selection of the plants for their nutritive value. It has been reported that the testa is poor in protein content, while embryo contains albumin and low molecular weight globulins associated with the nucleic acids and carbohydrates [3]. The albumin content in the total salt-soluble protein extract of cotyledon is low as compared to globulin. Hence the globulins hide the appearance of many of the albumin on the chromatogram of the total salt-soluble protein extract. More effective chromatographic analysis of albumin can be carried out by initially separating the albumin from the globulin of the total salt-soluble protein extract and by subjecting the albumin to chromatography on hydroxylapatite and electrophoresis in polyacrylamide gel which have already proved to be useful media for analysis of many plant proteins [4,5].

The present research work deals with a comparative study of the albumins of chick pea and lentil cotyledons by their chromatographic fractionation on hydroxylapatite and electrophoretic analysis in polyacrylamide gel.

METARIAL AND METHODS

The experiments were conducted at the laboratory of protein chemistry, Kishinev State University, USSR. The seeds of chick pea (*Cicer arietinum* L.), variety, Sowkhoz-14 and lentil lens esculanta Moench.), variety, Narodnaya were procurded from Biological Station Kishinev State University and Kishinev Agricultural Institute, Kishinev. The testa and embryo were removed. The cotyledons were ground into flour. The total salt soluble protein of both the varieties was extracted from the defatted flour with 1M NaCl phosphate buffer (pH 7.0) [6]. The extracts of chick pea and lentil cotylendons, were dialysed at 3°-5° against distilled water acdified to pH 4.1 and pH 4.0 respectively. These pH correspond to the isoelectric points of the globulins of the respective seeds at which all the globulin components were completely precipitated while the albumins remained in water solution.

The chromatographic separation of the albumins was carried out on a glass column of 1.4 x 30.0 cm packed with hydroxylapatite, as reported elsewhere.[7] The salt concentration in the eluate was determined graphically. The concentration of protein was determined spectrophotometrically in each tube based on absorption at 278 nm and was plotted on chromatogram. The nature of chromatographic fractions was determined on the basis of extinction correlation E-260/E-278 (nucleic acid/protein concentration ratio) at 260 nm and 278 nm. The chromatographic fractions were further analysed by gel electrophoresis using 7.5 per cent polyacrylamide gel and tris-buffer, pH 8.3.[8]

RESULTS AND DISCUSSION

The chromatograms of the albumins of chick pea and lentil cotyledons and electrophoretograms of the various

fractions are presented in Fig. 1. Extinction correlations (E-260/E-278) of chromatographic fractions are presented in Table 1.

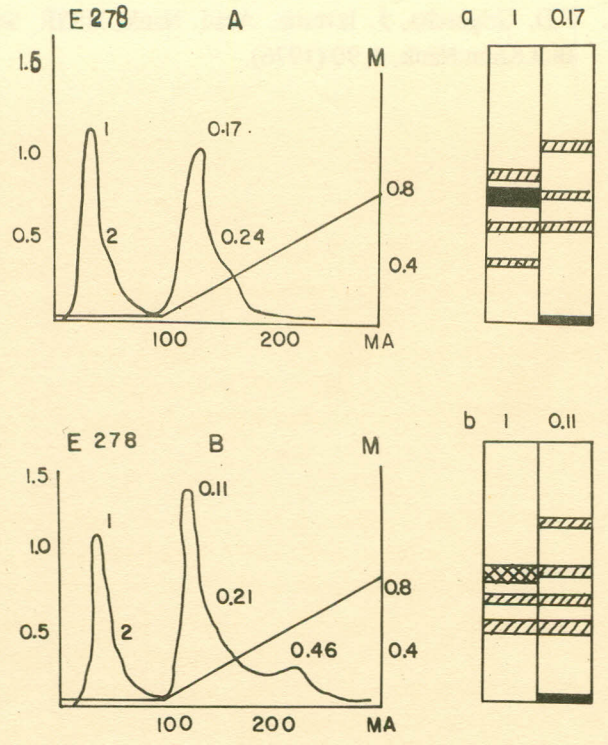

Fig. 1. Chromatography of albumins on hydroxylapatite column (A B) and electrophorisis of ablumins fractions in polyacrylamide gel (a,b) of Chick Pea and lentil cotyledons: A, a chickpea, B, b-lentil.

Table 1. Extinction correlations of chromatographic fractions, eluted by chromatography on hydroxylapatite, of albumins of chickpea and lentil cotyledons.

Chickpea		Lentil	
Chromato graphic fraction	E-260/E-278	Chromato-graphic fraction	E-260/E-278
1	1.18	1	1.20
2	1.04	2	1.02
0.17	0.97	0.11	0.91
0.24	0.99	0.21	0.98
–	–	0.46	0.75

It is evident from the chromatographic separation of albumins on hydroxylapatite that the albumins of chick pea and lentil cotyledons are composed of four and five chromatographic fractions respectively, out of which two fractions, idependent of the generic system of the investigated plants, are eluted with the starting buffer. Chromatographic fractions constituting peak-1 of the albumin of chick pea (Fig. 1B) dominate over the other peak s in their respective chromatograms. All the chromatographic fractions of the albumins of chick pea and lentil cotyledons are of mixed nature containing proteins and nucleic acids. However the higher values for protein are obtained in the chromatographic fractions 0.11-0.17, 0.21-0.24 and 0.46 (Table 1).

Electrophoretic analysis of the albumins of chromatographic fractions of chick pea and lentil cotyledons reveals that there exists an appreciable difference in the electrophoretic behaviour between the albumins of the chromatographic fractions of the investigated plants. Chromatographic fractions I, depending upon the nature of the seed, are separated in four and three electrophoretic components for the albumin of chick pea and lentil cotyledons respectively, while fractions 0.17 and 0.11- in four and five components, which differ in the relative mobility and staining intensity on the electrophoregram. Fractions I, independent of the nature of seeds, contain electrophoretic components posessing medium and fast mobility, whereas fractions 0.17 and 0.11 contain components possessing slow and medium mobility.

It is thus obvious from the chromato-electrophoretic analysis that the albumins of chickpea and lentil cotyledons are represented by a multicomponent system, which are further characterised by a complex electrophoretic composition. The differences observed in the chromatographic and electrophoretic behaviours of the albumins of chickpea and lentil cotyledons are due to the intergeneric differences between the investigated seeds.

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