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CHROMATOGRAPHY ON DEAE–CELLULOSE AND ELECTROPHORESIS IN POLYACRYLAMIDE GEL OF PROTEIN FRACTIONS FROM LENTILS SEEDS

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Total salt soluble protein extract of lentils seeds was fractionated with different concentrations of ammonium sulphate. Fractions were analysed by chromatography on DEAE–cellulose and electrophoresis in polyacrylamide gel. Seeds contained a highly complicated heterogeneous system of individual proteins. Fraction 0–30 was enriched with minor protein components and nucleic acids. Fraction precipitated with ammonium sulphate of below 60 % concentration was enriched with primary and secondary globulins. Fraction 60–100 was dominated by vicilin and legumin.

INTRODUCTION

The pulses are valuable source of plant protein[1]. Lentils seeds are one of the important pulses and contain between 21–31 % protein[2], which is composed of reserve proteins (globulins of primary and secondary type) and albumin[3]. The vicilin and legumin are the dominant globulins of the seeds and their quantitative distribution is determined by the type of plant as reported by Boulter *et al.* [4].

The qualitative and quantitative composition of protein is one of the basic factors important in the selection of plants for nutritive value. One of the most useful techniques, for analysis of proteins, particularly plant proteins is column chromatography on resin anion exchangers such as DEAE and TEAE celluloses[5–6]. Since the globulins elute in large quantities and hide the appearance of minor protein components on the chromatogram, more effective studies on the protein complex can be carried out initially by fractionating the total protein with ammonium sulphate. Azimove[5] employed the method of gradient extraction on column for the analysis of total protein of lentils seeds and on the basis of solubility curves determined the concentration interval of ammonium sulphate for the fractionation of total protiens.

The present study deals with the fractionation of total salt–soluble protein extract with ammonium sulphate and analysis of the fractions by chromatography on DEAE–cellulose and elecrophoresis in polyacrylamide gel.

MATERIAL AND METHODS

The experiments were conducted at the laboratory of protein chemistry, Kishinev State University, USSR. The seeds of lentils *(Lens esculenta* Moench), variety, Narodnaya, were used for protein fractionation. The cotyledons were ground into flour, defatted with ether and the total salt soluble proten extracted from the defatted flour with 1 M NaCl pH 7.9 phosphate buffer[8]. Calculated amount of crystaline ammonium sulphate[9] was gradually added to the extract[8]. so as to obtain protein fractions 0–30, 30–60 and 60–100.

The chromatographic separation of the initial fractions was carried out on a glass column of 1.4 X 30.0 cm packed with DEAE–cellulose[5]. The protein of the respective fraction was dissolved in phosphate buffer pH 7.9 and was subjected to chromatography using phosphate buffer of increasing NaCl concentration (0.1–1 M pH 7.9). 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. The nature of chromatographic fractions was determined on the basis of extinction correlation E–260/E–278 at 260 nm and 278 nm. The chromatographic fractions were analysed by gel electrophoresis using 7.5 % polyacrylamide gel and tris–buffer pH 8.3[10–11].

RESULTS AND DISCUSSION

The results of chromatographic separation on DEAE–cellulose and electrophoresis on polyacrylamide gel of protein fractions of lentils seeds are presented in the Figure. Extinction correlations E–260/E–278 of the chromatographic fractions are given in the Table.

It is evident from chromatographic studies on fraction 0–30 that the fraction is composed of eight chromatographic fractions, out of which three are eluted with the starting buffer (Fig. A). Chromatographic fractions consti–

tuting peaks 1, 2, 3 and 0.14, eluted with lower ionic strength of buffer, are of mixed and contain protein and a significant amount of substances non–proteinous in nature. Peaks 0.21 and 0.31 are of proteinous nature, whereas peaks, elute with higher ionic strength of buffer, are of non–proteinic nature and are enriched with nucleic acids (Table 1). Electrophoretic analysis reveal that the chromatographic fractions 1 and those eluted by 0.21–0.31 M NaCl (pooled together) are composed of five and ten components respectively which differ in the relative mobility and staining intensity in the electrophoregrams (Fig. a).

Fraction 30–60 is separated by chromatography in

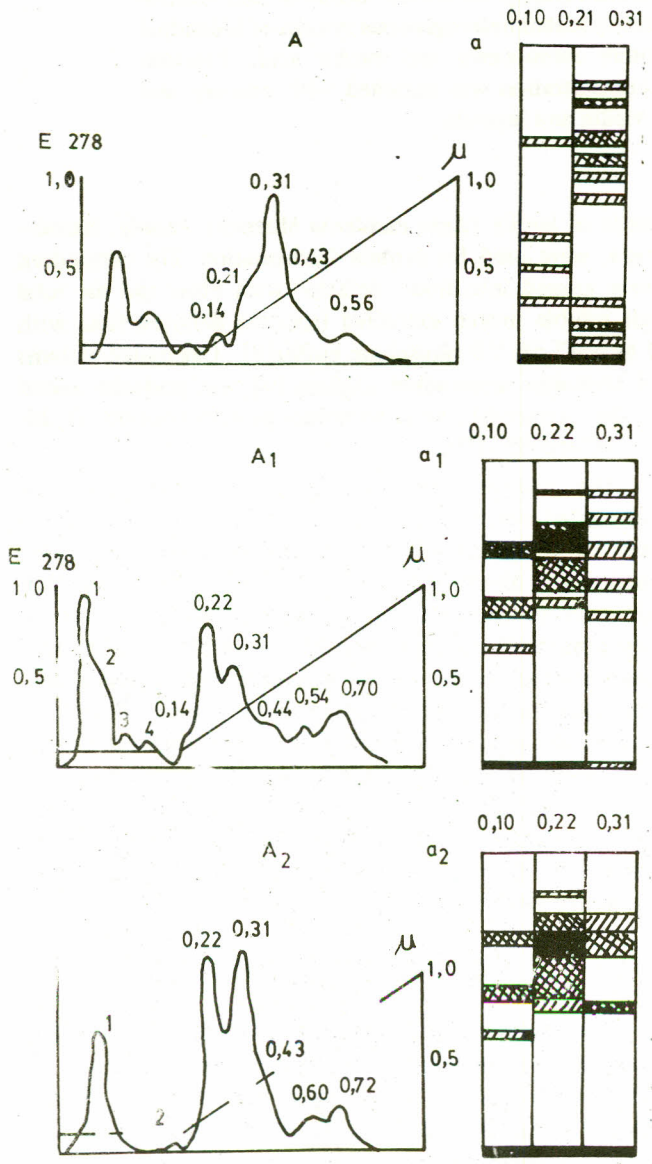

Fig. . Chromatography on DEAE–Cellulose (A) and electrophoresis in polyacrylamide gel (a) of protein fractions from lentils seeds. A, a – fraction 0–30, A_1, a_1 – fraction 30–60 and A_2 a_2 – fraction 60–100.

Table 1. Extinction correlations E–260/E–278 of the chromatographic fractions, eluted by chromatography on DEAE–Cellulose of protein fractions, of lentils seeds.

Concentration of ammonium sulphate in %	Chromatographic fraction	E–260/E–278
0–30	1	0.87
	2	1.03
	3	1.11
	0.14	0.90
	0.21	0.53
	0.31	0.66
	0.43	1.15
	0.56	1.42
30–60	1	0.97
	2	0.83
	3	0.88
	4	0.91
	0.14	1.03
	0.22	1.01
	0.31	1.04
	0.44	1.29
	0.54	1.29
	0.70	1.64
60–100	1	0.92
	2	0.89
	0.22	0.70
	0.31	0.55
	0.43	1.12
	0.60	1.62
	0.72	1.89

ten peaks, out of which four are eluted with the starting buffer (Fig. A_1). Chromatographic fraction constituting peak 1 is dominating on the remaining peaks of the chromatogram. All the peaks except peaks 0.44, 0.54 and 0.70 are of mixed nature. Peaks 0.44 and 0.54 and 0.70 are non–proteinic by nature (Table 1). Electrophoretic studies indicate that the chromatographic fraction 1 contains four components, whereas each of the peaks 0.22 and 0.31 possess six components. The components differ in the relative mobility and staining intensity on the respective electrophoretograms (Fig. A_1).

Fraction 60–100 is separated in seven peaks. Peaks 0.22 and 0.31 are dominating on the remaining peaks of the chromatogram (Fig. A_2). Peak 0.22 is enriched with vicilin of the seeds. Peak 0.31 contains legumin. Both the chromatographic fractions are of proteinic nature. Peaks 1 and 2, eluted with the starting buffer, are mixed by nature, whereas peaks eluting with higher ionic strength of

buffer are non–proteinic in nature (Table 1). Electrophoretic studies reveal that peak 1 contains four components. Peaks 0.22 and 0.31 contain six and four components respectively, which show a slow and medium mobility on the electrophoregrams (Fig. A_2). It is also obvious from the electrophoretic studies of the chromatographic fractions that the chromatographic fraction 0.31 containing legumin type of protein, is contaminated by the vicilin of chromatographic fraction 0.22.

It is thus evident from chromato–electrophoretic studies on the protein fractions of total protein complex of lentils seeds that the fractions are represented by a highly complicated heterogeneous system of protein components. Fraction 0–30 is enriched with minor protein components and nucleic acids. Fraction precipitating below 60 % ammonium sulphate saturation is dominated by primary and secondary globulins, whereas fraction 60–100 is enriched with the primary globulins – vicilin and legumin of the seeds. The chromatographic behaviour of vicilin and legumin of lentils seeds on DEAE–cellulose column is identical with the results reported by Azimov[5] and Lapteva[7]. However, the number of chromatographic fractions on chromatograms of initial fraction 0–30 and 30–60 is higher in the present study than those of the former who chromatographed the total protein. The variation may be due to the difference in the varieties climatic conditions, and because of the chromatographic analysis of the fractions of total protein, the appearance of minor protein components on the chromatograms becomes more conclusive.

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