Chromatography on Hydroxylapatite and Electrophoresis in Polyacrylamide Gel of Protein Fractions from Lentil Seeds.

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Summary: Total salt-soluble protein extract of lentil seeds was fractionated with differenct concentration intervals of ammonium sulphate. Fractions were separated by chromatography on hydroxylapatite column. Chromatographic fractions were analysed by electrophoresis on polyacrylamide gel.

The seeds contained a highly heterogeneous system of individual proteins. Fraction 0-30 was composed of minor protein components and nucleic acids. Fraction 30-60 was represented by primary and secondary globulins. The fraction which preciptated with ammonium sulphate of above 60% concentration was dominated by vicilin and legumin. Which were inseparable on hydroxylapatite column and had maximum elution at 0.29 M phosphate buffer.

Introduction

Pulses play an important role by increasing the resources of plant protein.[1] Lentils occupy a special position among the pulses. The lentil seed contains between 21-31% protein, which is composed of reserve proteins (globulins of primary and secondary type) and albumin, whereas about 80% of the total protein of the seed is composed of globulins (Koinov, 1968)[2] Vicilin and legumin are the primary globulins; their biosyntheses occur independent of each other while their quantitative distribution in the seed is determined by the type of plant (Boulter[3-4] et al).

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 media for analysis of proteins, particularly plant proteins, is column chromatography on hydroxylapatite (Ca 5 (PO4)3 OH)[5]. Since the major globulins elute, (by chromatography of total protein) in larger quantities and thus hide the appearance of minor protein components on the chromatogram, more effective studies on the protein complex can therefore be carried out initially by precipitating the total protein with ammonium sulphate in the fractions. Azimov[6] employed the method of gradient extraction on column for the analysis of total protein of lentil seed and on the basis of solubility curves determined the concentration intervals of ammonium sulphate for the fractionation of total protein.

The present study deals with the fractionation of total salt-soluble protein extract of lentil seeds with different concentrations of ammonium sulphate and analysis of the fractions by chromatography on hydroxylapatite and electrophoresis in polyacrylamide gel.

Methods

The experiments were conducted at the laboratory of protein chemistry, Kishinev State University, USSR. The seeds of lentil (*Lens esculenta* Moench), variety Narodynaya, were obtained. The cotyledons were ground into flour. The total salt-soluble protein was extracted from the defatted flour with 1 M NaCl phosphate buffer (pH 7.0)[9]. Ammonium sulphate was gradually added to the extract,[10] 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 and packed with hydroxylapatite.[5 8] The protein of the respective fraction was dissolved in the starting phosphate buffer (0.03 M, pH 7.6) and was subjected to chromatography using phosphate buffer of increasing salt concentrations (0.03 - 0.8 M, pH 7.6). 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 chromatograms. The nature of chromatographic fractions was determined on the bases 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 elctrophoresis using 7.5% polyacrylamide gel

and trisbuffer, pH 8.3.

Results and Discussion

The chromatagrams of the fractions, precipitated with different concentration intervals of ammonium sulphate from .total salt-soluble protein extract of lentil seeds, and electrophoregrams of the chromatographic fractions are presented in the Figure-1. Extinction correlations (E-260/ E-278) of the chromatographic fractions are presented in Table -1.

Chromatographic studies reveal that the fraction 0-30 is composed of seven chromatographic fractions out of which three are eluted with starting phosphate buffer (Fig. 1-A). Chromatographic fraction constituting peak 0.15 dominates over the other peaks of the chromatogram. Extinction correlations of the chromatographic fractions indicate that peak 0.15 contains protein and negligible amount of nucleic acids whereas the other peaks of the chromatogram contain a considerable amount of nucleic acids. (Table -1). Electrophoretic analysis reveals that peak 1 and 0.15 contain five and six components respectively, while peaks 0.34 - 0.43 (pooled together) possess four components which differ in the relative mobility and staining intensity on the electrophoregram (Fig. 1-a).

Table. 1. Extinction correlations of the chromatographic fractions, eluted by chromatography of initial fractions on hydroxylapatite, of lentil seeds.

Concentration of ammonium sulphate in %	Chromatographic fraction	E-260/E-278
0.30	1	1.19
	2	1.27
	3	1.14
	0.09	1.09
	0.15	1.00
	0.34	1.13
	0.43	1.18
30.60	1	0.97
	2	1.09
	0.09	1.14
	0.15	0.88
	0.24	0.93
	0.32	1.15
	0.44	1.17
60-100	1	1.18
	2	1.09
	0.09	1.04
	0.14	0.76
	0.29	0.75
	0.44	0.89

Fraction 30-60 is separated into seven chromatographic fractions, out of which two are eluted with the starting buffer (Fig. 1 - A). All the chromatographic fractions are of a mixed nature; however a higher value for protein is obtained for the chromatographic fraction 0.15 (TAble - 1). Peak 1 and 0.15 are composed of three and eight electrophoretic components respectively, whereas peaks 0.24 - 0.44 (pooled together) contain seven components only (Fig. 1-a_1).

Fraction 60-100 is composed of six chromatographic peaks, out of which two are eluted with the starting buffer (Fig. 1-A_2) Subfraction constituting peak 0.29 dominates over the others. Subfractions, eluting with lower concentration of buffer are of mixed nature, whereas peaks 0.14, 0.29 and 0.44 contain considerable amount of proteins. Electropheretic analysis further indicates that subfraction constituting peak 1 contains three components possessing medium and fast mobility (Fig. 1-a_2) and are identical with the electropheretic components of peak 1 of the fraction 30-60 (Fig. 1-a_1). Peaks 0.14, 0.29 and 0.44 contain between six to seven components. Some of the components found in the subfaction 0.29 are also noticed in the subfractions 0.14 and 0.44. Peak 0.29 is enriched with the major globulins of the total protein complex of lentil seeds.

The chromato-electropheretic studies on the protein fractions of lentil seeds thus reveal that the seeds contain a highly heterogeneous system of individual proteins. The fraction preciptiated above 60% ammonium sulphate saturation is dominated by primary globulins, vicilin and legumin, of the seeds. The fraction precipitated below 60% saturation contains primary and secondary globulins, whereas fraction 0-30 is enriched with minor protein components and nucleic acids. The chromatographic behaviour of major glabulins on hydroxylapatite column is identical to the one reported by Lapteva.[5] However the number of chromatographic fractions found on the chromatograms of fractions 0-30 and 30-60 in

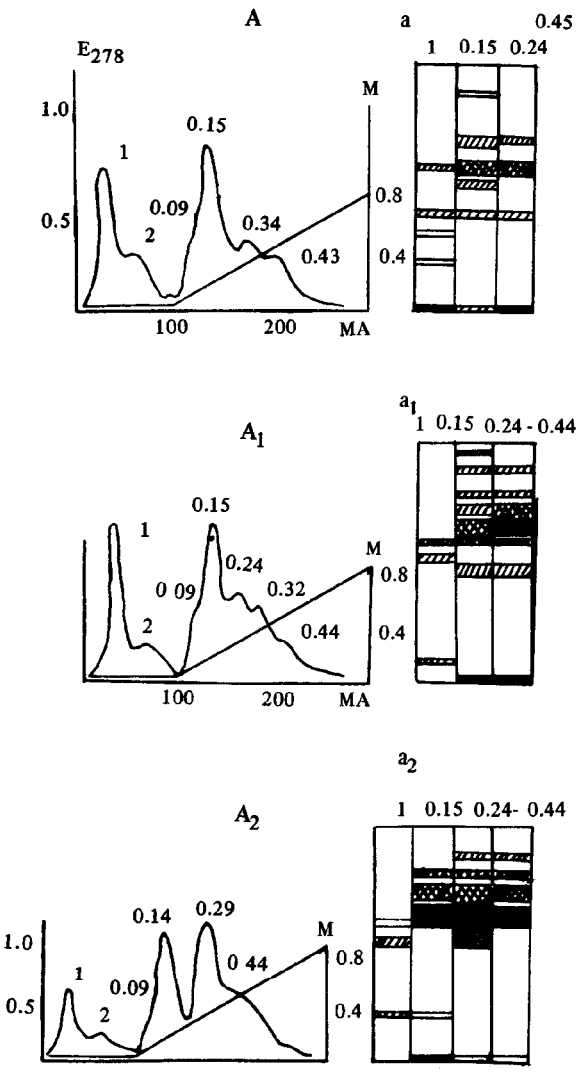

Fig. 1 . Chromatography on hydroxylapatite column (A) and polyacrylamide gel electropheresis (a) of protein fractions of lentil seeds: A, a– fraction 0–30 A_1, a_1– fraction 30–60 and A_2, a_2– fraction 60–100.

the present study is higher than the number obtained by Lapteva[5] on the chromatograms of the total salt-soluble protein extract of lentil seed. The variation may be due to the difference in varieties, climatic conditions and because in the chromatographic analysis of the fractions of total salt-soluble protein extract, the apperance of the minor protein components on the respective chromatograms of the fractions becomes more conclusive.

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