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# COMPARATIVE INVESTIGATION OF ZEIN ISOLATED FROM GLUTEN BY VARIOUS METHODS

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A comparative study has been made on zein preparations isolated from gluten by extraction with 70% ethanol and precipitation under various conditions; by dialysis, and by the addition of a 0.1 M solution of sodium sulfate or of acetone. An investigation of absorption spectra and of electrophoresis in polyacrylamide gel has shown the advantage of the method using acetone for precipitating the protein preparation.

Zein is the alcohol-soluble protein of maize [1]. Industrially, it is obtained by extraction with aqueous alcoholic solutions from gluten – a by-product of the maize starch industry [2]. The degree of purification and the characteristics of the zein depend on the conditions of extraction and precipitation [3]. There have been limited physicochemical investigations of preparations obtained by different methods [4].

In the present paper we give comparative characteristics of zein preparations isolated by extraction with 70% ethanol from gluten followed by precipitation under various conditions. Precipitation was performed by dialysis of the aqueous alcoholic extract obtained and also by the addition of 0.1 M NaCl solution or of acetone.

As an index of the degree of freeing of the zein from nonprotein substances it is possible to use the ratio of the optical densities at 277 and 255 nm ($D_{272}/D_{255}$) and also those at 277 and 320 nm ($D_{277}/D_{320}$) [5-7]. The value of 255 nm corresponds to the position of the minimum and that of 277 nm to the maximum in the UV spectrum of zein after purification [5], while at ~320 nm there is an inflection in the main absorption band connected with the pres-

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ence of nonprotein impurities. In the process of purifying zein the amount of carotenoids was monitored from the value of the optical density at 445 nm [7]. This wavelength corresponds to one of the absorption maxima of a hexane solution of zeinoxanthin isolated by extraction from maize gluten by hexane–acetone–water [8].

The degree of purification of the zeins obtained under the various experimental conditions was estimated from the relative optical densities $D_{277}/D_{255}$, $D_{277}/D_{320}$, and $D_{277}/D_{445}$, together with an analysis of the nature of the absorption spectra of solutions in 70% ethanol. For comparison, as standard we used a commercial preparation of zein. The values of the optical densities given below show that the use of acetone for precipitation enables a zein to be obtained which is less contaminated with nonprotein impurities than preparations obtained by precipitation with 0.1 M NaCl solution or by dialysis:

| Characteristics of the Zein Preparations | $D_{277}/D_{255}$ | $D_{277}/D_{320}$ | $D_{277}/D_{445}$ |
|---|---|---|---|
| Aqueous alcoholic extract | 1.12 | 1.50 | 16.62 |
| Precipitation by dialysis | 1.24 | 2.08 | 22.64 |
| Precipitation with 0.1 M NaCl | 1.23 | 2.20 | 17.35 |
| Precipitation with acetone | 1.42 | 4.69 | 253.96 |
| Standard zein | 1.22 | 1.78 | 5.05 |

This conclusion is in harmony with the nature of the absorption spectra. The absorption of an aqueous alcoholic extract in the UV region is characterized by a broad band with ill-defined maxima (280, 283, and 316 nm) and low-intensity maxima at 258 and 264 nm. The change in the nature of the UV absorption of zein in comparison with the extract appears in a decrease in the absorption of 316 nm and the appearance of a main absorption band at 278 nm characterized by a fine structure with maxima at 251, 258, 264, and 282 nm. The structured nature of the UV absorption band of zein as a tyrosine-containing protein is connected with a high content of phenylalanine residues [9].

Analysis of absorption in the visible region of the aqueous alcoholic extract showed the presence of carotenoids (group of maxima at 398, 420, 443, and 472 nm) [10, 11]. Absorption in this region was also characteristic for the zeins isolated by precipitation with 0.1 M NaCl solution or by dialysis, while for the preparation precipitated with acetone it was absent.

The parameters of the characteristic absorption bands amide A (~3300 $cm^{-1}$), amide I (1650-1658 $cm^{-1}$), and amide II (1536-1540 $cm^{-1}$) in the IR spectra of the standard zein and of preparations obtained by various methods agreed with those described previously [12].

The gel filtration on Sephadex LH-20 of the zein obtained by extraction from maize grain by the laboratory method permits the preparation to be freed from nonprotein impurities [7]. The nature of the elution curve after the gel filtration of the zein isolated from gluten using Sephadex LH-20 was similar to that described in [7]; the first main chromatographic fraction consisted of a complex of zein proteins, and the minor fractions consisted of proteins associated with nonprotein substances (Fig. 1).

Previously, electrophoresis in starch [3, 13] or polyacrylamide gel in the presence of sodium dodecyl sulfate [4], and also isoelectric focusing in agarose [4] had revealed some differences in commercial preparations from the zein obtained under laboratory conditions. To compare zein preparations isolated by different methods, we also used electrophoresis in polyacrylamide gel in acetate buffer [14] with the subsequent recording and comparative characterization of densitograms. A comparison of the densitograms of preparations permitted the singling out of three similar groups of zein components (A, B, and C) (Fig. 2) and the establishment of differences in their component compositions. The first group, A, contained five components. With respect to its protein content, group B was the main one and it contained four components, which were present in all the samples investigated.

In the zein precipitated by dialysis, the third group, C, was characterized by the presence of five protein zones. At the same time, in the zein precipitated with acetone, component $C_4$ was absent, and zone $C_2$ had an electrophoretic mobility different from that of the zein precipitated by dialysis. In the composition of the zein it was possible to single out the same three groups of components, the total number of which amounted to 15. The most considerable differences in the densitograms of the standard zein and the preparations that we had isolated from gluten were observed in group C, containing six components with changed electrophoretic mobilities.

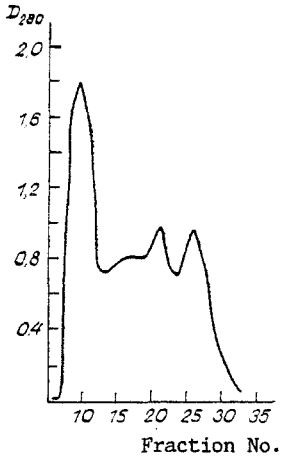


Fig. 1

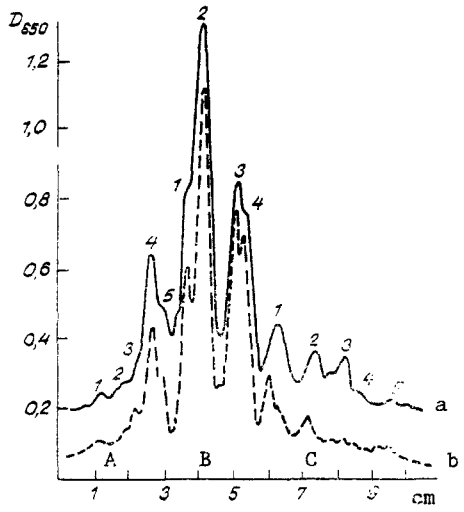


Fig. 2

Fig. 1. Gel chromatogram on Sephadex LH-20 of zein isolated from gluten.

Fig. 2. Densitograms of zein separated by electrophoresis in polyacrylamide gel: a) precipitation with 0.1 M solution of NaCl; b) precipitation with acetone.

For a comparative evaluation of the redistribution of the amounts of protein in the main group of components B we used the ratio of the optical densities of components $B_1$, $B_3$, and $B_4$ to the maximum optical density of component $B_2$ from the densitograms. These values of the relative optical densities changed for the zein preparations according to the method of isolation:

| Characteristics of the Zein Preparations | $D_{B_1}/D_{B_2}$ | $D_{B_3}/D_{B_2}$ | $D_{B_4}/D_{B_2}$ |
|---|---|---|---|
| Precipitation by dialysis | 0.53 | 0.60 | 0.57 |
| Precipitation with 0.1 M NaCl | 0.59 | 0.61 | 0.53 |
| Precipitation with acetone | 0.56 | 0.62 | 0.57 |
| Standard zein | 0.58 | 0.54 | 0.54 |

Thus, in the case of component $B_1$ the highest value of the optical density was characteristic for the preparation obtained by precipitation with 0.1 M NaCl, while for zone $B_4$ higher values of the ratio were found for the preparations precipitated by dialysis or by the addition of acetone. The value of the absorption for zone $B_3$ showed a complex nature of the redistribution of the protein content in the three methods of precipitating zein.

## EXPERIMENTAL

To isolate the zein we used gluten produced by the Verkhnedneprovsk Starch-Molasses Combine. The dry gluten (200 g) was extracted three times with 70% ethanol (one liter each time) for 1 h. The first and second extractions were carried out at 30°C, and the third at 50°C. The extracts were combined after centrifugation at 3500 rpm (20°C). The zein was precipitated from the extract (780 ml) with a threefold volume of 0.1 M NaCl solution [15]. After separation and drying, the yield of preparation was 15.2 g.

In precipitation by dialysis against water, 800 ml of extract yielded 14.9 g of product.

For the precipitation of the zein with acetone, one liter of the solvent was added to 110 ml of extract. The yield of dry preparation was 1.2 g.

For comparison we used a standard zein preparation (Fluka) without additional purification.

The IR spectra of the zeins were investigated in the form of unoriented films, which were prepared by depositing 1-ml portions of solutions of the preparations in 70% ethanol on

fluorite glass followed by drying in vacuum. The concentration of the solutions was 2.5 mg/ml. IR spectra were obtained on Specord 75 IR and Specord M 80 instruments in the 1100-4000 $cm^{-1}$ interval. The absorption spectra of zeins in the UV visible region were investigated on a Specord M 40 spectrophotometer in a 1-cm cell. To measure the optical densities of solutions, a program of measurements was drawn up which consisted of individual blocks according to the analytical wavelengths. The concentration of the solutions of zein in 70% ethanol was 1 mg/ml. To measure the absorption of an aqueous alcoholic extract of gluten, the initial solution was diluted 20-fold.

To perform gel filtration, 20 ml of extracts from gluten from 70% ethanol was deposited on a 2.6 × 60 cm column of Sephadex LH-20. The protein was eluted with 70% ethanol. The eluate was collected with the aid of a fraction collector and was analyzed on the basis of its absorption in the ultraviolet at 280 nm on a spectrophotometer.

Electrophoresis in polyacrylamide gel was performed by the procedure of [14] on a VÉS 2001 instrument. Densitograms were obtained by scanning the gel plates in a Specord M 40 spectrophotometer with a gel-scanning attachment at a wavelength of 650 nm.

## SUMMARY

A comparative investigation has been made of zein preparations isolated from gluten by various methods. An advantage of isolating the zein by precipitation with acetone from an aqueous alcoholic extract has been shown. Spectral and electrophoretic methods of evaluating the degree of purity of protein preparations have been proposed.

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