# Purification of Zein on a Laboratory Scale by Charcoal or Gel Filtration

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Although most studies of proteins are carried out in aqueous solution, much information can be obtained on certain proteins by use of nonaqueous solvent systems (1). Zein is soluble in a variety of nonaqueous solvents (2), but is virtually insoluble in water. It thus seemed to be an excellent protein to study and to compare with other proteins such as insulin, ribonuclease, and lysozyme which are soluble in both water and nonaqueous solvents. Pure zein is necessary for determinations such as amino acid composition, molecular weight, and optical rotary dispersion. Crude zein can be obtained by direct extraction from whole corn meal (unmodified zein) or can be purchased commercially as a 90% pure product. The object of this work was to find a fast, simple method for purifying both commercial and unmodified zein. Three procedures – ion exchange, gel filtration, and a new method involving treatment with charcoal – were compared as methods for purifying zein.

Ion-exchange chromatography was done with two different weakly cationic exchange resins and the method described by Craine et al. (3). Rexyn-102 (analytical grade, 16-50 mesh) was packed in a column 22 mm. in diameter and 480 mm. high, and Amberlite CG-50 (type 2, less than 200 mesh) was packed in a column 12 mm. in diameter and 330 mm. high. Both resins required extensive washing before packing. After the purified zein had been eluted, two dialysis procedures were required, the first to remove salt and the second to remove alcohol. Dry protein was recovered by lyophilization.

Gel filtration was done with a 22- by 440-mm. column of Sephadex LH-20 and elution with 70% ethanol. Zein eluted first, followed by impurities, in agreement

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with Landry et al. (4). Only one dialysis procedure (to remove the alcohol) was required prior to lyophilization.

Treatment with charcoal was tried in an attempt to simplify purification. Approximately 4 g. of Norit-A decolorizing carbon was added to 300 ml. of a 3% solution of crude zein in 70% ethanol. After 30 to 60 sec. the charcoal was rapidly removed by vacuum filtration. Recovery of protein material by this procedure was 95% or better.

Since zein has an absorption maximum at 277 nm. while an impurity absorbs in the region of 320 nm., the absorbance ratio 277:320 nm. (3) was used as one measure of the effectiveness of the purification procedures. These ratios are presented in Table I together with the nitrogen contents of some of the preparations. Electrophoresis was performed on the samples obtained in this study by use of Cellogel strips in an aluminum lactate buffer of pH 3.2 and nominal ionic strength of 0.05 (7) containing 8M urea (8). The patterns obtained were similar to those obtained by Turner et al. (8) and Boundy et al. (5) with starch-gel electrophoresis. In the case of unmodified zein, some material remained at the origin while two major and one minor band moved toward the negative terminal. The pattern for commercial zein was less well defined in that the bands were broader and poorly resolved. Little or no material remained at the origin. No difference was observed between the patterns for the unpurified zeins and those purified by Sephadex LH-20 or charcoal. The molecular weight of commercial zein purified with charcoal was 23,100 ± 600 as determined by osmotic pressure measurements with formamide as a solvent (unpublished data).

Although a good-quality purified zein is produced by ion-exchange chromatography in agreement with Craine et al. (3), the method has some undesirable features. Ethanol elutes a yellow material from the resin which absorbs in the same ultraviolet region as zein, and this material must be washed from the resin before it can be used. Moreover, after 24 hr. in 70% ethanol, the resin loses the ability to remove zein from solution (3). Also, two dialysis procedures are necessary.

TABLE I

| Sample | Ratio 277:320 | % N (dry basis)[a] |
|---|---|---|
| Crude commercial zein | 2.9 | 14.9 |
| Commercial zein purified on Amberlite (Craine et al., 3) | 6.8 | 16.0 |
| Commercial zein purified on Amberlite | 7.2 | |
| Commercial zein purified on Rexyn-102 | 10.3 | |
| Commercial zein purified on LH-20 | 8.8 | |
| Commercial zein purified with Norit-A | 11.8 | 16.1 |
| Unmodified zein purified on Amberlite (Craine et al., 3) | 5.2 | 16.4 |
| Untreated 70% extract from corn meal | 1.1 | 11.5 |
| Unmodified zein purified using LH-20 only | 3.2 | |
| Unmodified zein purified using Norit-A only | 6.6 | 12.8 |
| Unmodified zein purified using LH-20 and Norit-A | 7.0 | 15.3 |

[a]Nitrogen analyses were performed by Alfred Bernhardt Microanalytical Laboratory. Nitrogen content of zein has also been reported as 15.8 to 16.1% (5) and 16.3% (6).

In contrast, the LH-20 column can be used repeatedly, and only one dialysis procedure is required. Gel filtration alone produced good results with commercial zein but not with unmodified zein.

Treatment with charcoal is the fastest and easiest of the procedures tried, even though in some cases two or three treatments were required. It gave good results when used alone with commercial zein but was not as effective with unmodified zein.

The results of this study indicate that commercial grade zein can be purified by simple treatment with charcoal, or gel filtration. Unmodified zein can be purified by gel filtration followed by treatment with charcoal.

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