

## PREPARATION OF PURIFIED ZEIN BY ADSORPTION-DESORPTION<sup>1</sup>

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### ABSTRACT

A procedure was developed for preparing colorless zein that retains good solubility in aqueous ethanol. Zein was preferentially adsorbed from 70 volume-percent ethanol on a column of weak cation exchanger resin in the acid form and also was readily desorbed by salt in 70% ethanol. Isolation of the zein by slow precipitation from the aqueous ethanol through dialysis at 0° to 4°C. against water, followed by lyophilization, resulted in retention of good alcohol solubility. The nonhygroscopic, easily powdered product gives clear solutions in alcohol and merits consideration not only as a reference sample but also as a constituent of rations for animal feeding studies using zein. The purification procedure was shown to be applicable to unmodified zein extracted directly from corn meal and to commercial zein produced from gluten obtained in corn wet-milling.

Our need for reference samples in studies of the isolation and characterization of corn proteins led to a study of purification of zein. Two objectives were: the removal of nonprotein contaminants, including pigments, and the recovery of purified zein in a dry form retaining good solubility characteristics.

The removal of nonprotein impurities from zein generally has been accomplished by methods similar to those used by Chittenden and Osborne (1), who used ethanol and ether for extraction and precipitation. Removal of yellow pigments from zein by these solvents generally is incomplete. Extraction of pigments and lipids with ethylene dichloride was accomplished by Mason and Palmer (6); however, they gave no data on solubility characteristics of the zein which they prepared from commercial yellow corn gluten meal.

Incomplete solubility of zein preparations in aqueous ethanol has been noted in numerous reports on the purification and isolation of zein. In some cases insolubility may result from use of high temperature (1), repeated contact with different solvents, or a large number of extraction steps. Another important cause of low solubility appears to be the physical form in which the product is obtained. Precipitation of zein from an alcoholic solvent by addition of water or an organic solvent often yields a taffylike mass. Such a precipitate becomes horny

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on drying and requires mechanical grinding to reduce it to a powder. While small particle size after grinding improves solubility, such products often do not go into solution as readily as if the horny state were avoided. Some precipitation techniques that have been reported to give readily soluble zein, such as dispersing an alcohol solution of zein in cold water (4), did not give consistent results in our studies.

Some disadvantages of previous laboratory procedures for purifying and preparing dried samples of zein have been overcome in the present studies. A preferential adsorption of zein from alcoholic solutions by weakly acidic cationic exchange materials was observed. On this basis a method was developed for obtaining a highly purified white zein that gave clear, colorless solutions in ethanol. Retention of good alcohol solubility was achieved by modifying the procedure for precipitating and drying the zein.

Commercial zein and unmodified zein, which was extracted directly from ground corn, were included in these studies because of possible differences in their behavior. Commercial zein is prepared from corn gluten meal, obtained in wet-milling corn for starch. The sulfur dioxide used in the wet-milling process probably causes some chemical modification of the protein at disulfide (cystine) linkages. In addition, commercial corn gluten reportedly contains at least two types of zein protein, only one of which ( $\alpha$ -zein) is extracted in the commercial preparation of zein (7,10).

## Experimental

*Materials and Analytical Methods.* Commercial zein was supplied by the Corn Products Co.<sup>3</sup> as Lot No. HV-9 and had a nitrogen content of 14.7% (dry basis). This product may differ somewhat from current lots of commercial zein because of changes in processes.

Unmodified zein was prepared from whole ground yellow corn (Dyar 444 of the 1957 crop) which had been partially defatted with petroleum ether (2), dried, and then extracted with 70% (by volume) ethanol at 28°C. The extract contained about 35% of the total corn nitrogen. Although a second extraction at 60°C. removed an additional 8% of the total nitrogen, this fraction was not included in the present studies. The zein was isolated by dialysis and lyophilization as described below: about 90% of the nitrogen extracted was precipitated as zein. Aqueous ethanol having a concentration of approximately 70% by volume was prepared conveniently by diluting 70 ml. of 95% ethanol to 95 ml. with water.

<sup>3</sup>Mention of firm names or trade products is for identification only and does not imply endorsement by the U.S. Department of Agriculture.

The Amberlite IRC-50, a weak cation exchange resin, was cracked to smaller particle size in a laboratory Burr mill and then ground in a laboratory hammer mill so that about 95% passed through a 35-mesh screen. The screened resin was cycled twice through the sodium and hydrogen form and finally left in the hydrogen form after washing to remove excess acid. The resin was covered with water and stored at 5°C.

The amount of zein in the effluents from the resin columns was estimated by a turbidimetric method (3) which gives a comparative but not absolute value. Nitrogen was determined by a conventional micro-Kjeldahl procedure using mercuric oxide as a catalyst. Absorbance in the ultraviolet was determined in a 1-cm. cell of a Cary recording spectrophotometer, Model 11. Electrophoresis experiments were performed with an Aminco portable electrophoresis unit in a closed 7-ml. analytical cell at 1.5°C. using 15 ma. current.

*Adsorption-Desorption Procedures.* The adsorption columns were packed with IRC-50 resin (hydrogen form) which had been equilibrated with 70% ethanol by mixing with four changes of 70% ethanol and allowing 10 to 20 minutes' contact each time. The equilibrated resin was slurried with 70% ethanol into the column and allowed to pack by settling. A volume of 70% ethanol equal to two or three times the holdup volume of the column was allowed to percolate through the resin to ensure equilibration. The period of contact of resin with ethanol was kept to a minimum because long periods, such as 24 hours, caused nearly complete loss of ability for the resin to adsorb zein from solution.

The zein was applied to the column as a 1 to 3% solution in 70% ethanol. For preparative runs as much as 1.4 g. of zein were used for a column 18 × 600 mm.; smaller proportions were used for chromatographic fractionation. After the column was washed with 70% ethanol to remove unadsorbed material, the adsorbed zein was removed, either in one step by elution with a 0.5M solution of sodium chloride in 70% ethanol, or progressively by increasing concentrations of sodium chloride up to that level.

*Recovery of Dry Zein.* Zein was precipitated from solution in 70% ethanol by dialysis against water at about 2°C. Solutions containing salt were first dialyzed against 70% ethanol to remove salt and thereby avoid retention of salt in the precipitated zein (levels as high as 5% salt in the zein were encountered otherwise). The dialysis against ethanol and against water was conducted as a batch operation with a sufficient number of changes of ethanol or water to ensure less than 0.2% residual salt in the first case and less than 0.1% residual ethanol in the second case.

The flocculant precipitate of zein was separated by centrifuging. The precipitate was resuspended in about ten times its volume of water and the suspension was lyophilized. In some cases the entire nondialyzable contents of the dialysis tubing were mixed and lyophilized. In either case an easily powdered product was obtained which did not require grinding and was not hygroscopic. Some mechanical loss usually occurred during lyophilization because of the ease with which the light fluffy product was carried over into the receiver by the rush of water vapors, particularly when there was any vibration of the apparatus.

### Results and Discussion

*Purification of Commercial Zein by Adsorption-Desorption.* Commercial zein dissolved in 70% ethanol was separated into two fractions: (a) material not retained on the ion-exchange resin and (b) material eluted by 0.5M sodium chloride in 70% ethanol. In a typical preparation the recovery of total nitrogen was 101%; the unadsorbed material, which included the yellow pigment, accounted for 11% and the eluted fraction for 90%. The purified product obtained by dialysis of the eluted fraction against ethanol and then against water, followed by lyophilization, had a nitrogen content of 16.0% (dry basis). A 1% solution of the purified zein in ethanol was clear and very nearly colorless.

The nitrogenous material in the unadsorbed fraction was not characterized. The relatively large proportion may reflect interference of salts with adsorption of protein by the IRC-50 resin. Studies of gradient elution of zein showed that 0.003M sodium chloride in 70% ethanol could displace over 50% of the zein from the resin.

*Purification of Unmodified Zein.* The same adsorption-desorption procedure was applied to a preparation of unmodified zein having a nitrogen content of 13.5%. The unadsorbed fraction contained only 1% of the total nitrogen, whereas 86% of the nitrogen was in the fraction eluted with 0.5M sodium chloride in 70% ethanol. The nitrogen content of the resulting white powder was 16.4% and remained unchanged by a repetition of the adsorption-desorption procedure. The amount of unadsorbed nitrogenous material is very small in this case probably because of rather complete removal of salts by dialysis during zein preparation. The 13% nitrogen unaccounted for probably is material irreversibly adsorbed under the conditions used. No study was made of the nature of this portion of the nitrogenous components of the crude zein preparation, although it may correspond to beta-zein fraction, which is eliminated in the preparation of commercial zein (7).

*Properties of Purified Zein.* The ultraviolet absorption spectrum of a typical purified commercial zein is shown in Fig. 1, together with the

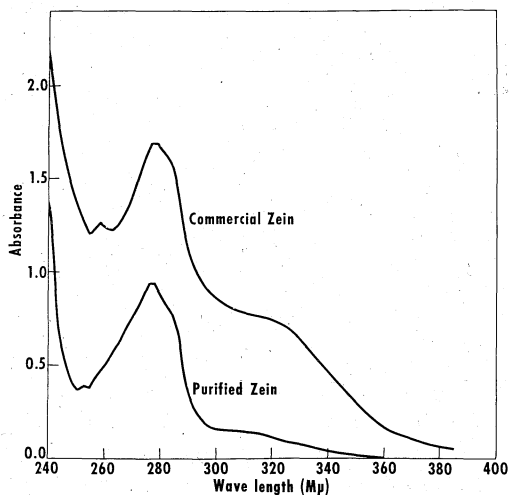


Fig. 1. Ultraviolet absorption spectra of commercial zein before (upper curve) and after (lower curve) purification by adsorption-desorption. Concentration 2 mg. zein per ml. and 1.75 mg. zein per ml. (determined turbidimetrically), respectively, in 70 volume-percent ethanol.

absorption curve of the original sample. The purified unmodified zein showed a similar ultraviolet absorption spectrum, whereas the crude unmodified zein contained larger amounts of contaminants absorbing in the ultraviolet (Fig. 2) than did the commercial zein.

The effectiveness of pigment removal is shown by the low absorbance in the region of 3,200 Å for purified zein. In the region of the protein absorption peak (around 2,770 Å) a strong background absorbance from pigments and other impurities in the crude zein shifts the peak upward to a considerable degree.

A quantitative comparison of the ultraviolet absorption characteristics of the crude and purified zeins is provided by the ratio of absorbance at 2,770 Å (the position of the absorption maximum for purified zein) to the absorbance at 3,200 Å (near the position of maximum absorption for samples of separated impurities high in the yellow pigment) and to the absorbance at 2,550 Å (the position of a minimum in the absorption curve for the purified zein and the approximate position of the minimum in typical protein absorbance curves). Absorbance ratios for representative preparations before and after purification are as follows:

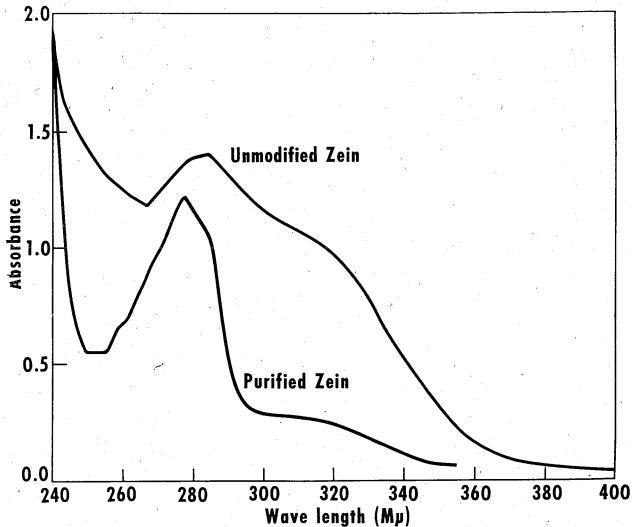


Fig. 2. Ultraviolet absorption spectra of unmodified zein extracted from corn meal, before (upper curve) and after (lower curve) purification by adsorption-desorption. Concentration 0.08 and 0.28 mg. nitrogen per ml. (the latter equivalent to about 2 mg. zein per ml.), respectively, in 70% ethanol.

	2,770 A/3,200 A		2,770 A/2,550 A	
	Crude	Purified	Crude	Purified
Commercial zein .....	2.3	6.8	1.4	2.5
Unmodified zein from meal extract	1.5	5.2	1.0	2.3

The marked change in the 2,770/3,200 A absorbance ratio reflects the considerable removal of pigments, although further purification appears possible for one or both samples since a difference exists between the two after purification.

Moving-boundary electrophoresis patterns for crude and purified zein are shown in Fig. 3. All zein samples were dissolved in sodium glycinate-sodium chloride buffers at pH 11.6 (5), since these conditions always gave complete solution. Zein from another corn sample was soluble in buffers at a pH as low as 10.5, possibly as a result of differences in the number of base-binding groups, such as free carboxyl groups. The use of the higher pH did not alter the electrophoretic properties of this zein. While purification had relatively little effect on the electrophoresis pattern of commercial zein, it changed that of unmodified zein considerably. The disappearance of the faster-moving component which had a mobility of about  $5.5 \times 10^{-5}$  cm<sup>2</sup>/v/sec. and comprised around 50% of the total pattern area, hardly can be explained entirely by the loss of 13% nitrogen during purification.

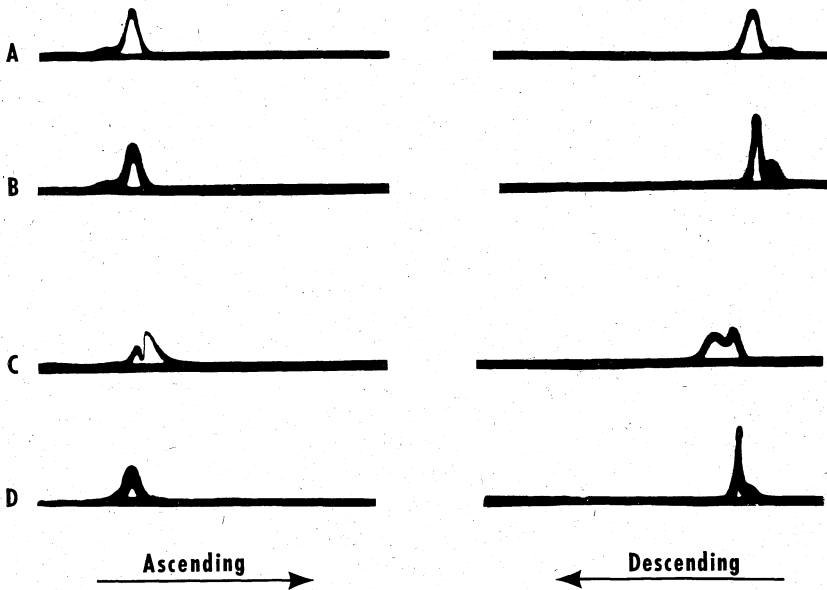


Fig. 3. Moving-boundary electrophoresis patterns of commercial zein, before (A) and after (B) purification, and of unmodified zein, before (C) and after (D) purification. Concentration of protein 0.5% in sodium glycinate-sodium chloride buffer, pH 11.6, ionic strength 0.1, 120 minutes; the delta and epsilon boundaries approximately coincide with the beginning of the patterns.

Possibly purification also removed protein-complexing impurities which altered the electrophoretic mobility of part of the zein protein. The mobilities of the main component in the samples of purified zein were in the range of 4.3 to 4.7 for both ascending and descending patterns and were in good agreement with the range of 4.0 to 4.3 reported by Mertz *et al.* (8) for the electrophoretic component which was predominantly in their zein fraction.

Solubility of the purified zein in 70% ethanol generally was complete at levels of 2 g. per 100 ml. Crude zein preparations generally were soluble to the extent of at least 85% after precipitation by dialysis and lyophilization. Occasional difficulties in redissolving the crude zein were attributed to leaving too high a level of ethanol (1 to 2%) in the solution when the precipitated zein was lyophilized with the entire contents of the dialysis bag.

Purification of zein for animal feeding studies might be done advantageously by the procedure described here. Not only the purity but also the physical properties of the product should be beneficial. Maximum digestibility of the protein would be expected as a result of

the finely divided state and the absence of denaturation or horniness as reflected in the solubility in 70% ethanol.

*Purification Based on Solubility.* Prior to the development of the adsorption procedure the possibility of purifying zein on the basis of solubility differences between the protein and the impurities was investigated. Among the methods tried were: extraction with solvents, reprecipitation, and prolonged dialysis against 70% ethanol.

None of these procedures were entirely satisfactory, as judged by the nitrogen content and color of the product. Ether extraction of one unmodified zein preparation raised the nitrogen content from 13.5% nitrogen to 14.6% nitrogen; while some color was removed, the product still was a yellow powder. Reprecipitation of another preparation from 70% ethanol by dialysis against water raised the nitrogen content from 13.5% only to 13.7%. Dialysis against 70% ethanol was somewhat more effective, as shown by an increase of nitrogen content from 13.5 to 15.2%, with simultaneous elimination of most of the pigmentation. Variable results were encountered in the extent to which color was eliminated during dialysis; some sizes of dialysis tubing permitted relatively rapid passage of the colored impurities while others permitted only slow removal or none at all. No attempt was made to determine whether this result was caused by differences inherent in tubing size or its age.

*Gradient Elution of Zein.* Since column chromatography has been used for fractionation of proteins, some trials were made of fractional elution of zein from IRC-50 resin columns. Sodium chloride gradients from 0 to 0.005M in 70% ethanol gave elution patterns that varied from a broad peak to partial resolution into two or more peaks and accounted for 50% or more of the adsorbed protein. A further quantity of protein was desorbed by raising the sodium chloride concentration in one step to 0.2M or 0.5M. Neither this final fraction nor the one from gradient elution appeared to be homogeneous, since rechromatography gave elution patterns similar to those for the original zein preparation. The results are suggestive of association-dissociation behavior comparable to that attributed to zein by Scallet (9) on the basis of fractional precipitation and electrophoresis studies.

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