MODIFICATION OF ZEIN BY DEAMIDATION

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Zein, the alcohol-soluble protein of corn, is insoluble in water below pH 11. Its solubility in aqueous alkali at pH 11.3 to 12.5 is due to the formation of sodium salt of the tyrosine hydroxyls (Croston, Evans and Smith, 1945). The calculated molecular weight from amino acid analysis is 25,190 (Cardinal and Hedrick, 1947), but the observed molecular weight may be greater depending upon the degree of polymerization caused by the formation of disulfide bonds between molecules (Laine, 1944). Pure zein contains 16.2% nitrogen, of which 36% is in the form of asparagine and glutamine—the monoamides of aspartic and glutamic acids. Thus, the non-peptide amide content (-CONH$_2$) is 0.206 equivalents per 100 gms. of zein, and this amide nitrogen accounts for 18% of the total nitrogen (Cardinal and Hedrick, 1947).

The insolubility of zein in water at neutral pH has been attributed to the absence of carboxyl groups. Gortner and Gortner (1949:328) have pointed out that it should theoretically be possible to prepare highly acidic proteins by splitting off the ammonia from the non-peptide amide groups and thus leaving the peptide linkages practically unaltered. They treated gliadin with dilute phosphoric acid under pressure and hydrolyzed as much as 96% of the non-peptide amide groups with only 6% of the peptide bonds being broken. Virtanen and Hamberg (1947) and Laine (1944) have shown that the non-peptide amide groups of zein are hydrolyzed more than three times as fast at pH 1.5-1.8 and 37° C. as the peptide bonds.

Steinhardt and Fugitt (1942) used detergents, such as dodecylsulfonic acid, to deamidate egg white and wool. However, we were unsuccessful in applying their technique to zein because of the difficulty in removing the large amount of detergent from the reaction product. Shildneck (1942) treated corn gluten with acid, solubilized both the starch and protein and then precipitated the protein by adjusting the pH to 4.5. This degraded protein was reported to be more soluble in aqueous alkali than before hydrolytic treatment. Cleland and Henke (1951) hydrolyzed corn gluten with lime at 25 to 45 p.s.i. steam pressure and precipitated the protein material by adjusting the pH to 4.5 to separate it from the solubilized starch. The resultant protein could be dissolved in aqueous alcohol.

This paper reports the preparation and some of the properties of partially deamidated zein which retains most of its molecular size.

MATERIALS AND METHODS

Commercial zein containing 15.6% nitrogen, 0.3% ash, 0.6% carbohydrates, and 1.0% oil was used in this study. Nitrogen analysis indicated a purity of 96%.

The rate of ammonia liberation was determined by placing 5 gms. of zein and 100 mls. of water containing the alkali in a flask equipped with a reflux condenser leading into boric acid solution containing bromcresol green and methyl red to absorb the ammonia. The reaction mixture was held at reflux temperature, and washed air was bubbled through the mixture to remove the ammonia. The boric acid solution was titrated at intervals as described by Croston (1943:13-15).

The conditions used for preparing deamidated zein are shown in Table 1. It was necessary to chill the mixture of water (or water-ethanol) and hydrolyzing agent before adding the zein to prevent formation of a gummy mass. The reaction flask was fitted with a reflux condenser and heated for the time and at the temperature shown in Table 1. The product was isolated by adjusting the pH to 7.0, centrifuging when necessary to clarify, adjusting the pH to 4.0 and centrifuging to remove the precipitate. The precipitate was washed with water, dissolved by adjusting the pH to 7.0 with sodium hydroxide, and dialyzed until free of salts. The dialyzer contents were then either lyophilized or evaporated to dryness under reduced pressure.

RESULTS

The rate of ammonia liberation from a 5% aqueous dispersion of zein at reflux temperature with 4 different levels of sodium hydroxide is shown in Figure 1, where 0.25 mole of sodium hydroxide is sufficient to disperse the zein and account for the primary amide groups in 100 gms. In Figure 2 the rate of ammonia liberation with barium hydroxide at two levels is compared with that for calcium hydroxide at two levels.

TABLE 1.—Preparation of Water-Soluble Zein.

Prep. No.	Ratio H_2O:zein	Agent used	Moles agent	Temp., °C.	Time, hrs.	Yield, percent	$-CONH_2$ hydrol., percent
1.	10:1	NaOH	0.25	75	8	46	. .
2.	1:1	NaOH	0.25	80	1	29	. .
3.	30:1	$Ca(OH)_2$	0.535	60	40	20[2]	88
4.	10:1[3]	$Ca(OH)_2$	0.10[4]	100	4	33	. .
5.	1:1	$Ca(OH)_2$	0.13	100	1	33	. .
6.	1:1	$Ba(OH)_2$	0.13	100	1	40	. .
7.	14:1[3]	$Ba(OH)_2$	0.625[4]	100	½	45	57
8.	10:1	$Sr(OH)_2$	0.625[4]	100	½	50[5]	25
9.	10:1	HCl	0.214	80	16	50	54

[1] Based on 100 gms. starting zein, dry basis. 100 gms. of commercial zein contained 0.20 equivalents of primary amide groups, and 0.05 equivalent of alkali was required to dissolve zein.

[2] Soluble in water at pH 5.5.

[3] 30% aqueous ethanol used as solvent.

[4] Zein dissolved in 0.05 mole NaOH before alkaline earth hydroxide was added.

[5] Cloudy solution at pH 7.0; soluble at pH 8.0.

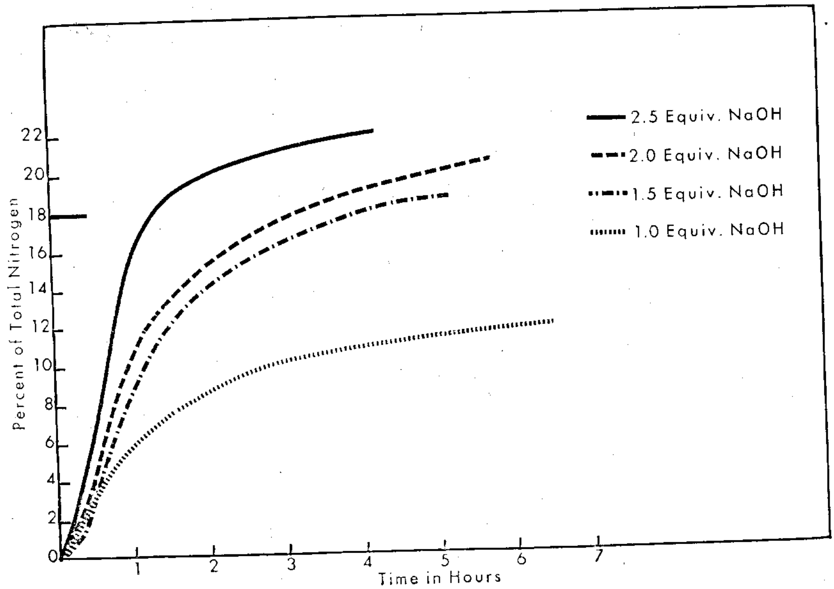


FIG. 1.—Rate of NH_3 liberation from zein by different equivalents of NaOH based on non-peptide amide groups.

The hydrolytic conditions used for preparing water-soluble zein are shown in Table 1. The moles of agent used are based on 100 gms. of zein which contains 0.20 equivalent of non-peptide amide groups ($-CONH_2$), and 0.05 equivalent of alkali is used up in dispersing the zein before hydrolysis (Cardinal and Hedrick, 1947; Croston, *et al.*, 1945). Thus, approximately 0.25 equivalent of alkali per 100 gms. of zein is required to hydrolyze the non-peptide amide groups on an equivalent basis. The percentage of total non-peptide amide groups hydrolyzed to carboxyl groups was calculated from the difference in total nitrogen content before and after hydrolytic treatment.

The ultracentrifuge patterns of zein and deamidated zein are compared in Figure 3.

DISCUSSION

Prolonged boiling of zein with strong alkali, as illustrated in Figures 1 and 2, results in considerable loss of ammonia by deamination, in addition to the deamidation as has been shown to occur for other proteins (Croston, 1943). Since the non-peptide amide nitrogen of zein is 18% of the total nitrogen, a reaction time of 1 hour or less is indicated for hydrolysis of these amide groups with strong alkali at reflux temperature. The lower curve of Figure 1 indicates that about one-half of these amide groups may be hydrolyzed by an equivalent amount of sodium hydroxide. The lower curve of Figure 2 indicates that increasing the amount of lime does not increase the rate of ammonia liberation, probably because of the relative insolubility of lime.

All of the preparations listed in Table 1, except No. 8, were soluble in water at pH 6 to 7. No. 8 gave a slightly cloudy dispersion at pH 7.0, but gave a clear dispersion at pH 8.0. From the data shown in the last column, it appears that zein becomes soluble in aqueous solution at pH 6 to 7 when about 40% of the non-peptide amide groups are hydrolyzed to carboxyl groups. Mecham and Olcott (1947) reported that soybean protein (16.0% total nitrogen) contains 0.114 mole of amide nitrogen (10% of the total nitrogen). According to Drackett (1955) there are 0.183 mole of dicarboxylic acids per 100 gms. in soybean protein. Calculations show that 38% of the dicarboxylic acids in this protein exist as free-carboxyl groups to which its solubility in mild alkali is attributed. Preparation No. 3, where 88% of the non-peptide amide groups were hydrolyzed, was soluble at pH 5.5. All of these preparations precipitated when the pH was adjusted to 4.0 and could be recovered by centrifugation. Whether the yield of high molecular weight, water-soluble zein can be increased is a matter of further research.

Hydrolysis with lime always resulted in denaturation or insolubilization of a portion of the zein. This denaturation is attributed to splitting out ammonia between non-peptide amide groups of adjacent molecules to form secondary amide linkages and thereby cross-linking to form 3-dimensional polymers: (pr= protein)

$$\text{Pr-CO-NH}_2 + \text{NH}_2\text{-CO-PR} \xrightarrow{\text{-NH}_3} \text{Pr-CO-NH-CO-Pr}$$

This type of a reaction is known to occur with polyacrylamide (Minsk and Kenyon, 1949), and the irreversible insolubilization of zein can also be explained on this basis. Undoubtedly, the formation of secondary amide cross-links is involved in the heat denaturation of most proteins. In the present study, insolubilization was not observed when strong alkalies were used, probably because the secondary amide linkages were hydrolyzed by the more drastic conditions.

Figure 3 compares the ultracentrifuge patterns of zein and the deamidated zein. From the sedimentation constant, the molecular weight is estimated at about 20,000 and the molecular size appears to be fairly uniform, considering the method of fractionation. Light-scattering measurements gave molecular weights of 17,000 to 20,000. Electrophoretic patterns showed a great deal of variance in the number of carboxyl groups on different molecules.

Solutions of the deamidated zein preparations showed no tendency to gel even at 30% concentrations. The aqueous solutions could be whipped into a meringue, and deamidated zein was successfully substituted for egg white in a recipe to make marshmallows. Five grams of Preparation No. 5 were dissolved in 75 mls. of water, 10 gms. of peanut oil added, and the mixture whipped into a stable emulsion. A 7% isotonic salt solution of Preparation No. 3 was repeatedly injected intravenously into rabbits and guinea pigs without any apparent toxic effects (Bollman,

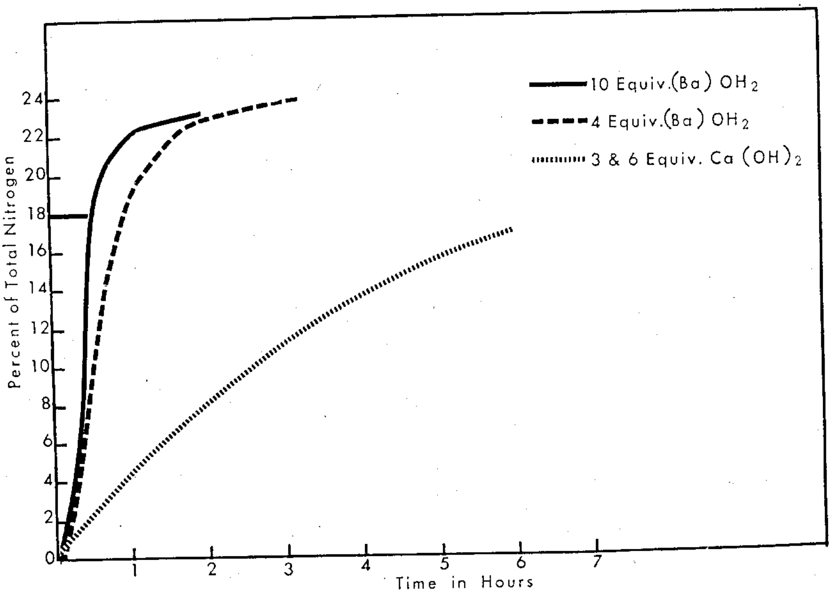
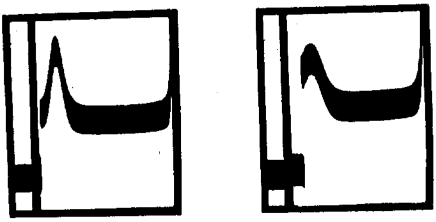


FIG. 2.—Rate of NH_3 liberation from zein by different equivalents of $Ba(OH)_2$ and $Ca(OH)_2$ based on non-peptide amide groups.


A. Zein (S_{20}=1.60)

B. Deamidated Zein (S_{20}=1.20)

FIG. 3.—Ultracentrifuge patterns, 255,000 g., 112 minutes, conc. 1.0% in 76% ethanol and 0.9% sodium acetate.

pers. comm., Feb. 7, 1951). The bland taste of the water-soluble zein indicates usefulness as a carrier or emulsifier in pharmaceutical preparations, as well as in bakery and pastry products.

SUMMARY

By controlled hydrolysis, the non-peptide amide groups of zein can be converted into carboxyl groups. When approximately 40% of these amide groups are hydrolyzed, zein becomes soluble in water at a neutral pH. This deamidated zein can be precipitated by adjusting the pH to 4.0 and recovered from solution.

Aqueous solutions of deamidated zein have whipping and emulsifying properties, indicating usefulness in food and drug products.

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