Water-Soluble Zein by Enzymatic Modification in Organic Solvents[1]

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ABSTRACT

Water-soluble zein was produced using a dual-phase, sequential enzymatic modification and ultrafiltration. Zein was first partially hydrolyzed in an organic solvent by a protease, followed by an aqueous phase hydrolysis with the same protease. Ultrafiltration of the reaction mixture with a 10,000 and/or a 30,000 molecular weight cut-off membrane yielded fractions with an average of 5,400 Da. A significant improvement in certain functional properties of zein was observed, controlled largely by the degree of hydrolysis (DH). Solubility increased from 0% for the un-

modified zein to 99% for enzyme-modified and ultrafiltered zein. At less than DH20, solubility was also a function of pH and membrane pore size. Foaming properties were affected by DH, membrane pore size, and pH of measurement. At a water activity of 0.97, moisture sorption was 2.6 g of water per gram of solids for the enzyme-modified and ultrafiltered hydrolysates, compared to less than 0.25 g of water per gram of solids for unmodified zein.

Today's formulated food products require cost-effective proteins with specific functional properties. Functional properties of proteins are those physicochemical attributes that lend desirable physical characteristics to a food and are responsible, in part, for certain textural and sensory properties of those foods. These functional properties include solubility, water and fat absorption, gelation, emulsion stability, whippability, and foaming and sensory properties (Kinsella 1976).

Compared to soy protein, corn protein has received little attention from the food industry as a potential food ingredient. No commercial corn protein concentrates or isolates exist, with the exception of zein produced in low quantities for very specialized applications (P. Freeman, *personal communication*). This may change in the near future, however. Large amounts of proteinaceous by-products are produced by corn refiners in the form of corn gluten meal (CGM, 69% protein db) and corn gluten feed (CGF, 24.5% protein db). These are primarily by-products of the manufacture of starch-based industrial products (e.g., fructose, dextrose, ethanol, etc.). The increasing volume of these industrial products results in a simultaneous increase in the protein by-products. Indeed, the viability of the corn ethanol industry relies heavily on the profits from the sale and marketing of the nonstarch products (protein, oil, and fiber). Thus, there is a need to find alternate uses for the nonstarch products that will yield a better return than animal feed does.

Zein is a storage protein located in "protein bodies" 1–2 μm in diameter (Wilson 1987, Abe 1989). In contrast to the albumins and globulins, zein has a high concentration of nonpolar amino acids such as leucine, isoleucine, valine, alanine, proline, and glutamine, rendering it soluble primarily in alcoholic solvents. Poor water solubility is a hindrance to the utilization of corn proteins as a food ingredient. Solubility in water is a key functional property because proteins generally have to be in a solution or in a fine suspension to exert other desirable properties (Kinsella 1976).

This article reports our studies on the enzymatic modification of zein, and the subsequent fractionation of the hydrolysis products by ultrafiltration, specifically for producing water-soluble zein. Enzyme hydrolysis was selected rather than chemical treatments because of milder process conditions, higher specificity, easier control of the reaction, and minimal formation of toxic by-products. Casella and Whitaker (1990) hydrolyzed zein using trypsin but obtained a very low degree of hydrolysis because the reaction was conducted in an aqueous environment. We have substantially improved the process by conducting the initial en-

zyme reaction in an organic solvent. Ultrafiltration (UF) membranes were used to separate the enzyme-modified proteins to produce corn protein and peptide fractions with improved functional properties.

MATERIALS AND METHODS

Zein (regular grade) was obtained from Freeman Industries Inc. (Tuckahoe, NY). Proximate analysis of zein was nitrogen (N) = 14.9%, ash = 0.93%, crude fat = 1.18% (all expressed as w/w db).

Enzymes selected for preliminary hydrolysis assays were: Alcalase (2.4L, type FG, serine protease extract, *Bacillus licheniformis*, 2.4 Anson units per gram) and SP-369 (an experimental, thermally stable bacterial protease). Both were obtained from Novo Laboratories, Wilton, CT. Pronase (a mixture of endo- and exopeptidases prepared from *Streptomyces griseus*) and papain (a plant protease from the latex of *Carica papaya*) were purchased from Calbiochem-Behring Co., La Jolla, CA. Milezyme APL 440 (a serine protease from *Bacillus licheniformis*) was obtained from Miles Biotechnology Products (Elkhart, IN).

Initial Enzyme Screening

The pH-drop method described earlier (Mannheim and Cheryan 1990) was used to measure the relative activity of the enzymes. Zein at 1% (w/v) was solubilized in 70% (v/v) ethanol, heated to 50°C, and adjusted to pH 8. The substrate-to-enzyme ratio (S/E, always expressed as w/w) was 23.4. The drop in pH during the reaction was recorded continuously for 10 min. Control experiments isolated pH-drop effects not caused by protease action. Instead of substrate in the reaction mixture, 1 mM phosphate buffer, preadjusted to the appropriate pH and temperature, was used. The enzyme was added and the pH-drop was monitored as above.

Batch Hydrolysis of Zein

The process involves several steps as shown in Figure 1. Zein was initially solubilized in an alcohol solution (e.g., 50–70%, v/v, isopropanol or 50–70% ethyl alcohol, as specified), adjusted to pH 9, and heated to 50°C. Alcalase was added at S/E 200, and the hydrolysis reaction was allowed to continue at a constant pH of 9 until no further alkali consumption was observed. The alcohol was removed by evaporation at 50°C. Water was added to bring the residue of the reaction mixture back to the original volume. The reaction was continued by redosing with enzyme (at S/E 200), maintaining pH 9 and 50°C. This second hydrolysis reaction was allowed to continue until the required degree of hydrolysis (DH) was obtained, followed by enzyme inactivation (at pH 4.2 for 30 min) and pH adjustment to 7 with 4N NaOH.

The extent of the reaction was monitored by the pH-Stat method (Adler-Nissen 1986). The amount of alkali consumed was measured and used to calculate the DH, defined as the ratio

of the number of peptide bonds cleaved to the total number of peptide bonds in the protein molecule (given in milliequivalents per gram of protein). DH was calculated using the following equation:

$$DH, \% = B \times N_b \times \alpha^{-1} \times M^{-1} \times (h_{tot})^{-1} \times 100 \qquad (1)$$

where B = base consumption (ml); N_b = base concentration (4N); α^{-1} = average degree of dissociation of α-amino groups = 1.01; M = mass of protein (g); h_{tot} = total number of peptide bonds in the protein substrate = 9.2 meq/g of protein.

The fractional conversion of protein in the batch reactor is expressed in terms of the nitrogen soluble in 10% trichloroacetic acid (TCA) in the reaction mixture as a percentage of total N present in the unhydrolyzed protein suspension. A correction was made for the initial TCA-soluble N content of the unhydrolyzed protein suspension (Mannheim and Cheryan 1990). The following equation was used to calculate conversion in the batch studies:

$$X = (P - P_o)/(S_o - P_o) \qquad (2)$$

where X = fractional conversion (percent conversion is 100X); P = product concentration (N in TCA-soluble fraction of hydrolysate); P_o = initial "product" concentration (N in TCA-soluble fraction of unhydrolyzed substrate); and S_o = initial substrate concentration (N in unhydrolyzed substrate).

Enzyme Stability in Organic Solvents

The stability of Alcalase in organic solvents was studied in a batch reactor using an enzyme concentration (E) of 0.5 g/L and pH 9 (adjusted with 4N NaOH). Samples (2 ml) were taken at 10-min intervals (for a total period of 90 min) and mixed with 8 ml of a corn gluten meal suspension (1.25%, w/v), preheated to the proper temperature (37 or 50°C), and adjusted to pH 9. After 10 min reaction, a 3-ml sample was withdrawn and promptly mixed with 3 ml of 20% (w/v) TCA. The N in the TCA-soluble

Fig. 1. Schematic of the two-phase sequential enzymatic modification of zein.

fraction was determined and used to calculate the relative activity of Alcalase.

Enzyme stability is expressed in terms of half-life ($t_{1/2}$):

$$t_{1/2} = 0.693/K_d \qquad (3)$$

where K_d = enzyme decay constant (hr^{-1}).

The enzyme decay constant was calculated as:

$$K_d = (2.303/t) \log (E_o/E_t) \qquad (4)$$

where t = assay time (hr); E_o = initial enzyme activity; E_t = enzyme activity at time t.

Fractionation of Hydrolysates by Ultrafiltration

The reaction mixture, after inactivation of the enzyme and adjustment to pH 7, was processed in series through two hollow-fiber UF membranes (A/G Technology, Needham, MA). First, the reaction mixture was recycled through a 30,000 molecular weight cut off (MWCO) membrane (UFP30-E-4). The permeate from this membrane (designated UFP30) was then passed through a 10,000 MWCO hollow-fiber membrane (UFP10-C-4). All products were dried in a vacuum oven and stored until needed for further analysis.

Analyses

Protein is expressed as total N $\times$ 5.7. Nitrogen was measured by the micro-Kjeldahl method (Method Ac-441, AOCS 1984). Nonprotein N is defined as soluble in 10% TCA (Bhatty 1973).

Molecular weight distribution of proteins and peptides was determined by size-exclusion high-pressure liquid chromatography (SE-HPLC) using a TSK G2000 SW column (7.5-mm i.d. $\times$ 30 cm, Tosoh Corp., Tokyo). The flow rate through the column was 1 ml/min, and the column temperature was 23 $\pm$ 2°C. A Spectronic 1001 UV-visible spectrophotometer was used as the detector (205 nm) and integrated with a personal computer. Dried samples were suspended in 0.067M phosphate buffer mobile phase (19%, v/v, monobasic sodium phosphate monohydrate and 81% dibasic sodium phosphate, pH 7.4) at a concentration of 10 mg/ml and filtered through a 0.45-μm filter. (In highly turbid solutions, the samples were first centrifuged at 1,500 $\times$ g for 10 min before microfiltration.)

The following standards (and molecular weights) were used to calibrate the column: bovine serum albumin (67,000), ovalbumin (43,000), chymotrypsinogen A (25,000), and ribonuclease A (13,700), all purchased from Pharmacia, Inc. (Piscataway, NJ). Trypsin inhibitor (6,700) was obtained from Accurate Chemicals (Westbury, NY). Hexa-L-tyrosine (997) was obtained from Sigma Chemical Co. (St. Louis, MO).

Functional Properties

The following products were evaluated: DH0—zein, unmodified, as received from Freeman Industries Inc. (Tuckahoe, NY); DH12—zein that had been enzyme-modified to a total (organic phase + aqueous phase) DH of 12; DH20—zein modified to a total (organic phase + aqueous phase) DH of 20. The permeate from zein hydrolysate purified by ultrafiltration through a 30,000 MWCO membrane is designated UFP30. The permeate from UFP30 passed through a 10,000 MWCO membrane is designated UFP10.

All the products had been adjusted to pH 7 with NaOH, dried in a vacuum oven, and stored in a refrigerator until analyzed.

Nitrogen solubility. The solubility of zein and its hydrolysate was measured by a modified protein dispersibility index (Method Ba 10-65, revised; AOCS 1984). The solubility was studied as a function of pH (3, 5, 7, and 9), DH, and pore size of UF membrane (10,000 or 30,000 MWCO). A 1% (w/v) aqueous suspension (100 ml) was prepared from the vacuum-dried sample. The pH was adjusted either by NaOH or HCl and the sample transferred into a 250-ml blender cup (Eberbach Corp., Ann Arbor, MI) placed on a Waring commercial blender (model 5011,
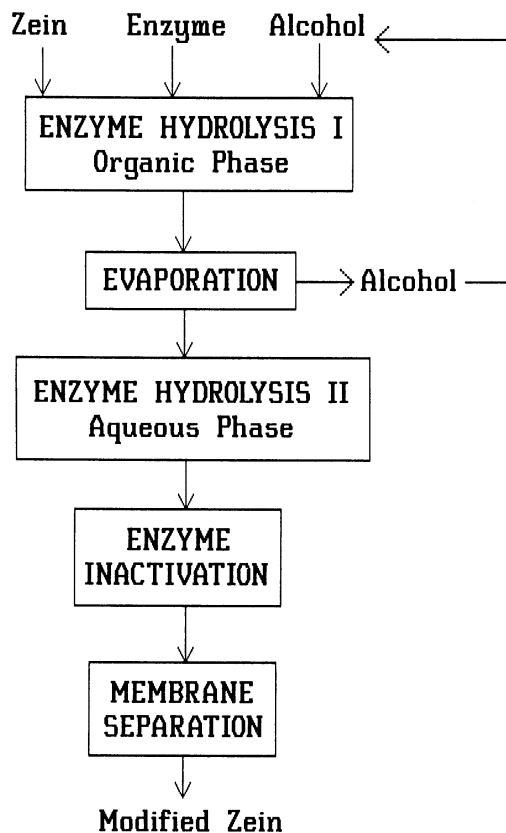
Waring Products, New Hartford, CT). After blending for 10 min, the suspension was held for 30 min in a 250-ml graduated cylinder before being transferred into two 50-ml centrifuge tubes. These tubes were centrifuged at 3,500 rpm (1,500 × g) for 10 min. The supernatant was analyzed by micro-Kjeldahl for total N. Nitrogen solubility is expressed as the percentage of the total N in the sample that was in the supernatant.

Turbidity. Turbidity of 1% (w/v) aqueous suspensions was evaluated by comparison of the optical density at 660 nm to deionized distilled water.

Foaming properties. Foaming properties of the 1% (w/v) aqueous suspensions were evaluated using the method suggested by Puski (1975), with slight modifications. After blending as described above for the solubility tests, the blended contents were transferred into a 250-ml graduated cylinder held at room temperature (23°C ± 1.0°C). The initial foam volume was measured promptly, and the residual foam volume was measured after 30 min. Foam stability is the percentage of the original foam volume left after 30 min.

Moisture sorption. The method of Lang et al (1981) using "mini-desiccators" was used to determine the sorption isotherms as a function of pH (3–9), DH, and UF membrane pore size.

RESULTS AND DISCUSSION

Enzyme Activity and Stability in Organic Media

The activity and stability of proteases in organic solvents is critical to the success of this concept. Preliminary experiments had indicated that 40–70% ethanol or isopropanol in water would be satisfactory for conducting the hydrolysis reaction (Mannheim 1991). Typical results of the pH-drop method of screening enzymes are shown in Figure 2. Alcalase, Milezyme, and Pronase showed the steepest pH-drop, whereas papain and SP-369 showed almost
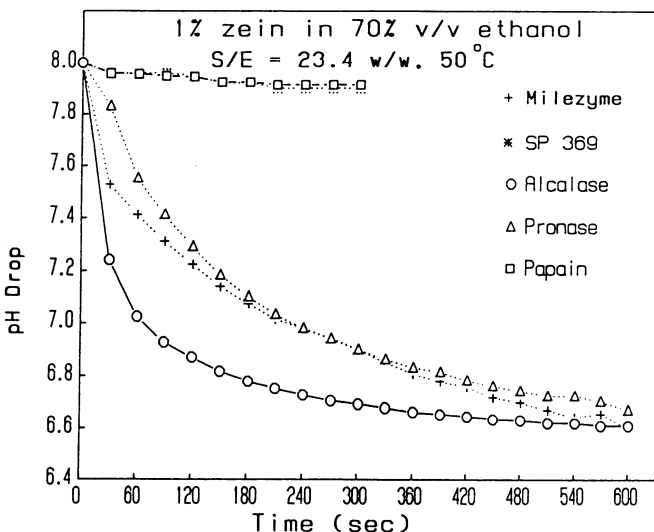
no pH drop. This indicated that the former enzymes were stable and active in 70% ethanol at pH 8. Alcalase was selected for further study.

The half-life of Alcalase, derived from decay coefficients (not shown here; see Mannheim 1991) are shown in Table I. There was essentially no enzyme decay in water at 37°C. Isopropanol
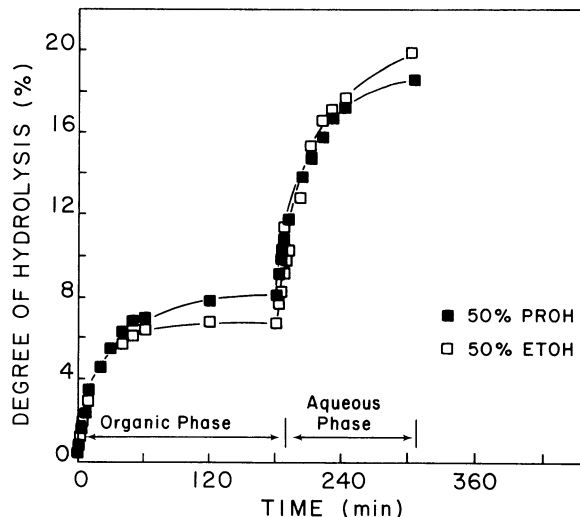
Fig. 3. Zein hydrolysis by Alcalase in the two-phase sequential process. Initial substrate concentration = 8% w/v, substrate-to-enzyme ratio = 200. First-stage reaction medium was either 50% isopropanol (PROH) or 50% ethanol (ETOH). Both stages were conducted at pH 9 and 50°C.
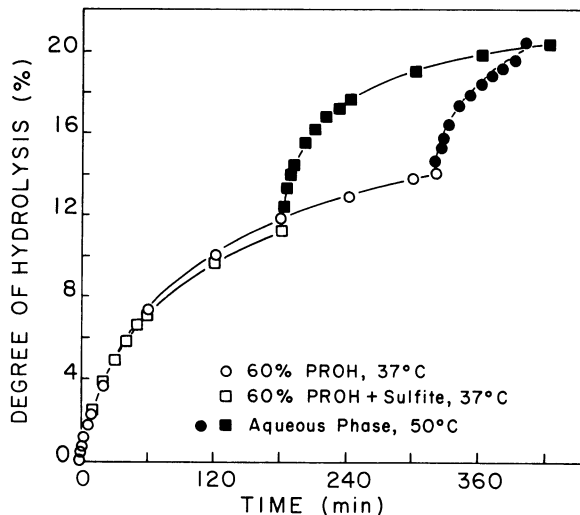
Fig. 2. Enzyme activity screening using the pH-drop method. S/E = substrate-to-enzyme ratio. Data for SP-369 is obscured by and is essentially the same as that of papain.

Fig. 4. Zein hydrolysis by Alcalase in the two-phase sequential process. Initial substrate concentration = 8% w/v, substrate-to-enzyme ratio = 200. First stage reaction medium was 60% propanol (PROH) at pH 9 and 37°C. The second stage (aqueous phase) reaction was done at pH 9 and 50°C.

TABLE I
Stability of Alcalase in Aqueous and Organic Solvents

Solution	Temperature (°C)	Half-life $t_{1/2}$ (hr)
Water	37	ND[a]
	50	15.3
50% isopropanol	37	10.2
	50	6.1
60% isopropanol	37	15.8
	50	3.4

[a] ND = not detectable, i.e., no enzyme decay observed during the period of the analysis.

TABLE II
Nitrogen Distribution in Zein-Alcalase Batch Hydrolysis Reaction
Conducted in 60% Isopropanol (Average of Two Runs)

Time (min)[a]	Total N (mg/ml)	Soluble N (mg/ml)	Insoluble N[b] (mg/ml)	Conversion (%)	Degree of Hydrolysis (%)
0	11.6	0.9	10.6	0.0	0.0
10	10.9	1.6	9.3	6.8	2.1
30	10.1	2.3	7.9	14.9	4.7
60	10.5	3.1	7.4	22.9	7.6
120	9.0	3.3	5.7	29.7	10.9
240	10.6	4.5	6.1	36.8	15.0

[a] After addition of enzyme to the reaction mixture.
[b] Insoluble N = total N − soluble N.

significantly reduced the stability of the enzyme. Temperature had a large effect. The manufacturer's recommended operating temperature for Alcalase in aqueous media is 50°C, but our results suggest that lowering the temperature of the reaction would be beneficial for the organic-phase reaction.

Batch Hydrolysis of Zein

Data obtained from our zein solubility and enzyme stability studies were used to design a unique two-stage process for the enzymatic modification of zein to yield a water-soluble product. The process (Fig. 1) is based on sequential hydrolysis conducted in two media: an organic solvent reaction followed by an aqueous phase reaction. This process was used because zein is insoluble in water, and no enzyme reaction takes place in aqueous media unless extreme pH conditions are used (e.g., pH = 1.6 or 12, conditions at which the enzyme is inactive). The organic solvent solubilizes some of the zein, allowing the enzyme access to the protein in the subsequent aqueous phase reaction.

Typical data for the sequential two-phase reaction is shown in Figure 3. In one experiment, the organic phase reaction was conducted in 50% isopropanol; in the other, 50% ethanol was used. An initial rapid hydrolysis (6.5% DH in the first hour) is followed by a very slow hydrolysis, reaching only 7–8% DH in 3 hr. As shown earlier, the enzyme appears to rapidly inactivate in the presence of the organic solvent. However, even this limited proteolysis was beneficial because the subsequent aqueous phase reaction was more rapid, going from 7–8% DH to 19–20% DH in the next 2 hr. In contrast, no zein hydrolysis occurred under similar conditions when only water was used as the reaction medium.

In Figure 4, the organic phase reaction was conducted at a lower temperature because the stability studies had shown the enzyme to be more stable at the lower temperature (Table I). This lower temperature was more effective: a higher degree of hydrolysis (12% DH) was obtained, under otherwise identical conditions, compared to that obtained at 50°C (8% DH). In one experiment shown in Figure 4, zein was solubilized in 60% isopropanol and treated with 0.4 mg of sodium sulfite per milliliter for 1 hr before hydrolysis. A similar sulfite treatment significantly improved hydrolysis of corn gluten meal proteins (Mannheim 1991), presumably by reducing disulfide bonds in the protein matrix and making the peptide bonds more susceptible to hydrolysis. However, for zein, no beneficial effect of sodium sulfite was observed in the organic phase reaction, perhaps because the zein contained mostly A and B fractions (Wilson 1987, 1988) that are low in cysteine and would not show a major effect.

The initial rapid hydrolysis rate (12% DH/hr in the first 30 min) was followed by a decreasing rate of reaction (4% DH/hr), until it reached 2.5% DH/hr at 5.5 hr reaction time. At the end of the organic phase reaction, the partially hydrolyzed zein was subjected to the aqueous phase reaction using otherwise identical conditions as the organic phase (i.e., same S/E and pH), except that the temperature was higher (50°C).

Nitrogen distribution and yield of soluble N in the zein-Alcalase reaction conducted in 60% isopropanol is shown in Table II. After 4 hr, Alcalase hydrolyzed about 37% of the zein at S/E 200. However, as the data in Figures 3 and 4 suggest, further zein hydrolysis is possible under the appropriate conditions (e.g., higher enzyme concentration, lower S/E ratios, longer reaction time of organic phase and/or aqueous phase).

Kinetic parameters of the zein-Alcalase reaction were calculated using the integrated form of the Michaelis-Menten equation known as the Henry equation (Segel 1976). In 60% isopropanol, the maximum rate (V_{max}) was 0.037 mg N(ml·min)$^{-1}$ versus 0.016 and 0.007 mg N(ml·min)$^{-1}$ for glutelin and CGM hydrolysis respectively (Mannheim 1991). The Michaelis constant (K_m) was 0.062% vs. 0.225 and 0.31% for glutelin and CGM, respectively.

Molecular Weight Distribution of Native and Modified Zein

Figures 5–9 show the SE-HPLC elution profile of native zein and the reaction products. Figure 5 shows the SE-HPLC profile of the soluble portion of unmodified zein, which is a very small proportion of the total zein in corn. (It should be remembered that this is not a profile of the entire zein fraction because it could not be solubilized for the HPLC.) It shows two major peaks at 3,700 and 27,700 Da, and several minor peaks in the 1,000–15,000 and 82,000–105,000 Da ranges.

Figure 6 shows the elution profile of a DH12 zein hydrolysate. Two peaks were observed at elution volume (E_v) = 7 ml and 13.8 ml, corresponding to molecular weights of 200,000 and 5,400, respectively. After the first ultrafiltration of the DH12 reaction mixture through the 30,000 MWCO membrane, the chromato-
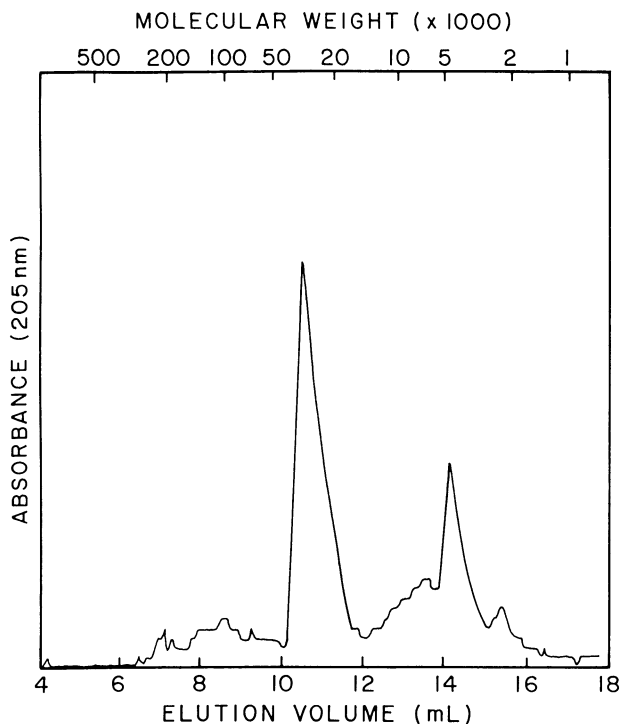

Fig. 5. Size-exclusion high-pressure liquid chromatography elution profile of native zein. Molecular weight distribution of portion of zein that is soluble in 0.067M phosphate buffer after centrifugation and filtration.
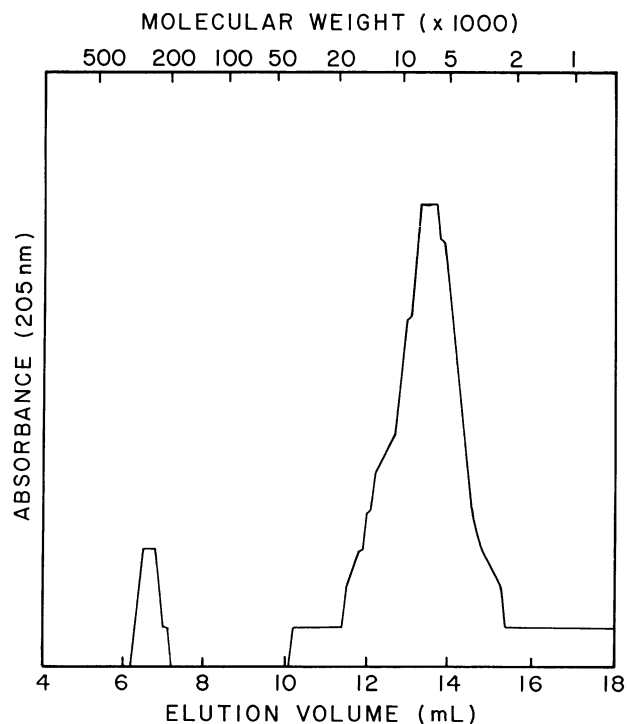

Fig. 6. Size-exclusion high-pressure liquid chromatography elution profile of DH12 zein hydrolysate.

gram of the DH12 zein hydrolysate (Fig. 7B) still showed the 5,400-Da peak but proportionally less of the 200,000+ Da peak. Further refining of the UFP30 permeate, by passing it through a 10,000 MWCO membrane, reduced this large molecular weight fraction further, as shown in Figure 7A, resulting in a fairly pure peptide fraction.
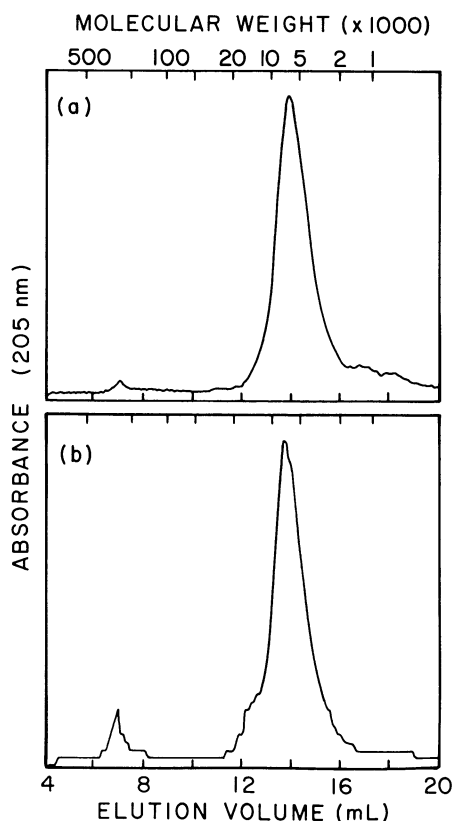
Figure 8 shows the SE-HPLC elution profile of a zein hydrolysate at DH20. The same two peaks appear in this chromatogram at $E_v = 7$ ml and 13.8 ml. The UFP10 permeate of the DH20 reaction mixture is shown in Figure 9. Ultrafiltration removed the high molecular weight components (the first peak shown in Figure 8 is absent in the UFP10 permeate shown in Figure 9), and the UFP10 permeate contained only the 5,400-Da peak. A similar elution profile was obtained for the UFP30 permeate of the DH20 reaction mixture (not shown here; see Mannheim 1991).

One unexpected aspect of these elution profiles is the presence of even a small amount of the high molecular weight fraction

Fig. 7. a, Size-exclusion high-pressure liquid chromatography elution profile of a DH12 zein hydrolysate ultrafiltered through both UFP30 and UFP10 membranes. **b,** Elution profile of a DH12 zein hydrolysate ultrafiltered only through UFP30 membrane. DH12 reaction mixture was the same as the reaction mixture shown in Fig. 6.
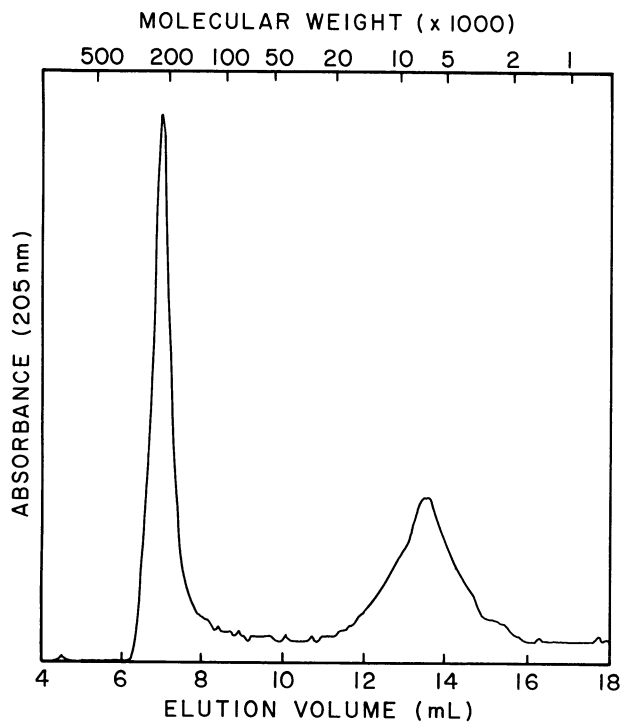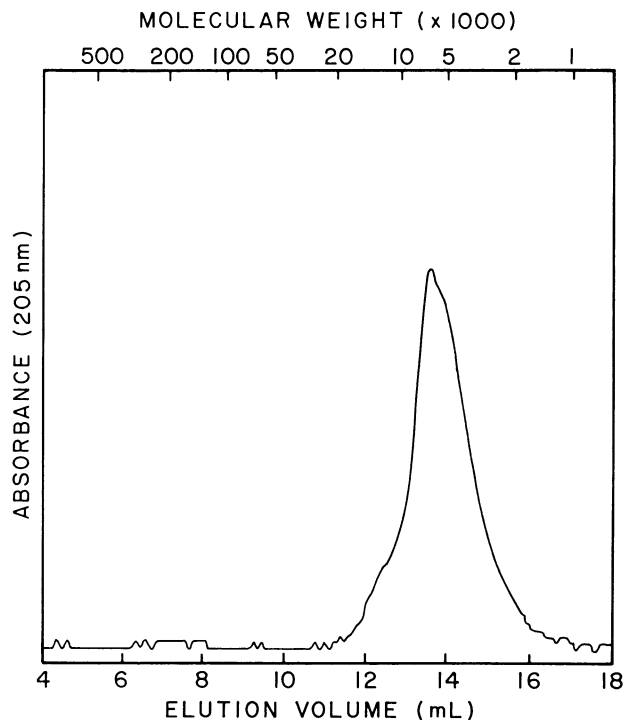

Fig. 9. Size-exclusion high-pressure liquid chromatography elution profile of DH20 zein hydrolysate ultrafiltered with both UFP30 and UFP10 membranes. The DH20 reaction mixture was the same as shown in Fig. 8.
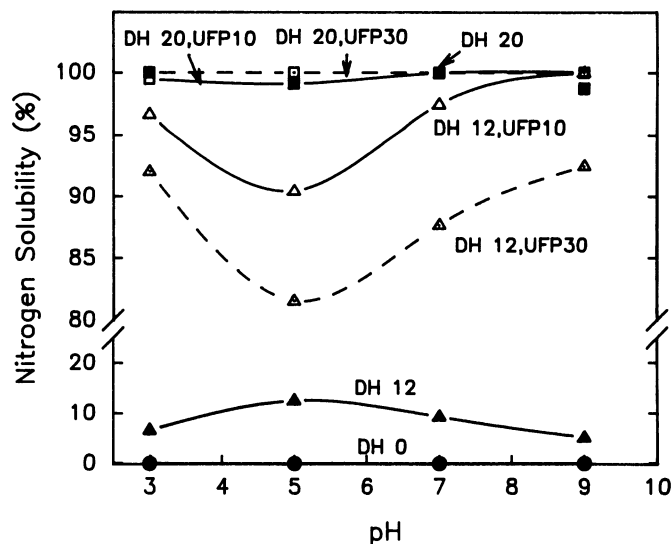

Fig. 8. Size-exclusion high-pressure liquid chromatography elution profile of zein hydrolysate at DH20.

Fig. 10. Nitrogen solubility of zein and its hydrolysates. Effect of degree of hydrolysis (DH), pH, and membrane pore size. ● = zein hydrolysate at DH0 with no ultrafiltration; ▲ = DH12 with no ultrafiltration; ■ = DH20 with no ultrafiltration; △ = DH12 ultrafiltered through UFP10 and UFP30; △ = DH12, ultrafiltered through UFP30; □ = DH20 ultrafiltered through UFP10 and UFP30; ⊡ = DH20 ultrafiltered through UFP30.

($E_v = 7$ ml) in the UF permeates. Such membranes are not expected to allow passage of such large protein molecules (Cheryan 1986). This could be due to postfiltration aggregation of peptides. Wilson (1988), using sodium-dodecyl sulfate-polyacrylamide gel electrophoresis, found two major fractions of 24,000–27,000 Da in Freeman zein. Paulis and Bietz (1988) suggested that the disulfide bonds cause oligomer formation in zein. Using reverse-phase HPLC, they found that these oligomers are composed of 22,000–24,000 Da fractions. These oligomers are apparently involved in extensive noncovalent (e.g., hydrogen) bonding that facilitates formation of oligomers. This phenomenon might be the reason for the appearance of the high molecular weight first peak (Fig. 7) after ultrafiltration (i.e., monomers of 22,000–24,000 Da permeate through the membrane and later form oligomers through disulfide and/or hydrogen bonds).

Functional Properties

Nitrogen solubility. Figure 10 summarizes nitrogen solubility of the unmodified zein (designated DH0 in the graphs) and zein hydrolysates, with and without ultrafiltration. It appears that DH12 is not sufficient to extensively render zein very soluble or dispersible in aqueous solutions. In contrast, 100% solubility

TABLE III
Turbidity (Absorbance at 660 nm) of Zein-Alcalase Hydrolysates as a Function of pH, Degree of Hydrolysis (DH), and Ultrafiltration (UF) Processing

Treatment	pH	DH (%)[a,b] 0	12	20
No UF	3	0.14 f	0.19 A	1.05 cB
	5	0.15 f	0.20 A	1.17 cC
	7	0.18	0.24 A	0.68 A
	9	0.17 f	0.21 A	0.50
UFP10	3	...	0.84	0.01 F
	5	...	1.63 bA	0.00 F
	7	...	0.80	0.00 F
	9	...	0.04 F	0.00 F
UFP30	3	...	1.86 aA	0.10
	5	...	2.37 aB	0.07
	7	...	2.37 aB	0.02 fA
	9	...	0.67	0.01 fA

[a] Same lower case letters indicate no significant ($P < 0.5$) difference.
[b] Different upper case letters indicate a significant ($P < 0.05$) difference due to treatment.

was achieved at DH20. The membrane had a very significant effect ($P < 0.05$; see Mannheim 1991 for statistical analysis) at DH12. Ultrafiltration increased solubility from an average of 8.4% for the nonfiltered DH12 to above 80% for the UF DH12 hydrolysates. The UFP30 product had slightly less solubility than the UFP10 product had, due perhaps to the larger molecular weight fractions in the UFP30 permeate. Chromatograms of the UF zein hydrolysates showed a distinct peak equivalent to 200,000+ Da, due perhaps to post-UF aggregation of peptides.

Because the DH20 product is already completely soluble across the entire pH range studied, membrane filtration had little effect on solubility.

Turbidity. Table III shows the turbidity of the zein hydrolysates as a function of pH, DH, and UF. Turbidity was significantly lower for DH0 samples than for DH20. Interestingly, the UF permeates at DH12 had a higher turbidity than that of the unfiltered samples. This is probably due to the occurrence of insoluble reaggregated peptides (as mentioned above).

Foaming properties. The effect of DH on foam volume of zein hydrolysates is shown in Figure 11. There was no foam formation for the DH0 and DH12 products, perhaps because they were not soluble enough (Fig. 10). The DH20 hydrolysate at pH 9 had a high foam volume. The UFP10 permeates of zein hydrolysates had a significantly higher initial foam volume than the unfiltered controls (at all pH values, except DH12/UFP10 at pH 3). The best foams were displayed by DH12/UFP30, a partially hydrolyzed zein ultrafiltered with a larger pore membrane. Thus, initial foam volume seems to be correlated with pH, solubility, and DH: the higher the pH and DH, the larger the foam volume. The ideal UF membrane would appear to be one that allows passage of primarily soluble, large molecular weight proteins and peptides.

However, the stability of the foams was poor. Almost all samples showed a complete collapse of the foam after 30 min (Mannheim 1991). Apparently, peptides of 5,400 Da are capable of film formation at the air-water interface but lack the strength to maintain the foam. Similar effects were reported by Casella and Whitaker (1990) with enzyme-modified zein and by Deeslie and Cheryan (1988) with enzyme-modified and UF soy protein.

Moisture sorption. Zein hydrolysates had much higher water sorption capacity than unmodified zein (Fig. 12). Enzymatic modification of the quarternary and tertiary structure of proteins exposes ionizable polar amino acids (containing free amino and carboxyl groups) such as glutamate, which is abundant in zein (166 mM per 100 g of protein, according to Wright [1987]). These
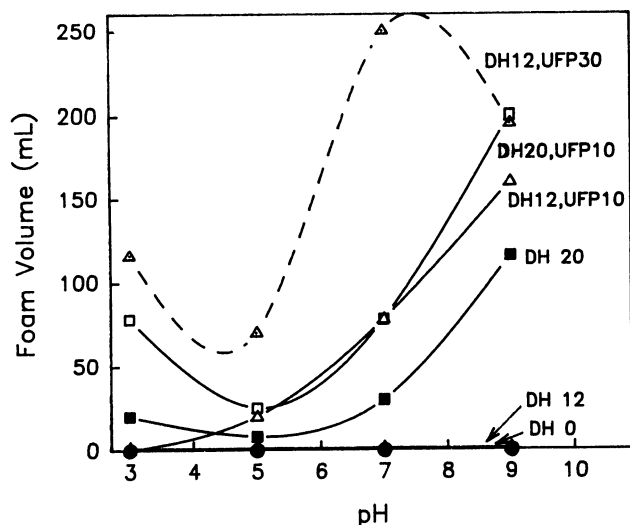
Fig. 11. Foaming properties of zein and its hydrolysates. ● = zein hydrolysate at DH0 with no ultrafiltration; ▲ = DH12 with no ultrafiltration; ■ = DH20 with no ultrafiltration; △ = DH12 ultrafiltered through UFP10 and UFP30; △ = DH12 ultrafiltered through UFP30; □ = DH20 ultrafiltered through UFP10 and UFP30.
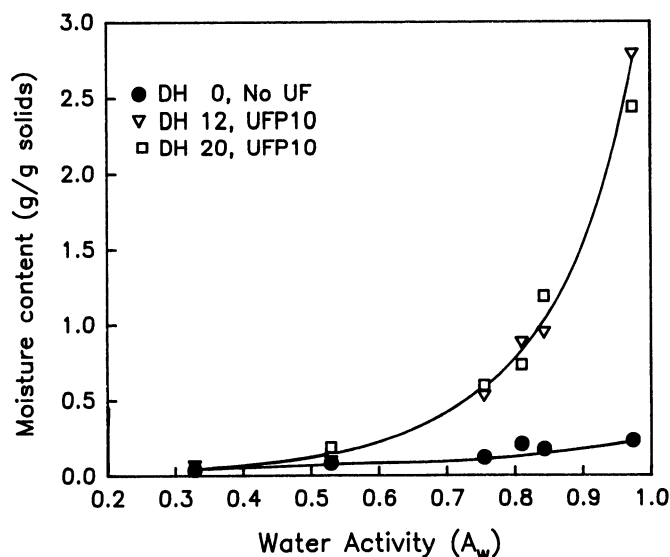
Fig. 12. Moisture sorption properties of zein and its hydrolysates. ● = zein hydrolysate at DH0 with no ultrafiltration; △ = DH12 ultrafiltered through UFP10 and UFP30; □ = DH20 ultrafiltered through UFP10 and UFP30.

amino acids are capable of binding almost three times more water than nonionized polar groups are (Chou and Morr 1979). In addition, in the intermediate water activity range, peptide linkages aid in binding water after the polar side chains have been saturated.

Improvement in moisture sorption by enzyme hydrolysis has been reported by Beuchat et al (1975) and Puski (1975). Similar beneficial effects of enzyme hydrolysis and membrane filtration were observed for soy protein hydrolysates (Deeslie and Cheryan 1988). However, at a water activity (A_w) of 0.973, moisture sorption was 50% higher for the zein hydrolysates than for UFP10 permeates of the soy hydrolysate.

CONCLUSIONS

Enzymatic modification combined with membrane technology was used to produce zein hydrolysates that are water soluble and possess improved functional properties. This should alleviate some of the problems hindering the utilization of corn proteins as functional food ingredients. Previous efforts at modifying corn proteins focused on chemical or physical methods that involved addition of chemicals or utilization of extreme processing conditions (e.g., temperature and pH) that precluded regulatory approval or were economically unfeasible, or did not result in products that were truly water soluble over a wide pH range. On the other hand, our water-soluble zein shows a substantial improvement in selected functional properties.

Enzyme modification by itself enhanced several functional properties. Ultrafiltration of the reaction mixture generally improved the properties further. Solubility of the zein increased from essentially from 0 to 99%. Foaming properties and moisture sorption are much better than those of the unmodified zein. Moisture sorption, in particular, may prove valuable in the food industry to prevent syneresis or water loss in breads, soups, cakes, sausages, and frozen products.

The yield of UF enzyme-modified zein in our experiments was 30–37% of the starting material. This is substantially higher than the 1.5–2% yields obtained by Casella and Whitaker (1990). The yield could be improved by continuing the reaction for a longer time or by recycling the larger peptides and/or unhydrolyzed protein for further reaction, e.g., in a continuous membrane reactor system (Cheryan 1986; Mannheim and Cheryan 1990; Deeslie and Cheryan 1988, 1992). Although not specifically studied in this research, the residue of the reaction mixture (i.e., the retentate) is expected to be of value because it contains a mixture of hydrolyzed and partially hydrolyzed zein. This mixture may have certain desirable functional properties such as foaming, emulsification, or gelation (Deeslie and Cheryan 1988, 1992; Casella and Whitaker 1990). Thus, an industrial process could generate a variety of functional, enzyme-modified zein fractions with high overall yields.

LITERATURE CITED

AMERICAN OIL CHEMISTS SOCIETY. 1984. Official and Tentative Methods of the AOCS, 3rd ed. Method Ac-441; Method Ba 10-65, revised. The Society: Chicago, IL.

ABE, M. 1989. Corn proteins. Page 93 in: Food Emulsifiers. Chemistry, Technology, Functional Properties and Applications. G. Charalambous and G. Doxastakis, eds. Elsevier: New York.

ADLER-NISSEN, J. 1986. Enzymic Hydrolysis of Food Proteins. Elsevier: Amsterdam.

BEUCHAT, L. R., CHERRY, J. P., and QUINN, M. R. 1975. Physicochemical properties of peanut flour as affected by proteolysis. J. Agric. Food Chem. 23:616.

BHATTY, R. S. 1973. Extraction of nonprotein nitrogen from oilseed meals with different solvents. Cereal Chem. 50:329.

CASELLA, M. L. A., and WHITAKER, J. R. 1990. Enzymatically and chemically modified zein for improvement of functional properties. J. Food Biochem. 14:453.

CHERYAN, M. 1986. Ultrafiltration Handbook. Technomic: Lancaster, PA.

CHOU, D. H., and MORR, C. V. 1979. Protein-water interactions and functional properties. J. Am. Oil Chem. Soc. 56:53A.

DEESLIE, W. D., and CHERYAN, M. 1988. Functional properties of soy protein hydrolyzates from a continuous ultrafiltration reactor. J. Agric. Food Chem. 36:26.

DEESLIE, W. D., and CHERYAN, M. 1992. Fractionation of soy protein hydrolysates using ultrafiltration membranes. J. Food Sci. 57:411.

KINSELLA, J. L. 1976. Functional properties of proteins in foods: A survey. CRC Crit. Rev. Food Sci. Nutr. 7:219.

LANG, K. W., MCCUNE, T. D., and STEINBERG, M. P. 1981. A proximity equilibrium cell for determination of sorption isotherm. J. Food Sci. 46:936.

MANNHEIM, A. 1991. Enzymatic modification and fractionation of corn protein. Ph.D. thesis. University of Illinois, Urbana-Champaign.

MANNHEIM, A., and CHERYAN, M. 1990. Continuous hydrolysis of milk protein in a membrane reactor. J. Food Sci. 55:381.

PAULIS, J. W., and BIETZ, J. A. 1988. Characterization of zeins fractionated by reverse-phase high-performance liquid chromatography. Cereal Chem. 65:215.

PUSKI, G. 1975. Modification of functional properties of soy proteins by proteolytic enzyme treatment. Cereal Chem. 52:655.

SEGEL, I. H. 1976. Biochemical Calculations. John Wiley and Sons: New York.

WILSON, C. M. 1987. Proteins of the kernel. Page 273 in: Corn: Chemistry and Technology. S. A. Watson and P. E. Ramstad, eds. Am. Assoc. Cereal Chem.: St. Paul, MN.

WILSON, C. M. 1988. Electrophoretic analysis of various commercial and laboratory-prepared zeins. Cereal Chem. 65:72.

WRIGHT, K. N. 1987. Nutritional properties and feeding value of corn and its by-products. Page 447 in: Corn: Chemistry and Technology. S. A. Watson and P. E. Ramstad, eds. Am. Assoc. Cereal Chem.: St. Paul, MN.