

Enzyme-Modified Proteins from Corn Gluten Meal: Preparation and Functional Properties

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Functional properties of proteins in corn gluten meal (CGM) can be improved by enzyme hydrolysis combined with membrane technology. CGM was treated with a protease (Alcalase), resulting in 30–50% of the proteins being converted to soluble peptides. Conversions were higher when CGM was pretreated with cysteine or sulfite. Solubility and clarity of the enzyme-modified proteins were better at higher degrees of hydrolysis (DH). Higher DH increased initial foam volume but decreased foam stability. Membrane filtration of the hydrolyzed CGM reaction mixture resulted in two peptide fractions, as determined by size-exclusion high-performance liquid chromatography. Protein solubility of the membrane-permeable fraction was 90–99% compared with 8% for unmodified proteins. Larger-pore membranes improved foaming but decreased solubility and clarity. Moisture sorption at a water activity of 0.97 was 3.75 g water per gram of enzyme-modified/ultrafiltered CGM, compared with 0.2 g/g for the unmodified CGM.

KEY WORDS: Corn gluten meal, functional properties, proteins, wet milling.

Corn gluten feed and corn gluten meal (CGM) are by-products of the corn processing industry. Their major market today is animal feed (1). Increasing demand for the starch component of corn, which is used to make sweeteners, ethanol and other biochemicals, imitation fats and other modified starch products, will require the simultaneous development of new uses for the nonstarch by-products in order for the corn refiners to maintain their economic viability.

CGM is the dehydrated protein stream resulting from starch separation in corn wet milling, containing a minimum of 60% total protein. The protein fraction of CGM is composed mainly of zein, a highly hydrophobic protein that is soluble in isopropanol or ethanol, and glutelin, which is soluble in aqueous alkaline solutions (2). Unfractionated corn proteins in their native state possess poor functionality compared to other commercial protein sources, such as milk, whey and soy. This is related primarily to their low solubility in aqueous systems under the conditions of pH and ionic strength occurring in most human food products. Proteins generally have to be in solution or in a fine suspension to develop their desirable functional properties (3).

To enhance the utilization of corn proteins in human food products, we have investigated the use of enzyme hydrolysis to modify certain functional properties. Enzyme hydrolysis may be preferable to chemical treatments because of milder process conditions, higher specificity, easier control of the reaction and minimal formation of by-products. Adler-Nissen (4,5) found that limited proteolysis of corn gluten significantly increased water binding capacity and solubility. Hard-

wick and Glatz (6) reported on the effect of enzyme concentration and size reduction on CGM hydrolysis, but functional properties of the enzyme-modified protein were not reported. Messinger *et al.* (7) partially hydrolyzed corn germ protein isolate with trypsin and pepsin.

The approach taken in this research was to use controlled enzyme hydrolysis and membrane technology to isolate specific fractions. These techniques were effective in producing hydrolyzates from soybeans with improved functional properties (8–11). The specific objective of our work was to develop a method for producing corn protein hydrolyzates and to evaluate selected functional properties, such as nitrogen solubility and clarity of aqueous solutions, foaming and moisture sorption as functions of the degree of hydrolysis (DH) and pore size of the ultrafiltration membrane used for fractionation.

EXPERIMENTAL PROCEDURES

Materials. CGM was obtained from A.E. Staley Manufacturing Co. (Decatur, IL). The following enzymes were selected for preliminary hydrolysis assays: Alcalase 2.4L, type FG [trademark for a serine protease made from *Bacillus licheniformis*, 2.4 Anson units (Au)/g], Neutrase 0.5L (trademark for metalloprotease made from *Bacillus subtilis*, 0.5 Au/g), chymotrypsin and SP-369 (experimental, thermally stable bacterial protease), all contributed by Novo Laboratories Inc. (Wilton, CT). Pronase [trademark for a mixture of endo- and exopeptidase(s) prepared from *Streptomyces griseus*] and papain were purchased from Calbiochem-Behring Corp. (La Jolla, CA). Trypsin (224 Units/mg) was purchased from Worthington Biochem. Corp. (Freehold, NJ), while Milezyme APL 440 (a serine protease from *Bacillus licheniformis*) was contributed by Miles Inc. (Biotechnology Products Division, Elkhart, IN). Glutathione reductase type IV (from baker's yeast), succinic acid and sodium sulfite were purchased from Sigma Chemical Corp. (St. Louis, MO).

Batch hydrolysis. The experimental procedure was similar to that described by Hardwick and Glatz (6). The substrate was prepared by suspending CGM in preheated (50°C) deionized water adjusted to the required pH with 4 N NaOH. The selected enzyme was added, and the reaction was allowed to proceed under constant stirring, temperature and pH. In the case of the controlled hydrolysis runs, the reaction was stopped at the desired end-point by lowering the pH to 4.2 with 4N HCl or heating at 100°C for 10 min. The reaction mixture was then air-dried and stored in a desiccator at room temperature until analysis.

The extent of the reaction was monitored by the pH-Stat method (5). 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 milli-equivalents/gram protein). DH was calculated from the following equation (5):

$$\text{DH (\%)} = B \times N_b \times \alpha^{-1} \times (M)^{-1} \times (h_{\text{tot}})^{-1} \times 100 \quad [1]$$

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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); and h_{tot} = total number of peptide bonds in the protein substrate = 9.2 milli-equivalents/gram protein.

The fractional conversion of the protein(s) in the batch reactor was calculated as the total nitrogen (N) soluble in 10% trichloroacetic acid (TCA) in the reaction mixture as a percentage of total nitrogen present in the unhydrolyzed protein suspension. A correction was made for the initial TCA-soluble N content present in the unhydrolyzed protein suspension (12). The following equation was used to calculate conversion in the batch studies:

$$X = (P - P_o)/(S_o - P_o) \quad [2]$$

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

Pretreatment of CGM. To improve hydrolysis rates, CGM was subjected to the following pretreatments: (i) L-Cysteine: The reaction mixture was adjusted to pH 9 and then heated to 50°C. L-Cysteine (Nutritional Biochem. Corp., Cleveland, OH) was added at either 0.5 or 1.5 mg/mL of the reaction mixture. The reaction was conducted with mixing for 60 min before enzyme addition. (ii) Reductase enzyme: The reaction mixture was adjusted to pH 5.5 at 25°C, and then disulfide glutathione reductase (0.05 or 0.1 mg/mL) was added. The reaction was allowed to proceed for 60 min with stirring. The pH was then adjusted to 9 and the temperature was adjusted to 50°C before adding the enzyme. (iii) Succinate: The reaction mixture was adjusted to pH 9 and then heated to 50°C. Succinic anhydride (10 or 20 g/100 g substrate) was then added, and the mixture was incubated for 60 min. (iv) Preheating: The reaction mixture was adjusted to pH 9, heated to 90°C and held at 90°C for either 15 or 60 min. (v) Sulfite: The reaction mixture was adjusted to pH 9, heated to 50°C, sodium sulfite (0.4 or 1.5 mg/mL of reaction mixture) was then added, and the mixture was incubated for 60 min.

The effectiveness of the above pretreatments (expressed in terms of kinetic parameters) were evaluated under the following reaction conditions: CGM concentration (S_o) = 1% wt/vol, substrate-to-enzyme (E) ratio (S_o/E) = 100 w/w, pH 9, 50°C and reaction time of 4 h.

Fractionation of hydrolyzates by ultrafiltration. The fractionation process used to obtain the CGM hydrolyzates (Fig. 1) used two hollow-fiber membranes obtained from A/G Technology (Needham, MA): UFP10-C-4 and UFP30-E-4, rated at 10,000 and 30,000 molecular weight cutoff (MWCO), respectively. The reaction mixture, after inactivating the enzyme as described earlier, was ultrafiltered first through the larger-pore membrane (UFP30). The permeate from this operation was then sent through the UFP10 membrane. The protein hydrolyzate in the permeates was designated "UFP30" and "UFP10", respectively.

Analytical methods. N content was measured by micro-Kjeldahl method Ac 4-41 of the AOCS (13). Protein content was estimated as $N \times 6.25$ unless specified other-

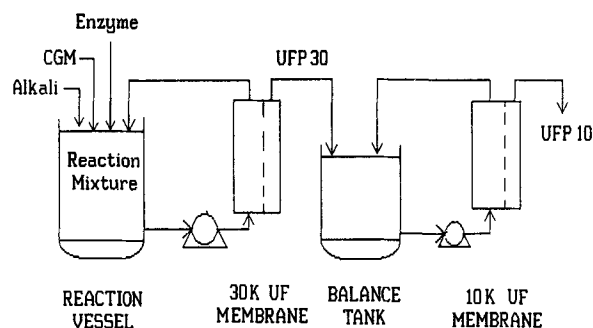


FIG. 1. Schematic of system for hydrolysis of corn gluten meal (CGM) and membrane filtration.

wise. Molecular weight distribution was determined by a high-performance gel filtration column (TSK G2000 SW, 7.5 mm \times 30 cm, Tosoh Corp., Tokyo, Japan). The high-performance liquid chromatography (HPLC) system was coupled to a Spectronic (Rochester, NY) 1001 ultraviolet (UV)-Visible spectrophotometer (as the detector) and an IBM (Armonk, NY) PC computer (for data acquisition). Dried samples of the reaction mixtures, UF retentates and permeates were suspended in a phosphate buffer mobile phase (0.067M, pH 7.4; 19% vol/vol monobasic sodium phosphate monohydrate, and 81% dibasic sodium phosphate) at a concentration of 10 mg/mL and filtered through a 0.45- μ m filter (highly turbid solutions were centrifuged at 1500 \times g for 10 min, prior to filtration). Samples were then injected into a 20- μ L injection loop of the injector. Operating conditions of the size-exclusion (SE) HPLC system were ambient temperature ($23 \pm 2^\circ\text{C}$); flow rate, 1.0 mL/min; absorbance, 205 nm.

Nitrogen solubility. The solubility of native and enzyme-modified CGM proteins were determined by a modified protein dispersibility index (PDI) method (14). Solubility was determined as functions of pH, pretreatment, DH and pore size of UF membrane (10,000 and 30,000 MWCO). A 1% (wt/vol) aqueous suspension was prepared, its pH preadjusted by adding either 1N NaOH or 1N HCl, and then blended at high speed in a Waring Commercial Blender (Model 5011, Waring Products Division, New Hartford, CT). After blending for 10 min, the suspension was held for 30 min and then centrifuged at 1500 \times g for 10 min. Samples of the supernatant were analyzed for total protein. Nitrogen solubility is expressed as the percentage of total nitrogen of the original sample present in the supernatant.

Clarity/turbidity. Dry samples were made to 1% (wt/vol) in deionized distilled water, the pH was adjusted with 1N HCl or 1N NaOH, and the optical density was determined against a deionized distilled-water blank in a spectrophotometer at 660 nm. Statistical analysis of the control and sulfite-treated samples was done separately from the UFP samples (15).

Foaming properties. Foam volume of the 1% (wt/vol) aqueous suspensions of the CGM or its hydrolyzates was evaluated by means of the method suggested by Puski (16), with slight modifications. The blended contents were transferred into a 250-mL graduated cylinder held at room temperature ($23^\circ\text{C} \pm 1.0^\circ\text{C}$). The initial foam volume was measured promptly and the residual foam volume was measured after 30 min. Foam stability is expressed as the

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percent of the original foam that was measurable after 30 min of standing.

Moisture sorption. Moisture sorption isotherms were determined by the method of Lang *et al.* (17).

RESULTS AND DISCUSSION

CGM hydrolysis. The pH-drop method (9,12) was used to measure the relative *in vitro* activity of the enzymes. This screening procedure is rapid and provides an indication of the relative activity of the enzyme. Based on these studies (15), Alcalase was selected because it appeared to possess the best combination of high activity and stability.

In the CGM-Alcalase batch hydrolysis (Fig. 2), the DH value obtained after 4 h with an So/E of 200 (w/w) was 27.7%. This was equivalent to a substrate conversion of 35.9%. In comparison, Hardwick and Glatz (6) achieved 53% substrate conversion after 48 h, with Alcalase at So/E ratios of 256–512 wt/vol.

The Michaelis-Menten parameters for the CGM-Alcalase reaction (Table 1) were determined from a Lineweaver-Burke analysis of data from kinetics experiments (15). The K_m value of 0.31% (wt/vol) compared well with the value of 0.379% obtained by Hardwick and Glatz (6) and was within the K_m range of 0.11–0.5% for casein-Alcalase batch hydrolysis (5,12). The V_{max} for CGM-Alcalase hydrolysis was 0.0068 mgN/mL·min, which was much lower than the V_{max} of 0.494 mgN/mL·min for casein-Alcalase hydrolysis (12). This large difference might be due to the greater steric hindrance of the CGM proteins.

The graph of enzyme concentration *vs.* CGM conversion (Fig. 3) showed two kinetic zones: an enzyme-dependent zone, where a dramatic increase in substrate conversion was observed at enzyme concentrations between 0.01 and 0.5 mg/mL; and an enzyme-independent zone, at concentrations greater than 0.5 mg/mL, where enzyme level had little or no effect on conversion. The effect of substrate concentration is shown in Figure 4. There is a sharp drop in substrate conversion as the substrate concentration increases, at a fixed enzyme concentration. The important operating parameter is the substrate-to-enzyme ratio (So/E) and its effect on the conversion (8–12). Our data indicate that, for maximum substrate conversions per unit weight of enzyme, the optimum So/E ratio is about 20.

Alcalase apparently cannot completely hydrolyze CGM proteins. The maximum we obtained (using sulfite-pretreated substrate) was about 50% conversion to TCA-soluble nitrogen, even at low So/E values. Alcalase is an alkaline serine-protease that attacks peptide bonds randomly, but has preference for peptide bonds adjacent to hydrophobic and/or aromatic amino acids (18). With corn proteins, a large proportion of the susceptible peptide bonds are apparently inaccessible to the enzyme. Glutelin has a large number of inter- and intramolecular disulfide bonds (19–21) that create a closed compact structure, which probably restricts access of the relatively larger enzyme molecule.

Hardwick and Glatz (6) suggested the existence of a "hydrolysis-resistant core" amounting to about 46.8% of the proteins in CGM. Argos *et al.* (22) described a structural model for zein in CGM, where this storage protein exists as "zein bodies", which are highly compact and ex-

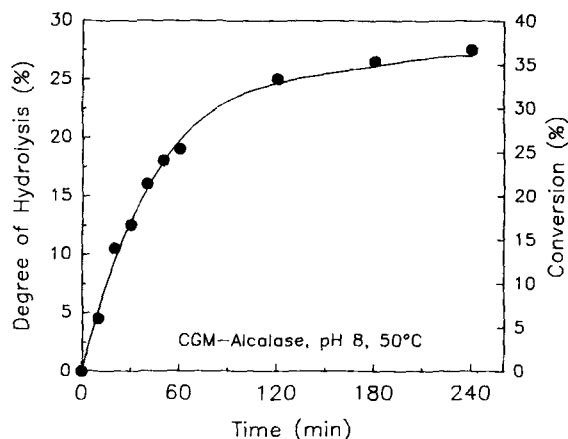


FIG. 2. Hydrolysis of corn gluten meal (CGM) by alcalase. Data are presented as degree of hydrolysis (% DH) and substrate conversion (%) *vs.* time of reaction. Initial substrate concentration (So) = 8% wt/vol CGM, So/E = 200 w/w. Reaction was conducted at pH 8, 50°C.

TABLE 1

Kinetic Parameters for CGM-Alcalase Hydrolysis^a

| Treatment | K_m (% wt/vol) | $V_{max} \times 10^3$ (mgN/mL·min) |
|---------------------------|---------------------|---------------------------------------|
| Control | 0.310 ^a | 6.8 ^d |
| Na-Sulfite (0.4 mg/mL) | 0.574 ^b | 16.8 ^e |
| Cysteine (1.5 mg/mL) | 0.796 ^c | 26.9 ^f |

^aParameters were obtained at E = 0.1 mg/mL, pH 9, 50°C, and So = 0.068–2.0 mgN/mL. (a–f) Data with different letters were significantly different from each other at the 5% level. Abbreviation: CGM, corn gluten meal.

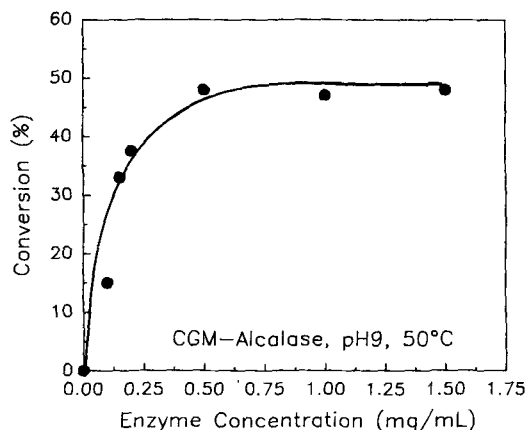


FIG. 3. Effect of enzyme concentration on corn gluten meal (CGM) conversion. So = 1% wt/vol, reaction was conducted at pH 9, 50°C for 120 min.

tremely hydrophobic. Furthermore, they are embedded in a disulfide-bonded glutelin matrix (21). Reduction of these disulfide bonds may open up the structure and allow better access, resulting in greater hydrolysis of the glutelin and liberation of the zein bodies, which tend to aggregate and form highly complex inaccessible protein structures in aqueous media.

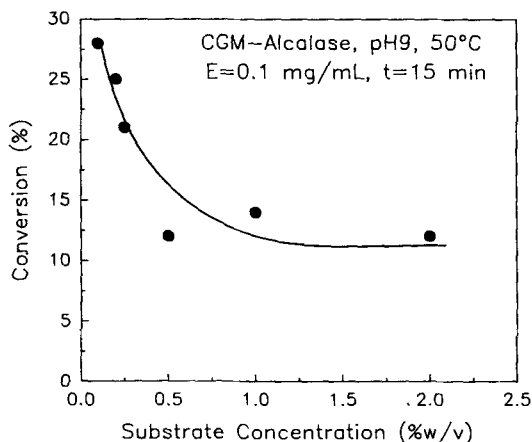


FIG. 4. Effect of substrate concentration on corn gluten meal (CGM) conversion. Enzyme concentration (E) = 0.1 mg/mL. Reaction was conducted at pH 9, 50°C, for 15 min.

Effect of pretreatments. The immediately preceding discussion is corroborated in part by the pretreatment studies, where the reduction of disulfide bonds had a positive effect on the kinetics and conversion of CGM. The cysteine and the sodium sulfite pretreatments had a (statistically) significant effect on the hydrolysis rates and maximum conversion levels (15). Compared with the untreated CGM, which had a maximum conversion of 38%, the cysteine pretreatment resulted in 47–50% conversion and the sodium sulfite resulted in 48.5–50.7% conversion. These pretreatments increased V_{\max} by almost 2–4 times (Table 1).

Molecular weight distribution. The elution profile of the UF permeate from a 30,000 MWCO membrane (UFP30) obtained from native (unhydrolyzed) CGM revealed the first small peak at an elution volume (E_v) of 7 mL, which corresponds to a molecular weight (MW) of about 217,700 Dalton (Da) (Fig. 5a). Theoretically, this should have been excluded by the < 30,000 MWCO membrane. It is possible that this large-MW fraction consists of proteins (perhaps “zein-bodies”) with MW 30,000 Da that permeate freely through the membrane, but later aggregate (during drying, storage and/or elution in the aqueous mobile phase) to yield the large fraction. To prove this hypothesis, the CGM was sulfite-treated prior to ultrafiltration. This treatment reduced disulfide bonds and resulted in a substantial increase in the peak size of the $E_v = 7$ mL fraction (Fig. 5b), indicating the possible release of proteins that had previously been embedded in the glutelin matrix.

Two peaks were identified from the elution profile of unfractionated peptides from a CGM reaction mixture at DH 10 (Fig. 6a). The first peak at E_v of 7 mL corresponded to fractions of about 217,700 Da. The second peak, at $E_v = 14$ mL, corresponded to fractions between 1000–12,000 Da, with a peak at 5345 Da. A greater degree of hydrolysis resulted in a breakdown of the large 217,700 fraction to at least one smaller fraction. The elution profile of an unfractionated DH 20 CGM hydrolyzate (Fig. 6b) shows that the $E_v = 7$ mL peak is smaller than the $E_v = 14$ mL fraction, and a new peak occurred at E_v of 10.5 mL, corresponding to a MW of 34,120 Da.

Both unfiltered and ultrafiltered CGM hydrolyzates had

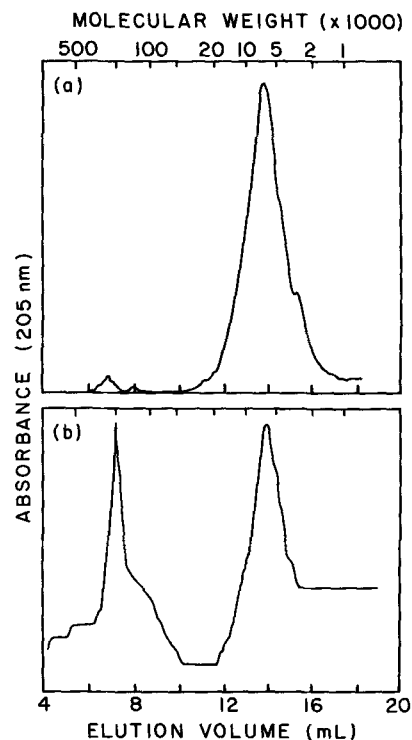


FIG. 5. Size exclusion-high-performance liquid chromatography elution profiles of UFP30 permeates of (a) unhydrolyzed corn gluten meal (CGM), and (b) unhydrolyzed CGM that had been pretreated with sulfite.

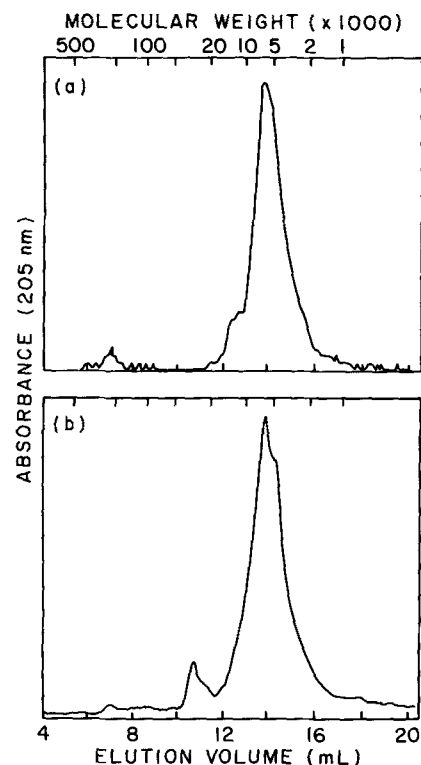


FIG. 6. Size exclusion-high-performance liquid chromatography elution profile of a corn gluten meal hydrolyzate at (a) degree of hydrolysis (DH) 10, and (b) DH 20.

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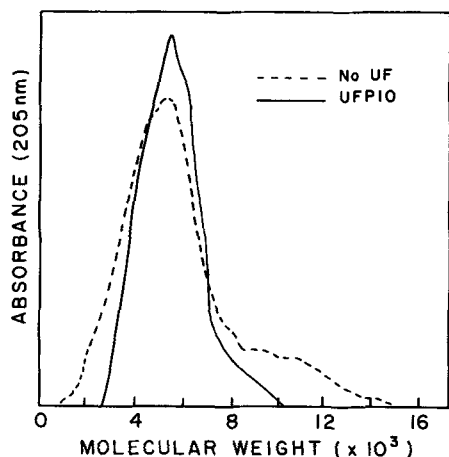


FIG. 7. Effect of ultrafiltration on molecular weight distribution of corn gluten meal hydrolyzate at degree of hydrolysis 10.

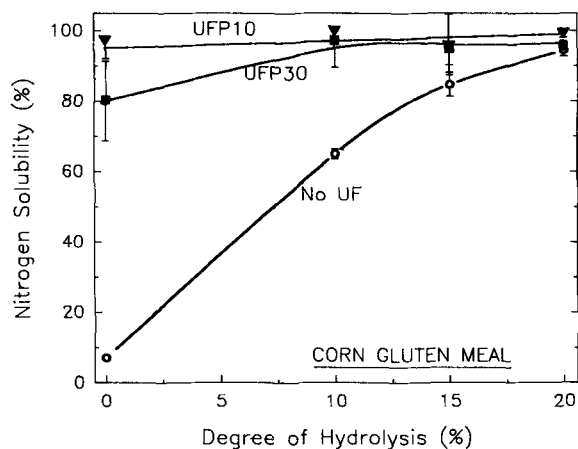


FIG. 8. Effect of degree of hydrolysis and membrane pore size on nitrogen solubility of corn gluten meal hydrolyzates: The data points are means of values at pH 3, pH 5, pH 7 and pH 9. The bar represents one standard deviation; in some cases, the size of the symbol is larger than the bar.

an average MW of about 5500 Da. However, ultrafiltration resulted in a narrower MW distribution of 2500–10,000 Da, vs. 1000–15,000 Da for the unfiltered reaction mixture (Fig. 7).

Nitrogen solubility. Enzyme hydrolysis increased the solubility of CGM proteins (Fig. 8). Pretreatment with sulfite had a small or negligible effect, and the pH at which the solubility test was done had no effect on solubility of unmodified or modified CGM proteins (15). Each point in Figure 8 is thus an average of all the data at a particular DH value. Protein solubility could be increased by proteolysis alone (*i.e.*, with no ultrafiltration) or by ultrafiltration alone of native CGM suspensions (*i.e.*, with no hydrolysis, designated as DH = 0 points). The latter is to be expected because the few fractions that are small enough to be permeable to such low MWCO UF membranes will probably be soluble. However, the yield would be extremely low because less than 10% of native CGM proteins are soluble at pH 3–9.

TABLE 2

Turbidity of Corn Gluten Meal (CGM) Hydrolyzates as a Function of Degree of Hydrolysis (DH) Membrane Pore Size and pH

| Treatment | pH | DH (%) | | | |
|-------------------------|----|----------------|-----------------------|----------|----------|
| | | 0 | 10 | 15 | 20 |
| None (Control) | 3 | — ^a | 2.550 aA ^b | 2.420 A | 2.120 a |
| | 5 | — | 2.535 a | 2.404 | 2.152 a |
| | 7 | — | 2.531 a | 2.416 | 2.088 b |
| | 9 | — | 2.531 a | 2.423 A | 1.957 cF |
| Sulfite pretreatment | 3 | — | 2.520 aB | 2.372 | 2.170 aA |
| | 5 | — | 2.532 a | 2.380 | 2.155 a |
| | 7 | — | 2.546 a | 2.378 | 2.162 a |
| | 9 | — | 2.550 aA | 2.311 B | 1.997 cF |
| UFP 10 | 3 | 0.001 fF | 0.005 F | 0.003 fF | 0.003 fF |
| | 5 | 0.003 f | 0.011 d | 0.003 fF | 0.003 fF |
| | 7 | 0.003 f | 0.007 aF | 0.005 F | 0.005 F |
| | 9 | 0.002 f | 0.008 bF | 0.006 F | 0.007 a |
| UFP 30 | 3 | 0.012 D | 0.005 fF | 0.021 aB | 0.012 |
| | 5 | 0.011 C | 0.016 | 0.008 | 0.006 |
| | 7 | 0.007 fA | 0.016 | 0.010 | 0.010 |
| | 9 | 0.008 fB | 0.017 | 0.011 | 0.012 |
| UFP 10(s) ^c | 3 | 0.001 fF | 0.002 fF | 0.004 F | 0.004 F |
| | 5 | 0.002 fF | 0.004 F | 0.003 fF | 0.003 fF |
| | 7 | 0.002 fF | 0.007 F | 0.008 f | 0.007 |
| | 9 | 0.003 f | 0.012 c | 0.011 c | 0.010 b |
| UFP 30(s) ^c | 3 | 0.005 f | 0.042 dE | 0.032 bE | 0.026 a |
| | 5 | 0.004 f | 0.019 | 0.011 a | 0.010 f |
| | 7 | 0.004 f | 0.022 A | 0.011 a | 0.016 B |
| | 9 | 0.005 f | 0.09 bB | 0.017 A | 0.018 B |

^aSamples showed extremely high optical densities (>3.0).

^bNumbers with different lower case letters (abcdef) are significantly ($P < 0.05$) different from each other due to differences in DH at the same treatment. Numbers with different upper case letters (ABCDEF) are significantly ($P < 0.05$) different from each other due to differences in treatment at the same DH.

^c(s), Refers to hydrolyzates produced from CGM pretreated with sulfite.

Ultrafiltration increased solubility of CGM hydrolyzates, notably at low DH values (Fig. 8). At DH = 0, unhydrolyzed UFP30 permeate was only 80% soluble on average, while unhydrolyzed permeate from the tighter membrane was essentially completely soluble. This is because permeating fractions were of lower MW compared with those of the reaction mixture, which contained both soluble and insoluble fractions. Smaller-pore membranes increased protein solubility only at the lower DH values. This correlates well with the SE-HPLC data, which also did not show any effect of membrane pore-size on MW distribution (15).

Turbidity. Table 2 summarizes the turbidity results for the CGM-*alcalase* hydrolyzates. The data were statistically analyzed across "treatments" (*e.g.*, sulfite pretreatment, DH, membrane pore size, etc.). UF permeates were much clearer than the unfiltered hydrolyzates.

Foaming properties. Figure 9 summarizes foam volumes obtained for CGM hydrolyzates as a function of DH of the enzyme reaction, ultrafiltration membrane pore size and pretreatments. Even a mild enzyme treatment (*e.g.*, DH = 10) resulted in a large increase in initial foam volume; this was especially notable for the ultrafiltered products. More extensive hydrolysis had little or no effect. Pretreatment had a beneficial effect on the ultra-

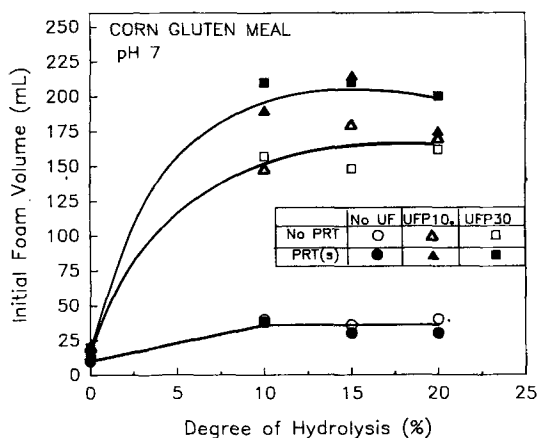


FIG. 9. Effect of degree of hydrolysis on foam volume of corn gluten meal (CGM) hydrolyzates [closed points: CGM pre-treated with sulfite. open points: not pretreated].

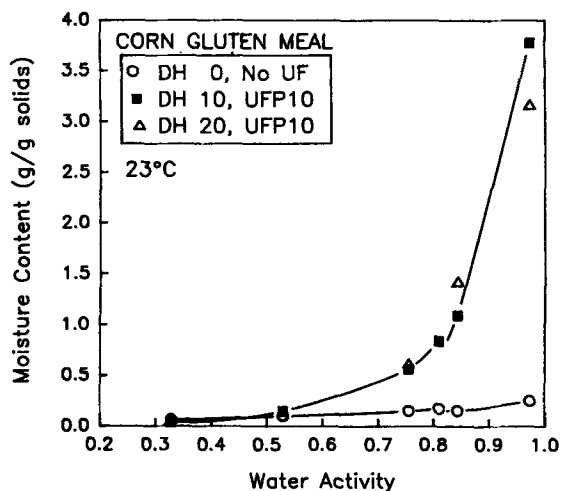


FIG. 10. Moisture sorption isotherms of corn gluten meal and its hydrolyzates.

filtered samples: initial foam volume of sulfite-treated samples were 20–50 mL higher than untreated CGM hydrolyzates.

The stability of the foams, however, was poor. Almost all samples showed a complete collapse of the foam after 30 min. Apparently, proteins/peptides of 5000 Da are capable of film formation at the air-water interface but lack the strength to maintain the foam. Similar results were reported by Deeslie and Cheryan (10) for UFP10 permeates of hydrolyzed soy protein isolate.

Moisture sorption. All CGM hydrolyzates showed higher moisture sorption capacities than the unhydrolyzed CGM (Fig. 10). Enzymatic modification of the quaternary and tertiary structure of proteins exposes ionizable polar amino acids (containing free amino and carboxyl groups) such as aspartic and glutamic acids that are abundant in CGM (4 and 15.5% dry basis, respectively) (23). These amino acids are capable of binding almost three times as much water as that of nonionized polar groups

(24). Extensive hydrolysis (*e.g.*, to DH20) exposes these groups, and if the peptides are small enough, prevents them from reforming random coils. Also, in the intermediate water activity range, peptide linkages aid in binding water after the polar side chains have been saturated.

The improvement in moisture sorption as a result of enzyme hydrolysis has been observed by Beuchat *et al.* (25) and Puski (16). Similar beneficial effects of enzyme hydrolysis and membrane filtration were observed for soy hydrolyzates (10). However, at a water activity of 0.973, moisture sorption by the corn protein hydrolyzate (sulfite-treated, DH20/UFP10) was double that of a soy protein hydrolyzate (90% conversion, UFP10) (10). High moisture sorption capabilities of a food ingredient can be utilized in the food industry to prevent syneresis or water loss in breads, soups and cakes, as well as increasing yields of cured sausages, canned fish and frozen products.

It may be possible to obtain a higher degree of conversion by using different enzymes (such as the cysteine proteases, bromelain or ficin) and/or lower So/E ratios, provided it is economical and has the desired effect on the functional properties. The process could be further refined by designing a continuous reactor system to improve productivity and lower costs of manufacture. It is possible that other enzymes and larger-pore membranes could result in corn protein hydrolyzates with better or other attractive functional properties. The resulting value-added products should enhance the utilization of the by-products of corn refining.

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