Preparation and Properties of Acid-Solubilized Gluten

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The best condition of acid hydrolysis was sought for preparing soluble gluten to utilize as emulsifying and foaming agents. A 5% gluten suspension is heated at 121 °C for 30 min with 0.02 N HCl or for 15 min with 0.05 N HCl. Isoelectric precipitation at 4.7–4.9 recovered approximately 78% of the original gluten. The molecular weight distribution determined by gel filtration chromatography and Trautman plot of approach-to-equilibrium sedimentation analysis in addition to analyses of amide-N, free amino groups, and SH and SS groups, proved that solubilization was a result of not only deamidation of glutamine and asparagine, but also rupture of a few peptide linkages in gluten molecules. The emulsifying capacity and stability were greatly improved by the acid treatments and were better than those of soybean protein isolate. A protein fraction extracted from the acid-treated gluten at pH 5.2 showed excellent foamability and foam stability. Acetic acid, 1.75 N, could replace 0.02 N HCl for preparation of soluble gluten of similar properties. There was no significant damage of essential amino acids during the acid treatments.

The insolubility of gluten in aqueous solutions is one of the major limitations for its more extensive use in food processing. This property of gluten is due to the high concentration of nonpolar amino acid residues such as proline, leucine, and glutamine, and to the low concentration of ionizable side chains such as lysine, arginine, glutamic acid, and aspartic acid. The interactions between glutamine and asparagine side chains through hydrogen bonds play an important role in promoting association of gliadin and glutenin molecules (Beckwith et al., 1963; Krull and Wall, 1966).

Holme and Briggs (1959) found that mild acid hydrolysis of amide groups in gliadin with hydrochloric acid at concentrations from 0.008 to 0.04 N caused an increase in the protein solubility and a decrease in protein interactions. When the degree of deamidation was greater than 10%, the deamidized gliadin was insoluble in water at pH 3.8 and ionic strength 0.01, and the precipitated protein was readily soluble in water at pH 7 or above. McDonald and Pence (1961) prepared deamidized gliadin by hydrolysis with 0.07 N hydrochloric acid and tested its foamability. They found that for meringue dessert shells, butter cream cake icing, fluffy cake icing, and divinity candy, deamidized gliadin performed as effectively as egg white. Gagen and Holme (1973) obtained deamidized gluten by refluxing with hydrochloric acid of various concentrations (0.15 to 1 N). The solubility of the treated gluten was greatly improved as deamidation proceeded to completion. The foamability, whippability, and baking performances of treated gluten were reduced by deamidation except for the whippability of the insoluble residue left after hydrolysis. Aranyi and Hawrylewicz (1972) prepared and isolated low molecular weight polypeptides from wheat gluten by acid-catalyzed hydrolysis with a mixture of 0.1 N hydrochloric acid and 4 N acetic acid at 60 °C for 24 h.

The purpose of this study was to investigate the conditions for solubilizing gluten using a mild acid treatment and to isolate a protein fraction having a bland taste with minimal changes in the molecular size and essential amino acids. Molecular weight distribution, deamidation, and peptide linkage hydrolysis were assessed. The emulsification and foaming capabilities of acid-modified gluten were also explored.

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MATERIALS AND METHODS

Source of Gluten. Vital gluten was obtained through the courtesy of the Research Laboratory of John Labatt Ltd., Montreal, Canada.

Preparation of Acid-Modified Gluten Samples. Fifty milliliters of a 5% gluten suspension in either hydrochloric acid or acetic acid was heated at 100 or 121 °C. A hot plate was used for refluxing at boiling temperature, and a Barnstead autoclave for heating at 121 °C. The concentration of acids varied from 0.02 to 0.5 N for hydrochloric acid and from 1.75 to 8.75 N for acetic acid. The pH of maximum precipitation was determined by measuring the absorbance at 280 nm of the supernatant after centrifugation (5000*g*, for 10 min) of acid-treated gluten at different pH values. The protein was recovered from the acid-treated gluten dispersions by adjusting to the pH of maximum precipitation and centrifuging for 10 min at 5000*g*. After decanting the supernatant, the precipitate was dissolved in the same volume of water by adjusting to pH 7.5. The solution was centrifuged at 5000*g* for 10 min and the supernatant was freeze-dried (Figure 1). An extra protein fraction was separated by extracting a 5% suspension of precipitate from the 0.02 and 0.05 N HCl-treated gluten with water at pH 5.2. Extraction was performed by agitating on a magnetic stirrer for 2 h. The supernatant after centrifugation was freeze-dried. The protein was also recovered by dialyzing the acid-treated gluten against water at 4 °C for 48 h. The dialysate adjusted to pH 7.5 was freeze-dried after centrifuging for 10 min at 5000*g*.

Deodorization of Modified Gluten by Activated Carbon Treatment. Granular activated carbon, Grade 209, 18 × 40 mesh, obtained from Witco Chemical Corp., New York, N.Y., was first washed to eliminate the floating fine particles and then packed into a column (1.2 × 8 cm). Deodorization was carried out by passing 5% modified gluten through this column at 25 or 90 °C.

Chemical Analyses. Moisture, ash, fat, and total nitrogen were determined according to the standard AOAC procedures. The protein was calculated using a factor of 5.7. Amide nitrogen was determined as distillable ammonia after refluxing 100 mg of gluten or modified gluten in 50 ml of 2 N HCl for 3 h (Wilcox, 1967). The chloranil method (Al-Sulimany and Townshend, 1973) was employed to determine amino acids and small peptides present in the effluents from the gel filtration columns. The same chloranil method and a method using 2,4,6-trinitrobenzenesulfonic acid (TNBS) (Fields, 1971) were

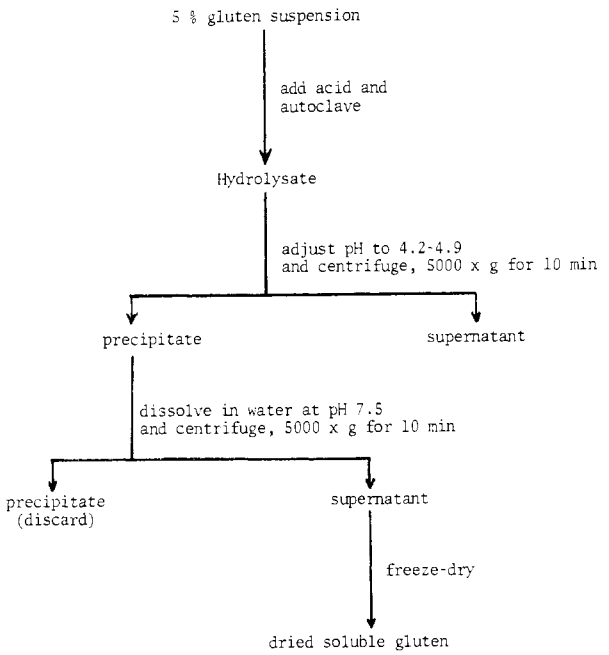

Figure 1. Procedure for separation of solubilized gluten by precipitation.

used for determining the amount of released amino groups as a result of hydrolysis of peptide bonds. Sulfhydryl and disulfide groups were determined by the method of Beveridge et al. (1974). Amino acids were analyzed on a Phoenix Micro Amino Acid Analyzer M 6800 equipped with a single column system of Durrum Chemical Corp., Palo Alto, Calif. The gluten samples were first treated with 4-vinylpyridine to protect cysteine as described by Cavins et al. (1972) and then hydrolyzed with *p*-toluenesulfonic acid (Liu and Chang, 1971).

Gel Electrophoresis. Disc polyacrylamide gel electrophoresis was performed according to the method of Chen and Bushuk (1970).

Gel Filtration Chromatography. Sephadex G-100 and Sepharose 6B purchased from Pharmacia Fine Chemicals were used to pack the columns which were eluted with the AUC solution (0.1 M acetic acid, 3 M urea, and 0.01 M hexadecyltrimethylammonium bromide). Immediately after autoclaving, the treated gluten was neutralized, freeze-dried, and applied to the Sepharose column after dissolving in the AUC solution. For the Sephadex column, the isoelectrically precipitated protein was applied after dissolving in the same eluent.

Trautman Plot of Approach-to-Equilibrium Technique in Ultracentrifuge. A Beckman L2-65B ultracentrifuge equipped with the uv scanner was used. The coordinates used by Erlander and Foster (1959) were modified as follows:

$$X = (C_0 - C_m)/X_a^2$$
$$Y = y/\omega^2 X_a$$

where C_0 is the initial concentration of the solute; C_m is the solute concentration at the meniscus; X_a is the distance from the center of rotation to the meniscus; y is the concentration gradient at the meniscus calculated by extrapolation of the slope of the uv pattern; and ω is the angular velocity.

Emulsifying capacity was measured according to the method of Swift et al. (1961) with minor modifications. Thirty milliliters of 0.75% protein solution was mixed with corn oil (Laboratory grade, Fisher Scientific Co.) using an Omni-Mixer Homogenizer (Ivan Sorvall Inc., Norwalk, Conn.) at a speed of 8600 rpm; corn oil was added at a rate of 5 ml/min while mixing.

Emulsion stability rating was measured by the method of Acton and Saffle (1970).

Foamability and foam stability were determined by the method of McDonald and Pence (1961).

Table I. Protein Content, pH for Maximum Precipitation, and Color of Acid-Modified Gluten

Acid concn	Heating time at 121 °C, min	Protein content, %	pH for max. ppt.	Color
0.02 N HCl	30	89.4	4.9	Pale yellow
0.05 N HCl	15	87.6	4.7	Pale yellow
0.1 N HCl	15	86.2	4.5	Pale yellow
0.5 N HCl	15	77.4	4.2	Brown
1.75 N acetic	30	85.5		Pale yellow
3.5 N acetic	15	85.4		Pale yellow
8.75 N acetic	15	85.5		Pale yellow
0.02 N HCl (pH 5.2 fraction)	30	99.5	4.9	White
0.05 N HCl (pH 5.2 fraction)	15	99.3	4.7	White

Table II. Yield of Solubilized Gluten after Autoclaving at 121 °C and Dialysis

Heating time, min	Solubilized gluten (% of original gluten)[a]						
	HCl, N				Acetic acid, N		
	0.5	0.1	0.07	0.02	8.75	3.5	1.75
5		88.5	92.1	89.8		89.8	60.2
15	66.1	89.8	88.5	92.1	90.9	93.3	83.9
30	44.9	87.4	85.1	90.9	86.2	92.1	88.5
60	41.3	85.1	86.2	82.6	85.1	88.5	87.4

[a] Dry weight basis, from duplicate samples.

RESULTS AND DISCUSSION

Preparation of Solubilized Gluten. *Recovery.* Maximum precipitation of protein was obtained from the acid-treated gluten by adjusting the pH to 4.2 to 4.9 depending on the concentration of acid used for hydrolysis (Table I). When gluten was hydrolyzed with acetic acid, the maximum precipitation occurred over the broad pH range of 3.0 to 9.0. This is in agreement with the observation of Krull and Wall (1966) that as the ratio of glutamine–glutamic acid in the copolymers increased, the minimum pH at which the copolymers were soluble in water increased.

Table II shows the yields of solubilized gluten prepared by the dialysis–freeze-drying method after autoclaving acid–gluten mixtures at 121 °C. The results show that the yield of gluten in the solubilized form was between 85 and 92% except for the treatments with 0.5 N HCl and 1.75 N acetic acid. The recovery of protein from the 0.5 N HCl treated gluten when heated at 121 °C for 15 min was only 66%. When the heating time was extended to 60 min, the recovery was further decreased to 41%. At a concentration of 0.5 N HCl, the gluten molecules undergo extensive hydrolysis with longer heating time. In the case of the 1.75 N acetic acid treatment with a heating period of 5 min, the recovery of protein was only 60%. When the heating time was extended to 30 min, the recovery of protein

Table III. Amide Nitrogen in Acid-Modified Gluten

Heating time, min	Amide nitrogen, mM per 100 g of protein[a]						
	HCl, N				Acetic acid, N		
	0.5	0.1	0.07	0.02	8.75	3.5	1.75
5		163.5	223.3	249.1		270.3	262.0
15	23.2	160.9	205.7	249.1	262.8	265.2	264.4
30	17.3	147.2	213.3	246.8	255.1	262.5	
60	14.5	120.7	181.5	246.9	252.6	249.7	261.9

[a] From triplicate samples; untreated gluten contained 279 mM amide per 100 g of protein.

increased because of increased solubility of the gluten. These data show that with lower heating temperatures, heating time had to be increased to obtain the equivalent degree of solubilization. For example, 6 h was needed to reflux the 5% gluten suspension containing 0.1 N HCl at 100 °C to achieve a yield of about 85%, a level which could be obtained in less than 5 min at 121 °C. In most subsequent experiments, autoclaving was used.

Although the yields of solubilized gluten from the 0.02 N HCl-treated gluten with heating times of 5 and 15 min were 89.8 and 92.1%, respectively, the solubility of the freeze-dried samples in water was inadequate. The 1.75 N acetic acid treated gluten with 15 min heating time showed the same result. However, all other acid-treated gluten samples in the freeze-dried form were almost completely water soluble.

Considering both the yield and the solubility characteristics of the freeze-dried samples, gluten was acid treated at 121 °C for 15 min for further studies except for 0.02 N HCl and 1.75 N acetic acid treated gluten which were heated for 30 min.

The yields of proteins isoelectrically precipitated from acid-treated gluten solutions were between 70 and 78% of the original gluten protein. The yield of the pH 5.2 fraction was 30–35%. However, if a slightly higher salt concentration, 3–6%, does not affect food processing, the whole acid-treated gluten solution can be directly dried after neutralization without causing any loss of nitrogen-containing compounds.

Carbon Column Treatment. The acid-modified gluten samples were almost odorless and pale yellow in color; however, when they were dissolved in water to make a concentrated solution, a wheaty odor (a wheat husk-like odor and taste) appeared. A possibility of eliminating this wheaty odor by activated carbon treatments was examined. Three solubilized gluten solutions were prepared and evaluated by specially trained panelists: (1) without the activated carbon treatment; (2) carbon treated at 25 °C; (3) carbon treated at 90 °C. Five of the six panel members judged sample 3 as having the most acceptable flavor and sample 2 as more acceptable than sample 1.

Physical and Chemical Properties. *Amide Nitrogen.* The amide nitrogen content of untreated gluten was 279.3 ± 7.2 mM/100 g of protein. Table III shows the amount of amide-N which remained in the acid-modified gluten. It is apparent from the results that only a small amount of amide-N remained when treated with 0.5 N HCl. The lower the HCl concentration, the larger the amount of amide-N remaining in the protein, and the acid concentration was more influential than the autoclaving time within the limit of experimental conditions used. Gluten treated with acetic acid had higher amide-N values than the HCl-treated gluten. Gluten was solubilized even when the amide-N was reduced by as little as 5% by the acetic acid treatment and by 10% by the HCl treatment.

Disc Gel Electrophoresis. The disc gel electrophoresis pattern of untreated gluten showed more than 12 clear bands as indicated in Figure 2. Acid modification of gluten brought about considerable changes in the electrophoresis pattern. With the 0.02 and 0.05 N HCl and the 1.75 and 3.5 N acetic acid treated gluten, the bands similar to those of the untreated gluten were obtained but with a slightly less discrete resolution. When the HCl concentration was increased to 0.1 N, a few bands near the origin disappeared and the rest of the bands became indistinct. With 0.5 N HCl, only a few poorly resolved bands were left at the front. The majority of the pH 5.2 fraction migrated at a slower rate but four minor fast-moving bands were noted in the disc gel electrophoretogram (Figure 2).

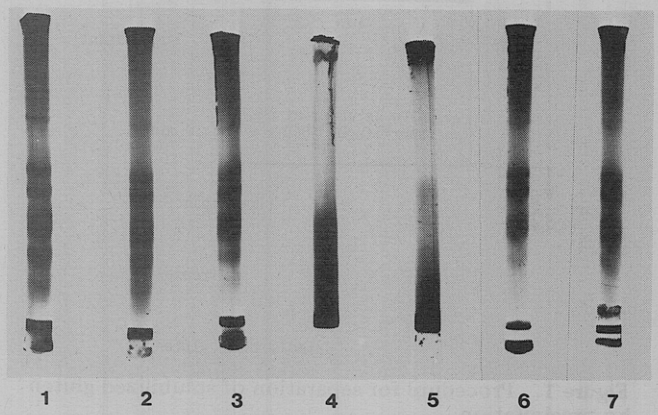

Figure 2. Polyacrylamide gel electrophoresis pattern of solubilized gluten: (1) untreated gluten; (2) 0.02 N HCl treated; (3) 0.05 N HCl treated; (4) 0.1 N HCl treated; (5) 0.5 N HCl treated; (6) pH 5.2 fraction from 0.02 N HCl treated; (7) pH 5.2 fraction from 0.05 N HCl treated; gel concentration, 7.5%; buffer, 4.5 M acetate (pH 3.8); sample, 50 μl of 1% protein in AUC solution; current, 4 mA/column; running time, 2 h; staining dye, Coomassie Brilliant Blue, R250.

Gel Filtration Chromatography. Three major peaks and one minor peak were observed in the elution pattern of untreated gluten from Sephadex G-100 (Figure 3A) which is similar to those of Wright et al. (1964) and Meredith and Wren (1966). The column was calibrated with standard proteins. The relationship between two columns, Sephadex G-100 and Sepharose 6B, was examined by applying the different fractions obtained from G-100 to 6B (Figure 3B). More detailed distribution patterns of high molecular weight fractions were demonstrated by Sepharose 6B gel filtration chromatography.

As the peak size of chloranil reactive small peptides and amino acids close to V_t was small, cleavage of peptide bonds must have been relatively moderate. Chloranil reacts only weakly with ammonium salts but not with urea (Al-Sulimany and Townshend, 1973); therefore the analysis of peptides and amino acids in the effluents containing AUC was feasible.

Trautman Plot of Approach-to-Equilibrium Sedimentation Analysis. The values of X and Y obtained at different centrifugal speeds and times are plotted (Figure 4). Y increases so rapidly as X approaches 0 that finding the Y intercept does not seem feasible for calculation of

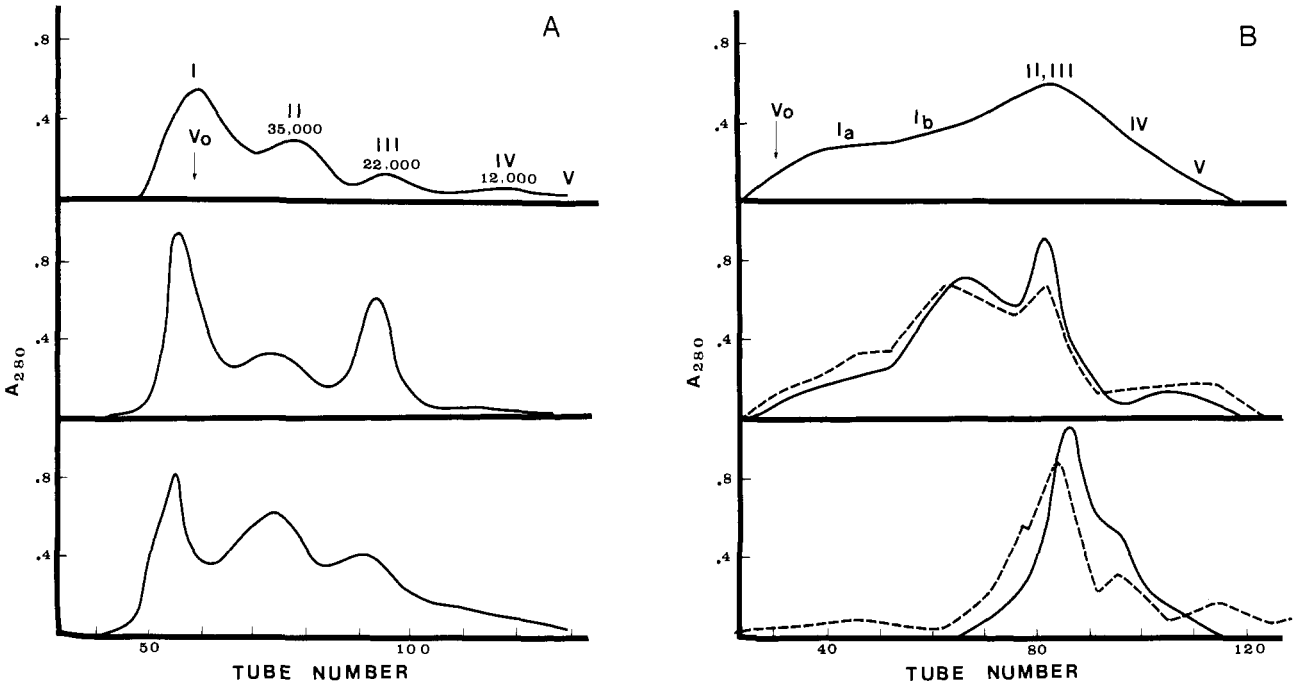

Figure 3. Gel filtration pattern of solubilized gluten: (A) Sephadex G-100; upper, untreated gluten; middle, 0.02 N HCl treated; lower, pH 5.2 fraction from 0.02 N HCl treated; column size, 4 × 72 cm; eluent, AUC; fraction size, 5 ml; V_0, void volume determined with blue dextran 2000, Pharmacia Fine Chemicals; sample, 5 ml of 2.5% solution; (B) Sepharose 6B; upper, untreated; middle, 0.02 N HCl treated; lower, 0.5 N HCl treated; column size, 2.5 × 80 cm; eluent, AUC; fraction size, 3.5 ml; sample, 4 ml of 2.5% solution; (—) absorbance at 280 nm; (- - -) absorbance at 350 nm after chloranil reaction.

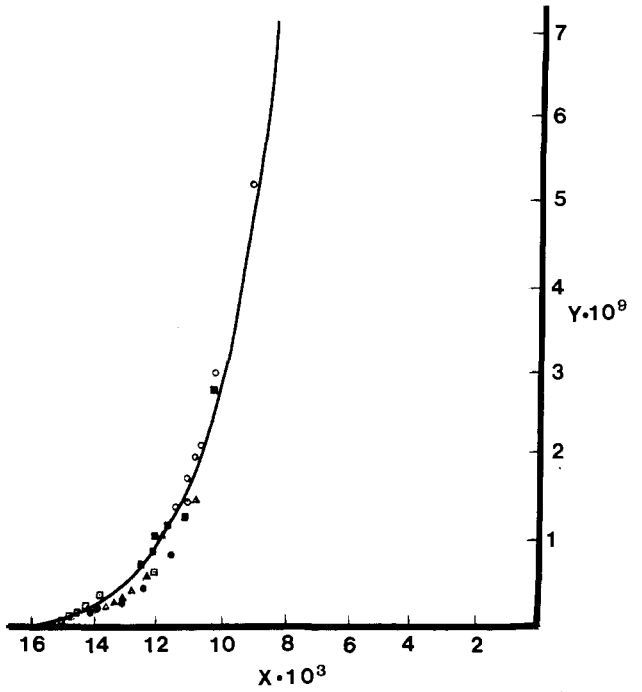

Figure 4. Trautman plot of 0.02 N HCl-treated gluten: $X = (C_0 - C_m)/X_a^2$ (mg/ml cm^2); $Y = y/\omega^2 X_a$ (mg sec^2/rad^2 ml); (○) 18080 rpm; (△) 38082 rpm; (□) 59148 rpm; (■) 27979 rpm; (●) 48253 rpm.

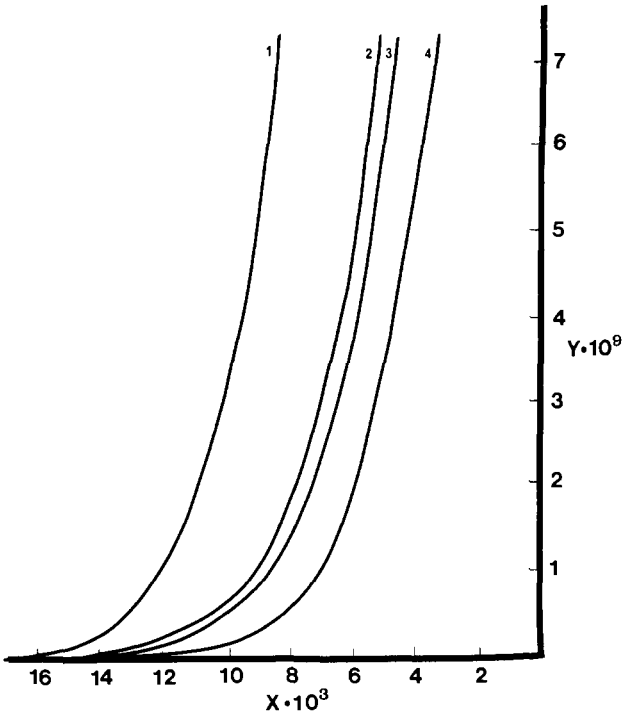

Figure 5. Trautman plot of solubilized gluten: (1) 0.02 N HCl treated gluten; (2) 0.05 N HCl treated; (3) 0.1 N HCl treated; (4) 0.5 N HCl treated.

the weight average molecular weight, implicating the presence of a significantly greater quantity of large molecular weight fractions in the treated gluten. More accurate plotting at X close to 0 was extremely difficult as at lower speeds of centrifugation the accuracy of the pattern reading progressively deteriorated and a mechanical instability developed in the scanner as well as in the centrifuge. To smoothen the X–Y plot, several transformation functions were used with the simple linear regression analysis as well as the polynomial regression analysis and the following regression equation was found to fit best: $\ln (y + c) = a + bx$. An iteration technique was used for finding the value for c. This c is important as it controls the slope at $Y = 0$ that corresponds to the molecular weight of the smallest components in the samples. The value c also decides the X intercept which is used for calculation of the weight average molecular weight of multicomponent systems. The regression curves

drawn for the HCl-treated gluten using this curve fitting are shown in Figure 5.

The Trautman curves indicate that all the treated gluten was heterogeneous and as the hydrochloric acid concentration increased the molecular weight of the largest as well as the smallest components decreased as the slopes at the highest Y and $Y = 0$ become less steep. This is a feature of nonselective hydrolysis.

Since the calculation of the overall weight average molecular weight of each treated gluten is difficult, an attempt was made to further interpret the Trautman curve in terms of molecular weight distribution of multicomponent systems. According to Erlander and Foster (1959):

$$(S/D)_w = \frac{Y^*}{(G_y/G_x)a'b'n_c^0 - X^*} \quad (1)$$

where $(S/D)_W$ is the weight average value of sedimentation-to-diffusion constant ratio; G_x and G_y are magnification constants; a' is the cell optical path; b' is the optical lever arm; n_c^0 are the refractive indices at the initial solute concentration; X^* and Y^* correspond to X and Y in this study. By simplifying:

$$(S/D)_w = Y/(AC_0 - X) = \Sigma(S/D)_i C_i/\Sigma C_i \quad (2)$$

where A is a constant. As S/D is proportional to molecular weight:

$$Y = KM_w(AC_0 - X) \quad (3)$$

$$= AK\Sigma M_i C_i - \frac{K\Sigma M_i C_i}{C_0} X$$

where K is a constant. In eq 3, when $Y = 0$

$$X = AC_0 \quad (4a)$$

and when $X = 0$

$$Y = AK(M_a C_a + M_b C_b + M_c C_c + \ldots) \quad (4b)$$

These relationships (eq 4a and 4b) were applied to the 0.5 N HCl-treated gluten (Figure 6). The slopes a, b, and c were arbitrarily chosen, representing three weight average molecular weight fractions. The concentration ratio of these fractions is approximately 13:9:10. The concentration ratio of the same fractions in the 0.02 N HCl-treated gluten is 38:11:10 which implies that the content of the largest molecular weight fraction in the 0.02 N HCl-treated gluten is almost triple that in the 0.5 N HCl-treated gluten. The slope c represents the molecular weight of the smallest component in the 0.02 N HCl-treated gluten; however, as seen in Figure 6, there are smaller fractions included in the 0.5 N HCl-treated gluten (a further stretch in the curve beyond A to reach $Y = 0$). Direct comparison of this result with that of gel filtration chromatography is unjustified as the AUC eluent which is a powerful dissociating solvent was used for gel filtration chromatography, whereas a milder phosphate buffer was used for Trautman plot.

The slope of Trautman curves can be calculated, once the best fit curve $\ln(y + c) = a + bx$ has been found, as $dy/dx = be^{a + bx}$. Several trials using standard proteins of known molecular weight will give a factor to convert the slope of Trautman plot to molecular weight; thereafter, using the slope at different X values on a Trautman curve for a multicomponent system, the molecular weight distribution may be calculated. However, recently more sophisticated approaches have been suggested for estimation of molecular weight distribution (Scholte, 1969; Wiff and Gehatia, 1972); therefore no further study beyond

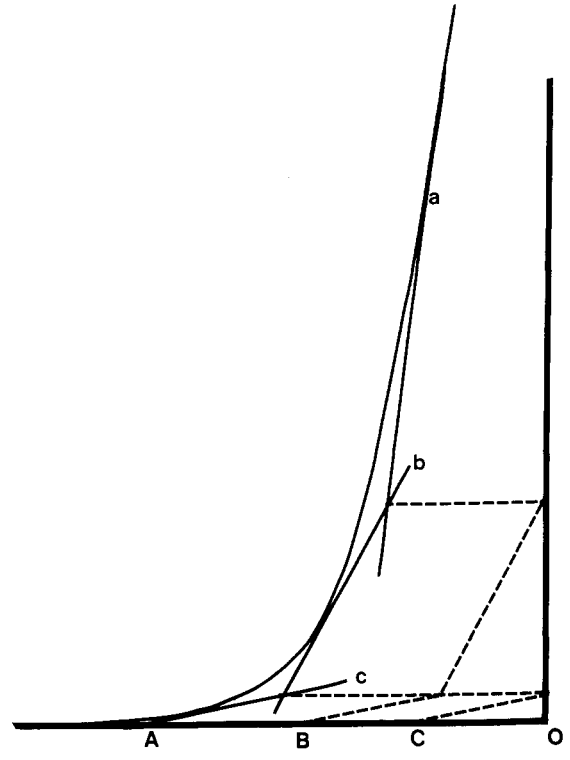

Figure 6. Molecular weight distribution analysis of Trautman curve for 0.5 N HCl-treated gluten: AB:BC:CO = 13.9:9.7:10.6. The broken lines were drawn parallel to lines b and c and to the X axis.

that stated in this paper has been attempted.

Amino Groups. As the untreated gluten is insoluble in water, the TNBS method was inapplicable. The AUC solution as well as sodium deoxycholate interfered with TNBS color development. Chloranil is the only reagent at the moment that has been proved to be insensitive to the AUC solution. However, the mechanism of chloranil color development is not well understood. Al-Sulimany and Townshend (1973) used tyrosinase as an example of protein giving a similar spectrum to that of amino acids. They reported that a solution containing 42 ppm of tyrosinase gave a maximal absorbance of 0.46 when subjected to the recommended procedure. Using a value of 130 as the average molecular weight of amino acids it was calculated from their data that the molar absorptivity at 350 nm for amino acids is about 10 to 20 times that of tyrosinase. This suggests that the chloranil color of protein could be derived from the reaction of chloranil with free α- and ϵ-amino groups. Assuming a molar absorptivity of 1500 for the amino groups in protein that is the average of the molar absorptivities of different amino acids reported by Al-Sulimany and Townshend (1973), the amino groups liberated on peptide rupture in gluten during acid treatment were calculated for comparison with the TNBS results (Table IV). As the concentration of acid increased for acid solubilization of gluten, more amino groups were liberated from gluten. This provides more evidence to prove the disruption of the peptide bonds in gluten during acid treatments. The effect of 1.75 N acetic acid on gluten was analogous with that of 0.02 N HCl, a similar tendency to the results obtained from gel filtration chromatography.

The total amino acid content in untreated gluten was determined as approximately 710 mM/100 g of protein. Table IV shows that the rupture of peptide bonds is approximately 5 to 8 mM/100 g, meaning the rupture of one peptide bond out of 89 to 140 upon 0.5 N HCl treatment. The molecular weight of the smallest fraction

Table IV. Amino Groups Exposed by Peptide Hydrolysis in Acid Modified Gluten Samples

Treatment[a]	Amino group content (mM/100 g of protein)[b] TNBS	Chloranil
0.02 N HCl	16.07	29.8
0.05 N HCl	16.64	30.4
0.1 N HCl	17.75	35.2
0.5 N HCl	21.02	37.3
1.75 N acetic	16.30	29.1
Untreated		29.0

[a] The heating for 0.02 N HCl and 1.75 N acetic acid was 30 min at 121 °C; for the other treatments the heating time was 15 min. [b] Average of triplicate analysis.

Table V. Emulsifying Capacity and Stability of Acid-Modified Gluten

Treatment[a]	Emuls. capac. (ml of corn oil/100 mg of protein)[b]	Stabil. rating[c] at corn oil level (%) 16.7	33.3	50.0
Untreated gluten	15.92 ± 0.46	5.22	15.31	47.32
0.02 N HCl	34.96 ± 0.67	10.26	24.83	70.84
0.05 N HCl	33.24 ± 0.58	9.32	21.37	67.59
0.1 N HCl	32.15 ± 0.51	8.23	19.83	54.21
0.5 N HCl	29.90 ± 0.67	7.49	18.52	50.30
1.75 N acetic acid	32.15 ± 0.63			61.59
3.5 N acetic acid	30.07 ± 0.51			59.35
Soy isolate	31.85 ± 0.25	10.20	24.19	70.53

[a] The heating time for 0.02 N HCl and 1.75 N acetic acid was 30 min, for other treatments 15 min. [b] At room temperature, triplicate samples. [c] Average of duplicate.

then could be about 12000 to 18000 and the chances of releasing amino acids or very small peptides are slight.

SH and SS Groups. Since the acid treatment of gluten dramatically decreased the viscosity, it is possible that the solubilization is a consequence of SS disruption. Analysis showed 8.28, 8.01, 7.91, 7.80, and 8.20 mM/100 g of protein for untreated, 0.02 N, 0.05 N, 0.1 N, and 0.5 N HCl-treated gluten, respectively, without detectable SH, whereas, half-cystine determined using an automatic amino acid analyzer was 18.3, 17.9, 19.0, and 18.9 mM/100 g of protein for untreated, 0.02 N, 0.05 N, and 0.5 N HCl-treated gluten. The changes in SS groups upon these acid treatments are nonsignificant and are inadequate to verify the possible solubility improvement due to SS disruption, although a possibility of the SS interchange reaction disrupting intermolecular SS groups still exists.

Amino Acid Composition. No great damage occurred to amino acids by the acid treatment except under more severe conditions, e.g. with 0.5 N HCl, that caused a loss of 40% in glycine and tryptophan, and 20% in aspartic acid, alanine, lysine, and arginine.

Functional Properties. *Emulsifying Capacity and Stability.* As shown in Table V, the untreated gluten revealed very low emulsifying capacity and stability rating. Both properties were remarkably improved by acid treatments. The gluten modified with 0.02, 0.05, and 0.1 N HCl or with 1.75 N acetic acid demonstrated an emulsifying capacity equal to or even better than that of soy isolates. The 0.5 N HCl and 3.5 N acetic acid treated gluten showed an emulsifying capacity slightly inferior to soy isolates, although far better than the untreated gluten. The acid modifications considerably improved the emulsion stability as compared to the untreated gluten, and the 0.02 N HCl-treated gluten showed almost the same emulsion stability as soy isolates.

For emulsification the greater molecular weight of solubilized gluten the better the emulsifying capacity. The fraction having a molecular weight of approximately 100000 (Ib in Figure 3B) seems to be desirable for this purpose.

Foamability and Foam Stability. Table VI shows the foamability and the foam stability of untreated gluten and acid-treated gluten. The untreated gluten showed a better foamability than the acid-treated gluten with the exception of the pH 5.2 protein fractions from the 0.02 and 0.05 N HCl-treated gluten. It was found that the lower the concentration of the acid used for the modification, the higher the foamability of the sample. The acetic acid modified gluten revealed greater foamability than the HCl-treated gluten.

With a starting volume of 50 ml, it was found that the untreated gluten had 10-ml drain volume at 0 time and the drain volume reached the maximum after 40 min, while the pH 5.2 fractions of both 0.02 and 0.05 N HCl-treated gluten had no drain at 0 time and the drain reached maximum after 80 min. It is clear that the pH 5.2 fractions had an excellent foamability and foam stability. High foamability of protein extracted at a pH close to the point of minimum solubility was reported for cottonseed flour (Lawhon et al., 1972). As seen in Figure 3, the pH 5.2 fraction contained the mol wt 35000 fraction at the highest relative concentration. Although there is no supporting

Table VI. Foamability and Foam Stability of Acid-Modified Gluten

Treatment	Foamability, % vol increase	Foam stability, drain vol (ml),[a] at min 0	5	10	20	30	40	50	60	80
Untreated gluten	460	10	25	30	40	44	46	46	46	46
0.02 N HCl	400	10	25	30	38	42	44	45	46	46
0.05 N HCl	300	15	30	40	46	46	46	46	46	46
0.1 N HCl	180	30	46	46	46	46	46	46	46	46
0.5 N HCl	80	40	45	46	46	46	46	46	46	46
0.02 N HCl pH 5.2 fraction	560	0	7	15	25	30	35	38	40	46
0.05 N HCl pH 5.2 fraction	560	0	7	15	25	32	38	40	42	46
1.75 N acetic acid	420	5	10	20	30	40	42	42	44	46
3.5 N acetic acid	380	5	15	25	32	40	44	46	46	46
8.75 N acetic acid	340	10	20	36	40	46	46	46	46	46

[a] Starting volume 50 ml.

evidence, it is reasonable to believe that a protein should have a certain molecular size to promote foaming due to decreasing surface tension, and be readily denaturable upon mechanical disturbance thereby acting as a foam stabilizer once foam has been formed.

In general, the larger the molecules the better the emulsification as well as whippability as long as gluten stays in solution. To prepare soluble gluten of large molecular size, acetic acid is suitable for hydrolysis because of mildness in acidity as well as the outstanding capability of protein dissociation. However, acetic acid after treatment should be recovered by evaporation and reused to reduce the cost of the acid. Furthermore, collecting protein by isoelectric precipitation was difficult as the acid-treated solution did not show a distinct pH of maximum precipitation.

Solubility of proteins was discussed by Bigelow (1967) who tried to correlate with hydrophobicity and charge density of the proteins. Hydrophobic groups are also strengthening the aggregation and secondary structure of glutamine–glutamic acid copolypeptides (Krull and Wall, 1966). However, changes in hydrophobicity of gluten during the acid modification used in this study must be slight as no significant change in amino acid composition was observed. Increased negative charges as a result of the acid modification are more likely to be a major cause for solubilization as a solution of glutamine–glutamic acid copolypeptides was primarily due to the electrostatic repulsion by negative charges upon ionization (Krull and Wall, 1966).

Much of the insolubility and cohesive nature of gluten proteins must arise from molecular associations caused by the numerous amide groups on the glutamine residues through hydrogen bonding that usually prevails (Holme and Briggs, 1959; Wall et al., 1968). Deamidation disrupts this extensive hydrogen bonding, thus increasing solubility as well.

In conclusion, the best acid hydrolysis condition was chosen from the aspect of functionality of solubilized gluten: a 5% gluten suspension in 0.02 N HCl is heated for 30 min at 121 °C or in 0.05 N HCl for 15 min. A mild hydrolysis such as this causes minimal changes in the molecular size of gluten and in its essential amino acids. For emulsion and foam formation, the retention of large molecular fractions, mol wt 100000 or above in AUC, in the water-soluble acid hydrolysate is required.

Solubilization of gluten by acid hydrolysis may be mainly due to the increased electrostatic repulsion and the decreased hydrogen bonding as a result of deamidation. Furthermore, contrary to Holme and Briggs (1959), there exists a possibility of peptide bond rupture contributing to solubilization depending on the acid concentration.

An activated carbon treatment eliminated objectionable wheaty odor from the solubilized gluten.

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LITERATURE CITED

Acton, J. C., Saffle, R. L., *J. Food Sci.* **35**, 852 (1970).
Al-Sulimany, F., Townshend, A., *Anal. Chim. Acta* **66**, 195 (1973).
Aranyi, C., Hawrylewicz, E. J., *J. Agric. Food Chem.* **20**, 670 (1972).
Beckwith, A. C., Wall, J. S., Dimler, R. J., *Arch. Biochem. Biophys.* **103**, 319 (1963).
Beveridge, T., Toma, S. J., Nakai, S., *J. Food Sci.* **39**, 49 (1974).
Bigelow, C. C., *J. Theoret. Biol.* **16**, 187 (1967).
Cavins, J. F., Krull, L. H., Friedman, M., Gibbs, D. E., Inglett, G. E., *J. Agric. Food Chem.* **20**, 1124 (1972).
Chen, C. H., Bushuk, W., *Can. J. Plant Sci.* **50**, 15 (1970).
Erlander, S. R., Foster, J. E., *J. Polym. Sci.* **37**, 103 (1959).
Fields, R., *Biochem. J.* **124**, 581 (1971).
Gagen, W. L., Holme, J., "Industrial Use of Cereals", Pomeranz, Y., Ed., American Association of Cereal Chemists, St. Paul, Minn., 1973, pp 88–90.
Holme, J., Briggs, D. R., *Cereal Chem.* **36**, 321 (1959).
Krull, L. H., Wall, J. S., *Biochemistry* **5**, 1521 (1966).
Lawhon, J. T., Cater, C. M., Mattil, K. F., *J. Food Sci.* **37**, 317 (1972).
Liu, T. Y., Chang, Y. H., *J. Biol. Chem.* **246**, 2842 (1971).
McDonald, C. E., Pence, J. W., *Food Technol.* **15**, 141 (1961).
Meredith, O. B., Wren, J. J., *Cereal Chem.* **43**, 170 (1966).
Scholte, Th. G., *Ann. N.Y. Acad. Sci.* **164**, 156 (1969).
Swift, C. E., Lockett, C., Fryar, A. J., *Food Technol.* **15**, 468 (1961).
Wall, J. S., Friedman, M., Krull, L. H., Cavins, J. F., Beckwith, A. C., *J. Polym. Sci., Part C* **24**, 147 (1968).
Wiff, D. R., Gehatia, M., *J. Macromol. Sci. Phys.* **B6**, 287 (1972).
Wilcox, P. E., *Methods Enzymol.* 11, 63–76 (1967).
Wright, W. B., Brown, P. J., Bell, A. V., *J. Sci. Food Agric.* **15**, 58 (1964).

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