Effects of Metal-Complexing Agents on Water Binding by Gluten

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ABSTRACT

Glutens from four soft red winter wheat cultivars were obtained by sedimentation (fractionation) of flours in water, in ethylenediaminetetraacetic acid (EDTA) at four concentrations (0.00125–0.0075*M*), and in 1*M* sodium chloride. Glutens then were repeatedly extracted with water by suspension of fragmented gluten in water followed by centrifugation at 1,000 × *g*. Volumes of sediments were measured after each centrifugation to determine water binding. Glutens obtained by sedimentation of flours in 0.0075*M* EDTA or in 1*M* sodium chloride became increasingly hydrophilic during water extraction, forming voluminous gels. Glutens from flours fractionated in 0.00125*M* EDTA did not swell. When flours were fractionated in 0.0025*M* EDTA, swelling of Becker gluten approached the response from fractionation of flour in 0.0075*M* EDTA, but Hillsdale gluten exhibited only slight swelling. Glutens from Caldwell and Compton flours showed intermediate swelling. Glutens obtained by fractionation of flours in water followed by treatment with sodium chloride or EDTA and lyophilized also exhibited measurable swelling when gluten powders were extracted with water. Wide differences between swelling responses of Becker and Hillsdale glutens, regardless of location or crop year, indicate a genetic basis for the differences. Results suggest that divalent cations bound to gluten are removed by EDTA (or by sodium chloride at high concentrations), resulting in increased interaction with water. Amounts of EDTA required to induce maximum response appear to vary with genotype.

The hydrophobic nature of gluten and the role of the hydrophobic character in the behavior of gluten and flour have been the subject of many studies. Much of the early work was devoted to investigations of gluten extractability and of factors that affect dispersibility in water (peptization). Results of numerous studies showed that inorganic salts affect gluten solubility to a great degree and that effects are dependent on the salt species (Dill and Alsberg 1924, Tague 1925, Blish and Sandstedt 1926, Sharp and Herrington 1927, Gortner et al 1929, Harris 1931, Rich 1933, Sinclair and Gortner 1933). More recently, Preston (1981, 1989) studied the effects of neutral salts on gluten and dough properties, noting that changes in protein extractability due to variation in salt concentration and anion species can be attributed to hydrophobic interactions that are a function of the inherent hydrophobic properties of the protein (rather than of the metal-protein complex). Results of these and other studies suggest that hydrophobic interactions between gluten fibrils contribute to gluten elasticity and therefore play an important part in rheological and baking behavior (Bernardin and Kasarda 1973, Kinsella and Hale 1984).

These studies have been concerned primarily with behavior of the proteins in the presence of a specific salt at a particular concentration. They have not been concerned with possible salt-induced effects that might be manifested after transfer of the gluten to water or to a more dilute salt solution. A previous report showed

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that gluten exposed to 1*M* sodium chloride and then washed with low-conductivity water became increasingly hydrophilic (Clements 1973). Treatment with 0.1*M* sodium chloride caused some swelling, while 0.01*M* sodium chloride had no visible effect. This drastic reversal of the normally hydrophobic character of gluten suggests that, at sufficiently high concentrations, sodium chloride removes or neutralizes a factor that hinders gluten-water interaction. Among the factors associated with gluten that might be sensitive to such treatment are divalent cations. Although more strongly bound to proteins than monovalent cations, divalent cations such as Ca^{2+} and Mg^{2+} might be displaced by monovalent cations such as Na^{+} when present in high concentrations. A consequence could be removal of inter- or intramolecular bridges, which in turn could lead to increased hydration (Haurowitz 1950).

Because of the possible implication of polyvalent metal ions in enhanced water binding by gluten after salt treatment, effects of metal-complexing (chelating) agents on gluten behavior were studied. Objectives were to compare effects of agents such as ethylenediaminetetraacetic acid (EDTA) with effects of sodium chloride, to compare different treatment and hydration conditions, and to establish possible differences among cultivars.

MATERIALS AND METHODS

Materials

Flour specimens were year and location samples on hand at the Wooster laboratory, milled either in the laboratory or by a commercial mill (Table I). All flours were straight-grade flours milled from pure cultivars of soft red winter wheats. Flours weights were not adjusted for moisture.

All reagents were reagent grade. Solutions of EDTA (Matheson, Coleman and Bell, Norwood, OH) and Chel CD (*trans*-1,2-diaminocyclohexane-N,N,N′,N′-tetraacetic acid monohydrate) (Fluka Chemical Corp., Ronkonkoma, NY) were adjusted to pH 6.0 with sodium hydroxide before use. House-deionized water was further purified by passage through two mixed-bed ion-exchange columns in series (Ion Exchanger, research model 2, Illinois Water Treatment Co., Rockford, IL). Conductivity ranged between 0.5 and 2.0 μS/cm.

Analytical Procedures

Protein was calculated from Kjeldahl nitrogen (N × 5.7). Ash was determined gravimetrically using a modification of the rapid ash method (AACC 1983, method 08-02) with addition of magnesium acetate but with incineration at 555°C for 15 hr. Particle size (mean volume diameter) was determined with a Microtrac Particle-Size Analyzer (model 799-0, Leeds & Northrup, Microtrac division, Largo, FL), using 2-propanol as a medium (AACC 1983, method 50-11).

Gluten Treatment and Extraction

Two approaches were used. The first procedure involved treatment of gluten proteins during isolation by sedimentation (fractionation) of flour in the treatment medium, followed by repeated water extraction of the freshly isolated and fragmented wet gluten. The second approach involved similar treatments but also included treatment of glutens after isolation. In this second series of experiments, treated glutens were lyophylized and ground, and the gluten powders were extracted sequentially with water. In both series, swelling of gluten during water extraction was determined by measuring the volume of sediment (±0.1 ml) after centrifugation at 1,000 × *g* after each step of extraction. Water and solutions were cooled to about 4°C before addition, and suspensions were maintained at 4°C during settling. All water extractions of gluten were by centrifugation at ambient temperature (IEC model V centrifuge with IEC 240 horizontal rotor accommodating 8- × 100-ml polycarbonate tubes, International Equipment Co., Boston, MA).

TABLE I
Flours Included in Gluten Hydration Study

Flour	Origin	Crop Year	Protein[a] (%)
Becker 1	Ohio	1988	9.8
2	Ohio	1988	9.5
3	Ohio	1985	7.5
4	Ohio	1985	9.0
5	Ohio	1986	8.7
Hillsdale 1	Michigan	1988	9.5
2	Michigan	1988	11.1
3	Washington	1988	9.1
4	Washington	1987	10.7
5	Michigan	1987	9.4
6	Michigan	1986	8.3
Caldwell	Indiana	1988	8.6
Compton	Indiana	1986	8.1

[a] Moisture basis, 14%.

Studies of Freshly Prepared Wet Glutens

Gluten preparation. A conventional aqueous fractionation procedure was employed (Yamazaki et al 1977), but only the gluten fraction was retained. Control glutens were prepared by fractionation of flours in water. For treatments, sodium chloride (0.01 and 1*M*), EDTA (0.00125, 0.0025, 0.005, and 0.0075*M*), or Chel CD (0.0025 and 0.005*M*) solutions were substituted for water. The basic procedure was as follows: Flour (500 g) was placed in a mixing bowl of a food mixer (model C-100, Hobart Corp., Troy, OH), 350 ml of water was added, and the dough was mixed for 3 min at low speed. An additional 650 ml of water was added and the dough liquified by mixing an additional 3 min at low speed. The batter was transferred to a blender (model PB-5, two-speed, with 1,250-ml glass jar, Waring Products Corp., New York, NY), blended for 1 min at high speed, divided between two centrifuge cups (bottom portions of 1-L polyethylene bottles), and centrifuged 15 min at 1,000 × *g* at 4°C (Sorvall model RC-3 refrigerated centrifuge with HG-4L rotor, DuPont Instruments, Wilmington, DE). Supernatant was decanted and tailings were scraped off and discarded. The gluten pads were peeled from the starch, and each was kneaded in 10 100-ml changes of 0.01*M* sodium chloride (4°C).

Water extraction of gluten and measurement of swelling. Fresh gluten (50 g) was cut into small pieces (8–12 g) with scissors. About 800 ml of 0.01*M* sodium chloride (4°C) was placed in a tall, graduated 1-L beaker with a neck (Fleaker, Corning Glass Works, Corning, NY). A homogenizer (Super Dispax model SD-45N with G-450 generator, Tekmar Co., Cincinnati, OH) was lowered into the flask with the blades 1 cm from the bottom of the flask. With the homogenizer operating at maximum speed (10,000 rpm), the gluten pieces were added and homogenized one at a time. Homogenization was continued for 30 sec after the last piece was dispersed. Additional 0.01*M* sodium chloride was added to fill the beaker, and the suspension was stirred and allowed to settle. Foam was broken by gentle intermittent stirring. If severe and stable, the foam was skimmed off and broken by subjecting it to vacuum, and gluten particles were returned to the beaker. The supernatant was decanted as soon as a sharp interface appeared (i.e., with the sediment occupying a volume of about 200 ml) and replaced with fresh 0.01*M* sodium chloride. The mixture was stirred vigorously to disperse the gluten and, after settling, again was decanted. The gluten was finally homogenized for 10–15 sec in 500 ml of 0.01*M* sodium chloride, and the volume was adjusted to 600 ml. With suspension maintained by stirring and swirling, the mixture was poured to fill six 90-ml glass centrifuge tubes to provide six aliquots, each containing about 6 g of wet gluten (determined by centrifugation of one aliquot). Two tubes were selected for extraction, and the remaining tubes were refrigerated for possible replicate extractions. Gluten in the selected duplicates was allowed to settle (5–10 min), and supernatants were decanted and replaced with water. The sediment was resuspended by stirring, and the tubes were centrifuged 5 min at 100 × *g*. Volume (±0.1 ml) of sediment was measured by reference to a graduated tube, and the supernatant again was decanted and replaced with water. After stirring, the suspension was centrifuged 5 min at 1,000 × *g*, and the volume of sediment

was again measured. This process, with centrifugation at 1,000 × *g*, was repeated until the swelling of the sediment attained a maximum volume and then decreased to the volume of the original wet gluten (2 ml).

Studies of Lyophilized Glutens

Gluten preparation. Gluten was isolated by fractionation of flour as described above and frozen until used. The frozen gluten (100 g) was thawed, homogenized in 0.01*M* sodium chloride, and washed twice by suspension in 0.01*M* sodium chloride as described under the procedure for wet gluten. Glutens that were not treated with 1*M* sodium chloride or complexing agent during the fractionation procedure then were treated by replacing the 0.01*M* sodium chloride with the specified medium (1*M* sodium chloride, or 0.0025 or 0.005*M* EDTA), stirring vigorously to resuspend, and then stirring 30 min with a magnetic stirrer. The suspension was allowed to settle, decanted, and then washed twice more by resuspension in 0.01*M* sodium chloride followed by settling and decanting (as above). Glutens that had been isolated by fractionation of flour in treatment media were subjected to the same washing procedure but with the preceding treatment step omitted. The sediment finally was suspended in about 800 ml of 0.01*M* sodium chloride and centrifuged 15 min at 1,000 × *g*. The gluten pad then was freeze-dried and ground in a burr mill (Falling Number type 3303 with "coarse" burrs, Falling Number AB, Stockholm, Sweden).

Gluten extraction and measurement of swelling. Gluten powder (1 g) was weighed into a 100-ml polycarbonate centrifuge tube, and 4 ml of 50% (by weight) sucrose was added. The powder was dispersed by agitating with a vortex-type mixer (Lab-Line Super Mixer, Lab-Line Instruments, Melrose Park, IL), and the suspension was deaerated by placing it in a vacuum chamber and evacuating it three times. The tube was filled with water, and the mixture was stirred vigorously to suspend the gluten and centrifuged 5 min at 300 × *g*. Volume of sediment was measured, the supernatant was decanted and replaced with water, and the process repeated with centrifugation at 700 × *g*. Extraction was continued by this procedure, but with all subsequent centrifugations at 1,000 × *g*. Extraction was discontinued when the volume of sediment attained a maximum and started to decrease.

RESULTS AND DISCUSSION

Studies of Freshly Prepared Wet Glutens

In an earlier study of the effects of salts on gluten behavior (Clements 1973), glutens were treated with sodium chloride after being isolated by conventional fractionation of flours in water. In preliminary studies of the effects of EDTA, water-extracted glutens treated with sufficiently high levels of EDTA showed effects similar to those shown by glutens treated with sodium chloride. However, an even more pronounced effect was observed when glutens were isolated by fractionation of flour in the treatment medium (aqueous EDTA or sodium chloride). This approach was followed for studies of wet (nondried) glutens. In this section, all data were obtained from glutens isolated by fractionation of flour in the treatment medium.

The swelling curves in Figure 1 obtained by water extraction of glutens isolated by fractionation of four flours in 0.00125*M* EDTA show negligible increases in water binding as a result of the treatment. At intermediate concentrations of EDTA, the flours showed differential effects. When flours were fractionated in 0.0025*M* EDTA, Becker gluten swelled to about 50 ml (at 1,000 × *g*). Hillsdale gluten, on the other hand, showed only slight swelling (7 ml). Glutens from Compton and Caldwell flours showed intermediate responses. Hillsdale gluten responded only slightly more when the flour was fractionated in 0.005*M* EDTA. However, glutens isolated by fractionation of the flours in 0.0075*M* EDTA all responded to about the same degree. After about seven water extractions of 6 g of wet gluten (about 3 ml),

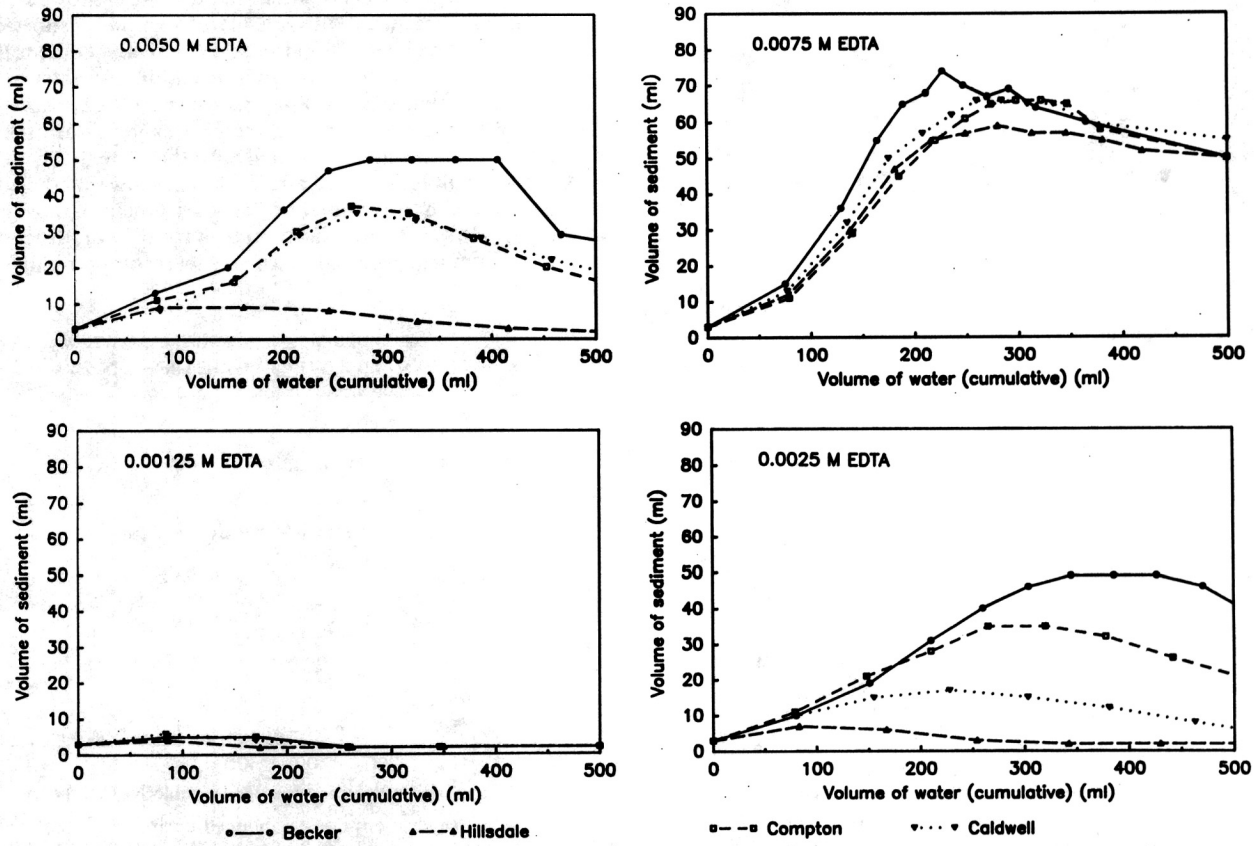

Fig. 1. Swelling (hydration) curves obtained during sequential water extraction of glutens isolated by fractionation of Becker, Hillsdale, Compton, and Caldwell flours in 0.00125, 0.0025, 0.005, and 0.0075*M* EDTA. Sediments (gels) were from repeated extraction of 6 g of wet gluten in 90 ml of water with centrifugation at 1,000 × *g*.

the residues had swollen to more than 60 ml (at 1,000 × *g*). At maximum swelling, residues were voluminous gels, forming sharp interfaces with the supernatant (Fig. 2).

The different responses exhibited by Becker and Hillsdale glutens from flours fractionated in 0.0025*M* EDTA suggested genetic differences in sensitivity to the complexing agent. When flours from Becker and Hillsdale wheats grown in several different locations and years were fractionated in 0.0025*M* EDTA and extracted with water, different degrees of swelling were still evident (Fig. 3). Only one of the six Hillsdale glutens exhibited a maximum volume exceeding 20 ml. Four of the five Becker glutens exhibited maximum volumes greater than 60 ml. In view of the range of locations, years, and protein levels in the Hillsdale wheat specimens (Table I), the lower sensitivity of the Hillsdale gluten to EDTA appears to be a function of genotype rather than of environment.

Fig. 2. Sediments (gels) from sequential water extraction of glutens from Caldwell (CD), Becker (B), Hillsdale (H), and Compton (CP) flours fractionated in 0.0025 and 0.0075*M* EDTA. Shown are maximum volumes obtained during repeated extraction of 6 g of wet gluten in 90 ml of water with centrifugation at 1,000 × *g*.

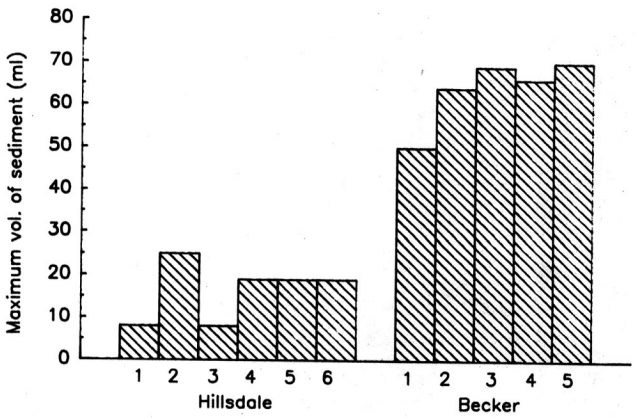

Fig. 3. Maximum volumes of sediments (gels) from sequential water extraction of glutens from Hillsdale and Becker wheats grown in different locations and years (Table I). Glutens were isolated by fractionation of flours in 0.0025*M* EDTA. Sediments were from repeated extraction of 6 g of wet gluten in 90 ml of water with centrifugation at 1,000 × *g*.

The different sensitivities of Hillsdale and Becker flours toward complexing agents also were evident when flours were fractionated in Chel CD, a diaminocyclohexane tetraacetic acid (Fig. 4). When flours were fractionated in 0.0025*M* Chel CD, Hillsdale gluten showed negligible swelling, but Becker gluten swelled to a maximum of about 40 ml. Gluten from the two cultivars showed comparable swelling (38 and 48 ml, respectively) when the flours were fractionated in 0.005*M* Chel CD.

Studies of Lyophylized Glutens

Because of the problems associated with quantitive studies of wet glutens, it seemed desirable to extend the studies to dry glutens. These experiments included glutens that were isolated by fractionation of flours in water and then treated, as well as glutens that were isolated by fractionation of flours in EDTA or sodium chloride. For swelling studies, the gluten powders were extracted with water. To prevent agglutination, the powders were first dispersed in 50% sucrose. Deaeration of these suspensions (under vacuum) was found to be necessary to avoid flotation of the gluten after water was added.

The data summarized in Figure 5 show maximum volumes of sediments during water extraction. The greatest swelling within each cultivar was shown by gluten isolated by fractionation of the flour in 1*M* sodium chloride, with Compton and Becker exhibiting the greatest responses (maximum volumes more than 50 ml). Glutens from flours fractionated in 0.005*M* EDTA also responded, but to a lesser degree. Glutens treated with 1*M* sodium chloride or 0.005*M* EDTA after isolation also showed varying degrees of swelling, with Hillsdale gluten exhibiting substantially less swelling than glutens from the other cultivars. Glutens treated with 0.01*M* sodium chloride after isolation in water did not respond, indicating that swelling is not a result of washing, i.e., removal of fine starch, gums, salt-soluble proteins, or other non-gluten factors physically associated with the gluten.

Analyses of glutens treated after isolation show that the washing process resulted in substantial protein enrichment of most glutens (Table II). However, protein in glutens from flours fractionated in 1*M* sodium chloride was also 3–9 percentage points above

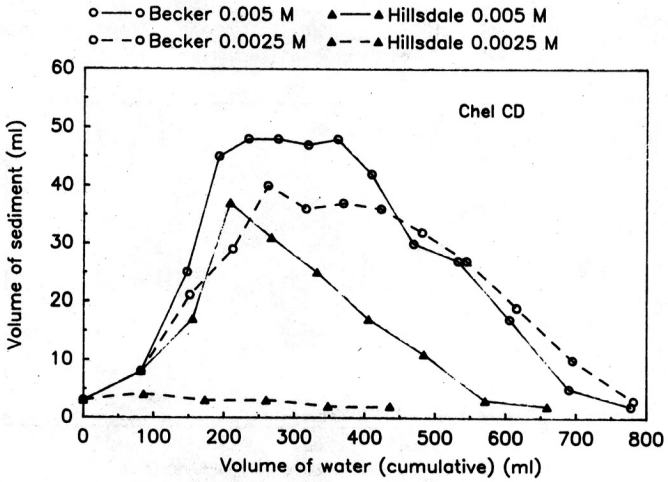

Fig. 4. Swelling (hydration) curves obtained during sequential water extraction of glutens isolated from Becker and Hillsdale flours by fractionation in 0.0025 and 0.005*M* Chel CD. Sediments were from repeated extraction of 6 g of wet gluten in 90 ml of water with centrifugation at 1,000 × *g*.

control levels. Ash levels ranged widely, possibly because of residual sodium chloride, but ash values for most glutens treated with EDTA or 1*M* sodium chloride after isolation were less than half of the control values. Because powders from milled, treated glutens appeared finer than powders from controls, particle size ranges were determined. Mean volume diameters of all powders from treated glutens were considerably less than the diameters of powders from controls. "Cementing" materials were probably washed from glutens treated after isolation. However, glutens from flours fractionated in treatment medium also milled to finer particles.

Early studies showed that hydration capacity is drastically reduced when gluten is dried (Sharp and Gortner 1922, Blish and Sandstedt 1926). However, although the dry glutens from flours fractionated in 0.005*M* EDTA did not exhibit the degree of swelling shown by the freshly prepared wet glutens, measurable swelling did occur. The results suggest that lyophylized gluten may be useful for quantitative compositional studies but that water-binding measurements on the gluten may not be truly indicative of the effects of EDTA treatment. Attempts to induce water binding by treatment of dry gluten with sodium chloride or EDTA have met with only limited success. Particle size and/or degree of hydration during treatment may be limiting factors.

General Discussion

Flours from several other soft red and soft white winter wheat cultivars were fractionated in EDTA. Water extraction of all glutens from flours fractionated in 0.0025*M* EDTA resulted in swelling comparable to that shown by Becker, Compton, or Caldwell. No other cultivar has shown the requirement for higher EDTA concentration shown by Hillsdale. However, differences among cultivars were noted. In many instances, effects of exposure to EDTA were evident before water extraction. During fractionation and kneading, glutens from some flours fractionated in EDTA behaved quite differently from the control glutens. Some EDTA glutens (e.g., Compton) tended to disperse and required considerable care during the early stages of kneading. Such observations are subjective, however, and difficult to quantify.

Although complexing agents and sodium chloride both induce water binding, it remains to be established whether the effects of these two different agents are based on a common mechanism. The effects of EDTA suggest that divalent metals are involved, and if so, the action of EDTA would be expected to be stoichiometric. If sodium chloride also is displacing metal ions, the effect would be through mass action, therefore requiring much higher concentrations than those required for EDTA. It may be significant that many of the EDTA- or sodium chloride-treated glutens fused during conventional dry ashing. Addition of magnesium acetate before ashing resulted in a typical ash.

Variable amounts of water- or salt-soluble proteins are normally associated with gluten isolated by aqueous sedimentation (Clements 1973). Dilute EDTA would be expected to extract small amounts of these proteins, and it is possible that chelation of cations results in solubilization and extraction of specific proteins that influence hydration properties of the gluten complex. Fullington (1967, 1974) reported a water-soluble flour protein that binds with Ca^{2+} and then complexes with phospholipids. He noted that dialysis of the water-soluble fraction against EDTA was necessary to remove cations bound to protein. He suggested that secondary structure of the protein is the determining factor in such bonds, bringing functional groups into a spatial orientation conducive to metal-protein binding. An extension of this suggestion is that such bonds could form bridges that would stabilize such a configuration (Haurowitz 1950). Although concerned with a specific water-soluble protein, this concept also may apply to gluten proteins. If such metal bridges do occur to an extent that affects water binding, it seems likely that they involve magnesium or calcium, each occurring at levels of about 0.03% in endosperm (Pomeranz 1988). However, magnesium chloride has been reported to have a specific solubilizing effect on gliadin (Tague 1925, Sharp and Herrington 1927).

A previous report (Clements 1973) showed that when gluten treated with 1*M* sodium chloride was extracted with water, swelling coincided with an abrupt extraction of large amounts of protein (presumably gliadin). Although extracted protein was not measured in this study, EDTA treatments apparently resulted in a similar phenomenon, evident as turbidity and heavy precipitate, when sodium chloride was added to extracts. Repeated water extraction of untreated gluten solubilizes gliadin as electrolyte level falls. However, because of the cohesive, agglutinated state of the gluten, gliadin is extracted very slowly. Apparently, EDTA treatment encourages interaction with water, resulting in dispersion of gluten particles and rapid solubilization of gliadin. In a recent study of adsorption of flour protein on wheat starch granules, Eliassson and Tjerneld (1990) made use of the effect of sodium chloride on gluten for protein isolation. Chelating agents may be even more useful for such applications, particularly if employed as a medium during flour fractionation.

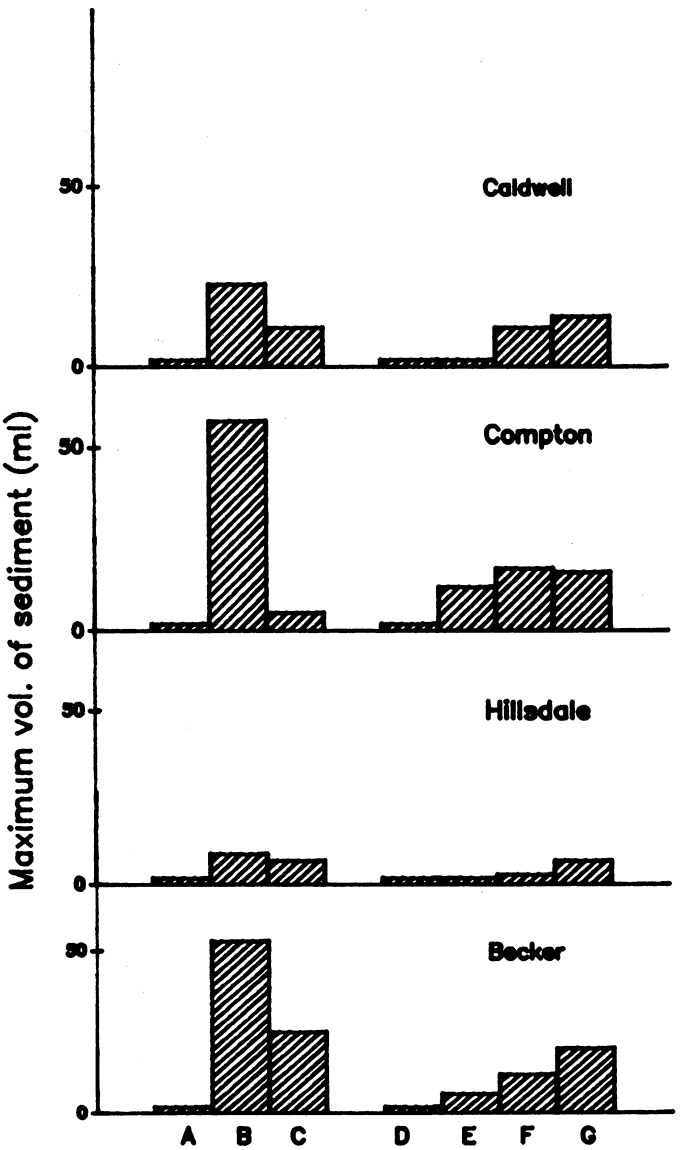

Fig. 5. Maximum volumes of sediments obtained during sequential water extraction of lyophylized glutens from Becker, Hillsdale, Compton, and Caldwell flours. Sediments were obtained during repeated extraction of 1 g of gluten powder in 100 ml of water with centrifugation at 1,000 × *g*. Glutens A–C were obtained by fractionation of flours in water (A), 1*M* sodium chloride (B), and 0.005*M* EDTA (C). Glutens D–G were obtained by fractionation of flour in water and then were treated with 0.01*M* sodium chloride (D), 1*M* sodium chloride (E), 0.0025*M* EDTA (F), and 0.005*M* EDTA (G).

TABLE II
Milled, Lyophylized Glutens Treated with Ethylenediaminetetraacetic Acid (EDTA) and Sodium Chloride[a]

Flour	Medium Used for Flour Fractionation			Medium Used for Treatment (Mechanical Dispersion + Water Extraction) of Gluten Obtained by Fractionation of Flour in Water			
				Sodium Chloride		EDTA	
	Water	1*M* Sodium Chloride	0.005*M* EDTA	0.01*M*	1*M*	0.0025*M*	0.005*M*
Becker							
Protein, %	70.5	79.1	66.9	81.8	78.9	79.6	77.8
Ash, %	0.55	1.43	0.57	0.42	0.25	0.35	0.32
Mean volume diameter, μm	93.0	85.3	72.6	75.4	76.4	69.0	71.5
Hillsdale							
Protein, %	69.5	72.9	70.9	76.9	76.1	75.4	76.0
Ash, %	0.75	1.56	0.57	0.40	0.32	0.37	0.34
Mean volume diameter, μm	96.5	83.6	80.5	73.5	73.9	70.7	69.1
Compton							
Protein, %	78.9	82.3	80.1	81.4	82.8	81.8	80.5
Ash, %	0.63	0.28	0.78	0.32	0.20	0.21	0.32
Mean volume diameter, μm	94.2	83.4	76.6	75.6	80.8	75.3	79.7
Caldwell							
Protein, %	76.9	80.4	76.0	81.1	80.5	80.4	78.4
Ash, %	0.61	0.61	0.59	0.42	0.26	0.30	0.26
Mean volume diameter, μm	96.5	80.1	78.7	78.4	77.3	75.5	77.1

[a]Compositions on dry basis.

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