Effects of Prior Salt Treatment on Gluten Dispersibility[1]

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ABSTRACT

Gluten was homogenized in 1M sodium chloride (NaCl) and then repeatedly extracted with water. Approximately 60% of the gluten protein was solubilized in the initial extracts as the salt concentration in the extraction medium declined to 10 mM. Continued extraction of the residue resulted in swelling to a voluminous gel, indicative of a highly hydrophilic nature. This swelling did not occur until salt concentration in the medium had declined to less than 5 mM. Exhaustive washing of the gel resulted in slow but steady extraction of a second protein fraction which accounted for approximately 30% of the gluten protein. The fraction solubilized in the initial extractions of the gluten was precipitated by salts to give a gliadin-like product, whereas the fraction precipitated from later extracts exhibited the gross characteristics of glutenin. Addition of traces of salts to the intermediate residue gel resulted in immediate clotting and loss of bound water. When gluten was treated with NaCl at concentrations below 1M, subsequent washing resulted in immediate extraction of the gliadin-like fraction, but the degree of residue swelling was directly related to the concentration of salt in the treatment medium. Gluten treated with 0.01M NaCl did not swell. Gluten treated with 1M potassium chloride, magnesium chloride, or calcium chloride exhibited effects similar to those resulting from treatment with 1M NaCl, but results suggest specific cations may have specific effects. When glutens from different varieties of wheat were treated with 1M NaCl and subsequently extracted, no pronounced differences were noted.

Much attention has been devoted to the physical and chemical nature of gluten, and a substantial proportion of these studies has been concerned with the extractability and solubility of gluten proteins. Because of the relatively low solubility of these components in neutral aqueous systems, most investigations have involved application of aqueous acids or bases, dispersants (such as urea or detergents), or organic solvents. Other studies have been devoted to the effects of various salts on dispersibility of gluten components in aqueous systems. These relationships have been discussed in several recent reviews (1,2,3). In spite of the great amount of literature on this subject, a certain degree of confusion and conflict is evident regarding the behavior of gliadin and glutenin in relatively simple aqueous systems. The insolubility of these proteins in water is often pointed out in discussions of gluten chemistry (4). On the other hand, many workers have noted an appreciable extractability of gluten protein when salt level is reduced sufficiently (5,6). Since the unique properties of wheat flour are primarily a function of gluten and its behavior in an aqueous environment, it is apparent that gluten-water relationships are of great practical importance.

One of the problems associated with gluten studies is removal of tailings and other nonprotein colloidal material without resorting to relatively drastic agents to

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effect dispersion. Among the approaches attempted in the present study was comminution of wet gluten in 1M sodium chloride (NaCl) (to suppress dispersion), followed by elutriation with salt solution to wash out nongluten material. It was assumed that removal of salt by subsequent washing with water would result in a "purified" gluten mass. However, upon removal of salt, a high degree of hydration and dispersion resulted. The following report presents the results of a preliminary investigation of this phenomenon. Glutens from several varieties of wheat were treated with NaCl and other salts at various concentrations, and repeatedly extracted with water or dilute salt solutions. Measurements were made of quantity of protein extracted by progressive washing, and of degree of water retention by the insoluble residue.

MATERIALS AND METHODS

General Procedure

The basic procedure involved comminution of wet gluten in aqueous salt solution, followed by repeated suspension in fresh salt solution and decantation to remove nongluten material. The resulting "salt-treated gluten" was then extracted with water by repeated extraction of a single sample with collection of extracts and a single final residue, or by serial extraction of several aliquots of a treated gluten, with one sample set aside after each extraction to represent the residue at that stage. Unless otherwise noted, extractions were carried out by suspending the wet gluten in freshly boiled, glass-distilled, de-ionized water at 5° to 10°C., and refrigerating for several hours (with frequent chopping and stirring), followed by centrifugation. Extracts and residues were freeze-dried and weighed to determine solids content. Protein was determined by a semi-micro Kjeldahl procedure, using 5.7 as a conversion factor. Salt concentration was routinely measured in extracts by conductivity measurements and reference to standard curves. In extracts containing high levels of salt (i.e., greater than 10 mM), salt concentration was estimated from the nonprotein content of the solids. Volumes or wet-weights of residues after centrifugation at 1,000 × g were used as indices of water retention.

The above outline describes the general procedure, but variations included suspension of the whole flour in aqueous salt solution, followed by isolation and extraction of the gluten, as well as treatment of gluten in the presence of tailings (isolated in combination with the gluten) with aqueous salt, followed by isolation and extraction of the gluten. Experiments were also performed to study the effects of treatment with different salt species and concentrations, and influence of salt concentration in the extraction medium. The experiments which follow are examples selected from many experiments based on the above procedures, all of which gave results similar to those presented here.

Experiment I—Water Extraction of NaCl-Treated Soft Wheat Gluten

Gluten was isolated from a composite soft wheat flour by a conventional wet-fractionation procedure (one part flour slurried in two parts water, followed by blending, centrifugation, and separation of layers). Duplicate 100-g. samples of wet gluten were homogenized in 1M NaCl (500 ml.) in a blender (1 min. at low speed plus 1 min. at high speed), and additional 1M NaCl was added to total 800 ml. The suspension was stirred, allowed to settle, and the supernatant was decanted. Suspension in fresh salt solution and decantation were repeated until the

supernatant was clear. The gluten duplicates were combined, the suspension (800 ml.) was blended, and aliquots of the homogenate (40 ml. each, containing approximately 7 g. wet gluten) were transferred to each of nineteen 100-ml. centrifuge tubes. NaCl solution (1M) was added to each tube to a total of 95 ml., and the samples were stirred, allowed to settle under gravity, and decanted. The first tube was set aside, and the remaining tubes were washed with 0.1M NaCl. This procedure was repeated until four tubes had been set aside. The remaining tubes were then extracted with water by the same procedure, allowing several hours for equilibration of each extraction. One tube was set aside after each stage, and the extractions were terminated when one tube remained. To obtain adequate sedimentation, the first two water extractions were centrifuged 10 min. at 200 × g and the remaining extractions were subjected to 1,000 × g for 30 min. Supernatants from each stage were pooled and freeze-dried. At the end of the extraction process, all tubes were centrifuged at 1,000 × g for 30 min., supernatants were decanted, and residues were photographed, weighed, and freeze-dried.

Experiment II—Extraction of NaCl-Treated Soft Wheat Gluten with NaCl Solutions of Various Concentrations

A soft wheat gluten was treated and washed with 1M NaCl as described under experiment I, and aliquots of homogenate containing approximately 12 g. wet gluten were transferred to each of twelve 100-ml. centrifuge tubes. Each sample was then extracted repeatedly with a medium containing a specified concentration of NaCl until residual salt had been extracted and equilibrium was attained (i.e., until salt concentration in the extract was not appreciably greater than salt concentration in the extraction medium). Extracts were collected and freeze-dried. The media were as follows:

Tube	*NaCl Concentration mM*	*No. of Extractions*
1	1,000	6
2	100	6
3	50	6
4	25	6
5	10	6
6	7.5	6
7	5.0	6
8	2.5	8
9	1.0	8
10	0.5	10
11	water	12
12	no extraction	0

Experiment III—Water Extraction of NaCl-Treated Glutens from Five Varieties of Wheat

Glutens were isolated from five varieties of wheat, treated with 1M NaCl as described under experiment I, and washed with two changes of 0.1M NaCl to reduce residual salt. The gluten from one variety (Thorne) was prepared in duplicate, and one of the NaCl-treated glutens was homogenized in 1M $CaCl_2$, and washed with 0.1M and 0.01M $CaCl_2$. Samples (30 g., wet) of each of the six treated glutens were weighed into polyethylene centrifuge cups (800 ml.), water was added

to a total of 600 ml., and the samples were refrigerated 24 hr. They were then centrifuged for 15 min. at 1,000 X g and supernatants were collected. Water was again added to a total of 600 ml., and the suspensions were equilibrated 24 hr. before centrifugation. A total of seven extractions was performed on each sample in this manner, with collection of each extract and of the final residue.

Experiment IV—Water Extraction of a Soft Wheat Gluten after Various Salt Treatments

Flour (250 g.) from a composite of four soft wheat varieties was slurried in water (600 ml.), blended (in sextuplicate), and centrifuged 15 min. at 1,200 X g. The samples were again blended (in the supernatants) and centrifuged. The starch layer was discarded, and the gluten and tailings were homogenized together in the supernatant and centrifuged. The supernatant was discarded, and the tailings and gluten from the six replicates were removed and combined. Salt treatment consisted of homogenizing the gluten-tailings mixture in various salt media (listed below), followed by centrifugation and isolation of the glutens. Each treated gluten was washed free of fines by repeated suspension in the corresponding medium, and those glutens treated with 1M salts were washed twice with the corresponding 0.1M salts. Samples (4 g., wet) of each gluten were weighed into sets of five 100-ml. centrifuge tubes as follows:

Set	*Treatment*	*Tube Numbers*				
I	None	1	7	13	19	25
II	0.01M NaCl	2	8	14	20	26
III	0.10M NaCl	3	9	15	21	27
IV	1.00M NaCl	4	10	16	22	28
V	1.00M $MgCl_2$	5	11	17	23	29
VI	1.00M $CaCl_2$	6	12	18	24	30

Tubes 1 to 6 were set aside to represent unextracted glutens. The remaining tubes were extracted with water by filling to the 95-ml. level and refrigerating 24 hr. After centrifugation at 1,000 X g and collection of supernatants, tubes 7 to 12 were set aside, and the remaining tubes were subjected to a second extraction. Tubes 13 to 18 were set aside, and the remaining samples extracted, etc., until one tube from each set remained (tubes 25 to 30). All extracts and residues were freeze-dried.

RESULTS

Experiment I

Figure 1 shows residues from the 19 gluten aliquots after extraction and centrifugation at 1,000 X g. After one extraction (tube 5, the first sample in the series subjected to water extraction), the residue demonstrated considerable swelling (from 8.2 to 19.6 g., wet). One additional extraction (tube 6) resulted in another twofold increase in wet weight (to 41.9 g.). Water retention increased after each of the following two extractions, resulting in almost 90 ml. of heavy gel (89.7 g.). Further extraction resulted in a very gradual decrease in viscosity (as judged by appearance), but volume remained relatively constant. During those extractions characterized by increases in residue volume, salt concentration in the extracts declined from 50 mM to approximately 1 mM, and there was an abrupt extraction of protein (Fig. 2). However, protein in the extract fell to a constant, low level

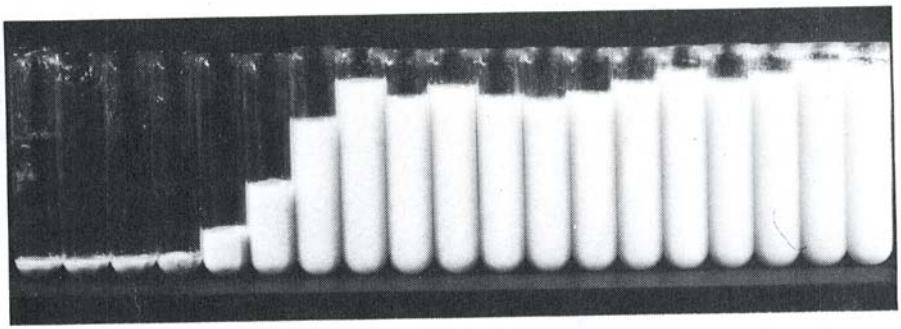

Fig. 1. Residues after successive water extractions of a soft wheat gluten treated with 1M NaCl. Each tube contained 7 g. wet gluten before extraction. Residues 1 to 4 are from initial washings with NaCl of decreasing normalities. Residue in tube 5 remained after first water extraction, residue in tube 6 remained after second water extraction, etc. (All residues after centrifugation at 1,000 × g.)

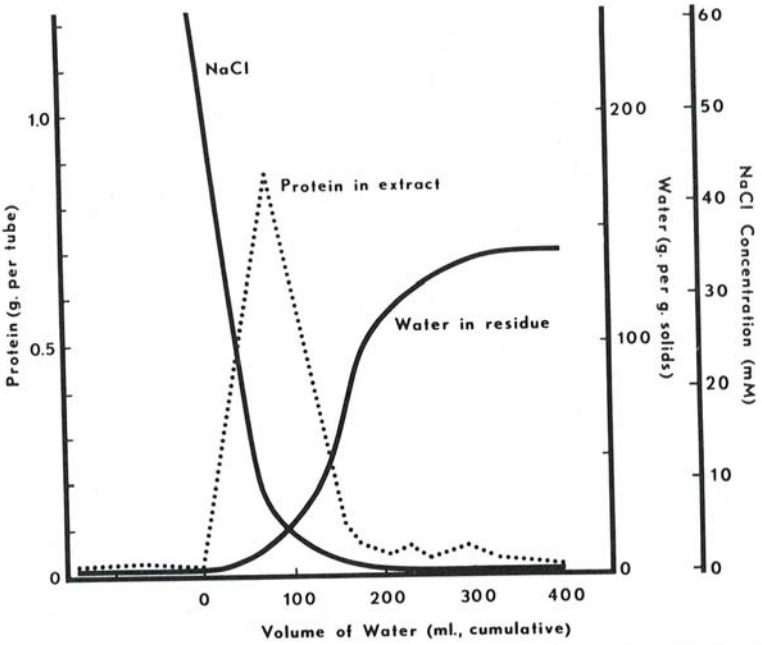

Fig. 2. Extraction of NaCl-treated gluten: Protein in extract and water in residue in relation to salt concentration in the extract.

during these initial extractions. It is noteworthy that the bulk of this protein fraction was soluble in extract containing 7.5 mM NaCl. Dry matter in the residue continued to decline after the initial protein extraction, but very slowly. The residue gel contained approximately 1% solids after four extractions, at which point maximum swelling had occurred. Nonprotein material in the residue (estimated by difference) remained at a uniform low level after the initial extractions (Fig. 3).

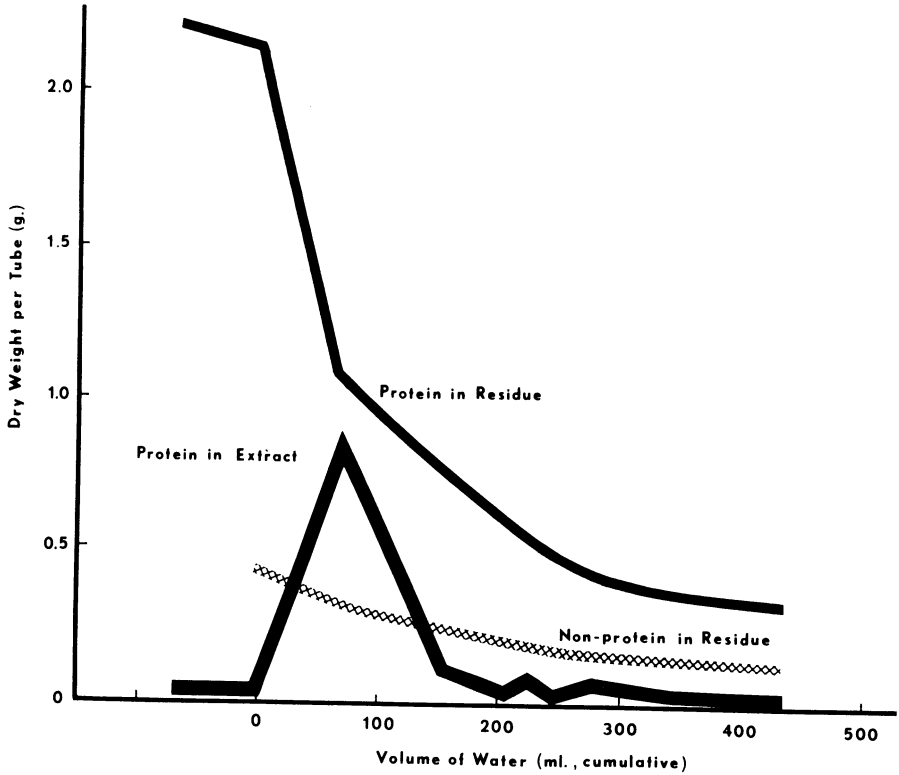

Fig. 3. Protein in extract and in residue after successive water extraction of NaCl-treated gluten.

The initial extracts which contained substantial amounts of protein were relatively clear, but with a slight opalescence and tendency to foam. Addition of a few drops of 1M NaCl to samples of these extracts resulted in immediate precipitation of a sticky, fluid, silver mass, with the general characteristics of gliadin. When freeze-dried, these extracts produced a fluffy white product containing 92 to 95% protein. Addition of traces of salt to residue gels resulted in immediate precipitation of tough, tan, elastic clots with the gross characteristics of glutenin. Extracts from later stages of extraction produced a similar product. Upon freeze-drying, these extracts gave a fibrous, tan product, generally containing less than 85% protein. Protein in the residue gel declined from 83.3 to 67.8% (on a dry basis) during the overall extraction procedure.

Experiment II

This experiment was designed to study the effects of salt concentration in the aqueous medium during extraction of NaCl-treated gluten. In previous experiments, involving water as a medium, significant protein solubilization occurred while salt concentration was still at a substantial level (from residual salt in the gluten). Residue swelling became evident at a lower but detectable level of salt. In this experiment, aliquots of NaCl-treated gluten were extracted at various fixed

concentrations of NaCl ranging from 0 to 1M. Figure 4 illustrates protein extraction curves for the range from 0 to 25 mM NaCl. No protein peak resulted from repeated extraction with 25 mM salt, whereas lower concentrations produced definite maxima. Thus, insofar as the gliadin-like fraction is concerned, there appears to be a solubility threshold between 10 and 25 mM NaCl. Figure 5 relates protein extraction to salt concentration during equilibration of the gluten against the extraction medium. When 10 mM salt was employed as a medium significant protein extraction did not occur until equilibration was approached. The 5 mM medium began to extract protein as concentration approached 10 mM, as did the

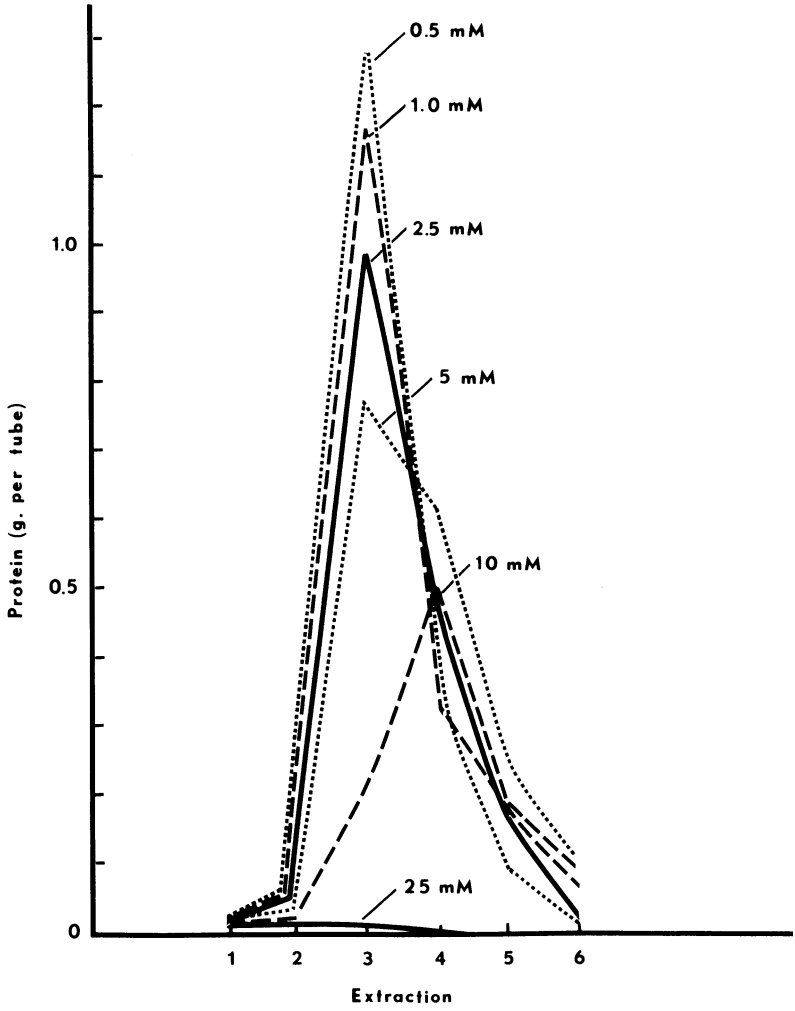

Fig. 4. Protein dispersed during serial extraction of NaCl-treated gluten with NaCl solutions of various concentrations.

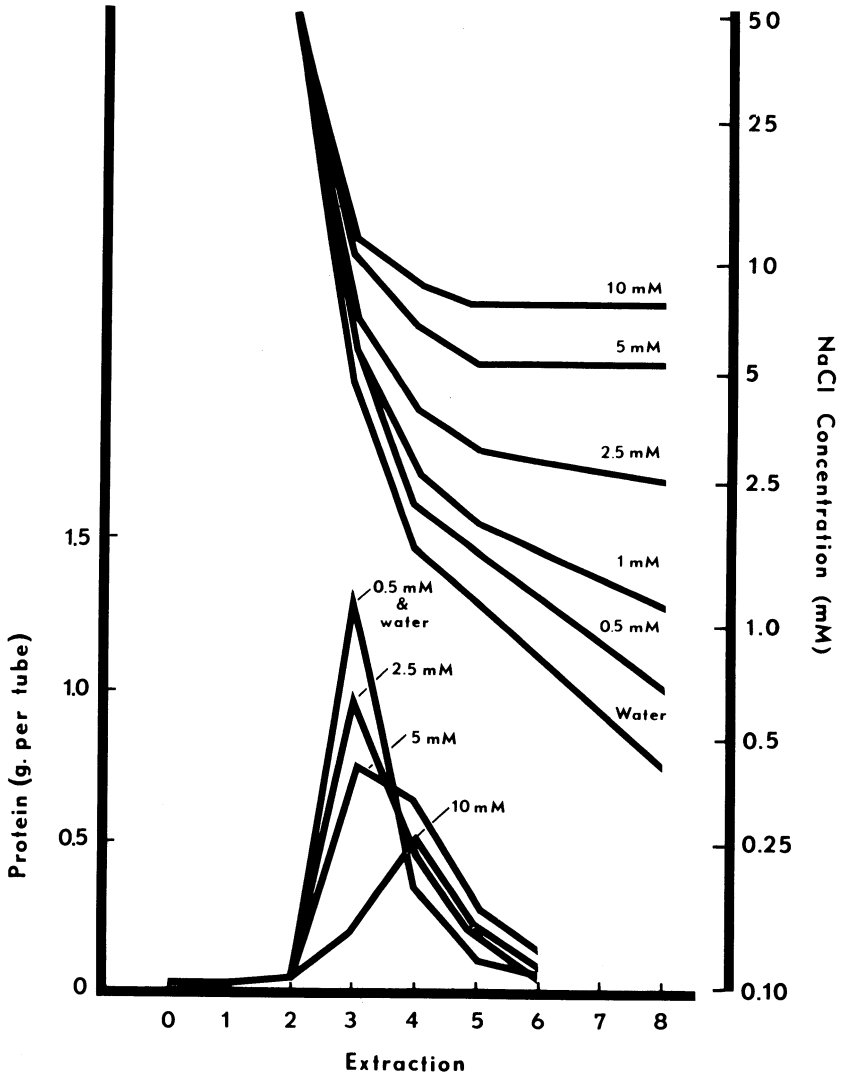

Fig. 5. Protein extraction as a function of salt concentration in the medium during serial extraction of NaCl-treated gluten with NaCl solutions of various concentrations.

more dilute media. Water and 0.5 mM salt gave essentially the same protein extraction curves. In general, extraction at the higher salt concentrations resulted not only in delayed extraction, but also in reduced total protein extracted (ranging from 1.03 g. dry protein extracted by 10 mM NaCl to 2.22 g. extracted by water).

Residue swelling is compared in Fig. 6. No swelling was evident after repeated extraction with 5 mM NaCl, but a definite increase in water retention was noted during extraction with 2.5 mM salt. Both rate and degree of swelling increased as salt concentration in the medium was reduced. Since extraction of the gliadin-like

fraction occurred at considerably higher concentrations of salt (up to 10 mM), it may be concluded that swelling does not coincide with protein extraction, but rather occurs only upon reduction of salt concentration in the medium. The threshold for this swelling apparently lies between 2.5 and 5.0 mM NaCl.

Experiment III

In this experiment, NaCl-treated glutens from five varieties of wheat were extracted with water. In addition, a duplicate NaCl-treated gluten from one variety (Thorne) was treated with $CaCl_2$ before extraction. In general, the results (Table I) were similar to those from previous experiments, and show no pronounced varietal differences. An exception was the gluten from Avon wheat which did not exhibit swelling. However, the unique behavior of this sample in this experiment is assumed to be the result of an unknown contaminant, since glutens from this same flour demonstrated swelling in several subsequent experiments. Conductivity measurements on the extracts indicated normal electrolyte levels in this instance, and failure to swell is an indication of the critical nature of the system. The gluten

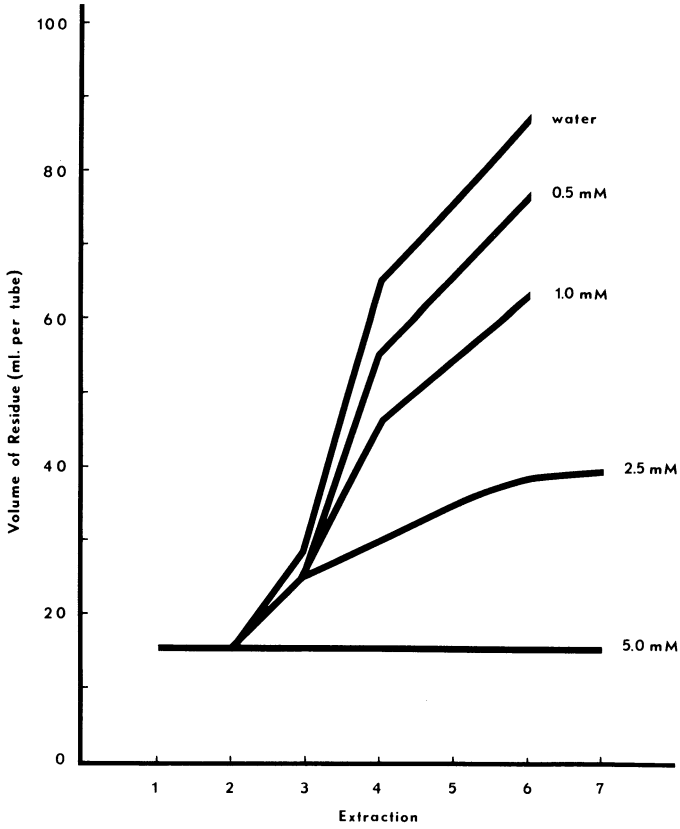

Fig. 6. Volumes of sediments after serial extraction of NaCl-treated gluten with NaCl solutions of various concentrations (after centrifugation at 1,000 × g.).

TABLE I. PROTEIN EXTRACTION AND RESIDUE SWELLING DURING AQUEOUS WASHING OF NaCl-TREATED GLUTENS FROM SEVERAL VARIETIES OF WHEAT. INITIAL GLUTENS (30 g., WET) WERE HOMOGENIZED IN 1M NaCl BEFORE EXTRACTION

Variety	Number of Extractions	Total Volume of Water ml.	Final Volume of Residue[a] ml.	Solids Extd. g.	Solids in Residue g.	Protein Distribution	
						Ext. %	Residue %
Comanche	7	2,470	290	5.2	4.8	60	40
Purkof	7	3,080	110	5.9	4.4	62	38
Trumbull	6	2,150	300	5.4	4.6	61	39
Thorne	7	2,540	240	5.2	4.6	60	40
Thorne[b]	6	3,020	90	4.4	5.0	50	50
Avon	6	3,310	10	3.5	4.8	48	52

[a]After centrifugation at 1,000 X g.

[b]Homogenized in 1M NaCl, followed by 1M $CaCl_2$.

from Thorne wheat which had been treated with $CaCl_2$ also demonstrated a greatly reduced tendency to swell (an effect which is substantiated by results from experiment IV).

Quantitatively, each 30-g. sample of wet gluten was extracted with a total of 2,000 to 3,000 ml. water, resulting in 50 to 60% of the total recovered protein appearing in the extract, and 40 to 50% appearing in the residue. In most instances, the soluble-protein peak occurred in the first extract (500 to 600 ml.).

Experiment IV

This experiment has provided further data regarding behavior of gluten treated with different concentrations of NaCl, and with 1M solutions of magnesium chloride and $CaCl_2$. Gluten treated with 0.01, 0.1, and 1M NaCl yielded approximately the same amounts of extractable protein, or approximately 50% more than that extracted from untreated gluten (Table II). An intermediate amount was extracted from glutens treated with magnesium chloride or $CaCl_2$. In general, extracted protein constituted about 55% and nonextracted protein 45% of the total recovered protein from salt-treated glutens. These values were 35 and 65%, respectively, for untreated gluten.

TABLE II. PROTEIN EXTRACTION AND RESIDUE SWELLING DURING AQUEOUS WASHING OF A SOFT WHEAT GLUTEN (10 g., WET) AFTER HOMOGENIZATION IN VARIOUS SALT SOLUTIONS

Treatment	Number of Extractions	Total Volume of Water ml.	Final Volume of Residue[a] ml.	Protein Extd. g.	Protein in Residue g.	Protein Distribution	
						Ext. %	Residue %
No salt	4	348	7	1.0	1.9	35	65
0.01M NaCl	4	348	7	1.5	1.2	56	44
0.1M NaCl	4	257	40	1.5	1.2	56	44
1M NaCl	4	194	75	1.4	1.1	55	45
1M $MgCl_2$	4	215	65	1.3	1.0	55	45
1M $CaCl_2$	4	260	45	1.3	1.1	53	47

[a]After centrifugation at 1,000 X g.

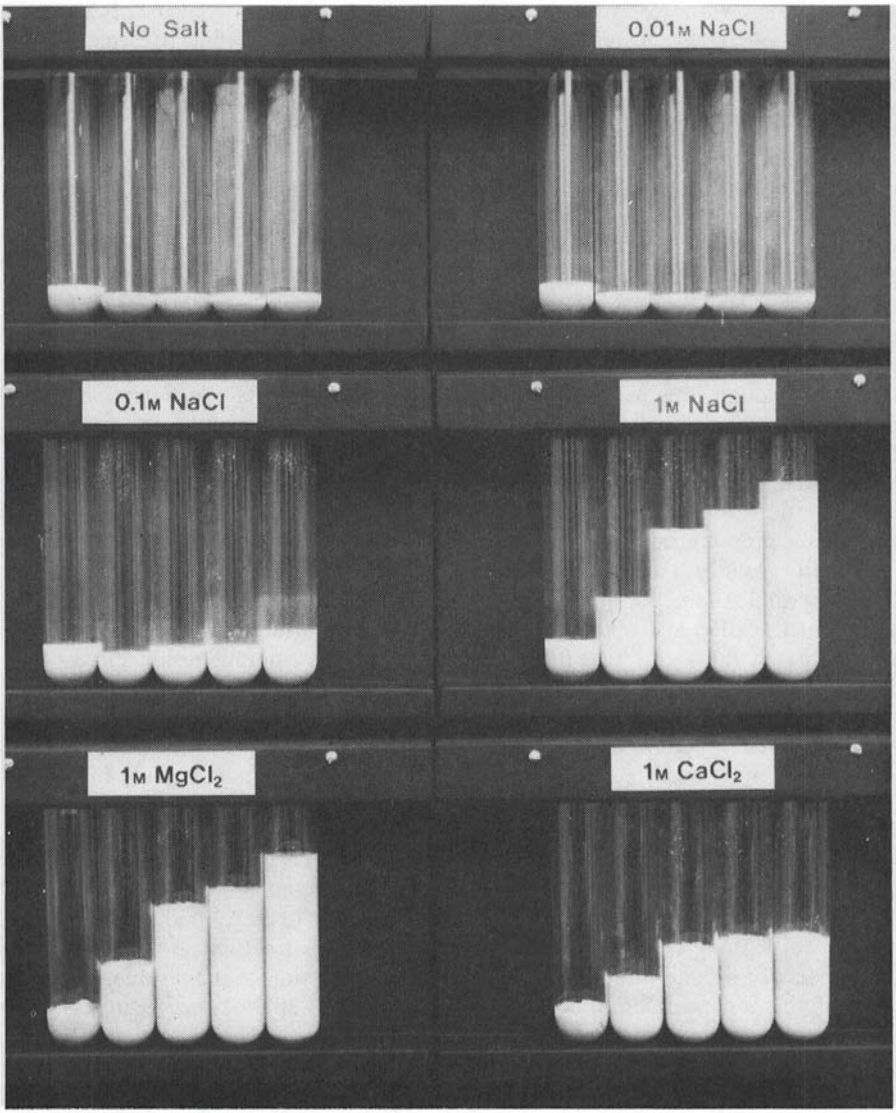

Fig. 7. Residues after successive water extractions of a soft wheat gluten subjected to various salt treatments. The labels indicate the media in which the gluten was homogenized before extraction. The first tube in each series represents the initial treated gluten (10 g., wet); succeeding tubes contain residues after successive water extraction and centrifugation at 1,000 × g.

Pronounced differences were noted in residue swelling (Fig. 7). No perceptible increase in volume occurred during extraction of untreated gluten, or gluten treated with 0.01M NaCl. However, a slight increase in volume was noted during extraction of gluten treated with 0.1M NaCl, and a drastic increase occurred during washing of

gluten treated with 1M NaCl. A large increase in volume was also noted during extraction of gluten treated with 1M magnesium chloride, while treatment with $CaCl_2$ resulted in an intermediate degree of swelling.

DISCUSSION

When a flour is repeatedly extracted with water, a limited amount of gluten protein is solubilized (presumably gliadin) as the natural electrolytes are removed (5,6). In experiments performed in connection with the present study, such extractions removed approximately 35% of the gluten, with most of the solubilized gluten appearing in the early extracts and declining to negligible values as washing continued. When untreated gluten was extracted with water, the residue of insoluble protein occupied less volume than the original gluten, and appeared to be relatively hydrophobic. However, when the gluten was exposed to aqueous NaCl before extraction, this pattern was altered, depending on the concentration of salt during treatment. When low concentrations (0.01M) were employed, 1.5 to 2.0 times as much protein was extracted as from untreated gluten, with the volume of the residue showing a slight decrease. When the gluten was exposed to a higher concentration of NaCl, the amount of extracted protein remained essentially the same, but pronounced increases in residue volumes were noted. Application of 0.1M salt produced only a slight increase in volume, but when the gluten was treated with 1M salt, the increase was more than tenfold (based on measurements after centrifugation at 1,000 × g). This voluminous, gelatinous sediment, containing approximately 1% solids, was indicative of a highly hydrophilic residue, in distinct contrast to the compact elastic residue obtained after extraction of untreated gluten.

The protein fraction which appeared in initial water extracts in these studies, and which has been referred to as "extractable protein," had the gross characteristics of gliadin, but no attempts have been made to establish this identity. This component, containing 92 to 98% protein, generally appeared in the extract as salt concentration declined to approximately 10 mM. On the other hand, swelling of the residue was not evident until salt level fell below 5 mM.

Although the gliadin-like protein has been referred to as "extractable protein," exhaustive washing of the residue after removal of this fraction resulted in steady extraction of a second fraction. After exhaustive extraction of the residue, the total yield of this second fraction accounted for more than 30% of the original gluten. This component, which was extracted as a stable, somewhat turbid dispersion, demonstrated the general characteristics of glutenin, but identity was not established. The freeze-dried product generally contained 70 to 80% protein, but the product from late stages of extraction often contained less than 70% protein. The ultimate residue, after extraction of this second component, was no longer viscous or sensitive to salt, and settled under gravity if sufficiently washed. Quantitative data regarding these exhaustive extractions are limited, however, because of the protracted washing required to effect the extraction. The process is summarized in Fig. 8.

Limited experimentation with salts other than NaCl produced similar effects, but results suggest specific effects may be associated with specific cations. Treatment with 1M potassium chloride resulted in behavior essentially identical to that resulting from NaCl. Treatment with $CaCl_2$ or magnesium chloride resulted in

different swelling patterns, but limited data do not permit definite conclusions.

The mechanism of this salt-induced behavior remains to be determined, but it might be speculated that in aqueous media of high ionic strength, ionic bonds are broken. The resulting exposure of polar sites, as well as possible reduction in size of aggregates, would tend to increase hydrophilicity and dispersibility in water. However, the extreme sensitivity of the fractions toward electrolytes suggests a scarcity of ionizable groups, presumably a general characteristic of the gluten proteins (2). In view of the lack of such ionic groups, it seems unlikely that the salt-induced effects can be attributed to displacement of polyvalent cations through

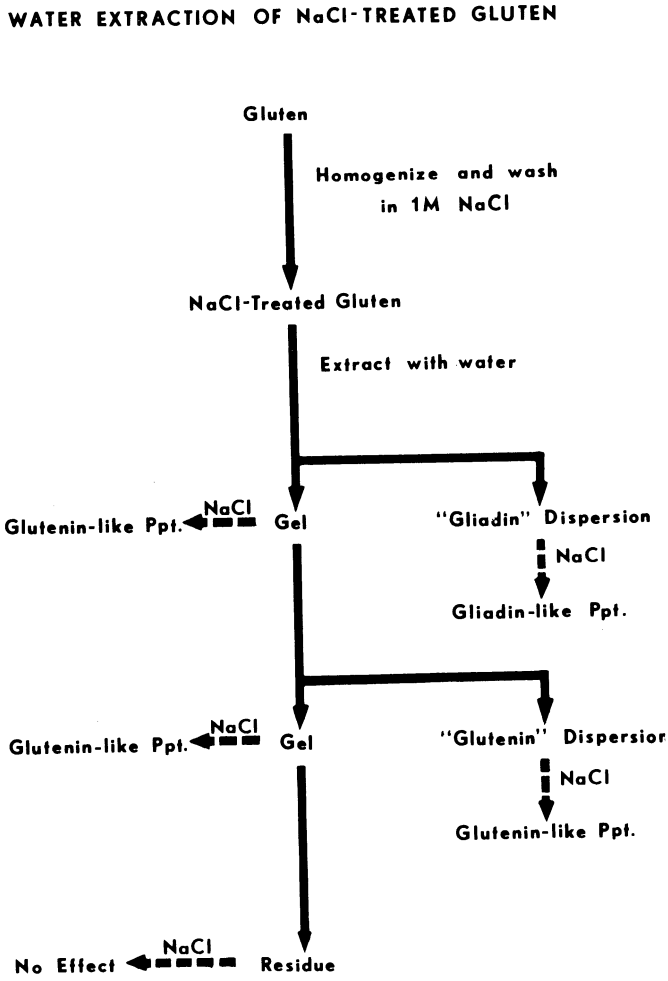

Fig. 8. Scheme for serial extraction of NaCl-treated gluten, showing effect of NaCl on various products and intermediates.

simple ion exchange. However, the increase in hydrophilic properties could result from disruption of complexes involving polyvalent metal ions, particularly if such complexes involved lipids. Such a complex has been described by Fullington (7). Reduction in gluten-lipid binding by NaCl has been reported in several studies, and ionic bonds were assumed to be involved (8,9,10). More recently, however, Pomeranz et al. (11) concluded such salt-induced increases in extractable lipid are unlikely to involve ionic bonds, and suggest NaCl exerts its effects on the gluten protein, i.e., the decreased solubility of gluten in the presence of a salt results in compaction, and exclusion of nonpolar lipids from the hydrophobic interior of the gluten. Current lipid studies, as well as investigations involving defatted flours, may demonstrate the extent to which lipids are involved in the behavior described in this report. In any event, it is evident from this study that the behavior of gluten in a low-salt or salt-free aqueous medium may be a function of past salt environments, and under certain conditions, the gluten proteins may become relatively hydrophilic.

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