Effects of Neutral Salts upon Wheat Gluten Protein Properties. I. Relationship Between the Hydrophobic Properties of Gluten Proteins and Their Extractability and Turbidity in Neutral Salts[1]

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ABSTRACT

The effects of increasing concentrations of neutral monovalent sodium salts upon the extractability and turbidity of wheat gluten proteins was studied. At low salt concentrations ($< 0.05M$) extractability was almost independent of anion type. However at higher salt concentrations ($0.5-4.0M$), gluten protein extractability was highly dependent upon anion type and followed the lyotropic anion series (in increasing order): F^-, Cl^-, Br^-, ClO_4^-, SCN^-. Similar results were obtained by measuring the turbidity of $0.05M$ acetic acid-soluble gluten proteins after addition of various levels of salts. At low salt concentrations turbidity increased in the presence of all salts, but at higher salt concentrations turbidity was dependent upon anion type. Electrophoretic and amino acid analyses showed differences in the properties of the gluten proteins extracted by the various salts. These results suggested that at low salt concentrations the solubility and aggregation properties of gluten proteins are largely determined by ionic interactions, whereas at higher salt concentrations hydrophobic interactions predominate. In addition, wheat gluten proteins appear to vary widely in their hydrophobic properties.

In recent years increased interest has been shown in the potentially important effect of hydrophobic bonding upon the properties of gluten proteins. Green and Kasarda (1971) showed the presence of hydrophobic regions on the surface of α-gliadin by means of fluorescence spectroscopy and equilibrium dialysis with the hydrophobic probe α-p-toluidinylnaphthalene-6-sulfonate. From these results and other studies, Bernardin and Kasarda (1973) suggested that hydrophobic forces may stabilize gluten structure and thus play an important role in the rheological and baking properties of wheat flour.

Unfortunately the measurement of gluten protein hydrophobic properties has been difficult because of the lack of techniques of adequate specificity and sufficient sensitivity to differentiate the extent of these interactions. One technique that has proven useful in this regard has been hydrophobic interaction chromatography. Caldwell (1979) and Papineau and Godon (1978) have reported the fractionation of gliadin proteins on the basis of apparent hydrophobicity by deabsorption with various buffers from octyl and phenyl Sepharose Cl-4B, respectively. Similar studies have also been reported by Chung and Pomeranz (1979), utilizing phenyl Sepharose Cl-4B. Their results showed that glutenins from a poor-baking wheat variety were less hydrophobic than glutenins from a good-baking variety. In contrast, gliadins from the poor variety were more hydrophobic than those of the good variety. The results also suggested that glutenins were, in general, more hydrophobic than gliadins. Unfortunately deabsorbing some of the proteins from the column for further study proved difficult or impossible.

A second technique that may prove useful in studying hydrophobic interactions of gluten proteins is differential extractability with soaps. Recent studies by Kobrehel and Bushuk (1977) and Kobrehel and Matignon (1980) have shown differences in the extractability of flour proteins with different soaps at various concentrations.

Previous studies by Gortner et al (1928, 1929) showed that flour protein extractability varied widely in the presence of various inorganic salts. For both anions and cations, extractability followed the lyotropic (Hofmeister) series, with the effects of anions being more pronounced. At the Grain Research Laboratory we have been investigating the use of simple neutral salts of the lyotropic series to study the hydrophobic properties of gluten proteins. The use of these salts is based upon theoretical protein studies, reviewed by Von Hippel and Schleich (1969), Franks (1978), and Melander and Horvath (1977), which have shown that at ionic strengths sufficient to minimize electrostatic interactions, changes in protein properties due to variations in the concentration and nature of anions of the lyotropic series can be directly attributed to hydrophobic interactions. A potential advantage of this technique, compared to those discussed above, is that the salts exert their effects upon water structure by altering the free energy associated with the transfer of an apolar protein residues from a nonpolar to aqueous environment rather than by direct binding to hydrophobic sites on the protein. Thus, changes in protein properties such as extractability can be directly attributed to changes in the inherent hydrophobic properties of the protein

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rather than to changes in the properties of hydrophobic ligand-protein complexes.

The present article reports the effects of altering the concentration and nature of anions of the lyotropic series upon the extractability and turbidity of chloroform-defatted gluten proteins isolated from a Canadian hard red spring wheat variety. Results are discussed on the basis of noncovalent bonding forces contributing to gluten protein structure.

MATERIALS AND METHODS

Preparation of Defatted Gluten

For the preparation of defatted gluten, a Canadian hard red spring wheat variety (*Triticum aestivum* L. cv. Neepawa) was milled to straight grade flour (approximately 75% extraction) on the Grain Research Laboratory pilot mill. A portion of the flour was then defatted with chloroform according to the procedure of MacRitchie and Gras (1973) and air dried. Gluten was prepared from the defatted flour by the method of Doguchi and Hlynka (1967), freeze-dried, and ground in a coffee grinder to a flourlike consistency. Nondefatted gluten was prepared by omitting the chloroform extraction. Protein was determined by Kjeldahl analysis (N × 5.7), and lipid contents were determined by the procedure of Drapron (1975). Table I shows the protein, moisture, and lipid contents of the flour, defatted gluten, and nondefatted gluten.

Extraction of Defatted Gluten

Sodium salt solutions were used at concentrations ranging from 0.05 to 4.0 *M*. Extractions with acetic acid (0.05*M*) and lactic acid (0.005*M*) were also made for comparison.

Defatted gluten (500 mg) was dispersed in 25 ml of aqueous salt solution (adjusted to pH 7.0) by shaking in a 40-ml capped centrifuge tube (IEC) and then rotated at 50 rpm on a rotator (Roto-Torque, Cole-Parmer) for 1 hr at room temperature. After centrifugation at 40,000 × *g*, the pellet was reextracted two more times according to the above procedure. Supernatants were combined, and salt was removed by dialysis against 0.005*M* lactic acid. Following lyophilization, protein levels (N × 5.7) of the soluble (supernatant) and insoluble (pellet) gluten fractions were determined by Kjeldahl analysis. All extractions were performed in duplicate. Coefficients of variability for yields of soluble and insoluble fractions were 1.49 and 1.88%, respectively.

Table I
Protein, Moisture, and Lipid Contents (%) of Neepawa Flour, Defatted Gluten, and Nondefatted Gluten

	Protein[a]	Moisture	Lipid
Flour	13.9	14.0	1.61
Defatted gluten	82.7	9.0	0.78
Nondefatted gluten	80.0	7.2	6.60

[a]N × 5.7 on an as is basis.

Table II
Effects of Neutral Sodium Salts (1.0*M*) on the Solubility (%) of Defatted and Nondefatted Gluten

	Gluten	
Salt	Defatted	Nondefatted
None (H_2O)	25.0	29.5
NaF	Trace	Trace
NaCl	5.8	5.2
$NaBrO_3$	5.4	6.4
NaBr	19.9	...[a]
$NaClO_4$	31.3	28.7
NaI	51.7	54.6
NaSCN	61.5	59.1
HAc (0.05*M*)	70.4	77.2
Lactic (0.005*M*)	71.4	75.8

[a]Not determined.

Determination of Turbidity

For the determination of the effects of salt on turbidity, defatted gluten (300 mg) was extracted with 20 ml of 0.05*M* acetic acid for 1 hr at room temperature on a rotator and then centrifuged at 40,000 × *g*. The resulting supernatant was diluted 1:50 with 0.05*M* acetic acid and filtered through a 0.45-μm membrane (Gelman). A 2-ml portion of the diluted gluten extract was placed in a spectrophotometer cuvette (10 × 10 mm); an equal volume of salt solution in 0.05*M* acetic acid was rapidly added and the contents shaken. The cuvette was placed in the primary cell position of the spectrophotometer (Pye Unicam SP 1750), and turbidity was determined as the increase in absorption at 350 nm after 10 min.

Electrophoresis

Electrophoresis was performed in polyacrylamide gels with 0.0085*M* aluminum lactate-lactic acid buffer (pH 3.1) on 0.15 × 17 × 12-cm slabs, using a Bio-Rad model 220 apparatus according to the procedure of Tkachuk and Mellish (1980). Samples (8 μl) containing protein (1mg/ml) in the same buffer plus 10% sucrose were loaded into preformed slots (20 slots per gel slab) and electrophoresed 90 min at 400 mv. Gels were stained overnight with 0.025% Coomassie brilliant blue R250 in 12.5% trichloroacetic acid.

Amino Acid Analysis

For amino acid anlaysis, lyophilized samples (10 mg) were hydrolyzed for 24 hr at 110°C in 2 ml of 6.0*M* HCl after flushing with N_2. Analysis was performed in duplicate with a Durrum amino acid analyzer (KIT MBN No. 26402) utilizing a 0.3 × 25-cm column of DC-5A resin with Na^+ buffers according to the procedure of Benson (1972). All duplicates that gave amino acid concentrations varying by more than 3% were repeated.

RESULTS

Extractability of Defatted Gluten in 1.0*M* Salts

Table II shows the effects of 1.0*M* monovalent neutral salts of Na^+ upon the extractability of defatted and nondefatted gluten. Extractabilities of the gluten in water, acetic acid, and lactic acid are also shown for comparison. Recoveries of gluten protein (soluble plus insoluble) for all extractions were between 96 and 99%.

With both the defatted and nondefatted gluten, altering the anion had dramatic effects upon protein extractability. The order of extractability of the defatted gluten proteins with the anions was, in increasing order, F^-, Cl^-, Br^-, Br^-, ClO_4^-, I^-, and SCN^-, which followed the lyotropic or Hofmeister series; nonchaotropic ions such as chloride, and in particular fluoride, reduced extractability compared to water, whereas chaotropic ("structure-disrupting") ions such as chlorate, iodide, and thiocyanate promoted extractability. Sodium thiocyanate, the most chaotropic salt used, was almost as effective as was lactic or acetic acid in extracting the defatted gluten proteins. The presence or absence of the chloroform–extractable lipids appeared to effect extractability to a greater extent with the acids than with the salts.

Electrophoresis of Defatted Gluten Proteins Solubilized in 1.0*M* Salts

Figure 1 shows the electrophoretic patterns of the defatted gluten proteins extracted in the 1.0*M* salts. Comparative patterns are also shown for gluten proteins solubilized in water, acetic acid, and lactic acid and for "Osborne" gliadin, glutenin, and albumin extracted from a Neepawa flour.

Because of the very low extractability of the defatted gluten proteins in sodium fluoride, electrophoretic analysis of these proteins was not performed. The gluten proteins solubilized in sodium chloride had a pattern similar to those of wheat albumins and/or fast-moving gliadins, consisting mainly of intermediate and fast-moving components. Sodium bromate, which was included because of its importance as an oxidant in the bread-making process, extracted a similar amount of defatted gluten protein (5.4%) and gave an electrophoretic pattern similar to that of sodium chloride. Proteins extracted with either sodium chloride or

sodium bromate gave considerably lower staining intensities than did the other extracts, even though equal amounts of protein nitrogen were added to the slots. Recent studies[2] suggest that this lower staining intensity is the result of a much higher proportion of low molecular weight peptides and proteins in the chloride and bromate extracts that migrate off the gel.

Sodium bromide showed an increase in slower moving components, suggesting an increase in gliadin extractability. The more chaotropic salts showed a further increase in the intensity of gliadinlike components and, in addition, the presence of gluteninlike components, as evidenced by the more heavily stained origin. The

[2]Unpublished data.

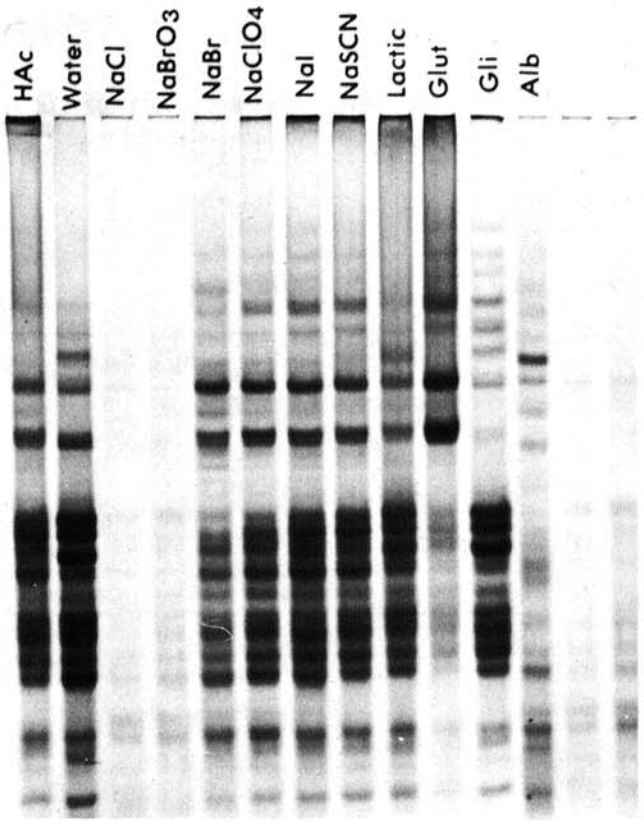

Fig. 1. Aluminum lactate polyacrylamide gel electrophoretic profiles of Neepawa defatted gluten proteins solubilized in 1.0*M* salt, 0.05*M* acetic acid, 0.005*M* lactic acid, and water. "Osborne" glutenin, gliadin, and albumin isolated from a Neepawa flour are shown for comparison. Unlabeled profiles on right represent more concentrated (2×) samples of defatted gluten proteins solubilized in 1.0*M* sodium chloride and 1.0*M* sodium bromate (far right).

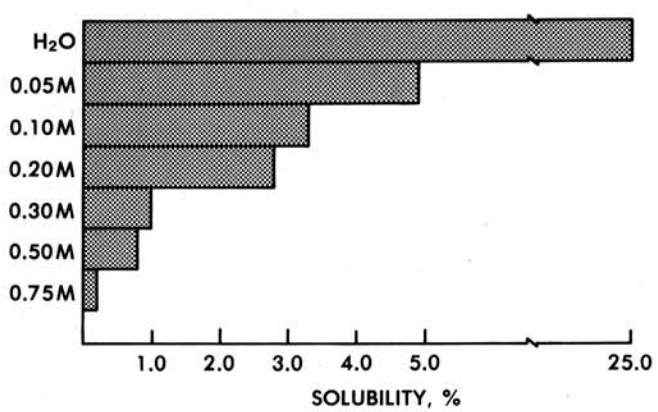

Fig. 2. Effects of increasing concentrations of sodium fluoride on the solubility of Neepawa defatted gluten.

electrophoretic patterns of the gluten proteins extracted by the most chaotropic salts (NaI and NaSCN) had patterns generally similar to that obtained with lactic or acetic acid, although some differences in the relative intensities of the slower moving bands were evident.

Effect of Salt Concentration on Extractability

The effects of salt concentration and anion type upon the extractability of the defatted gluten proteins were studied by determining nitrogen recoveries of the soluble proteins after dialysis. Both the salt concentration and the nature of the anion had large effects

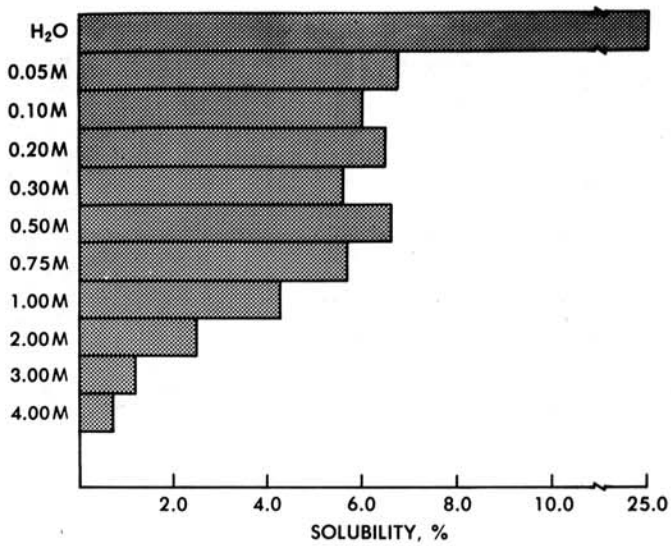

Fig. 3. Effects of increasing concentrations of sodium chloride on the solubility of Neepawa defatted gluten.

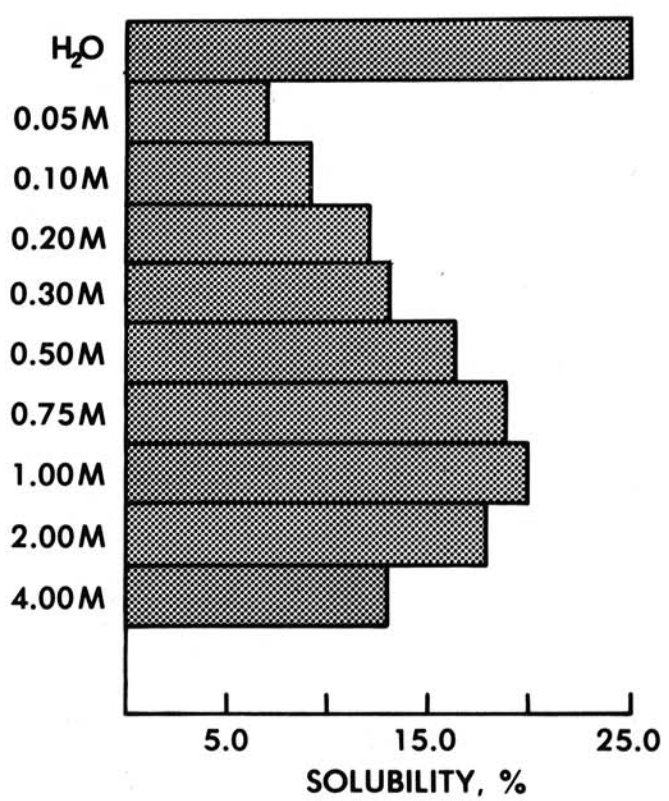

Fig. 4. Effects of increasing concentrations of sodium bromide on the solubility of Neepawa defatted gluten.

upon gluten protein extractability (Figs. 2–7).

The presence of low levels of all salts (0.05–0.2*M*) significantly reduced protein extractability compared to that in water. At the lowest concentration (0.05*M*), extractability appeared to be mainly dependent upon ionic strength and almost independent of the nature of the anion, as evidenced by the narrow range of protein solubilities (4.9–7.5%). However as salt concentration was increased, the protein extractability became highly dependent upon the nature of the anion. With 0.5*M* salts, extractabilities ranged from 0.8% for fluoride to 35% for thiocyanate, and with 1.0*M* salt, extractability ranged from less than 0.5% for fluoride to 61.5% for thiocyanate. The highest salt concentration (4.0*M*) gave the largest extractability range (0.7% for chloride to 70.2% for thiocyanate).

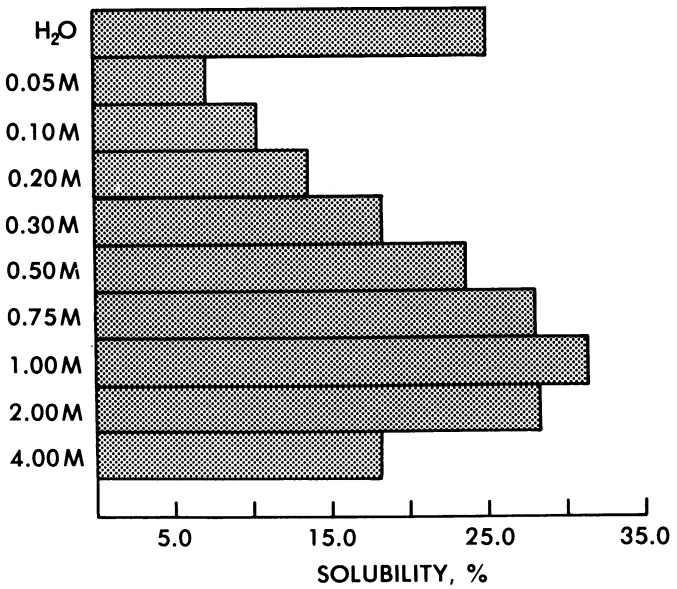

Fig. 5. Effects of increasing concentrations of sodium chlorate on the solubility of Neepawa defatted gluten.

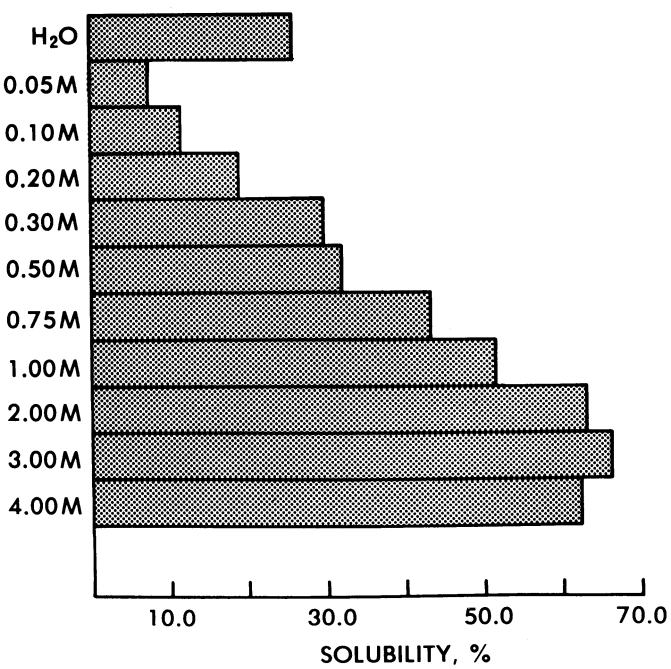

Fig. 6. Effects of increasing concentrations of sodium iodide on the solubility of Neepawa defatted gluten.

These latter results did not include sodium fluoride because this concentration exceeds its limit of solubility. With the exception of that for 0.05*M* salts, the order of extractability of the defatted gluten proteins at the various salt concentrations closely followed the lyotropic series for the anions.

Effects of Neutral Salts on Turbidity

The effects of increasing concentrations of neutral salts upon the turbidity (at 350 nm) of chloroform-defatted gluten proteins soluble in 0.05*M* acetic acid are shown in Fig. 8. Turbidity values were not corrected for refractive index and thus are not absolute values. The presence or absence of chloroform-extractable lipids did not appear to affect the light-scattering properties of the proteins extracted with the various salts (data not shown).

Addition of low levels of the various salts gave rapid increases in turbidity, suggesting that the acid-solubilized gluten proteins were sensitive to salt-induced aggregation. However at higher levels of

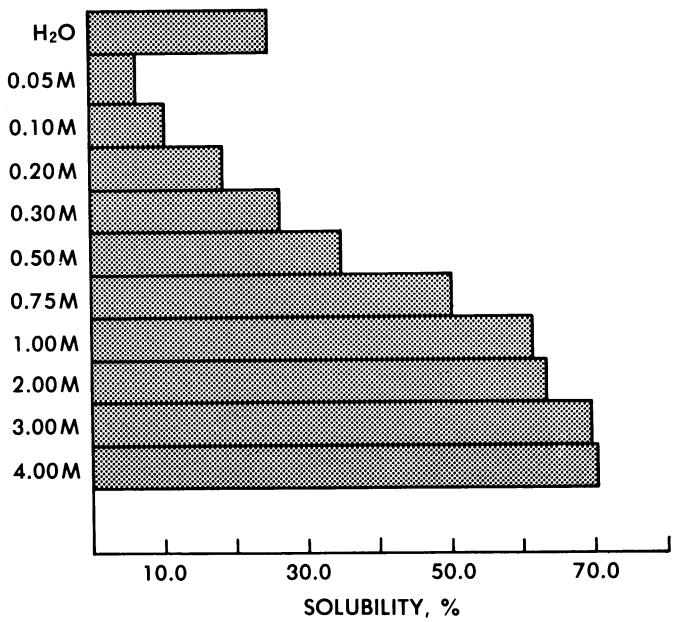

Fig. 7. Effects of increasing concentrations of sodium thiocyanate on the solubility of Neepawa defatted gluten.

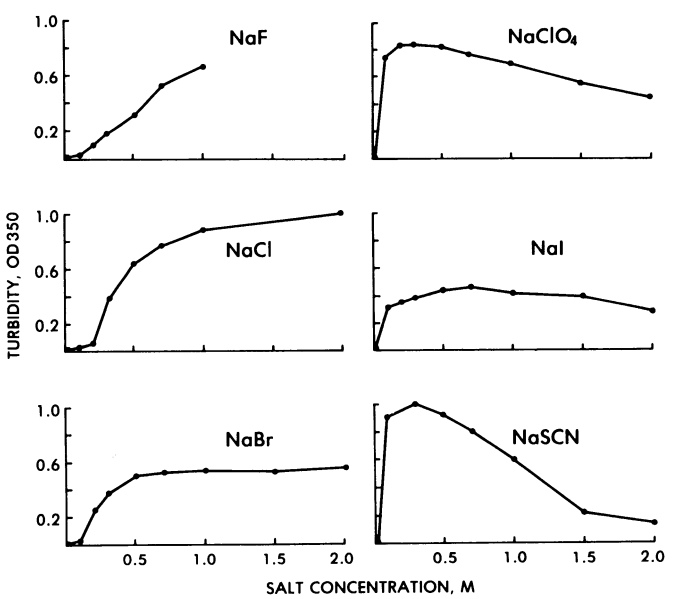

Fig. 8. Effects of increasing concentrations of neutral salts on the turbidity (at 350nm) of Neepawa defatted gluten solubilized in 0.05*M* acetic acid.

salt (0.5–2.0*M*), changes in turbidity became highly dependent upon the nature of the anion. In the presence of fluoride and chloride, increasing salt concentrations further increased turbidity, suggesting further aggregation of the proteins, whereas with bromide little change occurred in turbidity above 0.5*M*. In contrast, increasing concentrations (from 0.5 to 2.0*M*) of iodide, chlorate, and thiocyanate reduced turbidity, suggesting a reversal of the protein aggregation process that occurred at lower salt concentrations. In particular, increasing concentrations of thiocyanate gave large reductions in turbidity. With 2.0*M* thiocyanate, little turbidity was apparent.

The increase in turbidity with increasing concentrations of sodium fluoride (0.1–0.5*M*) was less rapid than that with the other salts, which could indicate that the gluten proteins were less sensitive to aggregation in the presence of low levels of this salt. However, with low levels of sodium fluoride protein rapidly precipitated to the bottom of the cuvette, which would not contribute to the turbidity. Thus, rather than being less sensitive to aggregation, the gluten proteins appear, in fact, to aggregate to such an extent that they precipitate out. Studies by Arakawa et al (1977) have shown a similar phenomenon. Their F1 glutenin fraction, in the presence of sodium chloride, aggregated to such an extent that turbidity values were reduced.

Results obtained with the turbidity experiments should be viewed with some caution. Recent studies in our laboratory,[3] involving the measurement of the wavelength dependence of the turbidity after addition of the various salts by the technique described by Camerino-Otero and Day (1978), suggest the presence of particles with dimensions of the order of $\lambda/2$. As outlined by Tanford (1961), the light-scattering intensity of particles with dimensions greater than approximately $\lambda/2$ is highly dependent upon scattering angle and can lead to significant changes in turbidity that are sometimes difficult to interpret.

Amino Acid Composition of Salt-Solubilized Defatted Gluten Proteins

Table III shows the amino acid compositions of defatted gluten proteins extracted in 1.0*M* neutral salt solutions of various anions. The table is arranged in order of increasing protein extractability and follows the lyotropic series. Amino acid data for the defatted gluten and the gluten proteins extracted in water and in 0.05*M* acetic acid are shown for comparison.

Large differences were evident in the amino acid compositions of the gluten proteins extracted with sodium chloride compared to those of proteins extracted by the other salts. The proteins extracted by sodium chloride had an amino acid composition similar to that of wheat flour albumins (Fish and Abbott 1969) whereas the proteins extracted by the other salts had an amino acid composition more similar to that of whole gluten (Wu and Dimler 1963a). Several trends appeared evident when the amino acid compositions were compared on the basis of increasing protein extractability. In particular, as the chaotropic properties of the salt increased across the lyotropic series from chloride to thiocyanate, levels of glutamic acid (including glutamine) and proline increased. Decreases in aspartic acid (including asparagine) and total basic amino acid were also evident. These trends, as in the case of the solubility data and electrophoretic profiles, suggested that increasing proportions of gliadinlike and gluteninlike proteins were being solubilized. The similarity of the amino acid composition of gluten proteins extracted by 0.05*M* acetic acid, which are known to contain high proportions of gliadins and glutenins, and that of the proteins solubilized by the more chaotropic salts is also consistent with this view. The amino acid composition of the water-extracted gluten proteins was most similar to that previously reported for wheat gliadins (Wu and Dimler 1963b) and is consistent with the electrophoretic profile (Fig. 1). Previous studies have demonstrated the ability of water at low ionic strength to extract gliadin proteins (Shogren et al 1969).

Increasing neutral salt concentrations had significant effects upon the amino acid compositions of the extracted gluten proteins. Tables IV and V show data for sodium chloride and sodium thiocyanate, salts at opposite ends of the lyotropic series for the anions studied. A larger sodium thiocyanate concentration range was analyzed because of the wider range of solubilities of the gluten proteins in this salt.

Glutamic acid and proline levels were lower in 0.05*M* sodium chloride than in deionized water, but the proportions of aspartic acid, glycine, alanine, and total basic amino acids were higher (Table IV). Further increases in sodium chloride concentration, which reduced protein extractability, led to further decreases in glutamic acid, proline, and phenylalanine and to further increases in aspartic acid, threonine, glycine, alanine, valine, and total basic amino acids. These changes suggested that as sodium chloride concentration increased (ie, extractability decreased), the relative proportion of albuminlike proteins increased and that of gliadinlike proteins decreased.

The defatted gluten proteins extracted in 0.05*M* sodium

[3]Unpublished data.

Table III
Amino Acid Composition (mole %) of Defatted Gluten Proteins Solubilized in 1.0*M* Neutral Salts

Amino Acid	Defatted Gluten	Gluten Solubilized in Salts NaCl	NaBr	$NaClO_4$	NaI	NaSCN	H_2O	0.05*M* Acetic Acid
Aspartic acid	3.4	6.8	3.8	3.4	3.3	2.9	2.9	2.7
Threonine	2.7	4.2	2.6	2.3	2.1	2.3	2.3	2.5
Serine	5.6	5.9	4.6	4.8	5.3	5.0	5.2	5.4
Glutamic acid	33.1	20.3	33.7	35.2	35.5	36.1	37.0	35.9
Proline[a]	15.1	9.0	15.6	16.3	16.8	16.5	17.6	16.0
Glycine	5.7	7.7	4.1	3.8	3.7	4.0	3.2	4.7
Alanine[a]	4.4	7.0	4.6	4.2	3.6	3.9	3.6	3.5
Half Cystine[a]	0.9	1.7	1.0	0.8	0.4	1.0	1.1	0.7
Valine[a]	4.4	6.1	4.5	4.0	4.3	4.1	4.1	4.2
Methionine[a]	1.3	2.7	1.9	1.7	2.0	1.8	1.6	1.9
Isoleucine[a]	3.5	3.6	3.7	3.9	3.9	3.7	3.8	3.6
Leucine[a]	7.0	7.5	6.4	6.6	6.9	6.8	6.9	6.8
Tyrosine[a]	2.2	3.2	2.2	2.4	2.2	2.3	1.9	2.4
Phenylalanine[a]	4.4	3.2	4.8	5.0	4.9	4.8	4.7	4.5
Lysine	2.1	3.6	1.6	1.3	1.1	1.0	0.8	1.2
Histidine	1.8	2.4	2.2	1.9	1.8	1.7	1.7	1.7
Arginine	2.5	5.5	2.9	2.4	2.3	2.3	2.0	2.3
Total hydrophobic	43.2	44.0	44.7	44.9	45.0	44.9	45.3	43.6
Total basic	6.4	11.5	6.7	5.6	5.2	5.0	4.5	5.2

[a]Hydrophobic amino acids.

thiocyanate (Table V) had an amino acid composition similar to that of the proteins extracted in 0.05*M* sodium chloride. Amino acid compositions of the gluten proteins extracted in 0.05*M* sodium fluoride, sodium bromide, sodium iodide, and sodium chlorate also gave very similar amino acid compositions (data not shown). These results, which suggested that at low salt concentrations similar proteins were extracted, are consistent with the similar extractabilities of the gluten proteins in the various 0.05*M* salts (Figs. 2–7).

As protein extractability increased with increasing concentrations of sodium thiocyanate (up to 0.5*M*), levels of glutamic acid and proline increased and of asparatic acid and total basic amino acids decreased. These changes suggested an increase in the concentration of gliadinlike and gluteninlike proteins. Comparison of the amino acid compositions of the defatted gluten proteins insoluble in sodium thiocyanate (data not shown) demonstrated that as salt concentration increased, glutamic acid and proline decreased and aspartic acid, threonine, glycine, and basic amino acids increased. The amino acid compositions of the gluten proteins insoluble in 2.0*M* and 4.0*M* sodium thiocyanate were similar to previously published values for wheat flour insoluble glutenin or "gel" protein (Jennings 1979).

Table IV
Effects of Increasing Concentrations of Sodium Chloride on the Amino Acid Composition (mole %) of Solubilized Defatted Gluten Proteins

		Concentration of NaCl			
Amino Acid	**Water**	**0.05*M***	**0.10*M***	**0.30*M***	**1.00*M***
Aspartic acid	2.9	4.3	5.0	5.5	6.8
Threonine	2.3	2.7	3.2	3.2	4.2
Serine	5.2	5.1	5.2	5.3	5.9
Glutamic acid	37.0	30.6	27.6	25.7	20.3
Proline[a]	17.6	14.8	13.6	13.0	9.0
Glycine	3.2	4.7	5.3	5.9	7.7
Alanine[a]	3.6	5.2	5.9	6.0	7.0
Half Cystine[a]	1.1	1.3	1.4	1.2	1.7
Valine[a]	4.1	4.8	5.1	5.2	6.1
Methionine[a]	1.6	1.8	1.9	2.4	2.7
Isoleucine[a]	3.8	3.7	3.8	3.6	3.6
Leucine[a]	6.9	7.2	7.6	7.3	7.5
Tyrosine[a]	1.9	2.4	2.4	2.9	3.2
Phenylalanine[a]	4.7	4.6	4.2	4.0	3.2
Lysine	0.8	1.8	2.3	2.4	3.6
Histidine	1.7	2.0	2.1	2.1	2.4
Arginine	2.0	2.9	3.2	4.1	5.5
Total hydrophobic	45.3	45.8	45.9	45.6	44.0
Total basic	4.5	6.7	7.6	8.6	11.5

[a]Hydrophobic amino acids.

DISCUSSION

Changes occurring in the extractability and related properties of proteins as a result of increasing concentrations of simple monovalent salts have been mainly attributed to effects upon electrostatic and hydrophobic bonding (Dandliker and de Saussure 1971, Franks 1978, Von Hippel and Schleich 1969). At low salt concentrations these effects are primarily dependent upon large changes in electrostatic free energy associated with the ionic shielding of charged amino acids on the surface of the protein. The magnitude of this effect is determined primarily by ionic strength and the charge density and distribution on the protein surface. The latter factor determines whether the extractability of the protein increases or decreases as salt concentration is increased. However at higher salt concentrations (depending upon the charge density and distribution on the protein), electrostatic interactions between charged amino acids are effectively neutralized by extensive ionic shielding, and the protein acts as a neutral dipole (Kirkwood 1943). In this case, increasing salt concentration can have two major effects upon protein extractability. These are an electrostatic "salting-in" effect dependent upon ionic strength and the dipole moment of the protein and a hydrophobic "salting-out" effect dependent primarily upon the inherent hydrophobic properties of the protein and the nature and concentration of the salt. The former effect is probably associated with nonspecific binding of salt ions to polar sites (peptide bonds and polar amino acids) on the protein. The latter effect is normally related to the effects of ion type and concentration upon the "ordering" of water structure, which can significantly alter the free energy resulting from entropy changes occurring when apolar amino acids are exposed to the solvent. In general, increasing salt concentration is believed to increase the ordering of water in such a way that the exposure of apolar groups to the solvent becomes less favored thermodynamically. However this ordering is highly dependent upon ion type, with nonchaotropic ions such as fluoride and chloride strongly promoting ordering of water structure and chaotropic ions such as iodide and thiocyanate having only small ordering effects. These differences are believed to form the basis of the lyotropic (Hofmeister) series.

Several authors (Melander and Horvath 1977, Pahlman et al 1977) have suggested that the effects of increasing concentrations of

Table V
Effects of Increasing Concentrations of Sodium Thiocyanate on the Amino Acid Composition (mole %) of Solubilized Defatted Gluten Proteins

		Concentration of Sodium Thiocyante						
Amino Acid	**Water**	**0.05*M***	**0.10*M***	**0.30*M***	**0.50*M***	**1.00*M***	**2.00*M***	**4.00*M***
Aspartic acid	2.9	4.3	4.0	3.6	3.2	2.9	2.8	2.9
Threonine	2.3	2.7	2.6	2.4	2.2	2.3	2.4	2.4
Serine	5.2	5.4	5.3	4.9	4.8	5.0	5.1	5.4
Glutamic acid	37.0	32.0	32.5	34.0	35.6	36.1	35.7	35.4
Proline[a]	17.6	15.9	15.5	16.3	17.1	16.5	17.0	16.4
Glycine	3.2	4.4	4.2	3.9	3.6	4.0	4.2	4.5
Alanine[a]	3.6	4.6	4.5	4.3	4.2	3.9	4.0	3.9
Half Cysinte[a]	1.1	0.9	1.3	0.9	1.0	1.0	0.9	0.9
Valine[a]	4.1	4.4	4.3	4.1	3.9	4.1	3.9	4.0
Methionine[a]	1.6	1.8	1.6	1.9	1.5	1.8	1.7	1.7
Isoleucine[a]	3.8	3.5	3.7	3.8	3.8	3.7	3.6	3.6
Leucine[a]	6.9	6.8	6.9	6.8	6.7	6.8	6.7	6.7
Tyrosine[a]	1.9	2.2	2.3	2.3	2.2	2.3	2.2	2.3
Phenylalanine[a]	4.7	4.9	5.0	5.1	5.1	4.8	4.6	4.7
Lysine	0.8	1.7	1.6	1.3	1.1	1.0	1.0	1.2
Histidine	1.7	1.8	1.9	1.9	1.8	1.7	1.7	1.7
Arginine	2.0	2.7	2.7	2.6	2.3	2.3	2.2	2.3
Total hydrophobic	45.3	45.0	45.1	45.5	45.5	45.0	44.6	44.2
Total basic	4.5	6.2	6.2	5.8	5.2	5.0	4.9	5.2

[a]Hydropholic amino acids.

salts of the lyotropic series upon the properties of proteins could be used to obtain an estimate of hydrophobic interactions. Thus large changes in the extractability or related properties of proteins with different neutral salts of the lyotropic series would indicate a large dependence upon hydrophobic bonding, whereas small variation with salt type would suggest that electrostatic (at low salt concentrations) or other factors such as hydrogen bonding (at higher salt concentrations) were predominant.

Results from the present study suggested that increasing concentrations of the neutral monovalent salts of the lyotropic series had two distinct effects upon the extractability and aggregation properties of the gluten proteins. At low concentrations (approximately 0.05–0.3 *M*), all the salts had strong salting-out tendencies, as evidenced both by significant decreases in gluten protein extractability compared to that in water and by large increases in the aggregation tendencies (turbidity) of the gluten proteins solubilized in acetic acid. Considering the relatively small dependence of extractability, especially at the lowest salt concentration (0.05 *M*), upon the anion present, these effects can probably by attributed mainly to electrostatic shielding of ionic amino acids on the surface of the proteins, which is dependent primarily upon ionic strength. Colloid titration studies by Yoshino and Matsumoto (1966) have demonstrated that wheat gluten proteins have more positively charged basic amino acids than negatively charged acidic amino acids at neutral and lower pH. This excess of positively charged amino acids would, therefore, lead to strong electrostatic repulsion between these proteins, which would account for their relatively high extractability in dilute acids (Shogren et al 1969) and in water (Shuey and Gilles 1973). Addition of low levels of salts would tend to neutralize this electrostatic repulsion and thus favor a reduction in extractability. This conclusion is similar to that proposed by Kasarda et al (1967) to explain the aggregation of α-gliadin when ionic strength was increased to about 0.005 *M* at pH 5.

At higher salt concentrations ($> 0.5\,M$), in which ionic strength appeared to be sufficient to neutralize electrostatic interactions between gluten proteins, increasing concentrations of the various salts had widely varying effects upon protein extractabilities and aggregation tendencies. Furthermore these effects followed the lyotropic series for the anions studied, with chaotropic anions such as thiocyanate, iodide, and chlorate strongly promoting extractability and nonchaotropic (salting-out) anions such as fluoride and chloride promoting aggregation and insolubility. Because these differences in extractability and aggregation tendency due to anion type can be attributed to hydrophobic bonding, several conclusions concerning the hydrophobic properties of wheat gluten proteins can be made.

First, the relatively high extractability (45% or more) of the gluten proteins in the presence of the chaotropic salts (I^- and SCN^-) above 0.5 *M* concentrations compared to their very low extractabilities with equivalent concentrations of the nonchaotropic salts bromide, chloride, and fluoride and the apparent large differences in the aggregation tendencies of the acid-soluble gluten proteins in the presence of these salts as measured by turbidity suggest that the bulk of these proteins have a strong inherent tendency to undergo interprotein hydrophobic bonding. This view is consistent with recent studies by Kobrehel and Bushuk (1977) that showed the extractability of glutenin to be strongly affected by sodium salts of long-chain fatty acids. Two major factors may account for this apparent strong tendency to undergo interprotein hydrophobic bonding. The most obvious factor is the presence of high levels of apolar amino acids, which are known to be present in wheat gluten proteins (Wu and Dimler 1963a,1963b). The other major factor may be the low proportion of ionic amino acids in gluten proteins. Bigelow (1967) and more recently Melander and Horvath (1977) have shown evidence that proteins with low charge densities tend to have significantly higher proportions of apolar amino acids on their surfaces, which would favor hydrophobically driven aggregation and the resulting insolubility. Studies by Green and Kasarda (1971), which showed the presence of accessible apolar regions on the surface of α-gliadin by means of a fluorescent probe, and studies by Pomeranz et al (1968), which showed a significant reduction of nonpolar lipid binding to gluten proteins when 2–4% sodium chloride was added during mixing, are consistent with this view of a highly hydrophobic surface.

Second, in view of the wide range of extractabilities obtained with the lyotropic salts at 1.0 *M* concentration and with increasing concentrations of sodium thiocyanate, gluten proteins appear to vary widely in their surface hydrophobicities. However, at the higher concentrations of sodium thiocyanate (2.0–4.0 *M*) large conformational changes may have occurred in the gluten proteins, which may have altered extractability independently of original surface hydrophobicity. From the electrophoretic results, which indicate that more chaotropic salts are required for the solubilization of glutenins than for gliadins, the strength of interprotein interactions, as determined by surface hydrophobicity, appears to be (in decreasing order):glutenins, gliadins, and albumins. This conclusion is similar to that of recent studies by Chung and Pomeranz (1979). These differences may be attributed to a number of factors, including differences in the proportion of apolar groups on the surface of these proteins and the size and shape of the proteins. At present no direct evidence is available that demonstrates differences in the proportion of apolar groups on the surface of gluten protein fractions. The amino acid composition data showed no major differences in the mole fraction of apolar amino acids in the gluten proteins solubilized with the various lyotropic salts (with the exception of 1.0 *M* sodium chloride) or at various concentrations of sodium thiocyanate. However, the amino acid data did show that the proportion of basic amino acids decreased as more protein was extracted, suggesting that the resulting lower charge density may favor a higher proportion of apolar groups on the surface of the proteins. Other factors, such as differences in the location and extent of disulfide bonding and amino acid sequences that could alter protein folding, probably also play important roles in determining surface hydrophobicity. Differences in the molecular size of the gluten proteins may also be closely related to their hydrophobic properties. Higher molecular weight proteins such as glutenins would be expected to interact more strongly than gliadins because of their larger total hydrophobic surface areas. In this context, the presence of interchain disulfide bonds, which effectively increases protein molecular weight, is particularly important. In fact, when 0.005 *M* 2-mercaptoethanol was included, over 90% of the gluten proteins could be extracted with 2.0 *M* sodium thiocyanate.

The order of sensitivity of various gluten protein fractions to salt-induced aggregation found by Huebner (1970) and by Arakawa and Yonezawa (1975) follows the same general order as the apparent hydrophobic aggregation tendencies of gluten proteins found in the present study. Furthermore the differences in salt sensitivities of glutens from strong and weak flours found by these workers may be explained by varietal differences in the hydrophobic properties of the gluten proteins, in particular the glutenins, which would alter aggregation properties and thus dough strength.

In conclusion, the present study suggests that the use of neutral salts of the lyotropic series may be useful for the study of the hydrophobic properties of gluten proteins and their relationship to rheological and baking properties.

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LITERATURE CITED

ARAKAWA, T., and YONEZAWA, D. 1975. Compositional differences of wheat flour glutens in relation to their aggregation behaviors. Agric. Biol. Chem. 39:2123.

ARAKAWA, T., YOSHIDA, M., MORISHITA, H., HONDA, J., and YONEZAWA, D. 1977. Relation between aggregation behavior of glutenin and its polypeptide composition. Agric. Biol. Chem. 41:995.

BENSON, J. R. 1972. Single column analysis of amino acids: Protein hydrolysates analysed in two hours. Durrum Resin Report No. 4.

BERNARDIN, J. E., and KASARDA, D. D. 1973. The microstructure of

wheat protein fibrils. Cereal Chem. 50:735.
BIGELOW, C. C. 1967. On the average hydrophobicity of proteins and the relation between it and protein structure. J. Theor. Biol. 16:187.
CALDWELL, K. A. 1979. The fractionation and purification of gliadins by hydrophobic interaction chromatography. J. Sci. Food Agric. 30:185.
CAMERINO-OTERO, R. D., and DAY, L. A. 1978. The wavelength dependence of the turbidity of solutions of macromolecules. Biopolymers 17:2241.
CHUNG, K. H., and POMERANZ, Y. 1979. Acid-soluble proteins of wheat flours. II. Binding to hydrophobic gels. Cereal Chem. 56:196.
DANDLIKER, W. B., and de SAUSSURE, V. A. 1971. Stabilization of macromolecules by hydrophobic bonding: Role of water structure and of chaotropic ions. Page 417 in: Hair, M. L., ed. The Chemistry of Biosurfaces, Vol. I. Marcel Dekker: New York.
DOGUCHI, M., and HLYNKA, I. 1967. Some rheological properties of crude gluten mixed in the farinograph. Cereal Chem. 44:561.
DRAPRON, R. 1975. Méthode rapide de détermination qualitative des lipides totaux dans les produits céréaliers. Ann. Technol. Agric. 24:117.
FISH, W. W., and ABBOTT, D. C. 1969. Isolation and characterization of a water soluble wheat flour protein. J. Sci. Food Agric. 20:723.
FRANKS, F. 1978. The hydrophobic interaction. Page 1 in: Franks, F., ed. Water: A Comprehensive Treatise. Vol. 4, Aqueous Solutions of Amphiphiles and Macromolecules. Plenum Press: New York.
GORTNER, R. A., HOFFMAN, W. F., and SINCLAIR, W. B. 1928. Physicochemical studies on proteins. III. Proteins and the lyotropic series. Colloid Symp. Monogr. 5:179.
GORTNER, R. A., HOFFMAN, W. F., and SINCLAIR, W. B. 1929. The peptization of wheat flour proteins by inorganic salt solutions. Cereal Chem. 6:1.
GREEN, F. C., and KASARDA, D. D. 1971. Apolar interactions of α-gliadin: Binding of α-*p*-toluidinylnaphthalene-6-sulfonate. Cereal Chem. 48:601.
HUEBNER, F. R. 1970. Comparative studies of glutenins from different classes of wheat. J. Agric. Food Chem. 18:256.
JENNINGS, A. C. 1979. The composition of a gel formed when wheat flour is suspended in phenol-acetic acid-water (1:1:1 w/v/v). J. Sci. Food Agric. 30:575.
KASARDA, D. D., BERNARDIN, J. E., and THOMAS, R. S. 1967. Reversible aggregation of α-gliadin to fibrils. Science 155:203.
KIRKWOOD, J. G. 1943. The theoretical interpretation of the properties of solutions of dipolar ions. Page 276 in: Cohn, E. J., and Edsall, J. T., eds. Proteins, Amino Acids and Peptides. Reinhold: New York.
KOBREHEL, K., and BUSHUK, W. 1977. Studies of glutenin. X. Effects of fatty acids and their sodium salts on solubility in water. Cereal Chem. 54:833.
KOBREHEL, K., and MATIGNON, B. 1980. Solubilization of proteins with soaps in relation to the bread-making properties of wheat flours. Cereal Chem. 57:73.
MELANDER, W., and HORVATH, C. 1977. Salt effects on hydrophobic interactions in precipitation and chromatography of proteins: An interpretation of the lyotropic series. Arch. Biochem. Biophys. 183:200.
MacRITCHIE, F., and GRAS, P. W. 1973. The role of flour lipids in baking. Cereal Chem. 50:292.
PAHLMAN, S., ROSENGREN, J., and HJERTEN, S. 1977. Hydrophobic interaction chromatography on uncharged sepharose derivatives. Effects of neutral salts on the absorption of proteins. J. Chromatogr. 131:99.
PAPINEAU, Y., and GODON, B. 1978. Application de la chromatographie d'interaction hydrophobe au fractionnement des prolamines du blé tendre. C. R. Hebd. Seances Acad. Sci. Ser. C 287:1051.
POMERANZ, Y., TAO, R. P., HOSENEY, R. C., SHOGREN, M. D., and FINNEY, K. F. 1968. Evaluation of factors affecting lipid binding in wheat flours. J. Agric. Food Chem. 16:974.
SHOGREN, M. D., FINNEY, K. F., and HOSENEY, R. C. 1969. Functional (breadmaking) and biochemical properties of wheat flour components. I. Solubilizing gluten and flour proteins. Cereal Chem. 46:93.
SHUEY, W. C., and GILLES, K. A. 1973. A note on the effect of water-flour ratios on flour protein extracted by employing a paint shaker. Cereal Chem. 50:161.
TANFORD, C. 1961. Light scattering. Page 275 in: Tanford, C., ed. Physical Chemical Properties of Macromolecules. John Wiley and Sons, Inc.: New York.
TKACHUK, R., and MELLISH, V. J. 1980. A rapid high voltage gel electrophoresis technique for characterizing wheat gliadins. Ann. Technol. Agric. In press.
VON HIPPEL, P. H., and SCHLEICH, T. 1969. The effects of neutral salts on the structure and conformational stability of macromolecules in solution. Page 417 in: Timasheff, S. N., and Fosman, G. D., eds. Structure and Stability of Biological Macromolecules. Marcel Dekker: New York.
WU, Y. V., and DIMLER, R. J. 1963a. Hydrogen in equilbria of wheat gluten. Arch. Biochem. Biophys. 102:230.
WU, Y. V., and DIMLER, R. J. 1963b. Hydrogen in equilibria of wheat glutenin and gliadin. Arch. Biochem. Biophys. 103:310.
YOSHINO, D., and MATSUMOTO, H. 1966. Colloid titration of wheat proteins, dough and flour. Cereal Chem. 43:187.

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