Effect of Protein Content and Wheat Variety on Relative Viscosity, Solubility, and Electrophoretic Properties of Gluten Proteins[1]

R. C. BUTAKI[2] and B. DRONZEK, Department of Plant Science, The University of Manitoba, Winnipeg, Canada R3T 2N2

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ABSTRACT

Viscosity, solubility, and electrophoretic properties of glutens of four wheat varieties that differed in mixing strength (from weak to very strong) and bread-making potential were studied to determine the basis for intrinsic differences in the glutens. Relative viscosity values of glutens determined in a highly dissociating mixed solvent (0.01*M* acetic acid, 3*M* urea, 0.01*M* cetyltrimethyl-ammonium bromide) differed markedly between the weak and the very strong mixing varieties. The relative viscosity values decreased with the decreasing mixing strength of the flours. Treatment of the glutens with 2-mercaptoethanol lowered the relative viscosity values. Exhaustive extraction of glutens with 0.05*N* acetic acid showed that stronger mixing wheats had more insoluble gluten than did wheats with less mixing strength. Polyacrylamide gel electrophoresis of the reduced glutens in the presence of sodium dodecyl sulfate showed differences in the subunit patterns in the high molecular weight region.

Gluten, the major protein in wheat, accounts for differences in bread-making quality of wheat varieties (Finney 1943). Sollars (1969) found gluten fractions to be responsible for viscosity differences among wheats of three different classes. Reducing agents lowered the relative viscosity of the gluten by fragmenting these proteins (Pence and Olcott 1952). Nielson et al (1962), studying the two main proteins of gluten, glutenin, and gliadin, reported that the intrinsic viscosity of glutenin was reduced markedly by disulfide-bond cleaving agents, whereas that of gliadin protein was essentially unaffected.

In 1965, Mullen and Smith reported major solubility differences in gluten proteins extracted from short-mixing and long-mixing flours. Gluten from the short-mixing flour was more soluble in water than was gluten from the long-mixing flour. Tanaka and Bushuk (1972) reported that, of two varieties examined, the one with the longer farinograph dough development time contained less acetic acid soluble-protein and more insoluble residue protein. The acetic acid insoluble protein in wheat gluten was found to have a high molecular weight and glutenin-like properties (Cluskey and Dimler 1967).

Our study examines the viscosity of gluten proteins of wheat varieties varying widely in mixing properties, using the highly dissociating AUC (0.1*M* acetic acid, 3*M* urea, 0.01*M* cetyltrimethyl-ammonium bromide) solvent first described by Meredith and Wren (1966). The solubility characteristics of the gluten proteins were determined by exhaustive extraction of the glutens with dilute acetic acid. In addition, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), which has been used to separate and estimate molecular weights of reduced gluten proteins (Bietz and Wall 1972, Khan and Bushuk 1976, Orth and Bushuk 1973), was used to compare the wheat varieties.

MATERIALS

The flour samples used for this study have been described (Butaki and Dronzek 1979). According to physical dough tests (farinograph, mixograph), the four varieties could be classified as: long-mixing or strong (Glenlea), strong (Norquay), medium-strong (Manitou), and weak (Talbot).

METHODS

Gluten Isolation

Gluten was recovered from the flour by washing a dough with dilute sodium chloride solution buffered to pH 6.8 with phosphate and using the Theby gluten washing machine (AACC Method 38-11). Excess water was removed from the wet gluten by rolling it between the hands to a predetermined consistency established by several previous trial runs.

Determination of Relative Viscosity Number

A 2-g portion of wet gluten was extracted for 15 min at room temperature, using a Potter and Elvejhem homogenizer, with 10 ml of AUC solvent of Meredith and Wren (1966) or with 10 ml of 0.05*N* acetic acid. The homogenate was then centrifuged at 27,000 $\times g$ for 20 min. The relative viscosity number of the gluten extract was determined using an Ostwald type viscometer immersed in a water bath maintained at 25° C. The relative viscosity number for glutens treated with 2-mercaptoethanol was determined similarly.

Exhaustive Extraction of Glutens with 0.5*N* Acetic Acid

A 1-g portion of wet gluten was sequentially extracted three times with 5-ml aliquots of 0.05*N* acetic acid in a Potter and Elvejhem homogenizer for 10 min. After each extraction, the homogenate was centrifuged at 20,000 $\times g$ for 10 min. Extraction and centrifugation were done at 0–4° C. The supernatants were combined and the total extracted protein was determined. The precipitate was uniformly dispersed in 0.1*N* NaOH and the total residue protein determined. The protein contained in 1 g wet gluten also was determined. All protein determinations were made using the micro-Kjeldahl procedure.

Electrophoresis of Reduced and Unreduced Glutens

Gluten was reduced with 2-mercaptoethanol as described by Orth and Bushuk (1973) complexed with SDS and examined by SDS-PAGE. A 5% polyacrylamide gel at pH 8.9 as first applied to cereal proteins by Bietz and Wall (1972) was used.

The unreduced gluten, dissolved in the SDS-containing buffer, was examined under the same conditions. For molecular weight estimation, the following proteins (with molecular weight in parentheses) were used to calibrate the gels: bovine serum albumin (65,000), human γ-globulin (153,100), equine heart cytochrome C (12,400) obtained from Calbiochem, and pepsin (35,000) from Sigma. The standards for the unreduced gluten proteins were not reduced.

RESULTS AND DISCUSSION

Relative Viscosity Number

The relative viscosity number of the gluten extracts and the reduced gluten extracts are shown in Table I. The relative viscosity number of Manitou glutens in AUC varied from 11.6 for the high protein flour to 12.8 for the medium protein flour. The AUC-soluble gluten of the low protein flour had a relative viscosity of 12.3. Thus, the gluten of the high protein flour had slightly lower viscosity than that of the medium protein flour. No linear trend of viscosity of gluten extracts with flour protein was detected in

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[2]On study leave from The National Plant Breeding Station, Njoro, Kenya.

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Manitou, however.

The AUC-soluble gluten of the strong variety (Glenlea) had the highest relative viscosity in AUC of the varieties examined. Within Glenlea the relative viscosity of the low protein flour was the lowest and that of the high protein flour was the highest, indicating a trend of increasing relative viscosity with increase in flour protein.

Norquay gluten had a relative viscosity in AUC almost as high as that of the Glenlea samples. Talbot gluten had the lowest relative viscosity of all the varieties.

By these results, the relative viscosity of the glutens could be classified as low, medium, or high. On such a scale, gluten of the weak variety Talbot would be low; those of Manitou medium, and those of the long-mixing flours Norquay and Glenlea high viscosity.

The relative viscosity of reduced glutens for the four varieties (Table I) was much lower than that of corresponding unreduced samples. However, significant differences between varieties were still discernible. Reduced Talbot gluten had the lowest and reduced Glenlea gluten the highest relative viscosity values among the varieties. The reduced glutens of Manitou and Norquay had relative viscosity values intermediate between those of Talbot and Glenlea.

Reduction with mercaptoethanol that cleaved all disulfide bonds of the gluten molecule, resulting in the subunits of gluten, lowered the relative viscosity substantially. These results underline the importance of disulfide bonds in the structure of the flour protein molecules and agree with earlier work by Pence and Olcott (1952), who reported that reducing agents lower relative viscosity by fragmenting the gluten proteins.

The differences in the relative viscosity of the reduced glutens in this study were taken to infer differences in the average molecular weight of the gluten polypeptide subunits. Our results suggest that the sizes of the reduced gluten polypeptide chains may be large in the long-mixing flours compared with that in weaker flours. Accordingly, the average molecular weight of the gluten polypeptide subunits for the four varieties decreased progressively in the following order: Glenlea, Norquay, Manitou, and Talbot.

The relative viscosity of the acetic acid-soluble gluten of Talbot and Glenlea was determined (Table I). The relative viscosity of the weak variety, Talbot, was essentially the same as of the strong variety Glenlea, demonstrating little difference between the acetic acid-soluble glutens of the weak wheat and the strong wheat. The relative viscosity differences of the acetic acid-soluble gluten protein were probably unrelated to flour strength.

Exhaustive Extraction of Gluten Proteins with 0.05*N* Acetic Acid

The solubility characteristics of the gluten proteins are presented in Table II. The nitrogen recovery of the soluble and insoluble gluten proteins extracted three times with 0.05*N* acetic acid from the glutens of the four wheat varieties ranged from 93.0 to 97.6%. This was considered adequate.

Talbot yielded the highest proportion of total protein (90.8%) in the soluble fraction. The other varieties yielded lower percentages in the soluble fraction in the following decreasing order: Manitou, Norquay, and Glenlea.

Manitou samples with increasing flour protein contained between 79.8 and 84.1% of the total protein in the soluble fraction. This variety showed an increase in the acetic acid-soluble fraction with increasing flour protein. Tanaka and Bushuk (1972) obtained similar results with two varieties differing widely in mixing characteristics. Glenlea samples with increasing flour protein contained between 68.7 and 70.2% of the total gluten proteins in the soluble fraction.

The weak-mixing variety, Talbot, contained the lowest and the long-mixing variety, Glenlea, contained the highest percentage of protein in the insoluble fraction. The Glenlea samples contained over 10% more protein in the insoluble fraction than the Manitou samples. Norquay had 16.9% of the total protein in the insoluble fraction. Cluskey and Dimler (1967) reported that the acetic acid-insoluble protein in wheat gluten had high molecular weight and gluten-like properties. Our results are consistent with those of Tsen (1967) and Orth and Bushuk (1972) who reported that weak flour contains a higher proportion of acetic acid-soluble protein than does strong flour.

SDS-PAGE of Unreduced and Reduced Glutens

The electrophoretic patterns of unreduced gluten complexed with SDS are shown in Fig. 1. Much of the protein did not enter the gel, as evidenced by the intense protein stain at the origin of the gel.

Four main protein bands were common to the gluten patterns of the four varieties. Starting with the slowest band, the molecular weights of these protein bands were: 152,000, 60,000, 30,000, and 12,000. The major band at about 30,000 has been reported to be gliadin (Bietz and Wall 1972). Talbot gluten had two more protein

TABLE I
Relative Viscosity of Glutens and 2-Mercaptoethanol-Treated Glutens of Four Wheat Varieties in AUC[a] and of Two Wheat Varieties in 0.05*N* Acetic Acid

	Glenlea			Norquay	Manitou			Talbot
% Flour protein[b]	12.6	13.9	14.6	12.0	10.2	11.6	13.4	10.9
Relative viscosity								
AUC-soluble glutens (AUC = 1)	19.9	21.5	22.5	18.3	12.3	12.8	11.6	6.3
AUC-soluble glutens (reduced) (AUC = 1)	...	5.2	...	.5	...	4.3	...	3.2
Acetic acid-soluble glutens (0.05*N* HAc = 1)	...	...	5.1	...	...	...	...	4.9

[a]AUC = 0.1*M* acetic acid, 3*M* urea, 0.01*M* cetyltrimethyl-ammonium bromide.
[b]N × 5.7 on a 14.0% mb.

TABLE II
Percent of Soluble and Insoluble Gluten Proteins Exhaustively Extracted with 0.05*N* Acetic Acid from the Glutens of Four Wheat Varieties

	Glenlea			Norquay	Manitou			Talbot
% Flour Protein[a]	12.6	13.9	14.6	12.0	10.2	11.6	13.4	10.9
% acetic acid-soluble gluten protein	68.7	70.2	70.2	80.1	79.8	80.4	84.1	90.8
% acetic acid-insoluble gluten protein	26.5	27.4	24.7	16.9	13.2	13.6	11.8	5.0
Total recovery (%)	95.2	97.6	94.9	97.0	93.0	94.0	95.9	95.8

[a]N × 5.7 on a 14.0% mb.

bands, with molecular weights of 110,000 and 108,000, respectively. The electrophoretic patterns for the three Manitou glutens were identical. Similarly, the patterns of Glenlea glutens were the same.

Electrophoresis of unreduced glutens did not reveal any characteristic patterns that could be related to baking quality. This finding is in general agreement with the observation of Danno et al (1974).

The results of SDS-PAGE of the reduced gluten proteins from four wheat varieties are depicted in Fig. 2. The quantity of protein applied to the gel was the same for each sample. In each case, most of the protein entered the gel.

The electrophoretic patterns of the varieties differed mainly in the molecular weight region of 60,000 and above. Below 60,000 mol wt, the four varieties had four protein bands of comparable mobility.

Manitou had six protein bands with molecular weights of 131,000, 110,000, 100,000, 82,000, 80,000, and 60,000. Photographic reproduction of the band at 82,000 resulted in a loss of detail. The protein bands of other varieties were similar to those of Manitou, but some differences were noted. Glenlea had an additional protein band of 70,000 mol wt. Talbot lacked the 82,000 mol wt protein band and its slowest subunit had a molecular weight of 134,000. Norquay, on the other hand, had two additional bands with molecular weights of 134,000 and 107,000. Consequently, in the region of molecular weight 60,000 and above, Manitou had six, Glenlea seven, Norquay eight, and Talbot five protein bands. In addition, all four varieties had four similar bands of fast-moving proteins with molecular weights less than 60,000.

The three Manitou glutens had identical subunit numbers and patterns regardless of the flour protein content. Glenlea samples also gave identical patterns in spite of differences in protein content of the flour from which the glutens were derived. Accordingly, flour protein had no qualitative effect on the subunit pattern or protein distribution among the reduced proteins of the two varieties. In spite of differences in subunit number and pattern of the reduced glutens for all four varieties, the electrophoretic characteristics could not be directly correlated with the rheological properties of the doughs.

The electrophoretic results are in general agreement with those of Orth and Bushuk (1973), and Bietz and Wall (1972). The molecular weight of the gluten subunits agree with the recently revised figures (Khan and Bushuk 1976) for glutenin subunits in hexaploid wheats.

SUMMARY

Several physicochemical techniques were applied to study the properties of the glutens of four wheat varieties known to differ in mixing strength and bread-making potential. Relative viscosity of glutens dissolved in AUC showed distinct differences between glutens from weak and strong varieties with larger relative viscosity

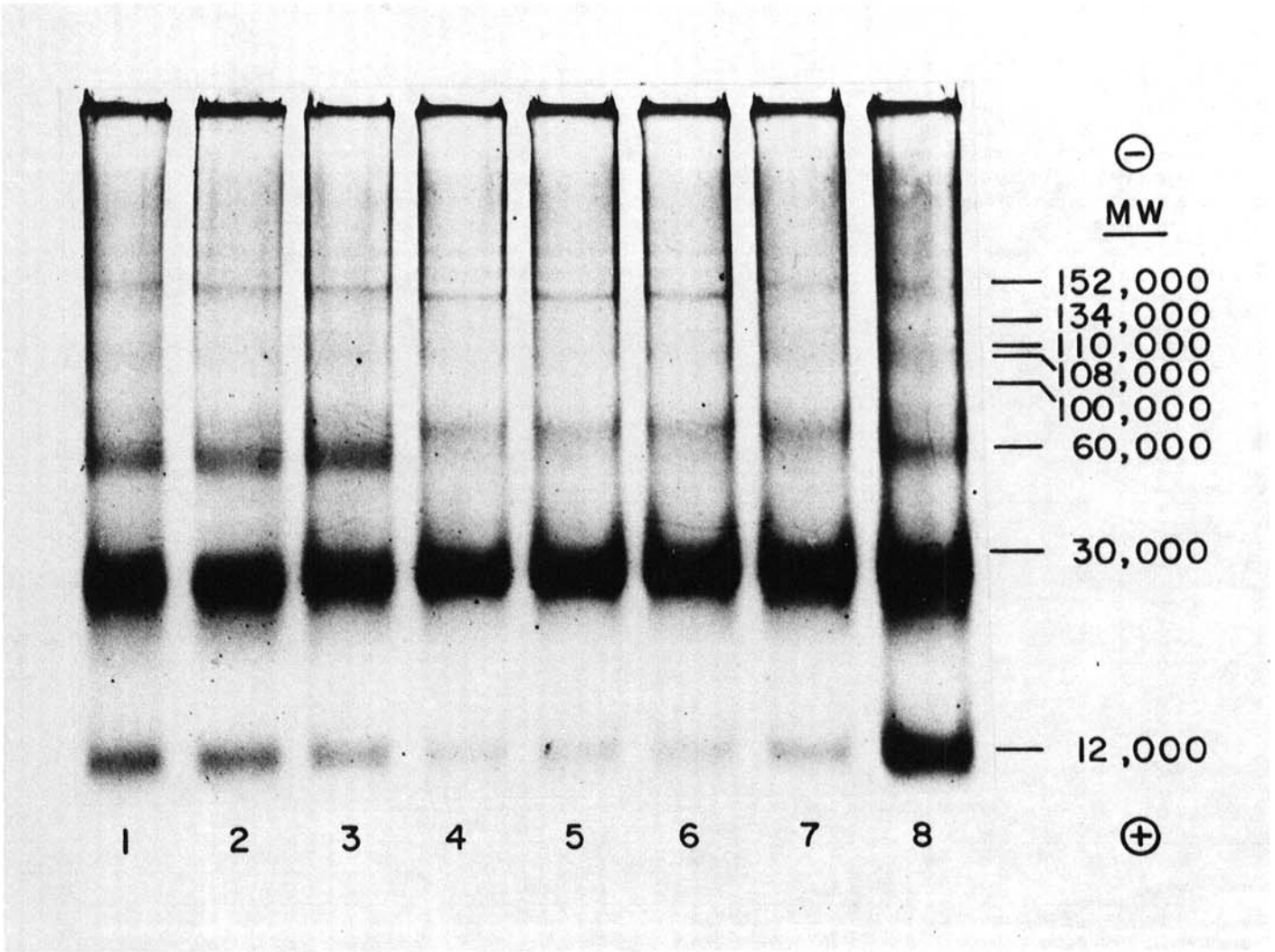

Fig. 1. SDS-PAGE patterns of unreduced glutens from four wheat varieties: Manitou of low (1), medium (2), and high (3) flour protein contents; Glenlea of low (4), medium (5), and high (6) flour protein contents; Norquay (7); and Talbot (8).

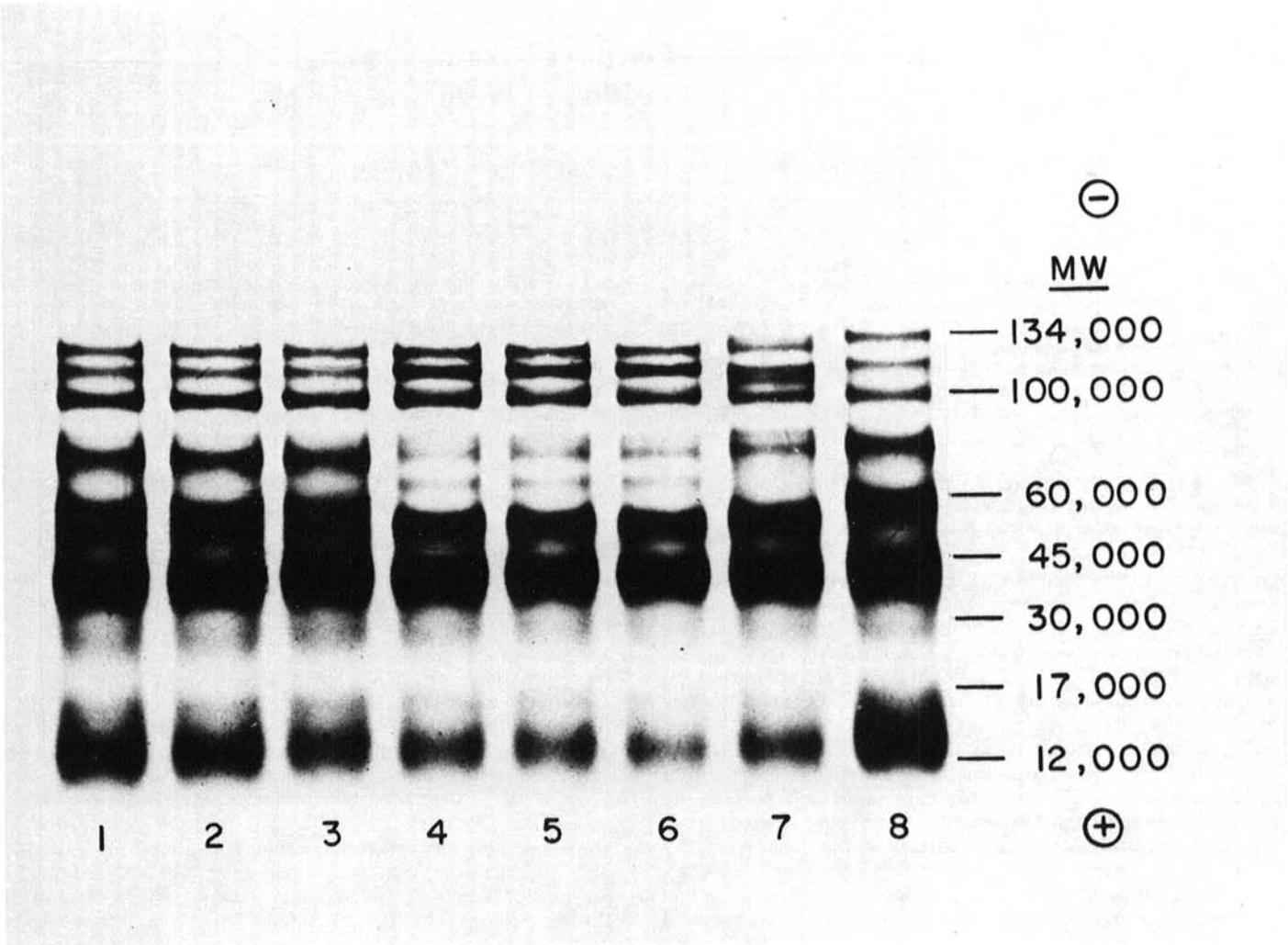

Fig. 2. SDS-PAGE patterns of reduced glutens from four wheat varieties: Manitou of low (1), medium (2), and high (3) flour protein contents; Glenlea of low (4), medium (5), and high (6) flour protein contents; Norquay (7); and Talbot (8).

values for strong varieties than for weak varieties. Extraction of wet glutens, with dilute acetic acid, showed that the gluten from a strong variety contained more insoluble protein than the same weight of the gluten from a weak variety. Electrophoresis of the gluten proteins revealed differences between varieties. These could not be associated directly with the mixing properties of the flour, however. This investigation confirmed that properties of the gluten are characteristic of a variety and are not affected by flour protein.

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