Polyacrylamide Gel Electrophoresis of Salt-Soluble Proteins of Soft Wheats from the Eastern United States

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ABSTRACT

Electrophoretic procedures were developed for the study of soluble proteins of soft wheats. Best results were obtained with a cationic system using 10–20% acrylamide gradient gels buffered at pH 5.3–6.0 with potassium acetate/β-alanine acetate, and with an anionic system using 8% acrylamide gels buffered at pH 8.0–8.5 with Tris-HCL/Tris glycine. Extraction medium had little effect on patterns, and good results were obtained with both meals and single kernels. Genotypical differences in patterns were noted, but major differences were quantitative rather than qualitative. Patterns of high- and low-protein specimens of selected cultivars showed only minor differences.

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The wheat proteins probably have received more attention than any other group of plant proteins, and Osborne's pioneering work (Osborne 1907) still serves as a basis for nomenclature and classification of proteins in general. Abundant literature in this area has stimulated several recent reviews (Kasarda et al 1976, Schofield 1986, Wrigley and Bietz 1988). Because of the historical role of hard wheats in bread production, most studies of wheat proteins have been carried out on hard wheats and have been concerned with functionality of the proteins in breads. To a great extent this information has been accepted as representative of wheats in general, without regard for class. Only recently have the soft wheats been recognized as a commodity apart from the hard wheats, and as having unique milling and baking properties (Hoseney et al 1988, Yamazaki and Greenwood 1981). There is little information pertaining specifically to the soft wheat proteins, and to the contribution of individual proteins to kernel texture or to functionality in soft wheat products.

Polyacrylamide gel electrophoresis (PAGE) has been used extensively for study of gliadins, and a previous study (Clements 1987) reported on electrophoretic patterns of gliadins of soft wheats. PAGE has been employed to a more limited extent for characterization of soluble wheat protein patterns, and such studies have been concerned primarily with hard wheats. In an earlier study, disc-electrophoretic patterns of soluble proteins in density fractions of hard and soft wheat flours were compared (Clements 1979).

The objectives of this study were to explore PAGE as a means for establishing profiles of soluble proteins of soft wheats, to study effects of various factors on electrophoretic patterns, and to determine whether soft wheat cultivars from the eastern United States exhibit genotypical differences. The study is intended to serve as a basis for establishing possible relationships between specific proteins and milling and baking characteristics of soft wheats.

MATERIALS AND METHODS

Wheats

Wheats were from specimens on hand at the Wooster Soft Wheat Quality Laboratory. Two of the cultivars (Abe and Logan) used in the protein-level study were from experiments involving foliar application of urea, and the the other two (Caldwell and Tecumseh) were location samples.

Reagents

Acrylamide was from Serva Fine Biochemicals, Inc., Westbury, NY ("2X crystallized") or Sigma Chemical Co., St. Louis, MO ("suitable for electrophoresis"). Bis-acrylamide was from Fisher Scientific Co., Fair Lawn, NJ. Stock solutions (32% total acrylamide, of which 3% was bisacrylamide) were treated with

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TABLE I
Formulations for Discontinuous Cationic and Anionic Systems for Polyacrylamide Gel Electrophoresis of Soluble Wheat Proteins

Component	Cationic Systems				Anionic System	
	Lower Gel			Upper Gel	Lower Gel	Upper Gel
	10%	12%	20%	8%	8%	6%
Acrylamide (32% T, 3% C)[a]	20 ml	24 ml	40 ml	2 ml	16 ml	1.5 ml
Buffer (4×)[b]						
0.5M Potassium acetate, pH 5.3	16 ml	16 ml	16 ml	...	...	...
0.5M Potassium acetate, pH 7.0	...	...	...	2 ml	...	...
0.95M Tris-HCl, pH 8.5	...	...	...	...	16 ml	...
0.16M Tris-H_3PO_4, pH 6.9	...	...	...	...	...	2 ml
Water	20 ml	16 ml	...	2 ml	28 ml	2.5 ml
TEMED[c]	80 μl	80 μl	80 ml	10 μl	80 μl	10 μl
Ammonium persulfate (0.2%)	8 ml	8 ml	8 ml	2 ml	4 ml	2 ml
Reservoir buffers						
Upper	0.065Mβ-alanine acetate, pH 4.3				0.38M Tris-glycine, pH 8.7	
Lower	0.04M acetic acid				0.063M Tris-HCl, pH 7.4	

[a] Acrylamide stock solution: 77.5 g of acrylamide + 2.5 g of bis-acrylamide made up to 250 ml.
[b] Molarity based on potassium and Tris.
[c] *N,N,N′,N′,*-Tetramethylenediamine.

mixed-bed ion-exchange resin as described previously (Clements 1987). Coomassie Brilliant Blue R-250 was Serva Blue R (Serva Fine Biochemicals). Other reagents were reagent grade and from various sources.

Protein Extraction

Proteins were extracted by suspension of meal or flour in medium (1:2, w/v), unless otherwise noted. Medium for the cationic system was a mixture of 0.5M potassium acetate, pH 7.0, and ethylene glycol (1:1, v/v). For the anionic system, the medium was a mixture of 0.16M Tris-H_3PO_4, pH 6.9, and ethylene glycol (1:1, v/v). Suspensions were agitated and after 2 hr at 0°C were centrifuged 15 min at 20,000 × *g*. Supernatants were applied to gels immediately or stored at −20°C. Single kernels were extracted by suspension of the crushed kernel in 100 μl medium for 18–24 hr at −20°C followed by centrifugation in a microcentrifuge (model HSC-10K, Savant Instruments, Hicksville, NY) for 5 min at 10,000 × *g*. Single kernel extracts were applied to gels immediately after centrifugation.

Electrophoresis

Electrophoresis was performed using a Hoefer model SE 600 unit (Hoefer Scientific Instruments, San Francisco, CA), employing 16 × 18 cm plates separated by 3-mm thick spacers. Electrophoretic systems were modifications of published systems (Maurer 1971); the formulations shown (Table I) represent most of the variations employed. Acrylamide concentration and pH were varied as indicated for specific electrophoregrams. Deaerated lower gel solutions were poured to a depth of 14 cm and over-layered with water. After polymerization, the water was decanted, the upper gel was poured, and the 10-place well former was inserted to a depth of 1 cm, providing an upper gel 1 cm deep.

For continuous gradient gels, a casting stand for the Hoefer model 600 was modified to permit gel addition from the bottom. For this purpose, a 14-gauge cannula with Luer fitting, 7 cm long, was bent at 90° and inserted into a hole drilled in the bottom of the stand to give a tight fit. The solid gasket in the bottom of the stand was replaced with a slotted gasket. A three-way valve with Luer fittings was attached to provide switching from addition to bypass.

Solutions for 10 and 20% acrylamide gels were made up to give final volumes of 32 ml each (after addition of 4 ml of ammonium persulfate). Before addition of persulfate, the deaerated acrylamide solutions were cooled until ice formed. The persulfate was added to each solution, these solutions were transferred to the chambers of a gradient mixer (model GM-1, Pharmacia, Inc., Piscataway, NJ), and the mixing valve was opened and stirring started. The mixture was pumped into the chilled sandwich at a rate of 2 ml/min by means of a peristaltic pump (Isco model 1612, Instrumentation Specialties Co., Lincoln, NE) until the gel was 2 cm from the top of the plates (about 30 min). The control valve was switched to bypass position, and the gel solution was immediately over-layered with water. After polymerization (30–45 min), the water was decanted, and the upper gel was poured as described above. Gels were sealed in plastic bags and stored at 4°C until used.

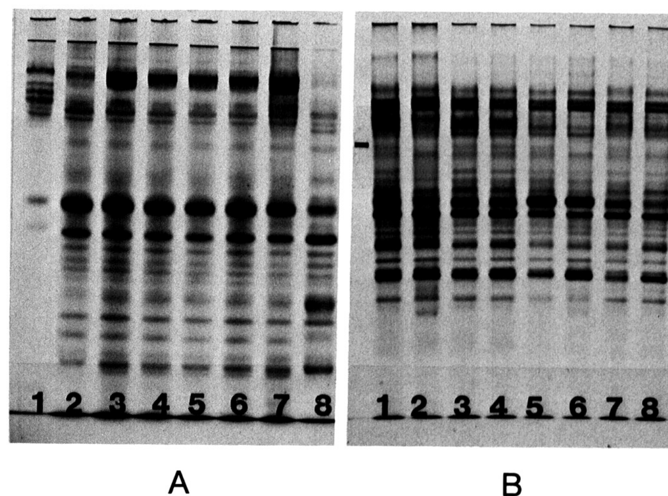

Fig. 1. Effects of extraction medium and wheat protein content on electrophoretic patterns of soft wheat proteins (cationic system). **A,** Patterns of proteins extracted from meal (Logan) using various media. Conditions: lower gel, 12% acrylamide, 0.125M potassium acetate, pH 6.0; upper gel, 8% acrylamide, 0.125M potassium acetate, pH 7.0. Load: (1) 10 μl, (2—7) 20 μl, (8) 30 μl. Lanes: (**1**) ethylene glycol; (**2**) aqueous 25% sucrose; (**3**) 0.1M sodium chloride/25% sucrose; (**4**) 0.1M potassium acetate, pH 6.0/25% sucrose; (**5**) 0.1M potassium acetate, pH 7.5/25% sucrose, (**6**) 0.1M potassium acetate, pH 6.0/25% sucrose/0.001M ethylenediaminetetraacetic acid; (**7**) 0.5M potassium acetate, pH 7.0/ethylene glycol (1:1, v/v), (**8**) freeze-dried water extract (1 mg). **B,** Patterns from low- and high-protein specimens of four soft wheat cultivars (protein, 14% mb). Conditions: lower gel, 10–20% acrylamide continuous gradient, 0.125M potassium acetate, pH 5.3; upper gel, same as A. Extraction medium: 0.5M potassium acetate, pH 7.0/ethylene glycol (1:1, v/v). Load: 40 μl. Lanes: (**1**) and (**2**) Abe (soft red winter [SRW]), 12.9 and 20.2%; (**3**) and (**4**) Caldwell (SRW), 9.4 and 12.3%; (**5**) and (**6**) Logan (SRW), 9.8 and 15.8%; (**7**) and (**8**) Tecumseh (soft white winter), 10.3 and 14.3%, respectively.

Sample size ranged from 20 to 50 μl. Methyl green (added to wells) and bromophenol blue (added to upper reservoir) served as tracking dyes for the cationic and anionic systems, respectively (Maurer 1971). Electrophoresis was performed at 40 mA per gel (current limiting) at 20°C, and was terminated when the dye

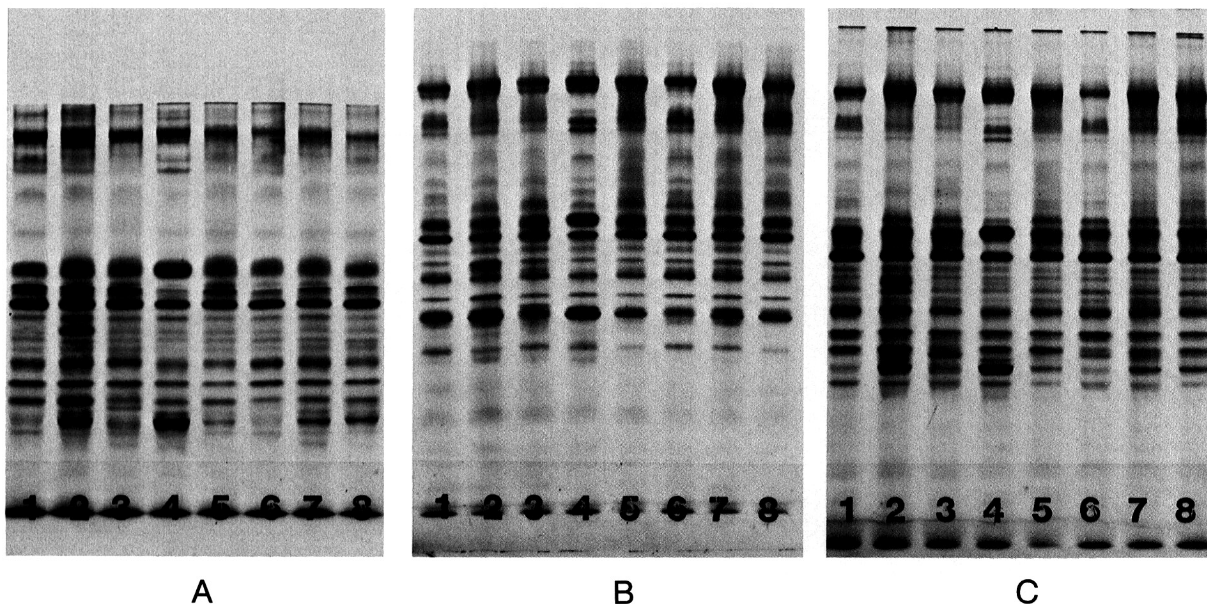

Fig. 2. Effects of electrophoretic conditions on patterns obtained from eight soft wheat cultivars: **(1)** Holley, **(2)** Houser, **(3)** Hunter **(4)** Logan, **(5)** McNair 1003, **(6)** Nelson, **(7)** Oasis, **(8)** Omega 78 (Houser soft white winter; all others soft red winter). Extraction medium: 0.5*M* potassium acetate, pH 7.0/ethylene glycol (1:1, v/v), 2 ml per gram of meal. Load: 30 μl. Electrophoretic conditions (all gels 3% cross-linked and buffered with 0.1*M* potassium acetate; upper gel, 8% acrylamide, pH 7.0): **A,** Lower gel: 8-12-14% acrylamide step gradient, pH 6.0. **B,** Lower gel: 10–20% continuous acrylamide gradient, pH 5.3. **C,** Lower gel: 10–20% continuous acrylamide gradient, pH 5.6.

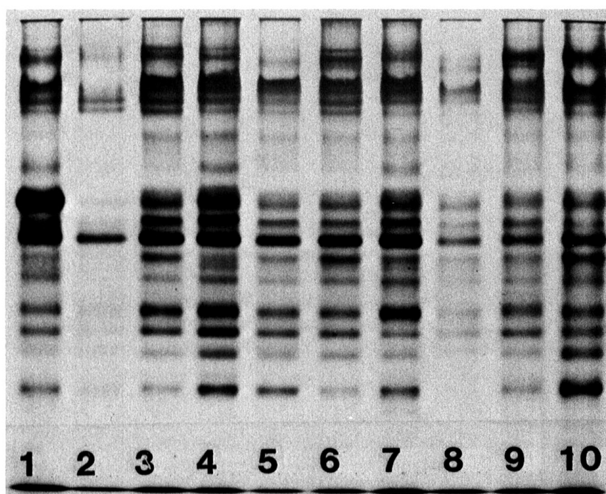

Fig. 3. Electrophoretic patterns of proteins obtained from single kernels of 10 soft wheat cultivars. Extraction medium: 0.5*M* potassium acetate, pH 7.0/ethylene glycol (1:1, v/v), 100 μl per kernel. Lower gel: 12% acrylamide, 0.125*M* potassium acetate, pH 6.0. Upper gel: 8% acrylamide, 0.125*M* potassium acetate, pH 7.0; Load: 50 μl. Lanes: **(1)** Adena, **(2)** Argee, **(3)** Augusta, **(4)** Fillmore, **(5)** Hart, **(6)** Hillsdale, **(7)** Scotty, **(8)** Wheeler, **(9)** Paha, **(10)** Houser (Augusta, Paha, and Houser, soft white winter; all others soft red winter).

marker had migrated about 12 cm. Running times ranged from 5–6 hr for the anionic systems to 7–8 hr for the cationic systems. Gels were stained and destained as previously described (Clements 1987).

RESULTS AND DISCUSSION

Electrophoretic conditions (buffer species, pH, acrylamide concentration) were varied in preliminary experiments with cationic systems. Best separations were obtained with gel pH in the range 5.3 to 6.0. Homogeneous gels containing 8 to 12% acrylamide (3% cross-linkage) gave good results, but 10 to 20% linear-gradient gels proved to be best for general application. Limited experiments also indicated that buffer species other than acetates (e.g., citrates) offered no advantages over the widely used acetate system (Maurer 1971).

The above conditions were employed for initial studies of effects of extraction medium on patterns (Fig. 1A). The results showed that choice of extraction buffer had little effect on patterns. Water, 0.1*M* sodium chloride, and 0.1*M* potassium acetate, pH 6.0 and pH 7.5, all containing 25% sucrose to provide density for application to gels, gave similar patterns (lanes 2–6). Ethylene glycol, a gliadin extractant (Clements 1987) (lane 1), combined with 0.5*M* potassium acetate, pH 7.0 (1:1, v/v), produced a pattern with somewhat heavier bands in the gliadin region (lane 7) than did other aqueous media. However, because of convenience and low freezing point (liquid at −20°C), the latter was selected as the medium for application to cationic systems in this study.

A comparison of patterns from low- and high-protein specimens of wheats showed relatively minor differences (Fig. 1B), the most noticeable difference being an increased intensity in certain high-mobility bands in patterns from the high-protein wheats. However, a band of intermediate mobility (arrow in Fig. 1B) was more prominent in the low-protein specimens of three of the four cultivars studied.

Application of cationic PAGE to soluble proteins of soft wheat genotypes, employing the range of conditions described above, showed that cultivars exhibit unique patterns. Differences among patterns, however, appear to be primarily quantitative. The patterns of the eight cultivars shown in Figure 2, obtained under different electrophoretic conditions, illustrate this generalization. They show that differences between specific patterns can be emphasized by selection of appropriate PAGE conditions. Increasing gel pH from 5.3 to 6.0 greatly increased mobilities, with the greatest increase occurring in the rates of the more mobile species. Among the cultivars illustrated, Logan (lane 4) is noteworthy because of the very heavy band near the center of the pattern. Certain other Ohio cultivars, e.g., Adena and Becker (results not shown), are also distinguished by this characteristic. Application of the cationic PAGE systems to single kernels also produced good patterns (Fig. 3).

Similar studies were made of conditions required for anionic PAGE of the soluble proteins. The patterns from several soft

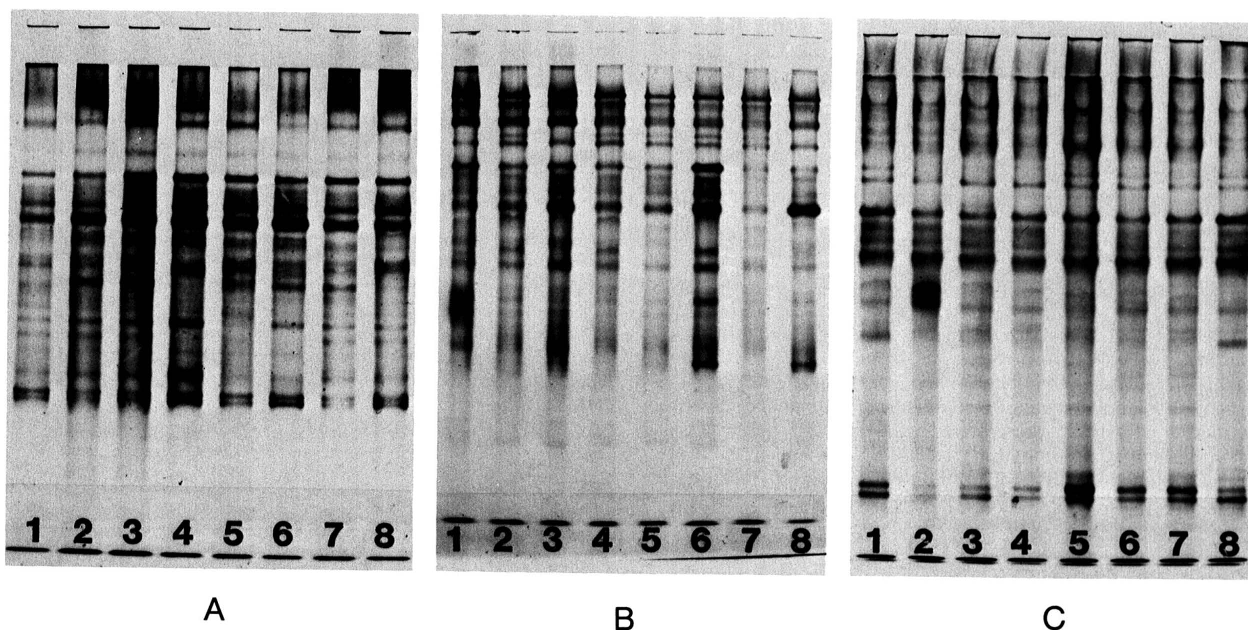

Fig. 4. Electrophoretic patterns of soft wheat proteins obtained using anionic systems. Extraction medium: 0.16*M* Tris-H_3PO_4, pH 6.9/ethylene glycol (1:1, v/v), 2 ml per gram of meal or 100 μl per kernel. Load: 40 μl. Buffers: lower gel, 0.25*M* Tris-HCl; upper gel, 0.04*M* Tris-H_3PO_4. **A,** Patterns from meals of eight cultivars: lower gel, 8% acrylamide, pH 8.5; upper gel, 6% acrylamide, pH 6.9. Lanes: **(1)** Arrow, **(2)** Auburn, **(3)** Beau, **(4)** Becker, **(5)** Blueboy, **(6)** Cardinal, **(7)** Charmany, **(8)** Coker 9323. (Arrow, soft white winter; all others soft red winter). **B,** Patterns from single kernels of eight cultivars: lower gel, 8% acrylamide, pH 8.0; upper gel, 6% acrylamide, pH 6.9. Lanes: **(1)** Titan, **(2)** Blueboy, **(3)** Hart, **(4)** Hillsdale, **(5)** Houser, **(6)** Frankenmuth, **(7)** Augusta, **(8)** Argee. (Houser, Frankenmuth, and Augusta, soft white winter; all others soft red winter). **C,** Patterns from single kernels of eight cultivars: lower gel, 8% acrylamide, pH 8.5; upper gel, 6% acrylamide, pH 6.9. Lanes: **(1)** Scotty, **(2)** Wheeler, **(3)** Fillmore, **(4)** Beau, **(5)** Caldwell, **(6)** Oasis, **(7)** Arthur, **(8)** Cardinal. (All soft red winter).

red and soft white winter cultivars shown in Figure 4 were obtained from meals with anionic PAGE at pH 8.5 (Fig. 4a), and from single kernels at pH 8.0 (Fig. 4b) and at pH 8.5 (Fig. 4c). Mobilities increased greatly as gel pH was increased from 8.0 to 8.5. Results, like those from cationic PAGE, show differences that are predominantly quantitative. However, close inspection of gels reveals many bands, and although the most prominent bands appear to be ubiquitous among cultivars, the weaker bands indicate definite qualitative differences. Some of these differences are evident in the photographs in Figure 4, but application of more sensitive detection methods should provide greater contrast among genotypes.

Because of sensitivity of systems to pH, acrylamide concentration, and other variables, reproducibility requires strict standardization of conditions. This is more difficult to accomplish with continuous gradient gels than with homogeneous gels, but the improved resolution justifies this approach. In any case, genotypes to be compared should be run on the same gel to ensure identical conditions.

Electrophoresis of soluble proteins of more than 50 soft wheat cultivars, several replicated from different locations, suggests patterns differ among genotypes. This study, however, was concerned primarily with development of appropriate methodology. Intensive application to selected cultivars grown at several locations will be required to establish genotypical differences.

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