Improvements in Cereal Protein Separations by Capillary Electrophoresis: Resolution and Reproducibility[1]

GEORGE L. LOOKHART[2] and SCOTT R. BEAN[3]

ABSTRACT

Cereal Chem. 73(1):81–87

Capillary electrophoresis rinsing protocols and buffer compositions were optimized to improve the resolution and reproducibility of cereal prolamin separations. Capillary cleaning protocols were varied to improve reproducibility. Four cleaning regimes were tested; the optimum was a 4-min rinse with 1*M* phosphoric acid. Several buffer modifiers were tested for use with 100 m*M* phosphate buffer (pH 2.5) containing 0.05% hydroxypropylmethylcellulose. Twenty percent acetonitrile provided optimal resolution, while maintaining excellent reproducibility. That buffer provided high resolution of wheat and oat prolamins. Resolution of rice prolamins also improved using that buffer, but best resolution of rice prolamins was found when lauryl-sulfobetain at its critical micelle concentration (26 m*M*) was also added.

Cereal proteins have been studied by many analytical techniques due to their relationships with quality and varietal identification. Polyacrylamide gel electrophoresis (PAGE) and high-performance liquid chromatography are currently the methods of choice. Gel electrophoresis, acid-PAGE, and sodium dodecyl sulfate-PAGE are the established methods for protein separations. However, these methods have several drawbacks, including the use of toxic reagents, long analysis times, and difficulties in quantifying and interpreting data. Electrophoretic methods and extraction conditions used to differentiate cultivars of all major cereal crops were recently reviewed by Lookhart (1990) and Lookhart and Wrigley (1995).

Reversed-phase high-performance liquid chromatography has also been used to differentiate cereal cultivars, alone and as a complement to acid-PAGE, for rice (Lookhart et al 1987, 1991; Huebner et al 1991; Lookhart and Juliano 1994), oats (Lookhart 1985, Lookhart and Pomeranz 1985, Bakhella et al 1992), barley (Marchylo and Laberge 1980, Heisel et al 1986), and wheat (Bietz 1983, Lookhart et al 1986, Lookhart and Albers 1988, Bakhella et al 1991, Huebener and Bietz 1994).

Recently, wheat proteins were characterized by a new technique, capillary electrophoresis (CE) (Bietz and Lookhart 1994; Werner et al 1994; Lookhart et al 1994, Bietz and Schmalzried 1995; Lookhart and Bean 1995a,b). An aluminum lactate buffer, in conjunction with capillaries coated with Microcoat (Perkin-Elmer), was used by Werner et al (1994) to separate gliadins. The other authors (Bietz and Lookhart 1994; Bietz and Schmalzried 1995; Lookhart and Bean 1995a,b) used primarily a low-pH (2.5) commercial (Bio-Rad) phosphate buffer to separate cereal proteins. Further modifications of this method reduced the time and optimized the resolution and reproducibility for separation of those proteins (Lookhart and Bean 1995a). Gliadins were separated in less than 10 min, with a relative standard deviation (RSD) of 0.6% for migration times.

CE of cereal proteins has recently undergone considerable development. It is a rapid technique, easily automated, that requires little sample or reagents (Chen 1991). We have shown that resolution and reproducibility could be further improved when wash procedures were optimized and buffers containing organic modifiers were used. These studies confirm that CE is a highly resolving, reproducible technique for rapid cereal cultivar identification and protein characterization.

MATERIALS AND METHODS

Samples

Wheat cultivars (TAM 107 and TAM 105) used in this experiment were from the Grain Quality and Structure Research Unit (Hard Winter Wheat Quality Laboratory) in Manhattan, KS. Flours from each wheat were produced by a Brabender Quadrumat Senior Experimental Mill.

One oat cultivar, Troy, was selected for analysis from those already analyzed by CE (Lookhart and Bean 1995b). Likewise, one rice cultivar (IR-28) was used (Lookhart and Bean 1995b).

Protein Extraction

Wheat proteins (gliadins) were extracted as previously described (Lookhart and Bean 1995a). Oat proteins (avenins) and rice prolamins were extracted as previously described by Lookhart and Bean (1995b). Glutenins were extracted and precipitated as described by Marchylo et al (1988).

Capillary Zone Electrophoresis

A Beckman P/ACE 2100 system was used to separate all extracts. Capillaries used were uncoated fused-silica, 27 cm long (20 cm separation length). Wheat and oat samples were analyzed on a 20-μm i.d. capillary, while rice samples were analyzed on a 50-μm i.d. capillary. Voltages and temperatures depended on the buffer system used (see figure legends). All samples were pressure-injected for 5 sec. Proteins were monitored by UV detection at 200 nm.

Capillary cleaning protocols. Four capillary cleaning protocols were used: 1) 0.1*M* NaOH, 1*M* phosphoric acid, water rinse; 2) 0.1*M* NaOH rinse; 3) water rinse; and 4) 1*M* phosphoric acid rinse.

[1]Cooperative investigations, U.S. Department of Agriculture, Agricultural Research Service and the Department of Grain Science and Industry, Kansas State University. Contribution 95-534-J, Department of Grain Science and Industry, Kansas State Agricultural Experiment Station, Manhattan, KS 66506.
Mention of firm names or trade products does not constitute endorsement by the U.S. Department of Agriculture over others not mentioned.

[2]Research Chemist, USDA-ARS, Grain Marketing Research Laboratory, Manhattan, KS 66502. Fax 913/776-2792.
[3]Research Assistant, Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506.

U.S. Department of Agriculture, Agricultural Research Service, Northern Plains Area, is an equal opportunity/affirmative action employer and all agency services are available without discrimination.

Publication no. C-1996-0111-01R.

Preinjection rinse. Before injection, capillaries were rinsed for 4 min with 0.1*M* phosphate buffer, pH 2.5, containing 0.05% hydroxypropylmethylcellulose. When an organic modifier was present in the buffer, capillaries were rinsed for 2 min with the above phosphate buffer and then for 4 min with buffer containing the organic modifier.

Buffer modifiers. Acetonitrile (ACN), dimethylsulfoxide, dimethylformamide, methanol, 2-methoxyethanol, and 2-propanol (each at 10, 20, and 30% v/v) and ethylene glycol (5 and 20% v/v) were tested as buffer modifiers. The ACN-modified phosphate buffer was further tested at 15, 25, 30, and 40%. Zwitterionic detergents CHAPS (3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate) (Sigma C-3023), lauryl-sulfobetain (SB-12) (*N*-dodecyl-*N,N*-dimethyl-3-ammonio-1-propanesulfonate) (Sigma D-4516), and octyl-sulfobetain (SB-8) (*N*-octyl-*N,N*-dimethyl-3-ammonio-1-propanesulfonate) (Sigma 0-6626) and the nonionic detergent Brij-35 (polyoxyethylene 23 lauryl ether) (Sigma P-1254) were tested with the 20% ACN buffer. Hexane sulfonic acid (HSA) (Sigma H-9026) was also tested. The phosphate buffer used in all analyses was made as previously described (Lookhart and Bean 1995b). In all cases, the phosphate concentration of the buffer was 100 m*M*.

RESULTS AND DISCUSSION

Cleaning Protocols

Cleaning protocols were varied to give optimal reproducibility with minimal wash time. We had previously rinsed capillaries with 0.1*M* NaOH, 1*M* phosphoric acid, and water (Lookhart and Bean 1995b). This procedure yielded good reproducibility but may not be the best possible method for several reasons. First, phosphate binds to silica (Mitsyuk 1972), forming partially deactivated capillaries (McCormick 1988). Furthermore, only alkaline washes, and not buffer or water washes, can remove these phosphate groups from the capillary walls (McCormick 1988). Thus, rinsing with NaOH may disrupt the uniformity of the capillary walls from run to run. Second, the inside surfaces of silica capillaries display a charge hysteresis when the pH is changed (Kohr and Englehardt 1991). Therefore, use of a high-pH rinse (e.g., NaOH) with a low-pH buffer may make it difficult to establish a uniform surface on the capillary walls between runs. Third, capillaries rinsed with NaOH and organic solvents produce unstable migration times (Smith et al 1991). This could lead to reproducibility problems when using NaOH rinses and organic modifiers in the buffers (see below).

Good results were obtained with all four cleaning procedures. However, water alone does not remove many adsorbed proteins from capillary walls and leads to long-term reproducibility problems. Likewise, NaOH rinses lead to problems in establishing a uniform capillary wall surface between runs, as discussed above. Use of 1*M* phosphoric acid as the only rinse avoids many of these problems. It removes most adsorbed proteins, does not perceptibly disturb the capillary walls, and maintains the bound phosphates between runs (Mitsyuk 1972). The best reproducibility found in this study was obtained using 1*M* phosphoric acid as the only rinse. The phosphoric acid rinse produced RSDs for migration times ranging from 0.1 to 0.2%, while the other rinses produced RSDs ranging from 0.6 to 2%. This reproducibility was better than that reported for wheat gliadins (Bietz and Schmalzreid 1995, Lookhart and Bean 1995a). Better reproducibility of peptide separation times was also obtained when capillaries using low-pH buffers were washed with acidic rinses (Strickland and Strickland 1990). Finally, use of the single postseparation rinse simplified the procedure and reduced the total analysis time by 20–25%.

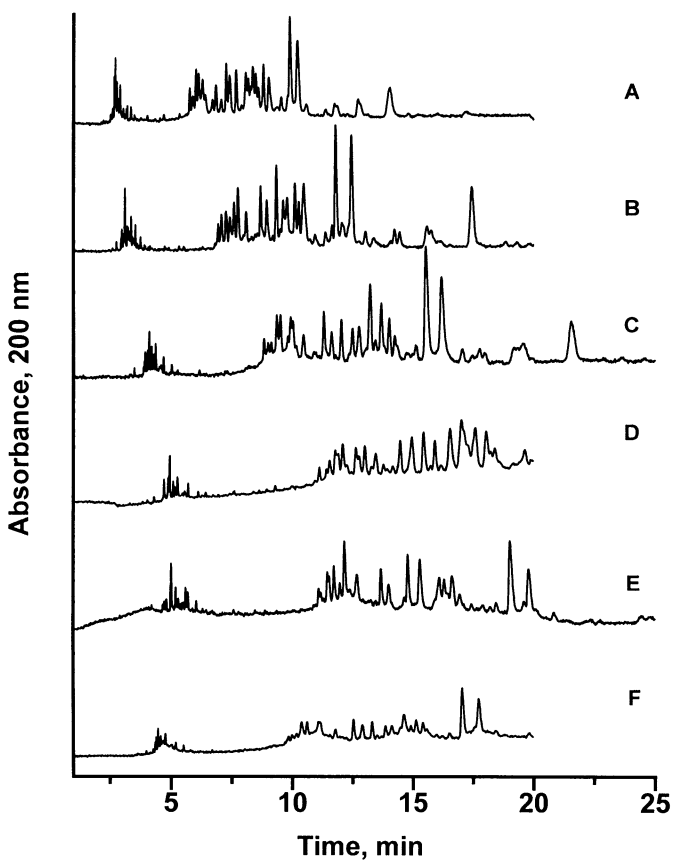

Fig. 1. Capillary electrophoresis gliadin patterns of TAM 107 separated with 100 m*M* phosphate buffer, pH 2.5, containing 0.05% hydroxypropylmethylcelluose (A), or buffer A plus an organic modifier; 20% acetonitrile (B), 20% methanol (C), 20% 2-methoxyethanol (D), 20% 2-propanol (E), or 20% ethylene glycol (F). Separations were carried out in 27-cm (20-cm separation length) × 20-μm i.d. capillaries at 15 kV (+ to -) and 45°C. Samples were pressure-injected (5 sec).

Buffer Modifiers

Effects of organic modifiers on cereal protein separations were tested with several buffer systems. Figure 1 shows CE patterns of TAM 107 gliadin extracts in the pH 2.5 phosphate buffer containing 0.05% hydroxypropylmethylcellulose modified with 20% of either ACN, methanol, 2-methoxyethanol, 2-propanol, or ethylene

TABLE I
Reproducibility of Migration Times of 15 TAM 107 Gliadin Peaks Using the P-ACN[a] Buffer System over a Period of 20 Runs

	Migration Time		
Peak No.	Average Minutes	Standard Deviation	Relative Standard Deviation (%)
1	6.81	0.0074	0.11
2	7.12	0.0088	0.12
3	7.60	0.0088	0.12
4	7.93	0.0094	0.12
5	8.50	0.0109	0.13
6	9.13	0.0132	0.14
7	9.54	0.0155	0.16
8	9.84	0.0150	0.15
9	10.19	0.0167	0.16
10	11.40	0.0203	0.18
11	12.02	0.0226	0.19
12	12.56	0.0247	0.20
13	13.70	0.0277	0.20
14	13.88	0.0273	0.20
15	16.58	0.0370	0.22

[a] 100 m*M* phosphate buffer (+0.05% hydroxypropylmethylcelluose) containing 20% acetonitrile.

glycol. No other modifier or concentration was as effective in resolving TAM 107 proteins as phosphate containing 20% ACN (P-ACN) (Fig. 1B). The buffer modified with P-ACN produced patterns in almost the same short time as the original buffer. Addition of 20% methanol separated all components well except peaks migrating in the 8–10 min and 17–20 min regions, where the patterns were more complex or broad (Fig. 1C) and required more time for separation. Addition of either 2-propanol (Fig. 1E) or ethylene glycol (Fig. 1F) was not as effective at improving the resolution of gliadins over that achieved by the addition of ACN (Fig. 1B) or methanol (Fig. 1C) or the original phosphate buffer (Fig. 1A). Ethylene glycol has a higher viscosity than the other modifiers, and, thus, less sample was injected into the capillary. Ethylene glycol also absorbs light in the UV region and thus reduces sensitivity (Gordon et al 1991). No peaks were seen with the buffers modified with dimethylsulfoxide or dimethylformamide (results not shown). These solvents absorb too strongly at 200 nm to be of use.

Comparison of data using various ACN concentrations showed that the optimum ACN concentration for separating gliadins was 20–25%. However, at 25% ACN, migration times were not as reproducible as at 20% ACN, so 20% was chosen as optimum. Cereal proteins are normally classified as relatively hydrophobic, and, since ACN is a relatively hydrophilic solvent, the resolution may be enhanced by altering the selectivity of the separation. Changing the hydrophobicity of the solvent will most likely change the protein internal interactions and thus change the separation. ACN may also decrease hydrophobic interactions among these naturally hydrophobic proteins by competing for hydrophobic sites on their surfaces (J. Bietz, *personal communication*). Addition of ACN to the buffer also affects the buffer pH. At 20%, the pH is ~2.7 and at 25% it is ~2.9. This change in pH may be the reason that 20% is more reproducible than 25%. The increased pH may allow for more interaction between the silica wall and the proteins. However, this pH change was not solely responsible for the increase in resolution. When a phosphate buffer (100 m*M*) containing no ACN was adjusted to pH 2.7, the resultant wheat protein electropherograms did not resemble those produced with the P-ACN buffer and gave a much lower resolution (data not shown). When the pH of the 20% ACN buffer was lowered to pH 2.5 with concentrated phosphoric acid, the separation was different from that of the normal buffer at pH 2.5 or from that of the unaltered 20% ACN buffer. If pH were the sole difference between buffers, one would expect this buffer to produce a pattern similar to that produced by the original phosphate buffer (no ACN).

ACN also affects electroendosmotic (EO) flow (Schwer and Kenndler 1991, Kenndler 1993). However, we do not believe that this phenomenon was responsible for the increased resolution with buffer containing ACN, since the other modifiers did not produce similar effects. Both methanol and 2-propanol slow EO flow more than ACN (Kenndler 1993). In addition, EO flow is minimal at low pH (McCormick 1988).

The presence of organic solvents in the buffer also decreases the current flow during a separation. This, in turn, would lead to less joule heating of the capillary. However, this factor was not responsible for the improvements in resolution of the ACN buffer, since phosphate buffers without organic modifiers were tested at the same current levels as the ACN buffers and there was no improvement in resolution at the lower current levels. Ohm's law plots for both buffer systems were prepared, and both buffers were operating in the linear portion of the plots, indicating that excessive heating of the capillaries was not occurring (Oda and Landers 1994).

Because of the improved separations found with the addition of organic solvents to aqueous buffers, separation conditions were optimized for buffers containing ACN. The best resolution with the ACN buffer occurred at 40–50°C. However, migration times were unstable above 45°C, so 45°C was chosen. Voltage levels of 15–18 kV provided best results. Above 18 kV, early gliadin peaks began to merge; therefore, 15 kV was chosen as optimum. At 15 kV, separations were still rapid, and resolution was uncompromised. The separation obtained with this buffer system (Fig.

TABLE II
Reproducibility of Peak Heights of 15 TAM 107 Gliadin Peaks Using the P-ACN[a] Buffer System over a Period of 20 Runs

	Peak Heights		
Peak No.	Average mAU[b]	Standard Deviation	Relative Standard Deviation (%)
1	0.0013	0.00011	8.46
2	0.0019	0.00019	9.70
3	0.0036	0.00036	10.25
4	0.0011	0.00010	6.19
5	0.0024	0.00011	4.40
6	0.0029	0.00022	7.43
7	0.0017	0.00010	5.12
8	0.0023	0.00011	4.78
9	0.0028	0.00010	2.12
10	0.0055	0.00019	3.45
11	0.0046	0.00016	3.40
12	0.0069	0.00006	8.19
13	0.0066	0.00005	6.93
14	0.0056	0.00004	7.40
15	0.0025	0.00010	4.20

[a] 100 mM phosphate buffer (+0.05% hydroxypropylmethylcelluose) containing 20% acetonitrile.
[b] Milliabsorbance units.

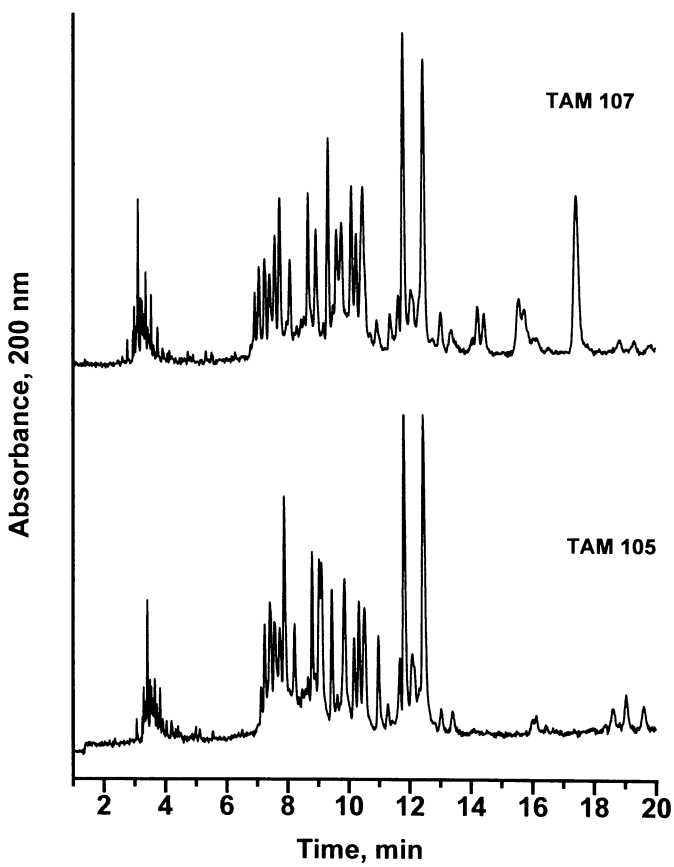

Fig. 2. Capillary electrophoresis gliadin separations of closely related wheat cultivars TAM 107 and TAM 105, using 100 m*M* phosphate buffer (+0.05% hydroxypropylmethylcelluose) containing 20% acetonitrile. Separation conditions as in Fig 1. Patterns were normalized for comparison.

1B) was compared to that previously used (Fig. 1A) (Bietz and Schmalzried 1995; Lookhart and Bean 1995a,b). The new buffer with ACN present (Fig. 1B) provided nearly baseline resolution.

Reproducibility of the separation with P-ACN buffer under optimal conditions was excellent. The highest reproducibility was found with the following procedure: before injection, the capillary was rinsed for 2 min with 100-m*M* phosphate buffer, pH 2.5 (containing no ACN) and then for 4 min with the phosphate/20% ACN buffer. Between separations, the capillary was rinsed for 4 min with 1*M* phosphoric acid. Using this protocol, RSDs for migration times of 15 major peaks ranged from 0.1 to 0.2% over 20 runs (Table I). RSDs for peak heights on the same peaks averaged 2–10% RSD (Table II). To ensure good reproducibility, fresh buffer was used every 20 runs (20 min per run, using 4.5-ml vials).

New equilibration procedures and daily washing programs were developed for this buffer system. New capillaries were first rinsed with 0.1*M* NaOH for 5 min. This was the only time NaOH was put through the capillaries. Capillaries were then rinsed 60 min with 1*M* phosphoric acid and 20 min with 100 m*M* phosphate buffer, pH 2.5 (with no ACN). A standard cultivar (TAM 107) was then analyzed (typically four to five runs) to ensure that the capillary was properly equilibrated and that migration times were stable. Electropherograms were rigorously inspected and compared to controls at this time to evaluate the state of the capillaries. Capillaries that were not fully equilibrated would sometimes produce stable migration times, but peaks would not be fully resolved. In particular, the two large gamma gliadin peaks (11–13 min) (Lookhart and Bean 1995c) often appeared smaller than normal in nonequilibrated capillaries. Some capillaries may take more than 24 hr for equilibration and for migration times and peak patterns to stabilize. Once fully conditioned, the capillary was ready for use. At the beginning of each day (or whenever a capillary had not been used for 1–2 hr), it was rinsed 30 min with 1*M* phosphoric acid and 10 min with 100 m*M* phosphate buffer (containing no ACN). At night, capillaries were rinsed 10 min with 1*M* phosphoric acid and 20 min with water and dried for 15 min with nitrogen. This prevented dissolution of the silica surface by water (Schwer and Kenndler 1991) and prevented disruption of bound phosphates (Mitsyuk 1972), eliminating the need for extensive conditioning when the column was next used. Capillaries were typically used in our laboratory until they broke or became plugged. We have successfully used capillaries for up to nine months (before plugging) without any breakdown in performance.

Standardization

A 30% ethanol extract of TAM 107 was used as a reference standard for all data sets. The TAM 107 extract was analyzed at the beginning and end of each set of analyses. This extract was prepared fresh every 24 hr (250 mg of flour to 1 ml of solvent). This allowed the visual assessment of resolution and standardization of data sets (Sapirstein et al 1989) day-to-day and from different capillaries or buffer lots, both of which can cause differences in migration times (Bietz and Lookhart 1994). This is similar to the methodology recommended by Bietz and Schmalzried (1995) and for A-PAGE analyses using Marquis as the control standard (Lookhart et al 1982).

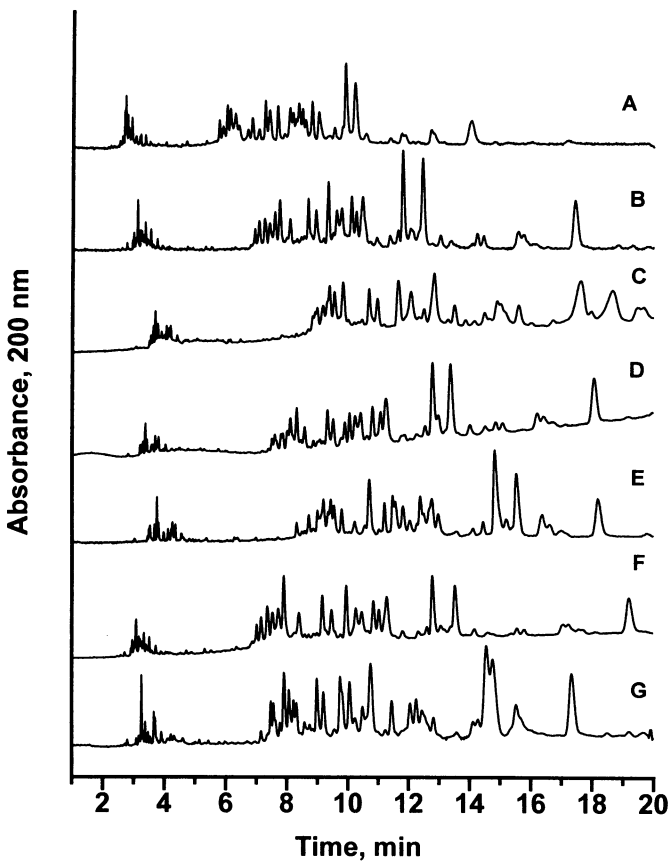

Fig 3. Capillary electrophoresis separations of TAM 107 gliadins using the following separation buffers: 100 m*M* phosphate buffer, pH 2.5, containing 0.05% hydroxypropylmethylcelluose (A); buffer A containing 20% acetonitrile (P-ACN) (B); P-ACN + 26 m*M* Brij-35 (C); P-ACN + 26 m*M* CHAPS (D); P-ACN + 26 m*M* hexane sulfonic acid (E); P-ACN + 26 m*M* octyl-sulfobetain (F); and P-ACN + 26 m*M* lauryl-sulfobetain (G). Separation conditions as in Fig. 1.

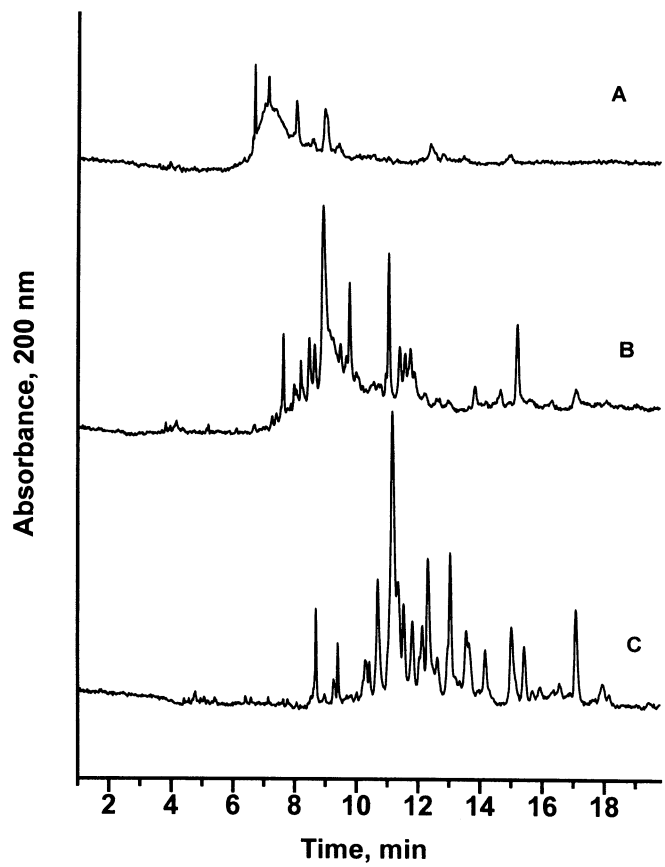

Fig 4. Capillary electrophoresis separations of TAM 107 glutenins in three buffers systems: 100 m*M* phosphate buffer, pH 2.5, containing 0.05% hydroxypropylmethylcelluose (A); buffer A containing 20% acetonitrile (P-ACN) (B); and P-ACN + 26 m*M* lauryl-sulfobetain (C). Separation conditions as in Fig. 1.

Resolution of Wheat Proteins

ACN buffer. When albumins and globulins were independently extracted by the method of Lookhart and Bean (1995c) and analyzed by CE using the P-ACN buffer system, slight increases in resolution of the albumins were found, but the globulin extracts, which were poorly separated in the original phosphate buffer (Lookhart and Bean 1995c), were also poorly separated in the P-ACN buffer (results not shown).

CE of gliadin extracts successfully differentiated even closely related wheat cultivars, like TAM 105 and TAM 107, using the P-ACN buffer system. The CE electropherograms of TAM 105 and TAM 107 were similar (Fig. 2), but unique differences were found in peaks migrating between 9 and 11 min and between 13 and 20 min. TAM 107 is a backcross of TAM 105 to Amigo and contains the 1AL/1RS rye translocation. The proteins from the 1RS rye segment transferred to TAM 107 are rye omega secalins and migrate in the 13–18-min range (Lookhart et al 1995).

ACN buffer + detergents. Detergents are widely used for micellar-based CE separations. Recently some surfactants have also been used in the separation of proteins (Moring and Nolan 1990) and polypeptides (Greve et al 1994). Lauryl-sulfobetain, a zwitterionic detergent, has been used to add hydrophobic selectivity to CE separations. This detergent provides a hydrophobic pseudostationary phase, with which molecules may interact during electrophoresis, thereby altering their normal electrophoretic mobilities (Greve et al 1994). HSA, although primarily an ion-pairing reagent, functions in a similar manner (Moring and Nolan 1990). These and other detergents were tested in the P-ACN system, to see if they provided increased resolution of wheat proteins.

The CE separations of gliadins from TAM 107 are shown in Figure 3. Separation buffers included phosphate buffer (Fig. 3A), P-ACN (Fig. 3B), P-ACN + 26 m*M* Brij-35 (Fig. 3C), P-ACN + 26 m*M* CHAPS (Fig. 3D), P-ACN + 26 m*M* HSA (Fig. 3E), P-ACN + 26 m*M* SB-8 (Fig. 3F), and P-ACN + 26 m*M* SB-12 (Fig. 3G). For gliadins, the best separation was found with the P-ACN buffer (Fig. 3B). However, differences in separation, solubility, and hydrophobic selectivity were apparent with each buffer-detergent system. The longest migration times were found with the Brij-35 system (Fig. 3C). Each detergent system appears to change the separation of different ranges (types of proteins) in the gliadin pattern.

Slight increases in resolution of albumins by CE were found in P-ACN buffer systems containing 10 m*M* HSA, 10 m*M* Brij-35, and 10 mM CHAPS (data not shown). The resolution of globulins was not materially affected by any of the detergent systems (data not shown).

The P-ACN buffer system also separates total glutenins (Fig. 4) and resolubilized precipitated glutenins (data not shown). Resolution of glutenins depended on the buffer systems: low resolution with phosphate buffer (Fig. 4A), higher resolution with P-ACN buffer (Fig. 4B), and optimally the highest resolution using P-ACN + 26 m*M* SB-12 (Fig. 4C). In most regions of the electropherograms, glutenin separations were improved with the P-ACN buffer containing 26 m*M* SB-12 (Fig. 4C).

Resolution of Oat Prolamins

The separation of 70% ethanol-soluble oat proteins from the oat cultivar Troy showed dramatically increased resolution with the P-ACN buffer (Fig. 5B) compared to that with the original

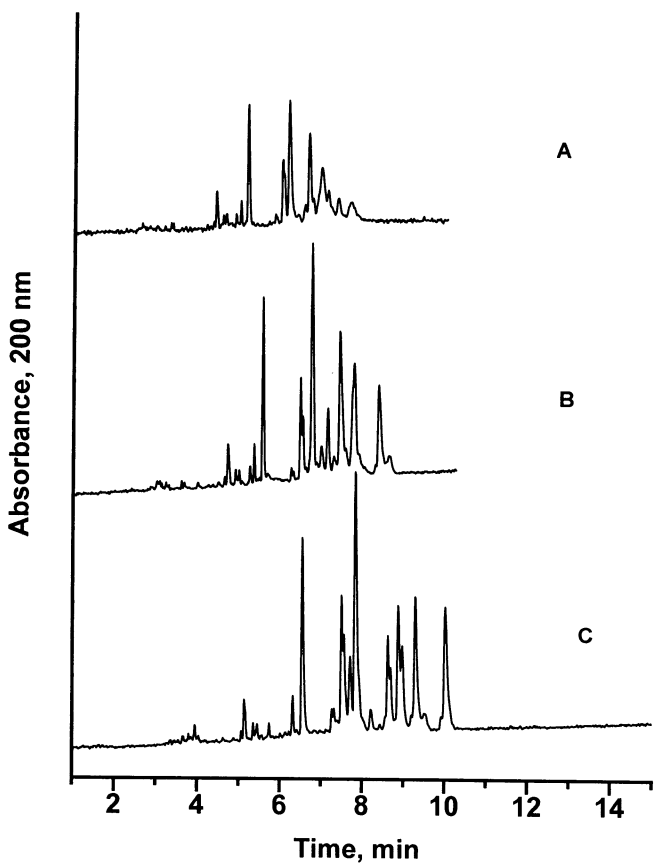

Fig 5. Capillary electrophoresis separation of oat cultivar Troy prolamins in three buffer systems: 100 m*M* phosphate buffer, pH 2.5, containing 0.05% hydroxypropylmethylcelluose (A); buffer A containing 20% acetonitrile (P-ACN) (B); and P-ACN + 26 m*M* lauryl-sulfobetain (C). Separation conditions same as for Fig. 1.

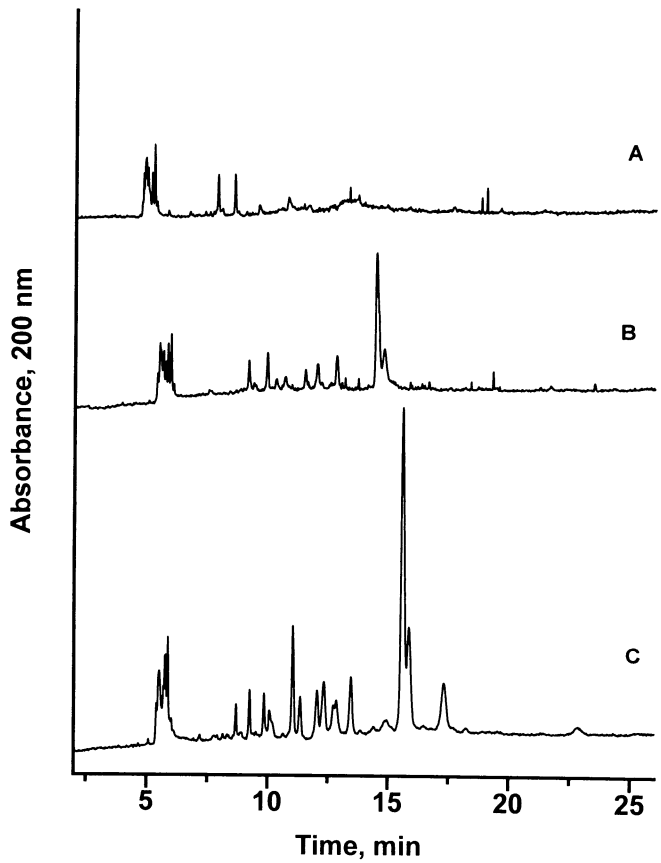

Fig 6. Capillary electrophoresis separation of rice cultivar IR-28 prolamins in three buffer systems: 100 m*M* phosphate buffer, pH 2.5, containing 0.05% hydroxypropylmethylcelluose (A); buffer A containing 20% acetonitrile (P-ACN) (B); and P-ACN + 26 m*M* lauryl-sulfobetain (C). Separations were carried out in 27-cm (20-cm separation length) × 50-μm i.d. capillaries at 10 kV (+ to -) and 40°C. Samples were pressure- injected for 5 sec each.

phosphate buffer (Fig. 5A). Resolution was near baseline for all peaks, and nearly all peaks were taller (Fig. 5B). Oat prolamin separations improved even further when SB-12 (Fig. 5C) was added at its critical micelle concentration (26 m*M*) (Greve et al 1994). This buffer further split some peaks, even those that were sharp singlets with the P-ACN buffer, into two or more components. No other detergent or additive produced patterns with greater separation than SB-12 (data not shown).

Resolution of Rice Prolamins

Separation of rice prolamins was also improved by the addition of organic additives. Improvements in the resolution of IR-28 prolamins were achieved upon addition of 20% ACN to the original phosphate buffer (Fig. 6B vs Fig. 6A). The resolution improved further when SB-12 was also added (Fig. 6C). However, the reproducibility with this buffer was not quite as good as with the P-ACN buffer. Migration times tended to drift with the P-ACN buffer containing SB-12. Extensive equilibration (60 min) of the capillary with P-ACN containing SB-12 before the first injection helped to stabilize migration times. More extensive conditioning and different rinsing protocols may have to be developed to optimally use buffers containing detergents.

CONCLUSION

The protocols presented in this article have improved existing methods of separating cereal proteins. The new buffer systems allow for rapid, high-resolution separation of cereal proteins with excellent reproducibility. The buffers used in this study do not require the use of coated capillaries for good results. This provides for rugged, dependable protocols for separating cereal proteins. Unlike uncoated capillaries, almost all coated capillaries have short lifetimes and are relatively expensive.

Different wheat protein classes have different hydrophobicities. Proteins with different hydrophobicities interact differently with detergents present in buffers. It appears that the separations of specific proteins or protein classes can be fine-tuned by the use of various detergents. Overall, however, resolution of gliadins was better with the P-ACN buffer than with the P-ACN buffer containing detergent additives. However, separation of glutenins was improved by the use of P-ACN + 26 m*M* SB-12.

Oat and rice prolamin separations can be improved by the use of phosphate buffers containing 20 % ACN and detergents. A judicious choice of buffer systems can expedite the separation of the individual protein classes.

LITERATURE CITED

BAKHELLA, M., LOOKHART, G. L., and HOSENEY, R. C. 1991. Identification of Moroccan wheats by reversed-phase high performance liquid chromatography and electrophoretic procedures. Actes Inst. Agron. Vet. (Maroc) 11:5.

BAKHELLA, M., LOOKHART, G. L., and HOSENEY, R. C. 1992. Identification of Moroccan oat cultivars by electrophoresis of their prolamins and total proteins. Actes Inst. Agron. Vet. (Maroc) 12:19.

BIETZ, J. A. 1983. Separation of cereal proteins by reversed-phase high-performance liquid chromatography. J. Chromatogr. 255:219.

BIETZ, J. A., and LOOKHART, G. L. 1994. Wheat varietal identification by capillary electrophoresis: An inter-laboratory comparison of methods. (Abstr.) Cereal Foods World 39:603.

BIETZ, J. A., and SCHMALZRIED, E. 1995. Capillary electrophoresis of wheat gliadin: Initial studies and application to varietal identification. Food Sci. Technol. (Zur.) 28:174.

CHEN, F. A. 1991. Rapid protein analysis by capillary electrophoresis. J. Chromatogr. 559:445.

GORDON, M. J., LEE, K., ARIAS, A. A., and ZARE, R. N. 1991. Protocol for resolving protein mixtures in capillary zone electrophoresis. Anal. Chem. 63:69.

GREVE, K. F., NASHABEH, W., and KARGER, B. L. 1994. Use of zwitterionic detergents for the separation of closely related peptides by capillary electrophoresis. J. Chromatogr. 680:15.

HEISEL, S. E., PETERSON, D. M., and JONES, B. L. 1986. Identification of United States barley cultivars by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of hordeins. Cereal Chem. 63:500.

HUEBNER, F. R., and BIETZ, J. A. 1994. RP-HPLC for varietal identification in cereals and legumes. Pages 97-120 in: High-Performance Liquid Chromatography of Cereal and Legume Proteins: Wheat. J. E. Kruger and J. A. Bietz, eds. Am. Assoc. Cereal Chem.: St. Paul, MN.

HUEBNER, F. R., HUSSAIN, A., LOOKHART, G. L., BIETZ, J. A., BUSHUK, W., and JULIANO, B. O. 1991. Discrimination of sister line IR rice varieties by polyacrylamide gel electrophoresis and reversed-phase high-performance liquid chromatography. Cereal Chem. 68:583.

KENNDLER, E. 1993. Organic solvents in capillary electrophoresis. Pages 161-186 in: Capillary Electrophoresis Technology. N. A. Guzmon, ed. Marcel Dekker Inc.: New York.

KOHR, J., and ENGELHARDT, H. 1991. Capillary electrophoresis with surface coated capillaries. J. Microcolumn Sep. 3:491.

LOOKHART, G. L. 1985. Identification of oat cultivars by combining polyacrylamide gel electrophoresis and reversed-phase high-performance liquid chromatography. Cereal Chem. 62:345.

LOOKHART, G. L. 1990. Cereal proteins: Composition of their major fractions and methods for identification. Pages 441-468 in: Handbook of Cereal Science and Technology. K. J. Lorenz and K. Kulp, eds. Marcel Dekker, Inc.: New York.

LOOKHART, G. L., and ALBERS, L. D. 1988. Correlations between reversed-phase high-performance liquid chromatography and acid- and sodium dodecyl sulfate-polyacrylamide gel electrophoretic data on prolamins from wheat sister lines differing widely in baking quality. Cereal Chem. 65:222.

LOOKHART, G. L., and BEAN, S. R. 1995a. A fast method for wheat cultivar differentiation using capillary zone electrophoresis. Cereal Chem. 72:42.

LOOKHART, G. L., and BEAN, S. R. 1995b. Rapid differentiation of oat cultivars and of rice cultivars by capillary zone electrophoresis. Cereal Chem. 72:312.

LOOKHART, G. L., and BEAN, S. R. 1995c. Separation and characterization of wheat protein fractions by high-performance capillary electrophoresis. Cereal Chem. 72:527.

LOOKHART, G. L., and JULIANO, B. O. 1994. RP-HPLC for varietal identification in cereals and legumes: Rice. Pages 175-183 in: High-Performance Liquid Chromatography of Cereal and Legume Proteins. J. E. Kruger and J. A. Bietz, eds. Am. Assoc. of Cereal Chem.: St. Paul, MN.

LOOKHART, G. L., and POMERANZ, Y. 1985. Characterization of oat species by polyacrylamide gel electrophoresis and high-performance liquid chromatography of their prolamin proteins. Cereal Chem. 62:162.

LOOKHART, G. L., and WRIGLEY, C. W. 1995. Variety identification by electrophoretic analysis. Pages 55-71 in: Identification of Food Grain Varieties. C. W. Wrigley, ed. Am. Assoc. of Cereal Chem.: St. Paul, MN.

LOOKHART, G. L., JONES, B. L., HALL, S. B., and FINNEY, K. F. 1982. An improved method for standardizing polyacrylamide gel electrophoresis of wheat gliadin proteins. Cereal Chem. 59:178.

LOOKHART, G. L., ALBERS, L. D., and BIETZ, J. A. 1986. A Comparison of PAGE and HPLC analyses of gliadin polymorphism in the wheat cultivar Newton. Cereal Chem. 63:497.

LOOKHART, G. L., ALBERS, L. D., POMERANZ, Y., and WEBB, B. D. 1987. Identification of U.S. rice cultivars by high-performance liquid chromatography. Cereal Chem. 64:199.

LOOKHART, G. L., JULIANO, B. O., and WEBB, B. D. 1991. The effect of solvent extraction, environment, and genetic background on differentiating milled rice by reversed-phase high-performance liquid chromatography. Cereal Chem. 68:396.

LOOKHART, G. L., BEAN, S. R., and AKKINA, S. K. 1994. High performance capillary electrophoresis (HPCE) optimization for wheat gliadin analyses and comparison of individual protein peak positions to high performance liquid chromatography (HPLC) peaks. (Abstr.) Cereal Foods World 39:627.

LOOKHART, G. L., BEAN, S. R., GRAYBOSCH, R. A., and CHUNG, O. K. 1995. Differentiation of 1AL/1RS from 1BL/1RS wheat-rye translocation lines by capillary electrophoresis. (Abstr.) Cereal Foods World 40:651.

MARCHYLO, B. A., and LABERGE, D. E. 1980. Barley cultivar iden-

tification by electrophoretic analysis of hordein proteins. Extraction and separation of hordein proteins and environmental effects. Can. J. Plant Sci. 60:1343.

MARCHYLO, B. A., HATCHER, D. W., and KRUGER, J. E. 1988. Identification of wheat cultivars by reversed-phase high-performance liquid chromatography of storage proteins. Cereal Chem. 65:28.

McCORMICK, R. M. 1988. Capillary zone electrophoretic separation of peptides and proteins using low pH buffers in modified silica capillaries. Anal. Chem. 60:2322.

MITSYUK, B. M. 1972. Mechanism of the reaction of silica with phosphoric acid in aqueous solutions. Russ. J. Inorg. Chem. (Engl. transl.) 17:471.

MORING, S. E., and NOLAN, J. A. 1990. Hydrophobic interaction capillary electrophoresis of peptides and proteins. Appl. Biosystems Inc. Res. News 2.

ODA, R. P., and LANDERS, J. P. 1994. Introduction to capillary electrophoresis. Pages 9-42 in: Handbook of Capillary Electrophoresis. J. P. Landers, ed. CRC Press: Boca Raton, FL.

SAPIRSTEIN, H. D., SCANLON, M. G., and BUSHUK, W. 1989. Normalization of high-performance liquid chromatography peak retention times of computerized comparison of wheat prolamin chromatograms. J. Chromatogr. 469:127.

SCHWER, C., and KENNDLER, E. 1991. Electrophoresis in fused-silica capillaries: The influence of organic solvents on the electroosmotic velocity and the ζ potential. Anal. Chem. 63:1801.

SMITH, S. C., STRASTERS, J. K., and KHALEDI, M. G. 1991. Influence of operating parameters on reproducibility in capillary electrophoresis. J. Chromatogr. 559:57.

STRICKLAND, M., and STRICKLAND, N. 1990. Free-solution capillary electrophoresis using phosphate buffer and acidic pH. Am. Lab. (Shelton, CT.) 22:60.

WERNER, W. E., WIKTOROWICZ, J. E., and KASARDA, D. D. 1994. Wheat varietal identification by capillary electrophoresis of gliadins and high molecular weight glutenin subunits. Cereal Chem. 71:397.

[Received July 26, 1995. Accepted September 2, 1995.]