Ultrafast Capillary Electrophoretic Analysis of Cereal Storage Proteins and Its Applications to Protein Characterization and Cultivar Differentiation†

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Free zone capillary electrophoresis conditions have been improved to allow rapid (2−8 min) separations of grain proteins from several cereals (wheat, oats, rice, barley, and rye) with high resolution and reproducibility. This new method utilized the isoelectric compound iminodiacetic acid (IDA) in conjunction with 20% acetonitrile and 0.05% hydroxypropylmethylcellulose. Cultivars of all cereals tested could be differentiated in 3 min, including wheat, using either prolamin or glutelin protein patterns. Resolution was similar to or higher than that of separations in other acidic buffers. Migration time repeatability was excellent with run-to-run variability <1% RSD, day-to-day <1.4% RSD, and capillary-to-capillary <3.3% RSD. Because larger inner diameter capillaries (50 μm) could be used with this buffer, sensitivity was improved and capillary rinse times could be reduced when compared to smaller capillaries (25 μm i.d.). This also served to reduce total separation time so that the majority of cereal storage protein from several types of cereals could be analyzed with total analysis times of 2−8 min with extremely high resolution and repeatability. This method would allow unattended, high-throughput (~180−400 samples/24 h) analysis of cereal proteins without the generation of much organic solvent waste as well as automated data analysis and storage.

Keywords: *Capillary electrophoresis; proteins; cereals; iminodiacetic acid; isoelectric buffers*

INTRODUCTION

Cereal storage proteins are an important class of proteins serving both nutritional as well as functional roles in many food products and animal feeds. In some instances, knowing the total amount of storage protein present is sufficient for scientific study. More often, though, it is desirable to fractionate the storage proteins with a high-resolution analytical separation, most often electrophoresis or chromatography.

One of the newest analytical techniques to be applied to cereal storage proteins is high-performance capillary electrophoresis (HPCE) [recently reviewed in Bean et al. (1998)]. Early work with HPCE produced several methods for successfully separating wheat (*Triticum aestiveum* L.), oat (*Avena sativa*), rice (*Oryza sativa*), and maize (*Zea mays* L.) proteins [reviewed in Bean et al. (1998)]. More recent developments have produced successful separations of barley (*Hordeum vulgare* L.) proteins (Lookhart et al., 1999) as well as new methods for the analysis of maize (Righetti et al., 1998; Bean et al., 1999) and sorghum (*Sorghum bicolor* Moench) (Bean et al., 1999) proteins.

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HPCE is capable of separating proteins by several different mechanisms, that is, size (SDS-CE), charge density (FZCE), and isoelectric point (cIEF). For cereal proteins, methods for both SDS-CE and FZCE have been developed as have two-dimensional HPLC × HPCE methods [reviewed in Bean et al. (1998)].

By far the most development has been done with FZCE. Early methods relied primarily on low-pH sodium phosphate buffers (Bietz and Schmalzried, 1995; Lookhart and Bean, 1995a) that utilized small-diameter (20 or 25 μm) capillaries to provide rapid, high-resolution separations (Lookhart and Bean, 1995a; Bean and Lookhart, 1997). Later work using special isoelectric buffers made up of aspartic acid (Capelli et al., 1998; Righetti et al., 1999) or low conductive buffers consisting of a phosphate−glycine buffer (Bean and Lookhart, 1998; Bean et al., 1999) improved the sensitivity by allowing larger capillary inner diameters to be used while maintaining rapid separations. These new buffers, combined with a better understanding of factors contributing to reproducibility problems and new, simple capillary equilibration procedures (Bean and Lookhart, 1998), have led to much improved FZCE methods for analyzing cereal storage proteins.

Although both the previous isoelectric and low conductive buffers allowed the use of larger capillaries and maintained relatively rapid separations, both of these buffer systems have limitations. The isoelectric aspartic acid buffers, due to their unique properties, allow very high voltage separations (30 kV in some cases), which produce relatively low currents and rapid separations (Capelli et al., 1998). However, these buffers work best

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with high concentrations of urea as a buffer additive (Capelli et al., 1998). Although it is certainly an effective protein solublization agent, high concentrations of urea are problematic in that it readily crystallizes, requiring frequent cleaning of instruments (Wehr et al., 1999). Urea also has substantial absorbance in the low-UV range and can modify proteins under certain circumstances (Wehr et al., 1999). Furthermore, when used with urea, the aspartic acid buffer had an apparent pH of 3.9, which could allow more protein interaction with uncoated fused silica capillaries than lower pH buffers. This is one possible factor for the migration time repeatability problems found when maize proteins were separated with the aspartic acid/urea buffer (Oliveiri et al., 1999; Bean et al., 1999).

Likewise, the low-conductive phosphate−glycine/ buffer used by Bean and Lookhart (1998), using acetonitrile (ACN) as a buffer additive, allowed separations to be carried out in larger diameter capillaries, although repeatability was reported to be better in the narrower capillaries, limiting sensitivity and increasing the time required to rinse the capillaries between separations. In addition, these buffers were not capable of extremely high voltage separations (only up to 17 kV), limiting how fast separations could be performed using this buffer.

To overcome the limitations of those buffer systems, an isoelectric buffer system composed of iminodiacetic acid (IDA) with acetonitrile (ACN) as a buffer additive was developed. Isoelectric buffers are unique compounds having p*I* values that are approximately equal to their pH values in solution, allowing them to provide good buffering capacity by themselves, without the need of a co-ion (Righetti et al., 1997). Furthermore, due to these unique properties, these buffers generate very little current, allowing extremely high voltages to be used during separations, resulting in rapid high-resolution separations (Righetti et al., 1997).

IDA was first reported by Bossi and Righetti (1997) for the separation of peptides. IDA has an apparent p*I* of ~2.33 and a pH range of 2.2−2.7, depending on the concentration and buffer additives (Bossi and Righetti, 1997). Thus, its working pH is lower than that of aspartic acid and closer to that of the phosphate buffers first used for wheat protein separations (Bietz and Schmalzried, 1995; Lookhart and Bean, 1995). IDA was also found to have good solubility in buffers containing an organic solvent (trifluoroethanol) (Bossi and Righetti, 1997).

ACN was recently shown to be equally as effective as urea for the solublization of maize and sorghum proteins and as a buffer additive for FZCE separations of these proteins (Bean et al., 1999). ACN has also been widely used in the separation of cereal proteins by RP-HPLC [for a review of HPLC of cereal proteins, see Kruger and Bietz (1994)] and has been used as a solvent for some cereals such as wheat (Morel and Autran, 1990). ACN has a low-UV cutoff, does not crystallize, and has low viscosity, making it an excellent buffer additive. Thus, the IDA/ACN combination overcomes the limitations of previous methodologies. This new method is capable of separating most storage proteins in <5 min with extremely high resolution, equal to or higher than that of previous, slower methods. Furthermore, excellent run-to-run, day-to-day, and capillary-to-capillary repeatabilities were achieved with this buffer. In addition, this buffer allows the use of larger diameter capillaries (50 μm i.d.), resulting in good sensitivity and fast capillary equilibration times.

MATERIALS AND METHODS

Capillary Electrophoresis. All separations were carried out using either a Beckman PACE 5510 or PACE 2100 instrument. Separations were done in 25 or 50 μm i.d. × 27 cm (20 cm L_d) uncoated fused silica capillaries (Polymicro, Phoenix, AZ). Capillaries were equilibrated before use by rinsing with separation buffer for 30 min (Bean and Lookhart, 1998). Between runs capillaries were rinsed with separation buffer for 2 min when using 25 μm i.d. capillaries or for 0.5 min when using 50 μm i.d. capillaries. When not in use, capillaries were rinsed with 500 mM acetic acid for 5 min followed by deionized water for 5 min and finally by nitrogen gas for 10 min.

Acetonitrile, methanol, ethanol, 1-propanol, 2-propanol, *tert*-butanol, and ethylene glycol were tested at concentrations from 5 to 20%. IDA concentrations were varied from 25 to 100 mM. Separation voltages were varied from 24 to 30 kV, and separation temperature was varied from 25 to 50 °C. For all buffers, 0.05% (w/v) hydroxypropylmethylcellulose (HPMC) was added to dynamically coat the capillary walls (Bean and Lookhart, 1998). All samples were injected into the capillaries with pressure (0.5 psi) for either 4 s when using 25 μm i.d. capillaries or for 1 s when using 50 μm i.d. capillaries.

Sample Preparation. Wheat gliadins were extracted with 50% 1-propanol after removal of albumins and globulins as described in Bean and Lookhart (1998). Glutenins were extracted with 50% 1-propanol plus 1% DTT after removal of soluble proteins with 50% 1-propanol as described in Bean et al. (1998). High molecular mass glutenin subunits (HMM-GS) were prepared by acetone precipitation as described in Bean and Lookhart (1998). Oat, rice, and rye prolamins were extracted with the same methodology as wheat gliadins, except in the case of rice a solvent-to-sample ratio of 3:1 was used. Oat, rice, and rye glutelins were extracted using the method described for wheat glutenins, except again for rice a solvent-to-sample ratio of 3:1 was used. Barley hordeins were extracted as described in Lookhart et al. (1999).

Optimum Separation Conditions. Optimized separation conditions for all cereals except rice were 50 mM IDA plus 20% ACN and 0.05% HPMC at 45 °C and 30 kV. For rice, the optimum buffer was the same as above, except for the addition of 26 mM lauryl sulfobetaine (SB 3-12).

Samples. Samples were from a collection of cereal grains at the USDA-ARS, Grain Marketing and Production Research Center, Manhattan, KS. For wheat, both flour and ground whole meal samples were used. All other cereal samples used were from ground whole meal. Ground whole meal samples were prepared by grinding whole kernels in a commercial coffee grinder. Samples were chosen at random from within the grain collection.

RESULTS AND DISCUSSION

Optimization. Although preliminary studies using 100 mM IDA as a buffer additive for wheat proteins using a urea/ACN mixture produced acceptable resolution, resolution was somewhat lower than that of phosphate−glycine/ACN buffers (Bean and Lookhart, 1998). To achieve higher resolution with IDA-based buffers and to find a buffer additive other than urea, several organic additives were tested at concentrations from 0 to 20% in 100 mM IDA. Organic solvents, particularly ACN, have produced high-resolution separations of wheat (Lookhart and Bean, 1996), barley (Lookhart et al., 1999), maize (Bean et al., 1999), and sorghum (Bean et al., 1999) in low-pH phosphate buffers.

As with the phosphate buffers, ACN produced the highest resolution separations, with 20% being optimum

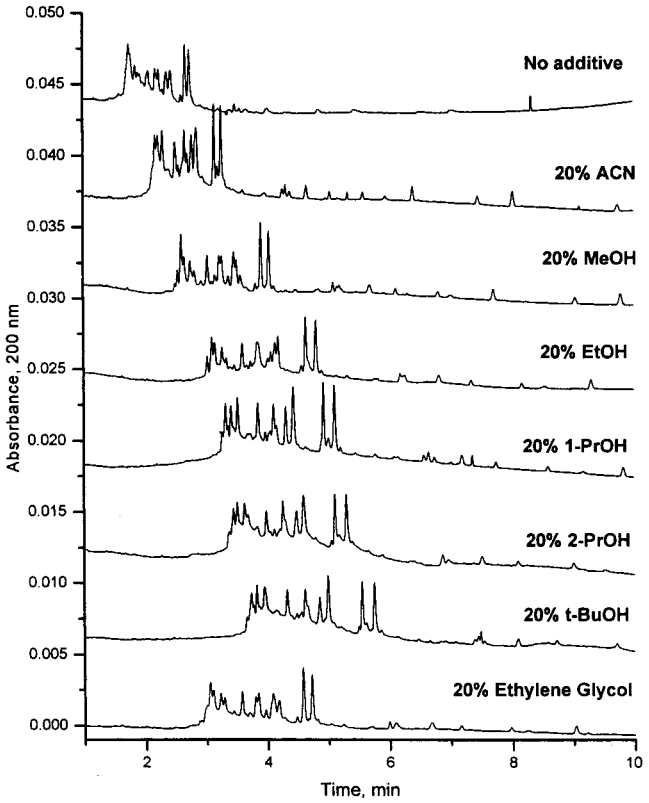

Figure 1. Effect of organic solvents on resolution of wheat gliadins. Additives were tested between 0 and 20% concentrations; only the 20% concentrations are shown in the figure. For all tests, 100 mM IDA + 0.05% HPMC was used as the buffer. Separation conditions were 30 kV and 45 °C in 25 μm × 27 cm uncoated capillaries. Detection was at 200 nm.

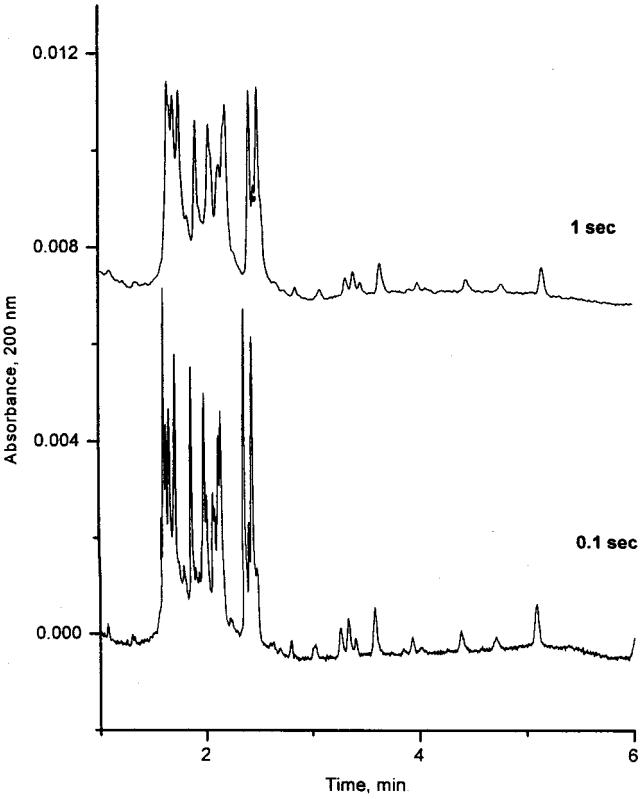

Figure 2. Effect of detector rise time on fast separation of wheat gliadins. Gliadins were separated in 50 mM IDA + 20% ACN and 0.05% HPMC at 30 kV and 45 °C in 50 μm × 27 cm uncoated capillaries. Detection was at 200 nm with two different detector rise times, 0.1 and 1 s.

(Figure 1). Ethanol also produced high-resolution separations, although the separation of the late migrating ω-gliadins was lower than in the ACN buffers. The other modifiers produced similar separations, although, again, resolution was not as high as with the ACN buffers (Figure 1). Less variation among the different solvents was noted, however, compared to a similar study performed using sodium phosphate buffers at pH 2.5 (Lookhart and Bean, 1996).

Concentrations of IDA from 25 to 100 mM were tested next. Resolution was judged to be optimum at 50 mM (data not shown). It should be noted that changes in concentration of isoelectric buffers result in slight changes in pH (Righetti et al., 1997), which may account for minor selectivity changes (Righetti et al., 1997). This is somewhat different from traditional buffers with a constant pH.

Separation voltage (from 24 to 30 kV) and separation temperature (from 25 to 45 °C) were also optimized. Optimum conditions were judged to be 45 °C and 30 kV (data not shown). One factor that was found to be critical was the rise time of the detector. Detector rise time is simply "the time required for the output to increase from 10 to 90% of its final value" (Lucy et al., 1998). Due to the high speed of these separations, the rise time of the detector had to be reduced from 1 s to 0.1 s. Detector settings can influence the separation efficiency and affect peak broadening (Lucy et al., 1998). Decreasing the detector rise time has been found to be beneficial in extremely fast separations, improving peak efficiency and resolution (Lucy et al., 1998). Resolution and separation efficiency of wheat gliadins were substantially improved when the detector rise time was decreased from 1 s to 0.1 s (Figure 2).

Due to the low currents generated by the IDA/ACN buffer system, 50 μm i.d. capillaries produced the same separation as 25 μm i.d. capillaries (data not shown), a major benefit of these type of buffers (Capelli et al., 1998). Thus, 50 μm i.d. capillaries were utilized for the remainder of this project.

Next, separations in the optimized IDA/ACN buffer system were compared to previous separations using phosphate−glycine (phos−gly)/ACN buffers (Bean and Lookhart, 1998) under optimum conditions (Figure 3). The IDA buffer produced a resolution similar to that of the phosphate−glycine buffer system, but separation times were much more rapid (Figure 3A), with the main body of gliadins separated in <3 min (Figure 3B). For the main body of gliadins (α/β and γ) there was a slight decrease in resolution, although roughly the same numbers of peaks were resolved (Figure 3B). Resolution of the ω-gliadins, however, was much improved in the IDA/ACN buffers (Figure 3C). Note that these two buffers are not at exactly the same pH, which could cause small selectivity differences. However, the overall patterns were quite similar and no large change in the subclass composition or migration order was visible from the separation in the phos−gly buffer. Thus, the IDA/ACN system could be used to rapidly quantify the gliadin subclasses.

Note that the separation time in the IDA/ACN buffer was faster than that reported when using aspartic acid/urea, in which the main body of gliadins was separated between 5 and 7 min (Capelli et al., 1998).

Repeatability. To test the run-to-run repeatability of the IDA/ACN buffer system, 35 consecutive injections

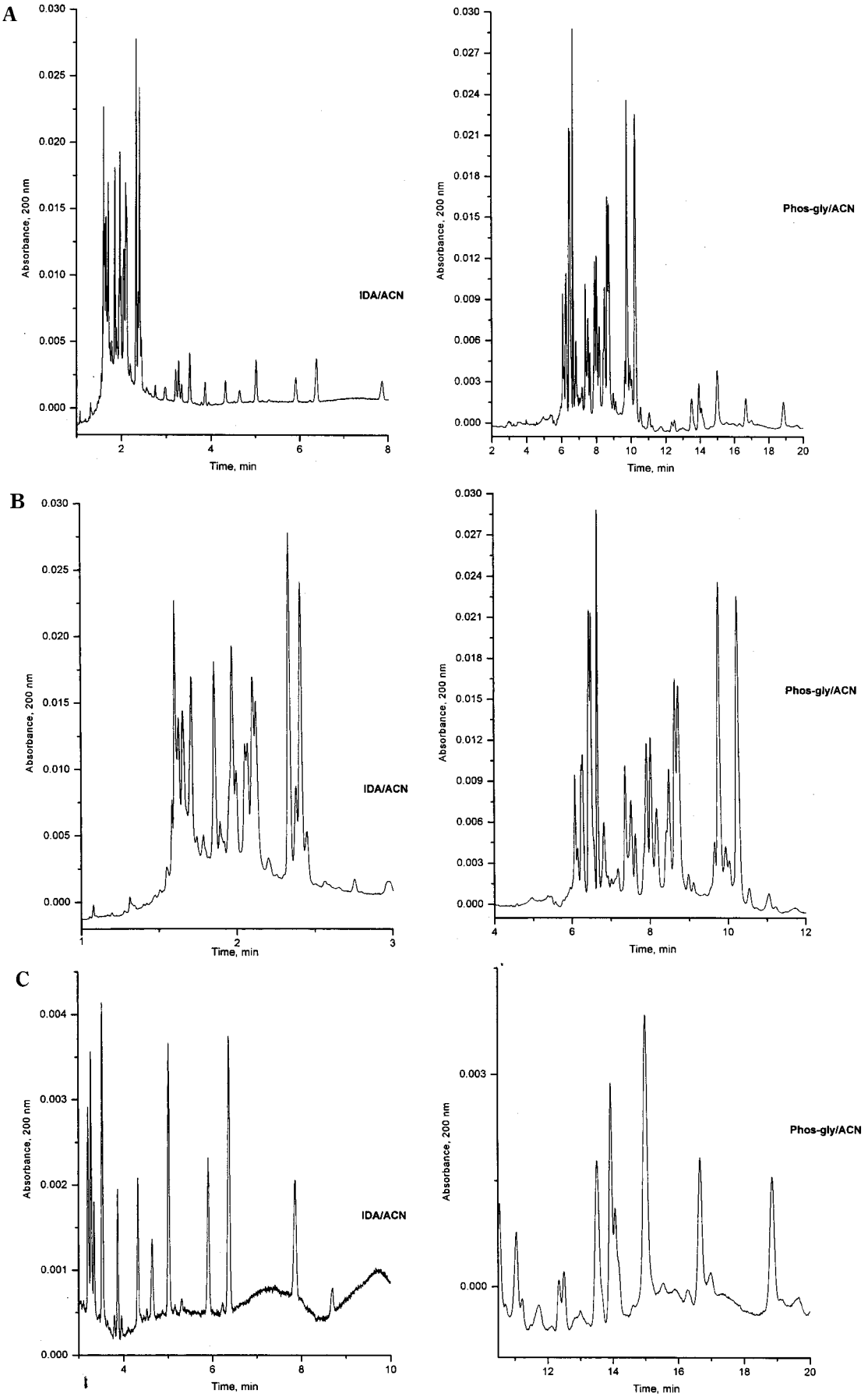

Figure 3. Comparison of IDA/ACN and phosphate−glycine/ACN buffers: (A) total electropherograms; (B) closeup of the main body of gliadins; (C) closeup of the late migrating ω-gliadins showing improved resolution in IDA/ACN buffers. IDA/ACN buffer was 50 mM IDA + 20% ACN and 0.05% HPMC; separation conditions were 30 kV and 45 °C. Phos−gly/ACN buffer was 100 mM phosphate−glycine + 20% ACN and 0.05% HPMC; separation conditions were 12.5 kV and 45 °C. Both buffers were used in 50 μm i.d. capillaries.

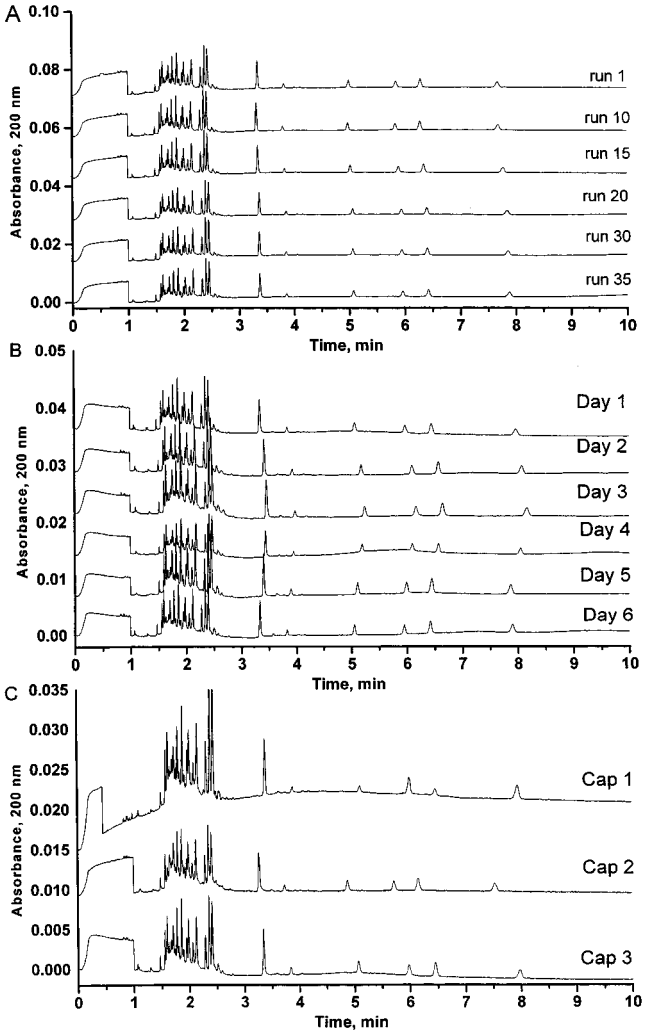

Figure 4. Repeatability of separations in IDA/ACN buffer: (A) run-to-run repeatability was tested over 30 consecutive injections; (B) day-to-day repeatability was measured over 6 consecutive days; (C) capillary-to-capillary repeatability was measured over three different capillaries. Separation buffer was 50 mM IDA + 20% ACN and 0.05% HPMC. Separation conditions consisted of using a 50 μm i.d. × 27 cm (20 cm L_d) uncoated capillary at 30 kV and 45 °C.

were made. The resulting reproducibility was excellent (Figure 4A). Peak migration times varied by <1% RSD. To further test the repeatability, separations were performed on six consecutive days. Again, the resulting repeatability was excellent (Figure 4B), with peak migration times varying by ~1% RSD. As a final check on repeatability, separations were performed on three different capillaries. Capillary-to-capillary variability was higher than day-to-day or run-to-run repeatability but still good (Figure 4C), with migration times varying <3.3% RSD. Previous work comparing results from two different laboratories also found that separation times varied slightly from capillary to capillary (Bietz and Lookhart, 1997).

Note that in this study all samples had their albumin and globulin proteins removed by pre-extraction (Bean and Lookhart, 1998). We have found that the IDA buffer does not tolerate the presence of the albumin and globulin proteins (or perhaps some other water or salt soluble compound present in flour), even if acid rinses are incorporated between runs. For the highest repeatability, pre-extraction of the samples with a water and salt combination is necessary. Note also that the capillary rinsing procedures used in this study consisted of only rinsing the capillary with separation buffer. This type of rinsing protocol is simple and rapid and does not disturb the capillary walls (Busch et al., 1995; Bean and Lookhart, 1998), resulting in excellent repeatability. Note also that with the larger diameter capillaries (i.e., 50 μm i.d.) shorter rinse times (0.5 versus 2 min for 25 μm i.d capillaries) could be used, resulting in very rapid total separation times (~8 min).

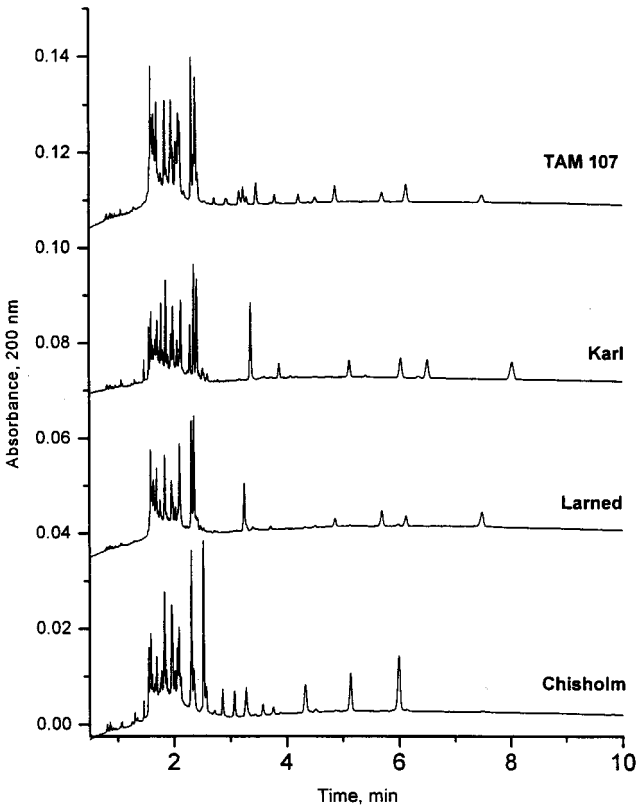

Figure 5. Separation of gliadins from hard red winter (HRW) wheats, TAM 107, Karl, Larned, and Chisholm. Separation conditions were as in Figure 4.

Wheat Protein Fractions. To test the IDA/ACN buffer system for wheat protein characterization, gliadins, glutenins, and isolated high molecular mass glutenin subunits (HMM-GS) from four hard red winter (HRW) wheats were separated and compared. Gliadins showed excellent separations with high resolution and rapid separation times (Figure 5). Resolution was excellent among the four samples, and all were easily differentiated from one another. Note the resolution of the ω-gliadins in cultivars Karl and Chisholm. Separations were extremely rapid, with the last proteins appearing at ~8 min. Combined with a short rinse time, 0.5 min, this results in a total separation time of only 8.5 min. This in turn would allow extremely high throughput, with ~160 samples in a 24 h period.

Glutenins of these cultivars also showed high resolution, with separations being complete in ~3 min (Figure 6A). Because these separations would have a total run time of only 3.5 min, this would allow ~400 samples to be analyzed in a 24 h period.

Previous separations of glutenins from wheat showed the highest resolution when the zwitterionic detergent lauryl sulfobetaine (SB 3-12) was added to the separation buffer (Lookhart and Bean, 1996) at approximately its critical micelle concentration, 26 mM (Greve et al., 1994). However, when SB 3-12 was added to the IDA/

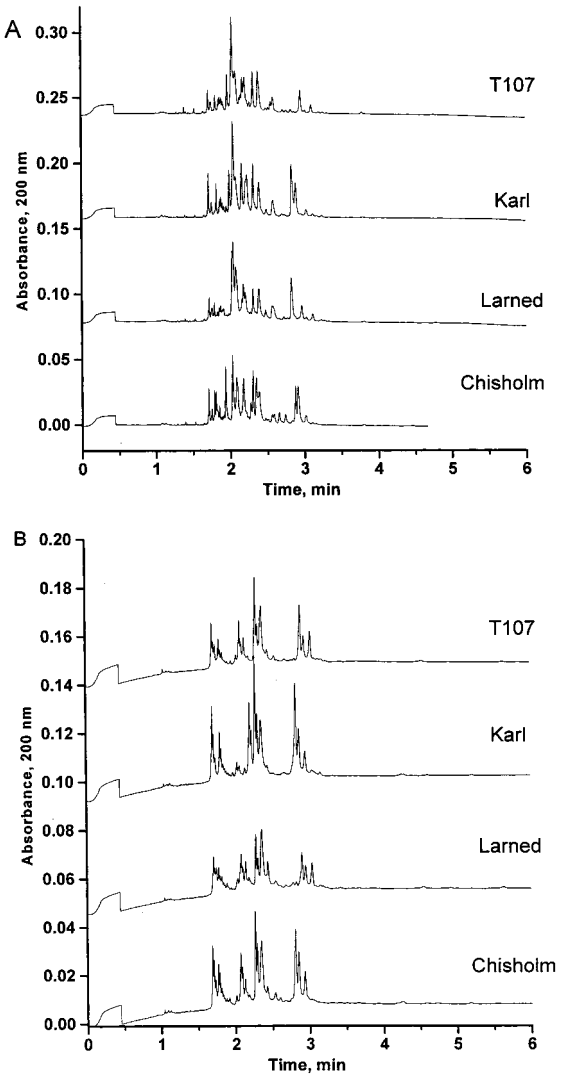

Figure 6. Separation of (A) glutenins and (B) high molecular mass glutenin subunits (HMM-GS) from HRW wheat cultivars TAM 107, Karl, Larned, and Chisholm. Separation conditions were as in Figure 4.

ACN buffer, resolution of glutenins was slightly decreased (data not shown). The exact reason for this is unknown but could have been a result of different pH values between the buffers.

As with previous buffer systems, HMM-GS were separated into multiple peaks (Figure 6B). From comparisons with previous separations of HMM-GS by FZCE (Lookhart and Bean, 1996; Bean and Lookhart, 1998) it appeared that the migration order of the HMM-GS is the same in the IDA buffers as it was in the previous separations. Note also that the migration time of the HMM-GS spanned the same range as total glutenins, meaning that the mobilities of the low molecular mass glutenin subunits (LMM-GS) and HMM-GS overlap, as in previous buffer systems (Bean and Lookhart, 1997).

Wheat Cultivar Differentiation. To test the ability of the IDA/ACN buffer system to successfully differentiate wheat cultivars, gliadins from five different classes of U.S. wheat were separated and compared. The four cultivars from the HRW class were easily differentiated (Figure 5). Cultivars from the hard white wheat (HWW) class were also easily differentiated (Figure 7) as were cultivars from the hard red spring (HRS) class of wheat (Figure 8). Again note the prominent ω-gliadin peaks, especially for the HWW cultivars Rio Blanco and Heyne (Figure 7).

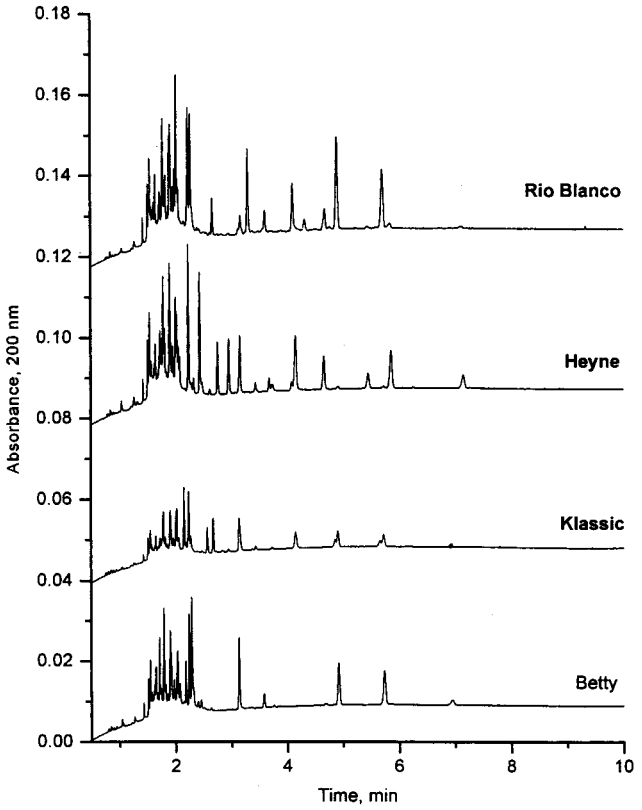

Figure 7. Separation of gliadins from hard white winter (HWW) wheats Rio Blanco, Heyne, Klassic, and Betty. Separation conditions were as in Figure 4.

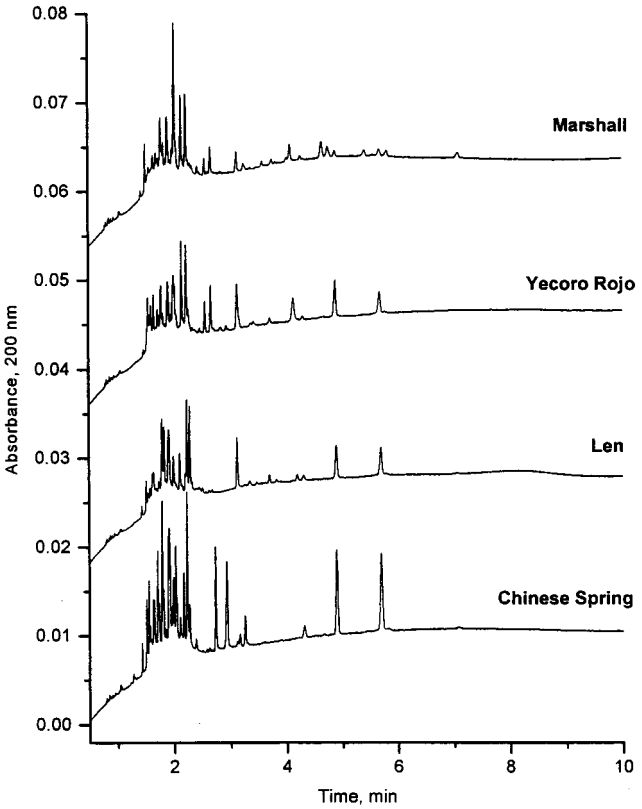

Figure 8. Separation of gliadins from hard red spring (HRS) wheats Marshall, Yecoro Rojo, Len, and Chinese Spring. Separation conditions were as in Figure 4.

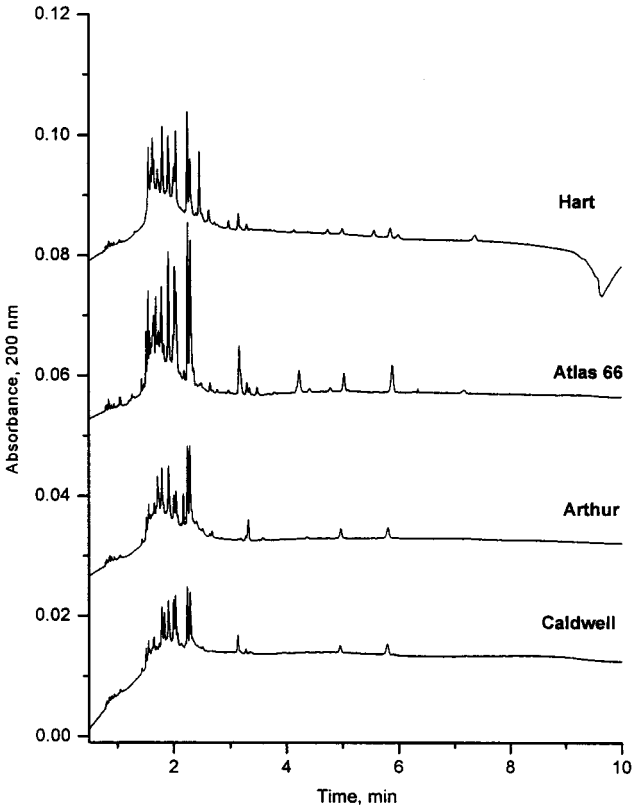

Figure 9. Separation of gliadins from soft red winter (SRW) wheats Atlas 66, Hart, Arthur, and Caldwell. Separation conditions were as in Figure 4.

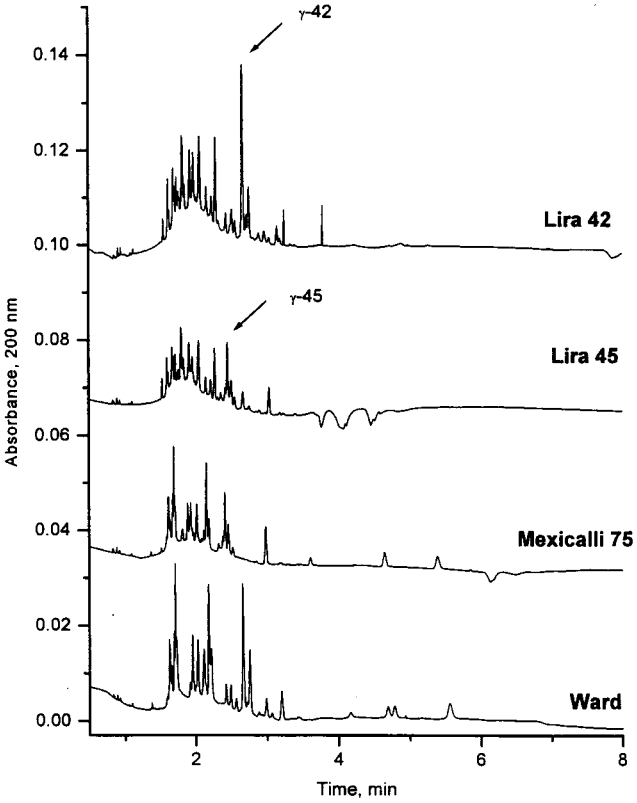

Figure 10. Separation of gliadins from durum wheats Mexicalli 75, Ward, Lira 42, and Lira 45. Arrows mark the position of γ-gliadins 42 and 45. Separation conditions were as in Figure 4.

All cultivars within each class of hard wheats were easily differentiated as were all cultivars between these classes (compare Figures 5, 7, and 8). Note that no proteins appeared later than ~8 min; therefore, wheat gliadins could be separated with high resolution in only 8.5 min. Note also that for the samples tested, all cultivars could be differentiated using only the main body of gliadins, with separation times of only 3−4 min, allowing high throughput (~360 samples/24 h).

In addition to hard wheats, gliadins from two other wheat classes, soft red winter (SRW) wheats (Figure 9) and durum wheats (Figure 10), were separated. As with the hard wheats, gliadins from these classes of wheats showed excellent resolution, and cultivars were easily differentiated from one another, again using only the main body of gliadins, requiring a separation time of only 3−4 min.

Two biotypes of the durum wheat cultivar Lira, Lira 42 and Lira 45, were separated. These biotypes contain two variants of γ-gliadins; either γ-42 or γ-45 (Masci et al., 1995). These two forms of γ-gliadins are known to be linked to durum wheat quality, with γ-42 related to higher quality (Damidaux et al., 1978; Payne et al., 1984). The two forms of γ-gliadins were easily differentiated in the Lira biotypes, with γ-45 migrating with a higher mobility than γ-42 (Figure 10). This is the same migration order as found in acid (A) PAGE separations of durum wheats (Pogna et al., 1990). Corresponding forms of these γ-gliadins were readily visible in the durum cultivars Mexicalli 75 and Ward (Figure 10). Separation of 13 additional durum cultivars also showed that these two forms of γ-gliadins were easily identified in the electropherograms (data not shown). Thus, the IDA/ACN buffer system provides a rapid method for screening durum wheat cultivars for quality, allowing extremely high throughput, unattended analysis.

Oat Proteins. In addition to wheat storage proteins, prolamins and glutelins from oats were separated in the IDA/ACN buffer. Oat prolamins showed very high resolution, and separations were completed in ~2 min, requiring a total separation time of only 2.5 min (Figure 11A), faster than previous reports with equal or higher resolution (Lookhart and Bean, 1995b, 1996). The cultivars were easily distinguished from one another. As with wheat glutenins, addition of 26 mM SB 3-12 to the separation buffer was found to decrease the resolution of the oat prolamin separations, unlike previous buffers (Lookhart and Bean, 1996).

Oat glutelins were also well separated in this buffer system (Figure 11B). The mobility of the oat glutelins was slightly slower than that of prolamins, with separations complete in roughly 2.75 min. As with the prolamins, the addition of SB 3-12 to the separation buffer did not improve oat glutelin separations (data not shown). The oat glutelin patterns were substantially different from the prolamin patterns, but, again, all oat cultivars were also successfully differentiated by the oat glutelin patterns.

Rice Proteins. Rice storage proteins, both prolamins and glutelins, were separated in the IDA/ACN buffer. However, unlike all of the previous cereals, rice prolamins and glutelins did show improved separations when 26 mM SB 3-12 was added to the separation buffer (data not shown), just as with previous buffer systems (Lookhart and Bean, 1996).

For prolamins, only a few peaks were noticed for most cultivars (Figure 12A), and several cultivars were not differentiated on the basis of their prolamin patterns

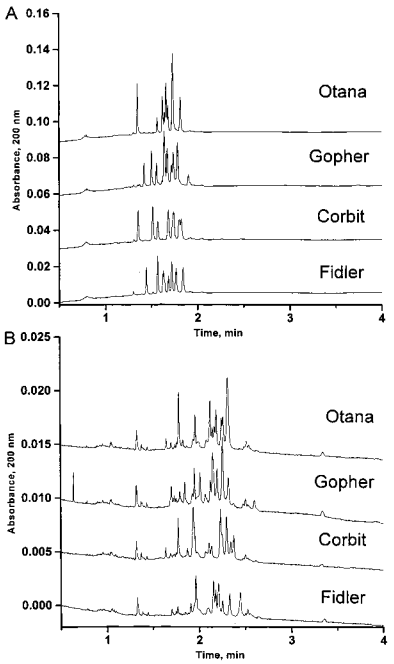

Figure 11. Storage protein separations from oat cultivars Otana, Gopher, Corbit, and Fidler: (A) prolamines; (B) glutelins. Separation conditions were as in Figure 4.

(data not shown). Rice is known to have very low levels of prolamins, however (Lookhart, 1991). Separations were completed much more quickly than previously reported FZCE separations (Lookhart and Bean, 1996), with no proteins visible after 2.5 min.

Rice glutelins tended to show more peaks (Figure 12B) than did prolamins, and in one case this allowed differentiation of cultivars L204 and Bengal that could not be differentiated on the basis of their prolamin patterns. This was not the case in all samples not distinguished by their prolamin patterns.

Barley Proteins. Barley hordeins were separated with high resolution in the IDA/ACN buffer system, and separations were complete in only 5 min, for a total separation time of only 5.5 min (Figure 13). Again cultivars were successfully differentiated, and the subclasses of hordeins previously determined in FZCE separations were easily identified (Lookhart et al., 1999). Separations were three times faster in this buffer system than previous separations and were complete in just under 5 min (Lookhart et al., 1999). Resolution was equal to that of previously used buffer systems (Lookhart et al., 1999). This method would allow rapid quantitation of hordein subclasses and identification of good malting quality barley cultivars.

Rye Proteins. Rye storage proteins were also separated in the IDA/ACN buffer; however, poor resolution of the prolamins was found (data not shown). In this study, albumins and globulins were pre-extracted with salt and water solutions. The prolamin storage proteins of rye, secalins, are known to be more water soluble than those of wheat (Dhaliwal et al., 1988); thus, some may have been removed during the pre-extraction. However, when a direct extraction of rye prolamins with 50% 1-propanol was attempted, resolution was still poor (data not shown). Rye glutelins extracted from a commercial rye flour, however, were well separated (Figure 14). More work with rye storage proteins is needed to develop successful prolamin separations.

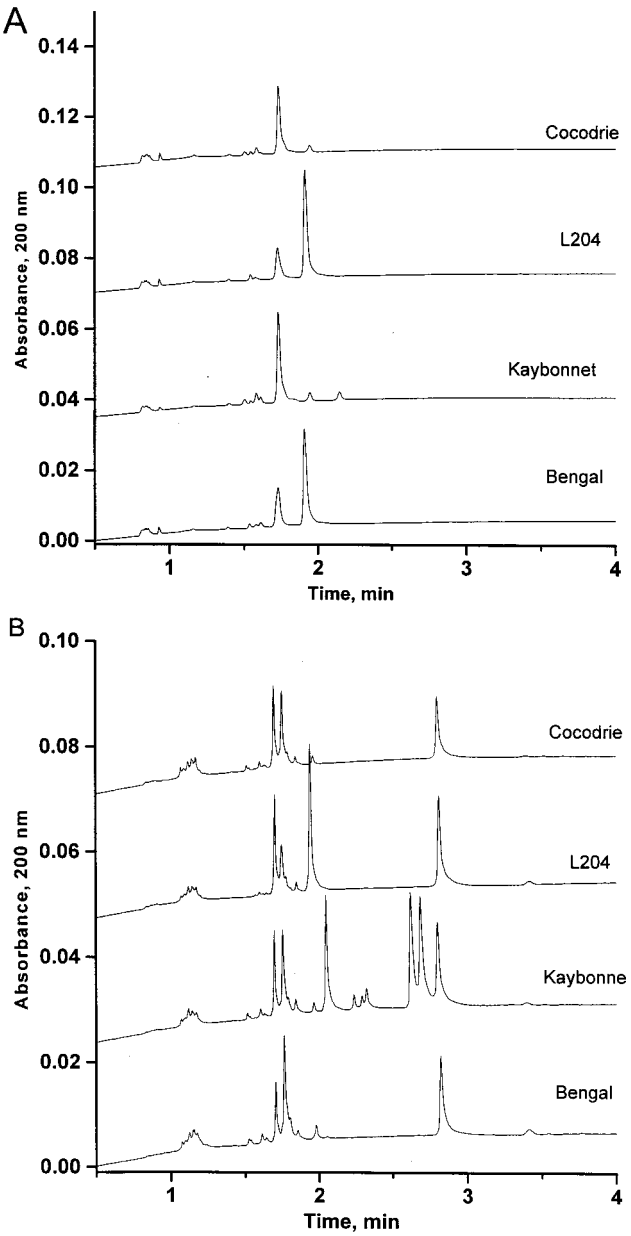

Figure 12. Storage protein separations of rice cultivars Cocodrie, L204, Kaybonnet, and Bengal: (A) prolamines; (B) glutelins. Separation conditions were as in Figure 4, except 26 mM SB 3-12 was added to the separation buffer.

Conclusions. Storage proteins of wheat, oats, rice, and barley were separated with extremely high resolution, most in <3 min, using a buffer based on the isoelectric IDA and ACN system. The new buffer system provided higher resolution and faster separations than other existing FZCE methods and avoided the use of urea as an additive. The new buffer system could be used in large-diameter capillaries (50 μm i.d.) with excellent run-to-run repeatability and reduced capillary rinse times. The new method, combined with simple, reliable capillary rinsing protocols, provided good day-to-day repeatability and capillary-to-capillary repeatability. Larger capillaries provide good sensitivity and are less prone to plugging.

FZCE is thus capable of providing extremely rapid separations with extremely high resolution without the cost of RP-HPLC columns and the solvent waste of HPLC. When combined with simple, reliable capillary equilibration procedures (Bean and Lookhart, 1998),

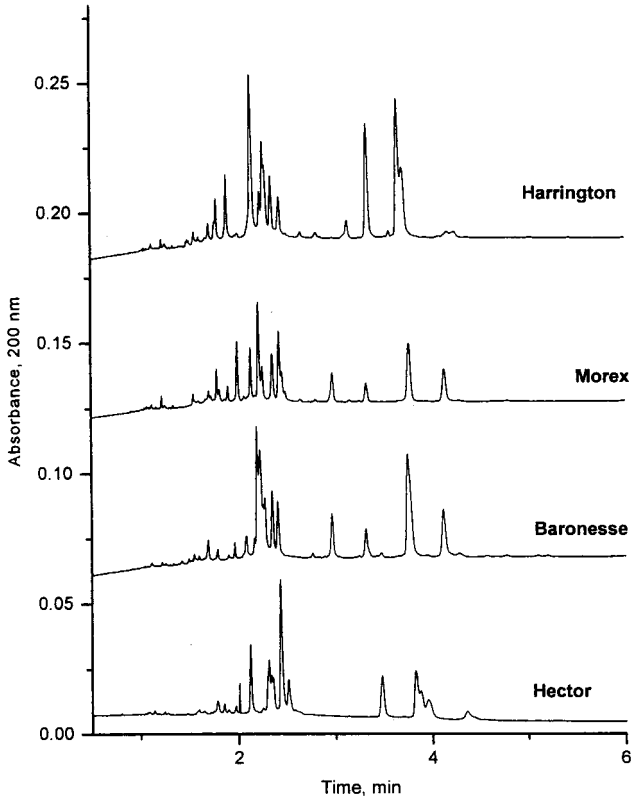

Figure 13. Storage protein separations of barley cultivars Harrington, Morex, Baronesse, and Hector. Separation conditions were as in Figure 4.

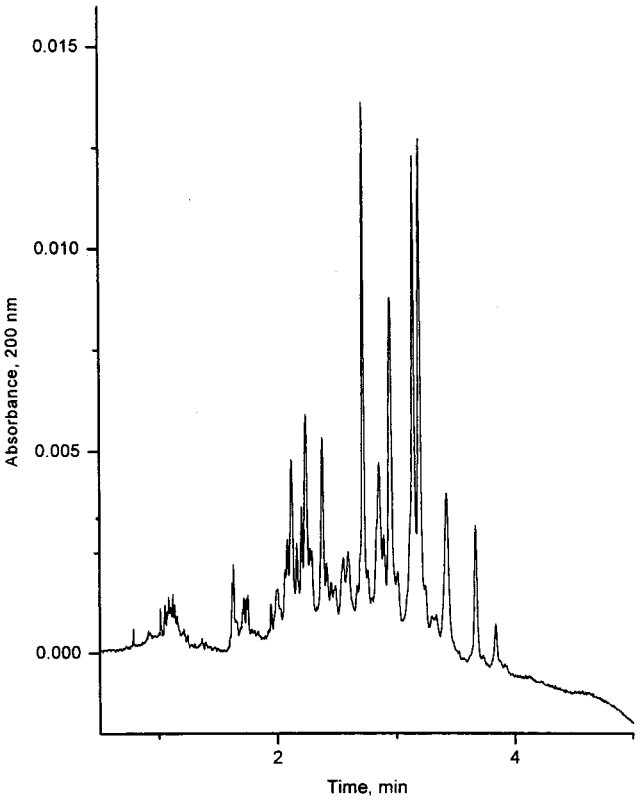

Figure 14. Example of separation of rye glutelins extracted from a commercial rye flour. Separation conditions were as in Figure 4.

this new buffer system is capable of high throughput and able to differentiate most cereal cultivars in 3−5 min. Because FZCE can be operated unattended, the IDA/ACN buffer system would allow between 180 and 480 samples to be analyzed in a 24 h period depending on the proteins being separated. This method would also allow rapid quantification of protein subclasses. The method was repeatable and rugged, demonstrating that FZCE is a valuable tool for rapid analysis of cereal proteins.

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