

Separation and Quantification of HMW Glutenin Subunits by Capillary Electrophoresis¹

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ABSTRACT

Cereal Chem. 78(6):737–742

The use of capillary electrophoresis in SDS (SDS-CE) for separation and quantification of HMW glutenin subunits (HMW-GS) was investigated. HMW-GS were precipitated with 40% acetone from 50% 1-propanol extract of flour under reducing conditions after removal of monomeric proteins with 50% 1-propanol. Poly (ethylene oxide) was used in the running buffer (3% w/v) for SDS-CE. The results indicated that HMW-GS could be well separated by SDS-CE, including subunits 7+8, 7+9, 2+12, 5+10, and 17+18. However, HMW-GS showed delayed migration times compared with molecular weight protein standards. Some HMW-GS were reversed in their mobilities in SDS-CE compared

with their mobility and molecular weights by SDS-PAGE. Therefore, the SDS-CE was unsuitable for MW determination of HMW-GS. A linear response was obtained from SDS-CE of a plot of the concentration of HMW-GS of the 40% acetone precipitate versus corrected areas for absorbance at 214 nm. Quantification of HMW-GS for the two biotypes (subunits 5+10 vs. 2+12) of an Australian wheat cultivar Warigal confirmed the differences between the two biotypes in their quantity of HMW-GS. Therefore, the technique could be used to quantify HMW-GS in conjunction with SDS-PAGE.

It has been established that HMW glutenin subunits (HMW-GS) were correlated in both composition and quantity with breadmaking quality (Payne et al 1979; Moonen et al 1982). In addition, the amount and size distribution of glutenin proteins significantly influenced breadmaking quality (Singh et al 1990; Huang and Khan 1997; Gupta et al 1993). The type of HMW-GS also was associated with the size distribution of glutenin proteins. For example, studies showed that the *Glu-D1* HMW-GS 5+10 could result in a higher molecular size distribution of glutenin in a cultivar than the 2+12 subunits (Gupta and MacRitchie 1994; Zhu and Khan 1999). SDS-PAGE has been used extensively for the identification and quantification of HMW-GS (Lawrence and Shepherd 1980; Payne et al 1981; Kolster et al 1992; Shewry et al 1992). However, quantification of HMW-GS by densitometry of SDS-PAGE gels has a few drawbacks such as poor reproducibility and accuracy. RP-HPLC was also employed to quantify HMW-GS in relation to breadmaking quality (Marchylo et al 1992; Kolster et al 1992). Recently, high-performance capillary electrophoresis (HPCE) has been introduced to analyze wheat proteins. Free-zone capillary electrophoresis (FZCE) was successfully used in separation of alcohol-soluble monomeric proteins, including albumins, globulins, and gliadins (Werner et al 1994; Bietz and Schmalzried 1995; Lookhart and Bean 1995a,b, 1996; Bean and Lookhart 1997, 1998, 2000; Lookhart et al 1999; Bean et al 2000). A size-based separation of SDS-proteins, which is analogous to SDS-PAGE, was developed for capillary electrophoresis (Ganzler et al 1992). SDS capillary electrophoresis (SDS-CE) also was used for separation of wheat glutenins (Ganzler et al 1992; Weegels et al 1995; Sutton and Bietz 1997; Bean and Lookhart 1999). The ProSort SDS-protein analysis kit (Applied Biosystems Division of Perkin-Elmer), which has a replaceable and entangled polymer solution as a matrix for separating SDS-proteins, was used with the SDS-CE to separate HMW-GS (Weegels et al 1995; Sutton and Bietz 1997). Besides this commercial polymer, other polymers such as Bio-Rad commercial polymer, dextran, poly (acrylamide), and poly (ethylene oxide) were studied to separate SDS glutenin proteins (Bean and Lookhart 1999). Results showed that all these polymers could provide good separations of wheat glutenins once the conditions were optimized. However, use of SDS-CE in separating wheat

glutenin proteins is still very limited. Capillary electrophoresis (CE) has the advantages of rapid separation and more accurate quantification. The potential of quantification by CE will be significant for study of glutenin proteins in relation to their functional properties. Therefore, the objective of this study was to optimize the SDS-CE method for identifying and quantifying HMW-GS.

MATERIALS AND METHODS

Wheat Samples

Wheat flour samples of three biotypes of Warigal and two other cultivars, Schom (HMW-GS 7+8, 5+10) and Lance (HMW-GS 17+18, 5+10), were obtained from Plant Breeding Institute, University of Sydney, Narrabri, Australia. The three biotypes of Warigal contained HMW-GS composition of 1) 7+8, 2+12; 2) 7+8, 5+10; and 3) 7+9, 2+12. All the HMW-GS were numbered according to the nomenclature of Payne and Lawrence (1983). The range of subunits would enable us to see the effectiveness of SDS-CE method employed in this study in separating various HMW-GS.

Extraction of HMW-GS

Flour (150 mg) was mixed with 1 mL of 50% 1-propanol and shaken for 30 min at room temperature. Samples were then centrifuged at $8,160 \times g$ for 5 min with an Eppendorf 5415C centrifuge. The supernatant was discarded and the pellet was extracted a second time to remove monomeric proteins. Glutenin proteins were extracted from the pellet by mixing with 1 mL of solvent of 50% (v/v) 1-propanol plus 1% (w/v) dithiothreitol (DTT) and shaken for 1 hr at 60°C. Samples were then centrifuged at $13,000 \times g$ for 5 min. The supernatant was collected and the pellet was then extracted one more time with the same solvent under the same extraction conditions. The two supernatants were combined and used for subsequent analysis. Acetone precipitation was used for the preparation of HMW-GS according to Melas et al (1994). A 400 μ L volume of pure acetone was added to 600 μ L of supernatant to give a final concentration of 40% (v/v) acetone. After a 10-min rest at room temperature, the solution was centrifuged at $13,000 \times g$ for 5 min. The acetone precipitate contained mainly HMW-GS. The precipitated HMW-GS were redissolved in 500 μ L solution of 1% (w/v) SDS and 1% (w/v) DTT vortexing for 30 min at 60°C. The mixture was heated in boiling water for 10 min before CE. Samples of precipitated HMW-GS were mixed in ratios of 100:0, 25:75, 50:50, 75:25, and 0:100 (v/v) from the two biotypes or the two other cultivars, which differed only in one pair of subunits, to see the relative elution times for the different subunits.

¹ Published with the approval of the Director, Agricultural Experimental Station, North Dakota State University, Fargo, ND 58105.

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Capillary Electrophoresis

Capillary electrophoresis of SDS-protein complexes was performed using a Beckman P/ACE system 5000 with an uncoated capillary (Poly Micro Technologies, Phoenix, AZ) with a 50 μm i.d. and 27 cm length (20 cm to detector). For the first use in a day, the capillary was flushed with 1M HCl for 15 min followed by a 15-min rinse by run buffer. The capillary was then rinsed with 1M HCl for 5 min and the run buffer for 5 min in each run before separation. The running buffer contained 100 mM Tris-CHES, pH 8.6, 0.1% SDS, and 3% (w/v) poly (ethylene oxide) (PEO) (MW 100,000) according to Ganzlar et al (1992) and Bean and Lookhart (1999). In this study, PEO was chosen because of good UV transparency and relatively low viscosity (Ganzlar et al 1992). Tris-CHES buffer was used with 0.1% SDS because this buffer has low conductivity and low UV absorbance (Ganzlar et al 1992). Samples were introduced into the capillary by pressure at 0.5 psi for 30 sec. The separation was conducted at 10 kV and 25°C for 30 min with detection of proteins by UV absorption at 214 nm.

Quantification of HMW-GS by SDS-CE

To evaluate the linear response of separation by SDS-CE of the PEO polymer (not done before), the standard protein lysozyme (MW 14.4 kDa, Sigma) was chosen for this purpose. A very good linearity response was found for lysozyme by SDS-CE (Fig. 1). The correlation between the corrected quantification of SDS-CE and the concentration was 0.998 ($P < 0.01$). The corrected area was used instead of area alone in CE for quantification (Altria 1993). The reason for this is that, in CE, peaks are moving in the capillary at both electrophoretic velocity in the forward direction and electro-

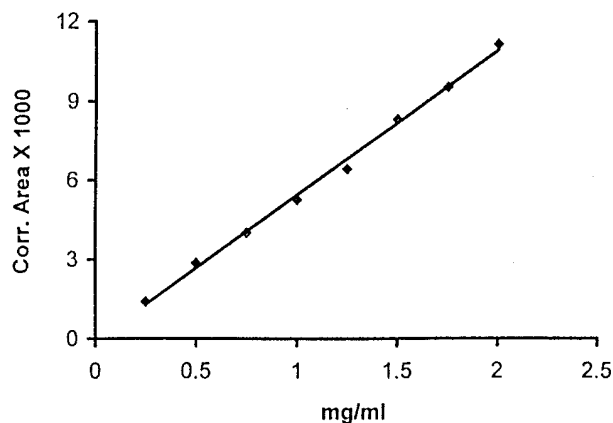


Fig. 1. Linear response for protein standard Lysozyme (MW 14,400) in SDS capillary electrophoresis.

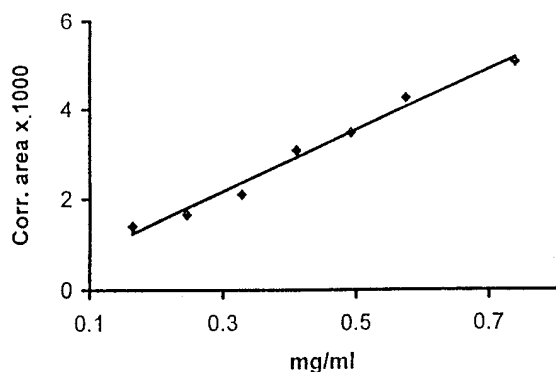


Fig. 2. Linear response of HMW glutenin subunit (GS) 7 from wheat cultivar Warigal in SDS capillary electrophoresis. HMW-GS 7+8 and 5+10 prepared in concentrations of 0.5, 0.75, 1.00, 1.25, 1.50, 1.75, and 2.25 mg/mL. Amount of HMW-GS 7 (mg/mL) given by relative percentage in total precipitate from SDS capillary electrophoresis elution profile.

osmotic flow in the opposite direction, resulting in the different residence time of each peak in the capillary (Altria 1993).

In addition, we studied the differences in migration time for HMW-GS by SDS-CE to compare linear response with molecular weight standards. The biotype of Warigal containing subunits 7+8 and 5+10 was used to prepare HMW-GS from 2.5 g of flour by 40% acetone precipitation (Melas et al 1994). The flour was mixed with 10 mL of 50% 1-propanol in 15-mL centrifuge tubes and shaken for 30 min to remove monomeric proteins. The sample was then centrifuged at 10,000 rpm with SS-34 rotor using RC-5 superspeed refrigerated centrifuge (Du Pont Instruments). The supernatant was discarded and the step was repeated once. The pellet was then extracted with 10 mL of 50% (v/v) 1-propanol plus 1% (w/v) dithiothreitol (DTT) and shaken for 1 hr at 60°C. Samples were then centri-

TABLE I
Reproducibility of Migration Times for Bio-Rad Protein Molecular Weight Standards Using 3% PEO Polymer^a

Proteins	CV (%)	No. Runs
Replacing running buffer after each run		
Lysozyme	0.41	9
Trypsin inhibitor	0.20	9
Carbonic anhydrase	0.42	9
Ovalbumin	0.24	9
Serum albumin	0.46	9
Phosphorylase B	0.30	9
β -Galactosidase	0.42	9
Myosin	0.28	9
Consecutive injections of running buffer		
Lysozyme	2.74	9
Trypsin inhibitor	2.63	9
Carbonic anhydrase	2.49	9
Ovalbumin	2.42	9
Serum albumin	2.21	9
Phosphorylase B	2.09	9
β -Galactosidase	2.08	9
Myosin	1.55	9

^a Running buffer: 100 mM Tris-CHES, pH 8.5, 0.1% SDS, 3% poly (ethylene oxide) (PEO); capillary: 50 m \times 27 (20) cm, uncoated; samples injected at 0.5 psi for 30 sec; detection at 214 nm; separation at 25°C and 10 kV.

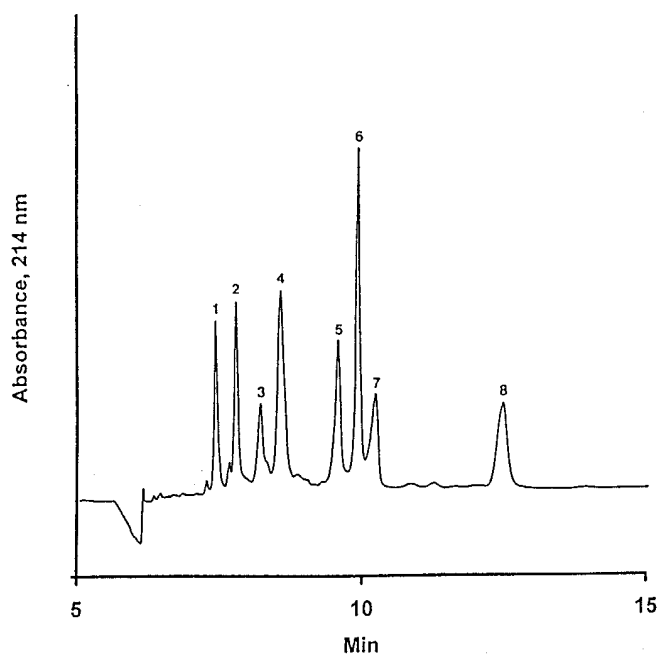


Fig. 3. Separation of molecular weight protein standards (Bio-Rad) by SDS capillary electrophoresis. Peaks: 1, Lysozyme; 2, Trypsin inhibitor; 3, Carbonic anhydrase; 4, Ovalbumin; 5, Serum albumin; 6, Phosphorylase B; 7, β -Galactosidase; 8, Myosin.

fused at 10,000 rpm for 15 min. The supernatant was collected and a second extraction was made with the remaining pellet. The supernatants were combined and precipitated with acetone for 10 min at room temperature as described above. The precipitate was obtained after centrifugation at 15,000 rpm for 15 min and freeze drying. Freeze-dried sample was used to obtain a standard curve by preparing solutions in 1% SDS + 1% DTT with different concentrations of the reduced HMW-GS. The concentrations (mg/mL) were 0.5, 0.75, 1.00, 1.25, 1.50, 1.75, and 2.25. The relative percentage of each HMW-GS was determined from the SDS-CE elution profile. From the average percentage of each HMW-GS, the concentration for each subunit in the solutions was calculated and used to set up the standard curve for the subunit from SDS-CE. Because it appears in the greatest quantity, HMW-GS 7 was representative for the standard curve for quantification of all HMW-GS. The average percentage for this subunit in the sample was 32.8% (CV = 0.84%). The response for HMW-GS 7 was linear (Fig. 2) with the correlation between the corrected area and concentrations of the samples of 0.991 ($P < 0.01$). Quantification of HMW-GS would be possible according to the linear response of the subunit as shown by the

standard curve in the concentration range used in this study. Because the response of HMW-GS to UV detection in the SDS-CE was different from that of standard proteins in migration times, it was necessary to use glutenin subunits to set up a standard curve to quantify glutenin subunits by SDS-CE.

RESULTS AND DISCUSSION

Separation of Bio-Rad SDS-CE Protein Standards

The Bio-Rad protein standards have a molecular weight range of 14,400–200,000 (Bio-Rad, Hercules, CA), including lysozyme (14,400), trypsin inhibitor (21,500), carbonic anhydrase (31,000), ovalbumin (45,000), serum albumin (66,000), phosphorylase B (97,000), β -galactosidase (116,000), and myosin (200,000). The Bio-Rad protein size standards (10 μ L) was mixed with 190 μ L of a 1% SDS (w/v) + 1% (w/v) DTT solution. Also, each molecular weight standard was prepared with SDS-DTT solution at 1 mg/mL for SDS-CE. All the samples were heated for 10 min before SDS-CE. Because of the reverse mobility for some HMW-GS in SDS-CE, it would be necessary to see if the molecular weight standards

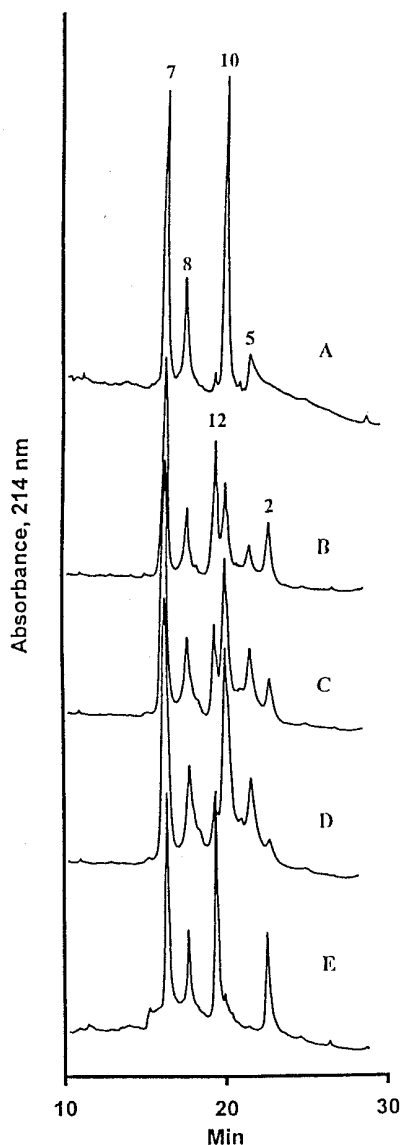


Fig. 4. Separation by SDS capillary electrophoresis of HMW glutenin subunits (GS) 5+10 vs. 2+12 from two biotypes of wheat cultivar Warigal. Mixture ratios (v/v): 100:0 (A), 25:75 (B), 50:50 (C), 75:25 (D), and 0:100 (E). HMW-GS designated A and B according to nomenclature of Payne and Lawrence (1983).

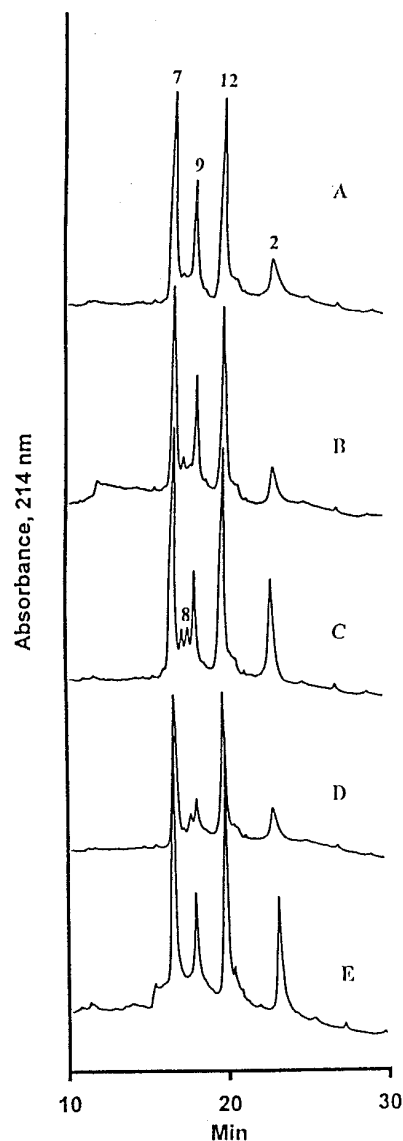


Fig. 5. Separation by SDS capillary electrophoresis of HMW glutenin subunits (GS) 7+8 vs. 7+9 from two biotypes of wheat cultivar Warigal. Mixture ratios (v/v): 100:0 (A), 25:75 (B), 50:50 (C), 75:25 (D), and 0:100 (E). HMW-GS designated A and B according to nomenclature of Payne and Lawrence (1983).

would separate according to molecular weights in SDS-CE. Samples for a single standard and the mixture of two standards with adjacent molecular weights were run on the SDS-CE. The mixture of two standards was prepared in ratios of 25:75, 50:50, and 75:25 (v/v). Therefore, each component could be identified with its relative migration time and peak height according to concentration. The relative migration times of the standards in the SDS-CE were compared and they were separated in the order of their molecular weights. Separation of the molecular weight standard proteins was achieved within 14 min (Fig. 3). The correlation coefficient between the log of molecular weights and migration times for the Bio-Rad standards was 0.973 ($P < 0.01$) when replacing running buffer after each run and 0.970 ($P < 0.01$) using consecutive injections of the running buffer. The reproducibility of migration times for Bio-Rad protein standards is shown in Table I. A higher reproducibility was achieved when replacing the running buffer after each run than with consecutive injections of the same running buffer.

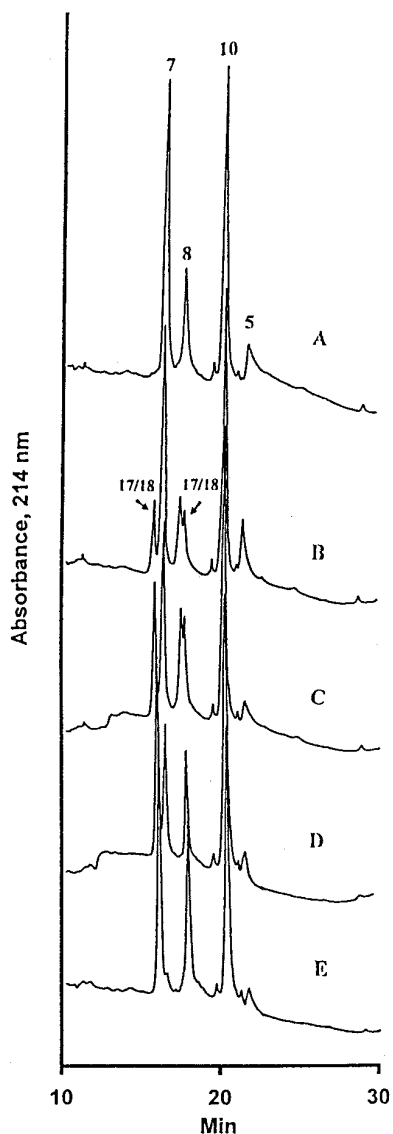


Fig. 6. Separation by SDS capillary electrophoresis of HMW glutenin subunits (GS) 7+8 vs. 17+18 from wheat cultivars Schom and Lance. Schom contains HMW-GS 7+8 and 5+10, while Lance contains HMW-GS 17+18 and 5+10. Mixture ratios of extract (v/v) from Schom 7 + 8 and Lance 17 + 18: 100:0 (A), 25:75 (B), 50:50 (C), 75:25 (D), and 0:100 (E). HMW-GS designated A and B according to nomenclature of Payne and Lawrence (1983). Peaks 17/18 cannot be distinguished.

Extraction and SDS-CE Separation of HMW-GS

The HMW-GS isolated by precipitation with 40% acetone were redissolved in 1% (w/v) aqueous SDS with 1% (w/v) DTT before CE. The dissolution was made at 60°C with constant vortexing for 60 min. The SDS-DTT solvent easily dissolved the precipitated glutenin subunits and generated a better separation compared with dissolution in 50% (v/v) 1-propanol plus 1% (w/v) SDS and 1% (w/v) DTT, or Tris-HCl buffer containing 0.06 mM Tris, pH6.6, 1% SDS (w/v), and 1% (w/v) DTT (results not shown). This indicated that sample matrix had a significant influence on the separation of proteins by SDS-CE. Sutton and Bietz (1997) also found that the use of aqueous SDS/DTT solvent allowed better dissolution of HMW-GS precipitated from 60% (v/v) 1-propanol, while excessive salt concentrations in the redissolving solution caused severe baseline disturbances in the CE elution profile when using the ProSort SDS-protein analysis kit.

The acetone precipitates containing HMW-GS were separated by SDS-CE. Figure 4 shows the separation of HMW-GS 5+10 and 2+12 from the two biotypes of Warigal. For each biotype, all the HMW-GS had a good resolution of separation. When the two biotypes were mixed, subunits 5+10 were well separated from subunits 2+12. The CE elution profiles from the different mixtures of the two biotypes were used to determine the differences in migration times for HMW-GS 5+10 versus 2+12. Within each pair, we could tell the x-type subunits (2 or 5) from the y-type subunits (12 or 10) in these elution profiles. The x- and y-types were distinguished

TABLE II
Proportion of *Glu-D1* HMW Glutenin Subunits (GS) 5+10 vs. 2+12 in Total HMW-GS and Quantity in Acetone Precipitate from Extract of 50% 1-Propanol Under Reduced Conditions After Removal of Monomeric Proteins by 50% 1-Propanol

Biotype of Warigal	Proportion of <i>Glu-D1</i> Subunits (%)	Protein Concentration of Acetone Precipitate (mg/mL)
HMW-GS 5+10	49.2a ^a	1.96a
HMW-GS 2+12	44.3b	0.34b

^a Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

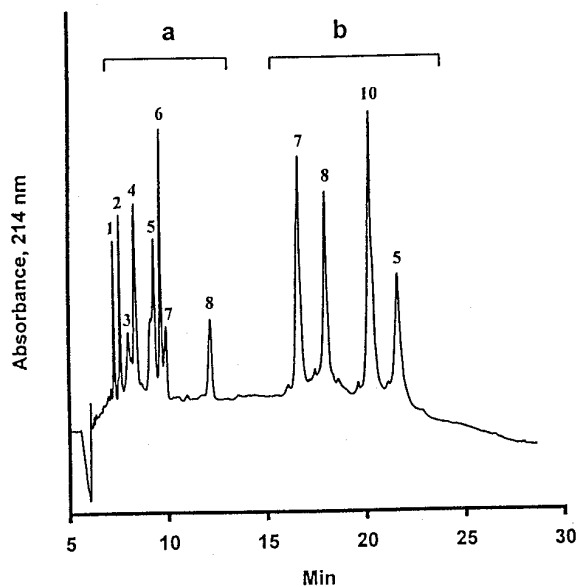


Fig. 7. Separation by SDS capillary electrophoresis of HMW glutenin subunits (GS) and Bio-Rad molecular weight protein standards. HMW-GS were 40% acetone precipitates from wheat cultivar Warigal containing HMW-GS 7+8 and 5+10. Peaks (a): 1, Lysozyme; 2, Trypsin inhibitor; 3, Carbonic anhydrase; 4, Ovalbumin; 5, Serum albumin; 6, Phosphorylase B; 7, β -Galactosidase; 8, Myosin. Peaks (b) HMW-GS named according to nomenclature of Payne and Lawrence (1983).

based on relative concentration, previously determined by densitometry of gels of SDS-PAGE in which y-type subunits 10 and 12 showed a significantly greater quantity than their respective x-type subunits 5 and 2 using the same extracts (results not shown). As a result, subunits 10 and 12 were eluted earlier than subunits 5 and 2 because the earlier eluted peaks constantly showed a significant and greater quantity than the later peaks. This is in accordance with the result of the study by Weegles et al (1995). The elution order of these HMW-GS also was consistent with the order of molecular weight by SDS-PAGE. In contrast, HMW-GS 7 eluted earlier than HMW-GS 8 and 9 in SDS-CE (Fig. 5). The migration order was reversed in SDS-CE from that in SDS-PAGE in which HMW-GS 7 migrated more slowly and gave a larger molecular weight than either HMW-GS 8 or 9. HMW-GS 8, could be well separated from HMW-GS 9 of subunits 7+9. Figure 6 shows that HMW-GS 17+18 (mixed with subunits 7+8) were resolved into two peaks even though we could not identify which peak was subunit 17 and which peak was subunit 18 at this point. However, HMW-GS 17 or 18 eluted earlier than subunits 7+8. The earlier elution of HMW-GS 17 or 18 indicated that the separation was not completely based on molecular sizes as in SDS-PAGE.

The reversed mobilities for some of the HMW-GS mentioned above indicated that the separation mechanism in the SDS-CE system for size separation of HMW-GS was different from the molecular weight sieving mechanism on SDS-PAGE for size separation of proteins. This difference between the two methods might be related to the response of the specific structural features of HMW-GS in a nonmatrix (CE) versus matrix (SDS-PAGE) system. This phenomenon also was noted in other studies (Werner et al 1994; Sutton and Bietz 1997; Bean and Lookhart 1999). Werner et al (1994) found that HMW-GS 1, which has a higher molecular weight by SDS-PAGE and would be expected to elute later in SDS-CE, instead eluted earlier in the capillary system than HMW-GS 5, which has a lower molecular weight by SDS-PAGE. In the present study, HMW-GS 7+8 or 7+9 were reversed in the SDS-CE separation system.

When HMW-GS were spiked with molecular weight standards, all the HMW-GS migrated much later than the standards (Fig. 7). This differed from Bean and Lookhart (1999) in that only the high end of HMW-GS was overestimated in their SDS-CE separations. According to Tatham et al (1990a,b), x-type HMW-GS have molecular weights of 83,000–88,000 and y-type subunits have molecular weights of 67,000–74,000 based on DNA sequence. The standard reference proteins, however, migrate in SDS-CE in the same order as molecular weight. The molecular weights by SDS-PAGE of HMW-GS fall below the standard phosphorylase B (97,000) and far behind myosin (200,000), but the HMW-GS migrated much later than those two standards on SDS-CE (Fig. 7). The anomalous mobility on SDS-CE indicates that the separation mechanism on SDS-CE is different for HMW-GS than for the standard proteins.

Comparison of SDS-CE with SDS-PAGE

Some HMW-GS reversed mobilities on SDS-CE, such as subunits 7+8 in the present study. However, the reason for the reversal is not clear. HMW-GS might be different from standard proteins in conformational structure and ability to bind SDS and, thus, the separation of HMW-GS by nongel sieving solution in SDS-CE may be somewhat different from the gel-sieving medium in SDS-PAGE. HMW-GS also had delayed migration times on SDS-CE compared with other molecular weight standards. Werner (1995) suggested that the anomalous migration might be related to the binding of SDS to HMW-GS, which binds SDS 15–20% less than standard proteins. The decreased binding efficiency for SDS might be due to the unusual amino acid sequences of these HMW glutenin proteins (Werner 1995). Thus, HMW-GS might have a lower mobility in the running buffer as shown in this study. Besides, it may be argued that the polymer used in this study binds SDS and may behave differently than other polymers, which do not bind SDS and remain uncharged (Takagi 1997). However, this binding to SDS seemingly did not affect the

separation of the protein standards used in the present study. The suggestion made by Werner (1995) that HMW-GS binds less SDS than standard proteins might be one explanation for the anomalous migration in SDS-CE. In addition, the type of polymer would have a significant effect on the mobility of HMW-GS (Bean and Lookhart 1999). Therefore, the SDS-CE is unsuitable for MW determination of HMW-GS.

SDS-CE for Quantification of HMW-GS 5+10 and 2+12.

In our previous study (Zhu et al 1999), the biotype with HMW-GS 5+10 had a greater amount of total protein concentration and HMW-GS 5 (vs. subunit 2) than the biotype with HMW-GS 2+12. We wanted to see if we could determine the differences by using the SDS-CE quantification method. The quantity and proportion of HMW-GS 5+10 versus 2+12 are given in Table II. There were significant differences between the two biotypes in proportion and amount of the *Glu-D1* subunits ($P < 0.05$). The biotype with 5+10 had a greater proportion and amount of *Glu-D1* subunits than the HMW-GS 2+12 in acetone precipitates of these proteins. This is in agreement with our previous study in which amounts of the two biotypes were compared with respective subunits by scanning densitometry of SDS-PAGE gels (Zhu et al 1999).

Therefore, the SDS-CE method used in the present study is another approach for separation and quantification of HMW-GS. HMW-GS showed a good separation including HMW-GS 7+8, 7+9, 2+12, 5+10, and 17+18. The relative quantity could be measured by SDS-CE with the advantage of digital control and computerization as compared with scanning densitometry of SDS-PAGE gels. However, it would be difficult to accurately identify the HMW-GS composition for an unknown wheat cultivar without a reference cultivar. This also was observed in previous studies (Werner et al 1994; Sutton and Bietz 1997; Bean and Lookhart 1999). Additionally, SDS-CE is unsuitable for MW determination of HMW-GS. Therefore, the CE method needs to be combined with the conventional SDS-PAGE to analyze HMW-GS composition.

CONCLUSIONS

In this study, SDS-CE was used to develop methodology to identify and quantify HMW-GS. HMW-GS could be well separated and quantified by SDS-CE. The precipitation procedure by 40% acetone after extraction by 50% 1-propanol under reduced conditions for glutenins proved to be good for CE separation of HMW-GS. HMW-GS in SDS-CE did not migrate by molecular weight as they do on SDS-PAGE. The SDS-CE is not suitable for MW determination of HMW-GS. Standard proteins tested, however, migrated on SDS-CE according to molecular weight. The delayed migration might be due to the specificity of glutenin protein structures and thus the binding of SDS to glutenin subunits was different in amount and type of polymer used. Conformational changes of HMW-GS might also occur in the capillary. Reversed mobility (molecular weight from SDS-PAGE) for some HMW-GS in SDS-CE also was observed. The reason for this is still unknown.

A method was developed to quantify HMW-GS using glutenin subunits to set up a standard curve instead of using globular protein standards as shown in Fig. 1. A very good linear response was achieved for the HMW-GS 7 by SDS-CE. This technique could permit rapid quantification of 40% acetone-precipitated HMW-GS. This method was used to confirm the differences between two biotypes, HMW-GS 5+10 and HMW-GS 2+12. SDS-CE could, therefore, be a useful tool for providing helpful information on the functionality of glutenin proteins in relation to end-use quality.

ACKNOWLEDGMENTS

The State Board of Agricultural Research and Education (SBARE) of North Dakota and the North Dakota Wheat Commission are thanked for financial support. We would like to thank Scott Bean and George Lookhart for their helpful discussions in SDS-CE analysis.

LITERATURE CITED

- Altria, K. D. 1993. Essential peak area normalization for quantitative impurity content determination by capillary electrophoresis. *Chromatographia* 35:177-182.
- Bean, S. R., and Lookhart, G. L. 1997. Separation of wheat proteins by two-dimensional reversed-phase high-performance liquid chromatography plus free zone capillary electrophoresis. *Cereal Chem.* 74:758-765.
- Bean, S. R., and Lookhart, G. L. 1998. Faster capillary electrophoresis separation of wheat proteins through modifications to buffer composition and sample handling. *Electrophoresis.* 19:3190-3198.
- Bean, S. R., and Lookhart, G. L. 1999. SDS-CE separations of wheat proteins. I. Uncoated capillaries. *J. Agric. Food Chem.* 47:4246-4255.
- Bean, S. R., and Lookhart, G. L. 2000. Ultrafast capillary electrophoresis analysis of cereal storage proteins and its applications to protein characterization and cultivar differentiation. *J. Agric. Food Chem.* 48:344-353.
- Bean, S. R., Bietz, J. A., and Lookhart, G. L. 2000. Electrophoresis of cereal storage proteins. *J. Chromatography A.* 881:23-36.
- Bietz, J. A., and Schmalzried, E. 1995. Capillary electrophoresis of wheat gliadin: Initial studies and application to varietal identification. *Lebensm. Wiss Technol.* 28:174-184.
- Ganzler, K., Greve, K. S., Cohen, A. S., and Karger, B. L. 1992. High-performance capillary electrophoresis of SDS-protein complexes using UV-transparent polymer networks. *Anal. Chem.* 64:2665-2671.
- Gupta, R. B., and MacRitchie, F. 1994. Allelic variation at glutenin subunit and gliadin loci, Glu-1, Glu-3 and Gli-1 of bread wheats: Biochemical basis of the allelic effects on dough properties. *J. Cereal Sci.* 19:19-28.
- Gupta, R. B., Khan, K., and MacRitchie, F. 1993. Biochemical basis of flour properties in bread wheats. I. Effects of variation in quantity and size distribution of polymeric protein. *J. Cereal Sci.* 17:23-41.
- Huang, D. Y., and Khan, K. 1997. Characterization and quantification of native glutenin aggregates by multistacking sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) procedure. *Cereal Chem.* 74:229-234.
- Kolster, P., Krecting, C. F., and van Gelder, M. J. 1992. Quantification of individual high molecular weight subunits of wheat glutenin using SDS-PAGE and scanning densitometry. *J. Cereal Sci.* 15:49-61.
- Lawrence, G. J., and Shepherd, K. W. 1980. Variation in glutenin protein subunits of wheat. *Aust. J. Bio. Sci.* 33:221-233.
- Lookhart, G. L., and Bean, S. R. 1995a. A fast method for wheat cultivar differentiation using capillary zone electrophoresis. *Cereal Chem.* 72:42-47.
- Lookhart, G. L., and Bean, S. R. 1995b. Rapid differentiation of oat cultivars and of rice cultivars by capillary zone electrophoresis. *Cereal Chem.* 72:312-316.
- Lookhart, G. L., and Bean, S. R. 1996. Improvements in cereal protein separations by capillary electrophoresis: resolution and reproducibility. *Cereal Chem.* 73:81-87.
- Lookhart, G. L., and Bean, S. R., and Jones, B. L. 1999. Separation and characterization of barley (*Hordeum vulgare* L.) hordeins by free zone capillary electrophoresis. *Electrophoresis* 20:1605-1612.
- Marchylo, B. A., Lukow, O. M., and Kruger, J. E. 1992. Quantitative variation in high molecular weight glutenin subunit 7 in some Canadian wheats. *J. Cereal Sci.* 15:29-37.
- Melas, V., Morel, M., Austran, J., and Feillet, P. 1994. Simple and rapid method for purifying low molecular weight subunits of glutenin from wheat. *Cereal Chem.* 71:234-237.
- Moonen, J. H. E., Sxcheepstra, A., and Graveland, A. 1982. Use of the SDS-sedimentation test and SDS-polyacrylamide gel electrophoresis for screening breeder's samples of wheat for bread-making quality. *Euphytica* 31:677-690.
- Payne, P. I., and Lawrence, G. J. 1983. Catalogue of alleles for the complex gene loci Glu-A1, Glu-B1 and Glu-D1 which code for high-molecular weight subunits of glutenin in hexaploid wheat. *Cereal Res. Commun.* 11:29-35.
- Payne, O. I., Corfield, K. G., and Blackman, J. A. 1979. Identification of a high-molecular-weight subunit of glutenin whose presence correlates with bread-making quality in wheats of related pedigree. *Theor. App. Gen.* 55:153-159.
- Payne, O. I., Corfield, K. G., Holt, L. M., and Blackman, J. A. 1981. Correlations between the inheritance of certain high-molecular-weight subunits of glutenin and bread-making quality in progenies of six crosses of bread wheat. *J. Sci. Food Agric.* 32:51-60.
- Shewry, P. R., Halford, N. G., and Tatham, A. S. 1992. High molecular weight subunits of wheat glutenin. *J. Cereal Sci.* 15:105-120.
- Singh, N. K., Donovan, R., and MacRitchie, F. 1990. Use of sonication and size-exclusion high performance liquid chromatography in the study of wheat flour proteins. I. Dissolution of total proteins in the absence of reducing agents. *Cereal Chem.* 67:150-161.
- Sutton, K., H., and Bietz, J. A. 1997. Variation among high molecular weight subunits of glutenin detected by capillary electrophoresis. *J. Cereal Sci.* 25:9-16.
- Tatham, A. S., Shewry, P. R., and Field, J. M. 1990a. Conformational studies of the repetitive domains of the cereal prolamins. Pages 376-388 in: *Proc. Int. Workshop on Gluten Proteins*, 4th. W. Bushuk and R. Tkachuk, eds. Am. Assoc. Cereal Chem.: St. Paul, MN.
- Tatham, A. S., Shewry, P. R., and Belton, P. S. 1990b. Structural studies of cereal prolamins, including wheat gluten. Pages 1-78 in: *Advances in Cereal Science and Technology*, Vol. 10. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.
- Weegels, P. L., Hamer, R. J., and Schofield, J. D. 1995. RP-HPLC and capillary electrophoresis of subunits from glutenin isolated by SDS and Osborne fractionation. *J. Cereal Sci.* 22:211-224.
- Werner, W. E. 1995. Ferguson plot analysis of high molecular weight glutenin subunits by capillary electrophoresis. *Cereal Chem.* 72:248-251.
- 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.* 71:397-402.
- Zhu, J., Khan, K., Huang, S., and O'Brien, L. 1999. Allelic variation at Glu-D1 loci for HMW glutenin subunits-quantification by multistacking SDS-PAGE of wheat grown under nitrogen fertilization. *Cereal Chem.* 76:915-919.

[Received March 5, 2001. Accepted July 30, 2001.]