# High throughput microchip-based separation and quantitation of high-molecular-weight glutenin subunits

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# High throughput microchip-based separation and quantitation of high-molecular-weight glutenin subunits

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*Keywords:* Wheat, Glutenin subunits, Microchip, Lab-on-a-Chip

*Abbreviations*: CE, capillary electrophoresis; DTT, dithiothreitol; HMW-GS, high-molecular-weight glutenin subunit; LMW-GS, low-molecular-weight glutenin subunit; RP-HPLC, reversed phase high performance liquid chromatography; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

## Abstract

Knowledge of glutenin subunit composition is important for the prediction of the genetic potential of breeding lines as these proteins are known to be responsible for the main differences in bread-making quality. In this study, a commercial high throughput microchip capillary electrophoresis-sodium dodecyl sulfate (microchip CE) platform, LabChip 90, was evaluated for qualitative and quantitative analysis of HMW-GS. 130 French common wheat varieties of known composition were analyzed for rapid identification and the allocation of individual HMW-GS. In addition, the HMW-GS were individually quantified and the ratio of HMW-GS to LMW-GS was determined for genotype comparison. The microchip CE analysis provide comparable resolution and sensitivity to conventional RP-HPLC for identification of the HMW-GS but at a time scale of approximately 100 times faster (45 sec per sample analysis versus 80 min for RP-HPLC). The results show high throughput microchip CE method can be used for routine identification and quantitation of glutenin subunits, in particular for screening wheat quality and wheat cultivar development activities where large numbers of samples are to be evaluated.

## 1. Introduction

Wheat endosperm proteins are divided into two main fractions, monomeric and polymeric proteins. The monomeric fraction includes gliadins, albumins, and globulins. Albumins and globulins are considered functional proteins, whereas gliadins are referred to as storage proteins. Polymeric fraction mainly consists of glutenin polymers composed of high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) associated together by intermolecular disulfide bonds.

The high molecular weight glutenin subunits (HMW-GS) are the most intensively studied protein fraction because their viscoelastic properties enable wheat dough to be made into bread, pasta, biscuit and other food products (Payne 1987; Shewry et al., 1992). The HMW-GS are encoded by genes at *Glu-1* loci on the long arm of the homoeologous group one chromosomes of the A, B and D-genomes (Payne et al., 1981; Payne 1987). Each homoeologous contains two closely linked genes encoding subunits of lower and higher molecular weight called *x*-type and *y*-type respectively (Forde et al., 1985; Harberb et al., 1986; Payne et al., 1981; Payne and Lawrence, 1983). Hexaploid bread wheat consists of six HMW-GS genes. However, cultivars contain three to five HMW-GS: zero or one encoded by *Glu-A1*, one or two by Glu-B1 and two by Glu-D1 (Lawrence and Payne, 1983). The variation in the number of expressed subunits is due to gene silencing. Considerable investigations have shown that allelic variation is closely related to bread-making quality of wheat flour (Shewry et al., 1992). However, the subunits of 1Ax1, 1Ay2*, 1Bx17 + 1By18 and 1Dx5 + 1Dy10 are associated with stronger dough and thus improve bread-making quality. The subunit pair 1Dx2 + 1Dy12 are associated with weaker dough (Gupta et al., 1996; Payne et al., 1979, Radovanovie et al., 2002) that results in poor dough quality.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) has been widely used for HMW-GS polymorphism investigation (Briggle and Curtis, 1987; Gupta and Shepherd, 1990; Gupta et al., 1991). SDS-PAGE requires multiple manual steps some of which could be hazardous and not practical for high throughput screening. In addition, achieving sufficient resolution, reproducibility, and quantitation by SDS-PAGE is difficult. SDS-PAGE has been supplanted by capillary electrophoresis (CE) separations using SDS and soluble linear polymer (Bean and Lookhart, 1998, 1999; Sutton and Bietz, 1997; Werner et al., 1994; Zhu and Khan, 2001). CE separates the SDS coated HMW-GS based on size differences similar to SDS-PAGE and the proteins are detected by UV absorbance which permits direct quantitation. Others (Huebner and Bietz, 1985; Sutton, 1991) have shown that reversed phase high performance liquid chromatography (RP-HPLC) is a useful analytical method for showing variability in pyridylethylated HMW-GS and determining the expression levels of HMW-GS (Marchylo, 1989). Naeem and Sapirstein (2007) recently used a new superficially porous silica-based column to develop a simple and fast (≈13 min) quantitative analysis by RP-HPLC.

Separations of HMW-GS by CE-SDS and RP-HPLC typically require 30 to 80 minutes per sample, respectively. The CE method often requires additional time between injections for rinsing and reloading of the polymer solution. The number of samples that can be analyzed per instrument in 24-hour day by these methods is less than 30. A cost effective high throughput analytical method is required in order to meet the demands of increasing number of samples that need to be analyzed at early stages of breeding.

Lab-on-a-chip technology, emerged in early 1990's (Harrison et al. 1993; Jacobson et al. 1994; Manz et al. 1992; Woolley and Mathies 1994) have demonstrated

great potential to increase throughput for CE-based separations by reducing the analysis time from minutes to seconds. Rapid separation in microchip devices is achieved from the ability to inject a very small sample plug on the order 10 μm to 100 μm in length. The small plug size is created by controlling the voltages and/or currents at four terminal wells of a cross-section of microfluidic channels. Recently, rapid separation and quantitation of glutenin subunits have been achieved using a planar microfluidic chip-based electrophoresis. Uthayakumaran et al. (2005, 2006) used a commercial microchip CE (Agilent 2100; Agilent Technologies) platform to rapidly separate and quantitate the HMW-GS. However, the planar chips used in these studies are limited to 10 samples per chip where each sample is added to individual chip wells. The time required to analyze 10 samples is 30 minutes. As stated by the authors, the platform is not suitable for high throughput analysis (>150 samples per 8-hour day) not only because of the limited number of samples per chip but the requirement of manual sample pipetting for each chip preparation. With increasing number of breeding lines comes greater demand for high throughput platform that rapidly and continuously analyzes large number of samples (>400 samples per 8-hour day).

In this paper, we report on the use of a high throughput microchip CE platform for analysis of HMW-GS in a large collection of French common wheat varieties. The accuracy in identification, quantitation of HMW-GS, as well as relative quantitation of HMW-GS to LMW-GS ratio, and the overall reproducibility of the platform was investigated.

## 2. Experimental

### *2.1. Wheat samples*

130 French common wheat cultivars representing a wide range of allelic combinations, were used for this investigation. These cultivars were grown under

conventional conditions (with fertilization and full fungicide protection) in 2004 at INRA (Station d'amélioration des plantes, Clermont-Ferrand). At maturity (53 DAA), grains were collected and ground in a Brabender Senior laboratory mill.

*2.2. Glutenin subunits extraction for the microchip analysis*

The purification and extraction of glutenin follows the protocol of Fu and Sapirstein (1996) with some modifications. Flour samples (30 mg) were stirred for 15 min at room temperature with 1 ml of 0.08 M Tris-HCl buffer (pH 7.5) containing 50% (v/v) propan-1-ol. Extraction was followed by centrifugation at 15900 *g* for 10 min at 15°C. The supernatant containing monomeric proteins (albumins, globulins and gliadins) was discarded. 600 µl of the Tris-HCl containing 2% (w/v) of SDS and 1% (w/v) of DTT was added to the pellet containing mainly polymeric glutenins and dispersed by sonication with amplitude of 30% for 15 s, which was performed using a stepped microtip prob of 3 mm of diameter (Ultrasonic Processor, Sonics, model 75038). The mixture was maintained at a constant temperature of 60°C for 30 min and then centrifuged at 12500 *g* for 10 min at 20°C.

Some optimizations of the sample denaturing condition was required in order to obtain consistent and reliable separation and detection of the HMW-GS on the LabChip 90. 5 µl of the supernatants was mixed with 4 µl of manufacturer's sample buffer containing 1 M DTT. The mixture was heat denatured at 95°C for 5 min and afterwards 36 µl of ultra high quality water was added to each sample well. The final percentage of SDS in the sieving matrix was also increased from 0.2% (w/w) to 0.27% (w/w) in order to consistently stain hundreds of samples with single preparation of the microchip. This protocol deviates from the sample preparation protocol given by the manufacturer.

*2.3. Glutenin extraction for the RP-HPLC analysis*

Procedure of purification and extraction of glutenin used here was achieved according to Fu and Kovacs (1999) with some modifications. 500 mg of flour samples was stirred for 30 min at room temperature with 2 ml of 0.3 M NaI, 7.5% propan-1-ol buffer to separate the albumins, globulins and gliadins from the starch and insoluble glutenin proteins. After centrifugation for 10 min at 15900 *g* at 15°C, the glutenins contained within the pellets were extracted with 1 ml of 0.08 M Tris-HCl buffer (pH 7.5) containing 50% (v/v) propan-1-ol and 1% (w/v) DTT. To disintegrate the pellets and improve protein extraction rate, the samples were sonicated (Ultrasonic Processor, Sonics, model 75038), followed by incubation for 30 min at 60°C. The supernatant obtained after centrifugation at 15900 *g* for 30 min at 15 °C was filtered through a 0.45 µm nylon filter. An aliquot (600 µl) was then alkylated with 40 µl of 70% (v/v) 4-vinylpyridine in 50% (v/v) propan-1-ol buffered with 80 mM Tris-HCl (pH 7.5) at 60°C for 20 min just prior to RP-HPLC analysis. Proteins were analyzed using a Surveyor system coupled to UV detector set at 214 nm (Thermo Electron Corporation, Courtaboeuf, France), and were separated in a Zorbax 300-SB-C8 analytical column, 150 x 4.6 mm, particle size 5 µm (Supelco Inc., Bellefont, PA, USA) preceded by a pre-column with the same characteristics. The column was maintained at 50°C during the run. Injection volumes were 30 µl in conjunction with a multiple 5 µl injection technique (Marchylo et al., 1989). Separation of glutenin subunits was carried out as described previously by (Carceller and Aussenac, 2001).

*2.4 Separation of glutenin subunits by the LabChip 90*

High throughput analysis of the glutenin subunits were performed on the LabChip 90 platform. Protein sizing and quantitation on a microchip is achieved by integration of the sample loading, staining, separation, destaining, and detection (Bousse et al., 2001; Chow, 2006). A schematic diagram of the microchannel layout of

the protein chip is illustrated in Figure 1. Unlike the planar chip the denatured proteins samples are introduced onto the chip directly from a 96- or 384-well microtiter plate through a sipper by applying vacuum pressure at well 1. Approximately 250 nl of the sample is introduced onto the chip and then mixed with lower marker from well 4 during the short time that the vacuum is applied. Sample and marker mixture is then electrokinetically loaded across the injection cross-section by applying voltage gradient across wells 3 and 6. A small volume of sample, 10 to 20 picoliter, is then injected into a separation channel 14 mm in length and 31 μm in width. The separation channel is filled with a high molecular weight polydimethyl methacrylate (pDMA) polymer solution in a Tris-Tricine buffer containing a non-covalent staining dye and 0.27% SDS. A subsequent sample is introduced onto the chip while the injected sample is being electrophoretically separated. The overlap sipping and separation are repeated for the entire microtiter plate. A ladder containing 6 proteins ranging in size from 14 to 120 kDa is sipped repeatedly every 12 samples. The system software automatically analyzes the data and determines protein size and concentration relative to a ladder.

## 3. Results and discussion

### *3.1 HMW-GS separation*

Protein samples were loaded onto the microchip sequentially from a 96- or 384-well plate. The electric field across the 14 mm separation channel is approximately 700 V/cm. An electrophoregram and the corresponding virtual gel view of the separation of purified and reduced wheat glutenins are shown Figure 2. The HMW-GS and LMW-GS are separated into two distinct fractions. The main peaks of the HMW-GS are clearly resolved. These results were comparable to those previously obtained by Uthayakumaran and co-workers (2006) using a similar low throughput microchip-based CE instrument (Agilent 2100 Bioanalyzer). The analysis time per sample was 45 s and

the total time for completion of a 96-well plate, including the time required for the preparation of chip (15 min) and sample plate (20 min), was about 120 min. A total of 100 to 200 samples could be analyzed with a single preparation of the microchip. Additional samples could be analyzed by simply replacing the destain reagents in wells 2 and 7, and the dye-SDS-polymer mixture in well 5.

Figure 3 shows comparison of the electrophoregrams of a wheat sample analyzed by RP-HPLC (A) and microchip CE (B). The HMW-GS 5, 7, 8, and 10 are identified by both methods but differ in order of elution. The resolution on microchip CE is similar to that observed by RP-HPLC but at much shorter analysis time, 45 s versus 80 min, which amounts to a throughput increase of about 100 fold. This rapid separation on microchip CE is due to the injection of a very small plug size, on the order of the channel width (Bousse et al. 2001; Chow 2006). In addition, the analysis on microchip CE is achieved at lower starting sample concentration (30 mg of flour in 600 µl of extraction buffer compared to 500 mg in 1000 µl in RP-HPLC) and 6 times less sample load for analysis (5 µl for microchip CE versus 30 µl for RP-HPLC).

To further examine the system performance and its ability for resolving wide range of HMW-GS, 130 common wheat cultivars were analyzed. Figure 4 represents gel view of twelve wheat samples where the identification of the HMW-GS was known. The bulk of the glutenin subunits were resolved and identified using the high throughput microchip CE platform. All the pairs of subunits encoded by *Glu-D1* and *Glu-A1*, and the wider range of subunits associated to *Glu-B1* were identified. According to their banding patterns, HMW-GS could be divided into two groups. The first group consists of 1, 2, 2*, 3, 4, and 5 subunits and the second group includes subunits 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, and 20. The elution order of HMW-GS is determined by their electrophoretic mobility in the sieving matrix. The first group, which contained mainly

x-type subunits, has a lower mobility than the second group of subunits. On microchip CE the order of migration of the HMW-GS were determined to be subunit 9, 12, 8, 18, 15, 16, 10, 17, 6, 7, 14, 13, 2*, 1, 4, 3, 5 and 2. The order of migration of the HMW-GS found is different than that reported by Sutton and Bietz (1997) when using conventional CE (i.e. 12, 10, 9, 8, 18, 17, 6, 7, 2*, 1, 5 and 2). Furthermore, we observed a difference in the order of elution than that reported by Uthayakumaran et al. (2006) using the planar-based microchips. Depending on the sample cultivars, two differences were observed. On microchip CE, subunits 9 and 16 migrated faster than 12 and 10, respectively. The order of migration for these pairs is reversed in the investigation reported by Uthayakumaran et al. (2006).

The most important combinations which are related to bread-making quality were easily detectable with microchip CE. These subunits include the identification of 2* and a combination of 5+10, 7+9, 14+15, 13+16 and 17+18. However, sample extracts containing the subunits 12 and 18, 16 and 10 or 1 and 4 were barely resolved where only a shoulder peak was observed. We were not able to resolve subunits 8 and 12.

*3.2 HMW-GS Sizing*

The reproducibility of sizing of glutenin subunits on microchip CE was evaluated by examining 12 replicates of each sample on three different chips. The apparent size for each glutenin subunit and the sizing reproducibility are summarized in Table 1. In general the apparent sizes determined by microchip CE were higher than values of molecular weights calculated from gene sequence or by mass spectrometry. Though microchip CE tends to oversize the subunits, the results were highly reproducible, with sizing CV less than 2%. The apparent sizes determined for some subunits were two times higher than the absolute sizes. The molecular weight of

subunits 1, 2, 5, 7, 9, 10 and 12 have been reported by mass spectrometry to be 87.5, 88.3, 88.9, 83.5, 72.5, 68.3, and 69.52 kDa, respectively (Hickman et al., 1995). The sizes based on gene sequence for the same subunits were 88.1, 87.4, 88.6, 83.2, 74.2, 67.9 and 69.3 kDa, respectively (Hickman et al., 1995).

Apparent sizes on LabChip 90 were slightly higher (5% to 20%) than those reported by Uthayakumaran et al. (2006) using planar microfluidic chips where an upper marker is used in addition to the lower marker for sizing. These authors reported apparent sizes of 128, 130, 138.5, 141, 144, 149, 162, 170, 175, 193, 199, 210 and 212 kDa respectively for HMW subunits 12, 9, 8, 18, 10, 16, 17, 7, 13, 2*, 1, 5 and 2. They suggested that the overestimation maybe due to factors such as variations in protein conformation in the specific running buffer or due to intrinsic characteristics of proteins such as hydrophobicity.

*3.3 Quantitative analysis*

About twenty five cultivars which represent a large allelic variation were used for quantitation of HMW-GS and estimation of the ratio HMW-GS to LMW-GS. Results are expressed as percentage of the time corrected area of individual HMW-GS to the total HMW-GS area (Table 2). To evaluate the reproducibility of this quantitation, the cultivar set was analyzed 5 times using multiple chips. The results show the quantitation to be highly reproducible and can be used to determine the variation of the relative amount of each individual HMW-GS.

The content of some HMW-GS (subunits 1, 13, 14, 16 and 17) fluctuated slightly among genotypes. Nevertheless, significant variations in the quantity of other HMW-GS (subunits 7, 2, 5 and 10) were detected. The determination of the amount of these subunits could be important because of the strong correlations established between the presence of the subunits and the quality of bread making. Payne et al. (1987) also

demonstrated the importance of the correlations of different alleles with dough properties and they assigned a system of quality score to HMW-GS. The HMW-GS pair 5+10 was assigned a score of 4 as this pair is found to be associated with increased dough strength. Pair 2+12 was assigned a score of 2, reflecting its association with dough weakness.

Furthermore, the quantitation of HMW-GS could be used to detect an over-expression of HMW-GS, for example, an over-expression of the subunit 7 is encountered in certain varieties.

The results of the ratio HMW-GS to LMW-GS for a large set of wheat cultivars are summarized in Table 3. Cultivars used herein, were divided into seven classes based on their allelic combinations. The ratio of HMW-GS to LMW-GS ranged from 20% to 60% and was highly reproducible in the major cases (i.e. CV less than 5%). It is established that HMW-GS to LMW-GS ratio affects the molecular weight distribution (MWD) of the polymeric glutenin proteins. Increasing the HMW-GS to LMW-GS ratio increases the relative proportion of polymers of high molecular weight (Southan and MacRitchie, 1999, Larroque et. al. 1997). They concluded that the molecular weight of subfractions decreased as the ratio decreased. Additional studies showed that the MWD of polymeric glutenin expressed as the proportion of unextractible or larger polymers in total polymeric protein were changed considerably using genotypes exempt of HMW or LMW subunits (Gupta et al. 1991, Gupta et al. 1995). In summary these studies show that the MWD is important in determining properties such as dough mixing and rheological characteristics (Southan and MacRitchie, 1999) and that the distribution is dependent on the HMW-GS to LMW-GS ratio.

Table 4 illustrates the comparison of the quantitative data of five wheat cultivars determined on microchip CE and RP-HPLC. The quantitative microchip CE data, when

expressed as a percentage of total HMW-GS peak area, is very similar to those determined by RP-HPLC. Statistical analysis of the data revealed no significant differences between the quantitation results from the two techniques. However, in spite of all reported advantages of RP-HPLC when compared to former methods, the low throughput is limiting for efficient analysis of large number of samples. Higher throughput RP-HPLC column have been reported (Naeem and Sapirstein 2007) but separation time remains significantly slower than microchip CE.

## 4. Conclusions

A high throughput microchip CE platform, LabChip90, was used for routine analysis of glutenin subunits. The results of the present study on the use of sipper format microchip CE for rapid identification and quantitation of individual HMW-GS are similar to those reported by Uthayakumaran et al. (2005, 2006) but at much higher throughput. Resolution, sensitivity, and reproducibility of the LabChip 90 were suitable for quality assessment of HMW-GS. In direct comparison with RP-HPLC, microchip CE shows similar electropherogram but at time scale of approximately 100 times fasters, 45 seconds versus 80 minutes. Additionally, the amount of HMW-GS and the ratio of HMW-GS to LMW-GS obtained were comparable with those of conventional techniques. The LabChip 90 is an efficient analytical platform for purposes of wheat quality screening and wheat cultivar development activities where large numbers of samples are to be analyzed (breeding programs). As discussed by Uthayakumaran et. al., the identification of LMW-SU is also of interest for the determination of wheat quality. In order to identify the LMW-SU, an improvement in resolution between subunit peaks is required. To improve the resolution, future studies will focus on the use of a microchip with an increase in separation channel, optimization of sieving matrix concentration and molecular weight, and dependence on extraction procedure.

## Acknowledgements

We thank Olfa Ben Abda and Amine Slim for their technical assistance.

## References


Bean, S.R., 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., Lookhart, G.L., 1999. Sodium dodecyl sulfate capillary electrophoresis of wheat proteins. I. Uncoated capillaries. Journal of Agricultural and Food Chemistry 47, 4246-4255.

Bousse, L.; Mouradian, S.; Minalla, A.; Yee, H.; Williams, K.; Dubrow, R. 2001. Protein sizing on a microchip. Analytical Chemistry 73, 1207-1212

Briggle, L.W., Curtis, B.C., 1987.Wheat worldwide. In E.G., Heyne (Ed.), Wheat and Wheat Improvement.American Society of Agronomy, Madison, WI, pp 1-31.

Carceller, J.L., Aussenac, T., 2001. SDS-insoluble glutenin polymer formation in developing grains of hexaploid wheat: role of the HMW-GS/LMW-GS ratio and the drying rate during ripening. Australian Journal of Plant Physiology 28, 193-201.

Chow, A. W., 2006. Protein Separations in Microfluidic Chips. Methods in Molecular Biology. 339 145-158.

Forde, J., Malpica, J.-M., Halford, N.G., Shewry, P.R., D, A.O., C, G.F., J, M.B., 1985. The nucleotide sequence of a HMW glutenin subunit gene located on chromosome 1A of wheat (*Triticum aestivum*). Nucleic Acids Ressource 13, 6817-6832.

Fu, B.X., Sapirstein, H.D., 1996. Procedure for isolating monomeric proteins and polymeric glutenin of wheat flour. Cereal Chemistry 73, 143-152.

Fu, B.X., Kovacs, M.I.P., 1999. Rapid single step procedure for isolating total glutenin proteins of wheat flour. Journal of Cereal Science 29, 113-116.

Gupta, R.B., Shepherd, K.W., 1990. Two step one dimensional SDS-PAGE analysis of LMG subunits of glutenin. I – Variation and genetic control of the subunits in hexaploid wheats. Theoretical and Applied Genetics 80, 65-74.

Gupta, R.B., MacRitchie, F., Shepherd, K.W., 1991. Relative contribution of LMW and HMW glutenin subunits to dough strength and dough stickiness of bread wheats. In : W. Bushuk and R. Tkachuk, (Eds), Gluten Proteins 1991. AACC, St Paul, MN, USA pp. 71-80.

Gupta, R.B., Masci, S., Lafiandra, D., Bariana, H.S., et MacRitchie, F., 1996. Accumulation of protein subunits and their polymers in developing grains of hexaploïd wheats. Journal of Experimental Botany 47, 1377-1385.

Harberb, N.P., Bartels, D., Thompson, R.D., 1986. DNA restriction fragment variation in the gene family encoding high molecular weight (HMW) glutenin subunits of wheat. Biochemical Genetic 24, 579-596.

Harrison, D.J.; Fluri, K.; Seiler, K.; Fan, Z.; Effenhauser, C.S.; Manz, A., 1993. Micromachining a Miniaturized Capillary Electrophoresis-Based Chemical Analysis System on a Chip. Science 261, 895-897.

Hickman, D.R., Roepstorff, P., Shewry, P.R., Tatham, A.S.,1995. Molecular weights of high molecular weight subunits of glutenin determined by mass spectrometry. Journal of Cereal Science 22, 99-103.

Huebner, F.R., Bietz, J.A., 1985. Detection of quality differences among wheats by high-performance liquid chromatography. Journal of Chromatography 327, 333-342.

Jacobson, S.C., Hergenroder, R., Koutny, L.B., Ramsey, J.M., 1994. Chip-based isothermal amplification devices and methods. Analytical Chemistry 66, 1114-1118.

Lawrence, G.J., Payne, P.I., 1983. Detection by gel electrophoresis of oligomers formed by the association of high-molecular-weigh glutenin protein subunits of wheat endosperm. Journal of Experimental Botany 34, 254-267.

MacRitchie, F., Lafiandra, D., 1997. Structure-Function relationships of wheat proteins. In: Damodaran, S., Paraf, A. (Eds.), Food Proteins and their Applications. Dekker, M, New York, pp. 293-324.

Manz, A.; Harrison, D.J.; Verpoorte, E.M.J.; Fettinger, J.C.; Paulus, A.; Ludi, H., Widmer, H.M., 1992. Capillary electrophoresis on a chip. Journal of chromatography 593, 253-258.

Marchylo, B.A., Kruger, J.E., Hatcher, D.W., 1989. Quantitative reversed-phase high-performance liquid chromatographic analysis of wheat storage proteins as a potential quality prediction tool. Journal of Cereal Science 9, 113-130.

Margiotta, B., Colaprico, G., D'Ovidio, R., Lafiandra, D., 1993. Characterization of high $M_r$ Subunits of glutenin by combined chromatographic (RP-HPLC) and electrophoretic separations and restriction fragment length polymorphism (RFLP) analysis of their encoding genes. Journal of Cereal Science 17, 221-236.

Naeem, H.A., Sapirstein, H.D., 2007. Ultra-fast separation of wheat glutenins by reversed-phase HPLC using a superficially porous silica-based column. Journal of Cereal Science 46, 157-168.

Payne, P.I., Corfield, K.G., 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. Theoretical and Applied Genetics. 55, 153-159.

Payne, P.I., Holt, L.M., N, L.C., 1981. Structural and genetical studies on the high-molecular-weight subunits of wheat glutenin. Theoretical and Applied Genetics 1981, 229-236.

388 Payne, P.I., Lawrence, G.J., 1983. Catalogue of alleles for the complex gene loci, Glu-
389 A1, Glu-B1, and Glu D-1 which code for high-molecular-weight subunits of
390 glutenin in hexaploid wheat. Cereal Research Communications 11, 29-35.
391 Payne, P.I., 1987. Genetic of wheat storage protein and the effect of allelic variation on
392 breadmaking quality. Annual Rewiew of Plant Physiology 38, 141-153.
393 Payne, P.I., Nightingale, M.A., Krattiger, A.F., Holt, L.M., 1987. The relation ship
394 between HMW glutenin subunit composition and the breadmaking quality of
395 British-grown wheat varieties. Journal of Sciences Food Agriculture 40, 51-65
396 Radovanovie, N., Cloutier, S., Brown, D., Humphreys, D.G., Lukow, O.M., 2002.
397 Genetic variance for gluten strength contributed by high molecular weight
398 glutenin proteins. Cereal Chemistry 79, 843-849.
399 Shewry, P.R., Halford, N.G., Tatham, A.S., 1992. High molecular subunits of wheat
400 glutenin. Journal of Cereal Science 15, 105-120.
401 Southan, M., MacRitchie, F., 1999. Molecular weight distribution of wheat proteins.
402 Cereal Chemistry 76, 827-736.
403 Sutton, K.H., 1991. Qualitative and quantitative variation among high molecular weight
404 subunits of glutenin detected by reversed-phase high-performance liquid
405 chromatography. Journal of Cereal Science 14, 25-34.
406 Sutton, K.H., Bietz, J.A., 1997. Variation among high molecular weight subunits of
407 glutenins detected by capillary electrophoresis. Journal of Cereal Science 25, 9-16.
408 Uthayakumaran, S., Stoddard, F.L., Gras, P.W., Bekes, F., 2000. Effects of incorporated
409 glutenins on the functional properties of wheat dough. Cereal Chemistry 77, 737-
410 743.

411 Uthayakumaran, S., Batey, I.L., Wrigley, C.W., 2005. On-the-spot identification of
412 grain variety and wheat-quality type by Lab-on-a-chip capillary electrphoresis.
413 Journal of Cereal Science 41, 371-374.
414 Uthayakumaran, S., Listiohadi, Y., Baratta, M., Batey, I.L., Wrigley, C.W., 2006. Rapid
415 identification and quantitation of high-molecular-weight glutenin subunits. Journal
416 of Cereal Science, 1-6.
417 Werner, W.E., Wiktorowicz, J.E., Kasarda, D.D., 1994. Wheat varietal identification by
418 capillary electrophoresis of gliadins and high molecular weight glutenin subunits.
419 Cereal Chemistry 71, 397-402.
420 Woolley, A.T., Mathies, R.A., 1994. Electrochemical detector integrated on
421 microfabricated capillary electrophoresis chips. Proceeding of the National
422 Academy of Sciences USA 91, 11348-11352.
423 Zhu, J., Khan, K., 2001. Separation and quantification of HMW glutenin subunits by
424 capillary electrophoresis. Cereal Chemistry 78, 737-742.
425 Zhu, J., Khan, K., 2002. Quantitative variation of HWM glutenin subunits from hard red
426 spring wheats grown in different environment. Cereal Chemistry 79, 783-786.
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## Figure Captions

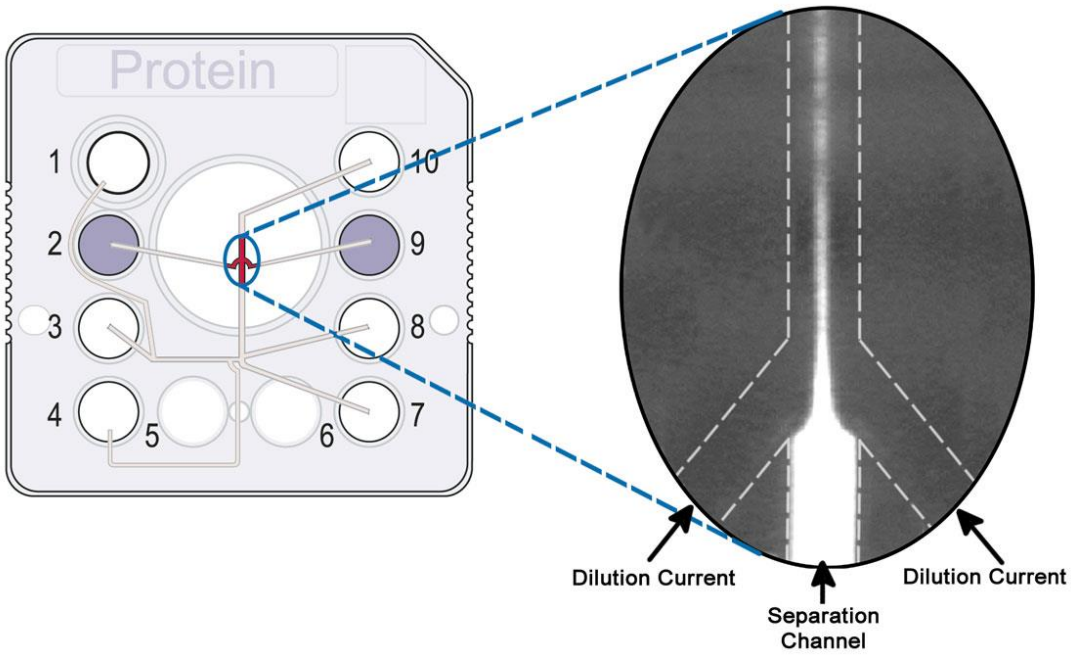
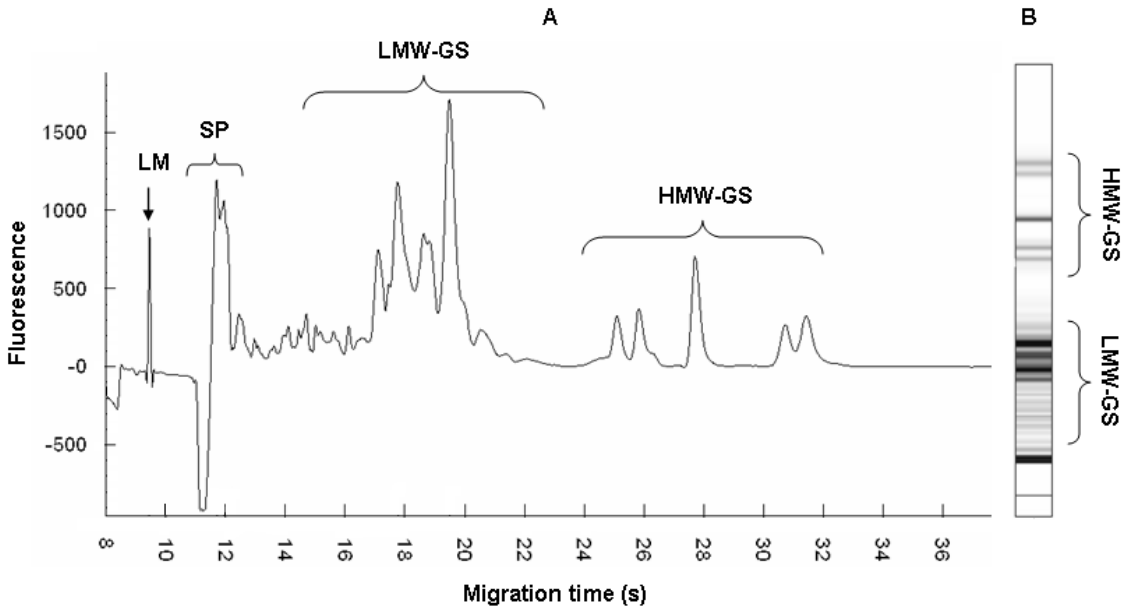



**Figure 1.** Schematic diagram of the layout of the microfluidic channels of the protein sipper chip.



433 **Figure 2.** (A) Elution profiles of wheat glutenin fraction under denatured and reduced

434 conditions and (B) its corresponding virtual gel. (LM : low marker ; SP: system peak).

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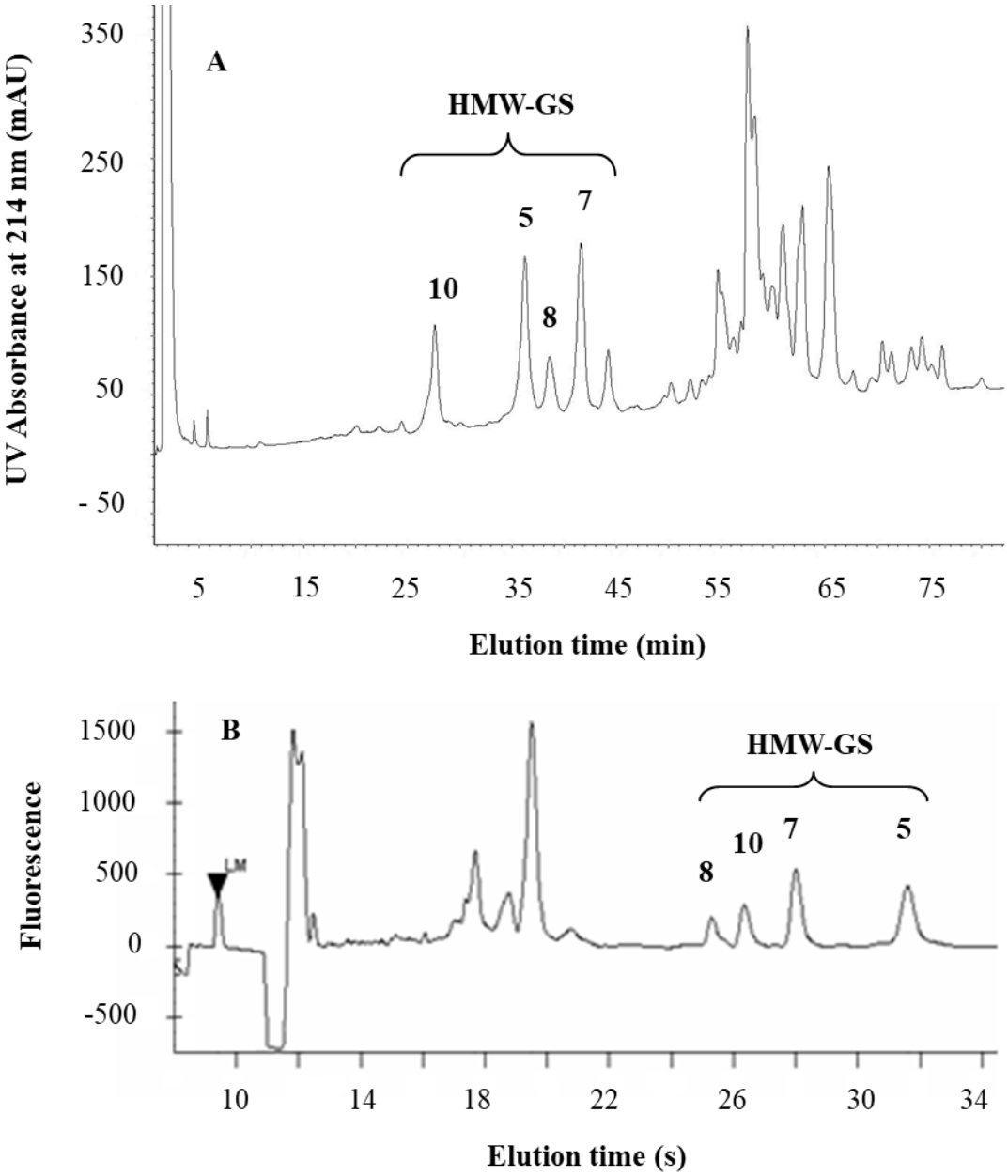


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440 **Figure 3.** Separation of denatured and reduced HMW-GS by (A) reversed-phase high-

441 performance liquid chromatography (RP-HPLC) and by (B) lab-on-a-chip method.

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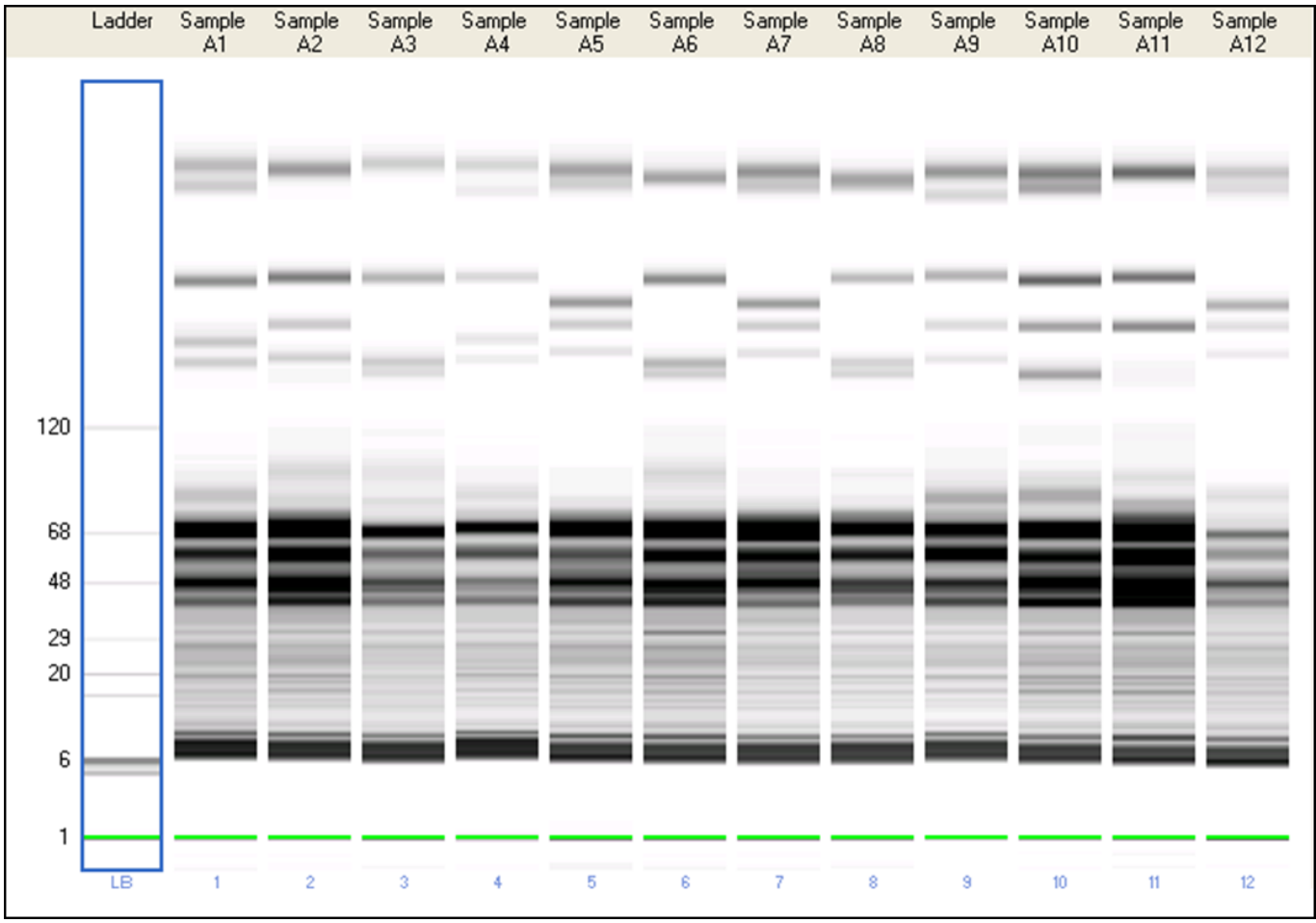


**Figure 4.** The gel image overlay of denatured and reduced glutenin subunits from known samples (1 to 12) and designation of HMW-GS.

**Table 1**
Apparent sizes and Reproducibility * of sizing the HMW-GS using LabChip 90 Caliper system

| HMW-GS | Mean MW (kDa) | MS | CV % |
|---|---|---|---|
| *Glu A1* | | | |
| 2* | 238.6 | 1.1 | 0.5 |
| 1 | 244.3 | 2.7 | 1.1 |
| *Glu B1* | | | |
| 9 | 148.0 | 2.2 | 1.5 |
| 8 | 155.3 | 2.7 | 1.8 |
| 18 | 157.0 | 1.1 | 0.7 |
| 15 | 163.8 | 2.3 | 1.4 |
| 16 | 164.5 | 0.3 | 0.2 |
| 17 | 184.1 | 2.1 | 1.1 |
| 6 | 190.3 | 0.8 | 0.4 |
| 14 | 196;7 | 2.5 | 1.3 |
| 7 | 198.4 | 2.4 | 1.2 |
| 13 | 202.0 | 0.2 | 0.1 |
| *Glu D1* | | | |
| 12 | 154.5 | 2.1 | 1.4 |
| 10 | 174.8 | 2.1 | 1.2 |
| 5 | 256.3 | 2.4 | 1.0 |
| 4 | 248.1 | 0.8 | 0.3 |
| 3 | 253.8 | 2.0 | 0.8 |
| 2 | 258.4 | 2.3 | 0.9 |

* Calculated as % CV

**Table 2**
Amounts of HMW-GS detrmined using microfluidic technique for twenty five cultivars

| HMW-GS | % HMW subunit | Min | Max |
|---|---|---|---|
| *Glu-A1* | | | |
| 2* | 17.31 | 15.8 | 18.6 |
| 1 | 19.05 | 18.7 | 19.5 |
| *Glu-B1* | | | |
| 6 | 14.86 | 14 | 15.4 |
| 7 | 32.4 | 26 | 40.8 |
| 8 | 12.32 | 10.1 | 14.5 |
| 13 | 34.56 | 34 | 36.00 |
| 14 | 30.22 | 29.5 | 31.00 |
| 15 | 12.53 | 11.5 | 13.30 |
| 16 | 9.66 | 9 | 10.20 |
| 17 | 30.43 | 29.6 | 32.00 |
| 18 | 8.07 | 7.5 | 8.70 |
| *Glu-D1* | | | |
| 2 | 33.91 | 27.4 | 42.2 |
| 5 | 41.06 | 31.7 | 50.7 |
| 10 | 12.9 | 9.7 | 16.3 |

**Table 3**
HMW-GS to LMW-GS ratio determined using the LabChip 90
Samples were analyzed five times

| Cultivars | *Glu-A1* | *Glu-B1* | *Glu-D1* | HMW-GS:LMW-GS | SD | CV (%) |
|---|---|---|---|---|---|---|
| 1 | - | 7 | 5+10 | 0.24 | 0.03 | 10.56 |
| 2 | - | 7 | 5+10 | 0.31 | 0.04 | 11.94 |
| 3 | - | 7 | 5+10 | 0.25 | 0.01 | 3.72 |
| 4 | - | 7 | 5+10 | 0.34 | 0.01 | 2.09 |
| 5 | - | 7 | 5+10 | 0.31 | 0.02 | 4.93 |
| 6 | 2* | 7+8 | 5+10 | 0.28 | 0.01 | 3.35 |
| 7 | 2* | 7+8 | 5+10 | 0.39 | 0.01 | 3.60 |
| 8 | 2* | 7+8 | 5+10 | 0.27 | 0.01 | 5.21 |
| 9 | - | 7+9 | 2+12 | 0.27 | 0.01 | 2.70 |
| 10 | - | 7+9 | 2+12 | 0.29 | 0.01 | 3.53 |
| 11 | - | 7+9 | 2+12 | 0.38 | 0.01 | 3.70 |
| 12 | - | 7+9 | 2+12 | 0.42 | 0.01 | 2.79 |
| 13 | 1 | 7+8 | 2+12 | 0.61 | 0.03 | 4.16 |
| 14 | 1 | 7+8 | 2+12 | 0.32 | 0.04 | 12.94 |
| 15 | 1 | 7+8 | 2+12 | 0.41 | 0.02 | 4.03 |
| 16 | 1 | 7+8 | 2+12 | 0.25 | 0.01 | 3.41 |
| 17 | 1 | 7+9 | 5+10 | 0.37 | 0.01 | 2.40 |
| 18 | 1 | 7+9 | 5+10 | 0.42 | 0.01 | 1.34 |
| 19 | 1 | 7+9 | 5+10 | 0.44 | 0.05 | 10.50 |
| 20 | - | 7+8 | 5+10 | 0.35 | 0.03 | 7.09 |
| 21 | - | 7+8 | 5+10 | 0.31 | 0.01 | 1.78 |
| 22 | - | 6+8 | 2+12 | 0.23 | 0.01 | 4.93 |
| 23 | - | 6+8 | 2+12 | 0.22 | 0.01 | 4.11 |