# Molecular Weight Distribution of Proteins in Hard Red Spring Wheat: Relationship to Quality Parameters and Intrasample Uniformity

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**ABSTRACT**

Molecular weight distribution (MWD) of proteins extracted from hard red spring wheat was analyzed by size-exclusion HPLC to investigate associations with wheat and breadmaking quality characteristics. Certain protein fractions were related to associations between wheat and breadmaking parameters, specifically when effect of quantitative variation of protein on those parameters was statistically eliminated by partial correlation analysis. SDS-unextractable high molecular weight polymeric proteins had positive partial correlations with percent vitreous kernel content and breadmaking parameters, including mix time and bread loaf volume. SDS-extractable protein fractions that were eluted before the primary gliadin peak had positive partial correlations with kernel hardness and water absorption parameters. The proportion of main gliadin fractions in total protein had a negative partial correlation with bread loaf volume and positive correlations with kernel hardness and water absorption parameters. Intrasample uniformity in protein MWD and kernel characteristics was estimated from three kernel subsamples that were separated according to single kernel protein content within individual wheat samples by a single-kernel near-infrared sorter. Wheat subsamples were significantly different in protein MWD. Intrasample uniformity in protein MWD did not differ greatly among wheat samples.

Size-exclusion high-performance liquid chromatography (SE-HPLC) has been applied to separate proteins according to protein molecular weight distribution (MWD) (Bietz et al 1984; Batey et al 1991). Research using SE-HPLC indicated that SDS-unextractable polymeric proteins could enhance dough strength while extractable polymeric proteins were associated with weak dough characteristics (Gupta et al 1993; Ciaffi et al 1996; Bangur et al 1997; Borneo and Khan 1999; Morel et al 2000; Tsilo et al 2010). Bean et al (1998) and Park et al (2006) reported similar results showing that mix peak time had a positive association with propanol-insoluble proteins and a negative association with propanol soluble proteins. Ohm et al (2006, 2008, 2009a) researched the association more specifically by calculating the correlation of quality parameters with SE-HPLC absorbance values measured at a narrow interval of retention time. They reported that specific protein fractions had distinct effects on quality characteristics, specifically that SDS-unextractable high molecular weight polymeric proteins had greater positive correlations with dough characteristics than other polymeric protein fractions. Tsilo et al (2010) reported similar findings using hard spring recombinant inbred lines.

Variation in proteins was also associated with wheat kernel characteristics such as kernel hardness (Huebner and Gaines 1992; Ohm et al 1998; Ohm and Chung 1999; Giroux et al 2000; Ohm et al 2006) and vitreousness (Dexter et al 1989; Gianibelli et al 1991; Samson et al 2005). Specifically, Huebner and Gaines (1992) found that specific fractions of gliadins had significant correlations with average particle size of ground wheat flour. Ohm et al (2009a) also reported that protein fractions rich in gliadins affected the variations of kernel hardness in soft winter wheat. Few reports have been published on associations of protein MWD and wheat characteristics in hard red spring wheat despite significant correlation found in other wheat classes.

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In the wheat marketing system, consistency in quality characteristics is a very important element for wheat procurement (Wilson and Dahl 2008). To ensure consistency in quality, the U.S. wheat industry is moving toward a system that requires the segregation of wheat of which identity is preserved by specifying variety, growing location, and quality characteristics (Wilson and Dahl 2008). Production of wheat with uniform distribution of quality characteristics among individual kernels is expected to enhance segregation of wheat according to quality standards and, consequently, promote consistency of wheat quality through identity preservation in commercial production and marketing systems.

Automated single-kernel near-infrared (SKNIR) technology has been successfully applied to determine the uniformity of distribution of quality characteristics in a wheat sample such as intravarietal variation of kernel protein content (Bramble et al 2006). Recently, uncertainty of consistency is specifically pointed out as a problem in wheat trading for mixing and breadbaking characteristics because of the requirements for large sample amounts and time-consuming and costly evaluation procedures (Wilson and Dahl 2008).

Because protein MWD has significant associations with dough mixing and breadmaking characteristics (Singh et al 1990b; Batey et al 1991; Larroque et al 2000; Morel et al 2000; Tsilo et al 2010), the analysis of protein in whole grain by SE-HPLC could be effective in gaining information on uniformity or consistency of mixing and breadbaking characteristics in hard red spring wheat samples. However, in previous studies by Ohm et al (2008, 2009a,b), protein extracted from whole grains of hard red spring wheat had not been analyzed by SE-HPLC to detail its associations with quality characteristics. The objectives of this study were to investigate associations of wheat and breadmaking quality characteristics with MWD of proteins in whole grain and to evaluate intrasample uniformity of protein MWD and kernel characteristics in hard red spring wheat. Specifically, the focus of this investigation was on determining whether SE-HPLC analysis of proteins extracted from whole wheat grain could be employed to further evaluate quality characteristics and consistency in hard spring wheat.

## MATERIALS AND METHODS

Twenty wheat samples consisted of 10 hard red spring wheat cultivars each harvested in North Dakota at two locations in 2006. Single kernel characteristics were measured with a Single Kernel Characterization System (SKCS) model 4100 (Perten Instruments, Huddinge, Sweden) according to the Approved Method 55-31.01

(AACC International 2010). All the abnormal, shrunken, and broken kernels and foreign materials were removed before SKCS analysis. Kernel hardness score was measured by a near-infrared analyzer (Infratec 1241 grain analyzer, Foss Tecator) according to AACC Approved Methods 39-70.02. Percent vitreous kernel content (PVKC) was visually determined as the percentage of dark hard vitreous kernels. Test weight was measured according to AACC Approved Method 55-10.01. For flour milling, wheat kernels were cleaned in a seed cleaner (Bulldog, Carter Day, Minneapolis, MN). Cleaned wheat was tempered to 16.5% moisture basis and conditioned for 16–18 hr. A Buhler experimental mill was used to mill the tempered wheat at an average feed rate of 175 g/min. Flour streams from the three break and two reduction sections of the mill were combined to patent flour and used in this research. Wheat and flour nitrogen contents were measured by the Dumas method using combustion apparatus (Leco, St. Joseph, MI) according to AACC Approved Method 46-30.01. Protein content was calculated as N × 5.7 on a 14% moisture basis (mb). Wheat and flour ash contents were determined according to AACC Approved Method 08-01.01. Flour mixing characteristics were analyzed by a computerized farinograph (Brabender, Duisburg, Germany) with a 50-g mixing bowl according to AACC Approved Method 54-21.01. Farinograph mixing peak time was determined at optimum water absorption, using Brabender software (Duisburg, Germany). Experimental breadbaking utilized the optimized, pup straight-dough method according to AACC Approved Method 10-10.03. Bake water absorption and mix time were determined from farinograph data but were adjusted by the feel and appearance of the dough because the baking formula included other ingredients in addition to flour and water. Bread loaf volume was determined by rapeseed displacement.

### Sorting Wheat Kernels by Protein Content

Wheat kernels were sorted into three subsamples according to single kernel protein content using the automated SKNIR sorter (Perten Instruments, Stockholm, Sweden). Single kernel characteristics and protein MWD were analyzed within the respective subsamples to compare intrasample uniformity between wheat samples. The calibration for protein content used in this study was developed by partial least squares regression (Grams AI v.7), using 97 hard red winter wheat samples of which quality characteristics were reported by Maghirang et al (2006). Each of the wheat samples was sorted into three categories: low, medium, and high protein content; sorting cutoffs varied from sample to sample. To determine the cutoff, 300 kernels that were randomly and automatically picked up by the SKNIR sorter were scanned and their protein contents were obtained. The low protein content category refers to the protein content range of the first 100 kernels with the lowest predicted protein content (1/3 of the total number of kernels). The medium protein content category refers to the protein content range of the next 100 kernels (right after the low protein category), while the high protein content category refers to the protein content range of the remaining 100 kernels, which has the highest protein content predictions for that specific sample. For each sample, we sorted at least 20 g each of low, medium, and high protein content subsamples and analyzed them using the SKCS 4100 and SE-HPLC.

### Extraction and SE-HPLC of Proteins

Wheat kernels were ground in a cyclone sample mill (Udy, Fort Collins, CO) using a 1-mm sieve. Protein was extracted from whole wheat flour as described by Gupta et al (1993) with minor modification (Ohm et al 2006). Extraction buffer was 1% SDS and 0.1*M* sodium phosphate buffer (pH 6.9). Flour (10 mg, 14% mb) was suspended in 1 mL of extraction buffer and stirred for 5 min at 2,000 rpm using a pulsing vortex mixer (Fisher Scientific) to solubilize SDS-extractable protein (EXP). The mixture was centrifuged for 15 min at 17,000 × *g* (Eppendorf Centrifuge 5424) and the supernatant was filtered through a 0.45 μm PVDF membrane (Sun Sri, Rockwood, TN). Immediately after filtering, the sample was then heated for 2 min at 80°C to suppress protease activity (Larroque et al 2000). The SDS-unextractable protein (UNP) was solubilized from the residue by 30 sec of sonication in 1 mL of buffer solution at the power setting of 10W output (Sonic Dismembrator 100, Fisher Scientific). The mixture was also centrifuged and filtered, and the filtered solution was heated as described for the EXP. SE-HPLC was performed using an chromatographer (1100 Series, Agilent Technologies, Santa Clara, CA). The 10 μL of EXP and UNP were separated by a narrow-bore size exclusion column (BIOSEP SEC S4000, Phenomenex, 300 × 4.5 mm, Torrance, CA) with guard cartridges (BIOSEP SEC S4000) (Batey et al 1991; Ohm et al 2009b). Proteins were eluted by 50% acetonitrile in water with 0.1% trifluroacetic acid at a flow rate of 0.5 mL/min and detected at 214 nm using a photodiode array detector (1200, Agilent Technologies, Santa Clara, CA). These experiments were duplicated and the mean values were used for data analysis.

### SE-HPLC Data Analysis

Absorbance data from SE-HPLC of protein extracts were analyzed using an in-house program (MATLAB 2008, The MathWorks, Natick, MA) (Ohm et al 2006, 2008, 2009a,b). Absorbance values were interpolated to 0.002-min intervals by a spline method in MATLAB. Absorbance area (AA) was calculated by mean absorbance by time interval of 0.002 min using the interpolated absorbance values. Data analysis was performed using the sum of AA for each retention time interval of 0.01 min between 3.6 and 7.7 min of runtime. The AA values for total proteins were mathematically estimated by adding AA values of EXP and UNP (Ohm et al 2009b). Absorbance area percentage (A%) values was also calculated for each retention interval of 0.01 min over the total AA (Ohm et al 2006). Simple linear correlation coefficient ($r$) was calculated between quality parameters and AA and A% values and presented as a continuous spectrum over retention time. SE-HPLC profiles were divided into five fractions: F1 (3.6–4.3 min), F2 (4.3–6.0 min), F3 (6.0–6.5 min), F4 (6.5–6.9 min), and F5 (6.9–7.7 min) (Morel et al 2000; Samson et al 2006; Ohm et al 2009b). Larroque et al (1997) showed electrophoresis patterns of protein fractions separated by SE-HPLC. Primary components of each fraction were HPP for F1; low molecular weight polymeric proteins for F2; ω-gliadin for F3; γ-, β-, and α-gliadins for F4; and albumin and globulins for F5 (Larroque et al 1997; Morel et al 2000; Samson et al 2005). The A% values of these five protein fractions were converted into percentage values based on wheat weight (% wheat) and total protein (% protein) (Park et al 2006).

### Statistical Analyses

All HPLC experiments were duplicated and statistical analysis was performed using the SAS System for Windows (v.9.1, SAS Institute, Cary, NC). Experimental design was randomized complete block design considering growing locations as replicates. Simple linear correlation and partial correlation coefficients were calculated using the CORR procedure in SAS. Uniformity was estimated by calculating variance values for protein fractions and single kernel characteristics among three kernel subsamples separated by an SKNIR sorter within individual wheat samples. Levene's test was performed to evaluate difference in the intrasample variance values of protein fractions and single kernel characteristics between wheat samples.

## RESULTS AND DISCUSSION

Variations in quality characteristics of wheat samples used in the current experiment were within the range typically found in hard spring wheat (Table I). Mean values of farinograph parameters were lower than those reported by Maghirang et al (2006) due

to the difference in flour characteristics or farinograph bowl size. For example, Maghirang et al (2006) used a 10-g bowl while a 50-g bowl was used in this experiment. Wheat and flour protein contents had significant ($P < 0.05$) $r$ values with PVKC but low $r$ values with SKCS hardness index and test weight (Table II). Vitreous kernels generally have higher protein content than mealy kernels in bread and durum wheat (Dexter et al 1989; Gianibelli et al 1991; Samson et al 2005). Flour protein content was also correlated with farinograph water absorption, arrival time and peak time, bake water absorption, and bread loaf volume, specifically suggesting that protein quantity affected mixing characteristics in this experiment. Test weight was positively correlated with SKCS hardness index and PVKC, indicating that harder and more vitreous kernels had a higher bulk density (Ohm et al 1998; Ohm and Chung 1999; Dobraszczyk et al 2002; Samson et al 2005). PVKC and SKCS hardness index seemed to be helpful for estimation of optimum water absorptions because they had greater $r$ values with farinograph and bake water absorptions than protein content in this experiment.

### Relationships of Kernel Characteristics with SE-HPLC Data

Although protein content did not have a significant correlation to the SKCS hardness index (Table II), variation in protein MWD was significantly associated with kernel hardness parameters. Kernel hardness parameters had significant ($P < 0.05$) and positive $r$ values with A% values of F3 in EXP and negative $r$ values with the A% values of the monomeric F5 in EXP (Fig. 1). These results indicate that high proportions of F3 and low proportions of F5 in wheat EXP could contribute to increasing kernel hardness in this sample set. These two protein fractions separated from total proteins by SE-HPLC were also indentified as affecting kernel hardness in soft winter wheat (Ohm et al 2006, 2009a).

The AA values of EXP that eluted around the F3 section had significant ($P < 0.01$) and positive $r$ values with PVKC (Fig. 2A). The A% values of monomeric gliadin (F3) and soluble protein fractions (F5) that had significant $r$ values with kernel hardness parameters (Fig. 1) were also significantly correlated with PVKC (Fig. 2B). This result suggests tha±t significant associations between the kernel hardness parameter and PVKC observed in this experiment (Table II) were directly related to the variations of these two protein fractions. Gianibelli et al (1991) and Samson et al (2005) also found that vitreous kernels had greater kernel hardness and contained proteins composed of higher proportions of gliadins and lower proportions of soluble proteins such as albumins and globulins when compared with mealy kernels. HPP that eluted around the F1 section had contrasting relationships with PVKC according to solubility in SDS buffer. HPP in UNP seemed to contribute positively to PVKC, while a high proportion of HPP in EXP had a negative influence as PVKC had negative $r$ values for A% values of HPP in EXP (Fig. 2B) and positive $r$ values for AA values for HPP in UNP (Fig. 2C).

### Mixing and Baking Parameters and SE-HPLC Data

The protein fractions that were associated with kernel characteristics also had significant association with mixing and breadmaking parameters. Protein fractions that eluted around F3 and F4 were associated with optimum water absorption (Fig. 3). SE-HPLC AA values of those fractions in EXP had a maximum $r$ of 0.88 for farinograph water absorption and 0.91 for bake water absorption. Variation in water absorption was primarily associated with quantitative variations of protein as was also confirmed by a significant $r$ with protein content in this experiment. Results obtained in this experiment indicate that the quantitative variation in gliadins was most likely responsible for the variation in water absorption. Ohm et al (2009b) also found that farinograph water absorption was significantly associated with gliadins in another hard spring wheat sample set. SE-HPLC A% values of EXP also had significant $r$ values with water absorption parameters (Fig. 3).

**TABLE I**
**Means and Standard Deviations (SD) of Quality Parameters**

| Quality Characteristics | Mean | SD |
|---|---|---|
| Test weight (lb/bu) | 60.5 | 1.6 |
| NIR hardness score | 75.9 | 9.2 |
| Single kernel characteristics | | |
| Hardness index | 74.0 | 8.5 |
| Hardness index SD[a] | 15.6 | 2.7 |
| Diameter (mm) | 2.33 | 0.13 |
| Diameter SD[a] | 0.40 | 0.06 |
| Weight (mg) | 32.5 | 2.1 |
| Weight SD[a] | 9.1 | 1.2 |
| Vitreous kernel (%) | 44.9 | 24.9 |
| Wheat protein (14% mb) | 14.3 | 0.8 |
| Flour yield (%) | 77.5 | 1.5 |
| Flour protein (14% mb) | 13.6 | 0.9 |
| Flour ash (14% mb) | 0.66 | 0.06 |
| Farinograph characteristics | | |
| Water absorption (14% mb) | 64.6 | 3.8 |
| Arrival time (min) | 3.4 | 0.8 |
| Peak time (min) | 6.4 | 1.1 |
| Dough stability (min) | 7.0 | 1.6 |
| Mechanical tolerance index (BU) | 37.6 | 9.8 |
| Time to breakdown (min) | 10.5 | 1.6 |
| Bake water absorption (14% mb) | 64.5 | 2.9 |
| Bread loaf volume ($cm^3$) | 890.2 | 84.1 |

[a] Standard deviation of single kernel characteristics ($n = 300$).

**TABLE II**
**Simple Linear Correlation Coefficients Between Wheat Kernel and Flour Quality Characteristics[a,b]**

| Quality Characteristics | Protein (14% mb) | | Test Wt | | |
|---|---|---|---|---|---|
| | Wheat | Flour | (lb/bu) | SKHI | VK |
| Flour protein | 0.98*** | – | | | |
| Test weight | ns | ns | – | | |
| SKHI | ns | ns | 0.48* | – | |
| VK | 0.59** | 0.58** | 0.82*** | 0.67** | – |
| Farinograph | | | | | |
| WA | 0.63** | 0.60** | 0.45* | 0.74*** | 0.74*** |
| Arr time (min) | 0.78*** | 0.80*** | ns | ns | |
| Peak time (min) | 0.49* | 0.47* | ns | ns | 0.56** |
| Breakdown (min) | ns | ns | ns | ns | 0.50* |
| Bake WA | 0.73*** | 0.70*** | 0.54* | 0.64** | 0.81*** |
| Bread LV ($cm^3$) | 0.80*** | 0.80*** | ns | ns | 0.61** |

[a] *, **, and *** indicate significance at $P < 0.05$, 0.01, and 0.001, respectively; ns indicates not significant at $P < 0.05$.
[b] SKHI, single kernel hardness; VK, vitreous kernel (%); WA, water absorption (14% mb); LV, loaf volume; .

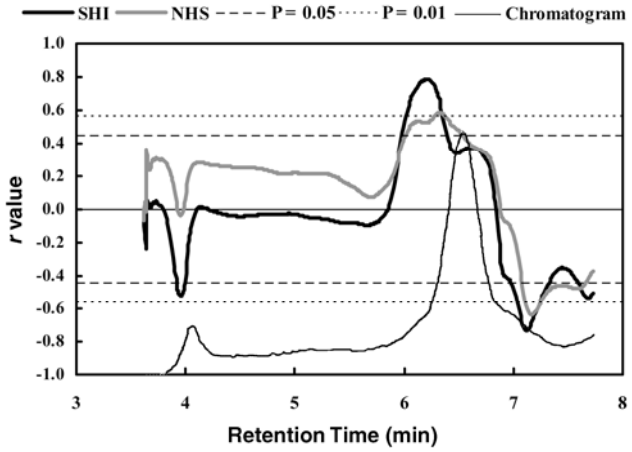


**Fig. 1.** Correlation coefficients ($r$) of size-exclusion HPLC absorbance area % values of SDS-extractable proteins with near-infrared hardness score (NHS) and single kernel hardness index (SHI).

Specifically, A% values of F1, F3, and F5 that were associated with kernel hardness parameters and PVKC (Fig. 1) also had significant *r* values with water absorption parameters. These results indicate that variations in the proportion of gliadin fractions in total protein also affected water absorption possibly due to relationships with kernel hardness and PVKC. Specifically, kernel hardness has a significant association with water absorption in relation to damaged starch granules (Pomeranz et al 1984).

Greater quantities and proportions of EXP eluted in F3 seemed to contribute to the increased mixing time and bread loaf volume as well as PVKC and water absorption. AA and A% values of EXP eluted around the F3 section had significant ($P < 0.05$) and positive *r* values with farinograph peak time and bread loaf volume (Fig. 4). The F3 in EXP consist primarily of ω-gliadins (Larroque et al 1997; Morel et al 2000) of which quantitative variation has a positive association with loaf volume but a negative influence on mixing times (Khatkar et al 2002; Uthayakumaran et al 2002). The positive correlations that occurred between the F3 and mixing time in this experiment were most likely confounded by the effects of other proteins (Uthayakumaran et al 2002).

A% values of EXP eluted between the retention time interval 5.6–5.9 min had negative *r* values with farinograph peak time. The A% values of HPP in EXP also had negative *r* values with farinograph peak time and bread loaf volume (Fig. 4B), indicating that a high proportion of polymeric EXP in total proteins negatively affected mixing time and loaf volume. A high proportion of soluble monomeric fractions (albumins and globulins) in protein was detrimental to wheat breadmaking quality because the A% values of EXP that eluted at the front of the F5 section had negative *r* values with water absorption, mix time, and bread loaf volume (Fig. 4).

In contrast with the polymeric proteins in EXP, HPP in UNP had positive *r* values with farinograph peak time and bread loaf volume (Fig. 5) that are in agreement with the results of other studies (Gupta et al 1993; Borneo and Khan 1999; Park et al 2006; Tsilo et al 2010). Quantity and properties of SDS, or acetic acid unextractable glutenin proteins (referred to as gluten macropolymer) are important predictors of breadmaking quality (Don et al 2006). Specifically, results obtained from this research indicated that quantitative variations of SDS-unextractable HPP in wheat had greater positive effects on mixing time and bread loaf volume than other polymeric protein fractions (Tsilo et al 2010).

### Partial Correlation Between Protein Fractions and Quality Characteristics

Wheat protein content greatly affects variations in protein fractions and certain quality characteristics (Table II). The complex

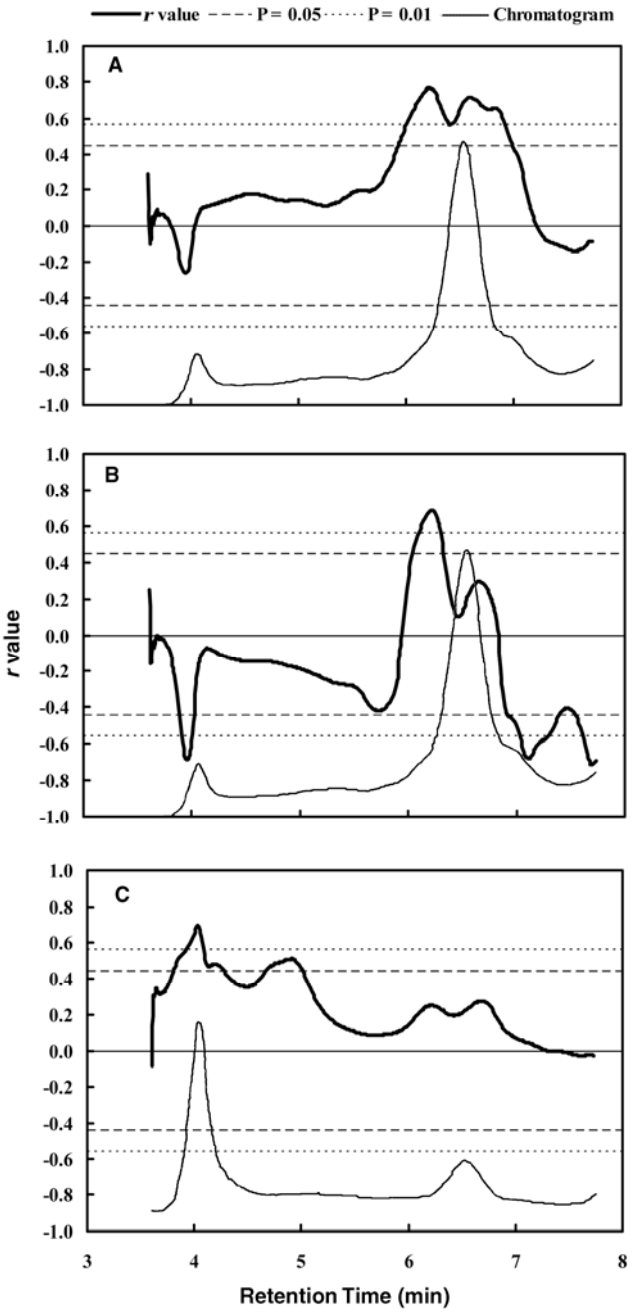


**Fig. 2.** Correlation coefficients (*r*) of % vitreous kernel content with size exclusion HPLC absorbance area (**A**) and area % (**B**) values of SDS-extractable proteins, and area values of SDS-unextractable proteins (**C**).

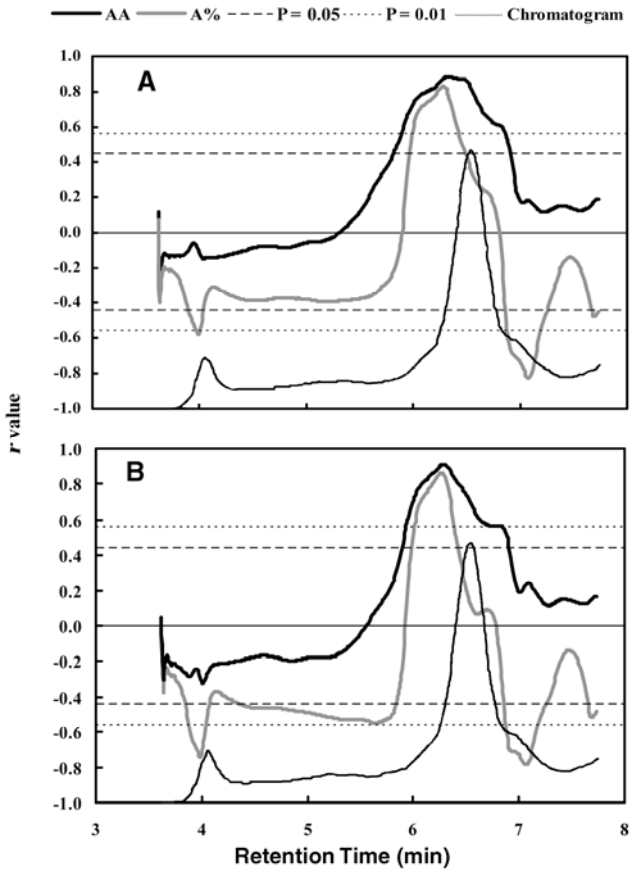


**Fig. 3.** Correlation coefficients (*r*) of size-exclusion HPLC absorbance area (AA) and area % (A%) values of SDS-extractable proteins with farinograph (**A**) and bake water absorption values (**B**).

interrelated associations prevented estimating the effect of individual protein fractions on quality characteristics. Partial *r* values between quality parameters and SE-HPLC data of protein fractions were calculated, statistically removing effect of quantitative variation of proteins in wheat (Table III). Partial *r* values revealed associations of quality characteristics with protein fractions at an equivalent level of wheat protein content, and aided in resolving the qualitative effect of protein fractions on wheat quality traits. Samson et al (2005) reported that kernel hardness and vitreousness had different responses to variations in proteins. Partial correlation also indicated differences in associations of protein fractions with kernel hardness and vitreousness in the current experiment. The F3 in EXP had significant partial *r* values with kernel hardness parameters but a low *r* value with PVKC. This result indicated that a greater quantity of extractable F3 equivalent to wheat protein content would contribute to increasing kernel hardness but significant simple *r* values that were estimated between F3 and PVKC (Fig. 2) most likely occurred due to interdependent associations of PVKC and the F3 with protein content (Table II). The F4 in EXP, which primarily consisted of gliadins (Larroque et al 1997; Morell et al 2002), had significant partial *r* values with both SKCS hardness index and PVKC.

Other researchers found that vitreous kernels had a greater proportion of gliadins than mealy kernels in bread wheats (Gianibelli et al 1991) and durum wheats (Dexter et al 1987; Samson et al 2005). Taken together, these results indicated that kernel hardness was primarily affected by proportional variations of SDS-extractable F3 and F5 fractions based on total protein content and kernel vitreousness was primarily associated with quantitative and proportional variations of SDS-extractable F4 and F5 fractions, and unextractable HPP.

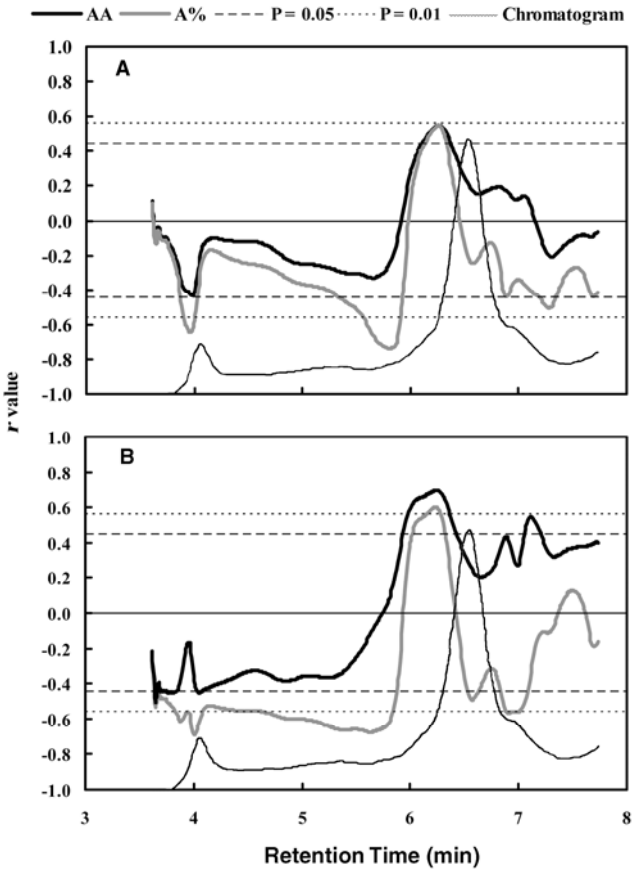


**Fig. 4.** Correlation coefficients (*r*) of size-exclusion HPLC absorbance area (AA) and area % (A%) values of SDS-extractable proteins with farinograph peak time (**A**) and bread loaf volume (**B**).

The F3 in EXP had significant partial *r* values with water absorption values but did not have a significant *r* value with farinograph peak time and bread loaf volume (Table III). Thus, the F3 fraction contributed to increasing water absorption but the significant associations with mixing time and loaf volume (presented in Fig. 5) could also be derived from interdependent associations with wheat protein content. Extractable F4 had a positive *r* value with farinograph water absorption but a negative *r* value with bread loaf volume, suggesting that greater quantity of F4 equivalent to protein content could have a detrimental influence on increased bread loaf volume.

Uthayakumaran et al (2002) also reported that a high proportion of gliadins in total protein had a detrimental effect on loaf volume. Water absorption values had a significant negative partial *r* value with F5% based on wheat weight. Farinograph peak time also had a significant and negative correlation with the F5% based on total protein, confirming that high proportion of soluble monomeric protein could be detrimental to breadmaking.

The F1 in UNP had a significant partial *r* value with farinograph peak time and loaf volume (Table III), also confirming that HPP in UNP should contribute to increasing breadmaking quality independently of quantitative variation of total wheat protein. The F1 in EXP did not have a significant partial *r* value with breadmaking characteristics (data not shown), suggesting that significant associations shown in Fig. 4 could be masked by variations in wheat protein content in partial correlation analysis. A% value of extractable F1 had a significant *r* value of –0.62 ($P < 0.01$) with wheat protein content that also had significant and positive r values with mixing times and loaf volume (Table II).

### Protein MWD and SKCS Characteristics of Kernel Subsamples

As expected, wheat protein content was significantly different between the kernel subsamples (Table IV). HPLC protein fractions were also significantly different ($P < 0.05$) in mean % wheat values between kernel subsamples. Quantity of all HPLC protein fractions in wheat was increased as protein content of kernel subsample increased. Quantitative variation of wheat protein by environmental effect is preferentially related to changes in monomeric gliadins (Saint Pierre et al 2008). The gliadin-rich F4 fraction in EXP also showed larger variation in % values based on wheat weight, which consequently resulted in larger variation in % values based on total protein than other protein fractions among kernel subsamples in this experiment. Samson et al (2005) reported

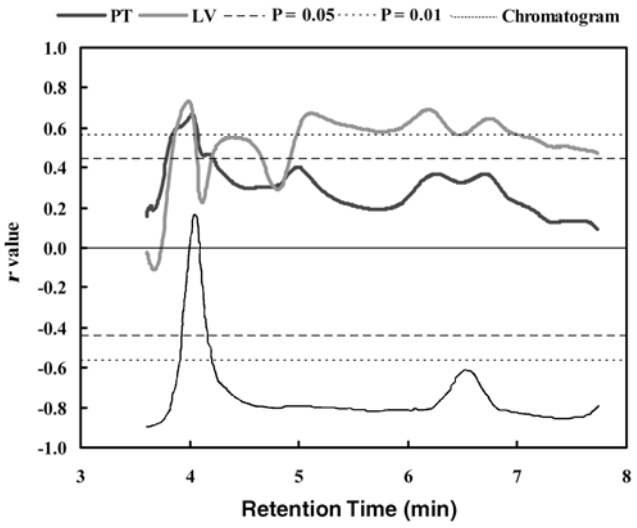


**Fig. 5.** Correlation coefficients (*r*) of size-exclusion HPLC absorbance area values of SDS unextractable proteins with farinograph peak time (PT) and bread loaf volume (LV).

similar results that the gliadin protein fraction showed the largest quantitative difference among protein fractions between vitreous and mealy kernels within a durum wheat cultivar. The F5 in EXP fraction that primarily consisted of water and salt-soluble proteins, (Larroque et al 1997; Morel et al 2000) showed smaller quantitative variation than other fractions in response to changes of protein content in kernel subsamples that resulted in decrease of % protein values as protein content of subsample increased (Saint Pierre et al 2008).

These results indicate that variation in single kernel protein content measured by the SKNIR sorter is associated with variation in protein MWD even within a wheat sample. As variation in wheat protein MWD is significantly related with quality parameters such as mixing and breadmaking characteristics, variation in single kernel wheat protein content is also most likely to have significant association with those parameters. Therefore, analysis of single kernel protein content using SKNIR sorter is expected to aid segregation of wheat samples that have uniform intrasample distribution of mixing and breadmaking characteristics in wheat breeding and industry.

Percent protein values of F3 in EXP that had significant *r* values with kernel hardness parameters were not significantly different among kernel subsamples. Although the single kernel hardness index increased as the protein content of kernel subsamples increased, the difference among the kernel subsamples was much smaller than that observed among wheat samples with a range of 62–82 for single kernel hardness index (Table IV). In addition, kernel hardness was not greatly affected by quantitative variation but by the variation of percent extractable F3 in total protein.

Variance values estimated for protein fractions and single kernel characteristics from kernel subsamples within individual samples are summarized as ranges among wheat samples (Table IV). Levene's *F*-test indicated that intrasample variance values for protein content and protein fractions were not significantly ($P < 0.05$) different between samples except for % protein values for the F2 and F5 in EXP and the F2 in UNP. Therefore, wheat samples tested in this experiment had fairly similar intrasample uniformity in protein quantity and composition. However, the intrasample variance values for single kernel hardness index and weight were significantly ($P < 0.05$) different between wheat samples.

**TABLE III**
**Partial Correlation Coefficients Between Wheat Kernel and Flour Quality Characteristics, and Protein Fractions Separated by Size Exclusion HPLC[a]**

| | Kernel Hardness | | Vitreous | Farinograph | | Baking | Bread |
|---|---|---|---|---|---|---|---|
| Protein Fraction[b] | NIR | SKCS | Kernel (%) | WA (%) | Peak Time (min) | WA (%) | LV ($cm^3$) |
| SDS extractable | 0.72*** | 0.78*** | | | | | |
| F3 (%W) | 0.72*** | 0.67** | ns | 0.87*** | ns | 0.72*** | ns |
| (%P) | ns | 0.56* | ns | 0.76*** | ns | 0.68** | ns |
| F4 (%W) | ns | 0.46* | 0.55* | 0.59** | ns | ns | –0.52* |
| (%P) | ns | ns | 0.47* | 0.52* | ns | ns | –0.52* |
| F5 (%W) | –0.56* | –0.59** | –0.52* | ns | —0.56* | ns | ns |
| (%P) | | | –0.67** | –0.50* | ns | –0.53* | ns |
| SDS unextractable | ns | ns | | | | | |
| F1 (%W) | ns | ns | 0.59** | ns | 0.60** | ns | 0.49* |
| (%P) | ns | ns | ns | ns | 0.67** | ns | 0.58** |

[a] Partial variable, protein content; *, **, and *** indicate significance at $P < 0.05$, 0.01, and 0.001, respectively; ns, not significant at $P < 0.05$. NIR, near infrared spectroscopy; SKCS, single kernel characterization system; WA, water absorption (14% flour mb); LV, loaf volume.
[b] F1–F5, protein fractions. %W, percentage of protein fraction based on wheat weight; and %P, percentage of protein fraction based on total protein.

**TABLE IV**
**Mean Values of Wheat Protein and Kernel Parameters for Kernel Subsamples of Low, Medium, and High Protein Levels and Intrasample Variance Ranges**

| | Subsample Protein Level[b] | | | Intrasample Variance | | |
|---|---|---|---|---|---|---|
| Quality Characteristics[a] | Low | Medium | High | Min | Max | Levene's *F*-Value[c] |
| WPC (%) | 13.1c | 14.2b | 15.3a | 0.681 | 2.036 | 0.57ns |
| SDS extractable | | | | | | |
| F1 (%W) | 0.76c | 0.86b | 0.91a | 0.004 | 0.013 | 0.67ns |
| (%P) | 5.76b | 6.03a | 5.99a | 0.010 | 0.060 | 0.90ns |
| F2 (%W) | 2.17c | 2.4b | 2.6a | 0.019 | 0.131 | 1.39ns |
| (%P) | 16.50b | 16.89ab | 17.04a | 0.003 | 1.293 | 3.53** |
| F3 (%W) | 2.24c | 2.44b | 2.58a | 0.013 | 0.085 | 1.23ns |
| (%P) | 17.01a | 17.1a | 16.93a | 0.001 | 0.413 | 2.24ns |
| F4 (%W) | 2.99c | 3.27b | 3.68a | 0.066 | 0.238 | 0.61ns |
| (%P) | 22.76b | 22.98b | 24.09a | 0.184 | 1.506 | 1.21ns |
| F5 (%W) | 2.01c | 2.02b | 2.08a | 0.000 | 0.018 | 3.02* |
| (%P) | 15.30a | 14.3b | 13.6c | 0.326 | 1.703 | 1.13ns |
| SDS unextractable | | | | | | |
| F1 (%W) | 1.51c | 1.69b | 1.79a | 0.014 | 0.043 | 0.73ns |
| (%P) | 11.50a | 11.83a | 11.7a | 0.020 | 0.315 | 1.20ns |
| F2 (%W) | 0.85b | 0.91ab | 0.94a | 0.001 | 0.025 | 1.95ns |
| (%P) | 6.50a | 6.42a | 6.19a | 0.002 | 2.408 | 3.62** |
| Single kernel | | | | | | |
| Hardness index | 71.3c | 74.1b | 76.1a | 1.158 | 48.323 | 2.53* |
| Diameter (mm) | 2.34ab | 2.37a | 2.31b | 0.000 | 0.010 | 1.98ns |
| Weight (mg) | 32.1b | 33.1a | 32.5ab | 0.003 | 4.323 | 2.59* |

[a] WPC, wheat protein content (14% mb); F1–F5, protein fractions separated by size-exclusion HPLC; %W, percentage of protein fraction based on wheat weight; and %P, percentage of protein fraction based on total protein.
[b] Mean values follwed by the same letter are not significantly different within the same row.
[c] *, and ** indicate significance at $P < 0.05$, and 0.01, respectively; ns, not significant at $P < 0.05$.

## CONCLUSIONS

Protein fractions that had significant associations with quality characteristics were identified based on correlation spectrum estimated with individual HPLC AA and A% values at 0.01 min of retention time. Furthermore, partial correlations revealed that certain protein fractions affected quality characteristics independently of quantitative variation of total wheat protein. HPP in UNP had a positive effect on PVKC, mixing time, and bread loaf volume. However, a high proportion of extractable HPP in total protein had negative effects on PVKC, mixing time, and bread loaf volume, which seemed to be associated with quantitative variations in total wheat protein due to insignificant ($P > 0.05$) partial $r$ values between them.

Gliadin fractions of EXP that primarily consisted of ω-gliadins positively affected variations in kernel hardness and water absorption parameters, mixing time, and loaf volume. Their associations with mixing time and loaf volume may be confounded with the effects of other proteins due to insignificant partial correlations. The main gliadin fraction had positive correlations with PVKC and water absorption, but a high proportion of the gliadin fraction in total protein was detrimental to an increase in bread loaf volume. A high proportion of albumin and globulin fractions in total proteins had negative effects on kernel hardness parameters, PVKC, water absorption, and mixing times. These results indicate that SE-HPLC of protein extracted from wheat grain is effective in evaluating breadmaking quality. Specifically, the protein fractions that had significant partial correlations are most likely to supplement wheat protein content in prediction of wheat breadmaking quality.

In a corresponding experiment, wheat kernels were separated into three subsamples according to protein content using an SKNIR sorter. The individual kernel subsamples were then analyzed for protein MWD to evaluate uniformity of kernel protein composition within a wheat sample. Kernel subsamples were significantly different for protein MWD within a sample. Specifically, the main gliadin protein fractions (F4) showed greater quantitative variations than other protein fractions among the three kernel subsamples. Hence, variation in single kernel protein content measured by an SKNIR sorter is associated with variation in protein MWD even within a wheat sample. As variation in wheat protein MWD is significantly related with quality parameters such as mixing and breadmaking characteristics, variation in single kernel wheat protein content is also most likely to have significant association with those parameters. Therefore, analysis of single kernel protein content using an SKNIR sorter is expected to aid further segregation of wheat samples that have uniform intrasample distribution of mixing and breadmaking characteristics in wheat breeding and industry.

Leven's $F$-test indicated that intrasample variance of protein MWD did not differ greatly between wheat samples in this experiment. However, wheat samples were significantly ($P < 0.05$) different in intrasample variance values for single kernel hardness index and weight. From all the results taken from this experiment, we conclude that SE-HPLC analysis of proteins extracted from whole wheat grain is effective for evaluating quality characteristics and their consistency or uniformity in hard spring wheat. Also, wheat single kernel characteristics and protein content may need to be evaluated using instruments such as the SKCS 4100 or SKNIR sorter to produce wheat cultivars wth uniform intrasample distribution.

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## LITERATURE CITED

AACC International. 2010. Approved Methods of Analysis, 11th Ed. Methods 08-01.01, 10-10.03, 39-70.02, 46-30.01, 54-21.01, 55-10.01, 55-31.01. Available online only. AACC International: St. Paul, MN.

Batey, I. L., Gupta, R. B., and MacRitchie, F. 1991. Use of high-performance liquid chromatography in the study of wheat flour proteins: An improved chromatographic procedure. Cereal Chem. 68:207-209.

Bean, S. R., Lyne, R. K., Tilley, K. A., Chung, O. K., and Lookhart, G. L. 1998. A rapid method for quantitation of insoluble polymeric proteins in flour. Cereal Chem. 75:374-379.

Bietz, J. A. 1984. Analysis of wheat gluten proteins by high-performance liquid chromatography. I. Baker's Dig. 58(1):15-17, 20-21, 32.

Borneo, R., and Khan, K. 1999. Protein changes during various stages of breadmaking of flour spring wheats. Cereal Chem. 76:711-717.

Bramble, T., Dowell, F. E., and Herrman, T. J. 2006. Single-kernel near-infrared protein prediction and the role of kernel weight in hard red winter wheat. Appl. Eng. Agric. 22:945-949

Ciaffi, M., Tozzi, L., and Lafiandra, D. 1996. Relationship between flour protein composition determined by size-exclusion high-performance liquid chromatography and dough rheological parameters. Cereal Chem. 73:346-351.

Dexter, J. E., Marchylo, B. A., MacGregor, A. W., and Tkachuk, R. 1989. The structure and protein composition of vitreous, piebald, and starchy durum wheat kernels. J. Cereal Sci. 10:19-32.

Dobraszczyk, B. J., Whitworth, M. B., Vincent, J. F. V., and Khan, A. A. 2002. Single kernel wheat hardness and fracture properties in relation to density and the modelling of fracture in wheat endosperm. J. Cereal Sci. 35:245-263.

Don, C., Mann, G., Bekes, F., and Hamer, R. J. 2006. HMW-GS affect the properties of glutenin particles in GMP and thus flour quality. J. Cereal Sci. 44:127-136.

Gianibelli, M. C., Arango, C., and Sarandon, S. J. 1991. Protein composition of vitreous and yellow berry bread wheat: Influence of nitrogen fertilization. Pages 765-771 in: Gluten Proteins 1990. W. Bushuk and R. Tkachuk, eds. AACC International: St. Paul, MN.

Giroux, M. J., Talbert, L., Habernicht, D. K., Lanning, S., Hemphill, A., and Martin, J. M. 2000. Association of puroindoline sequence type and grain hardness in hard red spring wheat. Crop Sci. 40:370-374.

Gupta, R. B., Khan, K., and MacRitchie, F. 1993. Biochemical basis of flour properties in bread wheats. I. Effect of variation in the quantity and size distribution of polymeric protein. J. Cereal Sci. 18:23-41.

Huebner, F. R., and Gaines, C. S. 1992. Relation between kernel hardness, environment, and gliadin composition. Cereal Chem. 69:148-151.

Khatkar, B. S., Fido, R. J., Tatham, A. S., and Schofield, J. D. 2002. Functional properties of wheat gliadins. I. Effects on mixing characteristics and bread making quality J. Cereal Sci. 35:299-306.

Larroque, O. R., Gianibelli, M. C., Batey, I. L., and MacRitchie, F. 1997. Electrophoretic characterisation of fractions collected from gluten protein extracts subjected to size-exclusion high-performance liquid chromatography. Electrophoresis 18:1064-1067.

Larroque, O. R., Gianibelli, M. C., Gomez Sanchez, M., and MacRitchie, F. 2000. Procedure for obtaining stable protein extracts of cereal flour and whole meal for SE-HPLC analysis. Cereal Chem. 77:448-450.

Maghirang, E. B., Lookhart, G. L., Bean, S. R. Pierce, R. O., Xie, F., Caley, M. S., Wilson, J. D. Seabourn, B. W., Ram, M. S., Park, S. H., Chung, O. K., and Dowell, F. E. 2006. Comparison of quality characteristics and breadmaking functionality of hard red winter and hard red spring wheat. Cereal Chem 83:520-528.

Morel, M.-H., Dehlon, P., Autran, J. C., Leygue, J. P., and Bar-L'Helgouac'h, C. 2000. Effects of temperature, sonication time, and power settings on size distribution and extractability of total wheat flour proteins as determined by size-exclusion high-performance liquid chromatography. Cereal Chem. 77:685-691.

Ohm, J. B., and Chung, O. K. 1999. Gluten, pasting, and mixograph parameters of hard winter wheats in relation to breadmaking. Cereal Chem. 76:606-613.

Ohm, J. B., Chung, O. K., and Deyoe, C. W. 1998. Single kernel characteristics of hard winter wheats in relation to milling and baking quality. Cereal Chem. 75:156-161.

Ohm, J. B., Ross, A. S., Ong, Y.-L., and Peterson, C. J. 2006. Using multivariate techniques to predict wheat-flour dough and noodle characteristics from size exclusion HPLC and RVA data. Cereal Chem. 83:1-9.

Ohm, J. B., Hareland, G., Simsek, S., and Seabourn, B. 2009a. Size exclusion HPLC of protein using a narrow-bore column for evaluation of

breadmaking quality of hard spring wheat flours. Cereal Chem. 86:463-469.

Ohm, J. B., Ross, A. S., Peterson, C. J., and Morris, C. F. 2009b. Relationships of quality characteristics with size-exclusion HPLC chromatogram of protein extract in soft white winter wheats. Cereal Chem. 86:197-203.

Ohm, J. B., Ross, A. S., Peterson, C. J., and Ong, Y.-L. 2008. Relationships of high molecular weight glutenin subunit composition and molecular weight distribution of wheat flour protein with water absorption and color characteristics of noodle dough. Cereal Chem. 85:123-131.

Park, S. H., Bean, S. R., Chung, O. K., and Seib, P. A. 2006. Levels of protein and protein composition in hard winter wheat flours and the relationship to breadmaking. Cereal Chem. 83:418-423.

Pomeranz, Y., Bolling, H., and Zwingelberg, H. 1984. Wheat hardness and baking properties of wheat flour. J. Cereal Sci. 2:137-143.

Saint Pierre, C., Peterson, C. J., Ross, A. S., Ohm, J., Verhoeven, M. C., Larson, M., and Hoefer, B. 2008. Winter wheat genotypes under different levels of nitrogen and water stress: Changes in grain protein composition. J. Cereal Sci. 47:407-416.

Samson, M. F., Mabille, F., Chéret, R., Abécassis, J. A., and Morel, M.-H. 2005. Mechanical and physicochemical characterization of vitreous and mealy durum wheat endosperm. Cereal Chem. 82:81-87.

Singh, N. K., Donovan, G. R., Batey, I. L., and MacRitchie, F. 1990a. 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.

Singh, N. K., Donovan, G. R., and MacRitchie, F. 1990b. Use of sonication and size-exclusion high performance liquid chromatography in the study of wheat flour proteins. II. Relative quantity of glutenin as a measure of breadmaking quality. Cereal Chem. 67:161-170.

Tsilo, T. J., Ohm, J. B., Hareland, G. A., and Anderson, J. A. 2010. Association of size exclusion HPLC of endosperm proteins with dough mixing and breadmaking characteristics in a recombinant inbred population of hard red spring wheat. Cereal Chem. 87:104-111.

Uthayakumaran, S., Tömösközi, S., Tatham, A. S., Savage, A. W. J., Gianibelli, M. C., Stoddard, F. L., and Bekes, F. 2002. Effects of gliadin fractions on functional properties of wheat dough depending on molecular size and hydrophobicity. Cereal Chem. 78:138-141.

Wilson, W. W., and Dahl, B. L. 2008. Procurement strategies to improve quality consistency in wheat shipments. J. Agric. Res. Econ. 33:69-86.

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