

Using Multivariate Techniques to Predict Wheat Flour Dough and Noodle Characteristics from Size-Exclusion HPLC and RVA Data

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ABSTRACT

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Flour proteins of hard and soft winter wheats grown in Oregon were characterized by size-exclusion HPLC (SE-HPLC). Flour pasting characteristics were assessed by a Rapid Visco Analyser (RVA). Principle component scores (PCS) were calculated from both RVA data and from absorbance area and % absorbance values from SE-HPLC. The PCS and cross-products, ratios, and squares were used to derive wheat classification and quality prediction models. A classification model calculated from PCS of SE-HPLC data could reliably separate these hard and soft wheats. The prediction models for mixing and noodle characteristics

showed better performance when calculated from PCS values of both SE-HPLC and RVA data than from SE-HPLC data only. The R^2 values of prediction models for mixograph absorption, peak time, and tolerance were 0.827, 0.813, and 0.851, respectively. Prediction models for noodle hardness, cohesiveness, chewiness, and resilience immediately after cooking had R^2 values of 0.928, 0.928, 0.896, and 0.855, respectively. These results suggest that multivariate methods could be used to develop reliable prediction models for dough mixing and noodle characteristics using just SE-HPLC and RVA data.

Wheat end-use properties are imprecisely determined by analyzing single components of flour; rather it requires observation of multiple components and interactions. In our laboratories, we routinely use a Rapid Visco Analyser (RVA-4, Newport Scientific, Warriewood, Australia) for flour pasting analyses and size-exclusion HPLC (SE-HPLC) analyses of wheat kernel proteins extracted using SDS-phosphate buffer with sonication. Both these techniques are relatively simple and do not require large sample amounts, analysis times, or extensive labor. To extend the utility of the techniques in our program, we applied multivariate analyses to develop improved prediction and classification models for targeted wheat end-use properties; these models also could be helpful for evaluating quality in wheat-breeding programs more generally.

A primary goal in our program was to develop wheat cultivars that are useful for the production of Asian noodles. It is necessary to observe more than protein-based parameters to understand, or predict, the full spectrum of quality characteristics in noodle end-use applications. In particular, flour or starch pasting or swelling characteristics are influential in determining Asian noodle texture. The predictive capacity of starch or flour pasting parameters for cooked noodle texture can be improved when a protein component is included with pasting or swelling data in multiple regression analyses. This has been shown for both salted (Konik et al 1993) and alkaline type noodles (Konik et al 1994; Ross et al 1997). Flour pasting and swelling characteristics are mainly influenced by starch properties (Konik et al 1994) such as amylose content (Zeng et al 1997). These pasting and swelling properties are useful in predicting texture of Asian noodles. In particular, flours with higher pasting peak viscosities and breakdown values produce softer and more cohesive noodles (Baik and Lee 2003; Guo et al 2003; Martin et al 2004; Sasaki et al 2004). Flour pasting characteristics are also related to breadmaking (Ohm and Chung 1999). In another study, breads from flours of hard spring Wx-B1 null type wheats that showed higher flour swelling properties had larger loaf volumes than loaves from hard spring Wx-B1 wild type wheats (Martin et al 2004). Given that models incorporating both protein and starch attributes were more effective as predictors of end-use performance, we opted to retain the multicomponent

attribute of the earlier models. However, we approached this issue with the idea that the prediction results for cooked noodle texture using multiple regressions and flour protein content presented by Konik et al (1993, 1994) and Ross et al (1997) could be improved by using a more refined analysis of the protein component along with more sophisticated multivariate statistical procedures. The multivariate procedures considered included principal component analysis and partial least squares, both of which have previously been used to calibrate prediction models of wheat end-use properties from near-infrared spectroscopy and conventional wheat quality data (Wikström and Bohlin 1996; Chung et al 2001, 2003; Sahlström et al 2003a,b). In addition to the prediction of end-use suitability, the distinction between the hard and soft classes is also important for white wheats in the marketing stream and in breeding programs. Huebner and Gaines (1992) reported that a gliadin fraction could be related to wheat kernel hardness, and significant correlations were observed between gluten characteristics and wheat kernel hardness parameters (Ohm and Chung 1999). These results suggest that SE-HPLC of wheat proteins might be used to categorize hard and soft white wheats.

This research was performed in an attempt to improve the evaluation of the quality of white winter wheats grown in Oregon, where wheats are mainly exported to Asian market. The main objective of this research was to investigate whether SE-HPLC chromatography of wheat proteins, in conjunction with RVA pasting parameters and multivariate analyses could provide useful predictions of dough mixing and noodle characteristics for hard and soft winter wheats. In addition, we investigated whether differences in SE-HPLC chromatograms of wheat proteins between hard and soft wheats were significant and could be applied to the development of a classification model that would reliably separate soft and hard white winter wheat classes.

MATERIALS AND METHODS

Eighteen white winter wheats were grown with two field replicates at Arlington, OR, in 2003 and included 15 hard white winter wheat elite lines and three soft white winter wheat cultivars from the Oregon State University breeding program. Soft wheats were originally included in the sample set for comparison with the hard wheats with respect to other aspects of the overall program goals of this research. They were retained in this iteration of the data analyses because they did not show any bias in model development and improved model performance.

Hardness index of wheat kernels was measured using a Single Kernel Characterization System (SKCS) (model 4100, Perten Instruments, Huddinge, Sweden) (Approved Method 55-31, AACC

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International 2000). Approximately 300 grains, free from nonwheat materials and defective and broken kernels were processed through the SKCS.

Wheat samples tempered to 15% moisture content were milled on an experimental mill (Quadrumat Sr., C. W. Brabender Instruments) (Approved Method 26-50, AACC International 2000). Soft wheats were milled using the same tempering condition as hard wheats because of their original inclusion as comparisons with hard wheats for other aspects of the overall program, which is mainly directed toward quality evaluation of hard white wheats. Flour protein content ($N \times 5.7$, 14% mb) was determined by a nitrogen determinator (Leco Corp., St. Joseph, MO) using Approved Method 46-30. Moisture content was determined by Approved Method 08-01 (AACC International 2000).

Pasting Characteristics

Flour pasting characteristics were measured by a Rapid Visco Analyser (RVA, Newport Scientific, Warriewood, New South Wales, Australia). Flour sample weight of 3.5 ± 0.05 g (14% mb) and 25 mL of 1 mM $AgNO_3$ in water were measured into aluminum RVA cans to make a total weight of 28.5 g. $AgNO_3$ was added to inhibit the small amount of activity of α -amylase present in sound grain (Crosbie et al 1999). The mixture was analyzed by the K-M 18-min noodle profile procedure as in Crosbie et al (2002). RVA parameters measured were pasting temperature, peak viscosity (PV), peak time, trough, breakdown, setback, and final viscosity.

Mixography

Mixography was performed using a 10-g bowl mixograph (National Mfg. Division, TMCO, Lincoln, NE) with optimum water absorption according to the procedure of Finney and Shogren (1972). Mixograms were obtained from flour (10 g, 14% mb) that was mixed with the optimum amount of distilled water for 8 min. Mixograph mixing time was determined on the mixograph. Midline peak time (MPT) was determined manually using an option in MIXSMART software (v. 3.80, National Mfg.). Mixing tolerance was also empirically graded by comparing with reference mixograms provided by the USDA ARS Western Wheat Quality Laboratory in which a score of 1 represents the weakest dough tolerance and 8 the strongest. Envelope right slope and midline band-

width at 6 min were other measures of mixing tolerance determined by MIXSMART (Ohm and Chung 1999; Chung et al 2001).

Salted Noodle-Making Procedure

Optimum water absorptions for noodle making were determined using the mixograph-based procedure described by Oh et al (1986). The "optimum" water addition was then validated by mixing flour (10 ± 0.05 g) and the predicted amount of water for 4 min in a 10-g pin mixer (National Mfg.). After mixing, the crumble was examined to see if it was too wet or too dry. If the dough was considered acceptable, the predicted optimum water addition was used for noodle making. If the dough crumble appeared too dry or too wet, the amount of water added was adjusted accordingly. Validation testing was repeated until the adjusted water addition gave dough with acceptable appearance and hand-feel as guided by an experienced noodle maker.

NaCl was dissolved in the appropriate amount of deionized water before addition to flour. Flour (100 ± 0.5 g) sample was dry mixed for 30 sec in a 200-g pin mixer (National Mfg.). Using a small rubber spatula, a well was made in the center of the flour mass in the bowl, and the salt solution in its entirety was poured into the well. Flour and salt solution were mixed for 1 min, the mixer stopped, and then the dough adhering to pins was scraped off with a rubber spatula, and the mixture was mixed again for 2.5 min. After resting 30 min in closed plastic bags, the crumble was compounded into a rough sheet using a noodle machine (Ohtake Mfg., Tokyo, Japan) with the roller gap set to 4 mm. The compounded sheet was folded once lengthways and compressed at the 4-mm gap in the same direction. This was repeated three times. After the dough sheet was rested for another 30 min, it was reduced in thickness through sequentially decreasing roll gaps of 3.5, 3.0, 2.0, and 1.5 mm. A final roll pass was made with the roll gap adjusted to give a final dough sheet thickness of 1.2 ± 0.05 mm (measured with Peacock thickness gauge). The final dough sheet was slitted through a no. 12 noodle slitter (2.5 mm width). The noodles were stored loosely in closed plastic bags at room temperature for 24 hr before cooking and subsequent texture measurement.

Noodles (50 g) were cooked in 500 mL of boiling deionized water for 6 min. After cooking, the noodle sample was rinsed with flowing tap water for 1 min and drained. The texture of cooked

TABLE I
Mean, Standard Deviation (SD), Minimum (Min), and Maximum (Max) Values of Kernel Hardness, Protein Content, Mixograph, and Noodle Characteristics

Quality Characteristics	Mean ($n = 36$)	SD	Min	Max
Single kernel hardness index	80.5	15.1	39.0	100.4
Protein (% , 14% mb)	11.12	1.8	9.1	13.9
Mixograph				
Water absorption (% , 14% mb)	62.7	2.8	56.9	67.7
Mix time (min)	2.7	0.7	1.5	4.5
Tolerance	3.7	1.2	2.0	6.0
Midline peak time (min) 3.0	0.7	1.7	4.6	
Bandwidth at 6 min	7.7	2.0	4.7	11.3
Envelope right slope	-6.8	2.4	-11.8	-2.6
Noodle characteristics				
1 min after cooking				
Hardness	731.0	54.2	624.6	851.4
Adhesiveness	-18.5	6.3	-35.6	-3.8
Springiness	0.948	0.150	0.926	0.988
Cohesiveness	0.665	0.190	0.576	0.697
Chewiness	460.4	30.2	396.0	524.8
Resilience	0.414	0.270	0.318	0.474
15 min after cooking				
Hardness	783.1	58.4	625.4	902.8
Adhesiveness	-25.8	7.7	-42.0	-11.3
Springiness	0.936	0.130	0.912	0.984
Cohesiveness	0.620	0.145	0.591	0.658
Chewiness	454.2	32.4	365.3	523.6
Resilience	0.350	0.246	0.302	0.401

noodle was tested by texture profile analysis using a texture analyzer (TA, TAXTPlus, Stablemicrosystems, UK). Testing was done immediately after draining the water from the noodles and after resting the noodles in an airtight container for 15 min. Thickness of the compression blade was 5 mm. Three strands of undamaged noodles of approximately the same length were tested at cross-head speed of 1.0 mm/sec and 70% strain on first compression. TPA parameters measured were hardness, adhesiveness, cohesiveness, springiness, chewiness, and resilience.

Extraction and SE-HPLC of Proteins

Flour proteins were extracted by the procedure of Morel et al (2000) with minor modifications. Flour sample (160 ± 0.05 mg, adjusted to 14% moisture content) was placed into 50-mL centrifuge tubes. To each sample, 20 mL of 1% SDS and 0.1M sodium phosphate buffer (pH 6.9) were added using a 10-mL automatic pipette. The mixture was sonicated (Fisher Scientific, sonic dismembrator 100) for 3 min at 30% (5W) power setting. After sonication, the mixture was heated in a water bath at 65°C for 30 min to inhibit protease activity and to stabilize the extract. The mixture was centrifuged for 40 min (Eppendorf centrifuge 5413) and the supernatant was filtered through a membrane filter (0.45- μ m HV Millipore DuraPore).

HPLC was performed using a Waters 2695 separations module (Waters, Milford, MA) according to the procedure of Batey et al (1991). A Phenomenex BIOSEP SEC S4000 size-exclusion column (600 \times 7.5 mm, Phenomenex, Torrance, CA) was used with a guard column (75 \times 7.5 mm). After injecting 20 μ L of sample, it was eluted using 50% acetonitrile in water with 0.1% trifluoroacetic acid for 30 min with a flow rate of 1 mL/min. Eluents were detected at 214 nm using a Waters 2996 photodiode array detector.

Data Analysis

All analyses were performed in duplicate for each of the 36 wheat samples. Mean values of duplicates for individual samples were used for data analyses, not the field replicate means.

The UV absorbance data of the SE-HPLC chromatography of protein extracts were transformed and analyzed using MATLAB (v. 6.5, The MathWorks, Natick, MA). Absorbance data were interpolated to retention time intervals of 0.01 min by the cubic spline interpolation methods in MATLAB. Using interpolated data, absorbance area (AA, absorbance \times sec) was calculated for each retention time interval of 0.05 min. Percentage of AA (A%) that could represent AA adjusted to the same level of protein content

was also calculated for each retention interval of 0.05 min over total AA.

Standardization of data was performed to transform data to have mean of 0 and variance of 1 by the equation: $(\chi_i - \mu)/\sigma$ where μ is the mean and σ is standard deviation of χ_i s. After standardization of AA and A% data, principal component analysis was performed using a chemometric software PLS_Toolbox (v. 2.1, Eigenvector Research, Manson, WA) that is a MATLAB-based program. Principal component scores (PCS) were also calculated from standardized values of RVA parameters. Twelve and five PCS were calculated and explained ~99% of variance in protein AA and A% data and RVA parameters, respectively (data not shown).

The PCS calculated from AA and A% data of protein extract were used to develop a model to classify hard winter wheats and soft winter wheats using SAS procedures (v. 8, SAS Institute, Cary, NC). The PCS values that were significant ($P < 0.05$) were included in the classification function by STEPDISC procedure of SAS. The performance of selected PCS values for classification was tested by cross-validation using DISCRIM procedure of SAS. In cross-validation, each sample was classified using a function that was computed again with selected PCS from the whole sample set, excluding the sample to be classified.

Prediction models were developed by continuum regression, using PLS_Toolbox and MATLAB softwares (Stone and Brooks 1990; Chung et al 2001). Data from all 36 wheat samples were used for development of prediction models because they were grown at different plots and could comprise environmental variation between blocks. Among PCS calculated from AA and A% data of protein extract and RVA parameters and transformed data including squares, cross-products, and ratios of PCS, a maximum of seven variables that showed higher correlation or partial correlation coefficients than others were selected as independent variables to develop prediction models and to prevent overfitting. Transformation of quality parameters that included square and cross-product terms of those parameters contributed to development of prediction models (Chung et al 2001), suggesting that nonlinear and interaction relationships could be important to explain relationships between quality components in addition to linear relationships.

Model performance was tested by cross-validation as described for classification analysis. The performance of a prediction model was also evaluated by coefficient of determination (R^2) and root mean square of error (RMSE). Analysis of variance was performed using SAS. Difference between hard and soft wheat was evaluated using contrast option in SAS.

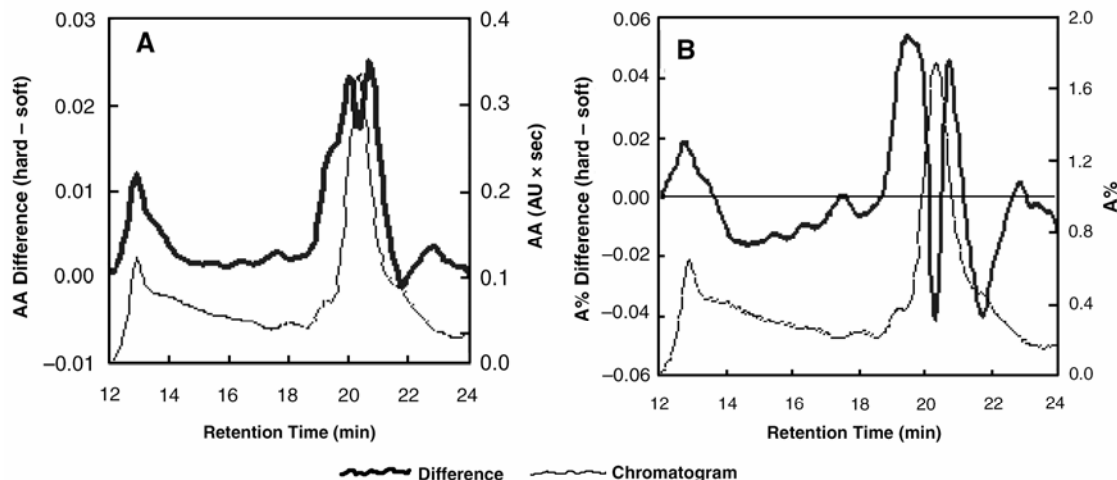


Fig. 1. Plot of difference of absorbance area (AA) (A) and area % (A%) (B) of size-exclusion HPLC of flour proteins between hard and soft white wheats. Differences were calculated as the values of hard wheats minus the values of the soft wheats. A typical SE-HPLC chromatogram of hard wheat is overlaid to assist interpretation.

RESULTS AND DISCUSSION

Classification of Hard and Soft Wheats Using SE-HPLC Chromatography

Mean, standard deviation, minimum, and maximum values of quality characteristics were shown in Table I. Kernel hardness index measured by SKCS showed mean of 80.5 with standard deviation of 15.1. The six soft wheat samples, which consisted of two replicates of Eltan, Stephens, and Madsen, showed a mean of 50.7 for hardness index with minimum of 39.0 and maximum of 59.0 in this sample set. Hardness indices of hard wheats had a mean of 86.5 and a range of 73.7–100.4.

Figure 1 shows the differences in AA and A% from SE-HPLC chromatography of proteins between hard and soft wheats over all retention times. The differences were calculated as the AA of the hard wheats minus the AA of soft wheats. The AA values represent quantitative variation in protein eluted at a given retention time. AA values of hard wheats were higher than those of soft wheats, except for protein fractions eluted at ≈ 21.8 min (Fig. 1A). This could be due to the higher protein content of hard wheat flours. Mean flour protein content was 11.3% (14% mb) for hard wheats and 10.3% (14% mb) for soft wheats. However, some hard wheats had similar or lower protein content than soft wheats. Protein fractions eluted at 18.8–21.4 min could be composed of mainly monomeric gliadins (Larroque et al 1997) and showed greater difference between hard and soft wheats when compared with other protein fractions. Protein fractions eluted between 12.4 and 14.3 min that include high molecular weight polymeric protein fractions (Larroque et al 1997) also showed high difference between hard and soft wheats. These results suggest that hard and soft wheats could have larger differences in quantities of monomeric gliadin protein and high molecular weight polymeric protein fractions than differences in other fractions.

The A% value that is the percentage of AA at a given retention time interval over total AA represents variation in protein compositions. For this group of samples, hard wheats showed higher mean A% values than soft wheats for the protein fractions eluted between 12.0 and 13.7, 18.8 and 20.1, and 20.6 and 21.2 min (Fig. 1B). For these samples, hard wheat had lower A% values for fractions between 13.8 and 18.7, 20.2 and 20.5, and 21.3 and 22.7 min than soft wheats. These results show that in this group of samples, hard wheat proteins contained a higher proportion of high molecular weight polymeric proteins that eluted between 12.0 and 13.7 min, but a lower proportion of low molecular weight polymeric proteins that eluted from 13.8 to 18.7 min.

Differences of AA and A% between hard and soft wheats were tested by analysis of variance (Fig. 2A). The F values were significant for all AA values, indicating that hard wheats had significantly higher mean AA values than soft wheats except for

protein fractions eluted from 14.2 to 17.0 and 21.4 to 22.3 min of retention time (Fig. 2A). Protein fractions that showed larger difference in AA values also showed higher F values. Protein fractions eluted from 19.0 to 19.7 min showed higher F values for AA than other protein fractions. These protein fractions also had much higher F values for A% than other protein fractions (Fig. 2A), suggesting that difference in quantity and % of the protein fractions eluted from 19.0 to 19.7 min could be most significant among protein fractions in determining the differences between these hard and soft wheats. The higher F values of A% for the 19.0 to 19.7 min protein fractions also suggests that, as we might intuitively reason, differences in protein composition are as significant, or more, than differences in quantity between hard and soft wheats, as illustrated by the chosen samples.

The protein fractions that had no significant difference in AA values between hard and soft wheats also showed high F values for A%. The high F values for these protein fractions could be caused by the lack of significant difference in their quantity that amplified the effects in the model of small changes in their proportions in the extracted proteins. These results suggest that monomeric gliadin protein fractions eluted from 19.0 to 19.7 min differ more significantly than other protein fractions between these hard and soft wheats. Huebner and Gaines (1992) also reported that a gliadin fraction could be related to wheat kernel hardness.

Varietal variations in AA and A% values were also tested by analysis of variance and the F values are shown in Fig. 2B. The F values of AA were statistically significant ($P < 0.05$) for all protein fractions except for those eluted after 23.3 min (Fig. 2B). The protein fractions eluted between 19.0 and 20.5 min had higher F values for AA than other protein fractions, suggesting that quantitative variations of these protein fractions were more significant than other protein fractions among cultivars in this sample set. The F values of A% were significant except for those protein fractions eluted from 22.4 to 22.9 min (Fig. 2B). The protein fractions eluted from 14.0 to 17.0 and 18.8 to 20.5 min showed higher F values for A% than AA. These results show that, in this sample set, varietal variations in quantity and proportions of many protein fractions separated by SE-HPLC are statistically significant and that some protein fractions could show more highly significant variations than other protein fractions.

The significant differences in protein fractions between these hard and soft wheats suggested that a function to classify hard and soft wheats could be developed using AA and A% data. Due to the large data size and high correlations between AA and A% values, principal component analysis was performed to compress data size and to overcome multicollinearity in modeling. Twelve principal component scores that explained 98.5% of variation in AA and A% (data not shown) were calculated and used for calculation of a classification model.

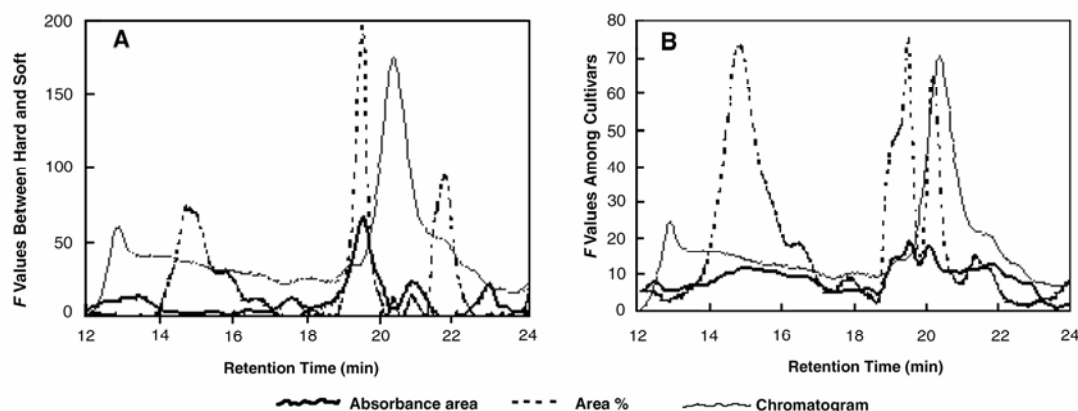


Fig. 2. F values calculated from analysis of variance between hard and soft white wheats (A) and among hard and soft cultivars (B). A typical SE-HPLC chromatogram is overlaid to assist interpretation.

Stepwise multiple discriminant analysis was performed using SAS software. Hard and soft winter wheats could not be classified by a single variable such as protein content or one PCS (Fig. 3A). The PCS 7 that showed partial R^2 of 0.365 (Table II) was selected as the most significant variable for classification but was still not enough for classification on its own (Fig. 3A).

Because PCS were calculated from standardized values of variables, the loadings indicate how the variables related each other and contribute to PCS. The loading values are not shown in this report due to the large size of data. The PCS loading indicated that PCS 7 is mainly related to protein fractions eluted from 22.1 to 22.3 min (data not shown). The PCS 7, 1, 6, and 5 and ratio of PCS 7 to PCS 5 were selected by stepwise multiple discriminant analysis for development of a classification function (Table II). The four PCS as well as the PCS ratio were significant at $P < 0.05$. The classification function was statistically significant at $P < 0.001$ and had an average square canonical correlation coefficient of 0.700. The developed function classified all of these hard and soft wheats without misclassification (Fig. 3B). These results suggest that as hard wheats are generally bred to have different protein composition than soft wheats, SE-HPLC chromatography could be used more broadly to classify hard and soft winter wheats, as they did successfully in this sample set.

Prediction of Flour Protein and Mixograph Parameters

Twelve PCS values calculated from AA and A% data and five PCS values calculated from RVA parameters and their transformed data were used to develop prediction models of protein content, mixograph parameters, and cooked noodle characteristics. The R^2 and RMSE values of prediction models for flour protein content

and mixograph parameters are summarized in Table III. Prediction model of protein content developed from SE-HPLC data showed R^2 values of 0.984 and 0.976 for model and cross-validation, respectively. This result confirms that variations in AA and A% in SE-HPLC analyses of flour proteins, when performed on a constant flour weight basis, explain quantitative variation in flour protein. This is a valuable tool for estimating flour protein when sample amounts and nitrogen analysis tools are limited.

The R^2 values of mixograph parameters of models calculated from AA and A% data had a range of 0.824–0.744. The R^2 values were 0.725–0.629 for the cross-validations that represent the performance of models (Table III, Fig. 4). Cross-validation also showed higher RMSE values than models. These R^2 values from AA and A% data indicated that prediction models explained over 60% of variations in mixograph parameters and could be used to estimate mixing characteristics.

The PCS loadings (data not shown) included in prediction equations indicated that mixograph parameters could have relationships with specific protein fractions eluted by SE-HPLC and their ratios. The PCS loadings indicated that protein fractions eluted from 21.6 to 23.6 min influenced variation in mixograph water absorption. Mixograph water absorption showed significant simple linear correlation coefficients (r) with AA ($r = 0.384$, $P < 0.05$) and A% ($r = 0.356$, $P < 0.05$) values of fractions from 21.6 to 23.6 min. The ratio of A% values of fractions from 13.2 to 18.6 min to fractions from 18.7 to 19.6 min had an r value of -0.346 ($P < 0.05$). These results suggest that there are two ways of detecting which protein fractions are more effective in determining flour water absorption than others, and that these fractions can be identified using multivariate techniques.

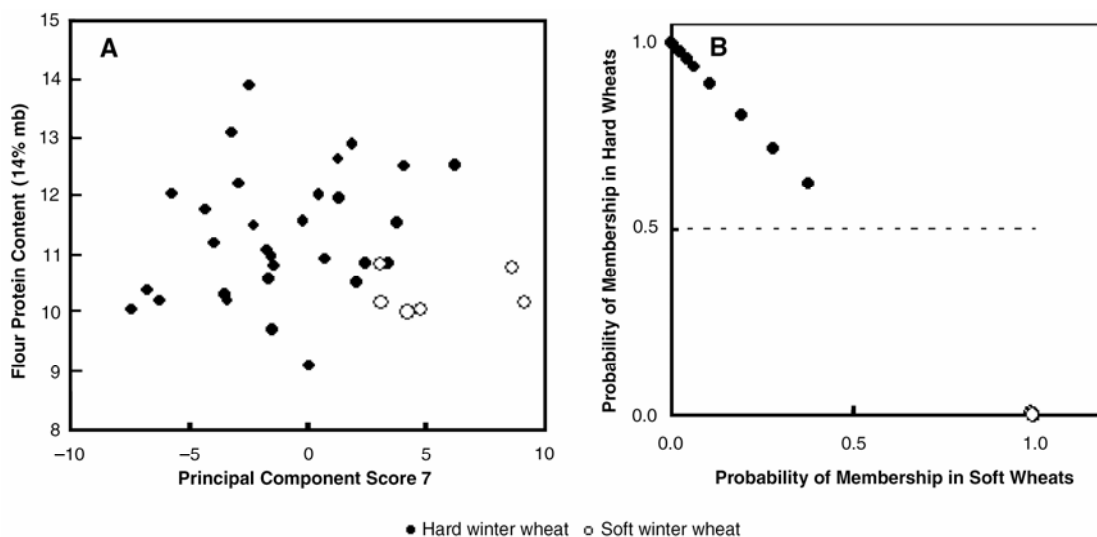


Fig. 3. Plot of flour protein content and principal component score 7 (A) and probability (B) of membership for each hard and soft wheat cultivars calculated from classification function developed using principal component scores calculated from absorbance areas and area % data of size-exclusion HPLC of flour proteins.

TABLE II
Results of Stepwise Multiple Discriminant Analysis

Step	Variable Entered ^a	Partial R^2	F -Value ^b	Pr > F ^c	ASCC ^d
1	PCS 7	0.365	19.53	<0.0001	0.365
2	PCS 1	0.172	6.83	0.013	0.474
3	PCS 6	0.196	7.79	0.009	0.577
4	PCS 5/PCS 7	0.167	6.23	0.018	0.648
5	PCS 5	0.149	5.26	0.029	0.700

^a Principle component score (PCS).

^b F statistic for entering the variable.

^c Probability level for the F statistic.

^d Average squared canonical correlation.

The PCS loadings also indicated that MPT was related to the very high molecular weight polymeric protein fraction that eluted at the front of chromatogram from 11.9 to 12.7 min. The A% of very high molecular weight fraction were positively correlated ($r = 0.389$, $P < 0.05$) with MPT. These results are consistent with the prevailing and widely supported hypothesis that HMW-GS form very high molecular weight glutenin macro polymers that have major influences on dough attributes. Varietal variation in relative amount of HMW-GS, specifically x -type, had significant correlations with dough development time (Wieser and Kieffer 2001). The influence of HMW-GS on resistance to dough extensibility was also shown by addition and incorporation studies (Verbruggen et al 2001). AA values of protein fractions eluted from 14.6 to 18.6 min had significant negative correlations with MPT ($r = -0.570$, $P < 0.01$) and tolerance ($r = -0.411$, $P < 0.05$), suggesting that these protein fractions in flour would contribute a negative term to any prediction equation and make the prediction more accurate.

The loadings of PCS included in equations indicated that ratios of protein fractions could contribute to prediction equation for MPT and midline bandwidth at 6 min. Specifically, the ratio of AA values of protein fractions that eluted from 11.9 to 13.2 min to those from 14.6 to 18.6 min were significantly correlated with MPT ($r = 0.406$, $P < 0.05$) and midline bandwidth at 6 min ($r = 0.376$, $P < 0.05$). The ratio of AA values of the protein fraction that eluted from 11.9 to 12.7 min to the protein fraction that eluted from 19.6 to 23.6 min also showed significant correlations with MPT ($r = 0.375$, $P < 0.05$) and midline bandwidth at 6 min

($r = 0.415$, $P < 0.05$). Protein fractions that eluted from 11.9 to 18.6 min mainly contain HMW-GS and LMW-GS and fractions from 19.6 to 23.6 min could contain gliadin proteins (Singh et al 1990a,b; Domenek et al 2002). Those significant correlations could be influenced by variations in HMW-GS and LMW-GS that influence molecular weight distribution of polymeric proteins. The ratios of quantities of protein subunits such as ratio of LMW-GS to HMW-GS and gliadins to x -type HMW-GS were reported to have significant correlations with dough development time (Uthayakumara et al 1999; Wieser and Kieffer 2001). These results suggested that ratios of specific protein fractions separated by SE-HPLC could contribute to the prediction of mixograph characteristics.

When PCS calculated from RVA parameters were included in the prediction equations, higher R^2 values were generated from the prediction models and cross-validations than those calculated from chromatography data alone, except MPT (Table III, Fig. 4). Although prediction models of water absorptions and midline bandwidth at 6 min from SE-HPLC and RVA data showed either equal or a little higher R^2 value than the model from SE-HPLC data only, tolerance, and envelope right slope showed both higher R^2 and lower RMSE values than those calculated from SE-HPLC data only.

The PCS loadings indicated that RVA PV could be related to variations in mixograph parameters. RVA PV had significant correlations with mixograph water absorptions ($r = 0.467$, $P < 0.01$), MPT ($r = 0.403$, $P < 0.05$), and envelope right slope ($r =$

TABLE III
Prediction of Flour Protein and Mixograph Characteristics Using SE-HPLC Chromatography and Rapid Visco Analyser (RVA)

Protein and Mixograph Characteristics	Model		Cross-Validation	
	R^2	RMSE ^a	R^2	RMSE ^a
SE-HPLC data only				
Protein (% , 14% mb)	0.984	0.2	0.976	0.2
Absorption (% , 14% mb)	0.824	1.3	0.725	1.5
Mix time (min)	0.770	0.4	0.635	0.4
Tolerance	0.744	0.7	0.629	0.7
Midline peak time (min)	0.819	0.3	0.673	0.4
Bandwidth at 6 min	0.782	1.0	0.701	1.1
Envelope right slope	0.809	1.2	0.697	1.3
SE-HPLC + RVA data				
Absorption (% , 14% mb)	0.827	1.3	0.752	1.4
Mix time (min)	0.813	0.4	0.711	0.4
Tolerance	0.851	0.5	0.796	0.5
Midline peak time (min)	0.819	0.3	0.682	0.4
Bandwidth at 6 min	0.797	1.0	0.640	1.2
Envelope right slope	0.841	1.1	0.734	1.2

^a Root mean square of error.

TABLE IV
Prediction of Noodle Texture (1 min after cooking) Using SE-HPLC Chromatography and Rapid Visco Analyser (RVA)

Noodle Characteristics	Model		Cross-Validation	
	R^2	RMSE ^a	R^2	RMSE ^a
SE-HPLC data only				
Hardness	0.855	23.0	0.794	24.3
Adhesiveness	0.744	3.5	0.626	3.8
Springiness	0.636	0.010	0.529	0.010
Cohesiveness	0.893	0.007	0.770	0.009
Chewiness	0.903	10.5	0.848	11.6
Resilience	0.799	0.013	0.694	0.015
SE-HPLC + RVA data				
Hardness	0.928	16.3	0.877	18.8
Adhesiveness	0.796	3.2	0.706	3.4
Springiness	0.623	0.010	0.483	0.011
Cohesiveness	0.928	0.006	0.874	0.007
Chewiness	0.896	10.9	0.858	11.2
Resilience	0.855	0.011	0.788	0.012

^a Root square of error.

0.542, $P < 0.01$), suggesting that components influencing peak viscosity could also have effect on mixing properties. Waxy wheat flour was reported to have longer farinograph arrival and development time than Chinese Spring flour and high amylose flour (Morita et al 2002). These results show that inclusion of RVA data marginally improved performance for prediction equations of mixograph parameters, and that in this sample set, flour pasting characteristics were related to mixing properties.

Similarly, RVA parameters have been reported to improve prediction of bread characteristics (Ohm and Chung 1999; Sahlström et al 2003a,b), although this seems more intuitive than the relations of mixing and pasting parameters, as the starch has gelatinized in finished breads. The relationships between RVA pasting parameters and dough mixing seen here could be an artifact of sample set, related to changes in starch/protein concentrations, or reflect some characteristics of starch granule surface or size that varies with paste characteristics and affects dough mixing.

Prediction of Cooked Noodle Characteristics

Prediction equations were developed for noodle characteristics using the PCS derived from SE-HPLC and RVA data. Noodle characteristics were measured by texture profile analysis at 1 min after cooking. When prediction equations were calculated from only SE-HPLC data, they accounted for >85% of variation in noodle hardness, cohesiveness, and chewiness (Table IV), and this predictive capacity is probably a result of the relationship between AA and flour protein, and modulated by proportional differences in some protein fractions. The R^2 values of cross-validation for these characteristics were higher than or equal to 0.770, suggesting that SE-HPLC data could be used to develop prediction equation of these noodle characteristics (Table IV).

Noodle adhesiveness and resilience showed R^2 values of 0.744 and 0.799 for models and 0.626 and 0.694 for cross-validations, respectively. However, springiness showed lower R^2 values than other noodle characteristics, suggesting that prediction of springiness using SE-HPLC was difficult in this sample set.

The PCS loadings included in the prediction models indicated that AA values of polymeric protein fractions eluted from 13.2 to 18.6 min influenced variations in cooked noodle hardness ($r = 0.592$, $P < 0.001$) and chewiness ($r = 0.573$, $P < 0.001$). The PCS loadings also indicated that the AA value of monomeric protein fractions eluted from 21.6 to 23.6 min could contribute to developing prediction equations for noodle hardness ($r = 0.577$, $P < 0.001$) and chewiness ($r = 0.608$, $P < 0.001$). Positive correlations have been reported between flour protein content and hardness of cooked noodle (Ross et al 1997; Park et al 2003). The results in this experiment indicated that quantitative variations in specific protein fractions influenced cooked noodle hardness more than other protein fractions.

The AA values of protein fractions eluted from 23.6 to 24.6 min at the end of separation were observed to negatively influence hardness and chewiness. The A% of these protein fractions also had negative correlations with hardness ($r = -0.498$, $P < 0.01$) and chewiness ($r = -0.436$, $P < 0.01$), suggesting that higher proportions of these protein fractions in flour protein decreased noodle hardness and chewiness. These protein fractions are mainly composed of albumin and globulin (Singh et al 1990a; Domenek et al 2002). The proportion of salt soluble proteins in protein had negative correlations with noodle hardness (Park et al 2003). These results indicated that high percentage of LMW monomeric proteins eluted at last stage of SE-HPLC could decrease noodle hardness and chewiness when flour protein content is constant.

When PCS values calculated from RVA data were included in prediction equation, R^2 values were higher and RMSE values were lower than those developed from SE-HPLC only, except for noodle chewiness and springiness (Table IV, Fig. 5). Prediction models developed from SE-HPLC and RVA data could explain at least 80% of variations in noodle texture characteristics, except for

springiness (Table IV, Fig. 5). Cross-validation also showed R^2 values from 0.788 to 0.877. The prediction model for chewiness that was developed from both SE-HPLC and RVA data showed lower R^2 and higher RMSE than those from SE-HPLC only. But higher R^2 and lower RMSE values were obtained from cross-validation of prediction equation from SE-HPLC and RVA data than SE-HPLC only. This cross-validation result indicated that the prediction of chewiness could be more robust when prediction equation was calculated from both SE-HPLC and RVA data.

These results showed that RVA parameters in addition to SE-HPLC data could contribute to improving accuracy and performance of prediction models of cooked noodle characteristics, except for springiness. This improvement in prediction could be due to the

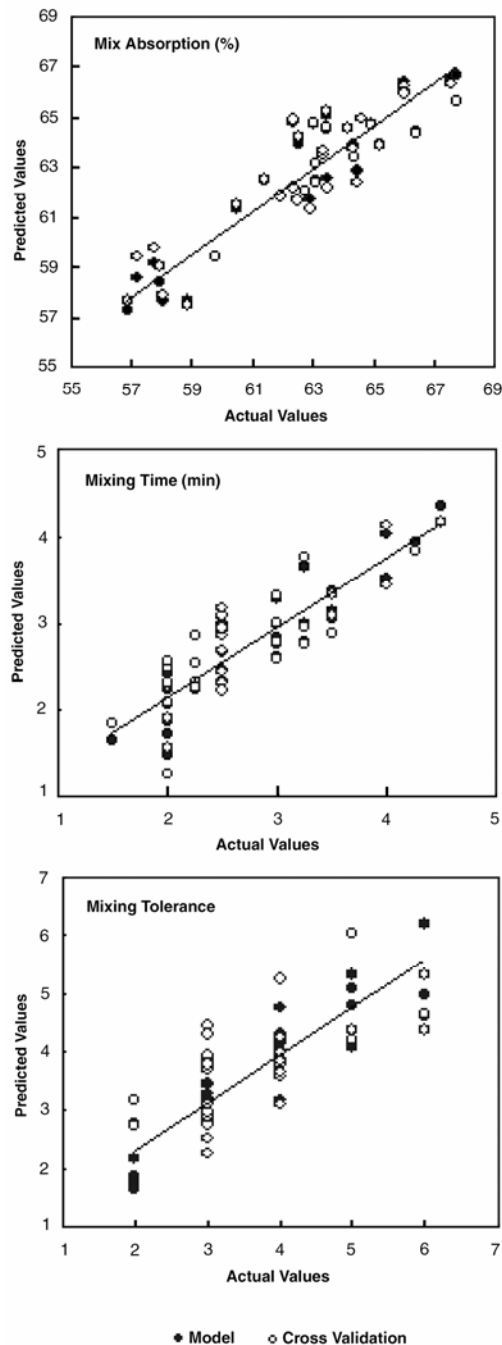


Fig. 4. Actual and predicted values of mixograph absorption, mixing time, and mixing tolerance for prediction model and cross-validation performed using chromatogram data of size-exclusion HPLC of flour proteins and pasting parameters.

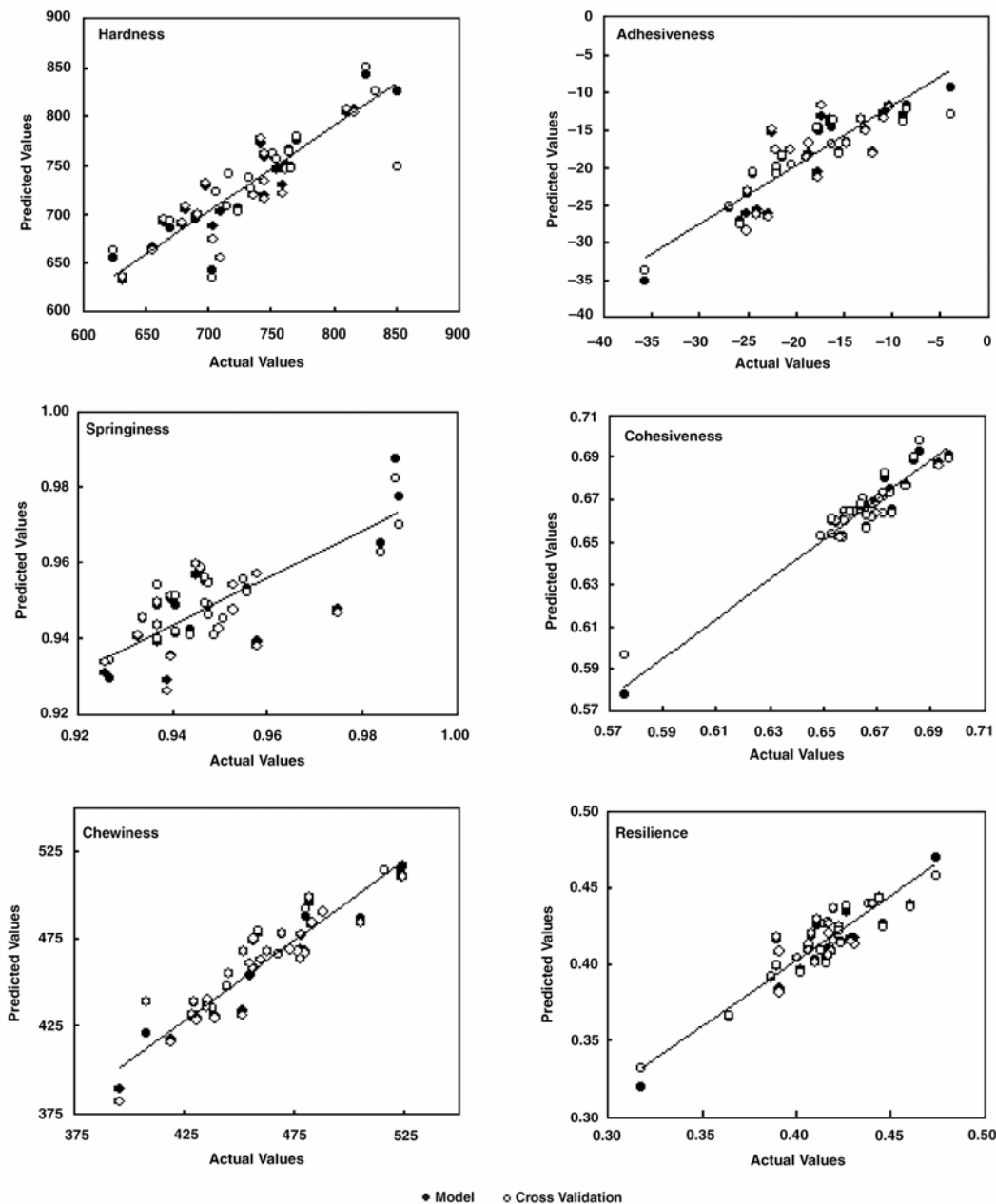


Fig. 5. Actual and predicted values of noodle texture characteristics measured at 1 min after cooking for prediction model and cross-validation performed using chromatogram data of size-exclusion HPLC of flour proteins and pasting parameters.

well-documented influence of starch properties on noodle texture characteristics (Baik and Lee 2003; Guo et al 2003; Martin et al 2004; Sasaki et al 2004).

The independent variables included in prediction equations also indicated that ratios of AA of specific protein fraction to RVA parameters could contribute to improve prediction of noodle characteristics. Specifically, the ratio of AA value from 13.2 to 18.6 min to RVA PV had significant positive correlations with noodle hardness ($r = 0.616$, $P < 0.0001$) and chewiness ($r = 0.479$, $P < 0.01$) and negative correlations with cohesiveness ($r = -0.506$, $P < 0.01$) and resilience ($r = -0.516$, $P < 0.01$). The ratios of AA values of protein fractions from 21.6 to 23.6 min to RVA PV also showed positive correlations with hardness ($r = 0.602$, $P < 0.0001$) and chewiness ($r = 0.488$, $P < 0.01$) and negative correlations with cohesiveness ($r = -0.463$, $P < 0.01$) and resilience ($r = -0.501$, $P < 0.01$).

The results in this experiment indicated that specific protein fractions could be more influential in interacting with starch to determine noodle texture.

SUMMARY

PCS values were calculated from absorbance and % absorbance values from SE-HPLC. The PCS and their cross-product, ratio, and square values were used to calculate a classification model to separate soft and hard wheats. Stepwise multiple discriminant analysis was performed and four PCS were selected for development of the classification function. The developed function classified all hard and soft wheats without misclassification. Monomeric gliadin protein fractions eluted from 19.0 to 19.7 min differed most significantly between hard and soft wheats in this experiment. These results from these sample sets suggest that differences in protein composition between hard and soft wheats are detectable by SE-HPLC analysis and could be used to differentiate hard and soft winter wheats more generally.

Prediction models were developed by continuum regression using seven variables selected from PCS and their transformed data calculated from AA and A% of SE-HPLC chromatograms, and tested by cross-validation for each quality parameter. The

prediction model for flour protein content showed R^2 values of 0.984 for the model and 0.976 for the cross-validation, suggesting that SE-HPLC data could be used to determine flour protein content. This is useful where sample size or access to other methods of determining N are limited. Prediction models indicated that SE-HPLC data could explain $\geq 80\%$ of variations in mixing and noodle characteristics, except for noodle springiness. Further prediction equations were developed by incorporating PCS attained from RVA data with the PCS derived from SE-HPLC. The R^2 and RMSE values indicated that prediction models of mixograph and noodle texture characteristics from SE-HPLC and RVA data could be more accurate and precise than those from SE-HPLC data only. Prediction models from both SE-HPLC and RVA data could explain at least 77% of variations in those characteristics, except for noodle springiness. This result suggested that addition of RVA parameters could help develop better prediction models for mixing and noodle characteristics. This result is intuitive for at least the noodle texture prediction, where it is well established that both protein and starch characteristics influence cooked noodle texture.

The PCS loading included in prediction equation indicated that specific protein fractions, ratios of protein fractions, specific RVA parameters, and the ratios of specific protein fractions to RVA parameters could be used to develop robust prediction models for dough mixing and cooked noodle textural characteristics using multivariate regression methods.

The multivariate models used in this report are available by contacting the corresponding author at the address listed in the footnotes on the title page.

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