NOTE

# Characterization of Hard Red Winter Wheat Storage Proteins by Two-Dimensional Electrophoresis and Their Correlations with Selected Quality Parameters[1]

G. YANG,[2,3] R. L. WEHLING,[2,4] M. G. ZEECE,[2] J. E. PARTRIDGE,[5] and D. R. SHELTON[6]

Cereal Chem. 72(6):568–570

The endosperm proteins of wheat are largely responsible for the unique breadmaking properties of this cereal grain. Numerous researchers have investigated the gliadin and glutenin components of wheat proteins and their relationships to the breadmaking potential of a flour. Studies on the relationships between breadmaking characteristics and the various storage protein subunits, including the high molecular weight (HMW) glutenins, have been reviewed by Wrigley and Bietz (1988), and more recently by Shewry et al (1992) and Autran (1993). In another related study, Dougherty et al (1990) separated and quantitated, without preliminary fractionation, storage proteins extracted from 14 cultivars of hard red winter (HRW) wheats using two-dimensional (2-D) electrophoresis coupled with digital image analysis. These results indicated that two HRW glutenin polypeptides and a gliadin protein were highly correlated with loaf volume of bread and mixograph peak time. Surprisingly high correlations were found ($R >$ 0.93) for the prediction of both loaf volume and mixing time using two-step multiple linear regression (MLR) analysis of the protein data. The purpose of this study was to 1) further characterize HRW wheat proteins by 2-D electrophoresis, and 2) test the validity of the relationships between individual proteins and baking properties previously reported by Dougherty et al (1990) using an expanded number of cultivars.

## MATERIALS AND METHODS

### Wheat Samples

Twenty-four HRW wheats were used in this study, including 21 North American commercial and experimental cultivars grown as part of the 1987 Nebraska varietal test. These included 11 cultivars previously analyzed (Dougherty et al 1990), plus the commercial cultivars Arkan, Centura, Colt, Recital, TAM 107, TAM 200, Trailblazer, and Turkey, and Nebraska experimental crosses 7846 and 78488. For each cultivar, samples were collected from five different growing locations in Nebraska and composited. The other three cultivars, Delta, Jasen, and MV14, were grown in Arizona in 1987 and were originally developed in Poland, Bulgaria, and Hungary, respectively.

[1]Published as Paper 10939, Journal Series, Agricultural Research Division, University of Nebraska, Lincoln.
[2]Department of Food Science and Technology, 143 Filley Hall, University of Nebraska, Lincoln, NE 68583-0919.
[3]Present address: Food Research Center, Kellogg Company, 235 Porter Street, Battle Creek, MI 49016-3423.
[4]Corresponding author. Fax: 402/472-1693.
[5]Department of Plant Pathology, University of Nebraska, Lincoln.
[6]Department of Agronomy, University of Nebraska, Lincoln.



### Methods

Experimental milling, mixograph analysis, experimental baking, 2-D electrophoresis, and image analysis were all conducted as previously described (Dougherty et al 1990). The electrophoretic system used nonequilibrium pH gradient electrophoresis (NEPHGE) in the first dimension, and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in the second dimension. The common spots (polypeptides) of all cultivars were matched by the computer software using Scout 66 as the reference. For each cultivar, reported results are the average percent integrated intensity (%II) values obtained from three replicate gels. Correlation and regression analyses were conducted using the Statistical Analysis System (SAS Institute, Cary, NC).

## RESULTS AND DISCUSSION

### Wheat and Flour Analysis

For the 24 wheat cultivars, flour yields ranged from 69.3 to 75.3%, flour protein from 10.8 to 13.3% (N × 5.7, 14% moisture basis), mixograph peak time from 1.80 to 6.50 min, and loaf volume (adjusted to 12% protein) from 628 to 1,000 $cm^3$.

### Electrophoresis

The 2-D electrophoretic separation performed on all cultivars, including those cultivars not examined in our previous work (Dougherty et al 1990), resulted in excellent resolution of most polypeptides. Endosperm storage proteins of four HRW wheat cultivars (NE78488, Delta, Rodeo, and Scout 66) as separated by 2-D electrophoresis are shown in Figure 1. The reproducibility of the protocol was tested by comparison of the 2-D separation and image analysis results currently obtained for Scout 66 with those of the previous study (Dougherty et al 1990). The current results were in very close agreement with those of our previous report. In addition, quantitative image analysis results (expressed as %II) using three replicate gels for each cultivar exhibited relative standard deviations of 10 and 15% for major and minor polypeptide spots, respectively. The agreement of results within samples, despite many opportunities for introduction of error, was excellent. This was due in part to the close control of separation conditions. For example, first dimension separations were conducted with a programmable power supply that controlled the applied voltage, amperage, and watts over a prescribed time course. It also integrated the Vhr function to provide consistent end points (4,800 Vhr). In this way, the nonequilibrium technique (NEPHGE) gave highly reproducible results. Additionally, the pattern recognition software of the image analysis system allowed for the $x$-$y$ locations of up to 100 spots to be stored and compensated for minor gel-to-gel variations in the location of equivalent polypeptide spots. Also, expressing image analysis results as percent integrated intensities (the ratio of integrated intensity for a

single spot to the total integrated intensity for all spots on the gel) compensated for variations in the amount of protein loaded and in staining.

It should be noted that the 2-D separation used here is not inclusive of all wheat protein components. In the protocol used (Dougherty et al 1989), polypeptides associated with the 2, 2*, and 5 HMW glutenin subunit bands, separated in one-dimensional electrophoretic procedures (nomenclature of Payne et al 1980, 1981a,b), migrate out of the capillary tube from the acidic side during focusing. However, the 2-D procedure does reproducibly resolve several components with significant relationships to baking quality. Some polypeptides that migrate as a single band in one-dimensional SDS-PAGE were separated into multiple spots based on isoelectric point or charge capacity differences. The four wheat cultivars in Figure 1 show considerable variation in their polypeptide profiles. Therefore, 2-D electrophoresis may have potential as a powerful tool for varietal identification.

Substantial differences in gliadin and low molecular weight (LMW) glutenin subunit patterns were observed between cultivars Delta and Rodeo, even though they have the same HMW glutenin subunit composition (2, 2*, 7, 9, and 12 according to the nomenclature of Payne et al 1980, 1981a,b).

## Correlation Study

The polypeptide spots found to be significantly correlated with loaf volume (adjusted to 12% protein content) and mixograph peak time at a 5% significance level are listed in Table I. Spot numbers were assigned by the image analysis system. For ease of comparison, the numbers previously assigned (Dougherty et al 1990) to equivalent spots are also listed parenthetically in Table I. The amount of gliadin spot 41 present (expressed as %II) had a very high positive correlation with loaf volume for the 24 samples analyzed. The previous study, using 14 of the 24 cultivars investigated here, also found that this spot was highly correlated with loaf volume (Dougherty et al 1990). The gliadin referred to as spot 39 (Dougherty et al 1990) was determined to be the same as spot 41 in the present study. When considering only the 10 cultivars not analyzed previously, the relationship remained significant at the 1% level ($r$ = 0.891). Earlier work (Dougherty et al 1990) had also shown that a minor protein (spot 10) in the HMW glutenin region had a strong negative correlation with loaf volume ($r$ = –0.917). When the same cultivars were evaluated in the present study, a similar correlation was obtained ($r$ = –0.901), but when the number of cultivars was expanded to 24, the strength of correlation for this peptide (spot 17 in the present study) decreased substantially to $r$ = –0.555, significant only at a 5% level. Other spots showing a significant correlation in this study were gliadins 45 and 77, and LMW glutenin spot 62. Dougherty et al (1990) had also identified this LMW glutenin (spot 64 in the previous study) as being positively correlated with loaf volume.

With respect to mixograph peak time, we found that the amount of spot 7 present in a sample has a strong negative correlation with mixing time (Table I). Spot 7 appears to be a HMW glutenin component coded by chromosome 1B. This spot was equivalent to spot 8 in the previous study (Dougherty et al 1990), where a strong negative correlation with mixing time was found when using the smaller group of 14 samples. When considering only the 10 samples not previously studied, the correlation was significant at the 5% level ($r$ = –0.641). Additionally, in the previous study a strong negative correlation between another HMW glutenin (spot 17) and mixograph peak time was reported, and also that the amounts of spots 8 and 17 were highly intercorrelated ($r$ = 0.882). We again found similar results when we analyzed the 14 samples previously reported (Dougherty et al 1990). However, when the number of samples analyzed in this study was expanded to 24, we found no significant correlation between spot 18 (the spot equivalent to 17 in the previous study) and mixing time, and the relative amounts of the two proteins present were not significantly intercorrelated at the 5% level ($r$ = 0.319). We did find that another HMW glutenin component, spot 5, was negatively correlated with mixing time at the 5% level ($r$ = –0.475). The previous study (Dougherty et al 1990) also reported that the gliadin identified as spot 39 had a high positive correlation with mixograph peak time ($r$ = 0.828). In this study, we again found that this gliadin (now identified as spot 41) was significantly correlated with mixing time at the 1% level, although the magnitude of the correlation was lower with the expanded sample set.

In the previous study (Dougherty et al 1990), MLR was used to develop models that related data from multiple polypeptides to loaf volume and mixograph peak time. The MLR equations explained a greater proportion of the variation in baking quality than could be explained by using a single polypeptide spot. We have developed equations based on the expanded set of 24 samples that again give higher correlations than can be obtained from the use of single spots. A forward stepwise regression program was used to select parameters for inclusion in the equations, and only spots with partial $F$-values significant at the 10% level were retained.

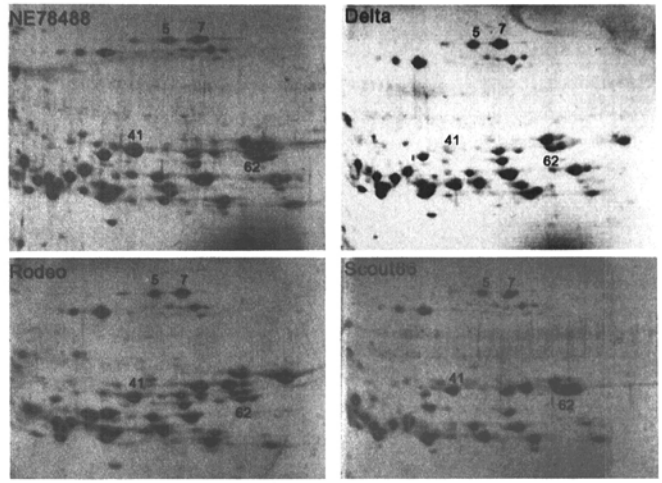


**Fig. 1.** Two-dimensional electrophoresis separations of storage proteins from the hard red winter wheat cultivars NE78488, Delta, Rodeo, and Scout 66. Numbers indicate those polypeptides that were used in developing multiple linear regression equations for predicting adjusted loaf volume and mixograph peak time. For the first dimension NEPHGE separation, the cathode is at the right of each gel. For the second dimension SDS-PAGE separation, the cathode is at the top of each gel.

**TABLE I**
**Correlations Between Percent Integrated Intensity of Polypeptides and Adjusted Loaf Volume and Mixograph Peak Time**

| Polypeptide Spot Number[a] | Correlation Coefficient ($r$) | Significance Level (%) |
|---|---|---|
| Adjusted loaf volume: | | |
| 41 (39) | 0.880 | 0.1 |
| 77 (91) | 0.638 | 0.1 |
| 45 (44) | 0.621 | 1.0 |
| 62 (64) | 0.621 | 1.0 |
| 17 (10) | –0.555 | 5.0 |
| Mixograph peak time: | | |
| 7 (8) | –0.782 | 0.1 |
| 41 (39) | 0.557 | 1.0 |
| 5 (6) | –0.475 | 5.0 |
| 67 (67) | 0.439 | 5.0 |

[a] Number in parentheses is the identification number assigned in the previous study (Dougherty et al 1990).

The equations, along with their multiple correlation coefficients ($R$) and standard errors of estimate (SEE) are:

$$\text{Adj. loaf volume } (cm^3) = 593.31 + 53.17(\%II_{spot\ 41}) + 45.59\ (\%II_{spot\ 62})$$

$R = 0.897$
SEE = 30.9 $cm^3$

$$\text{Peak time (min)} = 4.26 + 0.59\ (\%II_{spot\ 5}) - 0.985\ (\%II_{spot\ 7}) + 0.31\ (\%II_{spot\ 41})$$

$R = 0.872$
SEE = 0.68 min

The overall $F$-values for regression for both equations were significant at the 1% level. Again, we believe that the high $R$ values obtainable with the MLR models provide evidence that complex but quantitative relationships exist among various individual wheat storage proteins and baking quality characteristics.

In summary, the present study demonstrates that 2-D electrophoresis coupled with image analysis is a reproducible analytical method for quantitating wheat storage proteins. The relationship previously reported (Dougherty et al 1990) between an individual gliadin (spot 41) and loaf volume was shown to be valid with an expanded set of HRW cultivars. Likewise, the relationship between a HMW glutenin (spot 7) and mixing time was also confirmed. Research is continuing to further characterize these polypeptides and to determine whether these relationships remain valid across multiple crop years.

## LITERATURE CITED


AUTRAN, J.-C. 1993. Recent perspectives on the genetics, biochemistry and functionality of wheat proteins. Trends Food Sci. Technol. 4:358.

DOUGHERTY, D. A., ZEECE, M. G., WEHLING, R. L., and PARTRIDGE, J. E. 1989. High resolution two-dimensional electrophoresis of wheat proteins. J. Chromatogr. 480:359.

DOUGHERTY, D. A., WEHLING, R. L., ZEECE, M. G., and PARTRIDGE, J. E. 1990. Evaluation of selected baking quality factors of hard red winter wheat flours by two-dimensional electrophoresis. Cereal Chem. 67:564.

PAYNE, P. I., LAW, C. N., and MUDD, E. E. 1980. Control by homeologous group 1 chromosomes of the high-molecular-weight subunits of glutenin, a major protein of wheat endosperm. Theor. Appl. Genet. 58:113.

PAYNE, P. I., CORFIELD, K. G., HOLT, L. M., and BLACKMAN, J. A. 1981a. Correlations between the inheritance of certain high molecular weight subunits of glutenin and breadmaking quality in progenies of six crosses of bread wheat. J. Sci. Food Agric. 32:51.

PAYNE, P. I., HOLT, L. M., and LAW, C. N. 1981b. Structural and genetical studies on the high-molecular-weight subunits of wheat glutenin. Part 1: Allelic variation in subunits amongst varieties of wheat (*Triticum aestivum*). Theor. Appl. Genet. 60:229.

SHEWRY, P. R., HALFORD, N. G., and TATHAM, A. S. 1992. High molecular weight subunits of wheat glutenin. J. Cereal Sci. 15:105.

WRIGLEY, C. W., and BIETZ, J. A. 1988. Proteins and amino acids. Page 159 in: Wheat: Chemistry and Technology, 3rd ed. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.


[Received November 28, 1994. Accepted July 13, 1995.]