The relationship of Gliadin and glutenin subunits to breadmaking characteristics in winter wheat
by Mohamed Ali Al-Khawlani

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Crop and Soil Science
Montana State University

Abstract:
Storage proteins in wheat (Triticum aestivum L,) have major roles in baking quality characteristics. Baking quality improves with increased flour protein content. However, for a given protein content, variation among wheat cultivars for baking quality is a function of the qualitative nature of protein. The objective of this study was to determine which gliadin and glutenin subunits are correlated with breadmaking characteristics in winter wheat.

Twelve elite international winter wheat cultivars were planted in complete randomized block field experiments with four replicates in 2 locations and 2 years. Samples were analyzed for baking quality characteristics. Gliadin and glutenin proteins were analyzed on RP-HPLC using a gradient from 25% to 50% acetonitrile with the presence of 0.1% triflouroacetic acid over 55 minutes.

The results show significant differences among cultivars for all measured baking quality traits, flour protein content, and total gliadin and glutenin chromatogram areas. Loaf volume increased with increasing flour protein content, but the cultivars differed in the loaf volume response to increasing protein contents. This suggested that other factors affected the loaf volume.

Total area of peaks eluted during 10 minute intervals were used in linear regression analysis with baking quality traits. The 3 gliadin peaks eluted between 30 and 40 minutes were positively correlated with loaf volume (r=0.88), crumb score (r=0.87), and farinograph and mixograph absorption (r=0.59). Gliadin peaks eluted at 44 minutes were negatively correlated with baking quality traits (range of r=-0.24 to -0.57).

Glutenin subunits eluted from 20 to 30 minutes were positively correlated with loaf volume (r=0.86), crumb score (r=0.83), farino-graph and mixograph absorption (r=0.64), and peak time (r=0.52). Glutenin subunits eluted at 42 minutes were positively correlated with dough stability (r=0.58).

The positive and the negative correlation of some of the gliadin and glutenin subunits suggest the potential usefulness of these subunits for prediction of baking quality and selection of high quality wheats. The results show also that not all gliadin and glutenin subunits are needed for good baking quality. This indicates the possibility of improving the nutritional quality of wheat by increasing albumin and globulin fractions of protein at the expense of certain gliadins and glutenins.

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APPROVAL

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

Storage proteins in wheat (Triticum aestivum L.) have major roles in baking quality characteristics. Baking quality improves with increased flour protein content. However, for a given protein content, variation among wheat cultivars for baking quality is a function of the qualitative nature of protein. The objective of this study was to determine which gliadin and glutenin subunits are correlated with breadmaking characteristics in winter wheat.

Twelve elite international winter wheat cultivars were planted in complete randomized block field experiments with four replicates in 2 locations and 2 years. Samples were analyzed for baking quality characteristics. Gliadin and glutenin proteins were analyzed on RP-HPLC using a gradient from 25% to 50% acetonitrile with the presence of 0.1% triflouroacetic acid over 55 minutes.

The results show significant differences among cultivars for all measured baking quality traits, flour protein content, and total gliadin and glutenin chromatogram areas. Loaf volume increased with increasing flour protein content, but the cultivars differed in the loaf volume response to increasing protein contents. This suggested that other factors affected the loaf volume.

Total area of peaks eluted during 10 minute intervals were used in linear regression analysis with baking quality traits. The 3 gliadin peaks eluted between 30 and 40 minutes were positively correlated with loaf volume (r=0.88), crumb score (r=0.87), and farinograph and mixograph absorption (r=0.59). Gliadin peaks eluted at 44 minutes were negatively correlated with baking quality traits (range of r=-0.24 to -0.57).

Glutenin subunits eluted from 20 to 30 minutes were positively correlated with loaf volume (r=0.86), crumb score (r=0.83), farinograph and mixograph absorption (r=0.64), and peak time (r=0.52). Glutenin subunits eluted at 42 minutes were positively correlated with dough stability (r=0.58).

The positive and the negative correlation of some of the gliadin and glutenin subunits suggest the potential usefulness of these subunits for prediction of baking quality and selection of high quality wheats. The results show also that not all gliadin and glutenin subunits are needed for good baking quality. This indicates the possibility of improving the nutritional quality of wheat by increasing albumin and globulin fractions of protein at the expense of certain gliadins and glutenins.

INTRODUCTION

Wheat represents a major source of protein for both humans and animals despite a deficiency of some essential amino acids (mainly lysine). Storage proteins make major contributions to milling, dough formation, and baking characteristics of wheat flour. Baking quality improves with increased grain protein. However, for a certain protein percentage, variation of baking quality among wheat cultivars is a function of the qualitative nature of gliadin and glutenin compositions. Understanding the roles of gliadin and glutenin subunits in baking quality characteristics could be useful for predicting quality and breeding for improved wheat cultivars.

Many types of analyses are available to predict baking quality (mixograph, farinograph, and SDS-sedimentation method). None of these analyses can be used in early generation selection for baking quality because of the requirement of large quantities of seed. Prediction of baking quality by gliadin and glutenin subunits could eliminate these problems.

Improving wheat baking quality through plant breeding requires an increased understanding of the biochemical functions of gliadin and glutenin subunits in dough formation and baking quality. The objective of this study was to determine which gliadin and glutenin subunits are correlated with breadmaking characteristics in winter wheat.

LITERATURE REVIEW

Protein Content

Wheat protein could be fractionated by sequential extraction with different solvents. Osborne (1907) divided wheat proteins on the basis of solubility into four classes: albumins, soluble in water; globulins, soluble in salt solution; gliadin, soluble in 70% ethanol: and glutenin, soluble in acids or alkali. These solubility classes are still used to differentiate the four classes. Albumins and globulins are mainly enzymes, rich in essential amino acids (e.g., lysine). Gliadin and glutenin are the storage proteins and serve as a source of nitrogen and amino acids for the germinating seedling. They are high in glutamine and proline, but low in lysine (Kasarda et al., 1976).

Baking quality studies, using flour constituents which have been separated and reconstituted into dough, indicate that wheat proteins have major roles in breadmaking quality. Hoseney et al. (1969a) observed that addition of water soluble proteins from good or poor baking quality wheat flours did not alter the baking properties of standard flour. They concluded albumin and globulin fractions were not involved in breadmaking performance of wheats. Most of the present knowledge of the biochemistry and genetics of endosperm proteins resulted from electrophoresis analyses, which separate proteins on the bases of size, charge and molecular weight of proteins. Electrophoresis can be combined into two-dimensional methods of high resolving power (Wrigley and Shepherd, 1973; Lafiandra and Kasarda, 1985). Recently, Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) has been introduced for cereal protein analyses (Bietz, 1983; Burnouf and Bietz, 1987). RP-HPLC, which separates protein subunits on the basis of surface hydrophobicity combines speed, sensitivity, and high resolving power.

Gliadin Subunits and Baking Quality

Gliadin is the prolamine fraction of the wheat grain. It consists of a mixture of protein components with similar composition which is soluble in 70% ethanol. Jones et al. (1959) used electrophoresis to demonstrate the heterogeneity of gliadin. They found that gliadin consists of several subunits. They divided gliadin proteins into four arbitrary groups of subunits designated α-, β-, γ-, and ω-gliadin based on their mobility on the gel. Hoseney et al. (1969b) reported that reconstitution of glutenin fractions of good quality and poor quality wheats with a fixed gliadin-rich fraction controlled loaf volume.

Recent electrophoresis studies reported possible relationships between gliadin subunits and breadmaking characteristics. Wrigley et al. (1981) found that certain gliadin bands were significantly associated with baking quality. Branlard and Dardevet (1985a) reported that 18 electrophoresis bands were correlated significantly

(10 positively and 8 negatively) with baking quality technological criteria.

Huebner and Bietz (1986) used RP-HPLC to demonstrate that late-eluting gliadin subunits were inversely associated with baking quality. They speculated that gliadin may be linked to glutenin subunits coded by genes on the same chromosomes and may serve as markers of quality. Bietz and Burnouf (1985) used RP-HPLC of gliadin extracted from nullisomic-tetrasomic lines of Chinese Spring and found that all gliadin subunits are controlled by genes on the short arms of group 1 and group 6 chromosomes.

Glutenin Subunits and Baking Quality

Glutenin is made up of high molecular weight polymers consisting of a large number of subunits joined through disulfide, hydrogen, and hydrophobic bonds. It constitutes about 40% of the total endosperm proteins. Glutenin is difficult to study in its native form. It is insoluble in water, diluted salt solutions, or 70% ethanol. To solubilize glutenin it is necessary to disrupt non-covalent bonds with denaturants (urea), and to cleave and stabilize disulfide bonds through reduction and alkylation (Bietz, 1985).

Glutenin subunits play important roles in breadmaking characteristics in wheats. Significant differences among wheat cultivars in glutenin subunit composition were reported by Huebner (1970), Bietz and Wall (1972), Orth and Bushuk (1973a), and Payne et al. (1979). Huebner (1970) and Orth and Bushuk (1973a) found no obvious correlation between glutenin subunit composition and breadmaking characteristics. Orth and Bushuk (1973b) reported that bread wheat cultivars contained glutenin subunits of high molecular weight which were absent in durum wheats. They concluded that the presence of these subunits is not the only factor that controls baking quality, since all bread wheats contained them regardless of quality, but they appear to be necessary for the quality. Orth and Bushuk (1974) analyzed glutenin of nullisomic-tetrasomic lines of Chinese Spring wheat and showed that four of the glutenin subunits are coded by genes on the long arm of chromosome 1D. Huebner and Wall (1976) observed that dough strength was related to the total amount of glutenin. Higher correlation was obtained between dough strength and high molecular weight subunits of glutenin. It was established that the high molecular weight glutenin subunits are associated with dough formation and breadmaking characteristics in wheats (Payne et al., 1979; Burnouf and Boariquet, 1980; Payne et al., 1981; Branlard and Dardevet, 1985b; Payne et al., 1987).

Burnouf and Bietz (1984) described a RP-HPLC technique to separate high molecular weight glutenin subunits on the basis of their hydrophobicity. They demonstrated that the RP-HPLC is a valuable complement to other chromatographic and electrophoretic methods for analysis of glutenin subunits. Using RP-HPLC technique, Huebner and Bietz (1985) found that the high molecular weight subunits of glutenin eluted between 18 and 28 minutes were associated with good baking quality. Branlard and Dardevet (1985b) found that the quantity of 4 HMW subunits was positively correlated with baking quality.

MATERIALS AND METHODS

Cultivars

Twelve cultivars selected from International Winter Wheat Nurseries were used in this study. These cultivars originated from five countries (Table 1) and represent three different classes of wheats: Hard Red Winter Wheat (HRWW), Soft Red Winter Wheat (SRWW), and Soft White Winter Wheat (SWWW).

Table 1. Names, origin, and classes of cultivars.

Cultivar	Origin	Class
Atlas-66	USA, IN	SRWW
Odessa-4	USSR	HRWW
Redwin	USA, MT	HRWW
NE7060	USA, NE	HRWW
Bezostaya 1	USSR	HRWW
Doina	Romania	SRWW
Adams	Austria	HRWW
WWP4394	Austria	HRWW
Blueboy	USA, NC	SRWW
Houser	USA, NY	SWWW
Alcedo	E.Germany	HRWW
Centurk	USA, NE	HRWW

Experimental Design

The twelve cultivars were grown at Bozeman and Huntley, Montana in 1986, and Bozeman and Fort Ellis, Montana in 1987. Randomized complete block field experiments with four replicates were planted in all four location/year environments. Plots consisted of six rows 2.5 m long and 30 cm apart. The center four rows were harvested for yield and baking quality analysis. Sufficient grain was obtained from each experimental plot in all environments.

Flour Quality Analyses

Grain samples, tempered overnight to 16% moisture for hard wheats and 14% moisture for soft wheats, were milled with a Buhler Laboratory mill (AACC, 1983 method 26-10). Flour quality analyses including flour yield, flour protein, farinograph, and baking analyses were determined in the Cereal Quality Laboratory, Montana State University, Bozeman, MT. Approved methods of AACC were used for flour ash (AACC, 1983 method 08-12), farinograph (AACC, 1983 method 54-21), and straight-dough method of baking quality analysis (AACC, 1983 method 10-10B). Grain protein and flour protein content were determined using Near Infrared Reflectance (NIR) (Williams, 1979).

The following data were recorded for each experimental plot:

- Grain protein (%), flour protein (%), flour yield (% of grain), flour ash (%) - expressed as percent of the dry weight.
- Water absorption (ml) - the amount of water required to center the farinograph curve on the 500 B.U. line.
- Mixing time (minutes) - the time needed to bring the dough to the optimum consistency.
- Loaf volume (cc) was measured using the rape seed displacement.

- Crumb score was determined on a scale from 1 to 10 (1 = poor and 10 = excellent) by visual comparison with standard (loaves baked from commercial bakery flour Mello Judith).
- Peak time (minutes) or dough development - the time from the first addition of water to the dough's maximum consistency.
- Stability (minutes) - the time that the dough stays at the maximum consistency before signs of breakdown.
- Valorimeter value (VAL) - empirical quality score based on the dough development time and tolerance to mixing.

Gliadin Extraction

Gliadins were extracted from 25 mg flour samples with 70% (V/V) aqueous ethanol (Huebner and Bietz, 1985). Extraction time was 60 minutes at room temperature with periodic agitation. Following extraction, samples were centrifuged at 19,000 g for 5 minutes. The clear supernatant (20 μl) was used in the RP-HPLC analysis.

Glutenin Extraction

Glutenins were extracted and prepared for the RP-HPLC analyses as described by Burnouf and Bietz (1984). After the extraction of gliadins, dried pellets remaining from 25 mg flour samples were suspended in 750 μl of a medium containing 0.05 M Tris plus 8 M urea adjusted to pH 7.5 with nitric acid. Glutenins were reduced with 0.1% dithiothreitol for 2 hours at room temperature with occasional shaking. Glutenins were alkylated with iodoacetamide for 2 hours.

Reduced-alkylated glutenins were centrifuged at 19,000 g, and 20 μl of the clear supernatant were injected into the RP-HPLC.

RP-HPLC Analysis

Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) was used to separate both gliadin and glutenin subunits. RP-HPLC was described by Bietz (1983). For all analytical separations a 250 x 4.5 mm SynChropak RP-P (C18) column was used. Linear gradients were generated from two solvents: solvent A was HPLC grade water containing 0.1% trifluoroacetic acid (TFA), and solvent B was acetonitrile (ACN) containing 0.1% TFA. Protein subunits were eluted with linear gradient from 75% A and 25% B to 50% A and 50% B over 55 minutes with 1ml/min. flow rate. The column temperature was maintained at 70°C using a water bath. Eluted components were detected at wave length of 210 nm.

Statistical Analyses

Analyses of variance were computed for all traits combined over environments. The pooled mean square error was used to test the cultivar mean squares and cultivar x environment interactions (McIntosh, 1983). Since gliadin and glutenin subunits were detected at 210 nm wave length, which detects the carbonyl group of the peptide bonds, the areas under the peaks should reflect the amount of protein subunits (Bietz, 1983). The cultivar means of gliadin and glutenin peak areas over four replications (n = 48) were used in linear regression analyses with baking quality traits (Huebner and Bietz, 1987; Hobbs and Mahon, 1982).

RESULTS AND DISCUSSION

Growing conditions differed among location/year environments. At the Huntley location, moisture stress occurred early in the 1986 growing season reducing yield and grain protein quality. This was reflected in poor baking quality. At the Fort Ellis location (1987) severe lodging and disease resulted in reduced yield and grain protein. Several lodging and disease resistant cultivars gave very high yields. At the Bozeman location the environmental conditions were ideal for wheat growth in both 1986 and 1987. The soil fertility was high and water stress occurred only late in the growing season.

Protein Content and Baking Quality

The analyses of variance showed highly significant differences among cultivars for all baking quality traits. Significant cultivar x environment interactions were also observed. However, the cultivar mean squares were much larger than cultivar x environment interaction mean squares (Table 7 and 8, Appendix). Significant differences among the environments for baking quality traits were observed. Cultivar grain and flour protein varied with location/year environments.

Correlations between milling and baking quality traits are shown in Table 2. Water absorption was positively correlated with flour yield, flour protein, grain protein, and test weight. This suggests

Table 2. Correlation coefficients (r) between milling and baking quality traits of winter wheats combined over environments.

Traits	Flour yield	Flour protein	Grain protein	Flour ash	Test weight
Absorption					
Mixograph	.73**	.67**	.53**	ns	.69**
Farinograph	.74**	.73**	.58**	ns	.67**
Mixing time	.30*	ns	-.26*	ns	ns
Loaf volume	ns	.77**	.83**	ns	.37*
Crumb score	ns	.73**	.79**	ns	.26*
Peak time	.51**	.48**	.39*	ns	ns
Stability	.49**	.28*	ns	ns	ns
VAL	.52**	.47**	.36*	ns	ns

*,** = Significant at $P<0.05$ and $P<0.01$, respectively.
ns = Not significant.

that factors in addition to flour and grain protein affect water absorption.

Protein content in the grain and the flour were positively correlated with most of the quality traits. Grain protein plays a major role in determining the baking characteristics in wheats. Although loaf volume and crumb score were highly correlated with protein content, percent flour ash was not correlated with any of the baking quality traits. The positive correlation of protein content in the grain and the flour with loaf volume ($r = 0.83$ and 0.77) supports other research which showed protein affects baking quality in wheat (Hoseney et al., 1969b; Orth and Bushuk, 1972; Wall, 1979). However, at a given level of protein content variation among cultivars for baking quality was observed. For example, the plot of percent flour protein for three different cultivars with loaf volume illustrates the positive correlation between loaf volume and percent protein (Figure 1). At 14% flour protein the three cultivars had different loaf volumes. The response of loaf volume (and other baking quality traits) to increasing protein contents is different among cultivars. This could be due to the quality of the proteins in these cultivars.

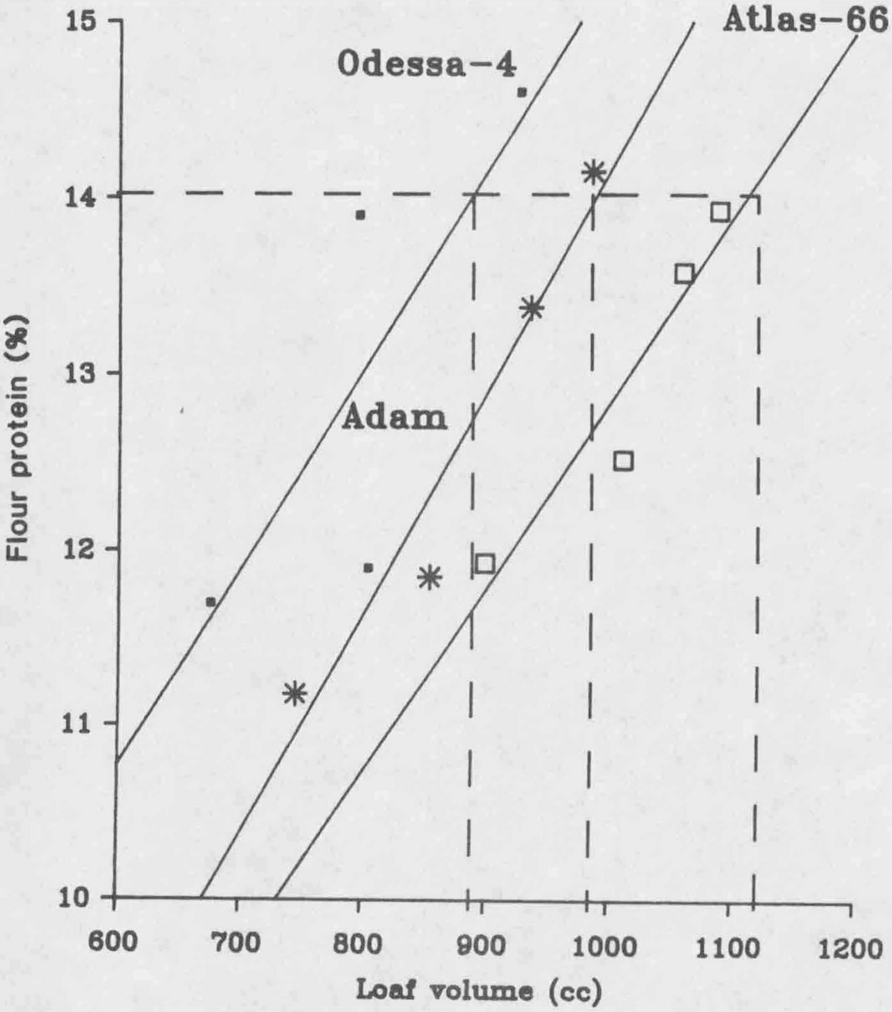

Figure 1. The relationships of loaf volume and percent flour protein of three winter wheat cultivars.

Gliadins and Baking Quality

An example of a RP-HPLC chromatogram of gliadin from the cultivar WWP 4394 is shown in Figure 2. Most of the gliadin subunits were eluted between 20 and 40 minutes (72 to 85% of total gliadin). Thirty to forty distinct major and minor (poorly resolved) peaks were observed. The gliadin peaks eluted at different time depending on surface hydrophobicity. Similar resolutions and gliadin distributions were reported by Bietz (1983) and Burnouf and Bietz (1987). Quantitative and qualitative differences among cultivars for gliadin composition were found. The gliadin composition is cultivar specific, and could be used for identifying wheats suitable for baking quality.

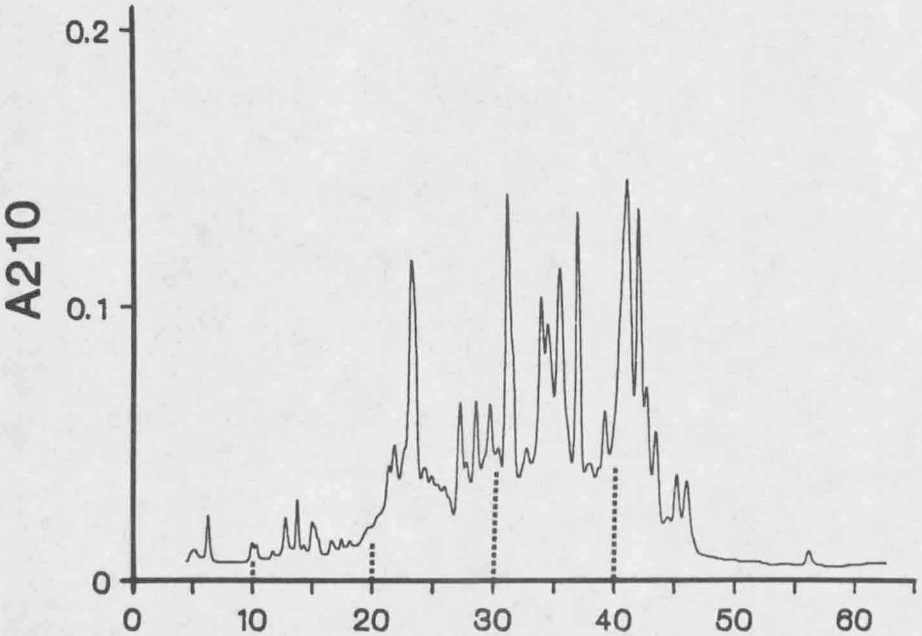

Figure 2. RP-HPLC chromatogram of gliadin of winter wheat cultivar WWP4394. The extraction and RP-HPLC analysis are as indicated in materials and methods.

Cultivar identification by gliadin composition has been reported by Wrigley et al. (1982) and Burnouf and Bietz (1987).

The gliadin chromatogram was divided into 5 intervals (indicated by dotted lines in Figure 2) in order to find which gliadin subunits are correlated with baking quality. The sum of all subunits eluted in these intervals was used in the linear regression analysis with baking quality traits. Correlation between gliadin subunits eluted in five time intervals and baking quality traits are shown in Table 3.

Table 3. Correlation coefficients (r) between quality traits and gliadin subunits of 12 winter wheat cultivars combined over environments.

		Gliadin subunit elution time (min)				
Traits	Total gliadin	0-10	10 -20	20-30	30-40	40-60
Absorption						
Mixograph	.58**	ns	ns	.30*	.57**	.30*
Farinograph	.64**	ns	ns	.53**	.59**	ns
Mixing time	-.44**	ns	ns	ns	ns	-.48**
Loaf volume	.63**	ns	.27*	.24*	.65**	.39*
Crumb score	.56**	ns	.26*	ns	.61**	.32*
Peak time	ns	ns	ns	.34*	.31*	ns
Stability	ns	ns	ns	ns	ns	-.33*
VAL	ns	ns	ns	.24*	ns	-.29*
Flour yield	-.30*	ns	ns	.30*	ns	-.54**
Ash (%)	ns	ns	ns	ns	ns	ns

*,** = Significant at P<0.05 and P<0.01, respectively.
ns = Not significant.

Total gliadin chromatographic area was positively correlated with absorption, loaf volume, and crumb score, but negatively correlated with mixing time and flour yield.

Gliadin subunits eluted between 20 and 30 minutes were significantly correlated with most of the baking quality traits, except mixing time, crumb score, and stability. However, the correlation coefficients were too low for predictive purposes. High positive correlations were noted between gliadin subunits eluted at 30-40 minute intervals and loaf volume and crumb score (r = 0.65 and 0.61 respectively). On the other hand, gliadin subunits eluted at 40-60 minute interval were negatively correlated with mixing time, mixing stability, valorimeter value (VAL), and flour yield (Table 3). Gliadin subunits eluted at the 0-10 and 10-20 minute intervals were not correlated with baking quality. This suggested that gliadin subunits could be divided into three groups: the first group consisted of subunits that were positively correlated with the baking quality (subunits eluted at 20-30 and 30-40 minute intervals); the second group included the subunits that were negatively correlated with baking quality (subunits eluted at 40-60 minute interval); and the third group included subunits that were not correlated with the baking quality (subunits eluted at 0-10 and 10-20 minute intervals). The positive and negative correlations of gliadin subunits and baking quality could be explained by either one of the following hypotheses: (1) the genes coding for these gliadin subunits could be linked to genes that code for other quality controlling proteins (glutenins).

It has been shown that some glutenin subunits (low molecular weight subunits) are coded by genes on the short arms of homologous group 1 chromosomes, where most of the genes coding for gliadin subunits are located (Bietz and Burnouf, 1985). (2) The correlations between the amount of gliadin subunits and baking quality could be due to causal relationships. Subunits that correlated positively may play a favorable role in determining baking quality, and subunits that negatively correlated may have unfavorable roles in baking quality. Various gliadin subunits could have different roles in dough formation and baking quality depending on the compositions, conformational structure, and interaction of these gliadin subunits. It has been demonstrated that gliadin subunits could be aggregated to form microfibrillar structure involving only secondary forces (hydrogen, ionic, and hydrophobic bonds) (Bernardin, 1975; Kasarda et al., 1976). Thus, gliadin subunit interactions may be reflected in the performance of the dough and in baking characteristics.

Gliadin subunits associated with baking quality were eluted in three time intervals. As a result, the study was then focused on the subunits eluted in these three intervals. The correlation of each individual subunit eluted in these three intervals with baking quality traits was examined. Table 4 shows the correlation of these subunits with milling and baking quality traits. Among the gliadin subunits eluted at 20-30 minute interval, subunit 29 was significantly correlated with seven of nine milling and baking quality traits. In the 30-40 minute interval, gliadin subunits 35, 37, and 39 were found to be highly correlated with baking quality. Gliadin subunit 44 had a significant relationship with eight of the nine milling and baking quality traits.

Table 4. Correlation coefficients (r) between baking quality traits and gliadin subunits of winter wheats combined over environments.

Traits	Gliadin subunit elution time (min)					
	29	35	37	39	Total 35+37+39	44
Absorption						
Mixograph	.50**	.57**	.50**	.43**	.58**	-.53**
Farinograph	.50**	.54**	.50**	.51**	.59**	-.57**
Mixing time	-.37*	ns	ns	ns	ns	ns
Loaf volume	.65**	.64**	.80**	.84**	.88**	-.26*
Crumb score	.58**	.61**	.81**	.83**	.87**	-.24*
Peak time	.24*	.37*	.41**	.37*	.44**	-.38*
Stability	ns	.29*	.26*	ns	.28*	-.27*
VAL	ns	.40**	.44**	.40**	.48**	-.45**
Flour yield	.26*	.44**	ns	ns	.31*	-.48**

*,** = Significant at P<0.05 and P<0.01, respectively.
ns = Not significant.

The gliadin subunit eluted at 29 minutes was positively correlated with absorption, loaf volume and crumb score, and negatively correlated with mixing time. This indicated that gliadin subunit 29 has an important influence on baking quality. Gliadin subunits 35, 37, and 39 were positively correlated with most of the baking quality traits, except mixing time (Table 4). High correlations occurred between these three subunits and loaf volume (r = 0.88) and crumb score (r = 0.87). The subunit eluted at 44 minutes was negatively correlated with most of the baking quality traits. These results suggested that prediction of baking quality could be achieved by examining these gliadin subunits. The positive and negative correlations of some of the gliadin subunits suggested their usefulness in breeding for high quality wheats. Early generation selection for lines having high amounts of gliadin subunits 29, 35, 37, and 39, and selection against lines having high amounts of gliadin subunit 44 should be useful in improving breadmaking quality in wheat.

For comparison, the gliadin chromatograms of three winter wheat cultivars varying in baking quality are shown in Figure 3. The loaf volume increased with increasing amounts of gliadin subunits 35-40 indicated by dotted lines in Figure 3. However, loaf volume was inversely associated with the amount of subunit 44, indicated by arrows in Figure 3.

Comparing these results with those reported in the literature is difficult due to different techniques used to separate protein subunits. However, the positive relationship of gliadin subunits with baking quality characteristics has been reported (Hoseney et al., 1969c, and Branlard and Dardevet, 1985a). The negative effect of some gliadin subunits also was reported (Branlard and Dardevet, 1985a, and Huebner and Bietz, 1986).

Protein content of wheat cultivars varied across the environments. The total chromatographic peak areas decreased with

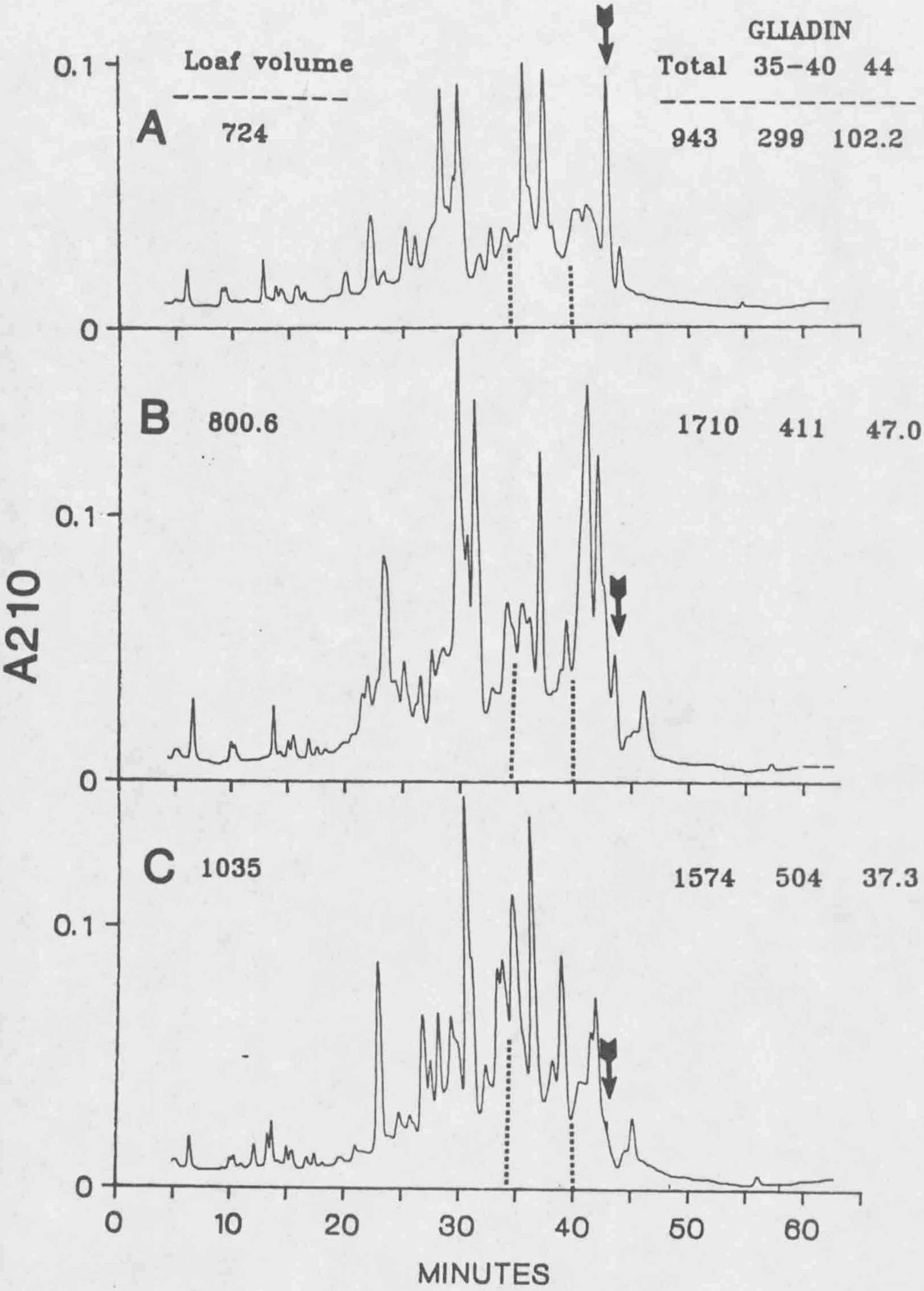

Figure 3. RP-HPLC chromatograms of three wheat cultivars varying in baking quality: (A) Houser, (B) Odessa-4 (C) NE7060, their loaf volumes (cc), and area under the curve of total gliadin and subunits 35-40 and 44.

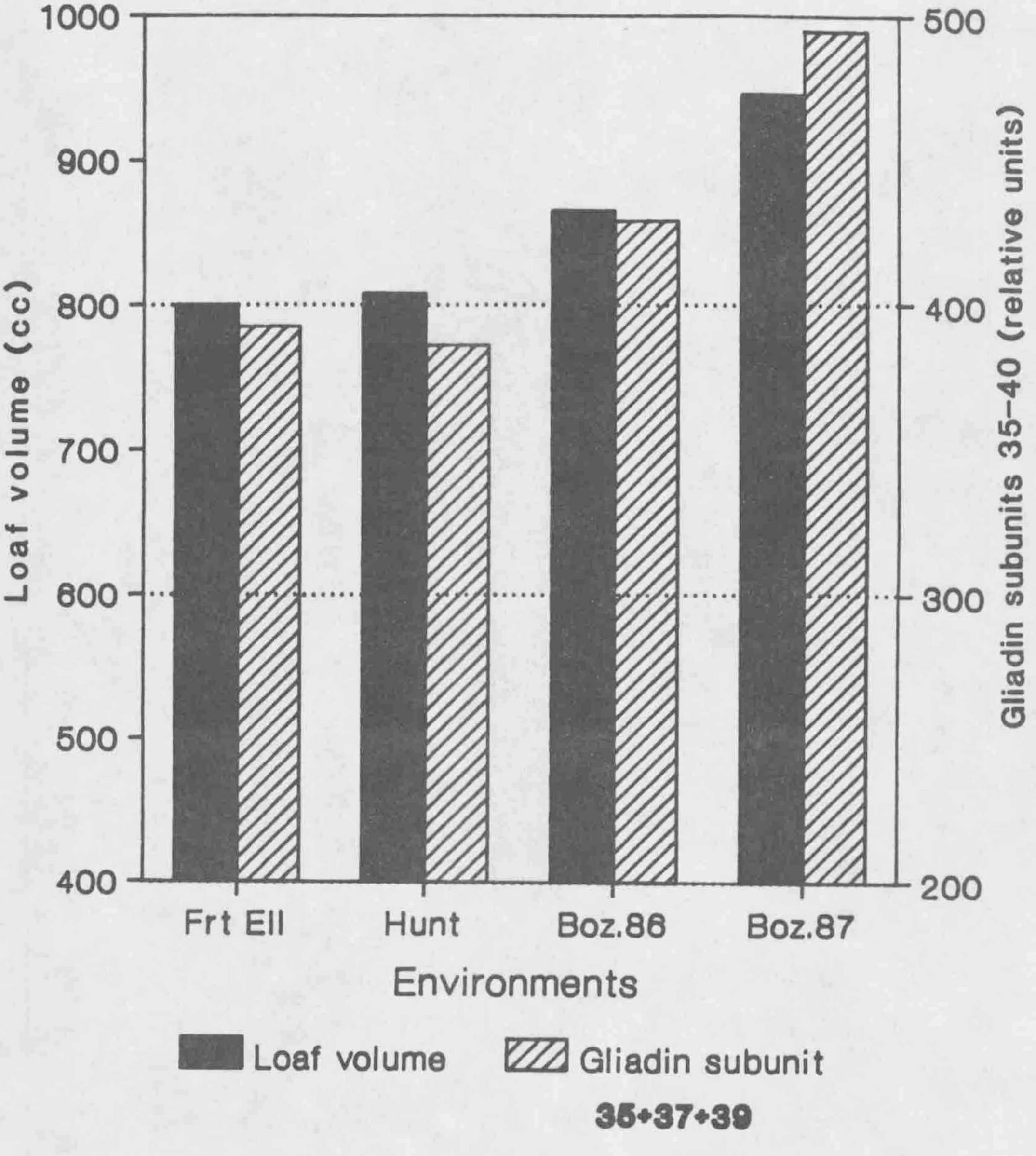

Figure 4. Relative amount of gliadin subunits 30-40 and loaf volume of winter wheat (mean of 12 cultivars) in four environments: Fort Ellis (Frt Ell), Huntley (Hunt), Bozeman 86 (Boz.86), and Bozeman 87 (Boz.87).

decreasing protein contents. This was reflected by the baking quality characteristics in different environments. The relationship of gliadin subunits 35-40 and loaf volume at four different environments is shown in Figure 4. A close association between gliadin subunits 35-40 and loaf volume was observed in every environment. The correlations between gliadin subunits and baking quality traits were similar among the four environments. For example, the correlation between gliadin subunits 35-40 and loaf volume were r = 0.82, 0.93, 0.95, and 0.89 in Bozeman 86, Huntley, Bozeman 87, and Fort Ellis, respectively. The correlations between gliadin subunits 35-40 and loaf volume (r = 0.88, Table 4) using the twelve cultivar means in all four environments (n = 48) were similar. This suggested that gliadin subunits could be used to predict this aspect of baking quality in any environment.

Each of the baking quality criteria was examined by multiple regression analysis, taking gliadin subunits as independent variables. Using loaf volume as dependent variable and gliadin subunits 27, 37, and 39 as independent variables accounted for 77% of the variation of loaf volume among cultivars. Using crumb score as the dependent variable and subunits 35, 37,and 39 as independent variables accounted for 78% of the variation. The R^2 values for the other baking quality traits ranged from 18% to 52% when 2 or 3 gliadin subunits were included in the regression. Some of the gliadin subunits were correlated with each other. As a result, adding more gliadin subunits to the multiple regression did not significantly increase R^2 (data not shown).

Glutenins and Baking Quality

Significant differences among cultivars for total glutenin were detected (Table 11 and 12, Appendix). Significant cultivar x environment interactions were also observed due to the diversity of the environments. However, the cultivar mean squares were 3 to 10 times larger than the cultivar x environment interactions. Visual examination of the RP-HPLC chromatograms of glutenin from 12 cultivars varying in baking quality showed major quantitative and qualitative differences in glutenin subunit composition and distribution. This suggested that glutenin composition could be used for cultivar identifications.

An example of a RP-HPLC chromatogram of glutenin is shown in Figure 5. Twenty-six to twenty-eight major and minor peaks were observed in the 12 cultivars. The elution time distribution of glutenin peaks was distinct and different from gliadin. Two distinct groups of peaks were eluted at different times. The first group of major peaks was eluted between 20 and 30 minutes (26% of total glutenin), and the second group of major peaks was eluted between 40 and 50 minutes (54% of total glutenin). Similar resolution and distribution of glutenin peaks have been reported by Huebner and Bietz (1985) and Burnouf and Bietz (1984). Burnouf and Bietz (1984) found four early eluted glutenin subunits coded by genes located on

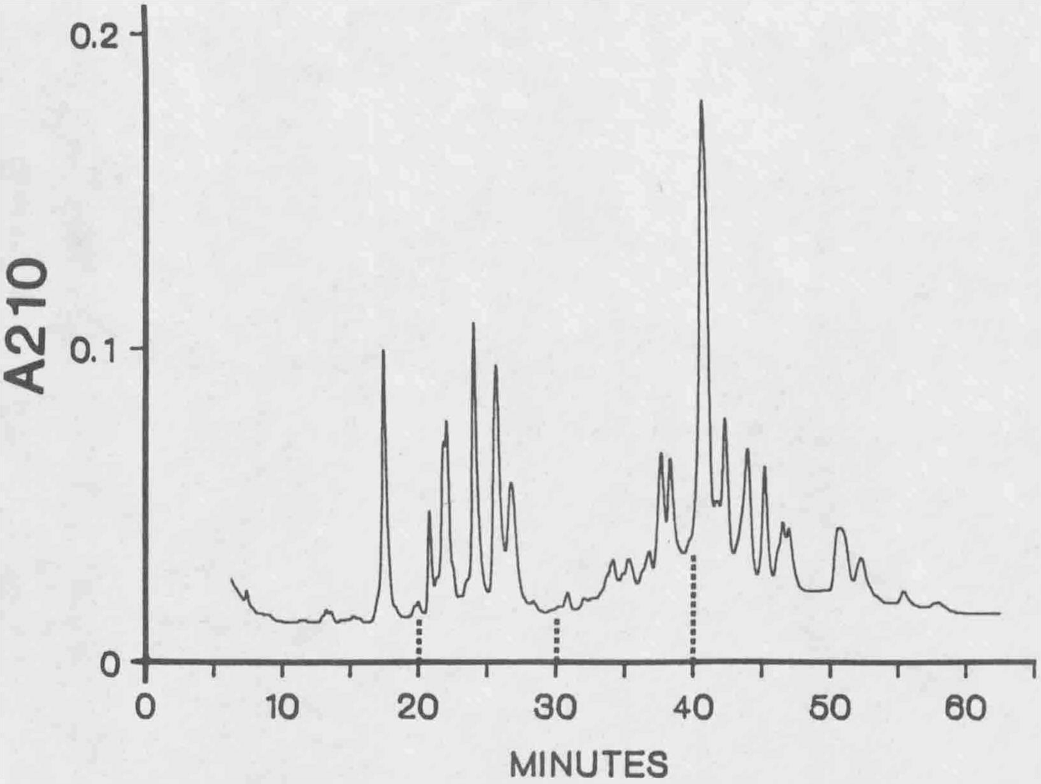

Figure 5. RP-HPLC chromatogram of glutenin from wheat cultivar Redwin.

the long arms of chromosomes 1D and 1B. They concluded from the SDS-PAGE analysis that these subunits (eluted between 10-30 minutes) corresponded to the high-molecular-weight subunits. They also found that the late-eluting subunits (eluted between 35-60 minutes) were the low-molecular-weight subunits and are regulated by genes on the short arms of homologous group 1 chromosomes. The early elution time of high-molecular-weight subunits indicated that these subunits have lower surface hydrophobicities than the low-molecular-weight subunits.

The RP-HPLC glutenin chromatogram could be divided into four time intervals (see dotted lines as exemplified in Figure 5). The areas under the curve for all subunits eluted in each interval were summed and used in linear regression analyses with all baking quality traits. These quantitative determinations are based on peptide bond absorbance at a wave length of 210 nm and give a fairly accurate determination of the amount of glutenin subunits (Huebner and Bietz, 1985). Quantification based on absorbance at 210 nm is less dependent on specific amino acids than is the absorbance at 280 or 254 nm. The correlations between glutenin subunits and baking quality are shown in Table 5. Total chromatographic areas of glutenin were positively correlated with most baking quality traits indicating the general importance of glutenin for baking quality. Glutenin subunits eluted between 0 and 20 minutes and 30 and 40 minutes showed very low association with baking quality traits (Table 5). Subunits eluted at these two time intervals are of lesser quantity and have little affect on the baking quality.

Glutenin subunits eluted between 20 and 30 minutes and 40 and 60 minutes were significantly correlated with most of the baking quality traits. High positive correlations between subunits eluted at the 20-30 minute interval and loaf volume (r = 0.86) and crumb score (r = 0.83) were observed. High positive correlations were also found between subunits eluted at 40-60 minute interval and peak time (r = 0.55), stability (r = 0.58), and VAL (r = 0.66). Mixing time and percent ash showed almost no correlations with most of the glutenin

Table 5. Correlation coefficients (r) between baking quality traits and glutenin subunits of winter wheats combined over environments.

Traits	Total glutenin	Glutenin subunit elution time (min) 0-20	20-30	30-40	40-60
Absorption					
Mixograph	.65**	.30*	.64**	.40**	.42**
Farinograph	.60**	ns	.64**	.30*	.49*
Mixing time	ns	ns	ns	ns	.25*
Loaf volume	.77**	.30*	.86**	.50**	.47**
Crumb score	.75**	.26*	.83**	.56**	.49**
Peak time	.49**	ns	.52**	ns	.55**
Stability	.40**	ns	.30*	ns	.58**
VAL	.56**	ns	.53**	ns	.66**
Flour yield	.38*	.24*	.33*	.28*	.25*
Ash (%)	ns	ns	ns	ns	ns

*,** = Significant at P<0.05 and P<0.01, respectively.
ns = Not significant.

subunits. These results suggested two groups of glutenin subunits are associated with baking quality. The first group was eluted at the 20-30 minute interval. These are the high-molecular-weight subunits of glutenin. The second group eluted at 40-60 time interval were the low-molecular-weight subunits of glutenin. Similar results using quantification of stained electrophoretic gels were reported by Payne et al. (1981) and Branlard and Dardevet (1985a). They concluded that high-molecular-weight subunits of glutenin were positively associated with baking quality. The importance of high-molecular-weight subunits of glutenin for the baking quality was also reported by Huebner and Bietz (1985), who used RP-HPLC analysis.

Mixing time was not significantly correlated with glutenin subunits eluted between 0 and 40 minutes and correlated with glutenin subunits eluted between 40 and 60 minutes (r = .25) (Table 5). These results are in contrast with those reported by Huebner and Bietz (1985). They found high-molecular-weight subunits were positively associated with mixing time.

The present study showed two groups of glutenin subunits: one group (eluted at 20-30 minute interval) was found to be associated with loaf volume and crumb score, and the other group (eluted at 40-60 time interval) was associated with peak time and dough stability. The correlation of individual subunits eluted in these two intervals with all baking quality traits was examined (Table 6). Glutenin subunits that showed high correlation with baking quality are reported. Subunits that were not significantly correlated were eliminated. Five major peaks which correspond to high-molecular-weight subunits of glutenin were found. One subunit was eluted at 16 minutes and the other four subunits were eluted between 20 and 30 minutes. Similar elution time and distribution of four high-molecular-weight glutenin subunits were reported by Burnouf and Bietz (1985). All five individual HMW subunits of glutenin (eluted at 16, 21, 24, 26, and 27 minutes) were positively correlated with most baking quality traits (Table 6) indicating their importance for breadmaking characteristics in winter wheats.

Table 6. Correlation coefficients (r) between baking quality traits and glutenin subunits of winter wheats combined over environments.

	Glutenin subunit elution time (min)						
Traits	16	21	24	26	27	Total	42
Absorption							
Mixograph	.68**	.63**	.49**	.73**	.52**	.72**	ns
Farinograph	.58**	.69**	.47**	.70**	.59**	.73**	ns
Mixing time	ns	ns	ns	ns	ns	ns	.27*
Loaf volume	.74**	.61**	.78**	.62**	.69**	.83**	.32*
Crumb score	.68**	.55**	.77**	.55**	.71**	.78**	.33*
Peak time	.34*	.54**	.38*	.52**	.48**	.54**	.33*
Stability	ns	ns	.29*	.37*	ns	.29*	.52**
VAL	.35*	.47**	.41**	.51**	.43**	.52**	.49**
Flour yield	.47**	.48**	ns	.60**	ns	.46**	ns
Ash (%)	ns	ns	ns	ns	ns	ns	ns

*,** = Significant at P<0.05 and P<0.01, respectively.
ns = Not significant.

High correlations were observed between the total of HMW subunits of glutenin and absorption (r = 0.73), loaf volume (r = 0.83) and crumb score (r = 0.78). The glutenin subunit eluted at 42 minutes (low molecular subunits) was significantly correlated with 6 of 10 baking quality traits. The correlations of subunit 42 with loaf volume and crumb score were lower than the correlations with stability and VAL (Table 6). These results suggested that low molecular weight subunits play important roles in dough stability, while high molecular weight subunits have a major role in determining both the dough stability and loaf volume.

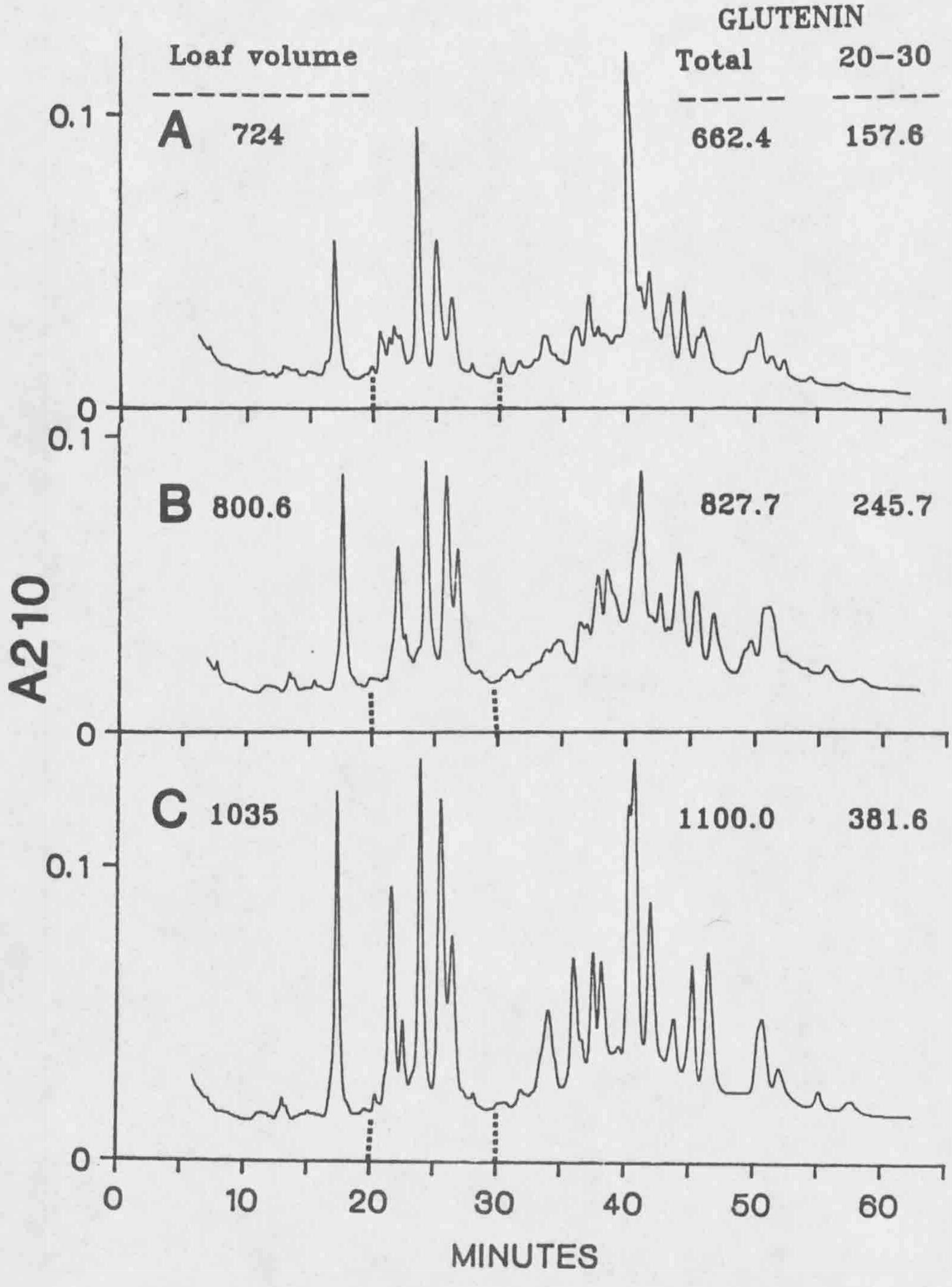

Figure 6. RP-HPLC chromatograms of three winter wheat cultivars varying in baking quality: (A) Houser, (B) Odessa-4, and (C) NE7060, their loaf volumes (cc), and area under the curve of total glutenin and subunits 20-30.

Figure 6 shows the chromatograms for glutenin from three selected wheat cultivars varying in breadmaking characteristics. (Gliadin chromatograms from these three cultivars were shown in Figure 3.) The loaf volume and other baking quality traits increased with increasing amounts of glutenin subunits eluted between 20-30 minutes (between the dotted lines). The correlation between the amount of high molecular weight subunits of glutenin and baking quality traits suggested that these subunits may have favorable roles in determining the baking quality in wheats (Table 6). The explanation of these positive relationships depends on the composition and amino acid sequences of glutenin subunits and on their conformational (three dimensional) structures. Various models and theories have been suggested to explain the molecular basis of the involvement of glutenin in dough formation and breadmaking characteristics (Bernardin and Kasarda, 1973; Wall, 1979). All of these models and theories emphasized the importance of both intersubunit disulfide bonds and non-covalent (hydrogen and hydrophobic) bonding.

The results of this study indicated quantities of high molecular weight glutenin subunits of different cultivars were positively associated with breadmaking quality in winter wheats. This suggested that high molecular subunits of glutenin could be used for prediction of baking quality. Because the RP-HPLC analysis requires very small flour samples, predication of quality could be achieved in early generations. Breeding for superior breadmaking quality wheat could be achieved by selecting wheat lines with a large amount of high molecular weight subunits of glutenin. Progeny having poor baking quality (small amount of HMW subunits) could be eliminated in early generations.

The results also show low non-significant correlations between the high molecular weight subunits and mixing times. This indicated that selecting for increased HMW subunits of glutenin would not change the mixing times significantly. Long mixing time is not associated with good baking quality. High loaf volume and crumb score are important breadmaking quality characteristics. A low crumb score indicates the presence of thick cell walls and large holes which reduce the quality of the bread. The high positive correlations of loaf volume and crumb score with HMW subunits of glutenin could be used for predicting these two important quality traits.

The relationships of glutenin subunits and baking quality traits were similar in all four environments. For example, the correlations of HMW glutenin subunits 20-30 were r = 0.78, 0.93, 0.87, and 0.92 in Bozeman 86, Huntley, Bozeman 87, and Fort Ellis, respectively. The correlations of HMW glutenin subunits 20-30 with loaf volume (r = 0.86, Table 5) using twelve cultivar means in all four environments (n = 48) were similar.

Each of the baking quality criteria was used in multiple regression analyses with glutenin subunits as explanatory variables. Using loaf volume as dependent variable and glutenin subunits 16 and 24 as independent variable accounted for 75% of total variation of loaf volume among cultivars. Using crumb score as dependent variable and glutenin subunits 16, 24, and 27 as independent variables accounted for 72% of the variation of crumb score among cultivars. The R^2 values for the other baking quality traits ranged from 44% to 56% when 2 or 3 glutenin subunits were included in the regression (data not shown). Adding more subunits to the regression did not significantly increase R^2.

SUMMARY

The results of this study corroborated the important role of protein content in baking quality in wheat shown in other studies. However, cultivars with the same percent protein differed in their baking quality characteristics. This suggested that the qualitative aspects of cultivar grain protein are important for determining baking quality.

The quantitative and qualitative differences among cultivars for gliadin subunit composition and distribution suggest that gliadin could be used for cultivar identification. The positive and the negative correlation of some gliadin subunits suggested their usefulness in predicting and improving various baking quality traits in wheat. Breeding for high quality wheat could be achieved by early generation selection of progeny having high amount of gliadin subunits eluted at 29, 35, 37, and 39 minutes, and imposing selection pressure against progeny having high amounts of the gliadin subunit eluted at 44 minutes.

This study also identified high molecular weight subunits of glutenin associated with breadmaking quality in winter wheats. Wheat cultivars varied in amounts of glutenin subunits.The positive correlation of glutenin subunits eluted at 20-30 minute interval with baking quality indicates their importance for baking quality. These subunits could be used for predicting flour quality and as screening tools in breeding for high quality wheat.

Both gliadin and glutenin subunits were important for baking quality in wheats. Dough formation and baking quality characteristics are primarily determined by the interaction of subunits of gliadin and glutenin positively correlated with baking quality. Therefore, both gliadin and glutenin subunits correlated with baking quality should be considered in breeding programs for improving baking quality in winter wheat.

The gliadin subunits that positively correlated with baking quality (subunits eluted at 29, 35, 37,and 39 minutes) constitute only 40.1% of total gliadins. The rest of the gliadins (59.9%) were either negatively correlated or not correlated with baking quality. Glutenin subunits that correlated with baking quality (subunits eluted at 16, 21, 24, 26, 27, and 42 minutes) constitute only 46.1% of total glutenin. The rest of glutenin (53.9%) were not correlated with baking quality. This indicates that all the gliadin and glutenin subunits are not needed for good baking quality. This suggests the possibility of increasing albumin and globulin fractions at the expense of specific gliadins and glutenins to improve the nutritional quality of wheats.

LITERATURE CITED

American Association of Cereal Chemists 1983. AACC approved methods (7th ed). Method 26-10, approved May, 1961; method 08-12, revised Oct., 1981; method 54-21, reviewed Oct., 1982; method 10-10B, revised Sep., 1985. The Association: St. Paul, Minn.

Bernardin, J.E. 1975. The rheology of concentrated gliadin solution. Cereal Chem. 52(3,II):136r-145r.

Bernardin, J.E. and Kasarda, D.D. 1973. The microstructure of wheat protein fibrils. Cereal Chem. 50:736-745.

Bietz, J.A. 1983. Separation of cereal proteins by RP-HPLC. Journal of Chromatography 255:218-238.

Bietz, J.A. 1985. HPLC: how protein look in cereals. Cereal Chem. 62:201-212.

Bietz, J.A. and Burnouf, T. 1985. Chromosomal control of gliadin: analysis by reversed-phase high-performance liquid chromatography. Theor. Appl. Genet. 70:599-609.

Bietz, J.A. and Wall, J.S. 1972. Wheat gluten subunits: Molecular weights determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Cereal Chem. 49:416-430.

Branlard, G. and Dardevet, M. 1985a. Diversity of grain proteins and bread wheat quality. I. Correlation between gliadin bands and flour quality characteristics. Journal of Cereal Science 3:329-343.

Branlard, G. and Dardevet, M. 1985b. Diversity of grain proteins and bread wheat quality. II. Correlation between high molecular weight subunits of glutenin and flour quality characteristics. Journal of Cereal Science 3:345-354.

Burnouf, T. and Bietz, J.A. 1984. RP-HPLC of reduced glutenin, a disulfide-bonded protein of wheat endosperm. Journal of Chromatography 299:185-199.

Burnouf, T. and Bietz, J.A. 1985. Chromosomal control of glutenin subunits in aneuploid lines of wheat: analysis by reversed-phase high-performance liquid chromatography. Theor. Appl. Genet. 70:610-619.

Burnouf, T. and Bietz, J.A. 1987. Identification of wheat cultivars and prediction of quality by reversed-phase high-performance liquid chromatographic analysis of endosperm storage proteins. Seed Sci. and Technol. 15:79-99.

Burnouf, T. and Boariquet, R. 1980. Glutenin subunits of genetically related European hexaploid wheat cultivars: Their relation to bread-making quality. Theor. Appl. Genet. 58:107-111.

Hobbs, S.R.A. and J.D. Mahon, J.D. 1982. Variation, heritability, and relationship to yield of physiological characters in peas. Crop Science 22:773-779

Hoseney, R.C., Fenney, K.F., Shogren, M.D. and Pomeranz, Y. 1969a. Functional (breadmaking) and biochemical properties of wheats flour components. II. Role of water-solubles. Cereal Chem. 46:117-125.

Hoseney, R.C., Fenney, K.F., Shogren, M.D. and Pomeranz, Y. 1969b. Functional (breadmaking) and biochemical properties of wheats flour components. III. Characterization of gluten protein fractions obtained by ultracentrifugation. Cereal Chem. 46:126-135.

Hoseney, R.C., Fenney, K.F., Shogren, M.D. and Pomeranz, Y. 1969c. Functional (breadmaking) and biochemical properties of wheats flour components. IV. Gluten protein fractionation by solubilizing in 70% ethanol and dilute lactic acid. Cereal Chem. 46:495-502.

Huebner, F.R. 1970. Comparative studies on glutenins from different classes of wheat. J. Agric. Food Chem. 18:256-259.

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

Huebner, F.R. and Bietz, J.A. 1986. Assessment of the potential breadmaking quality of hard wheat by reversed-phase high-performance liquid chromatography of gliadin. J. Cereal Sci. 4:379-388.

Huebner, F.R. and Bietz, J.A. 1987. Improvements in wheat protein analysis and quality prediction by reversed-phase high-performance liquid chromatography. Cereal Chem. 64:15-20.

Huebner, F.R. and Wall, J.S. 1976. Fractionation and quantitative differences of glutenin from varieties varying in baking quality. Cereal Chem. 53:258-269.

Jones, R.W., Taylor, N.W., and Senti, F.R. 1959. Electrophoresis and fractionation of wheat gluten. Arch. Biochem. Biophys. 84:363-376.

Kasarda, D.D., Bernardin, J.E., and Nimmo, C.C. 1976. Wheat protein. Pages 158-236 in: Advances in Cereal Science and Technology. Vol. 1. Y. Pomeranz, ed. Am. Assoc. Cereal Chem., St. Paul, MN.

Lafiandra, D. and Kasarda, D.D. 1985. One- and Two-dimensional (two pH) polyacrylamide gel electrophoresis in a single gel: separation of wheat proteins. Cereal Chem. 62:314-318.

McIntosh, M.S. 1983. Analysis of combined experiments. Agron. J. 75:153-155.

Osborne, T.B. 1907. The proteins of wheat kernel. Carnegie Inst. Washington: Washington, D.C.

Orth, R.A. and Bushuk, W. 1972. A comparative study of the proteins of wheats of diverse baking qualities. Cereal Chem. 49:268-275.

Orth, R.A. and Bushuk, W. 1973a. Studies of glutenin. II. Relation of variety, location of growth, and baking quality to molecular weight distribution of subunits. Cereal Chem. 50:191-197.

Orth, R.A. and Bushuk, W. 1973b. Studies of glutenin. III. Identification of subunits coded by the D-genome and their relation to breadmaking quality. Cereal Chem. 50:680-687.

Orth, R.A. and Bushuk, W. 1974. Studies of glutenin. VI. Chromosomal location of genes coding for subunits of glutenin of common wheat. Cereal Chem. 51:118-126.

Payne, P.I., Corfield, K.G., and Blackman, J.A. 1979. Identification of high-molecular weight subunit of glutenin whose presence correlates with bread-making quality in wheats of related pedigree. Theor. Appl. Genet. 55:153-159.

Payne, P.I., Corfield, K.G., Holt, L.M., and Blackman, J.A. 1981. Correlations between the inheritance of certain high-molecular weight subunits of glutenin and bread-making quality in progenies of six crosses of bread wheat. J. Sci. Food Agric. 32:51-60.

Payne, P.I., Nightingale, M.A., Krattiger, A.F., and Holt, L.M. 1987. The relationship between HMW glutenin subunit composition and bread-making quality of British grown wheat varieties. J. Sci. Food Agric. 40:51-65

Wall, J.E. 1979. The role of wheat proteins in determining baking quality. Pages 275-311 in: Recent Advances in the Biochemistry of Cereals. Eds. Laidman, D.L., Wyn Jones, R.G. London, New York, San Francisco: Acad. Press.

Williams, P.C. 1979. Screening wheat for protein and hardness by Near Infrared Reflectance spectroscopy. Cereal Chem. 56:169-172.

Wrigley, C.W., Autran, J.C., and Bushuk, W. 1982. Identification of cereal varieties by gel electrophoresis of grain proteins. Pages 211-259 in: Advances in Cereal Science and Technology, Vol. 5. Y. Pomeranz, ed. Am. Assoc. Cereal Chem., St. Paul, MN.

Wrigley, C.W. and Shepherd, K.W. 1973. Electrofocusing of grain protein from wheat genotypes. Ann. N.Y. Acad. Sci. 209:154-162.

Wrigley, C.W., Robinson, P.J., and Williams, W.T. 1981. Association between electrophoretic patterns of gliadin proteins and quality characteristics of wheat cultivars. J. Sci. Food Agric. 32:433-442.

APPENDIX

Table 7. Mean squares of grain yield, grain protein, and milling quality traits of 12 winter wheat cultivars combined over environments.

Source	d.f.	Grain yield	Grain protein	Flour yield	Flour protein	Flour ash	VAL
Environ.	3	32668000	67.4	140.7	49.92	0.035	2936.2
		**	**	**	**	*	**
BLK/ENV	12	1525600	5.87	7.7	1.15	0.0055	41.6
Cultivar	11	8303000	34.47	552.4	33.46	0.0075	3994.8
		**	**	**	**	**	**
C x E	33	2862100	3.46	30.8	2.37	0.0027	215.7
		**	**	**	**	ns	**
Error	132	365230	0.44	3.9	0.18	0.0027	15.3

*,** = Significant at P<0.05 and P<0.01, respectively.

Table 8. Mean squares of baking quality traits of 12 winter wheat cultivars combined over environments.

Source	d.f.	Absorp. (mix.)	Mixing time	Loaf volume	Score	Absorp. farin.	Peak time	Stability
Environ.	3	106.0	3.6	219460	20.9	228.6	429.8	1015.7
		**	**	**	**	**	**	**
BLK/ENV	12	2.5	0.05	9134	0.48	7.4	1.8	5.3
Cultiv.	11	180.0	8.8	151120	18.38	450.8	525.8	1053.1
		**	**	**	**	**	**	**
C x E	33	8.9	0.58	13065	1.43	9.9	81.3	109.6
		**	**	**	**	**	**	**
Error	132	0.82	0.04	941	0.13	1.5	1.6	4.6

** = Significant at P<0.01.

Table 9. Mean squares of gliadin subunits (eluted in 5 time intervals) of 12 winter wheat cultivars combined over environments.

Source	d.f.	Total gliadin	Gliadin subunit elution time (min)				
			0-10	10-20	20-30	30-40	40-60
Environ.	3	2632600 **	155.6 **	3751 **	116900 **	474130 **	670090 **
BLK/ENV	12	23333	3.3	105	3903	9085	1386
Cultiv.	11	1024900 **	50.2 **	2335.6 **	150700 **	278690 **	53424 **
C x E	33	100370 **	17.3 **	1284 **	32138 **	36420 **	30777 **
Error	132	8031	1.9	47.3	1558.6	2240.4	769

** = Significant at P<0.01.

Table 10. Mean squares of gliadin subunits (correlated with bread-making characteristics) of 12 winter wheat cultivars combined over environments.

Source	d.f.	Gliadin subunit elution time (min)					
		29	35	37	39	Total 35+37+39	44
Environ.	3	38617 **	4590.2 **	39454 **	23731 **	119830 **	1037.1 **
BLK/ENV	12	642	302.2	263	102	1090	64.8
Cultiv.	11	12137 **	11638 **	11698 **	11971 **	74969 **	6174.6 **
C x E	33	1883 **	1954.6 **	4147 **	5013 **	10711 **	451.1 **
Error	132	206	103.0	95	76	364	42.5

** = Significant at P<0.01.

Table 11. Mean squares of glutenin subunits (eluted in 4 time intervals) of 12 winter wheat cultivars combined over environments.

			Glutenin subunit elution time (min)			
Source	d.f.	Total glutenin	0-20	20.5-30	30.5-40	40.5-50
Environ.	3	911830 **	45644 **	101780 **	137750 **	434930 **
BLK/ENV	12	26369.6	329.6	3251	1101.8	14763.9
Cultiv.	11	309240 **	690.9 **	32704 **	15797 **	105440 **
C x E	33	49854 **	269.7 **	3085.6 **	5058.2 **	22212 **
Error	132	4698.6	91.1	430.7	694.5	3134.1

** = Significant at P<0.01.

Table 12. Mean squares of glutenin subunits (correlated with baking quality) of 12 winter wheat cultivars combined over environments.

		Glutenin subunit elution time (min)					
Source	d.f.	16	21	24	26	27	42
Environ.	3	7209.9 **	7328.9 **	7013.3 **	5344.7 **	2170.8 **	7518.5 *
BLK/ENV	12	158.1	96.7	398.2	282.9	109.9	916.5
Cultiv.	11	1328.3 **	2690.6 **	2191.1 **	2745.9 **	3139.6 **	40497 **
C x E	33	144.0 **	307.5 **	733.8 **	360.0 **	143.8 **	4540.4 **
Error	132	29.7	38.3	60.6	50.4	34.2	266.8

*,** = Significant at P<0.05 and P<0.01, respectively.

Table 13. Grain yield, grain protein, and milling quality trait means of 12 winter wheat cultivars combined over environments.

Cultivar	Flour yield %	Flour protein %	Grain yield kg/ha	Grain protein %	Flour Ash %
Atlas-66	55.1	13.0	3476	16.5	0.43
Odessa-4	66.0	13.0	5396	14.7	0.40
Redwin	67.2	12.6	4577	14.2	0.40
NE7060	67.4	13.3	5487	15.4	0.42
Bezos. 1	67.8	11.3	4895	13.2	0.37
Doina	55.1	11.1	5388	13.9	0.37
Adams	68.5	11.1	6194	13.1	0.39
WWP4394	62.6	12.6	6109	14.4	0.40
Blueboy	54.0	9.8	5155	12.2	0.37
Houser	54.3	8.5	5657	11.0	0.36
Alcedo	64.9	10.7	5332	12.7	0.37
Centurk	64.7	11.7	4957	13.5	0.38
Means	62.4	11.6	5218	13.7	0.39
C.V.	3.2	3.7	11.6	4.8	13.4
LSD.05	1.4	0.3	422.6	0.5	0.04
LSD.01	1.8	0.4	550.4	0.6	0.05

Table 14. Baking quality trait means of 12 winter wheat cultivars combined over environments.

Cultivar	Mixing time min.	Loaf volume cc	Crumb score	Absorb. ml	Peak time min.	Stabil. min.	VAL
Atlas-66	1.6	1015	5.9	59.7	5.5	8.0	52.6
Odessa-4	1.4	801	3.4	66.2	5.1	4.0	43.7
Redwin	2.5	932	5.4	66.4	15.3	22.6	79.5
NE7060	2.9	1037	5.8	67.7	20.5	20.0	86.4
Bezos. 1	3.1	785	3.2	64.9	14.1	23.5	75.1
Doina	2.2	788	3.3	53.6	3.3	6.7	45.7
Adams	2.5	813	3.4	62.0	6.4	15.8	58.7
WWP4394	1.4	880	4.2	64.1	4.9	4.6	45.2
Blueboy	1.9	834	3.8	55.0	3.0	4.7	45.1
Houser	2.0	724	2.8	52.2	2.1	3.3	37.4
Alcedo	3.7	775	3.3	58.9	10.1	21.5	64.9
Centurk	3.0	874	4.3	64.2	6.9	17.1	62.0
Means	2.3	855	4.1	61.3	8.1	12.7	58.0
C.V.	8.5	4	8.8	2.0	15.6	16.9	6.7
LSD.05	0.1	21	0.2	0.9	0.9	1.5	2.7
LSD.01	0.2	28	0.3	1.1	1.1	1.9	3.6

Table 15. Gliadin subunit means of 12 winter wheat cultivars combined over environments.

Cultivar	Total Gliadin	Gliadin subunit elution time (min)				
		0-10	10-20	20-30	30-40	40-60
Atlas-66	1667	10.5+	38.3	476.2	896.2	242.8
		0.6@	2.3	28.6	53.6	14.5
Odessa-4	1710	5.7	40.4	586.8	768.6	308.6
		0.3	2.4	34.3	44.9	18.0
Redwin	1509	8.4	30.7	512.2	733.9	222.8
		0.6	2.1	33.9	48.6	14.6
NE7060	1574	6.6	31.8	414.7	880.2	240.0
		0.4	2.0	25.9	55.9	15.3
Bezos. 1	1337	7.3	26.7	356.2	761.5	184.5
		0.5	2.0	26.3	57.0	13.8
Doina	1421	8.9	30.0	394.1	754.2	234.1
		0.6	2.1	27.3	53.2	16.5
Adams	1312	6.4	25.7	329.2	729.8	220.8
		0.5	2.0	25.0	55.7	16.8
W WP4394	1705	5.1	67.6	468.5	814.3	348.5
		0.3	3.9	27.5	47.7	20.4
Blueboy	1146	10.1	31.1	305.4	542.2	256.0
		0.9	2.8	26.1	48.0	22.0
Houser	942	5.9	22.4	260.2	463.5	191.2
		0.6	2.4	27.3	49.3	20.7
Alcedo	1095	8.6	45.9	358.3	540.0	142.8
		0.7	4.2	32.7	49.3	13.1
Centurk	1243	6.6	33.4	310.6	699.1	193.3
		0.5	2.7	24.9	56.5	15.3
Means	1388	7.5	35.3	396.0	714.1	226.7
		0.6	2.6	28.2	51.9	16.3
C.V.	6.5	18.4	19.5	10.0	6.6	12.2
LSD.05	62.7	1.0	4.8	27.6	33.1	19.4
LSD.01	81.6	1.2	6.3	35.9	43.1	25.2

+ = Area under the curve (relative units) X 1000

@ = % of total area

Table 16. Gliadin subunit means of 12 winter wheat cultivars combined over environments.

	Gliadin subunit elution time (min)					
Cultivar	29	35	37	39	Total 35+37+39	44
Atlas-66	177.9+	114.3	217.3	207.2	538.9	79.7
Odessa-4	165.1	116.3	171.0	123.8	411.1	46.8
Redwin	144.4	102.3	188.9	172.7	463.9	48.2
NE7060	143.9	162.9	169.9	171.7	504.5	37.3
Bezos. 1	138.6	117.7	149.0	158.2	424.9	49.7
Doina	150.3	125.5	152.6	147.7	425.8	72.9
Adams	118.1	145.8	172.7	171.4	490.0	60.1
WWP4394	131.0	134.0	132.2	142.5	408.7	73.6
Blueboy	100.5	94.1	136.9	152.5	383.5	58.1
Houser	87.2	60.1	126.6	111.6	299.0	102.2
Alcedo	113.3	88.1	129.5	115.4	333.0	86.9
Centurk	101.5	109.4	153.9	161.2	424.5	45.8
Means	131.0	114.3	158.4	153.0	425.6	63.4
C.V.	10.9	8.9	6.1	5.7	4.5	10.3
LSD.05	10.0	7.1	6.8	6.1	13.3	4.6
LSD.01	13.1	9.2	8.9	7.9	17.4	5.9

+ = Area under the curve (relative units) X 1000.

Table 17. Glutenin subunit (areas under the curve) means of 12 winter wheat cultivars combined over 4 environments.

Cultivar	Total glutenin	Glutenin subunit elution time (min) 0-20	20-30	30-40	40-60
Atlas-66	1041.0	40.5+	278.8	188.2	540.9
		4.0@	26.7	18.3	51.8
Odessa-4	865.2	46.8	245.7	147.6	442.6
		5.7	28.9	15.3	50.0
Redwin	1149.0	37.0	278.1	174.7	651.8
		3.5	24.1	15.3	56.4
NE7060	1100.0	53.4	329.3	176.9	543.6
		4.8	29.8	15.4	50.3
Bezos. 1	983.6	42.7	242.4	158.0	547.1
		4.3	24.4	16.0	56.1
Doina	883.9	36.5	216.7	160.1	458.6
		4.1	24.5	18.6	51.2
Adams	895.3	36.3	213.6	114.1	531.2
		4.1	23.9	12.7	59.4
WWP4394	900.3	44.4	257.6	173.3	424.6
		4.8	28.8	18.9	47.3
Blueboy	758.0	36.4	199.2	98.7	427.2
		5.1	26.1	12.9	56.2
Houser	662.4	27.1	157.6	99.7	376.1
		4.0	24.2	15.4	56.0
Alcedo	842.2	37.3	200.7	160.6	444.9
		4.5	23.7	19.7	52.2
Centurk	995.0	41.0	242.4	120.3	591.2
		3.9	24.3	11.7	60.0
Means	923.0	39.9	238.5	147.7	498.3
		4.4	25.8	16.0	54.0
C.V.	7.4	23.8	8.7	7.8	11.2
LSD.05	47.9	6.7	14.5	18.4	39.1
LSD.01	62.4	8.7	18.9	24.0	51.0

+ = Area under the curve (relative units) X 1000.
@ = % of total area.

Table 18. Glutenin subunit means (areas under the curve) of 12 winter wheat cultivars combined over environments.

	Glutenin subunit elution time (min)					
Cultivar	16	21	24	26	27	42
Atlas-66	47.84+	33.83	94.44	50.90	43.26	185.2
Odessa-4	47.37	48.00	70.36	72.84	39.27	86.1
Redwin	53.46	53.09	80.15	77.91	51.83	238.0
NE7060	64.13	68.04	90.35	94.46	52.25	169.9
Bezos. 1	46.59	42.79	66.85	69.33	38.90	218.7
Doina	37.78	29.43	64.88	63.91	19.54	193.8
Adams	38.80	32.32	78.29	69.79	10.69	224.4
WWP4394	41.38	44.70	75.32	73.63	40.26	105.5
Blueboy	34.46	34.37	58.37	52.39	27.73	143.5
Houser	30.84	19.11	60.31	46.14	29.81	137.1
Alcedo	37.37	37.61	60.71	64.84	12.08	114.7
Centurk	46.31	29.01	77.95	68.21	40.42	203.0
Means	43.86	39.36	73.16	67.03	33.84	168.3
C.V.	12.42	15.72	10.64	10.59	17.28	9.7
LSD.05	3.81	4.33	5.44	4.96	4.09	11.4
LSD.01	4.96	5.64	7.09	6.46	5.33	14.9

+ = Area under the curve (relative units) X 1000.

Table 19. Correlation coefficients (r) among baking quality traits of 12 winter wheat cultivars combined over environments.

Traits	Mixing time	Loaf volume	Crumb score	Absorp. (far.)	Peak time	Stability	VAL
Absorp. (mix)	ns	.51**	.45**	.90**	.44**	.38*	.47**
Mixing time		ns	ns	ns	.47**	.75**	.61**
Loaf volume			.97**	.51**	.39*	ns	.40**
Crumb score				.46**	.39*	.25*	.43**
Absorp. (far.)					.56**	.38*	.56**
Peak time						.74**	.93**
Stability							.87**

*,** = Significant at P<0.05 and P<0.01, respectively.
ns = Not significant.

Table 20. Correlation coefficients (r) of gliadin and glutenin subunits and flour and grain protein contents of 12 winter wheat cultivar means grown in four environments (n = 48).

Gliadin and glutenin subunits	Flour protein	Grain protein
Gliadin		
Total	0.83 **	0.83 **
Subunit 29	0.78 **	0.83 **
Subunit 35	0.72 **	0.65 **
Subunit 37	0.62 **	0.71 **
Subunit 39	0.71 **	0.77 **
Total 35+37+39	0.78 **	0.81 **
Subunit 44	-0.33 *	-0.18 ns
Glutenin		
Total	0.84 **	0.78 **
Subunit 16	0.69 **	0.63 **
Subunit 21	0.72 **	0.62 **
Subunit 24	0.74 **	0.77 **
Subunit 26	0.74 **	0.61 **
Subunit 27	0.67 **	0.63 **
Total	0.86 **	0.79 **
Subunit 44	0.19 ns	0.22 ns

*,** = Significant at $P<0.05$ and $P<0.01$, respectively.
ns = Not significant.