

Levels of Protein and Protein Composition in Hard Winter Wheat Flours and the Relationship to Breadmaking¹

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ABSTRACT

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Protein and protein fractions were measured in 49 hard winter wheat flours to investigate their relationship to breadmaking properties, particularly loaf volume, which varied from 760 to 1,055 cm³ and crumb grain score of 1.0–5.0 from 100 g of flour straight-dough bread. Protein composition varied with flour protein content because total soluble protein (SP) and gliadin levels increased proportionally to increased protein content, but albumins and globulins (AG), soluble polymeric proteins (SPP), and insoluble polymeric protein (IPP) levels did not. Flour protein content was positively correlated with loaf volume and bake water absorption ($r = 0.80$, $P < 0.0001$ and $r = 0.45$, $P < 0.01$, respectively). The

percent SP based on flour showed the highest correlation with loaf volume ($r = 0.85$) and low but significant correlation with crumb grain score ($r = 0.35$, $P < 0.05$). Percent gliadins based on flour and on protein content were positively correlated to loaf volume ($r = 0.73$, $P < 0.0001$ and $r = 0.46$, $P < 0.001$, respectively). The percent IPP based on flour was the only protein fraction that was highly correlated ($r = 0.62$, $P < 0.0001$) with bake water absorption followed by AG in flour ($r = 0.30$, $P < 0.05$). Bake mix time was correlated positively with percent IPP based on protein ($r = 0.86$) but negatively with percent SPP based on protein ($r = -0.56$, $P < 0.0001$).

Proteins have long been known as the unique component in wheat responsible for its breadmaking quality. Wheat flour proteins can be divided into two broad groups, the gluten and non-gluten proteins. Nongluten proteins include primarily albumins and globulins (AG), which are considered mainly metabolic proteins but may have some role in breadmaking (Hoseney et al 1969a). Gluten proteins (gliadins and glutenins) have been recognized as the major components responsible for variations in breadmaking characteristics. Gliadin proteins have little resistance to extension and are mainly responsible for the cohesiveness of dough, whereas glutenin proteins give dough resistance to extension (Dimler 1965; Hoseney 1992; Uthayakumaran et al 2000). Wheat proteins can also be classified based on molecular size, either polymeric or monomeric proteins. Polymeric proteins include mainly glutenins with minor amounts of high molecular weight AG, whereas monomeric proteins are gliadins with low molecular weight AG (MacRitchie 1992).

Many studies have attempted to relate wheat proteins to breadmaking quality (Bushuk 1985; Hoseney and Rogers 1990; MacRitchie 1992; Borneo and Khan 1999; Toufeili et al 1999; Uthayakumaran et al 1999; Wooding et al 1999; Khatkar et al 2002a,b; Tronsmo et al 2002; Cuniberti et al 2003). Pioneering work in this area was reported by Finney and Barmore (1948), who found bread loaf volume in hard red winter and spring wheat cultivars grown at several regions was related to protein quantity. Later, Finney and Yamazaki (1967) and Finney (1984) stated that both quantity and quality of proteins affected breadmaking properties such as mixing time, tolerance, dough handling properties, water absorption, oxidation requirements, loaf volume, and crumb characteristics of bread.

Recent research has been conducted to understand the role of wheat proteins in breadmaking quality, especially with regards to the large polymeric wheat proteins and dough strength (MacRitchie 1992; Wooding et al 1999; Cuniberti et al 2003). However, there is still debate as to the role of the various protein classes on breadmaking parameters such as absorption, mixing, loaf volume, and crumb grain. For example, gliadin proteins have been reported to be highly related to loaf volume by many researchers (Hoseney et al 1969a,b; Finney et al 1982; Branlard and Dardevet 1985; Weegels et al 1994; Khatkar et al 2002a,b). Others, however, have observed that gliadin proteins have an insignificant effect on loaf volume and that the glutenin proteins are the major components responsible for loaf volume (MacRitchie 1978, 1985; MacRitchie et al 1991; Gupta et al 1992; Borneo and Khan 1999; Toufeili et al 1999; Uthayakumaran et al 1999). Labuschagne et al (2004) also found that fractions with mainly gliadins negatively affect important quality traits. Large polymeric to monomeric protein ratio was related to better baking qualities.

Many studies have been conducted to relate dough strength or loaf volume with protein content or protein composition as a separate research object. However, limited research has been conducted to explain the effects of changes in protein content and composition together on breadmaking parameters such as water absorption and mix time requirements, loaf volume, and crumb grain. The objectives of this research were three-fold: to investigate the relationship between flour protein content and protein composition in hard winter wheat flours; to find individual effects of protein subclasses; and to find overall effects of flour protein content and protein composition on breadmaking parameters.

MATERIALS AND METHODS

Samples

Forty-nine hard winter wheats were provided by the U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), Grain Marketing and Production Research Center (GMPRC), Hard Winter Wheat Quality Laboratory, Manhattan, KS. Eight wheats were from the North Central Plains of the Northern Regional Performance Nursery, including SD94149, Jagger, Crimson, SD94241, SD94227, Tandem, Rose, and SD 93528. Forty-one wheats were from the Southern Regional Performance Nursery, including W94-245, OK94P549, W94-137, T89, W94-042, W94-320, W94-435, and T93 from the North Central Plains; W94-042, OK94P461, PI495594, NE93405, NE93427, XH1881, T93, NE93496, G1594, and G1720 from the North High Plains; W94-042, KS84W063-9-39-3MB, W94-435, T89, CO920696, NE93496,

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CO940700, CI13996, NE93405, TX95V4993, T94, OK94P461, TX94V2130, and CO910424 from the South Central Plains; and T89, WX95-2401, XH1877, CI13996, NE93405, NE94632, OK94P461, TX94V2130, and KS85W663-11-6-MB from the Southern High Plains region.

Protein Composition

Protein subclasses were measured using a combination of extraction, SE-HPLC, and nitrogen by combustion (Bean et al 1998). Briefly, wheat flour (250 mg, 14% mb) was mixed with 1 mL of 50% 1-propanol using a spatula. Samples were continuously vortexed for 5 min and centrifuged (Eppendorf 5415C) at 10,000 rpm for 5 min. This extraction procedure was repeated a total of three times. The first and second supernatants were pooled 1:1 for analysis of total soluble proteins (SP) using a Hewlett Packard 1090A HPLC system with a Waters ProteinPak 300SW SEC column (300 × 7.8 mm). Mobile phase was 50% acetonitrile plus 0.1% trifluoroacetic acid (w/v), column temperature was 40°C, and flow rate was 1 mL/min. All samples were filtered through 0.45-µm filters before analysis and 20-µL sample was injected. Proteins were detected with UV at 210 nm. Chromatograms were divided into three areas including soluble polymeric protein (SPP), gliadins, and albumins and globulins (AG) (Fig. 1) (Larroque et al 1997; Bean et al 1998). The pellets remaining after centrifugation were analyzed to determine the insoluble polymeric protein (IPP). The pellets were mixed with 1 mL of acetone, the mixture centrifuged, and the supernatant discarded. The pellets were then broken into pieces and dried in an oven at 130°C for 1 hr. Protein content in dried pellets was determined by combustion analysis (Approved Method 46-30, AACC International 2000) using a Leco FP-428 nitrogen determinator (St. Joseph, MI). All measure-

ments from SE-HPLC and combustion data were converted into milligram quantities and then reconverted into percentages for easy comparison among the various protein subclasses as described in Bean et al (1998).

Baking and Bread Quality Evaluation

An optimized straight-dough baking method (Approved Method 10-10B, Finney 1984) was used for an experimental breadmaking test. The bread formula contained 100 g of flour (14% mb), 11 mL of a solution containing 6 g of sucrose and 1.5 g of sodium chloride, 5 mL of aqueous malt mixture (0.25 g of dried malt), dry active yeast (1.0 g), shortening (3 g), and 1 mL of ascorbic acid solution (5 mg). Bake water absorption and mixing time were estimated based on mixograph data and finally optimized subjectively by the appearance and feel of the dough.

Doughs were fermented for 90 min at 86% rh and 30°C. They were baked at 218°C (425°F) for 18 min and were weighed immediately after removal from the oven. Loaf volume (cm³) was measured by rapeseed displacement immediately after weighing the loaf of bread. One-day-old breads were machine-sliced and crumb grain was graded by a baking expert. Crumb grain scores were graded and recorded on a scale of 0–6, where 0 is unsatisfactory; 1 is questionable to unsatisfactory; 2 is questionable; 3 is questionable to satisfactory; 4 is satisfactory; 5 is excellent; and 6 is outstanding. The descriptions for each grade were presented in detail in our previous study (Park et al 2004). In the present study, no breads showed a crumb grain score of 0 or 6.

Statistical Analysis

A completely randomized design was used and samples were analyzed in duplicate. Linear correlations were analyzed by the Statistical Analysis System (v.8.0, SAS Institute, Cary, NC). Statistical abbreviations were linear correlation coefficient (*r*) and significant at *P* < 0.05, <0.01, <0.001, and <0.0001 (*, **, ***, and ****, respectively).

RESULTS AND DISCUSSION

Protein Composition

The levels of protein in 49 hard winter wheat flours are reported based on flour weight, whereas those of protein components are based on flour or the protein in flour (Table I). Flour proteins were classified in two broad classes including total SP, which was the fraction soluble in 50% aqueous 1-propanol, and the IPP, which was not soluble. Total SP was further subdivided into three subclasses of AG, gliadins, and SPP. IPP are mostly high molecular weight glutenins and SPP are mostly low molecular weight glutenins.

The mean level of total SP was 7% of flour and 60% of protein in flour (Table I). There were wide variations in the levels of total SP based on flour and on protein. The AG contents averaged only

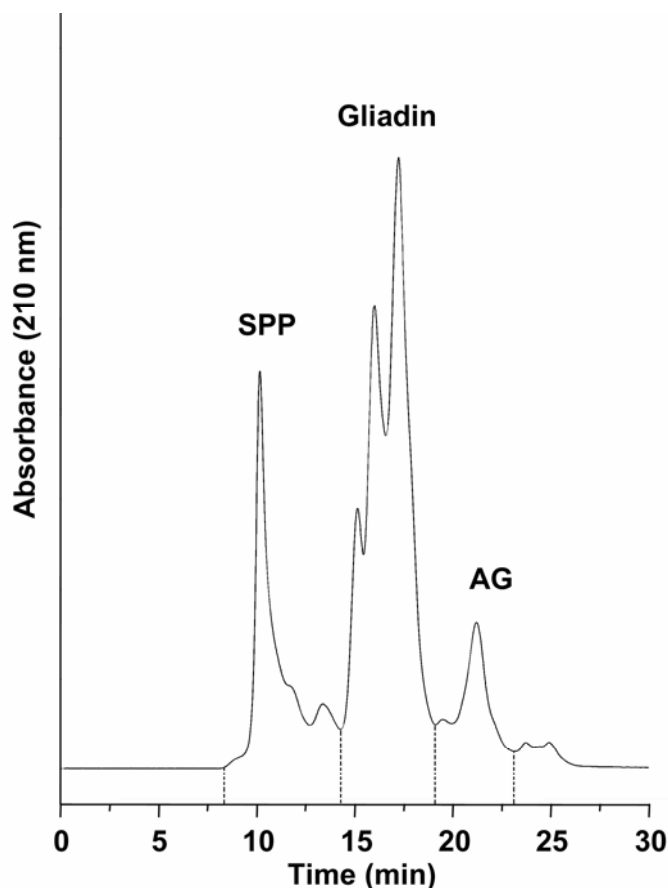


Fig. 1. Typical size-exclusion high-performance liquid chromatogram of wheat flour proteins soluble in 50% aqueous 1-propanol. Soluble polymeric protein (SPP); albumin+globulin (AG).

TABLE I
Protein and Protein Classes and Subclasses
in 49 Hard Winter Wheat Flours^a

Protein	% of Flour		% of Protein	
	Mean	Range	Mean	Range
Total protein	11.7	9.8–13.5	–	–
Total SP ^b	7.0	5.6–8.8	60.0	50.4–62.8
AG	1.1	1.0–1.3	9.6	7.9–11.8
Gliadin	4.0	3.0–5.1	34.3	30.6–39.1
SPP	1.4	0.9–1.9	12.2	7.3–15.0
IPP	4.7	3.6–5.5	40.0	35.9–45.3

^a Average values of two replicates.

^b SP, soluble protein; AG, albumin and globulin; SPP, soluble polymeric protein; IPP, insoluble polymeric protein.

1.1% with a relatively narrow variation of 1.0–1.3% of flour but showed a wider variation of 7.9–11.8% based on protein. Thus, while the level of AG in flour did not vary much, the proportion of AG in protein did. This result is accounted for by the large variation in the levels of the other protein subclasses in the various wheat lines as indicated by the protein content of 9.8–13.5%.

Gliadins were the major subclass of total SP with an average value of 4.0% of flour and 34.3% of protein in flour. Gliadin contents (% flour weight) varied by a factor of 1.7 (3.0–5.1% of flour) compared with a factor of 1.3 (30.6–39.1% of protein in flour). Thus, there is a wider variation in the level of gliadins when calculating based on flour than when calculating based on protein, as the level of other protein subclasses were changed with variation of flour protein contents. Therefore, the changes in absolute amount of a given protein subclass and its contribution to the protein composition will not always be parallel to each other. The gliadin percentages in flour reported here are generally lower than in previous studies (Hoseney et al 1969b; Orth and Bushuk 1972). In those using the same methods, gliadins were extracted with aqueous alcohols and quantitated gravimetrically, so both the AG and SPP fractions would have been counted together with the gliadins.

The SPP accounted for a small portion of the flour (1.4%) and the protein (12.2%), but with a twofold variation in level based on

flour (0.9–1.9%) and on protein (7.3–15.0%). The SPP showed the largest variation in level whether the level was calculated based on flour or protein in flour. For IPP, the mean value was 4.7% of flour and 40% of protein with a variation, respectively, of 3.6–5.5% and 35.9–45.3% (Table I).

Protein Classes and Subclasses in Relation to Flour Protein Content

The levels of protein class and subclass increased with increasing flour protein (Table II). This was especially evident for total SP and its gliadin fraction, which showed high correlations ($r = 0.95^{****}$ and $r = 0.93^{****}$, respectively). Levels of both AG and SPP based on flour were correlated to flour protein content but to a much lesser degree ($r = 0.36^*$ and $r = 0.44^{**}$, respectively). The AG content increased by only 0.3 percentage point with a 3.7 percentage point change of total protein content (9.8–13.5%) (Table I). As a result, AG (% total protein weight) showed a highly negative correlation ($r = -0.73^{****}$) with flour protein contents because the increase in the AG content was not as much as those in other protein subclass fractions (Table II). Our result is in agreement with a previous report by Singh et al (1990), who also observed a strong negative correlation ($r = -0.92^{****}$) of flour level with the level of AG based on protein. The declining levels of AG when protein increased in flour results from the building of storage proteins at the expense of the metabolically active AG proteins when wheat kernels mature in the field (Hoseney 1992; Eliasson and Larsson 1993; Tribotí et al 2003).

As mentioned earlier, flours with higher total protein contents would have also higher contents of all protein subclasses than flours with lower protein contents. However, the level of contribution of each subclass to the increase of total protein content is different. The SP class contributes more to an increase of protein content in any of the 49 hard wheat flours than does the IPP. That conclusion is based on the opposite correlations between protein in flour with the level of SP and IPP based on protein ($r = 0.39^{**}$ and $r = -0.38^{**}$, respectively, Table II). In addition, the range in the level of SP of 3.2% based on flour was by far more than for IPP of 1.9% based on flour (Table I). The gliadin fraction in the SP class increased more than the AG and SPP fraction as wheat protein increased (Table II). Gupta et al (1992) found similarly that the level of gliadins calculated based on flour or protein in flour were highly correlated with increased protein content, whereas the level of glutenin based on flour, but not on protein, correlated with protein content. Tronsmo et al (2002) also observed a higher ratio of monomeric to polymeric protein from the higher protein content flour when proteins were separated by SE-HPLC. Wieser and Seilmeier (1998) found, after different levels of nitrogen ferti-

TABLE II
Correlation Coefficients (r) of Flour Protein, Protein Class, or Subclass vs. Total Flour Protein (TFP), Loaf Volume (LV), and Crumb Grain Score (CGS)^a

Protein ^b	TFP	LV	CGS
Total protein	1	0.80 ^{****}	0.35 [*]
% Based on flour			
Total SP	0.95 ^{****}	0.85 ^{****}	0.35 [*]
AG	0.36 [*]	ns	ns
Gliadin	0.93 ^{****}	0.73 ^{****}	0.31 [*]
SPP	0.44 ^{**}	0.66 ^{****}	0.29 [*]
IPP	0.78 ^{****}	0.50 ^{**}	ns
% Based on protein			
Total SP	0.39 ^{**}	0.52 ^{***}	ns
AG	-0.73 ^{****}	-0.62 ^{****}	-0.40 ^{**}
Gliadin	0.65 ^{****}	0.46 ^{**}	ns
SPP	ns	ns	ns
IPP	-0.38 ^{**}	-0.52 ^{***}	ns

^a Significant at $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***), and $P < 0.0001$ (****); not significant (ns); ($n = 49$).

^b SP, soluble protein; AG, albumin and globulin; SPP, soluble polymeric protein; IPP, insoluble polymeric protein.

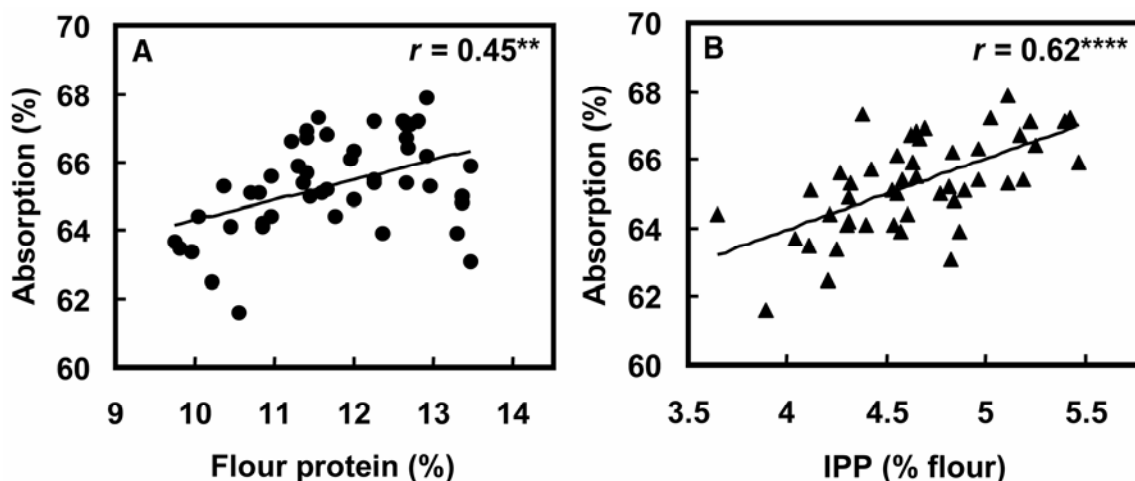


Fig. 2. Regression lines between baking water absorption (%) of flour and flour protein and insoluble polymeric protein (IPP, % based on flour weight).

lization treatments, a pronounced increase in the level of gliadins rather than the levels of AG and glutenin with an increase in protein content.

Protein Subclasses in Relation to Breadmaking Quality

Water absorption. Bake water absorption was significantly correlated with flour protein content ($r = 0.45^{**}$) (Fig. 2), % AG based on flour ($r = 0.30^*$, data not shown), and IPP based on flour ($r = 0.62^{****}$) (Fig. 2). In spite of high linear correlation of flour protein with both total SP and gliadin contents based on flour ($r = 0.95^{****}$ and $r = 0.93^{****}$, respectively), total SP and gliadin levels did not show significant correlations with bake water absorption (data not shown). Early in 1967, Finney and Yamazaki reported that bake water absorption increased as protein content increased, but they suggested also that some unknown quality of protein might be responsible for variation in bake water absorption. Data shown in Fig. 2 suggest that the IPP is the unknown responsible for the variation of bake water absorption. Bean et al (1998) also found significant correlations between IPP and bake water absorption.

Mix time. Bake mix time was negatively affected by the % SPP based on flour content ($r = -0.63^{****}$), followed by % total SP content ($r = -0.51^{***}$), and the % gliadins ($r = -0.38^{**}$) (graphical data not shown). Those correlation data indicate that the greater the SP content of flours, the shorter the mix time becomes. In agreement with that conclusion, the IPP content based on flour was positively correlated ($r = 0.30^*$) to bake mix time. Furthermore, the % IPP based on protein was highly positively correlated with bake mix time ($r = 0.86^{****}$), whereas the % SPP based on protein was negatively correlated ($r = -0.56^{****}$) (Fig. 3). Because of those contrasting effects among protein class and subclasses on bake mix time, flour protein content did not show a significant correlation with bake mix time ($r = -0.26$).

Because % IPP based on protein had a much higher positive correlation ($r = 0.86^{****}$) than % IPP based on flour ($r = 0.30^*$) to bake mix time, the relationship between increasing flour protein and decreasing the % IPP based on protein could shorten bake mix time when total flour protein content increased. Therefore, this suggests that the more dominant factor that controls bake mix time is ratio of protein fractions rather than absolute amount of each protein fraction, at least for our flour sample set.

Loaf volume. Loaf volume was positively affected by the levels (flour basis) of several protein classes and subclasses, including total SP ($r = 0.85^{****}$), gliadins ($r = 0.73^{****}$), SPP ($r = 0.66^{****}$), and IPP ($r = 0.50^{***}$) (Table II). Those positive correlations might be due to the positive relations of those variables with flour protein, which was highly correlated with loaf volume ($r = 0.80^{****}$). Even though the level of IPP based on flour was positively correlated with loaf volume, its level based on protein was negatively correlated ($r = -0.52^{***}$). That is because, as previously described, IPP content increased less than the other SP contents with increasing protein content of flour. MacRitchie et al (1991) reported that an increase in high molecular weight glutenin, which is a major component of IPP, led to an increase in dough strength, however no effect on loaf volume was observed. Even though the loaf volume was affected by levels of both SP and IPP based on flour, the SP content in flour caused a larger

effect on loaf volume as shown by its higher correlation with loaf volume than IPP content ($r = 0.85^{****}$ vs. $r = 0.50^{***}$).

The level of AG based on flour had a low but significant correlation with protein content but it was not significantly correlated with loaf volume (Table II). On the other hand, the level of AG and IPP based on protein were negatively correlated with loaf volume ($r = -0.62^{****}$ and $r = -0.52^{***}$, respectively), probably because the relative contents of those fractions were negatively correlated with flour protein content.

Borneo and Khan (1999) reported that loaf volume of 12 samples was correlated positively ($r = 0.73^{**}$) with the level of polymeric glutenin based on protein (peak I of SE-HPLC), and negatively ($r = -0.64^*$) with the level of AG (peak III of SE-HPLC). They did not find a significant relationship between the level of monomeric gliadin based on protein (peak II of SE-HPLC) and loaf volume, whereas our results showed that the level of gliadin, based on either flour or protein weight, was positively correlated with loaf volume ($r = 0.73^{****}$ or $r = 0.46^{***}$, respectively) (Table II).

Bean et al (1998) reported a higher correlation of loaf volume with the level of IPP based on flour compared with flour protein content. Gupta et al (1992) also reported significant correlations of total polymeric protein (SPP and IPP) to loaf volume, although the relationships showed a variation depending on the baking methodology used. They did not show a relationship between the level of gliadin and loaf volume. The exact roles of the polymeric and monomeric proteins in loaf volume are still not well understood and require further study.

In agreement with our study, Khatkar et al (2002) observed an increase in loaf volume and peak dough resistance when individual or total gliadins were added back to flour at 1% (w/w, flour basis). In our work, as gliadin content increased based on flour or protein in flour, the loaf volume increased. A higher correlation

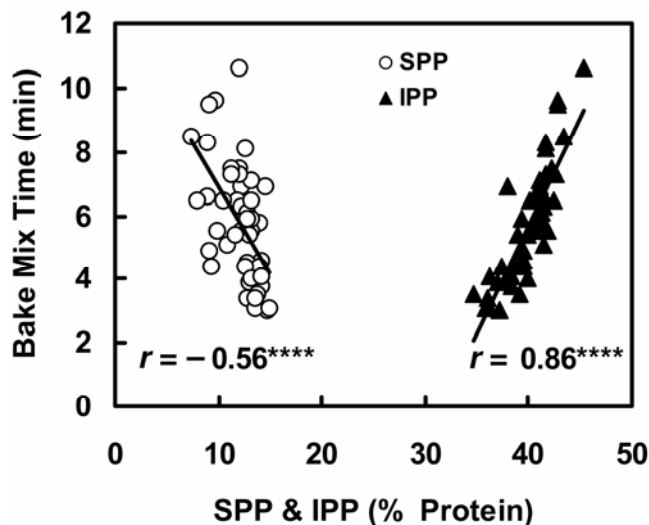


Fig. 3. Regression lines between bake mix time and soluble polymeric protein (SPP) and insoluble polymeric protein (IPP) % based on protein.

TABLE III
Baking Data of 49 Hard Winter Wheat Flours^a

Crumb Grain Score ^b	No. of Flours	Protein (%)	Water Absorption (%)	Mix Time (min)	Loaf Volume (cm ³)
1-2	10	9.8-12.7	62.5-67.1	4.5-9.5	795-980
2.5-3	15	9.8-13.4	63.4-67.3	3.1-10.2	760-1,050
3.5-4	9	10.6-13.5	62.0-67.9	3.0-7.1	875-1,010
4.2-5	15	10.9-13.5	63.9-67.2	3.5-8.5	900-1,055

^a Average values of two replicates.

^b 1 = poorest, 5 = best.

between the level of gliadin and loaf volume was found when its level was calculated based on flour rather than the protein ($r = 0.93^{****}$ vs. $r = 0.65^{****}$, Table II). The difference between those two correlations could be explained by an optimum ratio of gliadin to other protein subclasses. However, a higher order polynomial relationship between loaf volume and the ratios of gliadin to other protein subclasses (e.g., gliadin/SPP, gliadin/IPP, or gliadin/SPP+IPP) could not be found (data not shown). Thus, if gliadins are required at a certain ratio to other protein subclasses for optimum loaf volume, it is not clear which other subclasses are involved. These results indicate that another important factor is involved in the control of loaf volume in terms of protein composition. MacRitchie (2005) suggested two main factors control physical properties of dough; one is the ratio of glutenin/gliadin and the other is the molecular weight distribution of the glutenin fraction. This suggests that an optimum ratio of glutenin/gliadin is required for loaf volume, but that optimum ratio will vary with changes in the molecular weight distribution of the glutenin. This could be the reason that we found no significant polynomial relationship from the ratios of gliadins to the other protein subclasses.

Crumb grain. The ranges of breadmaking characteristics of the 49 wheat samples were grouped by different crumb grain scores (Table III). The groups still showed a wide variation in protein content, water absorption, mix time, and loaf volume. This suggested that crumb grain score must not be controlled by quality parameters such as flour protein content, bake absorption, mix time, and even loaf volume.

Crumb grain score showed a low correlation with flour protein content ($r = 0.35^*$) and also with the levels (% flour) of SP, gliadin, and SPP ($r = 0.35^*$, $r = 0.31^*$, and $r = 0.29^*$, respectively, Table II). In terms of the level of a protein class or subclass based on protein, the level of AG was the only one that showed a significant correlation with crumb grain scores ($r = -0.40^{**}$). Unlike loaf volume, the low correlation values between protein class or subclass and crumb grain score imply that protein content and composition are not the major determinants of crumb grain properties. Previous studies (Van Vliet et al 1992; Hayman et al 1998; Park et al 2004, 2005) have shown that starch granule size distribution influences the appearance and structure of crumb grain. In addition, puroindolines, which were reported as essential lipid-binding proteins giving foaming properties to dough liquor, may play a role in determining crumb grain (Dubreil et al 1998).

CONCLUSIONS

Protein composition was found to change with changes in flour protein content; that is, all protein subclasses did not increase or decrease to the same degree with changes in total flour protein. Individual protein classes or subclasses had different effects on mixing properties and loaf volume; for example, gliadin levels based on flour were highly correlated with loaf volume. It is suggested that an optimum glutenin/gliadin ratio is required for loaf volume, but the ratio could vary with changes in the molecular weight distribution of the glutenin.

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