# Relationship of Protein Fractions of Spring Wheat Flour to Baking Quality[1]

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**ABSTRACT**

The possible relationships between flour protein fractions and quality in hard red spring wheat were investigated. Eight cultivars differing widely in quality were comparably grown in 1976 at two locations in North Dakota. Average flour protein was 13.6 and 14.6% at the two locations, with a range between varieties at each location of 2–3 percentage points. Farinograph dough peak time and mixing stability varied widely between cultivars. Flour protein was fractionated into glutenin, gliadin, albumin, and nonprotein nitrogen by gel filtration on Sephadex G-150. A positive relationship appeared to exist between glutenin in flour protein and mixing strength, and a negative relationship appeared to exist between gliadin in flour protein and mixing strength. Glutenin was fractionated into a high molecular weight fraction (glutenin I) and a low molecular weight fraction (glutenin II) by gel filtration on Sepharose CL 2B using 5.5*M* guanidine hydrochloride as eluent. Glutenin I appears to be positively related to mixing strength. The loaf volume by a baking method that remixes the dough after fermentation was positively related to glutenin I. The protein fraction insoluble in 0.05*M* acetic acid (residue protein) also has the same relationships as that observed with glutenin I. Residue protein may be predominantly a high molecular weight glutenin like glutenin I.

Bread-making capacity of flour depends not only on the quantity of protein in the wheat flour but also on its quality (Bushuk et al 1969). The chemical and physical bases for baking quality have been extensively investigated for many years but are not yet completely understood. Results of recent research by several workers indicate that the size of protein molecules making up the gluten complex and/or their degree of association or aggregation are related to mixing and baking quality. Mecham (1968), for instance, explained differences in mixing properties by a protein-aggregation hypothesis. Cereal chemists have long known that the glutenin fraction of Osborne solubility classes imparts toughness and strength to gluten. The dependence of flour baking quality on the molecular weight distribution of gluten protein has been suggested by different investigators. Tanaka and Bushuk (1973) found that the gluten of wheat cultivars with long mixing time had components of higher molecular weight than cultivars of shorter mixing time. Lee and MacRitchie (1971) and MacRitchie (1973) reported that the addition of high molecular weight gluten fractions to a weak flour produced a stronger flour. Huebner and Wall (1976) observed that the amount or ratio of the highest and lowest molecular weight portion of the glutenin protein fraction appeared to be related to quality.

Protein fractions that are insoluble in dilute acetic acid are also related to quality. Gel protein (flour protein insoluble in 0.01*N* acetic acid) was reported by Mecham et al (1972) to be positively related to mixing strength; residue protein was reported by Orth and Bushuk (1972) to be positively related to mixing strength and bread loaf volume by the remix baking method.

Using these reports on the relationship of protein fractions to quality, we investigated bread-making quality of hard red spring wheat grown in North Dakota. Flour proteins of wheat cultivars with a broad spectrum of dough-mixing requirements and baking characteristics were fractionated through different methods. The relative amount of each fraction was related to flour quality, using statistical analysis.

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## MATERIALS AND METHODS

### Flour Samples

Eight hard red spring wheat cultivars were used. Cultivars were grown comparably in test plots at Carrington, ND (irrigated) and at Casselton, ND (dryland) in 1976. The cleaned wheat samples were tempered to 15.5% moisture and milled on a Buhler Experimental Mill (type MLV 202). The six flour streams were thoroughly blended and rebolted through 60- and 80-mesh sieves.

### Quality Evaluation

*Analysis and Dough Properties.* The flour samples were analyzed in duplicate for moisture, ash, and protein, using AACC methods 44-19, 08-01, and 46-11, respectively (1976). The dough rheological properties of flours were measured with a Brabender farinograph (AACC method 54-21), with the peak dough consistency centered at the 540 Brabender unit line of the farinogram.

*Experimental Baking Tests.* Flour samples were baked in duplicate, using a straight dough process described in AACC method 10-10. No bromate was needed in the baking formula.

Samples were also baked using the remix procedure of Irvine and McMullan (1960); dough was mixed for two min in a National mixer, fermented, and mixed again for 80 sec.

### Fractionation of Flour Proteins

*Protein Classes.* The AUC solvent of Meredith and Wren (1966), composed of 0.1*M* acetic acid, 3*M* urea, and 0.01*M* cetyltrimethylammonium bromide, was used to extract flour proteins. Wheat protein is 95–98% extracted by this solvent

(Bushuk and Wrigley 1971, Meredith and Wren 1966). Gel filtration in AUC solvent was performed on Sephadex G-150, using upward flow according to the method of Meredith and Wren and modified by Bushuk and Wrigley (1971). As shown in Fig. 1, the column was calibrated with proteins of known molecular weights. The relative proportions of glutenin (mol wt more than 100,000), gliadin (mol wt 25,000–100,000), albumin (mol wt 10,000–25,000), and nonprotein nitrogen (mol wt less than 10,000) as percent of total protein was calculated as the percent of the total absorption at 280 nm. A typical separation is shown in Fig. 2.

*Glutenin I.* The chromatographic procedure developed by Huebner and Wall (1976) was used for the fractionation of the AUC-extracted proteins on Sepharose CL 2B gel (2% cross-linked agarose) with 5.5*M* guanidine hydrochloride as eluent. Ultraviolet absorption again was used to measure relative protein concentrations. This agarose (Pharmacia) was employed because it could be used in guanidine HCl without partially dissolving, as occurred when Huebner and Wall used an agarose that was not cross-linked. The relative amount of the highest molecular weight glutenin fraction, glutenin I, which usually elutes as an initial peak, was calculated as the protein initially eluted in a volume of eluent corresponding to the distribution coefficient Kav = 0.30, in which $Kav = V_e - V_o / V_t - V_o$, where $V_e$ = elution volume (ml), $V_o$ = void volume (ml), and $V_t$ = total bed volume (186 ml in our 2.5-cm-diameter column). Because of the difficulty of calculation from the curve, glutenin II was not used here. A typical separation is shown in Fig. 3.

*Residue Protein.* The protein insoluble in 0.05*M* acetic acid is called residue protein (RP). This was determined in flour by the fast procedure of Orth and O'Brien (1976), which uses only one extraction with acetic acid instead of the sequential extraction with 0.5*M* NaCl, 70% ethanol, and 0.05*M* acetic acid used by Orth and Bushuk (1972). The RP protein was assumed to be the same as that produced by Orth and Bushuk (1972) because the albumins, globulins, and gliadins usually extracted first by NaCl solution and 70% ethanol are also soluble in 0.05*M* acetic acid. Three grams of flour (14.0% mb) was extracted by 75 ml of 0.05*M* acetic acid with mechanical shaking for 2 hr at room temperature and then centrifuged. The whole centrifuged cake was analyzed for nitrogen by the Kjeldahl procedure, and the result was corrected for protein solution trapped in the cake by the equation

$$\text{Percent RP} = \frac{A - \left[\left(\frac{B}{75}\right)(C)\right]}{1 - \left[\frac{B}{75}\right]}$$

where A = percent protein in cake on basis of flour weight (3 g, 14% mb), B = milliliters of solution in centrifuged cake (75 ml − ml of supernatant), and C = percent protein (14% mb) in flour.

The data were statistically analyzed on an IBM 360 computer using the statistical analysis system described by Barr and Goodnight (1972).

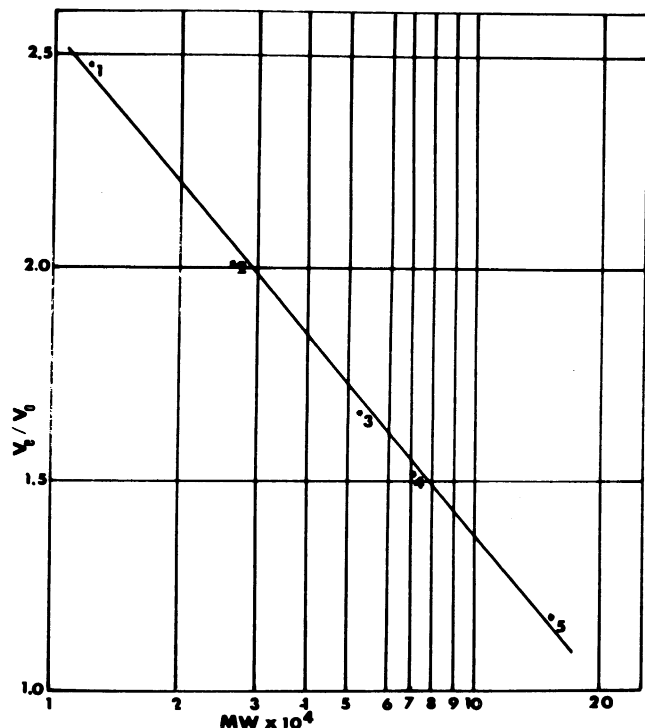


**Fig. 1.** Molecular weight calibration curve of Sephadex G-150 column. 1, Rubonuclease A (mol wt 13,700); 2, Chymotrypsinogen (mol wt 25,000); 3, ovalbumin (mol wt 45,000); 4, bovine serum albumin (mol wt 64,900); and 5, gamma globulin (mol wt 176,000).

## RESULTS AND DISCUSSION

### Flour Analysis and Bread-Baking Quality

The flour extraction from cultivars on milling ranged from 63.6 to 69.0%, with an average of 66.3%, and the ash from 0.31 to 0.47% with an average of 0.35%. The protein content and farinograph data for flour samples are shown in Table I. Cultivars are listed by increasing strength as judged from the development time, stability,

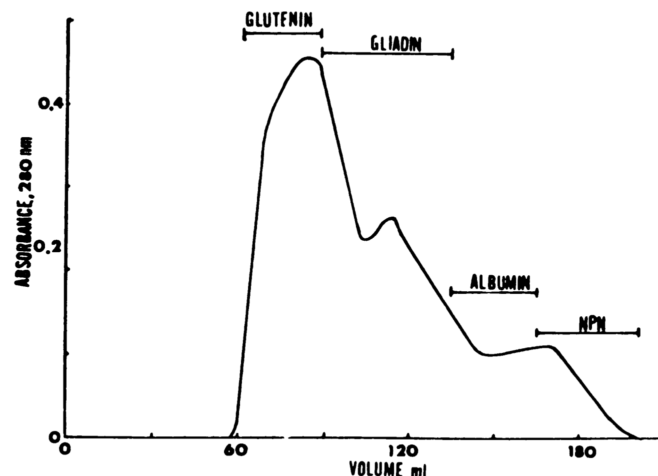


**Fig. 2.** Gel filtration of Olaf flour on Sephadex G-150 column. NPN = nonprotein nitrogen.

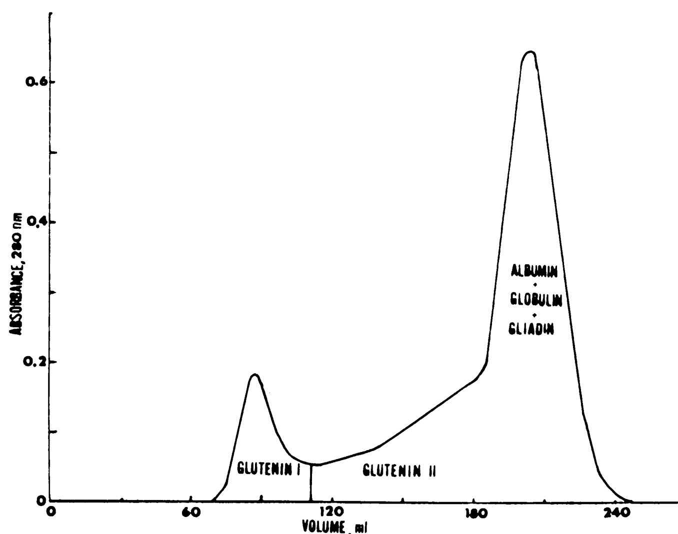


**Fig. 3.** Gel filtration of *n*-butanol defatted Manitou flour on Sepharose CL 2B column using 5.5*M* guanidine hydrochloride as eluent.

and mixing tolerance index (MTI). WS 6 is 1.5–1.8 percentage points lower in protein than Olaf and was considered stronger than Olaf even at Casselton, because lower protein contents produce a weaker curve. Flour samples were high in protein, with samples grown at Carrington averaging 14.6% and those at Casselton 13.4% at 14% mb. WS 6 was lowest in farinograph absorption, partly because it was about two percentage points lower in protein than other cultivars. Development time and mixing stability varied widely, with cultivar 12-144 as the lowest and Olaf and WS 6 the highest, whereas MTI was highest for 12-144 and lowest for Olaf and WS 6. Thus, 12-144 was the weakest, and Olaf and WS 6 were the strongest.

The loaf volume per percent flour protein of bread baked from cultivars by the AACC straight dough malt-phosphate method and the remix method are shown in Figs. 4 and 5. No significant differences in other bread properties were apparent. By the AACC baking method, almost all samples from Casselton yielded loaves having somewhat smaller volumes than loaves from the Carrington cultivars. This difference may have been caused by the differences in protein and the high temperatures of the growing season at Casselton (8° C above the average) that may have caused changes in protein composition or protein denaturation in wheat cultivars. The flour of the stronger cultivars performed more poorly than would be expected at their level of protein. The strong gluten evidently caused too much elasticity in the dough and produced oldness and reduced loaf volume in the bread. To overcome this problem, a remix baking procedure was developed by Irvine and McMullan (1960). Remixing after fermentation increases loaf volume of strong wheats but decreases that of weak wheats. The very strong or tough dough of cultivars Olaf and WS 6, however, still gave lower remix loaf volumes than weaker cultivars (Fig. 5). This decrease in volume by the strongest ones agrees with the results of Bushuk et al (1969), who also found that very strong cultivars gave low volumes by the remix method. They attributed this to undermixing with the fixed mixing time of the remix method.

The relationship of loaf volume to protein and gluten content and farinograph parameters are indicated in Table II. Flour protein

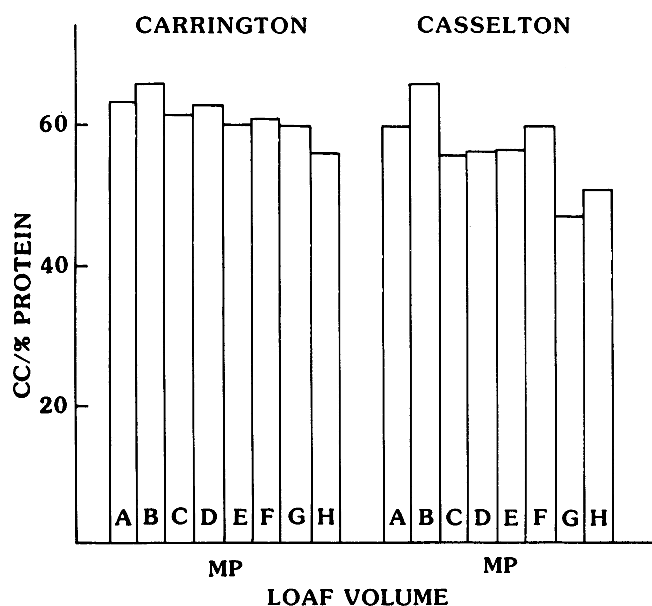


**Fig. 4.** Loaf volume per percent protein by the AACC malt phosphate (MP) method on flour samples of increasing mixing strength. Bars A–H represent, respectively, 12-144, WS 1812, Manitou, Waldron, Neepawa, Chris, Olaf, and WS 6 cultivars.

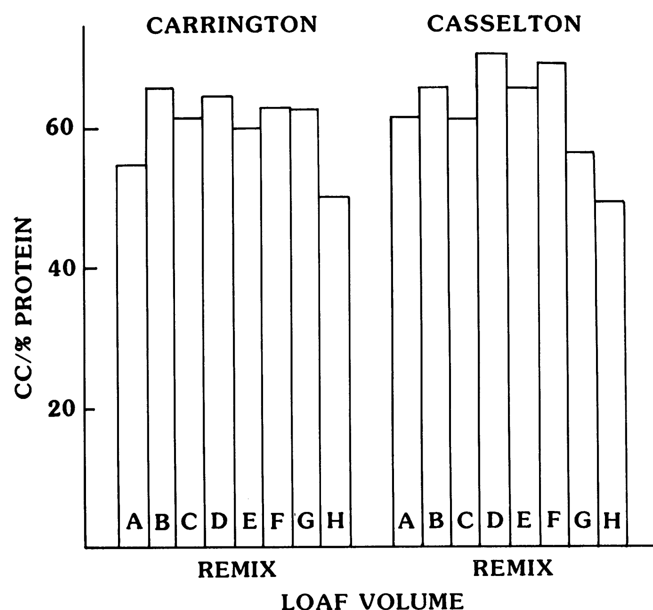


**Fig 5.** Loaf volume percent protein by the remix baking method on flour samples of increasing mixing strength. Bars A–H represent, respectively, 12-144, WS 1812, Manitou, Waldron, Neepawa, Chris, Olaf, and WS 6 cultivars.

**TABLE I**
**Protein Content and Farinograph Physical Dough Properties of Flour**

| Location | Cultivar | Protein[a] (%) | Absorption (%) | Development Time (min) | Stability (min) | Mixing Tolerance Index (BU) |
|---|---|---|---|---|---|---|
| Carrington, ND | | | | | | |
| | 12-144 | 14.3 | 62.1 | 3.0 | 3.1 | 58 |
| | WS 1812 | 13.5 | 62.2 | 5.3 | 14.4 | 30 |
| | Manitou | 15.1 | 63.4 | 5.1 | 9.5 | 35 |
| | Waldron | 15.5 | 63.9 | 5.8 | 7.8 | 35 |
| | Neepawa | 15.5 | 63.6 | 4.9 | 9.9 | 35 |
| | Chris | 16.0 | 65.7 | 6.0 | 12.0 | 35 |
| | Olaf | 14.3 | 65.1 | 20.9 | 24.9 | 10 |
| | WS 6 | 12.8 | 59.9 | 14.3 | 28.5 | 10 |
| Casselton, ND | | | | | | |
| | 12-144 | 13.8 | 60.7 | 3.8 | 3.3 | 60 |
| | WS 1812 | 12.6 | 62.3 | 4.4 | 3.9 | 55 |
| | Manitou | 14.2 | 63.3 | 6.4 | 7.0 | 18 |
| | Waldron | 13.5 | 62.6 | 7.0 | 8.0 | 23 |
| | Neepawa | 14.2 | 63.3 | 6.0 | 11.9 | 28 |
| | Chris | 14.1 | 62.9 | 6.8 | 10.1 | 20 |
| | Olaf | 13.5 | 65.2 | 13.6 | 18.0 | 13 |
| | WS 6 | 11.8 | 59.3 | 11.1 | 14.6 | 20 |

[a] 14% moisture basis.

and gluten content were positively related to loaf volume by both baking methods. The farinograph mixing qualities, however, gave correlations opposite to what would be expected; negative correlations with development time and stability and positive correlations for MTI were obtained for both baking methods. Because the two strongest wheats, Olaf and WS 6, appear to be too strong for optimum loaf volumes for even the remix method, the correlations were run without these samples. The remix loaf volume correlations with mixing characteristics are now in the correct direction, and a significant relationship exists in most cases.

### Protein Classes

The relationship of protein classes to wheat quality is indicated by the amounts of each protein class in flour protein (Figs. 6 and 7). Small differences occurred in the gliadin and glutenin fraction. Gliadin generally decreased, and glutenin generally increased in flour protein as mixing strength increased from cultivar 12-144 to WS 6 at both locations. This decrease, however, was not significant as indicated by analyses of variance (Table III). Some significant correlations of gliadin and glutenin existed with development time, mixing stability, and MTI (Table IV). Mixing strength appeared to relate negatively with gliadin and positively with glutenin. These results support the hypothesis that the stronger mixing wheats contain a higher proportion of high molecular weight proteins.

No significant correlations existed with loaf volume by either baking procedure. Even when the Olaf and WS 6 were omitted, the relationship to remix loaf volume was not significant.

### Glutenin I

The glutenin fraction was further investigated because it is the highest molecular weight class and/or the most highly associated class. The relative amounts of AUC-soluble protein from flour defatted by *n*-butanol in the highest and lowest molecular weight fractions of glutenin reportedly are related to mixing and baking quality (Huebner and Wall 1976). These workers reported that 10–23% of the defatted flour protein was not extracted. The procedure used by these workers was applied to our samples using an improved cross-linked agarose (Sepharose CL 2B, Pharmacia) in place of agarose. The weakest cultivar, 12-144, contained much less glutenin I in its AUC-extracted protein than did the other cultivars (Fig. 8). The relationship of glutenin I to quality in the other cultivars is not as clear because of the apparent significant differences between the two locations. Significant correlations also existed with glutenin I among cultivars between farinograph

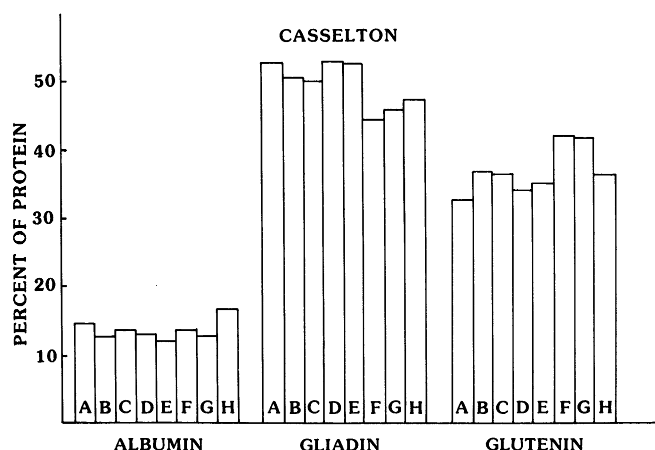


**Fig. 6.** Sephadex G-150 protein fractions in flour protein from Casselton samples. Bars A–H represent, respectively, 12-144, WS 1812, Manitou, Waldron, Neepawa, Chris, Olaf, and WS 6 cultivars.

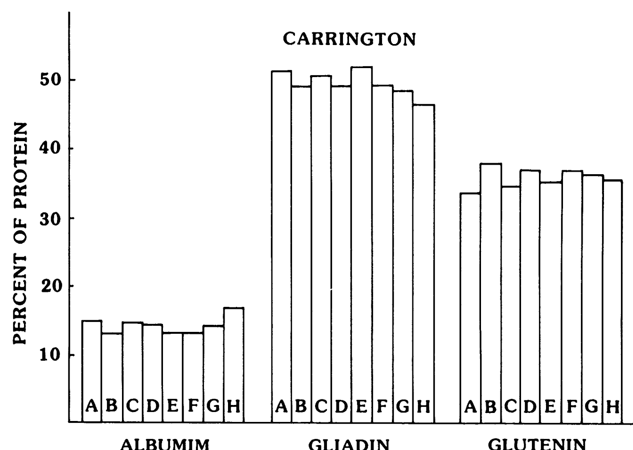


**Fig. 7.** Sephadex G-150 protein fractions in flour protein from Carrington samples. Bars A–H represent, respectively, 12-144, WS 1812, Manitou, Waldron, Neepawa, Chris, Olaf, and WS 6 cultivars.

**TABLE II**
**Correlation Coefficients of Protein Content and Farinograph Characteristics Versus Loaf Volume by Remix and Malt Phosphate Baking Procedures**

| | | Both Locations | | Casselton | | Carrington | |
|---|---|---|---|---|---|---|---|
| Samples | Correlation with | Remix Volume | Malt Phosphate Volume | Remix Volume | Malt Phosphate Volume | Remix Volume | Malt Phosphate Volume |
| All | Protein content | 0.75**[b] | 0.80** | 0.78** | 0.57* | 0.85** | 0.87** |
| | Gluten content[a] | ··· | ··· | 0.78** | 0.59* | 0.88** | 0.88** |
| | Development time | −0.37** | −0.47** | −0.56* | −0.90** | −0.32 | −0.58* |
| | Mixing stability | −0.38* | −0.37* | −0.42 | −0.76** | −0.48* | −0.78** |
| | Mixing tolerance index | 0.15 | 0.48** | −0.04 | −0.54* | 0.27 | 0.62** |
| | Remix versus malt phosphate loaf volume | r = 0.73** | | r = 0.76** | | r = 0.90** | |
| All but Olaf and WS 6 | Development time | 0.76** | −0.26 | 0.89** | −0.49* | 0.85** | 0.58* |
| | Mixing stability | 0.56** | 0.23 | 0.77** | −0.14 | 0.44 | 0.01 |
| | Mixing tolerance index x[b] | −0.62** | 0.24 | −0.77** | 0.49* | −0.67** | −0.23 |

[a] Gliadin plus glutenin.
[b] ** = significant at $P = 0.01$, * = significant at $P = 0.05$.

parameters or remix loaf volumes when Olaf and WS 6 were not included in the statistical analysis (Table V). Glutenin I gave a positive correlation to mixing strength and remix loaf volumes. The results again indicate that the higher molecular weight or more highly associated molecules are in stronger flours.

### Residue Protein

The relationship of dilute acetic acid-insoluble flour protein in our samples to baking quality was also investigated by determining RP (Orth and O'Brien 1976). In flour protein, the proportions of RP tended to increase as mixing strength increased in cultivars at both plot locations (Fig. 9). The relationships of RP content in protein of flour to mixing characteristics and loaf volumes are indicated in Table VI. Residue protein was significantly related positively with mixing strength, in agreement with the original work of Orth and Bushuk (1972) and later work of Orth (1976). Again, correlations with remix loaf volumes were in the wrong direction when Olaf and WS 6 were included, which occurs because they are too strong for the remix baking procedure. Without these two samples, significant positive relationships were observed with the remix loaf volumes but not with malt-phosphate loaf volumes. This remix relationship agrees with that of the original study of Orth and Bushuk (1972) in which the remix baking method was used.

**TABLE III**
**Analysis of Variance for Fractions in Flour Protein**

| Variable | F Value | |
|---|---|---|
| | Cultivar | Location |
| Albumin | 13.27[a] | 4.00 |
| Gliadin | 2.75 | 0.04 |
| Glutenin | 3.17 | 0.60 |
| Glutenin- to-gliadin ratio | 2.67 | 0.42 |

[a]Significant at $P = 0.01$.

**TABLE IV**
**Correlation Coefficients of Gliadin and Glutenin in Protein and Bread Quality Characteristics**

| Correlation with | Samples | | | |
|---|---|---|---|---|
| | Casselton | | Carrington | |
| | Gliadin | Glutenin | Gliadin | Glutenin |
| Development time | −0.60**[a] | 0.52* | −0.43 | 0.30 |
| Mixing stability | −0.56* | 0.53* | −0.56* | 0.40 |
| Mixing tolerance index | 0.50* | −0.50* | 0.53* | −0.04 |
| Loaf volume | | | | |
| Remix | 0.28 | ··· | 0.41 | −0.04 |
| Remix without Olaf and WS 6 | −0.30 | 0.38 | −0.21 | 0.34 |
| Malt phosphate | 0.38 | −0.18 | 0.28 | 0.13 |

[a]** = significant at $P = 0.01$, * = significant at $P = 0.05$.

**TABLE V**
**Correlation Coefficients of Glutenin I in Protein Extract and Baking Characteristics Without Olaf and WS 6**

| Correlation with | Glutenin I at | | |
|---|---|---|---|
| | Both Locations | Casselton | Carrington |
| Development time | 0.80**[a] | 0.81** | 0.85** |
| Mixing stability | 0.66** | 0.66** | 0.82** |
| Mixing tolerance index | −0.79** | −0.75** | −0.93** |
| Loaf volume | | | |
| Remix | 0.56** | 0.67** | 0.56* |
| Malt phosphate | −0.08 | −0.29 | 0.20 |

[a]** = significant at $P = 0.01$, * = significant at $P = 0.05$.

## CONCLUSIONS

Our results agree here with those of other workers (Bushuk et al 1969, Huebner and Wall 1976, Lee and MacRitchie 1971, MacRitchie 1973, Orth and Bushuk 1972); higher molecular weight gluten protein tends to increase dough mixing time and stability. This was indicated by the significant relations between the amount of glutenin, glutenin I, or RP in flour protein and mixing properties. The apparent higher molecular weight may be caused by larger molecules or by a high degree of association between glutenin protein molecules, as suggested by some workers (Kobrehel and Bushuk 1977, Wasik et al 1979). Apparently, no molecule or small fractions of the high molecular weight proteins contain most of the mixing quality; rather, a large number of molecules appear to be involved. A need may also exist for both large or highly associated molecules and small, weakly associated molecules; further work on RP indicates that it contains low molecular weight as well as high molecular weight, or highly associated, smaller molecules.

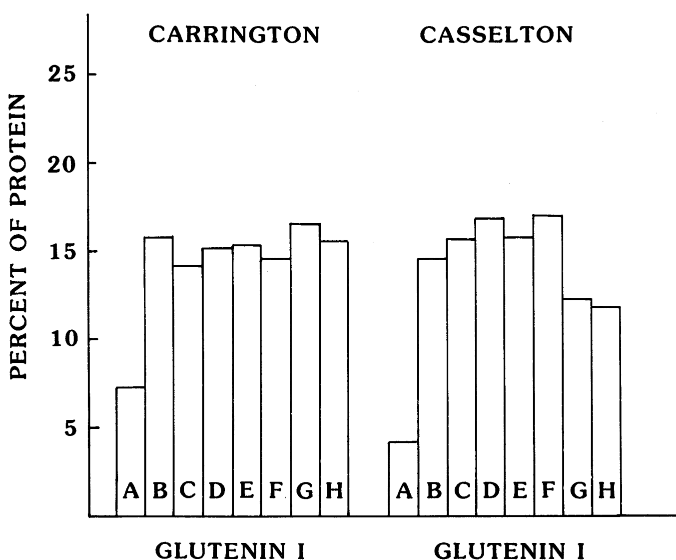


**Fig. 8.** Glutenin I from Sepharose CL 2B. Bars A–H represent, respectively, 12-144, WS 1812, Manitou, Waldron, Neepawa, Chris, Olaf, and WS 6 cultivars.

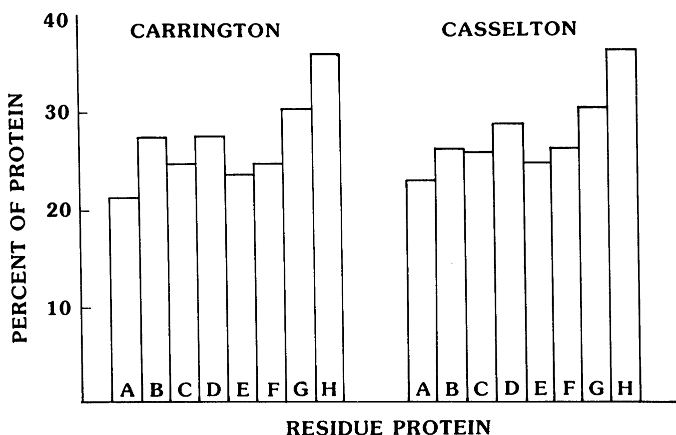


**Fig. 9.** Residue protein found in flour protein. Bars A–H represent, respectively, 12-144, WS 1812, Manitou, Waldron, Neepawa, Chris, and WS 6 cultivars.

**TABLE VI**
**Correlation Coefficients of Residue Protein in Protein and Quality Characteristics**

| Correlation with | Both Locations | | Casselton | | Carrington | |
|---|---|---|---|---|---|---|
| | All | Without Olaf and WS 6 | All | Without Olaf and WS 6 | All | Without Olaf and WS 6 |
| Mixing time | 0.72**[a] | 0.73** | 0.80** | 0.72** | 0.73** | 0.76** |
| Mixing stability | 0.74** | 0.39* | 0.69** | 0.29 | 0.90** | 0.64** |
| Mixing tolerance index | −0.69** | −0.60** | −0.52* | −0.56* | −0.88** | −0.79** |
| Loaf volume Remix | −0.65** | 0.51** | −0.65** | 0.66** | −0.53* | 0.51* |
| AACC Malt phosphate | − 0.72** | −0.25 | −0.90** | −0.52* | −0.78** | 0.22 |

[a] ** = significant at $P = 0.01$, * = significant at $P = 0.05$.

The relationship of protein fraction to bread loaf volume depends on the bread-baking procedure used. Because the AACC straight dough method gives optimum loaf volume with medium strength flour (Irvine and McMullan 1960), all but the weakest hard red spring wheats used here gave lower than expected volumes. With the remix baking procedure, strong wheats give optimum volumes (Bushuk et al 1969, Irvine and McMullan 1960), but two varieties that were used here are very strong and thus gave volumes that were lower than expected. When these two varieties were omitted, a positive relationship of RP and glutenin I in flour protein to remix loaf volumes was observed.

## ACKNOWLEDGMENTS

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