Comparative Studies on Glutenins From Different Classes of Wheat

Floyd R. Huebner

Glutenins from 11 varieties of wheat representing the five different classes commonly grown in the United States were compared in terms of protein composition and sensitivity to salt precipitation. Gluten obtained from the different wheats varied from 8 to 10.5% for soft wheats to about 9 to 11% for hard wheats and 17% for durums. Gliadin–glutenin ratios were similar in all varieties investigated: generally 46 to 50% gliadin to 50 to 54% glutenin. Electrophoretic patterns of the reduced and alkylated glutenins showed significant variations among varieties of the same class, but the greatest differences were among classes. Reduced-alkylated glutenin from durum wheats contained little or none of the slowest moving components present in the other wheats analyzed. Response of glutenins to precipitation with salt suggests that the quality of a gluten may be related to the sensitivity of its glutenin proteins to changes in ionic strength. Varieties recognized for higher quality in making bread or pasta products yield steeper precipitation curves than poorer quality wheats. The relationship is most valid for wheat varieties in the same class.

Considerable effort has been devoted to the study of wheat proteins and their relationship to the unique cohesive-elastic properties of wheat flour doughs. Nevertheless, identities of factors directly responsible for dough quality in breadmaking, for example, remain elusive, and predicting the behavior of a dough from measurements on purified fractions has not yet been possible. Earlier work (Aiken and Geddes, 1938, 1939; Finney, 1943) showed that increased levels of gluten or improved gluten quality are mostly responsible for increases in loaf volume. Lee and Lai (1967) suggest that an increase in masked sulfhydryls of HRS wheat protein also increases loaf volume, although not all varieties tested followed this generalization.

Relationships between gluten fractions or individual gluten proteins and dough quality have yet to be thoroughly explored. Previous work from this laboratory showed several qualitative and quantitative differences in the gliadin components from varieties of wheat of different dough properties (Huebner and Rothfus, 1968). In general, however, it was impossible to relate different gluten properties of wheats in the same class to differences in their gliadin composition. Either other factors are more important or small differences in gliadin composition can cause large variations in dough quality.

The purpose of this work is to extend the comparative analysis of gluten to the proteins of the glutenin fraction.

MATERIALS AND METHODS

Gluten was extracted from 2 grams of each variety of defatted (with dry butanol) wheat flour by stirring vigorously in 100 ml. of 0.05*N* acetic acid for 15 minutes. The residue was removed by centrifugation at 2500 × g. The solution was lyophilized and then from 70 to 90 mg. of the crude protein was purified by gel-filtration. Unless stated otherwise, all results were obtained with glutenins purified by the gel-filtration technique.

Carbohydrate was determined as glucose by the phenol–sulfuric acid method of Dubois *et al.* (1956). Glucose from the National Bureau of Standards served as the reference.

The glutenins were reduced in 6*M* urea and 0.1*M* potassium phosphate buffer, pH 8.0, with a 50 to 100*M* excess of 2-mercaptoethanol (Eastman Chemicals) based on the disulfide content. After 30 minutes, a fivefold excess of acrylonitrile (Eastman Chemicals) was added and alkylation was continued for 15 minutes, after which the pH was adjusted to 4.0 with acetic acid and the samples were dialyzed in 0.1*N* acetic acid and then lyophilized.

Electrophoresis was in starch gel with 3*M* urea aluminum lactate buffer at pH 3.1 (Huebner *et al.*, 1967).

Glutenin sensitivities to salt solutions were measured turbidimetrically (Beckwith *et al.*, 1963). Glutenin from each variety was dissolved in 2*M* urea (0.3% protein), 0.03*M* acetic acid. A series of dilutions (from 4 to 6 for each variety) was then made with additional urea–acetic acid and 1*M* NaCl–urea–acetic acid solution so the final concentration of glutenin was 0.06% and the salt concentration varied from 0 to 0.8*M*. The solutions were agitated rapidly during addition of the salt solution to prevent heavy localized precipitation. The absorbances of the resulting solutions were then read in a Beckman B spectrophotometer at 520 mμ two or three times during an hour.

RESULTS

As a mixture of proteins, the glutenin fraction from wheat flour is best identified in terms of both the methodology through which it is obtained and the properties exhibited by the majority of its components. Glutenin is commonly prepared by extraction from gluten with 0.1*M* acetic acid followed by precipitation in 70% ethanol (Jones *et al.*, 1959). This method was used to compare the 12 varieties shown in Figure 1; the soluble fractions were used for the previous gliadin study (Huebner and Rothfus, 1968). In Figure 1 differences in the chemical makeup of glutenins are apparent among varieties. The largest variations are among different classes. For example, compared to wheats in other classes, the durums have more bands in the center area and very few or no slower moving components. Similarly, gliadin proteins from durum wheats show greater differences in the slower migrating components (Huebner and Rothfus, 1968).

The other varieties all appear to have three main sets of bands labeled α, β, and γ, according to decreasing mobility. Generally, two prominent bands are present in each set; however, Red Chief, Selkirk, and Knox show only one band in the γ set and Selkirk, Lee, Knox, and Seneca only one major band in the β set. Other variations are the β- band in Lee, which is ahead of the β- bands in other hard and soft wheats. Also, Knox and Seneca, which appeared low in

Northern Regional Research Laboratory, U. S. Department of Agriculture, Peoria, Ill. 61604

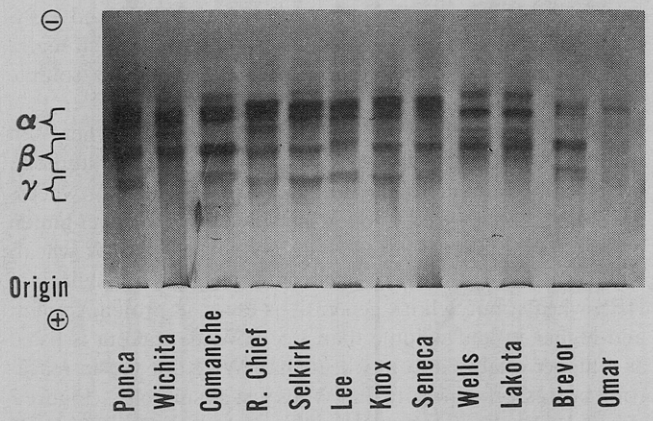

Figure 1. Starch-gel electrophoresis of reduced and alkylated glutenins. Buffer: 3*M* urea, aluminum lactate, pH 3.1. HRW: Ponca, Wichita, Comanche, and Red Chief. HRS: Selkirk and Lee. SRW: Knox and Seneca. Durum: Wells and Lakota. SWW: Brevor. Club: Omar

alpha-gliadins (Huebner and Rothfus, 1968), seem to have more fast-moving components in their reduced glutenins. The structures of these subunits and their characteristics are still to be determined; nevertheless, differences in the glutenin fraction among different varieties are apparent.

Chromatography of Gluten. Material prepared by precipitation from 70% ethanol was suitable for the chemical comparison of reduced and alkylated glutenins by electrophoresis, but too insoluble for use in salt precipitation experiments. A more soluble and more definitive (by molecular size) glutenin fraction resulted from gel-filtration chromatography similar to Meredith and Wren (1966), of whole gluten extracted from the defatted flour by 0.1*N* acetic acid. Generally, good separations of glutenin, gliadin, albumin, and nonprotein components in most of the wheats were obtained on a 2.2 × 58 cm. column of Sephadex G-100 in 0.1*M* acetic acid, 2*M* urea with 50- to 100-mg. samples. Glutens from Ponca, Seneca, and Knox, however, were not satisfactorily resolved under these conditions. Resolution was not improved by increasing the urea concentration, decreasing acetic acid, or adding 2*M* dimethylformamide. The chromatographic separations of gluten components from Ponca and Knox were best in 0.2*M* acetic acid, which was adopted for the fractionation of glutens from the other varieties.

A small fraction, usually only a few milligrams, was not completely soluble in the 4 to 5 ml. of 0.2*M* acetic acid used to apply the sample to the column. This residue was removed by centrifugation in a clinical centrifuge. Otherwise, the insoluble material soon plugged the column. This insoluble fraction, which usually contained from 15 to 50% carbohydrate, may represent glycoproteins or pentosans that were soluble in the more dilute solution used to extract the protein from the flour. Generally, gluten samples applied satisfactorily to the column could be 10 to 20% larger from the soft and durum wheats than from the hard wheats.

The elution pattern from gel filtration of gluten from Wells wheat (Figure 2) is an example of the resolution achieved on Sephadex G-100. Also shown is the starch-gel electrophoretic pattern of fractions 1 to 4, including a small area between the first two peaks which, according to the nomenclature of Woychik *et al.* (1961), contains mainly omega gliadins and some slightly faster moving gliadins. Fraction 5, which did not stain with nigrosine dye, was mainly carbohydrate.

The electrophoretic pattern for fraction 1 (glutenin) shows much of the material as streaks with a few bands. This pattern was common with soft and durum wheats, whereas with the hard wheats more of the glutenin stayed at the origin, and the bands were not so prominent. In the starch-gel electrophoresis of gluten proteins, Nielsen *et al.* (1968) report that the gluteninlike fraction from gliadin, which has a lower molecular weight than classic glutenin, enters the gel readily. Thus, the entry of soft and durum wheat glutenins into the gel may indicate that they contain more low molecular weight components, or there is less aggregation. Aside from these variations, the filtration patterns and the gel patterns from all varieties were similar.

Since the 280-mμ absorbancy of the gluten components appeared unequal in different fractions, all the samples were dried and weighed to estimate the amount of each fraction in defatted whole flour. Usual recovery of material from the column was between 90 and 95% by weight.

Table I shows the approximate percentages of gliadin and glutenin in each defatted wheat flour. As expected, the gluten levels were different in wheats from different classes, but the ratio of gliadin and glutenin was nearly 1 to 1 for all varieties studied.

Salt Precipitation. Although the salt sensitivities of gliadin

Table I. Gliadin and Glutenin in 11 Varieties of Wheat

Variety	Approximate % from Defatted Flour[a] Gliadin	Glutenin
HRW		
Ponca	3.8	4.2
Comanche	3.7	4.0
Red Chief	...	...
HRS		
Lee	4.7	5.3
Selkirk	4.8	4.8
SRW		
Knox	3.4	3.3
Seneca	3.4	4.0
SWW and Club		
Brevor	2.2	2.3
Omar	2.1	2.4
Durum		
Lakota	5.3	5.8
Wells	5.4	5.4

[a] Per cent by weight. Confidence limits ±10% with greater probability; the glutenin is low because of poor solubility.

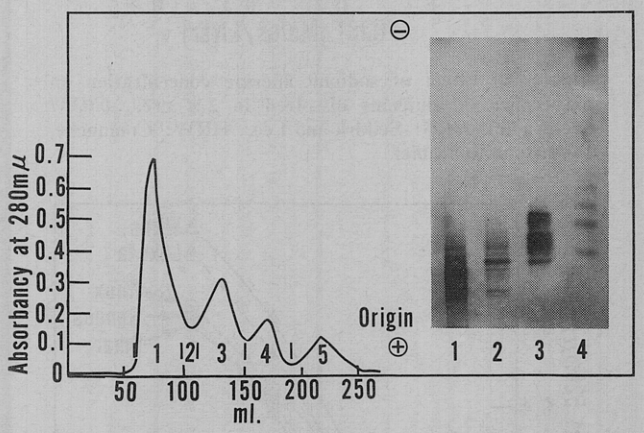

Figure 2. Left: Sephadex G-100 filtration of Wells gluten. Buffer: 0.2*M* acetic acid; fractions combined from area between vertical lines and numbered 1 to 5

Right: Starch-gel electrophoresis of fractions

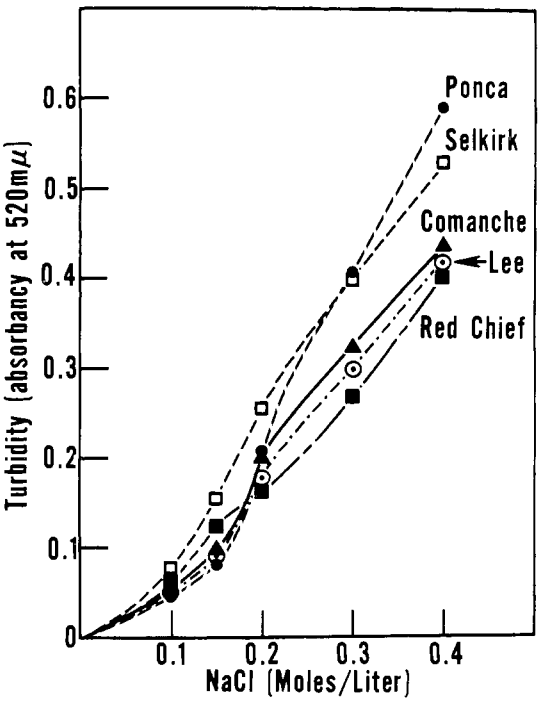

Figure 3. Effect of sodium chloride concentration on absorbance of glutenins dissolved in 2*M* urea, 0.03*M* acetic acid. HRS: Selkirk and Lee. HRW: Comanche, Ponca, and Red Chief

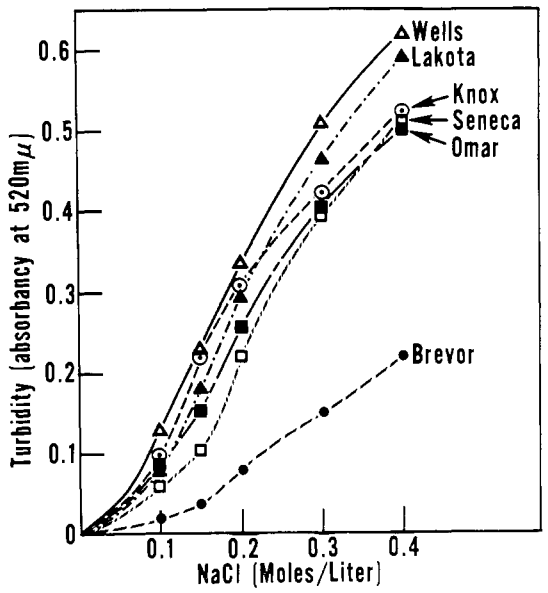

Figure 4. Effect of sodium chloride concentration on absorbance of glutenins dissolved in 2*M* urea, 0.03*M* acetic acid. SRW: Knox and Seneca. Durum: Lakota and Wells. SWW: Brevor. Club: Omar

and glutenin vary greatly in different ionic strength buffers (Beckwith *et al.*, 1966), the usefulness of precipitation measurements as indicators of subtle differences between gluten proteins from different varieties of wheat is relatively unexplored. Precipitation demonstrates the sensitivity of glutenin proteins to ionic strength differences more rapidly than standard solubility methods, which require equilibrium conditions.

The results of precipitation measurements on glutenins from different varieties of wheat are plotted in Figures 3 and 4. The steeper the curve the more sensitive the glutenin to increases in ionic strength. Figure 3 shows HRW and HRS wheats, in general, are the least soluble at higher ionic strength, whereas the SWW and club wheats are the most soluble (Figure 4).

Briggle and Reitz (1963) have rated various wheats in relation to their specific uses. According to these authors, Ponca and Comanche are both good quality HRW wheats for breadmaking: Red Chief, which gave a much lower gluten precipitation curve (Figure 3), is a poor baking HRW wheat. Selkirk and Lee are also listed as having good quality for HRS wheats, but Selkirk generally is lower in protein content and higher in loaf volume than Lee. While Lakota is listed as a higher quality durum wheat than Wells for use in macaroni, processors generally find Wells just as suitable. Figure 3 shows Wells as slightly higher than Lakota. Omar is listed as having excellent soft wheat milling and baking properties and Brevor as having only fair milling quality and satisfactory properties for general purpose and cake flours. Results in Figure 4 agree with this. Knox and Seneca are rated about equal, with Knox possibly slightly better.

On the basis of these data, better quality varieties within the same class yield the steepest glutenin precipitation curves. Comparison of varieties between classes is not meaningful because there is considerable variation in protein quantity between classes and the wheats are used for different purposes.

Figure 5 contains salt sensitivity curves for some gluten fractions prepared by precipitating the glutenin from 70% ethanol and then passing the gliadin fraction over Sephadex G-100 (Beckwith *et al.*, 1966). Low-molecular-weight gluten-inlike material (Woychik *et al.*, 1961) gives even steeper curves for all varieties shown (Figure 5, I) than the whole glutenin prepared by gel filtration (Figures 3 and 4). Crude omega-gliadin is much more soluble in salt solutions (Figure 5, II), similar to the gliadin fraction (Beckwith *et al.*, 1963, 1966). Crude omega-gliadin from Red Chief was repurified on a Sephadex G-100 column to remove the streaking material shown in gel electrophoresis. This purified omega fraction gave the lowest curve and is much more soluble in the presence of salt.

DISCUSSION

Measuring a property as elusive as gluten quality requires techniques that are sensitive to subtle differences in protein type, are easy to apply, and yield interpretable results. The gel electrophoretic studies reported here and by Huebner and

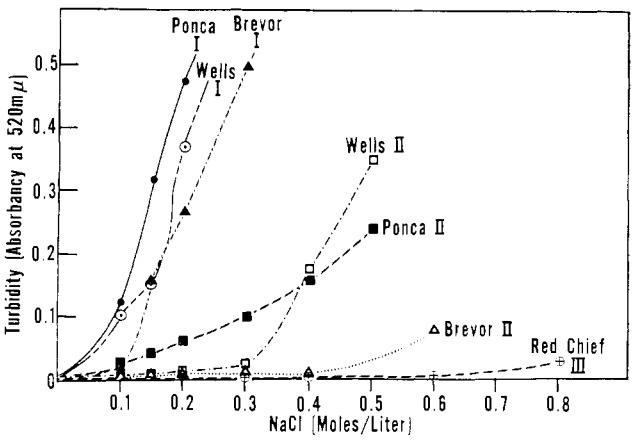

Figure 5. Effect of sodium chloride concentration on absorbance of gliadin fractions dissolved in 2*M* urea, 0.03*M* acetic acid. I. Gluten-inlike fraction. II. Omega fraction. III. Omega fraction, repurified

Rothfus (1968) show several distinct protein differences among varieties, but the relationship between gel patterns and gluten qualities of different varieties is obscured by limited knowledge of the individual proteins. The quantity, amino acid content (Thachuk, 1966), and viscosity (Cluskey *et al.*, 1961; Matsuo and McCalla, 1964) of protein in flour are, likewise, difficult to correlate directly to gluten or bread-baking quality. Simiarly, comparison of the glutenin viscosities for these varieties, while varying widely, had no correlation to dough quality or baking properties. Immunochemical comparisons, which show more antigenic similarities than differences in gluten proteins among wheat varieties (Nimmo and O'Sullivan 1967; Elton and Ewart, 1963; Benhamou-Glynn *et al.*, 1965), also do not relate clearly to gluten quality. Whereas the immunochemical method is sensitive to differences among water-soluble and salt-soluble proteins (Nimmo and O'Sullivan, 1967; Grabar *et al.*, 1965), it has not produced equally satisfactory results for the gliadins and glutenins. When the Ouchterlony double diffusion method is used, gamma-gliadins appear to give the same precipitin line (Huebner, 1967) even though their amino acid analyses differ slightly (Huebner *et al.*, 1967).

Separation of the gluten proteins from different varieties of wheat by gel filtration reproducibly gives glutenin fractions suitable for comparison. Here glutenin is defined as the first peak of Figure 2 or the high-molecular-weight material. This is a more precise means of identifying glutenin than other methods that depend upon differential solubility, which does not give complete fractionation.

The salt precipitation technique provides information that correlates well with published data on baking and gluten quality, particularly among wheat varieties within a specific class. This procedure has the advantage of comparing glutenin fractions at equal concentrations and is not affected by variations in protein quantity found in wheat from year to year or from different locations. The steeper the curves (Figures 3 and 4), the more sensitive the glutenin to salt and the higher the quality of gluten from varieties in the same class. The shape of each precipitation curve, especially the steep curves in Figure 5 of low-molecular-weight glutenin, suggests several components in glutenin with different sensitivities to salt. This low-molecular-weight fraction may have special properties that warrant further investigation. These results along with the differences noted in Figure 1 indicate that there is structural variation in the glutenins from the various classes of wheat.

Within the limits of the data, the relationship of glutenin salt sensitivity to gluten quality is striking and merits further investigation. The method could be of general benefit, since the precipitation can be done with the glutenin from as little as 1 gram of flour.

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