FRACTIONATION AND QUANTITATIVE DIFFERENCES OF GLUTENIN FROM WHEAT VARIETIES VARYING IN BAKING QUALITY[1]

F. R. HUEBNER and J. S. WALL, Northern Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture,[2] Peoria, IL 61604

ABSTRACT

Cereal Chemistry 53(2): 258-269

Properties of glutenin and other wheat proteins that may be responsible for varietal differences in wheat flour performance were investigated. The proteins from several wheat flours differing in mixing time and dough strengths were extracted with a solution of 0.1N acetic acid, 3M urea, and 0.01N hexadecyltrimethylammonium bromide buffer. After dialysis and lyophilization, the extract was dissolved in 5.5M guanidine hydrochloride and passed through an agarose gel filtration column. The glutenin protein was separated into two fractions: I) a very high mol wt fraction eluting at the void volume, and II) some lower mol wt proteins eluting as a broad peak. Next, the gliadins and water-soluble proteins were eluted in close sequence. Glutenin I and II differed only slightly in amino acid analysis and gel electrophoresis patterns of their subunits. However, the elution profiles (from the 4% agarose columns) of the extracts from the various wheats differed significantly, especially in the glutenin region. The ratio of glutenin I to glutenin II was generally higher in bread wheat flours exhibiting long mixing times and strong doughs. In accordance with observations by Orth and Bushuk, these flours also contained the highest amount of unextracted protein. Flours of weak dough wheats generally had lesser contents of both glutenin I and unextracted protein. A sufficient total amount of protein, and suitable proportions of the two glutenin fractions and the insoluble fraction, are necessary for a good baking flour.

Considerable research has been carried out on proteins of wheats to determine why certain varieties yield flours with superior breadmaking characteristics (1–3). Baking studies employing flour constituents which have been separated and reconstituted into doughs have established that flour proteins are prime factors governing wheat quality (4,5). While the total amount of protein in wheat flour must be adequate to ensure a strong dough, qualitative and quantitative differences in proteins of various classes among varieties influence their performance (6–8). Orth and Bushuk (7) found no significant correlations between loaf volume per unit protein *vs.* albumins, globulins, or gliadin content of a series of flours from wheats of different qualities. Hoseney *et al.* (8) observed that, in doughs prepared from reconstituted flours, introduction of water-soluble proteins from good or poor baking wheat flours did not significantly alter the baking properties of a standard flour. However, replacement of a gliadin-rich fraction from a good wheat for that of a poor one improved loaf volume (9,10). Although gliadin complexes in different varieties differ in their composition of constituent proteins (11), no physical differences have been reported that account for variations in gliadin performance in doughs.

Huebner (12) and Mattern *et al.* (13) observed positive correlations between mixing and breadmaking quality of wheat flours, and the quantity of glutenin in the flour. In contrast, Orth and Bushuk (7) observed a negative relation to glutenin content, and found a positive correlation between acetic acid-insoluble

[1]Presented at the 59th Annual Meeting, Montreal, Quebec, Oct. 1974.
[2]Mention of firm names or trade products does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

Copyright© 1976 American Association of Cereal Chemists, Inc., 3340 Pilot Knob Road, St. Paul, Minnesota 55121. All rights reserved.

protein and mixing strength and breadmaking quality. Since different extraction methods were used in those studies, heterogeneity of the proteins might be responsible for the conflicting results. Differences in the nature of the glutenin in flours of wheats having good baking characteristics from that of poor ones with equal protein contents were demonstrated by Hoseney *et al.* (9,10). Replacement of a portion of the glutenin from a fast mixing flour with that from a slow mixing flour increased the mixing time of the former flour.

Glutenin is composed of polypeptide chains of widely different amino acid compositions and mol wt, linked by disulfide bonds (14,15). Heterogeneity of glutenin may be due to different components consisting of different subunits, or to the fact that glutenin molecules contain the same ratio of these polypeptide chains but differ in degree of molecular size. The availability of new gel filtration media permitting separation of higher mol wt polymers facilitated fractionation of glutenin according to mol wt. Also, we examined the polypeptide composition of the different size fractions of glutenin by amino acid and electrophoretic analysis. Possible differences in mol wt distributions among glutenins from a number of wheat varieties were investigated. Analytical data on the glutenins were evaluated in terms of the recognized performances of the different flours in breadmaking.

MATERIALS AND METHODS

Sample Preparation

Almost all the wheat flour samples were the same as those used previously (11,12) and were prepared with a Buhler laboratory mill from grain obtained from commercial sources. Flours from varieties K-501099 and K-14042, described by Hoseney *et al.* (8), were generously supplied by them. Flour samples 66-2558 and 66-2560 are from the same variety, No. 13883, but grown in two different locations at Lincoln and Alliance, Nebr., respectively, and were donated by P. J. Mattern of the University of Nebraska, who also provided test data. Chinese Spring III-L came from W. C. Shuey of the Agricultural Research Service at North Dakota State University. All flours were stored at −20° C until used.

Flours were defatted with dry 1-butanol and then extracted three times with a solvent consisting of 0.1*N* acetic acid, 3*M* urea, and 0.01*N* hexadecyltrimethylammonium bromide (AUC) according to Meredith and Wren (16). Extracts were combined, dialyzed, and lyophilized.

Gel Filtration Chromatography

Proteins were separated on columns of Sepharose 4B (2.5 × 70 cm) and 2B (2.5 × 38 cm). Sepharose, manufactured by Pharmacia Co., is a beaded agarose free of agaropectin. The Sepharoses differ in agarose concentration and fractionation range for macromolecules: 4B contains 4% agarose and retains dextrans up to 5 million mol wt, whereas 2B contains 2% agarose and retains dextrans up to 20 million mol wt. The columns were prepared according to the manufacturer's direction using 5.5*M* guanidine hydrochloride (GHCl) for elution. Columns were operated in upward flow at room temperature. The optimum flow rate of both columns was 6.5 ml/hr, and a small increase in flow rate made a significant effect on compaction of the column. Generally, a 60-mg sample was applied in 5

ml of solvent. Proteins in the column effluent were determined by absorbance at 280 nm by measurement with a Beckman DU spectrophotometer.

Chemicals

GHCl from Eastman Organic Chemicals (white label, no longer available) was further purified by stirring with activated charcoal (Darco G-60, Atlas Chem. Ind.) for 2 hr and filtering through a 1.2 μ millipore filter. Also, ultrapure GHCl was purchased from Schwarz/Mann and used directly. The concentrations of GHCl solutions were adjusted to desired value based on refractive index (17). Because of the large amount of GHCl needed, high cost of the ultrapure product, and pollution problems, much of the GHCl was recovered from the column effluent. This step was accomplished by first dialyzing in 250- or 500-ml graduates to keep the dialysate volume low. The dialysate was filtered, concentrated under vacuum, and cooled to 5° C to permit GHCl to crystallize. After recrystallizing from hot 50% ethanol-50% methanol, the GHCl yielded a 6*M* solution with absorbance at 280 nm of less than 0.20 absorbance with water as the blank.

Other chemicals were either ACS grade or the purest commercially available.

Analytical Methods

Protein in flour samples was determined by automatic micro-Kjeldahl analysis according to the procedure of Bietz (18), based on the factor 5.7 times nitrogen content.

For amino acid analysis, proteins were hydrolyzed according to the method of Liu and Chang (19) with 3*M* aqueous *p*-toluene sulfonic acid (*p*-TSA) containing 0.2% 3-(2-aminoethyl) indole to permit quantitative recovery of tryptophan. Samples were hydrolyzed for 24 hr at 110° C in sealed tubes. Analyses were conducted directly on the hydrolysate solutions with a Beckman Model 121 automatic amino acid analyzer (20). L-α-Amino-β-guanidino-propionic acid (AGPA) (Calbiochem) and norleucine served as the internal standards. An Infotronics integrator and an IBM 1130 computer provided automatic computation of amino acid analyses (21).

Polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) was carried out as described by Bietz and Wall (22).

RESULTS

Gel Filtration

Because of the high mol wt of glutenin and its tendency to aggregate, a strong dissociating agent, such as GHCl solution, was required as eluent for gel filtration chromatography. A concentration of 5.5*M* GHCl proved satisfactory for the glutenin separation; however, some Sepharose dissolved in this concentration of GHCl. The dissolved Sepharose introduced problems in recovery of pure separated glutenin fractions. The proteins coprecipitated with the Sepharose during dialysis and no practical method was found for separating them. If the column were not used for even a day before sample application, considerably more Sepharose would be eluted with the proteins; therefore, the column was run continuously and, if it were necessary to halt flow, it was washed before another sample application. Yields of the various fractions were

calculated on the basis of their absorbance to avoid errors due to Sepharose impurities.

Protein extracts of Comanche, Red River, and K-14042 wheat flours were fractionated on Sepharose 4B and exhibited major quantitative differences in glutenin components (Fig. 1). The proteins of all three varieties were separated into four fractions differing in mol wt or conformation as indicated by the peaks in ultraviolet (uv) absorption. The initial peak (I), eluted at the void volume of the column, probably has a mol wt more than 5 million. A second fraction (II) appears as a smaller peak with a wide range of mol wt, probably from 5 million down to 100,000. Because of their high mol wt and behavior on electrophoresis, these proteins were designated glutenins I and II [(Glut-I) and (Glut-II)], respectively. Peak III was identified by electrophoresis as composed of gliadins and peak IV, albumins and globulins.

A fifth peak was found at the total hold-back volume when this work was started. No protein was associated with this peak, and contamination was found to be coming from the metal syringe needle used to apply the sample into the small tubing going to the column. Testing confirmed that high concentrations of GHCl will remove from various metals, even from stainless steel, ions which strongly absorb uv light at 280 nm. Therefore, when working with high concentrations of GHCl, care should be taken to avoid any contact with metals.

The larger pore size of the Sepharose 2B gel permits a better separation of higher mol wt proteins than does 4B. An AUC extract of Comanche flour proteins was separated on Sepharose 2B (Fig. 2). Although the 2B column was shorter (38 cm) than that prepared from 4B, the two glutenin fractions separated well. The first peak (Glut-I) eluting at the void volume may have a possible mol wt of more than 20 million. Because the second peak numbered 3 (Glut-II) is retained longer on the 2B column, resolution from gliadin is poor. The improved separation of higher mol wt Glut-I from Glut-II gave components that could offer greater contrast in electrophoretic and amino acid composition. The

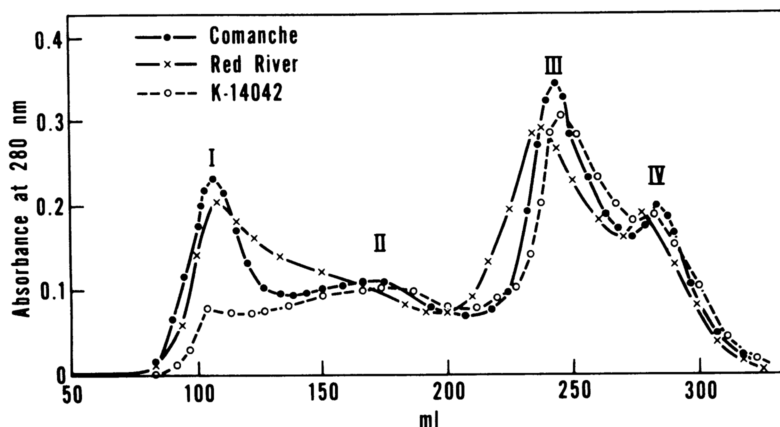

Fig. 1. Gel filtration of 0.1*N* acetic acid, 3*M* urea, and 0.01*N* hexadecyltrimethylammonium bromide (AUC) extracts from three wheat flours on Sepharose 4B. Solvent: 5.5*M* guanidine hydrochloride (GHCl).

shorter column and lower Sepharose content decreased Sepharose contamination of proteins by approximately one-half, and this decreased contaminant benefited electrophoretic study and amino acid analysis of the proteins.

Composition of Glut-I and II

In Fig. 3 are shown the SDS-polyacrylamide gel electrophoretic patterns of subunits from four fractions of Comanche proteins separated on Sepharose 2B as designated in Fig. 2. The SDS buffer contains reducing agents to cleave disulfide bonds, and subsequent migration of the resulting SDS-complexed subunits is inversely related to their mol wt. The only significant differences observed between the patterns of Glut-I and Glut-II (fractions 1 and 3, Fig. 2) are streaks in the gel and protein at the origin for Glut-I and a slight difference in intensity of some bands. The streaking in the gel was observed previously in reduced high-mol wt fractions of glutenin (14). The possible higher content of the 45,000 mol wt subunit in fraction 3 may be caused by the presence of high-mol wt gliadin, which consists primarily of these subunits.

Amino acid compositions of Glut-I and Glut-II (fractions 1 and 3, Fig. 2) are summarized in Table I. Negligible or small differences in amino acid contents, with the exceptions of lysine, glycine, and valine, exist between the two protein fractions. Together with results of the SDS-electrophoretic analysis, these findings suggest that although the glutenins differ in mol wt, they may be composed of a similar mixture of polypeptides.

Varietal Variations in Amounts of Glutenins

The elution profiles from Sepharose 4B columns of glutenin protein extracts from flours of three wheats differing considerably in mixing and baking

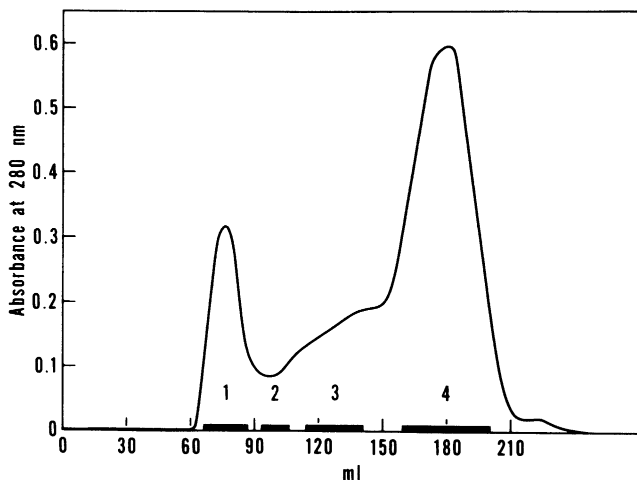

Fig. 2. Gel filtration of AUC extract from Comanche wheat flour on Sepharose 2B. Solvent: 5.5*M* GHCl. Solid lines at base designate fractions combined for further use. 1 = Glut-I, 3 = Glut-II, 4 = gliadin + albumins + globulins.

characteristics show marked variations (Fig. 1). Comanche is a good baking hard red winter wheat with desirable mixing characteristics. Its profile has a fairly sharp and large Glut-I peak. Red River 68, a semidwarf hard red spring, has long mixing requirements. Its pattern is unusual among those tested in having an appreciable amount of protein trailing the Glut-I peak. The K-14042 pattern is deficient in the Glut-I peak. This deficiency may be related to the weak dough characteristic of K-14042 flour.

The total uv absorption for the peaks produced from Sepharose 4B separations of proteins was used to compute yields of the four different extracted

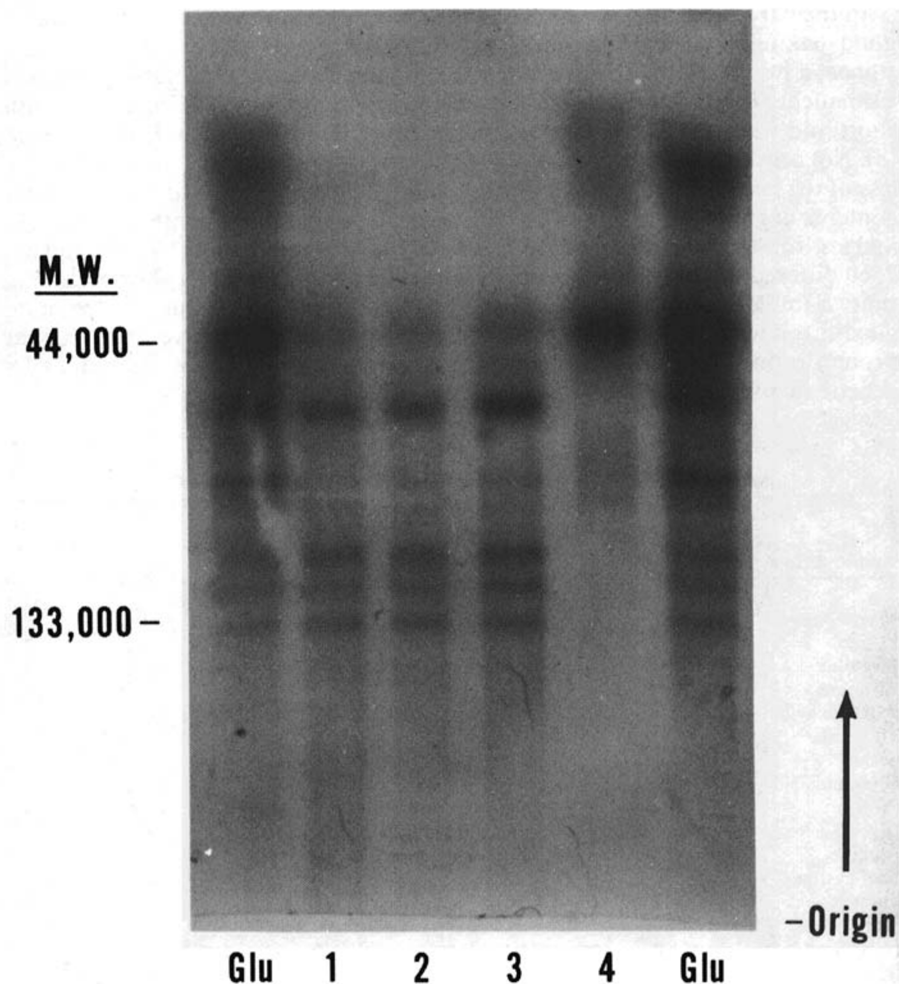

Fig. 3. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) of fractions from Fig. 2, numbered from 1 to 4. Glu = whole gluten. (Buffer is pH 8.9 tris borate, 0.1% SDS.) 1 = Glut-I, 3 = Glut-II, 4 = gliadin + albumins + globulins.

flour protein fractions from 14 representative varieties of wheat. The unextracted proteins of the flours were also determined. Data in Table II are presented as per cent of total protein, whereas data in Table III are given as g protein per 100 g of defatted flour. Comparison of the two tables shows how different presentations of data can appear to give conflicting results. For example, the percentage gliadin and lower mol wt proteins of Chinese Spring appear to be below average, whereas on a g protein per 100-g flour basis it has the highest amount of these proteins. Of course, it also has higher quantities of the other fractions as well. Also, for the soft wheats, *e.g.*, Brevor, the proportions of proteins (Table II) would seem favorable to good performance; however, the total protein per gram of flour is very low (Table III).

In the hard red winter wheat class, both Ponca and Comanche are considered good baking wheats. Comanche has a longer mixing time requirement than Ponca, a property which may relate to the higher content of insoluble protein in Comanche. K-501099 and K-14042 are poor and very poor in baking quality with short and very short mixing times, respectively, as described by Hoseney *et al.* (8). Not only do K-501099 and K-14042 have low levels of Glut-I, they also have less of the insoluble fraction than either Comanche or Ponca, and slightly higher contents of gliadins and low-mol wt proteins. K-14042 has less combined glutenin and less total protein than K-501099. The hard red winter wheats 66-2558 and 66-2560 differed in their mixing requirements; the latter required a shorter mixing time. Sample 66-2560 flour contained less Glut-I but more Glut-II; also more gliadin and lower mol wt proteins than 66-2558. Others (6,23) have observed that protein ratios are affected both by climate and soil conditions, as well as by genetic factors.

TABLE I
Amino Acid Composition of Fractions from Comanche Wheat Flour[a]

Amino Acid	Mol per 10^5 g Protein	
	Glut-I[b]	Glut-II
Tryptophan	4.7	4.2
Lysine	14.1	9.9
Histidine	12.7	12.6
Arginine	22.4	20.0
Aspartic acid	23.5	19.0
Threonine	26.6	25.2
Serine	60.3	60.3
Glutamic acid	280.5	297.0
Proline	105.0	108.0
Glycine	76.5	64.9
Alanine	33.9	30.3
Half-cystine	10.3	10.2
Valine	44.0	36.2
Methionine	16.0	13.5
Isoleucine	28.0	26.5
Leucine	57.8	56.5
Tyrosine	25.3	23.2
Phenylalanine	26.5	30.8

[a]Fractions from Fig. 2, column Seph-2B.
[b]Glut = Glutenin.

TABLE II
Relative Contents of Different Protein Fractions in Wheat Flour Differing in Functionality

Wheat	Mixing[a]	Loaf Volume	Total Protein %	% of Total Protein in Fractions: Glut-I	Glut-II	Gliadin	Albumin + globulin	Insoluble
HRW[b]								
Ponca	Medium long	Good	10.5	16.2	22.8	33.3	14.3	15.2
Comanche	Long	Good	11.0	15.5	18	32	13	21
K-14042	Very short	Very poor	11.7	6.8	25.0	38.4	15.4	9.4
K-501099	Short	Poor	13.6	6.6	26.4	50.0		9.6
66-2558	Medium long	Good	12.3	16.3	17.9	37.4	12.2	13.8
66-2560	Medium	Good	14.0	15.0	19.3	43.6	9.3	12.2
HRS								
Red River	Very long	Poor	12.7	17.3	13.4	33.0	11.8	22.8
Rescue	Long	Good	16.5	12.1	18.8	36.4	12.1	18.2
SRS								
Chinese Spring	Short	Poor	21.2	10.5	21.7	42.0		10.9
SRW								
Knox	Short	Poor	8.5	10.6	27.0	33.0	18.7	11.8
SWW								
Brevor	Short	Poor	8.8	14.8	22.7	35.2	10.4	17.1
Club								
Omar	Very short	Poor	6.5	9.2	24.6	35.4	15.4	18.5
Durum								
Wells	Medium	Poor	14.6	17.1	19.2	41.2	11.0	9.6

[a] Mixing times according to ref. 27. Long = 5.5–7.5 min, medium long = 4–5 min, medium = 3–3.5 min, short = 2–2.5 min, and very short = 1–1.5 min.

[b] HRW = hard red winter; HRS = hard red spring; SRS = soft red spring; SRW = soft red winter; SWW = soft white winter.

Rescue is a reasonably long mixing hard red spring wheat; its high protein content is distributed between the five protein fractions in proportions similar to that of the better HRW wheats. Red River 68 has a lower content of glutenin but a higher ratio of Glut-I to Glut-II than Rescue. It was the only flour in which Glut-I predominated over Glut-II. It also contained the highest proportion of insolubles. These components undoubtedly contribute to its high mixing requirements and tough dough qualities.

The flour of the soft red spring wheat, Chinese Spring, is relatively high in protein, but the high proportion of Glut-II and gliadins and the low percentage of Glut-I and insolubles render the protein rather weak. The low recovery of protein indicated considerable loss of nitrogen-containing components during dialysis, which could also contribute to its poor mixing and baking qualities.

In the soft winter wheats, Knox and Brevor, low total protein levels and low contents of Glut-I and insolubles result in weak doughs for bread-baking. The club wheat, Omar, is even poorer than the other soft wheats in proteins essential for making good dough.

Durum flours are high in protein but have a disproportionately high content of gliadin, low-mol wt proteins, and a low proportion of insolubles.

TABLE III
Content of Different Protein Fractions in Various Wheat Flours

<table>
<tr><th rowspan="2">Wheat</th><th colspan="5">Fractions, g Protein in 100 g Defatted Flour</th></tr>
<tr><th>Glut-I</th><th>Glut-II</th><th>Gliadin</th><th>Albumin + globulin</th><th>Insoluble</th></tr>
<tr><td>HRW</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Ponca</td><td>1.7</td><td>2.4</td><td>3.5</td><td>1.5</td><td>1.6</td></tr>
<tr><td>Comanche</td><td>1.7</td><td>2.0</td><td>3.5</td><td>1.4</td><td>2.3</td></tr>
<tr><td>K-14042</td><td>0.8</td><td>2.8</td><td>4.5</td><td>1.8</td><td>1.1</td></tr>
<tr><td>K-501099</td><td>0.9</td><td>3.6</td><td colspan="2">6.8</td><td>1.3</td></tr>
<tr><td>66-2558</td><td>2.0</td><td>2.2</td><td>4.6</td><td>1.5</td><td>1.7</td></tr>
<tr><td>66-2560</td><td>2.1</td><td>2.7</td><td>6.1</td><td>1.3</td><td>1.7</td></tr>
<tr><td>HRS</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Red River</td><td>2.2</td><td>1.7</td><td>4.2</td><td>1.5</td><td>2.9</td></tr>
<tr><td>Rescue</td><td>2.0</td><td>3.1</td><td>6.0</td><td>2.0</td><td>3.0</td></tr>
<tr><td>SRS</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Chinese Spring</td><td>2.2</td><td>4.6</td><td colspan="2">8.9</td><td>2.3</td></tr>
<tr><td>SRW</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Knox</td><td>0.9</td><td>2.3</td><td>2.8</td><td>1.6</td><td>1.0</td></tr>
<tr><td>SWW</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Brevor</td><td>1.3</td><td>2.0</td><td>3.1</td><td>0.9</td><td>1.5</td></tr>
<tr><td>Club</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Omar</td><td>0.6</td><td>1.6</td><td>2.3</td><td>1.0</td><td>1.2</td></tr>
<tr><td>Durum</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Wells</td><td>2.5</td><td>2.8</td><td>6.0</td><td>1.6</td><td>1.4</td></tr>
</table>

Protein compositions by class for seven wheat varieties are given in Fig. 4. Values were arranged for each protein class in the order of mixing requirements of the wheat flours, with those for longest mixing on the left. Although soft wheats contain too little total protein to be used for baking bread, they are included to demonstrate the generally lower quantities of nearly all protein fractions. While there are some exceptions due to the variations in total protein, the general decrease in content of Glut-I and insolubles and the increase in Glut-II with lower mixing time are evident. In previous work, the combination of Glut-I and -II would obscure this observation. Also, with less of the glutenin extracted leaving more insolubles, previous comparisons may not have been valid. In total quantity of protein per gram of flour, albumin and globulin fractions vary the least of the five.

These data indicate that a sufficient glutenin content is important to strong dough formation, and that a suitable proportion of high-mol wt glutenin is also essential. The amount of insoluble protein also contributes to dough quality.

DISCUSSION

The development of gel filtration materials for chromatography has contributed greatly to separation of wheat flour proteins on the basis of size, and to estimation of their mol wt. Initial studies with Sephadex G-100 and G-200 allowed separation of proteins of mol wt up to 200,000, and not only permitted distinction between glutenin and gliadins, but separation of a high-mol wt gliadin as well (24). Crow and Rothfus (25) used polyacrylamide gel filtration to separate glutenins but could achieve no distinct fractionation. The use of Sepharose columns and GHCl solvent appears to yield the first successful fractionation of high-mol wt glutenin. Unfortunately, recovery of pure proteins was hampered by solubility of Sepharose in the solvent.

In trials preliminary to this study, we tested porous glass beads Bio-Glas-500

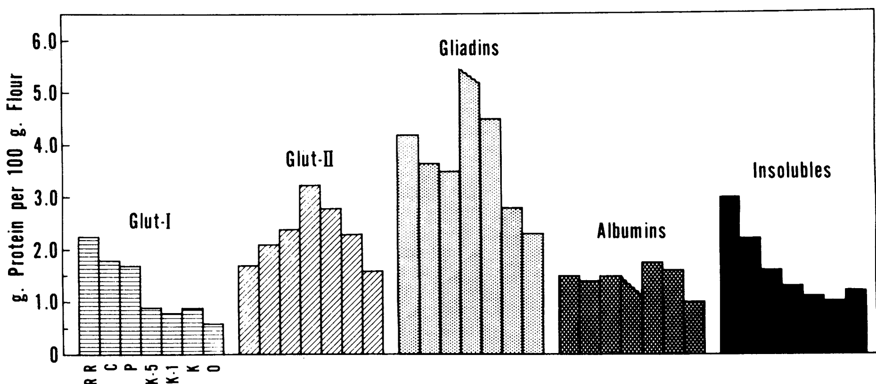

Fig. 4. Amounts of protein fractions for flours of selected varieties of wheats. RR = Red River, C = Comanche, P = Ponca, K-5 = K-501099, K-1 = K-14042, K = Knox, O = Omar. Slant lines at the top of columns for K-5 gliadins and albumins designate incomplete separation of those fractions.

and 1000 (Bio-Rad Laboratories, Richmond, Calif.) and CPG-10 (Electronucleonics, Fairfield, N.J.) as stable supports resistant to dissociation agents. Although these materials apparently separated high-mol wt glutenins, results were not reproducible. Coating the glass beads with Carbowax 20M improved recoveries, but 6*M* GHCl removed the Carbowax. The use of glass beads for permeation chromatography requires further study.

Although glutenin is composed of many different polypeptide chains linked by disulfide bonds, we have not been able to establish that glutenin molecules differ in their composition. In earlier work, Crow and Rothfus (25) found that fractions of glutenin obtained from polyacrylamide gel columns yielded similar proportions of different subunits after reduction, as shown by starch gel electrophoresis. Evidently, disulfide cross-links between glutenin subunits are randomly formed *in situ*.

While our results indicate that dough strength is related to the total amount of glutenin, including the insoluble fraction, the data also indicate that a better correlation exists with the higher mol wt fraction Glut-I plus the insolubles. Our observations do not contradict the findings of Orth and Bushuk (7) since the AUC solvent is a better extractant for total protein than acetic acid and some of our Glut-I may have been retained in their insoluble fraction. Bietz *et al.* (26) found evidence that much of the insoluble protein can be dissolved in SDS after reducing disulfide bonds; evidently, the major portion of the insoluble fraction resembles glutenin in its subunit composition. Perhaps a progressive increase of molecular size and disulfide cross-linkage occurs, proceeding from Glut-II to Glut-I to insoluble protein. Varietal variations in the ratios of these three protein fractions must depend on genetic differences in the subunit composition. Variations in subunit compositions among glutenins of different varieties have been established (15,22).

Literature Cited

1. MECHAM, D. K. Wheat and flour proteins. Baker's Dig. 47(5): 24 (1973).
2. POMERANZ, Y., FINNEY, K. F., and HOSENEY, R. C. Molecular approach to breadmaking. Baker's Dig. 44: 22 (1970).
3. BIETZ, J. A., HUEBNER, F. R., and WALL, J. S. Glutenin: The strength protein of wheat flour. Baker's Dig. 47(1): 26 (1973).
4. FINNEY, K. F. Fractionating and reconstituting techniques as tools in wheat flour research. Cereal Chem. 20: 381 (1943).
5. SHOGREN, M. D., FINNEY, K. F., and HOSENEY, R. C. Functional (breadmaking) and biochemical properties of wheat flour components. I. Solubilizing gluten and flour protein. Cereal Chem. 46: 93 (1969).
6. BUSHUK, W., BRIGGS, K. G., and SHEBESKI, L. H. Protein quantity and quality as factors in the evaluation of bread wheats. Can. J. Plant Sci. 49: 113 (1969).
7. ORTH, R. A., and BUSHUK, W. A comparative study of the proteins of wheats of diverse baking qualities. Cereal Chem. 49: 268 (1972).
8. HOSENEY, R. C., FINNEY, K. F., SHOGREN, M. D., and POMERANZ, Y. Functional (breadmaking) and biochemical properties of wheat flour components. II. Role of water-solubles. Cereal Chem. 46: 117 (1969).
9. HOSENEY, R. C., FINNEY, K. F., SHOGREN, M. D., and POMERANZ, Y. Functional (breadmaking) and biochemical properties of wheat flour components. III. Characterization of gluten protein fractions obtained by ultracentrifugation. Cereal Chem. 46: 126 (1969).
10. HOSENEY, R. C., FINNEY, K. F., POMERANZ, Y., and SHOGREN, M. D. Functional (breadmaking) and biochemical properties of wheat flour components. IV. Gluten protein

fractionation by solubilizing in 70% ethanol and in dilute lactic acid. Cereal Chem. 46: 495 (1969).
11. HUEBNER, F. R., and ROTHFUS, J. A. Gliadin proteins from different varieties of wheats. Cereal Chem. 45: 242 (1968).
12. HUEBNER, F. R. Comparative studies of glutenins from different classes of wheat. J. Agr. Food Chem. 18: 256 (1970).
13. MATTERN, P. J., SCHMIDT, J. W., MORRIS, R., and JOHNSON, V. A. A feasibility study of the use of a modified Maes protein extraction process and chromosome substitution lines for bread wheat quality identification. 3rd Int. Wheat Genet. Symp., ed. by K. W. Finlay and K. W. Shepard, p. 449. Plenum Publishing Corp.: New York (1968).
14. HUEBNER, F. R., and WALL, J. S. Wheat glutenin subunits. I. Preparative separation by gel-filtration and ion-exchange chromatography. Cereal Chem. 51: 228 (1974).
15. HUEBNER, F. R., DONALDSON, G. L., and WALL, J. S. Wheat glutenin subunits. II. Compositional differences. Cereal Chem. 51: 240 (1974).
16. MEREDITH, O. B., and WREN, J. J. Determination of molecular-weight distribution in wheat flour proteins by extraction and gel filtration in a dissociating medium. Cereal Chem. 43: 169 (1966).
17. ERLANDER, S. R., and TOBIN, R. The mechanism of dispersion of starch by urea and by guanidinium and lithium salts. Makromol. Chem. 107: 204 (1967).
18. BIETZ, J. A. Micro-Kjeldahl analysis by an improved automated ammonia determination following manual digestion. Anal. Chem. 46: 1617 (1974).
19. LIU, T. Y., and CHANG, Y. H. Hydrolysis of proteins with *p*-toluene-sulfonic acid. J. Biol. Chem. 246: 2842 (1971).
20. BENSON, J. V., Jr., and PATTERSON, J. A. Accelerated automatic chromatographic analysis of amino acids on a spherical resin. Anal. Chem. 37: 1108 (1965).
21. CAVINS, J. F., and FRIEDMAN, M. Automatic integration and computation of amino acid analyses. Cereal Chem. 45: 172 (1968).
22. BIETZ, J. A., and WALL, J. S. Wheat gluten subunits: Molecular weights determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Cereal Chem. 49: 416 (1972).
23. KOENIG, V. L., OGRINS, A., TRIMBO, H. B., and MILLER, B. S. The electrophoretic analysis of flour from several varieties of hard red winter wheat grown at several locations. J. Sci. Food Agr. 15: 492 (1964).
24. BECKWITH, A. C., NIELSEN, H. C., WALL, J. S., and HUEBNER, F. R. Isolation and characterization of a high-molecular-weight protein from wheat gliadin. Cereal Chem. 43: 14 (1966).
25. CROW, M. J. A., and ROTHFUS, J. A. Chromatography of proteins from wheat gluten on polyacrylamide gel. Cereal Chem. 45: 413 (1968).
26. BIETZ, J. A., SHEPHERD, K. W., and WALL, J. S. Single-kernel analysis of glutenin: Use in wheat genetic and breeding studies. Cereal Chem. 52: 513 (1975).
27. FINNEY, K. F., and SHOGREN, M. D. A ten-gram mixograph for determining and predicting functional properties of wheat flours. Baker's Dig. 46(2): 32 (1972).

[Received November 1, 1974. Accepted June 25, 1975]