Characterization of Wheat Protein Fractions Differing in Contributions to Breadmaking Quality

F. MacRITCHIE,[1] D. D. KASARDA,[2] and D. D. KUZMICKY[2]

ABSTRACT

Cereal Chem. 68(2):122–130

Protein fractions from six wheat cultivars, three of good quality and three of poor quality, were prepared by fractional extraction of gluten with dilute hydrochloric acid, and the relationship between breadmaking functionality and protein composition was investigated. Functionality was assessed by adding fractions to a base (reference) flour at a level of 1% (protein to flour weight) and measuring mixograph peak development times and loaf volumes in an optimized baking test. Protein composition was measured by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing and nonreducing conditions, followed by densitometry of the stained patterns. The pattern areas of reduced fractions, which corresponded to total proteins, were divided into subareas representative of five main protein classes: high molecular weight glutenin subunits; ω-gliadins; low molecular weight glutenin subunits; α-, β-, and γ-gliadins; and albumins plus globulins. Also, the proportion of glutenin in each fraction was estimated for some cultivars as the difference between the total area under the densitometer tracing of the pattern of a fraction reduced (total proteins) and the total area under the densitometer tracing of the pattern of the same fraction unreduced (from which essentially only monomeric proteins enter the gel). The proportion of high molecular weight glutenin subunits in the parent glutens increased in accordance with dough strength and, with one exception, loaf volume. As the fraction number increased, the proportion of glutenins generally paralleled the increases observed in dough development time and loaf volume, except for some of the later fractions.

In recent reports (MacRitchie 1987a,b), gluten protein fractions prepared from several wheat cultivars were evaluated for their contributions to dough mixing and breadmaking. The same approach had been used by a number of workers (Hoseney et al 1969, Booth and Melvin 1979, MacRitchie 1980, Preston and Tipples 1980), but a thorough characterization of the protein fractions giving rise to different effects usually was not performed. Chakraborty and Khan (1988) have stressed the need to do this. The fundamental aim of the present work was to relate functionality to the proportions of various types of endosperm proteins and thus approach an explanation for the variation in quality between wheat cultivars. In previous work concerning the proteins of the various fractions, certain inferences were drawn from amino acid compositions, but more information about the proteins of these fractions was needed to understand their breadmaking functionality. Accordingly, we now have studied the fractions by one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the reduced and unreduced fractions and by densitometry of the resulting protein patterns to provide qualitative and quantitative information about the proteins of these fractions.

[1]CSIRO Division of Plant Industry, Wheat Research Unit, P.O. Box 7, North Ryde, N.S.W. 2113, Australia.
[2]U.S. Department of Agriculture, Agricultural Research Service, Western Regional Research Center, 800 Buchanan Street, Albany, CA 94710.

The patterns of the reduced protein fractions, which correspond to the total endosperm proteins, were quantified by densitometry to provide information about the proportions in each fraction of five different protein classes as represented by five different molecular weight ranges in SDS-PAGE (Cole et al 1981, Fullington et al 1987). These classes, designated A1–A5 in order of decreasing relative molecular weight (M_r), are: A1, the high molecular weight glutenin subunits (HMW-GS); A2, ω-gliadins; A3, low molecular weight glutenin subunits (LMW-GS); A4, α-, β-, and γ-gliadins; and A5, albumins plus globulins. In addition, we attempted to estimate the total polymerized (by way of intermolecular disulfide bonds) protein in each fraction by subtracting the total area under the densitometric tracing of the SDS-PAGE pattern of the unreduced fraction from the total area under the tracing of the pattern of the reduced fraction. The rationale for this approach is that the patterns of the reduced fractions correspond to total protein, whereas the patterns of unreduced fractions correspond only to monomeric proteins. Comparisons of the proteins of the various fractions enabled us to reach some general conclusions about the relationship between function and composition for these fractions.

MATERIALS AND METHODS

Materials

Six wheat cultivars were used in the present study: four Australian (Cook and Mexico 8156, both of good quality; Halberd and Burgas, both of poor quality) and two California cultivars (Yecora Rojo and Anza, of good and poor quality, respectively). Protein fractions from the Australian cultivars were the same

as previously described (MacRitchie 1987a). The lots of the California cultivars were supplied by the California Wheat Commission and had designations CWC-141 (Yecora Rojo) and CWC-150 (Anza). The California cultivars were milled on a Brabender Quadrumat Senior mill at the Western Regional Research Center; the resulting flour protein contents were 11.0% (Yecora Rojo) and 10.0% (Anza) on a 13% moisture basis as measured by near-infrared reflectance spectroscopy at the U.S. Department of Agriculture Western Wheat Quality Laboratory, Pullman, WA. These analyses were in good agreement with duplicate Kjeldahl nitrogen analyses. The protein contents of the Australian flours were determined by Kjeldahl nitrogen analysis.

Preparation of Protein Fractions and Quality Assessment

Protein fractions were prepared and tested for functionality in breadmaking as reported earlier (MacRitchie and Gras 1973, MacRitchie 1985, MacRitchie 1987a). Powdered gluten containing 35 g of protein was sequentially extracted with dilute hydrochloric acid solutions; the first three extractions were made with 800 ml and subsequent ones with 400 ml of solution. This was followed by neutralization with sodium hydroxide and freeze-drying of the extracts. All fractions were added to a base flour at a level of 1% (dry protein to total flour weight), and the effects of these additions on mixing curves and loaf volume were measured.

As shown in Table I, a different base flour was used for each of the three pairs of cultivars because the studies of each pair were performed at different times, and there was not enough of the original or the second base flour for subsequent studies. Fractionation and reconstitution of the protein fractions with lipids, starch, and flour solubles (MacRitchie 1987a) resulted essentially in recovery of the original flour properties for all cultivars studied. Standard deviation of the mean for loaf volume should be 5 ml, and for mixograph peak development time, 0.2 min (MacRitchie 1987a).

Solubilization of Samples for SDS-PAGE

When reduced samples were required for electrophoresis, 1 mg of each fraction was dissolved in 250 μl of a sample buffer consisting of 0.0177M Tris-HCl (pH 6.8) that was 1% in SDS, 20% in sucrose, 6×10^3% in Pyronin Y, and 5% in β-mercaptoethanol. When unreduced samples were required, the same sample buffer mixture was used, but without any mercaptoethanol, and amounts of protein and solution were the same as those for reduced samples. Samples were usually agitated about three times over a period of several hours in a vortex mixer, examined for any residue, allowed to stand at 4°C overnight, reequilibrated to room temperature, and clarified in a microcentrifuge before use. Usually, 15 μl of such extracts were loaded into a gel slot, and in all cases, the amounts were the same for samples loaded on a single gel or on two gels that were to be compared (reduced versus unreduced).

All samples dissolved readily in reducing buffer, and fractions from the weaker flours dissolved in the SDS sample buffer without reducing agent, but later samples in the series from the stronger cultivars, such as Yecora Rojo, did not completely dissolve or disperse in the nonreducing buffer. The assumption was made that the dissolved proteins corresponded to all of the gliadins or other monomeric proteins, but only to part of the glutenin. When parent flour samples were analyzed, whether reduced or unreduced, 25 mg of flour was extracted with 1 ml of sample buffer by grinding the sample and the extracting buffer together in a small mortar and pestle. The mixture was transferred from the mortar with a Pasteur pipet to a microcentrifuge tube; the sample was then clarified by centrifugation in a microcentrifuge; and 10 or 15 μl of the clarified flour extract was loaded on the gel (the same amount was loaded in all cases where reduced samples were to be compared with unreduced samples).

SDS-PAGE

SDS-PAGE was performed in a Protean I apparatus (Bio-Rad Laboratories, Richmond, CA) according to the procedure of Laemmli (1970) in SDS-tris-glycine buffer. (The specific recipes are given in "Reagent and Gel Preparation for Laemmli SDS Polyacrylamide Gel Electrophoresis," Bulletin, Bio-Rad Laboratories, 1414 Harbor Way South, Richmond, CA 94804.) Slab gels with approximate dimensions of 16 cm (length), 14 cm (width), and 1.5 mm (thickness) were cast with 12 sample slots. The stacking gel was 3.88 %T (total gel concentration) and 1.33 %C (crosslinker concentration), whereas the running gel was 17.57 %T and 0.455 %C, where %T = (g of acrylamide + g of bis acrylamide) $\times$ 100/total volume; %C = (g of bis acrylamide) $\times$ 100/(g of acrylamide + g of bis acrylamide). Two gels were run simultaneously with tap-water cooling (approximately 18°C) at 50 mA for 50 min and then at 85–100 mA for 3–4 hr, usually until the tracking dye (Pyronin Y) ran off the end of the gel.

When unreduced samples were compared with reduced samples, all the reduced samples were usually placed on one gel and all the unreduced samples on the other. For some duplicate Anza fractions, both reduced and unreduced samples were run adjacently on the same gel to facilitate comparison of patterns, always leaving an empty slot between each sample to prevent partial reduction of unreduced samples that may result from diffusion of mercaptoethanol from one sample slot to another (Singh and Shepherd 1985).

Gel Staining and Preparation for Densitometry

Gels were stained with 0.02% Coomassie Brilliant Blue R250 in 6% trichloroacetic acid, 5% ethanol (500 ml per gel) overnight. Coomassie Brilliant Blue R250 (0.1 g) was dissolved in 25 ml of absolute ethanol, filtered, and added to the 6% trichloroacetic acid. The staining solution was changed three times over a two-day period at fairly regular intervals of about 12 hr. The gels were agitated gently at all times during staining. No change in staining intensity was obvious between the second and third changes of staining solution. When two gels were run, one gel remained in the stain an extra day (without change of stain) before densitometry was performed. After staining, the gels were rinsed five times with 500-ml portions of deionized water, photographed with a Polaroid MP-4 camera (Polaroid Type 55 sheet film), and cut into 12 strips corresponding to each of the sample tracks for densitometry.

Densitometry

Densitometry was performed with a Gilford gel scanner (model 2520) attached to a Gilford spectrophotometer (model 250). Gel strips were placed along one wall of a cuvette (the thin strip gel sticks to the wall through surface tension) that measured 20.0 $\times$ 1.0 cm (path length) $\times$ 1.5 cm (height). The cuvette was then filled with water to decrease light scattering and was scanned by transmission at a wavelength of 580 nm. The slit dimensions were 0.1 $\times$ 2.36 mm; scanning speed setting, 2; and chart speed,

TABLE I
Quality Data for Parent Flours

Variety[a]	% Protein (13%, mb)	Loaf Volume (ml)		Mixograph Peak Development Time (min)	
		Original	Reconstituted	Original	Reconstituted
Mexico 8156	12.1	197	196	7.2	7.6
Halberd	10.9	143	145	3.1	3.3
Oxley (base flour)	11.3	166	···	3.3	···
Cook	11.8	190	185	4.4	4.5
Burgas	15.0	127	131	1.4	1.3
Condor (base flour)	11.4	162	···	3.2	···
Yecora Rojo	10.9	205	198	4.7	4.9
Anza	09.9	158	154	2.1	2.4
Timgalen (base flour)	11.1	183	···	4.4	···

[a] Base flours are the flours to which fractions were added at the 1% level to obtain loaf volumes and mixograph peak development times shown in Tables III–VIII.

5 cm/min. The arrangement of the slit relative to the gel strip was such that only the central part of a pattern was scanned, thus avoiding the distortions that often occur near the edges of the protein bands in an SDS-PAGE pattern. Baselines were drawn arbitrarily according to the following general approach: For unreduced fractions, the baseline was begun approximately at the beginning (the end with higher molecular weight) of A2 to exclude most of the glutenin streaking into the gel from the slot. Densitometer scans were photocopied, and areas corresponding to the desired relative molecular-weight ranges (A1–A5) were cut out from the photocopies (enabling us to use a more suitable, uniform paper) and weighed. Assignment of relative molecular-weight ranges corresponding to each of the areas A1–A5 was made on the basis of past studies (Cole et al 1981, Fullington et al 1983, 1987).

The boundaries of the ranges were adjusted slightly to fit with minima in the densitometric scans of the SDS-PAGE patterns, but for all cultivars, the ranges were close to the following: A1, $\geq 80 \times 10^3$(80K); A2, 80–51K; A3, 51–40K; A4, 40–28K; and A5, $\leq$ 28K. A1 corresponds almost exclusively to HMW-GS. A2 corresponds to ω-gliadins, which have the same mobilities in the patterns of the reduced and unreduced fractions because they have no cystine, and to some HMW-albumins and globulins (Gupta and Shepherd 1987), which appear to be polymerized through intermolecular disulfide bonding as they appear mainly in the reduced patterns. A3 corresponds mainly to the B group of the LMW-GS (Tao and Kasarda 1989), but some higher molecular weight gliadins have mobilities in this range (Kasarda et al 1987). A4 corresponds mainly to gliadins, but some of the C group of LMW-GS appear in A4 (Tao and Kasarda 1987). A5 corresponds to LMW albumins and globulins. Monomeric proteins with intramolecular disulfide bonds, such as the α-, β-, and γ-gliadins, tend to migrate slightly faster in the unreduced form in SDS-PAGE, presumably because the proteins are constrained into a more compact form by the intact disulfide bonds. The differences in mobility are usually small, however, and are not likely to have caused any errors in our assignment of the proteins to the appropriate mobility ranges. For example, Figure 4B of Singh and Shepherd (1985) (not shown) shows a two-dimensional SDS-PAGE pattern of an SDS extract of wheat flour, with the first dimension performed without reducing agent and the second dimension performed after equilibration of the first dimension with SDS plus reducing agent. The α-, β-, and γ-gliadins appear above the diagonal in the pattern, indicating that they move slightly faster in the first dimension (unreduced), but we are fairly certain on the basis of having run similar two-dimensional patterns (results not shown) that our assignments to mobility groupings are essentially correct.

For reduced proteins of a fraction, percent of the total area (total protein) for each subarea was calculated as $A_i \times 100/A_T$, where A_i = the area of fraction i (i = 1–5) and A_T = the total area of the densitometric scan of the pattern of reduced proteins. Some indication of the precision of the method can be noted in Table II. The proportion of polymerized protein (or proteins strongly interacting with the polymerized proteins through secondary forces thus excluded from the gel) was calculated as $[A_T \text{ (reduced)} - A_{TU} \text{ (unreduced)} \times 100]/A$ (reduced), where A_T is as defined above, and T_{TU} = total area under the densitometric scan of the pattern of the unreduced proteins. This quantity should represent mainly glutenins, and we sometimes refer to it as glutenin, but it should be kept in mind that some albuminlike and globulinlike proteins are found in this fraction (Gupta and Shepherd 1987).

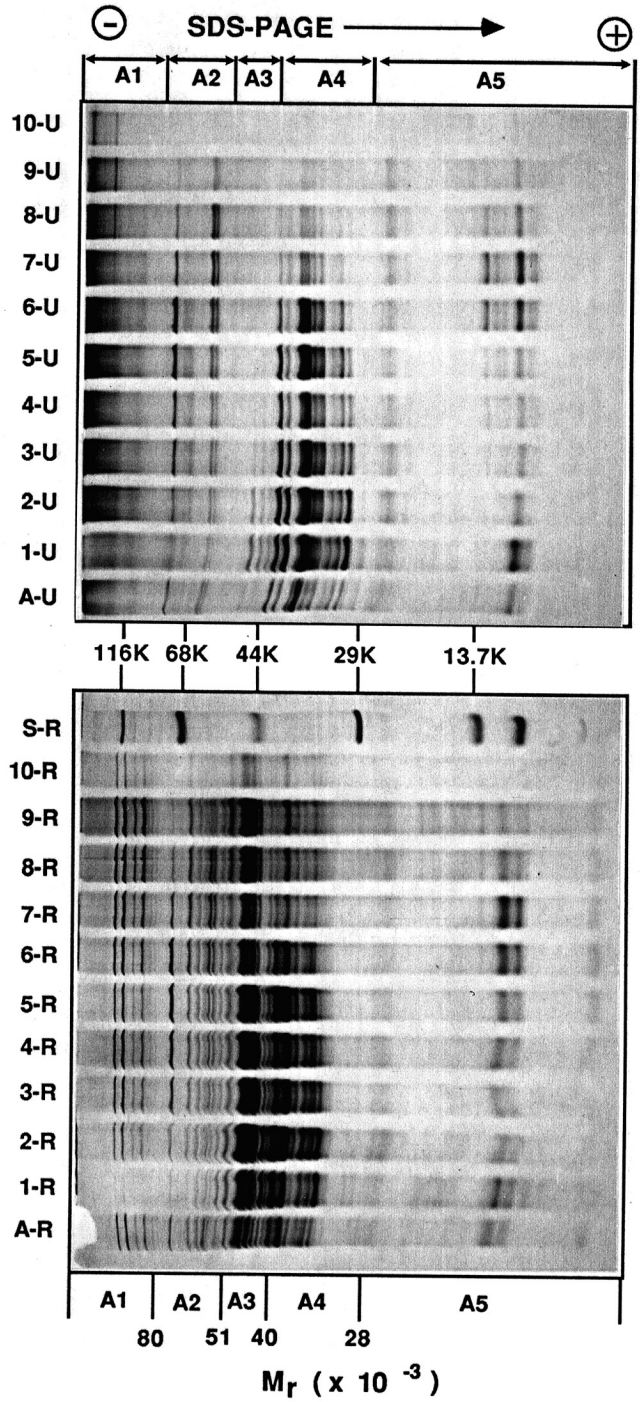

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of unreduced (1-U–10-U) and reduced (1-R–10-R) fractions of the cultivar Anza. A-U indicates the pattern of an unreduced extract of flour proteins; A-R is the pattern of a flour extract made under reducing conditions. The bottom scale, M_r ($\times 10^{-3}$), indicates the molecular weight range of separation on the gel in terms of areas A1–A5, with boundaries for the areas indicated in terms of molecular weights. The molecular weights of the standard (reduced) proteins are shown in between the two gel patterns: 116K, β-galactosidase; 68K, bovine serum albumin; 44K, ovalbumin; 29K, carbonic anhydrase; and 13.7K, ribonuclease. Cytochrome C (12.5K) is also visible although not indicated.

TABLE II
Precision for Measuring Areas A1–A5 (% of Total Area) for Flour Proteins and Percent Glutenin

Variety	A1	A2	A3	A4	A5	Glutenin (%)
Anza	9.4 ± 1.0[a]	13.4 ± 0.5	17.8 ± 1.2	34.0 ± 1.2	25.4 ± 1.5	30.4 ± 6.5
Yecora Rojo	12.0 ± 0.9	11.6 ± 0.9	21.2 ± 0.9	32.9 ± 2.3	22.4 ± 2.3	36.1 ± 6.2

[a] Values following numbers are 95% confidence intervals.

For the calculation of polymerized protein percentage by difference between the areas of densitometric scans of SDS-PAGE patterns of reduced and unreduced proteins to be valid, it is necessary that the proteins bind dye to the same degree, whether reduced or unreduced. Although this was not studied extensively, a densitometric comparison of the reduced and unreduced patterns of a gliadin mixture that had been freed of glutenins by gel permeation chromatography indicated no difference in area between the patterns when stained according to our method (and that no error was made in our assignment of gliadins to mobility groupings, whether the proteins were run reduced or unreduced).

RESULTS

In our initial studies, only SDS-PAGE patterns of reduced proteins were obtained for fractions from Mexico 8156 and Halberd (patterns not shown; see MacRitchie 1989). Subsequently, patterns were obtained for both reduced and unreduced

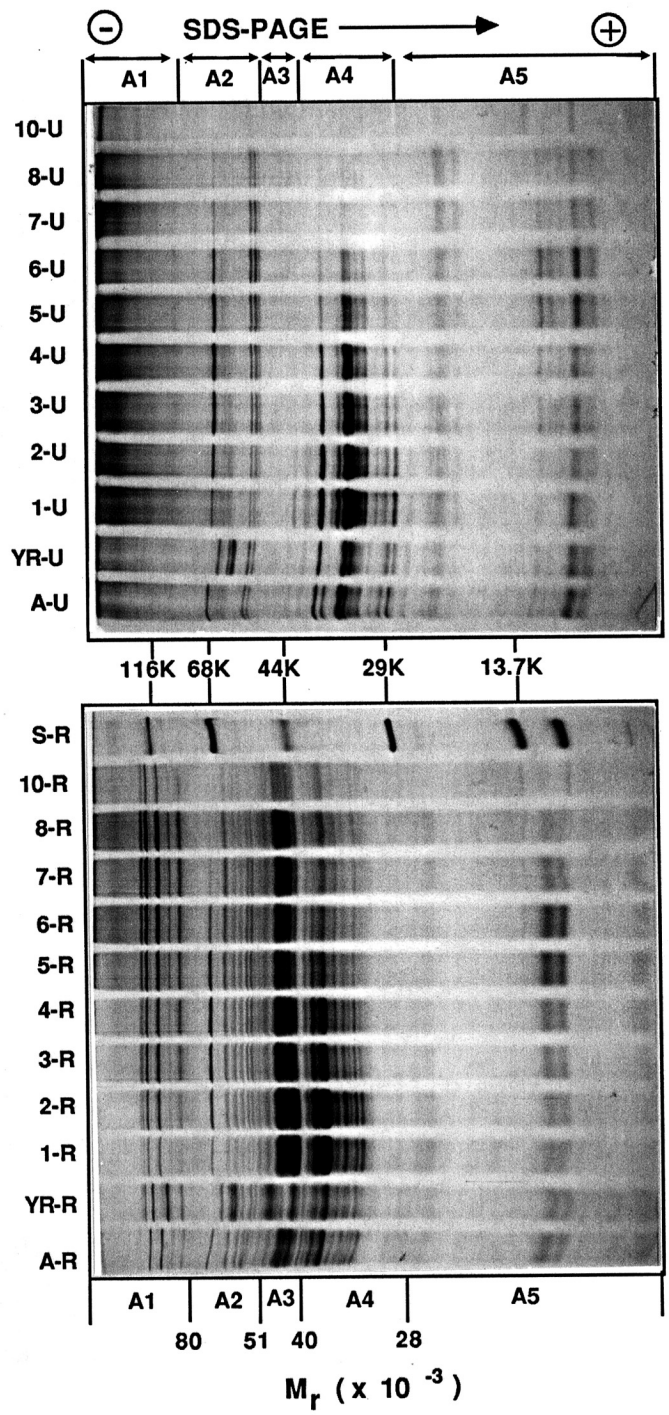

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of unreduced (1-U–10-U) and reduced (1-R–10-R) fractions of the cultivar Cook. Fraction 9 was not included. A-U and YR-U indicate the patterns of unreduced extracts of flour proteins from Anza and Yecora Rojo, respectively; A-R and YR-R indicate the patterns of extracts of Anza and Yecora Rojo flours made under reducing conditions. Other conditions are the same as described in Figure 2.

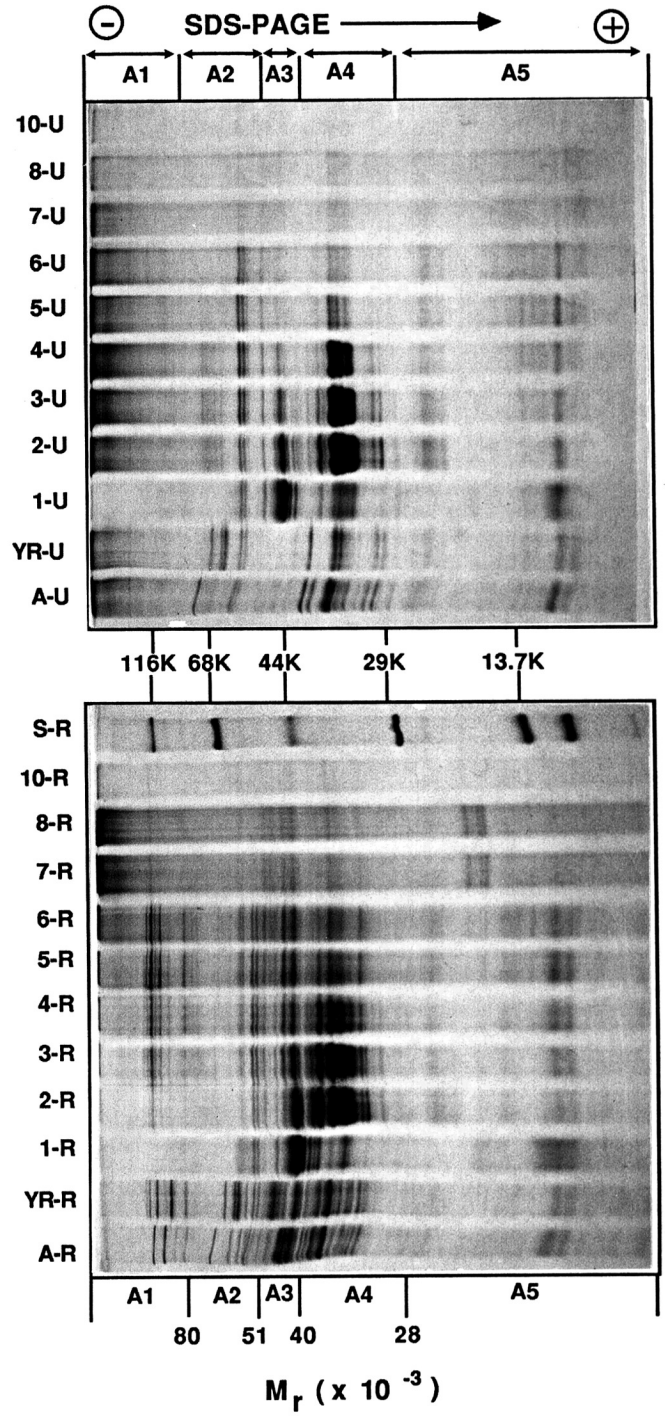

Fig. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of unreduced (1-U–10-U) and reduced (1-R–10-R) fractions of the cultivar Burgas. Fraction 9 was not included. A-U and YR-U indicate the patterns of unreduced extracts of flour proteins from Anza and Yecora Rojo, respectively; A-R and YR-R indicate the patterns of extracts of Anza and Yecora Rojo flours made under reducing conditions. Other conditions are the same as described in Figure 2.

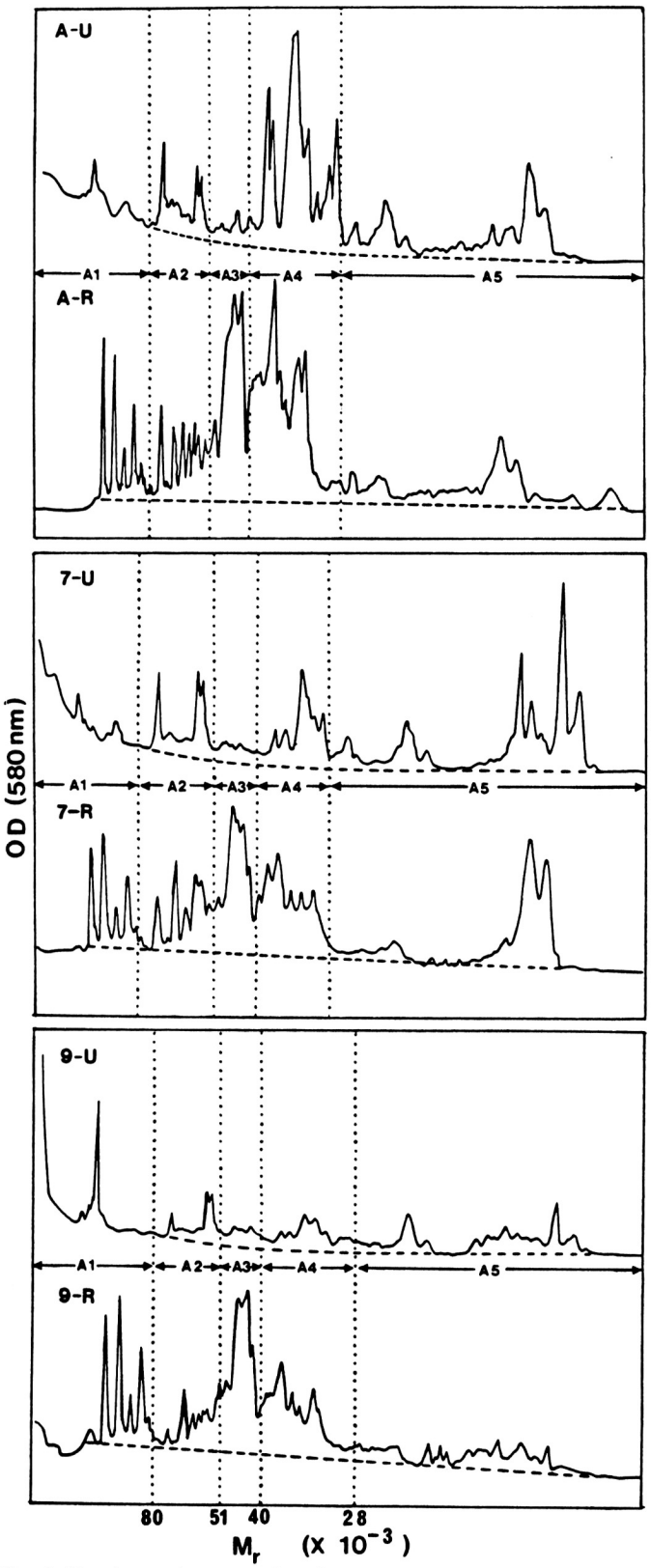

Fig. 4. Densitometric scans of sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of Anza flour (A-U, no reducing agent; A-R, reducing agent included) and of Anza fractions 7 and 9 (U, unreduced fraction; R, reduced fraction). The patterns are divided into molecular weight ranges; boundaries for A1–A5 are indicated on the bottom scale and by vertical dotted lines. These lines do not coincide in all patterns because, although the patterns of each fraction (reduced and unreduced) and the parent flour were taken from a single gel, the results for different fractions were obtained from different gels, in which the proteins had slightly different absolute mobilities (the densitometric scans were not normalized from gel to gel).

proteins of the other cultivars (Anza, Yecora Rojo, Cook, and Burgas) in an attempt to provide more information about the content of glutenin and other polymerized proteins in the fractions. The SDS-PAGE patterns for Anza fractions are shown in Figure 1. Similar comparisons of patterns are shown for Cook (Fig. 2) and Burgas (Fig. 3) fractions. Patterns for the fractions of the other cultivars are not shown but were generally similar to those in Figure 1, with differences that will be considered in the discussion section. (Fraction 9 of Cook and fraction 9 of Burgas were omitted from Figures 2 and 3 because we wished to include several reference patterns on the 12-slot gel; these fractions were analyzed on other gels. The reference patterns of Cook and Burgas flours were not included in Figures 2 and 3 because the supply of the original flours had been exhausted by the time these figures were prepared.)

Figure 4 illustrates the type of results obtained by densitometry of the SDS-PAGE patterns for proteins extracted directly from Anza flour without (A-U) and with (A-R) reducing agent, and for unreduced and reduced proteins of two selected Anza fractions (7-U, 7-R and 9-U, 9-R, respectively). Baselines were generally drawn as straight lines for reduced samples (A-R, 7-R, and 9-R) and slightly curved lines for unreduced samples (A-U, 7-U, and 9-U). The reason for the curved lines is that lower molecular weight polymers (oligomers) of LMW-GS enter the gels to some degree, causing streaking that declines curvilinearly in intensity from the slot, disappearing in A-2. This disappearance corresponds to the mobility of dimers of LMW-GS, the smallest possible native glutenin molecules, which have molecular weights of 60–80K (D. D. Kasarda and H. W. Jones, *unpublished data*). A somewhat diffuse band corresponding to various possible dimers of LMW-GS can be seen in Figures 1 and 2 near the position of the 68K standard protein and just ahead of the slowest-moving ω-gliadin band of A2 in the patterns of unreduced proteins (for example, note lane 5-U in Fig. 1 and lane 1-U in Fig. 2) for most of the early fractions analyzed in the unreduced form. Higher-level oligomers (trimers, tetramers, and so on) can be seen in A1 of patterns of unreduced proteins. Because we wished to exclude contributions to the area of the patterns of unreduced proteins from any proteins linked by intermolecular disulfide cross-links, the baseline was drawn to exclude contributions to the absorbance by these LMW-GS oligomers (with the exception of the small contribution from dimers, which usually had slightly higher mobility than the slowest-moving ω-gliadin in most of the cultivars we examined). Another feature of the patterns of the unreduced proteins of some cultivars was the presence in late fractions of a sharp band in A1 (see Fig. 1, 7-U–10-U) that disappears from the patterns of the reduced proteins. This band is likely to result from triticin (trimer) aggregates (Singh and Shepherd 1985, Singh et al 1987), although two-dimensional electrophoresis would have been necessary to confirm this possibility.

Areas A1–A5 were obtained by densitometry of the patterns of reduced proteins similar to those shown in Figures 1–3 (other data not shown). These percentage areas (relative to the total area) are given in Tables III–VIII. To facilitate comparison with composition data, quality data (effect of 1% additions of protein fractions on the properties of the base flour) for the various acid-extracted fractions from each cultivar are given in Tables III–VIII. In addition, the percentages of total protein (% protein) corresponding to each fraction, the percent of total protein extracted for each fraction, and the final pH of the supernatant solution corresponding to each fraction are included in the tables. Also given in Tables V–VIII is the percent of glutenin determined from the differences between the areas of the densitometric scans of patterns for reduced and unreduced proteins (as mentioned, reduced patterns were not obtained for Mexico 8156 and Halberd). For example, the difference between areas 9-R and 9-U in Figure 1 indicates that Anza fraction 9 consists of 71.8% glutenin (and other polymerized proteins) (Table VIII). The value for the percent glutenin may tend to be high if some proteins are noncovalently bound by the truly polymerized proteins and thus do not migrate into the gel.

The proportions of A1–A5 and percent glutenin were compared for extracts of the parent Anza and Yecora Rojo flours. The results, averaged for at least three replicates, are shown in Table II. Although for some of the wheat cultivars, single analyses of A1–A5 were done for the acid fractions in Tables III–VIII, an indication of the reproducibility of the method may be gained from the confidence intervals of Table II.

DISCUSSION

General Quality-Composition Relationships

Some general conclusions regarding relationships between functionality and composition may be reached by inspecting Tables III–VIII. As the fraction number increases for each cultivar, an increase generally occurs in loaf volume and mixograph peak

TABLE III
Quality and Composition Data for Gluten Protein Fractions from Mexico 8156

Fraction	Final pH of Supernatant	Total Protein (%)	Protein in Fraction (%)	Mixograph[a] Peak Development Time (min)	Loaf[a] Volume (ml)	Relative Areas[b] (%)				
						A1	A2	A3	A4	A5
Control[a]	...	...	...	3.27	166	...	...	...	...	...
1	5.65	20.2	91.4	3.08	166	8.0	7.9	28.3	48.4	7.4
2	5.44	19.0	86.2	2.70	168	9.9	9.3	25.9	41.6	13.3
3	5.12	14.8	90.3	2.92	166	11.4	11.4	23.4	36.1	17.7
4	5.01	9.8	90.7	3.55	173	15.4	14.6	24.5	26.0	19.5
5	4.84	7.7	88.5	3.90	187	18.0	13.9	25.6	25.9	16.6
6	4.61	5.7	84.9	4.21	181	15.9	14.0	26.0	19.1	25.0
7	4.10	5.4	87.3	4.91	185	20.8	13.4	31.0	23.2	11.6
8	3.65	6.1	85.7	5.41	190	17.5	10.2	30.2	22.2	19.9
9	3.31	2.5	80.6	4.75	179	18.2	10.4	31.8	23.6	16.0
10	...	8.7	54.2	3.33	164	17.9	5.8	30.0	29.6	16.7
Composite of all fractions[c]						13.2	10.5	26.9	34.4	15.0

[a] The base flour (control) was Oxley, to which fractions 1–10 were added at the 1% level to obtain the quality data shown (loaf volumes and mixogram peak development times).
[b] Relative areas were obtained from densitometry of sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of fractions 1–10.
[c] Composite values represent a weighted average of all 10 fractions, with weighting based on the percent of total protein contributed by each fraction.

TABLE IV
Quality and Composition Data for Gluten Protein Fractions from Halberd

Fraction	Final pH of Supernatant	Total Protein (%)	Protein in Fraction (%)	Mixograph Peak Development Time (min)	Loaf Volume (ml)	Relative Areas (%)				
						A1	A2	A3	A4	A5
Control (Oxley)	...	...	...	3.27	166	...	...	...	...	...
1	5.91	17.0	84.6	2.96	162	6.0	10.1	25.9	40.4	17.6
2	5.69	28.7	90.5	2.83	160	8.2	10.6	23.7	44.3	13.2
3	5.28	19.0	87.4	2.70	171	10.5	11.7	21.9	39.0	16.9
4	5.10	9.8	86.4	3.40	175	13.0	10.6	21.6	31.7	23.1
5	4.75	8.5	85.0	4.15	181	14.3	10.9	25.4	26.7	22.7
6	4.30	4.3	79.4	4.21	188	15.0	11.5	24.4	23.1	26.0
7	3.56	3.4	75.0	...	...	15.5	11.4	29.6	23.3	20.2
8	3.25	3.2	81.0	4.40	169	11.3	10.8	25.8	22.6	29.5
9	3.16	1.4	76.2	...	...	10.6	10.8	26.0	23.1	20.5
10	...	4.7	32.1	3.62	146	9.9	10.8	26.3	23.5	29.5
Composite of all fractions						10.0	10.8	24.1	36.3	18.7

TABLE V
Quality and Composition Data for Gluten Protein Fractions from Cook

Fraction	Final pH of Supernatant	Total Protein (%)	Protein in Fraction (%)	Mixograph Peak Development Time (min)	Loaf Volume (ml)	Relative Areas (%)					Glutenin[a] (%)
						A1	A2	A3	A4	A5	
Control (Condor)	...	...	...	3.21	162	...	...	...	...	...	...
1	5.59	23.0	88.8	2.64	154	6.2	5.5	28.9	44.3	15.1	35.7
2	5.50	20.4	91.2	3.02	167	9.4	7.1	26.6	43.9	13.0	49.1
3	5.21	16.4	89.9	3.02	179	12.0	10.2	24.5	36.4	16.9	36.9
4	5.06	7.7	88.6	3.02	178	11.8	10.2	25.4	34.6	18.0	52.1
5	4.84	6.0	87.5	3.90	187	13.3	11.6	22.2	26.8	26.2	49.0
6	4.66	5.2	87.3	4.28	195	15.4	11.7	23.7	23.0	26.1	54.2
7	4.16	7.1	87.0	5.03	207	18.9	10.6	27.5	21.3	21.7	66.5
8	3.65	5.7	81.2	6.04	209	18.7	7.9	30.3	23.6	19.5	68.9
9	3.29	2.4	81.4	6.04	192	16.9	7.3	28.4	24.7	22.7	70.7
10	...	6.1	36.7	3.46	167	15.1	6.8	26.6	25.7	25.9	72.5
Composite of all fractions						11.6	8.3	26.6	35.6	18.0	48.8

[a] Percent glutenin was obtained from the densitometry of sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns for the reduced and unreduced fractions (see text).

development time, accompanied by an increase in the proportion of glutenins. This is indicated by the percentage of fraction A1 (Tables III–VIII), which includes only the HMW-GS, and by glutenin (Tables V–VIII), which is based on the differences between patterns of reduced and unreduced samples. There was a tendency for the proportions of glutenins to stabilize or drop in the later fractions, especially in the last (residue) fraction, which paralleled to some extent the decreases in development time and loaf volume. However, this does not completely explain the negative effects of the latest fractions. Although these fractions had SDS-PAGE patterns similar to those of glutenin (results not shown), other proteins were present, particularly in regions A2 and A5, which should have no glutenin components. This is supported by the amino acid compositions of these fractions, which were not in accord with the compositions usually associated with wheat gluten proteins (MacRitchie 1987a); for example, the values of glutamine and glutamic acid were rather low. The presence of high levels of nonstorage proteins in these late fractions has been shown by two-dimensional electrophoresis (P. I. Payne and S. A. Forsyth, *personal communication*) These fractions, especially the residue fraction, had relatively low protein contents and probably contained significant amounts of carbohydrates, although we did not analyze the nature of the nonprotein components.

When we compared the results for composite values of A1–A5 for the different varieties, A1 (HMW-GS) seemed to fit best with

TABLE VI
Quality and Composition Data for Gluten Protein Fractions from Burgas

Fraction	Final pH of Supernatant	Total Protein (%)	Protein in Fraction (%)	Mixograph Peak Development Time (min)	Loaf Volume (ml)	Relative Areas (%)					Glutenin (%)
						A1	A2	A3	A4	A5	
Control (Condor)	...	...	...	3.21	162	...	...	...	...	...	...
1	6.00	4.7	62.6	2.89	157	3.6	9.2	27.2	30.8	29.3	19.3
2	5.68	31.0	90.6	2.64	157	5.3	6.0	15.0	56.6	17.1	17.4
3	5.46	29.3	90.5	2.70	157	8.8	7.7	13.1	53.9	16.5	24.3
4	5.26	11.3	88.6	2.83	170	11.7	9.5	12.3	45.0	21.6	31.9
5	4.87	13.1	87.0	3.14	187	14.6	11.2	14.1	32.0	28.1	53.2
6	4.25	5.3	85.2	3.33	177	13.2	12.3	15.2	29.5	29.8	56.2
7	3.38	1.4	79.4	...	...	7.5	10.4	14.6	28.0	39.5	36.1
8	3.14	0.7	70.6	3.14	176	6.6	9.4	13.6	27.6	42.8	48.5
9	3.13	0.5	59.6	...	...	7.0	11.4	12.4	28.5	40.7	54.8
10	...	2.7	11.6	3.14	138	7.3	13.4	11.3	29.3	38.7	61.1
Composite of all fractions						8.7	8.4	14.5	47.1	21.3	29.7

TABLE VII
Quality and Composition Data for Gluten Protein Fractions from Yecora Rojo

Fraction	Final pH of Supernatant	Total Protein (%)	Protein in Fraction (%)	Mixograph Peak Development Time (min)	Loaf Volume (ml)	Relative Areas (%)					Glutenin (%)
						A1	A2	A3	A4	A5	
Control (Timgalen)	...	...	...	4.4	183	...	...	...	...	...	...
1	5.45	36.0	94.1	3.7	176	8.7	8.5	23.5	44.3	15.0	20.3
2	5.11	36.6	90.9	4.7	199	15.0	13.7	20.3	35.9	15.1	38.9
3	4.45	15.2	92.5	5.7	202	15.7	16.6	19.6	27.4	20.6	63.1
4	4.01	3.2	94.9	6.7	195	16.6	12.1	22.9	27.4	21.0	66.9
5	3.80	1.7	74.9	6.3	187	16.1	7.5	24.5	28.6	23.2	83.1
6	3.56	...	...	...	...	...	...	...	...	...	...
7	3.33	1.3	64.4	5.2	165	9.7	6.5	25.8	26.8	31.1	68.6
8	3.20	...	...	...	...	...	...	...	...	...	...
9	3.13	...	...	...	...	...	...	...	...	...	...
10	...	5.9	38.0	4.5	168	8.9	9.9	18.5	25.4	37.2	64.3
Composite of all fractions						12.5	11.8	21.5	36.5	17.7	39.4

TABLE VIII
Quality and Composition Data for Gluten Protein Fractions from Anza

Fraction	Final pH of Supernatant	Total Protein (%)	Protein in Fraction (%)	Mixograph Peak Development Time (min)	Loaf Volume (ml)	Relative Areas (%)					Glutenin (%)
						A1	A2	A3	A4	A5	
Control (Timgalen)	...	...	...	4.4	183	...	...	...	...	...	...
1	5.80	7.7	72.5	3.7	162	2.4	8.2	22.9	45.4	21.1	−2.8
2	5.69	18.7	91.2	3.7	170	5.6	9.6	23.4	47.9	13.4	35.2
3	5.40	21.4	93.1	4.3	182	8.8	11.4	21.9	44.1	13.8	37.7
4	5.28	12.2	91.5	3.5	188	9.9	13.6	21.1	41.1	14.0	52.1
5	5.14	7.0	91.4	4.2	189	7.6	14.4	20.5	41.9	15.6	41.2
6	4.98	6.2	90.0	4.7	189	12.4	16.9	16.6	33.3	20.8	26.3
7	4.46	7.3	88.4	5.2	192	13.9	19.0	16.2	23.1	27.7	43.4
8	4.02	10.4	88.1	6.2	190	14.6	17.7	20.2	24.4	23.1	59.3
9	3.50	4.3	91.2	4.8	173	14.7	15.3	21.7	25.4	22.9	71.8
10	...	4.8	23.9	6.0	168	17.5	16.6	19.3	24.1	22.6	65.8
Composite of all fractions						9.6	13.3	21.0	38.4	17.7	40.9

the known characteristics of the cultivars studied. The percentages of A1 calculated from densitometry (composite value for each cultivar) were 13.2 (Mexico 8156), 12.5 (Yecora Rojo), 11.6 (Cook), 10.0 (Halberd), 9.6 (Anza), and 8.7 (Burgas), which are in the correct ordering for dough strength as measured by mixograph peak development time for the original flours. A similar ordering occurred when A1 was compared with loaf volumes of the original flours, except that Yecora Rojo gave the largest loaf volume and Mexico 8156 the second largest. However, the difference in loaf volumes was small for these two cultivars. Lawrence et al (1988) reported that breadmaking quality declined dramatically when the HMW-GS content of flour protein was progressively decreased through the use of wheat lines that were null for specific subunits. The HMW-GS make up only about one fourth of the glutenin (Gupta and Shepherd 1987), but evidence suggests that they contribute more to dough strength of bread wheats than do the LMW-GS (MacRitchie et al 1989).

Data for composite values of percent glutenin calculated from the differences in total areas from densitometry are rather limited, but it is clear that the cultivar Cook has a much higher value (48.8%) than its poorer counterpart Burgas (29.7%). Yecora Rojo and Anza had similar values (39.4% and 40.9%, respectively), despite the fact that Yecora Rojo had better quality parameters. However, as we have seen, Yecora Rojo shows a substantially higher content of A1 components (HMW-GS) than Anza, which helps to rationalize the differences in quality.

The cultivar Burgas had the poorest quality. Burgas is a substitution line in which the entire chromosome 1B has been replaced by rye chromosome 1R. Because chromosome 1B usually codes for two HMW-GS (long arm) and a group of LMW-GS (short arm), the loss of this chromosome severely depletes the complement of wheat glutenin subunits. These two HMW-GS appear to be replaced by a single, weakly expressed rye HMW subunit, which may not be equivalent to a wheat HMW-GS in contributing to quality. The short arm of the 1R chromosome contributes gliadinlike secalin components (Shewry et al 1984) in place of the wheat 1B-coded LMW-GS and gliadins. None of these secalins participate in the formation of the disulfide-linked glutenin network. The substitutions of rye proteins for wheat proteins in Burgas seem a likely explanation for the poor quality observed for Burgas.

Early fractions (usually 1 and 2) were notable for large amounts of gliadins, especially in A4, and generally resulted in decreased loaf volume and mixing strength, but fraction 2 of Yecora Rojo (Table VII) produced an improvement in the base flour for both of these characteristics. The protein of Yecora Rojo was exceptionally extractable relative to the other cultivars studied, so that, apparently, most of the gliadin was extracted in fraction 1 and a significant amount of glutenin was contained in fraction 2, as evidenced by A1 (15%). We cannot satisfactorily explain the ready extractability of the the protein of Yecora Rojo; it may result from a lesser amount of buffering substances in the endosperm of this cultivar relative to the others, which results in a more rapid pH drop during the extraction process. The resulting lower pH would tend to facilitate protein extraction.

The data in Tables III–VIII and Figures 1–3 indicate that substantial amounts of LMW albumins and globulins corresponding in mobility to A5 of our SDS-PAGE patterns were sometimes present in the fractions that contributed most strongly to improvement in loaf volume or peak mixing time of the base flour (for example, see results for fraction 7 of Anza in Table VIII and Fig. 1). This was also largely the case for ω-gliadins that appear in A2, which can best be observed in the patterns of the unreduced proteins in Figures 1 and 2. Our results do not permit us to conclude that the albumins and globulins of A5 and the ω-gliadins of A2 contribute to the quality-improvement effects shown by these fractions; they may only extract coincidentally with quality-contributing glutenins. On the other hand, however, it seems unlikely that they have strong detrimental effects on quality.

The SDS-PAGE patterns of fractions from the cultivar Mexico 8156 (good quality) and the cultivar Halberd (poor quality) have been shown (MacRitchie 1989) and are of interest because of their similar HMW-GS composition. Both cultivars have the same chromosome 1A-coded subunit (subunit 1 according to the system of Payne et al 1985) and the same 1D-coded subunits (Payne numbers 5 and 10). The 1B-coded subunits of Mexico 8156 are 7 + 8, and of Halberd, 7 + 9 for one biotype and 20 for the other. The results of Lawrence (1986) indicate that the 7 + 9 biotype made up 80% of Halberd and the 20 biotype made up 20%, but our patterns (results not shown; MacRitchie 1989) indicate that the proportions of the two Halberd biotypes were reversed in the particular sample we studied. The lower quality of Halberd (Table I) might result from subunit 20 being a poor-quality allele, but it might also result from a lower level of expression of HMW-GS in the predominant subunit 20 biotype as a consequence of there being only one 1B-coded subunit (which itself might be expressed poorly). Densitometry results shown in Tables III and IV indicate that A1 (HMW-GS) was greater for Mexico 8156 (13.2%) than for Halberd (10.0%), which supports the possibility that differences in quality between these two cultivars result from a quantitative effect rather than from specific allelic effects on quality.

In conclusion, our results underscore the complexity and overlapping nature of the solubility fractions of wheat proteins. However, they support the view that glutenin proteins are especially important to quality and that a quantitative approach is needed to understand their role.

ACKNOWLEDGMENTS

We thank A. E. Adalsteins for performing the comparisons of dye binding by reduced and unreduced gliadins, H. P. Tao for assistance in computerizing and printing some of the densitometric data, S. Huang for the Anza and Yecora Rojo flours, and G. Rubenthaler for some analytical data on these flours.

LITERATURE CITED

BOOTH, M. R., and MELVIN, M. A. 1979. Factors responsible for the poor breadmaking quality of high yielding European wheat. J. Sci. Food Agric. 30:1057.

CHAKRABORTY, K., and KHAN, K. 1988. Biochemical and breadmaking properties of wheat protein components. I. Compositional differences revealed through quantitation and polyacrylamide gel electrophoresis of protein fractions from various isolation procedures. Cereal Chem. 65:333.

COLE, E. W., FULLINGTON, J. G., and KASARDA, D. D. 1981. Grain protein variability among species of *Triticum* and *Aegilops*: Quantitative SDS-PAGE studies. Theor. Appl. Genet. 60:17.

FULLINGTON, J. G., COLE, E. W., and KASARDA, D. D. 1983. Quantitative sodium dodecyl sulfate-polyacrylamide gel electrophoresis of total proteins extracted from different wheat varieties: Effect of protein content. Cereal Chem. 60:65.

FULLINGTON, J. G., MISKELLY, D. M., WRIGLEY, C. W., and KASARDA, D. D. 1987. Quality-related endosperm proteins in sulfur-deficient and normal wheat grain. J. Cereal Sci. 5:233.

GUPTA, R. B., and SHEPHERD, K. W. 1987. Genetic control of LMW glutenin subunits in bread wheat and association with physical dough properties. Pages 13–19 in: Proc. of the Third International Workshop on Gluten Proteins. R. Lasztity and F. Bekes, eds. World Scientific Publishing Co.: Singapore.

HOSENEY, R. C., FINNEY, K. F., SHOGREN, M. O., and POMERANZ, Y. 1969. Functional (bread-making) and biochemical properties of wheat flour components. III. Characterization of gluten protein fractions obtained by ultracentrifugation. Cereal Chem. 46:126.

KASARDA, D. D., ADALSTEINS, A. E., and LAIRD, N. F. 1987. γ-Gliadins with α-type structure coded on chromosome 6B of the wheat (*Triticum aestivum* L.) cultivar Chinese Spring. Pages 20–29 in: Proc. of the Third International Workshop on Gluten Proteins. R. Lasztity and F. Bekes, eds. World Scientific Publishing Co.: Singapore.

LAEMMLI, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680.

LAWRENCE, G. J. 1986. The high-molecular-weight glutenin subunit composition of Australian wheat varieties. Aust. J. Agric. Res. 37:125.

LAWRENCE, G. J., MacRITCHIE, F., and WRIGLEY, C. W. 1988. Dough and baking quality of wheat lines deficient in glutenin subunits controlled by the Glu-A1, Glu-B1, and Glu-D1 loci. J. Cereal Sci.

7:109.
MacRITCHIE, R. 1980. Studies of gluten protein from wheat flours. Cereal Foods World 25:382.
MacRITCHIE, F. 1985. Studies of the methodology for fractionation and reconstitution of wheat flours. J. Cereal Sci. 3:221.
MacRITCHIE, F. 1987a. Evaluation of contributions from wheat protein fractions to dough mixing and breadmaking. J. Cereal Sci. 6:259.
MacRITCHIE, F. 1987b. Identification of "quality" components of gluten protein using fractionation and reconstitution methods. Page 247 in: Proc. of the Third International Workshop on Gluten Proteins. R. Lasztity and F. Bekes, eds. World Scientific Publishing Co.: Singapore.
MacRITCHIE, F. 1989. Identifying the baking quality related components of wheat flours. Cereal Foods World 34:548.
MacRITCHIE, F., and GRAS, P. W. 1973. The role of flour lipids in baking. Cereal Chem. 50:292.
MacRITCHIE, F., GUPTA, R. B., and WRIGLEY, C. W. 1989. Chemical aspects of wheat proteins related to breadmaking potential. Page 115 in: Proc. International Cereal Chemistry Symp. Wheat End-Use Properties. H. Salovaara, ed. Univ. of Helsinki, Finland.
ORTH, R. A., and BUSHUK, W. 1972. A comparative study of the proteins of wheats of diverse baking qualities. Cereal Chem. 57:314.
PAYNE, P. I., HOLT, L. M., JARVIS, M. G., and JACKSON, E. A. 1985. Two-dimensional fractionation of the endosperm proteins of bread wheat (*Triticum aestivum*): Biochemical and genetic studies. Cereal Chem. 62:319.
PRESTON, K. R., and TIPPLES, K. H. 1980. Effects of acid-soluble and acid-insoluble gluten proteins on the rheological and baking properties of wheat flours. Cereal Chem. 57:314.
SHEWRY, P. R., BRADBERRY, D., FRANKLIN, J., and WHITE, R. P. 1984. The chromosomal locations and linkage relationships of the structural genes for the prolamin storage proteins (secalins) of rye. Theor. Appl. Genet. 69:63.
SINGH, N. K., and SHEPHERD, K. W. 1985. The structure and genetic control of a new class of disulphide-linked proteins in wheat endosperm. Theor. Appl. Genet. 71:79.
SINGH, N. K., SHEPHERD, K. W., LANGRIDGE, P., GRUEN, L. C., and SKERRITT, J. H. 1987. Characterization of the "triplet proteins" (TRITICIN) from wheat endosperm. Pages 339–342 in: Proc. of the Third International Workshop on Gluten Proteins. R. Lasztity and F. Bekes, eds. World Scientific Publishing Co.: Singapore.
TAO, H. P., and KASARDA, D. D. 1989. Two-dimensional gel mapping and N-terminal sequencing of LMW-glutenin subunits. J. Exp. Bot. 40:1015.

[Received October 3, 1989. Revised September 11, 1990. Accepted September 12, 1990.]