Quantitative Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis of Total Proteins Extracted from Different Wheat Varieties: Effect of Protein Content[1]

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ABSTRACT

Total proteins were extracted from flour or seed samples differing in protein content for each of four wheat (*Triticum aestivum* L.) varieties Prodax, Wanser, Atlas 66, and Nugaines, with buffers containing sodium dodecyl sulfate and mercaptoethanol. The reduced proteins were fractionated according to molecular weight by two methods of sodium dodecyl sulfate-polyacrylamide gel electrophoresis, one giving much higher resolution than the other, and the results quantified by densitometry of the stained patterns. The patterns were considered in terms of five different molecular weight ranges that corresponded, to an extent, to the solubility fractions of wheat flour proteins. The proportion of low molecular weight albumins and globulins decreased significantly with increase in protein content for all four varieties, whereas the proportion of gliadins increased significantly for all but Atlas 66, both samples of which had relatively high protein content. The two methods yielded significantly different results for some of the molecular weight ranges considered; methods of staining and destaining gels that affected the amount of sodium dodecyl sulfate remaining complexed with the proteins also affected the results.

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Increasing the protein content of wheat grain improves its total lysine content but not the proportion of lysine (Johnson and Mattern 1977, Lawrence et al 1958), and usually improves baking quality (Finney and Barmore 1948, Pence et al 1954, Tipples et al 1977). Grain protein content can be increased by nitrogen fertilization, and consistent varietal responses indicate differences in the genetic control of this character (Johnson et al 1975, Konzak 1977). Studies of the amino acid composition of wheat flour with increase in protein content showed that glutamine (or glutamic acid) and proline increased in proportion, whereas lysine decreased in proportion (McDermott and Pace 1960). This change probably resulted from the decrease in the proportion of albumin and globulin proteins, which have high lysine contents (Pence et al 1964) relative to the storage proteins that make up most of the gluten protein. The corresponding solubility fractions change accordingly with increase in protein content (Bell and Simmonds 1963, Dexter and Matsuo 1977, Johnson and Mattern 1977, Pence et al 1954).

In a previous study (Fullington et al 1980), we extracted all proteins from flours of several different wheat varieties using solutions containing sodium dodecyl sulfate (SDS) and mercaptoethanol and fractionated the extracted proteins according to the molecular weights of their reduced, dissociated polypeptide chains by polyacrylamide gel electrophoresis (PAGE) in a buffer containing SDS. Peaks in densitometric scans of the gels corresponding to molecular weights near 12×10^3 (12k) were contributed mainly by low molecular weight albumins, which constitute a major part of the albumin solubility fraction, and to a lesser degree by low molecular weight globulins; peaks corresponding to molecular weights near 30k were contributed almost entirely by gliadins; and peaks corresponding to molecular weights near 100k were contributed by proteins that are characteristic of the glutenin fraction. Cole et al (1981) (Fig. 1) showed the relationship of the subunits to solubility fractions and demonstrated that glutenins contribute only a minor portion to A4 (lesss than one third).

This approach (Fullington et al 1980) to quantification of proteins thus bore some relationship to quantification by solubility fractionation but differed importantly in analyzing the intrinsic molecular weight distribution of reduced, dissociated polypeptide chains. It should not be affected by variables such as aggregation of protein subunits through disulfide bonding or secondary bonding forces, by complexation of proteins with lipids or other nonprotein constituents, and reactions or conformational changes of proteins brought about by high temperatures during grain development or storage, or other environmental factors.

We used the approach of Fullington et al (1980) to look for intrinsic differences in the molecular weight distribution of the reduced, dissociated polypeptide chains or subunits of the proteins from two different samples, differing in protein content, of each of four different wheat varieties. If such differences were found, we intended to consider their implications for wheat quality. In addition, we compared a different approach to SDS-PAGE that gives a much higher resolution (Cole et al 1981, Payne and Corfield 1979) than our previous method (Fullington et al 1980). We examined the effects of two different methods of staining and destaining the polyacrylamide gels that apparently affected the amount of SDS remaining complexed with the stained proteins in the gels to see what effects these procedures had on the quantitation of the gel patterns.

MATERIALS AND METHODS

Wheat Samples

Hard red spring wheat (*Triticum aestivum* L.) of the variety

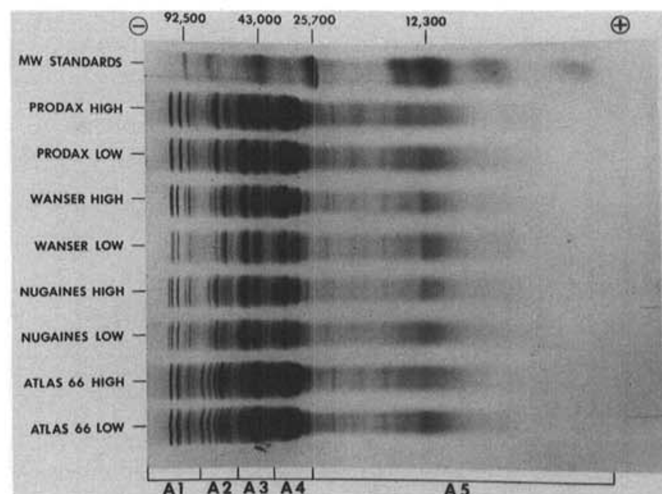

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of high- and low-protein accessions of four wheat varieties. From top to bottom, the patterns correspond to molecular weight standards, Prodax samples, Wanser samples, Nugaines samples, and Atlas 66 samples.

[1]Reference to a company or product name does not imply its approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may also be suitable.

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Prodax was supplied (H. A. R. Houlton and C. F. McGuire, Montana State University, Bozeman). The samples had been produced in a fertilizer trial in the Gilford-Hingham area of Hill County, MT. Flours milled from two of these samples had protein contents of 9.5 and 14.9% and moisture contents of 6.8 and 7.1%, respectively. The lower-protein sample had received no fertilizer; the other had been grown on plots treated with 110 kg/ha of nitrogen. A sample of Atlas 66, a soft white winter wheat with a flour protein content of 16.5% and moisture content of 12.6%, was used in our previous study (Fullington et al 1980). A higher-protein sample that milled into flour with 20.3% protein and a moisture content of 10.3% also was supplied (E. G. Heyne, Kansas State University, Manhattan).

Two samples each of hard red winter wheat variety Wanser and soft white winter wheat variety Nugaines were supplied (R. L. Warner and C. F. Konzak, Washington State University, Pullman). Those samples were also from fertilizer trials performed at Lind, WA. The lower-protein samples had received no fertilizer, whereas the higher-protein samples had received 200 kg/ha of nitrogen at planting time. Seeds were sorted according to size; those of the lower-protein samples and higher-protein samples of both varieties averaged 47 mg per seed. The samples were examined for quality (G. L. Rubenthaler, Western Wheat Quality Laboratory, U.S. Department of Agriculture, Pullman, WA), and mixing and baking test results were published (Cochran et al 1978). Because we had only small amounts of these samples, they were not milled into flour, but single seeds were analyzed and the results averaged. The lower-protein seeds of Wanser had about 8.8% protein and the higher ones about 15.0% protein. The lower-protein sample of Nugaines contained about 8.7% protein and the higher one about 13.6% protein. All the Wanser and Nugaines samples were about 9% moisture.

Samples of Atlas 66 and Prodax were milled in a Brabender Quadrumat Jr. mill; the endosperm fraction was used in this study. Protein contents were determined by multiplying Kjeldahl nitrogen by 5.7. Results are reported on a dry-weight basis.

High-Resolution SDS-PAGE

In this article, we refer to the procedure of Payne and Corfield (1979) as high-resolution SDS-PAGE. Extractions for electrophoresis were made by mixing 30 mg of flour or grinding seeds with 1.00 ml of 0.062*M* Tris-(hydroxymethyl)aminomethane-HCl (pH 6.8) that included 2% SDS (Sigma Chemical Co., 67% dodecanoic, 27% tetradecanoic, 5% hexadecanoic), 5% 2-mercaptoethanol, and 0.01% Pyronin Y (tracking dye). After grinding the flour or seed in a mortar and pestle for a few minutes, solid sucrose (about 100 mg) was added, and the mixture allowed to stand for about 1 hr, with occasional agitation. The mixture was then centrifuged, and sample amounts of 15–30 μl were taken for electrophoresis. Electrophoresis was performed in a vertical slab gel apparatus (Hoefer Scientific Inst. Co., San Francisco) for 15 hr at a current of 8 mA. Separation gels were 17 × 16.5 cm × 1.5 mm and contained 17.5% acrylamide and 0.08% N,N'methylene-*bis*-acrylamide (bis); stacking gels were 3.5 cm in height and contained 3% acrylamide with 0.043% bis. Although gels of lesser acrylamide concentration can produce a greater spread of the major bands of flour protein patterns, 17.5% was used to allow direct comparisons to previous work of our own and others. Each gel contained 10 sample slots. Gels were stained for 24 hr with 0.02% Coomassie brilliant blue R250 in water-methanol-acetic acid (80:20:7, v/v) containing 6% trichloroacetic acid. Gels were destained with 6% trichloroacetic acid for 48 hr, with one change of trichloroacetic acid solution at 24 hr. Gels were then soaked in water for 2 hr before being photographed on Polaroid type 55 film (black and white) with a Wratten G-15 filter (yellow-orange). Each pattern (corresponding to one gel slot) was then sliced from the gel, placed in a 20-cm cuvette, and scanned densitometrically with a Gilford apparatus (Fullington et al 1980). Sample loading was adjusted so that the absorption between baseline and peak maxima was 1.0 optical density unit or less; previous results indicated that response was linear in that range (Fullington et al 1980).

A computer program written for a Tektronix 4052 computer was used to transform data for quantitative comparisons. Patterns were normalized to equivalent mobilities, then to equivalent total areas, and each of the total areas was divided into five molecular weight ranges that defined subareas of the pattern. We refer to these subareas simply as areas A1 through A5; they correspond to the following molecular weight ranges: A1, 80k and higher; A2, 50–80k; A3, 38–50k; A4, 28–38k; and A5, 10–28k. These were defined in reference to the following standards (BRL, Bethesda, MD): phosphorylase b, 92.5k; BSA, 68k; ovalbumin, 43k; α-chymotrypsinogen, 25.7k; β-lactoglobulin, 18.4k; and cytochrome C, 12.3k. These molecular weight ranges differ somewhat from those used in a previous study (Fullington et al 1980) because the method of Payne and Corfield (1979) gives much higher resolution but also results in a somewhat different grouping of bands. The ranges we chose for high-resolution SDS-PAGE avoided divisions that fell within major peak areas.

Low-Resolution SDS-PAGE

We refer to the method used by Fullington et al (1980), which is similar to the method of Koenig et al (1970), as low-resolution SDS-PAGE. It gives much less resolution for wheat proteins than the method of Payne and Corfield (1979). Extractions were made,

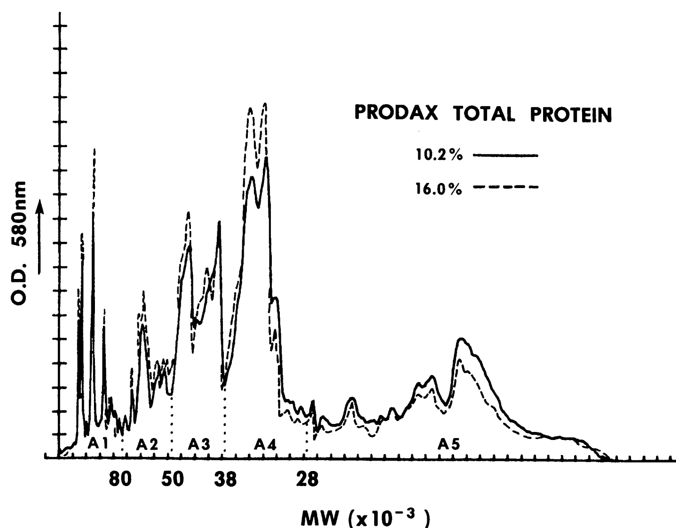

Fig. 2. Densitometric tracings of the electrophoretic patterns of Prodax high- (---) and low-protein (——) samples.

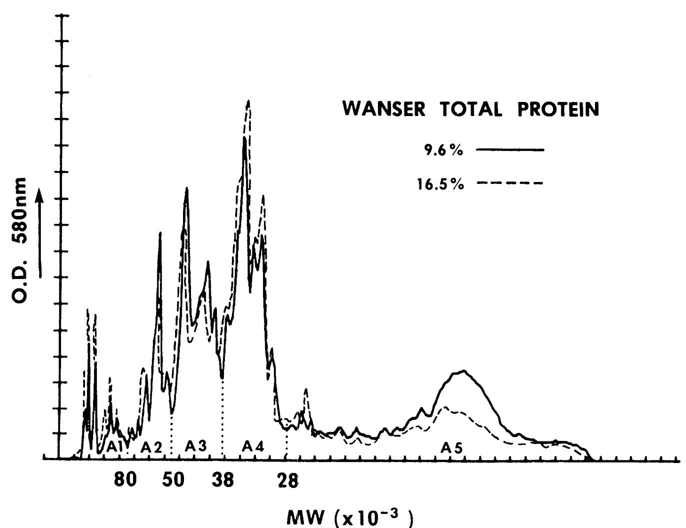

Fig. 3. Densitometric tracings of the electrophoretic patterns of Wanser high- (----) and low-protein (——) samples.

SDS-PAGE performed, and gels stained and destained as previously described (Fullington et al 1980).

Comparison of Staining and Destaining Methods

A high-resolution SDS-PAGE experiment was performed with 10 samples of a Prodax extract on a single gel, which was then divided into two pieces, each of which included the patterns of five samples, for comparison of two staining methods. One was identical to that described for our high-resolution approach (vide supra), and the other was indicated (Fairbanks et al 1971) as being effective in extracting SDS from the gels. This latter procedure was as follows: the gel was stained with 0.05% Coomassie brilliant blue R250 in isopropanol-acetic acid-water (25:10:65, v/v) overnight; then in 0.005% dye in isopropanol-acetic acid-water (10:10:80, v/v) for 8 hr; then in 0.0025% dye in 10% acetic acid overnight. Each set of samples was then analyzed with the densitometer and the results averaged.

Solubility Fractions

Solubility fractions were prepared from Prodax flour as previously described (Cole et al 1981). The three fractions used in this study were the albumin fraction, the gliadin fraction, and the glutenin (residue) fraction. Protein content of each fraction was determined by Kjeldahl nitrogen analysis.

Dye-Binding by Solubility Fractions

Carefully weighed portions of each solubility fraction were dissolved in Tris-(hydroxymethyl)aminomethane-borate-SDS-mercaptoethanol buffer, or extracted with this buffer in the case of the residue fraction, for dye-binding analysis by the Millipore filter technique described previously (Fullington et al 1980). In this method, dye is bound to the solubilized SDS-protein complexes, and the SDS-protein dye complexes are precipitated with trichloroacetic acid solutions and collected as spots on a Millipore filter. Each spot is cut from the filter, the dye is solubilized in a known volume, and the optical absorbance of the resulting dye solution is determined spectrophotometrically.

RESULTS AND DISCUSSION

High-Resolution SDS-PAGE

High-resolution SDS-PAGE patterns of the eight samples analyzed (two of each variety) are shown in Fig. 1. Approximately equal amounts of protein were applied to the gel so that differences

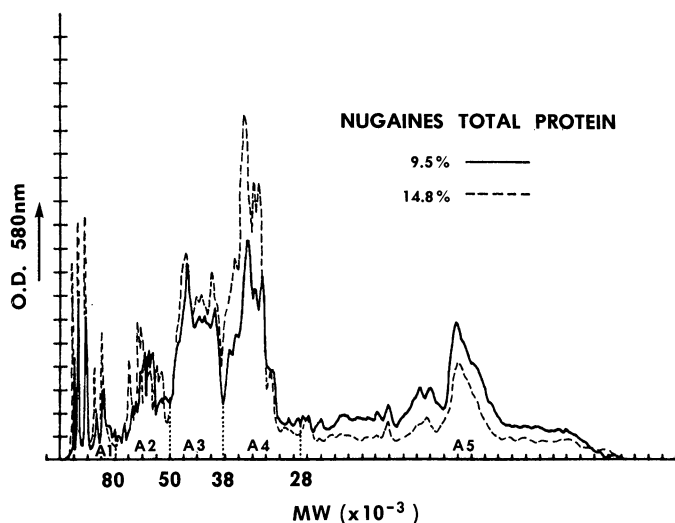

Fig. 4. Densitometric tracings of the electrophoretic patterns of Nugaines high- (----) and low-protein (——) samples.

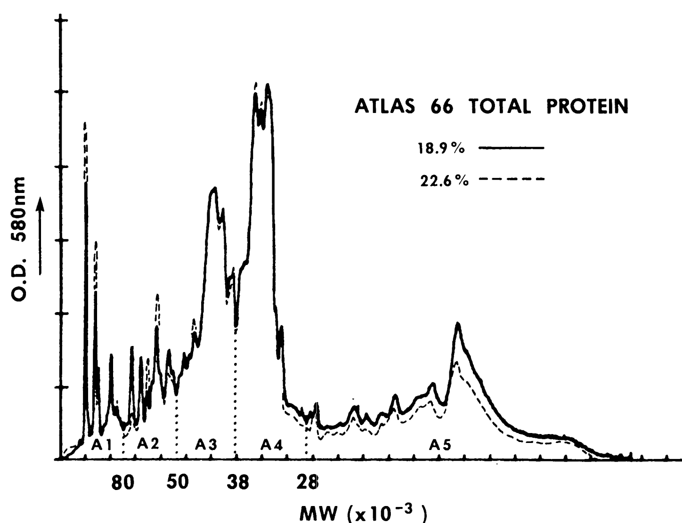

Fig. 5. Densitometric tracings of the electrophoretic patterns of Atlas 66 high- (----) and low-protein (——) samples.

TABLE I
Distribution of Proteins Among Five Molecular Weight Fractions
High-Resolution Subareas: Precent of Total Area and Confidence Limits[a]

Variety	Percent Protein (dry-weight basis)	A1	A2	A3	A4	A5
Prodax						
1	10.2	7.3 (±0.5)	8.9 (±0.6)	20.0 (±0.7)	29.7 (±1.3)	33.2 (±1.7)
2	16.0	6.8 (±0.6)	9.3 (±0.3)	21.1 (±0.8)	32.2 (±1.1)	30.5 (±2.2)
	5.8	−0.5	0.4	1.1	2.5	−2.7
Wanser						
1	9.6	4.8 (±1.1)	9.0 (±0.3)	20.1 (±0.9)	30.7 (±1.4)	35.4 (±0.6)
2	16.5	5.9 (±0.8)	9.2 (±0.5)	20.7 (±0.7)	34.9 (±0.5)	29.3 (±0.4)
	6.9	1.1	0.2	0.6	4.2	−6.1
Atlas						
1	18.9	5.9 (±0.7)	9.3 (±0.9)	22.9 (±0.8)	31.8 (±0.7)	30.2 (±1.5)
2	22.6	6.6 (±0.4)	9.9 (±0.5)	23.7 (±0.7)	31.8 (±0.9)	28.1 (±0.8)
	3.7	0.7	0.6	0.8	0.0	−2.1
Nugaines						
1	9.5	4.9 (±0.5)	8.1 (±0.5)	19.6 (±1.1)	26.2 (±1.5)	41.2 (±2.6)
2	14.8	6.2 (±0.7)	9.3 (±0.4)	20.0 (±1.1)	32.2 (±1.1)	32.3 (±1.8)
	5.3	1.3	1.2	0.4	6.0	−8.9

[a]95% level.

TABLE II
Distribution of Proteins Among Molecular Weight Fractions
Low Resolution Subareas: Percent of Total Area and Confidence Limits[a]

Variety	Percent Protein (dry-weight basis)	A1	A2	A3	A4	A5
Prodax						
1	10.2	14.7 (±2.6)	19.3 (±3.0)	15.3 (±2.1)	24.5 (±1.2)	26.2 (±1.2)
2	16.0	12.7 (±1.7)	18.6 (±0.5)	16.8 (±1.6)	28.4 (±2.3)	23.6 (±0.7)
	5.8	−3.0	−0.7	1.5	3.9	−2.6
Wanser						
1	9.6	7.9 (±2.7)	18.3 (±3.2)	12.0 (±2.4)	32.8 (±4.4)	28.8 (±1.0)
2	16.5	9.7 (±3.2)	19.8 (±2.3)	14.4 (±1.0)	34.0 (±5.0)	21.9 (±1.4)
	6.9	1.8	1.5	2.4	1.2	−6.9
Atlas 66						
1	18.9	8.0 (±3.3)	16.4 (±1.1)	16.2 (±2.8)	31.2 (±3.0)	28.3 (±1.8)
2	22.6	11.2 (±1.0)	19.3 (±1.6)	17.7 (±1.3)	29.0 (±1.0)	21.4 (±1.0)
	3.7	3.2	2.9	1.5	−2.2	−6.9

[a]95% level.

TABLE III
Relative Dye-Binding by Prodax Solubility Fractions

Fraction	Optical Density/50 μg Protein[a]
Albumin	0.45 (±0.02)
Gliadin	0.19 (±0.00)
Glutenin	0.32 (±0.01)

[a]95% confidence level.

TABLE IV
Distribution of Dye Among Molecular Weight Fractions as Affected by Different Staining Solutions

Staining Solvent	Prodax Subareas: Percent of Total Area and Confidence Limits[a]				
	A1	A2	A3	A4	A5
Trichloroactic acid	6.2 (±0.8)	8.2 (±0.5)	21.6 (±1.1)	32.6 (±0.8)	31.4 (±1.2)
i-PROH	6.1 (±1.3)	8.0 (±0.6)	18.9 (±0.9)	26.7 (±1.8)	40.3 (±1.9)

[a]95% level.

in the intensity of bands in the patterns of samples from a given variety would reflect differences resulting from the different protein contents. These samples were somewhat heavily loaded, and the only obvious differences that can be seen in the patterns were for proteins with molecular weights near 12k, which gave more intense bands for the lower-protein samples. Varietal differences can be noted in the patterns of the four varieties.

Typical densitometric scans of the electrophoretic patterns of the higher- and lower-protein samples of each variety are shown in Figs. 2–5. The percentage of total area for areas A1–A5 are given in Table I, along with confidence limits at the 95% level, based on 10 replicates for each sample. The densitometric scans were derived from single patterns and differ slightly in the proportions of areas A1–A5 from the areas given in Table I, which are averages. These values and those in Table II are not percentages of protein, but are percentages of total area and are dependent on the amount of dye bound.

We noted few disproportionate changes in particular peaks of the densitometric scans relative to the other peaks in any range of molecular weight corresponding to our arbitrary division. Such changes were noted occasionally in A2 and A3. We suspect that disproportionate changes result from peaks in the densitometric scans that are complex and include both albumin or globulin components and storage protein components such that when one component decreases, the other increases.

A5: Low Molecular Weight Albumins and Globulins

Area A5 was usually comparable to A4 or larger, but because we were using dye-binding by proteins to measure their proportions, the effects on differences in ability to bind dye must be considered. Area A5 corresponds to low molecular weight albumins and globulins (Cole et al 1981, Fullington et al 1980), which have greater dye-binding capability than gliadins and other storage proteins, mainly because of a greater number of basic groups per unit weight. Some indication of the extent of this difference can be obtained from the results given in Table III; dye-binding by the albumin solubility fraction was about twice as great as that of the gliadin solubility fraction and half again as much as the glutenin fraction. The glutenin fraction actually corresponded to residue proteins remaining after albumins and globulins had been extracted with salt solution, and gliadins with alcohol-water solution. These residue proteins would correspond mainly to storage proteins, but significant amounts of albumin and globulin proteins should be present that were not extracted under the conditions used. Membrane proteins should also be present in the fraction, and these proteins might have dye-binding capabilities similar to those of albumins and globulins. Membrane proteins have been estimated to make up 3–6% of the proteins of wheat endosperm (Kasarda et al 1976) and should contribute about twice that much to our residue fraction. The greater dye-binding we observed for our glutenin fraction may result from the presence of such nonstorage proteins in the fraction.

The results of Table III were obtained for SDS-protein complexes in solution and should approximate the dye-binding characteristics of SDS-protein complexes in our SDS gels. Detailed information about the dye-binding characteristics of the separate components in the mixture is not available; if we assume that the results of Table III are applicable, then areas corresponding to A5 in Table I should be reduced by about one-half and the other areas increased proportionately (we shall ignore possible contributions from nonstorage proteins to A1, A2, and A3 for the purposes of this discussion). For the high-protein sample of Prodax, the areas A1 through A5 would become, respectively, 8.3, 11.3, 25.7, 39.3, and 15.3% of the total area. The value of 15.3% for A5 agrees reasonably well with albumin-globulin contents of 13.6–21.4% estimated by Pence et al (1954) for wheat samples ranging from 14.2 to 7.0% protein.

In an earlier paper (Fullington et al 1980), we had assumed that albumins and globulins corresponding to A5 would bind about three times as much dye as gliadins. We did not consider possible effects of complexed SDS on dye-binding by the proteins. When we compared two different methods of staining and destaining the polyacrylamide gels after SDS-PAGE, the method that seemed to be more effective in removing SDS from the gels (Fairbanks et al 1971) resulted in A5 making up a greater proportion of the total area when compared with the other method as shown in Table IV for SDS-PAGE of Prodax extracts; A5 also increased absolutely in area. We noted also that the method of Fairbanks et al (1971) produced color differences among some of the bands in the SDS-PAGE patterns, whereas our normal method of staining and

destaining gels produced a uniform blue color for all bands in the patterns. This may be a further indication of fairly complete removal of SDS from the proteins in the gels, as we noted similar color differences among albumin and globulin components separated by PAGE methods that do not include SDS (Caldwell and Kasarda 1978). The SDS-protein complexes that probably remained intact in the trichloroacetic acid method used in this study seemed to diminish differences in the extent and nature of dye-binding characteristics of various wheat endosperm proteins. Although it is difficult to rule out the possibility of a slight solubilization of gliadin components corresponding to A3 and A4 in the isopropanol-acetic acid system before enough dye has bound to the protein to form an insoluble complex, some preliminary studies of this possibility do not support it.

The areas corresponding to A5 in Table I decreased significantly for three of the varieties with increase in protein content. These results are in good agreement with those of solubility fractionation studies (Bell and Simmonds 1963, Dexter and Matsuo 1977, Johnson and Mattern 1977, Pence et al 1954), which indicate that albumins and globulins decrease in proportion as total protein percentage increases, although Tanaka and Bushuk (1972) found such a decrease for only one of two varieties studied. Although the portion of salt-soluble proteins (albumins and globulins) decreases with increase in protein percentage, their absolute amount increases slightly (Bell and Simmonds 1963, Pence et al 1954). In contrast, Soliman et al (1980) found no decrease in the proportion of soluble proteins when total protein content of wheat was raised by chromosome addition.

The areas (from Table I) corresponding to A5 for the different varietal samples are plotted as a function of protein percentage in Fig. 6. We have joined the points corresponding to the lower and higher protein samples of each variety by dashed lines, but this is not meant to imply that the change with protein content is linear, although it may be. The relationship between the area of A5 and protein content as shown in Fig. 6 might be interpreted as indicating a linear relationship between the amount of low molecular weight albumins (and low molecular weight globulins) and protein content that was independent of variety when the results for Wanser, Prodax, and Atlas 66 are considered. The different areas at equivalent protein content for Nugaines, which resulted in a different slope for the connecting line, does not fit with this possibility. Our study did not determine whether the difference for Nugaines resulted from an intrinsic varietal difference between it and the other three varieties, or from some environmental effect on the amounts (proportions) of the low molecular weight albumins and globulins in this particular lot of grain.

A4-Gliadins

Area A4 corresponds largely to gliadin proteins (Cole et al 1981, Fullington et al 1980), although gliadins (ω-gliadins) also contribute to A2 and A3. The results in Table I combined with indications that A5 should be reduced by one-half and other fractions increased by the distributed difference because of differences in dye-binding indicate that the gliadins of A4 compose up to about 40% of the total protein. Gliadins are clearly the largest protein fraction of wheat endosperm. The proportions of gliadins as indicated by A4 increased significantly with increased protein content for three of the varieties studied, but not for Atlas 66. The protein content of both Atlas 66 samples was above 15%, which is about the level of protein above which the percentage of lysine in grain ceases to decrease with increased protein (Lawrence et al 1958, Vogel et al 1973). This decrease in lysine probably results from a decrease in the proportion of albumins and globulins relative to storage proteins. At higher levels of protein, the leveling-off of the decrease in lysine may indicate that all fractions increase proportionately. We do not consider the barely significant decrease in A5 we noted for Atlas 66 to conflict seriously with this possibility.

Johnson and Mattern (1977) found that alcohol-water-soluble proteins (largely gliadins) increase with protein content. Dexter and Matsuo (1977) found a significant increase in the proportion of alcohol-water-soluble protein for one durum wheat variety, but not for another, when total protein increased. Tanaka and Bushuk (1972) found no significant increase in alcohol-water-soluble proteins, however, for two spring wheat varieties. Our results support an increase in the proportion of gliadins with increase in protein content: at least for protein levels less than about 15%.

A1, A2, and A3—Glutenins

We found a small increase in the proportions of proteins corresponding to areas A1–A3, with increase in protein content for all four varieties except for A1 of Prodax, but the increases were not always significant. The proteins of areas A1–A3 comprise mainly glutenin components (Bietz and Wall 1972, Cole et al 1981, Huebner et al 1974), although gliadins contribute somewhat less than half of area A3 (based on Fig. 8 of Kasarda et al 1976 and Fig. 9a of Cole et al 1981), and ω-gliadins should contribute slightly to A2 (based on molecular weights of Charbonnier 1974). In addition, we would expect a small contribution from membrane proteins and other nonstorage proteins to these areas, but it is difficult to make an estimate of the size of this contribution. It seems reasonable to us to expect all storage proteins, including those that contribute to A1–A3, to increase in proportion with increase in protein content, as do gliadins, but our data are not sufficiently precise to show this. The situation may be complicated by a decrease in nonstorage proteins concomitant with the increase in storage proteins both contributing to A1–A3.

Relationship to Baking Quality

Because the areas A1–A3 correspond predominantly to components usually found in glutenin preparations, whereas A4 corresponds predominantly to gliadin proteins, we examined the possibility that the ratio (A1 + A2 + A3)/A4 might correlate with baking quality. This ratio decreased with increasing protein content for Prodax (1.22–1.15), Wanser (1.11–1.03), and Nugaines (1.24–1.10), and increased for Atlas 66 (1.20–1.26), but these changes were of borderline significance. The higher-protein sample of Wanser had good baking quality (Cochran et al 1978), and the higher-protein sample of Prodax would be expected to have good baking quality, whereas Atlas 66 and Nugaines would be expected

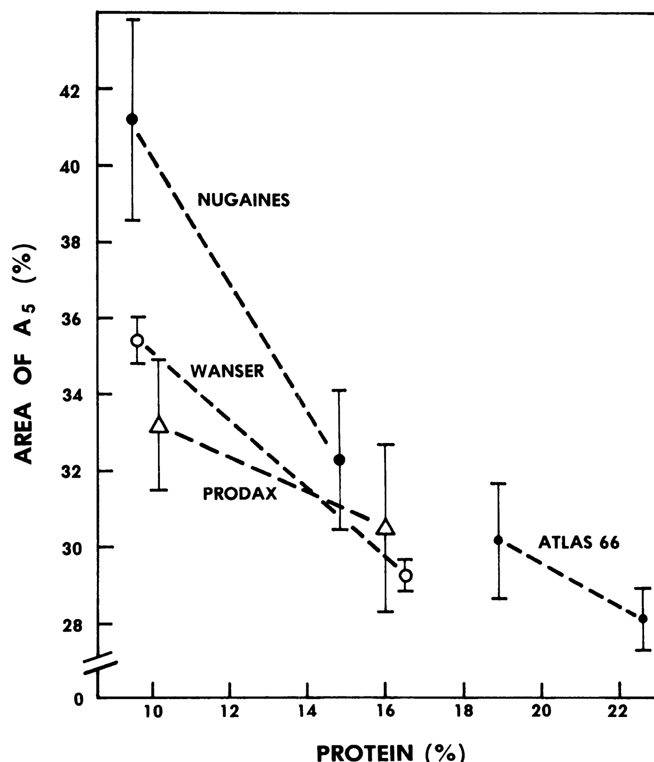

Fig. 6. Percent of total protein found in A5 for each sample plotted against the flour protein content. Vertical lines show the confidence limits at the 95% level for each point.

to have poor baking quality. Our results do not support the possibility that the glutenin/gliadin ratio, as measured by our ratio of (A1 + A2 + A3)/A4, correlates with baking quality. Our approach measures the proportions of dissociated polypeptide chains; aggregates of glutenin proteins that exist in the native state (unreduced) may be more important in determining varietal differences in baking quality than the proportions of reduced-dissociated polypeptide units in the various molecular weight ranges.

Low-Resolution SDS-PAGE

We performed SDS-PAGE by the low-resolution technique (Fullington et al 1980) for comparison with the high-resolution technique. Patterns are not shown, but the results of analysis of densitometric scans into areas A1–A5 are given in Table II, along with confidence limits based on five replicates. The areas of Table II represent slightly different breakdowns of the molecular weight ranges used to define areas A1–A5 than were used for the high-resolution patterns: A1, 63k and higher; A2, 48–63k; A3, 40–48k; A4, 31–40k; and A5, 8–31k. This was done because the bands grouped somewhat differently, and mobilities changed slightly relative to the standards for the two methods. If the same molecular weight ranges were used for both methods, a few of the dividing lines would intersect major peaks in the densitometric scans of one of the methods.

The results shown in Table II are less precise than those of Table I, partly because of the fewer replications involved, but also because of problems with streaking in the low-resolution gels. Only the decrease in A5 was significant for all three varieties (Prodax, Wanser, Atlas 66) compared, whereas the increase in A4 was significant only for Prodax. In general, the proportions of proteins in the various fractions were similar to those obtained by use of the high-resolution method (Table I), but there were some puzzling differences. For example, A2 of the low-resolution method corresponds to a smaller range of molecular weight yet constitutes almost twice the proportion of the total area as A2 of the high-resolution gels. Also, the area of A5 was considerably smaller for the high-protein sample of Atlas 66 in the low-resolution method than in the high-resolution method. We cannot readily explain these differences.

Comparison of the Two Methods

Both of the procedures we compared for SDS-PAGE have been used considerably by cereal chemists and others for the study of proteins. Our high-resolution method is a variation of the Laemmli (1970) method adapted by Payne and Corfield (1979) for cereal proteins, and our low-resolution method is similar to that of Koenig et al (1970), which we have used for the study of cereal proteins (Bietz and Wall 1972, Fullington et al 1980, Orth and Bushuk 1973). We consider the high-resolution method much more suitable for the study of wheat proteins—especially for quantitation of patterns by densitometry. In addition to having much higher resolution, there is much less streaking in the gels, which results in improved baseline determination.

One method resulted in a slightly different grouping of bands in the patterns compared with the other. We suspect that this indicates slight, nonuniform changes in the mobilities of the various proteins relative to one another when the procedure is changed. This might be demonstrated by comparing purified components. We noted that A-gliadin corresponds to a lower molecular weight in the high-resolution method than in the low-resolution method when compared to the same set of standards (Cole et al 1981).

We did not systematically study the dependence of results from SDS-PAGE on reagents, buffers, and polymerization conditions, but we believe, based on eight years of experience with SDS-PAGE, that results can be significantly affected by these variables. We have demonstrated that two different methods can yield significantly different results and that the approach to staining and destaining of gels can also significantly affect results. Comparisons, especially those involving quantitative results, will be generally meaningful only when all procedures and reagents are the same.

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