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[1]

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ABSTRACT

Three hard red spring wheat varieties, grown under similar environmental conditions, were fractionated by three protein solubility procedures and compared for quantitative protein recovery, subunit composition, and molecular weight and size distribution. Variations in the isolation conditions and types of solvent used had a major effect on the amount of protein extracted in each fraction. Polyacrylamide gel electrophoresis of the various protein fractions isolated by the different solubility procedures showed distinct differences in subunit composition for the same class of protein. Contamination of gliadin with glutenin was found in two of the three isolation procedures. The acetic acid soluble, acetic acid insoluble, and lactic acid soluble fractions from the different procedures, although referred to as glutenin, were different in composition. Some residue fractions (those containing starch) contained substantial amounts of protein.

The proteins of wheat flour have long been associated with the baking performance of flour and have been studied intensively in this regard for many decades by cereal chemists (Pence 1962). Significant advances in the knowledge of these components have occurred in the past 15 years, but the advent of powerful new research tools within only seven to eight years has accelerated progress considerably (Kasarda et al 1976).

Previous studies by Preston and Tipples (1980) focused on the solubility and molecular weight distribution of the gluten proteins, which were suggested to be major factors in determining gluten quality. Hoseney et al (1969a,b) showed that the lactic acid soluble proteins or gliadin were positively correlated with loaf volume, whereas the glutenin protein was responsible for the mixing properties of a flour. On the other hand, Orth et al (1972) used a modified

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Osborne (1907) procedure and studied the solubility distribution of 26 bread wheats varying in breadmaking quality. They showed that remix loaf volume of the flours of the 26 varieties was positively correlated with residue protein, which they termed insoluble glutenin, and negatively correlated with acetic acid soluble glutenin. Another researcher, MacRitchie (1978), carried out reconstitution breadmaking studies and reported that acetic acid insoluble protein or glutenin was responsible for loaf volume potential between poor and good bread wheat varieties.

The above three groups of researchers have indicated that three different wheat protein fractions were responsible for loaf volume. The fractionation procedures used by these researchers were quite different. The obvious question we raised was whether the protein fractions (albumins, globulins, gliadins, glutenins, and residue proteins) from the various procedures were of the same composition.

The objectives of the present study were to isolate and quantitate the protein fractions from the procedures of Chen and Bushuk (1970), MacRitchie (1978), and Hoseney et al (1969a,b), and to use polyacrylamide gel electrophoresis (PAGE) and sodium dodecyl sulfate (SDS)-PAGE to analyze these fractions for compositional differences in an attempt to address the controversy concerning functional properties of specific wheat protein fractions.

MATERIALS AND METHODS

Flour

Three hard red spring (HRS) wheat varieties (Prodax, Len, and Coteau) of poor, good, and medium breadmaking quality (loaf volume), respectively, were grown in Dickinson, ND, and were milled into straight-grade flour (73–75% extraction) on a Miag pilot mill (Miag, Braunschweig, Switzerland). The protein contents (14.0% mb) of the flours from Prodax, Len, and Coteau were 12.4, 13.2, and 14.5%, respectively.

Analytical Procedures

Moisture and protein were determined by AACC approved methods (1983). Protein content was obtained by multiplying the nitrogen content by the conversion factor 5.7 (Tkachuk 1969).

PAGE

PAGE was carried out at pH 3.1 in sodium lactate buffer according to the method of Khan et al (1985), using the E-C470AX vertical slab gel electrophoresis apparatus (E-C Apparatus Corporation, St. Petersburg, FL).

SDS-PAGE

SDS-PAGE was carried out at pH 8.3 according to a modified method of Laemmli (1970), using LKB 2001 vertical slab gel electrophoresis apparatus (LKB, Broma, Sweden, manufactured by Hoefer Scientific, San Francisco, CA).

Fractionation of Flour into Protein and Starch (Residue) Fractions

Flours were fractionated into solubility protein fractions and starch (residue) by the procedures described by Chen and Bushuk (1970, Fig. 1), MacRitchie (1978, Fig. 2), and Hoseney et al (1969a,b; Fig. 3). All fractions were freeze-dried except for starch fractions from MacRitchie's procedure in which the starch is air-dried. The fractions were then ground with a mortar and pestle in an ice bath, sifted through a sieve (U.S. no. 70), and stored at −40°C.

RESULTS AND DISCUSSION

Fractionation by Chen and Bushuk's Procedure

The quantitative solubility protein data of the three HRS wheat varieties Prodax, Len, and Coteau of poor, good, and medium breadmaking quality, respectively, fractionated by Chen and Bushuk's (1970) procedure are shown in Table I. The protein distribution for each solubility class and standard deviation of recovered fractions are also reported in Table I. Although the three varieties belong to the same HRS wheat class, they did not yield the same quantity of protein in the respective solubility classes. The poor breadmaking variety Prodax, even though low in protein content, yielded higher quantities of albumin (12.5%), globulin

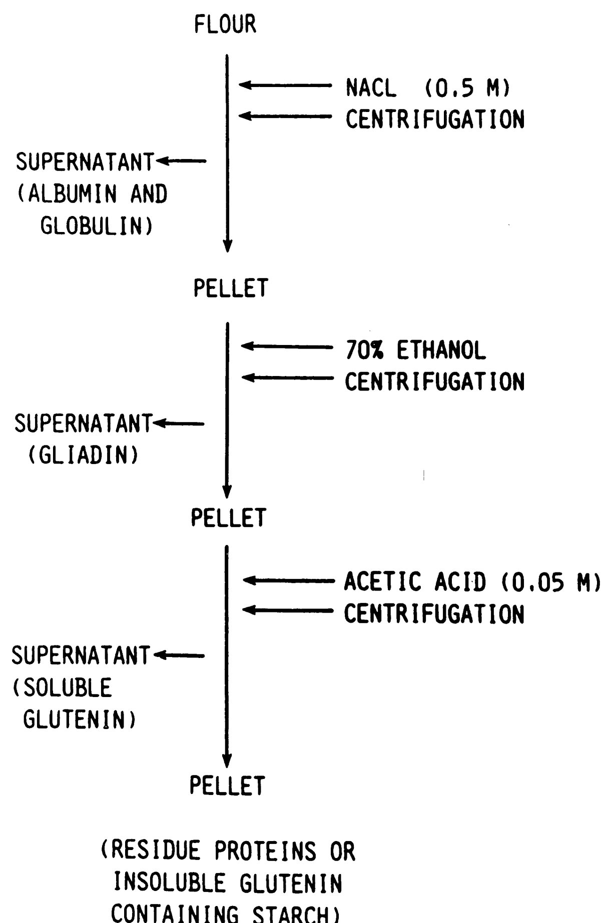

Fig. 1. Schematic of the protein fractionation procedure of Chen and Bushuk (1970).

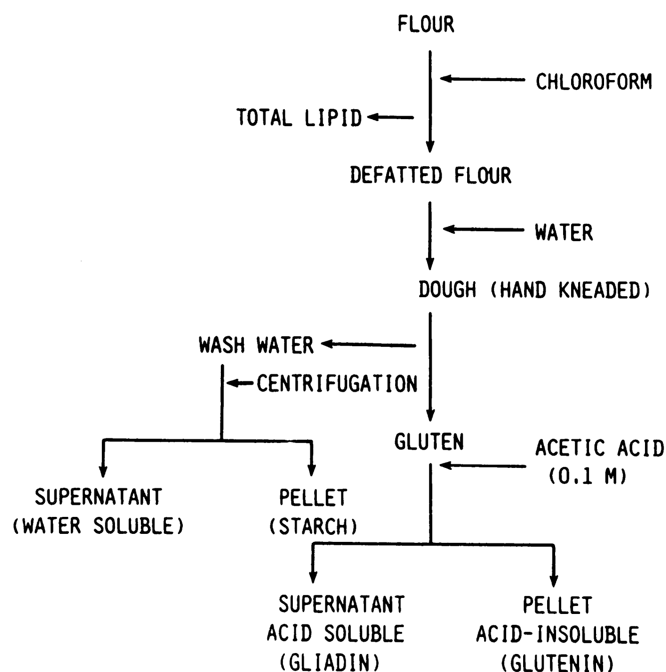

Fig. 2. Schematic of the protein fractionation procedure of MacRitchie (1978).

(4.7%), and gliadin (33.8%) than Len and Coteau, which are of better breadmaking quality. The amount of residue protein was higher for Len than for Prodax. Coteau showed a solubility distribution that was intermediate between Len and Prodax. These results are in good agreement with those of Orth et al (1972), who showed that poor breadmaking varieties such as Prodax contain more gliadin and less residue proteins than good breadmaking varieties such as Len. The residue fraction includes the weight of insoluble glutenin and starch.

Fractionation by MacRitchie's Procedure

The varieties Prodax, Len, and Coteau were fractionated by MacRitchie's (1978) procedure. Protein solubility distribution and standard deviation of recovered fractions are shown in Table II. The protein recovery of water-soluble, gliadin, glutenin, and starch fractions from Prodax and Len were similar to each other except for Coteau, of medium quality, which showed lower values for these fractions and a slightly higher value for gliadin (79.1%). In Chen and Bushuk's (1970) fractionation procedure, gliadin (acetic acid soluble) contributed the highest percent of total flour protein of the soluble fractions. MacRitchie separated the gluten proteins, dissolved in 0.1 M acetic acid, into 60% gliadin and 40% glutenin. However, in our study the gluten proteins were separated into 92–94% gliadin and 6–8% glutenin. This wide difference between the two studies may be mainly due to the difference in the type of blender used to solubilize the gluten proteins. MacRitchie used a Janke and Kunkel Ultraturrax mixer at high speed, whereas we used an overhead electric stirrer (model 106, Talboys Instrument Corp., Emerson, NJ).

Fractionation by the Procedure of Hoseney et al

Likewise, Prodax, Len, and Coteau varieties were fractionated by the procedure of Hoseney et al (1969a,b). The solubility protein recovery data and the standard deviations of recovered fractions are shown in Table III. The results show that the poor breadmaking variety Prodax yielded higher quantities of water solubles (10.8%), insoluble (12.7%), and starch (10.9%) fractions than Len. However, glutenin (lactic acid insoluble) was higher in Len (15.2%) and Coteau (15.0%) and least in Prodax (10.9%). Similar to MacRitchie's procedure, the gliadin fraction from the Hoseney et al (1969a,b) procedure contributed the highest amount of total flour protein (48.0, 47.6, and 50.6% for Prodax, Len, and Coteau, respectively) as shown in Table III. Prodax gave the highest protein recovery followed by Coteau and then Len. Protein content of fractions from Coteau were close to those of Len, except for the gliadin (50.6%) and starch (4.0%) fractions, which were

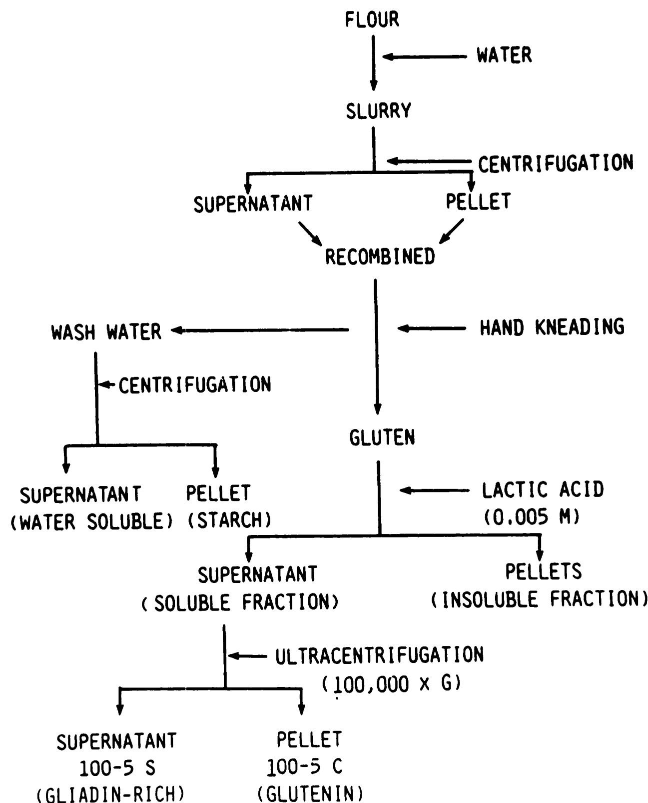

Fig. 3. Schematic of the protein fractionation procedure of Hoseney et al (1969a,b).

TABLE I
Quantitation[a] of Protein Fractions of Three Hard Red Spring Bread Wheats Isolated by Chen and Bushuk's (1970) Procedure

Protein Fraction	Wheat Varieties		
	Prodax	Len	Coteau
Water-soluble fraction (albumin)			
Weight, g	2.9	2.7	2.8
Protein content,[b] % db	53.4	52.3	52.7
Percent of total protein	12.5	10.7	10.2
SD of fraction recovery	±0.12	±0.10	±0.08
Salt-soluble fraction (globulin)			
Weight, g	0.8	0.8	0.8
Protein content, % db	72.7	63.1	70.0
Percent of total protein	4.7	3.8	3.9
SD of fraction recovery	±0.10	±0.04	±0.05
70% Ethanol-soluble fraction (gliadin)			
Weight, g	5.4	5.2	5.6
Protein content, % db	77.6	72.8	74.8
Percent of total protein	33.8	28.7	28.9
SD of fraction recovery	±0.35	±0.54	±0.10
Acetic acid soluble fraction (soluble glutenin)			
Weight, g	1.9	2.4	2.1
Protein content, % db	75.1	75.3	80.3
Percent of total protein	11.5	13.7	11.6
SD of fraction recovery	±0.30	±0.10	±0.11
Residue (insoluble glutenin)			
Weight, g	74.2	74.7	73.7
Protein content, % db	6.25	7.3	7.8
Percent of total protein	37.4	41.3	39.6
SD of fraction recovery	±0.76	±0.30	±0.87
Protein recovery, %	99.8	98.2	94.2

[a] On 100 g flour basis.
[b] N × 5.7 (Tkachuk 1969); db = dry basis.

TABLE II
Quantitation[a] of Protein Fractions of Three Hard Red Spring Bread Wheats Isolated by MacRitchie's (1978) Procedure

Protein Fraction	Wheat Varieties		
	Prodax	Len	Coteau
Water-soluble fraction (albumin and globulin)			
Weight, g	5.1	5.1	4.3
Protein content,[b] % db	22.4	23.4	25.5
Percent of total protein	9.2	9.0	7.6
SD of fraction recovery	±0.63	±0.21	±0.26
Acetic acid soluble fraction (gliadin)			
Weight, g	11.2	12.3	13.4
Protein content, % db	84.4	84.2	85.6
Percent of total protein	76.2	78.5	79.1
SD of fraction recovery	±2.32	±0.15	±1.7
Acetic acid insoluble fraction (glutenin)			
Weight, g	1.9	3.6	2.9
Protein content, % db	16.7	10.9	11.5
Percent of total protein	2.6	3.0	2.3
SD of fraction recovery	±1.05	±1.16	±0.57
Starch			
Weight, g	78.7	75.3	73.7
Protein content, % db	0.5	0.6	0.5
Percent of total protein	3.2	3.4	2.5
SD of fraction recovery	±1.40	±1.13	±3.26
Protein recovery, %	91.2	94.0	91.5

[a] On 100 g flour basis.
[b] N × 5.7 (Tkachuk 1969); db = dry basis.

highest and lowest, respectively.

Tables I–III clearly indicate that all three procedures gave different protein recovery for the same class of protein for the same variety. This is obviously because the fractionation steps were very different in each of the three procedures.

Comparison of Protein Fractions from the Three Isolation Procedures

Total protein recovery of the varieties Prodax (poor), Len (good), and Coteau (medium) from each of the three fractionation procedures are summarized in Table IV.

Water-Soluble Fraction

The Chen and Bushuk (1970) procedure gave higher quantities of water-soluble fractions for the three varieties than the procedure of MacRitchie or Hoseney et al. In Chen and Bushuk's procedure, flour was extracted directly with various solvents, whereas in the other two procedures gluten was first formed and the wash water was centrifuged to recover water solubles. It is possible that during gluten formation, albumins and globulins were bound in the gluten complex, resulting in the recovery of lower amounts of these proteins. It is interesting to note also that a larger weight of water-soluble fraction (5.1 g, Tables II and III) was obtained in the procedures of MacRitchie and Hoseney et al than in Chen and Bushuk's procedure (2.9 g, Table I), yet the protein content was higher in the latter procedure (compare values in Tables I, II, and III).

TABLE III
Quantitation[a] of Protein Fractions of Three Hard Red Spring Bread Wheats Isolated by the Procedure of Hoseney et al (1969a,b)

Protein Fraction	Wheat Varieties		
	Prodax	Len	Coteau
Water-soluble fraction (albumin and globulin)			
Weight, g	5.1	4.2	4.5
Protein content,[b] % db	26.3	25.6	28.1
Percent of total protein	10.8	8.1	8.7
SD of fraction recovery	±0.26	±0.62	±0.27
Insoluble fraction			
Weight, g	2.9	3.5	3.0
Protein content, % db	54.5	36.8	51.7
Percent of total protein	12.7	9.7	10.6
SD of fraction recovery	±0.67	±1.31	±0.47
Lactic acid soluble fraction (100-5S)			
Weight, g	7.1	7.7	8.8
Protein content, % db	83.8	81.7	83.5
Percent of total protein	48.0	47.6	50.6
SD of fraction recovery	±1.09	±1.02	±0.45
Lactic acid insoluble fraction (100-5C)			
Weight, g	2.0	2.9	3.0
Protein content, % db	67.9	69.3	72.6
Percent of total protein	10.9	15.2	15.0
SD of fraction recovery	±0.28	±0.74	±0.32
Starch			
Weight, g	67.5	66.6	65.2
Protein content, % db	2.0	1.3	0.9
Percent of total protein	10.9	6.6	4.0
SD of fraction recovery	±2.34	±0.96	±1.01
Protein recovery, %	93.5	87.2	88.9

[a] On 100 g flour basis.
[b] N × 5.7 (Tkachuk 1969); db = dry basis.

Gliadin Fraction

For all three varieties, MacRitchie's procedure contained the highest amount of gliadin followed by those of Hoseney et al, then Chen and Bushuk. MacRitchie's higher gliadin (acetic acid soluble) recovery over Hoseney et al could be due to the following reasons: 1) acetic acid probably solubilizes gluten proteins better than lactic acid (Hoseney et al 1969a,b); 2) acetic acid (0.1*M*) used by MacRitchie (1978) was at a concentration 20 times higher than the lactic acid (0.005*N*) used by Hoseney et al (1969a,b); 3) lipid-protein association, because in MacRitchie's procedure flour was defatted before fractionation; and 4) as already mentioned, the use of a different stirrer that provided more vigorous stirring. The lower amount of gliadin in Chen and Bushuk's procedure was perhaps due to the use of 70% aqueous ethanol solvent, which solubilized most of the gliadins but very little glutenin. On the other hand, the acid solvents used in the procedures of MacRitchie and Hoseney et al solubilized most of the gliadin and also a portion of the glutenin proteins, as will be shown in our gel electrophoresis study.

Glutenin Fraction

The procedures of Chen and Bushuk and Hoseney et al showed similar amounts of glutenin, except for Coteau, which showed slightly higher differences (probably a varietal difference) (Table IV). However, MacRitchie's procedure showed considerably less glutenin. This is due to the fact that a large portion of glutenin was solubilized with the gliadin fraction (acid soluble protein) in MacRitchie's procedure, thereby resulting in less glutenin recovery than in the other procedures. MacRitchie's acid-soluble fraction from our study would be more similar to the acid-soluble fraction of Preston and Tipples (1980), who solubilized about 80% of the gluten proteins in 0.05*M* acetic acid.

It is interesting to note that even though the weight of material recovered in the glutenin fractions in each of the three procedures for the respective varieties was very similar, the protein content in the respective materials was quite different (Tables I–III). This

TABLE IV
Quantitation of Protein Fractions of Bread Wheats from Various Isolation Procedures (percent of total protein[a])

Isolation Procedures/ Varieties	H_2O Soluble	Gliadin	Glutenin	Residue and Starch	Insoluble Fraction	Percent Recovery
Chen and Bushuk[b]						
Prodax	17.2	33.8	11.5	37.4	···	99.9
Len	14.5	28.7	13.7	41.3	···	98.2
Coteau	14.1	28.9	11.6	39.6	···	94.2
MacRitchie[c]						
Prodax	9.2	76.2	2.6	3.2	···	91.2
Len	9.0	78.5	3.0	3.4	···	93.9
Coteau	7.6	79.1	2.3	2.5	···	91.5
Hoseney et al[d]						
Prodax	10.8	48.0	10.9	10.9	12.7	93.3
Len	8.1	47.6	15.2	6.6	9.7	87.2
Coteau	8.7	50.6	15.0	4.0	10.6	88.9

[a] Standard deviation of recovered fractions provided in Tables I, II, and III.
[b] 1970.
[c] 1978.
[d] 1969a,b.

difference was especially striking for MacRitchie's procedure, which contained the lowest protein content for glutenin, even though the weight of the fraction was quite similar to those of the other two procedures.

Residue and Insoluble Fractions

The residue fraction of Chen and Bushuk contained large amounts of acetic acid insoluble proteins or insoluble glutenin together with starch. On the other hand, the starch fractions of MacRitchie and Hoseney et al contained considerably less protein, because in these procedures starches were removed at the beginning of the fractionation procedure by washing out gluten with water.

A lactic acid insoluble fraction, which Hoseney et al (1969b) called "insoluble fraction," consisted of 9–12% of the total protein. This unique fraction was reported only by Hoseney et al (1969b).

The quantitative protein fractionation data clearly showed compositional differences among the various classes of proteins. These differences are obviously caused by the differences in the solubility procedures (Figs. 1–3) employed to isolate the various protein fractions.

PAGE Studies

Various fractions were further analyzed by PAGE and SDS-PAGE for compositional differences at the molecular level. PAGE patterns of water solubles and gliadins of the varieties Prodax, Len, and Coteau isolated by the three previously mentioned fractionation procedures are shown in Figures 4 and 5. In order to compare qualitative and quantitative differences, the same amount of protein was applied for each sample on each gel. Patterns 1, 2, and 3 are water solubles (Fig. 4) and gliadins (Fig. 5) from Chen and Bushuk, MacRitchie, and Hoseney et al procedures, respectively, of Prodax (A), Len (B), and Coteau (C). Patterns in Figure 4 and 5 clearly show several qualitative and quantitative compositional differences in the three procedures for the same wheat variety.

In the pattern of water solubles (Fig. 4) there seems to be material remaining at the origin in each slot. The material is most likely soluble starch, gliadin, small amounts of glutenin, and probably glycoprotein described by Kundig et al (1961a,b), Hoseney et al (1969a), and Wrench (1965). The quantitative compositional differences are indicated by differences in band intensity such as bands 7, 15, 17, 18, 23, 24, and 25. Qualitative differences are evident by the presence or absence of bands such as bands 5, 8, 11, 12, and 24. Major differences were seen in the region of lowest mobility, that is, from the origin (point of sample application) to band 5.

Similarly in the patterns of gliadin (Fig. 5) large amounts of material, most likely glutenin (Hoseney et al 1969b), did not enter the polyacrylamide gel but remained at the origin of sample application, especially evident in the samples from MacRitchie's procedure. The quantitative compositional differences, indicated by differences in staining intensity, were seen in the ω- and γ-gliadin regions. MacRitchie's gliadin (pattern 2, Fig. 5) showed much streaking, perhaps caused by the large amounts of material (most likely glutenin) at the origin.

SDS-PAGE Studies

The water-soluble, gliadin, glutenin, and residue fractions were analyzed for size and molecular weight distribution by SDS-PAGE. SDS-PAGE of the water-soluble proteins (Fig. 6) shows many qualitative and quantitative differences in banding patterns among the three fractionation procedures for the same variety. For example, bands 3 and 12 are present in all varieties in Chen and Bushuk's procedure but absent in the other two procedures. Quantitative differences are especially evident in the 25,000–12,000 molecular weight (mol wt) region (bands 14 to 18). Bands 14 to 16 are more intensely stained in Chen and Bushuk's procedure, whereas bands 17 and 18 are more intensely stained in the procedures of MacRitchie and Hoseney et al for all varieties.

In the SDS-PAGE electrophoregram of gliadins (Fig. 7), the

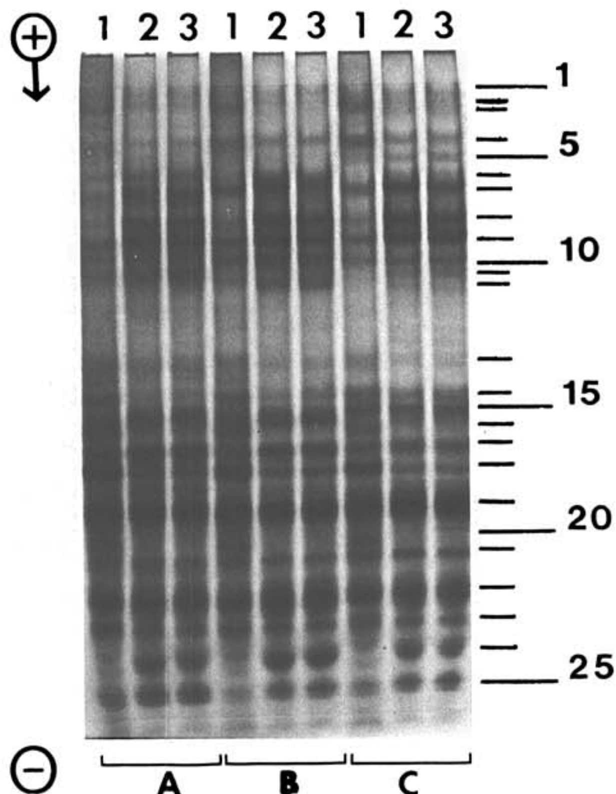

Fig. 4. Polyacrylamide gel electrophoregrams at pH 3.1 (Na lactate buffer) of water solubles isolated according to the procedures of **1,** Chen and Bushuk (1970); **2,** MacRitchie (1978); and **3,** Hoseney et al (1969a,b) from the following cultivars: **A** = Prodax, **B** = Len, **C** = Coteau. Migration is from the positive electrode.

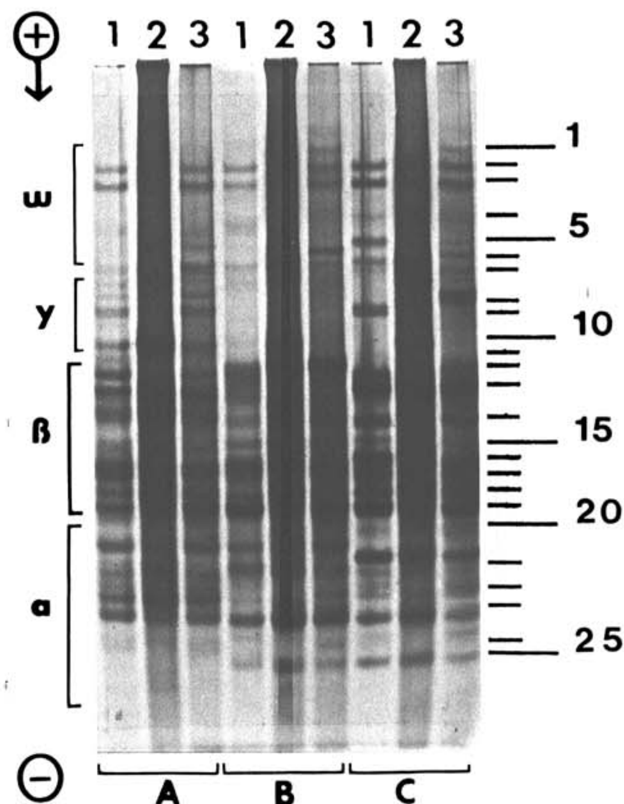

Fig. 5. Polyacrylamide gel electrophoregrams at pH 3.1 (NA lactate buffer) of gliadins isolated according to the procedures of **1,** Chen and Bushuk (1970); **2,** MacRitchie (1978); and **3,** Hoseney et al (1969a,b) from the following cultivars: **A** = Prodax, **B** = Len, **C** = Coteau.

presence of glutenin, as indicated by subunits of 134,000–68,000 mol wt, were clearly evident in all patterns. These bands appear only when gliadin is treated with reducing agent such as β-mercaptoethanol. These bands were not evident in the patterns of Figure 5, because they appear only when reducing agents are used to obtain those patterns as in Figure 7. Chen and Bushuk's gliadin contained the least glutenin followed by that of Hoseney et al, then MacRitchie's fractions for all varieties as indicated by staining intensity of bands in the 134,000–68,000 mol wt region. The subunits in the 35,000 mol wt region are more intensely stained in Chen and Bushuk's fraction. Also, below 35,000 mol wt several faint bands were missing in Chen and Bushuk's fraction, lightly stained in Hoseney et al, but more intense in MacRitchie's fractions.

The SDS-PAGE pattern of glutenin is shown in Figure 8. It is quite obvious that Chen and Bushuk's glutenin is different from the glutenin of the other two procedures. The glutenins of MacRitchie and Hoseney et al seemed more similar to each other. The glutenins of MacRitchie and Hoseney et al showed a more even distribution of bands, whereas in that of Chen and Bushuk bands in the 45,000–35,000 mol wt region predominated.

Several bands in the 134,000–45,000 mol wt region and the region below 30,000 mol wt were missing in Chen and Bushuk's glutenin but were present in glutenins of the other two procedures. These differences are mainly due to the differences in the three fractionation procedures themselves as discussed earlier. It is interesting to observe that, in the procedures of MacRitchie and Hoseney et al, in which gluten was first formed before extraction of gliadin and glutenin, the subunits in the 25,000–12,000 mol wt region (subunits 22 to 27) are more intensely stained. Note, in contrast, that subunits of similar molecular weight in the water solubles of Figure 6 were more intensely stained in Chen and Bushuk's procedure, in which gluten was not formed, than in the other two procedures. These results would indicate that, perhaps, some albumins and globulins became associated with the gluten proteins during gluten formation resulting in more intensely stained bands in the 25,000–12,000 mol wt region.

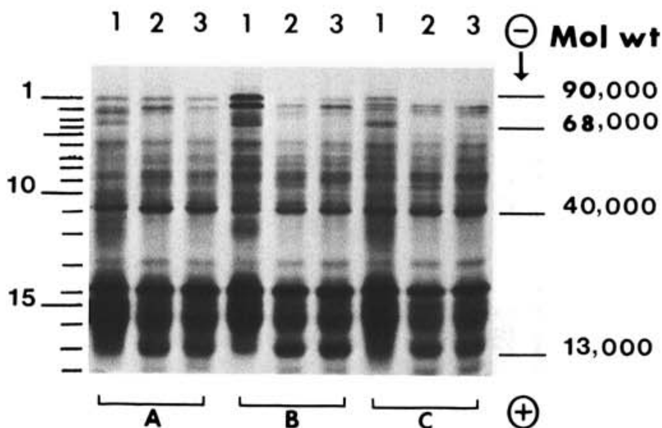

Fig. 6. Sodium dodecyl sulfate-polyacrylamide gel electrophoregrams at pH 8.3 (0.075*M* Tris, 0.192*M* glycine buffer) of water solubles isolated according to the procedures of **1**, Chen and Bushuk (1970); **2**, MacRitchie (1978); and **3**, Hoseney et al (1969a) from the following cultivars: **A** = Prodax, **B** = Len, **C** = Coteau.

SDS-PAGE of Residue and Starch Fractions

Isolation of residue and starch fractions differed depending on the fractionation procedure. In Chen and Bushuk's procedure, the fraction remaining after sequential extraction of flour with salt, alcohol, and acids was called the residue fraction. This fraction contained a large amount of proteins (insoluble glutenin) (Table I) together with the insoluble starch. On the other hand, starch and water-soluble fractions of MacRitchie and Hoseney et al were recovered as a slurry at the beginning of the fractionation procedures after washing a dough ball with distilled water. The slurry was centrifuged to recover starch. The protein quantitation data (Tables II and III) showed that a small amount of protein also was present in starch fractions of the MacRitchie and Hoseney et al procedures. SDS-PAGE of the residue (pattern 1) and starches (patterns 2 and 3) are shown in Figure 9. It is speculated that subunits present in the residue and starch fractions were most likely bound to the starch portion of flour, and therefore, could not be separated either by sequential extraction, or by washing the dough ball with water. The manner in which this binding exists between the glutenin subunits and starch is not yet established (Cluskey and Dimler 1967).

After treatment with reducing agent, several subunits in the 134,000–35,000 mol wt region of the Chen and Bushuk and Hoseney et al procedures (patterns 1 and 3, Fig. 9) of Prodax and Len showed mobility similar to those of the glutenin protein fraction (Fig. 8). Coteau, however, showed faint subunits in the 134,000–75,000 mol wt region (bands 4 to 7) in the Hoseney et al procedure (pattern 3). The results indicated that gluteninlike proteins are associated with the starch fractions. MacRitchie's procedure, however, showed a broad band in the 13,000 mol wt region which was more intensely stained in Prodax and least stained in Coteau. On the other hand, bands 7–20 were absent in Prodax, faint in Len, and more brightly stained in Coteau. The starch fraction from MacRitchie's procedure, therefore, seemed to have different subunits associated with it. It should be noted that in MacRitchie's procedure flour was defatted with chloroform before gluten was washed. These results further indicate that the solubility

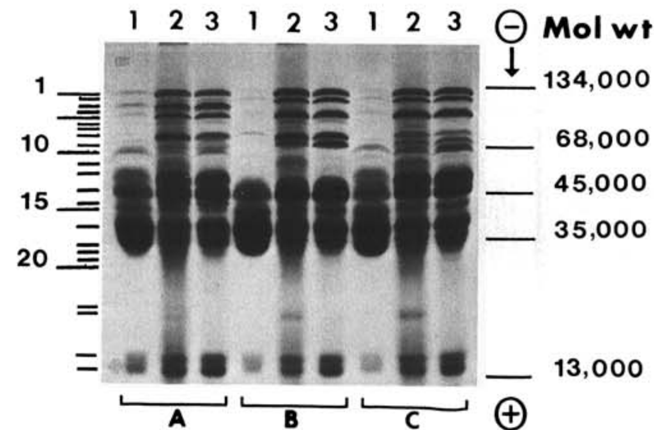

Fig. 7. Sodium dodecyl sulfate-polyacrylamide gel electrophoregrams at pH 8.3 (0.075*M* Tris, 0.192*M* glycine buffer) of gliadins isolated according to the procedures of **1**, Chen and Bushuk (1970); **2**, MacRitchie (1978); and **3**, Hoseney et al (1969b) from the following cultivars: **A** = Prodax, **B** = Len, **C** = Coteau.

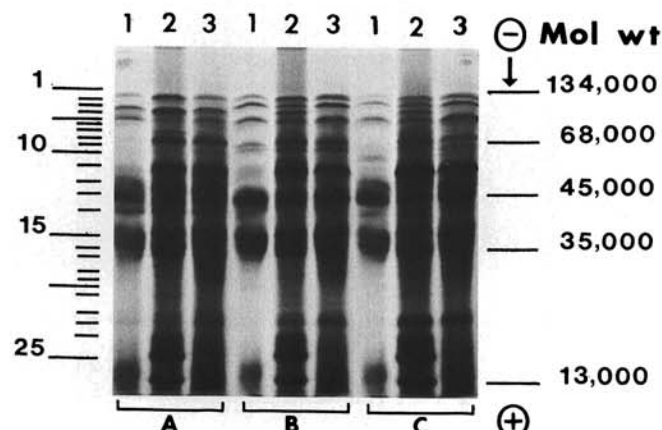

Fig. 8. Sodium dodecyl sulfate-polyacrylamide gel electrophoregrams at pH 8.3 (0.075*M* Tris, 0.192*M* glycine buffer) of glutenins isolated according to the procedures of **1**, Chen and Bushuk (1970); **2**, MacRitchie (1978); and **3**, Hoseney et al (1969b) from the following cultivars: **A** = Prodax, **B** = Len, **C** = Coteau.

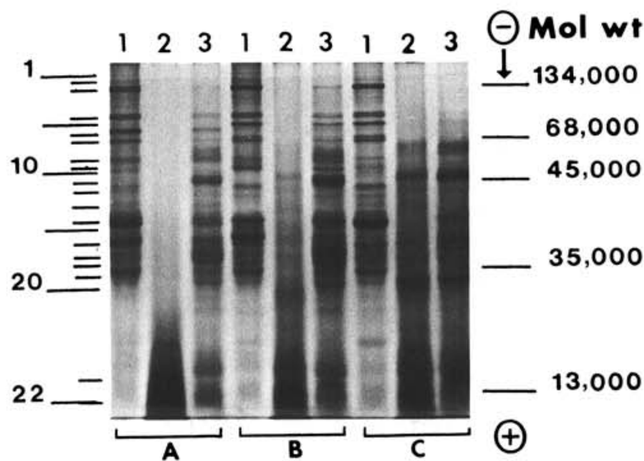

Fig. 9. Sodium dodecyl sulfate-polyacrylamide gel electrophoregrams at pH 8.3 (0.075*M* Tris, 0.192*M* glycine buffer) of residue isolated according to the procedures of **1,** Chen and Bushuk (1970); **2,** MacRitchie (1978); and **3,** Hoseney et al (1969a) from the following cultivars: **A** = Prodax, **B** = Len, **C** = Coteau.

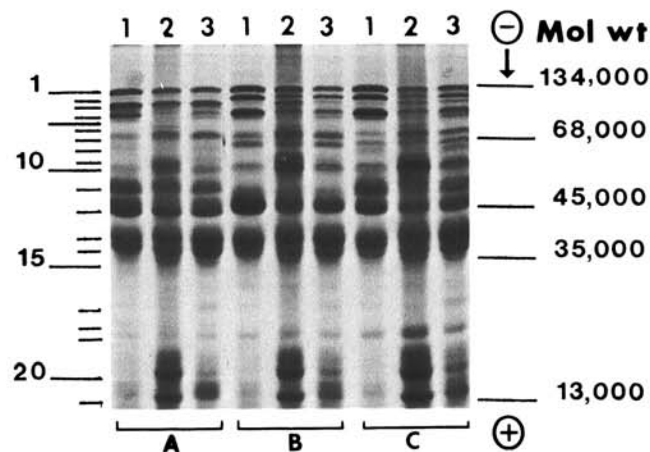

Fig. 10. Sodium dodecyl sulfate-polyacrylamide gel electrophoregrams at pH 8.3 (0.075*M* Tris, 0.192*M* glycine buffer) of residue, acid-insoluble (glutenin), and insoluble fractions isolated according to the procedures of **1,** Chen and Bushuk (1970); **2,** MacRitchie (1978); and **3,** Hoseney et al (1969b), respectively, from the following cultivars: **A** = Prodax, **B** = Len, **C** = Coteau.

of gluten proteins will be different depending on the extraction procedure employed.

SDS-PAGE of Insoluble Fraction of Hoseney et al

At the beginning of the Hoseney et al fractionation procedure, an insoluble fraction is separated by centrifugation from gluten dispersed in lactic acid. This insoluble fraction is said to have no specific role in breadmaking (Hoseney et al 1969b). Figure 10 compares this insoluble fraction (pattern 3) with the residue fraction (acetic acid insoluble) of Chen and Bushuk (1970) (pattern 1) and the acetic acid insoluble fraction (glutenin) of MacRitchie (1978) (pattern 2), respectively. The SDS-PAGE pattern of the insoluble fraction of Hoseney et al and the residue fraction of Chen and Bushuk were qualitatively and quantitatively very similar for all three varieties. MacRitchie's acid-insoluble fraction, however, showed major quantitative differences from the other two procedures, for example, band 3 of Len, band 4 of Prodax and Coteau, and bands 10, 20, and 21 of all three varieties. The SDS-PAGE pattern of the Hoseney et al insoluble fraction showed a subunit pattern typical of a glutenin fraction.

CONCLUSION

The protein fractionation and gel electrophoresis results of this study showed that variations in the procedures used to isolate the different wheat protein fractions such as albumins, globulins, gliadins, and glutenins can result in compositional differences in these protein classes. Variations in protein fractions could have resulted from the type of solvent used, the starting material such as flour or dough, and the type of stirrer used to solubilize the gluten proteins. Researchers such as Hoseney et al (1969a,b), Orth and Bushuk (1972), and MacRitchie (1978,1985) have used the protein fractions from their isolation procedures to evaluate the functional properties of the wheat protein classes. These researchers report conflicting results for the functionality of the gliadin and glutenin fractions. Because the composition of the various fractions would differ due to the various reasons shown in this study, it would be reasonable to assume that the functional properties of these fractions would also be different. The functional properties of the various fractions isolated in this study were evaluated through reconstitution baking studies. These results are reported in a companion paper (Chakraborty and Khan 1988).

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LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods of the AACC. Methods 44-40, approved April 1961; Method 46-10, approved April 1961, revised September 1985; Method 46-12, approved October 1976, revised October 1986. The Association: St. Paul, MN.

BIETZ, J. A., and WALL, J. S. 1973. Isolation and characterization of gliadinlike subunits from glutenin. Cereal Chem. 50:537-547.

CHAKRABORTY, K., and KHAN, K. 1988. Biochemical and breadmaking properties of wheat protein components. II. Reconstitution baking studies of protein fractions from various isolation procedures. Cereal Chem. 65:340-344.

CHEN, C. H., and BUSHUK, W. 1970. Nature of proteins in triticale and its parental species. I. Solubility characteristics and amino acid composition of endosperm proteins. Can. J. Plant Sci. 50:9-14.

CLUSKEY, J. E., and DIMLER, R. J. 1967. Characterization of the acetic acid insoluble fraction of wheat gluten protein. Cereal Chem. 44:611-619.

HOSENEY, R. C., FINNEY, K. F., SHOGREN, M. D., and POMERANZ, Y. 1969a. Functional (breadmaking) and biochemical properties of wheat flour components. II. Role of water solubles. Cereal Chem. 46:117-125.

HOSENEY, R. C., FINNEY, K. F., SHOGREN, M. S., and POMERANZ, Y. 1969b. Functional (breadmaking) and biochemical properties of wheat flour components. III. Characterization of gluten protein fractions obtained by ultracentrifugation. Cereal Chem. 46:126-135.

KASARDA, D., BERNARDIN, J., and NIMMO, C. 1976. Wheat proteins. Pages 158-236 in: Advances in Cereal Science and Food Technology. Y. Pomeranz, ed. Am Assoc. Cereal Chem.: St. Paul, MN.

KHAN, K., HAMADA, A., and PATEK, J. 1985. Polyacrylamide gel electrophoresis for wheat variety identification: Effect of variables on gel properties. Cereal Chem. 62:310-313.

KUNDIG, W., NEUKOM, H., and DEUEL, H. 1961a. Untersuchungen uber getreideschleimstoffe. I. Chromatographische franktionierung von wasserloslichen wezenmehlpentosanen an diethylaminoethylaminoathyt cellulose. Helv. Chim. Acta. 44:823-829.

KUNDIG, W., NEUKOM, H., and DEUEL, H. 1961b. Untersuchungen uber getreideschleimstoffe. II. Uber die gelierung waseriger losungen von weizenmehlpentosanen dusch oxydationsmittel. Helv. Chim. Acta. 44:969-976.

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

MacRITCHIE, F. 1978. Differences in baking quality between wheat flours. Food Technol. 13:187-194.

MacRITCHIE, R. 1985. Study of the methodology for fractionation and reconstitution of wheat flours. Cereal Sci. 3:221.

ORTH, R. A., and BUSHUK, W. 1972. A comparative study of the proteins of wheats of diverse baking quality. Cereal Chem. 49:268.

ORTH, R. A., BAKER, R. J., and BUSHUK, W. 1972. Statistical evaluation of techniques for predicting baking quality of wheat cultivars. Can. J. Plant Sci. 52:139-146.

OSBORNE, T. 1907. The Proteins of the Wheat Kernel. Publ. 84. Carnegie

Institute of Washington: Washington, DC.
PENCE, J. 1962. Recent research on components and primary structures of the flour proteins. Cereal Sci. Today 7:178-180, 208.
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. 54:314-320.
TKACHUK, R. 1969. Nitrogen to protein conversion factors for cereal and oilseed meals. Cereal Chem. 46:419-423.
WRENCH, P. M. 1965. The role of wheat flour pentosans in baking. III. Enzymatic degradation of pentosan fractions. J. Sci. Food Agric. 16:51-54.

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