ACID-SOLUBLE PROTEINS OF WHEAT FLOURS
I. EFFECT OF DELIPIDATION ON PROTEIN EXTRACTION[1]

K. H. CHUNG[2] and Y. POMERANZ,[3] U.S. Grain Marketing Research Center, U.S. Department of Agriculture,[4] Agricultural Research Service, Manhattan, KS 66502

ABSTRACT

Cereal Chem. 55(2): 230-243

Extractability and composition of proteins from flours of good and poor baking quality, and from these flours after delipidation by acetone and isopropanol, were examined. Proteins were extracted with 0.05*N* acetic acid from nondefatted and defatted flours. Extracts were dialyzed, lyophilized, and fractionated by gel filtration on a Sephadex G-100 column. The extracts and the fractions were characterized by starch and sodium dodecyl sulfate polyacrylamide gel electrophoresis. Both the nondefatted and the defatted poor (Chiefkan/Tenmarq) flours showed higher protein extractability than did the good flours (Shawnee). More proteins were extracted from good and poor control flours than from the corresponding defatted flours. In elution profiles from the gel column, all protein extracts showed similar retention times for the glutenin, gliadin, and water-soluble fractions. Quantitatively, the glutenins were the most variable among the gel-separated fractions in the acetic acid extracts from the flours. Whereas concentrations of gliadins were similar in all extracts, concentrations of glutenins were dependent on the flour samples and the delipidation solvent. Delipidation decreased glutenin/gliadin ratios. Glutenin/gliadin fractions showed differences between strong and weak flours and between nondefatted and defatted flours in number and intensity of electrophoretic bands.

The importance of wheat proteins in breadmaking has been well recognized (1,2). Consequently, considerable research has been done to correlate breadmaking quality of wheat with qualitative and quantitative protein differences (3–7). In most of these investigations, researchers have tried to strike a compromise between desirability of extracting and characterizing practically all wheat flour proteins and recognition that harsh treatments may modify the extracted proteins considerably and complicate the interpretation of differences in fractions separated by column chromatography or electrophoresis. Scientists have had many differences, however, as to what constitutes a reasonable compromise.

Whereas some studies have directly demonstrated contributions of wheat flour components to breadmaking, others have postulated such contributions on the basis of statistical inferences. Several investigators have not distinguished between the effects on breadmaking of protein quantity and quality and have claimed differences in protein quality without considering the effects on loaf volume of up to threefold differences in protein quantity. Some have drawn inferences on the basis of "expected" performance of wheat classes and cultivars

[1]Contribution No. 193 j, Department of Biochemistry, Kansas Agricultural Experiment Station, Manhattan, KS 66506.

Cooperative investigations between the Agricultural Research Service, U.S. Department of Agriculture, and Kansas Agricultural Experiment Station, Manhattan, KS.

[2]Research associate, Biochemistry Department, Kansas State University, Manhattan, KS. Present address: Paniplus Co., Olathe, KS.

[3]Director, U.S. Grain Marketing Research Center, Manhattan, KS.

[4]Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be available.

0009-0352/78/000027$03.00/0
© 1978 The American Association of Cereal Chemists

in breadmaking. Finally, in many cases no clear distinction has been made between mixing requirement and tolerance and loaf volume potential under optimized conditions.

Dilute acetic or lactic acid solutions have been used most frequently in extracting wheat flour proteins. Those solutions, however, do not extract or solubilize part of the protein. Mecham *et al.* (8) reported that after repeated extraction of flours with 0.01*M* acetic acid, much of the "gel" protein could be extracted with 0.04m*M* mercuric chloride in acetic acid. In seven flours ranging in protein content from 7.9 to 14.7%, gel protein amounting to 12–28% of the total protein was solubilized. In general, the flours that were least stable to mixing yielded the smallest percentage of solubilized gel protein.

Tanaka and Bushuk (9) found that poor-quality varieties contained less alcohol- and acetic acid-soluble proteins and more insoluble residue proteins than did good-quality varieties. In a subsequent report, however, Orth and Bushuk (4) postulated that proteins in good breadmaking flour should contain at least 25% insoluble protein and a small proportion of acetic acid-soluble protein. A high gliadin/glutenin ratio was considered beneficial but not critical.

Bietz and Wall (10) studied the effect of various extractants on the subunit composition and associations of wheat glutenin. They emphasized that the sequence in which the solvents are used, the mode of extraction, and the starting material (gluten or flour) affect extraction yields of proteins. More effective solvents, which may modify the proteins and lead to inconsistencies, can extract glutenins that are undissolved in dilute acetic acid.

Huebner and Wall (11) recently extracted proteins from 13 defatted flours ranging in protein from 6.5 to 21.2% with a solvent consisting of 0.1*N* acetic acid, 3*M* urea, and 0.01*N* hexadecyltrimethylammonium bromide. The proteins were separated on Sepharose 4B and 2B columns and eluted with 5.5*M* guanidine HCl. The eluates were characterized by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. Column chromatography gave two glutenin, one gliadin, and one albumin plus globulin fractions. In attempting to reconcile their findings with those of Orth and Bushuk (4), Heubner and Wall (11) suggested that a sufficient glutenin content is important to strong dough formation, that a suitable proportion of high molecular weight glutenin is also essential, and that the amount of insoluble protein also contributes to dough quality. In some instances, poor breadmaking quality was attributed to suboptimal ratios among the high- and low-molecular weight glutenins, gliadins, albumins plus globulins, or insoluble proteins.

In studying distribution of lipids between gliadin and glutenin of wheat proteins, Olcott and Mecham (12) found that more than 80% of the lipids were linked to the glutenins. Ponte *et al.* (13), however, found that the major part of lipids was associated with the gliadin fraction. Later, Hoseney *et al.* (14) found that association of lipids with gliadin or glutenin depended on extracting conditions. Simmonds and Wrigley (15) reported that much less protein was extractable with 6*M* urea from gluten than from "storage protein" that had been prepared with organic solvents and was depleted in lipid. Reconstitution experiments suggested that the reduced protein solubility in gluten was due to lipid-protein association during dough formation. Charbonnier (16) showed that solubility of part of the glutenins in 55% ethanol depended on interaction with lipids. The proteins were rendered insoluble after treating the flour with

ethanol/ether/water (2:2:1, v/v/v). Effects of delipidation on protein extractability have not been studied extensively, but such effects could explain the mechanisms of protein-lipid interactions in wheat flours.

We reexamined chemical interactions among proteins and between proteins and lipids. Proteins were extracted with dilute acetic acid from untreated and delipidated flours differing in breadmaking quality. We selected extraction with dilute acetic acid, because investigations in our laboratories have shown that solubility of wheat proteins in mild extractants (*i.e.*, dilute acetic acid) is higher in poor than in good breadmaking flours, the mild extractant does not damage end-use properties of wheat proteins in breadmaking, and the dilute acid extracts most of the gliadins (which control loaf volume potential) and some of the glutenins (which govern mixing properties). We recognize that dilute acetic acid leaves some glutenin unextracted, but believe that the selected conditions are a reasonable compromise.

MATERIALS AND METHODS

Sample Preparation

The untreated straight-grade flours were experimentally milled (Allis) from two selections— Chiefkan/Tenmarq (KS501097) and Shawnee (CI 14157)—of hard red winter wheats that were harvested in 1973. The two flours had these characteristics:

Flour sample	*Protein ($N \times 5.7$) (%, 14% mb)*	*Ash (%)*	*Loaf volume (100 g flour, cc)*	*Mixing time (min)*
Shawnee (CI 14157)	12.7	0.40	1,051	4-5/8
Chiefkan/Tenmarq (KS501097)	13.4	0.43	523	7/8

Shawnee was a good and Chiefkan/Tenmarq an extremely poor breadmaking flour. The flours were defatted with acetone or 2-propanol by a Soxhlet apparatus as described previously (17); the defatted flours were kept at 4°C until used.

Proteins were extracted three times with 0.05 *N* acetic acid; 4 g (db) of flour was suspended in 60 ml of 0.05 *N* acetic acid, and the mixture stirred with a magnetic stirrer for 30 min. The suspension was centrifuged (type SS-34 head, Sorvall centrifuge) at $21,713 \times g$ for 30 min. Two more such extractions were made with the pelleted centrifugate. The combined supernatants were dialyzed against 0.05 *N* acetic acid at 4°C and lyophilized.

Analytic Methods

All analyses, including separations and fractionations, were run in duplicate. Moisture and protein contents of the flours were determined whenever a sample of flour was used (18). Protein contents of lyophilized acid extracts were determined by the AOAC micro-Kjeldahl method (19), and protein content was calculated as Kjeldahl N × 5.7. Unless specified otherwise, lyophilized protein

samples were kept in desiccators (over P_2O_5) for 24 hr under vacuum before being weighed.

Gel Filtration Chromatography

Lyophilized protein extracts were dissolved in 0.05*N* acetic acid (40 mg protein/5 ml) and were separated at room temperature on a 2.6 × 83-cm Sephadex G-100 column (Pharmacia Inc., Uppsala, Sweden). The column, equilibrated with 0.05*N* acetic acid, was operated in an upward flow. The average flow rate on the column was 20 ml/hr. Volume of separated protein sample was measured by the number of drops injected (70 drops). Proteins in the column effluent were determined by absorbance at 280 nm with a UV monitor (2089 UIVCORD III, LKB Instruments, Inc., Bromma, Sweden). Appropriate fractions were collected by a fraction collector (LKB 7000 ULTRORAC fraction collector), and molecular weights of the fractions were determined according to a calibration kit instruction manual (Pharmacia) with standard proteins of known molecular weight (ribonuclease A, chymotrypsinogen A, ovalbumin, aldolase). Each fraction was dialyzed against 0.05*N* acetic acid, and total absorbance at 280 nm was measured with a Carry 118 C spectrophotometer. Lyophilized fractions were kept in a desiccator for determination of dry weight.

SDS Polyacrylamide Gel Electrophoresis

Gel electrophoresis was performed in the EC 470 cell for vertical slab electrophoresis (E-C Apparatus Co., St. Petersburg, FL) with 0.125*M* tris-borate buffer, pH 8.9 (20). Bietz and Wall (21) used this method in a study of wheat gluten subunits that involved determination of molecular weights with SDS polyacrylamide gel electrophoresis. For preparation of the gel (12.0 × 20.0 × 0.6 cm), 5.6 ml of a freshly prepared 2% ammonium persulfate solution (E-C Apparatus Co.), 100 mg of sodium sulfite (Fisher Scientific Co., Waltham, MA), and 0.7 ml of *N,N,N′,N′*-tetramethylethylenediamine were mixed with 280 ml of a solution containing 6% acrylamide, 0.13% N,N-methylene bisacrylamide, and 0.1% SDS (Bio-Rad Laboratories, Richmond, CA) in the electrophoresis buffer.

The fractionated proteins were dispersed (10 mg/ml) in pH 8.9 tris-borate buffer containing 1% SDS and 1% 2-mercaptoethanol to reduce disulfide bonds; then they were incubated overnight at 37°C. Reduced proteins (100 μl) were mixed with one drop of glycerin and one drop of 0.3% bromphenol blue, and placed in the slots of the electrophoresis apparatus. Electrophoresis was at 280 V and 60 to 90 mA for 3 hr, with the positive electrode in the lower chamber. After electrophoresis, the gels were stained overnight in a freshly prepared mixture of acid in acetic acid/methanol/water (7:20:80, v/v/v) (20). For destaining, a mixture of acetic acid/methanol/water (10:60:140, v/v/v) was used (20). We estimated molecular weights of protein subunits by running reduced proteins of known molecular weight in gels parallel to the samples. The standard proteins used as molecular weight markers included ribonuclease A (13,700), chymotrypsinogen A (25,000), ovalbumin (45,000), and bovine serum albumin (68,000) (22).

Starch Gel Electrophoresis

A stock solution of 0.008*M* aluminum lactate buffer was prepared with 7.10 g of aluminum lactate powder (Pfaltz and Bauer, Inc., Stamford, CT) in 3 L of

deionized water; pH was adjusted to 3.1 with lactic acid and then brought to a final volume of 3 L with deionized water. A starch gel was made from 34 g of hydrolyzed starch (Connaught Medical Research Lab., Toronto, Ont.) and 300 ml of aluminum lactate buffer (pH 3.1) containing 3*M* urea according to the method that Woychik *et al.* (23) described.

The gel was prepared in the Buchler starch gel vertical electrophoresis mold (Buchler Instrument Inc., Fort Lee, NJ) after starch-buffer solutions had been heated according to Smithies' (24) method. After the gel had cooled overnight in a refrigerator, the samples (30 mg/0.5 ml of 0.008*M* aluminum lactate containing 8*M* urea) were applied and electrophoresis was done at 500 V for 10 hr in a cold room (4°C). Gels were sliced horizontally with a gel slicer (Buchler) and were stained for 12 hr in a solution containing 0.05% nigrosine and 30% acetic acid. The stained gels were destained in 2 L of a solution consisting of 966 ml of methanol, 172 ml of glacial acetic acid, and 862 ml of water (25).

RESULTS AND DISCUSSION

Protein Extractability

Protein extracts from six samples of nondefatted and defatted (by acetone and isopropanol) Shawnee and Chiefkan/Tenmarq flours were analyzed; the results are summarized in Table I.

The protein contents of the extracts from the six flours were fairly uniform (77.8–79.8%); the percentages of extracted proteins were somewhat higher for the control flours than for the defatted flours. The low protein yield (expressed as percent of flour protein) resulted from incomplete extraction (protein inextractable in 0.05*N* acetic acid), losses during dialysis of acid extracts, and nitrogenous materials extracted by the organic solvents. Isopropanol extracted 3.4 and 9.6% of the total N compounds in the good and poor flours, respectively.

Protein extractability was much higher (62.3%) in the poor breadmaking (Chiefkan/Tenmarq) flour than in the good breadmaking (Shawnee) flour (54.1%). Extractability of the defatted good flour was higher after acetone than

TABLE I
Protein Contents of Control and Defatted Flours and Their 0.05*N* Acetic Acid Extracts

	Shawnee (Good Breadmaking)			Chiefkan/Tenmarq (Poor Breadmaking)		
	Control	Flour Defatted With		Control	Flour Defatted With	
Measurements/Samples	Flour	Acetone	Isopropanol	Flour	Acetone	Isopropanol
Flours						
Protein content (%)[a]	14.8	15.0	14.3	15.6	15.5	14.1
Extracts						
Extract amount (mg)	400.8	378.7	319.9	487.0	473.7	434.2
Protein content (%)	79.8	77.9	77.8	79.8	78.2	78.1
Protein yield (% of flour protein)	54.1	49.2	43.5	62.3	59.7	60.1

[a]On a dry matter basis.

after isopropanol treatment. The results indicate that delipidation reduced protein solubility. Thus, protein extractability could have been related to the amount of extracted lipids and to the associated structural modification of the flour components. Such modifications can be detrimental to the breadmaking properties of the flour (26). The effect of defatting on protein yield was greater for

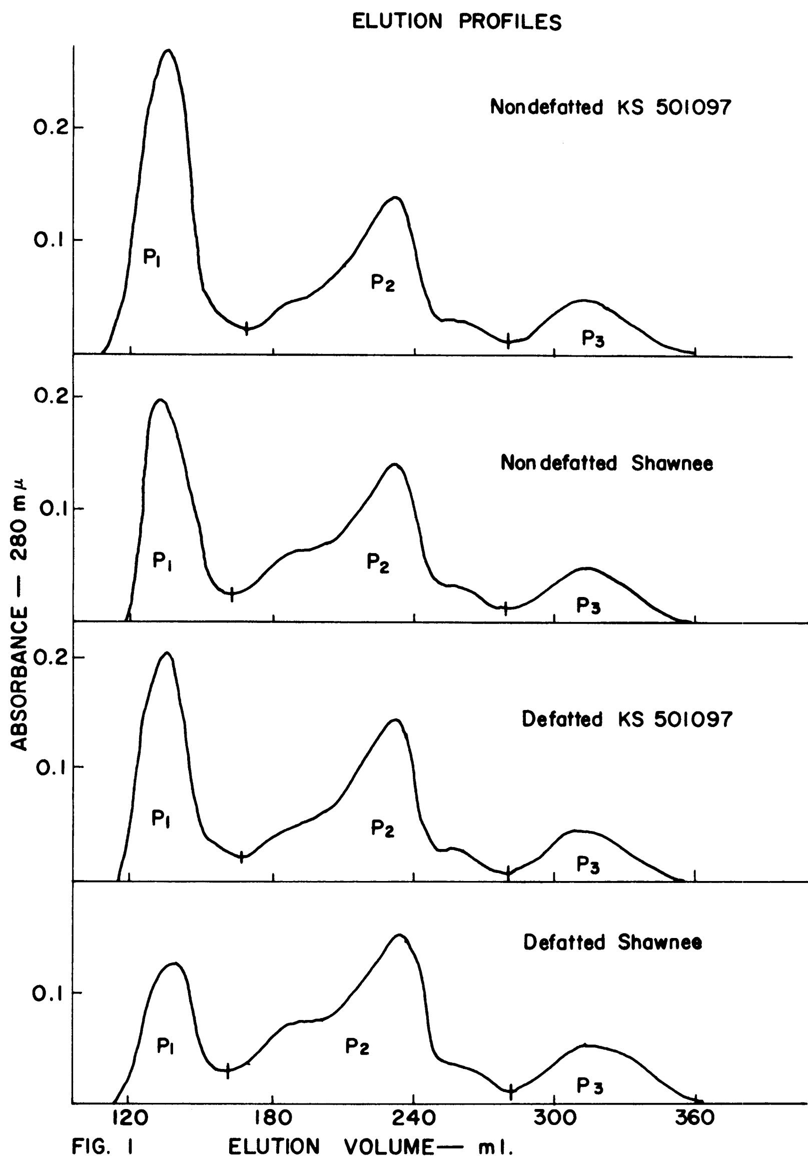

Fig. 1. Profiles of acid-soluble protein extracts of nondefatted and isopropanol-defatted wheat flours eluted from Sephadex G-100 column with 0.05N acetic acid.

the good than for the poor flour. Also, the difference in protein solubility between acetone- and isopropanol-treated flours was much greater for the good than for the poor flour. The high, stable (less affected by organic solvent treatment) protein solubility of the poor flour after delipidation could be attributed to a high concentration of available charged polar groups or to less interaction between lipids and proteins than in the good flour. If binding between proteins and lipids had been hydrophobic, we would have expected defatting to lower extractability of proteins in 0.05N acetic acid by exposing nonpolar protein sites. Our findings and above considerations indicate that good breadmaking flour had either more available nonpolar proteins or more hydrophobic bonds between proteins and lipids than did poor breadmaking flour. Consequently, the reduction in protein solubility by isopropanol delipidation was greater for Shawnee than for Chiefkan/Tenmarq.

Gel Filtration

Figure 1 shows elution profiles of protein extracts fractionated on a Sephadex G-100 column. Approximate molecular weights of the three large fractions—glutenin, gliadin, and water-soluble proteins (albumin plus globulin)—were estimated on the basis of the calibration kit instruction manual

TABLE II
Protein Fractions (Separated on Sephadex G-100) of 0.05N Acetic Acid Extracts of Good and Poor Breadmaking Wheat Flours

	Shawnee (Good Breadmaking)			KS501097 (Poor Breadmaking)		
Fractions	Control Flour	Acetone Defatted Flour	Isopropanol Defatted Flour	Control Flour	Acetone Defatted Flour	Isopropanol Defatted Flour
				Absorbance (280 nm)[a]		
Glutenin	5.4	4.3	3.5	7.3	5.8	4.8
Gliadin	6.1	6.7	7.1	5.6	5.7	5.9
Water soluble	1.2	1.3	1.0	1.1	1.2	1.2
Total	12.7	12.3	11.6	14.0	12.7	11.9
				Composition (%)[b]		
Glutenin	42.9	35.4	29.7	51.8	45.6	40.6
Gliadin	47.9	54.5	61.3	40.2	45.3	49.5
Water soluble	9.2	10.1	9.0	8.0	9.1	9.9
Glutenin/gliadin ratio[c]	0.90	0.65	0.49	1.29	1.01	0.82

[a]Total absorbance at 280 nm = absorbance of fractionated solution times volume of eluted fraction.
[b]Percentages = total absorbance of a fraction divided by total absorbance of all fractions.
[c]Glutenin/gliadin ratio was calculated from total absorbance values of all fractions.

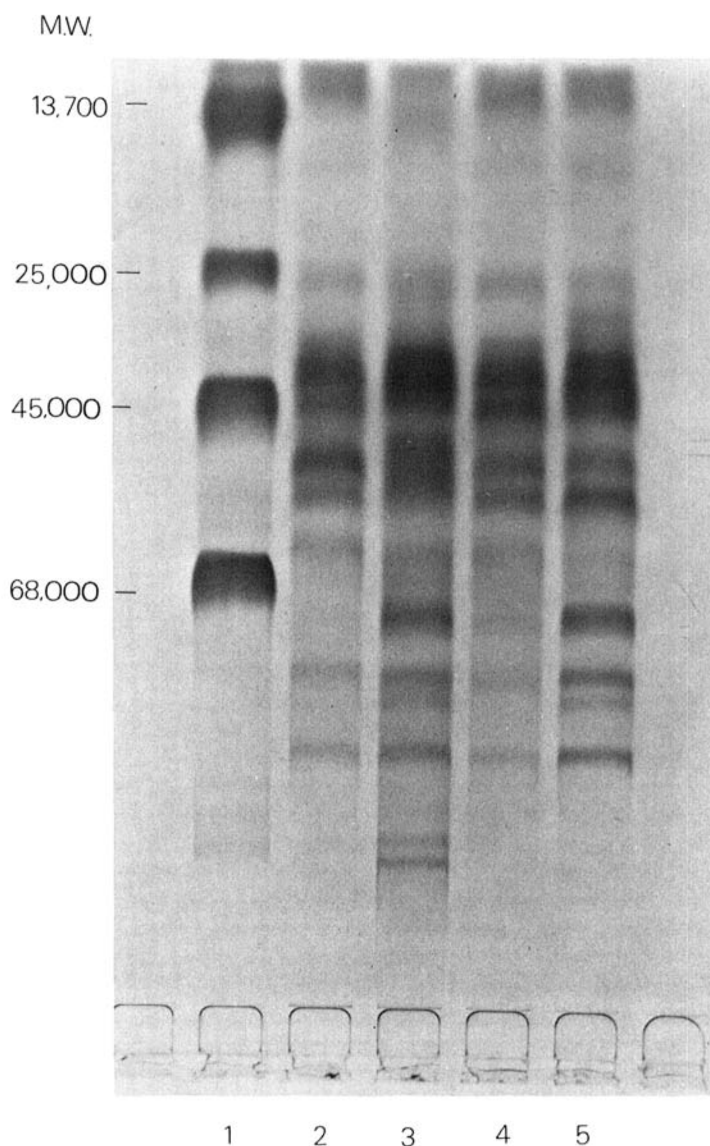

Fig. 2. SDS polyacrylamide gel electrophoresis of 0.05*N* acetic acid-soluble protein extracts from nondefatted and isopropanol-defatted good (Shawnee) and poor (Chiefkan/Tenmarq) breadmaking flours. 1: Standard proteins: bottom to top, bovine serum albumin (68,000 mol wt), ovalbumin (45,000 mol wt), chymotrypsinogen A (25,000 mol wt), ribonuclease A (13,700 mol wt). 2,3: Acid-soluble protein extracts from nondefatted good and poor flours, respectively. 4,5: Acid-soluble protein extracts from defatted good and poor flours, respectively.

(Pharmacia) and were found to be more than 100,000 (P_1), 30,000 (P_2), and 10,000 (P_3), respectively. The first fraction was eluted at the void volume and was shown to contain small and large molecular weight glutenins and some gliadin (16). Calculated molecular weight (K_{av}) of fractions P_2 and P_3 from extracts of the six samples differed little. Ratios of the glutenin/gliadin fractions of protein extracts, however, varied widely among the samples (Table II). Among extracts of the six flours, variation in total 280-nm absorbance (absorbance times volume) was greatest for the glutenin fraction and smallest for the water-soluble proteins. Protein content (as determined by absorbance at 280 nm) of gliadin fractions increased slightly after delipidation. The increase in absorbance could have been caused by a disaggregation of glutenin or an increase in the concentration of aromatic amino acids or both (27). Consequently, the data on compositional ratios are semiquantitative at best.

The glutenin/gliadin ratios of control flours decreased drastically with delipidation (Table II). The decreases in extracts from Shawnee were from 0.90 to 0.65 and to 0.49 and from Chiefkan/Tenmarq, from 1.29 to 1.01 and to 0.82 in acetone- and isopropanol-defatted flours, respectively. The decreases in ratios were mainly due to decreases in glutenin content. The results indicate that delipidation highly affected glutenin. The glutenin/gliadin ratio of protein extracts was highly correlated ($r = 0.886^{**}$) with total acid extractable protein (protein yield in Table I). The results are consistent with the findings of Hoseney *et al.* (14) and Wehrli and Pomeranz (28) that some glutenins are hydrophobically associated with lipids, and with the results of this study that delipidation of flour decreases glutenin solubility in 0.05*N* acetic acid.

SDS Electrophoresis

The SDS polyacrylamide gel patterns of reduced proteins in extracts of good and poor breadmaking flours are shown in Figs. 2 and 3; molecular weight of bands were estimated from those of standard proteins (Fig. 2). Migration of the SDS-complexed subunits was inversely related to the log of their molecular weight (29). Molecular weights determined from gel patterns are reasonably reproducible, but their accuracy is limited (±10%) (22).

Electrophoretic patterns for 0.05*N* acetic acid extracts from the good and poor breadmaking flours differed little in intensity and numbers of bands; more glutenin subunits (more than 68,000 mol wt) were present in the poor than in the good flour (slots 2 and 3, Fig. 2; slots 3 and 6, Fig. 3). Similarly, intensities of bands in the region of high-molecular weight proteins (larger than 45,000) in the two flours differed. In the defatted flours, some components with a molecular weight larger than 68,000 were either absent or reduced in concentration (slots 4 and 5, Fig. 2). The change could have resulted from defatting by isopropanol, which reduced protein solubility, especially in the strong flour. Some of the electrophoretic bands in the glutenin fraction were identical in mobility to some components of the gliadin fraction (slots 3 and 4 and 6 and 7, Fig. 3). Small amounts of gliadin may have been eluted with glutenins from the Sephadex G-100 column, or small gliadin-like proteins may have been liberated from glutenin proteins on reduction (30). Although glutenin proteins play a major role in dough mixing (6,7), the glutenin fractions on the 0.05*N* acetic acid extracts of Shawnee and Chiefkan/Tenmarq flours differed little in band intensity and

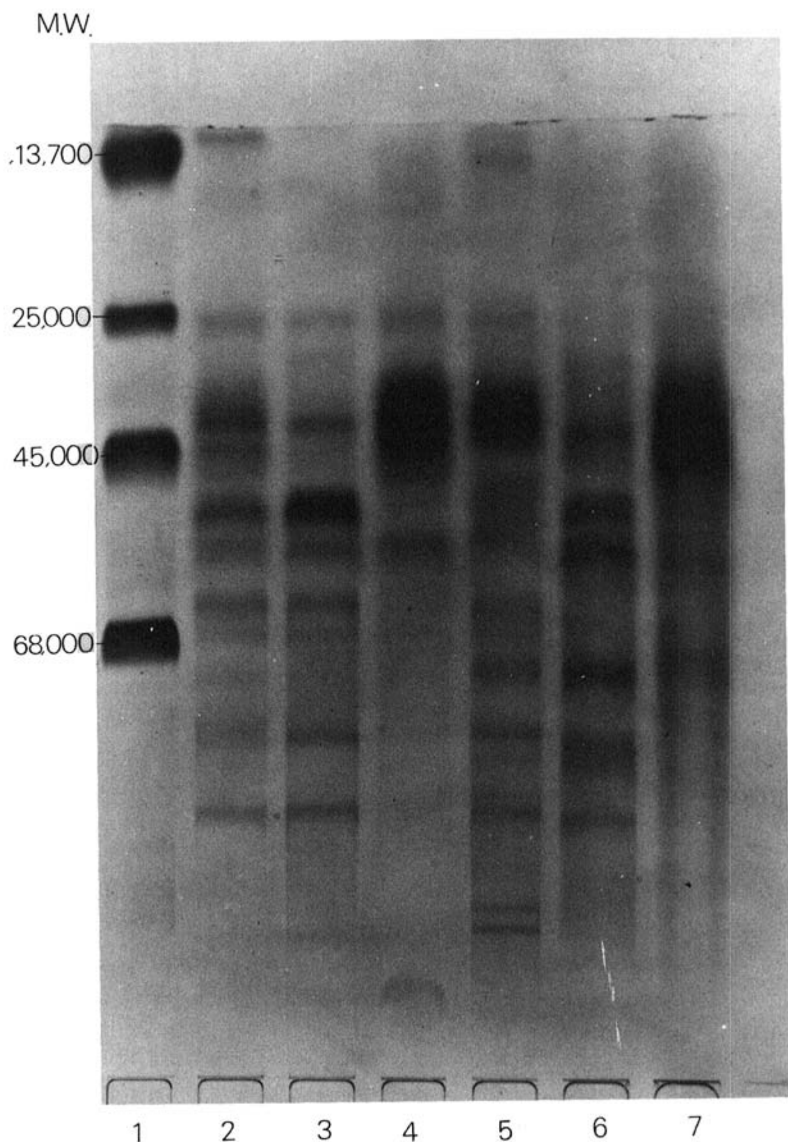

Fig. 3. SDS polyacrylamide gel electrophoresis of fractions from nondefatted good (Shawnee) and poor (Chiefkan/Tenmarq) breadmaking flours. 1: Standard proteins as in Fig. 2. 2, 3, 4: Whole extract, glutenin, and gliadin fractions, respectively, of nondefatted good flour. 5, 6, 7: Whole extract, glutenin, and gliadin fractions, respectively, of nondefatted poor flour. MW = molecular weight.

number. It is possible that the lack of differences in electrophoretic patterns resulted from differences in amounts of total extracted proteins.

Starch Gel Electrophoresis

In starch gel electrophoresis, the nonreduced, acid-soluble protein extracts showed differences in number and intensity of bands between good and poor

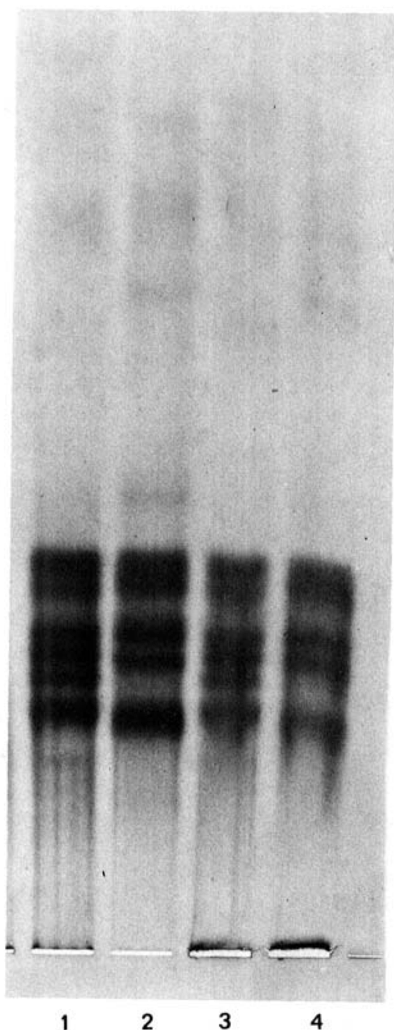

Fig. 4. Starch gel electrophoresis of 0.05*N* acetic acid-soluble protein extracts from nondefatted and isopropanol-defatted good (Shawnee) and poor (Chiefkan/Tenmarq) breadmaking flours. 1, 2: Acid-soluble protein extracts from defatted poor and good flours, respectively. 3, 4: Acid-soluble protein extracts from nondefatted poor and good flours, respectively.

breadmaking flours (Fig. 4). Two high molecular weight gliadin components were absent in the good flour, and one lower molecular weight water-soluble protein component was absent in the poor flour (slots 1 and 2, Fig. 4). Differences in nonreduced gliadin proteins detected by starch gel electrophoresis

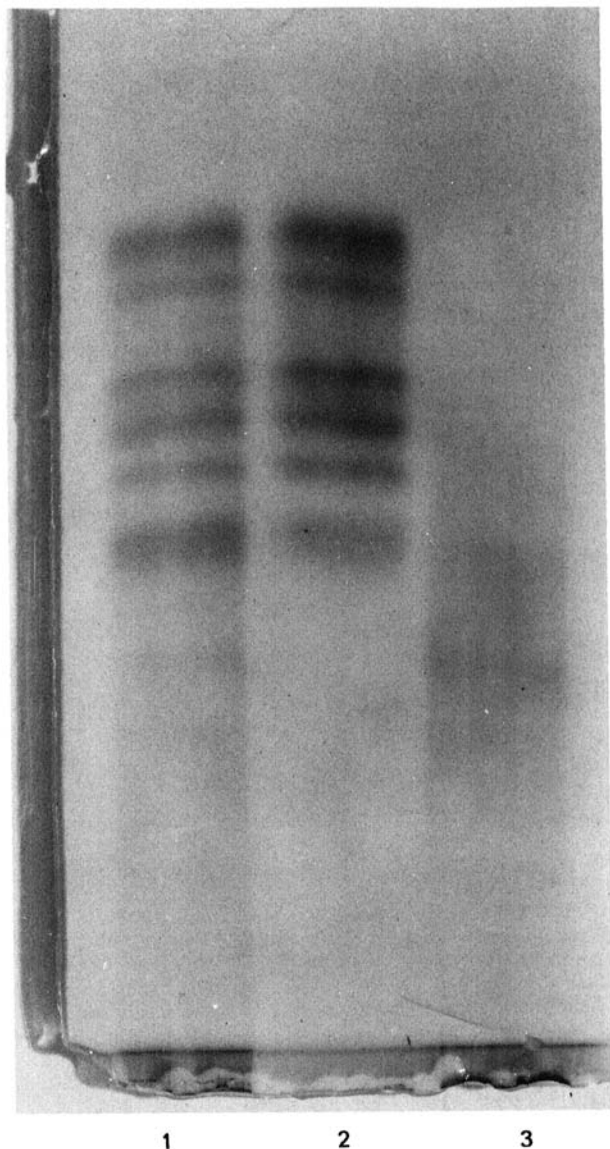

Fig. 5. Starch gel electrophoresis of fractions from 0.05N acetic acid extracts of nondefatted poor breadmaking flour (Chiefkan/Tenmarq). 1, 2, 3: Whole extract, gliadin, glutenin fractions, respectively.

(Fig. 4) were not discernible in samples of reduced gliadin proteins separated by SDS polyacrylamide gel (Fig. 3). We offer the possible explanation that when the protein extract was reduced for SDS polyacrylamide gel electrophoresis, small proteins were formed and were then detected as one of the gliadin subunits. Such a formation of small proteins would contribute to differences in intensities of certain bands observed in SDS polyacrylamide gel electrophoresis as compared with starch gel electrophoresis. Defatting flours did not influence appreciably the starch electrophoresis patterns of wheat proteins (slots 1 and 2, Fig. 4); however, it made the bands sharper with less protein at the origin.

The patterns of nonreduced gliadin and glutenin fractions from the poor flour were further examined in a separate starch gel (Fig. 5). The gliadins separated well without either tailing or a heavy mark at the starting line. The glutenins left a heavy mark at the starting line, with much tailing, and separated into only one or two faint bands. Such typical patterns as marking and tailing of nonreduced glutenin proteins in starch gel electrophoresis might be due to the molecular distribution of glutenin proteins (30,31).

Results of this study point to differences in gluten proteins in dilute acetic acid extracts of good and poor flours and to the effects of delipidation on those differences. The differences were demonstrated by chromatography on Sephadex G-100 columns and by starch gel and SDS polyacrylamide gel electrophoresis. Further characterization of the proteins will be reported elsewhere.

Acknowledgment

The authors thank Dr. O. K. Chung for the defatted flours.

Literature Cited

1. POMERANZ, Y., FINNEY, K. F., and HOSENEY, R. C. Molecular approach to breadmaking. Science 167: 944 (1970).
2. POMERANZ, Y. Relation between chemical composition and breadmaking potentialities of wheat flour. Advan. Food Res. 16: 335 (1968).
3. FINNEY, K. F. Fractionating and reconstituting techniques as tools in wheat flour research. Cereal Chem. 20: 381 (1943).
4. ORTH, R. A., and BUSHUK, W. A comparative study of the proteins of wheats of diverse baking qualities. Cereal Chem. 49: 268 (1972).
5. HUEBNER, F. R. Comparative studies on glutenins from different classes of wheat. J. Agr. Food Chem. 18: 256 (1970).
6. HOSENEY, R. C., FINNEY, K. F., SHOGREN, M. D., and POMERANZ, Y. Functional (breadmaking) and biochemical properties of wheat flour components. III. Characterization of gluten protein fractions obtained by ultracentrifugation. Cereal Chem. 46: 126 (1969).
7. HOSENEY, R. C., FINNEY, K. F., POMERANZ, Y., and SHOGREN, M. D. Functional (breadmaking) and biochemical properties of wheat flour components. IV. Gluten protein fractionation by solubilizing in 70% ethanol and in dilute lactic acid. Cereal Chem. 46: 495 (1969).
8. MECHAM, D. K., COLE, E. W., and NG, H. Solubilizing effect of mercuric chloride on the "gel" protein of wheat flour. Cereal Chem. 49: 62 (1972).
9. TANAKA, K., and BUSHUK, W. Effect of protein content and wheat variety on solubility and electrophoretic properties of flour proteins. Cereal Chem. 49: 247 (1972).
10. BIETZ, J. A., and WALL, J. S. The effects of various extractants on the subunit composition and associations of wheat glutenin. Cereal Chem. 52: 145 (1975).
11. HUEBNER, F. R., and WALL, J. S. Fractionation and quantitative differences of glutenins from wheat varieties varying in baking quality. Cereal Chem. 53: 258 (1976).

12. OLCOTT, H. S., and MECHAM, D. K. Characterization of wheat gluten. I. Protein-lipid complex formation during doughing of flours. Lipoprotein nature of the glutenin fraction. Cereal Chem. 24: 407 (1947).
13. PONTE, J. G., Jr., De STEFANIS, V. A., and COTTON, R. H. Studies of gluten lipids. I. Distribution of lipids in gluten fractions separated by solubility in 70% ethanol. Cereal Chem. 44: 427 (1967).
14. HOSENEY, R. C., FINNEY, K. F., and POMERANZ, Y. Functional (breadmaking) and biochemical properties of wheat flour components. VI. Gliadin-lipid-glutenin interaction in wheat gluten. Cereal Chem. 47: 135 (1970).
15. SIMMONDS, D. H., and WRIGLEY, C. W. The effect of lipid on the solubility and molecular weight range of wheat gluten and storage proteins. Cereal Chem. 49: 317 (1972).
16. CHARBONNIER, L. Etude des proteines alcoolo-solubles de la farine de ble. Biochimie 55: 1217 (1973).
17. CHUNG, O. K., POMERANZ, Y., FINNEY, K. F., HUBBARD, J. D., and SHOGREN, M. D. Defatted and reconstituted wheat flours. I. Effects of solvent and Soxhlet types on functional (breadmaking) properties. Cereal Chem. 54: 454 (1977).
18. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Approved methods of the AACC. Method 44-15, approved 10-30-75; Method 46-10, approved 4-13-61. The Association: St. Paul, MN (1962).
19. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. Official methods of analysis (11th ed.). Method No. 42014-42016. The Association: Washington, DC (1970).
20. KOENIG, R., STEGEMANN, H., FRANCKSEN, H., and PAUL, H. L. Protein subunits in the potato virus X group. Determination of the molecular weights by polyacrylamide electrophoresis. Biochim. Biophys. Acta 207: 184 (1970).
21. BIETZ, J. A., and WALL, J. S. Wheat gluten subunits: molecular weights determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Cereal Chem. 49: 416 (1972).
22. WEBER, K., and OSBORN, M. The reliability of molecular weight determination of dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244: 4406 (1969).
23. WOYCHIK, J. H., BOUNDY, J. A., and DIMLER, R. J. Starch gel electrophoresis of wheat gluten proteins with concentrated urea. Arch. Biochem. Biophys. 94: 477 (1961).
24. SMITHIES, O. Zone electrophoresis in starch gels: Group variations in the serum proteins of normal human adults. Biochem. J. 61: 629 (1955).
25. GRAHAM, J. S. D. Starch gel electrophoresis of wheat flour proteins. Aust. J. Biol. Sci. 16: 342 (1963).
26. FINNEY, K. F., POMERANZ, Y., and HOSENEY, R. C. Effects of solvent extraction on lipid composition, mixing time, and bread loaf volume. Cereal Chem. 53: 383 (1976).
27. JANKIEWICZ, M., and POMERANZ, Y. Isolation and characterization of wheat flour protein. II. Effects of urea and N-ethylmaleimide on the behavior of wheat proteins during extraction and fractionation. J. Sci. Food Agric. 16: 652 (1965).
28. WEHRLI, H. P., and POMERANZ, Y. A note on the interaction between glycolipids and wheat flour macromolecules. Cereal Chem. 47: 160 (1970).
29. SHAPIRO, A. L., VINUELA, E., and MAIZEL, J. V., Jr. Molecular weight estimation of polypeptide chains by electrophoresis in SDS-polyacrylamide gels. Biochim. Biophys. Res. Commun. 28: 815 (1967).
30. ELTON, G. A. H., and EWART, J. A. D. Glutenins and gliadins: Electrophoretic studies. J. Sci. Food Agric. 17: 34 (1966).
31. SMITHIES, O. Zone electrophoresis in starch gels and its application to studies of serum proteins. Advan. Protein Chem. 14: 65 (1959).

[Received June 28, 1977. Accepted October 11, 1977]