Modification of the Maes Continuous-Extraction Process for Fractionation of Hard Red Winter Wheat Flour Proteins[1]

PAUL J. MATTERN, ALI SALEM, and **GEORGE H. VOLKMER**[2], University of Nebraska, Lincoln, Nebraska

ABSTRACT

Fractions obtained by the Maes continuous process of extracting proteins from wheat flour mixed with sand in columns were examined by starch-gel electrophoresis and amino acid analyses to ascertain the distribution of components in the fractions. A new solvent sequence was selected after changes were made and results compared. This new solvent sequence consisted of 40% isopropyl alcohol, 2% NaCl, 3.85% lactic acid, and 0.1% potassium hydroxide. The efficiency of protein extraction was improved by grinding flour with pumice. The final procedure was precise and provided 94–99% recovery of protein. The final procedure is recommended for determining protein differences among wheat varieties accurately and rapidly.

Fractionation of wheat proteins for investigation has been a problem in cereal protein research. The classic fractionation of wheat protein by solubility (1) into five fractions has been replaced recently by more sophisticated techniques such as ion-exchange cellulose column chromatography (2,3,4), gel filtration (5,6), and moving-boundary electrophoresis (7). However, a total extraction or crude fractionation of wheat protein is always required. Fractional precipitation of wheat gluten (7) and dispersion in various solvents with urea, guanidine, or detergent (2) generally were used. The possible artifact of entraining soluble proteins, fat, or starch in a crude gluten fraction isolated from dough by conventional washing procedures has prompted investigation of methods to extract directly from defatted flour which has been washed with dilute salt solution (8). Removal of lipids usually precedes extraction of protein to avoid protein denaturation and must be performed at a low temperature. The solubility of wheat gluten is increased significantly by prior extraction of lipids. However, only 70% of the total nitrogenous material in the flour was extracted.

Wheat samples are small during early phases of a breeding program. It is not convenient to prepare gluten from small flour samples. Maes (9) presented a novel approach of fractionating flour protein based on solubility, to obtain data on the baking quality of European wheat. The method has the advantages of crude simple fractionation of nondefatted flour with small samples, and investigating the whole spectrum of wheat protein. This paper reports on evaluation of the original method and modification to fractionate U.S. bread wheats.

MATERIALS AND METHODS

Nitrogen was determined by macro or micro Kjeldahl methods (10).

[1]Contribution from the Department of Agronomy, Nebraska Agricultural Experiment Station, Lincoln, and Crops Research Division, ARS, USDA. Published with the approval of the Director as Paper No. 2146, Journal Series, Nebraska Agr. Expt. Sta. The research was supported by a grant provided by the USDA, ARS, Northern Utilization Research and Development Division, Peoria, Ill.

[2]Associate Professor, Research Associate, and Research Technician, respectively, Agronomy Department.

Aliquots of liquid fractions to be analyzed for N were predried in dialyzing tubing, with forced air to eliminate foaming during acid digestion.

Protein fractions from the column (except the water-solubles) were dialyzed against 0.01*N* acetic acid before lyophilization.

Protein fractions were reduced and alkylated essentially according to the method of Woychik *et al.* (11). Modifications included the use of 0.1*M* *tris*-acetate buffer at pH 8.5. Solutions were deaerated with nitrogen and kept in a nitrogen atmosphere during reduction and alkylation.

Preparation of Fractionating Column

A reduced sample size and increased quantity of sand were necessary to process U.S. hard red winter wheats without plugging the column. Five grams of flour was ground in a mortar with 10 g. of pumice (medium grit FF Dental Laboratory Italian Pumice). This mixture was incorporated thoroughly with 125 g. of sand (Fisher S-25, washed and ignited sea sand) and placed in the cylinder at the position indicated in Fig. 1. The column was packed gently.

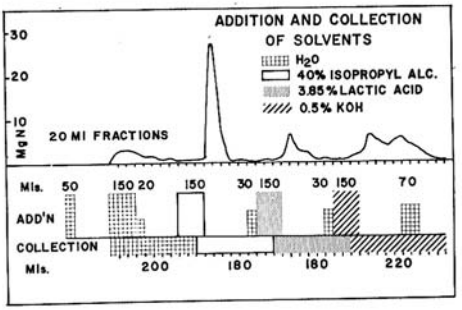


Fig. 1 (left). Dimensions and preparation of Maes column.
Fig. 2 (right). Procedure for adding and collecting a typical solvent system.

Additions and collections of the solvent fractions were made according to instructions in Fig. 2. Solvents, unless specifically noted, were distilled water, 40% isopropyl alcohol (v./v.), 3.85% lactic acid (w./v.), and 0.5% KOH (w./v.). Six to ten columns could be used simultaneously.

Starch Gel-Urea Electrophoresis

A horizontal starch-gel tray divided into eight sections, each 0.6 × 2.0 × 10 cm., was used throughout this investigation. Electrical contacts to the gels were placed directly into the buffer solution in the electrode compartments.

The starch was a commercial hydrolyzed starch prepared by Connaught Laboratories, Toronto, Canada.

The buffer solution in the starch-gel and electrode compartments was 0.038*M* aluminum lactate at pH 3.1 (ionic strength 0.06 unless stated otherwise).

Gels were prepared by heating 15 g. of starch in 100 ml. of buffer in a 500-ml. vacuum filtering flask. The starch was heated over an open flame

with continuous swirling until the starch gelatinized (approximately 65°C.). Solid urea was added slowly and dissolved by continuous swirling and heating until a concentration of 3*M* was obtained (12). The clear viscous solution was deaerated by vacuum until the solution boiled vigorously for 30 sec. The hot gel was poured into the gel tray, smoothed, and covered with a plastic wrap. The plastic wrap was lowered to the gel surface after 1 hr. and the gel placed in the refrigerator overnight for a stronger and clearer gel with higher resolving power. The protein sample was applied to the gel by soaking a thick filter paper (2 × 0.5 cm.) in a solution of 5% lyophilized protein material in 3*M* urea buffer and inserting the strip into a transverse cut in the gel bed. The gel was covered with a plastic wrap and a voltage applied to produce 45 ma. (unless otherwise specified), with a constant current power supply. The buffer was changed between runs.

After electrophoresis, the gel was cut horizontally into four slices with a thin wire cutter. Two middle sections were immersed into 0.5% solution of nigrosin in aluminum lactate buffer for 3 hr. Gel strips were destained in running tap water overnight.

Hydrolysis for Amino Acid Analysis

A 25-mg. sample of the lyophilized protein fraction of flour was hydrolyzed with 10 ml. of 6*N* HCl in an evacuated sealed tube for 24 hr. at 110°C. The hydrolysates were filtered through Whatman No. 42 filter paper and washed carefully with 50 ml. of deionized water. Excess HCl in the hydrolysate was removed by repeated evaporation under vacuum at 45°C. The hydrolysates were kept over NaOH pellets and a desiccant under vacuum for removal of residual HCl. Hydrolysates were made to volume with 0.2*N* citrate buffer at pH 2.2 and filtered through Whatman No. 42 filter paper. The hydrolysates were very light tan, indicating little humin formation. Amino acid analyses were made by ion-exchange column chromatography with a Beckman-Spinco amino acid analyzer, model 120C. Cysteine and tryptophan were not determined in this preliminary study.

RESULTS AND DISCUSSION

Effect of Grinding with Pumice on Fractionation Efficiency

The original Maes method provides for grinding the flour sample with pumice. This should ensure better dispersion of the individual flour particles after mixing with sand. A comparison of fractionation efficiency using the Maes solvents sequence on a flour sample was made by grinding and mixing with pumice prior to blending with sand (Fig. 3). The sample ground with pumice gave more efficient fractionation and sharper separation with less tailings than the sample mixed with pumice. Large flour agglomerates in a sample loosely mixed with pumice were not extracted efficiently. The use of adequate volumes of solvents gave similar total protein extractions of 93.6 and 91.1% for the sample ground and that mixed with pumice, respectively.

Two samples of flour were analyzed before and after normal grinding with pumice to determine the increase in level of damaged starch. The samples increased in damaged starch from 5.6 to about 9.5%. Hard U.S.

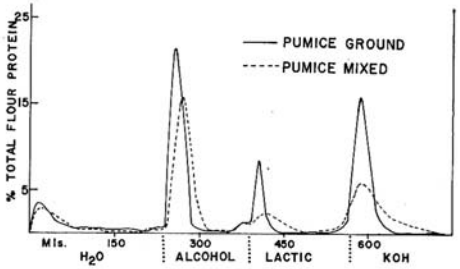


Fig. 3. Comparison of the efficiency of solvent fractionation with the pumice and flour either ground or mixed.

wheats usually produced stoppage with the original procedure. Dilution of the sample in the column was necessary to offset the slow flow rate due to swelling of damaged starch.

Solvent Sequence

The percolation approach offers complete flexibility in the sequence of solvents during extraction. Several solvent sequences were studied and the fractions were evaluated for purity by electrophoresis. The original method of Maes used distilled water, isopropyl alcohol, lactic acid, and potassium hydroxide.

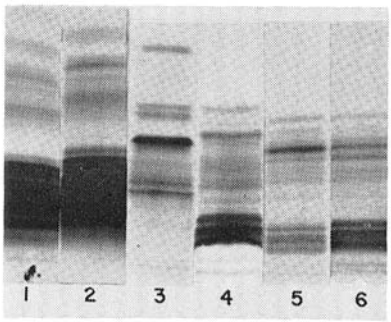


Fig. 4. Starch-gel electrophoresis of water-solubles from Bison variety prepared by various procedures. 3–6, 2% NaCl. 1–2, 2 hr., 45 ma.; 3–6, 1 hr. 25 min., 45 ma. 1, Jones *et al.* (7); 2, Gehrke *et al.* (8); 3, Maes (9), following IPA; 4, Maes, 1st solvent; 5, Maes, 1st solvent (1st half of fraction); 6, Maes, 1st solvent (2nd half of fraction).

Figure 4 shows electrophoretic patterns of crude water-soluble protein from Bison wheat prepared by methods of Jones *et al.* (7), Gehrke *et al.* (8), and Maes (9). Gels 1 and 2 are water-soluble proteins prepared from dough and flour, respectively (7,8). A considerable amount of gliadin contamination was present, although gliadin is less soluble in solutions of higher ionic strength. Gel 3 has the electrophoretic pattern of water-solubles by the Maes procedure with 2% NaCl after isopropyl alcohol. The pattern is almost without contamination from gliadin.

Gel 4 was prepared with distilled water as the first solvent. The gliadin proteins were in higher concentrations than in gel 3. Preliminary data indicated that gliadin was also present when 2% NaCl was used to extract the

soluble proteins before isopropyl alcohol. The electrophoretic pattern of the water-solubles extracted with 2% NaCl or water were different. Two water fractions of 100 and 80 ml. as the first solvent were collected sequentially for comparison. Gels 5 and 6 (Fig. 4) show relatively more gliadin in the second fraction. Gliadin may account for 50% of the fraction on the basis of relative intensity of electrophoretic bands. The high content could be attributed to intermixing with the alcohol solvent.

The amount of nitrogenous material extracted with distilled water or NaCl as the first eluant and a stepwise concentration of salt or 2% salt as the second eluant is shown in Table I. Sodium chloride (2%) as the first solvent extracted 8.1% more nitrogenous material than water. Increasing

TABLE I

NITROGEN RECOVERY WITH DIFFERENT SEQUENCES OF SOLVENTS ON BISON FLOUR (5 g.)

SOLVENT SEQUENCE[a]	WATER-SOLUBLE	IPA-SOLUBLE	ACID-SOLUBLE	ALKALI-SOLUBLE	TOTAL
	%	%	%	%	%
H:A:L:K	15.44	31.40	15.32	32.44	94.60
S:A:L:K	23.30	30.10	11.80	31.30	96.50
A:S:L:K	3.00	52.20	7.70	31.60	94.50
A:(SS):L:K	7.40	51.40	7.72	30.80	97.32

[a] H, distilled water; A, 40% isopropyl alcohol (IPA); S, 2% NaCl; SS, stepwise concentration of NaCl 0.0, 0.1, 2.0, 5.0, 10.0%; L, 3.85% lactic acid; and K, 0.5% potassium hydroxide.

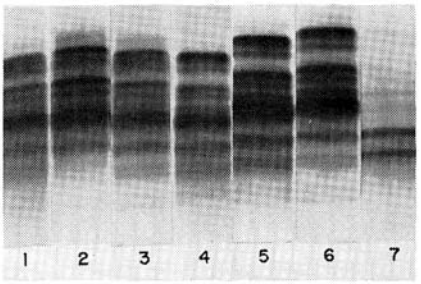
concentrations of NaCl, as the second solvent following IPA, extracted 7.4% of the protein. This was 15.9% less than with 2% NaCl as the first solvent.

It appeared that a 2% NaCl solution as the second solvent extracted the most simple protein mixture. On this basis the recommended solvent sequence was alcohol, 2% NaCl, lactic acid, and potassium hydroxide.

The gliadins isolated from Bison flour were prepared by fractional precipitation methods of Elton and Ewart (13), Gehrke *et al.* (8), and Jones *et al.* (7) and isopropyl alcohol extraction from the Maes (9) column. The gel strip patterns 1–4 in Fig. 5 are essentially similar. Gels 5 and 6 show similar patterns for gliadin extracted by isopropyl and ethyl alcohol on the Maes column. The order of isopropyl alcohol solvent in extraction did not affect the pattern; however, the percentage of gliadin, extracted first, increased, since only traces of gliadin were lost in the water-solubles (Table I). Ethyl alcohol following isopropyl alcohol extracted more of the slow-moving bands (gel 7).

Figure 6 compares gliadin from defatted and nondefatted flours. Gel 1 was the electrophoretic separation of gliadin from a fat-extracted flour using the first portion of the acetic acid extraction from the gluten ball made with Ponca flour. Gel 2 was the 40% isopropyl alcohol fraction from the Maes column. Samples prepared by the Maes method were resolved more clearly. Gliadin from the nonextracted flour with the method of Jones *et al.* (7) caused streaking (gel 3).

The lactic acid fraction, which was assumed to contain only glutenin, contained a considerable amount of water-solubles (gel 1, Fig. 7). To check



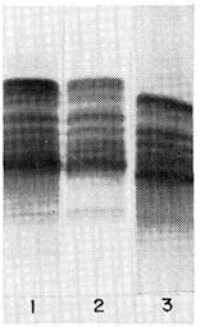
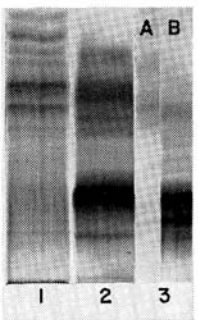


Fig. 5 (left). Starch-gel electrophoresis of gliadin prepared from Bison flour by various procedures. 3 hr., 30 min., 45 ma. 1, Elton and Ewart (13); 2, Gehrke *et al.* (8); 3, Jones *et al.* (7); 4, Maes (9), IPA 2nd solvent; 5, IPA, 1st solvent; 6, EtOH 1st solvent; 7, EtOH following IPA.

Fig. 6 (right). Comparison of gliadin fractions from Ponca variety from fat-extracted and nonextracted flour. 3 hr. 30 min., 45 ma. 1, Jones *et al.* (7), defatted 1st gluten extraction; 2, Maes (9), nonextracted; 3, Jones *et al.* (7), nonextracted.



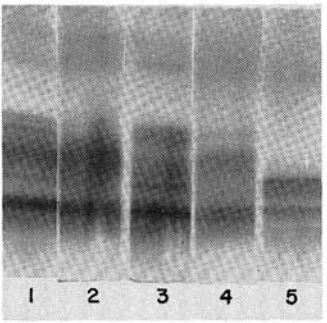


Fig. 7 (left). Starch-gel electrophoresis of 3.85% lactic acid solubles, following 2% NaCl. 2 hr., 45 ma. 1, lactic acid, Maes (9); 2, lactic acid, reduced; 3a, glutenin, nonreduced; 3b, glutenin, reduced.

Fig. 8 (right). Starch-gel electrophoresis of KOH and DMAE solubles. 3 hr., 45 ma. 1, 0.1% KOH solubles, Bison; 2, 0.5% KOH solubles, Bison; 3, 0.1*M* 2-dimethylaminoethanol; 4, 0.1% KOH Chinese Spring, nonreduced; 5, 0.1% KOH Chinese Spring, reduced.

the possibility of a globulin contamination in the lactic acid fraction, a flour sample was extracted with separate solvents of increasing concentrations of NaCl prior to lactic acid. Although not shown, the electrophoretic pattern was typical for a normally prepared lactic acid fraction.

The lactic acid fraction contained "gluteninlike" material which did not migrate during electrophoresis (gel 1). The lyophilized material of lactic acid solubles and conventionally prepared glutenin were reduced and alkylated. The "modified glutenin" penetrated the gel with no trace at the origin (gel 2) and the major band corresponded to that found in a conventionally reduced glutenin preparation (gel 3b). However, the second band from a reduced glutenin (gel 3b) was not present. Gel 3a is the nonreduced glutenin sample.

The electrophoretic pattern of the KOH solubles was not distinct (Fig. 8). The indistinct pattern may be attributed to a degree of protein denaturation. When the KOH concentration was reduced from 0.5% (gel 2) to 0.1% (gel 1), the pattern and the bands were more distinct. However, 0.5% extracted more protein than the 0.1%. The substitution of 2-dimethylaminoethanol (14) as a final solvent to replace KOH produced a pattern (gel 3) similar to that of 0.1% KOH. More of the 0.1% KOH soluble protein penetrated the gel (gel 1) than the 0.5% KOH soluble material (gel 2). This indicated increased electrophoretic mobility which could result from a change in molecular size or configuration. The reduced and alkylated 0.1% KOH solubles had two-thirds of the electrophoretic mobility of that which was not reduced (gel 5). This response was also observed by Woychik *et al.* (11). Reduction in mobility generally was true with gliadin and whole gluten fractions.

The precision of the modified Maes method with the variety Cheyenne is shown in Table II. The duplicates agreed reasonably well. Very careful collection of different eluants was necessary to reproduce the different con-

TABLE II
PRECISION OF FRACTIONATION OF CHEYENNE FLOUR PROTEIN

TRIAL	40% IPA	2% NaCl	3.85% LACTIC	0.5% KOH	TOTAL RECOVERY
	%	%	%	%	%
1	52.9	3.3	7.2	35.7	99.1
2	53.2	3.6	7.3	34.5	98.6

tents of the fractions. Total protein recovered on duplicate runs will check within $\pm$ 2%.

Wheat samples from the breeding or cytogenetic research are in short supply during the early part of a development program. The Maes fractionation method would appear to offer potential in reducing the sample size necessary for fractionation preceding electrophoretic studies. To investigate the feasibility of reducing the size of a sample, comparisons were made on 1.0-, 2.5-, and 5-g. samples of Bison flour (Table III).

TABLE III
NITROGEN RECOVERY OF BISON FLOUR PROTEIN

FRACTIONS	FLOUR SAMPLE		
	1 g.	2.5 g.	5 g.
	mg.	*mg.*	*mg.*
40% IPA	11.49	26.35	59.53
2% NaCl	0.56	1.61	2.98
3.85% Lactic acid	1.66	4.23	8.23
0.1% KOH	6.75	18.85	33.30
Total recovered	20.46	51.04	104.04
Total nitrogen	21.84	54.60	109.20
% Recovery	93.70	93.50	95.20

The 5- and 2.5-g. samples were placed on the same size of column as previously described. However, the 1-g. sample was placed on a propor-

tionately smaller column. The recovery data show reasonable agreement, especially with 1- and 5-g. samples. Recovery of the isopropyl alcohol solubles was less with the 2.5-g. sample on the large column, whereas recovery of the KOH solubles was more. Protein contents of the fractions isolated from the Maes column and with conventional methods are shown in Table IV. The protein content of 26.8% for the water-solubles prepared

TABLE IV

COMPARISON OF PROTEIN CONTENTS OF WHEAT PROTEIN FRACTIONS BY THE MAES AND CONVENTIONAL PROCEDURE

FRACTION[a]	PERCENT PROTEIN[b]	
	Water-Soluble	Gliadin
	%	%
Jones *et al.* (7)	72.5	83.6
Gehrke *et al.* (8)	74.2	80.3
Maes column	26.8	81.2

[a] From Bison flour 12.5% protein, 14% m.b.
[b] "As-is" on lyophilized sample.

by the Maes procedure was considerably lower than the 72.5 and 74.2% in the fractions obtained by conventional washing procedures. The higher values could be due to the removal of starch by centrifugation in the conventional procedures. Preliminary analyses for carbohydrates showed substantially more glucose in the Maes water-soluble fraction than with conventional methods. This was probably due to high levels of soluble starch eluted with the water-soluble material.

Amino Acid Composition of Fractions

The protein contents and the amino acid composition of Bison flour and various fractions isolated from the column are summarized in Table V. Nitrogen recovery following ion-exchange chromatography of the hydrolysates was 95 ± 5%. No corrections were applied for loss of threonine or serine during hydrolysis, and values are not reported for cysteine and tryptophan. The values for the amino acid composition of flour agree satisfactorily with those reported in the literature (15). The water-solubles were high in the basic amino acids, half-cystine, and methionine, but low in glutamic acid and proline.

Protein and amino acid levels for the water and 2% NaCl soluble fractions were variable, particularly the amino acids lysine, histidine, threonine, serine, glutamic acid, proline, and glycine. The amino acid composition of the 2% NaCl fraction was also in agreement with water-soluble composition reported in the literature (16).

The isopropyl alcohol fraction was characterized by low lysine, glycine, alanine, and aspartic acid content and high glutamic acid and proline content which is similar to reports for gliadin (16). The lactic acid fraction contained less proline and more serine than the flour. The amino acid composition of the lactic acid fraction differed from the KOH protein fractions (glutenin) in lysine, arginine, aspartic acid, glutamic acid, and proline. There-

TABLE V

AMINO ACID COMPOSITION OF ISOLATED PROTEIN FRACTIONS OF BISON FLOUR[a]

AMINO ACID	FLOUR	WATER 1ST	NaCl 1ST	NaCl 2ND	IPA[b] 1ST	LACTIC[c]	0.1%[c] KOH	0.5%[c] KOH
Protein[d], %	12.44	26.80	42.50	30.10	79.60	84.20	42.20	75.60
	g.	g.	g.	g.	g.	g.	g.	g.
Lysine	2.11	2.69	3.52	4.63	1.21	4.09	2.02	2.01
Histidine	2.21	2.06	2.48	3.48	2.11	2.55	1.90	1.89
Ammonia	3.17	3.23	3.24	3.30	4.08	3.12	3.30	3.31
Arginine	3.85	5.08	5.54	6.16	3.15	5.03	3.42	3.37
Aspartic acid	4.43	7.28	6.31	6.92	3.21	5.55	3.74	3.22
Threonine	3.10	3.07	3.45	4.25	2.62	3.59	3.59	3.50
Serine	5.27	3.43	3.91	5.40	4.80	6.65	5.84	6.03
Glutamic acid	36.26	27.30	24.50	23.20	36.81	29.90	34.50	32.05
Proline	12.60	12.24	11.34	9.10	15.81	9.06	12.60	12.12
Glycine	3.70	3.41	3.70	4.67	2.45	4.40	5.09	5.10
Alanine	3.07	4.49	4.55	4.67	2.65	4.06	3.33	3.31
½-Cystine	1.88	3.47	3.73	3.99	3.07	1.89	1.77	1.64
Valine	4.52	5.31	5.42	5.51	4.40	4.92	4.78	4.81
Methionine	1.54	2.01	2.20	2.15	1.88	1.84	1.88	1.99
Isoleucine	3.78	3.91	4.08	3.91	4.29	3.75	3.81	3.87
Leucine	7.07	7.14	7.51	7.16	7.19	7.09	7.29	6.62
Tyrosine	3.07	3.89	3.92	3.65	3.30	3.24	4.25	4.30
Phenylalanine	5.48	4.36	4.64	5.09	6.45	4.94	5.11	5.00

[a] Expressed as g. amino acid/16 g. total N.
[b] Isopropyl alcohol.
[c] Modified methods (2% NaCl).
[d] 14% m.b.

fore, the comparative electrophoretic studies of the reduced lactic acid fraction and glutenin, reported earlier, may be misleading. The major component of the reduced lactic acid may not be identical with that of the reduced glutenin, although the electrophoretic patterns are similar. The basic amino acids were high in the lactic acid fraction; this probably was due to the characteristic fast-moving bands.

The KOH fraction was high in glycine content. The general composition of the KOH fraction was similar to that of the gliadin. The amino acid compositions of 0.1 and 0.5% KOH fractions were similar. The data indicated general agreement in relative amino acid values of the water-solubles and alcohol solubles with those reported (16). The differences in method of preparation, varietal differences, and precision of the analytical methods account for some of the variation.

Literature Cited

1. OSBORNE, T. B. The proteins of the wheat kernel. Carnegie Inst. Wash. Publ. 84 (1907).
2. HUEBNER, F. R., and WALL, J. S. Improved chromatographic separation of gliadin proteins on sulfoethyl cellulose. Cereal Chem. 43: 325–335 (1966).
3. SIMMONDS, D. H. Proteins of wheat and flour. The separation and purification of the pyrophosphate-soluble proteins of wheat flour by chromatography on DEAE-cellulose. Cereal Chem. 40: 110–120 (1963).
4. WOYCHIK, J. H., DIMLER, R. J., and SENTI, F. R. Chromatographic fractionation of wheat gluten on carboxymethylcellulose columns. Arch. Biochem. Biophys. 91: 235–239 (1960).
5. JONES, R. W., BABCOCK, G. E., TAYLOR, N. W., and DIMLER, R. J. Fractionation of wheat gluten by gel filtration. Cereal Chem. 40: 409–414 (1963).

6. WRIGHT, W. B., BROWN, P. J., and BELL, A. V. A method of fractionation of flour proteins by means of gel filtration on sephadex G-100. J. Sci. Food Agr. 15: 56–62 (1964).
7. JONES, R. W., TAYLOR, N. W., and SENTI, F. R. Electrophoresis and fractionation of wheat gluten. Arch. Biochem. Biophys. 84: 363–376 (1959).
8. GEHRKE, C. W., OH, Y. H., and FREEARK, C. W. Chemical fractionation and starch gel-urea electrophoretic characterization of albumins, globulins, gliadins and glutenins in soft wheat. Anal. Biochem. 7: 439–460 (1964).
9. MAES, E. Mehleiweissloslichkeit und Backqualitat. III. Continuierliche, progressive Extraktion. Getreide Mehl 6(12): 70–72 (1962).
10. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Cereal laboratory methods (7th ed.). The Association: St. Paul, Minn. (1962).
11. WOYCHIK, J. H., HUEBNER, F. R., and DIMLER, R. J. Reduction and starch gel electrophoresis of wheat gliadin and glutenin. Arch. Biochem. Biophys. 105: 151–155 (1964).
12. WOYCHIK, J. H., BOUNDY, JOYCE A., and DIMLER, R. J. Starch gel electrophoresis of wheat gluten proteins with concentrated urea. Arch. Biochem. Biophys. 94: 477–482 (1961).
13. ELTON, G. A. H., and EWART, J. A. D. Starch-gel electrophoresis of cereal proteins. J. Sci. Food Agr. 13: 62–72 (1962).
14. MECHAM, D. K., INAMINE, E. S., and NOBLE, ELAINE. Gel filtration of the acetic acid-insoluble protein of wheat flour. Abstr., 51st Annual Meeting, American Association of Cereal Chemists (1966).
15. TKACHUK, R. Amino acid composition of wheat flours. Cereal Chem. 43: 207–223 (1966).
16. WOYCHIK, J. H., BOUNDY, JOYCE A., and DIMLER, R. J. Amino acid composition of proteins in wheat gluten. J. Agr. Food Chem. 9: 307–310 (1961).

[Received June 26, 1967. Accepted March 18, 1968]