Functional (Breadmaking) and Biochemical Properties of Wheat Flour Components. II. Role of Water-Solubles[1]

R. C. HOSENEY, K. F. FINNEY, M. D. SHOGREN, and Y. POMERANZ, Hard Winter Wheat Quality Laboratory, Department of Grain Science and Industry, Kansas State University, Manhattan 66502

ABSTRACT

When gluten was washed from flour, a portion of the flour became soluble in the wash water. The amount and composition of the material solubilized depended on the salt concentration in the wash water and therefore on the flour-to-water ratio. When a flour-water ratio was selected to exclude gliadin proteins, the water-soluble fraction of flour was not responsible for loaf-volume differences; nevertheless, it is required to produce a normal loaf of bread. Increasing the amount of water-solubles above the amount normally present in flour did not increase loaf volume. The albumin or globulin proteins were not involved in breadmaking performance. The water-solubles were found to have a dual role of (a) contributing to gassing power and (b) modifying the physical properties of the gluten. The dialyzable fraction (dialysate) of the water-solubles contributed as much to gas production as the total water-solubles, but not so much to loaf volume. One of the nondialyzable fractions contained the water-soluble pentosans and glycoproteins and was involved in the modification of gluten.

When flour is slurried with water, part of it becomes soluble. Finney (1) found that the water-soluble material was not responsible for differences in baking quality. However, the water-solubles were necessary for normal baking characteristics in two of his three reconstituted flours. Pence et al. (2) also found that soluble components were required for maximum performance of all glutens studied, except for a durum wheat. A crude albumin fraction isolated from the water-solubles was responsible for the largest volume response. Pence (3) stated that albumins are implicated in the baking performance of flours and may account for a significant part of the differences in baking characteristics. However, how the albumins influence rheological properties of dough has not been established (4).

Pence and Elder (5) found that albumins consist of at least six individual components of approximately the same molecular size but differing in electrophoretic mobility. The albumins were characterized by higher tryptophan and lower amido-nitrogen contents than other wheat proteins. The apparent average molecular weight of the albumins was about 28,000; however it decreased to about 17,000 in the presence of urea or sodium salicylate.

Pence et al. (6) found that both the albumin and globulin contents, as well as the ratio of albumins to globulins, varied significantly among 32 flours of widely varying types and baking qualities. The amount of soluble proteins increased with increasing flour protein, but decreased with increasing flour protein when expressed as percent of total flour protein. Electrophoretic patterns of albumins prepared from durum wheat flour differed from electrophoretic patterns of albumins from club and common wheat flour (7).

---

[1]Co-operative investigations between the Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, and the Department of Grain Science and Industry, Kansas Agricultural Experiment Station, Manhattan. Contribution No. 644. Presented at the 53rd Annual Meeting, Washington, D.C., April 1968.

Authors, respectively: Research Chemist, Research Chemist, Research Cereal Technologist, and Research Chemist, Crops Research Division, ARS, U.S. Department of Agriculture, Kansas Agricultural Experiment Station, Manhattan 66502.

Pence (3) found that omission of the globulin proteins from reconstituted doughs impaired breadmaking potentialities of the flour. However, it was not clear whether the globulins were essential, or whether the salt used to remove the globulins was detrimental. The globulins were characterized by low tryptophan, low amide-nitrogen, and high arginine contents (5). Koenig et al. (8) found that good-quality flours, which also had longer mixing times, contained more salt-soluble proteins than poor-quality flours. Mullen and Smith (9) obtained similar amounts of salt-soluble nitrogen from short- and long-mixing flours; however, electrophoresis showed quantitative differences in protein composition.

Water-extracts of flour contain a third group of proteins, probably the proteose Osborne reported (10). That group has been identified as glycoproteins by Kundig et al. (11, 12). Four glycoproteins were separated by fractionation on DEAE columns. The major glycoprotein fraction was shown to be responsible for the gelation of aqueous extracts of wheat flour in the presence of oxidizing agents. The principal glycoprotein fraction was found to contain, in addition to pentosans, about 15% protein, together with small amounts of phenolic compounds including ferulic acid (13). The ferulic acid could not be detected after oxidative treatment. Tracey (14) and Wrench (15) found that the carbohydrate part of the glycoprotein was necessary for normal baking characteristics.

The purpose of our present study was to determine if water-solubles from flours of widely varying baking quality differed in baking response. In addition, the role (in breadmaking) of certain fractions obtained from the water-solubles was investigated.

MATERIALS AND METHODS

Flours

Four flours were used. Quivira-Tenmarq X Marquillo-Oro, C.I. 12995, was a composite flour that possessed good loaf-volume potential and a relatively long mixing time. Regional baking standard (RBS) was a composite flour that had good loaf-volume potential and medium mixing time. Chiefkan X Tenmarq, K501099, was a composite flour having poor loaf-volume potential and short mixing time. Those three flours were milled from composites of wheats harvested at many locations throughout the southern and central Great Plains. Ottawa Selection, K14042, was milled from wheat harvested at Manhattan, Kansas. It had unusually poor loaf-volume potential and extremely short mixing time. Mixograms of the four flours are reproduced in Fig. 1.

Analytical Procedures

Protein and moisture were determined as described in *Cereal Laboratory Methods* (16). Mixograms were obtained as described by Finney and Yamazaki (17). Gassing powers were determined at 30°C., with gage-type pressuremeters, and on 10 g. flour with the same formula employed in baking except that shortening was omitted and water absorption was increased to 100%. The baking procedure described by Finney and Barmore (18,19,20) and Finney (21) was adapted by Shogren et al. (22) for 10 g. of flour. Standard deviation for the average of duplicate loaf volumes (10 g.) was 1.75 cc.

Starch-Gel Electrophoresis

The starch-gel electrophoretic technique was a modification of the procedure

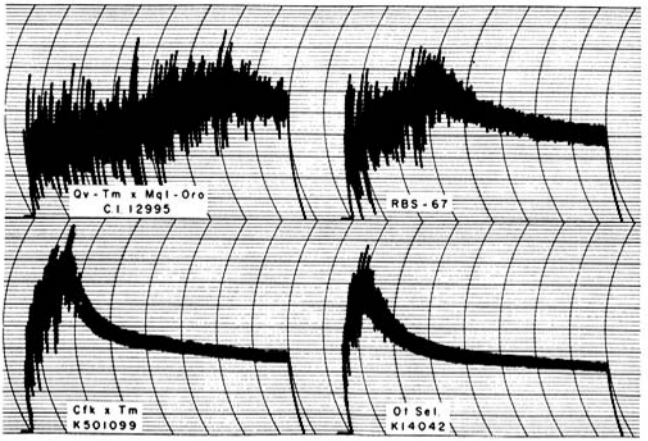


Fig. 1. Mixograms of typical hard winter wheat varieties that have excellent (C.I. 12995), good (RBS), poor (K501099), and extremely poor (K14042) mixing properties.

described by Woychik et al. (23). A vertical starch-gel apparatus eliminated electrodecantation and absorption effects inherent in the horizontal apparatus. Although both Ag-AgCl and platinum electrodes were used successfully, platinum electrodes were preferred for their greater stability and longer life. The buffer used, both in the electrophoretic tanks and to prepare gels, was 0.017M aluminum lactate and lactic acid pH 3.2 made to contain 3.0M urea. Improved separations were obtained when fresh buffer was prepared daily. Commercially available "acid-hydrolyzed" starch from Fisher Scientific Co., St. Louis, Mo., was used. To obtain gels rigid enough to stand in the vertical apparatus, starch was increased to 15% above manufacturer's recommendations of 10.1 to 10.6%. Urea in the buffer system improved separations but weakened the gel and greatly increased gelling time. The starch was pasted as recommended by the manufacturer, except that degassing of the starch paste by vacuum was found to be impractical, probably because of the large amount of carbon dioxide dissolved in the acidic buffer. As an alternative, the pasted starch was not poured until the large, trapped air bubbles rose to the top and were skimmed off with a spatula. The small bubbles either redissolved or rose to the top of the gel where they did not interfere. Handling properties of the gel were improved markedly when it was allowed to set, preferably 48 hr., at 4°C. prior to electrophoresis. Protein solution, 50 microliters, containing about 2 mg. protein dissolved in the aluminum lactate, lactic acid, and urea buffer, was pipetted into the preformed slots. The protein-buffer solution was frozen when stored for 12 hr. or more. The protein solutions were sealed in the slots with melted Vaseline. The exposed surface of the gel was covered with Saran Wrap to prevent loss of moisture. Protein migrated downward in a vertical gel. Reproducible patterns were obtained when a constant current was used (30 mA for the 12 × 32-cm. gels).

After electrophoresis was completed, the gel was removed from the mold and sliced to reduce its thickness one-half. The bottom half was stained and the top half discarded. Several dyes and dyeing techniques were studied. The adopted technique was to soak the gel overnight in 0.1% aqueous Amido Black 10B. Excess dye was removed by repeated changes of distilled water during about 2 days. The electro-

phoretic patterns were photographed immediately after washing, because they fade out completely in a few days. Amido Black 10B gave greater resolution than other dyes tested. In addition, less protein was leached from the gel when water was used as a dye solvent and for washing, instead of the generally recommended 5% acetic acid.

Fractionating Flour into Gluten, Starch, and Water-Solubles

Flour was fractionated into gluten and starch plus water-solubles by the procedure described by Shogren et al. (22). In addition, starch and water-soluble fractions were prepared by centrifuging the suspension of starch plus water-solubles at 1,000 g for 20 min. to separate the starch from the water-solubles. The starch was slurried with approximately 500 cc. distilled water and centrifuged again, and the combined supernatants (water-soluble fraction) of the two centrifugations were shelled and lyophilized. The starch centrifugate (starch fraction) was frozen in full-length sections on the inside wall of a 1-qt. jar, and lyophilized.

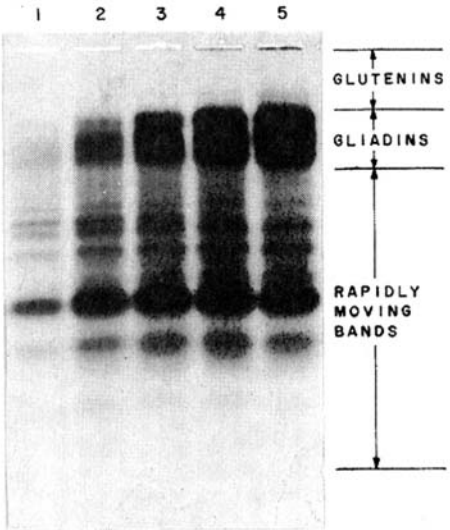


Fig. 2. Starch-gel electrophoretic patterns of proteins extracted with flour-to-water ratios of 1:3 (pattern 1), 1:5 (pattern 2), 1:10 (pattern 3), 1:20 (pattern 4), and 1:50 (pattern 5).

RESULTS AND DISCUSSION

Water-Solubility of Flour Proteins

Since flour is a biological system containing considerable salt (ash), any flour extract is a salt solution. Starch-gel electrophoretic patterns of extracts made with flour-to-water ratio (left to right) of 1:3, 1:5, 1:10, 1:20, and 1:50 are reproduced in Fig. 2. As the flour-water ratio decreased and, therefore, the salt concentration decreased, more and more gliadin was solubilized. At the lowest ratio, a small amount of glutenin was extracted. There also were several changes in the rapidly moving bands. Thus, the ratio of water to flour distinctly alters the amount and type of protein solubilized.

The flour-water ratio of about 3 to 1 (22) solubilized only trace quantities of

gliadin in the water-soluble fraction. The water-soluble fraction of flour was fractionated by the scheme given in Fig. 3, and characterized electrophoretically (Fig. 4). The water-solubles (pattern 1) were dialyzed and centrifuged. The salt-soluble and water-insoluble globulins (pattern 2) were precipitated. The supernatant (pattern 3) then contains small amounts of the gliadins plus the albumins and other water-soluble proteins. Since albumins are coagulated by heat, the dialyzed supernatant was boiled, cooled, and centrifuged to remove the albumins. The proteins that remained in solution (pattern 4), in addition to small amounts of gliadins, probably are glycoproteins as described by Kundig et al. (11,12) and Wrench (13). It appears that proteins solubilized when flour is extracted with water can be represented diagrammatically, as in Fig. 5.

Comparison of Flour Water-Solubles from Varieties of Widely Varying Baking Quality

The water-soluble fractions obtained from the three varieties, C.I. 12995 (good quality), K501099 (poor quality), and K14042 (extremely poor quality), were reconstituted with gluten and starch fractions from the RBS and baked into bread; baking data are given in the table below. All reconstitutes contained 13.1% protein, the protein of the unfractionated RBS flour. Water-solubles from varieties of widely varying flour quality were not significantly different. All flours had a water absorption of 65.0% and an oxidation requirement of 30 p.p.m. potassium bromate.

Source of Water-Solubles	Mixing Time	Loaf Volume
	min.	*cc.*
RBS (unfractionated flour)	4	83
RBS	3	83
C.I. 12995	3	82
K501099	3	82
K14042	2-7/8	81

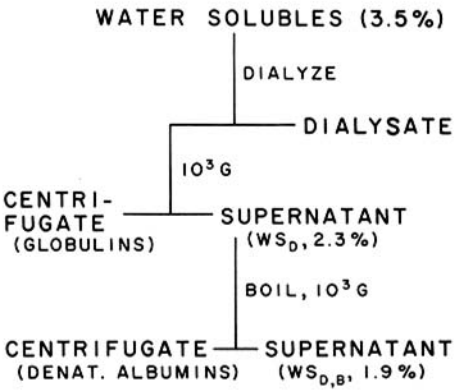
TABLE I. BAKING RESULTS WHEN RBS GLUTEN AND STARCH WERE RECONSTITUTED WITH 0, 0.5, 1.0, and 2.0 TIMES THE NORMAL LEVEL OF WATER-SOLUBLES

Original or Reconstituted Flour	Mixing Time *min.*	Baking Absorption %	Potassium bromate Requirement *p.p.m.*	Loaf Volume *cc.*
RBS flour	3¾	65.0	30	83
Gluten + starch	3	67.0	30	60
Gluten + starch + 3.6% WS[a]	2-5/8	66.0	30	83
Gluten + starch + 1.8% WS	2	66.0	30	70
Gluten + starch + 7.2% WS	2½	65.0	40	85

[a]The normal level of water-solubles (WS) was 3.6% of flour weight.

Fractionation of Water-Solubles

The water-soluble fraction from RBS flour represented approximately 3.5% of the total flour weight and contained about 27% protein that represented approximately 8.5% of the total flour protein. To investigate the role of specific RBS water-soluble fractions in breadmaking, the fractions were reconstituted with RBS



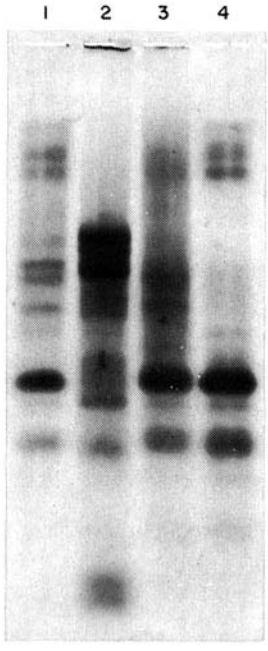


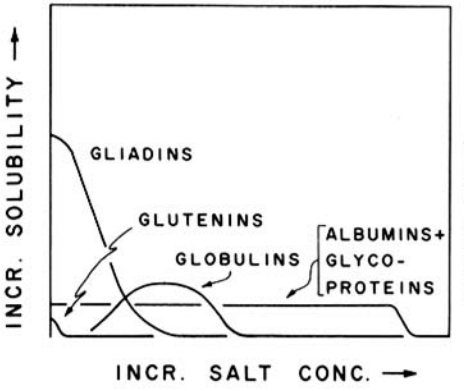
Fig. 3(left). Fractionation scheme employed to obtain certain water-soluble fractions of flour. Percentages are based on total flour weight. WS and subscripts D and B are abbreviations for water-solubles, dialyzed, and boiled, respectively.

Fig. 4(right). Starch-gel electrophoretic patterns of the following protein fractions that were obtained from the water-solubles: water-soluble proteins (pattern 1), globulins (pattern 2), albumins and glycoproteins (pattern 3), and glycoproteins (pattern 4).

gluten and starch and baked into bread. Gluten plus starch with no water-solubles gave a loaf volume of 60 cc. (Table I), whereas the complete reconstitute (gluten, starch, and water-solubles) gave a loaf volume of 83 cc. that was comparable to the loaf volume of the control RBS flour. Reconstitution of the gluten plus starch with 1.8% (half the normal level) water-solubles gave a loaf volume of 70 cc. Twice the normal level of water solubles (7.2%) gave no higher loaf volume than that of the normal level of 3.6%. Adding the water-soluble fraction increased loaf volume 23 cc. When the base volume of 23 cc. (the volume of flour containing no protein) was subtracted from each loaf volume, the gluten with water-solubles contributed 60 cc. (83–23), which was 62% more than the 37 cc. (60–23) contributed without the water-solubles.

Gassing powers were determined on the gluten plus starch, with and without water-solubles, as well as on the control flour and on baking ingredients without flour (Fig. 6). Flour materially increased the rate of gas production, and more than 50% of the increase was due to the water-solubles. The dialysate (material passing through the dialysis bag and recovered by lyophilization) increased gas production as much as the total water-solubles.

Baking data for reconstituted flours containing the water-soluble fractions are given in Table II. Reconstitution of the dialysate with the gluten and starch in-



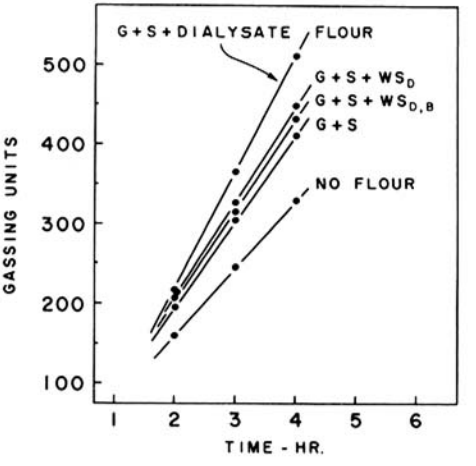


Fig. 5(left). Diagrammatic representation of proteins that were solubilized when flour was extracted with various concentrations of salt.

Fig. 6(right). Gas production vs. fermentation time of reconstituted RBS flours in which the water-soluble fraction and one or more of its components were omitted or replaced by yeast food. G, S, and WS and subscripts D and B are abbreviations for gluten, starch, water-solubles, dialyzed, and boiled, respectively. Other reconstituted flours (not shown) that had gas production equal to the original flour included the following: G + S + WS, G + S + 17.5 mg. Difco nitrogen base, and G + S + 5 mg. ammonium chloride.

creased loaf volume only to 72 cc., or about 55% of the total water-soluble response. Reconstituting the dialysate with the dialyzed water-solubles and gluten plus starch gave normal loaf volume and gassing power, even though the material

TABLE II. BAKING RESULTS FOR THREE WATER-SOLUBLE FRACTIONS THAT WERE RECONSTITUTED WITH GLUTEN AND STARCH FROM RBS FLOUR

Original or Reconstituted Flour	Mixing Time *min.*	Baking Absorption %	Potassium bromate Requirement *p.p.m.*	Loaf Volume *cc.*
RBS flour	3-3/4	65.0	30	82
Gluten + starch	3	67.0	30	60
Gluten + starch + WS[a]	2-5/8	66.0	30	83
Gluten + starch + dialysate	2-5/8	66.0	30	72
Gluten + starch + WS_D	2-5/8	66.0	30	73
Gluten + starch + WS_D + dialysate	2-5/8	66.0	30	82
Gluten + starch + $WS_{D\ \&\ B}$	2-5/8	66.0	30	70
Gluten + starch + $WS_{D\ \&\ B}$ + dialysate	2-3/4	66.0	30	81
RBS flour + YF	3-3/4	65.0	30	81
Gluten + starch + YF	3-3/4	67.0	30	73
Gluten + starch + WS_D + YF	3-1/2	66.0	30	84
Gluten + starch + $WS_{D\ \&\ B}$ + YF	3	66.0	30	80

[a] Abbreviations used:

WS = water solubles; WS_D = dialyzed water-solubles; $WS_{D\&B}$ = water-solubles dialyzed and boiled; YF = 17.5 mg. of yeast food.

precipitated upon dialysis (globulin proteins) was omitted. Since the dialysate appeared to be involved in gas production, a synthetic yeast food (Bacto yeast nitrogen base, Difco Lab., Detroit, Mich.) was used in its place. The dialysate could be replaced by 17.5 mg. of the yeast food per 10 g. flour.

The dialyzed water-solubles were boiled, cooled, and centrifuged to denature and remove the albumin proteins. The supernatant was lyophilized to recover the water-soluble fraction that was dialyzed and boiled. When that fraction was reconstituted with gluten plus starch and supplemented with dialysate or synthetic yeast food, loaf volumes and gassing powers were comparable to those of the control flour. Thus, after removal of the two major groups of water-soluble proteins (globulins and albumins), the remaining fraction plus a suitable yeast food still gave the entire water-soluble response. The dialyzed and boiled water-soluble fraction contained 26% protein and probably consisted of water-soluble pentosans and glycoproteins.

Although the water-soluble fraction was not involved in quality differences, it was required to obtain a normal loaf of bread with optimum volume. The role of the water-solubles in baking appeared to be twofold: first, the dialyzable fraction contributed to gas production, a contribution that could be replaced by suitable yeast food; and second, the fraction that contained soluble pentosans and glycoproteins contributed to gas retention and/or gluten extensibility.

Literature Cited

1. FINNEY, K. F. Fractionating and reconstituting techniques as tools in wheat flour research. Cereal Chem. 20: 381-396 (1943).
2. PENCE, J. W., ELDER, ANGELINE H., and MECHAM, D. K. Some effects of soluble flour components on baking behavior. Cereal Chem. 28: 94-104 (1951).
3. PENCE, J.W. The flour proteins. Cereal Sci. Today 7: 178-180, 208 (1962).
4. PENCE, J. W., and NIMMO, C. C. New knowledge of wheat proteins. Baker's Dig. 38(1): 38-42 (1964).
5. PENCE, J. W., and ELDER, ANGELINE H. The albumin and globulin proteins of wheat. Cereal Chem. 30: 275-287 (1953).
6. PENCE, J. W., WEINSTEIN, N. E., and MECHAM, D. K. The albumin and globulin contents of wheat flour and their relationship to protein quality. Cereal Chem. 31: 303-311 (1954).
7. PENCE, J. W., WEINSTEIN, N. E., and MECHAM, D. K. Differences in the distribution of components in albumin preparations from durum and common wheat flours. Cereal Chem. 31: 396-406 (1954).
8. KOENIG, V. L., OGRINS, A., TRIMBO, H. G., and MILLER' B. S. The electrophoretic analysis of flour from several varieties of hard red winter wheat grown at several locations. J. Sci. Food Agr. 15: 492-497 (1964).
9. MULLEN, J.D., and SMITH, D. E. Studies on short- and long-mixing flours. I. Solubility and electrophoretic composition of proteins. Cereal Chem. 42: 263-274 (1965).
10. OSBORNE, T. B. The protein of the wheat kernel. Publ. No. 84. Carnegie Inst. of Wash.: Washington, D.C. (1907).
11. KUNDIG, W., NEUKOM, H., and DEUEL, H. Untersuchungen uber Getreideschleimstoffe. I. Chromatographische Fraktionierung von wasserloslichen Weizenmehlpentosanen an Diethylaminoathyl-cellulose. Helv. Chim. Acta 44: 823-829 (1961).
12. KUNDIG, W., NEUKOM, H., and DEUEL, H. Untersuchungen uber Getreideschleimstoffe. II. Uber die Gelierung wasseriger Losungen von Weizenmehlpentosanen durch Oxydationsmittel. Helv. Chim. Acta 44: 969-976 (1961).
13. FAUSCH, H., KUNDIG, W., and NEUKOM, H. Ferulic acid as a component of a glycoprotein from wheat flour. Nature 199: 287 (1963).
14. TRACEY, M. V. The role of wheat flour pentosans in baking. I. Enzymatic destruction of pentosan in vitro. J. Sci. Food Agr. 15: 607-611 (1964).
15. WRENCH, P. M. The role of wheat flour pentosans in baking. III. Enzymatic degradation of pentosan fractions. J. Sci. Food Agr. 16: 51-54 (1965).

16. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Cereal laboratory methods (7th ed.). The Association: St. Paul, Minn. (1962).
17. FINNEY, K. F., and YAMAZAKI, W. T. *In* Wheat and wheat improvement, ed. by Quisenberry and Reitz. American Society of Agronomy: Madison, Wis. (1967).
18. FINNEY, K. F., and BARMORE, M. A. Yeast variability in wheat variety test baking. Cereal Chem. 20: 194-200 (1943).
19. FINNEY, K. F., and BARMORE, M. A. Varietal responses to certain baking ingredients essential in evaluating the protein quality of hard winter wheats. Cereal Chem. 22: 225-243 (1945).
20. FINNEY, K. F., and BARMORE, M. A. Optimum vs. fixed mixing time at various potassium bromate levels in experimental bread baking. Cereal Chem. 22: 244-254 (1945).
21. FINNEY, K. F. Methods of estimating and the effect of variety and protein level on the baking absorption of flour. Cereal Chem. 22: 149-158 (1945).
22. SHOGREN, M. D., FINNEY, K. F., and HOSENEY, R. C. Functional (breadmaking) and biochemical properties of wheat flour components. I. Solubilizing gluten and flour protein. Cereal Chem. 46: 000-000 (1969).
23. 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).

[Received April 15, 1968. Accepted August 20, 1968]