Functional (Breadmaking) and Biochemical Properties of Wheat Flour Components. IV. Gluten Protein Fractionation by Solubilizing in 70% Ethyl Alcohol and in Dilute Lactic Acid[1]

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

ABSTRACT

Gluten was fractionated into gliadin and glutenin with 70% ethanol. The ratio of gliadin to glutenin was essentially constant at 53% gliadin and 47% glutenin for four wheat varieties that varied widely in breadmaking quality. The glutenin proteins retained their functional properties, provided that certain rheological properties were restored by remixing. Reconstitution of the glutenin fractions of good-quality and poor-quality varieties with a fixed gliadin-rich fraction has shown that the gliadin proteins controlled the loaf-volume potential of a wheat flour. Similar reconstitution studies have shown that the glutenin fraction governed the mixing requirement of a wheat flour. Additional evidence supporting the above conclusions was obtained by reconstituting and baking protein fractions that were obtained by partially solubilizing gluten in dilute (0.002N) lactic acid. The fractions were characterized as gliadin-rich (soluble) and glutenin-rich (insoluble) by starch-gel electrophoresis.

Osborne (1) concluded that gluten was about 80% of the total flour protein and consisted of about equal amounts of gliadin, a prolamine soluble in 70% ethanol, and glutenin, a glutelin insoluble in 70% ethanol but soluble in dilute acid or base. Many workers proposed that the gliadin-to-glutenin ratio was important in determining gluten quality. Because of mounting evidence that neither gliadin nor glutenin is a homogeneous protein, this concept has been generally abandoned (2).

Finney (3) used fractionating and reconstituting techniques to show that the gluten proteins were responsible for baking-quality differences. Recent work (4) has indicated that the gliadin proteins were responsible for differences in loaf-volume potential between good-quality and poor-quality varieties.

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[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 State University. Contribution No. 662, Kansas Agricultural Experiment Station, Manhattan.

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Authors, respectively: Research Chemist, Research Chemist, Research Chemist, and Research Cereal Technologist, Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas 66502.

In the present study we fractionated gluten proteins into the classic gliadin and glutenin fractions with 70% ethanol. We then determined the contribution of each fraction to rheological properties and to loaf-volume potential, and whether any of the fractions was denatured by ethanol.

MATERIALS AND METHODS

Flours

The first three of the four flours mentioned below were milled from composites of wheat samples harvested at many locations in the Southern and Central Great Plains. Regional baking standard (RBS) was a composite of many wheat varieties, and had a flour protein content of 12.9% (14% m.b.), good loaf-volume potential, and a medium mixing requirement of 3-7/8 min. Quivira-Tenmarq X Marquillo-Oro, C.I. 12995, had a flour protein content of 12.5%, good loaf-volume potential, and a relatively long mixing requirement, 6-1/2 min. Chiefkan X Tenmarq, K501099, had a flour protein content of 13.7%, poor loaf-volume potential, and a short mixing requirement, 1-7/8 min. Ottawa Selection K14042 was milled from samples harvested at Manhattan, Kansas. It had a flour protein content of 17.1%, unusually poor loaf-volume potential, and an extremely short mixing requirement of 1 min. Mixograms of the four flours have been given previously (5).

Analytical Procedures and Starch-Gel Electrophoresis

Protein and moisture were determined as described in AACC Approved Methods (6). The baking procedure described by Finney and Barmore (7,8,9) and by Finney (10) was adapted by Shogren et al. (11) for 10 g. of flour. The standard deviation for the average of duplicate loaf volumes was 1.75 cc. The starch-gel electrophoretic technique was a modification (5) of the procedure described by Woychik et al. (12).

Fractionation of Flour into Gluten, Starch, and Water-Solubles

Flour was fractionated with distilled water into gluten and a mixture of starch and water-solubles by the procedure described by Shogren et al. (11). In addition, fractions of starch and water-solubles were prepared by centrifuging the mixture of starch and water-solubles at 1,000 X g for 20 min. The starch was slurried with approximately 500 cc. distilled water and centrifuged, and the combined supernatants (water-soluble fraction) of the two centrifugations were shelled and lyophilized. The starch centrifugate (starch fraction) was frozen on the inside wall of a 1-qt. jar, and lyophilized.

Preparation of Total Lipid

Total lipids were extracted from RBS flour. Five grams of flour was ground with 100 ml. of water-saturated n-butanol for 4 min. in a Stein mill, allowed to stand for 1 min., ground a second time for 2 min., allowed to stand for 1 min., and then ground a third time for 2 min. The butanol was decanted and replaced with fresh water-saturated butanol. The above procedure was repeated two more times. The extracts were filtered, combined, and evaporated under vacuum (below 40°C.). The residue was dissolved in petroleum ether and centrifuged, and the supernatant was evaporated to recover the total lipids. Lipids were reconstituted with the other flour components by mixing in a mortar.

RESULTS AND DISCUSSION

Fractionation with 70% Ethyl Alcohol

Glutens washed from the four flours of widely varying quality were fractionated into gliadins and glutenins. The ratio of gliadin to glutenin was essentially constant at 53% gliadin and 47% glutenin for the four varieties, regardless of mixing requirement. These values are in good agreement with those reported by Huebner (13). Reconstitution of the two fractions at the 53:47 ratio with starch-plus-water-solubles failed to result in a normal dough, and a poor loaf of bread was produced (Table I). Thus, one or both of the protein fractions appeared to be denatured.

As reported earlier (4), gluten can be fractionated without loss of its functional properties by solubilizing in 0.005N lactic acid and centrifuging at 100,000 X g. The centrifugate (100-5C) comprised 15% of the total protein and consisted of glutenin contaminated with globulins. The supernatant (100-5S) contained the remaining 85% of the total protein. Since the ratio of gliadin to glutenin, based on alcohol fractionation, was 53% gliadin and 47% glutenin, it was obvious that the 100-5S fraction contained about 68% of the glutenin as well as all the gliadin. The 100-5C glutenins were necessary to obtain a loaf volume that was comparable to the original unfractionated flour (4). Thus, it was possible to determine whether the glutenins (47% of total protein) obtained by fractionation with 70% ethanol had retained their functional properties in being substituted for the 100-5C glutenins. The fraction insoluble in 70% ethyl alcohol was reconstituted at an 85:15 ratio with the 100-5S fraction (obtained by ultracentrifugation) and a mixture of starch and water-solubles, and baked into bread (Table I). Although mixing produced a

TABLE I. BAKING DATA FOR RECONSTITUTED RBS FRACTIONS OBTAINED WITH 70% ETHYL ALCOHOL

Reconstituted Flour and Fraction Ratios	Mixing Time min.	Baking Absorption %	$KBrO_3$ Requirement p.p.m.	Loaf Volume cc.
RBS Flour	3-3/4	65.0	30	83
Soluble 70% ETOH 53 / Insoluble 70% ETOH 47	...	63.0	30	54
100-5S 85 / Insoluble 70% ETOH 15	1-3/8	62.0	30	56
100-5S 85 / Insoluble 70% ETOH 15 + TL[a]	1-3/8	62.0	30	66
[100-5S 25 / Insoluble 70% ETOH 75 + 100-5S (85/15)]	4-1/2	63.0	30	68
[100-5S 25 / Insoluble 70% ETOH 75 + 100-5S (85/15) + TL]	1-1/2	64.0	30	82
[100-5S 25 / 100-5S Glutenins 75 + 100-5S (85/15) + TL]	1-3/4	63.0	30	81

[a]TL is an abbreviation for 1.5% total lipid.

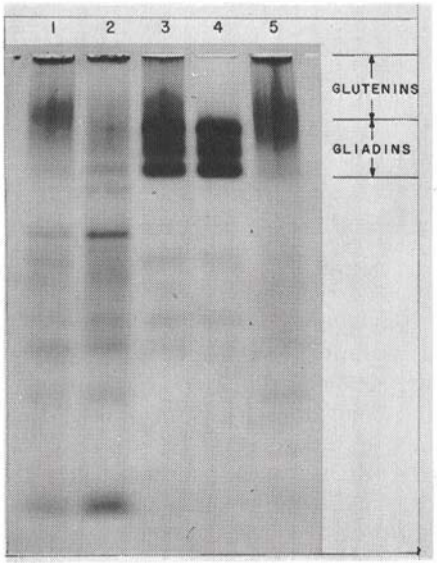
normal dough, the loaf volume (56 cc.) was still reduced substantially. Disregarding the obvious conclusion that the glutenins were denatured, we reasoned that 70% ethyl alcohol (being a relatively good lipid solvent) removed some of the essential lipids from the glutenin. Confirmation was obtained by subsequent lipid analysis. The fraction soluble in 70% ethanol contained 9% lipid, compared to 1.3% in the 100-5S fraction. Reconstitution of the 100-5S fraction and that insoluble in 70% ethyl alcohol at a ratio of 85:15 with starch and water-solubles and 1.5% total wheat-flour lipids gave a loaf volume of 66 cc., 10 cc. greater than that obtained without the lipids. Loaf volume, however, was still about 15 cc. below the control. The lumpy appearance of the dough and the mottled crust of the bread suggested that the glutenin had not interacted with the gliadins to form a homogeneous gluten-complex. Thus, it appeared that mixing time (point of minimum mobility) should be extended to allow interaction of the two fractions.

Mixing time was increased when the amount of glutenin was increased in the dough (11). Therefore, 10-g. reconstituted doughs were prepared from the 100-5S fraction and the fraction insoluble in 70% ethyl alcohol at a ratio of 15:75, plus starch and water-solubles, and mixed as a flour-water dough to optimum consistency (about 20 min.). This premixed dough was then frozen, lyophilized, and ground, after which 100-5S and starch-plus-water-solubles were added to give 10 g. of flour (14% m.b.) containing the protein content of the control flour and the 85:15 ratio of 100-5S and the fraction insoluble in 70% ethyl alcohol. When this partially premixed and reconstituted flour was supplemented with total lipids and baked, bread with normal loaf volume (82 cc.) and normal crumb characteristics was obtained (Table I). Thus, the glutenins (insoluble in 70% ethyl alcohol) retained their functionality, provided certain rheological properties were restored by premixing.

Comparison of the starch-gel electrophoretic patterns (Fig. 1) of the 100-5C fraction (pattern 2) and the fraction insoluble in 70% ethyl alcohol (pattern 1) shows one major difference. The fraction insoluble in 70% ethyl alcohol has two faint bands (in the glutenin area) that correspond to the two slowest-moving bands of the 100-5S fraction (pattern 3) prepared by ultracentrifugation. Yet, when glutenins were prepared on the basis of their insolubility in 70% ethanol, the two bands would be considered glutenin proteins by the classic definition. A pertinent question relates to whether the glutenins represented by those two bands performed the same function in baking as those (100-5C) retained at the origin, or whether their baking characteristics were similar to those of the gliadin proteins.

To answer the above questions, glutenins were prepared from the 100-5S fraction with 70% ethyl alcohol as described previously. The 100-5S glutenin fraction (Fig. 1, pattern 5) was premixed and reconstituted with the 100-5S fraction and starch-plus-water-solubles, and mixed with total lipids as described above for the fraction insoluble in 70% ethyl alcohol. Loaf volume of the bread (81 cc., Table I) was equal to that of the control. Therefore, since the 100-5S glutenins can replace the 100-5C glutenins in baking, they are glutenins with respect to their role in baking.

Previous work (4) has shown that the glutenin proteins sedimented at 100,000 X g (100-5C fraction) were not responsible for differences in loaf volume potential of good- and poor-quality varieties of wheat. Since the glutenins separated from the



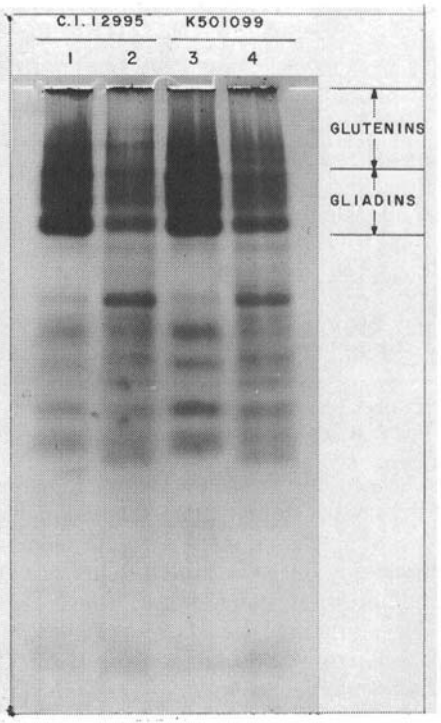


Fig. 1(left). Starch-gel electrophoretic patterns of protein fractions obtained by ultracentrifugation and solubilization in 70% ethyl alcohol of the pH 4.7-soluble gluten. The patterns represent the following fractions: insoluble in 70% ethyl alcohol (glutenins, pattern 1), 100-5C (pattern 2), 100-5S (pattern 3), soluble in 70% ethyl alcohol (gliadins, pattern 4), insoluble in 70% ethyl alcohol from 100-5S (100-5S glutenins, pattern 5).

Fig. 2(right). Starch-gel electrophoretic patterns of protein fractions obtained by partial solubilization of gluten in 0.002N lactic acid. The patterns represent the following fractions: 0.002N-S (supernatant, patterns 1 and 3) and 0.002N-C (centrifugate, patterns 2 and 4) from C.I. 12995 and K501099.

100-5S fraction can replace the 100-5C fraction, it can be assumed that the total glutenin was not responsible for differences in loaf-volume potential. That was checked by preparing glutenins (insoluble in 70% ethyl alcohol) from varieties that had good and poor loaf-volume potentials. Reconstitutions of glutenins with the 100-5S fraction and starch-plus-water-solubles from C.I. 12995 (Table II) show that the glutenin fraction of the variety with poor loaf-volume potential was just as good as that of the variety with good loaf-volume potential.

Fraction Responsible for Mixing Requirement

Mattern and Sandstedt (14) concluded that the factor responsible for mixing time was water-soluble. Smith and Mullen (15) and Shogren et al. (11), although obtaining similar data, showed that both the glutenin and gliadin fractions are required for normal mixing. In addition, as the ratio of gliadin to glutenin decreased, the mixing requirement became longer.

TABLE II. BAKING DATA FOR RECONSTITUTED FLOURS CONTAINING THE GLUTENIN FRACTIONS FROM GOOD- AND POOR-QUALITY VARIETIES RECONSTITUTED AT 12.5% PROTEIN WITH THE 100-5S FRACTION AND STARCH-PLUS-WATER-SOLUBLES FROM C.I. 12995

Original or Reconstituted Flour	Mixing Time min.	Absorption %	$KBrO_3$ Requirement p.p.m.	Loaf Volume cc.
C.I. 12995	6-1/2	65.0	20	79
K501099	1-7/8	65.0	40	61[a]
C.I. 12995 100-5S / C.I. 12995 Insol. 70% ETOH $\frac{25}{75}$ + 100-5S $\frac{85}{15}$ + TL[b]	3-1/4	65.0	20	80
C.I. 12995 100-5S / K501099 Insol. 70% ETOH $\frac{25}{75}$ + 100-5S $\frac{85}{15}$ + TL	3	65.0	30	79

[a]Corrected to 12.5% protein.

[b]TL is an abbreviation for 1.5% total lipids.

Since the ratio of gliadin to glutenin does not vary from short-mixing to long-mixing flours, the mixing requirement apparently is governed by differences in one of the fractions, or by the mode of interaction of the fractions. During the premixing of reconstituted flours, the time required to reach a point of minimum mobility was much longer for the long-mixing variety (C.I. 12995) than for short-mixing K501099 (Table III). The flours were reconstituted with starch-plus-water-solubles from C.I. 12995 to 12.9% protein. Half of the gluten protein was contributed by the 100-5S fraction and half by the fraction insoluble in 70% ethyl alcohol. Thus, the ratio of gliadin to glutenin was held constant at about 31:69, a considerably lower ratio of gliadin to glutenin than that (53:47) of the original flours. The high amount of glutenin was required to obtain a normal interaction of the two fractions, as discussed earlier. Since the gliadin and glutenin composition of the reconstituted flours was constant, it is obvious that mixing time was a function of differences in the glutenin proteins.

Undoubtedly, the mixing process involves an interaction of the gliadin and glutenin protein fractions, not only with each other but also with the other flour constituents; and altering the ratio of those constituents undoubtedly would alter mixing time.

Fractionation of Gluten by Partial Solubilization

Wet gluten, cut into small pieces and stirred in 0.002N lactic acid for 5 hr., was only partially solubilized. The insoluble protein (about 35% of total) was recovered after centrifugation at 1,000 X g for 20 min. After the supernatant and centrifugate were adjusted separately to pH 6.1 with 0.1N Na_2CO_3, the precipitate from the supernatant (0.002N-S) and the neutralized centrifugate (0.002N-C) were lyophilized.

Starch-gel electrophoretic patterns for those two protein fractions from the good-quality C.I. 12995 and the poor-quality K501099 are reproduced in Fig. 2. It is apparent that the gliadin proteins were solubilized to a greater extent than were the glutenin proteins. Since a constant amount of protein was applied to each starch-gel pattern, the proteins represented in patterns 2 and 4 (centrifugates) are

TABLE III. MIXING TIMES FOR RECONSTITUTED FLOURS CONTAINING 12.1% PROTEIN, 50% FROM THE 100-5S FRACTION AND 50% FROM THE FRACTION INSOLUBLE IN 70% ETHYL ALCOHOL, AND STARCH-PLUS-WATER-SOLUBLES FROM C.I. 12995

Source of 100-5S	Source of Insol. 70% ETOH	Mixing Time[a] min.	Source of 100-5S	Source of Insol. 70% ETOH	Mixing Time[a] min.
C.I. 12995	C.I. 12995	9	RBS	K501099	4
C.I. 12995	RBS	4-7/8	RBS	K14042	1-3/4
C.I. 12995	K501099	5-5/8	K501099	C.I. 12995	9
C.I. 12995	K14042	1-7/8	K501099	RBS	5-1/4
RBS	C.I. 12995	8-7/8	K501099	K501099	4
RBS	RBS	5-3/8	K501099	K14042	1

[a]Mixing times of the unfractionated flours were: C.I. 12995, 6-1/2 min.; RBS, 3-7/8 min.; K501099, 1-7/8 min.; and K14042, 1 min.

about twice as concentrated as those in patterns 1 and 3 (supernatants), in comparison with their relative concentrations in gluten.

The four fractions (0.002N-S and 0.002N-C from the poor-quality flour K501099, and 0.002N-S and 0.002N-C from the good-quality flour C.I. 12995) were reconstituted within each variety and interchanged between varieties. The two fractions 0.002N-S and 0.002N-C were reconstituted at a 65:35 ratio and to 12.5% protein with starch and water-solubles from C.I. 12995. Reconstituted loaves containing the 0.002N-S fraction from C.I. 12995 have volumes of 79 and 77; those containing 0.002N-S fraction from K501099 have volumes of 62 and 70 cc. (Table IV). It is noteworthy that the loaf of 77 cc. contained a small amount of K501099 gliadin in the centrifugate, and that the loaf of 70 cc. contained a small amount of C.I. 12995 gliadin in the centrifugate. Thus, the factor responsible for loaf-volume potential is located primarily in the 0.002N-S (gliadin-rich) fraction.

Doughs containing the 0.002N-C fraction from C.I. 12995 have mixing times of

TABLE IV. BAKING DATA OBTAINED WHEN THE 0.002N-S AND 0.002N-C PROTEIN FRACTIONS FROM C.I. 12995 and K501099 WERE RECONSTITUTED AT A 65:35 RATIO AND AT 12.5% PROTEIN WITH STARCH AND WATER-SOLUBLES FROM C.I. 12995

Unfractionated or Reconstituted Flour	Mixing Time min.	Baking Absorption %	$KBrO_3$ Requirement p.p.m.	Loaf Volume cc.
C.I. 12995, unfractionated	6-1/2	65.0	20	79
K501099, unfractionated	1-7/8	65.0	40	61[a]
C.I. 12995, 0.002N-S and -C	4-3/4	65.0	20	79
K501099, 0.002N-S and -C	1-7/8	63.0	40	62
C.I. 12995, 0.002N-S / K501099, 0.002N-C	2-3/4	65.0	30	77
K501099, 0.002N-S / C.I. 12995, 0.002N-C	3-1/2	64.0	30	70

[a]Loaf volume adjusted to 12.5% protein.

4-3/4 and 3-1/2 min. compared to 1-7/8 and 2-3/4 min. for doughs containing the 0.002N-C fraction from K501099. It is also noteworthy that the reconstituted dough that required 3-1/2 min. of mixing contained some K501099 glutenin in the supernatant, and that the reconstituted dough that required 2-3/4 min. contained some C.I. 12995 glutenin in the supernatant. Thus, the factor responsible for mixing time appears to be located in the insoluble (glutenin-rich) fraction.

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[Received September 9, 1968. Accepted April 4, 1969]