Functional (Breadmaking) and Biochemical Properties of Wheat Flour Components. III. Characterization of Gluten Protein Fractions Obtained by Ultracentrifugation[1]

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ABSTRACT

The gluten protein fraction governed the quality factors of mixing time and loaf volume. Solubilization of the gluten in 0.005N lactic acid (pH 4.7) left an insoluble fraction consisting of 5 to 8% of the total flour protein. The insoluble fraction had no specific role in breadmaking.

The pH 4.7-soluble gluten was fractionated by ultracentrifugation at 100,000 X g for 5 hr. Approximately 15% of the protein was recovered as centrifugate (100-5C) and 85% remained in the supernatant (100-5S). Starch-gel electrophoresis characterized the fractions as (a) proteins retained at the origin (centrifugate), and (b) proteins migrating into the starch gel (supernatant). The two fractions, when reconstituted and baked into bread, had loaf volumes and crumb grains equal to those of the original flour. The proteins migrating into the starch gel were responsible for loaf-volume potential when the two fractions from good- and poor-quality varieties were interchanged.

Since Finney (1) demonstrated that the gluten proteins were responsible for baking-quality differences, much progress has been made in protein fractionation techniques. Recent reviews (2,3,4) have emphasized the great amount of work being carried out on the gluten proteins. Relatively little work has been reported concerning the relation of specific gluten fractions to baking-quality differences. Mattern and Sandstedt (5) concluded that the principal factor responsible for determining the mixing requirement of wheat flour was water-soluble. Although Smith and Mullen (6) obtained similar data, they concluded that both water-soluble and water-insoluble proteins were involved.

The purpose of our study was to fractionate the gluten proteins into meaningful fractions and to determine the contribution of each to quality differences (primarily loaf-volume potential). The study was based on two premises: 1) The gluten protein complex is a functional protein system. Its function is to interact with itself and with other flour constituents to form a dough that will retain gas and produce bread of acceptable loaf volume and crumb grain. 2) No meaningful conclusion can be drawn unless the fractionated system can be reconstituted and its original functionality restored.

MATERIALS AND METHODS

Flours

Four flours were used. Quivira-Tenmarq x Marquillo-Oro, C.I. 12995, was a composite flour that had good loaf-volume potential and 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,

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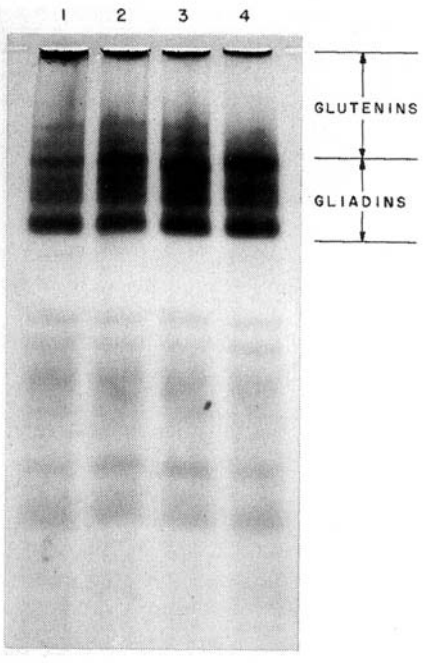


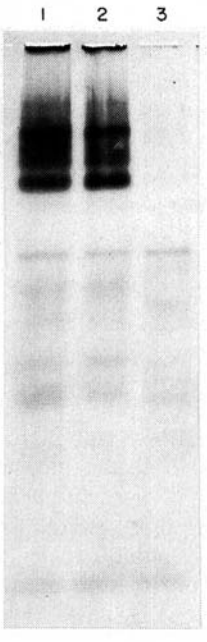


Fig. 1(left). Starch-gel electrophoretic patterns of glutens washed from hard winter wheat flours that have good (C.I. 12995, pattern 1, and RBS, pattern 2), poor (K501099, pattern 3), or extremely poor (K14042, pattern 4) quality characteristics.

Fig. 2(right). Starch-gel electrophoretic patterns of crude gluten (pattern 1), pH 4.7-soluble gluten (pattern 2), and the insoluble fraction (pattern 3).

was a composite flour that had poor loaf-volume potential and short mixing time. Those three flours were milled from a composite of wheats that were harvested at many locations throughout the southern and central Great Plains. Ottawa Selection, K14042, was milled from wheat that was harvested at Manhattan, Kansas. It had unusually poor loaf-volume potential and extremely short mixing time. Mixograms of the four flours were given previously (7).

Analytical Procedures

Protein and moisture were determined as described in *Cereal Laboratory Methods* (8). The baking procedure described by Finney and Barmore (9,10,11), and Finney (12) was adapted by Shogren et al. (13) for 10 g. of flour. The standard deviation for the average of duplicate loaf volumes by the 10-g. method was 1.75 cc.

Starch-Gel Electrophoresis

The starch-gel electrophoretic technique employed was a modification (7) of the procedure described by Woychik et al. (14).

Fractionation of 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. (13). In addition, starch and water-soluble fractions were prepared by centrifuging the starch plus water-soluble suspension 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.

RESULTS AND DISCUSSION

Starch-Gel Electrophoresis

Starch-gel electrophoresis was used as an analytical tool to follow the progress in fractionating proteins and to determine if the separations being made were meaningful.

Starch-gel electrophoretic patterns of crude glutens washed from flours milled from hard winter wheat varieties of widely varying quality are reproduced in Fig. 1. There were several differences among good-quality C.I. 12995 (pattern 1), good-quality RBS (pattern 2), poor-quality K501099 (pattern 3), and extremely poor-quality K14042 (pattern 4). Protein remaining at the origin appeared to decrease and gliadin proteins appeared to increase with decreasing quality. The extremely poor-quality variety K14042 (pattern 4) did not have the two slowest moving bands; in addition, the second gliadin band from the origin appeared to differ in mobility from the other three varieties. It cannot yet be stated whether any of the differences are causatively related to the observed differences in breadmaking quality.

Flour Fractions Responsible for Baking Quality

The fractionating procedure yielded dry, storable fractions that retained original baking quality when reconstituted and baked into bread (Table I).

To determine which fraction (or fractions) contains the factor (or factors) responsible for quality, the fractions obtained from C.I. 12995 (good-quality) and K501099 (poor-quality) were interchanged (Table I). Reconstituted flours with C.I. 12995 gluten contained the protein content (12.5%) of the original, unfractionated C.I. 12995 flour. Those containing K501099 gluten contained 13.7% protein, that of the original, unfractionated K501099 flour.

The quality factors governing mixing time and loaf volume were found in the gluten fraction. The starch fraction from K501099 had high water absorption, and K501099's gluten had relatively low absorption. The bromate requirement appeared to depend on both the gluten and water-soluble fractions.

Solubilization and Stability of Gluten

To study the gluten proteins, it is imperative that all or nearly all of the proteins be solubilized without impairing the loaf-volume potential of the gluten. No meaningful results concerning protein quality can be obtained unless the protein can be reconstituted with the other flour constituents and baked into a loaf of bread equal to that from the original, unfractionated flour.

On the basis of work by Shogren et al. (13), dilute lactic acid (0.005N) was used to dissolve the gluten, and the protein was recovered by adjusting pH to 6.1 with 0.1N sodium carbonate. The wet, crude gluten was scissored into small pieces and allowed to fall into 0.005N lactic acid. The suspension was stirred 5 hr. at 25°C. and then centrifuged at about 1,000 × g for 20 min. The centrifugate, representing

TABLE I. BAKING DATA FOR THE THREE ORIGINAL (UNFRACTIONATED) FLOURS AND RECONSTITUTED FLOURS IN WHICH THE FRACTIONS OF GOOD AND POOR VARIETIES WERE INTERCHANGED

Flours	Source of Gluten	Source of Starch	Source of Water-Solubles	Mixing Time	Absorption	Potassium Bromate Requirement	Loaf Volume
				min.	%	p.p.m.	cc.
Unfractionated	RBS			3-3/4	65.0	30	83
	C.I. 12995			6-1/2	65.0	20	79
	K501099			1-7/8	65.0	40	65
Reconstituted	RBS	RBS	RBS	3	65.0	30	82
	C.I. 12995	C.I. 12995	C.I. 12995	5-1/2	65.0	20	78
	K501099	K501099	K501099	1-3/8	65.0	40	64
	C.I. 12995	C.I. 12995	K501099	4-5/8	65.0	30	78
	C.I. 12995	K501099	C.I. 12995	5-3/8	69.0	20	79
	K501099	C.I. 12995	K 501099	1-1/8	62.0	40	66
	K501099	K501099	C.I. 12995	1	65.0	30	66

the "insoluble fraction" (Fig. 2, pattern 3), consisted of 5 to 8% of the total flour protein, together with starch not removed during the washing process. The supernatant (pH 4.7) was adjusted to pH 6.1 with 0.1N sodium carbonate, and the precipitated protein was collected by centrifugation. The "purified" gluten (pH 4.7 gluten fraction) was then frozen and lyophilized.

The pH 4.7-soluble gluten was investigated for stability after storing for various periods at 25°, 4°, and −18°C., and after heating to 90°C. for 5 min., followed by rapid cooling. The solution's stability to acid was studied by means of progressive lowering of the pH by addition of 0.1N lactic acid. In each case after the solution was adjusted to pH 6.1, the protein was collected by centrifugation and then frozen, lyophilized, ground, analyzed, and reconstituted with starch plus water-solubles and baked into bread.

Storing the pH 4.7-soluble gluten as little as 16 hr. at room temperature (Table II) lowered loaf-volume potential. At 4°C. the solution was stable 16 hr., then slowly lowered in loaf-volume potential. Heating to 90°C. for 5 min., to inactivate enzymes, seriously damaged loaf-volume potential. Storing the frozen pH 4.7 solution 30 days did not impair loaf-volume potential. Lowering the supernatant's pH below 4 impaired loaf volume.

Problems arose when the solubilization technique was applied to the varieties C.I. 12995 and K501099. Insoluble protein from C.I. 12995 was two to three times that from RBS flour, and appeared to be quite different from that obtained from other varieties because it centrifuged down as a gel. Those differences were overcome by allowing the C.I. 12995 gluten to "relax" in a closed container 5 hr. and then stirring it into lactic acid. The pH 4.7 gluten fraction from K501099, when reconstituted with starch and water-solubles and baked into bread, consistently gave loaf volumes 10 to 15 cc. less than that of the control. Since the K501099 gluten dispersed extremely rapidly in 0.005N lactic acid, lower concentrations of acid were tried. When the gluten of K501099 was stirred 5 hr. with 0.003N instead of 0.005N lactic acid, insoluble protein and loaf volume of reconstituted loaves were normal.

Baking data for reconstituted flours, in which the insoluble fraction from C.I.

TABLE II. EFFECTS OF STORAGE TIME, STORAGE TEMPERATURE, AND ACIDITY ON THE LOAF-VOLUME POTENTIAL OF THE pH 4.7-SOLUBLE GLUTEN. FLOURS WERE RECONSTITUTED FROM PRECIPITATED RBS GLUTEN AND STARCH PLUS WATER-SOLUBLES

	Mixing Time	Baking Absorption	Potassium Bromate Requirement	Loaf Volume
	min.	%	p.p.m.	cc.
Treatment				
Hours at 25°C.				
0	3	65.0	30	82
16	1-7/8	65.0	30	75
40	1-3/8	55.0	30	50
Hours at 4°C.				
16	2-3/8	65.0	30	83
40	2	65.0	30	81
80	1-5/8	65.0	30	77
Days at −18°C.				
30	2-7/8	65.0	30	82
Min. at 90°C.				
5	7	64.0	30	66
pH of solution				
4.7 (control)	3	65.0	30	82
4.0	2-5/8	65.0	30	83
3.5	3-3/8	65.0	30	79
3.0	6-3/8	67.0	20	71

12995 and K501099 were interchanged, are given in Table III. Loaf-volume potentials of the insoluble fractions of the two varieties did not differ. Therefore, loaf-volume potential is governed by the soluble gluten fraction. When the insoluble fractions were omitted in the reconstituted flours, loaf volumes were at least equal to those of corresponding unfractionated flours. Thus, the insoluble fraction has no specific role in breadmaking.

TABLE III. BAKING DATA FOR RECONSTITUTED FLOURS CONTAINING SOLUBLE GLUTEN (IN LACTIC ACID), INSOLUBLE FRACTION OF GLUTEN, AND STARCH PLUS WATER-SOLUBLES OF C.I. 12995 AND K501099, AND FOR RECONSTITUTED FLOURS IN WHICH THE INSOLUBLE FRACTIONS WERE OMITTED OR INTERCHANGED

Unfractionated and Reconstituted Flour		Mixing Time	Baking Absorption	Potassium Bromate Requirement	Loaf Volume
		min.	%	p.p.m.	cc.
C.I. 12995 unfractionated flour		6-1/2	65.0	20	79
K501099 unfractionated flour		1-7/8	65.0	40	65
C.I. 12995 soluble gluten[a] and	C.I. 12995	7-1/2	65.0	20	78
starch + WS, reconst. at 12.5%	None	6	65.0	20	80
protein with insolubles from:	K501099	6-1/2	65.0	20	78
K501099 soluble gluten[b] and	K501099	1-1/4	65.0	40	64
starch + WS, reconst. at 13.7%	None	1-1/2	65.0	40	66
protein with insolubles from:	C.I. 12995	1-3/4	65.0	40	64

[a]Soluble in 0.005N lactic acid. WS = water-solubles.

[b]Soluble in 0.003N lactic acid.

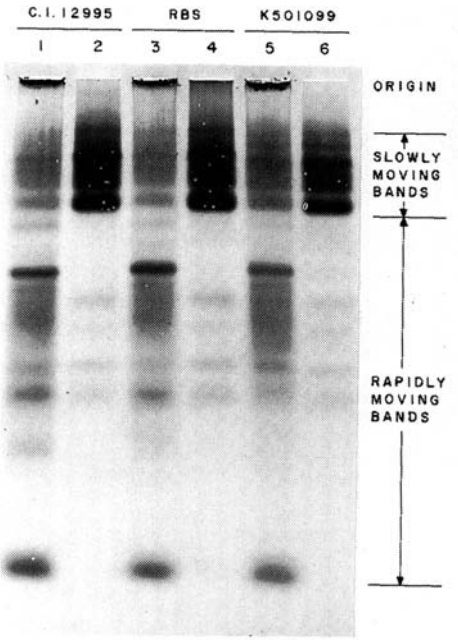
Fractionation of pH 4.7 Gluten by Ultracentrifugation

The work described thus far has shown the factor or factors responsible for quality to be in the gluten solubilized at pH 4.7. Thus, subsequent studies were concerned with developing fractionating techniques that did not denature the pH 4.7-soluble protein fractions (as measured by baking), and separating of the pH 4.7 gluten into meaningful fractions (as determined by starch-gel electrophoresis).

The starch-gel electrophoretic patterns for the pH 4.7 gluten (Fig. 2, pattern 2) point to two meaningful fractions, (a) proteins retained at the origin and (b) proteins migrating into the gel.

The pH 4.7-soluble gluten was fractionated on a preparatory ultracentrifuge. Ultracentrifugation at 100,000 × g for 5 hr. gave essentially the desired separation. Approximately 15% of the protein in suspension was thrown down as centrifugate and 85% remained in the supernatant. The two fractions, labeled 100-5C (centrifugate) and 100-5S (supernatant), were adjusted to pH 6.1, lyophilized, ground, and analyzed. Typical Kjeldahl protein values (14% moisture basis) were 65% for the 100-5C fraction and 85% for the 100-5S fraction.

Starch-gel electrophoretic patterns for the 100-5C and 100-5S fractions of C.I.



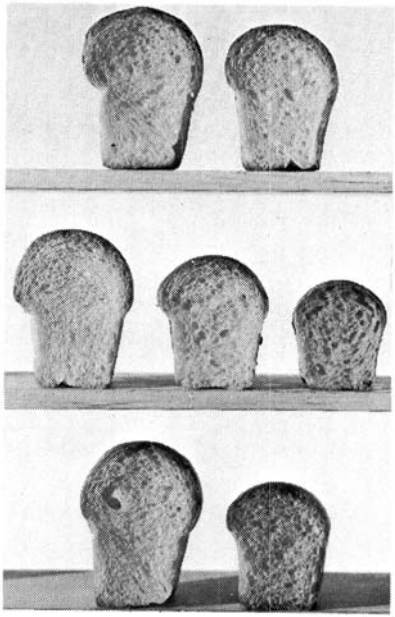


Fig. 3(left). Starch-gel electrophoretic patterns of the 100-5C (patterns 1, 3, 5) and 100-5S fractions (patterns 2, 4, and 6) of C.I. 12995, RBS, and K501099.

Fig. 4(right). Cut loaves of bread baked from reconstituted flours containing the 100-5S and 100-5C fractions of C.I. 12995, and reconstituted flours in which those two fractions of C.I. 12995 and K501099 were interchanged. All reconstituted flours contained 12.5% protein and C.I. 12995 starch and water-solubles. Loaves represent, from left to right: first row, original flours of C.I. 12995 and K501099; second row, 100-5S plus 100-5C, 100-5S, and 100-5C fractions of C.I. 12995; third row, 100-5S of C.I. 12995 plus 100-5C of K501099 and 100-5S of K501099 plus 100-5C of C.I. 12995.

TABLE IV. BAKING DATA FOR RECONSTITUTED FLOURS CONTAINING VARIOUS RATIOS OF THE 100-5S AND 100-5C FRACTIONS FROM RBS FLOUR

Original or Reconstituted Flour	Ratio	Mixing Time	Baking Absorption	Potassium Bromate Requirement	Loaf Volume
		min.	%	p.p.m.	cc.
RBS flour	.....	3-3/4	65.0	30	83
100-5S	100/0	1	61.0	30	70
100-5S/100-5C	94/6	1-1/2	62.0	30	79
	93/7	1-1/2	62.0	30	83
	92/8	1-3/8	62.0	30	80
100-5S/100-5C	91/9	1-1/8	62.0	30	79
	90/10	1-1/2	62.0	30	84
	89/11	1-1/4	62.0	30	83
	88/12	1-1/4	62.0	30	81
100-5S/100-5C	87/13	1-3/8	62.0	30	83
	86/14	1-3/8	62.0	30	82
	85/15	1-1/8	62.0	30	85
	84/16	1-3/8	62.0	30	84
100-5S/100-5C	80/20	1-3/8	62.0	30	81
	75/25	1-3/8	64.0	30	82
	50/50	2-5/8	66.0	30	76
100-5C	0/100	∞	70.0	30	43

12995, RBS, and K501099 are reproduced in Fig. 3. Nearly all protein too large to enter the starch gel was in the 100-5C fractions (patterns 1, 3, and 5). The six or seven slowest-moving bands were concentrated in the 100-5S fractions (patterns 2, 4, and 6).

The rapidly moving bands in both fractions of each variety are considered to be water-soluble and salt-soluble proteins that were trapped during gluten formation and therefore are impurities. Because a constant amount of protein was used for each electrophoretic pattern, the 100-5C fraction is about seven times as concentrated as it occurs in gluten.

Various ratios of the two fractions, 100-5C and 100-5S, from RBS were reconstituted with starch and water-solubles and baked into bread. The original 85/15 ratio and all others from 75/25 through 94/6 gave essentially normal loaf volumes (Table IV). The 100-5S fraction, when reconstituted with starch and water-solubles, gave below-normal loaf volume of only 70 cc. The 100-5C fraction, when reconstituted with starch and water-solubles, gave an extremely low loaf volume (43 cc.) and poor crumb grain. The dough appeared to have an infinite mixing time. The extremely short mixing times of the reconstituted flours composed of the 100-5S and 100-5C fractions are attributable, at least in part, to the rapid hydration rate of the lyophilized fractions. The 100-5S fraction absorbed water extremely fast. Air-drying the 100-5S fraction materially increased mixing time.

Similarly, the 100-5S and 100-5C fractions from the good-quality variety, C.I. 12995, and the poor-quality variety, K501099 (Fig. 3), were reconstituted at various ratios with their respective starch and water-solubles to the same protein contents of the original flours and were baked into bread. For both varieties, approximately 15% of the protein was collected as centrifugate. Ratios (100-5S/100-5C) of 80/20 to 90/10 for C.I. 12995 gave loaf volumes comparable to

TABLE V. BAKING DATA FOR RECONSTITUTED FLOURS CONTAINING VARIOUS RATIOS OF THE 100-5S AND 100-5C FRACTIONS FROM C.I. 12995 AND K501099 FLOURS

Original or Reconstituted Flour	Ratio	Mixing Time	Baking Absorption	Potassium Bromate Requirement	Loaf Volume
		min.	%	p.p.m.	cc.
C.I. 12995 Flour	.....	6-7/8	65.0	20	79
pH 4.7-soluble gluten	.....	6	65.0	20	80
100-5S	100/0	1-3/4	61.0	20	63
100-5S/100-5C	90/10	1-3/4	61.0	20	78
	87/13	2-7/8	61.0	20	79
	84/16	2-5/8	61.0	20	81
100-5S/100-5C	80/20	2-5/8	61.0	20	82
	50/50	5-3/4	67.0	20	69
100-5C	0/100	∞	70.0	20	40
K501099 Flour	.....	2-3/8	65.0	40	65
pH 4.7-soluble gluten	.....	1-7/8	64.0	40	55
100-5S	100/0	7/8	63.0	40	56
100-5S/100-5C	90/10	3/4	63.0	40	50
	87/13	7/8	64.0	40	56
	84/16	1	64.0	40	53
100-5S/100-5C	80/20	7/8	64.0	40	53
	50/50	1-5/8	67.0	40	56
100-5C	0/100	∞	70.0	40	43

that of the control (Table V). In general, the results were very similar to those obtained with RBS flour. The reconstituted flours of the poor-quality K501099 variety, however, were 10 to 15 cc. less than that of the control flour. As mentioned earlier, lower volumes were attributable to treatment with 0.005N lactic acid. The 0.005N instead of 0.003N lactic acid was used to obtain comparable fractions for both varieties. When gluten protein was replaced by the 100-5C fraction, the reconstituted flours had strikingly similar baking results, irrespective of variety.

It is of particular interest that the blends of the 100-5S and 100-5C fractions from RBS and C.I. 12995, at all ratios, gave loaf volumes 15 to 20 cc. higher than those calculated from the components of the blends. This was taken as evidence of interaction between the two fractions. No synergism was obtained with fractions from K501099. The loaf volumes were essentially equal to those calculated from components of the blends. Thus, when the poor variety was treated with 0.005N lactic acid, one or both fractions were altered in such a way that they did not interact.

The 100-5S and 100-5C fractions from C.I. 12995 and K501099 were interchanged as indicated in Table VI. All samples were reconstituted at an 85/15 ratio of 100-5S/100-5C, at 12.5% protein, and with starch and water-solubles from C.I. 12995. The baking results (see Table VI and Fig. 4) show conclusively that the 100-5S fraction controlled the volume of the loaf. The 100-5C fraction from K501099 was just as good as that from C.I. 12995 when each was baked with the 100-5S fraction from C.I. 12995. The 100-5C fraction from C.I. 12995 was no better than from K501099 when each was baked with the 100-5S fraction from

K501099. Thus, the factor or factors responsible for differences in loaf-volume potential were in the 100-5S fraction. The 100-5S, not the 100-5C, fraction from K501099 was damaged by 0.005N lactic acid.

TABLE VI. BAKING DATA FOR RECONSTITUTED FLOURS CONTAINING AN 85/15 RATIO OF THE 100-5S/100-5C FRACTIONS OF C.I. 12995 AND K501099, AND RECONSTITUTED FLOURS IN WHICH THE FRACTIONS OF THE TWO VARIETIES WERE INTERCHANGED. THE INTERCHANGED FRACTIONS WERE RECONSTITUTED TO 12.5% PROTEIN WITH C.I. 12995 STARCH AND WATER-SOLUBLES

Original or Reconstituted Flour	Mixing Time	Baking Absorption	Potassium Bromate Requirement	Loaf Volume
	min.	%	p.p.m.	cc.
C.I. 12995 unfractionated flour	6-1/2	65.0	20	79
K501099 unfractionated flour	1-7/8	65.0	40	61[a]
C.I. 12995 100-5S / 100-5C	2-5/8	61.0	20	81
K501099 100-5S / 100-5C	7/8	64.0	40	53
C.I. 12995 100-5S / K501099 100-5C	2	61.0	30	79
K501099 100-5S / C.I. 12995 100-5C	1-3/4	59.0	30	52

[a]Loaf volume adjusted to the protein content (12.5%) of C.I. 12995.

The 100-5S fraction was less complex than the original gluten because it contained fewer protein entities. Since all the proteins in the 100-5S fraction migrate electrophoretically into the starch gel, they apparently also represent lower-molecular-weight proteins of the gluten. The 100-5S fraction contains all the gliadin proteins, along with a significant part of the glutenin proteins. If the glutenin proteins in the 100-5S fraction perform a role in baking similar to that of the glutenin proteins in the 100-5C fraction, then the gliadin proteins would be responsible for differences in loaf-volume potential between good- and poor-quality flours.

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