STUDIES ON SHORT- AND LONG-MIXING FLOURS

II. Relationship of Solubility and Electrophoretic Composition of Flour Proteins to Mixing Properties[1]

D. E. SMITH and J. D. MULLEN

ABSTRACT

Mixing properties of doughs prepared from salt-soluble, water-soluble, and protein-starch residue fractions of short- and long-mixing flours were determined with the farinograph. Fractions were recombined and interchanged to determine (a) the role of the fraction in dough-mixing properties and (b) the fraction responsible for mixing differences between the flours. The mixing results were interpreted in terms of protein composition as determined by moving boundary electrophoresis and –SH and S–S content. Salt-soluble fractions (albumins and globulins) had little effect upon mixing characteristics. Protein-starch residues (glutenins) had long-mixing requirements, whereas the addition of water-solubles (gliadins) markedly shortened the mixing requirements. Combinations of these two latter fractions in the ratios obtained during fractionation resulted in doughs similar to the original flours. Short-mixing flour contained more water-soluble protein initially and more was produced during mixing. Additives which decreased protein solubility markedly increased mixing requirements. Study of –SH and S–S content of fractions indicated that mixing properties were not controlled by the total amount of these functional groups. The mixing differences of these two flours appear to be determined by undefined characteristics of the protein-starch residues and the quantity and molecular-weight distribution of the water-solubles.

The commercial acceptability of bread wheat flours is closely related to their mixing behavior. Considerable work has been done to learn the reason for different mixing characteristics of flours (1). An important contribution was made by Mattern and Sandstedt (2), who found that removal of water-solubles from flours markedly increased their mixing times, whereas readdition of these solubles or a gliadin preparation decreased the mixing requirements. Further evidence of the importance of solubles to mixing was that short-mixing flours produced more soluble protein than long-mixing flours. Work of Mecham *et al.* (3,4,5) has shown that the solubility of flour protein in 0.01*N* acetic acid increased during mixing. Characteristic differences in mixing requirements of flours were reflected in protein solubility changes. A short-mixing flour produced the most acid-soluble protein during mixing. More-vigorous mechanical action also produced more-soluble proteins with some flours. Mecham and co-workers also showed that addition of N-ethylmaleimide, (NEMI), a –SH

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blocking agent, to flour dough decreased the mixing time and increased the solubility of protein in 0.01*N* acetic acid but did not alter the solubility in water.

Considerable emphasis has been placed upon the relationship of mixing behavior to –SH reactions in the dough system (6). At present, sulfhydryl-disulfide interchange reactions are thought to be involved in changes in dough properties (7,8). Sulfhydryl-blocking agents decrease the mixing time and tolerance of doughs mixed in the farinograph, supposedly by removal of –SH groups, causing less interchange and greater rupture of disulfide bonds (6,9,10). Extensive cleavage of disulfide bonds during mixing to nonreducible groups, however, has not been observed (11,12). Losses of sulfhydryl occurring during mixing (12,13) appear to be related to the mixing properties of the flour.

A differential effect of NEMI, iodate, and oxygen upon the mixing behavior of flour and gluten doughs and upon the gel proteins of flour doughs has been observed by Bushuk and co-workers (10,14). They suggest that all of these agents are involved with –SH reactions but that reaction occurs to a different extent with each reagent.

Recent work of Sullivan *et al.* (15) and Tkachuk and Hlynka (16) emphasizes the difference in the reactivity of –SH groups in flour. About one-half of the –SH is quite labile and more readily oxidized than the other half. The most labile –SH groups appear to be water- and salt-soluble and react readily with NEMI. Because of the large effect of NEMI upon mixing, it is suggested that the soluble protein is most important in determining a farinogram pattern.

The work to be reported in this paper relates mixing properties of a short- and a long-mixing flour to the protein-solubility and electrophoretic data which were presented in the previous paper in this series (17). The sulfhydryl and disulfide contents of the fractions are also considered in relation to the dough properties.

Materials and Methods

Flours and flour fractions were prepared as described in the previous paper (17). In this fractionation process, preliminary separations were made into salt-soluble and salt-insoluble fractions. Later, the salt-insoluble fraction was further separated into a water-soluble fraction and a protein-starch residue fraction. The salt-soluble fraction contained albumins, globulins, and pentosans. The water-soluble fraction was rich in gliadin, whereas the protein-starch residue was rich in glutenin.

Farinograms were obtained with the 80-g. Brabender stainless-steel bowl at the second speed. Dry mixtures, when used, were

blended together in the bowl before the water was added in the usual manner. When salt, NEMI, or other additives were added, these were first dissolved in the water. A constant dough weight of 80 g. was used for all of the tests. Absorptions have been calculated on a material at 14% moisture basis. If possible, doughs were mixed within 20 B.U. of the 500 line.

The –SH and S–S values were determined by a modification of the method of Axford *et al.* (18). The flour was dispersed directly in the titrating solvent in a nitrogen atmosphere without the preliminary pasting. In addition to the reagents used by Axford, 1 ml. of 0.03*M* ethylene diamine tetraacetic acid solution was added and the titrations were carried out at 2°–4°C.

Water-solubilities of the proteins were determined on materials that had been ground in a Wiley mill to pass through a 40-mesh screen. A 2-g. sample of the dry material was magnetically stirred into 50 ml. of distilled water at 25°C. for 10 min. followed by centrifugation at 27,600 × *g* for 10 min. at 0°–5°C. The nitrogen content of a 25-ml. aliquot of the supernatant was obtained by the Kjeldahl method.

Results

Effect of Salt-Soluble Proteins upon Dough Properties. Farinograph results are given in Table I for defatted flours and recombined salt-insoluble and salt-soluble fractions. Levels of salt-solubles as recovered during fractionation were used. The effect of salt was also determined. As 20% of the salt-soluble fraction was ash, a level of NaCl was used equal to 20% of the amount of salt-solubles.

The Willet salt-insoluble fraction contained 15.6% protein and the Rodco 14.5% protein on a dry basis. These salt-insoluble fractions containing about 90% of the total protein in the flour had essentially the same mixing properties as the original flours. Thus, the salt-insoluble fraction contained the factor causing the major differences between the mixing properties of the flours.

Addition of NaCl to either the original flour or the salt-insoluble fraction decreased the absorption and increased the mixing time and tolerance of the dough. This effect of NaCl has also been described by Hlynka (19). Readdition of the salt-solubles to the salt-insolubles had an effect similar to that of adding salt.

Interchange of the salt-solubles showed that Rodco solubles resulted in somewhat higher absorption and stability. The higher absorption may be due to the higher pentosan content (17). The presence of more water in the system would increase the stability (19).

TABLE I

EFFECT OF SALT-SOLUBLES AND SALT UPON FARINOGRAMS

MATERIAL	ABSORPTION	MAXIMUM	TIME TO MAXIMUM	STABILITY
	%	*B.U.*	*min.*	*min.*
Defatted Willet flour	59.7	490	5	6
Defatted Willet flour + NaCl	57.2[a]	515	10	13
Willet salt-insolubles	63.3	500	5	6
Willet salt-insolubles + NaCl	61.0	525	8	10
Willet salt-insolubles + *Willet* salt-solubles	59.0	490	7	11
Willet salt-insolubles + Rodco salt-solubles	59.7	505	8	15
Defatted Rodco flour	63.6	500	23	20
Defatted Rodco flour + NaCl	61.6	520	30	20
Rodco salt-insolubles	65.2	500	19	14
Rodco salt-insolubles + NaCl	62.6	500	20	17
Rodco salt-insolubles + Willet salt-solubles	59.0	490	18	14
Rodco salt-insolubles + *Rodco* salt-solubles	59.9	500	21	21

[a] Values on doughs containing salt or salt solubles are not corrected for the amount of salt present in the system.

Effect of Water-Solubles and Protein-Starch Residues upon Dough Properties. As differences between the two flours were primarily found in the salt-insoluble fraction, the site of the differences was investigated further. The salt-insoluble fraction was separated into water-soluble and protein-starch residue fractions (17). These fractions were recombined at several water-soluble levels and tested. The results are given in Table II. Doughs are combinations of protein-starch residues and water-solubles except for the salt-insoluble controls. The water-solubles used were the Willet B fraction (second and third water extractions) and Rodco C fraction (fourth to sixth water extractions) except for the reconstituted samples (see footnote). These contained all of the water-solubles in the proportions obtained during fractionation of the salt-insoluble fractions and represent completely reconstituted systems. Protein contents of the residue and water-soluble fractions used were as follows (dry basis): Willet residue, 10.9%; Rodco residue, 11.9%; Willet solubles B, 91.7%; Rodco solubles C, 83.4%. The total quantity of protein present in each dough is shown.

The protein-starch residues of each flour had greatly extended mixing requirements. The Willet residue had a mixing time of 30 min. and the Rodco, 120 min. A Willet residue fraction with less water-solubles present had a mixing time of 110 min., showing that the mixing time of the residue depended upon the amount of water-soluble protein removed from it.

Readdition of water-solubles to the residues decreased the mixing

TABLE II

EFFECT OF WATER-SOLUBLES AND PROTEIN-STARCH RESIDUES UPON FARINOGRAMS

PROTEIN-STARCH RESIDUE	LEVEL OF SOLUBLES [a]	TOTAL PROTEIN	RATIO: HIGH MW/ LOW MW	ABSORPTION	MAXIMUM	TIME TO MAXIMUM	STABILITY
	%	g.		%	B.U.	min.	min.
A. Willet fractions							
Willet salt-insoluble control		6.6	1.6	63.3	500	5	6
Willet	0	4.4	2.2	69.1	500	30	18
Willet	1.75	5.0	1.9	70.2	480	18	14
Willet	3.5	5.5	1.8	70.2	500	12	10
Willet	7.2 [b]	6.6	1.6	68.4	520	8	8
Willet	14.0	9.0	1.3	70.6	510	6	10
B. Rodco fractions							
Rodco salt-insoluble control		6.0	2.0	65.2	500	19	14
Rodco	0	4.9	3.1	66.8	480	120	..
Rodco	1.75	5.4	2.5	66.7	510	66	42
Rodco	3.5	5.9	2.1	67.4	495	45	26
Rodco	6.5 [b]	6.4	1.8	65.3	510	28	16
Rodco	14.0	9.0	1.3	68.0	510	13	6

[a] Based on weight of dry material in system.
[b] Reconstituted samples.

times and stabilities in proportion to the level added. The effect on mixing times is shown in Fig. 1. Arrows show results at the level

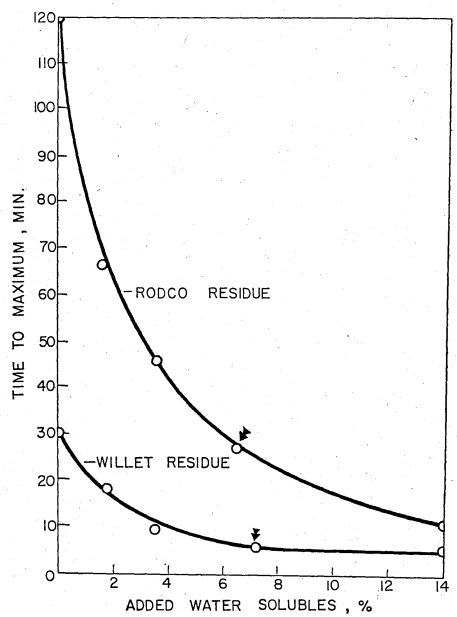

Fig. 1. Effect of water-solubles upon the mixing times of protein-starch residue doughs.

of solubles present in the flour.

Electrophoretic data were used to calculate a ratio of the high to low molecular weight components in the system using information on molecular weights of Jones *et al.* (20). This ratio relates the total alpha components plus the acid-insoluble material to the sum of beta, gamma, and omega components. Figure 2 shows the relationship between this ratio and the mixing time. Data with interchanged fractions are also included on the figure. As the ratio increases, the mixing time increases. The type of water-solubles used did not change the relationship, but two curves were obtained for the two different residues. These residues have different mixing requirements that are not related to differences in their observed electrophoretic composition. The Rodco protein-starch residues contain more acid-insoluble protein, possibly of higher molecular weight than Willet residue protein. This may account for the longer mixing requirements of doughs containing Rodco residues.

Water-solubles used in these experiments were of quite similar electrophoretic composition. These were obtained by extracting salt-insolubles from Willet flour three times and from Rodco flour six

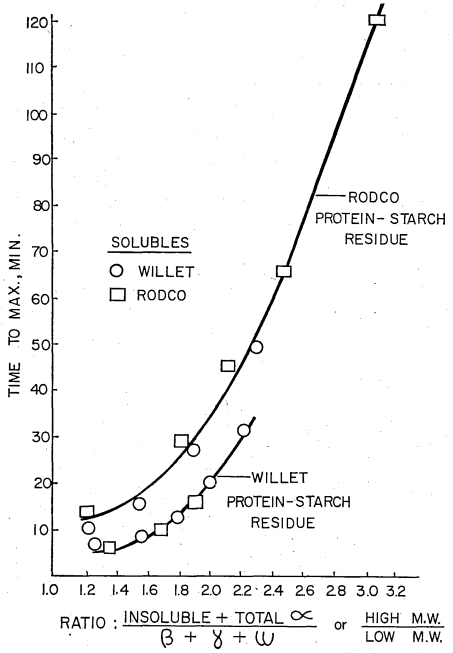

Fig. 2. Relation between farinograph time to maximum and ratio of high to low molecular weight components present in the doughs.

times. When water-solubles were prepared by extracting each salt-insoluble fraction four times, water-solubles were obtained with different composition. The latter Willet water-solubles contained more of the alpha component than the Rodco. Mixing results were obtained with these water-solubles and protein-starch residues. When these results were plotted as in Fig. 2, two curves were again obtained. This shows that Willet and Rodco water-solubles were similar in their effect upon mixing when the molecular weight ratio was considered. Thus, water-solubles have an identical functional relation between mixing time and molecular weight ratios, but protein-starch residues have a dissimilar relation between these properties.

Proteolysis furnished further evidence that the molecular size of the water-solubles had an effect upon the mixing properties. Willet solubles were partially digested with a proteolytic enzyme (Pronase), boiled to destroy the enzyme, and dried. Mixing properties of doughs made up of 95% Willet residues and 5% of untreated or Pronase-treated solubles were redetermined. Times to maximum were 8 min. for untreated solubles and 3 min. for the Pronase-treated. Mixing times decreased as the molecular size of the solubles decreased.

Untreated solubles were also taken up in water, boiled, and lyophilized. This material had essentially the same effect upon doughs as the original solubles, indicating that the mix-shortening effect of water-solubles is probably not enzymatic (2).

Changes in Solubility during Mixing. Willet and Rodco flour doughs were mixed for varying times, dried, and ground, and the water-soluble protein was determined. Results (Fig. 3) show that Willet initially contains more water-soluble protein and this increased further during mixing. Changes are much less with Rodco flour. The addition of certain anionic polysaccharides and detergents to flour doughs decreases the solubility of Willet protein and increases the mixing requirements (21). Conversely, the addition of certain dextrans to doughs decreases mixing requirements and appears to increase protein solubility (22). Changing of protein solubility, therefore, causes changes in dough-mixing behavior. Possibly Willet flour contains carbohydrates which could act as dextrans, or Rodco flour contains anionic materials which decrease protein solubility.

Relations of –SH and S–S Content of Fractions to Their Mixing Properties. The –SH and S–S contents of the Rodco fractions used in the reconstitution studies are given in Table III. Similar data were obtained for the Willet fractions.

The –SH values are markedly different for the three main flour fractions. Disulfide values differ less. Salt-solubles are much higher

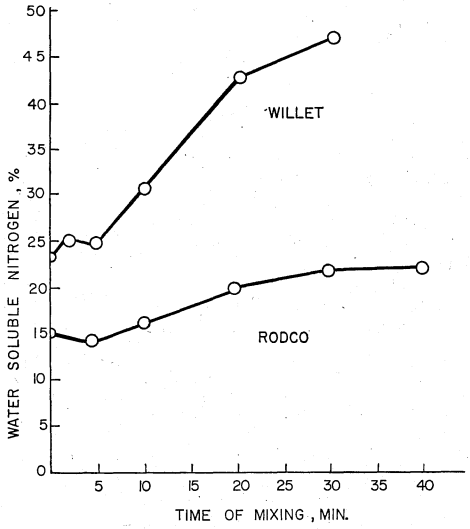

Fig. 3. Effect of farinograph mixing time upon water-solubility of proteins of Willet and Rodco doughs.

than water-solubles in both values on a protein basis. Salt-solubles contain about 55% of the –SH and 20% of the S–S present in the fractions recombined in the proportions in which they were obtained. Mixing properties of recombined Rodco fractions in relation to the amount of –SH in the system are shown in Table IV.

These data show that the proportion of the total –SH in the system does not determine the mixing requirement of the dough. Short or long mixing is determined by the type of protein-starch residues and the presence or absence of the water-solubles, and not by the level of –SH in the system.

TABLE III

–SH AND S–S VALUES ON RODCO FLOUR FRACTIONS

Fraction	In Material		In Protein		Percent of Total	
	–SH	S-S	–SH	S-S	–SH	S–S
	μeq./g.	*μeq./g.*	*μeq./g.*	*μeq./g.*	%	%
Rodco salt-solubles	3.94	26.6	15.6	105.1	55.1	21.8
Rodco water-solubles (A)	0.58	22.2	2.5	94.5	1.3	2.8
Rodco water-solubles (B)	0.45	37.3	0.7	59.2	1.6	6.2
Rodco water-solubles (C)	0.39	50.5	0.5	63.1	2.3	17.5
Rodco protein-starch residue	0.24	5.4	2.1	47.4	39.7	51.7

TABLE IV
MIXING PROPERTIES AND –SH VALUES OF RECOMBINED RODCO FRACTIONS

MATERIAL	ABSORPTION	MAXIMUM	TIME TO MAXIMUM	TOTAL –SH IN SYSTEM	–SH AS PERCENT OF –SH IN RECONSTITUTED DOUGH
	%	*B.U.*	*min.*	*g.*	%
Reconstituted Rodco	61.0	510	33	0.00078	100
Rodco residue and water-solubles	65.3	510	28	0.00036	46
Rodco residue	66.8	480	120	0.00034	44
Rodco residue and salt-solubles	55.1	505	130	0.00078	100

Effect of NEMI upon Mixing Properties and Protein Solubility of Doughs. The addition of NEMI to flour results in markedly decreased mixing requirements and stabilities (9). This effect is thought to be due to blocking of –SH groups, which would prevent interchange reactions. This results in greater strains in protein S–S linkages during mixing. It has been shown by Mecham *et al.* (4) that addition of NEMI to doughs increases the solubility of protein in dilute acids but does not appreciably change solubility in water. The previous paper (17) shows that addition of NEMI to flour doughs does not alter the distribution of gluten components in the acid-soluble material, nor does it appreciably change the amount of acid-solubles when the Waring Blendor is used for dispersion.

Results as given in Table V show the effect of NEMI upon the mixing properties of flour doughs or protein-starch residue doughs. Solubilities of the protein in water and 0.02*M* formic acid using the water-solubility method described earlier are also given. Willet and Rodco doughs were mixed 20 min. and 30 min., respectively.

TABLE V
EFFECT OF NEMI UPON DOUGHS

MATERIAL	–SH [a]	NEMI ADDITION	ABSORPTION	TIME TO MAXIMUM	STABILITY	WATER-SOLUBLE N_2	ACID-SOLUBLE N_2
	%	*mg.*	%	*min.*	*min.*	%	%
Willet flour	0.0022	..	58.4	3.5	3.5	41	85
Willet flour	0.0022	7	59.0	2	2	43	90
Rodco flour	0.0024	..	61.0	22	22	22	70
Rodco flour	0.0024	7	61.0	6	5	20	83
Willet residue	0.0010	..	69.1	30	18		
Willet residue	0.0010	7	69.1	9	4		
Rodco residue	0.0008	..	66.8	120	..		
Rodco residue	0.0008	7	65.6	35	18		

[a] –SH content of material before mixing.

The NEMI had a marked effect upon both the flour and the protein-starch residue doughs. The low level of –SH in the residues did not appear to decrease the effect of the NEMI. Solubility of the protein was not altered appreciably in water but was increased in acid when mild dispersion techniques were used. It is possible that molecular size may have been altered sufficiently by the NEMI to increase the solubility in acid but not in water.

Discussion

The results presented show that there are two major flour fractions that are primarily responsible for determining mixing properties. These are the water-soluble (gliadin) proteins and the more insoluble residue proteins. Recombination of these two fractions in different proportions results in mixtures with a range of mixing properties greater than that normally encountered in the most different flours.

Water-solubles affect mixing properties of doughs in relation to the ratio of high to low molecular weight components that they contain. The short-mixing flour contains proteins that are much more readily soluble in water than those from the long-mixing flour. The greater solubility of Willet flour protein undoubtedly is an important factor in causing this flour to be short-mixing. Some additives which decrease protein solubility have been shown to increase the mixing requirements of doughs (21).

Willet and Rodco protein-starch residues, combined with similar amounts of solubles, show considerable differences in their mixing properties. These residues contain factors that are also responsible for differences between the flours. Their electrophoretic analyses are similar and indicate large amounts of the alpha component. As alpha glutenin contains materials with a broad range of molecular weights (20), it seems probable that there might be molecular weight differences between the alpha glutenins of the two flours. Such differences could be responsible for mixing differences, but would not be detected by electrophoretic analyses. These differences may also account for protein-solubility differences. The difference in the mixing properties and protein-solubilities of these flours might also be due to the presence of different amounts of bound anionic materials.

Although our experimental observations agree with those of Mattern and Sandstedt (2), we do not agree with their conclusion that "the principal factor responsible for determining the mixing requirement of wheat flour is water-soluble." Our work shows that both the water-soluble and residue proteins are involved.

Salt-soluble fractions are high in –SH and S–S content but have

minor effects upon dough-mixing properties. Combinations of fractions with differing –SH contents indicate that the content of total –SH in the dough system does not control its mixing requirement. Furthermore, water-solubles, the fraction with the least amount of –SH, has the most dramatic effect upon dough properties.

There is considerable evidence in the literature that changes in the –SH and S–S content of doughs are related to changes in dough properties caused by mixing or oxidation. Determinations have usually measured changes in the total –SH and S–S content of the system. The present results show that at least one-half the –SH content of flour doughs is not of importance in relation to dough-mixing properties. This –SH not affecting mixing, which is present in the salt-soluble fraction, is the more labile –SH of flour (15). If any of the –SH present in flour is involved in changes that occur during mixing, it must be chiefly the –SH present in the protein-starch residue fraction, the least labile –SH fraction. Changes in the –SH and S–S content of the protein-starch residue fraction occurring during mixing have not been determined. The Willet and Rodco protein-starch residues, as prepared, have low and rather similar –SH and S–S contents.

The addition of NEMI to the protein-starch fractions has a very marked effect upon their mixing properties in spite of their low –SH content. This does not agree with the suggestion that NEMI acts primarily upon the –SH of the soluble protein (15).

Future workers relating reaction of –SH groups to dough-mixing properties should recognize that some of the –SH groups are undoubtedly not involved. Only farinograph curves were used to measure dough properties in these studies. A study should be made of the effects of oxidation and mixing upon other dough properties and baking behavior in relation to salt-soluble and salt-insoluble proteins and their –SH content.

It is interesting to speculate as to how these flour fractions determine farinograph patterns. Protein-starch residue doughs and long-mixing flours require oxygen to give their long-mixing curves (23). A gradual increase in dough viscosity occurs until the peak is reached. This may be caused by gradual accessibility and oxidation of –SH groups in the more insoluble glutenin proteins to form linkages as suggested by Meredith and Hlynka (24). The addition of water-soluble gliadin to these doughs decreases their mixing times. The mode of this action is not known, but the effect is related to the molecular size of the soluble proteins but apparently not to their –SH content. Such doughs with added solubles and short-mixing flour doughs give essentially the same mixing curves whether mixed in nitrogen or oxygen.

Addition of dextran sulfate to short-mixing doughs, however, decreases the amount of water-soluble gliadin produced during mixing and increases the mixing requirement of the dough, making the system sensitive to oxygen.

The mixing requirements of a dough appear to be controlled by two reactions: (a) a nonoxygen-sensitive mix-shortening effect of water-soluble gliadin proteins and (b) an oxygen-sensitive mix-lengthening effect involving water-insoluble glutenin proteins or associated materials. The mixing properties of a flour appear to be determined by the relative amount of each action which occurs.

The present results may serve as a link between the earlier characterization of proteins using solubility techniques (2,25,26), and the present, using electrophoretic techniques. By using quantitative methods with the total protein system of wheat flour and combining solubility and electrophoretic techniques, it has been possible to define some of the protein components that are important in determining dough properties. Additional studies are needed to explain the greater solubility of proteins from short-mixing flour and the greater mixing requirement of the protein-starch residues from long-mixing flour.

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