DOUGH-MIXING PROPERTIES OF CRUDE AND PURIFIED GLUTENS[1]

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ABSTRACT

Doughs were mixed from blends of glutens and low-protein flours in a recording dough mixer. Doughs containing crude glutens required the longest mixing to reach maximum resistance; progressively shorter times were required with glutens subjected successively to dispersion in dilute acetic acid and centrifuging to remove poorly dispersed material. These treatments of glutens also decreased dough stability. The effects of gluten treatments were similar with different flours; with changes in the ratio of gluten to flour fraction; with glutens from n-butyl alcohol-extracted flour; and with N-ethylmaleimide added to the doughs. The observations support a suggestion that dough mixing decreases the size of protein aggregates in flour.

When doughs are developed to an optimum state by mechanical mixing, the desirable bread characteristics that result are generally attributed to maximum formation of thin, continuous films of protein surrounding gas bubbles and starch granules. The films are considered to be made up of wheat flour proteins in a three-dimensional network of cross-linked protein chains. Sulfhydryl-disulfide interchange and hydrogen-bonding through amide groups (1,2,3) have been proposed as the most significant contributors in building up the network.

Axford, Campbell, and Elton (4) suggested another factor in dough development, but it has received little attention. In flour, wheat protein exists in an "undispersed" form. Presumably, aggregates of protein molecules are present; on addition of water these aggregates start to break up, and the protein molecules only then can be incorporated into a continuous network. Evidence that such aggregates are indeed present in flour has been presented (5,6). When protein was extracted with dilute acetic acid from a strong flour and from its flour-water doughs, the amount of protein removed was least from the flour and increased with dough development. The increase was obtained without change in extracting conditions; that is, pH, salt concentration, and other factors governing protein solubility were the same for flour and dough samples. It therefore is reasonable to assume that the large size of protein aggregates originally present in the flour prevented their dissolving, and that dough mixing decreased the size of

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aggregates until they became soluble. A voluminous, highly hydrated residue remained after the extraction of protein from a strong flour, whereas much smaller volumes of residue, decreased rapidly by mixing, were obtained from doughs. Conversion of the highly hydrated residue material to a soluble form thus appeared to occur.

It was thought that some protein aggregates might persist during the usual washing-out of gluten, because it has been observed repeatedly that after crude gluten is suspended in acetic acid solutions by blender treatment the suspension contains protein material that can be easily sedimented with low centrifugal forces. The poor solubility of the sedimented material indicates that it is more gluteninlike than gliadinlike; but regardless of its composition, its presence suggested that it might correspond in some degree to protein aggregates present in flour. In the experimental work presented in this paper, glutens were prepared which were expected to differ in the proportion of protein aggregates they contained. The glutens then were combined with a low-protein air-classified flour fraction, and dough mixing curves of the combinations were compared. The expected differences were observed, in that glutens subjected to the disaggregating effect of blender treatment and glutens recovered from dispersions after aggregates had been removed by centrifugation gave doughs less stable to mixing than crude gluten.

Materials and Methods

The flours used as sources of gluten were commercially milled unbleached long patents. Protein contents were 14.7, 13.4, and 10.7%, 14% moisture basis, for two hard red spring (HRS) wheat flours and a hard red winter (HRW) wheat flour, respectively. Corresponding ash contents were 0.39, 0.45, and 0.41%. These flours will be referred to as flours A, B, and C, respectively. Flour from Brevor wheat grown in Washington was air-classified to obtain the low-protein flour fractions (7). The fractions used in the present work contained 1.5, 1.6, and 2.1% protein, 14% moisture basis.

The procedures used for gluten preparation were essentially those of Jones *et al.* (8). They were used because glutens so obtained have been characterized by various physical and chemical methods (9,10,11).

Extraction of Flour Lipids. Flour (250 g., 13.6% moisture) was slurried in 500 ml. dry n-butyl alcohol at room temperature. The slurry was poured into a large filter paper in a ribbed funnel. The transfer of flour to funnel was completed with portions of n-butyl alcohol until a total of 1,250 ml. had been used. After drainage of the alcohol was completed, the washed flour was spread in an aluminum

pan in a hood and residual free alcohol allowed to evaporate. No attempt was made to remove bound alcohol before gluten was washed from the flour.

Washing of Gluten. Flour (200 g.) and 130 ml. 0.1% sodium chloride solution were mixed 1.5 min. in a Swanson-type mixer. The dough was covered with 0.1% salt solution, allowed to stand for 15 min., and kneaded in changes of salt solution until the wet gluten ball weighed 100 to 110 g. in the case of the HRS wheat flours or 77 to 81 g. in the case of the HRW wheat flour.

Preparation of Gluten Samples. To provide uniform starting material, wet gluten balls totaling 405 g. were kneaded together. An 81-g. portion then was removed, pressed between dry ice cakes, and freeze-dried to provide *crude gluten.*

The remaining 324 g. wet gluten was dispersed in four 846-ml. portions of 0.01*N* acetic acid by blending each portion for 5 min. in a closed 1-qt. jar (to restrict foam formation) on a kitchen blender. The portions were combined, their total volume and nitrogen content determined, and one-fourth the dispersion was freeze-dried to provide *dispersed gluten.*

The remaining steps in treatment of the gluten dispersion were centrifuging at 2,000 × *g* for 30 min.; centrifuging at 20,000 × *g* for 30 min.; and heating to inactivate proteolytic enzymes. The heat-treatment was carried out on approximately 400 ml. of centrifuged dispersion in a 1-liter Erlenmeyer flask, using a hot plate, with constant stirring. Heating was stopped when the dispersion reached 98°C. (in approximately 8 min.). Nitrogen contents of the dispersions were determined after each step and portions of the dispersions were freeze-dried. The glutens obtained from these steps are referred to as *2,000* × g *gluten, purified gluten,* and *heated-purified gluten.*

The fractions freeze-dried from dispersion were very fluffy and porous. To bring them to approximately the same physical state as the crude gluten, they were wetted with the minimum of water required to work them into gluten balls, and these were pressed between dry ice cakes and again freeze-dried.

The dried materials were ground in a Wiley mill and exposed to room air for 2 days to permit them to absorb moisture and approach equilibrium with the ambient air. Nitrogen determinations were made again at this point.

The gluten samples finally were combined with a Brevor low-protein flour fraction in proportions given below. The mixtures were sifted through a 60-mesh screen.

Dough Mixing Curves. Mixing curves were obtained using a mixograph recording dough mixer or a farinograph. The glutens recovered from acetic acid dispersions retained some acetic acid. In making doughs with these glutens, enough 0.1*N* sodium hydroxide was added to raise the dough pH to that of the crude gluten. To match any effects of the sodium and acetate, to the crude gluten dough was added a portion of 0.1*N* sodium hydroxide approximately equal to that added to the refined gluten doughs, and an equal amount of 0.1*N* acetic acid which maintained the pH.

All mixograph and farinograph doughs contained 2% sodium chloride, flour basis. When N-ethylmaleimide (NEMI) was added to mixograph doughs, the amount was 2.94 mg. (35 μequiv.) to each 35 g. of gluten-flour fraction mixture.

Experimental Results

Gluten Preparations. The percentages of gluten protein recovered after the acetic acid dispersions were centrifuged, and the protein contents of the freeze-dried preparations, are given in Table I. The centrifuging steps removed a maximum of 10% of the protein from the dispersions, divided usually about equally between the 2,000 and

TABLE I

GLUTEN RECOVERY AND PROTEIN CONTENTS OF GLUTEN PREPARATIONS

FLOUR	GLUTEN	RECOVERY OF GLUTEN N AFTER CENTRIFUGATION	PROTEIN[a] CONTENT
		%	%
HRS-A	Crude	..	69.8
	Dispersed	..	73.0
	2,000 × *g*	99	..
	Purified (20,000 × *g*)	93	84.6
HRS-B	Crude	..	73.0
	Dispersed	..	71.5
	2,000 × *g*	96	83.5
	Purified (20,000 × *g*)	92	84.1
	Heated-purified	..	83.9
n-BuOH — washed	Crude	..	75.2
	Dispersed	..	74.7
HRS-B	2,000 × *g*	95	87.0
	Purified (20,000 × *g*)	90	89.0
	Heated-purified	..	89.7
HRW-C	Crude	..	76.1
	Dispersed	..	75.2
	2,000 × *g*	96	86.4
	Purified (20,000 × *g*)	93	86.9
	Heated-purified	..	85.2

[a] Not corrected for moisture content. At equal moisture content, crude and dispersed glutens would be expected to have equal contents, as would purified and heated-purified. Values are given only to show that the 2,000 × *g* centrifuging step removes appreciable nonprotein material, and the 20,000 × *g* removes little nonprotein.

20,000 × *g* treatments. The protein levels in the recovered glutens indicate that essentially all starch and other sedimentable nonprotein material was removed by the 2,000 × *g* centrifuging, and that the second centrifuging removed largely protein. Protein levels in the glutens from n-butyl alcohol-washed flour were higher (after centrifuging) than in glutens from washed flour, indicating the expected lower content of lipids.

Mixing Curves: Glutens from Flour A (HRS). Crude, dispersed, and purified glutens were prepared from the HRS flour A. Glutens at these three stages of the preparative procedure were chosen after trials had indicated they definitely differed in properties. The crude gluten contained all the gluten proteins and served as a reference; the dispersed gluten had the same composition as the crude gluten, but it had been subjected to the shearing action of a blender and its proteins were presumed to be in a less aggregated state; and the purified gluten was the dispersed gluten from which the larger remaining aggregates had been removed.

Mixtures of the glutens with the Brevor flour fraction of 1.6% protein content were made to contain 12.3% protein, 14% moisture basis. Absorptions determined in the farinograph were 57.0, 56.2, and 53.8% (14% m.b.) for the crude, dispersed, and purified samples, respectively. Curves are shown in Fig. 1. The peak time on all three samples was short, with differences too small to be significant (read

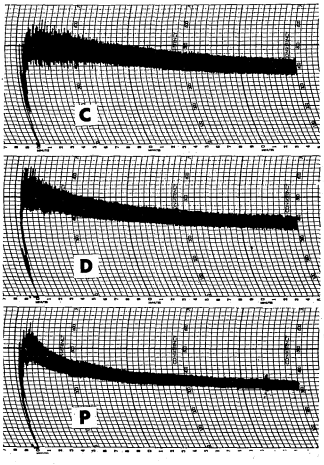

Fig. 1. Farinograph curves for blends of glutens with low-protein flour fraction. Crude gluten (C, top), dispersed gluten (D, center), and purified gluten (P, bottom) from flour A (HRS) mixed with flour fraction of 1.6% protein content to give blends of 12.3% protein content, 14% moisture basis. Absorptions, 57.0% (C); 56.2% (D); and 53.8% (P). All doughs contained 2% sodium chloride, flour weight basis.

as 2.0, 1.8, and 1.5 min., respectively). The rate of breakdown, however, was increased by the dispersion and purification steps; for example, after 15 min. of mixing the curve heights were 420, 400, and 345 Brabender Units.

Two series of mixograph curves then were obtained. In the first series, 35 g. of sample on 14% m.b. was used with the absorption as determined by the farinograph; in the second, 35 g. sample on an "as is" basis was used with a constant amount of added water that gave absorptions of 54.0 to 54.2%. Results are given in Table II and Fig. 2. In the Series I doughs, peak height remained essentially unchanged,

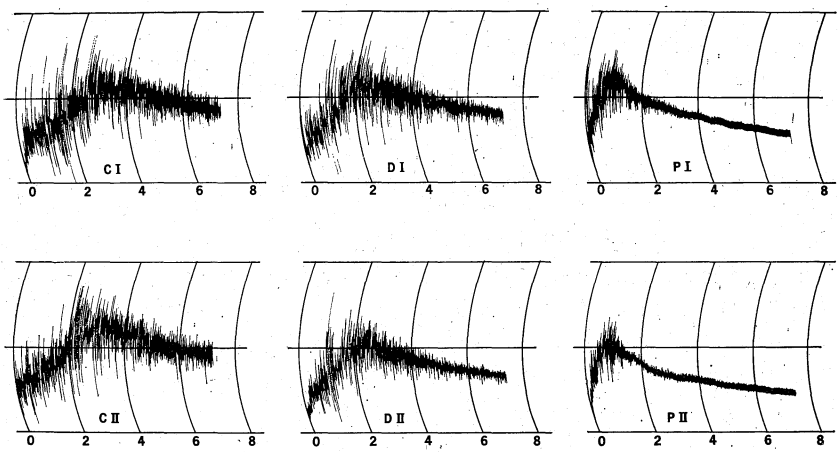

Fig. 2. Mixograph curves for blends of glutens with low-protein flour fraction. Crude gluten (C), dispersed gluten (D), and purified gluten (P) from flour A (HRS) mixed with flour fraction of 1.6% protein content to give blends of 12.3% protein content, 14% moisture basis. Series I curves were obtained using absorptions as determined on the farinograph, 14% moisture basis (57.0, 56.2, and 53.8% for C, P, and D, respectively). Series II curves were obtained using 35 g. gluten-flour fraction blend, "as is" moisture basis, and the same amount of added water in all doughs (giving 54.0 to 54.2% absorption, 14% moisture basis).

but time to peak decreased from 3 to 2 to 1 min. Breakdown also was faster in the dispersed and purified glutens. In Series II, at nearly constant absorption,[2] peak heights decreased with treatment. Time to peak also decreased nearly as much as with adjusted absorption, but there was little change in rate of breakdown. Comparison of the two sets of results indicates that either procedure would reflect effects of treatment; changes in peak height in the second set roughly correspond to differences in absorption in the first.

[2]Moisture contents of the flour fraction-gluten blends varied only from 9.2 to 9.6%; the blends contain about 85% of the low-protein flour fraction, so that differences in moisture arise only from the 15% of gluten preparation that is added.

TABLE II

CHARACTERISTICS OF MIXOGRAPH CURVES OF DOUGHS CONTAINING GLUTENS FROM HARD RED SPRING WHEAT FLOUR A

GLUTEN	CURVE CHARACTERISTICS [a]		
	Time to Maximum	Maximum Height	Height after 5 min. Mixing
	min.	*m.u.*	*m.u.*
Series I			
Crude	3.0	580	500
Dispersed	2.0	580	470
Purified (20,000 × g)	1.0	600	360
Series II			
Crude	2.5	620	520
Dispersed	1.5	540	390
Purified (20,000 × g)	0.8	520	280

[a] 12.3% protein (14% moisture basis) in gluten-flour fraction blend. Series I: 35 g. blend (14% moisture basis), 2% sodium chloride, water, acid and base added as described in text using farinograph absorptions. Series II: 35 g. blend ("as is" moisture basis; moisture contents ranged from 9.2 to 9.6%), 2% sodium chloride, water, acid and base added, as described in text using the same amount of added water for all samples (which gave 54.0–54.2% absorption).

Mixing Curves: Glutens from Flour B (HRS). Glutens from this flour were compared at different protein levels and with added NEMI, and were prepared also after the flour had been washed with n-butyl alcohol. Incomplete series or pairs of samples were used, but the observations extend the range of conditions covered and support those described in the preceding section.

For example, crude and dispersed glutens were mixed with the Brevor flour fraction of 1.5% protein content in ratios of 6:29 and 5:30. At both ratios, the crude gluten blends required a longer mixing time in the mixograph to reach maximum resistance than the dispersed gluten blends – 4.8 *vs.* 2.0, and 3.0 *vs.* 2.1 min., respectively. (These comparisons between crude and dispersed glutens were made at equal ratios because no protein or other constituent is removed in the dispersing process. The calculated levels of protein were 11.6% at the 5:30 ratio and 13.6% at the 6:29 ratio on an "as is" basis. Equal amounts of water were added to all doughs in the set. Differences in moisture content between pairs of the gluten-flour fraction blends would affect results somewhat, but in view of results given in the preceding section and in footnote 2, the differences were not considered likely to be large enough to obscure effects of gluten treatments.)

Similar comparisons were made with glutens obtained from flour B after it had been washed with n-butyl alcohol. These glutens gave somewhat longer mixing times than those from untreated flours, but the effect of the dispersing treatment was much the same – 5.3 and

3.1 min. to peak in the mixograph with crude and dispersed glutens, respectively. The sulfhydryl-blocking reagent, NEMI, was added to some doughs also. As expected (6), mixing time to peak was shortened with both crude and dispersed gluten doughs, but a difference persisted. With the glutens from the n-butyl alcohol-washed flour, times to mixograph peak were 3.9 and 2.3 min. for the crude and dispersed samples respectively.

Comparisons of purified (centrifuged at 20,000 × *g*) glutens with crude or dispersed glutens are not satisfactory on a ratio basis because of the higher protein content of the purified samples. However, heated-purified samples could be compared directly with unheated ones, since again no constituents are removed. With glutens from flour B, heated samples gave lower peak heights, both with and without added NEMI and when prepared from n-butyl alcohol-washed flour as well as from unwashed. As examples, maximum heights for various pairs of unheated *vs.* heated samples were: 850 *vs.* 720; 860 *vs.* 720; 760 *vs.* 660; and 800 *vs.* 760. While this effect appeared regularly with these HRS glutens, heating had little effect on a HRW gluten (next section), so that no generalization is possible.

Mixing Curves and Baking Tests: Glutens from Flour C (HRW). Glutens were prepared from the HRW flour, including portions recovered after all the steps of the Jones *et al.* procedure (8). These glutens then were blended with a Brevor flour fraction of 2.1% protein content to give mixtures containing 13% protein, as is, and doughs were mixed with a constant amount of added water. To a second set, NEMI was added. Results are given in Table III. The time to maxi-

TABLE III

CHARACTERISTICS OF MIXOGRAPH CURVES OF DOUGHS CONTAINING GLUTENS FROM HARD RED WINTER WHEAT FLOUR C

GLUTEN	NEMI	CURVE CHARACTERISTICS[a]		
		Time to Maximum	Maximum Height	Height after 5 min. Mixing
	μequiv.	*min.*	*m.u.*	*m.u.*
Crude	0	3.2	740	600
Dispersed	0	1.4	700	500
2,000 × *g*	0	1.2	730	490
Purified (20,000 × g)	0	1.0	660	440
Heated-purified	0	1.0	640	400
Crude	35	2.8	700	490
Dispersed	35	1.3	700	360
2,000 × *g*	35	1.1	700	350
Purified (20,000 × g)	35	0.8	680	300
Heated-purified	35	0.8	700	300

[a] 13% protein in gluten-flour fraction blend; 35 g. blend, 0.7 g. sodium chloride, 27.0 ml. water in doughs.

mum of the curves decreased at successive steps in the preparation of the glutens. The change in maximum height was not as consistent as in the set of glutens from HRS flour B, probably from lack of close control of moisture and absorption, but trended downward; and the extent of breakdown consistently increased.

The doughs containing NEMI did give uniform peak heights, and again showed the decrease in time to maximum and in stability after the peak. The trends in the HRW sets are in general quite similar to those with the HRS glutens. An exception in the particular glutens used was the lack of sensitivity of the HRW gluten to heating.

Micro-loaves were baked from crude and heated-purified HRW gluten samples, primarily to make certain that the doughs were capable of forming a loaf of bread and that the mixing curves for this type of dough (made up of gluten and a low-protein flour fraction) could be interpreted in the same way as curves from flour doughs. The formula of Finney and Barmore (12) was used, with a straight dough, 3-hr. fermentation procedure, and scaling at 50 g. dough. Both under- and overmixed doughs were prepared in a mixograph. Maximum resistance to mixing was reached just before 4.25 min. with doughs containing crude gluten and just before 2.75 min. with doughs containing heated-purified gluten. Loaf volume changes with mixing time are given in the table (averages from two bakes):

Crude		*Heated-Purified*	
Mixing Time	*Loaf Vol.*	*Mixing Time*	*Loaf Vol.*
min.	*ml.*	*min.*	*ml.*
2.75	205	1.5	200
3.5	212	2	212
4.25	205	2.75	205
5.75	195	4	182

With both glutens, largest loaf volume was reached before doughs were mixed to maximum resistance, and in general, loaf volumes at comparable stages of mixing were essentially equal. The relation of loaf volume to mixing curve characteristics thus appeared normal. However, it was noted particularly that purified gluten doughs lost nearly all elasticity when slightly overmixed, whereas the crude gluten doughs remained quite elastic. Overmixing of the purified gluten doughs also produced a noticeable breakdown of crumb structure, whereas overmixing of the crude gluten doughs by one-third beyond peak time did not adversely affect crumb structure. The crumb of the purified gluten loaves was definitely whiter than that of crude gluten loaves.

Discussion

The observations reported above show that more stability to mixing is imparted to a dough by crude gluten than by gluten that has been dispersed in dilute acetic acid. Removal of a fraction of large particle size from the gluten dispersion decreases stability further.

It has been usual to interpret the effects of mixing on the physical properties of doughs and glutens as showing the building-up of a protein network, or at least some degree of association of proteins during mixing to form films. Attention then has been centered on mechanisms that could lead to network or film formation from protein molecules. The present results suggest that disruption or dissociation of protein particles or aggregates should be given more consideration; but it is not believed that the two viewpoints are contradictory. Instead, a subdivision of aggregates may be necessary before the proteins in aggregates can participate in film formation.

Some comments of Bloksma and Hlynka (13) appear to be pertinent. In discussing mechanical work as a factor in dough rheology, they state that dough development and breakdown probably are due to "two simultaneous processes, one creating bonds and the other breaking either the same or other bonds. If the rate of the first process gradually decreases as a consequence of an exhaustion of potential sources of bonds, it can be easily explained that, for example, the dough consistency first increases with mixing, goes through a maximum, and gradually decreases again." While the observations in this paper give no indication of the types of bonds that might be created, certainly the disaggregation of particles of protein could be considered to provide a source of bonds, and as such the supply would gradually diminish as the number of unchanged particles was decreased. In this way the disaggregation step could exert a controlling influence on the rate of development of doughs, presumably along with other processes that are directly involved in the formation of new bonds.

The aggregates that could be involved in the changes observed in this work may be similar to the protein bodies that have been described in the developing wheat endosperm by Morton and his coworkers (14,15), with allowance for modification of the nature of these bodies in maturation of the kernel and in the flour-milling process. The protein bodies contain gluten proteins, and each body is surrounded by lipoprotein (or at least alternating lipid and protein) layers. If it were necessary to disrupt such bodies before a continuous film of protein could be formed in a dough, the process could well

account for changes which have been referred to here as disaggregation or dissociation.

In some respects it was surprising that the differences in gluten properties at different steps in the preparations were so pronounced. The amount of protein sedimented by centrifuging the acetic acid dispersion was never more than 10% of the total. Also, the crude gluten was not obtained without some mixing and kneading of dough, so that comparison of it with the dispersed gluten cannot be considered to show as large a difference as would be possible if gluten proteins could be isolated without subjecting any aggregates to disrupting treatments. Nevertheless, in every case the changes observed seem consistent with the idea that aggregates or particles of protein in flours have a pronounced influence on the mixing stability of doughs; when such aggregates are reduced in size or removed, stability to mixing is decreased.

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