Changes in Flour Proteins during Dough-Mixing. II. Gel Filtration and Electrophoresis Results[1]

K. TANAKA[2] and W. BUSHUK, Department of Plant Science, University of Manitoba, Winnipeg, Canada R3T 2N2

ABSTRACT

Gel filtration of AUC (0.1M acetic acid, 3M urea, 0.01M cetyltrimethylammonium bromide) extracts of doughs on Sephadex G-150 showed that during mixing under conditions of accentuated dough breakdown there was a marked decrease in the amount of the high-molecular-weight (MW) glutenin. Concomitantly, there was an increase in the amount of low-MW glutenin and gliadin. Dough-mixing under normal and accentuated breakdown conditions produced only minor changes in the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of the protein fractions obtained by Osborne-type fractionation. The patterns of the alcohol-soluble fraction (gliadin) of doughs that suffered extensive mixing breakdown, e.g., those containing 2.0 μeq. *N*-ethylmaleimide, had several new bands in the high-MW region. Components of similar MW were observed in the patterns of the reduced acetic acid-soluble and the reduced residue proteins. The SDS-PAGE patterns of reduced acetic acid-soluble and residue proteins were essentially identical and were not affected by mixing under the conditions investigated.

Changes in solubility properties of flour proteins during dough-mixing under a variety of conditions were discussed in the first paper of this series (1). This paper will describe the changes in the proteins of the same doughs determined by gel filtration chromatography and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

MATERIALS AND METHODS

The flours and doughs used for this study were described in the first paper of this series (1).

Gel Filtration

Freeze-dried ground doughs and flours (1 g.) were extracted in a Potter and Elvejhem homogenizer for 15 min. with 20 ml. of the AUC solvent (AUC = 0.1M acetic acid, 3M urea, 0.01M cetyltrimethylammonium bromide) of Meredith and Wren (2). The mixture was centrifuged 25 min. at 20,000 × g and the supernatant further clarified by centrifuging 30 min. at 100,000 × g. The clarified supernatant was used for gel filtration chromatography on Sephadex G-150. Preparation of the chromatographic columns and their use for fractionating AUC extracts of flour or freeze-dried doughs were as described by Bushuk and Wrigley (3).

SDS-PAGE

The SDS-PAGE technique that was used in this study to determine the molecular weights (MWs) of the components of the Osborne fractions was described by Orth and Bushuk (4). Where a comparison between reduced and intact protein was required, an additional electrophoresis experiment was carried out. For this

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[2]Present address: Nisshin Flour Milling Co., Central Research Laboratories, Saitama, Japan.

experiment, the sulfhydryl groups of the intact protein were blocked by reaction with *N*-ethylmaleimide (NEMI). For complete reaction, 10 mg. protein was allowed to react with 10 mg. NEMI in 1 ml. solution overnight at 40°C.

For electrophoresis of the insoluble residue proteins, 2 g. of the starchy residue was extracted with 20 ml. of AUC by stirring overnight in a cold room (4°C.). This suspension was centrifuged for 25 min. at 20,000 × g and the supernatant was freeze-dried. The freeze-dried protein was reduced with β-mercaptoethanol and complexed with SDS according to Orth and Bushuk (4).

Myoglobin (10 μl. of 1 mg. per 1 ml. solution) was used as the reference protein in each SDS-PAGE experiment.

RESULTS AND DISCUSSION

Gel Filtration Results

The highly dissociating solvent AUC dissolves about 95% of flour protein (2,3). It has been assumed (2) that the extracted flour proteins in this solvent exist as a molecular solution and not as aggregates. Fractionation of the proteins in this solution can be achieved by gel filtration on Sephadex. Accordingly, this technique was selected for the present study in an attempt to distinguish between the "disaggregation" and "depolymerization" hypotheses of dough breakdown during mixing. If dough breakdown occurs by the former mechanism, then there would be no change in the gel filtration elution profiles of extracts of doughs that showed different degrees of breakdown. On the other hand, if depolymerization of proteins

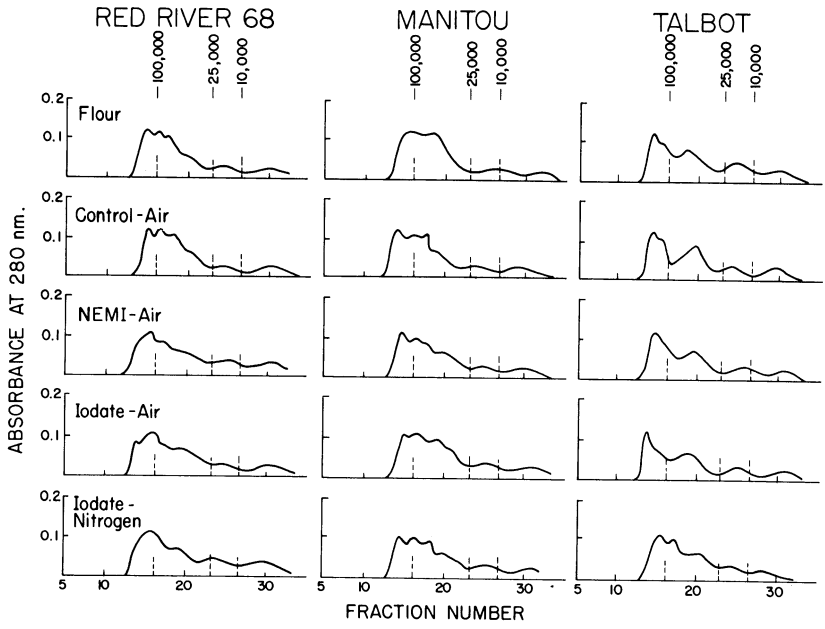

Fig. 1. Gel filtration elution curves of AUC extracts of the flours and selected doughs for the three wheat varieties. Figures on top indicate elution position of proteins of given MW.

occurs, then the elution profiles should show gradual shifts indicating an increase in the lower-MW components and a decrease in the higher-MW components.

Figure 1 shows the gel filtration elution curves for the extracts of the flours of three wheat varieties and dough samples of each selected to represent extremes in mixing breakdown. The four doughs selected were the control mixed for 15 min. in air, the same dough treated with the higher concentration (2.0 μeq. per g.) of iodate and of NEMI, and the dough mixed for 15 min. in nitrogen with iodate (2.0 μeq. per g.). Gel filtration results for the other doughs were also obtained, but are not included in Fig. 1, since they were only slightly different from the results for the control dough mixed for 15 min. in air.

For all three varieties, the elution profiles for the control doughs were essentially the same as the profiles for the extracts of the flours. Furthermore, the extracts of the 5 min. doughs (not shown) gave the same profiles as the extracts of the 15 min. doughs. Accordingly, it is concluded that the small drop in consistency of flour-water doughs mixed from 5 to 15 min. in air or in nitrogen is mainly caused by disaggregation of hydrated flour (and protein) particles. As shown by Mecham (5) and Tsen (6) and confirmed by the present study (1), this disaggregation leads to an increase in the amount of acetic acid-soluble protein but, as shown here, it does not affect the MW distribution of the proteins dissolved by AUC as determined by gel filtration on Sephadex G-150.

Doughs that contained iodate and NEMI showed changes in elution profiles which suggest depolymerization of higher-MW components. These changes were particularly notable for the NEMI-treated doughs mixed in air and in nitrogen, and for the iodate-treated doughs mixed in air. The decrease in the number of higher-MW components can be visualized by comparing the elution profiles of Fig. 1 but can be expressed more precisely in terms of the proportion of the component above 80,000 daltons in MW. These data for the samples represented in Fig. 1 are given in Table I. It is seen that, for all three varieties, 15 min. mixing of doughs containing iodate or NEMI produced a significant decrease in the proportion of the high-MW protein. This can only occur by some form of depolymerization. The results in Table I also show the additive (synergistic) effect of iodate and atmospheric oxygen noted previously (1).

The main difference between the profiles for the stronger mixing varieties (Red River 68 and Manitou) and the weaker mixing variety (Talbot) is that the former contained a considerably higher proportion of the components with MWs above 25,000. This is in general agreement with the solubility fractionation results (1) if it is assumed that, for each variety, the solubility of the protein decreases with increasing MW.

TABLE I. PROPORTIONS OF PROTEIN COMPONENTS WITH MWs ABOVE 80,000 IN FLOURS AND DOUGHS MIXED FOR 15 MIN.

	Red River 68	Manitou	Talbot
Flour	0.57	0.54	0.47
Control–air	0.54	0.52	0.45
Iodate–nitrogen	0.50	0.47	0.44
Iodate–air	0.43	0.44	0.40
NEMI-air	0.43	0.45	0.40

SDS-PAGE Results

Polyacrylamide gel electrophoresis (PAGE) was used to examine flour and dough proteins after complexing with sodium dodecyl sulfate (SDS) directly or after reduction with β-mercaptoethanol. This procedure eliminates the effects of intrinsic charge differences on the proteins and separates the components according to MW.

With Osborne fractions of flour proteins, SDS-PAGE without prior reduction could only be used to examine the water-, salt-, and alcohol-soluble fractions. The intact protein of the acetic acid-soluble or the insoluble residue fractions is too large to enter the gel.

Water- and Salt-Soluble Proteins. The electrophoretic patterns for water- and salt-soluble protein fractions were not affected by mixing under the conditions examined. These patterns were determined but are not shown.

Alcohol-Soluble Proteins. The electrophoretic patterns for the intact (nonreduced) alcohol-soluble proteins of the flour and the doughs were not affected by mixing. For each variety, the patterns for the alcohol-soluble fractions of the doughs were identical with the pattern for the same fraction from flour. The patterns for the doughs mixed in nitrogen were identical with those for doughs mixed in air.

To facilitate intervarietal comparisons, the electrophoretically distinguishable components (determined from a single gel) of the alcohol-soluble protein for the three varieties are listed in Table II according to MW. Red River 68 had seven components (bands) ranging in MW from 20,000 to 150,000. In addition, this variety had three bands in the MW region above 300,000, which is beyond the resolving region of the gel used. Manitou also had seven bands in the 20,000 to 150,000 region and three bands with MWs of 300,000 or higher. Talbot had six bands in the measurable range and none in the 300,000 region.

Examination of the results in Table II shows that the SDS-PAGE pattern of the intact alcohol-soluble protein is a varietal characteristic. Only the lowest-MW component was common to the three varieties. Five components were common to two of the three varieties. Red River 68 had four specific components, Manitou had

TABLE II. DISTINGUISHABLE SDS-PAGE COMPONENTS IN NONREDUCED, ALCOHOL-SOLUBLE PROTEIN FOR THREE FLOUR VARIETIES

MW of Component	Red River 68	Manitou	Talbot
20,000 to 22,000	+	+	+
29,000	+		
36,000		+	+
43,000		+	+
48,000	+		
56,000			+
62,000		+	
65,000	+		
73,000		+	+
110,000	+	+	
120,000	+		
140,000			+
150,000	+	+	
300,000		+	
>300,000	3 bands	2 bands	

one, and Talbot had two. It would be necessary to examine a larger number of wheats varieties in order to determine if the degree of specificity observed here is generally applicable to common wheats.

Results in Table II suggest that stronger mixing varieties have more higher-MW components in the alcohol-soluble fraction. In the range above 80,000, the strong variety (Red River 68) had six components, the medium variety (Manitou) had five, and the weak variety (Talbot) had only one component.

The SDS-PAGE patterns of the reduced alcohol-soluble proteins from various doughs showed minor qualitative and quantitative differences when compared with the patterns of the intact proteins of the same fraction. In general, reduction decreased the MWs of most of the components. For example, the fastest components for the intact proteins were in the 16,000 region. However, the apparent decrease in MW is not sufficient to indicate that these components contain disulfide linkages combining polypeptide subunits. Reduction completely eliminated the components that were in the 300,000 region for the intact alcohol-soluble proteins of Red River 68 and Manitou. It is presumed that these high-MW alcohol-soluble proteins comprise several subunits held together by disulfide linkages and in this respect are similar to the acetic acid-soluble and the residue proteins of flour (see below).

For each variety, the patterns of the reduced alcohol-soluble proteins for the

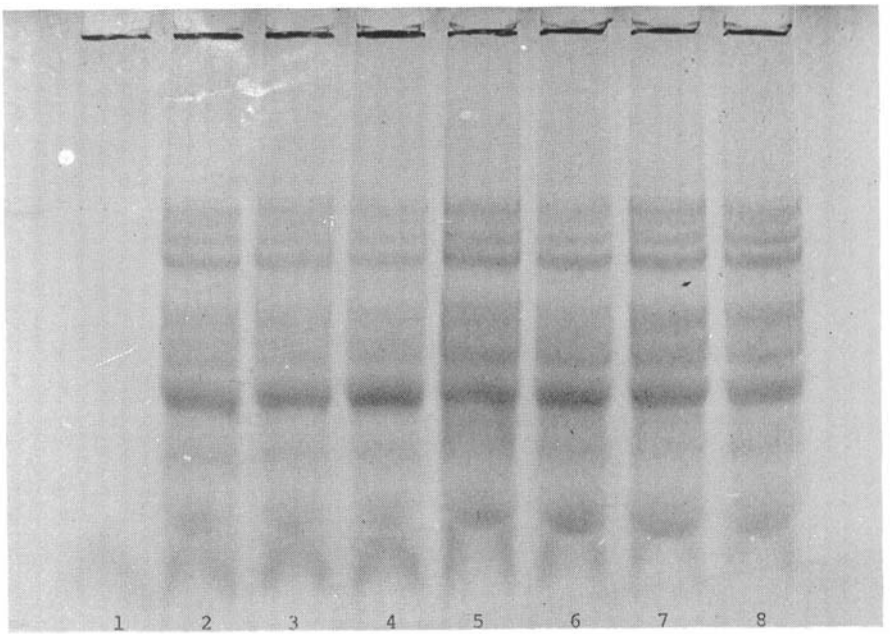

Fig. 2. SDS-PAGE patterns for reduced acetic acid-soluble proteins from Manitou flour and doughs mixed in air: 1, myoglobin (MW 17,000); 2, flour; 3, control dough mixed for 5 min.; 4, control dough mixed for 15 min.; 5, dough-NEMI (2 μeq. per g. mixed for 5 min.); 6, dough-NEMI (2 μeq. per g. mixed for 15 min.); 7, dough-iodate (2 μeq. per g. mixed for 5 min.); 8, dough-iodate (2 μeq. per g. mixed for 15 min.).

doughs mixed in air were essentially the same, except for the doughs containing NEMI and iodate (which showed considerable mixing breakdown). Two doughs had three and one had two additional "new" bands in the higher-MW region. These new components, identified by their approximate weight, produced under high breakdown conditions are as follows:

Red River 68	75,000	100,000	140,000
Manitou	80,000	110,000	160,000
Talbot	70,000	100,000	

The appearance of these new components under conditions of extensive breakdown is taken as further evidence of depolymerization of the higher-MW gluten in proteins under these conditions. Results, to be presented below, were obtained that indicate that these new components are of similar MW (by SDS-PAGE) as the subunits of the acetic acid-soluble and the insoluble residue protein fractions.

Acetic Acid-Soluble Proteins. Acetic acid-soluble proteins of flour can only be examined by SDS-PAGE after reduction of disulfide groups. The results for the flours and the six selected doughs mixed in air are shown in Fig. 2. By way of example, results are shown for one variety (Manitou) only. The patterns for the flour and the doughs were identical; that is, mixing under conditions investigated had no effect on the pattern of the reduced acetic acid-soluble proteins. The patterns for doughs mixed in air and in nitrogen were the same. Analogous results were obtained for the other two varieties.

To facilitate intervarietal comparison, the SDS-PAGE components of the reduced acetic acid-soluble proteins are listed in Table III in order of increasing MW. All three varieties had 10 components. Three of the first four lower-MW

TABLE III. DISTINGUISHABLE SDS-PAGE COMPONENTS IN REDUCED ACETIC ACID-SOLUBLE PROTEIN FOR THE THREE VARIETIES

MW of Component	Red River 68	Manitou	Talbot
19,000	+	+	+
26,000 to 27,000	+	+	+
29,000			+
33,000	+		
35,000 to 37,000	+	+	+
41,000		+	
43,000	+		+
46,000 to 47,000		+	+
50,000 to 51,000	+	+	
53,000			+
63,000	+		
80,000			+
84,000		+	
89,000	+		
110,000		+	+
120,000	+		
130,000		+	
160,000			+
170,000	+		
200,000		+	

components were common for the three varieties. However, there were numerous components that were specific for each variety, especially in the higher-MW region. In the region above 51,000, Red River 68 had four specific components, Manitou had three, and Talbot also had three. Presumably the varieties could be "fingerprinted" on the basis of these characteristic components. There was no apparent relation between mixing strength and the number or the MWs of components of reduced acetic acid-soluble proteins for the flours examined.

Insoluble Residue Proteins. SDS-PAGE patterns for the reduced residue proteins of the flour and the six selected doughs mixed in air for the variety Manitou are shown in Fig. 3. All the patterns were identical; pattern 5 was too faint to reproduce distinctly on the photograph. This was also true for other varieties. The results for doughs mixed in nitrogen were the same as for doughs mixed in air. Accordingly, mixing and conditions of mixing investigated in the present study did not affect the SDS-PAGE patterns of the reduced residue proteins. The amount of residue protein obtained from the NEMI-treated doughs was quite low; therefore the bands were quite faint and are indistinct in the photograph (pattern 5). However, the bands were sufficiently distinct for a visual comparison of the stained gels.

The SDS-PAGE patterns for the reduced residue protein and the reduced acetic acid-soluble proteins showed only minor differences (see below). It appears that the difference in solubility between the acetic acid-soluble and residue proteins is due to a difference in MW (e.g., the number of subunits held together by disulfide bonds).

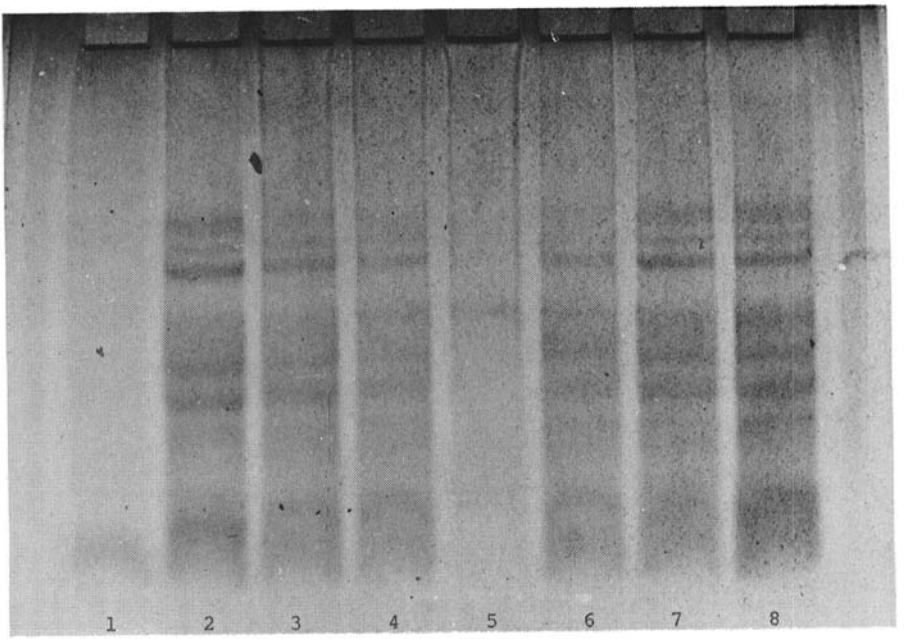

Fig. 3. SDS-PAGE patterns for reduced residue proteins from Manitou flour and doughs mixed in air. Identity of patterns 1–8 is same as in Fig. 2.

TABLE IV. DISTINGUISHABLE SDS-PAGE COMPONENTS IN REDUCED RESIDUE PROTEIN FOR THE THREE VARIETIES

MW of Component	Red River 68	Manitou	Talbot
19,000	+	+	+
26,000 to 27,000	+	+	+
29,000 to 30,000		+	+
33,000	+		
35,000 to 37,000	+	+	+
41,000		+	
43,000	+		+
47,000			+
50,000 to 51,000	+	+	
63,000	+		+
80,000			+
84,000		+	
89,000	+		
110,000		+	+
120,000	+		
130,000		+	
160,000			+
170,000	+		
200,000		+	

The SDS-PAGE components in the reduced insoluble residue proteins are listed in Table IV according to MW. Each variety had 10 distinguishable components. Three of the four components of lowest MW were common to the three varieties. Several components were common to two of the varieties. In the high-MW region, each variety had a number of characteristic components.

Comparison of the results of Tables III and IV shows that the number and MWs of the components of the reduced residue protein are essentially the same as those of the components of the reduced acetic acid-soluble protein. Four minor differences were observed.

1. For the variety Manitou, reduced residue protein had a component of 30,000 daltons, that was absent in the pattern for the reduced acetic acid-soluble protein.
2. For Manitou, reduced acetic acid-soluble protein had a component of 46,000, that was absent from the pattern of the reduced residue protein.
3. For Talbot, reduced residue protein had a component of 63,000, that was absent in the reduced acetic acid-soluble protein.
4. For Talbot, reduced acetic acid-soluble protein had a component of 53,000, that was absent in the reduced residue protein.

The patterns of the reduced proteins of the acetic acid-soluble and residue fractions had components of essentially the same MW as the three "new" components of the alcohol-soluble fraction of the doughs that suffered drastic mixing breakdown. However, further work is required to establish complete identity of these components.

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