Changes in Flour Proteins during Dough-Mixing.I. Solubility Results[1]

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ABSTRACT

Changes in solubility of flour proteins during mixing under a variety of conditions were studied, using three flours of widely different mixing properties. Extended mixing in the presence of relatively high concentrations of *N*-ethylmaleimide and potassium iodate was used to produce accentuated mixing breakdown. Exhaustive extraction of the proteins from freeze-dried doughs with 0.05N acetic acid indicated that under mixing conditions where doughs showed a marked decrease in consistency, the amount of protein soluble in this solvent increased markedly. This was attributed to the depolymerization of the high-molecular-weight glutenin. Results of Osborne-type solubility fractionation were consistent with the depolymerization hypothesis. Concomitantly with the decrease in the amount of the insoluble protein, increases were observed in the amount of the alcohol-soluble and the acetic acid-soluble protein fractions.

Dough-mixing is the most critical step in the breadmaking process. In continuous breadmaking processes, mixing plays a prominent role, since much of the dough development is achieved by the mechanical action of mixing. Many physicochemical changes in the dough components go together to produce the overall effect of mixing; the flour proteins undergo the most important of these changes.

Mecham and co-workers (1-4) found that the amount of protein extracted from dough by dilute acetic acid increased with increased mixing. The increases were different for flours of different mixing characteristics. To explain this increase in extractability, these workers postulated that mixing decreased the size of protein aggregates in the flour particles. This mechanism was later supported by Tsen (5,6). Mecham (7,8) also showed that extractability increased much faster and to a higher maximum if sulfhydryl-blocking reagents were added to dough.

Mullen and Smith (9,10) and Smith and Mullen (11) showed that the main difference between short- (weak) and long-mixing (strong) flours was that the latter contained less acetic acid-soluble protein. The salt-soluble fractions (albumins and globulins) had little effect on mixing characteristics. The protein-starch residue, after extraction with acetic acid, increased the mixing requirements to optimum in the farinograph, whereas the water-soluble gliadins markedly shortened the mixing requirements.

There are a number of possible mechanisms by which the protein particle or aggregate size could decrease. It could involve physical breakdown of flour particles, dissociation of physical or noncovalent bonds such as hydrogen bonds, hydrophobic interactions and salt linkages, and/or depolymerization of the protein macromolecules as a result of cleavage of covalent bonds. The primary objective of the present study was to extend the previous studies on mixing breakdown in order to determine which of these mechanisms gives rise to the increased extractability or solubility of flour proteins during dough-mixing.

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MATERIALS AND METHODS

Three wheat varieties of widely different dough mixing characteristics but of similar protein content were selected for this study. They are: Red River 68 (strong, 10.9% protein); Manitou (medium, 10.8%); and Talbot (weak, 11.3%). The first two are hard red spring wheats, and the third is a soft white winter wheat. The wheats were milled into a straight grade flour on an experimental Buhler mill, using an overnight tempering to 16.5% moisture. The farinograph dough development times for the three varieties were 10, 4.0, and 2.5 min. for Red River 68, Manitou, and Talbot, respectively.

Preparation of Doughs

Doughs containing 0, 0.4, and 2.0 μeq. of iodate or *N*-ethylmaleimide (NEMI) per g. of flour were mixed in air and in nitrogen for 5 or 15 min. in a farinograph. For the exhaustive extraction experiment, an additional dough containing 2.0 μeq. of cysteine per g. of flour was mixed.

After the appropriate mixing time, the dough was quickly frozen by immersion in liquid nitrogen. The frozen dough was freeze-dried, pulverized by hand, and ground in a coffee grinder. The ground samples were kept in a laboratory refrigerator (4°C.) and removed as required for analysis.

Exhaustive Extraction of Protein with 0.05N Acetic Acid

One gram of freeze-dried ground dough or flour was dispersed in 17 ml. of 0.05N acetic acid and extracted for increasing periods in a cold room (4°C.) in a Potter and Elvejhem homogenizer. Extraction times used were 5, 10, 30, 60, and 120 min. The suspensions were centrifuged and the supernatants were freeze-dried. The protein content of the dried solids was determined by the macro-Kjeldahl method.

Extraction and Fractionation of Proteins

The proteins of ground dough and flour samples were extracted and fractionated by a modified Osborne procedure as described by Chen and Bushuk (12). All extractions were made in a cold room (4°C.) to minimize effects of proteolytic enzymes or thermal denaturation.

RESULTS AND DISCUSSION

Effect of Dough-Mixing on Exhaustive Extractability of Proteins by 0.05N Acetic Acid

The effects of iodate and NEMI on farinograph mixing properties were the same as obtained by others (see, for example, Meredith and Bushuk, 13). The farinograms for the control doughs (mixed in air, no additives) showed that the three varieties selected covered a relatively wide range of mixing properties from very strong through medium strong to weak.

Figure 1 shows the amount of extractable protein (as a percentage of total) as a function of extraction time obtained by the exhaustive extraction procedure. The top part of the figure gives the curves for the strongest variety (Red River 68), the middle for the next strongest (Manitou), and the bottom for the weakest (Talbot). Results shown are for doughs containing 2.0 μeq. of iodate, NEMI, or cysteine mixed for 15 min.

Results for the control doughs will be considered first. The equilibrium extractability for the strongest variety after 15 min. of dough mixing was 61%, only 3 percentage units above the value for the flour. Analogous data for the two weaker varieties, in order of decreasing strength, were 66 and 69% for extractability, and 15 and 20 percentage units for the increased extractability after 15 min. of dough mixing. Similar increases in extractability of the flour proteins by dough-mixing were observed by others (1,6). However, the marked differences among flours of different strength were not emphasized.

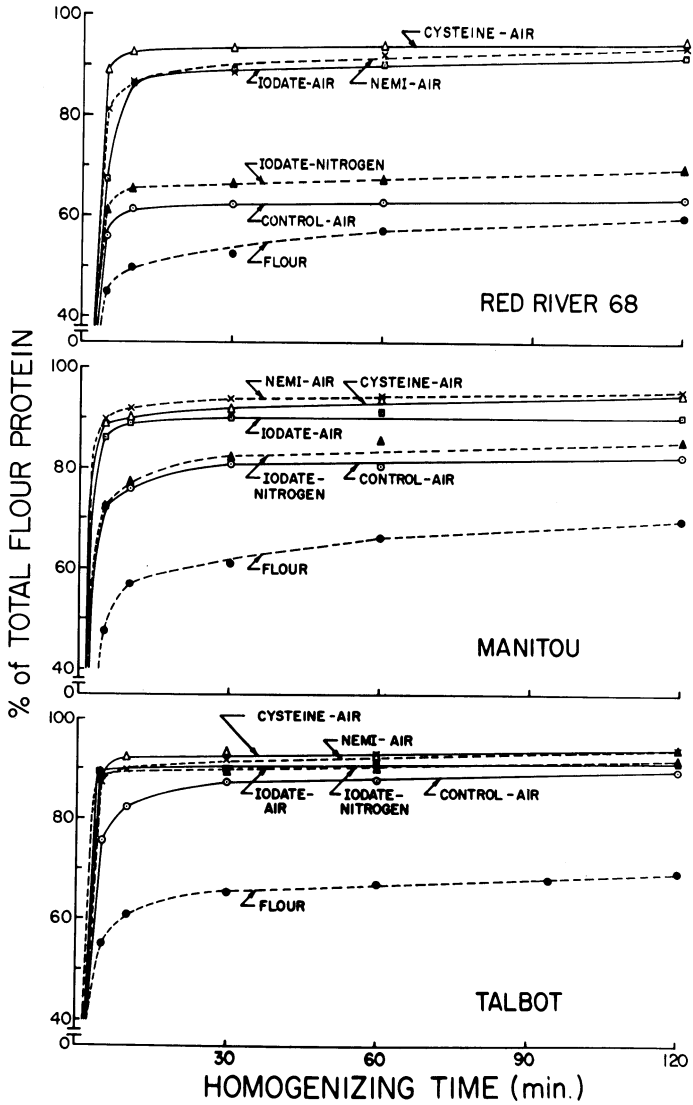

Fig. 1. Exhaustive extraction of the protein with 0.05N acetic acid from the flours and various doughs mixed for 15 min.

The results for the control doughs suggested that the main, direct effect of mixing is the disaggregation of flour particles and protein aggregates, which was postulated by Mecham and co-workers (1-4) and Tsen (6). Comparison of the curves for the flours and the 15 min. doughs mixed in air shows that the equilibrium protein extractability is higher for the doughs than for the flours. Also, the extractability increased faster with extraction time for the control doughs than for the flours. Additions of chemicals such as iodate, NEMI, or cysteine produced additional effects. These effects seem to depend on the degree of mixing.

When the doughs were mixed in the presence of cysteine, iodate, or NEMI, there was an additional marked increase in extractability. Under these conditions equilibrium extractabilities of over 90% were obtained for all doughs except the iodate-containing doughs of the two stronger varieties mixed in nitrogen.

The magnitude of the effect of added chemicals depended on the mixing strength of the flour (wheat variety). In doughs from the weakest flour, the additional effect of the additive was small, mainly because the dough already exhibits a marked breakdown without the additive. It was greatest in doughs from the strongest flour and intermediate in the Manitou doughs which were also of intermediate strength. In the doughs of the two stronger varieties, the effects of NEMI and iodate-air were analogous to that of cysteine. The effect of NEMI on protein extractability was the same in doughs mixed in air and in nitrogen. On the other hand, iodate without atmospheric oxygen increased the extractability only slightly above the values for the control doughs. These results are generally similar to those of Meredith and Bushuk (13), who showed that the effects of iodate and oxygen on mixing breakdown appear to be synergistic.

The results presented above suggest that the additional increase in extractability produced by mixing in the presence of iodate and air or NEMI results from a depolymerization of rheologically important gluten proteins. The similarity of the effects of these chemicals to the effect of cysteine strongly suggests that disulfide bonds are involved in this depolymerization.

Effect of Dough-Mixing on Solubility Fractionation of Endosperm Proteins

The amounts of water-soluble protein and salt-soluble protein were not affected by dough-mixing under the conditions used in this study. Accordingly these data will not be considered further.

The changes in the amount of the other protein fractions with mixing in the control doughs and those treated with 2.0 μeq. iodate or NEMI are shown in Fig. 2. Results for the lower concentration of iodate or NEMI were generally intermediate between the analogous values for the controls and the doughs that were treated with 2.0 μeq. of each reagent. These were not included in Fig. 2.

In the control doughs mixed in air, there was a gradual small increase in the amount of alcohol-soluble protein with mixing. Additions of iodate or NEMI produced a large increase in the amount of this fraction. Most of this increase occurred during the first 5 min. of mixing. Additional 10 min. of mixing produced very little further increase, except in the case of the Manitou dough containing NEMI. This dough showed a continuous, almost linear, increase in the amount of this protein with mixing.

The behavior of the doughs mixed in nitrogen was quite different. In some cases, there was a slight decrease in the amount of alcohol-soluble protein during the first 5 min. of mixing. Again, the control doughs showed little change. Addition

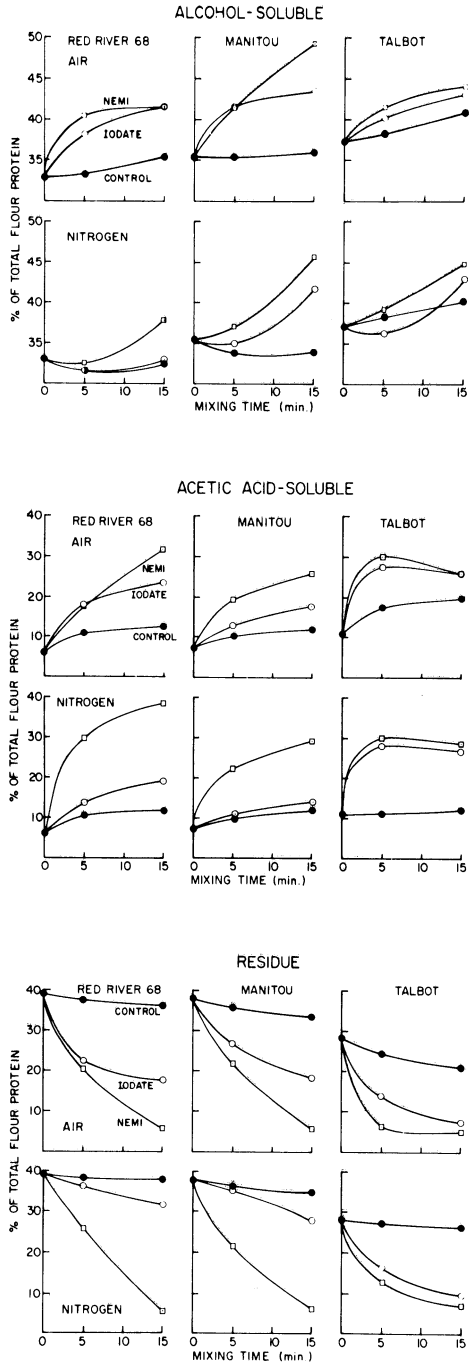

Fig. 2. Changes in the amount of alcohol, acetic acid-soluble, and insoluble residue protein during mixing of various doughs from flours of three wheat varieties.

of iodate or NEMI increased the amount of this fraction. The increase was small after 5 min. of mixing, but became quite large after 15 min. of mixing; that is, the effects of the two chemical additives and mixing appear to be synergistic. On basis of the amount of alcohol-soluble protein, the analogous 5 min. doughs mixed in air and nitrogen appear to be quite different. These differences became negligible after 15 min. of mixing, except for the iodate dough, which contained significantly more alcohol-soluble protein when mixed in air than when mixed in nitrogen.

Comparison of the results of the three wheat varieties showed that the magnitude of the effects of iodate and NEMI depends on the intrinsic mixing strength of the variety. The weak flour seems to have an endogenous factor that acts during mixing in a way similar to iodate (+air) and NEMI. These differences in susceptibility to breakdown during mixing might well be related to subtle differences in physicochemical properties, as yet undefined, that are important to functional quality, e.g., breadmaking.

The amount of acetic acid-soluble protein increased with mixing in all the doughs examined. Oxygen, iodate, and NEMI each produced additional increases. For the weakest variety, Talbot, the amount of this fraction reached a maximum after 5 min. mixing in the doughs containing iodate and NEMI. Subsequently there was a slight decrease as mixing was extended to 15 min. The effect of oxygen is synergistic with iodate, but not so with NEMI. Also, the effect of iodate in doughs mixed in air and in nitrogen increased in the ascending order: Manitou, Red River 68, Talbot. This result may be due to differences in accessibility of the component or components that react with iodate. This factor does not appear to be important in the reaction with NEMI.

The amount of residue protein decreased with increasing mixing time. Although the decrease for the control doughs mixed in nitrogen was extremely small, the decreasing trend was quite obvious. Oxygen, iodate, and NEMI each produced additional decreases in the amount of residue protein. The effects of the higher concentrations (2.0 μeq. per g.) of iodate or NEMI were extremely drastic. Again, the synergistic effect of oxygen with iodate was particularly evident, especially for the two stronger wheats.

The major shift in the solubility distribution on mixing is from the insoluble residue to acetic acid-soluble protein, and in some cases to the alcohol-soluble fraction. It should also be noted that the amount of acetic acid-soluble protein in the flour was greater and the amount of residue protein less for flours of lower strength mixing. Red River 68 flour contained 6.2% of the acetic acid-soluble fraction, and 39.2% of the residue fraction. Analogous figures for Manitou and Talbot flours are 7.3 and 38.0, and 11.0 and 28.3%, respectively. These results are consistent with similar results obtained by Mullen and Smith (9) and with those of Bushuk and co-workers (14,15).

Average total recoveries of protein (for all samples including flours and doughs) by the solubility fractionation were 95.4, 95.0, and 92.5% for Red River 68, Manitou, and Talbot, respectively. These recoveries appear to be related to intrinsic dough-mixing strength of each variety, but were not affected by mixing conditions or by the additives investigated in this study. The weaker flours apparently contain larger quantities of low-molecular-weight nitrogen compounds (free amino acids and peptides), so the lower recovery could be attributed to losses of such compounds during dialysis in the separation of the salt-solubles from the water-solubles.

In general, the results of the solubility fractionation experiment are consistent with the hypothesis that protein depolymerization occurs during mixing breakdown caused by excessive mixing in air or in the presence of sulfhydryl reagents such as iodate and NEMI. However, the same results could also be explained on the basis of the disaggregation hypothesis, if it is assumed that the disruption of the insoluble gluten complex will increase the extractability of the protein fractions that are removed by the sequence of solvents used. Presumably, mixing in air or in the presence of certain chemicals such as iodate or NEMI could disrupt the gluten complex, and thereby make the previously unextractable proteins become available to the extracting solvent. On the other hand, exhaustive extraction results support the depolymerization hypothesis for the decrease of dough consistency under conditions of extensive mixing breakdown.

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