Effects of Oxidizing and Reducing Agents on Changes of Flour Proteins during Dough Mixing[1]

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ABSTRACT

The amount of flour protein extractable with acetic acid and the distribution of protein components in the extract were changed when dough was mixed. The changes became intensified when dough was treated with one of the oxidizing and reducing agents. Iodate, bromate, and N-ethylmaleimide all could increase the protein extractability and the glutenin fraction in the extract, as shown by separation curves with gel filtration. On the other hand, reducing agents including sodium sulfite, cysteine, glutathione, and dithiothreitol exerted the similar, but faster, effect for increasing the extractability through disaggregation of flour protein aggregates. They could increase not only the glutenin fraction but also the gliadin fraction. Mixing did not enhance the effect of reducing agents as markedly as that of oxidizing agents. Results from experiments on flour-water suspensions treated with various oxidizing and reducing agents supported the above findings with doughs. The present findings are discussed in relation to the effect of oxidizing agents (flour improvers) on dough and the reactions of cysteine and bromate in dough for the chemical dough development process.

Previous studies (1) showed that the distribution of flour protein components in the acetic acid extract was changed when dough was mixed. The glutenin fraction (component I) increased with mixing through the disaggregation of large protein aggregates; the other components did not change significantly. The change was related to the mixing properties of flours. With mixing the glutenin fraction of weak flours increased faster than that of strong flours.

It is also known that this mixing effect can be modified by treating dough with an oxidizing or sulfhydryl (SH)-blocking agent. Meçham et al. (2) have found that N-ethylmaleimide (NEMI) can increase the rate and extent of the extractability of flour proteins with mixing, as does potassium iodate. Mamaril and Pomeranz (3) have shown that flour proteins dispersible in 3M urea increase with mixing, exhibiting the same trend as with acetic acid. The dispersibility is markedly increased with doughs treated with iodate and mixed in air. The mechanism by which NEMI or iodate can exert such an effect is, however, not clear (2). In view of the importance of the mixing properties of flour, the function that flour improvers perform in breadmaking, and the reduction-oxidation system used for chemical development of dough, the present study was undertaken to explore the effects of oxidizing agents as well as reducing agents on changes of flour proteins with mixing. Results of this study are reported and discussed in this paper.

MATERIALS AND METHODS

Flour

An untreated straight-grade flour milled from a blend of HRS wheat was used throughout the study. The protein (N × 5.7) and ash contents of the flour were 13.6 and 0.45% (14% m.b.), respectively.

Flour Suspensions Treated with Various Oxidizing and Reducing Agents

An oxidizing or reducing agent (300 μeq.) was dissolved in 10 ml. water (nitrogen-saturated). Two grams of flour (14% moisture basis) was dispersed in this

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freshly prepared solution in a flask under nitrogen to minimize oxidation by air. The flask was shaken vigorously with a wrist-action shaker for 15 min. Then, 20 ml. of 0.075N acetic acid was added to the flask. After another 15 min. of shaking, the suspension was centrifuged at 30,000 X g and 4°C. for 30 min. The supernatant is referred to as the acetic acid extract used for gel filtration in this study.

The absorbance at 280 mμ of the diluted extract (1:20 dilution) was measured with a Beckman DU spectrophotometer. Total nitrogen content of the extract was determined by the Kjeldahl method. For gel filtration, 5 ml. of the extract was used.

The procedures for dough preparation and extraction, and for gel filtration, were those described previously (1). For doughs treated with various agents, the agent was first dissolved in 31.6 ml. water (farinograph absorption was 63.2%) and mixed with flour. If a reducing agent was used, then nitrogen-saturated water was used, and the mixing was done under nitrogen.

RESULTS AND DISCUSSION

Iodate Action

The results (Table I) show that the amount of protein extracted from flour-water dough by 0.05N acetic acid increased with mixing, and the increase was enhanced by treating the dough with iodate at 0.92 μeq. per g. of flour. Since flour contains 0.92 μeq. of SH groups per g., the added iodate would be expected to oxidize all the SH groups available for the reaction with iodate in the flour protein. These results confirm those obtained by Mecham et al. (2) under different experimental conditions.

Effects of Various Concentrations of Iodate, Bromate, and NEMI on Protein Extractability

In view of the iodate action, further experiments were undertaken to ascertain the effects of various concentrations of iodate, bromate, and NEMI on the protein extractability. Table II summarizes the results, showing that the amount of protein extracted from doughs treated with various concentrations of these agents increases with the higher treatment level. NEMI is the most effective, bromate the least, among the agents tested for increasing protein extractability. Their comparative effects correspond well with their improving actions on dough (4,5). However, when a dough was treated with an excess of one of the reagents, the extent of the increase leveled off (Table II).

TABLE I. EFFECTS OF IODATE AND DOUGH MIXING ON PROTEIN EXTRACTABILITY

Treatment	Mixing Time min.	Extractable Protein % total protein
Control	3.0	62.8
	6.5	70.4
	15.5	75.1
	31.0	80.9
KIO_3 (0.92μeq./g.flour)	3.0	70.4
	6.5	78.4
	15.5	87.3
	31.0	89.0

TABLE II. EFFECTS OF SH-OXIDIZING AND -BLOCKING AGENTS ON PROTEIN EXTRACTABILITY OF DOUGHS MIXED FOR 6.5 MIN.

Agent	Amount Added μeq./g.flour	Extractable Protein % total protein	Agent	Amount Added μeq./g.flour	Extractable Protein % total protein
KIO_3	0.00	70.4	$KBrO_3$	1.84	74.2
	0.46	71.3		18.40	76.7
	0.92	78.4	NEMI	0.46	80.4
	1.84	78.2		0.92	81.6
	18.40	78.1		1.84	82.0

Gel Filtration of Proteins Extracted with Acetic Acid from Iodate-Treated Doughs

The effect of these agents on the change in protein distribution was also studied with gel filtration. Since the pattern of elution curves is similar for all the extracts obtained from the treated doughs, typical results with the extracts from the iodate-treated doughs only are presented in Fig. 1.

As reported previously (1), four UV-absorbing components were separated by filtration on Bio-Gel P150. The glutenin fraction (component I–the first peak) in the extract from the dough treated with iodate (0.92 μeq. per g. flour) increased as time of mixing was extended from 3.0 to 15.5 min. When these elution curves are compared with those for the extract from the untreated dough under the same conditions as presented previously (1), it is obvious that iodate can enhance the mixing effect on the disaggregation of large protein particles or aggregates in flour, as reflected by the increase in the glutenin fraction.

Similarly, the glutenin fraction increased with higher levels of iodate treatment from 0.46 to 1.84 μeq. per g. flour (not shown). The effect, however, was not as marked as that with mixing.

Effects of Reducing Agents

In view of the effects of the SH-oxidizing or -blocking agents on protein extractability and protein distribution in the extract, it seemed desirable to study

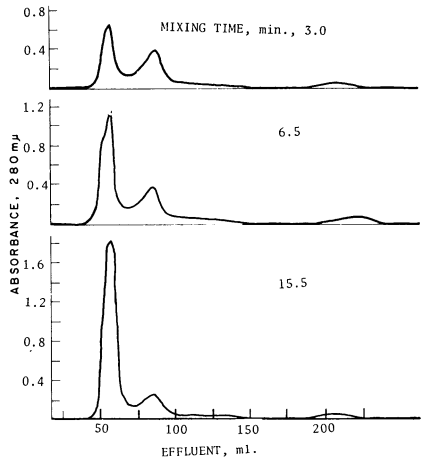

Fig. 1. Gel filtration of acetic acid extracts from doughs treated with iodate (0.92 μeq./g. flour) and mixed for various periods.

TABLE III. EFFECTS OF REDUCING AGENTS AND DOUGH MIXING ON PROTEIN EXTRACTABILITY

Agent	Amount Added μeq./g. flour	Mixing Time min.	Extractable Protein % total protein
Sulfite	1.5	3.0	85.8
	1.5	6.5	86.7
	1.5	15.5	86.8
	1.5	31.0	87.9
	3.0	6.5	87.0
	6.0	6.5	88.2
Dithiothreitol	1.5	6.5	88.1
	3.0	6.5	89.7
	6.0	6.5	90.3

also the effect of reducing agent, to aid toward elucidation of oxidative and reducing actions on flour proteins during dough mixing.

Table III shows the effect of treatment of dough with sodium sulfite on protein extractability with mixing. It can be seen from these data that neither prolonging the mixing time from 3.0 to 31.0 min., nor increasing the sulfite treatment level from 1.5 to 6.0 μeq. per g. of flour, affects protein extractability markedly.

Additional experiments were run, to separate protein components by gel filtration of the extracts from sulfite-treated doughs. Results, given in Fig. 2, present two major features: (a) the glutenin and gliadin fractions are increased, so far as their peak heights are concerned, when the treatment level of sodium sulfite is raised from 1.5 to 6.0 μeq. per g. of flour; (b) the effect of extending the time of mixing from 3.0 to 15.5 min. on the change of flour proteins is less marked in sulfite-treated dough than in iodate-treated dough.

To confirm these findings, further studies were undertaken with other reducing agents including cysteine, dithiothreitol, and glutathione. Results of these studies show that all these reducing agents exert a similar action on dough, except that the effect of dithiothreitol or cysteine is stronger than that of sulfite. As an illustration, the data concerning the dithiothreitol-treated dough are presented here.

The lower portion of Table III shows that treatment of dough with dithiothreitol, like that with sulfite, increases the amount of protein extractable

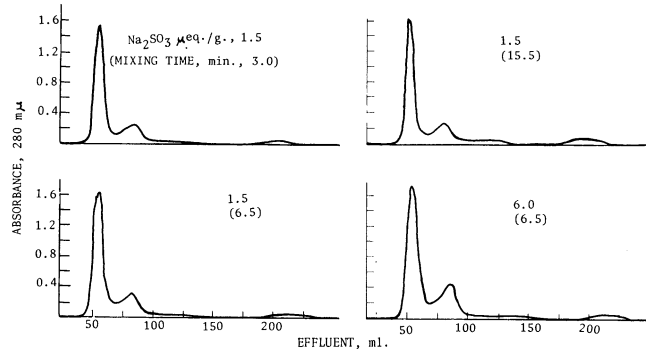

Fig. 2. Gel filtration of acetic acid extracts from doughs treated with sodium sulfite and mixed for various periods.

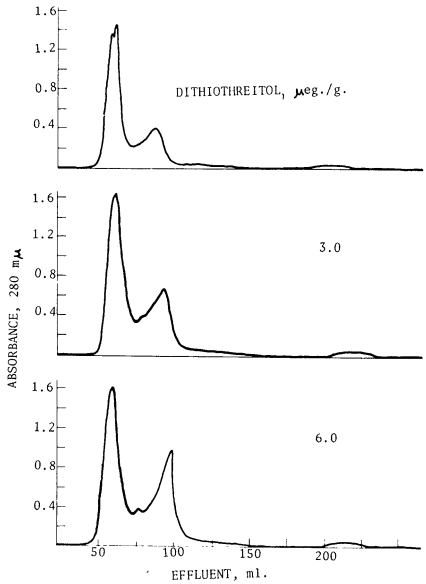

Fig. 3. Gel filtration of acetic acid extracts from doughs treated with dithiothreitol.

from dough. The elution curves for the extracts from the dithiothreitol-treated doughs (Fig. 3) demonstrate that the gliadin fraction, in addition to the glutenin fraction, increases as larger amounts of dithiothreitol are added to the dough, confirming the results with sulfite-treated dough. Furthermore, the elution curve for the extract of the dough treated with 6.0 μeq. dithiothreitol per g. of flour shows an additional small peak between the glutenin and gliadin fractions.

All these results indicate that the effect of reducing agents differs from that of oxidizing agents, on two accounts: (a) the mixing action does not intensify the effect of reducing agents as it does the effect of oxidizing agents on protein disaggregation; (b) the reducing agents, unlike oxidizing agents, can affect the gliadin fraction besides the glutenin fraction.

Effects of Sulfhydryl-blocking, Oxidizing, and Reducing Agents on Proteins in Flour Suspension

In view of the difference in mixing effects on the actions of oxidizing and reducing agents in dough, further studies were made to investigate this difference by testing flour suspension instead of dough with these agents. Extraction data from these studies are summarized in Table IV and separation curves for the extracts are presented in Fig. 4.

Table IV shows that adding iodate, bromate, or NEMI to a flour suspension increases the percent of extractable protein in total protein only about 2%. A much greater increase results from adding reducing agents, ranging from 13.6% more with cysteine HCl to 22.1% for dithiothreitol. The difference in the effects on protein extractability between oxidizing or -blocking agents and reducing agents confirms the results with dough; i.e., the effect of an oxidizing agent without mixing is small but is greatly enhanced by mixing; whereas the action of the reducing agent starts

TABLE IV. EFFECTS OF REDUCING AGENTS ON PROTEIN EXTRACTABILITY FROM FLOUR-WATER SUSPENSIONS

Agent	Extractable Protein 6% total protein	Agent	Extractable Protein 6% total protein
None	66.0	Cysteine HCl	73.6
Bromate	67.9	Cysteine	88.0
Iodate	68.0	Thioglycerol	87.9
NEMI	68.7	Dithiothreitol	88.1
Glutathione	75.5		

as soon as the flour protein and reducing agent are brought together and is little affected by mixing. The data in Table IV also indicate the free base form of cysteine to be more effective than the HCl form in increasing protein extractability. This is, of course, due to the greater reactivity of SH groups at a higher pH. Wren and Nutt (6) also have observed that cysteine and dithiothreitol can raise the extractability of proteins from flour.

The separation data in Fig. 4 also support the findings with dough. The elution curves for extracts of iodate-, bromate-, or NEMI-treated flour suspensions show a slight increase in the glutenin fraction; in general, the curves do not appear significantly different from that for the extract of the untreated flour suspension. The elution curve for the NEMI-treated flour suspension displays a high peak around the 200-ml. effluent fraction which is related to the residual NEMI present in the extract.

On the other hand, the elution curves for the extracts of flour suspension treated with reducing agent show an increase not only in the glutenin fraction but in the gliadin fraction as well. In addition, the elution curve of the extract with dithiothreitol exhibits, again, a shoulder peak between the glutenin and gliadin fractions. It seems that the reducing agent performs two functions under the present experimental conditions: One is to reduce (scission) interdisulfide bonds of some flour protein aggregates, thus splitting the aggregates to small units which are available for extraction. As a result, the glutenin fraction is increased with the treatment of a reducing agent. The second is to reduce some of the glutenin

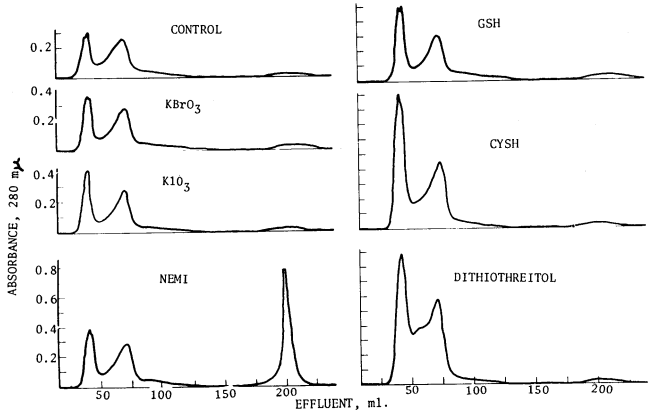

Fig. 4. Gel filtration of acetic acid extracts from flour suspensions treated with various agents.

fraction, also through scission of the disulfide bonds, to a group of proteins with a molecular-size range corresponding to that of the gliadin fraction as separated by gel filtration. However, the increase in the gliadin fraction could also arise directly from disaggregation of protein aggregates (particles) in flour.

GENERAL DISCUSSION

During the mixing stage, flour protein, together with some other flour constituents, is first hydrated. Then, through the tearing and shearing action of mixing, the hydrated protein particles (or aggregates) are disaggregated to form protein films and a protein network (gluten matrix) in dough development. Reducing agents can expedite the disaggregation by scission of disulfide bonds between protein aggregates through reduction, thus alleviating the mixing requirement for dough development, as shown by the results of the present study.

Although the net effect of sulfhydryl-oxidizing or -blocking agents is similar to that of the protein-reducing agents in causing disaggregation with mixing, the mechanism of the action of oxidizing agents must be different from that of reducing agents and cannot be explained easily.

The SH-oxidizing or -blocking agent may exert its effect through the disulfide interchange mechanism – a mechanism which presumably occurs in dough (7). It is known that the disulfide interchange is catalyzed by SH groups, and once the SH group is oxidized or blocked, the interchange would slow down in dough. As a result, the dough is tightened because there is less disulfide interchange to release the stress in dough. Under these conditions, some protein bonds may be cleaved so as to bring about the disaggregation.

Another way to understand the action of SH-oxidizing or -blocking agents is to consider the function of the SH group itself in the stabilization of protein structure. Ample evidence concerning this SH function has been accumulated with studies of enzymes, viruses, and other proteins during recent years (8). For example, reaction of muscle phosphorylase with *p*-hydroxymercuribenzoate (PHMB) results in dissociation of the enzyme into four subunits. Removal of PHMB with cysteine restores the tetramer and also its enzyme activity. The dissociation is not peculiar to PHMB alone but also occurs in the presence of methyl mercuric nitrate and iodoacetamide. Such dissociation is also found with other enzymes, viruses, and other proteins. Thus it is reasonable to suppose that the SH group plays an important role in maintaining the protein structure. The increase in protein extractability from dough or flour with the addition of the SH-oxidizing or -blocking agents may indicate that the dissociation of flour protein aggregates is caused by these agents. Prolonged mixing can intensify the effect on disaggregation, probably because more SH groups are oxidized or blocked with mixing so as to make more protein aggregates labile to disaggregation. Further work is in progress to obtain additional evidence about the function of SH groups in regard to protein disaggregation in dough.

Results of this study also provide useful information for explaining chemical dough development. Recent work of Henika et al. (9,10), in developing the Reddi-sponge process, advocates the use of cysteine, bromate, and whey to develop dough. As mentioned before, cysteine, through its reductive action, can facilitate disaggregation and promote disulfide interchange so that a dough can be developed with less mixing requirement. The dough, thus developed, is matured (oxidized) by

the addition of a relatively high level of bromate. Since cysteine itself can also be oxidized by bromate, one would doubt whether there is any direct mutual destruction when these two agents are added together to dough. Present results show that cysteine exerts its reducing action on dough proteins immediately upon mixing, whereas bromate oxidizes the SH groups of dough proteins very slowly until the initial baking stage, where the high temperature favors SH oxidation by bromate, as reported previously (11). Thus it is reasonable to presume that the reactions of cysteine and bromate are separated in time to permit their respective actions in dough development and maturation.

Acknowledgment

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