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MODIFICATION OF FLOUR PROTEINS BY DOUGH MIXING: EFFECTS OF SULFHYDRYL-BLOCKING AND OXIDIZING AGENTS[1]

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ABSTRACT

Part of the protein in wheat flour cannot be extracted with 0.01*N* acetic acid, but is converted to an extractable form by dough mixing. Both the rate and extent of this conversion are increased by adding the sulfhydryl-blocking reagent, N-ethylmaleimide (NEMI), to doughs.

Doughs from six flours (5 HRW, 1 HRS) were mixed in a farinograph in air and freeze-dried. In the absence of NEMI, maximum conversion of protein required about 20 minutes of mixing, or longer. With additions of NEMI equivalent to the sulfhydryl contents of the flours, maximum conversion was reached in 5 to 10 minutes. In the absence of NEMI, maximum extractable N ranged from 76 to 94% of total flour N; with NEMI added, maximums ranged from 90 to 96%. Potassium iodate produced smaller changes than NEMI.

Rapid modification of protein solubility properties by NEMI (and potassium iodate) begins as soon as dough mixing starts, although changes in farinograph mixing curves are large only after doughs have reached maximum resistance to mixing.

With one of the flours, dough mixing in the absence of NEMI produced little change in the amount of N extractable by 5% sodium chloride, water, or 60% ethanol. In the presence of NEMI, the amounts extractable with 60% ethanol increased with dough mixing as with 0.01*N* acetic acid, but with water they decreased slightly.

In a previous paper, the amounts of protein extracted with dilute acetic acid from flours and doughs were compared (6). Changes occurred during dough mixing which increased the amount of extractable protein and prevented formation of a highly hydrated gelatinous residue from the insoluble material, as occurred with flours that were not mixed. The increases in extractable protein differed in extent and

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rate among flours in such a way as to suggest that the changes were related to the mixing characteristics of the flours.

Numerous observations have indicated that sulfhydryl groups in flour proteins also play a part in determining the mixing properties of doughs (4,5,11,13). For example, sulfhydryl-blocking reagents added to doughs have a marked effect on the recording mixer curves obtained. An effect of such reagents on the change of nonextractable to extractable protein brought about by dough mixing seemed probable and has now been observed.

Materials and Methods

The flours used are described in Table I. All were unbleached. The

TABLE I
SOURCE AND ANALYTICAL DATA (DRY BASIS) OF FLOURS

FLOUR	SOURCE	ASH	PROTEIN	SULFHYDRYL CONTENT
		%	%	μeq/g
Commercial (HRS)	Montana	0.45	15.6	1.12
Wasatch (HRW)	Montana	0.44	13.6	0.75
Pawnee (HRW)	Nebraska	0.60	12.6	0.85
Nebraska No. 1 (HRW)	Nebraska	0.49	12.8	0.69
Nebraska No. 2 (HRW)	Nebraska	0.54	14.4	0.82
Nebraska No. 3 (HRW)	Nebraska	0.61	15.4	1.23

hard red spring wheat flour was commercially milled. The Nebraska No. 3 and Pawnee were milled on a Brabender Quadrumat Junior mill and were consequently of larger average particle size than the other flours. The remaining flours were experimentally milled on Buhler or Allis-Chalmers laboratory mills.

Preparation and Extraction of Doughs. The procedures used were those described earlier (6). N-ethylmaleimide (NEMI) was dissolved in water before it was added to flour; the amounts added were equivalent to the sulfhydryl contents of the respective flours. Individual points in the figures represent single determinations. When duplicate determinations were carried out, they were made on different days.

Experimental Results

NEMI Additions. Addition of NEMI to doughs consistently increased both the rate and extent of conversion of flour protein during mixing to a form extractable with 0.01N acetic acid. This is shown in Figs. 1 to 5 inclusive for five of the flours and their doughs mixed in a farinograph in air. The NEMI exerted its effect quickly, with a definite increase having occurred in four of the flours during

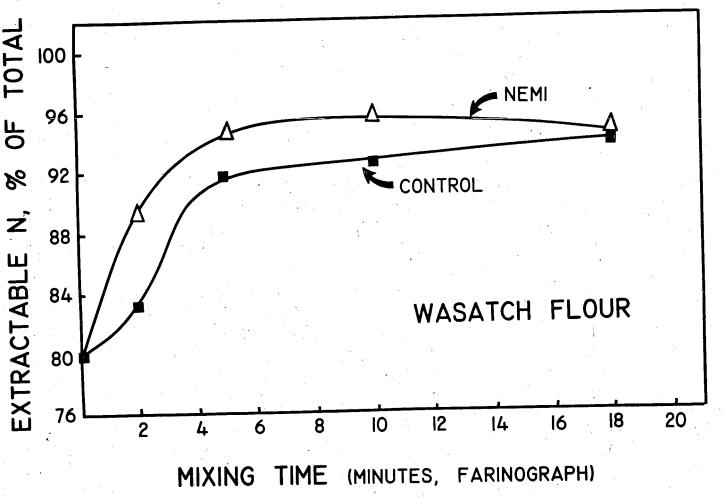


Fig. 1. Effect of NEMI on changes in extractable nitrogen in doughs. (Wasatch flour, 0.75 μeq. NEMI per g. flour; extraction with 0.01*N* acetic acid.)

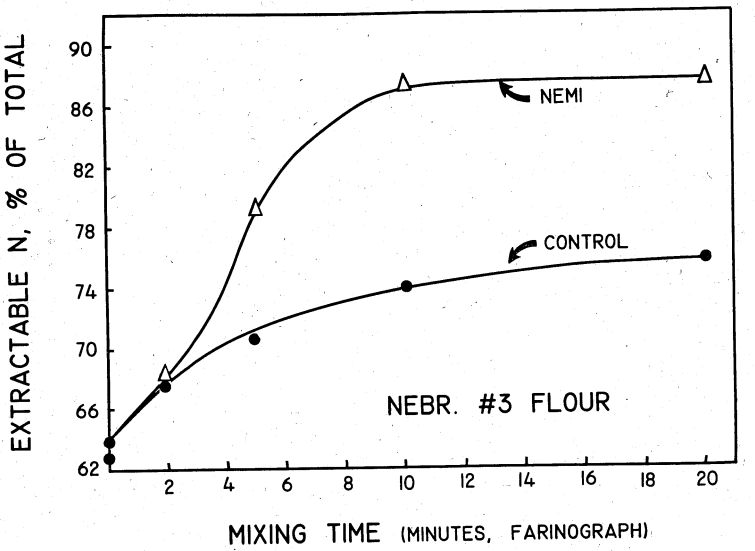


Fig. 2. Effect of NEMI on changes in extractable nitrogen in doughs. (Nebraska No. 3 flour, 1.23 μeq. NEMI per g. flour; extraction with 0.01*N* acetic acid.)

the first 2 minutes of mixing. The other two flours, Nebraska No. 3 (Fig. 2) and Pawnee (Table II), were slower to respond, showing no effect in 2 minutes. Because the latter two flours were much coarser than the other four, the slow response may reflect inaccessibility of

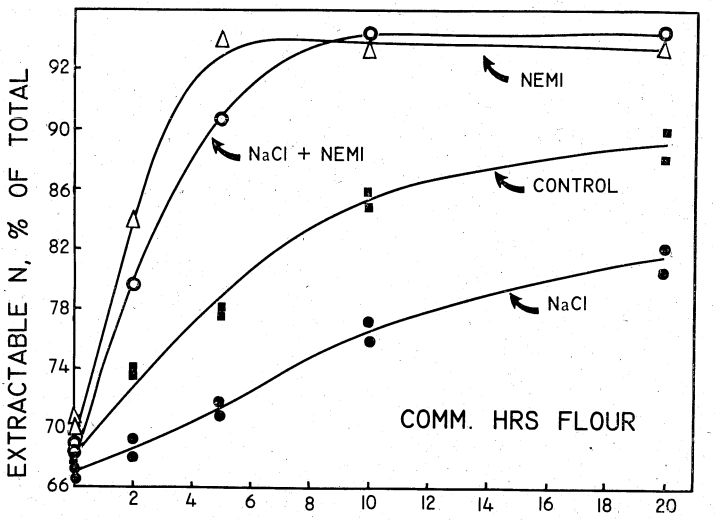


Fig. 3. Effects of NEMI and sodium chloride on changes in extractable nitrogen in doughs. (Commercial spring wheat flour, 1.12 μeq. NEMI per g. flour, 2% sodium chloride, flour basis; extraction with 0.01*N* acetic acid.)

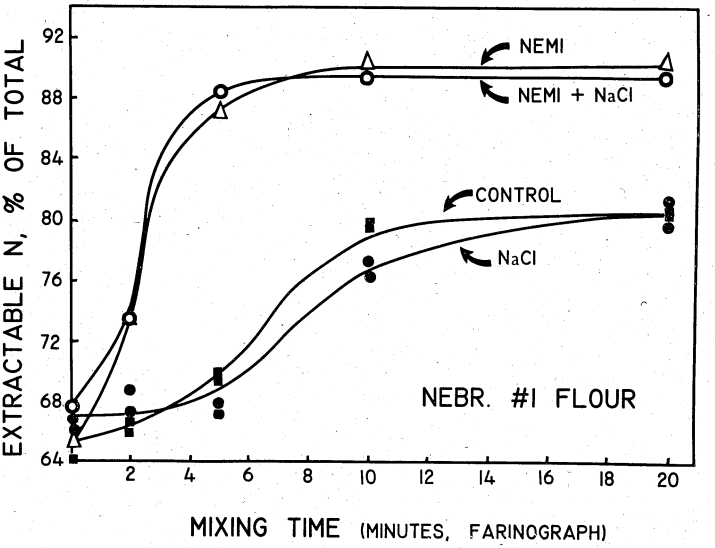


Fig. 4. Effects of NEMI and sodium chloride on changes in extractable nitrogen in doughs. (Nebraska No. 1 flour, 0.69 μeq. NEMI per g. flour, 2% sodium chloride, flour basis; extraction with 0.01*N* acetic acid.)

sulfhydryl groups. For example, Bushuk (1) has found that granulation affects the availability of sulfhydryl groups to iodate. Similarly, the full effect of the NEMI addition was shown within 5 minutes by

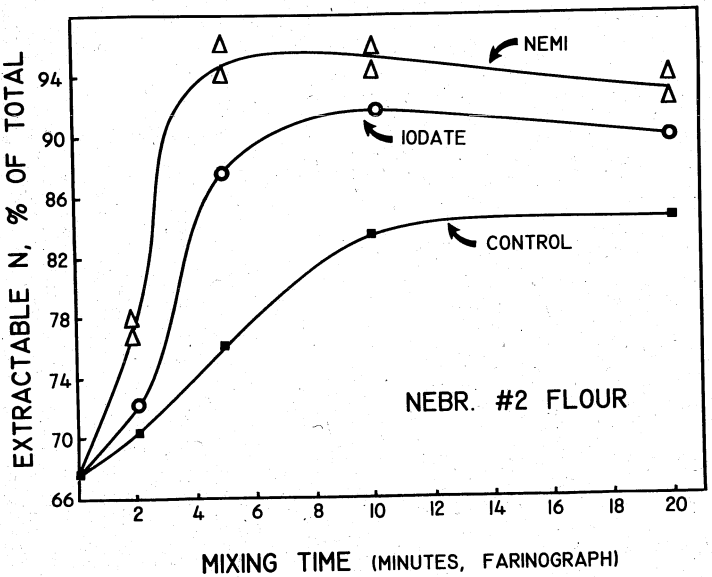


Fig. 5. Effects of NEMI and potassium iodate on changes in extractable nitrogen in doughs. (Nebraska No. 2 flour, 0.82 μeq. NEMI per g. flour, 2% sodium chloride, flour basis; extraction with 0.01*N* acetic acid.)

TABLE II

EXTRACTABILITY OF N IN FLOUR AND DOUGH SAMPLES BY THREE SOLVENTS
(Pawnee flour; doughs mixed in farinograph; NEMI additions equivalent to sulfhydryl content)

	Acetic Acid, 0.01*N*		Ethanol, 60%		Water	
	No Additive	+NEMI	No Additive	+NEMI	No Additive	+NEMI
	%	%	%	%	%	%
Flour	67	...	60	60	39	39
2 minutes' mixing	68	70	62	61	41	34
5 minutes' mixing	76	86	65	70	43	36
10 minutes' mixing	80	92	66	75	42	36
20 minutes' mixing	83	94	65	76	41	35

the finer flours, but was delayed to about 10 minutes with the two coarser flours.

The extent of response to NEMI was least with the Wasatch flour (Fig. 1). It is thus of interest that this flour and its doughs contained a considerably higher proportion of extractable N in the absence of NEMI than the other flours and their doughs. The Wasatch flour also had very poor stability to mixing (see reference 8 for the farinograph curve).

The presence of sodium chloride in doughs of a hard red spring

wheat flour had been observed earlier to retard the increase in extractable N brought about by mixing (6). This is again shown in Fig. 3, together with the effect of NEMI in both the presence and absence of sodium chloride. In contrast to the retarding effect of salt in the absence of NEMI, none was observed in its presence. An instance of a flour that does not show a retarding effect of salt is shown in Fig. 4. The addition of NEMI again had about equal effects in the absence and presence of salt.

Potassium Iodate Additions. Potassium iodate is known to oxidize sulfhydryl groups rapidly in doughs (1,4,14), and it can also modify recording dough mixer curves in a way similar to that done by small additions of NEMI (2,5). The effects of potassium iodate on extractable N changes with mixing therefore were determined. Results with one flour are shown in Fig. 5. The effect of iodate was similar to, but less than, that of NEMI, although the amount of iodate added was 200 p.p.m. (roughly a sixfold excess) to make certain that all sulfhydryl groups accessible to iodate were reacted (1). Meredith and Bushuk (8) have shown that oxygen may have an effect additional to that of iodate even in the presence of an excess amount of iodate, but this was not observed with NEMI except at low levels. The present results appear consistent with their observations, i.e., excess iodate and air together had less effect than NEMI and air, even though no attempt was made to observe the maximum effect of NEMI. An effect of potassium iodate similar to, but less than, that of NEMI was also observed with two other flours and with doughs mixed in a mixograph rather than a farinograph.

Extractions with Water, 5% Sodium Chloride, and 60% Ethanol. The additional protein extractable by 0.01*N* acetic acid from doughs, as compared to flours, with or without added NEMI, was first assumed to be derived from the more insoluble gluten proteins, because gliadin and albumin components would be expected to be extracted by the acetic acid before dough mixing, although globulin proteins would not be. Nevertheless, the solubility of these other groups of proteins also might be modified by mixing or by NEMI. To obtain information on this point, samples of the Pawnee flour and its doughs were extracted with water, 5% sodium chloride, and 60% ethanol.

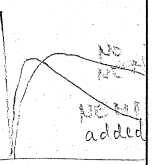
Neither mixing nor the addition of NEMI affected the amount of protein extracted with 5% sodium chloride solutions; all values fell within the range of 22.8 to 24.1% of total N extracted. With the other extractants, however, significant changes were observed, as shown in Table II. With water extraction, effects of mixing were small, but the addition of NEMI decreased extractable N. With

60% ethanol extraction, a small increase in extractable N occurred in doughs mixed in the absence of NEMI; in the presence of NEMI, however, changes were appreciably larger and in the same direction as with 0.01*N* acetic acid extraction.

The results with 60% ethanol appear consistent with the assumption that the extractability of the more insoluble gluten components is predominantly affected by mixing and by NEMI. The results with 5% NaCl indicate no effect of either mixing or NEMI on the extractability of globulin and albumin components. The amount of protein extractable by water is high enough that nearly half of it would be expected to be gliadinlike, and the amount changes little with mixing. In contrast to the other extractants, however, water extracted somewhat less protein from the NEMI-treated doughs. This decrease was confirmed by replication of the experiment. Differences among flours were observed, however; the Nebraska No. 3 gave a decrease of only 3%, while the Nebraska No. 2 gave an increase of 2%.

Discussion

The large effect of sulfhydryl-blocking reagents on recording dough mixer curves prompted the experimental work reported in the present paper. In the case of the mixing curves, NEMI additions increased slightly the rate of development and the maximum resistance reached, but shortened the time of mixing to maximum resistance (5). However, the most pronounced effect was shown after the peak had been reached, the rate and extent of decline being much accelerated. The observations reported here show, in contrast, that much of the increase in extractable protein attributable to the presence of NEMI occurs rapidly and well before the most marked change in the mixing curve. That is, the effect of NEMI on extractability of protein occurred largely during the mixing development of doughs, whereas the effect on the mixing curve was most evident after maximum resistance had been reached.



It has been suggested that the development and breakdown of a dough during mixing involves the incorporation of a protein gel fraction into gluten and formation of a gel structure throughout a dough, followed by breakdown of this structure (7,6). On the basis of results presented in this paper, NEMI then might be considered to break down the gel fraction in the first stages of mixing, leading to the increase in extractable protein. After destruction of the gel structure, no stability to mixing would remain.

The mechanism by which NEMI could exert such effects is not clear, however. The insoluble or gel protein would be expected to

consist predominantly of glutenin components because of its lack of solubility in dilute acetic acid, and thus to contain protein subunits bonded together through disulfide bonds (16). Lower-molecular-weight particles of increased solubility then could be produced by scission of the proper disulfide bonds. Such scission might occur through disulfide-sulfhydryl radical interchanges which have been postulated to occur in doughs (4,5,13). However, the results presented above do not appear to support such a mechanism; blocking of sulfhydryl groups should decrease the rate or prevent scission of disulfide bonds by interchange. NEMI does not react with all the sulfhydryl groups of flour, however, even under conditions (6*M* urea, pH 7.4) that would seem to be more favorable than those in doughs (10). Consequently a small, much reduced proportion of sulfhydryl groups probably was present in all the doughs.

Alternatively, the presence of NEMI may lead to scission of some disulfide bonds in the flour proteins during dough mixing. NEMI has been reported to enhance the cleavage of disulfide bonds in oxidized glutathione at pH 6.2 (12), and Bushuk and Hlynka (2) have suggested that the effects of NEMI on physical properties of doughs might be so explained. However, the latter authors were referring to conditions of excess of NEMI over sulfhydryl equivalents, and to doughs subjected to prolonged mixing. In the present work the effect on extractability is evident with added NEMI equivalent to flour sulfhydryl content and with very little mixing.

The decrease in water-extractable N in some doughs containing NEMI also is not readily explained. Each of the extractants used removes several protein components, as has been shown by zone electrophoresis (3,9,15), so that differing responses (in solubility) to mixing and NEMI perhaps could be expected. It is apparent that further studies are required to determine the protein components modified by the mixing and NEMI treatments and the nature of the changes that occur.

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