THE DOUGH-MIXING BEHAVIOR OF GLUTEN AND OTHER FLOUR FRACTIONS TREATED WITH N-ETHYLMALEIMIDE[1]

D. K. MECHAM, H. A. SOKOL, and J. W. PENCE

ABSTRACT

The modification of dough-mixing behavior by N-ethylmaleimide (NEMI) appears to result from reaction with sulfhydryl groups in the less soluble portion of gluten as indicated by mixing curves of doughs that contained various flour fractions treated with NEMI.

After extraction of a hard red spring wheat flour with 0.5*M* sodium chloride, the gluten-starch residue retained a marked sensitivity to NEMI additions. The dialyzed solids from the salt extract did not affect this sensitivity. Also, NEMI-treated and untreated extracts had equal and small effects on mixing behavior of the residue in the absence of added NEMI.

After extraction with water, the gluten-starch residue differed markedly in mixing properties from the original flour or salt-extracted residue. Nevertheless, the water-extracted residue remained sensitive to the addition of NEMI. NEMI treatment of the water-soluble constituents did not modify their effects on mixing-curve characteristics.

The sustained interest of cereal chemists in the sulfhydryl groups in flours and doughs has been based largely on the possibility that "maturing" or "improving" agents affect physical properties of doughs by oxidation of the sulfhydryl groups (5). Recently it was suggested that sulfhydryl groups might be critically involved, also, in determining the mixing characteristics of a flour (3). This suggestion was based on the observed effects of some highly specific sulfhydryl-binding reagents on the mixing behavior of doughs. The present paper reports the results of efforts to determine whether the effects of N-ethylmaleimide (NEMI), in particular, on mixing behavior of doughs can be attributed to reaction with the sulfhydryl groups in a specific constituent or group of constituents of flour.

Observations of this kind seemed necessary because typical straight-grade flours contain only about 1 microequivalent (1×10^{-6} equivalent) of sulfhydryl per g. of flour, or, in a flour of 10% protein content, 10 microeq. of sulfhydryl per g. of protein. In contrast, the protein would contain about 100 or 125 micromoles of cystine per g. and 2400 of glutamic acid. Because of the very small proportion of sulfhydryl residues, measurement of reaction products of sulfhydryl groups and binding reagents in flour and doughs seemed likely to be most difficult, so that it would be advantageous to be able to fractionate the NEMI-treated dough and discard those portions not involved in the effect

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of NEMI. Furthermore, there is no reason to suppose that all the sulfhydryl groups of the gluten components, albumins, or globulins, even though they total only 1 microeq. per g. of flour, are equal in their effects on mixing properties or that they react equally readily with NEMI. Elimination of any of these constituents as participants would simplify study of the problem of accounting for the effects of NEMI on mixing characteristics.

Materials and Methods

Three hard red spring wheat flours were used. Two were milled commercially from Montana wheats, one in 1955, the other in 1958. Their protein contents, dry basis, were 14.8% and 15.6%, respectively. The third flour was milled experimentally from a composite of pure variety lots of Lee wheat grown in 1956; it contained 17.5% protein, dry basis. The flours were stored at − 10°F. (− 23.3°C.) in friction-top cans; small portions were removed as needed, allowed to equilibrate to room temperature at least overnight, and then used within 2 weeks.

Separations by Gluten Washing. In some exploratory experiments a conventional gluten-washing procedure was used. Doughs were mixed in a laboratory dough mixer until all flour was moistened and the mixture cohered, but dough development was avoided. Water and water solutions of NEMI were used to form doughs. The doughs were allowed to stand 30 minutes under water at room temperature, then were kneaded gently to separate starch and gluten in the usual way. The volume of water was restricted, however, to about 250 ml. per 100 g. flour. Doughs containing NEMI were very soft and sticky in the early stages of washing and had to be kneaded very carefully, but the gluten eventually cohered well enough to permit normal handling. The gluten was frozen in thin sheets by pressing onto blocks of solid carbon dioxide; and the frozen sheets were broken into pieces and lyophilized.

The starch suspension was centrifuged and the supernatant liquid retained. The starch precipitate was present as two distinct layers; the lower prime starch and upper tailings starch were separated with a spatula, and each was suspended in water. After centrifuging, the wash liquids were added to the first supernatant liquid, and the starch fractions were washed again in the same way. The final washings were discarded. The washed starch fractions were dried in a vacuum oven at 37°C.

The soluble constituents present in the wash liquids were recovered by vacuum concentration to a small volume in a rotating-flask evaporator followed by lyophilization. The various lyophilized fractions

were ground through a 40-mesh screen in a small Wiley mill and exposed to atmospheric humidity for at least 24 hours before nitrogen and moisture contents were determined.

The amounts of the fractions recovered and their nitrogen contents are given in Table I. Reconstituted doughs were prepared with the

TABLE I

FRACTIONS FROM 1955 HRS FLOUR BY GLUTEN-WASHING PROCEDURE

DOUGH	FRACTION	WEIGHT RECOVERED PER 86 G. FLOUR SOLID	TOTAL NITROGEN (DRY BASIS)
		g	%
Control	Gluten	13.3	13.7
	Prime starch	46.3	0.0
	Tailings starch	15.0	0.3
	Solubles	4.8	3.4
+NEMI	Gluten	11.5	14.3
(6.6 mg. per	Prime starch	46.4	0.0
86 g. flour solids)	Tailings starch	14.5	0.5

fractions combined in proportion to the amounts recovered (dry weight) from control doughs. Thus 7.2 g. gluten, 25.1 g. prime starch, 8.1 g. tailings starch, and 2.6 g. solubles were combined to give 43.0 g. flour solids (equivalent to 50 g. flour solids, 14% moisture basis). These quantities were used to obtain mixing curves in a farinograph, using a 50-g. bowl. Although the proportions of fractions recovered from the NEMI doughs were slightly different (as were their nitrogen contents), the differences were considered to be relatively minor in view of marked differences in the mixing curves. Fractions from the NEMI-treated doughs therefore were substituted for control fractions on an equal-weight basis.

Extraction of Soluble Constituents. In addition to the gluten-washing procedure, flour fractions were obtained by the following procedure. Eighty-gram portions of flour were weighed into polyethylene bags and placed under vacuum to remove air; the vacuum was released with purified nitrogen, and the samples were let stand under nitrogen overnight. Eight hundred milliliters of water or other extractant were placed in a 1-qt. Mason jar and brought to freezing by holding the jar in a Dewar flask containing some powdered dry ice. During the cooling period, the extractant was flushed with a stream of purified nitrogen. An 80-g. portion of the flour then was transferred to the jar, and the jar was closed with an Osterizer blending assembly (John Oster Mfg. Co., Racine, Wisconsin).[2] These amounts of extractant and flour

[2] Mention of trade names or equipment does not constitute endorsement by the U. S. Department of Agriculture over others of a similar nature not mentioned.

were chosen to minimize head space in the jar. The blender was run at full speed for 1 minute, then slowly for 4 minutes. (By allowing some ice to form when the extractant is cooled, the extraction can be maintained at 0°C. throughout the mixing.) The suspension then was centrifuged for 20 minutes in precooled cups; the centrifuge also was cooled by additions of powdered dry ice.

The extracts were decanted from the starch-gluten residue. NEMI-treated solubles were obtained by adding NEMI equivalent to 20 times the sulfhydryl content of the flour represented by the extract. The extracts were held 5.5 hours in an ice-water bath, then were transferred to cellophane dialysis tubing. Dialysis was carried out at 2° to 5°C. Dialyzed solids were recovered by placing the dialysis sacks in an air blast to evaporate most of the water, then lyophilizing.

The starch-gluten residues were frozen rapidly by spreading on dry ice blocks; frozen residues were broken into pieces, lyophilized, and ground through a 40-mesh screen.

Mixing curves were obtained with these fractions in a Swanson-Working recording mixer. In operating the mixer, a spring setting of 10 was used.

Also, mixing was stopped briefly after 1 minute and the dough was scraped down from the sides of the bowl. The absorption used was that required by the original flour to give a maximum of 500 Brabender units in a farinograph. Thirty-five grams of starch-gluten residue (14% moisture basis) were used in each dough. Additions of soluble constituents were made without reducing the quantity of starch-gluten residue.

The starch-gluten residues obtained by extracting flour with 0.5*M* sodium chloride contained about 2.1% of salt (dry basis), based on conductivity measurements of water extracts of the residues. Accordingly, sufficient starch-gluten residue (30.7 g. dry weight) was weighed out for each dough to provide 30.1 g. dry flour solids; this quantity contained 0.6 g. sodium chloride. Additional salt, 0.1 g., was added to give 2% salt (flour basis) in each dough. To doughs prepared from water-extracted flour residues, 0.7 g. salt was added.

Sulfhydryl analyses were carried out by amperometric titrations with silver nitrate (1) as adapted for use with flour samples (4).

Results and Discussion

Fractions by Gluten Washing. The fractions described in Table I were combined into reconstituted doughs and mixed in the farinograph. The composition of the doughs and some characteristics of the mixing curves are given in Table II. Substitution of prime starch or

TABLE II

EFFECTS OF N-ETHYLMALEIMIDE TREATMENT PRIOR TO GLUTEN WASHING ON FARINOGRAPH CURVE CHARACTERISTICS OF RECONSTITUTED DOUGHS [a]

DOUGH CONSTITUENTS			MIXING TIME TO MAXIMUM	MAXIMUM RESISTANCE	DECREASE IN RESISTANCE 20 MINUTES PAST MAXIMUM
Gluten	Prime Starch	Starch Tailings			
			minutes	*B.u.*[b]	*B.u.*[b]
Control	Control	Control	6.0	515	110
NEMI	Control	Control	3.3	510	140
Control	NEMI	Control	6.5	495	100
Control	Control	NEMI	6.3	495	95
NEMI	NEMI	NEMI	3.0	560	170

[a] All doughs contained "control" solubles. Water was added to give 62% absorption on a 14% moisture basis; this absorption with the original flour gave a curve maximum of 500 B.u.
[b] Brabender units.

tailings starch fractions from the NEMI-treated dough for the corresponding control fractions had no significant effects on the mixing behavior of the reconstituted doughs, but gluten from the NEMI dough decreased mixing time to 3.3 minutes. The latter value also is not significantly different from the 3.0-minute value for the dough containing gluten and both starch fractions from NEMI-treated dough.

Because the starch fractions were found not to be involved in the effect of NEMI on mixing, further studies were simplified, because gluten need not be separated from starch. This facilitated both the preparation of dough fractions and the mixing of reconstituted doughs.

The above observations and other similar exploratory experiments suggest that most of the effect of NEMI is due to its reaction with gluten sulfhydryl groups. However, the washing procedure that had been used for the separation of the dough fractions undoubtedly left appreciable amounts of globulins, and perhaps albumins, in the gluten. These soluble proteins then could have been involved in the NEMI effects. In addition, many of the sulfhydryl groups in the dough fractions were lost, presumably by oxidation, during the gluten-washing process. For example, one set of determinations indicated only one-fourth the sulfhydryl groups of the flour to be present in the fractions. To minimize these difficulties in subsequent work, extraction of solubles from flour suspensions was conducted at low temperatures and with nitrogen-flushed extractants.

Extractions with 0.5M Sodium Chloride. Gluten-starch residues and dialyzed solubles were prepared from the 1958 commercial flour, using a 10:1 (v/w) extractant-to-flour ratio as described above. Mixograph curves obtained with the original flour and its gluten-starch residue, without and with added NEMI, are shown in Fig. 1. Comparison of curves A and B shows the typical effects of addition of 1

microeq. of NEMI per g. flour. In the presence of NEMI (curve B), the mixing curve reaches a slightly higher maximum more rapidly and then breaks down very rapidly. The very narrow band beyond the maximum is quite typical; the contrast in width before and after breakdown is more pronounced in mixograph than in farinograph curves, however.

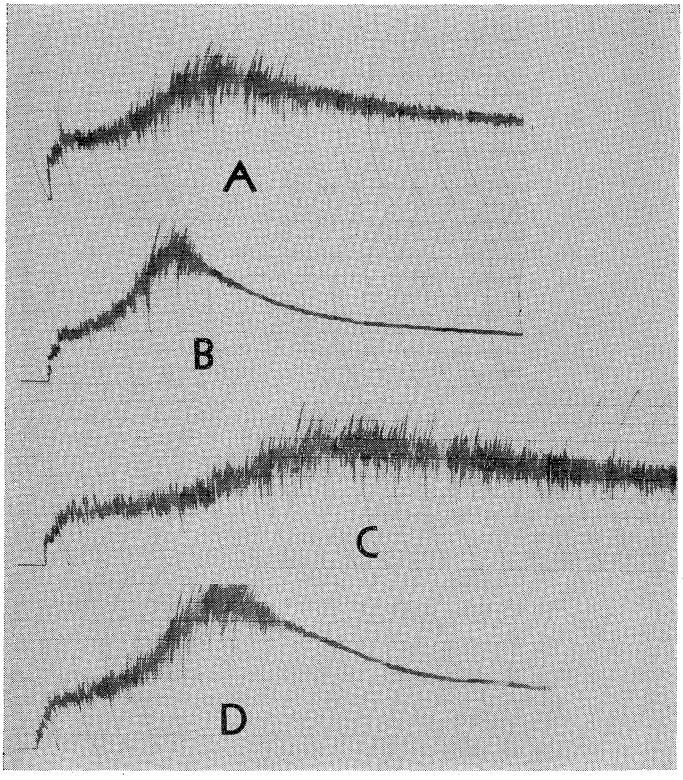

Fig. 1. Effect of extraction with 0.5*M* sodium chloride and of N-ethylmaleimide on mixograph curves of a hard red spring wheat flour (commercial milling, 1958). All doughs contained 35.0 g. flour or flour residue on a 14% moisture basis, 0.70 g. sodium chloride, and 22.8 ml. water. A, untreated flour; B, untreated flour plus 4.4 mg. N-ethylmaleimide; C, residue after extraction of flour with 0.5*M* sodium chloride; D, as C plus 4.4 mg. N-ethylmaleimide.

The residue from flour extracted with 0.5*M* sodium chloride (curve C) gave a longer mixing time to peak and somewhat slower breakdown than the original flour. In fact, the dough was tougher, more elastic, and in general stronger than that from the original flour. However, it is quite evident that the extracted flour retains a marked sensitivity to NEMI (curve D). The sulfyhydryl groups retained in the glu-

ten of the residue thus are sufficient to show the effect of NEMI, although the residue contains only about two-fifths of the sulfhydryl content of the original flour (0.48 vs. 1.12 microeq. per g.).

In contrast to the gluten-starch residue, the soluble constituents recovered by dialysis and lyophilization could not be shown to be involved in the effect of NEMI on doughs, as illustrated in Fig. 2. When

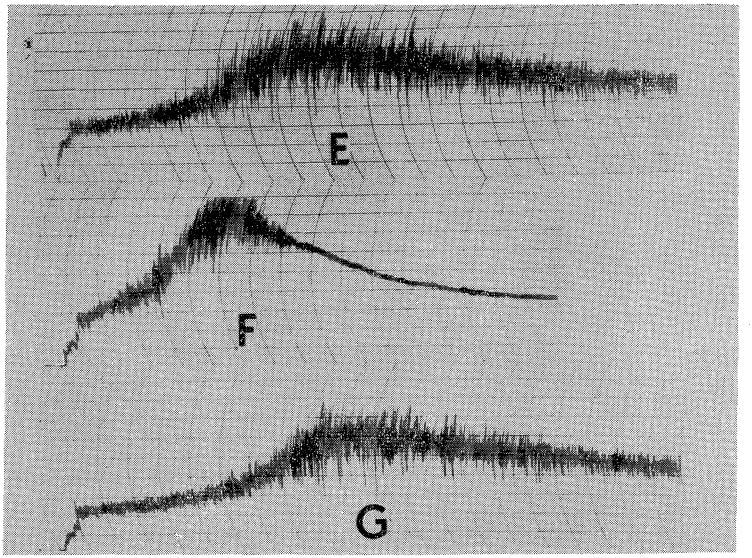

Fig. 2. Effect of additions of dialyzed extract solids and of N-ethylmaleimide on mixograph curves of gluten-starch residue extracted with 0.5*M* sodium chloride (hard red spring wheat flour, commercial milling, 1958). All doughs contained 35.0 g. flour residue or flour residue plus solubles on a 14% moisture basis, 0.70 g. sodium chloride, and 22.8 ml. water. E, salt-extracted residue plus dialyzed extract solids; F, as E plus 4.4 mg. N-ethylmaleimide; G, salt-extracted residue plus N-ethylmaleimide-treated dialyzed extract solids.

the control solubles were added to the gluten-starch residue, the mixing curve was little changed (compare curves C and E), and no diminution or enhancement of the effect of an NEMI addition was observed (compare the change from curve E to F with that from C to D). Also, treatment of the solubles with NEMI had, at most, a small effect on mixing curve characteristics (compare curves E and G).

The recovery of sulfhydryl groups in the residue and extract solubles, based on the actual weights of material recovered so that the value is minimal, was 52% of the original flour sulfhydryl groups — 39% in the residue and 13% in the extracted solids. The largest losses probably occurred in the extracted solids, because of the prolonged dialysis required to remove salt. Also, the sulfhydryl content of ex-

tracted solids after NEMI treatment was 4.4 microeq. per g. compared to 5.7 microeq. per g. in the untreated extract solids. This relatively small difference suggests that in the untreated extract sulfhydryl groups were oxidized to nearly the same extent they were blocked by NEMI in the treated extract, and that only less reactive groups remain. Despite these unsatisfactory aspects of the observations, the marked sensitivity to NEMI retained by the 0.5*M* sodium chloride-extracted gluten-starch residue makes it unlikely that the more labile sulfhydryl groups in the extracted solids are of any considerable importance to the NEMI effect.

Extractions with Water. Mattern and Sandstedt (2) observed that the mixing characteristics of flours were altered markedly by extraction with water. The removal of water-soluble constituents increased mixing time; the constituents principally responsible appeared to be gliadinlike in composition, i.e., high in amide-N content. Mattern and Sandstedt noted also that extraction with sodium chloride solution did not alter mixing characteristics significantly. With the different flour and somewhat different procedures used in work described above, the gluten-starch residue extracted with 0.5*M* sodium chloride gave somewhat longer mixing times and slower breakdown than the original flour, as shown in Fig. 1. However, in agreement with Mattern and Sandstedt, these changes were relatively minor compared to those observed after water extraction of the same flour, as shown in Fig. 3.

Curve H, Fig. 3, was obtained with the gluten-starch residue from water-extracted flour. Nearly 1.25 hours of mixing were required before the residue particles cohered well enough to form a rather weak dough; then, however, a definite development took place. Breakdown of the dough likewise was very slow.[3] Addition of dialyzed water-extractable material shortened mixing time to about 25 minutes, but NEMI treatment of the extractable material appeared to have no significant effect. Mixing time was still about 23 minutes. The original mixing properties of the flour were not restored as completely as Mattern and Sandstedt (2) observed, but the effect was certainly pronounced and in the same direction. The incomplete restoration, in the present work, may arise in part from the removal of constituents of low molecular weight, by dialysis.

The recovery of sulfhydryl groups in the water-extracted flour residue and extract solubles, again based on the actual weights of material

[3] Appreciable evaporation of water from the dough occurred during this long mixing; control experiments with other doughs indicated that about 2.5 g. were lost in 1.5 hours of mixing, equivalent to 7.1% absorption. When a dough was mixed with the water-extracted gluten-starch residue with absorption reduced 10% below that used to obtain curve H, Fig. 3, development time was shortened to about 20 minutes. The long mixing time to maximum therefore might be attributed to the use of too high an absorption, after removal of an appreciable portion of the flour proteins. As shown later, however, the absorption used is not too high for rapid dough development when NEMI is added.

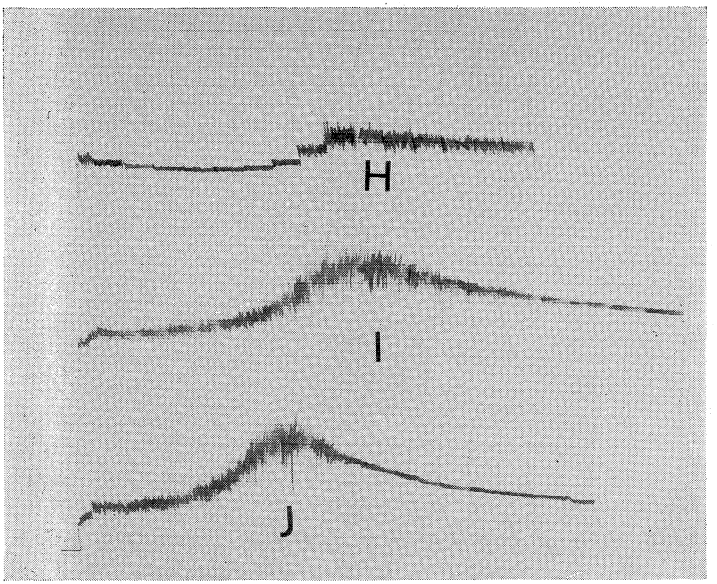

Fig. 3. Effect of extraction with water and of N-ethylmaleimide on mixograph curves of a hard red spring wheat flour (commercial milling, 1958). All doughs contained 35.0 g. flour residue or residue plus solubles on a 14% moisture basis, 0.70 g. sodium chloride, and 22.8 ml. water. H, residue after extraction of flour with water (the mixing curve for H only was recorded 1 minute of each 10 minutes of mixing; the total time represented on the chart is 2.5 hours); I, as H plus 4.4 mg. N-ethylmaleimide; J, water-extracted residue plus dialyzed water-extracted solids plus 4.4 mg. N-ethylmaleimide.

recovered, was 61% — somewhat higher than with 0.5*M* sodium chloride extraction. The distribution was markedly different, however, with 58% in the residue. The residue contained 0.75 microeq. per g.; the extract solids, only 1.4 microeq. per g., despite the fact that the flour nitrogen extracted by water (29% of the total) was nearly twice as much as extracted by 0.5*M* sodium chloride. NEMI treatment of the water extract did not lower the sulfhydryl content of the recovered solids; 1.5 microeq. per g. were found.

Because the dialyzed water extract of this flour contained 29% of the flour nitrogen, extensive changes in mixing behavior of the gluten-starch residue perhaps could be expected. The return toward original flour properties upon restoration of the extract solubles then could be expected also. The marked effect of NEMI on the gluten-starch residue was unexpected, however. As shown by curve I, Fig. 3, one microeq. per g. changed the dough from one that did not develop for more than an hour to one that coheres, develops to a peak in 10 minutes, and breaks down at a moderate rate. In the presence of added

NEMI, supplementation of the residue with dialyzed solubles had a moderate additional effect in shortening mixing time to peak resistance (curve J).

Important as the water-extractable constituents are to the normal mixing behavior of flours, these observations on the water-extracted gluten-starch residue and water-soluble constituents consistently indicate that the effects of NEMI are not exerted through the water-extractable material.

NEMI Treatment during Extraction. As described above, the gluten-starch residues from either 0.5*M* sodium chloride or water extraction show marked responses to NEMI additions. Nevertheless the recovery of sulfhydryl groups indicates that many of the more easily reacted sulfhydryl groups were lost during extraction and drying. In order to obtain residues in which these sulfhydryl groups might be expected to have combined with NEMI, extractions were conducted with 10 volumes of water and salt solutions, each of which contained a twofold excess of NEMI based on flour sulfhydryl content. Extraction conditions otherwise were as described above. A comparison of some of the mixing curves obtained with untreated and these treated residues is given in Table III.

The gluten-starch residue after extraction with NEMI-0.5*M* sodium chloride gave a curve nearly identical with that obtained from the

TABLE III

COMPARISON OF MICRO-RECORDING DOUGH MIXER CURVES OF 1958 COMMERCIAL FLOUR RESIDUES EXTRACTED WITH WATER OR 0.5*M* SODIUM CHLORIDE WITH AND WITHOUT ADDITION OF N-ETHYLMALEIMIDE

DOUGH CONSTITUENTS	MIXING TIME TO MAXIMUM	RESISTANCE[a]	
		Maximum	10 Minutes Past Maximum
	minutes		
1. 0.5*M* sodium chloride-extracted residue; no NEMI present	10.5	670	530
2. 0.5*M* sodium chloride-extracted residue; NEMI present during extraction	10.5	610	420
3. As 2, plus 4.4 mg. NEMI in dough	7.2	680	310
4. Water-extracted residue, no NEMI present	about 90	330	330
5. As 4, plus 4.4 mg. NEMI in dough	10.3	600	360
6. Water-extracted residue, NEMI present during extraction	13.5	500	390
7. As 6, plus dialyzed water-extracted solubles	8.8	630	420
8. As 6, plus NEMI-treated dialyzed water-extracted solubles	8.8	620	410

[a] Units on the basis of 1000 for full width of chart.

residue extracted in the absence of NEMI, and the dough remained sensitive to further NEMI additions. The gluten-starch residue after NEMI-water extraction, in contrast, was changed in mixing behavior as compared to the residue after water-extraction. A dough was obtained similar to that from the water-extracted flour to which NEMI was added during dough mixing. The sulfhydryl contents of these residues were definitely lower than those of residues extracted in the absence of NEMI. Values were 0.34 microeq. per g. for the NEMI-0.5*M* sodium chloride sample (vs. 0.48 in the absence of NEMI), and 0.52 for the NEMI-water sample (vs. 0.75 in the absence of NEMI). Reaction of sulfhydryl groups thus was slightly more extensive in the water-extracted residue, but the difference does not seem sufficiently large to account for the differing response of the two preparations. Taken with the mixing results, the observations suggest that in the presence of salt the insolubility of the gliadinlike protein may protect certain critical sulfhydryl groups in the gluten complex from reaction with NEMI during extraction of the flour suspension.

Behavior of Other Flours. Observations with fractions prepared from the flour from Lee wheat are summarized in Table IV. The resi-

TABLE IV

COMPARISON OF MICRO-RECORDING DOUGH MIXER CURVES OF LEE FLOUR AND GLUTEN-STARCH RESIDUES [a]

DOUGH CONSTITUENTS	MIXING TIME TO MAXIMUM	RESISTANCE [b]	
		Maximum	10 Minutes Past Maximum
	minutes		
1. Untreated flour	8.0	700	480
2. As 1, plus 4.4 mg. NEMI	5.5	770	340
3. 0.5*M* sodium chloride-extracted residue	14.5	760	560
4. As 3, plus 4.4 mg. NEMI	8.5	810	380
5. Water-extracted residue	... [c]	...	...
6. As 5, plus 4.4 mg. NEMI	21.5	480	350

[a] 35 g. flour or gluten-starch residue and 67% absorption on a 14% moisture basis.
[b] Units on the basis of 1000 for full width of chart.
[c] After about 1.5 hours the residue particles cohered to some extent but showed little indication of dough development.

dues recovered after 0.5*M* sodium chloride and water extraction differed in mixing behavior in the same manner as those from the 1958 commercial HRS flour. Their response to NEMI additions also was similar.

The Lee flour contained 0.97 microeq. sulfhydryl per g. Extraction with 0.5*M* sodium chloride removed 14% of the flour nitrogen, and the residue contained 0.59 microeq. sulfhydryl per g. In contrast, extraction with water removed 48% of the flour nitrogen, but the residue contained 0.78 microeq. sulfhydryl per g. The retention of sulfhy-

dryl groups in the residues was higher with this flour than with the 1958 flour. The differences between salt and water extractions in sulfhydryl content and amount of nitrogen extracted are similar, however.

Doughs made up of a commercial gum gluten and starch also showed the typical effect of NEMI on mixing curve characteristics.

General Discussion

The results reported above consistently point to the gluten fraction of flour as the medium through which NEMI additions exert their effect on dough-mixing properties. Despite the presence of reactive sulfhydryl groups in the soluble constituents, treatment of the solubles with NEMI could not be demonstrated to change dough-mixing characteristics. In contrast, all the gluten or gluten-starch preparations were sensitive to this addition of NEMI. In the case of the residues after extraction with salt solution, the effect of the NEMI additions was deleterious. With water-extracted residues, however, small additions of NEMI appeared to compensate to some extent for the changes brought about by removal of water-soluble substances. The proportion of protein extracted by water was high with the two flours used. In our experience this is typical of HRS flours. The behavior of other types of flours has not been investigated in this regard.

Comparison of the results from water- and salt solution-extracted flours suggests first that the effects of NEMI are exerted by reaction with the nongliadin, or at least nonwater-dispersible, portion of the gluten complex. A somewhat different suggestion also can be offered, however. The appearance of mixing curves of doughs to which NEMI has been added is similar to that of doughs to which an excess of gliadin has been added, as published by Mattern and Sandstedt (2). In both cases, a fairly short mixing time to maximum resistance is observed, followed by a sudden and pronounced narrowing of the recorded curve as mixing is continued. This suggests that NEMI reacts with the water-extracted residue to liberate some gliadinlike material and so tends to restore mixing properties of the flour residue to its original pattern. The sulfhydryl groups responsible for the NEMI effect then could occur in either the nongliadin or the liberated gliadinlike fractions.

Further investigations, particularly chemical studies, to determine the site of the NEMI-sulfhydryl reaction product in doughs mixed with added NEMI are needed to clarify the mechanism of the changes produced by NEMI. Information of this kind may help to provide an understanding of the chemical basis for the varied mixing characteristics of different flours.

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