

THE EFFECT OF IODATE AND N-ETHYLMALEIMIDE ON EXTENSIGRAPH PROPERTIES OF DOUGH¹

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ABSTRACT

N-ethylmaleimide (NEMI), an -SH-blocking reagent, and iodate, an oxidizing agent, both give the normal improver effect when used at comparable concentrations in doughs mixed for 2.5 minutes under nitrogen in the GRL mixer. In doughs mixed longer than 5 minutes NEMI gives the reverse effect when it is added at a rate higher than the accessible -SH content of the dough. This reversal appears to be due to the breakdown of the dough by the mixing action and is only partially caused by the reagent. In doughs treated with iodate in excess of the -SH content, physical breakdown during mixing is partly inhibited by salt, so that the reverse effect could not be obtained by mixing up to 15 minutes. In salt-free, iodate-treated doughs, mixing for 15 minutes can produce the reverse effect. Physical breakdown by mixing seems to be particularly rapid in doughs in which all the -SH groups have been blocked.

Until quite recently, the consensus among cereal chemists has been that the over-all effect on certain rheological properties of dough of sulfhydryl(-SH)-blocking reagents was similar to that of oxidative flour improvers. Goldstein (3) showed that the effect of *p*-chloromercuribenzoate (PCMB) on the extensigram was similar to that of bromate or ascorbic acid, and that bromate had no effect if sufficient PCMB is first added to the dough to block all the -SH groups. On the basis of this evidence he postulated the -SH-blocking mechanism for the improver action. Mecham (5) and Mecham, Sokol, and Pence (6) used the farinograph and the Swanson-Working recording mixer respectively and showed that N-ethylmaleimide (NEMI), PCMB, and iodoacetamide shortened the time to maximum resistance and increased the rate of breakdown. Frater, Hird, Moss, and Yates (2) showed that an increase in the rate of breakdown in the farinograph can also be produced by additions of iodate and cysteine.

The results in the references cited above are all in general agreement with the -SH-blocking mechanism for the so-called improver effect. According to this mechanism the effect of -SH-blocking reagents on the physical properties of doughs measured by the extensigraph is similar to that of fast-acting oxidizing agents such as iodate; both types of reagents decrease extensibility and increase resistance to extension. On the other hand, Sullivan, Dahle, and Nelson (8)

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have recently reported that "specific -SH reagents, such as NEMI and PCMB . . . increase the extensibility and decrease the resistance to extension; whereas common maturing agents produce a reverse effect."

Results obtained in this Laboratory and described in this paper elucidate conditions under which iodate and NEMI behave similarly and differently. A possible explanation is also offered for the difference in behavior occurring under certain conditions.

Materials and Methods

This study was made on an untreated straight-grade flour milled commercially from hard red spring wheat. The protein ($N \times 5.7$) and ash contents of the flour were 13.2 and 0.43% respectively, both expressed on a 14% moisture basis. The accessible -SH content determined by the iodate method (1) was 0.7 μ eq. per g. of flour.

NEMI was obtained from Nutritional Biochemicals and was used without further purification. All other chemicals were reagent grade.

The extensigrams reported were obtained as follows: Doughs of 60 and 55% absorption containing 2% (flour basis) sodium chloride (or as otherwise indicated) were mixed under nitrogen in the GRL mixer (4) for 2.5 minutes, or other mixing times which will be indicated in the text. The doughs were given a reaction time of 180 minutes, then were rounded and shaped, and stretched after a rest period of 20 minutes. During the reaction time and the rest period the doughs were kept in the constant-humidity cabinet at 30°C.

The following working definition of the *improver effect* based on the extensigram will be used in the discussion that follows: An *improver effect* is obtained by the extensigraph technique when resistance to extension increases and (or) extensibility decreases relative to the magnitude of the same parameters for the control dough.

Results

Figure 1 shows extensigrams for doughs of 60% absorption mixed for 2.5 minutes under nitrogen and treated with various amounts of iodate and NEMI. Control curves for untreated doughs are shown as broken lines. Reagent concentrations were selected to represent approximately 0.5, 1, 1.5, and 4 times the molar equivalent of the accessible -SH content of the dough (0.7 μ eq. per g. of flour) determined by the iodate method (1).

Contrary to the observations of Sullivan *et al.* (8), the effects of iodate and NEMI used at comparable concentrations are similar. With 2.5 minutes of mixing under nitrogen in the GRL mixer and

with the extensigraph procedure described, curves for both reagents at all concentrations shown in Fig. 1 show an increase in resistance and a decrease in extensibility over the same extensigraph properties

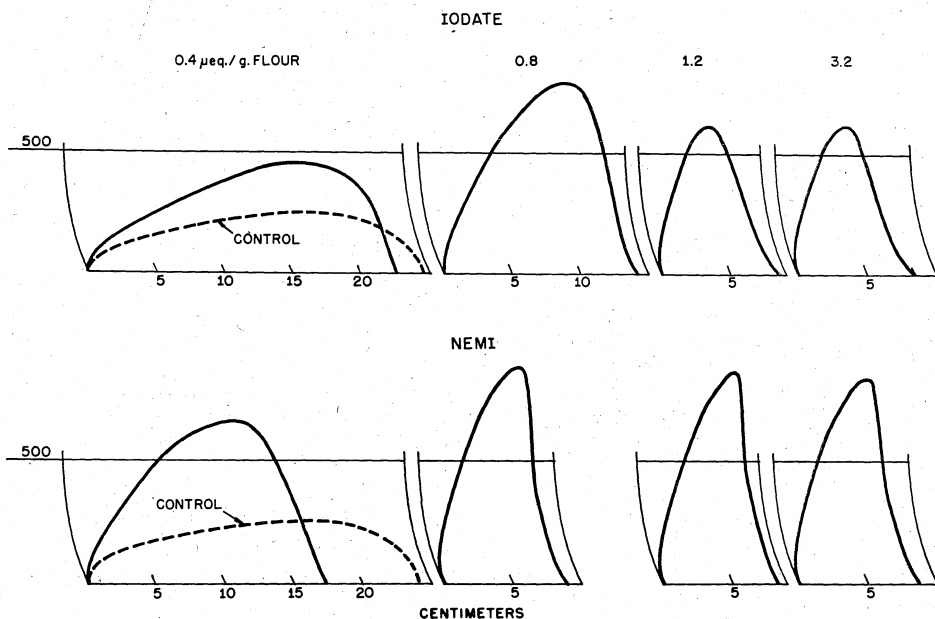


Fig. 1. Extensigrams for doughs containing various amounts of iodate and N-ethylmaleimide for the following experimental conditions: Absorption, 60%; mixing time (under nitrogen), 2.5 minutes; reaction time, 180 minutes; rest period, 20 minutes. Control extensigrams for the same conditions — broken lines.

for the control doughs. That is, both reagents give an improver effect under the experimental conditions used. The results (not shown in Fig. 1) were essentially the same when the doughs were mixed for 2.5 minutes under atmospheric conditions.

In another type of experiment, a study was made of the effect of prolonged mixing under nitrogen on doughs containing various amounts of NEMI. Results obtained for two concentrations, 0.4 and 1.2 μ eq. per g. and three mixing times, 5, 7.5, and 10 minutes, are shown in Fig. 2. Control extensigrams are shown as broken curves. To increase the height of the extensigrams, doughs of 55% absorption were used in this experiment.

At the lower concentration of NEMI (upper curves) there is essentially no decrease in resistance or increase in extensibility with increasing mixing time, i.e. these NEMI-treated doughs exhibit a mixing tolerance similar to the control doughs. (A marked improver

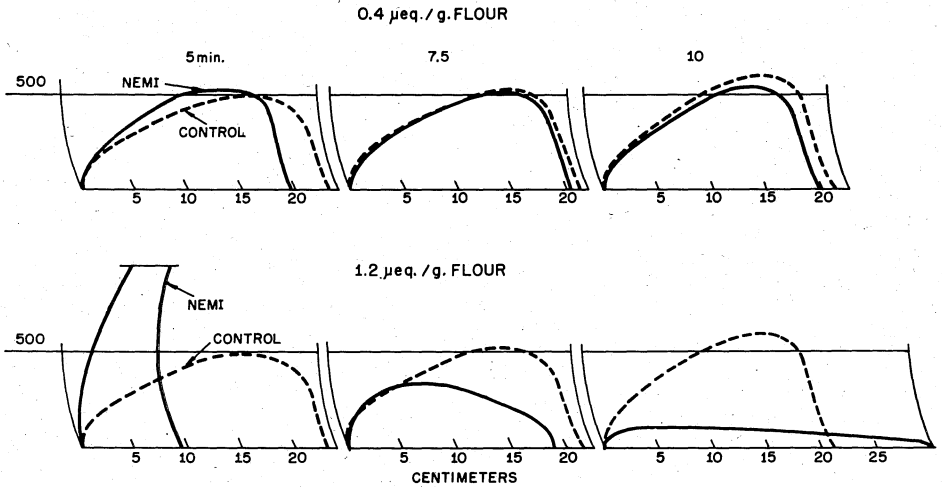


Fig. 2. Extensigrams for doughs containing 0.4 and 1.2 $\mu\text{eq.}$ NEMI per g. of flour and mixed under nitrogen for 5, 7.5, and 10 minutes. Other conditions as follows: Absorption, 55%; reaction time, 180 minutes; rest period, 20 minutes. Control extensigrams — broken lines.

effect was actually obtained at this level of NEMI after 2.5 minutes of mixing in the dough of slightly higher absorption represented in Fig. 1.) At the higher concentration of NEMI (lower curves, Fig. 2),

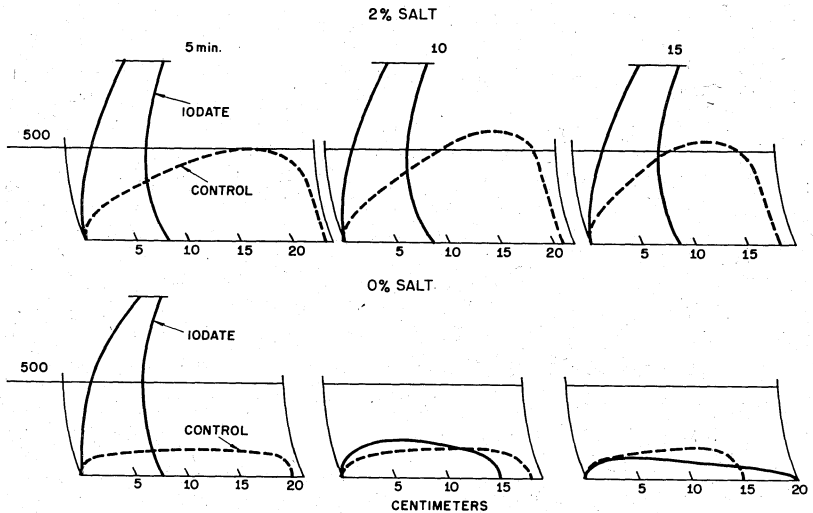


Fig. 3. Extensigrams for doughs treated with 1.7 $\mu\text{eq.}$ iodate per g. flour and mixed under nitrogen for 5, 10, and 15 minutes. Upper curves, doughs containing 2% salt; lower curves, salt-free doughs. Other conditions: Absorption, 55%; reaction time, 180 minutes; rest period, 20 minutes. Controls — broken lines.

normal improver effect is obtained for the dough mixed for 5 minutes, whereas the reverse effect is obtained for the doughs mixed for 7.5 and 10 minutes. The results for the two longer mixing times are similar to the results obtained by Sullivan *et al.* (8) for NEMI- and PCMB-treated doughs.

Effect of prolonged mixing in nitrogen of doughs treated with iodate in excess of the $-SH$ content is shown in Fig. 3. Upper curves represent doughs containing 2% salt (flour basis) mixed for 5, 10, and 15 minutes. Lower curves are for salt-free doughs subjected to the same amount of mixing. Although extended mixing beyond 15 minutes (not shown) did reduce the amount of improver effect in the salt-containing doughs, it was not possible to produce the reverse effect by mixing up to 40 minutes. With salt-free doughs the reverse effect is readily demonstrable (see lower curves).

Discussion

Results of this study showed that for normal (short) mixing times both iodate and NEMI give the improver effect over a wide range of concentrations (Fig. 1). By combining high reagent concentrations with prolonged mixing, results which show an improver effect for iodate (Fig. 3) and the reverse effect for NEMI can be obtained (Fig. 2). This reverse effect of NEMI seems to be due partially to physical breakdown of dough by mixing after blockage of all the $-SH$ groups and partially to a reaction of NEMI that occurs when it is present in excess of the $-SH$ content. This secondary reaction of NEMI apparently facilitates breakdown of the dough structure during mixing.

Similar physical breakdown can also occur in doughs treated with sufficient iodate to react with all the $-SH$ groups. However, this breakdown is inhibited by salt, to the extent that the reverse effect cannot be demonstrated in iodate-treated doughs by mixing up to 15 minutes. When the stabilizing effect of salt is removed, the reverse effect can be readily demonstrated (see Fig. 3). In NEMI-treated doughs, the stabilizing effect of salt is not nearly as marked as in iodate-treated doughs.

The results obtained by Sullivan *et al.* (8) are consistent with ours, but their conclusions require modification. Their flour had a $-SH$ content of $0.41 \mu\text{eq. per g.}$, and their mixing procedure was relatively severe. In their Fig. 2, the iodate concentration was only $0.28 \mu\text{eq. per g.}$; whereas the concentrations of PCMB (0.79 and $0.92 \mu\text{eq.}$) and especially of NEMI ($1.2 \mu\text{eq.}$) were much higher. With such widely different concentrations of reagents, doughs exhibit different mixing

characteristics. The iodate-treated doughs still contained $-SH$ groups, were tolerant to mixing, and showed an improver effect. But in doughs treated with excess of PCMB or NEMI, all $-SH$ groups were blocked, mixing tolerance was sharply reduced, and the doughs broke down and showed a reversal of the improver effect. The conclusions drawn by Sullivan *et al.* (from experiments that were not strictly comparable — i.e., their flour and mixing conditions were different) seem too sweeping; specific $-SH$ reagents and common maturing agents behave similarly or differently depending on the concentrations of reagents and the severity of mixing.

It should also be pointed out that Sullivan *et al.* (8) mixed all doughs in air, whereas the results of the present paper were obtained with doughs mixed in nitrogen. Experiments of the type described by Fig. 1 with doughs mixed in air for 2.5 minutes showed that the results described are not markedly influenced by atmospheric oxygen during this short mixing time. However, in doughs mixed for longer periods, atmospheric oxygen seems to increase the rate of dough breakdown. This effect of oxygen becomes apparent at shorter mixing times in doughs of which the $-SH$ groups are initially blocked (or reacted with) by specific $-SH$ reagents. The role of oxygen in the stability of doughs during mixing will be discussed in greater detail in a subsequent paper.

Finally, a comment should be made about the difference in the effects of NEMI and iodate on the physical properties of doughs. As indicated above, salt-containing doughs break down during mixing much faster when treated with NEMI than when treated with iodate. This difference between the effects of the two reagents is amplified further by prolonged mixing. Doughs treated with NEMI at a dosage larger than the $-SH$ content and subjected to prolonged mixing (10 or 15 minutes) in nitrogen or in air appear, by touch and sight, like doughs treated with small amounts of reducing agents such as reduced glutathione. There is this definite suggestion that NEMI might facilitate breakage of disulfide bonds. It has been demonstrated recently that in solution of pH 6.2, NEMI can actually enhance the hydrolytic cleavage of oxidized glutathione (7). Further experiments are necessary to demonstrate if this effect of NEMI is operative in doughs.

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