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THE EFFECTS OF IODATE, N-ETHYLMALEIMIDE, AND OXYGEN ON THE MIXING TOLERANCE OF DOUGHS¹

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ABSTRACT

The effect of two sulfhydryl reagents, N-ethylmaleimide (NEMI) and iodate, added to doughs mixed in nitrogen, air, and oxygen, was studied using the farinograph, the extensigraph, and a technique to estimate the acid-insoluble part of washed gluten. Both reagents cause dough breakdown during mixing in nitrogen, but NEMI is more effective. The effect of iodate seems to reach a saturation point where more is ineffective; additional NEMI continues to show effect but at a diminishing rate. In doughs mixed in air or oxygen, the effect of incorporated oxygen is superposed on that of iodate or NEMI; oxygen alone has only slight effects during analogous mixing times. Dough breakdown occurs with less iodate or NEMI when mixed in air or nitrogen. Oxygen has an added effect regardless of how much iodate is present; this effect occurs only at lower dosages of NEMI. NEMI added to iodate-treated dough causes additional breakdown; there is no similar effect when the reagents are reversed. The results are therefore discussed in terms of the role of the sulfhydryl groups in chemical and physical changes in dough structure.

The mixing tolerance of dough is considered one of the prime factors in assessing quality of flour for breadmaking by conventional processes. Doughs with low mixing tolerances require more rigid control of mixing than do doughs with greater mixing tolerances. The importance of mixing tolerance may have been increased in recent years by the introduction of new continuous processes of dough preparation such as the Baker Dō-maker and the Amflow and Chorleywood processes.

We attempt to define some conditions under which dough breakdown occurs. Oxygen, potassium iodate, and N-ethylmaleimide (NEMI), reagents of current interest because of their reaction with

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sulfhydryl groups, have been used together with prolonged mixing to produce breakdown. Two well-known techniques, the extensigraph and the farinograph, were supplemented by a relatively new technique based on the amount of gel protein in the washed gluten (9), to examine changes indicative of breakdown that occurred during mixing.

The relationship between the amounts of iodate and NEMI necessary to produce breakdown and the determined sulfhydryl group content of the flour allows an interpretation of the mechanisms of relaxation and breakdown in terms of disulfide interchange mediated by sulfhydryl groups.

Some parts of the present work repeat earlier investigations by Smith and Andrews (10) in 1952 on the effects of oxygen in mixing; by Mecham (8) in 1959 on the effect of NEMI in air; by Sullivan *et al.* (11) in 1961 on the effects of iodate and NEMI in air; by Bushuk and Hlynka (2) in 1962 on the effects of iodate and NEMI in nitrogen. This repetition was necessary in order to present completely comparable data.

Materials and Methods

HRS
An untreated, straight-grade flour milled commercially from hard red spring wheat was used. Its protein ($N \times 5.7$) was 13.2% and ash 0.43%, both expressed on 14% moisture basis. The accessible sulfhydryl (-SH) content in a dough of 60% absorption determined by the iodate method (1) was 0.70 $\mu\text{eq.}$ per g. flour. Amperometric titration with methylmercuric iodide of a dispersion of the flour in urea solution (1) gave 0.80 $\mu\text{eq.}$ per g. for the -SH content. For the gel protein experiments, the flour was extracted with Skellysolve F95 in a Soxhlet-type extractor to remove free lipids. Results for original and extracted flours were similar, but extracted flours gave cleaner fractionations. For doughs mixed under nitrogen, flour was first stored overnight under nitrogen after repeated evacuation and replacement with nitrogen.

Oxygen and nitrogen were commercial grade (99.9%) and were used without further purification. All other chemicals were reagent grade. Versene was the disodium form. Concentrations of iodate and NEMI are expressed in $\mu\text{eq.}$ per g. flour (14% m.b.). The equivalent of iodate has been taken as one-sixth of the molecular weight.

The extensigraph procedure is as follows: Doughs of 55% absorption and containing 1% sodium chloride are mixed in the GRL mixer (7) at 30°C. All doughs are given a reaction time of 10 minutes, then are rounded and shaped, and stretched after a rest period of 20 min-

utes. This procedure and absorption level, which do not conform to normal practice, were selected for convenience in obtaining extensigrams over a wider range of reagent dosages than might otherwise be possible. A decrease in resistance to extension and an increase in extensibility are used as the index of dough breakdown.

The method used to determine the amount of gel protein in washed gluten was similar to that described previously (9). Briefly, the experimental procedure is as follows: Doughs of 60% absorption and containing 0.6% sodium chloride and 60 p.p.m. Versene (flour basis) are mixed in the GRL mixer for the required length of time in either nitrogen or air and then rested for 1 hour in an aqueous solution containing 1% sodium chloride and 100 p.p.m. Versene and bubbled with nitrogen when appropriate. Gluten is washed from the dough under a stream of tapwater, rested for 1 hour, and freeze-dried without prior freezing to obtain an open structure which facilitates hydration. To analyze for gel protein content, 0.5 g. of ground, freeze-dried gluten is extracted twice with 15- and 10-ml. portions of 0.01*N* formic acid solution. The extract is evaporated to dryness and weighed as "soluble" protein. The gelatinous protein in the residue is solubilized by oxidation with 2 ml. of 30% hydrogen peroxide and 10 ml. of 0.2*N* formic acid, and the residual starch and bran are removed by centrifugation. The "gel" protein, dried and weighed, is expressed as percentage of the sum of the soluble and gel proteins obtained. Decrease in the amount of gel protein is taken as an indication of dough breakdown. The amounts of gluten recovered from the doughs were so nearly constant that the trends of the analyses would not be affected.

Farinograms were obtained with the small bowl at 63 r.p.m. and 30°C. using 50 g. flour (14% m.b.) and 64% absorption. A special bowl cover with vents to permit circulation of gas and addition of the liquids was fabricated. For doughs mixed under nitrogen, the farinograph bowl is flushed with nitrogen before the flour is introduced and the flow of nitrogen is continued for 5 minutes with the mixer running prior to the addition of the liquids while nitrogen is bubbled through the liquids for 5 minutes. Air is excluded from the bowl throughout the mixing by a continuous flow of nitrogen. Similar procedure was used to obtain farinograms for doughs mixed in oxygen. To change the atmosphere during mixing, the original gas is turned off and the flow of the desired gas is directed into the farinograph bowl. A decrease in farinograph consistency and a narrowing of the farinogram are used as indicators of dough breakdown. For convenience, the absorption has been kept constant rather than varied to give constant maximum consistency. The curves presented

by Hlynka (6) for a similar flour show that the effect on dough development time caused by the observed changes in consistency would be slight.

Results

The results of this investigation will be presented in several subsections. First, the extensigraph investigations show the general trend of improvement followed by breakdown as mixing time is increased; the rapidity of gain of this optimum in dough properties is a function of the amounts of improver and atmospheric oxygen present. Secondly, determinations of the proportion of gel protein in glutens provides additional evidence of the amounts of improvers that are effective in producing marked structural breakdown during mixing. A recovery of structure following the breakdown is also demonstrated in certain reagent concentration ranges, but the initial improver effect is not detected by this technique. Thirdly, the farinograph enables more direct demonstration of many of the effects and, in particular, shows the effects of successive additions of reagents during mixing. Finally, a direct comparison of the mixing actions of the farinograph and GRL mixers is described.

Extensigraph Results

Mixing in Nitrogen, Air, or Oxygen. (Fig. 1, long-dashed curves.) Extensigrams of Fig. 1 show that there is little change of dough structure as reflected by our extensigraph technique during mixing under nitrogen for 20 minutes. Mixing in nitrogen beyond this point produces some physical breakdown giving extensigrams (not shown) showing a slightly lower resistance. Doughs mixed in air show the improver effect, the amount of which increases gradually with increasing mixing time. Doughs mixed in oxygen show an improver effect to 7.5 minutes of mixing; further mixing produces gradual breakdown of dough structure, as indicated by the decrease in resistance to extension and increase in extensibility. With mixing beyond 20 minutes a weakening effect, compared with results for analogous doughs mixed in nitrogen, is obtained. Smith and Andrews (10) have compared mixing for varying periods in nitrogen and in air. In all cases (3 through 30 minutes) an improver effect of air was demonstrated, the maximum improvement being at 15 minutes for the farinograph.

Effect of Iodate. Figure 1 also compares, by means of extensigrams, the effects of mixing in nitrogen, in air, and in oxygen of doughs containing 0 (long dashes), 0.4 (short dashes) and 1.2 (solid curves) $\mu\text{eq. iodate per g. of flour}$. Comparison within each row of curves

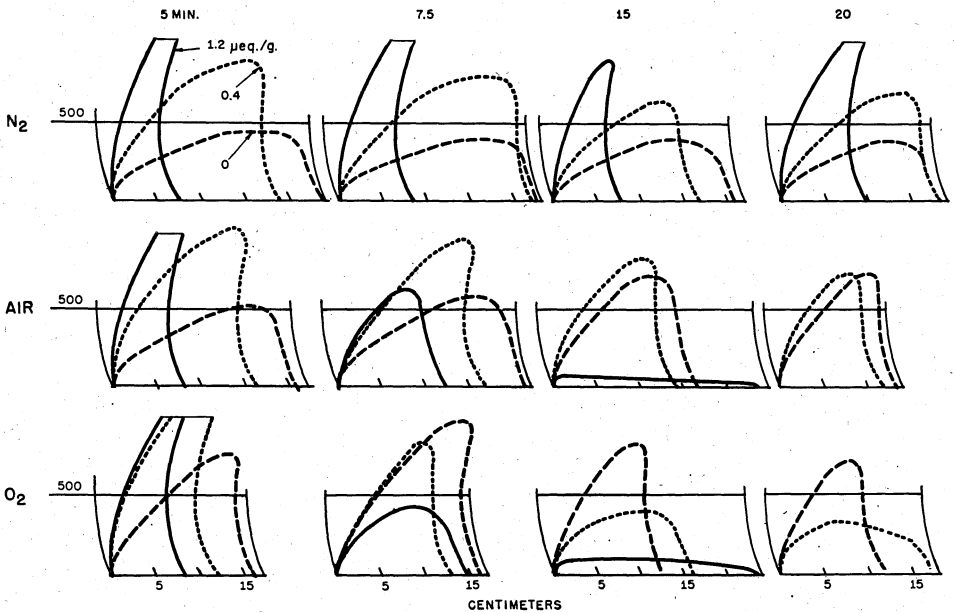


Fig. 1. Extensigrams of doughs mixed in GRL mixer for varying periods, with differing amounts of iodate, in nitrogen, air, and oxygen.

shows the effect of increasing mixing time at three concentrations of iodate; whereas a comparison within each column of curves gives the effect of increasing oxygen concentration at three concentrations of iodate and at one mixing time.

Doughs mixed in nitrogen show an improver effect in comparison with the controls, for both concentrations of iodate; the magnitude of the effect at one mixing time increases with increasing iodate concentration. There is a suggestion that with the two concentrations of iodate used, mixing in nitrogen produces a slight, gradual breakdown during the first 15 minutes and then a strengthening with additional 5 minutes of mixing; e.g., 20-minute extensigrams are higher than 15-minute. Our theories do not account for this initial slight breakdown.

Doughs mixed in air show an additional effect of atmospheric oxygen, the magnitude of which increases with mixing time. This additional effect of oxygen can show up as an increase in the amount of improver effect (compare nitrogen curves with air curves for 0.4 $\mu\text{eq. per g.}$) or as an increase in rate of breakdown, weakening, or overoxidation (compare analogous curves for 1.2 $\mu\text{eq. per g.}$), depending on the initial iodate concentration and the amount of mixing.

The combined effects of iodate, oxygen, and mixing can be so marked that under certain conditions a complete reversal of effects can be obtained; e.g., 1.2 μ eq. iodate per g. gives a definite improver effect after 5 minutes of mixing in air, whereas after 15 minutes of mixing the control dough shows a much higher resistance to extension than the iodate-treated dough.

In doughs mixed in oxygen, the additional effect of oxygen is so large that in all doughs except one the effect is to increase the rate of breakdown. The one exception is the 5-minute dough treated with iodate at the rate of 0.4 μ eq. per g. which showed an additional improver effect compared with analogous doughs mixed in nitrogen and in air. In doughs mixed in oxygen, the mixing "reversal" from improver to weakening occurs between 5 and 7.5 minutes for both concentrations of iodate used. The doughs mixed for 20 minutes in air or in oxygen were soft and extremely sticky and could not be formed into extensigraph test pieces.

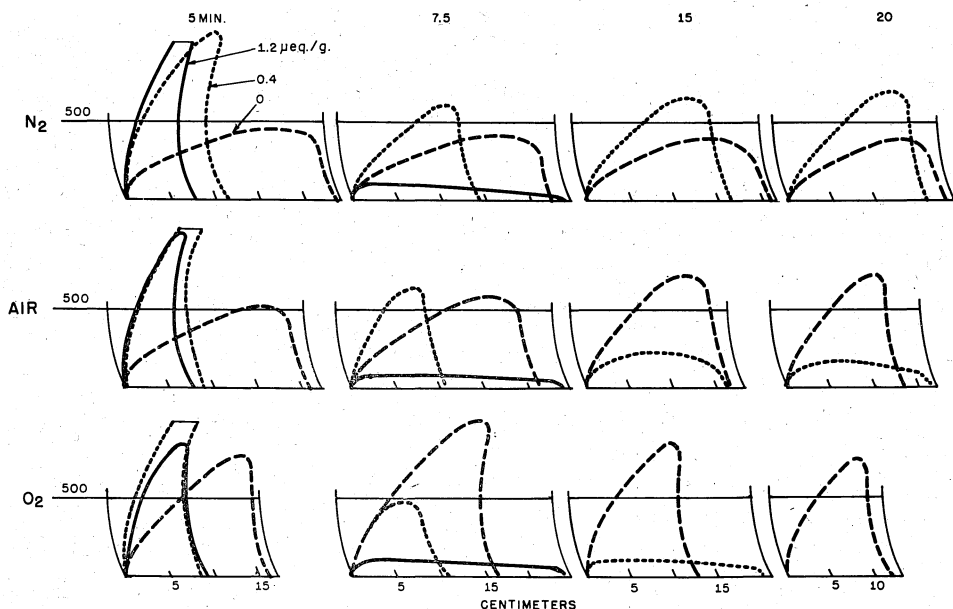


Fig. 2. Extensigrams of doughs mixed in GRL mixer for varying periods, with differing amounts of NEMI, in nitrogen, air, and oxygen.

Effect of NEMI. Figure 2 shows extensigrams for NEMI-treated doughs analogous to those of Fig. 1 for iodate-treated doughs. In the previous paper (2) it was shown that the reversal from improvement to weakening is readily obtainable in doughs treated with NEMI at

1.2 $\mu\text{eq.}$ per g. after about 7 minutes of mixing in nitrogen. Figure 2 shows similar results for doughs treated with the same amount or less of NEMI when the mixing is in air or in oxygen. Comparison of Figs. 1 and 2 shows that at equivalent concentrations, NEMI is considerably more effective than iodate in producing dough breakdown. Some curves for higher concentrations and mixing times were not obtained, because the doughs had broken down so far that they could not be formed into test pieces.

Gel Protein Analysis Results

Mixing in Nitrogen, Air, and Oxygen. (Fig. 3.) The amount of gel protein in the gluten from doughs mixed in nitrogen, air, or oxygen up to 40 minutes remained constant. That is, no changes in dough properties under these conditions could be detected by this technique.

Effect of Iodate. In nitrogen at iodate concentrations up to 1.2 $\mu\text{eq.}$ per g. flour, the doughs showed no breakdown up to 40 minutes of mixing. As the iodate concentration was further increased, there was initial dough breakdown followed by a recovery with increased mixing time. At 1.8 $\mu\text{eq.}$ per g. iodate concentration and above, the dough breakdown persisted through at least 40 minutes of mixing.

In air a pattern of changes was observed similar to that for nitrogen atmosphere but at lower iodate concentrations; 0.4 $\mu\text{eq.}$ per g. produced no apparent breakdown and 0.8 $\mu\text{eq.}$ per g. produced breakdown persisting through 40 minutes of mixing. Thus there was a sparing effect for iodate due to the presence of air.

Effect of NEMI. In nitrogen the pattern of changes was similar to that for iodate, the concentrations required being about half of those for iodate in nitrogen.

In air even less NEMI was required to produce similar changes, again showing a sparing effect of air in the reaction.

Farinograph Results

Mixing in Nitrogen, Air, and Oxygen. It is generally assumed that the effect of atmospheric oxygen on the farinogram is quite small, and accordingly it has been usually neglected. In the present studies the following observations were made: Nitrogen doughs reach optimum development about 1 minute faster than air doughs, which in turn develop only slightly faster than oxygen doughs. Doughs of 64% absorption had maximum consistencies of 490, 520, and 550 B.U. when mixed in nitrogen, air, and oxygen respectively. The rates of decrease in consistency after the optimum were 4, 9, and 12 B.U. per minute for doughs mixed in nitrogen, air, and oxygen respectively. It

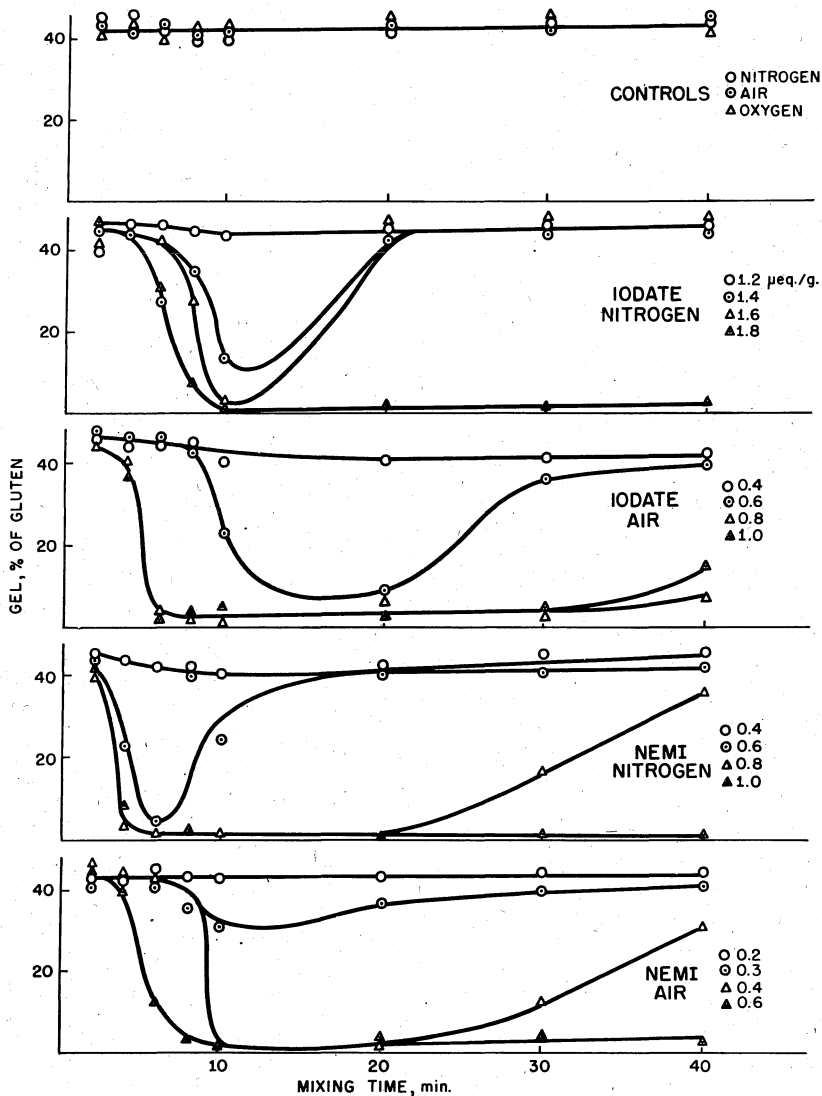


Fig. 3. The effects of reagent concentration and mixing time, in nitrogen and air, on the proportion of gel in gluten.

appears that the effect of oxygen is to increase the time to maximum development, increase the maximum consistency, and increase the rate of breakdown after maximum consistency when constant absorption is considered.

Effect of Iodate in Doughs Mixed in Nitrogen. (Fig. 4A.) Development time and maximum consistency increased with increasing iodate

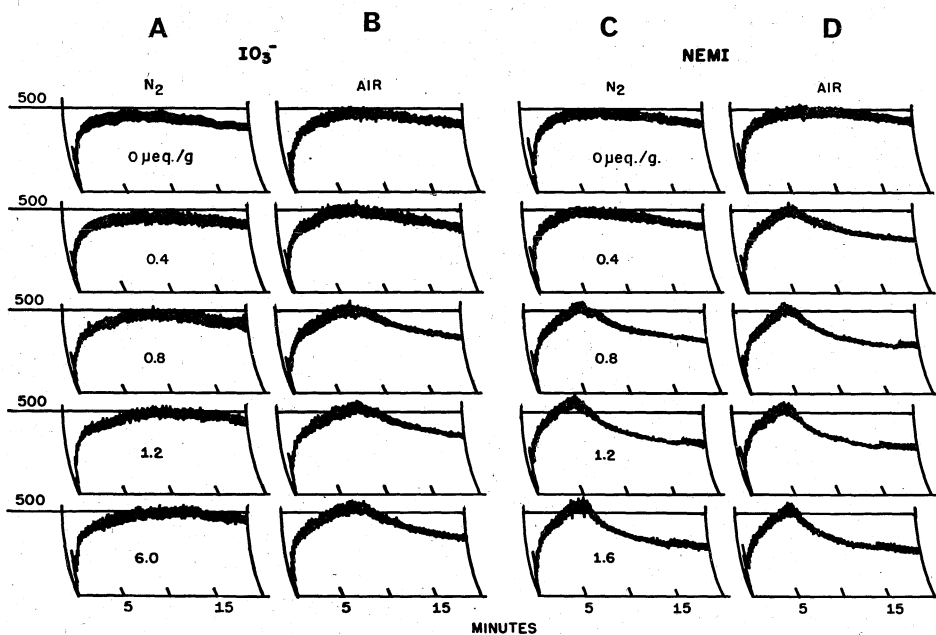


Fig. 4. Farinograms for various concentrations of iodate and NEMI, mixing in nitrogen and in air.

concentration, reaching a maximum at 1.2 $\mu\text{eq.}$ per g. iodate. Increase in concentration to 6.0 $\mu\text{eq.}$ iodate did not produce any additional change in these parameters. The rate of decrease in consistency after the maximum was reduced slightly when 0.4 $\mu\text{eq.}$ per g. was used and then increased as the amount of iodate used approached the $-\text{SH}$ content, to the extent that at 1.2 $\mu\text{eq.}$ per g. the rate of decrease was somewhat higher than for the control dough. Further increase of iodate to 6.0 $\mu\text{eq.}$ per g. did not produce any detectable additional change in the rate of decrease in consistency. That is, the effect of iodate in doughs mixed in the farinograph under nitrogen becomes saturated in the concentration range 0.8–1.2 $\mu\text{eq.}$ per g. of flour. Increase of iodate concentration to a relatively high level (20 $\mu\text{eq.}$ per g.) produced a detectable change.

Effect of Iodate in Doughs Mixed in Air. (Fig. 4B.) When iodate-treated doughs are mixed in air, they develop at about the same rate as the nitrogen doughs containing the same amounts of iodate, but the rate of decrease in consistency after maximum development is much higher than in nitrogen. The effect becomes saturated between 0.8 and 1.2 $\mu\text{eq.}$ per g.; however this saturation occurred at slightly lower iodate concentration in doughs mixed in air than in doughs

mixed in nitrogen. These results show that oxygen is extremely effective in causing dough breakdown, particularly when $-SH$ groups are removed by iodate.

The effect of oxygen in addition to that of iodate indicated above can be demonstrated by another type of experiment. Doughs containing initially 2 or 20 $\mu\text{eq.}$ iodate per g. flour were mixed in nitrogen for 10 or 15 minutes, at which times the nitrogen was turned off and

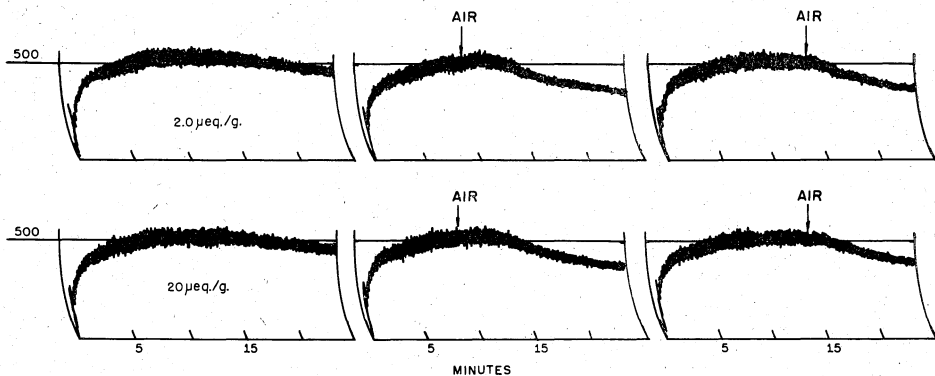


Fig. 5. Farinograms, initially in nitrogen, at two iodate concentrations, showing effect of air addition at 10 and 15 minutes.

the bowl was opened to the atmosphere. Results of this experiment (Fig. 5) show that the rate of decrease in consistency suddenly increases when the dough is exposed to atmospheric oxygen. This type of dough breakdown apparently results from oxygen reacting either with a reaction product of $-SH$ groups and iodate or with some other component which is necessary for mixing tolerance. Experiments in which the dough was mixed in oxygen instead of air did not show any marked effect of the additional oxygen on the farinograms. The amount of oxygen that is mixed into the dough during mixing in air does not seem to be a limiting factor in the oxygen effect on the farinograph properties in the presence of iodate.

Effect of NEMI in Doughs Mixed in Nitrogen. (Fig. 4C.) Doughs treated with NEMI develop rather faster than control doughs, go to a higher maximum consistency, and show a higher rate of decrease of consistency after the optimum. The rate of decrease of consistency becomes markedly greater with increase of NEMI concentration up to about 0.8 $\mu\text{eq.}$ per g. Further additions increase the breakdown rate more slowly, and there does not seem to be a level of NEMI at which its effect on the farinograph properties becomes completely saturated, as obtained with iodate-treated doughs.

It is of interest to note that the doughs treated with NEMI at a rate in excess of $0.8 \mu\text{eq. per g.}$ and mixed for 15 or 20 minutes were quite sticky and fluid, and by touch and sight appeared like doughs treated with reducing agents such as thioglycollic acid or reduced glutathione.

Effect of NEMI in Doughs Mixed in Air. (Fig. 4D.) Changes occur similar to those observed in nitrogen, but at lower NEMI concentrations. At concentrations of NEMI of $0.8 \mu\text{eq. per g.}$ or higher, additional effects of oxygen are not noticeable.

Effect of Iodate in the Presence of NEMI, and Vice-Versa, in Doughs Mixed in Nitrogen. The following experiments were designed to check further the possibility that, in addition to its reaction with $-\text{SH}$ groups, NEMI reacts with another functional group which reacts with iodate either very slowly or not at all. Furthermore, this secondary reaction which seems to occur during mixing, rather slowly and only after all the $-\text{SH}$ groups have been removed or blocked, has a marked effect on the tolerance of doughs to mixing. The farinograph procedure used in this experiment is as follows: Doughs initially containing $1.6 \mu\text{eq. per g.}$ of iodate or NEMI are mixed for 4.5 or 9.0 minutes, at which time an additional quantity of the second substance (as solid), $1.6 \mu\text{eq. per g.}$, is added to the dough without stopping the mixing. The doughs are mixed in nitrogen to eliminate the effects of oxygen.

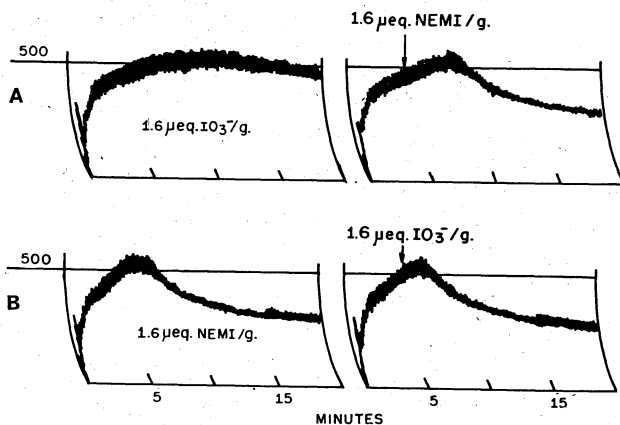


Fig. 6. Farinograms, in nitrogen, to show: (a) effect of NEMI in the presence of iodate; (b) effect of iodate in the presence of NEMI.

Comparison of farinograms shown in Fig. 6 by direct superposition gives the following effects: With iodate added first and NEMI second there is a definite additional effect due to NEMI. This addi-

tional effect of NEMI occurred even when the concentration of iodate was increased to 6.4 $\mu\text{eq.}$ per g. (results not shown). With NEMI added first and iodate second, iodate has no effect additional to that of NEMI. In other words, NEMI completely inhibits, presumably by a competitive mechanism, the action of iodate. Sullivan *et al.* (11) observed that the maturing effect of iodate was eliminated if NEMI had been added first.

Comparison of the Mixing Action of the Farinograph and GRL Mixers

Discussion of the interrelationship of the results presented in this paper made necessary a direct comparison of the mixing actions of the GRL and the farinograph mixers. The extensigraph technique was selected for this comparison. 55% absorption was used in both mixers.

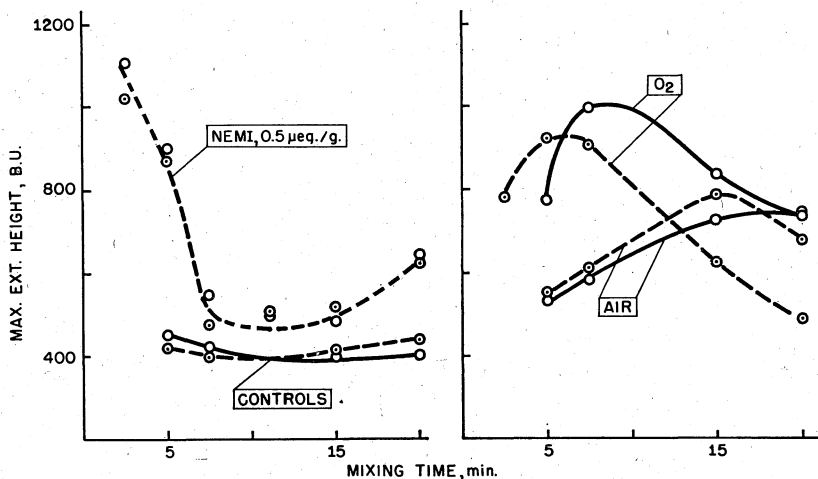


Fig. 7. Extensigrams comparing the actions of farinograph (filled circles) and GRL (open circles) mixers.

The results shown in Fig. 7 give the variation of the maximum extensigraph height with mixing time for various doughs. On the left are data for untreated doughs and doughs treated with 0.5 $\mu\text{eq.}$ NEMI per g. of flour mixed in nitrogen. The results for these two types of dough were the same for the two mixers. Untreated doughs did not show any tendency to break down during mixing for up to 20 minutes in either mixer. Doughs treated with NEMI at the rate of 0.5 $\mu\text{eq.}$ per g. showed a decrease in resistance to extension at first and then an increase in the same parameter as mixing was increased. There is a definite reversal of the effect of mixing in this type of dough i.e., the dough starts to weaken at first and then it seems to strengthen as mix-

ing continues. This strengthening effect was obtained with both mixers.

On the right of Fig. 7 are data for untreated doughs mixed in air and in oxygen. Doughs mixed in air by the GRL mixer showed a gradual increase in the amount of improver effect as mixing time increased to 20 minutes; doughs mixed by the farinograph mixer showed an increase in the improver effect with mixing time up to 15 minutes of mixing; 20 minutes of mixing produced a slight weakening effect in comparison with 15 minutes of mixing. These results suggest that the farinograph mixer exposes dough to the atmosphere more than does the GRL mixer, and that the observed difference is due to the additional oxygen that is incorporated by the farinograph mixer. A similar conclusion can be drawn from the results for the doughs mixed in oxygen. Smith and Andrews' (10) experiments, previously referred to, compared the actions of two mixers in nitrogen and in air. These authors also concluded that the mechanical action of mixing played a part in dough development additional to the incorporation of atmosphere. Thus the effects of oxygen, whether it is improvement that occurs in the early stages of mixing or weakening that occurs in the latter stages, will become apparent at a shorter mixing time in doughs mixed with the farinograph than with the GRL mixer. It is necessary to bear this factor in mind when results for doughs mixed in air or oxygen by different mixers are compared.

General Discussion

For the purpose of this discussion, it is assumed that dough breakdown can arise from two different, though not necessarily unrelated, mechanisms: a physical mechanism in which both covalent and other linkages are ruptured by purely physical forces, and a chemical mechanism in which structural cross-linkages are broken by reductive, oxidative, or hydrolytic cleavage. Both mechanisms apparently involve the $-SH$ and $-S-S-$ groups (2,3,11).

Normal, untreated doughs possess a specific mixing tolerance for particular mixing conditions. If the number of $-SH$ groups in the dough is decreased by additions of $-SH$ -blocking reagents (NEMI) or oxidizing agents (iodate, oxygen), tolerance to mixing seems to decrease (2,8,11). It is speculated that in a dough with high mixing tolerance, the stress that is introduced into the dough by the mixing action is dissipated by a rearrangement of protein chains mediated by $S-S$ interchange reactions which are initiated by $-SH$ groups. This is essentially the same mechanism that has been suggested for relaxation of resting doughs (3).

Untreated doughs mixed in nitrogen are relatively stable during mixing. Additions of iodate or NEMI increase the rate of breakdown. However, considerable amounts of these reagents must be added to produce marked breakdown, and NEMI appears more effective than iodate in causing the breakdown. The finding of Hird and Yates (5) that the equivalence of iodate in reaction with sulfhydryl groups is 4 rather than 6 would explain this apparent difference in effective concentration. Extensigraph evidence of the difference has already been published (2,11). Farinograms show that the effect of iodate in doughs mixed in nitrogen becomes saturated at about 1.2 $\mu\text{eq.}$ per g. This is approximately equivalent to the *accessible* -SH content of the dough, taking into account the effect of mixing on accessibility of -SH (1). The gel protein method, however, showed a requirement of 1.8 $\mu\text{eq.}$ per g. for full effect. This difference in requirement by the two methods may be due to removal of flour lipids enhancing the accessibility of -SH groups. The effect of higher doses of NEMI does not become saturated in the same way; from this it may be inferred that a reaction other than with the -SH groups occurs.

At levels of iodate or NEMI just lower than the -SH content of the doughs, the doughs appear to break down or weaken at first and then work-harden or strengthen with further mixing, as observed most distinctly with the gel technique and also with the extensigraph. Under similar conditions an upswing in the farinogram has also been observed in this laboratory. It appears that breakdown of dough can set in immediately if the -SH content of the dough is drastically reduced. When the dough has no -SH groups, and in the absence of a reagent that can give rise to chemical breakdown, the breakdown continues by the physical mechanism. If the dough contains a small number of residual -SH groups, these apparently can facilitate subsequent strengthening of the dough structure. The most obvious mechanism for this strengthening during mixing is the rearrangement of S-S bonds by the reaction of -SH groups with S-S bonds.

Oxygen initially acts as an improver, but if it is continually incorporated into the dough, as in extended mixing, oxygen can produce the reverse or weakening effect. There is considerable indirect evidence that the oxygen-improver effect results from the removal of -SH groups, and evidence has been obtained (12) which suggests that the reaction of oxygen that causes dough breakdown involves the S-S linkages.

Oxygen alone had only a weak effect on the mixing tolerance of dough but oxygen in the presence of iodate had a marked effect, such that the requirement of iodate for irreversible breakdown, assessed

by the gel technique after 10 minutes of mixing, was reduced from 1.8 to 0.8 μ eq. per g. This may be interpreted in terms of chemical breakdown by oxygen when -SH groups have been made ineffective by iodate. Alternatively, it could be that the initial product of the reaction between iodate and -SH is susceptible to easy oxidation by oxygen or by more iodate. Such a two-stage reaction of -SH cannot be excluded on the present evidence.

The effect of oxygen incorporated into dough is superposed on the effects of reagents added initially to the dough. Thus with NEMI or iodate at a dosage lower than equivalent to the -SH content, oxygen produces an additional effect, and the direction of this effect depends on the residual -SH content and on the amount of oxygen incorporated into the dough (duration of mixing). When the amounts of NEMI or iodate are in excess of -SH content, the NEMI-treated doughs show no additional effect of oxygen, but the iodate-treated doughs do show an additional effect of oxygen even when the amount of iodate added to the dough is as high as 20 μ eq. per g. Indirect evidence that was invoked previously (2) to explain the breakdown effect of NEMI suggests the cleavage of S-S bonds. Similar arguments may also be used to explain the secondary effect of oxygen (and of iodate which occurs to a limited extent at relatively high concentrations).

It seems that the secondary or breakdown reaction that occurs with oxygen can occur somewhat more efficiently with NEMI. Hence if NEMI is added to the dough in sufficient quantity, it apparently can competitively inhibit the analogous reaction of oxygen. According to farinograph results, iodate cannot do this. Farinograms of Fig. 6 show that physical properties of dough can be affected by NEMI (as well as by oxygen as shown in Fig. 5) through a reaction which occurs either very slowly or not at all with iodate under similar conditions. The effect of iodate can be completely inhibited by NEMI. Results have already been published on the inhibition of the effect of bromate by NEMI (4,11). On the other hand, the effect of NEMI cannot be completely eliminated by iodate, even when excessive amounts of iodate are added initially to the dough. The reason for this may well be in the rates at which these reagents react.

It should be emphasized that this paper makes no attempt to deal with the chemistry, in molecular detail, of reactions that determine changes in physical properties of doughs during mixing, but it is hoped that the results will be of some value as a starting point for studies of chemical reactions that might be involved. Reference to possible reactions has been purely speculative.

Conclusions

1. Iodate affects mixing tolerance primarily by reaction with -SH groups.
2. NEMI and oxygen affect mixing tolerance by reaction with -SH groups and by a second, much slower reaction. Indirect evidence suggests that the second reaction of these reagents involves the S-S bonds.
3. The results presented are compatible with the following ideas:
 - (a) Changes in the mixing tolerance of doughs can be due to either physical or chemical cleavage of structural bonds, or both.
 - (b) Relaxation of stress introduced into doughs by mixing can be achieved by rearrangement of protein chains facilitated by reactions of -SH groups with S-S bonds.
 - (c) Mixing tolerance implies a balance between the physical forces which introduce the stress and the rate of -SH-S-S reactions which dissipate the stress. Disruption of this balance will lead to decrease in mixing tolerance.

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