THE BROMATE REACTION IN DOUGH
II. Inhibition and Activation Sudies[1]

W. BUSHUK AND I. HLYNKA

ABSTRACT

The amperometric titration method was used to study effects of seventeen different chemicals on the bromate reaction in dough. Results indicate that: 1) Iodate, chlorine dioxide, and oxygen react with the same reactive components as bromate but at much greater rates and, accordingly, inhibit both phases of the bromate reaction; 2) N-ethylmaleimide, and copper(II), mercury(II), silver and selenite ions inhibit the bromate reaction probably because of their ability to react with sulfhydryl groups; 3) vanadate and versene activate the bromate reaction; the former probably acts as a normal catalyst whereas the latter probably removes inhibiting ions; 4) urea and guanidine hydrochloride have no effect on the bromate reaction, indicating that the rate of the linear reaction is probably not diffusion-controlled.

This paper, the second of the present series (1) on the bromate reaction in dough, continues the kinetic studies by examining the effects of inhibiting and activating reagents. The reagents were selected because they either affect the physical properties of dough or are known to interact with protein sulfhydryl groups which are believed to be involved in the bromate reaction.

Materials and Methods

The flour used in this study was described previously (1). The chemicals used as inhibitors or activators and the grade or origin are listed in Table I.

The method for following the disappearance of the bromate ion in dough was described in the first paper (1). All the experiments reported in this paper were made with initial bromate concentration of 18.75 mg. per kg. of dough (30 p.p.m. of flour) mixed to 60% absorption. The doughs were mixed in nitrogen with the exception of the experiments on the effect of oxygen. To study the effect of oxygen, the flours were first stored overnight under air (20% oxygen) or under pure oxygen, and the doughs were mixed under corresponding atmospheres. Treatment of flour with chlorine dioxide was made by the acetic anhydride method of Parker and Fortmann (8).

In experiments with solid reagents, the desired amount of the reagent, in aqueous solution, was added to the liquid used to mix the dough. The bromate solution was added to the same liquid.

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TABLE I

LIST OF CHEMICALS USED

CHEMICAL	GRADE OR ORIGIN
Oxidizing agents	
Potassium iodate	Reagent
Chlorine dioxide	Prepared in laboratory (8)
Oxygen	Canadian Liquid Air
Sulfhydryl-blocking agents	
N-ethylmaleimide	Nutritional Biochemicals, Inc.
Iodoacetic acid	Eastman Organic Chemicals
Sodium iodoacetate	Eastman Organic Chemicals
Inorganic ions	
Copper(II) chloride	Reagent
Silver nitrate	Reagent
Mercury(II) chloride	Analytical
Calcium chloride	Reagent
Lead nitrate	Reagent
Selenious acid	Reagent
Sodium vanadate (meta)	C.P.
Ammonium molybdate	Analytical
Complexing agent	
Versene (disodium salt of ethylene diamine tetraacetic acid)	Analytical
H-bond-breaking agents	
Urea	Reagent
Guanidine hydrochloride	Reagent

Blank titrations of extracts from doughs, which contained the inhibitor but no bromate, served as controls. The possibility of direct reaction between the added reagent and the bromate ion was checked for reagents which were suspected of such a reaction.

Results and Discussion

The effects of some seventeen different chemicals on the bromate reaction in dough were studied. To simplify the presentation of results, it is convenient to discuss the various reagents in groups according to their type or function as indicated in Table I. Results for each group will be discussed separately.

As in the earlier paper (1), the bromate reaction in dough will be characterized by the amount of initial reaction and the rate of the secondary or linear reaction. Effects on both reactions will be considered.

Oxidizing Agents. The effects on bromate reaction of three oxidizing agents commonly used for improving the baking quality of flour, namely, potassium iodate, chlorine dioxide, and oxygen, were studied. Figure 1 compares the results that were obtained. The upper curve for each reagent shows the effect on the initial reaction; the bottom curve gives the corresponding effect on the secondary or linear reaction. It is apparent that the oxidizing agents used are strong inhibitors of both phases of the bromate reaction in dough.

Control experiments with potassium iodate showed that, up to about 18.75 mg. per kg., this improver reacts completely in the normal dough during the mixing stage. At higher concentrations small amounts of iodate were recovered; the bromate recoveries for the high iodate concentrations were accordingly corrected for residual iodate which titrates as bromate.

It was not possible to produce 100% inhibition by iodate, even with relatively high concentrations of the inhibitor. This may result from a competitive mechanism in which an equilibrium is established between the reactants and the inhibitor, or it may be due to magnification of experimental errors when bromate recoveries are corrected for large amounts of residual iodate.

In the experiments with chlorine dioxide-treated flour it was observed that if the flour was used immediately after treatment a small amount of reactant, presumably unreacted chlorine dioxide which titrates as bromate, can be detected in control doughs. This residual chlorine dioxide seems to react rapidly after mixing, since no chlorine dioxide could be extracted from the dough after a reaction time of 1 hour. No chlorine dioxide could be recovered immediately after mixing if the flour was stored for several days after treatment.

A comparison of the efficiency of iodate and chlorine dioxide as inhibitors of the bromate reaction on an equivalent basis (assuming that iodate and chlorine dioxide are reduced to the corresponding halide ion) shows that iodate is apparently more efficient. However, this may be because part of the chlorine dioxide reacts in the bleaching reaction. The treated flour was considerably whiter than the original flour.

Figure 1 shows also that oxygen is a highly efficient inhibitor of both phases of the bromate reaction. These results are apparently different from those of Cunnigham and Hlynka (2), who showed that oxygen seems to increase the amount of initial reaction and inhibits only the linear reaction. The reason for this discrepancy is not known.

Sulfhydryl-Blocking Agents. Of the various sulfhydryl-blocking agents that are available, N-ethylmaleimide (NEMI) was selected because it seems to react specifically with sulfhydryl groups (3,9); it affects dough in a way similar to that of iodate; and it is conveniently soluble and adequately stable at the normal pH of dough. Figure 2 compares the inhibition of the bromate reaction by NEMI and iodate.

It is apparent that the two agents are quite similar in their effects on the bromate reaction, although they are quite different chemically. With NEMI, as with iodate, it was impossible to produce 100% inhibition.

The effect of iodoacetic acid or its sodium salt, which may also be classified as a sulfhydryl-blocking agent, was not as clear-cut as that of

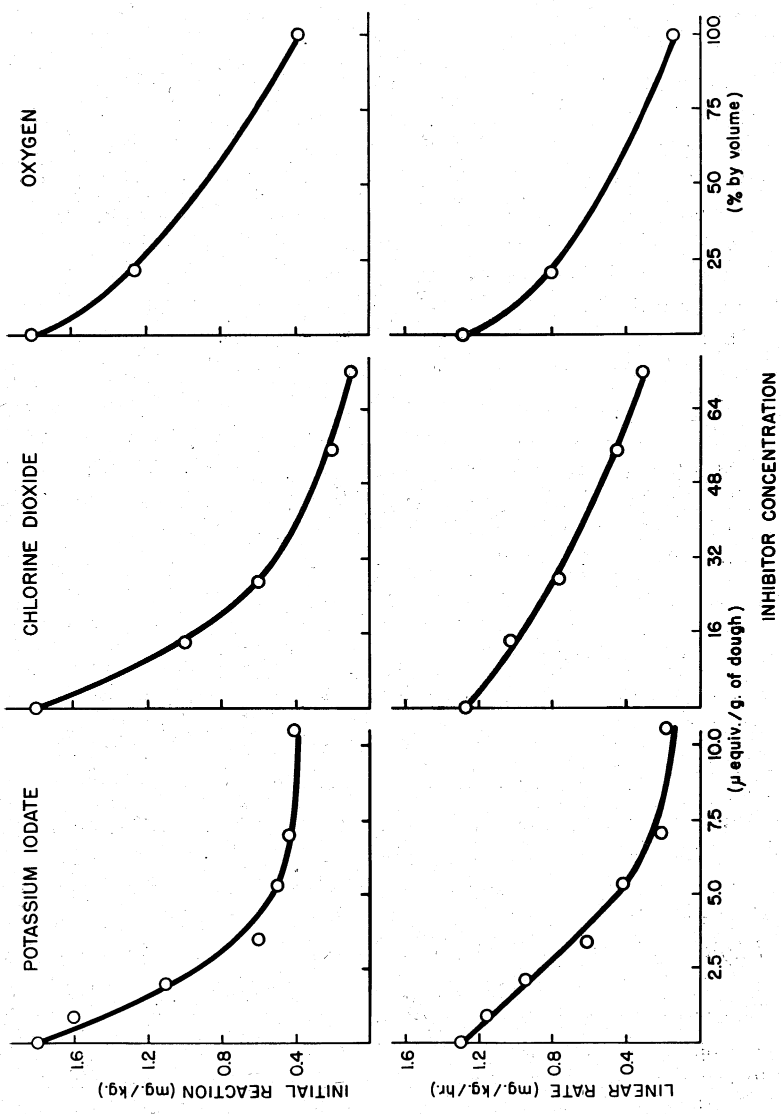

Fig. 1. Inhibition of the bromate reaction in dough by iodate, chlorine dioxide, and oxygen. Upper curves, initial reaction; lower curves, linear reaction.

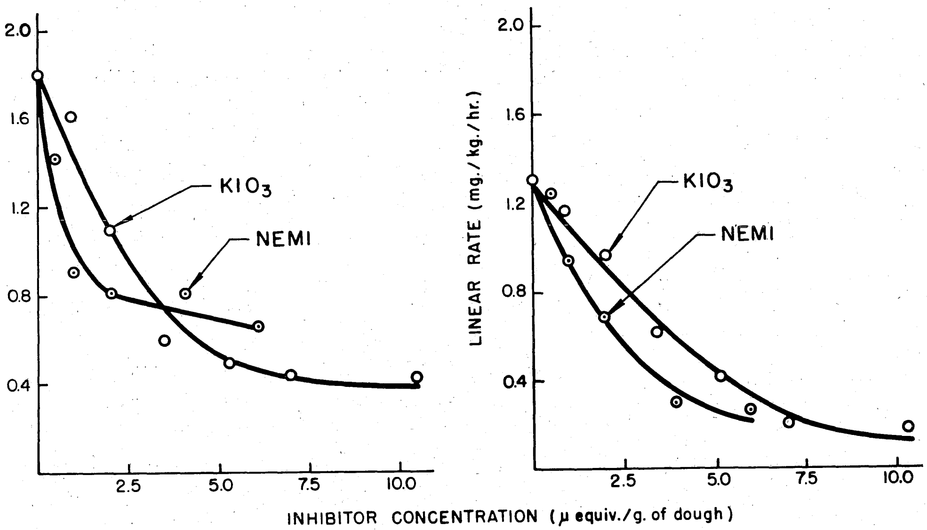

Fig. 2. Comparison of the inhibition of the bromate reaction in dough by N-ethylmaleimide with the inhibition by iodate. Upper curves, initial reaction; lower curves, linear reaction.

any of the inhibitors discussed so far. The maximum inhibition that could be produced with these inhibitors was only about 20%; this maximum occurred at an inhibitor concentration of 150 μmoles per kg. of dough. Increasing the inhibitor concentration further did not increase the amount of inhibition. In the experiments with sodium iodoacetate, an apparent reversal in inhibition was actually obtained. It seems that the results with iodoacetic acid and its sodium salt are complicated by some additional reaction. For example, if hydrogen iodide is produced, it may subsequently react as a reducing agent and consequently counteract the effect of the initial inhibition.

Inorganic Ions. Some inorganic cations show very marked effects on the physical properties of dough. Among these, copper(II), silver, and mercury(II) ions are probably the most active. The mechanisms of these effects are still not known, although it is suspected that the sulfhydryl group may be involved, either through oxidation or mercaptide formation. It was therefore of fundamental interest to study the effects of these ions on the bromate reaction. The effects of two less active cations, calcium and lead, were studied also.

Experiments were also made with three anions. Selenite ion was used because it seems to be a strong catalyst for the oxidation of the sulfhydryl group in glutathione, but is only a weak inhibitor of some sulfhydryl-containing enzymes (10,11); vanadate was used because it is known to catalyze the bromate reaction in dough as measured rheologically (6); and molybdate was used because it is an accepted catalyst for the oxidation of iodide by bromate in acidic solution.

Of the five cations studied, copper(II), silver, and mercury(II) ions inhibited the bromate reaction very markedly; calcium and lead ions showed very little effect and will not be discussed any further. Of the three anions studied, selenite showed a very strong inhibitive effect; vanadate ion seemed to accelerate both phases of the bromate reaction; and molybdate had no effect on the initial reaction but showed a slight inhibitive effect on the linear reaction.

Figure 3 gives the amounts of initial reaction and the rates of the linear reaction as a function of inhibitor concentration. Figure 3, A, compares the inhibition curves for copper (II), mercury (II), and silver ions, with the ion concentrations expressed in equivalents per g. of dough. Figure 3, B, gives the same data for the silver and mercuric ions, except that the ion concentrations are now in moles per g. This comparison assumes that the reacting ion for mercury (II) chloride is $HgCl^+$ and not Hg^{++} as is implied in Fig. 3, A. It is not known definitely which ion is the active one in dough. Experiments in which sodium chloride was added in conjunction with mercury(II) chloride showed that the additional chloride ion had very little effect on the amount of inhibition. Accordingly, it seems that $HgCl^+$ might be the reacting ion. Figure 3, C, gives the inhibition curves for the selenite ion.

The results (Fig. 3) show that the inhibition mechanism is essentially the same for the four ions. Again the mechanism appears to be competitive. The differences in the degree of inhibition shown in Fig. 3, A and B, are not considered as highly significant, except for the inhibition of the initial reaction by copper(II), in which case 100% inhibition was obtained with fairly low concentrations of the ion. Selenite ion seems to be the strongest inhibitor of the bromate reaction of all the inhibitors studied so far. Of the two ions, silver and mercury(II), silver seems to be a stronger inhibitor on the equivalent basis. On a molar basis, $HgCl^+$ appears to show a slightly higher inhibitive effect on the initial reaction.

It may be speculated as to why only copper(II) can inhibit the initial reaction 100%. One possible explanation that can be advanced is the ability of this ion to catalyze the oxidation of sulfhydryl groups in addition to its ability to form mercaptides or fairly stable complexes. In view of the fact that it is somewhat difficult to recover all the bromate from dough, either as bromide or bromate (5),

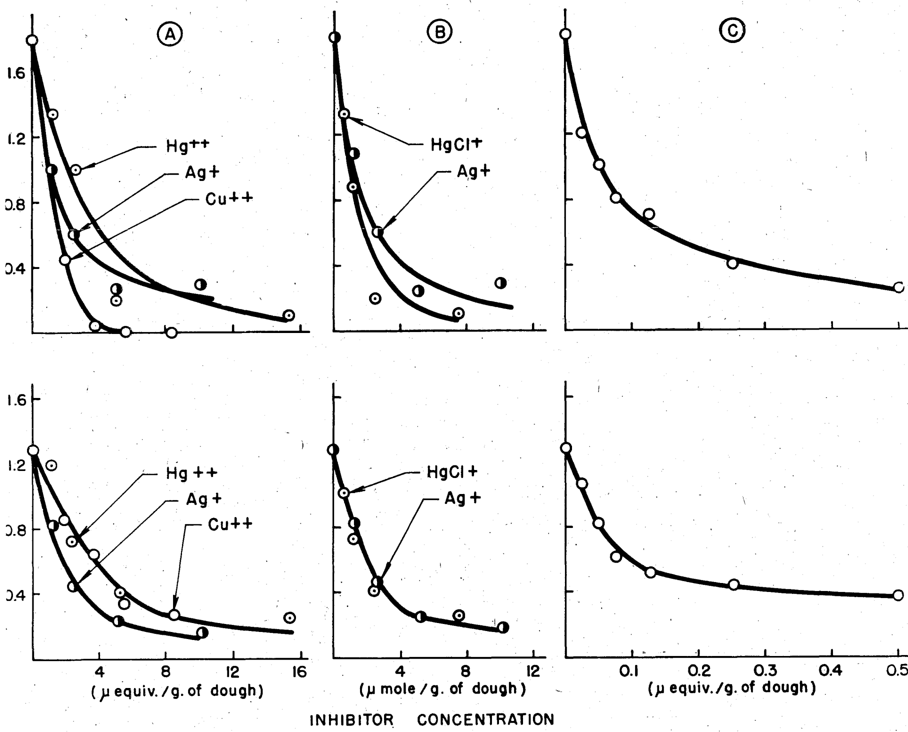

Fig. 3. Inhibition of the bromate reaction in dough by various inorganic ions. A, copper(II), mercury(II), and silver, with ion concentrations in μequiv. per g. of dough; B, mercury(II) chloride and silver nitrate with concentrations in μmoles per g. of dough; C, selenite ion. Upper curves, initial reaction; lower curves, linear reaction.

it is impossible to attach too much significance to the fact that copper-(II) ion can cause 100% inhibition of the initial reaction, whereas most of the other inhibitors can only produce 80 to 90% inhibition.

Table II gives the amounts of initial reaction and the rates of the linear reaction for the disappearance of the bromate ion in four doughs containing 0, 6.25, 12.50, and 18.75 mg. per kg. of sodium meta vanadate. The positive catalytic effect is quite apparent. These findings confirm the conclusions on the effect of vanadate on the bromate reaction based on studies of physical dough properties (6).

Complexing Agents. Table III gives the amounts of initial reaction and the rates of the linear reaction for doughs containing 0, 25, and 50 mg. per kg. of versene. These data show that versene has an apparent positive catalytic effect. This effect is probably due to the ability of versene to complex certain ions, some of which may be inhibitors

TABLE II

EFFECT OF SODIUM VANADATE (META) ON THE BROMATE REACTION

Sodium Vanadate Concentration		Initial Reaction	Linear Reaction
ppm flour	*mg/kg of dough*	*mg/kg*	*mg/kg/hour*
0	0.0	1.80	1.28
10	6.25	3.26	1.65
20	12.50	3.95	1.85
30	18.75	4.80	2.00

of the bromate reaction as was suggested by Hlynka (4) and confirmed in this study.

H-Bond-Breaking Agents. Additions of up to 6*M* urea (based on liquid used to mix the dough) and 4*M* guanidine hydrochloride had essentially no effect on the bromate reaction in dough, although the handling properties of the dough were altered considerably. Accordingly, it seems that urea and guanidine hydrochloride do not uncover any additional reactive groups and that the rate of the linear reaction, despite the magnitude of the activation energy (1), is not a diffusion-controlled process.

TABLE III

EFFECT OF VERSENE (DISODIUM SALT OF ETHYLENE DIAMINE TETRAACETIC ACID) ON THE BROMATE REACTION

Versene Concentration		Initial Reaction	Linear Reaction
ppm flour	*mg/kg of dough*	*mg/kg*	*mg/kg/hour*
0	0	1.80	1.28
40	25	2.36	1.57
80	50	3.18	1.84

General Discussion

Although the iodate and the bromate ions have essentially the same chemical properties, the rates at which they affect physical dough properties differ widely. Extensigraph studies show that iodate reacts almost entirely during mixing, so that its effect on the physical properties is perceptible immediately, whereas bromate requires a definite reaction time to show its effect. Results reported in this paper suggest that iodate and bromate react with the same group(s) in flour, except that iodate reacts much faster. A difference in the reaction rates would be sufficient to account for the inhibitive effect of iodate on the bromate reaction. It is actually possible to slow down the iodate reaction in dough considerably by lowering the temperature to 10°C. and raising the pH of the dough to 8.7. The mechanism of chlorine dioxide and oxygen inhibition is apparently the same as that of the iodate inhibition.

The ability of the other chemicals, namely, NEMI, iodoacetic acid and its sodium salt, copper(II), silver, mercury(II) and selenite ions, to inhibit the bromate reaction seems to parallel their ability to react with sulfhydryl groups. Indeed, NEMI is considered to be a highly specific reagent for sulfhydryl groups (3,9). Accordingly, it appears that at least 80 to 90% of the bromate reaction (both phases) in dough occurs with free sulfhydryl groups. The remaining 10 to 20% of the bromate disappearance may represent experimental error involving a physical loss inherent in the reaction system and the method of extraction of the unreacted bromate ion from dough. It should be mentioned in this connection that Matsumoto and Hlynka (7) found that NEMI decreased the sulfhydryl content of water-soluble components of flour by only 62% and of the acid-soluble components by only 40%.

Results reported in this paper add to the weight of evidence, obtained in this and other laboratories, that sulfhydryl is the main group involved in the bromate reaction in dough. Nevertheless, clear-cut results relating sulfhydryl content and disappearance, with disappearance of bromate have not yet been obtained, and the stoichiometry of the over-all reaction remains elusive.

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