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THE BROMATE REACTION IN DOUGH

IV. Effect of Reducing Agents¹

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ABSTRACT

The rate of bromate ion reaction in dough is increased by thioglycolic acid, L(+) cysteine hydrochloride, glutathione (reduced), thiolated gelatin, sodium bisulfite, and sodium borohydride if these reagents are incorporated into the dough before addition of potassium bromate. For each reagent, the increase in the reaction rate is proportional to the amount added. Increases in the rate are similar for the first four reagents, slightly lower for bisulfite, and much lower for borohydride. These results are consistent with the hypothesis that the bromate ion reacts principally with -SH groups and that the number available is increased by the reagents studied.

Reducing agents, such as bisulfite and thioglycolate, are known to split disulfide linkages in proteins to produce one or sometimes two sulfhydryl groups (8); this reaction is considered to be responsible for the changes in the physical properties of dough caused by reducing agents (5,7,9). Since bromate ions react principally with sulfhydryl groups in dough, the rate of this reaction should be increased by prior addition of reducing agents. Quantitative results consistent with these postulates are reported in the present paper, which continues a larger study seeking information on various aspects of the bromate reaction in dough (1,2,3).

Materials and Methods

The flour used in this study was an untreated, straight-grade flour commercially milled from hard red spring wheat. Its protein and ash contents were 13.3 and 0.46% on a 14% moisture basis. The reducing agents used and their grade or origin were as follows:

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<i>Reagent</i>	<i>Grade or Origin</i>
Sodium bisulfite	Reagent
Sodium borohydride	98+%; Metal Hydrides Inc.
Thioglycolic acid	80% in water; analytical
L(+) Cysteine hydrochloride	General Biochemicals Corp.
Glutathione (reduced)	Nutritional Biochemicals, Inc.
Thiolated gelatin	Schwarz Thiogel A.

Ordinary gelatin, used in control experiments on thiolated gelatin, was the Knox unflavored type.

The normal method for preparing doughs was modified to obviate the rapid reaction of the reducing agent with bromate ions when they are added to the water used to prepare the dough. This was accomplished by two-stage mixing. The flour was first mixed for 2.5 minutes with 90% of the water containing the reducing agent. The remaining 10% of the water, containing the required amount of potassium bromate, was then added and mixing was continued for another 2.5 minutes. All doughs were mixed under nitrogen to 60% absorption.

Residual bromate ion in dough was determined by the method described in the first paper of this series (1). The initial potassium bromate concentration used in this study was 18.75 mg. per kg. of dough (30 p.p.m. of flour).

Results and Discussion

Preliminary Experiments. In studies seeking to determine the effect of a reducing agent on the reaction of an oxidizing agent with a third substance, it is difficult to design unambiguous experiments. Because of the rapid reaction of most of the reducing agents used with bromate ions in aqueous solution, the two substances could not be added to the flour simultaneously and the dough mixed in the usual manner. Two-stage mixing was designed to overcome this difficulty. The development of stickiness in the doughs with all reagents except thiolated gelatin (see below) after the first 2.5 minutes of mixing was taken as evidence of a definite reaction between the reducing agent and flour proteins. Furthermore, aqueous extracts of the doughs prepared immediately after the first stage of mixing did not show any residual reducing agent for the highest concentrations used. Unreacted reducing agent was extracted from doughs which contained approximately two times the highest concentration of reducing agent (except thiolated gelatin) used in this study. In another type of experiment, the dough was allowed to rest in the mixer bowl for 7.5 minutes after the first 2.5 minutes of mixing and prior to the addition of the bromate. The rate of bromate disappearance in this dough was the same as that in the dough to which bromate was added im-

mediately after the initial 2.5 minutes of mixing. Accordingly, on the basis of this evidence, it was concluded that all of the originally added reducing agent reacts in the dough in the 2.5 minutes of mixing. However, these results do not completely rule out the possibility that some reducing agent remains in the dough in an adsorbed, unextractable form and might thus be available for direct reaction with bromate ions.

Effect of Reducing Agents on the Bromate Reaction. Figure 1 shows the bromate reactions in doughs which were initially treated with various amounts of sodium bisulfite. The rate of bromate reaction in the bisulfite-treated doughs increased with increasing concentration of reducing agent. When the addition of bisulfite (1 μ mole per g.) and bromate to the two-stage dough was reversed, the amount of bromate that reacted at each reaction time in this dough was about 15% higher than that shown in Fig. 1 for the same concentration of bisulfite. This 15% increase can be attributed to a small amount of direct reaction of bisulfite with bromate ion which probably occurs along with the reaction of bisulfite with protein disulfide.

Results (Fig. 1) show that the reaction of bromate ion in dough increases with increasing amount of bisulfite initially incorporated into the dough. It has been postulated that bisulfite reacts with disulfide groups of wheat proteins to produce one sulfhydryl and one S-sulfonate (8); accordingly, it is concluded that the increase in the bromate reaction is the direct result of the increase in the number

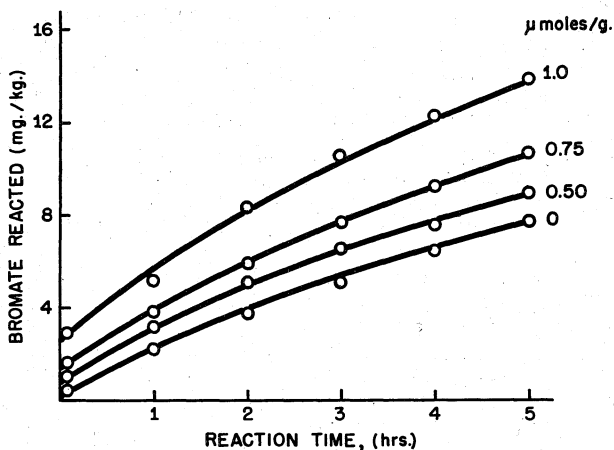


Fig. 1. Reaction of bromate in doughs treated with various amounts of sodium bisulfite. Initial potassium bromate concentration was 18.75 mg. per kg. Bisulfite concentrations given in μ moles per g. of dough.

of sulfhydryls available for reaction. These results can therefore be cited as additional evidence consistent with the hypothesis that bromate ion reacts in dough with sulfhydryl groups.

Figure 2 gives first-order plots for the data of Fig. 1. The data give good straight lines; accordingly, these reactions can be classified as first-order. The slopes of the first-order lines increased with increasing concentration of reducing agent, and this makes possible the use of the specific rate constant, which is directly related to the slope, as an index of the effect of the reducing agent.

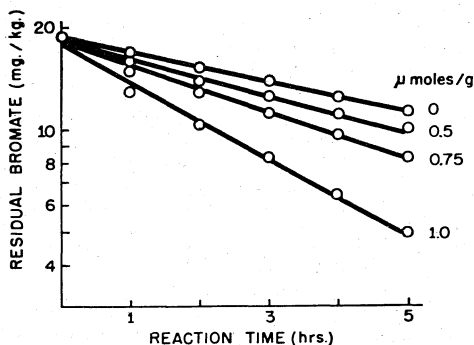


Fig. 2. First-order plots for the reactions shown in Fig. 1.

Results analogous to those obtained with bisulfite were also obtained with the other reducing agents used in this study. Table I gives the specific rate constants for the bromate reactions in doughs containing various amounts of the reducing agents. The concentrations of reducing agents are given on a per g. of dough basis, and the rate of bromate reaction is expressed in terms of the first-order specific rate constant. Control experiment for thiolated gelatin, in which 5 g. of ordinary gelatin were included in 320 g. of dough, showed that the increase in protein alone does not affect the bromate reaction. That is, it is the introduction of sulfhydryl groups *per se* or by reaction that gives the increase in the bromate reaction.

The increases in the specific rate constant (Δk) for the bromate reactions in doughs treated with reducing agents are plotted in Fig. 3 as a function of the reducing agent concentration. The point for the highest concentration of thiolated gelatin was not included in this graph. With all reagents, considerable increases in the bromate reaction were obtained. The change in the rate constant increased approximately linearly with concentration. The slight deviation from linearity for some of the reagents is not considered as highly significant.

TABLE I
FIRST-ORDER SPECIFIC RATE CONSTANTS AT INCREASING CONCENTRATIONS OF
VARIOUS REDUCING AGENTS
(Potassium bromate concentration = 18.75 mg/kg of dough)

CONCENTRATION	$k \times 10^5$			
	Bisulfite	Thioglycolate	Glutathione	Cysteine
$\mu\text{moles/g}$	sec^{-1}	sec^{-1}	sec^{-1}	sec^{-1}
0	2.82	2.82	2.82	2.82
0.25	...	4.45	4.79	5.24
0.50	3.56	7.57	9.05	9.57
0.75	4.38
1.0	7.15	14.82	14.03	16.57

THIOLATED GELATIN			SODIUM BOROHYDRIDE	
Concentration	$k \times 10^5$		Concentration	$k \times 10^5$
mg/g	$\mu\text{moles SH/g}^a$	sec^{-1}	$\mu\text{moles/g}$	sec^{-1}
0	0	2.82	0	2.82
0.78	0.125	4.40	4.13	5.31
1.56	0.25	6.21	8.26	6.86
3.10	0.50	8.95	16.52	10.97
7.80	1.25	17.05		
15.60	2.5	26.10		

^a Calculated on the basis of -SH content of 16 per 100,000 molecular weight.

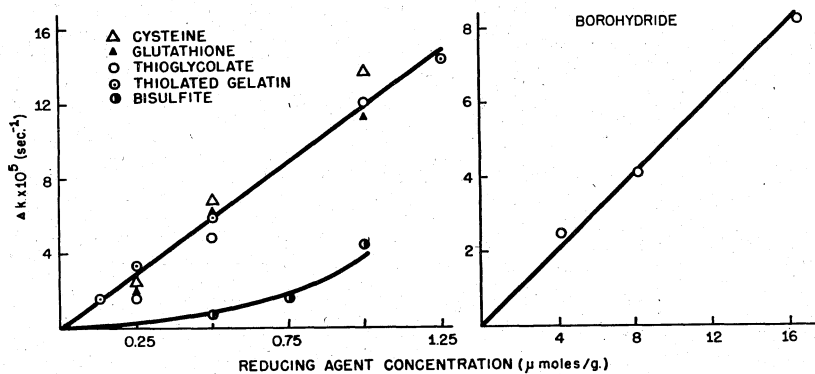


Fig. 3. The increase in the first-order rate constant for bromate reaction in doughs treated with increasing amounts of various reducing agents, plotted as a function of reducing agent concentration (per g. of dough).

Effect of Reducing Agents on Handling Properties of Dough. This study presented an opportunity to examine the effect of the various reducing agents used on the handling properties of dough, and, although the observations that were made are qualitative, it seemed desirable to put them on record.

All the reducing agents used except thiolated gelatin affected the physical properties of dough to such an extent that it was difficult

to handle them in the usual manner. The doughs were slack, sticky, and highly extensible. As the bromate reaction proceeded, a definite decrease in stickiness was observed; however, it was not entirely overcome even after a reaction time of 5 hours. This suggests that after 5 hours the sulfhydryl content has not yet decreased to the original level in the unreduced dough.

This conclusion can be supported by calculating the amount of sulfhydryl remaining in a dough treated with cysteine at the rate of 1.6 μ mole per g. of flour. If it is assumed that the original flour had a sulfhydryl content of about 1 μ mole per g. (10) and that all the cysteine reacts with disulfide groups to produce two sulfhydryls, i.e., 1.6 μ mole per g., the total sulfhydryl content is of the order of 2.6 μ mole. During 5 hours, the loss of bromate in such a dough was 28 p.p.m. of flour, which is equivalent to 1.01 μ mole of sulfhydryl per g. of flour. Thus, after 5 hours, the dough would still contain 1.6 μ mole of sulfhydryl as compared to an original content of about 1 μ mole.

The effect of thiolated gelatin on the physical properties of dough is significantly different from that produced by the other reducing agents and deserves special comment. Doughs containing as much as 5 g. of thiolated gelatin (2.5 μ moles -SH per g. of dough) did not feel different from control doughs. Two hypothetical explanations, both based on the high molecular weight of the thiolated gelatin used, can be offered at present to explain the absence of a reducing effect of this reagent on dough. First, it might be that the rate of the interchange reaction is negligible because of the high molecular weights of the components involved; the increase in the bromate reaction would then be the result of the reaction of bromate ion with the sulfhydryls of thiolated gelatin. This latter reaction, in aqueous solution of neutral pH, proceeds at about the same rate as the normal bromate reaction in dough. Second, it might be that the physical properties of the protein that results from the interchange are similar to the properties of the original protein, because the relative size of the molecules produced is not very different from the original size.

General Discussion

The main difference between the effects of the various reducing agents shown in Fig. 3 is in the magnitude of the increase in the bromate reaction per equivalent weight of each reagent. Thioglycolate, cysteine, and glutathione have approximately the same effect (thiolated gelatin is not included in this comparison for reasons given below). This is slightly higher than the effect of bisulfite. The small

difference between the effects of the former three reagents and that of bisulfite probably reflects a difference in the equilibrium constants for the reactions involved. It is known that under some conditions bisulfite produces one sulfhydryl and one S-sulfonate group from each disulfide (8). This possibility, together with the slow rate of the bromate reaction in dough, may explain the observation that the effect of bisulfite on the physical properties of gluten cannot be entirely overcome by bromate (5).

Thiolated gelatin was not included in the comparison made above because it is difficult to ascertain from the present study whether the observed increase in bromate reaction reflects a reaction of bromate ions with the sulfhydryls of thiolated gelatin or with the sulfhydryl produced by reaction of thiolated gelatin with gluten disulfide. It may well be that, so far as the bromate reaction is concerned, the origin of the sulfhydryl groups is immaterial.

Sodium borohydride, which apparently reduces disulfide groups of wheat protein to two sulfhydryls (4), seems to be an order of magnitude less effective (note the change in scale in Fig. 3) than the other reagents in its ability to increase the bromate reaction. A possible explanation of this observation may be the instability of borohydride in acid solution or its reaction with some extraneous substance. If this is true, then the results of the present study indicate that the amounts of the reagent that actually react in dough with disulfide groups are only about 5% of the amounts used (given in Table I and Fig. 3).

The technological implications of the results presented in this paper deserve comment. It has already been reported that doughs having certain desirable mixing characteristics can be produced by using L-cysteine in conjunction with bromate (6). Accordingly, with the increased understanding of the bromate reaction and its role in determining the physical properties of dough, it might be worth while to re-examine the use of combinations of reducing and oxidizing reagents in baking technology.

From the theoretical viewpoint, the results obtained are consistent with the hypothesis that bromate ions react principally with sulfhydryl groups and the number available is increased by the reagents studied. Moreover, the study provides further evidence that both the sulfhydryl and the disulfide groups are implicated in the improver action of bromate in breadmaking.

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