THE BROMATE REACTION IN DOUGH

V. Effect of Flour Components and Some Related Compounds[1]

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ABSTRACT

The disappearance of bromate in dough from a high-grade flour occurs by the reaction of sulfhydryl groups of flour proteins with the bromate ion. Starch and pentosans are not directly involved in the bromate reaction. Crude lipids seem to be involved indirectly. In doughs mixed in air, the lipids use up part of the available oxygen in the dough, probably by the lipoxidase-catalyzed peroxidation reaction, and, accordingly, decrease the inhibitory effect of the oxygen on the bromate reaction. Cumene hydroperoxide added to a dough mixed under nitrogen inhibits the bromate reaction; thus, lipid hydroperoxides, if produced in sufficient quantity, probably can compete with bromate for the sulfhydryl group. It is postulated that the involvement of lipids in the improver effect is through the reaction of sulfhydryl with hydroperoxides produced by the lipoxidase-catalyzed oxidation of lipids. n-Propyl gallate and butylated hydroxyanisole, common antioxidants, exert an inhibitory effect on the bromate reaction in doughs mixed in air but are inactive in doughs mixed in nitrogen. A reaction scheme explaining the role of various flour components in the sulfhydryl-bromate reaction in dough is proposed.

Previous studies of the disappearance of bromate in dough have shown that the reaction seems to depend on the protein content of the flour (1,5). There is some evidence that the ash-producing com-

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ponents (5) and lipids (6,9) might be involved in this reaction, also. It is now established that, in a dough from a high-grade flour, bromate ion reacts with sulfhydryl groups (2,4,7); however, it is not clear whether these groups are entirely located in the flour proteins, as has been presumed, and whether other flour components are involved in the bromate reaction, either directly or indirectly.

The study described in this paper re-examines the effect on the bromate reaction in dough of proteins and lipids and examines the effect of starch, pentosans, cumene hydroperoxide, and two commercial antioxidants, n-propyl gallate and butylated hydroxyanisole.

Materials and Methods

The flour used for most of this study was a hard red spring, straight-grade flour of 13.2% protein and 0.46% ash (14% moisture basis). In one series of experiments on the effect of protein content, low- and high-protein fractions from a hard red winter flour prepared by the air-classification process and supplied by The Pillsbury Company were used. The low and the high fractions had protein contents of 10.8 and 20.3% and ash contents of 0.40 and 0.68% (14% moisture basis). The average size of the particles comprising these fractions was 21.6 and 4.8 microns, respectively.

The flour components used in this study and the methods used for their separation from the hard red spring flour are described in the paragraphs below.

Granular Starch. The flour was mixed into a dough, and the starch was washed out by working the dough by hand in a beaker of tap-water. Excess water was decanted after centrifugation for 30 minutes at 2,000 r.p.m. The squeegee starch was separated from the granular starch by resuspending in water and centrifuging. The granular starch was then air-dried to a moisture of about 12%. This method yielded granular starch having protein and ash contents of 0.2 and 0.15%, respectively.

Gluten. The gluten was washed out by hand, dried by lyophilization to about 9% moisture, and ground on a Wiley mill to pass through screen No. 30. Two types of gluten were used in this study. One type, henceforth referred to as nitrogen-washed gluten, was prepared in the total absence of oxygen. The dough was mixed under nitrogen and subsequently worked up in a beaker of distilled water, which was initially purged of oxygen by bubbling nitrogen through it. The bubbling of nitrogen was continued throughout the washing. The protein content of the nitrogen-washed gluten was 87% (dry basis).

The second type of gluten, which will be referred to as air-washed gluten, was prepared from a dough mixed in air. The gluten was washed out in distilled water containing normal amount of atmospheric oxygen. The protein content of the air-washed gluten was 88% (dry basis).

Pentosans. The pentosans were prepared by the method of Pence, Elder, and Mecham (10). Nine grams of pentosan material were obtained from 1 kg. of flour.

Lipids. Crude fat was extracted from the flour with petroleum ether (Skellysolve F 95) in a large Soxhlet extractor. The solvent was initially purged of oxygen by bubbling nitrogen through it. Oxygen was excluded from the apparatus during extraction by a continuous flow of nitrogen directed into the top end of the condenser. The extracted fat was recovered by evaporation of the petroleum ether *in vacuo,* and the residual flour was dried in a stream of nitrogen. The fat extracted by this method was about 1% of original flour.

Other materials used in this study were cumene hydroperoxide (Monomer-Polymer, Inc., Leominster, Mass.), n-propyl gallate (practical, Eastman), and butylated hydroxyanisole (Nutritional Biochemicals).

The effects of the flour components, with the exception of lipids, cumene hydroperoxide, and the two antioxidants, were studied by determining the rate of bromate disappearance in doughs prepared from flours obtained by blending together the required amount of the dry component in question and the parent flour. With the high- and low-protein fractions of the hard red winter flour, the protein range was extended by adding granular starch to the low- and the high-protein flours. Two flours, with protein contents between the low- and the high-protein fractions, were prepared by blending required amounts of the low- and high-protein flours. The lipids, cumene hydroperoxide, n-propyl gallate, and butylated hydroxyanisole were added directly to the flour at the time of the preparation of the dough.

Protein contents (N $\times$ 5.7) of the blended flours were determined by the Kjeldahl method and will be given in the next section on a dry matter basis.

The doughs in this investigation were all mixed to an absorption of 60% and in an atmosphere of nitrogen except for a small number of doughs used to study the effect of lipids and antioxidants, which were mixed in air. All experiments were made at 30°C.

The initial potassium bromate concentration used was 18.75 mg. per kg. of dough (30 p.p.m. of flour), and the residual bromate was

determined by the method described previously (1). The first-order specific rate constant was used as a measure of the reaction rate (3).

Results of this investigation will be presented in two main sections. First subsection under "Results and Discussion" will describe and briefly discuss the results obtained with flours of different protein content, regardless of whether they were prepared by adding gluten to the parent flour or by diluting the parent flour with starch or pentosan material, or by blending high- and low-protein flours. The second subsection will deal with the results of the experiments on the effect of lipids and the related substances. A second main section will be a general discussion of the interrelationship of the results of the entire investigation.

Results and Discussion

Effect of Protein Content. Table I gives the results for the reaction of bromate ion in doughs from hard red spring flours of different protein content. The components and the protein contents of the blended flours are given in the first and second columns, and the third column gives the specific rate constants. Figure 1 shows graphically the relation between the rate constant and the protein content. The points for the flours enriched with nitrogen-washed gluten fall on the same straight line that passes through the points for the flours prepared by dilution with starch, as well as the point at the origin which represents the bromate reaction in a concentrated starch-water mixture. The two points for the flours enriched with air-washed gluten, together with the point for the parent flour, also give a straight line. However, the increase in the bromate reaction produced by the air-washed gluten is only about 50% of the increase produced by the nitrogen-washed gluten.

These results suggest that about 50% of the sulfhydryl groups of

TABLE I

SPECIFIC RATE CONSTANTS FOR HARD RED SPRING FLOURS OF DIFFERENT PROTEIN CONTENTS

FLOUR OR BLEND	PROTEIN CONTENT	$k \times 10^5$
	%, dry basis	*sec^{-1}*
Flour + N_2 gluten	36.2	5.66
Flour + N_2 gluten	28.1	4.30
Flour + air gluten	39.5	4.10
Flour + air gluten	30.2	3.49
Flour	15.3	2.82
Flour + starch	11.1	1.82
Flour + starch	5.27	0.76
Starch	0.24	0.0

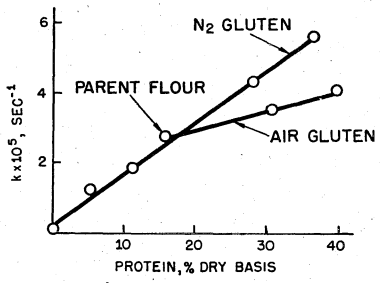

Fig. 1. Rate constants for bromate reaction in doughs from hard red spring flours of various protein content obtained by dilution with starch or by fortification with nitrogen-washed or air-washed gluten.

gluten are lost during the preparation of the dough and the removal of the gluten by washing, if these operations are made under normal atmospheric conditions. This estimate of the sulfhydryl loss agrees with the 50% loss obtained by Sokol, Mecham, and Pence (11) from direct measurements of sulfhydryl content in doughs subjected to prolonged mixing in air. Nitrogen-washed gluten, on the other hand, appears to have retained all of the original sulfhydryl content. These results suggest that it is necessary to use anaerobic conditions to prepare gluten that will retain its natural properties.

Table II gives the results of the experiments with the low- and the high-protein fractions obtained from the same parent hard red winter flour by the air-classification process, two flours obtained by blending the low- and high-protein fractions, and the flours obtained by diluting the low- and high-protein fractions with starch.

Figure 2 shows the variation of the specific rate constant as a function of the protein content for the flours listed in Table II. Linear relationships again result for each set of flours prepared by blending

TABLE II

SPECIFIC RATE CONSTANTS FOR THE LOW- AND HIGH-PROTEIN HARD RED WINTER FLOURS AND BLENDS OBTAINED FROM THEM

FLOUR OR BLEND	PROTEIN CONTENT	$k \times 10^5$
	%, dry basis	*sec^{-1}*
High-protein	23.8	3.81
High-protein + low-protein	20.2	2.78
High-protein + low-protein	16.9	2.07
High-protein + starch	15.8	2.31
High-protein + starch	7.9	1.04
Low-protein	12.4	0.91
Low-protein + starch	9.7	0.74
Low-protein + starch	5.4	0.39

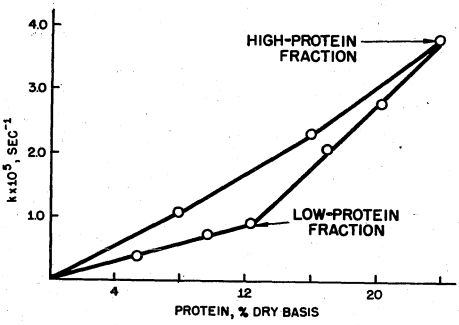

Fig. 2. Rate constants for bromate reactions in doughs from hard red winter flours of various protein content. Upper curve, high-protein fraction diluted with starch; left portion of lower curve, low-protein fraction diluted with starch; right portion of lower curve, blends of high- and low-protein fractions.

any two components. An interesting feature of the results of Fig. 2 is the difference in the slopes of the lines for the flours obtained by diluting the low-protein fraction with starch and those obtained by blending various amounts of the low- and high-protein fractions. Apparently the specific reactivity (the number of sulfhydryls available or accessible for reaction) of the protein in the high-protein fraction is higher than that of the low-protein fraction. This higher reactivity might be a direct result of a shift of the protein of higher sulfhydryl content to the high-protein fraction during the air-classification process, or it might be due to the higher specific surface of the same fraction. It was shown previously that physical accessibility of sulfhydryls, i.e., the specific surface area, plays a definite role in controlling the rate of the bromate reaction in a resting dough (3).

Pentosans do not seem to play any direct role in the bromate reaction other than as a relatively inert diluent similar to starch. Additions of 0.5 and 2% (flour basis) of the pentosan material, to give flours of approximately two and four times the natural pentosan content, did not decrease the protein concentration sufficiently to affect the rate of bromate reaction. It is of interest to note that the added pentosan material produced a definite change in the handling properties of the dough. Doughs from pentosan-rich flours were more sticky than normal doughs.

Effects of Lipids and Related Substances. Figure 3 compares the bromate reactions in the following doughs from hard red spring flour: control doughs from the original flour mixed in nitrogen and in air; doughs from defatted flour mixed in nitrogen and in air; and a dough mixed in nitrogen from defatted flour after reconstitution of the recovered crude fat. The main role of the crude fat (Fig. 3) seems to be

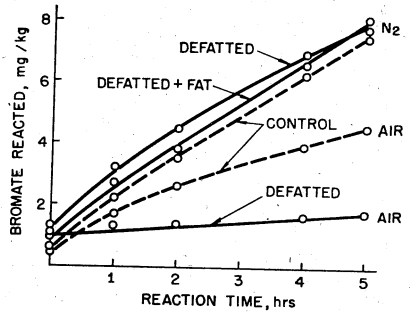

Fig. 3. Effect of crude lipids in the presence and absence of oxygen on the bromate reaction in doughs from hard red spring flour.

related primarily to the effect of oxygen. When the doughs are mixed in nitrogen (top three curves), fat has little effect. In the presence of oxygen (lower two curves), the depression of bromate reaction is much greater in the dough from defatted flour than in the dough from the control flour. The crude fat, probably through the lipoxidase-catalyzed reaction (13), seems to compete with protein sulfhydryl for the available oxygen in the dough, and, hence, the oxygen inhibition of the bromate reaction in the presence of lipids is not as great as in their absence.

Previous studies (6,9) showed that the removal of crude fat in the presence of atmospheric oxygen produced a slight depression in the bromate reaction in a dough from the defatted flour. When the fat is extracted with petroleum ether in an atmosphere of nitrogen the bromate reaction is not affected; actually, a slight increase was observed in the present study. This is ascribed to removal from the flour of certain inhibitory substances, e.g., hydroperoxides (see below).

Attempts to produce a totally defatted flour by extraction with water-saturated n-butanol were unsuccessful because it was not possible to remove the last traces of butanol from the residual flour without damaging its dough-forming properties.

To determine the possible role of lipid hydroperoxides in the bromate reaction, experiments were made with doughs containing up to 2.5 mg. per g. of flour of cumene hydroperoxide, which might be expected to compete with bromate for the available sulfhydryl groups. As was expected, a definite competitive inhibition of the bromate reaction was obtained. Accordingly it is concluded that lipid hydroperoxides, if produced in sufficient quantity, would react with protein sulfhydryl and in this way bring about an improver effect.

The role of antioxidants in the bromate reaction also seems to involve atmospheric oxygen. Figure 4 suggests that n-propyl gallate has

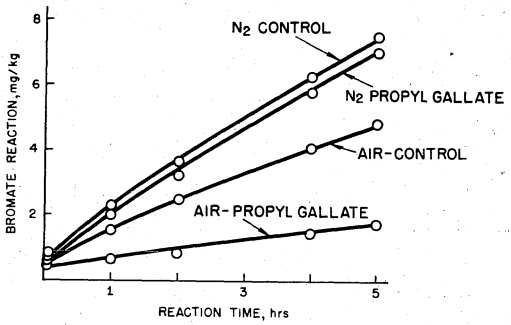

Fig. 4. Effect of n-propyl gallate in the presence and absence of oxygen on the bromate reaction in doughs from hard red spring flour.

essentially no effect in a dough mixed in nitrogen, whereas in a dough mixed in air it acts as an inhibitor. Similar results were obtained with butylated hydroxyanisole. Accordingly, any antioxidants occurring naturally in flour might be expected to behave similarly.

General Discussion

Results of this investigation are in agreement with the hypothesis that the sulfhydryl groups which react with the bromate ion in doughs from a high-grade flour are an integral part of the flour proteins. Starch and pentosans are not involved in the bromate reaction except as inert diluents of the protein. Lipids play a secondary role by reacting with part of the available oxygen and thus lessening the inhibitory effect of oxygen on the bromate reaction. By analogy with the effect of cumene hydroperoxide, which apparently competes with bromate for the available sulfhydryls, the hydroperoxides produced by the lipoxidase-catalyzed oxidation of fats might also be expected to react with sulfhydryl groups in dough. Antioxidants, in the presence of oxygen, depress the bromate reaction; accordingly it is the oxidation product that reacts with the sulfhydryl, as was the case with lipids.

Although the kinetics of the bromate reaction in dough seem to be complex, the results that have been obtained in the present investigation of this reaction can be summarized schematically by the following sets of competing reactions:

Reactions competing for sulfhydryl:

$$\text{Protein–SH} + \begin{cases} BrO_3^- & (1) \\ O_2 & (2) \\ \text{hydroperoxides} & (3) \\ \text{oxidized antioxidants} & (4) \end{cases} \longrightarrow \text{Products}$$

Reactions competing for oxygen:

$$\text{Oxygen} + \left\{\begin{array}{l}\text{Protein -SH} \\ \text{Lipids} \\ \text{Antioxidants}\end{array}\right\} \longrightarrow \text{Products} \qquad \begin{array}{l}(2)\\(5)\\(6)\end{array}$$

In a bromated dough mixed in nitrogen, the main reaction is undoubtedly that given by equation 1. However, in the more normal situation where the dough is mixed in air, with incorporation of a certain amount of oxygen, all six reactions probably occur. The most important reactions seem to be 1, 2, and 5, although the others may well occur to a limited extent.

The involvement of the sulfhydryl group of the cysteine residues of flour proteins in the chemical improvement of flour quality seems to be well established. Whether this improver effect is produced by the cross-linking mechanism first proposed by Sullivan, Howe, Schmalz, and Astleford (12), the blocking mechanism of Goldstein (8), or some other mechanism is still a matter of conjecture. Final decision as to which mechanism predominates for a particular improver must await further basic study of this problem.

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