Cereal Chemistry

Vol. 45 November, 1968 No. 6

Oxidation of Sulfhydryl Groups of Flour by Bromate under Various Conditions and during the Breadmaking Process[1]

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ABSTRACT

Bromate is a slow-oxidizing agent for cystine and glutathione, particularly for the sulfhydryl (SH) group of flour. The oxidation of SH groups in flour-water suspension by bromate can be increased slightly by lowering the pH of the suspension, but markedly by raising the temperature of the suspension. This temperature effect is confirmed with the determination of SH groups oxidized by bromate in nonfermented and fermented doughs heated at various temperatures. It is also supported by the results of baking tests with bromated and unbromated doughs, whether fermented or not. The effect of dough fermentation on the oxidation by bromate is insignificant. The present results indicate that the major bromate effect on oxidation of SH groups occurs when dough is heated during the early stage of baking. The synergistic action through the use of a combination of flour improvers is discussed.

Previous studies on the chemical reaction of various flour improvers, including potassium bromate and iodate (1), acetone peroxides (2), azodicarbonamide (3), and ascorbic acid (4), all show that sulfhydryl (SH) groups of flour proteins are involved in the reactions with these improvers. The reaction rate varies with different improvers. Comparatively, bromate is a slow SH-oxidizing agent among the improvers, and its effect on the rheological properties of dough is also slow (5), but it is the most widely used improver in the milling and baking industries. From the standpoint of cereal science and the baking industry, it is worth exploring how and when bromate exerts its improving action during the entire breadmaking process. Dough fermentation is commonly presumed to bring out or accelerate the bromate effect. Evidence is, however, lacking to support such a presumption.

This research was designed to study the improving action of bromate by investigating the oxidation of SH groups of simple SH compounds, flour suspensions, and dough by bromate under various conditions and during the breadmaking process. Results of this study are reported and discussed herein.

MATERIALS AND METHODS

Flour and Dough

An untreated straight-grade flour commercially milled from a blend of Kansas and Nebraska hard winter wheats was used throughout the study.

[1] This study was supported by the Baking Research Foundation.

The protein (N × 5.7) and ash contents were 12.3 and 0.47% respectively (14% m.b.).

Unless otherwise stated, dough, consisting of 1,000 g. flour, 25 g. compressed yeast, 20 g. salt, 30 g. lard, 60 g. sugar, 1 g. ammonium dihydrogen phosphate, potassium bromate (variable amounts), and 660 ml. ice-cold water, was mixed in a Hobart mixer with McDuffee bowl and fork for a total of 6 min. Flour-water dough was mixed from 1,000 g. flour, bromate (variable amounts), and 660 ml. ice-cold water under the same conditions. After mixing, the dough was panned and placed in a fermentation cabinet at 27°C. (81°F.) for various periods. For the baking test, the dough, after fermentation for 3 hr., was moulded in a drum moulder and then proofed at 37°C. (98°F.) for 50 min. The baking was done at 232°C. (450°F.) for 20 min. Dough or loaf samples, taken from the middle portion (slice) of a loaf, were frozen in a freeze-dryer (–35°C.), freeze-dried, ground to pass a 40-mesh sieve, and stored in a refrigerator for subsequent analyses.

Oxidation of L-Cysteine, Glutathione, and SH Groups of Flour

For these oxidations, the reaction system consisted of 1 ml. L-cysteine (CySH) or glutathione (GSH), 1 ml. bromate or water, and 8 ml. water. Variable concentrations of CySH, GSH, and bromate were used for different experiments. For the oxidation of SH groups in flour, the suspension comprising 0.800 g. flour, 1 ml. bromate or water, and 9 ml. water was used. Oxidation time was 20 min. All chemicals used were reagent grade. Distilled water was passed through a "Deeminizer" before use.

Sulfhydryl Determinations

Sulfhydryl contents of CySH, GSH, flour-water suspensions, and dough were determined according to the modified method of Sokol, Mecham, and Pence (6), as described previously (7). However, for CySH, GSH, and flour-water suspensions, determinations were carried out in the absence of 6*M* urea. The total SH content of the flour used was 1.03 μmole; the reactive SH content, determined in the absence of urea, was 0.77 μmole per g. of flour.

RESULTS

Oxidation of Cysteine, Glutathione, and SH Groups of Flour-Water Suspension by Bromate

Experimental results concerning the oxidation of CySH, GSH, and SH groups of flour-water suspension are summarized in Fig. 1; part A shows that the oxidation of CySH and GSH increases with increasing bromate

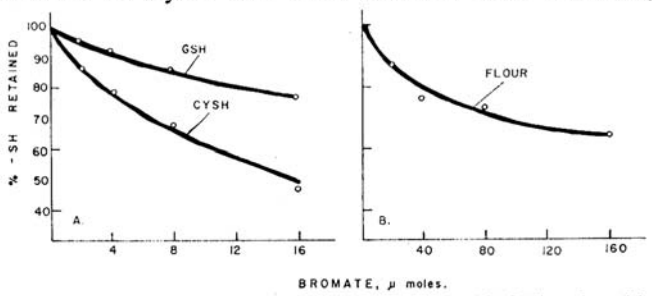

Fig. 1. Oxidation of sulfhydryl groups of cysteine (CySH), glutathione (GSH), and flour by bromate.

concentration. The extent of oxidation is higher for CySH than for GSH. For oxidation of SH groups of flour-water suspensions, the bromate concentration has to be increased 10 times above that used for oxidation of CySH or GSH to make the SH loss measurable, as shown in Fig. 1, B. The results are in general agreement with the findings of Hird and Yates (8,9).

Oxidation of SH Groups in Flour-Water Suspension by Bromate at Different pH Values

The table below presents the results of experiments on flour-water suspensions with pH varying from 3.0 to 5.0 as measured by the Beckman Zeromatic pH meter. The pH was adusted by adding predetermined quantities of hydrochloric acid. The table shows that the oxidation of SH groups in flour-water suspension by bromate is increased by lowering the pH of the suspension.

pH of Suspension	*SH Content*	
	No Bromate	*Bromate (20 μmoles)*
	μmole/g. flour	*μmole/g. flour*
5.0	0.69	0.64
4.0	0.64	0.56
3.0	0.63	0.51

Oxidation of SH Groups in Flour-Water Suspension by Bromate at Various Temperatures

Table I shows the effect of reaction temperature on the oxidation of SH groups of flour suspensions by air and by bromate. The oxidation by bromate increases with rising temperatures from 25° to 75°C.; the increase is greater than that by the air oxidation. This indicates that the improving action of bromate is probably affected by temperature.

TABLE I

OXIDATION OF SH GROUPS IN FLOUR-WATER SUSPENSION BY BROMATE AT VARIOUS TEMPERATURES

TEMP.	REACTION TIME	SH CONTENT		TEMP.	REACTION TIME	SH CONTENT	
		No Bromate	Bromate (20 μmoles)			No Bromate	Bromate (20 μmoles)
°C.	min.	μmole/g. flour	μmole/g. flour	°C.	min.	μmole/g. flour	μmole/g. flour
25	10	0.77	0.62	55	10	0.75	0.46
	20	0.70	0.52		20	0.66	0.42
	40	0.64	0.49		40	0.60	0.35
	80	0.63	0.46		80	0.52	0.30
35	10	0.75	0.52	75	10	0.74	0.39
	20	0.66	0.49		20	0.64	0.34
	40	0.64	0.45		40	0.53	0.26
	80	0.57	0.39		80	0.53	0.22

Oxidation of SH Groups in Fermented Dough by Bromate

The preceding sections reported the oxidation of SH groups in flour-water suspension, serving as a model or simple system to show the effect of bromate on SH oxidation. Though results obtained from such a model system give good indications about the oxidation, they may deviate from those with dough. Additional experiments were therefore undertaken to study the SH oxidation by bromate directly in dough.

Fermented dough is, of course, much more complex than a flour-water suspension. The dynamic yeast growth or division, depending on fermentation time and conditions, complicates the experimental results, for yeast contains SH compounds and proteins which can enlarge the SH value and mask the actual data. Since it is impossible to differentiate the SH groups of flour from those of yeast by the present amperometric titration, parallel experiments on doughs with or without the bromate treatment were run.

The table below summarizes the results of experiments on oxidation of SH groups in fermented doughs treated with various bromate levels. All doughs were fermented for 3 hr. Oxidation of SH groups does increase with increasing levels of bromate added to dough, but the extent of the increase is small. These results support those obtained from nonfermented doughs by Tsen and Bushuk (1) and by Sokol, Mecham, and Pence (10).

Bromate Added p.p.m.	*SH Content* μmole/g. dough
0	0.99
30	0.97
120	0.92
480	0.86

Effect of Fermentation Time on SH Oxidation by Bromate in Dough

In view of the finding that the low pH could increase the oxidation of SH groups by bromate, it appeared that fermentation might speed up oxidation by bromate because of the drop in pH during dough fermentation. Further experiments were then conducted to evaluate the effect of fermentation on the SH oxidation by bromate in dough.

The results (see table below) show that the SH content decreases slightly with fermentation whether the dough is treated with 120 p.p.m. bromate or not.

Fermentation Time hr.	*SH Content*	
	Unbromated μmole/g. dough	*Bromated (120 p.p.m.)* μmole/g. dough
0	1.13	1.11
1	1.04	1.02
2	1.01	0.95
3	0.99	0.92

Effect of Temperature on SH Oxidation in Dough

Since the oxidation of SH groups in flour-water suspension increased markedly by raising the reaction temperature, further experiments were run to examine the temperature effect on SH oxidation in dough. The dough samples were treated with and without 120 p.p.m. bromate and fermented for 3 hr. These fermented doughs were then heated in a humidified oven at various temperatures for 20 min.; the samples were then frozen, freeze-dried, and ground for SH determinations.

The results (Fig. 2, A) confirm that the high heating temperatures, particularly 75° and 95°C., sharply accelerate the SH oxidation by bromate; whereas without bromate, oxidation proceeds at a rather slow and linear rate.

To confirm the above results, flour-water doughs were mixed under the same conditions and then heated in a humidified oven at various tem-

peratures for 20 min. The results (Fig. 2, B) clearly point to the same conclusion drawn from experiments with flour-water suspensions and with fermented dough, that temperature plays an essential role in controlling the oxidation of SH groups of flour proteins by bromate.

Further evidence about temperature effect is obtained directly from the baking test, for in practice it is during baking that the high temperature sets in to play the role.

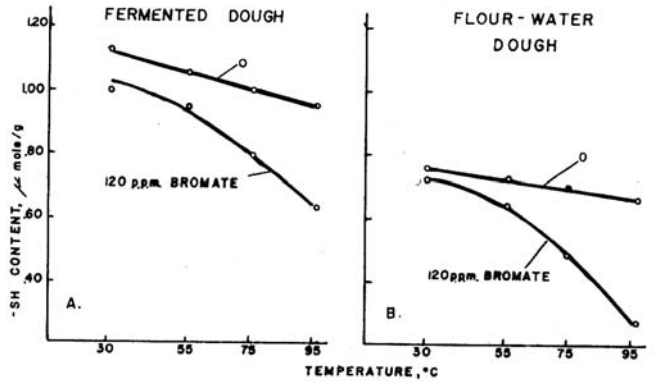

Fig. 2. Oxidation of sulfhydryl groups of fermented and nonfermented doughs by bromate at various temperatures.

A comparison of the SH contents in loaf samples (see table below) reveals a conspicuous difference in SH content between the samples prepared from bromated and unbromated doughs, whether fermented or not. Since only a small variation in SH content is observed for bromated and unbromated doughs before baking, the difference confirms the finding that oxidation of SH groups by bromate mostly takes place during the initial baking stage, where the high temperature favors oxidation. This table also shows that the residual SH contents of these loaf samples, particularly the unbromated ones, are rather high. This reflects that some SH groups of flour proteins can stand the baking temperature without oxidation or decomposition.

Loaf Sample	*SH Content*[a]	
	Unbromated μmole/g. loaf	*Bromated (120 p.p.m.)* μmole/g. loaf
Nonfermented	0.59	0.12
Fermented	0.91	0.44

[a] SH contents before baking are 0.74 (unbromated) and 0.65 (bromated) μmole/g. nonfermented dough, and 0.99 (unbromated) and 0.92 (bromated) μmole/g. fermented dough.

DISCUSSION

This study presents evidence to support previous studies that bromate is a slow SH-oxidizing agent for dough. There are two approaches to measure the oxidation of SH groups of flour by bromate in dough. One approach is to determine the bromate loss. This has been employed by

Cunningham and Anderson (11), and extensively by Bushuk and Hlynka (12–16). In both fermented and nonfermented doughs mixed in nitrogen, a small initial loss of bromate is followed by a continuing loss at uniform rate. The rate, according to Cunningham and Anderson, was 1.07 and 1.16 p.p.m. per hr. for nonfermented and fermented doughs prepared from the patent flour treated with 15 p.p.m. bromate (11). Bushuk and Hlynka also found that the over-all reaction of bromate in dough was characterized by two distinct phases: a rapid initial reaction that occurs almost completely during mixing, and a slower secondary reaction that proceeds at a constant rate (12). These findings all show that the bromate loss is very slow in dough. Bushuk and Hlynka further found from their study on the disappearance of bromate during baking of bread that most of the added bromate (5–40 p.p.m.) in dough was lost during the initial baking stage. Essentially no bromate was found in the bread crumb after a 10-min. baking period (17).

The other approach is to measure the oxidation of SH groups by bromate, as done by Sokol *et al.* (10) and by Tsen and Bushuk (1).

The present study with the latter approach provides evidence that the SH group of flour is not as easily oxidized by bromate as that of cysteine or glutathione. The lower reactivity of flour SH groups than that of other SH compounds is most likely due to several physical barriers, including effects of flour particle size, insolubility of flour gluten, steric hindrance, and neighboring groups or side chains of flour proteins.

The lowering of pH or fermentation increases the oxidation of SH groups in flour by bromate, as shown by the present study. This finding is in accord with those of Bushuk and Hlynka (12) based on measurements of bromate loss in doughs of different pH values, and of Hlynka and Chanin (18) on the effect of pH on the rheological properties of bromated and unbromated doughs. However, the increase in the oxidation does not seem to reach such an extent as to support the commonly held presumption that fermentation brings out the bromate effect.

The present study does show that oxidation of SH groups by bromate is accelerated by raising the temperature of flour suspensions and of fermented or nonfermented doughs. In practice, it is during the initial baking stage that most SH groups of flour are oxidized by bromate. This conclusion is also sustained by baking tests through measurements of SH groups oxidized in the present study, and of bromate loss in the study of Bushuk and Hlynka (17).

Bromate is a slow SH-oxidizing agent or a slow-action improver. The baking industry, however, makes good use of this property. A combination of bromate and iodate in the ratio of 4:1 is widely used in continuous-dough processes such as the Baker Dō-Maker and Amflow. This combination provides a synergistic (complementary) action of the slow-acting bromate and iodate which acts rapidly (1,19). The rates of oxidation of SH groups by these improvers also correspond well with their improving effects as shown by rheological measurements and baking data (5). Azodicarbonamide (Maturox) or acetone peroxides (Keetox) can replace or reduce iodate in

the combination (20,21). The replacement or reduction is largely because azodicarbonamide, acetone peroxides, and iodate all are fast SH-oxidizing agents and so they can be interchanged (1,2,3) to exert the expected improving effect. The synergistic action is also found for the combination of bromate and ascorbic acid in our previous study (4,22). Perhaps the synergistic action can be visualized as oxidation of part of the SH groups of flour immediately during dough mixing by a fast SH-oxidizing agent such as iodate or azodicarbonamide (1,3). Under a normal treatment level of flour improver, all the added fast-oxidizing agent is likely consumed according to its stoichiometric relation with SH groups in dough during mixing. Though bromate contributes some oxidative action during dough mixing, its contribution is relatively slight. During dough fermentation and proof period, bromate slowly exerts its action until the initial baking stage, where most of the added bromate is consumed in the oxidation to complete the improving action synergistically.

Acknowledgments

It is a pleasure to acknowledge the competent assistance of Greta Kestenbaum and the Dixie-Portland Flour Mills, Inc., who supplied the flour used in this study.

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[Received September 27, 1967. Accepted February 16, 1968]