# DECOMPOSITION OF POTASSIUM BROMATE IN WHEAT-FLOUR DOUGHS

by

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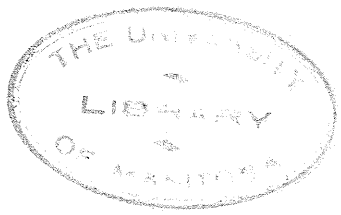


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## INTRODUCTION

Wheat flour does not exhibit its maximum baking potentialities if it is used immediately after milling. However, if the flour be stored for a period of several months, or if certain chemical reagents be added to the fresh flour, the optimum baking qualities will manifest themselves. This phenomenon is known to the baking trade as "improvement" and the chemical reagents are termed "improvers". Since the storage method of flour improvement is time consuming and demands greater storage facilities and extra handling of the flour, it has given way to the more expedient chemical method.

From the time it was first discovered that chemical agents could preclude the need for a storage (or natural aging) period for flour, numerous compounds have been tried as flour improvers. From the number (which included such substances as ammonium persulfate, nitrogen trichloride, potassium bromate, and ascorbic acid) potassium bromate has risen to highest popularity. This is because it is convenient to handle, is required in only very minute amounts (around 10 p.p.m. of flour) to produce a maximum amount of improvement and it has no secondary objectionable qualities that deter the consumption of bread that has been baked with it.

Since potassium bromate is widely used as an improver it is desirable to know as much as possible about its physico-chemical reactions in the dough. The outward physical changes in dough and bread that are effected by added bromate are obvious to the trained eye: e.g., a more elastic dough, greater loaf volume, finer crumb texture, silkier appearing crumb. However the exact

chemical nature of the reaction of bromate, that causes these changes, is not clear. This has been the subject of much investigation and speculation in the literature.

At present the literature reveals that the generally accepted hypothesis is that bromate acts as an oxidizing agent in its role of improver. Decomposition or reduction of bromate in bread dough has been reported as evidence for this hypothesis. If oxidation-reduction is, in fact, the mechanism of improver action then a more detailed study of the reaction is required. On the other hand, if the decomposition of bromate is not concerned with the improving action, but is merely a concurrent reaction, this aspect, too, merits study. In either case the study undertaken here will give greater insight into the mechanism of improver action by inquiring into the various issues that arise out of the hypotheses.

Some of the fundamental and important aspects of such a study are the effects of time, temperature and concentration. Bromate decomposition with time at a constant temperature and different concentrations of reactants is studied here. The effect of mechanical action, such as mixing or working in the extensograph machines is also investigated. One other important facet of such a study is the influence of pH on the reaction. This too has been approached. There is also a report given on the work done at the Grain Research Laboratory in Winnipeg, which prefaced this thesis.

However, there is more to be gained by such studies. Bread constitutes a major portion of the world's diet and as such should be under continual study with regard to improving it as a nutriment source. Any scientific investigation that is concerned with the constituents of bread and their interaction will add to the stockpile of knowledge. It is superfluous to say that the more we know about a food substance the better use we can make

of it and the more we can do to improve it. It is felt that the work presented here constitutes a positive contribution to the knowledge of the improvement of wheat-flour dough by potassium bromate.

# LITERATURE REVIEW

There is little reported in the literature directly concerning the mechanism of the decomposition of bromate in dough but numerous investigations dealing with the nature of the improving action of potassium bromate in wheat-flour doughs as it is related to the oxidizing action of bromate, are to be found. This review outlines the work and opinions of those investigators who seek an answer to the enigma of the improvement of bread dough by bromate.

One of the first hypotheses on the nature of the improving action of bromate was the one concerning oxidation. Early mention of this was made by Geddes (14). He reasoned that bromate oxidized, and thereby inactivated, certain wheat germ constituents, presumably the phosphatides, which had a deleterious effect on the dough. More recently, Sullivan, Howe, Schmalz and Astleford (35) agree that the germ has harmful effects on the flour but attribute it to its glutathione content. However, they say that all flours which responded to bromate did show the amount of reducing substances to be expected.

Saunderson (32) conducted a series of comparative baking tests on flour germ mixtures using $NaBrO_3$, $KBrO_3$, $KIO_3$, $CaO_2$ and $K_2S_2O_8$. These were added in amounts which were calculated to yield the same quantities of oxygen. The effect of the several improvers was not proportional to their oxidizing power, but seemed to be a specific effect of the salt used.

Ziegler (39, 40) says that bromate acts gradually as an improver due to its slow rate of oxidation of glutathione. He also found that small amounts (1 in 25,000) of oxidized glutathione had a beneficial effect on dough and so improvement caused by bromate is not merely due to suppression of the

harmful effect of the protease activator reduced glutathione. He also notes that at temperatures below 40°C. an enormous overdose of bromate is necessary for the rapid oxidation of glutathione. Further studies by Ziegler (41) on the oxidation of glutathione by various chemicals and the improver action of these revealed that only bromate showed the improver effect.

Another approach to the question of the nature of the improver action of potassium bromate concerns proteolysis. Jorgensen (24) as well as Balls and Hale (5) believe the flour contains "powerful but latent" proteolytic enzymes of the papain type which are activated by SH compounds and inactivated by bromate. The extremely small amount of oxidant required, said Balls and Hale, is not surprising because its action is not on the main constituents of the system but on a catalyst, itself present only in traces. Jorgensen suggests that over improvement results when the bromate depresses the proteolysis in the dough below a certain optimum amount. Jorgensen supports his theory by pointing out the depression in the amount of soluble nitrogen caused by bromate in a dough treated with papain.

Shen and Geddes (33) found that bromate considerably depressed proteolytic activity as indicated by the lower amino nitrogen levels at corresponding fermentation times. They also found that the bromate requirements to produce a satisfactory dough increased as the flour contained more reducing matter and protease.

However, Swanson (37, 38) found from a study of mixogram curves that those obtained from autolyzed doughs were similar to those obtained with use of papain but, if this change were due to enzyme action, it was only slightly influenced by the presence of $KBrO_3$. Even when using bromate in amounts as high as 72 mg. for doughs made with 35 g. of flour, it could not be shown from the characteristics of the recording mixer curves that the

bromate had any inhibiting effect on proteolysis.

Sullivan, Howe, Schmalz and Astleford (35) point out that not all chemists are agreed that the effect of improvers such as bromate is to be attributed to their effect in inhibiting the proteolytic enzymes. They believe that glutathione and other SH compounds acted mainly per se on the gluten and not indirectly as an enzyme activator. They review their work on the influence of oxidizing agents on the lipids, gassing power, diastatic activity, starch, sugars, fermentation and gluten. They conclude that changes in the sulfur linkages of the gluten proteins are responsible for many of the effects described as improvement. Baker, Parker and Mize (4) also indicated that the reaction of oxidizing agents on dough is located either in the gluten or in the water-soluble portion of the dough. Sullivan (36) elaborates on this by stating that some if not all of the disulfide groups of the proteins of flour form cross linkages between polypeptide chains. These cross linkages exert a profound influence on the physical properties of gluten. Reducing agents effect the dough structure by virtue of destroying such linkages; oxidizing agents, by helping to form new linkages.

This question of the mechanism of bromate action in dough has been approached from other angles. Among these are the effect of bromate on carbon dioxide production, on carbon dioxide retention, on the colloidal properties of the dough, and as an oxidizing agent in the dough.

With regard to the effect of bromate on carbon dioxide production and retention Harris (16) noticed changes in loaf volume when diastatic malt was included in the baking formula along with bromate. Geddes and Larmour (15), Larmour and Brockington (27) and Saunderson (32) found that bromate does not influence the rate nor the amount of $CO_2$ production in bread doughs but modifies their gas retaining capacity. Studies on wheat germ by these

workers indicate that the action of bromate on gluten quality is largely indirect. But they also found that oxidation is not the only or indeed the principal effect of chemical improvers in bread doughs.

Freilich and Frey (12) deal with the aspect of oxidation and mixing effects. They found that even when mixing in excess of oxygen with the dough no excess-bromate effect was observed. There seems to be a fundamental difference between the effects of oxygen and bromate on dough. They also found that bromate and prolonged fermentation tend towards "over improvement", but remixing of the dough eradicates this effect. They say (10) that this suggests that the improvements may be due mainly to a physical change in the colloidal properties of the dough. Saunderson (32) makes a similar suggestion saying that the effect of potassium bromate is directly on the colloidal nature of the gluten proteins and the effect which it has on loaves baked with phosphatides present is concurrent with the effect of those substances rather than directly on them.

In recent work by Holme and Spencer (18) the effect of bromate on sulfhydryl groups from different flour fractions was measured by means of a titration using iodosobenzoate which is specific for sulfhydryl groups. Bromate caused no apparent reduction in the sulfhydryl content of both gluten washings and flour slurries when held at room temperature. Added glutathione however was partially oxidized.

The addition of $KBrO_3$ to a flour slurry showed no decrease in the apparent sulfhydryl content at the end of 21 hours at room temperature. With these results in mind it would appear that flour improvers added to yeasted doughs must be used up in reaction with some other groups than the sulfhydryl groups which occur in the flour protein as determined with iodosobenzoate, during the stages of dough make up and fermentation.

Non-uniform response of different flours to improvement by potassium bromate could be due to different rates of oxidation.

And now some work that deals more directly with the reduction of bromate in dough, is as follows. In a series of experiments to show that bromate's improver action may not be due to retardation of proteolytic activity, Freilich and Frey (9) added excessive amounts of $KBrO_3$ to doughs. They measured, among other things, the amount of undecomposed bromate present in baked loaves and found that when 100 mg. was the original dosage, only slight amount of bromate could be recovered[a] but when 500 to 1000 mg. was the dosage, large amounts were recovered. They also found that the excess bromate effect reached a maximum at a level of 100 mg. per 100 g. of flour. The amount of acid, they say, developed in the dough as a result of fermentation was probably a factor limiting the amount of bromate decomposed.

Sullivan, Howe, Schmalz and Astleford (35) found that over half of the bromate added to a straight dough was lost merely by mixing. A further slight loss was noted during fermentation and the baked loaf contained no bromate. The method used for bromate determination involved a titration with standard thiosulfate.

The only other work relating to the reduction of bromate in dough was analogous work done by Conn, Hollenbeck, Rosenblum and Woodbury (7) on the reduction of $KIO_3$ in dough. Using radioactive iodine in potassium iodate they found less than 7.5% of the original 3.5 to 4.0 p.p.m. iodate in baked bread. The major decomposition product they determined to be iodide.

Naturally, the action of bromate in yeasted doughs holds the most interest for the majority. There seems to be a close connection between improver action and fermentation of a yeasted dough. Baker and Mize (3) mention that

one of the chief functions of the four hour sponge is the activation of the bromate by the motion which the fermentation produces.

Smith and Geddes (34) found that addition of wheat germ to flour resulted in poor quality doughs and bread. However, increasing the fermentation time from 1.5 to 4.5 hours or the addition of $KBrO_3$ improved the dough and loaf characteristics. This is in agreement with the work of Freilich and Frey (12). Smith and Geddes (34) and Hullett and Stern (21) also found that prefermentation of the germ suspensions or addition of $KBrO_3$ to the suspensions resulted in reduction of the harmful effects as well. However, Hullett and Stern attribute this to the destruction of glutathione by an enzyme mechanism rather than to oxidation. Smith and Geddes say that the much greater efficiency of $KBrO_3$ noted when added to fermenting germ-flour dough rather than to fermenting germ sponge suggests that it exerts a direct action on the gluten proteins.

Both Jorgensen (26) and Smith and Geddes (34) agree that over-fermentation and $KBrO_3$ together can cause "overtreatment" although either one by itself causes improvement.

Finally, Shen and Geddes (33) remark that although increased fermentation time and bromate produce a satisfactory loaf from flour which contains a large amount of reducing matter, the reducing matter content of the dough increases with longer fermentation periods while it decreases when bromate is added.

Last in this review is the work on the connection between the acidity of the dough and the bromate effect. The first direct mention of a chemical change of the $KBrO_3$ added to a dough seems to have been made by Kosmin. Read and Haas (30) allude to this work. They say that the suggestion of Kosmin that bromic acid may be slowly set free in the dough batch and thus

attack the proteinase contained in the flour would seem to be rather improbable. It was found that the hydrogen ion concentration necessary for the presence of bromic acid is never reached in a normal dough.

Jorgensen (26) and Freilich and Frey (12) draw attention to the importance of the acidity of the dough, produced during fermentation mainly by the carbon dioxide evolved, in the effects of bromate on bread. The baking strength of a flour may be improved by lowering the pH of the dough, but Jorgensen warns that overtreatment may result with a combination of low pH and bromate treatment.

In the foregoing work on the subject of bromate as a dough improver various suggestions are made regarding the mechanism of the improving action. Yet no intensive study has ever been made where the suspected mechanism was investigated with regard to the fate of the bromate. For this reason, partly, this intensive study of the decomposition of bromate in dough was undertaken. Another reason for the study is that the aforementioned work does not adequately account for all the observed phenomena. It is clear that there is an insufficiency of data on the mechanism of bromate reduction and related subjects. This thesis should help to erase this insufficiency.

# MATERIALS AND METHODS

The analytical method used for the various studies undertaken concerning the decomposition of potassium bromate in dough is that of Johnson and Alcock (23) modified slightly to enable its use for dough analysis. The original method is for the determination of bromate in flour. Slight changes (e.g. sample weight, volume of extracting solution, use of Waring Blendor) enable one to use the method for dough analysis and it was found to be applicable to an analysis of yeasted doughs and doughs with other constituents. A description of the method as it was used is given here as well as a resume of an alternative method given by Howe (20). A comparison of the two is also made and reasons are given for abandoning the Howe method.

## Adaptation of the Johnson and Alcock Method

A 14.0 g. dough sample is dispersed in 80 ml. of an extracting solution (made up of approximately 74.8 ml. of a 25% KCl solution in water and 5.2 ml. of a 0.035% iodine solution) in the Waring Blendor for 3/4 minutes. The mixture is centrifuged for 10 minutes in the No. 2 International centrifuge using 250 ml. centrifuge tubes at 1800 r.p.m. A 5.0 ml. aliquot of the supernatant liquid is diluted to 35 ml. with 25% KCl solution in 2% acetic acid. One packed level teaspoonful of celite analytical filter-aid is added and stirred in for 1 minute. The mixture is centrifuged for 10 minutes in the clinical centrifuge at 1800 r.p.m. From this clarified extract three solutions are made up in separate colorimeter tubes.

| Tube 1 (Reference Solution) | Tube 2 | Tube 3 |
|---|---|---|
| 3 ml. distilled water | 1 ml. distilled water | 1 ml. 5 µg. per ml. standard bromate soln. |
| 1 ml. 0.5% starch soln. | 1 ml. 0.5% starch soln. | 1 ml. 0.5% starch soln. |
| 10 ml. extract | 10 ml. extract | 10 ml. extract |
| 3 ml. 1% potassium iodide soln. | 3 ml. 1% potassium iodide soln. | 3 ml. 1% potassium iodide soln. |
| 1 ml. dilute iodine soln. | 1 ml. dilute iodine soln. | 1 ml. dilute iodine soln. |
| | 2 ml. 5% sulfuric acid | 2 ml. 5% sulfuric acid |

Each addition is made in rotation, as rapidly as convenient and in the order given. Exactly 4 minutes after adding the acid to tubes 2 and 3 the optical density is read at a wavelength of 575 mµ. using a Beckman model DU spectrophotometer with test tube attachment. The difference in optical density between tubes 2 and 3 is due to 5 µg. of bromate and when 5 ml. of the first extract is taken, the concentration of bromate in the original dough is obtained as follows.

$$\text{Concentration of bromate p.p.m.} = \frac{A}{B} \times \frac{35}{10} \times \frac{80 + x}{5} \times \frac{1}{y} \times 5$$

A is the optical density of the unknown in tube 2

B is the difference in optical density between tubes 3 and 2

x is the mls. of water contained in the dough sample taken

y is the weight of flour in the dough sample in grams

The reagents used are made up according to Johnson and Alcock's specifications and are as follows:

(1) 25% potassium chloride solution (500 g. KCl in 1500 ml. distilled water).

(2) Iodine, stock solution (0.35 g. of iodine dissolved in 7 ml.

of ethyl alcohol and diluted to 1 litre with 1% potassium iodide solution. This is kept in a brown bottle).

(3) 25% potassium chloride in 2% acetic acid (500 g. KCl in 1470 ml. distilled water and 30 ml. glacial acetic acid).

(4) Standard potassium bromate solution, 5.0 µg. per ml. Prepared as required from a solution containing 100 µg. per ml. which is stable.

(5) 0.5% starch solution, prepared as follows. While stirring approximately 30 ml. of a 20% sodium hydroxide solution were added to a suspension of 2 g. of soluble starch in 300 ml. of distilled water and was allowed to stand for 1 hour. This was neutralized with concentrated hydrochloric acid using litmus as an indicator and then 1 ml. of glacial acetic acid was added. The solution was diluted to 400 ml. with water. This can be used for an indefinite period.

(6) 1% potassium iodide solution (2 g. KI in 200 ml. of solution). This was prepared fresh weekly.

(7) Iodine solution, approximately 17 µg. per ml. 5 ml. of stock iodine solution was diluted to 100 ml. This was prepared fresh daily.

(8) 5% sulfuric acid. (12.5 ml. concentrated $H_2SO_4$ in 250 ml. of solution).

While this project was under way, using the Johnson and Alcock method a rapid method for the determination of potassium bromate in flour was published by Howe (20). It was desirable to know if this method could yield results of greater precision than could the Johnson and Alcock method. For this reason a comparison of the merit of the two analytical methods was

made. It is described following a review of the Howe method.

The Howe Method

The Howe method was easily adapted to the determination of potassium bromate in dough. Essentially it is as follows: A 14.0 g. dough sample (approx. 9 g. of flour) is dispersed in 200 ml. of distilled water by mixing for 3/4 minutes in the Waring Blendor. Two drops each of GE 81106 silicone anti-foam emulsion and n-octyl alcohol are added to eliminate foam. The organic substances are precipitated with 25 ml. each of 0.18 N NaOH and 0.18 N $ZnSO_4$ and a clear extract is obtained by stirring in a teaspoonful of celite filter aid and centrifuging in the No. 2 International Centrifuge for 10 minutes. To a 50 ml. aliquot of the supernatant are added 3 ml. of 30% potassium iodide solution, 1 ml. starch solution and 10 ml. 10% sulfuric acid solution. Titration to a colorless end point is done using 0.002 N sodium thiosulfate solution. The blank is obtained by running a parallel analysis on an unbromated dough sample. Each 50 ml. aliquot contains the bromate present in 1.68 g. of flour and

$$\text{Potassium Bromate, p.p.m.} = \frac{27.84 \times N \times \text{vol.}}{1.68}$$

Comparison of Methods

Both the Howe method and the Johnson and Alcock method were given preliminary trials. It was found that the best way to come to a decision as to which method was to be used for the most of the analyses was to run a series of comparative tests on recoveries of bromate from water solutions and from flour. Actually what was to be decided was which method was better for recovery of small amounts of bromate such as 1.0 p.p.m. The results of this series are given in Table I.

Table I. Recoveries of Bromate in p.p.m. by Two Methods

| $KBrO_3$ Added | Johnson and Alcock | | Howe | |
|---|---|---|---|---|
| | Flour | Water | Flour | Water |
| 1 p.p.m. | 0.81 | 0.00 | 0.00 | 0.00 |
| | 0.68 | 1.50 | 0.00 | 1.00 |
| | 0.39 | 1.20 | 0.00 | 1.00 |
| | | 0.70 | | 0.00 |
| | | 0.40 | | 1.00 |
| | | 0.90 | | 1.00 |
| | | 1.20 | | 2.00 |
| | | 0.00 | | |
| 2 p.p.m. | 0.10 | | 1.77 | |
| | 1.05 | | 1.52 | |
| | | | 0.56 | |
| 3 p.p.m. | 4.76 | | 2.55 | 1.66 |
| | 2.95 | | 2.55 | 3.40 |
| | 3.30 | | 2.16 | 1.86 |
| | 3.10 | | | |

These results obviously favour neither method. To enable a choice to be made between the two, it was decided to run two parallel series of experiments on bromate recovery from sponge doughs using each method exclusively for each series. The one that gave the most consistent results was to be adopted for all of the investigations of the thesis. It was found that the results obtained by the Johnson and Alcock method showed a slightly greater precision than those by the Howe method. For this reason the Howe method was dropped. An outline of the series is as follows.

Fermenting sponges were made up without bromate and allowed to rest for 3 hours in one instance, and 12 hours in another. After this, the sample was doughed-up with the remainder of the flour and water and 30 p.p.m. bromate. Samples of these doughs were analyzed for bromate after reaction periods from 0 to 15 hours. The dough's temperature was maintained

at about 30°C. all this time by storing it in a constant temperature cabinet.

The formula used for the doughs in this series is given in Table II.

Table II. Sponge Dough Formula

| Ingredient | Sponge Dough | Added to Sponge to Dough-Up |
|---|---|---|
| Flour | 70.5 g. | 30.2 g. |
| Yeast[i] | 3.0 g. | - |
| Salt[ii] | 1.0 g. | - |
| Malt[iii] | 0.3 g. | - |
| Phosphate[iv] | 0.1 g. | - |
| Bromate[v] | 0.0 | 3.0 mg. |
| Water | 8.2 ml. | 15.3 ml. |

[i] Added in water soln. (25 ml. of a suspension of 30 g. Fleischmann's yeast in 225 ml. distilled water)

[ii] Added in water soln. (10 ml. of a 10% soln.)

[iii] Added in water soln. (1 ml. of a solution of 60 g. Standard Brands Diastatic Malt Syrup made up to 200 ml. with water)

[iv] Added in water soln. (1 ml. of water solution containing 25 g. monobasic ammonium phosphate in 250 ml. of solution)

[v] Added in water soln. (3 ml. of a solution containing 1 mg. $KBrO_3$ per ml. of solution)

The results as obtained by both methods were plotted as bromate recovery vs. rest period after doughing up. The difference between the plots by each method was small and could be ignored by virtue of being comparable in size to experimental error. Both analyses showed exactly

similar trends for bromate decomposition. However, the precision within the Johnson and Alcock method was slightly greater than that in the Howe method. This can be seen from Table III where the mean deviations are seen to be on the average slightly larger with the Howe method than with the Johnson and Alcock method.

Table III. Recoveries of Bromate Added to Sponge Doughs at Doughing-Up Stage

| | 3 Hr. Sponge | | | | 12 Hr. Sponge | | | |
|---|---|---|---|---|---|---|---|---|
| | Howe Method | | Johnson and Alcock Method | | Howe Method | | Johnson and Alcock Method | |
| Rest Period (hrs) | % Recovery | Mean Deviation | % Recovery | Mean Deviation | % Recovery | Mean Deviation | % Recovery | Mean Deviation |
| 0 | 82.0 | | 88.0 | | 85.3 | | 89.3 | |
| | 100 | | 94.2 | | 76.3 | | 80.0 | |
| | 98.0 | 7.6 | 98.3 | 3.7 | 94.0 | 5.9 | 95.0 | 5.4 |
| 3 | 61.3 | | 60.0 | | 53.3 | | 67.0 | |
| | 65.0 | | 67.0 | | 67.0 | | 64.3 | |
| | 83.7 | 9.1 | 62.7 | 2.5 | 46.0 | 7.7 | 64.7 | 1.1 |
| 8 | 59.7 | | 47.4 | | 45.0 | | 35.7 | |
| | 62.3 | 1.3 | 32.0 | | 45.0 | 0.0 | 47.7 | |
| | | | 41.3 | 6.6 | | | 34.7 | 5.6 |
| 12 | 34.3 | | 29.0 | | 36.3 | | 23.3 | |
| | 41.3 | | 20.3 | | 28.0 | | 25.7 | |
| | 24.1 | 6.1 | 25.7 | 3.1 | 25.0 | 4.4 | 14.8 | 4.3 |
| 15 | 18.0 | | 9.0 | | 17.3 | | 11.7 | |
| | 20.0 | 1.1 | 4.2 | 2.4 | 4.7 | 6.3 | 5.9 | 2.9 |

The foregoing means of comparing the methods was preferable to a more formal one as it saved valuable time by accomplishing two tasks with one investigation. First, it enabled the comparison of merit of the Howe and Johnson and Alcock methods; second, it forms part of the complete study of the influence of yeast on bromate decomposition which is reported fully in the section dealing with results.

Specificity of the Method

Both methods of analysis depend on the oxidation of iodide ion to iodine and subsequent color development with starch. The question therefore arises as to how specific the analysis is for potassium bromate. Howe states that her method is applicable when only potassium bromate is present. Other oxidizing substances, such as potassium iodate or potassium persulfate, but not chlorine or chlorine dioxide, interfere with this determination. This would also apply to the Johnson and Alcock method. In all the experiments conducted on the reduction of bromate in dough, it is known that no other strong oxidizing agents were added to the system. The only factor that could influence results would be oxidizing agents that are inherent in the flour, or that are produced by mixing the dough. One example of such oxidants is the fat peroxides.

With the object of investigating the specificity of the Johnson and Alcock method for bromate, an experiment was carried out in which excessive amounts of fat peroxides were produced in the dough by means of added lipoxidase in the form known as "wytase". If such peroxides or similar substances were extracted by the Johnson and Alcock method they would show up as bromate in the final result. By adding "wytase" to the dough, an excess of such substances was produced. If this excess did not give high bromate recoveries, then the analytical method could be considered specific for bromate.

The wytase used was a commercial product made from fat extracted soy flour. The dosage was two grams per hundred grams of flour. The whiteness of the doughs was evidence enough that the reaction had produced a large amount of oxidizing substances.

There were four stages in the test for the effect of peroxides. In each stage 100 g. of untreated flour was mixed for 8 minutes with sufficient water to give a dough of proper consistency for baking as determined by the farinograph. The bromate and wytase were mixed with the water in each case that they were added. The mixing was prolonged to 8 minutes to assure ample production of fat peroxides. The procedures in the four stages of the experiment were as follows.

(1) The dough was mixed 8 minutes without bromate or wytase. A sample was taken and mixed with the KCl extracting solution in the Waring Blendor. Wytase in KCl solution was added to the mixture in the blender and mixed in. The procedure as usual for bromate determination was then carried out.

(2) The dough was mixed 8 minutes with 2 g. wytase added, but no bromate. The procedure as usual for determination of bromate was carried out.

(3) The dough was mixed 8 minutes with 30 p.p.m. bromate, but no wytase. A sample was taken and mixed in the Waring Blendor. Then the procedure was as in (1) above.

(4) The dough was mixed 8 minutes with 30 p.p.m. bromate and 2 g. wytase. Then the procedure as usual for the determination of bromate was carried out.

The object of stage (1) was to determine the effect of wytase that has not reacted with the dough. Stage (2) was to determine the effect of wytase-produced peroxides on bromate recovery from a non-bromated dough. Stage (3) was to determine the effect of unreacted wytase on bromate recovery from a bromated dough and stage (4) was to determine the effect of

wytase-produced peroxides on bromate recovery from a bromated dough.

The results are given in Table IV.

Table IV. Tests for Bromate on Dough after Reaction with Wytase

| Stage | Bromate Added | Recovery of Bromate |
|---|---|---|
| | (p.p.m.) | % |
| 1 | none | 0 |
| | none | 0 |
| 2 | none | 0 |
| | none | 0 |
| 3 | 30.0 | 102 |
| | 30.0 | 92.3 |
| 4 | 30.0 | 102 |
| | 30.0 | 105 |

The recoveries from unbromated doughs with and without wytase should be proof enough that fat peroxides do not show up as bromate in the analysis. Further evidence is given by stages 3 and 4, where the results indicate that the large excess of fat peroxides produced in the dough do not show up as bromate.

It was concluded that fat peroxides or similar substances are not carried in the bromate extracting solution of the Johnson and Alcock method for bromate analysis and therefore the analysis is quite specific for bromate.

Materials

Three different flour samples were used in the study of bromate decomposition. The particulars of each are given in Table V. A fourth sample (#9053) is also mentioned because it is used for the sake of comparison in one of the studies of the influence of yeast concentration on

bromate decomposition.

Table V. Particulars on Flours used in the Investigation of the Decomposition of Potassium Bromate in Wheat Flour Doughs

| Sample No. | Wheat Grade | Crop Year | Flour Protein* % | Flour Moisture* % | Absorption* % |
|---|---|---|---|---|---|
| 9053 | Comp. #1 and #2 Northern | 1950 | 13.1 | 13.3 | 64.7 |
| 9718 | Comp. 2/3 #2 & 1/3 #3 Northern | 1951 | 13.0 | 13.4 | 62.6 |
| 57 | #1 Northern | 1951 | 12.2 | 14.6 | 61.0 |
| 1714 | #3 Northern | 1951 | 13.1 | 14.2 | 60.8 |

* 14% moisture

An attempt was made to use only one flour sample throughout each phase of the work since the flour peculiarities have an effect on bromate decomposition, but this was not always possible. However, each sample was given similar handling. Milling was done on a Buhler to about 70% extraction from each hard red spring wheat sample. The flour was kept in cold storage except for a working sample of about 40 pounds which was kept at room temperature while in use. Such a sample lasted about two to three months so storage effects were minimized. All flour samples were used without any pretreatment such as bleaching or improving by any means.

The protein contents of the samples were determined by the official Kjeldahl method (1). The moistures were determined by the standard air oven method (2). Absorptions were determined on the Farinograph by mixing to a consistency of 540 Brabender Units.

Dough Formulae

The specific formula used for each study of bromate decomposition is better placed under the particular discussion of each experiment. However,

it can be said here that in each case the total amount of flour used was 100 g. (on 14% moisture basis) and the total absorption was the one given in Table V.

The mixing of all doughs (except those for the study of acidified doughs) was done in the Hobart for 3 minutes at medium speed. The mixer used for the acidified doughs was of laboratory construction and has a gentle mixing action intermediate between the Hobart and Hobart-Swanson type.

## RESULTS AND DISCUSSION

The topics of investigation of this thesis constitute a continuation of work that was begun in the Grain Research Laboratory, Winnipeg. In view of this, and because that work was not published, a short resume of it will be given here.

The first investigations of the decomposition of bromate in dough that were of interest were concerned with flour water doughs as used in extensogram studies, and bread doughs made up according to a basic AACC method. A useful terminology used by Dempster (8) gives the name "reaction time" to the elapsed time after mixing a dough and before shaping it on the extensograph machines. The term implies that during this interval the chemical reactions involving the dough constituents take place. It is during this time presumably that the bromate reacts in the dough and effects the changes which are subsequently observed as improvement. In early investigations it was learned that no loss of bromate could be detected from doughs containing 30 p.p.m. bromate given zero reaction time even though the physical treatment of the dough was quite rigorous, e.g., up to 8 minutes of mixing as well as rounding and shaping on the extensograph machines. Similarly, bread doughs mixed with 30 p.p.m. bromate and given zero reaction time were found to contain almost 100% of the initial concentration of bromate. However, if these non-fermenting, and fermenting doughs were given 3 hours or more of reaction time a definite bromate loss was detectable. When the rate of this bromate reduction was followed it was found that it was greater in fermenting doughs than in non-fermenting doughs and was increased by higher bromate concentration (rate for 30 p.p.m. > rate for 10 p.p.m.) and by heat (baking the bread). Kinetically it was defined

as a zero order reaction since the relationship of decomposition with time was linear.

On perusal of these results it was thought that bromate reduction in dough was a slow, yeast dependent reaction. Reduction in non-yeasted doughs could be accounted for in part by microbiological activity since flour is not sterile. This work suggested the investigations to be undertaken for this thesis, viz, the influence of yeast, the effect of prefermentation of the dough, the influence of yeast inhibitors, and considerations of pH in connection with the decomposition of bromate in dough.

## Influence of Yeast on Bromate Decomposition

### 1. Rate of Decomposition of Bromate at Three Yeast Levels

It had already been demonstrated that bromate was reduced faster in a yeasted dough than in a non-yeasted dough. It was decided that a series should be run in which the effect on bromate reduction of varying the yeast concentration could be demonstrated. Flour #9718 was used for this series, which covered the loss of bromate from doughs that contained 10 p.p.m. and 30 p.p.m. potassium bromate and yeast concentrations of 0, 1 and 3% with reaction times of 0 to 20 hours. With each bromated dough in the series an unbromated dough was treated and analyzed in an identical manner to serve as a blank.

The formula for the doughs was conventional except that no sugar was used. It is given in Table VI.

The doughs were mixed for 3 minutes in the Hobart mixer at No. 2 speed. They were then stored for the various reaction times in a cabinet at constant temperature (30°C). The doughs were kept in covered crocks so that there would be no appreciable loss of moisture. The analysis was carried out

Table VI. Dough Formulas

| | Yeasted Doughs | Non-Yeasted Doughs |
|---|---|---|
| Flour | 100 g. (on 14% moisture basis) | Flour 100 g. |
| Salt[I] | 1.0% | Water 62.6 ml. |
| Malt[I] | 0.3% | Bromate 10 p.p.m. or 30 p.p.m. |
| Phosphate[I] | 0.1% | |
| Yeast[I] | 1%, 3% | |
| Water | to make total absorption 62.6% | |
| Bromate[I] | 10 p.p.m. or 30 p.p.m. | |

[I] All added as described in Table II.

by the Johnson and Alcock method as described in the section on Materials and Methods. Two aliquots were analyzed from the extract of each dough sample and each sample was done in duplicate. This means that the values given in Table VII are averaged from four results. These values indicate the concentration of bromate recoverable from the dough after the elapsed time shown and the stated treatment.

Table VII. Bromate Recoveries (p.p.m.) From Doughs Given Long Fermentation Periods at Various Yeast Concentrations

| Time | 10 p.p.m. Bromate Added | | | 30 p.p.m. Bromate Added | | |
|---|---|---|---|---|---|---|
| (hrs) | 0% yeast | 1% yeast | 3% yeast | 0% yeast | 1% yeast | 3% yeast |
| 0 | 9.1 | 9.5 | 8.7 | 28.8 | 28.8 | 30.1 |
| 2 | 7.0 | 5.4 | 9.1 | - | 25.8 | - |
| 4 | 5.7 | 5.1 | 5.4 | 23.1 | 25.5 | 20.4 |
| 6 | 6.1 | 5.6 | 0.9 | 22.0 | 22.0 | 17.1 |
| 8 | 5.0 | 3.3 | 2.4 | 22.9 | 13.2 | 10.5 |
| 12 | 3.9 | - | 0.0 | 16.9 | 5.4 | 4.2 |
| 14 | 2.8 | 0.0 | 0.0 | 14.2 | 5.4 | 2.4 |
| 16 | 0.9 | 0.0 | 0.0 | 11.7 | 3.0 | 3.0 |
| 18 | 0.9 | 0.0 | 0.0 | 7.5 | 0.0 | 0.7 |
| 20 | 0.0 | 0.0 | 0.0 | 4.7 | 0.0 | 0.0 |

The results in Table VII are plotted in Fig. 1 as bromate concentration vs. reaction time. Also in Fig. 1 is a plot showing bromate decomposition with time in a flour-water dough made with a different flour sample (#9053).

The following are the features brought out by Fig. 1. The rate of decomposition of bromate in unyeasted doughs as shown in graphs on the left is relatively slow indicating that only a small amount decomposes with short reaction times. The rate was slower for flour #9053 than for flour #9718 indicating clearly that the flour constituents are contributing factors in the reduction of bromate. Second, the presence of yeast in dough definitely increases the rate of bromate decomposition. Thus 30 p.p.m. of bromate disappeared from doughs containing no yeast at 0.6 p.p.m. per hour for flour #9053 and at 1.2 p.p.m. per hour for flour #9718; from doughs containing 1% yeast at 1.8 p.p.m. and from doughs containing 3% yeast at 2.1 p.p.m. per hour. Similarly the rate of disappearance of 10 p.p.m. bromate was 0.5, 0.9 and 1.2 p.p.m. per hour for the 0, 1 and 3% yeast levels. Third, the rate of bromate decomposition in yeasted doughs seems to be greater at the higher bromate level. Fourth, the rate of bromate decomposition appears to be linear with time. The significance of some of these observations will be considered in the "General Discussion".

2. Increase in Bromate Decomposition with Increasing Yeast Concentration

The above work showed that the rate of reduction of bromate was greater for the higher yeast level and it was thought desirable that a detailed investigation of the relationship between yeast concentration and bromate decomposition be carried out. The work involved bromate analysis of doughs that had various yeast concentrations up to 6% and that were allowed reaction times of 0 and 12 hours.

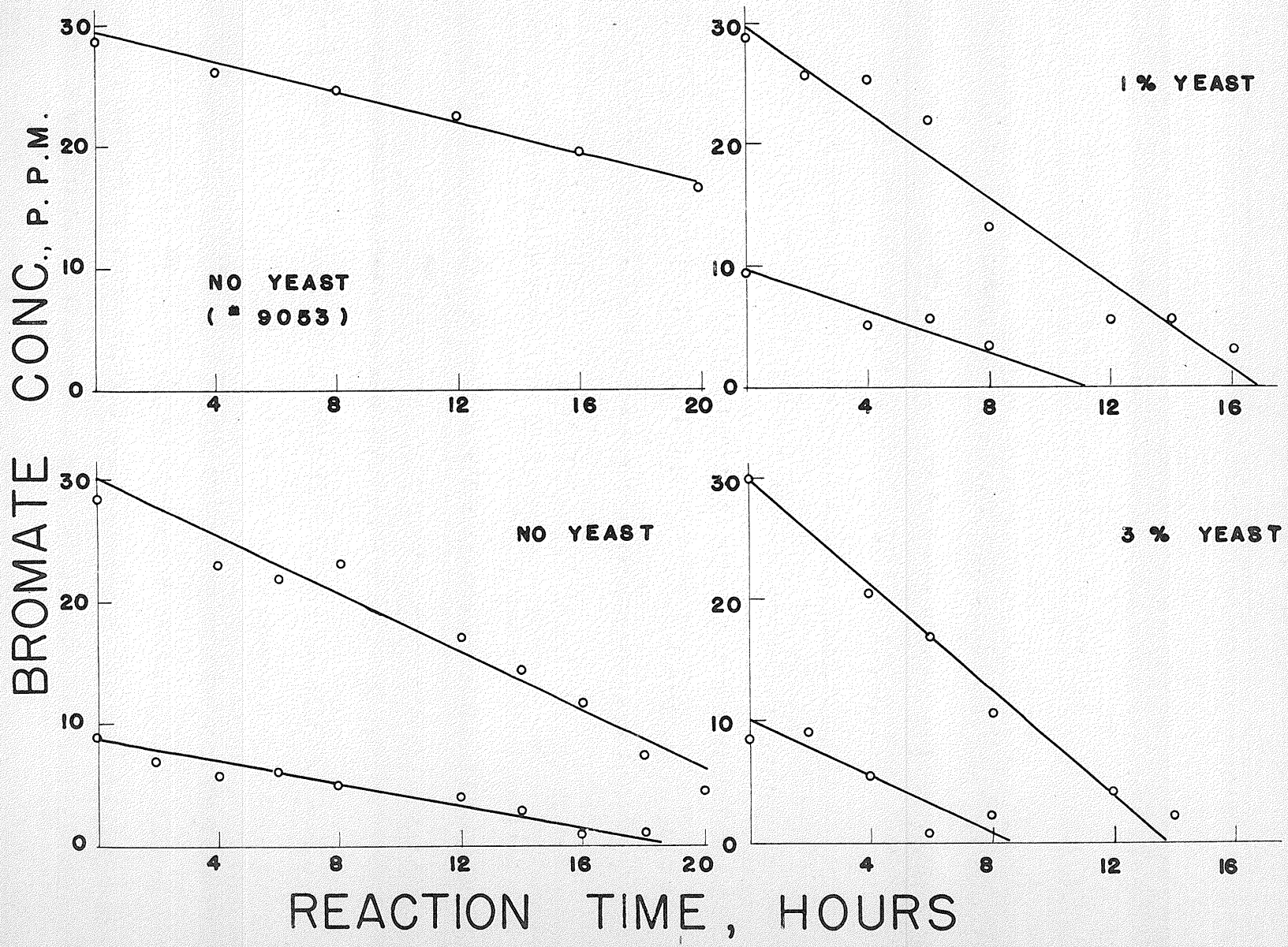


Figure I

Bromate Decomposition With Time in Yeasted and Unyeasted Doughs

Doughs were mixed from flour #57 for 3 minutes in the Hobart mixer at medium speed. The standard flour, water, salt, malt, phosphate, formula was used as previously described. Bromate was added at a level of 30 p.p.m. and yeast in concentrations of 0 to 6.0%. There is a slight difference in dough formula here from that shown in Table VI in that the doughs with zero yeast concentration contained all other ingredients that the doughs with positive yeast concentrations contained. (In Table VI, the doughs with zero yeast were merely flour-water doughs). The doughs were kept in closed crocks in a cabinet at 30°C. for 0 and 12 hours. The pH of each dough was measured at the time of sampling. Table VIII shows the analytical data which were obtained.

Table VIII shows that for zero reaction time there does not appear to be any particular trend in bromate decomposition. The recovery at the 6.0% yeast level is lower than the others. This might mean that at such a high yeast concentration even with a very short reaction time (such as the time between mixing and sampling the dough as soon as possible afterward) there is slightly more reduction of bromate than at lower yeast levels. The trend is more obvious in the results for a 12 hour reaction time. As the yeast concentration increases the bromate decomposition rate increases, i.e., more bromate is decomposed in 12 hours in a dough with 6.0% yeast than in doughs with lesser amounts.

Table VIII also demonstrates that the pH of the doughs given zero reaction time gradually falls as the yeast content of the dough is increased. In the case of doughs given a 12 hour reaction time there is a sudden drop of pH from 5.7 in a non-yeasted dough to 4.8 in a dough with 0.5% yeast. After this initial drop there is not much change in pH with increased yeast concentration. Even with 6.0% yeast the pH is only 0.2 points below that for a dough with 0.5% yeast.

Table VIII. Bromate Recoveries from Doughs with Various Yeast Contents Fermented for 0 and 12 Hours

| Yeast Conc. % | Reaction Time: 0 hr. Bromate (p.p.m.) | Av. | pH | Av. | 12 hr. Bromate (p.p.m.) | Av. | pH | Av. |
|---|---|---|---|---|---|---|---|---|
| 0 | 29.1 | | 5.8 | | 18.0 | | 5.7 | |
| | 28.5 | | 5.8 | | 18.3 | | 5.7 | |
| | 28.7 | 28.8 | 5.8 | 5.8 | 18.3 | 18.2 | 5.8 | 5.7 |
| 0.5 | 27.6 | | 5.8 | | 12.3 | | 4.9 | |
| | 30.6 | | 5.8 | | 15.5 | | 4.8 | |
| | 28.3 | 28.8 | 5.7 | 5.8 | 14.0 | 13.9 | 4.7 | 4.8 |
| 1.0 | 30.0 | | 5.7 | | 15.3 | | 4.8 | |
| | 30.3 | | 5.7 | | 15.2 | | 4.9 | |
| | 31.2 | 30.5 | 5.7 | 5.7 | 14.9 | 15.1 | 4.7 | 4.8 |
| 2.0 | 32.1 | | 5.6 | | 10.8 | | 4.7 | |
| | 31.5 | | 5.6 | | 10.0 | | 4.8 | |
| | 28.8 | 30.8 | 5.6 | 5.6 | 11.1 | 10.6 | 4.8 | 4.8 |
| 3.0 | 28.4 | | 5.7 | | 12.1 | | 4.9 | |
| | 29.2 | | 5.7 | | 13.2 | | 4.9 | |
| | 28.7 | 28.8 | 5.6 | 5.7 | 12.5 | 12.6 | 4.7 | 4.8 |
| 4.5 | 28.5 | | 5.4 | | 9.81 | | 4.6 | |
| | 27.9 | | 5.4 | | 10.1 | | 4.8 | |
| | 30.3 | 28.9 | 5.4 | 5.4 | 10.0 | 9.97 | 4.7 | 4.7 |
| 6.0 | 27.2 | | 5.4 | | 6.81 | | 4.6 | |
| | 27.5 | | 5.4 | | 5.31 | | 4.5 | |
| | 28.7 | 27.8 | 5.5 | 5.4 | 6.60 | 6.24 | 4.6 | 4.6 |

The Beckman pH meter was used to measure the pH of the doughs. Even though the instrument can be read accurately to two decimal places the results here are only extended to tenths of a unit because it was found that when measuring the pH of dough, the accuracy is only about 0.05 of a pH unit.

The results in Table VIII are summarized graphically in Fig. 2. Fig. 2 shows that the accuracy of measurement of bromate is low. Although the trend is obvious, the points do not fall in too great regularity along the lines.

This work on the effects of varying yeast concentration on bromate decomposition leads to two possible conclusions. First, bromate reduction is effected by the releasing of reducing substances as a result of yeast metabolism, and therefore the more yeast present, the more reducing substances and the more bromate that is reduced. Second, the lowering of the pH is the responsible factor for bromate reduction. These two will be discussed again in separate sections.

## The Effect of Prefermentation on Bromate in Dough

### I. Analysis by Johnson and Alcock Method

Previous work on the decomposition of bromate added to fermenting and non-fermenting doughs showed that there was a fairly rapid reduction of bromate in the yeasted doughs compared to unyeasted doughs. A second observation was the linearity of the relationship between bromate loss and fermentation time. The data just presented indicate that reduction of bromate is related closely to the fermentation process. The purpose of the present investigation was to ferment doughs prior to adding bromate and then to compare the decomposition of bromate with time in such doughs with that described earlier. If the reduction was associated with the releasing of

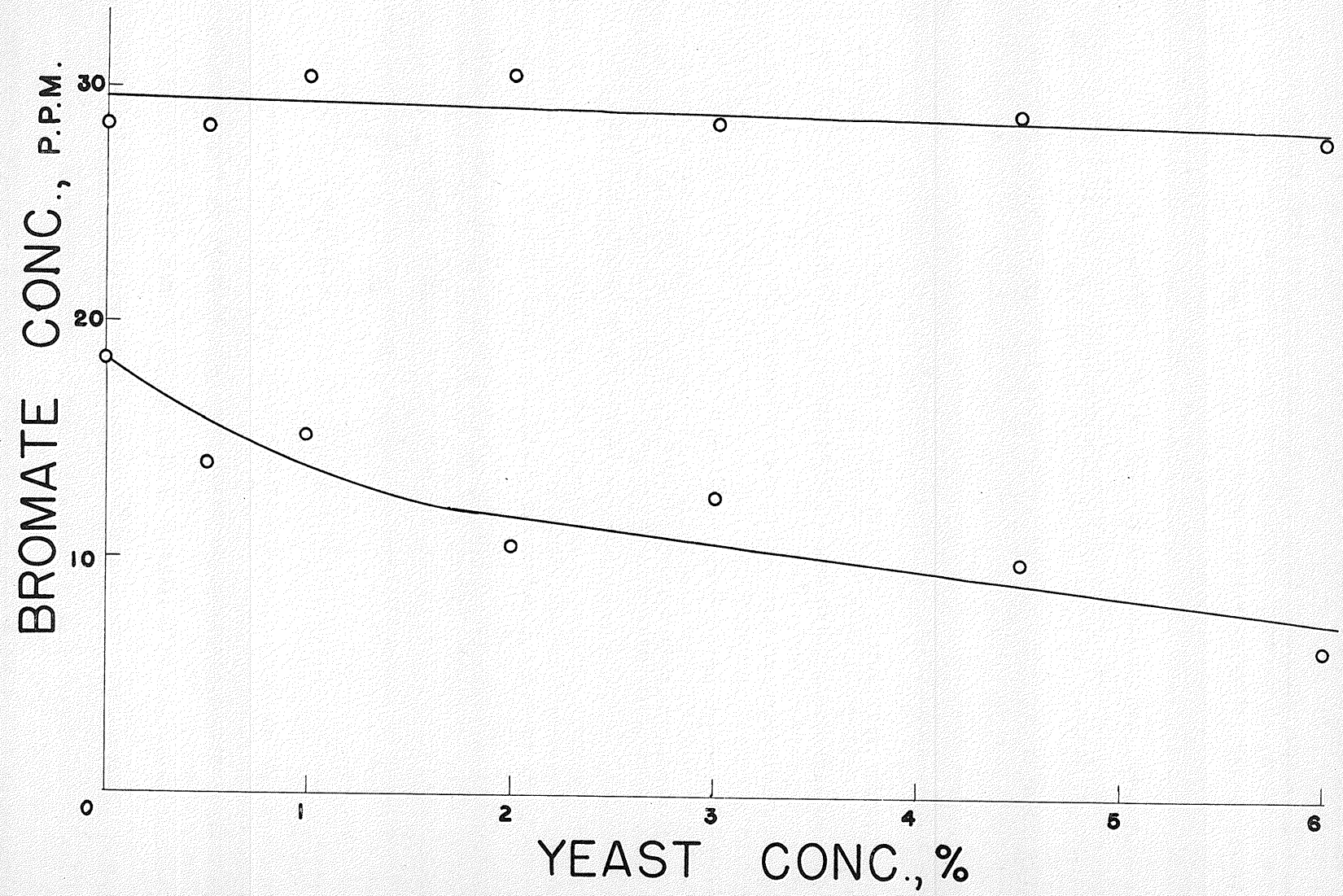


Figure 2

Bromate Decomposition After Zero and Twelve Hour Reaction Times With Varying Yeast Concentration

reducing substances in the dough in conjunction with fermentation it might be expected that fermentation prior to addition of bromate could result in an accumulation of reducing substances which would cause sudden reduction of the bromate when it was added to the dough. If this were the case, the reduction of bromate with time would be more rapid than previously observed and the linear relationship would, perhaps, no longer hold. The data obtained from this study did not bear this out.

Table IX contains the sponge dough formula used.

Table IX. Sponge Dough Formula

| | | |
|---|---|---|
| Flour #57 | 70.5 g. | 70% of total baking formula |
| Yeast | 3.0 g. | 100% of total baking formula |
| Salt | 1.0 g. | 100% of total baking formula |
| Malt | 0.3 g. | 100% of total baking formula |
| Ammon. Phosphate | 0.1 g. | 100% of total baking formula |
| Absorption | 42.7% | 70% of total baking formula |

When doughing up the remainder of the flour (30.2 g.) and water (18.3 ml.) were added. Bromate (30 p.p.m.) was added with the water.

The sponge was mixed for 3 minutes. Sponge times at 30°C. were 3 hours duration for one series and 12 hours for a second series. The sponge plus the remainder of the water was mixed for 1-1/4 minutes to a gruel-like consistency, then the remainder of the flour was added and the dough mixed for 3/4 minute. This gave a fairly elastic and smooth dough which was stored at 30°C. for times of from 0 to 15 hours. Bromate analysis by the Johnson and Alcock method was carried out as described previously. Table X summarizes the results. Each value is an average of two aliquot analyses.

The precision of the analysis is not very high as Table X illustrates. However, it can be seen that the longer the reaction time between bromate and the yeasted dough the greater is the amount of bromate

Table X. Recoveries of Bromate Added to 3 and 12 Hour Sponge Doughs at Doughing-Up Stage
(Johnson and Alcock Analysis)

| Reaction Time (hrs) | 3 Hour Sponge | | | 12 Hour Sponge | | |
|---|---|---|---|---|---|---|
| | p.p.m. | % | Av.% | p.p.m. | % | Av.% |
| 0 | 26.4 | 88.0 | | 26.8 | 89.3 | |
| | 28.3 | 94.2 | | 24.0 | 80.0 | |
| | 29.5 | 98.3 | 93.5 | 28.5 | 95.0 | 88.1 |
| 3 | 18.0 | 60.0 | | 20.1 | 67.0 | |
| | 20.1 | 67.0 | | 19.3 | 64.3 | |
| | 18.8 | 62.7 | 63.2 | 19.4 | 64.7 | 65.3 |
| 8 | 14.2 | 47.4 | | 10.7 | 35.7 | |
| | 9.60 | 32.0 | | 14.3 | 47.7 | |
| | 12.4 | 41.3 | 36.9 | 10.4 | 34.7 | 39.4 |
| 12 | 8.70 | 29.0 | | 7.00 | 23.3 | |
| | 6.10 | 20.3 | | 7.70 | 25.7 | |
| | 7.74 | 25.7 | 25.0 | 4.44 | 14.8 | 21.2 |
| 15 | 2.70 | 9.0 | | 3.50 | 11.7 | |
| | 1.26 | 4.2 | 6.6 | 1.76 | 5.9 | 8.8 |

reduced. The recovery of bromate after a 3 or 12 hour sponge time and zero reaction time is around 90% which shows that there is not a great accumulation of reducing substances that can react immediately to reduce the bromate. This is again illustrated when the recoveries from doughs made from 3 hour sponges are compared with those made from 12 hour sponges. The recoveries, within experimental error, are identical in both cases for any reaction time. (Compare column 4 with column 7) These results are plotted in Fig. 3.

In Fig. 3 the two curves from the data of Table X are compared with a curve obtained earlier when work was done on straight bread doughs in which the bromate and yeast were both added at the initial mixing. The plots show great similarity. The rate of decomposition of bromate does not appear to be accelerated by an accumulation of reducing substances which should occur by allowing fermentation to proceed prior to adding the bromate. The plots for the sponge dough experiments cannot be compared strictly with the other since different flour samples were used for each series.

2. Analysis by Howe Method

This same experiment was carried out again with all details identical except that the bromate analysis was done by the Howe method as described previously. The use of two methods for the same series of experiments should be explained. The Johnson and Alcock method had been used for all work previous to this but when the more recent Howe method for bromate determination was published it was felt that it should be given a trial to test its superiority or inferiority. Although different trends in the results of the sponge dough series are indicated by the two methods, neither method seemed precise enough to give a definite answer and the Johnson and Alcock method seemed to be slightly more precise. Table XI contains the

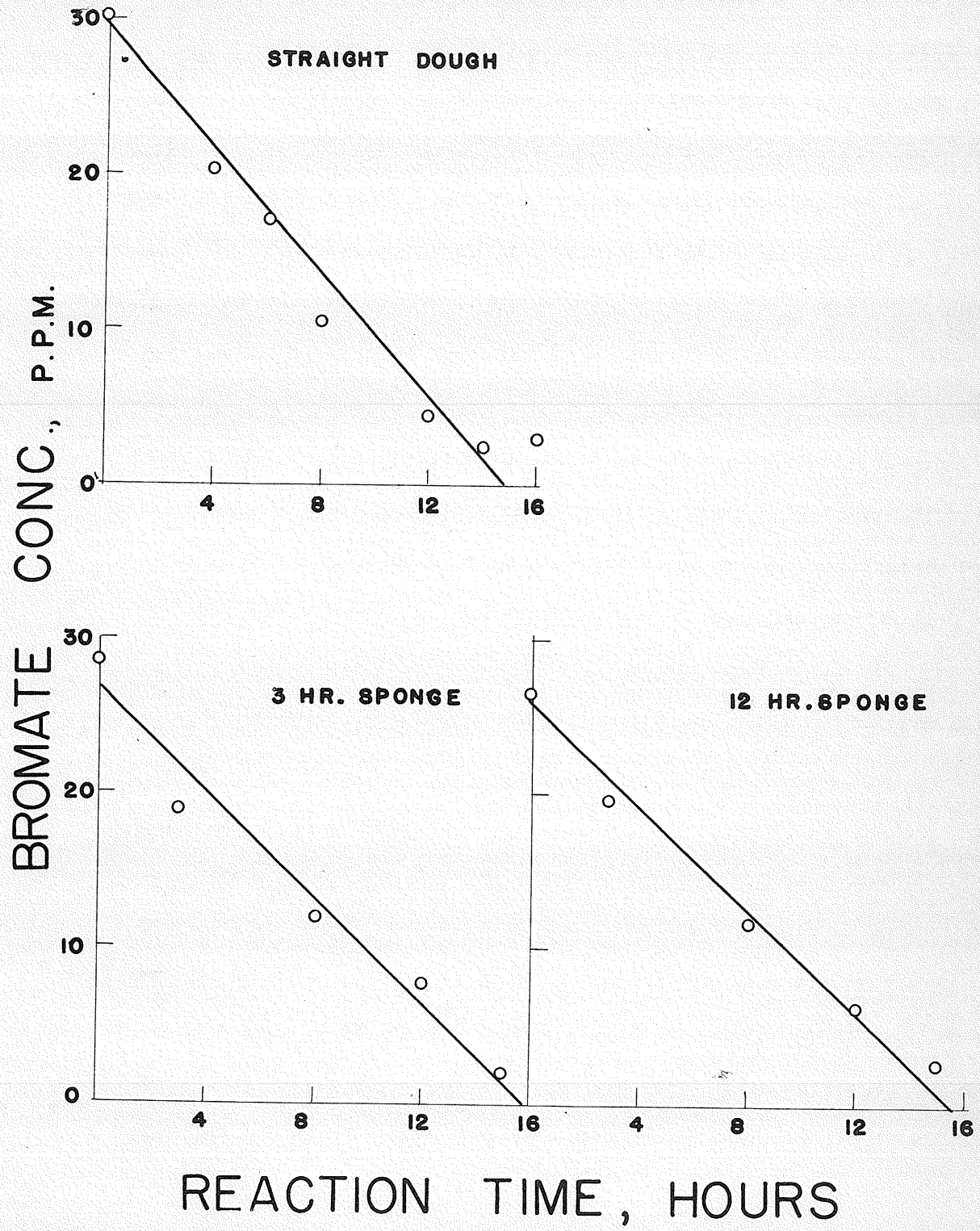


Figure 3

Decomposition of Bromate Added to Bread Dough at Time of Doughing-Up from Fermented Sponge (1)

Table XI. Recoveries of Bromate Added to Sponge Doughs at Doughing-Up Stage (Analysis by Howe Method)

| Reaction Time (hrs) | 3 Hour Sponge | | | 12 Hour Sponge | | |
|---|---|---|---|---|---|---|
| | p.p.m. | % | Av.% | p.p.m. | % | Av.% |
| 0 | 24.6 | 82.0 | | 25.6 | 85.3 | |
| | 30.0 | 100 | | 22.9 | 76.3 | |
| | 29.4 | 98.0 | 93.3 | 28.2 | 94.0 | 85.2 |
| 3 | 18.4 | 61.3 | | 16.0 | 53.3 | |
| | 19.5 | 65.0 | | 20.1 | 67.0 | |
| | 25.1 | 83.7 | 70.0 | 13.8 | 46.0 | 55.4 |
| 8 | 17.9 | 59.7 | | 13.5 | 45.0 | |
| | 18.7 | 62.3 | 61.0 | 13.5 | 45.0 | 45.0 |
| 12 | 10.3 | 34.3 | | 10.9 | 36.3 | |
| | 12.4 | 41.3 | | 8.4 | 28.0 | |
| | 7.2 | 24.1 | 33.2 | 7.5 | 25.0 | 29.8 |
| 15 | 5.4 | 18.0 | | 5.2 | 17.3 | |
| | 6.1 | 20.2 | 19.1 | 1.4 | 4.7 | 11.0 |

results given by the Howe method.

Corresponding data in columns 4 and 7 of Table XI show that after a 12 hour sponge period, there is more bromate decomposed subsequent to the doughing up stage than after a 3 hour sponge period under similar conditions. This was not found when the Johnson and Alcock analysis was used. The variation in replicate determinations is as great if not greater than with the Johnson and Alcock method however. It might be said that there is an accumulation of reducing substances over a 12 hour sponge period and that this could account for the more rapid reduction of the bromate, but the evidence in one series only opposes the evidence of the other. It would seem that there is a difference in the specificity of the two analytical methods. Either the Johnson and Alcock method measures oxidizing agents other than bromate, thus giving high bromate recoveries after a 12 hour sponge period, or the Howe method of extraction might carry over some reducing substances which further reduce bromate, thus giving the observed low recovery after a 12 hour sponge period. The results of Table XI are plotted in Fig. 4, along with the results of Table X, so that a ready comparison can be made.

According to the graphs in Fig. 4 the rate of decomposition of bromate is slightly faster as measured by the Johnson and Alcock analysis than as measured by the Howe analysis. Which one of the rates is more nearly correct cannot be said at this time, but the difference is only slight and may even be ignored in view of the large experimental error of both methods.

Influence of Yeast Inhibitors on Bromate Decomposition

So far the evidence indicates that the decomposition of bromate in dough is closely connected with the yeast content. Since even in unyeasted

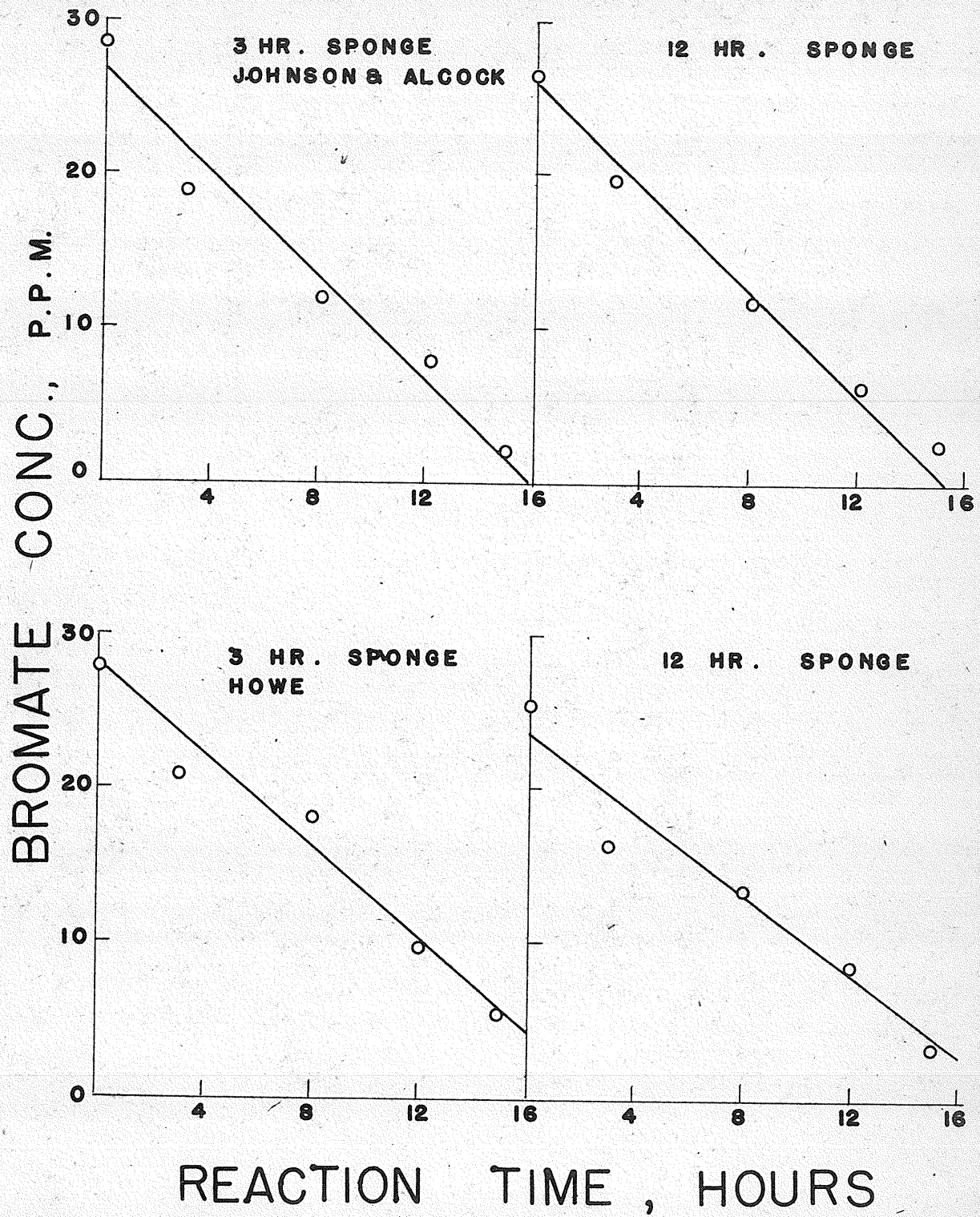


Figure 4

Decomposition of Bromate Added to Bread Dough at Time of Doughing-Up from Fermented Sponge (2)

doughs there is some microbiological activity, as mentioned previously, it was thought that if yeast inhibitors (chemical substances that would halt the fermenting action of yeast) were added to flour-water doughs, they could stop entirely the decomposition of bromate or at least slow down the rate of decomposition.

Freilich and Frey (13) found that n-octyl alcohol was effective in inhibiting yeast fermentation without retarding protease activity. Jorgensen (26) says that a 0.3% NaF solution is a powerful antiseptic. These were the two yeast inhibitors chosen for the study which was conducted as follows.

Bromated (30 p.p.m.) flour-water doughs (flour #57) were mixed 3 minutes in the Hobart mixer. In one set, 0.6 ml. n-octyl alcohol was added per 100 g. of flour, in a second set, 0.3 g. sodium fluoride per 100 g. of flour was added and in a control set, no inhibitor was added. The doughs were stored in closed crocks at 30°C. for various reaction times up to 24 hours before being analysed for bromate. The pH of the doughs was measured at the time of analysis by means of the Beckman pH meter. The results are presented in Table XII.

The first noticeable fact in Table XII is that the yeast inhibitors have actually stopped fermentation. This is demonstrated by the pH of the three doughs after the reaction period. The pH did not change in the two doughs with added inhibitor but in the flour-water dough it dropped to 5.6 after 20 hours.

N-octyl alcohol apparently causes a decrease in the rate of reduction of bromate in the dough. After 20 hours only about 25% of the added bromate remains in the flour-water dough but in the dough with n-octyl alcohol there remains over 50% of the added bromate. This favours the hypothesis that bromate reduction in dough is closely linked with the fermentation process. It

Table XII. Bromate Recoveries from Doughs Containing Yeast Inhibitors and Given Long Reaction Times

| Reaction Time (hrs) | Flour-Water | | Flour-Water-n-octyl Alcohol | | Flour-Water-Sodium Fluoride | |
|---|---|---|---|---|---|---|
| | pH | $BrO_3$ (p.p.m.) | pH | $BrO_3$ (p.p.m.) | pH | $BrO_3$ (p.p.m.) |
| 0 | 5.8 | 30.9 | 5.8 | 28.4 | 5.9 | 30.6 |
| | 5.7 | 30.9 | 5.9 | 30.6 | 5.9 | 30.3 |
| | 5.9 | 31.5 | 5.9 | 27.9 | 5.9 | 29.5 |
| 2 | | | | | 6.0 | 23.3 |
| | | | | | 5.9 | 25.4 |
| | | | | | 5.9 | 23.0 |
| 4 | 5.9 | 25.0 | 5.9 | 24.4 | 6.0 | 18.1 |
| | 5.9 | 23.7 | 6.0 | 26.0 | 6.0 | 18.6 |
| | 5.9 | 22.3 | 5.9 | 26.0 | 6.0 | 17.3 |
| 8 | | | | | 6.0 | 15.8 |
| | | | | | 6.0 | 16.0 |
| 12 | 5.8 | 16.8 | 5.8 | 18.9 | 5.9 | 12.9 |
| | 5.9 | 15.1 | 5.9 | 18.9 | 5.9 | 13.8 |
| | 5.9 | 15.6 | 5.9 | 20.5 | 6.0 | 13.9 |
| 16 | 5.9 | 16.4 | 5.9 | 19.6 | 6.1 | 8.25 |
| | 5.8 | 12.9 | 6.0 | 18.1 | 6.0 | 7.29 |
| | 5.9 | 13.0 | 5.9 | 19.9 | 6.1 | 7.23 |
| 20 | 5.5 | 8.80 | 5.9 | 17.3 | 5.9 | 8.19 |
| | 5.6 | 5.70 | 5.8 | 16.3 | 6.2 | 6.45 |
| | 5.6 | 7.55 | 6.0 | 15.6 | 6.0 | 6.96 |

also favours the hypothesis that low pH is responsible for bromate reduction. However, there is the result of the sodium fluoride study to be considered.

Table XII shows that the pH of the doughs with added sodium fluoride is kept constant and one must assume that fermentation has been halted. But the bromate is reduced at a much greater rate for the first 16 hours (until the bromate concentration is quite low) and at 20 hours the bromate concentration in the control and the inhibited dough is about the same. The rate of decomposition in both doughs seems to have levelled off at this point. It is difficult to say any more about this or to investigate the decomposition past 20 hours to get more information about the rate of decomposition since low concentrations of bromate are not measured accurately by the method in use.

The results are summarized in Fig. 5.

It is obvious from the slopes of the curves in Fig. 5 that n-octyl alcohol inhibits bromate decomposition markedly whereas sodium fluoride does the opposite; it accelerates the decomposition. When the logarithm of concentration of bromate is plotted against time using the data from the sodium fluoride study, the plot is a quite good straight line which is a criterion for first order reactions. Therefore, the addition of sodium fluoride to the dough has resulted in changing the apparent order of the reaction from zero to first order.

## Considerations of pH in Connection with Bromated Doughs

The importance of fermentation of doughs in connection with the bromate improving effect has been mentioned in the literature and the low pH of fermenting doughs has been mentioned as an important factor in the effects of bromate on bread dough. For these reasons, special attention was given to pH in some of the studies already discussed in this thesis. This

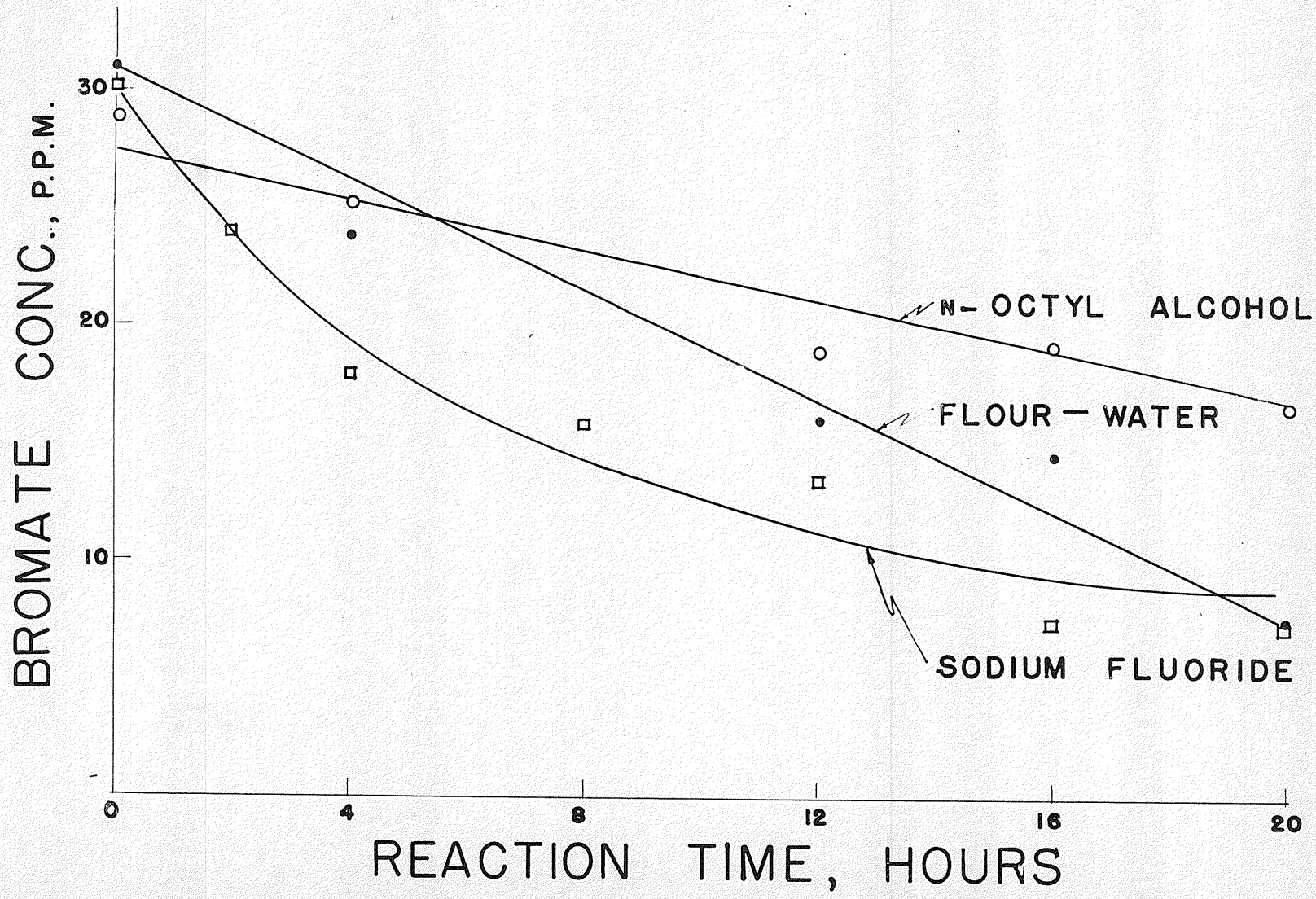


Figure 5

Decomposition With Time of Bromate in Doughs
With Yeast Inhibitors Added

will be reviewed here along with a report on studies of bromated doughs acidified by addition of carbonic, lactic and hydrochloric acids.

1. Changes in pH with Various Yeast Concentrations

It has been suggested that the rate of decomposition of bromate in a dough is controlled by the pH of the dough. From Table VIII (page 29) it can be seen that although the pH decreases with greater yeast concentration, with zero reaction time the amount of bromate decomposed does not become greater with greater yeast concentration. This does not reveal very much, however, since there was, as mentioned, no reaction time. When a 12 hour reaction time was allowed the results were somewhat different. With 0.5% yeast, the pH of the dough dropped to 4.8 and remained at this point with yeast concentrations up to 3.0%. With 4.5% yeast the pH dropped to 4.7 and with 6.0% yeast, to 4.6. The average bromate recovery at pH 4.8 was slightly lower than that at 5.7 (0% yeast) and then the recovery dropped steadily through pH 4.7 to pH 4.6 where the investigation was terminated. It is difficult to conclude that the bromate decomposition increases with decreasing pH due to the results at pH 4.8, but it is equally difficult to ignore the trend. The alternate explanation, however, that bromate decomposition increases with increased yeast concentration accounts for the observed results just as well.

2. pH Considerations in Doughs with Added Yeast Inhibitors

In Table XII it is seen that for flour water doughs given long reaction times the pH changes only slightly after 20 hours and yet the bromate is decomposed at a fairly steady rate. Also in the doughs containing n-octyl alcohol, the pH remains constant around 5.9 and the bromate still decomposes at a slow but steady rate. Finally, in the doughs with sodium fluoride added the pH stays at 5.9 - 6.0 and yet the bromate is reduced at an accelerated rate.

The foregoing does not support the hypothesis that bromate decomposition increases with decreasing pH. Bromate decomposition appears to be independent of the pH of the dough since doughs with low, as well as doughs with high pH values have been found to have high rates of bromate decomposition.

The object of the final series of experiments to be reported was to simulate the pH of yeasted doughs by adding acid and then to determine the effect on bromate decomposition. It was also decided that the study could be given greater interest if a simultaneous extensogram study of the doughs was made. Reversal of the order of importance and lack of time have resulted in the completion of the extensogram study but only partial completion of the bromate study. A new flour sample (#1714) was required.

3. Extensogram Studies of Doughs with Added Acids

It was thought best to acidify the doughs with acids indigenous to normal yeasted doughs and also with one mineral acid. The ones chosen were carbonic, lactic and hydrochloric as it was believed that these could have no direct effect on bromate. The pH levels selected for study were from 4.80 to 5.90, the latter being the pH of a flour-water dough made from sample #1714. The desired pH levels for the doughs were obtained by an essentially trial and error method.

The doughs were mixed as follows: 200 g. of flour (14 per cent moisture basis) were mixed for 3 minutes with water, acid, and bromate in a laboratory constructed mixer which has a gentle mixing action intermediate between the Hobart and Hobart-Swanson types. It required 115 ml. of an approximately 0.04 M hydrochloric acid solution, in a dough which had a total absorption of 121.6 ml., to obtain a pH of 4.8. Lesser amounts gave pH values of 5.3 and 5.5 units. It required 30 ml. of an

approximately 0.27 M lactic acid solution to obtain a pH of 4.9. Lesser amounts gave pH values of 5.0 and 5.3 units. In the dough with added carbonic acid an attempt was made to add the maximum amount in order to obtain a low pH. However, even with the most drastic means the pH could only be lowered to about 5.7. This was effected by adding $CO_2^3$ saturated water to the flour and mixing the dough in an atmosphere of $CO_2$.

The extensogram procedure was as follows. After mixing, two 150 g. dough samples were scaled off; one was put in a cabinet at 86°C. and 90% relative humidity, the other was rounded and shaped and put away in a holder in the cabinet for 45 minutes and then stretched to give the extensogram. The first dough sample was given a reaction time of 135 minutes, then was shaped, put in a holder, rested 45 minutes, and then stretched. The results of this study are illustrated in Figs. 6 and 7.

The significance of the results shown in Figs. 6 and 7 may be summarized as follows.

The height of the horizontal lines and of the bars above the zero level represents resistance to extension, in Brabender units, exerted by a dough when stretched and when the reading is taken at 7 cm. on the extensogram paper. (Method suggested by Dempster (8). The lines correspond to the resistance to extension of unacidified doughs given the treatment specified by the titles in the squares. These values can be designated as follows.

| | | |
|---|---|---|
| Fig. 6 | Upper half | F |
| | Lower half | F + B |

The heights of the bars in each square represent the resistance to extension of acidified doughs given the specified treatment and which have pH values as indicated. These resistances to extension can be designated as follows.

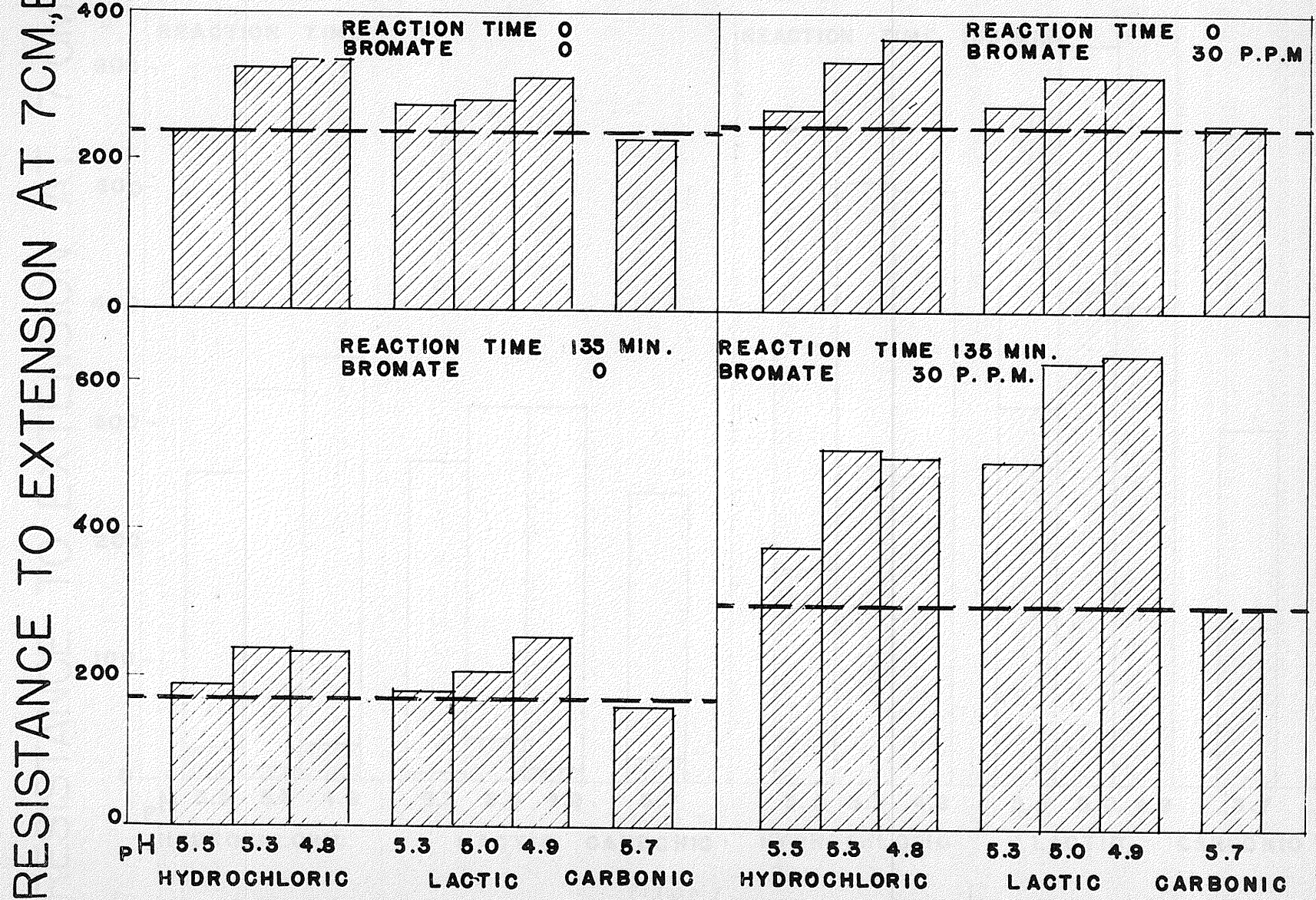


Figure 6

Summary of Extensogram Studies of Bromated-Acidified-Doughs

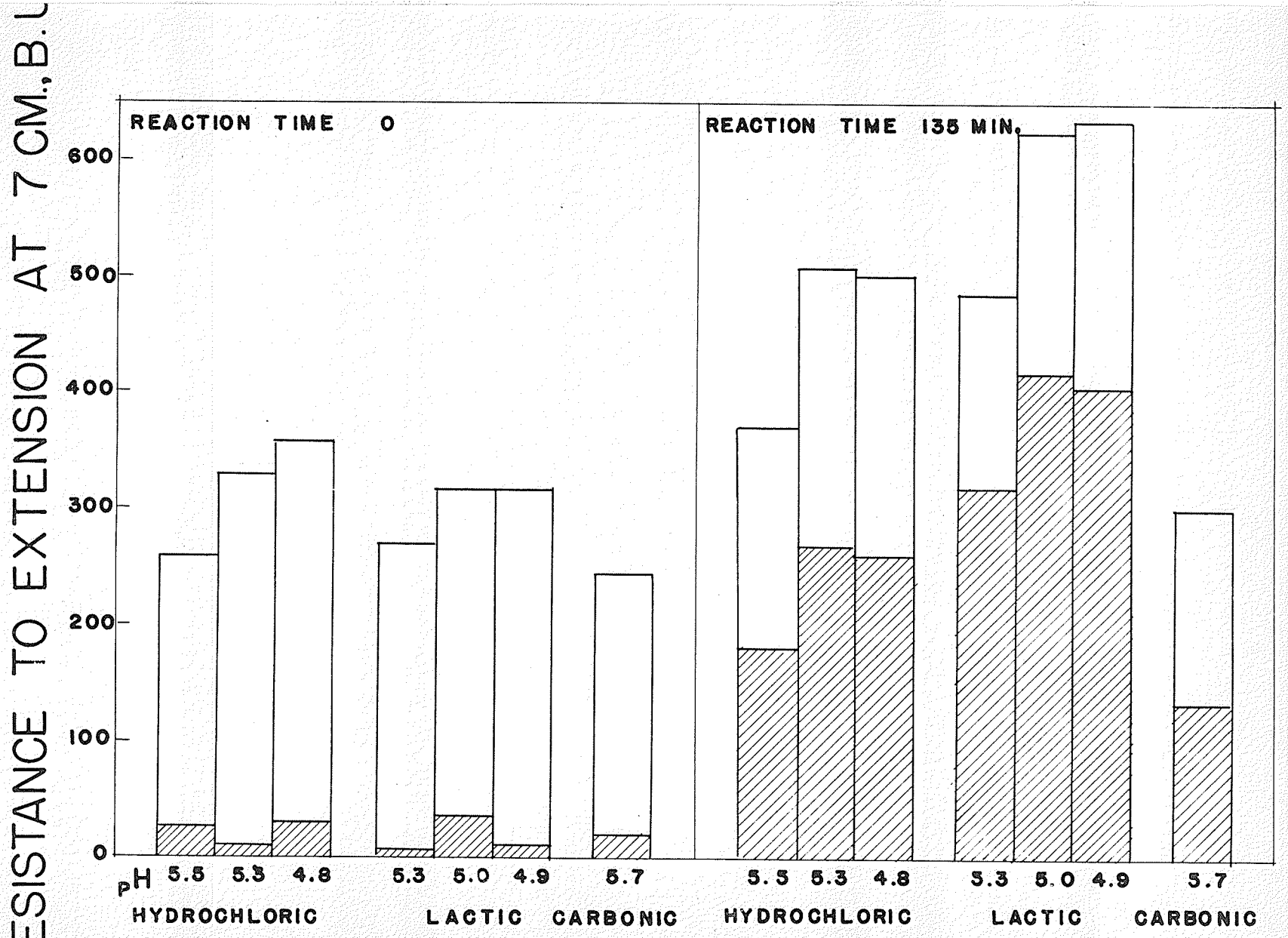


Figure 7

Effect of Acid on the Bromate Effect on Flour Water Doughs

Fig. 6 Upper half $F+A$
Lower half $F+A+B_A$

The letters above stand for the effects on resistance to extension as follows.

F - flour effect (normal flour-water dough)

B - bromate effect

$B_A$ - bromate effect in an acidified dough

A - acid effect

The effect of the various treatments on the resistance to extension of the doughs can be summarized as follows.

With o reaction time

Immediate Effect of Acid

Fig. 6 Upper left

| | |
|---|---|
| $F+A$ | height of bar above zero |
| $-F$ | -height of line above zero |
| $A$ | height of bar above line |

Acid has a small immediate tightening effect on the dough. The effect is characteristic of the acid used and different for different pH levels.

Immediate Effect of Bromate

Fig. 6 Upper half

| | |
|---|---|
| $F+B$ | height of line on right |
| $-F$ | -height of line on left |
| $B$ | difference in heights |

The usual bromate effect is observed.

Immediate Effect of Bromate in Acidified Dough

Fig. 7 Left

| | |
|---|---|
| $F+A+B_A$ | height of white bar above zero |
| $-(F+A)$ | -height of white bar above hatched bar |
| $B_A$ | height of hatched bar |

The bromate effect is increased in an acidified dough. The amount of increase is dependent more on the acid used than on the pH of the dough.

With 135 minutes reaction time

Effect of Acid

Fig. 6 Lower left

| | |
|---|---|
| F+A | height of bar above zero |
| -F | -height of line above zero |
| A | height of bar above line |

After a 135 minute reaction time the "acid effect" is lessened a great deal.

Effect of Bromate

Fig. 6 Lower half

| | |
|---|---|
| F+B | height of line on right |
| -F | -height of line on left |
| B | difference in heights |

The usual bromate effect is observed.

Effect of Bromate in an Acidified Dough

Fig. 7 Right

| | |
|---|---|
| $F+A+B_A$ | height of white bar above zero |
| -(F+A) | -height of white bar above hatched bar |
| $B_A$ | height of hatched bar |

The "acid-bromate effect" becomes more apparent after a reaction period. It appears that there is an optimum pH value for maximum bromate effect but this is not well defined by the data obtained.

It might also be mentioned that the acidified doughs were quite short. That is, they were not very extensible and tore after relatively short extensions. This was particularly noticeable in the bromate-acid doughs given 135 minutes of reaction time.

4. Bromate Decomposition in Doughs with Lactic Acid Added

As time was getting short it was decided that the study of bromate decomposition in acidified doughs would be done only on doughs containing lactic acid. Lactic acid was selected from the three acids (hydrochloric, lactic, carbonic) because, first, hydrochloric acid is foreign to bread doughs; second, even though carbonic acid is the main contributing factor in determining the pH of a fermenting dough, the pH attainable by the use of carbonic acid was not low enough for this study; third, Johnson (22) found that lactic acid occurs in bread doughs to the extent of about 75% of the organic acid present. Bromate recoveries were made after reaction times corresponding to those allowed in extensogram studies, i.e., zero and 135 minutes. Recoveries were also made on doughs with and without lactic acid that were given a long reaction period (16 hours). This was done in order to show up any difference in the rate of bromate decomposition that may exist between the acidified and non-acidified doughs. If the decomposition showed the usual linear relationship and the rate of decomposition was greater in the acidified dough, then this would show up better after a 16 hour reaction period than it would after only 135 minutes of reaction time. The results are presented in Table XIII.

Zero reaction time means, in the case of extensograms, that the dough was worked as soon as possible after mixing and, in the case of bromate recoveries, that the dough was sampled as soon as possible after mixing. However, there is a finite reaction period actually involved here. Its effect is observed in extensograms in so far as a bromated dough given "zero" reaction time shows slightly greater resistance to extension than does an unbromated dough given identical treatment. Nevertheless on looking at Table XIII, there appears to be no definite bromate loss with a "zero" reaction time. Table XIII also shows that for reaction times of 0, 2-1/4 and 16 hours,

Table XIII. Bromate Recoveries from Doughs Acidified with Lactic Acid (30 p.p.m. Bromate Added)

| pH | Bromate p.p.m. | | | | | |
|---|---|---|---|---|---|---|
| | 0 Min. | | 135 Min. | | 16 Hr. | |
| | Reaction | Average | Reaction | Average | Reaction | Average |
| 5.9 | 28.7 | | 25.2 | | 8.2 | |
| | 27.0 | | 24.5 | | 8.6 | |
| | 29.5 | | 28.3 | | 8.5 | |
| | 43.2 | | 24.7 | | 7.9 | |
| | 24.8 | | 22.7 | | 10.1 | |
| | 27.0 | 30.0 | 20.1 | 24.3 | 11.4 | 9.1 |
| 5.3 | 31.4 | | 21.7 | | | |
| | 26.5 | | 22.7 | | | |
| | 28.0 | | 21.9 | | | |
| | 23.9 | | 22.1 | | | |
| | 24.6 | | 24.9 | | | |
| | 26.7 | 26.9 | 23.8 | 22.9 | [illegible] | |
| 5.0 | 31.0 | | 22.7 | | | |
| | 24.2 | | 29.3 | | | |
| | 27.9 | | 29.6 | | | |
| | 24.7 | | 24.9 | | | |
| | 31.7 | | 24.5 | | | |
| | 38.0 | | 23.7 | 25.8 | | |
| | 46.0 | | | | | |
| | 31.7 | 31.9 | | | | |
| 4.9 | 24.7 | | 21.0 | | 11.6 | |
| | 39.5 | | 24.7 | | 8.2 | |
| | 30.5 | | 24.3 | | 10.8 | |
| | 41.1 | | 31.6 | | 14.5 | |
| | 28.9 | | 28.7 | | 9.7 | |
| | 27.5 | 32.0 | 17.3 | 24.6 | 12.6 | 11.2 |

the pH of the dough does not effect the rate of bromate decomposition in any regular way. The recoveries for various pH levels and for any constant reaction time are (within the experimental error) identical. (It should be mentioned that the pH of the dough rested for 16 hours dropped to 5.4).

Combining the knowledge from Fig. 6 and Table XIII it is seen that although the resistance to extension of a bromated acidified dough is greater than that of a bromated unacidified dough, this property cannot be attributed to a greater reduction of the bromate in the former. A tentative conclusion is that there is no direct relationship between the amount of bromate improvement and the amount of bromate reduced. This will be discussed more fully in the next section.

## GENERAL DISCUSSION

Various aspects of the decomposition of bromate in dough have been discussed separately along with pertinent experimental results. It is now desirable to consider the whole of the work as a unified picture of the mechanism of bromate reduction. It was not intended that this thesis would constitute a complete answer for the mechanism of improvement of dough by bromate but because it deals with the reduction of bromate in dough it must necessarily touch on the current hypothesis that bromate acts as an oxidizing agent in dough improvement. First, limitations of the analytical method will be discussed since all the results are in terms of this method. Next will follow a discussion of the mechanism of reduction of bromate showing how the oxidation hypothesis of improvement or alternate hypotheses may be tenable in the light of the results. Finally, certain conclusions will be drawn concerning the mechanism of improvement and suggestions for future investigation will be put forward.

The Johnson and Alcock method for bromate analysis was designed for the determination of bromate in flour. The adaptation of the method for dough analysis has not proved entirely satisfactory because, although it was convenient in its simplicity, its precision was not very high. For this reason it was felt that the accuracy of some of the determinations might have suffered.

The reasons for low precision are as follows. It is quite conceivable that the extraction of the bromate from the dough was not always complete in some of the experiments. The extraction technique of the original method was modified only by introduction of the use of the Waring Blendor to break up the dough sample and it was naturally more difficult to extract bromate from a dough where it was added in water solution than from a flour sample where it exists as unreacted solid.

Perhaps the largest source of possible error lies in the colorimetric part of the analysis. First of all, the starch-iodine reaction is not a good quantitative reaction. Its exact nature is not known and hence it is not an entirely reliable means of quantitative measurement of bromate. Further, the density of the starch-iodine blue color was measured while it was changing (getting denser). The original method stated that after 4 minutes the density of the color changed only slowly, but on investigation one found this change rather rapid. Therefore, precision depended too much on exact timing. Another point that made the colorimetric determination uncertain and difficult was that the solutions as used in the spectrophotometric measurements were not optically clear. The starch solution and the nature of the dough extract always gave a solution which had slight opalescence in spite of means taken to clarify it. This of course does not make for good colorimetric work. Lastly, the blanks were large with respect to the concentrations being measured. When unbromated doughs were analyzed bromate "recoveries" of about 3.5 p.p.m. were obtained. This represents over 10% of the total bromate determined at any time and indicates shortcomings in the method.

Other factors effecting the precision of the analysis were, the difficulty in measuring such small concentrations of bromate (at most 0.3 mg. in a 14 g. dough sample), likelihood of sampling error (unless the whole dough was used it was often difficult to select a representative sample since the physical properties of a dough have such variance), and the dilutions estimated when extractions of the dough were carried out were subject to error.

However, the outlook should be optimistic since in each experiment the analysis was repeated on two or three samples and two aliquots of the KCl extract were analyzed for further checks. Each point on any of the graphs

then represents an average value of six analyses (except in Fig. I where each point is derived from four analyses) and should therefore be fairly reliable. Moreover, it is merely the trends that are of interest, rather than individual bromate recoveries, and these observed trends, being controlled by several points on each graph, could not be very far from the truth. Finally, it is admitted that a more precise method of analysis would have been preferable, but there was no choice. Corroboration of the findings of the thesis await an improved method for bromate analysis in dough.

At the outset it is well to mention the different possible modes of action of bromate on dough since it will be shown how each is more or less tenable in the light of this work on bromate decomposition. First, the most widely accepted view is that potassium bromate as an oxidizing agent acts by various means as an improver. Second, an alternative hypothesis is that improvement of dough and reduction of bromate are unrelated and bromate acts in some way other than oxidation in improving the dough (such as co-ordination complex formation). Third, perhaps only a small fraction of the observed reduction of bromate in dough is related to improvement, the major part being only a concurrent and unrelated reaction. As an example, Dempster (8) found that the bromate effect as measured by dough extensograms increases with increased bromate concentration. This can be interpreted on the oxidation hypotheses of improvement as being attributable to increased oxidation, but in terms of the alternate non-oxidation hypothesis it could mean that a larger concentration of bromate leads to an increased number of affected chemical groups and therefore to more improvement. The writer believes that the major reduction of bromate in doughs is connected with the metabolism of yeast and not with other dough constituents and that the rate of the reduction is controlled by a step involving diffusion of bromate

to reactive centres. The following discussion supports this contention.

The first investigations of bromate decomposition carried out on yeasted and unyeasted doughs showed immediately the relation between reduction of bromate and yeast. It was found that the decomposition rate increased with increased yeast concentration up to 6% yeast. The linear relationship between bromate concentration and time indicated that the reaction is kinetically zero order. A priori it would have been expected to be first order, i.e., a linear relationship between logarithm of bromate concentration and time. The observed linearity suggests that there is some rate controlling process involved, such as diffusion. This could be the diffusion of bromate into the yeast cell. If a diffusion process was involved there would always be a finite amount of reaction time necessary before reduction of bromate could be detected even though the yeast concentration was high. This was borne out by experiment and Fig. 2 (page 31) shows this. When zero reaction time is allowed, even 6% yeast has little effect on bromate concentration. Therefore a diffusion step seems to be involved in the reduction of bromate in dough.

It has been suggested that low pH will cause a more rapid reduction of bromate. In fact, supporters of the oxidation hypothesis of improvement point out that increased bromate effect at lower pH is due to the greater oxidation potential of bromate in acid media. However, the first experiments on yeast variation showed that yeast concentration rather than pH was the determining factor in bromate reduction. Table VIII shows that with 1 2 hours of reaction time the pH of the dough gets as low as 4.8 with only 0.5% yeast, but it remains around this value as yeast concentration increases and bromate decomposition rate increases. Assuming that bromate improves by oxidizing certain dough constituents it is questioned why its reduction should depend on the concentration of a dough addendum such as yeast as is shown above. If,

however, improver action and reduction of bromate are considered merely concurrent but unrelated phenomena, then it may make little difference what is responsible for the reduction as long as it is known what that is.

Further evidence in support of the hypothesis that attributes bromate reduction to the yeast content of the dough comes from the experiments where yeast inhibitors were added to flour-water doughs. It was noticed that bromate was decomposed slowly in doughs without added yeast and this was attributed to microbiological activity which is inherent in the flour. It was thought that if this activity could be stopped altogether there would be no bromate decomposition if the decomposition was due to the fermentation process. Therefore yeast inhibitors were added to flour-water doughs and the decomposition of bromate was followed. With n-octyl alcohol a stationary pH indicated that fermentation had ceased and the decomposition of the bromate was slowed considerably. When a second effective fermentation inhibitor, sodium fluoride, was used, however, the decomposition of bromate was not halted but tended toward a first order reaction. No conclusion could be drawn. Whether bromate decomposition was closely connected with fermentation by yeast or connected with some other aspect of yeast metabolism could not be decided from these experiments, although the latter seemed more probable since it is known that whereas n-octyl alcohol acts as a very good disinfectant, fluorine compounds inhibit the activities of many enzymes without interfering with certain other specific cellular catalysts.

Two more facts are brought out by this work. One is further evidence that pH has no direct effect on bromate decomposition since the pH of the dough with added sodium fluoride remained at 6.0 while the decomposition of bromate continued at an accelerated rate. The second point is that the diffusion process as a limiting factor, still evident in doughs with n-octyl alcohol seems to have been by-passed when sodium fluoride was added to the

dough and the reaction became first order (linear relationship between logarithm of bromate concentration and time). The explanation for this is far from obvious at present.

The above study again shows the association of the larger amount of bromate reduction with yeast rather than with the major structural constituents of the dough, and so favours the alternate hypothesis of improvement over the oxidation hypothesis. But since there was a small bromate loss from doughs with n-octyl alcohol added, it could be that there was some oxidation of dough constituents other than yeast. This oxidation could be associated with improvement.

The experiments on decomposition of bromate added to sponge doughs again illustrate the interaction between bromate and yeast but more important information is obtained concerning the postulated diffusion process. It will be recalled that fermentation was allowed prior to the addition of bromate to the dough. This could quite conceivably have resulted in an accumulation of reducing substances in the dough which might have effected a sudden reduction of the bromate added when "doughing-up" from the fermented sponge. However, it was observed that this procedure did not result in an increase in the rate of bromate reduction. This is in good agreement with the postulated diffusion mechanism. This also points out that reduction of bromate may not be connected with fermentation but with some other phase of yeast metabolism.

There seems to be sufficient evidence now to cast doubt on the oxidation hypothesis of improver action. It appears that reduction of bromate and improvement are not related unless as mentioned, a small amount of bromate does oxidize certain dough constituents while the major reduction of bromate is effected by yeast.

The extensogram study showed no relationship between oxidation and improvement. Fig. 7 shows that for doughs with lactic acid, bromate improvement as illustrated by greater resistance to extension of the dough, is greatest for the dough with pH 5.0 and yet the bromate reduced at this pH is essentially the same as the amount decomposed at pH levels of 5.9, 5.3 and 4.9. Two inferences can be drawn from this study. One, the pH of the dough again is seen to have no effect on bromate reduction. Two, bromate reduction and improvement are not connected. It is seen however that bromate improvement is different at different pH levels.

The foregoing discussion shows fairly conclusively that the reduction of bromate in dough is connected with the yeast content of the dough. There seems to be a rate controlling step connected with this reduction and it is concluded that this is a diffusion process involving diffusion of bromate to reducing centres, perhaps in the yeast cell.

It appears, then, that oxidation by bromate is not related to improvement of dough. In this connection it should be recalled that the reduction of bromate is small in the relatively short dough time in bread-making and that most of the reduction may be attributed to the action of yeast. There is one possibility, however, which cannot be dismissed. It is possible that improvement by oxidation requires only a very small fraction of the added bromate and that such small amounts were not detectable by the method used for analysis. In other words, the oxidation-reduction hypothesis may be tenable but at an entirely different level of concentration. At this time a choice cannot be made between the hypothesis that improver action is the result of oxidation by bromate at a much smaller concentration than has hitherto been supposed, and the alternate hypotheses that unreduced bromate acts directly on the dough. A final decision awaits the advent of a more accurate analysis for bromate in dough.

Suggestions for future investigation that could clear up this point are; to show that no bromate is decomposed in some instance where improvement is observed, or else to show that a large amount of bromate is reduced in a dough where there is no contemperaneous improvement.

## SUMMARY

1. The Johnson and Alcock method and the Howe method for the determination of potassium bromate in flour have been adapted to analysis of potassium bromate in dough by using the Waring Blendor to disperse the dough in the extracting solution. The Johnson and Alcock method was preferred to the Howe method for most of the analyses of the investigation.

2. An estimated margin of error of the adaptation of the Johnson and Alcock method is 6%. This precision is rather low but since only average values of several replicate analyses were used to indicate the trends in bromate decomposition and since the trends rather than the individual results were of interest, the conclusions drawn can be considered valid.

3. Potassium bromate added to a flour-water dough decomposes steadily with time. The rate is about 1.5 - 2.0 p.p.m. per hour depending on the flour sample used.

4. The rate of bromate decomposition is accelerated if yeast is included in the dough (1.6 p.p.m. per hour for 3% yeast); the greater the yeast concentration, the greater the decomposition rate within experimental limits of 0 and 6% yeast.

5. The bromate decomposition reaction in flour-water doughs and yeasted doughs is kinetically of zero order, i.e., there is a linear relationship between the concentration of bromate and time.

6. Fermentation of the dough prior to addition of bromate does not result in an accumulation of reducing substances that could cause a sudden reduction of the bromate when it is mixed into the dough. This was determined by following the decomposition of bromate added at the

doughing-up stage of sponge doughs that had been fermented as sponges for 12 hours.

7. When n-octyl alcohol, a yeast inhibitor, is added to a flour-water dough the rate of decomposition of bromate is slowed down to about 0.7 p.p.m. per hour.

8. When sodium fluoride, a yeast inhibitor, is added to a flour-water dough the rate of decomposition of bromate changes to first order, i.e., a linear relationship between logarithm of concentration and time.

9. The pH of the dough has no direct bearing on the bromate decomposition, e.g., in a dough acidified with lactic acid, after 16 hours of reaction time the bromate recovery at pH 5.9 was essentially the same as that at pH 4.9.

10. The pH of the dough as well as the particular acid added to the dough have characteristic effects on the physical properties as exhibited by resistance to extension of the dough on the extensograph. The pH of the dough and the acids also influence the bromate effect as exhibited by extensograms. There seems to be an optimum pH for maximum resistance to extension. The three acids tested, in order of dimishing effect are, lactic, hydrochloric and carbonic.

11. It is concluded that reduction of bromate in dough is directly connected with the yeast in the dough but not necessarily with alcoholic fermentation and that the rate of the decomposition is controlled by the rate of diffusion of bromate to reducing centres, perhaps inside the yeast cell.

12. The conclusion is drawn also that reduction of bromate in dough and improvement of dough by bromate are concurrent but independent phenomena.

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