THE EFFECT OF METHYL BROMIDE FUMIGATION ON FLOURS

by

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INTRODUCTION

Control of insect pests in food-processing plants, storehouses, and in stored products by means of fumigation is a very widely accepted practice. Elimination of these pests in the food industries is a matter of considerable importance both from the economic as well as the public health standpoint.

Successful fumigation practice, of course, is dependent not only on efficient destruction of the insect but also on doing this in such a manner that toxic or undesirable residues do not remain in the finished product.

Among the many commercial fumigants used at present methyl bromide is one of the most recently developed. Its use has shown a tremendous increase within the past few years because of its advantageous handling and toxic characteristics to insect life.

In 1884 Perkin (1) reported the preparation of methyl bromide; its early use was in the medicinal field, and for the synthesis of methyl compounds employed in the manufacture of aniline dyes. Later on, it attained considerable popularity as a fire extinguishing agent in airplanes. Also, it was used mixed with other fumigants to prevent fire hazards. In 1932 Le Goupil (2), an entomologist of the French government, working with a mixture of this organic halide and ethylene oxide found that the former, supposedly only a fire retardant, was more toxic to animal life than the latter. Later, Vayssière (3), de Francolini (4), Lepigre (5), and Shepard et al. (6) did work on its insecticidal properties and relative toxicity, and found them to compare favorably with those of the most potent insect fumigants such as hydrocyanic acid, chloropicrin, and ethylene oxide.

Among the fumigants, high vapor pressure is one of the outstanding features of methyl bromide. Its boiling point is 4.6°C and it has a vapor pressure of 760 mm. at 25°C. These characteristics make methyl bromide a gas at normal room temperature, with an excellent rate of diffusion. According to Dean and Cotton (7) methyl bromide, because of its remarkable powers of penetration, is the most efficient fumigant known for the treatment of warehouses filled with bagged commodities. It has the ability to reach those insects embedded in the product that usually escape the action of less volatile fumigants.

Several investigations have demonstrated that there are no harmful effects from residues in foodstuffs. However, complaints occasionally do occur regarding the unpleasant odor and taste of baked products produced from flour fumigated with methyl bromide. It seems to be very likely that these alterations occur when flour becomes exposed to excessive amounts of fumigant.

The object of this research was to determine the nature of the modifications occurring in methyl bromide fumigated flour with particular reference to the constituents responsible for the development of undesirable odor and taste. Further, it was desired to study the effects of moisture on the sorption (combined adsorption and absorption) of the methyl bromide, and any alterations of the physical properties of dough subsequent to fumigation.

REVIEW OF LITERATURE

To date most of the papers on methyl bromide deal with techniques of commercial fumigation, toxicity on laboratory insects, and effects of bromide residues in foodstuffs on laboratory animals.

Mackie and Carter (8) presented some of the first evidence in favor of the use of methyl bromide in the fumigation of infested fresh vegetables. They claimed that using normal dosages the vegetables were not damaged. Mackie (9) explained that the toxic effect is due to the rapid hydrolysis of the fumigant in the presence of moisture to form hydrobromic acid, methanol, and formaldehyde. On the other hand, Phillips, Munro, and Allen (10) found that methyl bromide is practical in the destruction of insects in harvested apples, although under certain conditions the treatment results in both external and internal injury to the fruit.

Dudley et al. (11, 12) found that dried fruits, fresh fruits, and vegetables absorbed minor quantities of the fumigant, but milled grains, cheese, nuts, and nut meats absorbed greater amounts. These authors believed that milled grains sorbed more methyl bromide because of their greater surface area, while the oily and fatty foods sorbed large quantities of the fumigant because of its solubility in fats. They also determined that the bromine residues normally encountered in foods fumigated with methyl bromide are harmless to laboratory animals. Other authors (13, 14) also have found that the incorporation of inorganic bromides in experimental diets did not produce deleterious effects.

Shepard and Buzicky (15) presented evidence on the toxicity of different fumigants; they were able to show that methyl bromide has a delayed effect on insects; those exposed at concentrations higher than necessary to kill 100 percent seemed naturally active immediately after exposure. Hydrocyanic acid, on the contrary, does not exhibit delayed action. These authors reported that baking tests with flour fumigated with methyl bromide at two pounds per 1,000 cubic feet showed no detectable injury. Young and Bayfield (16) have shown, on the other hand, that hydrocyanic acid when used as a flour fumigant, produced an appreciable and detrimental influence upon the bread quality. This effect was not apparent after thorough aeration of the flour.

Methyl bromide is being used even for the fumigation of tobacco, but Brubaker and Reed (17) feel that it might combine with the waxes, fats, and oils in the tobacco leaf and likely affect the aroma.

To the best of the writer's knowledge, the type of odoriferous reaction given by methyl bromide and flour has not been reported except by Searls (18) who said that it has been known that this fumigant produced a disagreeable odor on some furs and leathers. However, all furs and leathers do not respond similarly.

Several other papers on the determination of bromide residues in fumigated foodstuffs have been published. They will be reviewed under the section Determination of Bromine Residues.

MATERIALS AND METHODS

Fumigation. The first part of these experiments was done with a commercial flour, with a protein content of 11.95 percent, which had been bleached and malted. Methyl bromide in one-pound cans was used, with a purity of 99.4 percent.

Fumigations were carried on in a laboratory scale fumigator with a capacity of 116 liters (4.1 cubic feet) and equipped with a fan. All fumigations were done at room temperature.

The samples of flour were placed in sacks in the same manner in order to minimize variations, such as the degree of packing, which might affect the sorption of the fumigant.

The dosage used in most of the experimental fumigations was 25 pounds per 1,000 cubic feet for 24 hours. The reason for this high dosage (12 to 25 times greater than the normal) was to insure overfumigation. It is very likely that troubles in commercial practice arise when milled cereals are overfumigated. The volume of the liquid fumigant was calculated as follows: (sp. g. of CH_3Br : 1.732)

$$1 \text{ lb.}/1{,}000 \text{ cu. ft.} : 0.016 \text{ g./lt.} : \frac{0.016}{1.732} : 0.009238 \text{ ml./lt.}$$

$$1 \text{ lb.}/1{,}000 \text{ cu. ft. in } 116 \text{ liters} : 0.009238 \times 116 : 1.072 \text{ ml.}$$

$$25 \text{ lb.}/1{,}000 \text{ cu. ft. in } 116 \text{ liters} : 1.072 \times 25 : 26.800 \text{ ml.}$$

The fumigant was measured in a graduated cylinder and poured into a beaker. This in turn was placed in the fumigator, the fumigator lid was rapidly tightened and the fan started.

In the study of the sorption of methyl bromide as related to the moisture content of flour, the desired moisture content was obtained and the samples were placed in two 2,100-gram sacks. The ratio of fumigant to product so obtained in fumigation was about 4.5 times greater than the ratio used in commercial practice for the same dosage (23). Immediately after fumigation the contents of one sack were blended in a sealed blender for 20 minutes and then placed in a sealed can. The other sack was left to aerate for seven days at room temperature. After this period of time the flour was blended in the same blender and again stored in sealed cans.

Petroleum Ether Extract. Flour was extracted with Skelly solve F (boiling point 35°C) at room temperature. Extractions on 1-kg. samples were carried out with two 1,000-ml. portions of solvent, followed by four 500-ml. portions of the same extracting agent.

Flour and solvent were placed in glass jars and mechanically shaken. Supernatant liquids were collected and concentrated under vacuum at room temperature. The oily extracts were stored in the refrigerator.

Fractionation of Flour. The fat-free flour was thoroughly aerated to eliminate as much of the solvent as possible. Then it was made into a dough using the appropriate amount of water, and fractionated into starch, gluten, and water soluble material according to the technique described by Finney (19).

An attempt to find a less tedious procedure was unsuccessful. It was observed that when a dough was suspended in water by means of a Waring Liqui-Mixer, most of the gluten came to the surface and was easily separated from the starch water suspension with a separatory funnel. Starch could then be recovered by centrifuging, but always a layer of starch and fine gluten settled, rather loosely, on top of the starch cake. This procedure, although more rapid than the usual technique of hand washing, did not render pure fractions and so could not be employed.

The liquid fraction, after the separation of starch, was placed in a round-bottom flask and concentrated under vacuum. A 90-cm. copper condenser was employed with diluted alcohol as the cooling fluid. It was continuously pumped through a copper coil surrounded by cracked ice and salt.

Alcoholic Potassium Hydroxide Test. The experiments reported in this work demonstrated that when fumigated flour is treated with a solution of potassium hydroxide or sodium hydroxide in ethyl alcohol, a characteristic, rather unpleasant odor is produced. This test was successfully employed to detect bromide residues due to chemical reactions in fumigated fractions of flour. On the other hand, when methyl bromide was bubbled through a solution of potassium hydroxide in ethyl alcohol precipitation of potassium bromide took place, but there was no noticeable odor.

Baking Test. A straight dough method was used, with the following formula:

	percent
Flour (14% moisture basis)	100.0
Dry milk solids	2.0
Diamalt	0.25
Shortening	3.0
Bromate	0.002
Yeast	2.0
Sugar	6.0
Salt	1.5
Absorption	As required

Absorption was determined with the Brabender Farinograph and the optimum mixing time with the Swanson-Working recording dough mixer. As it will be indicated later on, the mixing time was always shorter for the fumigated samples than for the untreated controls.

The weight and volume of the loaves were determined immediately after they were taken from the oven. They were then placed in sealed cans, and scored for grain and texture and for odor on the following day.

Determination of Bromine Residues. Several methods for determining small quantities of bromine in different materials have been published. Before methyl bromide became a widely used fumigant, considerable effort had been devoted to the development of reliable micro-methods for the estimation of bromine in biological materials. All the procedures involve an initial destruction of the organic matter by wet or dry ashing, followed by selective oxidation of the inorganic halides, the quantitative separation of bromine, and its determination by titrimetric or colorimetric methods.

The increasing use of methyl bromide has raised questions about the residues left in fumigated foodstuffs, and many papers have been given on methods for this analytical determination.

The accuracy of bromine estimation is affected by several factors such as loss of the halogen during ashing, incomplete oxidation of bromide, and partial oxidation of the chlorides present.

Generally speaking, the methods may be grouped into two types: a) those that oxidize bromide to bromine by means of chromic acid, and b) those that effect the oxidation of bromide to bromate with sodium hypochlorite. Either bromine or bromate is estimated by the amount of iodine set free from potassium iodide.

Brodie and Friedman (20) recommended a special procedure for ashing tissues and biological fluids to avoid losses of bromine. This technique, more or less modified, has been adapted for most of the methods used at present.

In the method originated by Baughman and Skinner (21) chromic acid is used for the oxidation of bromide to bromine. Several modifications of this method used as microchemical procedures have been proposed by Neufeld (22) and by Yates (23). Dudley (24) has employed variations of the same method for determining the bromine content of a variety of fumigated foodstuffs.

According to van der Meulen (25) bromides can be quantitatively oxidized to bromates by two and a half times the theoretical amount of hypochlorite if the solution contains a buffer and is half saturated with sodium chloride. This is illustrated by the following equation:

$$3\ ClO^- + Br^- \rightleftharpoons BrO_3^- + 3\ Cl^-$$

The reaction from left to right is completed if the oxidation is carried out at 85°C and the excess of hypochlorite is removed. Kolthoff and Yutzy (26) used this method for the volumetric determination of bromide; they introduced several modifications such as phosphate buffer instead of borate, and sodium formate instead of hydrogen peroxide to destroy excess hypochlorite. They were able to show that formate does not reduce bromate on boiling.

Stenger, Shrader, and Beshgetoor (27) applied this method to the determination of bromine in fumigated foodstuffs. Using the same procedure Roehm, Shrader, and Stenger (28) studied the residues of bromine in cereals and dairy products fumigated with methyl bromide. They found that after seven days of aeration the volatile halide had been completely eliminated from the samples, but that a residue of bromine was permanently bound to the product.

In the present work the analytical procedure described by Shrader et al. (29) for total bromide was used, with some modifications. The method, as described by the authors, is as follows:

Reagents.

(1). Alcoholic potassium hydroxide, 2.5 grams of potassium hydroxide per 100 ml. of 95 percent ethyl alcohol.

(2). Sodium hydroxide, analytical reagent.

(3). Sodium peroxide, analytical reagent.

(4). Hydrochloric acid, about 6 N (1+1). This should be as free from bromide as possible.

(5). Hypochlorite solution, 1 N sodium hypochlorite in 0.1 N sodium hydroxide. The solution is prepared by dissolving 71 g. of chlorine in 1,500 ml. of water containing 88 g. of sodium hydroxide. The gas is slowly passed into the alkaline solution that is cooled with ice. The solution is diluted to two liters.

(6). Sodium formate solution, 50 g. of the salt per 100 ml. of solution.

A sample of 5 to 10 grams is treated in a 100-ml. nickel crucible with 40 ml. of alcoholic potassium hydroxide, allowed to stand for an hour, and evaporated to dryness on a steam bath. It is then dried for a short time at 110°C and is covered with 10 grams of sodium hydroxide pellets. The crucible is kept for an hour or two on a hot plate until the bubbling or smoking diminishes, after which it is placed in a muffle at 600°C. Fusion should be carried out without excessive burning or foaming; if the charge becomes ignited, the crucible should be removed from the muffle until the flame is extinguished. It is then returned to the muffle and this process repeated until the volatile gases have been removed. Sodium peroxide is added to the melt, a few milligrams at a time, to complete the oxidation of the remaining carbon or organic matter. The peroxide must be added cautiously while the crucible is removed from the furnace; bromide is lost if the charge burns with a flare when too much peroxide is added.

Complete combustion of the organic matter can be effected best by returning any organic matter that has raised above the sodium hydroxide to the bottom of the crucible, where it mixes with the melt and is easily destroyed by addition of the peroxide. This is accomplished by carefully rotating the hot crucible to wash down the organic matter and adding 0.5 gram more of peroxide. If no burning or bubbling takes place, the oxidation is complete. A few carbon particles which may remain after the final addition of sodium peroxide do not affect the accuracy of the results.

The crucible is rotated to allow the melt to solidify on the sides, cooled, and the contents are dissolved in 75 ml. of water. Solution of the sodium compounds is hastened by placing the crucible on a hot plate for several minutes. The solution is transferred to a 400-ml. beaker and partially neutralized with about 50 ml. of 6 N hydrochloric acid. The solution is boiled to destroy peroxides and to reduce the volume to 100 to 125 ml. Nickel hydroxide and other insoluble hydroxides are removed by filtering through a

No. 2 Whatman paper, collecting the filtrate and washings in a 500-ml. wide-mouthed Erlenmeyer flask. The filtrate is made slightly acid with 6 N hydrochloric acid, then neutralized with sodium hydroxide solution, adjusting to the color change of methyl red. The volume at this point should be approximately 150 ml.

About 2 grams of sodium acid phosphate (monobasic) and 5 ml. of hypochlorite solution are added and the mixture is heated to boiling. After a minute or so 5 ml. of sodium formate solution are introduced and boiling is continued for 2 minutes. The sample is cooled and treated with a few drops of 1% sodium molybdate solution, 0.5 gram of potassium iodide, and 25 ml. of 6 N sulfuric acid. Titration should be made immediately with standard 0.01 N sodium thiosulfate solution, starch indicator being added just before the end point. A blank on all the reagents should be carried through the entire procedure and subtracted. One milliliter of 0.01 N sodium thiosulfate is equivalent to 0.1332 mg. of bromide ion.

Young, Carter, and Soloway (30) gave a paper on the bromine residues from methyl bromide fumigation of cereal products. They described a method that is more tedious than that of Shrader et al. and it does not seem to present any advantage over the existing procedures.

Determinations of bromide residues were run in duplicates. In case of doubt, when differences between single determinations were greater than 15 p.p.m., a third or fourth analysis was done.

It is interesting to point out here that since methyl bromide constitutes a hazard for human beings, its identification and estimation in the atmosphere have given rise to much concern. This organic halide may be detected in the air using the Beilstein test. The test is based on the decomposition of organic halides by heat and the combination of the halogen with copper. The copper halides are readily volatile, and color a flame green. For commercial purposes a methanol torch is available, sold under the name of "Frigidaire halide leak detector".

With methyl bromide the color of the flame varies from faint green for 50 p.p.m. by volume in the atmosphere to strong blue for 1,000 p.p.m. (27). This test gives a rough indication of the concentration of the fumigant in the air. When more exact estimations are required, methods based on hydrolysis and subsequent determination of the halogen are necessary. Some of the latest papers on the subject provide extensive literature reviews and up to date procedures (31, 32, 33, 34).

Determinations of Moisture. The standard air-oven method of the American Association of Cereal Chemists (35) was used. It is as follows:

> Weigh accurately approximately 2 grams of the well mixed sample into a covered dish that has been dried previously at 130°C (±3°C), cooled in the desiccator, and weighed soon after attaining room temperature. Uncover the sample and dry the dish, cover, and contents in the oven at 130°C (±3°C) for one hour after the oven regains a temperature of 130°C. Cover the dish while in the oven, transfer to the desiccator and weigh soon after room temperature is attained. Report the flour residue as total solids and the loss in weight as moisture.

In all cases determinations of moisture were run in triplicates.

Fumigation with CO_2 - CH_3Br Mixture. Two 2,100-gram sacks of flour with 13.1 percent moisture were placed in the fumigator with 36 grams of dry ice (four times the weight equivalent of methyl bromide for a dosage of 25 pounds per 1,000 cubic feet) for two hours. Then methyl bromide was introduced as usual (25 pounds per 1,000 cubic feet) and fumigation continued for 24 hours. Other than noted, the handling of the flours was the same as with other samples.

Physical Dough Testing. The Brabender Farinograph was used to determine the exact water absorption of the samples, to obtain doughs of the same consistency, and to observe alteration in the physical dough properties due to fumigation. The amount of sample employed in every test was corrected to 14 percent moisture basis.

The optimum mixing times were estimated with the Swanson-Working recording dough mixer, using the absorption found with the Farinograph. This also permitted additional observations on physical dough properties.

Nitroprusside Test. A small amount (app. 10 mg.) of material is placed on a porcelain spot plate and one drop of ammonium hydroxide (one to one) and one drop of freshly prepared five percent sodium nitroprusside ($Na_2Fe(CN)_5(NO) \cdot 2\ H_2O$) solution added. Pink color on the surface within 30 seconds is indicative of the presence of sulfhydryl groups.

Gas Production and Gas Retention. In these tests the machine and procedure described by Working and Swanson (36) were used.

Bacterial Count. The estimation of the killing power of methyl bromide on the microflora of flour was done according to the technique recommended by Kent-Jones and Amos (37), and by Smith and Dawson (38) for bacterial and fungal counts.

pH Determinations. The electrometric method recommended by the American Association of Cereal Chemists for the determination of hydrogen-ion concentration in flour is as follows:

> Place 10 g. flour (or some multiple thereof) in a dry Erlenmeyer flask and add for each 10 g. flour 100 ml. cool, recently boiled distilled water at a temperature of 25°C. Agitate the flask until an even suspension free from lumps is obtained. Allow suspension to stand at 25°C for 30 minutes, agitating continuously or intermittently in such a manner as to keep the flour particles in suspension. Let stand quietly for 15 minutes, then decant the supernatant liquid through a folded, hardened, dry filter paper into a suitable vessel discarding first 5 ml., and determine hydrogen-ion activity electrometrically.

In the course of this work it was found that the pH values obtained with the above mentioned method were in all cases one tenth of a pH unit greater than those values obtained from direct determinations on water-flour suspensions. These latter values were considered to be closer to the true hydrogen-ion concentration.

EXPERIMENTAL RESULTS

Preliminary Studies. Preliminary baking tests with flours fumigated at a dosage of 25 pounds per 1,000 cubic feet gave products with a sharp, very objectionable odor. Upon cooling the loaves, the odor was not nearly so well marked, but became strong again when slices of the bread were toasted. Flour fumigated with a heavy dosage of methyl chloride produced baked loaves with a very objectionable odor but different from that given by methyl bromide fumigated flours.

The work was begun with the fumigation of flour, followed by fractionation into four parts, namely, the petroleum ether extract, the starch, the gluten, and the water soluble materials. The same procedure was planned with nonfumigated flour, and a recombination of fractions from both sources to study the effect of each fumigated fraction on the properties of the flour. However, at the onset of this work the alcoholic potassium hydroxide test showed that only the protein-containing fractions reacted with the fumigant. Results obtained with the different fractions were as follows:

Fraction	Odor
Petroleum ether extract	negative
Starch	negative
Gluten	positive
Water soluble materials	positive

The petroleum ether extract from flour was included because according to Balls et al. (39, 40) it contains a lipoprotein with high content of sulfur. It was thought that mercapto groups could be responsible for the odor obtained in the baked products of fumigated flours. But subsequently the nitroprusside test demonstrated that nonfumigated flour, fumigated flour, fumigated flour treated with alcoholic KOH, and an aqueous solution of methionine treated with liquid methyl bromide did not have free sulfhydryl groups. The nonexistence of free sulfhydryl groups in flour confirms the findings of Myers and Working (41).

Baking tests were made with nonfumigated flour, nonfumigated extracted flour, fumigated flour, and fumigated extracted flour. Both fumigated flours, extracted and unextracted, produced products with the same unpleasant odor and, apparently, of similar intensity. The odor was present in the loaves not only shortly after they were taken from the oven but also on the following day after storing in sealed cans.

Petroleum ether extracts from nonfumigated and fumigated flours were treated with 95 percent ethanol, and then these two extracts and the same extracts without treatment were fumigated with a heavy dosage of methyl bromide. After fumigation all of them were treated with alcoholic potassium hydroxide and they gave negative reactions, i.e., lack of pungent odor.

Gluten washed from fumigated flours was fractionated into gliadin and glutenin by means of ethanol, 70 percent by volume. Both fractions gave positive reactions with alcoholic potassium hydroxide, apparently of the same intensity.

When separated gluten was given a severe treatment with methyl bromide and then added to flour it had a detrimental effect on the baking qualities. Gluten was washed from a dough, dried, and finely ground. A portion of this powdered gluten was treated with liquid methyl bromide in a porcelain dish. The powder was thoroughly aerated at room temperature until it was considered to be free from volatile fumigant. Then, two portions of the same flour that was used to obtain the gluten were baked, one with the addition of five percent of treated gluten, the other with untreated gluten; plain flour, without addition of gluten, was also baked. Loaf volumes obtained (averages) were as follows:

Flour plus 5% gluten	857 cc.	Increase due to gluten addition
Plain flour	745 cc.	Decrease due to detrimental effect
Flour plus 5% treated gluten..	610 cc.	

When an attempt was made to wash out gluten from flour treated with liquid methyl bromide a glue-like substance was obtained; glutens from fumigated flours also showed alterations, but not so marked.

Miscellaneous Materials Fumigated with Methyl Bromide. Twelve different materials were fumigated with methyl bromide at a dosage of 25 pounds per 1,000 cubic feet. The alcoholic potassium hydroxide test gave positive results for all but gelatin (Table 1). This gelatin, the only material that did not give the characteristic odor with the reagent, according to Schmidt (42), lacks the amino acids valine, beta-hydroxyglutamic acid, tyrosine, methionine, and tryptophane.

Sorption of Methyl Bromide as Related to the Moisture Content of Flour. Adsorption refers strictly to the existence of a higher concentration of any particular component at the surface of a liquid or solid phase than is present in the bulk; it should, theoretically, be clearly distinguished from

Table 1. Results of the alcoholic KOH test on different materials fumigated with methyl bromide.

Material	Alcoholic KOH test
Corn flour	positive
Rye flour	positive
Oat flour	positive
Flax flour	positive
Barley malt	positive
Navy beans	positive
Soya beans	positive
Sorghum	positive
Dried gluten	positive
Dried blood fibrin	positive
Egg albumin	positive
Gelatin	negative

absorption as applied to solids, the latter referring to a more or less uniform penetration. In practice it is impossible to separate the effects of adsorption from those of absorption, particularly for systems of gases and solids; hence the term "sorption" is used.

The flour and technique used have been described under Materials and Methods. Moisture contents ranged from 9 to 16 percent. Samples were numbered from 1a to 8b. The number corresponds to the run (moisture content before fumigation) and the letters a and b to the samples after fumigation and after aeration respectively.

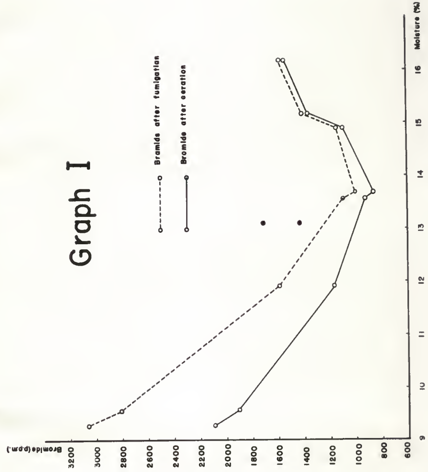
Results obtained in the determination of bromide residues are listed in Table 2 and plotted in Graph I. The fractions of the total bromide sorbed in fumigation that were retained after aeration were calculated and are shown as percentage values in Table 2. These results were plotted (Graph II) and the regression line and regression coefficient were calculated. According to the latter the results are highly correlated (r : 0.97). These data indicate that at a moisture content of 16.43 percent the bromide sorbed during fumigation was retained 100 percent even after aeration.

Several factors control the sorption of methyl bromide by flour. If it is assumed that flour is a system formed by granules of starch and protein globules, it is easy to visualize the existence of voids and channels among these particles. Gases diffuse into flour through these free spaces. Water vapor present in the atmosphere of the void spaces hinders diffusion

Table 2. Relationship between moisture of flour and bromide residues after fumigation and after aeration (in percent).

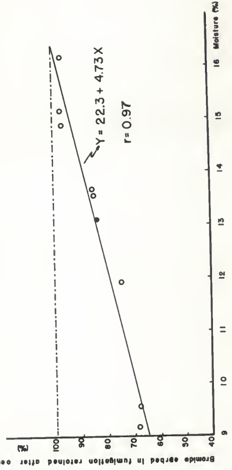
Run	Moisture			Bromide*		Bromide sorbed in fumigation retained after aeration
	before fumigation	after fumigation	after aeration	(a) after fumigation	(b) after aeration	
1	9.29	9.25	9.30	.3063	.2099	68.5
2	9.57	9.61	9.74	.2810	.1914	68.1
3	11.93	11.93	11.01	.1582	.1175	74.3
4	13.56	13.55	11.54	.1100	.0934	84.9
5	13.69	13.45	10.78	.1009	.0861	85.3
6	14.90	14.90	12.45	.1143	.1099	96.6
7	15.18	14.90	11.53	.1415	.1374	97.1
8	16.19	15.92	13.00	.1592	.1543	96.9
CO_2-CH_3Br	13.10	12.96	11.78	.1722	.1434	83.3

* Percent on dry matter.


Bromide after fumigation
Bromide after aeration
Bromide (p.p.m.)
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
9
10
11
12
13
14
15
16
Moisture (%)

Graph I

Graph II



Y = 22.3 + 4.73X
r = 0.97
Moisture (%)
Bromide sorbed in fumigation retained after aeration
(%)
9
10
11
12
13
14
15
16
40
50
60
70
80
90
100

of gases, and at the same time it forms a film around the particles. To a great extent, such films must control sorption of the fumigant. This accounts for the high sorption of methyl bromide and the comparatively low retention after aeration when flour is on the dry side. It might be assumed that retention is primarily due to chemical reactions between methyl bromide and proteins and only secondarily to hydrolysis.

When moisture increases, the absolute value of sorption decreases but retention is higher due to greater hydrolysis. After a certain moisture content is reached (about 13.7 percent) sorption due to hydrolysis seems to prevail. At this point it is of the utmost importance to insure a uniform technique, especially in the amount of flour and exposed surface, because it is very likely that most of the bromide would be sorbed in the outer layers of the sacks without further diffusion.

For the above reasons the results shown in Graph I are considered to represent a composite picture of the retention of methyl bromide by flour during fumigation. Changes in the technique used would bring about different results but the general pattern should be the same.

Plate I shows farinograms and mixograms prepared from the samples (weights corrected to 14 percent moisture basis). Table 3 shows abrorptions, and valorimeter readings of the curves. All of the curves for the treated flours indicate faster breaks and shorter mixing times than does the curve for the check flour. Further, valorimeter readings for the fumigated flours are lower than for the control. It is evident that

Table 3. Flour absorptions and valorimeter readings determined with the Farinograph.

Sample no.	Absorption (percent)	Valorimeter reading
Control	61.1	63
1a	61.3	60
1b	61.0	62
2a	59.8	55
2b	59.9	59
3a	60.5	60
3b	61.0	62
4a	61.3	62
4b	61.5	62
5a	60.4	61
5b	60.4	62
6a	61.3	60
6b	61.4	58
7a	61.9	59
7b	61.9	60
8a	62.2	58
8b	61.2	53
CO_2-CH_3Br a	60.8	62
CO_2-CH_3Br b	60.5	65
Flour + cystine	61.1	56
Flour + tryptop.	61.1	58
Flour + tyrosine	61.1	56

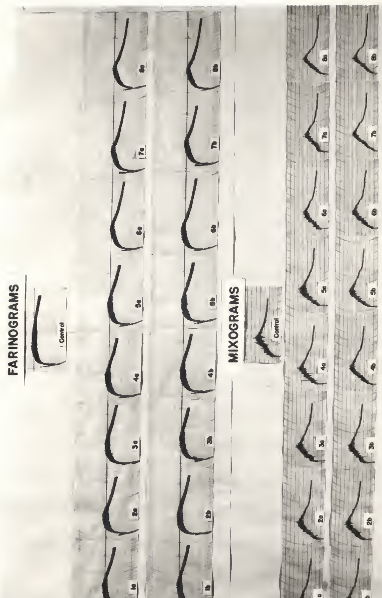

FARINOGRAMS
Control
1a
2a
3a
4a
5a
6a
7a
8a
1b
2b
3b
4b
5b
6b
7b
8b
MIXOGRAMS
Control
1a
2a
3a
4a
5a
6a
7a
8a
1b
2b
3b
4b
5b
6b
7b
8b

PLATE 1

changes have occurred in the physical properties of the fumigated flour. The kind and degree of the alterations seem to be regulated to a great extent by the moisture content of flour. As a matter of fact, samples 7 and 8 (high moisture flours) present narrower traces at the end of the farinograms than the other samples. They also showed lower consistency at the end of the mixograms than the rest of the fumigated samples.

In connection with the alterations shown by the farinograms and mixograms of fumigated flours, it was considered pertinent to give the data for the hydrogen-ion concentrations of some of the samples. The following values were obtained:

Sample	pH value
Control	5.9
1b (dry; high bromide residue)	5.4
4b (normal; lowest bromide residue)	5.6
8b (wet; medium bromide residue)	5.4

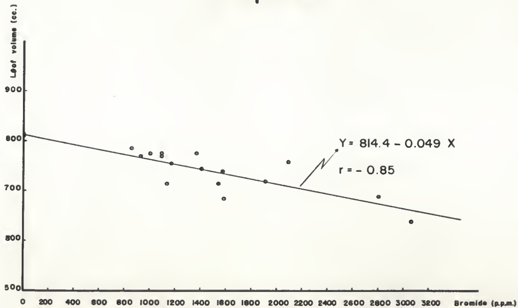
From these and other data (unpublished) it is evident that the hydrogen-ion concentration is related to the bromide residue in the sample. In addition, again in unpublished work, a relationship between pH and the shape of farinograms and mixograms was found. However, there seems to be a limit for the effect of hydrogen-ion concentration. The high bromide residue flour showed a hydrogen-ion concentration no lower than the medium bromide residue flour. However, it will be noted from Plate I that the mixogram and farinogram of the high moisture (medium bromide residue) flour are markedly different from either the check flour or the low moisture (high bromide residue) flour. The whole picture appears to be a balance between the chemical action of methyl bromide on the protein molecules, the effect of moisture on hydrolysis, the effect of the hydrolysis products on the flour properties, and the influence of the acidity developed by these products.

The samples were baked and the finished loaves were scored for odor, and grain and texture. Loaf volume data were plotted in Graph III and the regression line and regression coefficient were calculated. The results are significant and indicate that the greater the bromide content the lower the loaf volume. Samples 4, 5, and 6, with relatively low bromide content had less intense odor than the other samples; sample 5b with the least bromide residue showed the best grain and texture.

Fumigation with CO_2-CH_3Br. It was thought that it might be possible to use some other gas to react selectively with the proteins of flour and thus prevent methyl bromide from reacting. Carbon dioxide was employed in a tentative experiment because its use in mixtures with other fumigants has been recommended (43).

Results for bromide residues are listed at the bottom of Table 2 and plotted in Graph I as two isolated points. It should be noted that the values obtained are higher than the corresponding values for the same moisture content with bromide alone. But Graph II shows that that percentage of the bromide sorbed in fumigation which was retained after aeration agreed with the general curve.

Graph III


Løof volume (cc.)
900
800
700
800
500
0
200
400
800
800
1000
1200
1400
1600
1800
2000
2200
2400
2600
2800
3000
3200
Bromide (p.p.m.)
Y = 814.4 - 0.049 X
r = - 0.85

The farinograms obtained with these samples are shown in Plate II. Here again the letter a indicates the sample without aeration, and the letter b the sample after seven days' aeration. The addition of carbon dioxide to the fumigant does not appear to reduce the sorption of methyl bromide; apparently this precludes its use to prevent alterations of a chemical nature.

Addition of Amino Acids. It is probable that reactions of methyl bromide with the flour proteins are dependent on the presence of a specific group in the molecules of amino acids, or on a specific amino acid itself. In order to throw some light on the latter assumption to three samples of flour (13.3 percent moisture) were added respectively 0.1 percent cystine, 0.1 percent tryptophane, and 0.05 percent of a crude preparation of tyrosine. They were then fumigated in the usual manner. Farinograms shown in Plate II do not indicate greater alterations than obtained with fumigated plain flour.

Aqueous solutions of methionine and tryptophane were treated with liquid methyl bromide, the solutions added to non-fumigated flours, and the flours baked. Methionine-treated flour gave loaves with a very strong objectionable odor while the tryptophane-treated was normal. But, the same odor obtained with treated methionine was produced when a plain water solution of the amino acid was used. It is evident that the objectionable odor in this experiment was due to decomposition of methionine, and not to methyl bromide action.

Treatment of Fumigated Flours with Oxidizing Agents. Farinograms and mixograms prepared with fumigated flours


CO_2-CH_3Br a
CO_2-CH_3Br b
Flour + Cystine
Flour + Tryptophane
Flour + Tyrosine
8b + 3 ml. I_2 0.01 N
8b + 5 ml. I_2 0.01 N
Control + 5 ml. I_2 0.01 N
8b + 0.026 % KIO_3
8b + 0.02 % $KBrO_3$
8b + 0.2 % $KBrO_3$
Control + 0.2 ml. conc. HBr
Control + 0.4 ml. CH_3OH
Control + 0.1 ml. HBr + 0.2 ml. CH_3OH
Dough + liquid CH_3Br

PLATE 2

resembled those obtained with the addition of reducing substances like cysteine (44). For this reason the action of oxidizing agents on the pattern of the curves was determined. Sample 8b was used for these experiments.

Curves were determined on the Farinograph with the appropriate amount of sample, and with added iodine, potassium bromate, and potassium iodate. The curves obtained for these variously treated fumigated flours are presented in Plate II. Addition of three ml. of iodine in potassium iodide (0.01 N) resulted in a curve with good recovery. A better recovery was obtained after using five ml. of the same solution. On the other hand, 0.026 percent of potassium iodate and either 0.02 percent or 0.2 percent of potassium bromate did not show satisfactory improvement.

The favorable effect obtained with iodine solution might be due to the salt effect. In fact, nonfumigated flour treated with five ml. of the iodine solution showed that the dough had been toughened (Plate II).

Treatment of Flour with HBr and CH_3OH. It was desired to observe the effect of hydrobromic acid and methyl alcohol on flour because these two compounds might be the possible hydrolysis products of methyl bromide. Plate II shows the curves obtained when these reagents were added to the water used in the Farinograph. Hydrobromic acid produced a graph that resembles those obtained with fumigated flours. Methyl alcohol decreased the consistency of the dough but did not produce as fast a break as does methyl bromide. The addition of the two reagents to the same flour resulted in an additive effect.

A nonfumigated flour treated with 0.5 ml. of concentrated hydrobromic acid and one treated with 0.5 ml. of methyl alcohol were baked according to the procedure already outlined. Neither one of these reagents produced loaves with objectionable odors, although they showed a detrimental effect on the volume. This was especially apparent with hydrobromic acid because it produced a decrease in loaf volume of 25 percent.

These results clearly indicate that methyl bromide has a two-fold effect on flour. One is the specific reaction of the organic halide with a certain part or parts of the protein-containing fraction. The other is the action of the hydrolysis products, mainly hydrobromic acid, on the physical dough properties.

Treatment of Dough with Liquid Methyl Bromide. Reactions between methyl bromide and gluten proteins might take place with those parts of the molecules that are responsible for the formation of gluten. It was determined whether or not the fumigant could react with the proteins after the gluten network was established. Plate II shows the curve produced when the dough was treated with liquid methyl bromide just as it reached its peak consistency in the mixer. The sudden increase in consistency may be due to the cooling action of evaporation.

Gas Production and Gas Retention. It was of interest to determine whether or not bromine residues in methyl bromide fumigated flours affect the yeast used in baking and also if the ability of the gluten network to retain the carbon dioxide produced during fermentation is influenced. Measurements of the rate of gas production furnish information regarding the fermentation capacity of the dough. Gas retention is useful to determine, very roughly, alterations in the physical properties of the proteins. In the former determination the total volume of carbon dioxide produced during fermentation is measured while in the latter only the gas retained in the dough is evaluated. The machine used in these experiments furnishes the results in the form of two curves that represent gas pressure against time. Gas pressure multiplied by the apparatus constant gives the results in units of volume.

Superimposed curves obtained in one experiment are shown in Graph IV. The upper curves show the rates of gas production and their changes after certain times (period of constant rate of fermentation). The curves at the bottom indicate the amounts of gas retained at any time. A duplicate experiment gave very similar results. Table 4 shows the periods of constant rate of fermentation, the gas produced at the end of this period, i.e., at the time when the slope of the curve changes, and the gas retained at the time of maximum production for these two experiments.

The fumigated sample gave a longer period of constant fermentation rate than the nonfumigated. The presence of more fermentable sugar could account for this difference.

The percentage of total gas produced which is retained by the dough shows a small decrease for fumigated flour. This confirms what was found in the baking tests. For the dosages

Graph IV

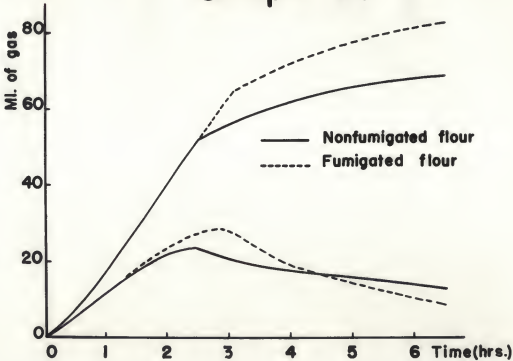

Ml. of gas
80
60
40
20
0
0
1
2
3
4
5
6
Time(hrs.)
Nonfumigated flour
Fumigated flour

Table 4. Total gas produced and retained at the point where the rate of gas production changes.

Sample		Period of constant rate of fermentation	Gas produced	Gas retained	Gas retained x 100 / gas produced
		hours	ml.	ml.	%
Exp. 1	Check	2.5	51.6	23.8	46.2
	4b	3.1	63.5	27.0	42.5
Ex. 2	Check	2.8	57.2	26.2	45.8
	4b	3.3	67.5	25.4	37.6

used in these fumigations the fumigant had a definite but not pronounced detrimental action on the loaf volume.

Selenium Bearing Flours. It is well known that selenium compounds tend to form products with very sharp odors. It was of interest to determine whether a selenium bearing flour showed more pronounced reaction products than a normal flour. For this purpose, a wheat[1] containing 26.6 p.p.m. of selenium was milled. Two samples of the flour were fumigated with methyl bromide, one with two pounds per 1,000 cubic feet and the other with 25 pounds per 1,000 cubic feet, and baked. The odor of the loaves was no more objectionable than that of common flours fumigated at the same dosages.

Microbicidal Effect of Methyl Bromide. During the course of this work it was found that flours fumigated with methyl bromide appeared to be relatively free from microflora. In order to corroborate this observation microfloral counts were run.

The results obtained, although of a preliminary nature, are considered of importance and significance. In these experiments four samples of the same flour were used for enumeration of bacteria and fungi. Two of the samples had been fumigated with methyl bromide at dosages of two and 25 pounds per 1,000 cubic feet, another one had been treated with a heavy dosage of methyl chloride, and the fourth was the nonfumigated control. The data obtained (see Table 5) show that even a

1/ This wheat was grown near Dixon, South Dakota, in 1941. It was kindly furnished by the Agricultural Experiment Station of South Dakota State College.

Table 5. Microfloral counts on fumigated flours.

Sample	Incubation: 4 days; dilution 1/100 microorganisms per gm.	
	Nutrient agar	Rose bengal 1/
Check	approx. 30,000*	1,700 - 2,700
25 lbs./1,000 cu. ft.	0 - 100 *	0 - 300
2 lbs./1,000 cu. ft.	200 - 400	0 - 100
Methyl chloride	700 - 1,200	100 - 300

1/ Rose bengal medium preferentially inhibits bacteria growth.

* Incubation period: 2 days.

moderate dosage of fumigant had a pronounced microbicidal effect. Methyl chloride likewise appreciably decreased the number of microorganisms per gram but it should be borne in mind that a heavy dosage of this compound had been used.

DISCUSSION OF EXPERIMENTAL RESULTS

All the evidence gathered during the various stages of this study indicates that methyl bromide and, or, its hydrolysis products react with the protein fractions of flours. Methyl chloride also seems able to react with flour. However, the products of these reactions apparently are different because the odors of the loaves baked from flours treated with both organic halides did not resemble each other.

The reaction of methyl bromide with the proteins of flours also is given by other protein-containing materials. Of those tested, gelatin was the only one that gave a negative reaction. It is worthwhile to repeat here that gelatin does not have the amino acids valine, beta-hydroxyglutamic acid, tyrosine, methionine, and tryptophane. This may have some significance and future work should be directed towards determination of why gelatin does not react, and whether or not the absence of one or several of the above mentioned amino acids is responsible for this lack of reactivity. However, it may be that the deficiency of these regular components of proteins may result in a special structure of the protein molecules with the absence of particular types of linkages or reactive spots.

The previously unobserved reaction between fumigated protein and alcoholic potassium hydroxide might be of further importance. It could be used as a rapid method to establish the fumigant-protein reaction instead of the more laborious tests. Probably it could find a practical application as a test for overfumigation of foodstuffs. The specificity of the reaction between methyl bromide and proteins could also be studied with the aid of the alcoholic potassium hydroxide test.

From the study of the sorption of methyl bromide as related to the moisture content of flour it can be concluded that the moisture factor is very important. For a certain dosage, it controls the amount of methyl bromide fixed by the flour and also the mode of action of the fumigant. As a matter of fact, when moisture is low methyl bromide is mostly bound by unknown chemical reactions with the protein fractions; converse-ly, when moisture is rather high it hinders these reactions and favors hydrolysis phenomena. The products of these two modes of action are different and the effects also are different. It is of particular significance that minimum sorption of the bromide takes place in the neighborhood of 14 percent moisture (Graph I). If moisture contents other than approximately 14 percent are used it appears that they should be above rather than below this value.

There is no question but that the high bromide residues left after overfumigation decrease the baking quality of flour. This is true not only because of the objectionable odor that develops in the baked loaves but also because of alterations in the physical properties of the dough. Accordingly, methyl bromide fumigation of flour should be performed with considerable care, such that this condition of overfumigation does not arise.

One of the objectives of this work was to find some means to avoid the above noted difficulties. However, no way has been discovered to alleviate the damage to the flour once the overfumigation occurs. Aeration, of course, helps to eliminate the volatile fumigant that has not reacted.

Any further work should be directed to finding out just what the reaction between methyl bromide and cereal proteins is. If this were known for certain it might be possible to correct the difficulty, although the work done appears to indicate that the reaction is severe enough to irreparably alter the protein.

It was thought that the fumigant might attack the disulfide linkages (-S-S-) and liberate sulfhydryl groups (-SH). This should weaken the protein structure, especially the cross linkages, and at the same time the sulfhydryl groups should contribute to the off odors associated with baking. But the nitroprusside test has shown that there is no liberation of sulfhydryl groups, even if the fumigant acts directly on an amino acid like cystine or methionine. The latter amino acid was chosen because, as previously noted, it is not present in gelatin. Further, the $-SCH_3$ group of this amino acid could react with methyl bromide to give an addition compound (sulfonium compound) that could be decomposed by heat. However, these experiments did not confirm this possibility. Furthermore, it was found that when cysteine (containing a free -SH group) is used in baking it markedly affects the baking quality of flour but does not give objectionable odor.

Tryptophane was another amino acid tested because of its deficiency in gelatin and because of its intimate relation with skatole and other indole derivatives that are usually very odoriferous. But it also failed to give any reaction with the fumigant.

During the fermentation of nonfumigated flour the normal microflora may use part of the sugar present for their own metabolism. With destruction of the microflora this sugar would remain in the doughs and be available to the yeast. The longer maintenance of peak fermentation rate with the fumigated flour could therefore be explained in this manner. However, a change in the starch-amylase relationship in the dough due to methyl bromide fumigation is not excluded. No investigations have been made in this direction but it is possible that the bromide reaction could result in either the starch being made more available to hydrolysis or the amylases becoming more available for hydrolysis. In either case there would be a net increase in the amount of fermentable sugar.

Complete or partial elimination of the microorganisms present in flour might be of great value in preventing the development of "rope" in bread. In future studies with methyl bromide fumigation of flours it would be very desirable to determine whether or not flours fumigated at normal dosages can develop rope. Thus an important contribution to the baking industry could be made. Furthermore, methyl bromide could be used as a tool for disinfection of materials when heat can not be employed.

The effects of methyl bromide fumigation on flour properties therefore are twofold. If used in excessive amounts it is detrimental to baking quality. On the other hand, in addition to serving as an insecticidal agent, it appears to have definite promise as an effective germicide.

SUMMARY AND CONCLUSIONS

The action of methyl bromide on the different fractions of flours was studied. A test was developed to detect reactions between the fumigant and proteins. In addition, some investigation was done on the sorption of this organic halide as related to the moisture content of flour. Alterations of the physical dough properties and of baking quality due to bromide residues were investigated. Fumigation with carbon dioxide and methyl bromide was tried. Further, the effects of the addition of several amino acids, oxidizing agents, hydrobromic acid, and methyl alcohol were studied. Finally, some work was done on the microbicidal action of the fumigant and the influence of this total or partial sterilization on the gas production and gas retention of doughs.

The following conclusions can be made:

1. Methyl bromide reacts with the protein fraction of flour in an unknown way. The reaction is also given by proteins contained in other materials, both of cereal and non-cereal nature.

2. When proteins react with methyl bromide and are subsequently tested with a solution of alcoholic potassium hydroxide they give a characteristic, rather unpleasant odor.

3. Moisture content is a very important factor in the sorption of methyl bromide by flour. It is advisable, for practical purposes, to fumigate in the neighborhood of 14 percent moisture content.

4. Excess bromide residues affect the physical properties of flour doughs. This results in a significant detrimental action on the baking quality of flour. The effect of normal fumigation is negligible.

5. Methyl bromide shows a pronounced microbicidal action on the microflora of flour.

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