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# Methyl Bromide as a Microbicidal Fumigant for Tree Nuts

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Methyl bromide (MeBr) has broad microbicidal activity, but its use as a disinfectant for food is limited by the resulting bromide residues. Increasing the MeBr concentration, exposure temperature, or exposure period of a treatment tended to increase both the microbicidal efficacy of MeBr and the bromide residues. Its sporicidal activity was less at high than at low relative humidity within the range of 20 to 99%. Both the efficacy and the resulting residues of a MeBr treatment varied inversely with the load of product in a fumigation chamber due to sorption of the fumigant. Fumigation tests with almond kernels inoculated with *Escherichia coli* or *Salmonella typhimurium* indicated that MeBr can be used to disinfect whole nut kernels without resulting in excessive bromide residues, although the MeBr level necessary is higher than that normally used for insect control.

The sanitary quality of commercially processed nutmeats has been a subject of considerable concern for many years and was reviewed by Hall (6). The studies of Kokal and Thorpe (14) and King et al. (12) indicate that almond meats become contaminated with coliform bacteria primarily by exposure to insects and field dirt before and during harvesting. King et al. (12) found that a statistically significant relationship exists between insect damage and bacterial contamination, and there appears to be a similar relationship between insect damage and mold (7, 22). Exposure to field dirt is especially serious with modern methods of mechanical harvesting, which involve knocking the nuts to the ground, sweeping them into rows, and then brushing them onto a conveyor belt that leads to a collecting bin. Obviously, the shells and any exposed nutmeats become contaminated with field dirt.

*Escherichia coli* is widely used as an indicator organism of fecal pollution of nutmeats as well as other foods (6). However, the persistence of *E. coli* on nutmeats can be expected to vary with conditions and type of nut (2, 13, 19). In recent years, concern has arisen about the presence of mycotoxins from the growth of molds on various products (10). As a result, the food industry has given more attention than every to avoiding mold contamination and spoilage.

Nuts and other agricultural products are commonly fumigated to destroy insects soon after harvesting and periodically thereafter during storage. Such control measures help to minimize microbial contamination incurred from insects and rodents (7). Furthermore, some insecticidal fumigants (e.g., ethylene oxide, propylene oxide, and methyl bromide [MeBr]) can destroy microorganisms, as well as insect vectors (3, 9, 15, 18, 27). The epoxides of ethylene and propylene have been used for years to reduce microorganisms on several food products. These epoxides are effective and, until recent years, were thought to leave no objectionable residues (27). At present, they are considered to be potentially dangerous due to residues of chlorohydrin that may result from their use on certain food products (26). MeBr, a common insecticidal fumigant for agricultural products, has received relatively little attention as a microbicidal fumigant. Lack of interest in MeBr as a microbicide evidently has been due to its lower activity than that of ethylene oxide or propylene oxide and the likelihood that its use for this purpose would result in objectionably high bromide residues. From a review of the properties and use of MeBr (25), it seems that the use of MeBr as an insecticidal fumigant for foodstuffs presents little risk to consumers' health. In view of its general use as a fumigant and its apparent lack of highly toxic residues, MeBr seemed worthy of reevaluation as a microbicidal fumigant.

## MATERIALS AND METHODS

**Test organisms.** *E. coli* H-23 and *Salmonella typhimurium* TM-1 were grown on Trypticase soy broth (BBL) for 1 to 3 days at 35°C. *Saccharomyces bisporus* var. *mellis* (ATCC 28252) was grown on

Sabouraud maltose broth (Difco) for 3 days at 28°C. The conidiospores of *Aspergillus flavus* NRRL 3145 were obtained from a 2-week-old culture grown on malt agar (Difco) at 24°C. The spores of *Bacillus megaterium* B-938 and *Bacillus subtilis* var. *niger* were prepared by the method of Alderton and Snell (1). In both cases the bacterial spores had been stored in closed bottles at 1 to 2°C for several years.

**Nut samples.** The nutmeats of sweet almonds (*Prunus amygdalus*) and English walnuts (*Juglans regia*) were obtained from commercial packing houses before routine fumigation with MeBr. Nutmeats were inoculated with *E. coli* or *S. typhimurium* by spraying 300 to 400 g of nutmeats with 3 to 4 g of a 3-day-old broth culture. The nuts were sprayed while being tumbled within a 16.7-liter tin can containing agitator blades and an orifice in its lid for the spray nozzle. To assure uniform distribution of the inoculum, the nuts were mixed thoroughly by rolling the can at 15 rpm for 40 min. The inoculated nuts were stored for 2 or 3 days at room temperature before use to allow the moisture to equilibrate and the less resistant bacterial cells to die, so that the counts would be fairly stable.

**Inoculated filters.** Test organisms were suspended on sterile membrane filters (47-mm diameter, 0.45-$\mu$m pores, type HA; Millipore Corp., Bedford, Mass.). The filters were inoculated by filtering a suspension of the desired number of cells or spores in 99 ml of sterile suspending menstruum. The bacterial and yeast suspensions were prepared by direct serial dilution of the culture with sterile 0.1% peptone-water. The bacterial spore suspensions were prepared by serial dilution with 0.01% Tween 20 in water, starting with a heavy suspension of spores prepared with a Teflon homogenizer. The mold conidiospores were suspended in aqueous 0.01% sodium lauryl sulfate and serially diluted for application to the filters.

**Fumigation.** Fumigations were carried out in either 9.5-liter glass vacuum desiccators or wide-mouth 1-quart (950-ml) glass jars. With desiccators, MeBr was added as a liquid from a chilled graduated pipette connected to an evacuated desiccator with a short rubber tube. The material to be fumigated was protected from the liquid MeBr by a large watch glass. In some tests the vacuum was broken by admitting air after delivery of MeBr. With 1-quart jars, gaseous MeBr was injected through a syringe septum in one of two ports of a special lid, using a backfill gas syringe connected to a MeBr cylinder with plastic tubing. The gas was mixed with the bottle atmosphere by a special magnetic stirrer equipped with vanes. The material to be fumigated was placed on special wire shelves or in a wire basket above the stirrer.

After fumigation, MeBr was removed from the test chambers by using vacuum and filtered fresh air. With desiccators, a high vacuum was drawn three or four times, breaking the vacuum with filtered air each time; then the fumigated filters were exposed to air in petri dishes for 1 to 2 h before being used to determine survivors counts. MeBr was withdrawn from the 1-quart jars by vacuum through one port extending to the bottom of the jar while filtered air was admitted through the other port at the top; the fumigated materials were aired in this way for 45 to 60 min. The slight vacuum that existed in the jars during this flushing with air favored the removal of MeBr from the fumigated product. The nuts fumigated and aired in this manner were then stored for 4 days or more in the closed jars before being used to determine survivors, so that any trace of MeBr would be desorbed and would not interfere with the counts.

**Humidity control.** A selected relative humidity (RH) was attained in each desiccator by adding a calculated amount of water, for a given storage temperature, to the thoroughly evacuated (less than 0.5 mm of pressure) chamber before adding MeBr. With 1-quart jars, the RH was assumed to be the same as the ambient RH with which the jar was equilibrated in the storage room before sealing. The ambient RH was measured by sling psychrometer. In a few cases the RH in the jar was increased by adding a calculated amount of water. Fumigations of nuts were conducted at the equilibrium RH of the nuts.

**MeBr dosage and analysis.** MeBr dosage is given in milligrams per liter, which, for all practical purposes, is equal to ounces (avoirdupois) per 1,000 cubic feet of treated space. Gas concentration remaining after any period of fumigation is also given in milligrams per liter. (A concentration of 1 mg of MeBr per liter is equivalent to approximately 258 ppm [vol/vol] at 25°C and 760 mm of pressure). The MeBr concentration in the chamber atmosphere was determined by gas chromatographic analysis. A 250- to 500-$\mu$l sample of gas from the chamber was injected into a gas chromatograph equipped with a hydrogen flame ionization detector. A stainless-steel column (152 cm by 3 mm, outside diameter) packed with Poropak R (80/100 mesh) was used with a column temperature of 127°C; the injection port temperature was 144°C, and the detector temperature was 196°C. The MeBr concentration was estimated by comparing peak areas of the samples with peak areas of samples taken from similar chambers (without a product load) containing a known level of MeBr.

**Bromide residues.** Total bromide (inorganic and organic) residues were determined by an X-ray fluorescence method similar to that reported by Getzendaner et al. (5). It is assumed that these residues were essentially inorganic bromide residues, since very little MeBr is retained as such after airing and storage of the fumigated product (5, 17, 25). MeBr reacts with various constitutents of the treated product, so that the bromide residue is almost entirely inorganic bromide (25). The tolerances in the United States for residues resulting from fumigation with MeBr are for inorganic bromides calculated as Br.

**Survivor counts.** The number of organisms surviving the treatments on membrane filters was determined by incubating the filters in duplicate or triplicate: bacterial spores for 16 to 18 h or longer at 35°C on 1% tryptone-0.5% glucose-0.1% starch agar; *E. coli* and *S. typhimurium* for 18 to 24 h at 35°C on

plate count broth (Difco); *S. bisporus* for 2 days at 28°C on yeast broth (Difco); and *A. flavus* for 4 to 7 days at 25°C on potato dextrose agar (Difco).

Microbial counts on nuts were made by adding an equal weight of sterile 0.1% peptone-water to 50 g of nutmeats in a sterile bottle, shaking for 30 s, allowing the sample to soak for 3 min, and shaking again for 30 s before plating. Aerobic bacterial counts of nuts were made with plate count agar (Difco) containing 100 mg of Acti-dione per liter. The plates were incubated for 2 or more days at 28°C. Fungal counts were made by using acidified (pH 3.5) potato dextrose agar (Difco) and incubation at 28°C for 2 or more days.

Difco violet red bile agar (VRB) was used to determine the recovery of both *E. coli* and *S. typhimurium*, individually, from nutmeats inoculated with these organisms (23). Several samples of uninoculated nutmeats that were examined had no coliforms and had essentially no other bacterial counts on VRB. Since *S. typhimurium* does not produce colonies characteristic of the coliform group on VRB, all visible colonies were counted as *S. typhimurium*. Difco brilliant green agar and Difco bismuth sulfite agar were also used to determine *S. typhimurium* survivors from inoculated nutmeats (23).

## RESULTS

**In vitro study of MeBr fumigation variables.** Several tests were conducted to determine the microbicidal efficacy of MeBr against selected microorganisms on membrane filters. Even the mildest MeBr treatment (16 mg/liter for 4 h at 26°C) caused 99 and 100% destruction of *S. typhimurium* and *E. coli*, respectively, as compared to air-exposed controls, which had $2.9 \times 10^2$ and $2.1 \times 10^2$ cells per filter, respectively. A mild treatment of 16 mg/liter for 17 h at 24°C resulted in complete destruction of *S. bisporus* cells ($5.4 \times 10^2$ cells per filter), whereas a similar treatment at 35°C resulted in 99.9% destruction of *A. flavus* conidiospores ($1.7 \times 10^5$ spores per filter). In view of these results, bacterial spores were used to study conditions affecting the biocidal activity of MeBr, because bacterial spores are more resistant than vegetative cells to ethylene oxide and MeBr (21).

Concentration of MeBr has a direct effect on its sporicidal activity, as shown for two sets of conditions in Table 1. Increasing the temperature by 8°C increased the sporicidal activity of MeBr about as much as doubling the MeBr concentration, even though the exposure time was reduced from 24 to 18 h. Only the highest MeBr level at the higher temperature (35°C) showed appreciable activity against bacterial spores.

The effects of temperature and RH on the sporicidal activity of MeBr at low concentrations were examined in desiccators under vacuum in a series of four tests, summarized in Table 2. Temperatures between 10 and 35°C and humidities of 20 to 80% RH probably include the limits of conditions that occur seasonally in nut storage rooms in California. High temperature and moderately low humidity (e.g., 20% RH) appear to favor the sporicidal activity of MeBr (Table 2). Although there was fair sporicidal activity at 35°C, there was very little activity at 10 or 20°C; and there was noticeably less activity at 50 to 80% RH than at 20 to 30% RH. Additional tests with spores of *B. megaterium* and *B. subtilis* were conducted in jars without vacuum to check the effect of humidity (Table 3); these tests confirmed the observation that the sporicidal activity of MeBr is reduced by high humidity.

TABLE 1. *MeBr activity against bacterial spores on membrane filters*

| Treatment | | No. of surviving spores | |
|---|---|---|---|
| Conditions[a] | MeBr (mg/liter) | *B. megaterium* | *B. subtilis* |
| 24 h, 27°C, 40% RH | 0 | 85 | |
| | 16 | 80 | |
| | 32 | 48 | |
| | 64 | 18 | |
| 18 h, 35°C, 32% RH | 0 | 109 | 101 |
| | 32 | 16 | 4 |
| | 64 | 0.3 | 0 |

[a] Exposed without vacuum at ambient RH in glass desiccator.

The effect of load (type and amount of product being fumigated) on the efficacy of MeBr as a microbicide was also evaluated with bacterial spores on filters (Table 4). An increase in product load decreased the efficacy of a given treatment, even though the loads in this test occupied only a small percentage of the available space. In a similar test, using a milder treatment (32 mg/liter, 24°C, 4 h), the destruction of *E. coli* (99.99%) on filters was not affected by the presence of 350 g of walnut kernels, due to the great sensitivity of *E. coli* to MeBr.

**MeBr activity against natural flora of almonds.** Tests were made to determine the microbicidal activity of MeBr against the natural flora of almonds, using various MeBr concentrations at temperatures higher than those normally used for insect fumigation. MeBr reduced the total microbial count on almonds to a very low level when a high Me Br concentration was used at a fairly high temperature (Table 5). However, severe treatments like this are likely to leave high bromide residues in the fumigated product. Since there is a tolerance of 200 $\mu$g of bromide per g in tree nuts (4), fumigation is limited by the bromide residue.

**MeBr sorption and bromide residues.** To estimate the degree of MeBr exposure that is possible without exceeding the tolerance for bromide residue, a study was made of MeBr sorption and bromide residue on whole almond kernels. The load size was varied to determine its effect on bromide residue (Table 6) and on atmospheric MeBr concentration during the exposure period (Fig. 1). The maximum load in the test chamber (950-ml jar) was 50% of its capacity, due to the space occupied by the circulation system. At first, the load, by its displacement of space, tended to concentrate the gaseous MeBr, but very soon the gas concentration was decreased by sorption on the product. The results in Fig. 1 show that increasing the load increases the rate of MeBr removal from the chamber atmosphere. Most of the gaseous MeBr was sorbed within the 24-h exposure period by the large (50%) load but not by the small (17%) load. Thus, if microbicidal activity is proportional to atmospheric MeBr concentration, increasing the exposure period would be of little value in increasing effectiveness with the large load, but it would be beneficial in the case of the very small load.

The temperature of fumigation also was varied to determine its effect on MeBr sorption and bromide residues. Increasing the temperature resulted in a net increase in MeBr sorption and bromide residues after 24 h (Table 6 and Fig. 2), although the amounts of MeBr sorbed after 4 or 5 h of fumigation did not vary much with temperature.

**Disinfection of inoculated nutmeats.** Several tests were made with almond kernels inoculated with enteric bacteria (Tables 7 and 8). MeBr could be used to destroy *E. coli* on whole almonds without exceeding the tolerance for bromide residues (Table 7). For example, an 8-h exposure at 27°C to 48 mg of MeBr per liter with

TABLE 2. *Effect of temperature and RH on sporicidal activity of MeBr*

| MeBr concn[a] (mg/liter) | RH (%) | No. of surviving *B. megaterium* spores at: | | | |
|---|---|---|---|---|---|
| | | 10°C | 20°C | 29°C | 35°C |
| 0 | 20 | | | 80 | 83 |
| | 30 | | | 81 | |
| | 50 | | 81, 63 | 87 | 105 |
| | 80 | | | | 127 |
| 32 | 20 | | 31 | 6 | 2, 0.5 |
| | 30 | | | 5 | |
| | 50 | 84 | 86, 68 | 63 | 19 |
| | 99 | 70 | | | |
| 64 | 20 | | | | 0.5 |
| | 50 | | | | 6 |
| | 80 | | | | 3 |

[a] Exposed for 24 h in vacuum on membrane filters.

TABLE 3. *Effect of RH on sporicidal activity of MeBr*

| Treatment[a] | | | | No. of surviving spores | |
|---|---|---|---|---|---|
| Sample | Time (h) | Temp (°C) | RH (%) | *B. megaterium* | *B. subtilis* |
| Control | 70 | 24 | 30 | 86 | |
| MeBr | 70 | 24 | 30 | 6 | |
| MeBr | 70 | 24 | 90 | 60 | |
| Control | 18 | 35 | 23 | | 76 |
| MeBr | 18 | 35 | 23 | | 1.5 |
| MeBr | 18 | 35 | 55 | | 20 |
| MeBr | 18 | 35 | 90 | | 32 |

[a] Spores exposed on membrane filters to air (control) or 32 mg of MeBr per liter in a 950-ml jar without vacuum.

TABLE 4. *Effect of load on sporicidal activity of MeBr*

| Treatment[a] | Load (of walnuts) | | Surviving[b] *B. megaterium* spores |
|---|---|---|---|
| | Wt | % of capacity[c] | |
| Control | None | 0 | 86.3 |
| MeBr | None | 0 | 1.2 |
| MeBr | 100 g of kernels | 3 | 51.7 |
| MeBr | 350 g of kernels | 11 | 61.7 |
| MeBr | 222 g of in-shell nuts | 8 | 8.0 |
| MeBr | 1,013 g of in-shell nuts | 40 | 80.0 |

[a] Exposed to 64 mg of MeBr per liter at 35°C for 18 h in glass desiccator without vacuum.

[b] Spore count on membrane filters in presence or absence of nuts.

[c] Estimated percentage-of-capacity load (by volume).

TABLE 5. *MeBr effectiveness against natural flora on almond kernels*

| Treatment[a] | | Survival (%)[b] | |
|---|---|---|---|
| MeBr concn (mg/liter) | Temp (°C) | Bacteria (PCA) | Fungi (PDA) |
| 0 (control) | 35 | 100 ($6.2 \times 10^3$) | 100 ($2.2 \times 10^3$) |
| 16 | 35 | 74.2 | 14.5 |
| 64 | 35 | 30.6 | 5.5 |
| 0 (control) | 35 | 100 ($>2.0 \times 10^4$) | 100 ($3.0 \times 10^3$) |
| 160 | 35 | <2.0 | 1.0 |
| 0 (control) | 51 | 100 ($3.9 \times 10^3$) | 100 ($2.0 \times 10^3$) |
| 160 | 51 | 0.5 | 0.25 |

[a] Exposed for 18 h in glass jar containing a 26%-capacity load.

[b] Survival is given as percentage of control count; count per gram (in parentheses) is shown for controls only. PCA, Plate count agar; PDA, potato dextrose agar.

TABLE 6. *Effect of chamber load and temperature on bromide residue in almonds fumigated for 25 h with 24 mg of MeBr per liter*

| Variable | Load (% of capacity) | Temp (°C) | Total bromide residue (μg/g) | |
|---|---|---|---|---|
| | | | Potential[a] | Found[b] |
| Load | 17 | 35 | 210 | 140 |
| | 33 | 35 | 105 | 80 |
| | 50 | 35 | 70 | 64 |
| Temp | 33 | 20 | 105 | 71 |
| | 33 | 35 | 105 | 82 |
| | 33 | 54 | 105 | 110 |

[a] Maximum residue possible (calculated).
[b] X-ray fluorescence analysis; average of four assays.

a 33%-capacity load resulted in 99.6% destruction of *E. coli*, relative to the control, with a residue of 98 μg of bromide per g. Fumigation treatments are often described in terms of the product of fumigant concentration ($c$) and exposure time ($t$) under specific conditions, since the "$c \times t$ product" required for a successful treatment of a given load is almost constant within certain limits of concentration and time (17). A 4-h exposure with 96 mg of MeBr per liter (Table 7) gave approximately the same amount of destruction of *E. coli* as the 8-h exposure with 48 mg of MeBr per liter (99.7 versus 99.8% destruction). However, the shorter exposure to high concentration appears to be preferable to the longer exposure to low concentration, since it resulted in a lower bromide residue (83 μg/g). The total counts also were reduced by about 99% by these treatments, because the total count was dominated by the inoculum. A comparison of the results of the tests in Tables 7 and 8 shows that *S. typhimurium* is not as sensitive as *E. coli* to MeBr. For example, a $c \times t$ product of 384 mg·h per liter destroyed 99.7% of the *E. coli* but only 95.6% of the *S. typhimurium*, according to counts made on VRB 4 days after fumigation. Counts of *S. typhimurium* were repeated after 1 month of storage of the fumigated nuts to determine the change that occurred during this period at room temperature and to compare the counts obtained on VRB with those obtained on salmonella-selective media. The counts on VRB agreed quite well with those obtained on brilliant green agar. Counts on bismuth sulfite agar were slightly lower than those on brilliant green agar or VRB.

A similar fumigation test was made with walnut kernels inoculated with *S. typhimurium* (Table 9). The heavy wetting with the inoculum and the tumbling of the fragile walnut kernels during the inoculation resulted in a very heavy coat of nut debris. The total count (plate count agar) and salmonella count (VRB) on the inoculated walnuts were several times higher than those found on the inoculated almonds (cf. Tables 9 and 8). A 4-h MeBr treatment with a $c \times t$ product equal to that used to treat almonds (384 mg·h per liter) was less effective than expected. Although the natural fungal count (potato dextrose agar) was reduced by 99.9%, the total count and salmonella count were reduced by only 93.1 and 79.0%, respectively. Evidently, the layer of nut material protected the inoculum somewhat from the MeBr. A more severe treatment to improve MeBr penetration may have been permissible, since the bromide residue due to the treatment was 42 μg/g, about half of that found in almonds similarly treated.

## DISCUSSION

The fungicidal activity of MeBr has received more attention than its bactericidal activity (11, 16, 18, 27). The results of this study show

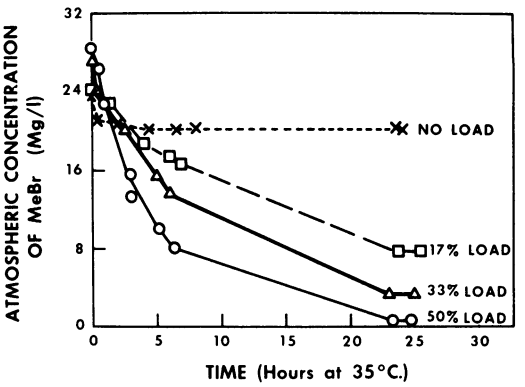


FIG. 1. *Effect of load size on MeBr sorption by almond kernels (24 mg of MeBr per liter).*

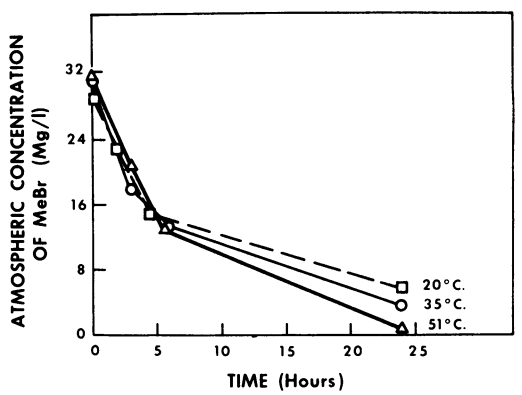


FIG. 2. *Effect of temperature on MeBr sorption by almond kernels (33%-capacity load, 24 mg of MeBr per liter).*

TABLE 7. *MeBr activity against E. coli on almond kernels*

| Treatment[a] | | | Survival (%)[b] | | Bromide residue (μg/g) |
|---|---|---|---|---|---|
| MeBr concn (c; mg/liter) | Time (t; h) | c × t (mg·h per liter) | PCA | VRB | |
| 0 (control) | 4 | 0 | 100 (2.1 × 10⁵) | 100 (1.0 × 10⁵) | 0 |
| 32 | 4 | 128 | 7.9 | 3.6 | 50 |
| 96 | 4 | 384 | 0.8 | 0.3 | 83 |
| 0 (control) | 8 | 0 | 100 (1.2 × 10⁵) | 100 (3.9 × 10⁴) | 0 |
| 48 | 8 | 384 | 1.2 | 0.2 | 98 |

[a] Exposed at 27°C, using a 33%-capacity load.
[b] Survival is given as percentage of control count, which is shown in parentheses. Counts were made 4 days after treatment and aeration, using plate count agar (PCA) and VRB.

TABLE 8. *MeBr activity against S. typhimurium on almond kernels*

| Treatment[a] | | Survival (%)[b] | | | | |
|---|---|---|---|---|---|---|
| MeBr concn (c; mg/liter) | c × t (mg·h per liter) | PCA, 4 days | VRB | | BGA, 30 days | BSA, 30 days |
| | | | 4 days | 30 days | | |
| 0 (control) | 0 | 100 (5.8 × 10⁵) | 100 (2.5 × 10⁵) | 100 (8.6 × 10⁴) | 100 (7.8 × 10⁴) | 100 (6.9 × 10⁴) |
| 16 | 128 | 52 | 76 | 15 | 18 | 9.6 |
| 48 | 384 | 3.3 | 4.4 | 0.7 | 1.1 | 0.5 |

[a] Exposed for 8 h at 27°C, using a 33%-capacity load.
[b] Survival is given as percentage of control count (in parentheses) shown for each medium. Counts were made 4 and/or 30 days after treatment, using plate count agar (PGA), VRB, brilliant green agar (BGA), and bismuth sulfite agar (BSA).

TABLE 9. *MeBr activity against S. typhimurium on walnut kernels*

| Treatment[a] (c × t product) | Survival (%)[b] | | |
|---|---|---|---|
| | PCA | VRB | PDA |
| 0 | 100 (6.1 × 10⁶) | 100 (1.1 × 10⁶) | 100 (3.6 × 10³) |
| 384 | 6.9 | 21 | 0.1 |

[a] Exposed for 4 h at 27°C with 33%-capacity load.
[b] Counts made 4 days after treatment, using plate count agar (PCA), VRB, and potato dextrose agar (PDA).

that MeBr has reasonable biocidal activity against vegetative bacteria as well as fungi and even shows some activity against bacterial spores. Appreciable microbicidal activity is evident with MeBr concentrations as low as those equivalent to the dosages used to fumigate nuts and similar agricultural products for insect control (16 to 64 mg/liter or 1 to 4 pounds/1,000 cubic feet). Fumigant dosage is usually expressed as weight of chemical added per volume of space or product treated (17, 25). Although dosage in terms of MeBr per product volume is most valid, in this study dosage is expressed as MeBr per chamber volume, since chamber volume is most convenient and, therefore, very commonly used. Although in practice a distinction between product volume and chamber volume often is not made, a product is exposed to higher MeBr concentration than that recommended whenever a large difference exists between these two volumes. Since MeBr is sorbed by the product being fumigated, the MeBr concentration in the chamber atmosphere, after several hours of exposure, varies inversely with the size of the product load, expressed here as the percentage of a full-capacity load (Fig. 1). Since product load in these tests was often smaller than usual in industrial practice, the MeBr concentration was often greater than that experienced in commercial fumigations with the same dosage.

The severity of MeBr exposure that can be used to treat foodstuffs is limited by the resulting bromide residues (4) and/or adverse flavor effects in the foodstuffs (7, 20, 25). Conditions that improved the microbicidal effectiveness of MeBr were found to increase bromide residues as well. For example, decreasing the load size in a given chamber resulted in both an increase in free MeBr (gas) concentration (Fig. 1) and an increase in bromide residue (Table 6). Similarly, increasing the temperature of MeBr fumigation, which had a profound positive effect on its biocidal activity (Table 2), resulted in an increase in bromide residue (Table 6). However, increasing the exposure temperature did not seem to increase residue as much as it increased the effectiveness of MeBr. Therefore,

for disinfection it may be wise, or even necessary, to use higher temperatures than those (10 to 20°C) normally adequate for MeBr fumigation to control insects during storage. Obviously, maximum care must be exercised if microbicidal effects are to be achieved without concurrent adverse effects from excessive MeBr treatment.

Temperature was considered by Monro (17) to be the "most important environmental factor" influencing the insecticidal action of fumigants, whereas humidity was considered to be "not so important in practice" as temperature. The microbicidal action of MeBr is influenced greatly by both temperature and humidity (Tables 2 and 3), but it is easier and more desirable to control temperature than humidity. For microbial disinfection with MeBr, it seems best to fumigate at as high a temperature as possible, since at low temperatures physical adsorption of MeBr increases, penetration of the product load decreases, and biocidal activity is low. If the product is removed from cold storage, sufficient time must be allowed for equilibration at the higher temperature, because it is the temperature of the product, not that of the atmosphere, that is important. Since it is difficult, and usually undesirable, to modify RH and the concomitant moisture content of large loads of product, it is probably best to fumigate nuts at or near their equilibrium RH, as suggested for the disinfection of nuts with propylene oxide (2). Although the effect of RH on sorption and bromide residues was not determined in this study, in fumigation studies with groundnuts Somade (24) found that MeBr sorption increased somewhat with equilibrium RH (22.5, 75.4, and 86.4%) and moisture content of the nuts, so that fumigation at low RH would likely result in both good microbicidal action and low bromide residues.

Since *E. coli* and salmonellae are of particular importance in sanitation and safety but are rarely found on tree nuts (12, 14), it was necessary to use nutmeats inoculated with these bacteria. Exceptionally high counts of these bacteria were used to assure an adequate assessment of MeBr activity. The severity of the challenge was increased by the fact that the bacteria were added in culture media, which might protect the cells from MeBr much like organic materials do in fecal suspensions (8). The results of these tests (Tables 7 and 8) indicate that MeBr might be used to disinfect almonds without exceeding the legal tolerance for bromide residues. Disinfection with the lowest bromide residue for a given treatment, i.e., $c \times t$ product, can likely best be achieved by using a higher MeBr concentration for a shorter time than those normally recommended for insect control. For example, the results in Table 7 show that a 99.7% destruction of *E. coli* was achieved by using 48 or 96 mg of MeBr per liter for 8 or 4 h, respectively, whereas the treatment often recommended for insect control at the same temperature (27°C) is 16 to 24 mg/liter for 16 to 24 h (17). Although the recommended fumigation has about the same $c \times t$ product (384 mg·h per liter), in practice it is usually applied to a chamber that is more fully loaded (e.g., 60 to 85% of capacity) than that used in the test (33% of capacity). Thus, the test treatment was two or three times more severe than the treatment recommended for insect control. Also important is the finding that of the two equivalent test treatments, the one with the shorter exposure resulted in the lower bromide residue (Table 7). Thus, not only does it appear necessary to use a treatment with a $c \times t$ product two or three times that recommended for insect fumigation but, apparently, it is best to use higher MeBr concentrations and shorter exposure periods than those normally recommended for insect control.

In addition to suitable MeBr concentration (in relation to load), exposure time, and product temperature, it is necessary to use some means to assure a rapid, even distribution of MeBr to achieve a successful fumigation. The use of fans or other means to obtain an even distribution of MeBr throughout the chamber (load) is especially important when using short exposures to high MeBr concentrations, which seem to offer the most promise for microbial disinfection. It is apparent from this study that the common use of MeBr as a fumigant for agricultural products can serve to reduce microbial contaminants directly, by microbicidal action, as well as indirectly, by destruction of vectors.

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