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Effect of Propylene Oxide Treatment on the Microflora of Pecans

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Studies were conducted to determine the effectiveness of propylene oxide (PO) treatment in controlling the microflora of pecans. As used commercially, PO has little effect on internal bacteria and fungi in pecan halves. Tests of surface washings of commercially treated pecans showed a reduction of 96% in coliform bacteria following PO treatment. Under controlled laboratory conditions, PO gave 80 to 92% reduction of surface microflora and at least 64% reduction of internal flora, but neither bacteria nor fungi could be eliminated completely, even with high dosages. Current assay methods for determining bacterial content of nutmeats were shown to be inadequate because they utilize only surface washings and thus do not give an accurate picture of the total bacterial population of the nutmeat. Consequently, such assays do not permit an accurate assessment of any potential health hazard related to these organisms.

The spoilage of food products due to microbial contamination is a serious problem in the food industry. One method of reducing the microflora of products like spices and nuts has been to disinfect them with ethylene oxide gas. This treatment is no longer permitted by the Food and Drug Administration (FDA), however, because of the presence of toxic ethylene chlorhydrins in treated products (8). Consequently, attention has focused on propylene oxide as an alternate fumigant, because its residual product, propylene glycol, is not considered harmful as a food additive.

Because of the need to control the presence of coliform bacteria on nutmeats, many pecan shellers began using propylene oxide (PO) as a fumigant after processing. With the report by Lillard et al. (6) that pecans provided a favorable substrate for the growth of *Aspergillus flavus* Link ex Fr. and for aflatoxin production, it became of interest to determine whether PO treatment would also control fungi. Hanlin (4) reported that various fungi, including *A. flavus*, could be present in pecan nutmeats at harvest time, indicating a good source of mold damage during storage if some means of arresting fungal growth was not used. After this report, our laboratory was asked to determine the effectiveness of a PO treatment being used by a commercial sheller. A pilot study indicated that very little control of either fungi or bacteria was being obtained. When these data were reported to a meeting of research workers and industry representatives, our results were challenged by others who claimed up to 90% reduction in bacterial count. Consequently, the present study was undertaken to determine (i) the effect of PO treatment in controlling fungi and bacteria in pecans, and (ii) the reason for the discrepancy between our results and those of other workers.

MATERIALS AND METHODS

Regulations of the FDA governing the use of PO on foods (3) state in part that PO shall be applied "not more than one time and not in excess of 4 hours' duration at a temperature not in excess of 125° F." The assumption is made that the regulations refer to pure PO gas. Commercial shellers, however, do not use pure PO, but purchase gas from commercial suppliers of fumigants. These are commonly supplied as 8% PO in 92% CO_2. One brand frequently used is UCAR F8-92 fumigant gas manufactured by Union Carbide Corp., and shellers using this product follow the manufacturer's directions in doing so. Under Union Carbide's interpretation of FDA regulations, they give the maximum allowable dosages as 35 lb of fumigant (8% PO) per 1,000 ft^3 at 100 F for 48 h on the theory that because the concentration of their gas (8%) is only one-twelfth the maximum allowed by the FDA, the time can be extended by a factor of 12, or to 48 h maximum. These figures were transposed to a laboratory maximum dosage of 0.27 ml of PO/6 liters at 37.7 C for 48 h; this is referred to as the standard treatment. Because the total exposure of the product

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to the gas is the critical factor, time of exposure and dosage can presumably be varied so long as the maximum is not exceeded.

A total of six experiments was conducted during this study. Tests 1 to 3 were performed on pecan halves selected before and after PO treatment by a commercial sheller. These pecans were packed in cardboard boxes, 28 lb/box, sealed, and stacked on pallets, and then they were loaded into a 20,000 lb capacity tank and treated with UCAR F8-92 fumigant gas at 37.7 to 43.3 C. Tests 4 to 6 were performed on three separate lots of market pecans obtained from local retailers. Pecan halves were used for two reasons: (i) they are larger than pieces and thus more difficult to sterilize because of their bulk; and (ii) they are the most valuable type of product and represent the greatest potential loss to the sheller.

Various combinations of PO, time, and temperature were tested to try to duplicate conditions used commercially and to determine whether any amount of PO would kill fungi and bacteria in pecan halves. For each laboratory experiment, six pecan halves were put into each of five small brown paper bags (only 25 halves were subsequently used). The bags were placed in a 6-liter vacuum desiccator, and a small glass petri dish was introduced to hold the PO, which was added in selected amounts as indicated in the various tests. The desiccator was then placed in an incubator maintained at the proper temperature. In elevated temperature experiments, a water trap was used to allow escape of air as the PO evaporated, after which the desiccator was sealed for the remainder of the experiment. Some experiments were conducted under reduced pressure of 50 cm of mercury to see if this would increase the effectiveness of the PO treatment. All experiments were run in duplicate, and the data are the average of both replicates.

All pecans studied were surface sterilized in a solution of commercial bleach (20%), 95% ethyl alcohol (20%), and distilled water (60%) (percentages by volume). Pecan halves were immersed in this solution for 2 min before being plated on malt extract agar (MEA) (1); this assured that any microorganisms growing out of the pecans were internal and not surface contamination. In those tests where surface washings were made, the pecan was washed before being surface sterilized. Washing was accomplished as follows: each pecan half was aseptically transferred from the shipping container to a 25-mm test tube containing 10 ml of sterile, distilled water with 0.01% Tween-20. Each test tube was agitated for 30 s on a test tube vibrator. A sterile pipette was used to remove 1 ml of solution which was spread over an MEA plate. One test was conducted with pecan halves inoculated with *A. flavus*. Inoculation was accomplished by placing 27 pecan halves in a large petri dish containing moist filter paper. A culture of *A. flavus* was inverted over the pecans and tapped on the bottom, causing conidia to fall onto the pecans. The pecan halves were then incubated for 7 days to allow the mycelium to penetrate them.

The presence of coliform bacteria was determined by plating the wash with Difco deoxycholate agar (5). All plates except the coliform test were periodically examined for 4 weeks for growth of bacteria, *A. flavus*, and mycelium other than *A. flavus*. *A. flavus* was selected for individual analysis because of its potential as a toxin producer. Coliform plates gave results after 24 h of incubation. Bacterial colonies and *A. flavus* were verified by microscopy examination. No attempt was made to quantitate the observations since this seemed irrelevant; from the viewpoint of product sanitation, the presence of any microorganisms is undesirable. For this reason, a record was also kept of the percentage of pecan halves which contained either bacteria or fungi; this figure is given in the right-hand column of the tables.

RESULTS AND DISCUSSION

Commercial PO treatment: test 1. In test 1, PO treatment of pecan halves from nine separate commercial samples of the same storage age (from the January 1971 shelling, 8 weeks postharvest) showed very little effect on the control of microorganisms within pecan meats (Table 1). By averaging all samples, the only noticeable effect appeared to be in a reduction of *A. flavus*, from 16 to 9%. The presence of bacteria increased slightly after treatment, as did mycelium other than *A. flavus*. Only 1% of the 900 pecan halves examined had no microorganisms, and these were from untreated samples. It is apparent from this study that the PO treatment used commercially fails to control internal microorganisms in pecan halves, as evidenced by the fact that 100% of the treated halves contained fungi or bacteria. Although there was a reduction of 43.75% in the *A. flavus* content of treated halves (from 16 to 9%), in view of the zero tolerance on *A. flavus* content imposed by FDA, the treatment must be considered ineffective.

Test 2. Test 2 was similar to test 1 except that the nine samples of pecans were selected periodically from January to June 1971, thus representing pecans varying in storage age from 8 to 34 weeks. PO treatment was again ineffective in controlling internal microflora (Table 1). When the data from the nine samples were averaged, the presence of bacteria increased

TABLE 1. *Effect of commercial PO treatment on internal microflora of pecan halves*

Test	Pecan halves with microflora (%)[a]							
	Total bacteria		*A. flavus*		Other mycelium		With bacteria or fungi	
	B[a]	A[b]	B	A	B	A	B	A
1	37	38	16	9	83	86	98	100
2	67	75	2	2	37	21	92	88

[a] B, Before PO treatment; A, after PO treatment.

slightly after PO treatment and the presence of mycelium decreased somewhat. *A. flavus* was present at the same low level both before and after treatment. Storage time apparently affected the types of organisms present, because all nutmeats from the last three samples contained bacteria but very little mycelium, whether treated or not. Because of this, the decrease in the percentage of halves containing mycelium is attributed to length of time in storage rather than to the PO treatment. Also, the fact that 88% of the treated halves still contained bacteria or fungi emphasizes the ineffectiveness of the commercial PO treatment.

Test 3. Although the presence of bacteria in food products may generally be considered undesirable, it is the coliform bacteria that are used as the basis for judging the sanitary quality of nutmeats. Six samples of pecan halves of the same storage age (from the January 1971 shelling) were examined for the presence of coliform bacteria (test 3). The level of coliform bacteria was reduced from 28% before PO treatment to 1% after treatment. This reduction of 96% agrees with figures presented by other laboratories and confirms that PO is an effective surface fumigant.

Laboratory PO treatment: test 4. Since the commercial PO treatment was shown to be ineffective in controlling internal microflora in pecan halves, a laboratory experiment was set up to determine whether PO would kill internal microorganisms in pecans under high and low levels of PO (Table 2). Only the effect on the total microflora was recorded. Treatments were performed under partial vacuum at 21 C; the amounts of PO used were based on the maximum allowable dosage at 37.7 C. Untreated market pecans (control) showed microflora present in all surface-sterilized pecan halves. Treatment A, with 0.48 ml/16 h (one-half of the standard treatment), gave no reduction in microflora, with 100% of both washings and halves containing microorganisms. Treatment B, with 5.0 ml/16 h (6 times the standard), gave complete reduction of surface organisms, but only 72% reduction of internal organisms. Thus although complete control of internal microflora was not obtained, even at a level of PO too high for commercial use, there was a marked reduction in microfloral content and consequent improvement in the sanitation of the pecans.

Test 5. Test 5 was conducted to determine the effects of time and temperature on the effectiveness of PO in reducing pecan microflora. Three regimes were examined: A, with 0.27 ml/48 h at 37.7 C at atmospheric pressure; B, with 0.54 ml/24 h at 37.7 C at atmospheric pressure; and C, with 0.81 ml/16 h at 21 C under 50-cm mercury vacuum, all of which were within the maximum allowable dosage as recommended by the manufacturer. All washings and 88% of the halves in the control sample contained microflora. Treatments A and B showed a marked reduction in total bacteria (from 52 to 8%) and other mycelium (from 68 to 8%); the level of *A. flavus* was also reduced, but the level in the control was only 4%. The percentage of washings containing microflora decreased from 100% in the control to 20% in treatment A and 8% in treatment B. A similar decrease occurred in pecan halves, from 88% in the control to 16% in treatment A and 8% in B. In treatment C, however, the reduction in microflora was much less, from 100 to 72% in control washings and from 88 to 72% in control halves.

These results indicate that although permissible PO treatments can markedly decrease microfloral contamination of pecan halves, they do not eliminate it, thus leaving a source of contamination that can spread throughout stored pecans. Temperature also appears to be an important factor in the greater effectiveness of treatments A and B as compared with C.

Test 6. Because the level of *A. flavus* in

TABLE 2. *Effect of laboratory PO treatment on microflora of pecan halves under variable time and temperature regimes*[a]

Test	Treatment		Pecan halves with microflora (%)			
			Bacteria	*A. flavus*	Other mycelium	With bacteria or fungi
4	Control	W	nd	nd	nd	100
		N				100
	A, 0.48 ml/16 h at 21 C, vacuum	W	nd	nd	nd	100
		N				100
	B, 5.0 ml/16 h at 21 C, vacuum	W	nd	nd	nd	0
		N				28
5	Control	W	80	4	96	100
		N	52	4	68	88
	A, 0.27 ml/48 h at 37.7 C	W	8	4	8	20
		N	8	0	8	16
	B, 0.54 ml/24 h at 37.7 C	W	4	0	4	8
		N	0	0	8	8
	C, 0.81 ml/16 h at 21 C	W	28	4	64	72
		N	24	4	56	72

[a] W, Wash; N, surface-sterilized nutmeat; nd, no data collected.

market pecans was unpredictable and tended to be low, experiments were run on pecan halves that had been inoculated with *A. flavus* to insure the presence of the fungus in the pecans (test 6). PO treatments were 3.24 ml/4 h at 48 C (A, standard treatment) and 3.24 ml/16 h at 48 C (B, 4 times the standard). Uninoculated pecan halves served as controls for those inoculated with *A. flavus*. All washings and halves in the control sample contained microflora (Table 3). There was a reduction from 100 to 0% in the level of internal *A. flavus* in inoculated halves in both treatments A and B; in the uninoculated halves, the level remained at 4% in treatment A but dropped to 0% in treatment B. With other mycelium the reduction was less, from 100% in the inoculated control to 12 and 16% in treatments A and B and from 96% in the uninoculated control to 20 and 4% in treatments A and B.

The inoculation with the fast-growing *A. flavus* made readings of bacteria nearly impossible, except in the uninoculated samples. The total bacterial level in washings dropped from 56% in the control to 8 and 4% in treatments A and B. In the halves, the level increased from 8% in the control to 12% in treatment A and dropped to 0% in treatment B. Total microflora decreased from 100% in uninoculated washings to 18% in treatment A and 16% in treatment B. With pecan halves there was a decrease from 100% in the inoculated control to 28% in treatment A and 16% in treatment B; in uninoculated halves, the decrease was from 100% in the control to 56 and 4% in treatments A and B.

These data indicate that PO treatment can greatly reduce *A. flavus* content in pecan halves in 4 h by using standard dosage at an elevated temperature. Higher than permissible amounts of PO appeared to eliminate *A. flavus* from pecan halves. Unfortunately, the same was not true for either bacteria or other mycelium, because some contamination remained even after excessive amounts of PO were used. It should also be noted that elimination of *A. flavus* from pecan halves by PO treatment would not eliminate any aflatoxin already in the pecan, but would prevent further toxin production.

Conclusions. (i) Examination of pecan halves commercially treated with PO indicate that very little control of internal fungi is obtained and that total bacterial contamination may actually increase. (ii) Propylene oxide treatment gives a marked reduction in the level of coliform bacteria, as determined by tests of surface washings. (iii) In laboratory tests, PO treatment eliminated 80 to 92% of the surface microflora of pecans. (iv) Laboratory tests indicate reductions of at least 64% in internal microflora by using maximum allowable amounts of PO. Even with dosages four times the maximum allowable amount, however, some organisms still survive. Tyner (7) showed similar results in PO treatment of barley seeds whereby manipulation of moisture content had an effect on fungal control, but no treatment could eliminate all bacteria. (v) *A. flavus* appeared to be controlled better than other fungi. (vi) High temperature appeared to increase the effectiveness of PO treatment. (vii) Because presently used commercial methods of PO treatment are ineffective, processors need to evaluate the economic feasibility of using the process. Even partial reduction of the microfloral content, however, results in a more sanitary food product. Engineering studies are needed to improve the commercial operation.

TABLE 3. *Effect of laboratory PO treatment on the control of microflora in pecan halves inoculated with A. flavus*[a]

Test	Treatment		Pecan halves with microflora (%)							
			Total bacteria		*A. flavus*		Other mycelium		With bacteria or fungi	
			I	U	I	U	I	U	I	U
6	Control	W	nd	56	96	16	100	100	100	100
		N	nd	8	100	4	100	96	100	100
	A, 3.24 ml/4 h at 48 C	W	0	8	12	0	16	20	28	18
		N	16	12	0	4	12	20	28	56
	B, 3.24 ml/16h at 48 C	W	0	4	0	0	0	12	0	16
		N	0	0	0	0	16	4	16	4

[a] W, Wash; N, surface-sterilized nutmeat; I, inoculated with *A. flavus*; U, uninoculated; nd, no data collected.

(viii) A technical conflict exists between FDA regulations and manufacturer's recommendation covering the use of PO, because FDA regulations say nothing about dosage in restricting the use of PO to 4 h. So long as residue levels are kept below the prescribed 300 parts per million, little difficulty is likely to be encountered. (ix) This study has shown that current assay methods for determining bacterial content of nutmeats are inadequate. The procedure used (2) involves only surface washes of nutmeats and consequently does not take into account internal microorganisms. Our studies show that surface-disinfected pecan halves frequently contain high levels of bacteria inside the nutmeat; under favorable conditions these bacteria can grow out and spread. Thus, a negative reading obtained by present procedures may be inaccurate, because even surface-disinfected nutmeats may still contain microorganisms internally that can represent a health hazard.

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