

Salmonella Survival on Pecans as Influenced by Processing and Storage Conditions

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Survival of *Salmonella senftenberg* 775W, *S. anatum*, and *S. typhimurium* during exposure to currently practiced, as well as abusive, pecan processing and storage conditions was studied. Thermal treatments normally carried out during the processing of pecans are inadequate to consistently destroy salmonellae in highly contaminated inshell nuts. Pecan nut packing tissue was toxic to salmonellae, thus affording some protection against high initial contamination and subsequent survival of the organisms. Examinations of inoculated inshell pecans stored at -18, -7, 5, and 21 C for up to 32 weeks revealed that the extent of survival was inversely correlated to the storage temperature. *S. senftenberg* 775W and *S. anatum* were not detectable on inshell nuts after 16 weeks of storage at 21 C. Little decrease in viable population of the three species was noted on inoculated pecan halves stored at -18, -7, and 5 C for 32 weeks. Due to organoleptic quality deterioration in pecan nutmeats at elevated temperatures, sterilization methods other than thermal treatment appear to be required for the elimination of viable salmonellae from pecan nuts.

The production and utilization of pecans has increased significantly in recent years, mainly due to the advent of mechanical processing equipment and improved storage practices. With this increase, pecan processors and food manufacturers using pecans as product ingredients have become increasingly concerned with the presence of potentially pathogenic microorganisms. Concern is especially intense among manufacturers of certain confectionery, dairy, and snack products wherein pecans are incorporated without processing treatments which would be lethal to microorganisms. Research attention has been given to the incidence of these microorganisms on pecans and other nuts (3, 7, 8, 12) and to their destruction during processing (2, 11) and storage (1).

The present study was initiated to determine the behavior of salmonellae as they are exposed to commercial pecan processing and storage conditions. Pilot plant experiments were designed to simulate currently practice processing and storage procedures as well as conditions which represent process abuse.

MATERIALS AND METHODS

Pecans. Standard grade Stuart and Schley cultivar pecans used in this study were a gift from Nut Tree Pecan Co., Albany, Ga. Nuts in 28-pound (ca. 13 kg)

sealed boxes had received a double propylene oxide treatment to reduce natural microflora counts and simplify salmonellae enumeration in planned inoculation studies.

Test organisms. *Salmonella senftenberg* 775W, *S. anatum*, and *S. typhimurium* were maintained on nutrient agar slants at room temperature. Working cultures were transferred daily in tryptic soy broth containing 0.5% yeast extract (TSY) and grown at 30 C on a gyratory shaker (150 rpm).

Inoculation of pecans. Four separate tests were carried out to determine the behavior of salmonellae during processing and storage of pecans. These tests are illustrated in Fig. 1, together with a schematic diagram of the procedures generally followed in processing and handling pecans after the nuts are delivered from the grove. Test 1 was made to determine the survival of salmonellae on inshell pecans during prolonged storage. Test 2 was run to determine the lethality of the hot water treatment to salmonellae. Test 3 was included to determine if a water-floatation vacuum treatment resulted in a decrease in recoverable organisms. Finally test 4, yielding information concerning potential public health problems which might be associated with pecans contaminated with salmonellae, was carried out to assess the survival of the organisms on nutmeats which would be distributed and possibly consumed without further processing. Both Stuart and Schley cultivars were examined in tests 2 and 3, whereas Stuart alone was used for test 1 and Schley was used for test 4.

Cells from 20-h cultures of the three salmonellae

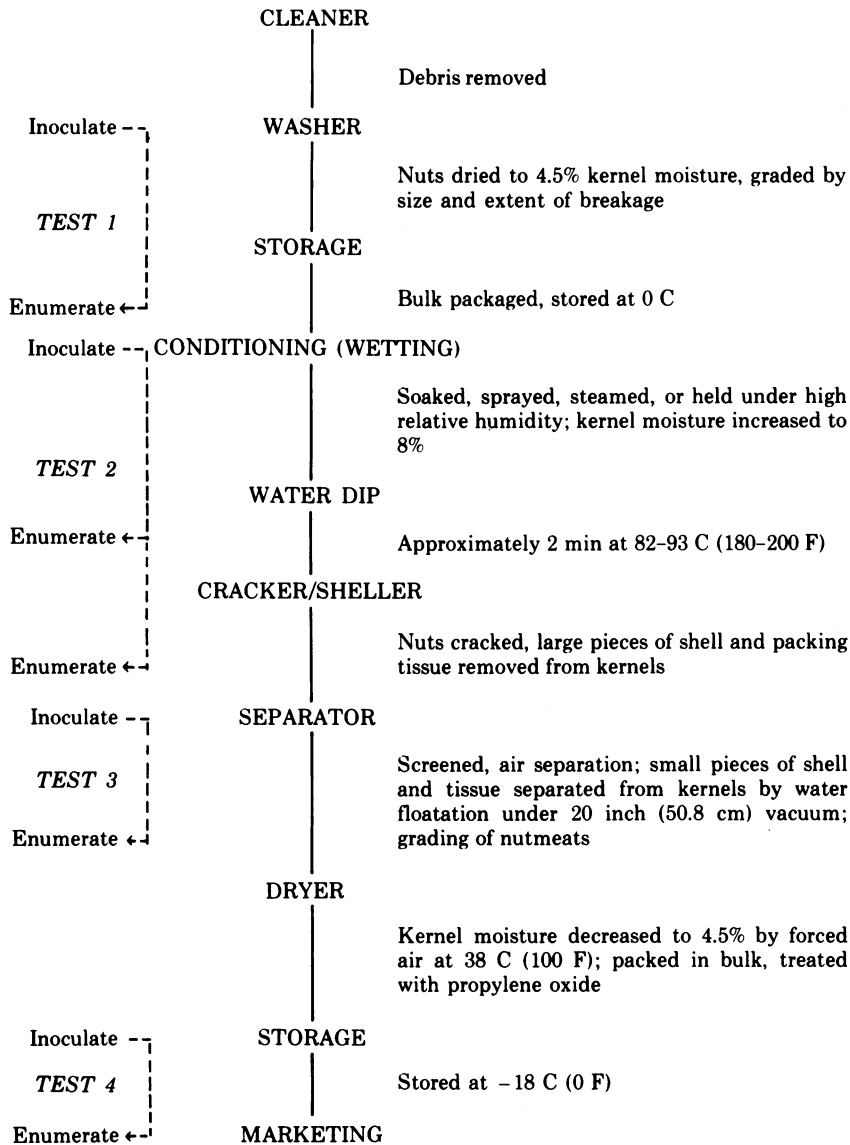


FIG. 1. Flow diagram of a general pecan processing scheme. Test numbers indicate laboratory and pilot plant investigations pertaining to the survival of salmonellae. See text for details.

species grown in TSY at 30 C on a gyratory shaker were collected by centrifuging for 15 min at $5,000 \times g$. The cells were resuspended in sterile tap water and applied to inshell pecans (test 1) and halves (test 4) using an aerosol spray. Nuts were dried at 40 C in a forced-air oven, and 50-g quantities were deposited in 28-ounce (ca. 1.8 kg) glass jars. Sealed jars were stored at -18, -7, 5, and 21 C for 2, 4, 8, 16, 24, and 32 weeks.

For test 2, *S. senftenberg* 775W was studied. Inshell pecans were submerged for 5 min in a tap water suspension containing 4×10^7 viable salmonellae per ml. Nuts were drained briefly and sealed in a plastic

bag at 22 C overnight, thus simulating the conditioning treatment a sheller would carry out prior to cracking. Conditioned nuts were submerged in tap water at 60, 71, 82, 93, and 99 C (104, 160, 180, 200, and 210 F, respectively) for 1, 2, 3, 4.5 and 6 min, cooled, and examined for viable *S. senftenberg* 775W. Conditioned nuts inoculated with *S. senftenberg* 775W were also submerged in tap water at 71, 82, and 93 C for 2 min, cracked and shelled (Champion Pecan Machine Co., model C, San Antonio, Tex.), and examined for viable organisms.

Test 3 consisted of subjecting pecan halves which had been freshly inoculated with a tap water suspen-

sion of *S. senftenberg* 775W to two alternate 3-min treatments of 13-inch (33 cm) vacuum and atmospheric pressure. Viable cells were then enumerated.

Enumeration of salmonellae. Fifty grams of in-shell pecans, pecan halves, or shells were combined in a 28-ounce glass jar with 50 ml of sterile 0.1% peptone and shaken on a mechanical shaker for 3 min. Serial dilutions of the peptone wash were plated in TSY containing 1.5% agar, and counts were made after 24 h incubation at 35 C. Supplementation of tryptic soy broth with yeast extract has been reported to enhance the recovery of cold-injured bacteria (9, 10). The initial natural microflora population was less than 10 per 100 g. For this reason, traditional salmonellae enrichment and identification procedures were not required in this study. All samples were brought to room temperature before viable count procedures were initiated. Counts reported are averages from at least two samples run in duplicate for each test.

Antimicrobial activity of pecan packing tissue. Packing tissue (see Fig. 2) was removed from noninoculated Stuart pecans, ground with a mortar and pestle, and passed through a 30-mesh screen. The material was added to 100-ml quantities of TSY (pH 7.0) at levels of 0.05, 0.1, 0.2 and 0.4% (wt/vol) and dispensed in 250-ml Erlenmeyer flasks. A 16-h culture of *S. senftenberg* 775W was harvested by centrifuging at $5,000 \times g$ for 15 min. Cells were resuspended in 0.1 M phosphate buffer (pH 7.2), and 1 ml of the suspension was used to inoculate each of the test media. Cultures were incubated at 35 C on a gyratory shaker (150 rpm). Aliquots were withdrawn over a 7.5-h growth period, serially diluted, and plated on TSY containing 1.5% agar. Colony counts were made after 24-h incubation at 35 C.

Heat penetration of inshell pecans. Holes, $\frac{1}{16}$ inch (ca. 0.159 cm) in diameter, were drilled at one of three positions (see Fig. 2) in inshell Stuart pecans. Thermocouples were inserted and sealed by applying an epoxy glue. Nuts were submerged in a water bath at 60, 71, 82, and 93 C for up to 5.2 min, and temperatures were recorded on a Honeywell Elektronik 16 Multipoint recorder.

Analytical. Procedures for moisture determination were reported earlier (1). Data presented throughout this paper are averages from at least two independent trials run in duplicate.

RESULTS AND DISCUSSION

A flow diagram of the processing and handling procedures which would generally be followed by a pecan processor is shown in Fig. 1. The information presented in the diagram reflects practices considered to be optimum for maintenance of high quality pecans. Substantial deviation from these procedures would result in decreased product quality. Realizing that process deviations do exist within the industry, specific tests were designed to include possible conditions of abuse. These conditions, in addition to those recognized as acceptable for

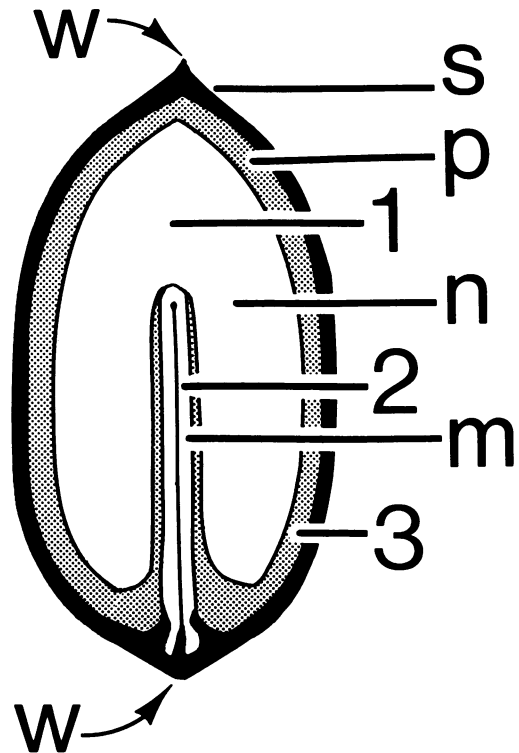


FIG. 2. Longitudinal section of pecan nut at right angles to the plane of the middle septum. Symbols: s, shell; p, packing tissue; n, nutmeat; m, middle septum; w, most likely ports of entry for water. Numbers indicate thermocouple positions during heat penetration studies.

the preservation of high quality pecans, were studied with regard to their effect on the survival of salmonellae.

Figure 3 shows the survivor curves for three species of *Salmonella* on inshell Stuart variety pecans stored at -18 , -7 , 5 , and 21 C for up to 32 weeks. Data were derived from test 1 (Fig. 1). Although initial counts for the three species were different, their behavior throughout the examination period was similar. No *S. senftenberg* 775W or *S. anatum* were detected after 16 weeks of storage at 21 C. *S. typhimurium* counts dropped significantly on nuts stored at 21 C, but detectable levels were still observed after storage for 32 weeks. Populations surviving the storage period were inversely related to the storage temperature. Only slight reductions in viable salmonellae were noted for inoculated inshell nuts stored at -18 C for 32 weeks.

Data from test 2 (Fig. 1) are shown in Tables 1 and 2. Table 1 lists viable *S. senftenberg* 775W

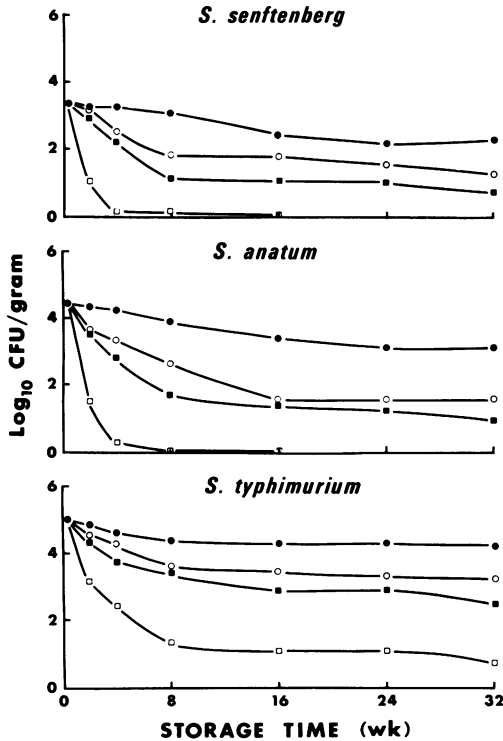


FIG. 3. Survival of salmonellae on inshell pecans. Symbols: storage at -18°C (●); -7°C (○); 5°C (■); and 21°C (□).

on inshell Stuart and Schley pecans heated in tap water for periods of time ranging to 6.0 min. Control experiments showed that low numbers of microflora naturally present on the nuts did not proliferate during the overnight holding period and that less than 50 viable cells per 100 g of inshell pecans were contained on nuts run through the cracking and shelling operations. The initial salmonellae population on both varieties was $6 \times 10^5/\text{g}$. A trend toward reduction in viable population at the higher temperature, longer treatment times is weak at best. The inconsistent data reveal a lack of uniformity among the nuts within sample lots with respect to initial contamination levels and/or a lack of uniformity with respect to rates of heat penetration in the nut. To test these theories, thermocouples were used to measure the internal temperature of sound inshell pecans heated at 60, 71, 82, and 93 C. Temperatures were monitored in the nutmeat, middle septum and packing tissue as illustrated in Fig. 2. Surprisingly, only small differences were noted in temperatures at the three positions after exposure to the same time-temperature treatment. The nutmeat temperature was always about 2 F

(ca. 1 C) lower than septum and tissue temperatures. The data were averaged and plotted as heat penetration curves (Fig. 4). Come-up times were not reached within sound pecans even after 5.2-min exposure to heat. The 2-min water dip treatment at 180 and 200 F (82 to 93 C) as carried out by processors would result in internal nut temperatures of approximately 160 and 185 F (71 and 85 C), respectively. Poor heat conductivity of the porous packing tissue accompanied by the high lipid content of kernels apparently retards heat transfer within the pecan shell. A portion of the salmonellae which were imbibed by nuts in various quantities during inoculation remained viable and thus resulted in part for the inconsistent data presented in Table 1. Dissolved solids within the nut may also have afforded some protection against thermal inactivation of *S. senftenberg* 775W. Salmonellae are reported to have increased heat resistance as the water activity of

TABLE 1. Viable *S. senftenberg* 775W on inshell pecans subjected to thermal treatment

Pecan cultivar	Water temperature		Viable salmonellae per g of pecan after heat treatment (min) ^a				
	C	F	1.0	2.0	3.0	4.5	6.0
Stuart	60	140	— ^b	16,300	5,000	5,750	2,500
	71	160	25	700	152,750	0	0
	82	180	20,750	53	3	4	5
	93	200	585	54	5	0	0
	99	210	135,500	93	0	—	—
Schley	60	—	—	59,000	161,000	1,520	17,850
	71	—	1,770	760,000	1,100	15	60
	82	—	82	15	0	38	18
	93	—	525,000	25	12	6	—
	99	—	0	0	0	—	—

^a Initial population was 600,000/g.

^b —, No test performed.

TABLE 2. Viable *S. senftenberg* 775W on pecan shells and nutmeats after 2-min thermal treatment followed by cracking and shelling

Pecan cultivar	Water temperature		Viable salmonellae per g ^a	
	C	F	Shell	Nutmeat
Stuart	71	160	189	124
	82	180	19	37
	93	200	57	363
Schley	71	—	4,230	24,300
	82	—	133	183
	93	—	334	517

^a Initial population was 600,000/g of inshell pecan.

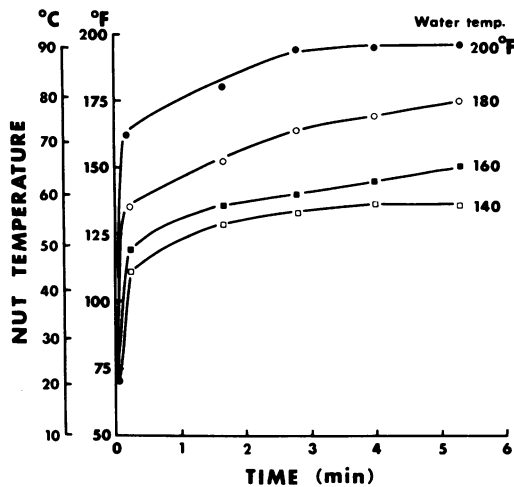


FIG. 4. Heat penetration curves for pecans submerged in water at 140 to 200 F (60 to 93 C).

heated menstrua is decreased (5). In any case, when present in the internal portions of inshell pecans, *S. senftenberg* 775W survives the 2-min water dip treatment at 180 to 200 F.

Test 2 was extended to determine the fate of surviving *S. senftenberg* 775W as the nuts were passed through a cracker-sheller (Fig. 1). Data are summarized in Table 2. Counts are again slightly higher for the Schley pecans, possibly indicating greater numbers of nuts with hairline cracks which may have initially facilitated the entrance of higher populations of salmonellae. Apparently, the viable population tends to equilibrate between shell and nutmeat samples, probably due to surface contact.

Results from test 3 (Fig. 1) revealed that the alternating vacuum-atmospheric pressure treatment had no effect on the number of salmonellae recoverable from pecan halves.

Figure 5 shows results from test 4 (Fig. 1). The three salmonellae test species were enumerated from Schley pecan halves stored at -18, -7, 5, and 21 C for times ranging to 32 weeks. With the exception of persistent survival of *S. anatum* at 21 C, the three species behaved similarly. Neither *S. senftenberg* 775W nor *S. typhimurium* were detectable after 24 weeks of storage at 21 C. Slight losses in viability were noted for the duration of the study for all species when storage was at -18, -7 or 5 C. Unfortunately these lower temperatures are required if high organoleptic qualities of pecans are to be maintained over prolonged storage.

During initial experiments in this study, attempts were made to inoculate nutmeats of inshell pecans by submerging the nuts in heavy suspensions of salmonellae followed by drying.

The inshell pecan will readily absorb liquid through fibrovascular bundles at its base and through suture separations at its apex as illustrated in Fig. 2, thus providing ports of entry for salmonellae. Repeated trials using this inoculation procedure, however, were unsuccessful. Aseptic separation and analysis of nutmeats from inoculated inshell pecans after drying yielded low numbers of viable salmonellae. These results prompted an investigation of the packing tissue for possible bacteriocidal activity (Fig. 6). Packing tissue, when present in TSY at a level as low as 0.05%, retards the growth of *S. senftenberg* 775W. At 0.2%, tissue exerts a bacteriocidal effect. The toxic effect is probably due to tannins and polyphenolic compounds which are present in high concentrations in the packing tissue.

The moisture contents of nutmeats and shells have a pronounced effect on the ability of salmonellae to remain viable during prolonged storage. The moisture contents of nutmeats studied in tests 1 and 2 were 4.5% at the beginning of the storage period. Previous studies indicated that inshell pecans having a nutmeat moisture content of 4.5% have an outer-

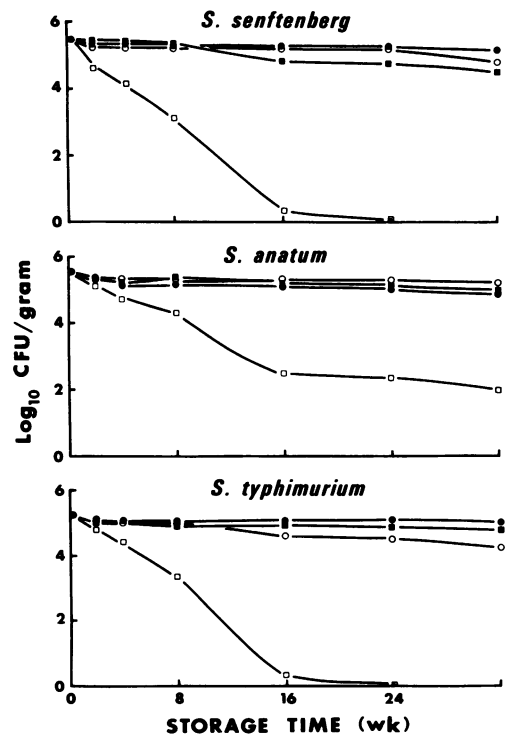


FIG. 5. Survival of salmonellae on pecan halves. Symbols: storage at -18 (●); -7 (○); 5 (■); and 21 C (□).

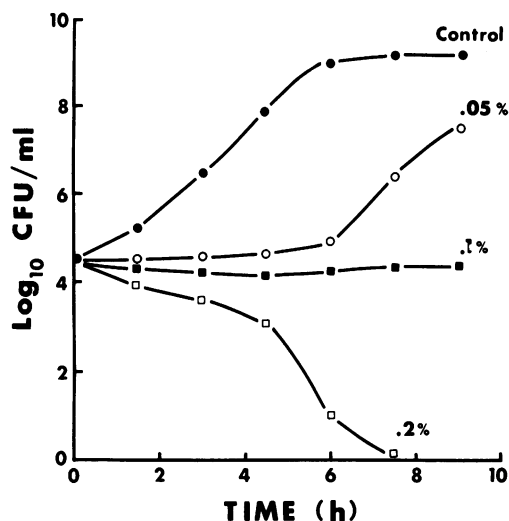


FIG. 6. Antimicrobial effect of pecan packing tissue on *S. senftenberg* 775W. Percentages indicate levels of tissue in TSY growth medium.

shell moisture content of 11.5% (6, 13). This higher moisture level is probably accompanied by higher water activity which, according to a report on the survival of *S. newport* in "dry" foods over a range of water activity (4), is likely to have a lethal effect. This line of reasoning may explain in part the decline in viable salmonellae on inshell pecans at all storage temperatures in test 1 compared to relatively little decline in population on nutmeats stored at -18 , -7 , and 5 C in test 2.

Data from this study point up the necessity for considering several factors when and if microbiological limits are established for pecans and possibly other tree nuts. Due to morphological differences among pecan cultivars, susceptibility to cracking and, hence, the potential for water uptake with subsequent microbial contamination differs greatly. Realizing that inshell pecans are often marketed as mixed variety lots, it will be important to design sampling schemes which reflect the true incidence of contamination over large quantities of product. Results from test 2 of the present study indicate that this criterion was not achieved. Storage conditions after processing affect the viability of all microbial contaminants on pecans. Response to environmental conditions by particular groups of organisms may differ, however, thus rendering total aerobic counts of questionable value in setting up microbiological limits on pecans. It is not reasonable to extrapolate from survival data presented here for sal-

monellae, or from survival studies involving other pathogenic organisms, a maximum total microbiological count which would insure pecans as being microbiologically safe from a public health standpoint. Additional research is required to determine the behavior of naturally occurring bacterial and fungal contaminants during pecan processing and storing.

On a practical level, results from this study indicate that presently established heat treatment procedures used during pecan processing are not adequate for the elimination of salmonellae from internally contaminated inshell nuts. However, morphological features and composition of nut packing tissue and shell do afford protection against high initial contamination and subsequent survival of salmonellae on the nutmeat. Surface to surface contact between contaminated shells and "clean" nutmeats during cracking and shelling is likely to result in nutmeats harboring salmonellae which are extremely tolerant to commercial refrigerated storage conditions. Destruction of these salmonellae will need to be achieved by means other than thermal treatment, such as gassing with propylene oxide, due to organoleptic quality deterioration in pecans subjected to temperatures sufficient for pasteurization.

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