Factors Affecting Infiltration and Survival of *Salmonella* on In-Shell Pecans and Pecan Nutmeats

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ABSTRACT

A study was done to determine the infiltration and survival characteristics of *Salmonella* in pecans. The rate of infiltration of water into in-shell nuts varied among six varieties evaluated and was significantly ($\alpha = 0.05$) affected by the extent of shell damage. The rate of infiltration at $-20$ or $4^\circ$C was lower than the rate of infiltration into nuts at $21$ or $37^\circ$C when nuts were immersed in water at $21^\circ$C. In-shell nuts immersed in a suspension of *Salmonella* (8.66 or 2.82 log CFU/ml) for 1 h contained populations of 6.94 to 6.99 and 1.85 to 1.95 log CFU/g, respectively. *Salmonella* that infiltrated in-shell nuts reached the kernel and remained viable after drying and during subsequent storage at 4°C. Initially high (5.78 log CFU/g) and low (1.53 log CFU/g) populations of *Salmonella* did not significantly decrease in in-shell pecans stored at $-20$ and 4°C for 78 weeks (18 months). Significant reductions of 2.49 and 3.29 log CFU/g occurred in in-shell nuts stored for 78 weeks at 21 and 37°C, respectively. High (6.16 log CFU/g) and low (2.56 log CFU/g) populations on pecan halves and high (7.13 log CFU/g) and low (4.71 log CFU/g) populations on medium pieces stored for 52 weeks at $-20$ and 4°C decreased slightly, but not always significantly. Significant reductions occurred on nutmeats stored for 52 weeks at 21 and 37°C, but the pathogen was detectable, regardless of the initial inoculum level. Results emphasize the importance of applying process treatments that will inactivate *Salmonella*.

Salmonellosis has historically been attributed to consumption of foods of animal origin. Outbreaks associated with fresh fruits and vegetables and other foods of plant origin, however, have been documented with increased frequency in recent years (24, 38). Several outbreaks of salmonellosis linked to consumption of foods with water activity (a$_w$) below the minimum for growth of *Salmonella* have been documented (45). Examples of low-a$_w$ foods implicated as vehicles in these outbreaks include almonds (11, 28), chocolate (19, 27, 30), potato chips seasoned with paprika (35), in-shell peanuts (33), a savory peanut snack (31), and peanut butter (12, 13, 44). *Salmonella* can survive at reduced a$_w$ for long periods (29, 32, 34), thereby representing a safety risk throughout the intended shelf life of a wide range of dry foods and food ingredients.

*Salmonella* has been isolated from almonds (11, 17, 21), cashew nuts and Brazil nuts (22, 37), macadamia nuts (46), walnuts (43), pistachio nuts (36), and mixed nuts (almonds, Brazils, cashews, peanuts, and walnuts) (37). Outbreaks of *Salmonella* Enteritidis infection associated with consumption of contaminated raw almonds in 2000 to 2001 (28), 2003 to 2004 (11), and 2005 to 2006 (41) have sparked interest in better understanding preharvest and postharvest conditions that may result in contamination and affect survival and inactivation of *Salmonella* on tree nuts in general. Reports on the prevalence and populations of *Salmonella* on raw almonds (17), persistence of *Salmonella* in almond orchards for at least 5 years (49), conditions affecting survival of the pathogen on stored almonds (48), and infiltration (15) and growth on shell and hull substrates (25, 51) have helped to define behavioral characteristics of the pathogen on almonds and in environments to which they are exposed. Thermal inactivation of *Salmonella* on almond shells and kernels has been described (20, 50). From a risk assessment model developed with data from the two almond-associated outbreaks (11, 28), it was concluded that a 4-log CFU/g reduction would result in an appropriately low safety risk for consumers (16).

A study on survival characteristics of *Salmonella* on surface-inoculated in-shell pecans (*Carya illinoinensis*) dates back to the 1970s (4). Decreases in populations of three serotypes of *Salmonella* on spray-inoculated, in-shell pecans and pecan nutmeats stored at freezing and refrigeration temperatures for 32 weeks were minimal. One of the three serotypes survived on pecan kernel halves stored at 21°C. Orchard contamination of pecans with *Escherichia coli* (39) and infiltration of in-shell pecans with microorganisms (6) have been reported. There are many variations in pecan growing, cleaning, storing, conditioning (tempering), shelling, and drying practices, which have potential for either enhancing or compromising microbiological safety. However, conditions affecting contamination, infiltration, and survival of *Salmonella* on and in in-shell pecans and pecan nutmeats have been given only meager research attention.

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To better understand the behavior of *Salmonella* on pecans and to develop background information of value when designing subsequent studies focused on determining the efficacy of processing practices in killing *Salmonella* on in-shell pecans and pecan nutmeats, we undertook a study to determine water and *Salmonella* infiltration characteristics of in-shell nuts and the effect of temperature on survival of the pathogen on and in in-shell pecans and nutmeats during long-term storage.

**MATERIALS AND METHODS**

**Source and varieties of pecans.** In-shell pecans (Elliott, Desirable, Moneymaker, Eastern Schley, Stuart, and Sumner varieties) and pecan nutmeats (Desirable variety) were obtained from commercial shelling companies in Georgia. Selection of varieties was based on factors such as high average tonnage produced annually and differences in shell thickness, shell-out percentage (percent of in-shell nut consisting of nutmeat), size, and shape of nuts.

Two U.S. Department of Agriculture standard grades (52) of nutmeats (kernels) were used, mammoth halves (250 halves or fewer per pound [551 or fewer per kg]) and medium pieces (maximum diameter, \(\gamma_{4/5} \) in. [0.95 cm] and minimum diameter, \(\gamma_{3/6} \) in. [0.48 cm]; i.e., will pass through a round opening 0.95 cm in diameter but not a round opening 0.48 cm in diameter).

In-shell pecans, mammoth halves, and medium pieces were stored at 4°C until used in various experiments. Moisture content, \(a_w\) (shell-out, percent kernel), and volume of the six varieties of in-shell pecans were measured. All six varieties of undamaged in-shell nuts at 20, 4, 21, and 37°C were used in experiments designed to determine the rate of infiltration of water at 21°C. Desirable variety in-shell pecans and nutmeats were used in all other experiments.

**Measurement of moisture content.** The moisture content of nutmeats in in-shell pecans as well as nutmeat halves and pieces was determined before, during, and after subjecting to various treatments. In-shell pecans were cracked with a mechanical cracker designed for home use, and nutmeats were separated from the shells, middle septum, and packing tissue. Nutmeats removed from in-shell pecans in the laboratory, as well as mammoth halves and medium pieces obtained from commercial shellers, were chopped in a One-Touch chopper (model HC306, Black and Decker, Towson, MD). The moisture content of halves and pieces was determined with a Mettler Toledo moisture analyzer (model HB43-S, Mettler Toledo, Greinfensee, Switzerland). Samples (5 g) were dried at 130°C for 5 to 7 min, depending on the initial moisture content. Weight loss was attributed to removal of water during drying. The percent moisture in nutmeats was calculated.

**Measurement of \(a_w\).** Subsamples of nutmeats prepared for moisture analysis were used to determine \(a_w\). Measurement of \(a_w\) was made with 3-g samples and an AquaLab water activity meter (model CX2, Decagon Devices, Inc., Pullman, WA).

**Measurement of percent kernel.** Twenty nuts each of six varieties (Elliott, Desirable, Moneymaker, Schley, Stuart, and Sumner) of pecans were weighed and mechanically cracked in the laboratory. Nutmeats were separated from the shells and middle septum tissue and weighed. The percent shell-out (percent kernel, by weight) was calculated.

**Measurement of in-shell pecan volume.** Groups of 20 undamaged pecans at 21°C of each of six pecan varieties were separately immersed in 1 liter of water at 21°C in a 2-liter graduated cylinder. The number of milliliters of water displaced by the pecans within 10 s was calculated by subtracting 1,000 from the level of water in the cylinder after immersing pecans. The average volume of each pecan within each variety was determined by dividing this value by 20. Values are reported as cubed centimeter per nut.

**Uptake of water by undamaged in-shell pecans.** It was hypothesized that the rate of infiltration of water, and any microorganisms it may contain, into in-shell pecans during cleaning and conditioning might vary among varieties and be affected by temperature differential between the nuts and the water in which they were immersed. The rate of uptake of water by six varieties (Elliott, Desirable, Moneymaker, Schley, Stuart, and Sumner) of undamaged in-shell pecans as affected by temperature differential between the nuts and the water in which they were immersed was determined. Nuts (200 g, 2.7 to 3.0% kernel moisture) were placed in bags fabricated in our laboratory from polypropylene mesh (Volv Bag Company, Inc., Guntersville, AL). Each bag was secured with a plastic-coated metal wire. Bags were placed in 1-gal (3.79-liter) Snap n’ Seal freezer bags (Kroger Co., Cincinnati, OH) and held at 20, 4, 21, and 37°C for 20 to 24 h. Nuts in mesh bags at each temperature were separately immersed, without agitation, in tap water (50 liters) at 21°C for 0, 1, 3, 8, 16, and 24 h. At the end of each immersion time, bags were drained and the pecans were weighed. The percent weight gain and the amount of weight gained (gram[s] per nut) were calculated.

In a second set of experiments, the effect of temperature of hot water on rate of uptake of water by undamaged in-shell pecans was determined. In-shell Desirable variety pecans (200 g) were placed in polypropylene mesh bags and adjusted to 4°C before immersing in tap water (50 liters) at 66, 71, 77, 82, 88, and 93°C for 0, 10, 20, 30, and 60 min. Bags of pecans were drained and weighed after each immersion time. The percent weight gain and the amount of weight gained (gram[s] per nut) were calculated.

**Uptake of water by damaged in-shell pecans.** In-shell Desirable variety pecans with visual damage were separated from undamaged nuts. Damaged nuts were separated into two categories, i.e., those with a cracked shell but no part of the shell missing, and those with part of the shell missing. Experiments were done to determine the rate of uptake of water (as described above) for undamaged in-shell pecans, with the exception that they were not immersed in water at 71, 77, and 88°C. The percent weight gain and the amount of weight gained (gram[s] per nut) were calculated.

**Uptake of water by nutmeats in undamaged in-shell pecans.** Experiments were done to determine the percent moisture in nutmeats in undamaged in-shell Desirable variety pecans affected by immersion in water at 21, 66, 82, and 93°C. The procedures for preparing and immersing nuts were as described above for determining water uptake by undamaged in-shell pecans in water at 21°C and in hot water. On removal from immersion water, nuts were cracked and the nutmeats were removed. The moisture content of chopped nutmeats was determined.

**Salmonellae used and preparation of inocula.** The inoculum contained a mixture of five *Salmonella* serotypes: Anatum, strain 6802, isolated from raw peanuts; Enteritidis, strain ATCC BAA-1045, from raw almonds; Oranienburg, strain 1839, from pecans; Sundsvall, strain 1659, from pecans; and Tennessee, strain K4643, a clinical isolate from a patient in an outbreak of salmonellosis associated with consumption of peanut butter.
All serotypes were grown at 37°C for 24 h in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) supplemented with nalidixic acid (50 µg/ml) (TSBN). One milliliter of culture of each serotype was surface spread on each of four large (150 by 15 mm) petri plates containing 60 ml of TSBN supplemented with agar (TSAN; 15 g/liter). Plates were incubated at 37°C for 24 to 26 h. Cells were grown on an agar medium rather than in broth, because at least one of the strains (Salmonella Enteritidis) used in the study appears to have increased resistance to drying on almonds when cells are grown on TSA rather than in TSB (48). To harvest cells, 5 ml of sterile 0.1% peptone was deposited on the lawn that had developed on the surface of each plate, and cells were suspended in the peptone by gently rubbing the lawn with a sterile glass rod. Cell suspensions harvested from four plates of each serotype were pooled and analyzed for populations of Salmonella (see below). Equal volumes (17 to 20 ml, depending on the experiment) of each serotype suspension were combined to give 85 to 100 ml of a five-serotype mixture. The population of Salmonella in this mixture was also determined. The suspension was used to inoculate in-shell pecans and nutmeats within 1 h after preparation.

Uptake of Salmonella by undamaged in-shell pecans. Uptake of salmonellae by undamaged Desirable variety in-shell pecans as affected by the temperature differential between the pecans and cell suspension in which they were immersed was determined. Undamaged Desirable variety in-shell pecans were immersed in suspensions containing high and low numbers of Salmonella. A high-population cell suspension was prepared by combining 12 ml of the five-serotype cell suspension prepared as described above, with 1,200 ml of sterile deionized water; a low-population suspension was prepared by serially diluting the five-serotype suspension by 10⁻⁶ in sterile deionized water and adding 12 ml of the diluted suspension to 1.2 liters of sterile deionized water. Populations of Salmonella in high and low inocula were determined (see procedure described below). Undamaged in-shell pecans (600 g) at 4 or 21°C were immersed in 1,200 ml of high- or low-inoculum suspension (21°C) for 1, 2, 4, 6, and 24 h. Pecans were removed from the cell suspension and drained. The weight of in-shell pecans and a₀ of nutmeats were determined. Duplicate samples (five pecans per sample, ca. 50 g) were analyzed for populations of Salmonella.

Location of Salmonella in inoculated undamaged in-shell pecans. A study was done to determine the number of Salmonella that reached the kernels of undamaged Desirable variety in-shell pecans immersed in a suspension of the pathogen. A high-population suspension (ca. 8 log CFU/ml) was prepared by adding 50 ml of the five-serotype mixture harvested from TSAN plates, as described above, to 5,000 ml of sterile deionized water. Pecans (2,500 g) at 21°C were immersed for 5 h in the suspension (21°C), drained, and dried at 30°C for 2 h in a forced-air Fisher Scientific Isotemp oven (model 851; Fisher Scientific, Dubuque, IA).

Moisture content and a₀ of nutmeats were determined immediately after immersing in-shell nuts in the suspension and after drying. Inoculated dry pecans were placed in 1-gal Snap n’ Seal bags and stored at 4°C for 3 to 5 weeks before analyzing the nutmeats and inedible portions for the presence and populations of Salmonella. With a Moto-Tool (model 395, type 4, Dremel, Racine, WI), a transverse cut was made around the circumference of the shell of five pecans (one five-pecan sample, ca. 50 g) midway between the base and apex. The two cotyledons (nutmeats), which constitute approximately 55% (wt/wt) of the in-shell nut, were removed from pecans in a way that they did not come in contact with the shell and had minimal contact with the middle septum tissue, and placed in a Stomacher 400 bag (Seward Medical Ltd., London). The inedible portion (shell, packing material, and middle septum tissue) of the five-pecan sample was placed in a second bag. Analyses for presence (by enrichment) and populations of Salmonella were done as described below.

Survival of Salmonella on and in undamaged in-shell pecans during long-term storage. A five-serotype suspension of Salmonella was harvested from TSAN plates, as described above. A high-population suspension was prepared by adding 80 ml of the suspension to 8 liters of sterile deionized water; a low-population suspension was prepared by serially diluting the five-serotype suspension by 10⁻⁶ and adding 80 ml of the diluted suspension to 8 liters of sterile deionized water. Populations of Salmonella in both suspensions were determined. Undamaged Desirable variety in-shell pecans (4,000 g) at 21°C were immersed in high- or low-inoculum suspension (21°C) for 4 h. Pecans were drained, and weight and a₀ were determined.

The wet pecans were placed in aluminum mesh baskets and dried, with occasional mixing, in a forced-air Fisher Scientific Isotemp oven at 30°C for 24 h. Triplicate samples (ca. 50 g), each consisting of five inoculated, dried pecans were analyzed for presence (by enrichment) and populations of Salmonella as described below. Counts obtained from these pecans were considered 0-day storage counts. Samples, each consisting of five pecans, were placed in 1-qt (0.95-liter) Snap n’ Seal freezer bags, sealed, double bagged in a second 1-qt freezer bag, placed in sets according to intended storage temperature in 1-gal Snap n’ Seal freezer bags, and stored at −20, 4, 21, and 37°C for up to 78 weeks (18 months) before analyzing for the presence (by enrichment) and populations of Salmonella.

Survival of Salmonella on pecan halves and medium pieces during long-term storage. The effect of temperature on survival of Salmonella on dry pecans (Desirable variety) nutmeats during long-term storage was determined. Five-serotype suspensions containing high or low populations of Salmonella were prepared (as described above) for survival studies using in-shell pecans. Mammoth halves (1,600 g) and medium pieces (1,600 g) at 21°C were separately placed in bags fabricated in our laboratory from fiberglass insect screen (Phifer, Inc., Tuscaloosa, AL) and immersed for 30 s, with constant gentle agitation, in 3,200 ml of either a low-population inoculum or a high-population inoculum, both at 21°C. Nutmeat halves and pieces were then placed in aluminum mesh baskets and dried, with occasional mixing, in a forced-air oven at 30°C for 20 and 27 h, respectively. Samples were analyzed for moisture content and a₀. Triplicate samples of dried halves and pieces were analyzed for the presence (by enrichment) and populations of Salmonella. Counts obtained from these nutmeats were considered 0-day storage counts. Samples of inoculated nutmeats, each consisting of 25 g, were placed in 1-qt Snap n’ Seal freezer bags, sealed, double bagged in a second 1-qt freezer bag, placed in sets according to intended storage temperature in 1-gal Snap n’ Seal freezer bags, and stored at −20, 4, 21, and 37°C up to 52 weeks before analyzing for the presence (by enrichment) and populations of Salmonella.

Detection and enumeration of Salmonella in in-shell pecans. Duplicate samples, each consisting of five undamaged Desirable variety in-shell nuts (ca. 50 g), were analyzed for presence (by enrichment) and populations of Salmonella. For studies focused on determining uptake of Salmonella as affected by the temperature differential between in-shell pecans and the immersion suspension, samples were analyzed immediately after
immsersing nuts in inoculum for 1, 2, 4, 6, and 24 h. In studies designed to determine survival of *Salmonella* on dry in-shell pecans stored for 2, 5, 10, 16, 24, 36, and 78 weeks, as affected by storage temperature (−20, 4, 21, and 37 °C), nuts were removed from storage and brought to 22 ± 1 °C for 1 to 2 h before analysis.

Each in-shell pecan in the five-pecan sample was crushed with a hammer, and the contents of each bag were transferred to a Stomacher 400 bag. Two hundred milliliters of lactose broth (Difco, Becton Dickinson) supplemented with nalidixic acid (LBN; 50 µg/ml) was added to each bag, and the mixture was shaken vigorously by hand for 30 s. After 3 to 5 min without shaking, the mixture was again shaken vigorously for 30 s before removing samples of the LBN wash for sprial plating (WASP2, Microbiology International, Frederick, MD) or surface plating (quadruplicate 0.25-ml samples and duplicate 0.1-ml samples) on TSAN, and bismuth sulfite agar (BSA; Difco, Becton Dickinson) supplemented with nalidixic acid (BSAN; 50 µg/ml). Samples of LBN wash (0.1 ml, in duplicate) from high-inoculum pecans were also serially diluted in sterile 0.1% peptone water and surface plated (0.1 ml, in duplicate) on TSAN and BSAN. Bags containing the mixture of crushed pecans and LBN, as well as TSAN plates, were incubated at 37 °C for 24 h; BSAN plates were incubated at 37 °C for 48 h. Colonies formed on TSAN and BSAN that were presumptive positive for *Salmonella* were counted. If colonies presumptive for *Salmonella* did not develop on TSAN, the preenriched LBN was streaked on BSAN. Plates were incubated at 37 °C for 48 h before examining for colonies presumptive for *Salmonella*. Cells from selected presumptive-positive colonies were subjected to confirmation tests by using BBL Enterotube II (Difco, Becton Dickinson) or API 20E (bioMérieux Vitex, Hazelwood, Mo.) assays, and the *Salmonella* latex agglutination test (Oxoid, Ltd., Basingstoke, UK). For samples anticipated to contain *Salmonella* at populations not detectable by direct plating on TSAN or BSAN, preenriched LBN cultures were enriched by transferring 1.0 ml and 0.1 ml to 10 ml of tetrathionate broth (Difco, Becton Dickinson) and 10 ml of Rappaport-Vassiliadis broth (Difco, Becton Dickinson), respectively; tetrathionate and Rappaport-Vassiliadis broths were incubated at 37 and 42 °C, respectively, for 24 h before streaking on BSAN. Plates were incubated at 37 °C for 48 h before examining for *Salmonella* colonies and confirming identity by using Enterotube II or API 20E assay kits and the agglutination test. The detection limit for enumerating *Salmonella* by direct plating was 4 CFU/g of in-shell pecans. The detection limit by enrichment was 1 CFU per five in-shell pecans (1 CFU per ca. 50 g of pecans).

**Detection and enumeration of *Salmonella* in halves and pieces.** In studies involving determination of survival of *Salmonella* on inoculated halves and pieces, duplicate 25-g samples stored at −20, 4, 21, and 37 °C for 2, 5, 10, 16, 24, 36, and 52 weeks were brought to room temperature (22 ± 1 °C) before analyzing for the presence and populations of *Salmonella*. Each sample was placed in a Stomacher 400 bag with 100 ml of LBN and pummeled for 1 min at normal speed. Populations of *Salmonella* in the homogenate were determined by plating samples on TSAN and BSAN as described above. Enriched samples were streaked on BSAN to determine the presence of *Salmonella*. Positive-presumptive colonies detected by direct plating or enrichment were subjected to confirmation tests. The detection limit for enumerating *Salmonella* in nutmeats by direct plating was 4 CFU/g. The detection limit by enrichment was 1 CFU/25 g.

**Detection and enumeration of internalized *Salmonella*.** In studies designed to determine the location of *Salmonella*, i.e., inedible portion versus nutmeat of in-shell pecans that had been immersed in a suspension containing the pathogen, dried, and stored at 4 °C for 3 to 5 weeks, the inedible portions (ca. 25 g), and the nutmeats (ca. 25 g) of five-pecan samples were separately placed in Stomacher 400 bags containing 100 ml of LBN. The mixtures were shaken vigorously by hand before removing samples of the LBN wash and analyzing for presence and populations of *Salmonella*, as described above. The detection limit for enumerating *Salmonella* by direct plating was 4 CFU/g of inedible material or nutmeat. The detection limit by enrichment was 1 CFU per ca. 25 g of inedible material or nutmeat.

**Statistical analysis.** Experiments were replicated three to six times. Values from duplicate or triplicate samples representing each test parameter combination in each replicate trial were analyzed with a general linear model on SAS software (version 8.0, SAS Institute Inc., Cary, NC). The least significant difference test was used to determine significant differences (α = 0.05) in mean values.

**RESULTS AND DISCUSSION**

**Physical characteristics.** The size, shape, shell thickness, and other physical and sensory characteristics of pecans differ greatly among varieties. These differences could result in different rates of infiltration of water, and presumed uptake of *Salmonella* and other microorganisms that may be in the water, during preharvest exposure to rain or soil surface water, and during postharvest cleaning and conditioning operations. Initial experiments were focused on measuring physical characteristics and shell-out percentage of the six pecan varieties selected for water-infiltration studies.

Some of the physical characteristics of the six varieties of in-shell pecans evaluated are listed in Table 1. These varieties are among those most commonly grown commercially in the United States. Varieties are listed in order of number of nuts per pound or kilogram, with the Elliott variety being the smallest (174 nuts per kg) and the Stuart variety the largest (104 nuts per kg). Average weights for these two varieties are 5.8 g per nut and 9.6 g per nut, respectively. Nut volumes ranged from 9.7 cm³ (Elliott variety) to 15.0 cm³ (Desirable variety). Nut volume of test varieties is not directly correlated with the number of nuts per kilogram or weight per nut. Likewise, the shell-out percentage is not directly correlated with number of nuts per kilogram, weight per nut, or nut volume. Schley nuts, which have a characteristically thin shell, had a significantly (α = 0.05) higher shell-out percentage (61.2%) than had the other varieties. The shell-out percentage (46.4%) of Moneymaker nuts was significantly lower than that of Stuart nuts, which in turn was significantly lower than the other four varieties. While these characteristics are not entirely independent of each other, they do reflect morphological, structural, and size differences among the six varieties, which may affect the rate of infiltration of water and microorganisms it may contain.

**Rate of water uptake.** Shortly after pecans are harvested by mechanical sweepers, they are subjected to a cleaning process to remove sticks, stones, and other debris.
Separation is based on differences in density of pecans and inedible materials, and is achieved by immersion in water or by an air flotation process. Just prior to cracking and shelling, nuts are conditioned (tempered) by spraying with water or immersing in chlorinated water at ambient or elevated temperature for up to 24 h, depending on the water temperature, during which the moisture content of kernels increases to 5 to 9%, making them less prone to shattering during cracking. The rate of uptake of water by in-shell pecans, and thus the potential for uptake of *Salmonella* and other microorganisms that may be present, might be affected by pecan variety and would likely affect the conditions needed for effective cleaning by water immersion and for the conditioning process. The rate of uptake of water could also affect the amount of potentially contaminated water taken up by pecans in contact with orchard soil before harvesting.

A series of experiments was done to determine if the rate of water uptake is affected by varietal differences in undamaged in-shell pecans and by temperature differential between pecans and water. Each pecan variety was adjusted to \(-20, 4, 21, \) and \(37^\circ C\) before immersing in water at \(21^\circ C\) for up to 24 h. Results are shown in Figure 1. At the same initial nut temperature, water uptake by Moneymaker, Stuart, and Sumner nuts tended to occur at a slower rate, as compared with uptake by the other varieties, indicating that water-infiltration characteristics are not strongly correlated with any of the specific physical attributes listed in Table 1. Overall, the rate of uptake water, as well as the total amount of water that infiltrated pecans, tended to be higher in Desirable pecans, regardless of the initial nut temperature.

Shown in Figure 2 are water-infiltration characteristics, presented as mean values for all six varieties at initial temperatures of \(-20, 4, 21, \) and \(37^\circ C\). Undamaged in-shell pecans at temperatures initially lower (\(-20 \) and \(4^\circ C\)) than the temperature of the immersion water (\(21^\circ C\)) took up significantly (\(\alpha = 0.05\)) less water, as reflected by percent weight gain, as compared with pecans initially at \(21\) or \(37^\circ C\). Enhanced infiltration of microorganisms in water suspensions at temperatures lower than the temperatures of several raw fruits and vegetables, e.g., apples (9), mangoes (8), tomatoes (1), and lettuce (47), immersed in these suspensions has been described. The negative pressure in internal tissues of produce caused by this temperature

<table>
<thead>
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<th>Variety</th>
<th>No./lb</th>
<th>No./kg</th>
<th>Wt (g/nut)</th>
<th>Vol (cm³/nut)</th>
<th>Nutmeat (% of in-shell nut)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elliott</td>
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<td>174 A</td>
<td>5.8 D</td>
<td>9.7 E</td>
<td>53.4 B</td>
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<td>9.6 A</td>
<td>13.3 B</td>
<td>49.0 C</td>
</tr>
</tbody>
</table>

* Mean values in the same column that are not followed by the same letter are significantly (\(\alpha = 0.05\)) different.
* Shell-out percentage, i.e., percent (by weight) of in-shell nut consisting of nutmeat.

**FIGURE 1.** Weight gain (percentage) in six varieties (Elliott, Desirable, Moneymaker, Schley, Stuart, and Sumner) of undamaged in-shell pecans as affected by initial temperatures \(-20, 4, 21, \) and \(37^\circ C\) when immersed in water at \(21^\circ C\) for up to 24 h.
differential results in enhanced uptake of the suspension. A positive differential, i.e., the temperature of the suspension is higher than the temperature of the produce, on the other hand, acts to limit uptake of the suspension. It appears that this law of physics also applies to in-shell pecans. Movement of water contaminated with E. coli into black walnuts by internal aspiration resulting from lower ambient temperatures in the environment, as when warm walnuts are exposed to cold water, has also been offered as an explanation for infiltration (40).

Conditioning of pecans is sometimes done with hot water rather than cool water at temperatures that fluctuate, depending on the source and season. The amount of time needed to increase the moisture content of kernels to levels that will result in minimal shattering during cracking can be reduced substantially by using hot-water rather than cool-water conditioning, thereby facilitating throughput in cracking and shelling operations. Theoretically, the use of hot water to condition nuts may have an added advantage of causing thermal inactivation of Salmonella and other microorganisms that might be present on the shell surface or on internal tissues, including nutmeats. Shown in Figure 3 are weight gains (percentage) by undamaged in-shell Desirable pecans immersed in water at 66, 71, 77, 82, 88, and 93°C for up to 1 h. The initial temperature of nuts was 4°C. Depending on the water temperature, weight gains increased by 4.0 to 4.3% within 10 min, and 6.3 to 8.7% within 1 h. This compares to a weight gain of 4.3% after immersion of Desirable nuts initially at 4°C in water at 21°C for 1 h, and subsequently 6.7% after immersion for 3 h (Fig. 1). On a commercial level, the presence of viable Salmonella in hot treatment water, even at the low end of a range of 66 to 93°C, is unlikely. However, the effectiveness of conditioning pecans in hot water as a process for killing Salmonella that may have infiltrated nuts during some point previous to the treatment remains to be determined.

As much as 40% of some varieties of pecans may have cracked shells at the point they enter the conditioning treatment (42). Early harvesting or early maturity of thin-shelled varieties with a tendency toward high percentage of kernel fill will often crack when nuts are dislodged from the trees on sunny days (53). Damage may also occur during harvesting or during subsequent cleaning and handling. Uptake of water potentially contaminated with Salmonella or other microorganisms would be predicted to be more rapid and extensive in damaged nuts than in undamaged nuts. Figure 4 shows water-infiltration characteristics of undamaged in-shell Desirable pecans, pecans with cracked shells, and pecans with pieces of the shell missing. The initial temperatures of nuts were −20, 4, 21, and 37°C, and the temperature of the immersion water was 21°C. Regardless of the extent of damage, infiltration was enhanced, in some cases significantly (α = 0.05), when the initial temperature of the nuts was at 21 or 37°C, as compared with initial temperatures of −20 or 4°C. This is in general agreement with observations on the effects of temperature differential on rate of uptake of water by undamaged nuts (Fig. 2). Not surprisingly, water was taken up at a significantly (α = 0.05) more rapid rate by nuts with pieces of shell missing than it was by cracked nuts which, in turn, took up water more rapidly than did undamaged nuts. Depending on the initial temperature of the nuts, weight gains by undamaged pecans, pecans with cracked shells, and pecans with pieces of shell missing were 15.7 to 20.0%, 34.7 to 42.3%, and 49.0 to 53.3%, respectively, after immersion in water for 24 h. Percent weight gains (means of nuts at four initial temperatures) by undamaged in-shell Desirable pecans, cracked pecans, and pecans with pieces of shell missing when immersed in water 21°C for up to 24 h are shown in Figure 5. Clearly, damaged in-shell nuts allow larger amounts of water to infiltrate during immersion, thereby potentially resulting in a higher risk of microbial contamination of nutmeats. To reduce this risk, attempts should be made to efficiently segregate damaged nuts from undamaged nuts before the shelling operation.

Shown in Figure 6 are changes in kernel moisture content and a_w of nuts initially at −20, 4, 21, and 37°C on immersion in water at 21°C for up to 24 h. The moisture content increased from 2.7%, to 5.0 to 7.0% within 8 h, 7.3 to 12.3% within 16 h, and 8.9 to 11.9% within 24 h. Breakage of kernels during cracking is minimal in a
moisture range of 5 to 9%, depending on the variety, shell-out percentage, type of cracking equipment, and other factors. The initial aw (0.35) increased to 0.96 within 24 h.

We also determined the amount of water taken up by damaged in-shell Desirable pecans (4°C) immersed in hot water (66 to 93°C). As with nuts immersed in water at 21°C (Figs. 4 and 5), immersion of damaged pecans initially at 4°C in water at 66 to 93°C resulted in larger amounts of the water taken up, as compared with the amount taken up by undamaged pecans (Fig. 7). Infiltration was more rapid and extensive in nuts immersed in hot water for 60 min versus 21°C water for 24 h. A larger amount of water, as evidenced by weight gain, was taken up by undamaged pecans and pecans with pieces of shell missing when pecans were immersed in water at 93°C than in water at 66°C. To reduce the risk of infiltration of microorganisms, attempts should be made at a commercial level to greatly minimize the percentage of damaged nuts before they are subjected to the conditioning treatment, regardless of the temperature of the conditioning water.

Changes in moisture content and aw of nutmeats in in-shell pecans initially at 4°C when immersed in water at 66, 82, and 93°C are shown in Figure 8. The initial kernel moisture content of 2.6% increased to 4.1, 4.9, and 5.7% within 60 min in nuts immersed in water at 66, 82, and 93°C, respectively. Immersion times of 30 and 60 min at 93 and 82°C, respectively, were needed to increase the moisture content to a level that would minimize shattering of kernels during cracking and shelling. In addition, subjective evaluation of kernels revealed that immersion of in-shell nuts in water at 82 and 93°C for 60 min caused the kernel testae to darken in color, thereby compromising aesthetic quality.

Uptake of Salmonella. Throughout the study, higher numbers of Salmonella on in-shell pecans and pecan nutmeats were recovered on TSAN than on BSAN. In some instances, counts obtained on the two media were significantly (α = 0.05) different. This is attributed in part to the inability of desiccation- and cold-stressed cells to

FIGURE 4. Weight gain (percentage) by undamaged in-shell Desirable pecans, pecans with cracked shells, and pecans with pieces of the shell missing when immersed in water at 21°C for up to 24 h. The initial temperatures of nuts were −20, 4, 21, and 37°C.

FIGURE 5. Weight gains (percentage, means of nuts at initial temperatures of −21, 4, 21, and 37°C) by undamaged in-shell Desirable pecans, cracked pecans, and pecans with pieces missing when immersed in water at 21°C for up to 24 h.

FIGURE 6. Changes in moisture content (open symbols) and aw (closed symbols) of nutmeats of undamaged Desirable in-shell pecans initially at −20, 4, 21, and 37°C when immersed in water at 21°C for up to 24 h.
resuscitate and form colonies on BSAN. Only counts obtained from plating samples on TSAN are reported.

Attachment and infiltration of *Salmonella* into undamaged in-shell Desirable pecans as affected by initial nut temperature (4 and 21°C) when nuts were immersed in suspensions (21°C) containing high (8.66 log CFU/ml) and low (2.82 log CFU/ml) numbers of the pathogen were studied. Nuts immersed in a high-population inoculum for 1 h contained a high number of *Salmonella* (6.94 to 6.99 log CFU/g), regardless of the initial temperature of the nuts, and there was little change in population after immersion for an additional 23 h (Table 2). In-shell pecans immersed in a low-inoculum suspension for 1 h had *Salmonella* counts of 1.85 to 1.95 log CFU/g, which did not change significantly after immersion for an additional 5 h. Counts increased significantly (α = 0.05) to 3.14 to 3.39 log CFU/g between 6 and 24 h of immersion. The initial temperature (4 or 21°C) of pecans did not significantly affect the number of *Salmonella* recovered after immersion in suspension for a given time.

These results, along with observations of water uptake by nutmeats in in-shell pecans, suggest that *Salmonella* might reach the nutmeats in undamaged in-shell nuts immersed in water containing the pathogen. We did experiments to determine if *Salmonella* reaches the nutmeats of in-shell Desirable pecans immersed in a suspension of the pathogen. Inoculated pecans (nutmeat moisture of 3.78%, aw of 0.50) were stored at 4°C for 3 to 5 weeks before shells and middle septum tissues were separated from the kernels, taking care to not cross-contaminate the kernels. The number of *Salmonella* recovered from the nutmeats (4.25 log CFU/g) was significantly (α = 0.05) lower than was the number recovered from the inedible portions of nuts (5.62 log CFU/g). Results show, however, that *Salmonella* can infiltrate undamaged in-shell pecans, reach the nutmeats, and survive on the nutmeats for at least 5 weeks at 4°C. This is in agreement with observations that *Salmonella Enteritidis* PT30, one of the strains in the five-serotype inoculum used in our study, can migrate through almond hulls and shells (15). Motile as well as nonmotile strains of *Salmonella Typhimurium* migrated through the almond shell, indicating that infiltration may be a passive process. *Salmonella Enteritidis* migrated through whole almonds, i.e., intact hull, shell, and kernel, to reach the kernels during immersion in a cell suspension for 24 to 72 h.

The interior of English walnut shells can become contaminated with *Salmonella* when exposed to contaminated water or wet, contaminated soil (7). Contamination rates are higher when shells are damaged or when nuts are exposed to higher levels of moisture. Meyer and Vaughn (40) conducted experiments to determine if *E. coli* could penetrate to nutmeats of black walnuts that, by all outward appearances, were sealed. Nutmeats of all hand-hulled walnuts soaked in a suspension of *E. coli* for several days were positive for the bacterium. Infiltration was observed within 4 h. It was concluded that movement of microorganisms into in-shell black walnuts could be by capillary

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**FIGURE 7.** Weight gain (percentage) by undamaged in-shell Desirable pecans, pecans with cracked shells, and pecans with pieces of shells missing when immersed in water at 66 to 93°C for up to 60 min. The initial temperature of nuts was 4°C.

**FIGURE 8.** Changes in moisture content (open symbols) and aw (closed symbols) of nutmeats of undamaged Desirable in-shell pecans initially at 4°C when immersed in water at 66, 82, and 93°C for up to 60 min.
TABLE 2. Number of Salmonella recovered from undamaged Desirable variety in-shell pecans initially at 4 and 21 °C and immersed in high- and low-inoculum suspensions (21 °C) for up to 24 h

<table>
<thead>
<tr>
<th>Salmonella in immersion suspension (log CFU/ml)</th>
<th>Initial nut temp (°C)</th>
<th>Salmonella recovered from pecans (log CFU/g)*</th>
</tr>
</thead>
</table>

*Comparison of immersion time: Mean values in the same row that are not followed by the same letter are significantly (α = 0.05) different. Comparison of nut-immersion suspension temperature differential: Within the same immersion suspension population, mean values in the same column that are not preceded by the same letter are significantly different.

action or by internal aspiration resulting from a lower ambient temperature in the environment, as when warm walnuts are exposed to cold contaminated water. Our observations indicate that infiltration of water into undamaged in-shell pecans at temperatures initially lower (−20 and 4 °C) than the temperature (21 °C) of water in which they are immersed is less than that of pecans at temperatures initially higher (21 and 37 °C) than the immersion water. Observations on almonds, walnuts, and pecans support the hypothesis that microorganisms can infiltrate undamaged in-shell nuts and reach the nutmeats.

Others, however, have reported that bacteria do not infiltrate in-shell pecans. Nutmeats aseptically removed from pecans that had been immersed in a suspension of E. coli were negative for the organism (39). In the same study, the shells of 24% of in-shell pecans soaked in water opened along shell suture lines. One would expect that microorganisms in the water would enter the in-shell nut and reach the nutmeat because of this loss of shell integrity. In another study, bacteria were not isolated from nutmeats aseptically removed from in-shell pecans (14). In these studies (14, 39), in-shell pecans were "surface sterilized" by treating in mercuric chloride solutions before nutmeats were subjected to microbial analysis. Based on our observations on infiltration of water containing Salmonella into pecans, it is likely that mercuric chloride solution would infiltrate the nuts, thereby also killing microorganisms on the surface of the kernels. If this occurred, it is not surprising that nutmeats were negative for E. coli and other microorganisms. Blanchard and Hanlin (6) concluded that analysis of surface washings from pecan nutmeats does not give an accurate picture of the total bacterial population. Internalized bacteria were detected in up to 75% of pecan kernels (halves) that had been surface sterilized by hypochlorous acid and ethanol, and treated with propylene oxide.

Survival of Salmonella on dry in-shell pecans. Survival curves for Salmonella on undamaged in-shell Desirable variety pecans stored at −20, 4, 21, and 37 °C for up to 78 weeks are shown in Figure 9. The moisture contents of nutmeats in these nuts in the three replicate trials were 3.2 to 3.6%, and the aw values were 0.43 to 0.51. The number of Salmonella recovered from high-inoculum (5.78 log CFU/g) and low-inoculum (1.53 log CFU/g) in-shell pecans stored at −20 and 4 °C for 78 weeks did not decrease significantly (α = 0.05). Populations of Salmonella on high-inoculum pecans stored at 21 and 37 °C decreased significantly within 36 and 10 weeks, respectively. Reductions of 2.49 and 3.29 log CFU/g occurred in high-inoculum nuts stored at 21 and 37 °C, respectively, for 78 weeks. Salmonella was detected in low-inoculum pecans stored at 21 and 37 °C for 78 weeks, but only by enrichment (≥1 CFU/50 g).

In a previous study (4), we observed that populations of salmonellae on the surface of spray-inoculated in-shell pecans stored at −18, −7, and 5 °C for 32 weeks decreased by approximately 1.5 to 3.3 log CFU/g. Decreases were greater as the storage temperature was increased. Initial populations of Salmonella Senftenberg and Salmonella Anatum at 3.3 and 4.2 log CFU/g, respectively, decreased to undetectable levels on nuts stored at 21 °C for 16 weeks. Salmonella Typhimurium decreased from an initial population of 5.1 log CFU/g to populations of 1.2 log CFU/g within 16 weeks, and 0.8 log CFU/g after storage of pecans for 32 weeks. These trends in loss of viability of Salmonella on surface-inoculated in-shell pecans as affected by temperature are in general agreement with observations from our current study using immersion-inoculated pecans.
Salmonella. Storage of in-shell pecans at refrigeration or freezing temperatures preserves the viability of Salmonella. Storage at these temperatures also preserves the sensorial quality of pecans (2, 26), however, so storage at elevated temperature is not a realistic solution to reduce or eliminate Salmonella. The pathogen also has been shown to survive on in-shell walnuts (7). Salmonella Enteritidis PT30 was observed to survive on walnuts stored at 23°C for more than 1 year.

Survival of Salmonella on dry pecan halves and pieces. Shown in Figure 10 are survival curves for Salmonella on mammoth pecan halves and medium pieces (aw of 0.51 to 0.63, 3.3 to 3.9% moisture) stored at −20, 4, 21, and 37°C for up to 52 weeks. The initial population was 6.16 log CFU/g. As with in-shell pecans (Fig. 9), survival of Salmonella on nutmeats was enhanced at refrigeration or freezing temperatures (Fig. 10). Significant (α = 0.05) decreases (0.48 to 0.69 log CFU/g) in numbers of the pathogen recovered from high-inoculum halves stored at −20 and 4°C occurred within 36 weeks; significant decreases occurred within 10 and 2 weeks on pecans stored at 21 and 37°C, respectively. Reductions of 0.31, 0.42, 2.13, and 2.83 log CFU/g occurred within 52 weeks on pecan halves stored at −20, 4, 21, and 37°C, respectively. After a significant decrease (0.69 to 1.58 log CFU/g) in Salmonella on low-inoculum halves stored at −20, 4, and 21°C for 2 weeks, populations did not change significantly during storage for an additional 22 weeks. Counts on low-inoculum halves stored at 37°C decreased significantly within 2 weeks and again between 2 and 24 weeks. The initial population (2.56 log CFU/g) on low-inoculum halves stored at 21 and 37°C for 52 weeks steadily decreased to levels detectable only by enrichment. Trends in loss of viability of Salmonella on pecan pieces as affected by temperature during long-term storage were similar to those observed for pecan halves. Other microorganisms are known to survive on pecan nutmeats stored at −20°C for 25 years (23).

Salmonellae have been reported to survive in high numbers on spray-inoculated pecan halves stored at −18, −7, and 5°C for at least 32 weeks (4). Populations of two of three serotypes decreased by approximately 5.3 log CFU/g of halves stored at 21°C for 24 weeks, which is substantially greater than the reduction (1.5 to 2.0 log CFU/g) observed for immersion-inoculated halves in the present study. Cells used in the spray inoculum were grown in TSB supplemented with yeast extract rather than on TSA, as was done in the present study, thereby possibly being less tolerant to desiccation during storage. In addition, the extent of infiltration of cells into tissues may be different in spray-versus immersion-inoculated halves, thereby affecting viability during long-term storage. No significant reductions in Salmonella Enteritidis PT30 were observed on almond kernels stored at −20 and 4°C for 550 days (approximately 18 months) (48). Cooler temperatures also favor survival of Salmonella in almond-orchard soils (19). Biphasic survival curves for Salmonella Enteritidis were reported for almonds stored at 23 and 35°C (48). Our observations on survival of salmonellae on pecan nutmeats stored at 21 and 37°C are in agreement with these findings. Populations of Salmonella Enteritidis have been reported not to change significantly on walnut kernels stored at 23°C for 3 weeks (7). Initially, at 1.5 log CFU/g of peanut butter, Salmonella survives for at least 24 weeks at 5°C (10). At a higher initial population (5.7 log CFU/g), the pathogen decreased by 4.2 to 4.5, and 2.9 to 4.3 in peanut butter and peanut spreads stored for 24 weeks at 21 or 5°C, respectively.

As with pecans, E. coli and Salmonella survives best on and in other nuts and nut products at reduced aw and at refrigeration or subfreezing temperatures. The moisture contents of pecan halves and pieces used in our study were 3.4 to 3.8%, and 3.3 to 3.9%, respectively; aw values were 0.51 to 0.58, and 0.55 to 0.63, respectively. Pecan nutmeats containing about 3.4 and 3.9% moisture and 76 and 72% oil, respectively, equilibrate at aw 0.68 at 21°C (3), suggesting that the nutmeats used in our study contained less than 72% oil. Water activity less than 0.68 will prevent or greatly retard the growth of molds on pecans (5). Survival of E. coli on pecan halves is enhanced at −7, 0, and 14°C, as compared to storage at 21 or 30°C (2). Survival was also favored in halves containing 3.5% moisture versus halves containing 4.5 or 6.2% moisture.
Observations on infiltration characteristics of pecans as well as on the behavior of *Salmonella* in pecans and other types of nuts and nut products further demonstrate the pathogen’s ability to survive for extended periods in low-a_\text{w}\_ foods and reinforces the importance of applying process treatments that will result in its inactivation. Information on the effectiveness of chlorinated water in killing *Salmonella* and other foodborne pathogens on pecans subjected to cleaning, conditioning, and flotation treatments used in the pecan industry is lacking. Reports describing the effectiveness of hot-air drying in killing *Salmonella* on pecan nutmeats after shelling operations have not appeared in the scientific literature. Studies to determine the behavior of *Salmonella* on in-shell pecans and pecan nutmeats on exposure to conditions mimicking those used in the pecan industry are underway in our laboratory.

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