Survival of *Salmonella, Escherichia coli O157:H7*, and *Listeria monocytogenes* on Raw Peanut and Pecan Kernels Stored at $-$24, 4, and 22°C

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ABSTRACT

Cocktails of lawn-collected cells were used to determine the survival of *Salmonella, Escherichia coli O157:H7*, and *Listeria monocytogenes* on the surface of raw peanut and pecan kernels. Kernels were inoculated with mixtures of four to five strains at 3 or 6 log CFU/g, dried at room temperature, and then stored at $-$24 $\pm$ 1, 4 $\pm$ 2, and 22 $\pm$ 1°C for 28 or 365 days. In most cases, rates of decline of the pathogens did not differ significantly between the two inoculum concentrations in the 28-day study. At 6 log CFU/g, populations of all pathogens were reduced by 0.5 to 1.6 log CFU/g during an initial 3-day drying period on both peanuts and pecans. The moisture content of peanuts and pecans remained stable at $-$24 $\pm$ 1 and 22 $\pm$ 1°C; at 4 $\pm$ 2°C, the moisture content increased from 3.8 to 5.6% on peanuts and from 2.6 to 3% on pecans over 365 days. Pathogen populations were stable on pecans stored under frozen and refrigerated conditions, except for *L. monocytogenes*, which declined at a rate of 0.03 log CFU/g/30 days at 4 $\pm$ 2°C. *Salmonella* populations were stable on peanuts stored at $-$24 $\pm$ 1 and 4 $\pm$ 2°C, but *E. coli O157:H7* and *L. monocytogenes* declined at rates of 0.03 to 0.12 log CFU/g/30 days. At 22 $\pm$ 1°C, *Salmonella, E. coli O157:H7*, and *L. monocytogenes* declined at a rate of 0.22, 0.37, and 0.59 log CFU/g/30 days, respectively, on peanuts, and at 0.15, 0.34, and 1.17 log CFU/g/30 days, respectively, on pecans. *Salmonella* counts were above the limit of detection (0.30 log CFU/g) throughout the study. In most cases during storage, counts obtained from pecans were higher than from peanuts.

Almonds, cashews, coconut, hazelnuts, pine nuts, pecans, pistachios, sesame seeds, and peanuts, as well as several processed nut products, have been associated with foodborne outbreaks and/or recalls after isolation of foodborne pathogens (19, 28). The majority of these outbreaks and recalls have been associated with *Salmonella*, and many of the outbreaks have lasted for months and have included cases from multiple states in the United States (10–12, 14–17) and/or from other countries (9, 20, 22, 24). Outbreaks and recalls due to *Escherichia coli O157:H7* (8, 13) and recalls due to *Listeria monocytogenes* contamination (35) also are documented for some nuts and nut products. The low water activity of nuts and nut products prevents multiplication of microorganisms (4); however, the survival of *Salmonella, E. coli O157:H7*, and *L. monocytogenes* has been documented on almond kernels for at least 365 to 550 days at $-$19, 4, 24, and 35°C (21), on pistachios for at least 365 days at $-$19, 4, and 24°C (21), and on walnut kernels for at least 365 days at $-$20, 4, and 23°C (5). *Salmonella* can survive in peanut butter and peanut butter spreads for at least 168 days at 5 and 21°C (6). At $-$20, 4, 21, and 37°C, *Salmonella* survived on pecan halves and pieces for 365 days and on in-shell pecans for 550 days (3).

The shelf life of peanuts and pecans is influenced by initial moisture as well as humidity and temperature of storage. Pecan handlers generally store bulk raw product under controlled conditions to maintain quality. The common storage temperatures (and suggested storage times) for pecans, both in-shell and kernels, include $-$18°C (for up to 6 to 8 years), 0°C (for about 12 to 18 months), or ambient (for about 3 to 6 months) (30). Common storage temperatures (and suggested times) for raw peanuts, both in-shell and kernels, include $-$18°C (for 2 to 10 years), 1 to 5°C (for approximately 1 year), or ambient (up to 6 months) (26). After processing and packaging, both raw and roasted nuts are typically stored under ambient conditions at the retail level; consumers may store nuts under ambient, refrigerated, or freezer conditions for extended periods (25). In general, *Salmonella* survives on nut surfaces at lower temperatures (i.e., refrigeration or freezer conditions) without significant declines in populations over time, whereas slow, but significant, reductions are typically observed at ambient temperatures, 21 to 25°C (3, 5, 21, 33).

Data on the behavior of foodborne pathogens on peanut and pecan kernels at common storage temperatures are

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MS 14-327: Received 10 July 2014/Accepted 28 September 2014
limited. The objectives of this study were to determine the survival of *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* on inoculated peanut and pecan kernels during ambient, refrigerated, and frozen storage.

**MATERIALS AND METHODS**

**Nuts.** Raw pecan (*Carya illinoinensis*) halves and peanut (*Arachis hypogaea* *L. hypogaea*) kernels were obtained from nut shellers in the southeastern United States. Raw pecan halves were mammoth size (14 to 15 halves per 28 g); raw peanut kernels were medium runner type splits (40 to 50 kernels per 28 g). All nuts were stored at 4°C, for no more than 2 weeks before use.

**Bacterial cultures.** The strains used by Kimber et al. (21) for a similar study on almonds and pistachios were used in the current study to inoculate peanuts and pecans. Five serotypes of *Salmonella enterica* were used: Anatum, strain 1715, isolated from an almond survey; Enteritidis PT 30, strain ATCC BAA-1045, isolated from raw almonds associated with an outbreak; Enteritidis PT 9c, strain RM4635, a clinical isolate from an almond-associated outbreak; Oranienburg, strain 1839, isolated from pecans (provided by Dr. Beuchat, University of Georgia); and Tennessee, strain K4643, a clinical isolate from a peanut butter–associated outbreak. The five strains of *E. coli* O157:H7 used were all clinical isolates from patients in the associated foodborne outbreaks: Odwalla strain 223 (apple juice); CDC 658 (cantaloupe); H1730 (lettuce); F4546 (alfalfa sprouts); and EC4042 (spinach). Four strains of *L. monocytogenes* were used: 101M (serotype 4b), isolated from beef from a beef-associated outbreak; Scott A (serotype 4b), a clinical isolate from a milk-associated outbreak; V7 (serotype 1/2a), isolated from milk associated with an outbreak; and LCDC81-861 (serotype 4b), isolated from raw cabbage associated with an outbreak. The moisture of peanuts and pecans was determined during storage on nuts that were inoculated with *E. coli* K-12, a nonpathogenic organism.

To enumerate pathogens in the presence of relatively high background microbial populations on the raw nuts, we used a stepwise procedure (29) to isolate mutants of all strains that were able to grow on media supplemented with nalidixic acid (Sigma-Aldrich, St. Louis, MO). Unless otherwise specified, all media were from Difco, BD (Franklin Lakes, NJ) and were supplemented with nalidixic acid (N) at 50 μg/ml. The isolates were stored at −80°C in tryptic soy broth (TSB) supplemented with 15% glycerol.

**Inoculum preparation.** Frozen cultures of *Salmonella* and *E. coli* O157:H7 were streaked onto tryptic soy agar (TSAN; nonselective media), and *L. monocytogenes* cultures were streaked onto brain heart infusion (BHI) agar (nonselective media); all plates were incubated at 37 ± 2°C for 24 h. Each strain was subcultured twice in TSB supplemented with nalidixic acid at 50 μg/ml (*Salmonella* and *E. coli* O157:H7) or BHI broth (*L. monocytogenes*) and was incubated at 37 ± 2°C for 24 h. After overnight growth, 1 ml of culture was spread over large TSAN or BHI plates (150 by 15 mm) and was incubated at 37 ± 2°C for 24 h to produce a bacterial lawn. Sterile 0.1% peptone water. Pathogen populations from cocktails were determined by plating onto nonselective media, namely TSAN (*Salmonella* and *E. coli* O157:H7) or BHI (*L. monocytogenes*), at 37 ± 2°C for 24 h and by plating onto selective media, namely bismuth sulfite agar (BSAN) for *Salmonella*, sorbitol MacConkey agar (SMACN) for *E. coli* O157:H7, and modified Oxford agar (MOXN) for *L. monocytogenes*, at 37 ± 2°C for 48 h.

**Inoculation and storage of peanuts and pecans.** Nuts were inoculated as described by Uesugi et al. (33) for almonds, with a ratio of 25 ml of inoculum for 400 g of peanut or pecan kernels. Inoculation with each pathogen cocktail was carried out separately. Nuts (400 g) were weighed into a plastic bag (30.5 by 30.5 cm; Bitran Com-Pac International, Carbondale, IL). 25 ml of inoculum cocktail was added, and the bag was sealed. Each bag was shaken and massaged by hand for 1 min to ensure that the nuts were evenly coated with the inoculum. Inoculated nuts were spread onto four layers of filter paper (two sheets folded in half; Qualitative P8 Grade sheets, Fisher Scientific, Pittsburgh, PA) placed on a metal rack inside a large lidded plastic container; the lid was left ajar to allow the nuts to dry at ambient temperature and relative humidity (RH) conditions in the laboratory (~22°C, 56% RH). After the drying period, the inoculated nuts were transferred into sterile plastic bags (30.5 by 30.5 cm), which were sealed using the built-in ‘‘zipper’’ before the nuts were placed into storage.

The survival of pathogens was investigated during short-term (28 days) and long-term (365 or 550 days) storage. Vacuum or modified atmosphere storage was not considered in the study because it is not a common practice for bulk storage of raw nuts. The short-term storage study was carried out to evaluate the influence of inoculum levels on pathogen survival. Nuts were inoculated with the *Salmonella*, *E. coli* O157:H7, or *L. monocytogenes* cocktail at inoculum levels of 6 log CFU/g (high) and 3 log CFU/g (low) and then were dried for 24 h at ambient conditions. For the long-term storage study, nuts were inoculated with the *Salmonella*, *E. coli* O157:H7, or *L. monocytogenes* cocktail at 6 log CFU/g and were dried for 3 days at ambient conditions. These dried nuts were then held in sealed zipper-top bags for an additional 4 days at room temperature to ensure moisture equilibration, for the long-term study only. Inoculated, uninoculated, and control nuts in sealed bags were stored at −24 ± 1, 4 ± 2, or 22 ± 1°C. Temperature and RH (%) in each storage area were monitored with data loggers (TempTale 4, Sensitech Inc., Beverly, MA) throughout the storage periods.

**Moisture analysis.** The moisture content of inoculated nuts was determined monthly throughout storage at each temperature for up to 12 months. The nuts used for moisture analysis were inoculated with *E. coli* K-12 at 6 log CFU/g, dried at ambient conditions for 3 days, and held for an additional 4 days before storage. Peanut or pecan kernels (~10 g) were finely ground in a food processor for 30 s and then were placed into foil pans, weighed, and dried in an oven at 100°C for 24 ± 2 h. The moisture content was determined by measuring the weight lost as a result of heating.

**Recovery and enumeration.** Nuts were sampled weekly for the short-term study and monthly for the long-term study. At each sampling time, three 10-g subsamples from each of two replicates of inoculated nuts (*n* = 6) were added to 207-ml Whirl-Pak bags (Nasco, Fort Atkinson, WI) with 20 ml of 0.1% peptone and then were macerated (Smasher, AES Chemunex, Cranbury, NJ) for 2 min. Serial dilutions were prepared with 0.1% peptone. Appropriate dilutions were plated (0.1 ml) in duplicate onto nonselective media

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Inoculation with some modifications. For each inoculum concentration (6 log CFU/g) for the long-term study and for both inoculum levels (P > 0.05), with two exceptions: at ambient storage for peanuts inoculated at the low level and for pecans inoculated at the high level (data not shown). L. monocytogenes populations enumerated on BHIN (selective) and MOXN (nonselective) were not significantly different for peanuts (P > 0.05), but counts on MOXN were significantly higher than on BHIN for pecans (P < 0.05) for both inoculum levels (data not shown). Significant differences in counts on selective and nonselective media were independent of initial inoculum concentration.

In most cases, the rates of pathogen population decline on both nut types were not significantly different between the two inoculum levels (P < 0.05) (Tables 1 and 2). Significantly slower rates of decline were observed with peanuts that were inoculated at log 3 CFU/g with Salmonella and L. monocytogenes at storage temperatures of 4 ± 2 and 22 ± 1°C, respectively. Significantly greater rates of decline were observed with pecans that were inoculated at log 3 CFU/g with Salmonella and E. coli O157:H7 and stored at −24 ± 1°C. We used the high inoculum concentration (6 log CFU/g) for the long-term study and believe that this approach provided a conservative

### RESULTS

**Temperature and RH during storage.** The median temperature and RH values at the three storage conditions were as follows: −24 ± 1°C and 69% ± 5% RH in frozen storage, 4 ± 2°C and 94% ± 9% RH in refrigerated storage, and 22 ± 1°C and 56% ± 8% RH in ambient storage. Fluctuations in humidity were greater than fluctuations in temperature, with maximum fluctuations observed at ambient storage (Fig. 1). Climatic conditions can impact laboratory humidity during events such as rainfall (21). Rainfall is common in central Florida; hot temperatures and high humidity are typical, and air conditioning is used to mediate temperature and humidity. This combination of factors likely resulted in the greater fluctuations in RH we observed at ambient conditions.

**Influence of inoculum level on pathogen survival.** Pathogen cocktails were inoculated at 6 log CFU/g (high level) and 3 log CFU/g (low level) onto peanuts and pecans; nuts were stored for 28 days under frozen, refrigerated, and ambient conditions. Salmonella populations enumerated on BSAN (selective) and TSAN (nonselective) did not differ significantly for peanuts and pecans at any of the storage temperatures or at either inoculum level (P > 0.05) (data not shown). E. coli O157:H7 populations enumerated on TSAN were significantly higher than on SMACN (selective) for both nut types during storage and for both inoculum levels (P < 0.05), with two exceptions: at ambient storage for peanuts inoculated at the low level and for pecans inoculated at the high level (data not shown). L. monocytogenes populations enumerated on BHIN (selective) and MOXN (nonselective) were not significantly different for peanuts (P > 0.05), but counts on MOXN were significantly higher than on BHIN for pecans (P < 0.05) for both inoculum levels (data not shown). Significant differences in counts on selective and nonselective media were independent of initial inoculum concentration.

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### Statistical analysis.** All experiments were replicated twice with three samples per replication (n = 6), and pathogen populations were analyzed by analysis of variance (ANOVA) and Tukey-Kramer tests with JMP 8 software (SAS Institute Inc., Cary, NC). Differences between mean values were considered significant at P < 0.05. The best-fit models were generated with the aid of DMFit and were selected on the basis of $R^2$ values (2), and pathogen reductions per month were calculated from the curves obtained. The decline rates from high and low inoculums observed during the 28-day study were analyzed by linear regression with an interaction term in the model. The decline rate was considered significant at P < 0.05.

### FIGURE 1. (A) Temperature (°C) and (B) relative humidity (%) in each storage condition as recorded monthly by data loggers over 365 days. Whiskers denote minimum and maximum values, the box denotes the 75th and 25th percentile, the solid line in the middle denotes the median, and the dotted line denotes the mean.
prediction of the behavior of pathogens that may be present on nuts at lower concentrations.

**Moisture content of inoculated nuts during storage.** Kimber et al. (21) found very minor differences in moisture content between inoculated and uninoculated pistachios and almonds; here, the changes in moisture content of inoculated peanuts and pecans during long-term storage are shown in Figure 2, whereas those of the uninoculated nuts were not recorded. The initial moisture content of the peanuts and pecans used in this study was 3.8 and 2.0%, respectively. Immediately after inoculation, the moisture content increased to 8.0% in peanuts and to 6.5% in pecans; after the 3-day inoculum-drying period, the moisture content decreased to 3.8 and 2.6% in peanuts and pecans, respectively. During storage at 4 ± 2°C, the

<table>
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<tr>
<th>Pathogen cocktail</th>
<th>Storage temp (°C)</th>
<th>Inoculum level</th>
<th>Initial (day 1) population level (log CFU/g)</th>
<th>Final (day 28) population level (log CFU/g)</th>
<th>Rate of change (log CFU/g/30 days)</th>
<th>R²</th>
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<td>0.29</td>
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* Short-term storage lasted 28 days. H, high inoculum level (6 log CFU/g); L, low inoculum level (3 log CFU/g) before 24 h of drying. Population values are mean ± standard deviation, n = 6; values shown represent enumeration on nonselective media only. Different letters represent significant differences between the rates of decline at high and low inoculum (P < 0.05).
Pathogen survival on inoculated nuts during drying and holding periods. Before long-term storage, kernels were inoculated (8.0 ± 0.0, 7.3 ± 0.4, and 8.2 ± 0.1 log CFU/ml for *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes*, respectively) to 5.4 ± 0.3, 5.3 ± 0.2, and 5.7 ± 0.2 log CFU/g on pecans and 5.4 ± 0.1, 5.4 ± 0.4, and 5.4 ± 0.4 log CFU/g on peanuts, for *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes*, respectively. Nuts were dried at ambient temperature for 3 days. During the drying period, *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* populations decreased significantly (*P* < 0.05) to 4.7 ± 0.1, 4.7 ± 0.9, and 5.0 ± 0.8 log CFU/g on pecans and 4.5 ± 0.1, 3.9 ± 0.2, and 3.8 ± 0.5 log CFU/g on peanuts, respectively. Nuts were held in sealed bags at ambient temperature for 4 days, and no significant changes in pathogen populations (<0.5 log CFU/g) occurred on either nut type during the holding period (*P* > 0.05). Populations of *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* at the initiation of storage were 5.1 ± 0.1, 4.7 ± 0.6, and 5.3 ± 0.2 log CFU/g on pecans, respectively, and 4.4 ± 0.3, 3.6 ± 0.1, and 4.1 ± 0.2 log CFU/g on peanuts, respectively.

*Salmonella* survival during storage. *Salmonella* populations enumerated on BSAN were significantly lower (*P* < 0.05, up to 0.3 log CFU/g) than those on TSAN for peanuts and pecans stored at −24 ± 1°C and 4 ± 2°C (Fig. 3); there were no significant differences in *Salmonella* populations enumerated on TSAN and BSAN for the nuts stored at 22 ± 1°C (Fig. 3). *Salmonella* populations declined slightly but not significantly (*P* > 0.05) on peanuts and pecans at −24 ± 1 and 4 ± 2°C (Fig. 3); reductions were 0.3 and 0.4 log CFU/g on peanuts and 0.5 and 0.1 log CFU/g on pecans, respectively, over 365 days.

*Salmonella* populations on peanuts declined by 2.8 log CFU/g over 365 days of storage at 22 ± 1°C (Fig. 3A). After being fit to a best-fit model, levels declined linearly at the rate of 0.22 log CFU/g/30 days (*R*² = 0.88; Table 3). On pecans, *Salmonella* populations declined by 2.0 log CFU/g over 365 days (Fig. 3B); a linear decline at the rate of 0.15 log CFU/g/30 days (*R*² = 0.90; Table 3) was calculated from the best-fit model.

*Salmonella* levels remained above the limit of detection (0.3 log CFU/g) throughout 365 days on both nut types. Cell counts obtained from the peanuts and pecans were not significantly different at −24 ± 1 and 4 ± 2°C (*P* > 0.05), but significantly higher counts were obtained for pecans.
than for peanuts at the ambient storage condition ($P < 0.05$).

**E. coli O157:H7 survival during storage.** *E. coli* O157:H7 populations enumerated on SMACN were significantly lower ($P < 0.05$; up to 1.2 log CFU/g) than those on TSAN for peanut and pecans at the three storage temperatures (Fig. 4), with one exception for pecans stored at 22 ± 1°C (Fig. 4B). *E. coli* O157:H7 populations declined significantly ($P < 0.05$) by 0.6 and 0.7 log CFU/g on peanuts at −24 ± 1°C and 4 ± 2°C over 365 days (Fig. 4A), at a rate of 0.03 (R$^2 = 0.42$) and 0.12 (R$^2 = 0.71$) log CFU/g/30 days, respectively (Table 3). *E. coli* O157:H7 populations did not decline significantly on pecans at −24 ± 1°C and 4 ± 2°C (Table 3); reductions of 0.5 and 0.2 log CFU/g were observed over 331 and 365 days, respectively (Fig. 4B).

At 22 ± 1°C, *E. coli* O157:H7 populations declined by 3.2 log CFU/g over 272 days on peanuts (Fig. 4A); when data were fit to the no-lag model, a decline rate of 0.37 log CFU/g/30 days (R$^2 = 0.93$) was calculated (Table 3). On pecans, *E. coli* O157:H7 populations declined by 4.3 log CFU/g over 365 days (Fig. 4B) at a linear rate of 0.34 log CFU/g/30 days (R$^2 = 0.89$) (Table 3).

*E. coli* O157:H7 levels on SMACN were below the limit of detection for peanuts and pecans after 211 and 331 days of storage, respectively, at 22 ± 1°C. Population levels on TSAN were not below the limit of detection at any time at all three temperatures for both nut types. Significantly higher population counts were obtained from the pecans stored at −24 ± 1°C and 4 ± 2°C ($P < 0.05$), but no significant differences in counts between peanuts and pecans were observed at 22 ± 1°C ($P > 0.05$).

**L. monocytogenes survival during storage.** *L. monocytogenes* populations enumerated on MOXN were significantly lower ($P < 0.05$; up to 0.8 log CFU/g) than those on BHIN for peanuts and pecans stored at −24 ± 1°C for 365 days. ND, not done; there was no significant change in population levels over the storage period.

**TABLE 3.** Calculated rates of decline for inoculated pathogen populations on raw peanut kernels and pecan halves during storage$^a$

<table>
<thead>
<tr>
<th>Pathogen cocktail</th>
<th>Nut</th>
<th>Storage temp (°C)</th>
<th>ANOVA P value</th>
<th>Model$^b$</th>
<th>Rate of change (log CFU/g/day)</th>
<th>Rate of change (log CFU/g/30 days)</th>
<th>R$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em></td>
<td>Peanut</td>
<td>−24 ± 1</td>
<td>0.88</td>
<td>ND</td>
<td>−0.007</td>
<td>−0.22</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>Peanut</td>
<td>4 ± 2</td>
<td>0.09</td>
<td>ND</td>
<td>−0.007</td>
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<td>0.88</td>
</tr>
<tr>
<td></td>
<td>Peanut</td>
<td>22 ± 1</td>
<td>&lt;0.0001</td>
<td>Linear</td>
<td>−0.007</td>
<td>−0.22</td>
<td>0.88</td>
</tr>
<tr>
<td><em>E. coli</em> O157:H7</td>
<td>Peanut</td>
<td>−24 ± 1</td>
<td>0.09</td>
<td>ND</td>
<td>−0.007</td>
<td>−0.22</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>Peanut</td>
<td>4 ± 2</td>
<td>0.06</td>
<td>ND</td>
<td>−0.007</td>
<td>−0.22</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>Peanut</td>
<td>22 ± 1</td>
<td>&lt;0.0001</td>
<td>Linear</td>
<td>−0.007</td>
<td>−0.22</td>
<td>0.88</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>Peanut</td>
<td>−24 ± 1</td>
<td>0.59</td>
<td>ND$^c$</td>
<td>−0.007</td>
<td>−0.22</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>Peanut</td>
<td>4 ± 2</td>
<td>0.06</td>
<td>ND</td>
<td>−0.007</td>
<td>−0.22</td>
<td>0.88</td>
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<tr>
<td></td>
<td>Peanut</td>
<td>22 ± 1</td>
<td>&lt;0.0001</td>
<td>Linear</td>
<td>−0.007</td>
<td>−0.22</td>
<td>0.88</td>
</tr>
</tbody>
</table>

$^a$ Peanuts and pecans were stored at −24 ± 1, 4 ± 2, or 22 ± 1°C for 365 days. ND, not done; there was no significant change in population levels over the storage period.

$^b$ Model (DMfit) was chosen based on R$^2$ value and the shape of the curve.

$^c$ Counts obtained from last sampling day were not included in the analysis.
and 4 ± 2°C (Fig. 5); there were no significant differences in colony counts on the two media for both nut types stored at 22 ± 1°C. L. monocytogenes populations declined by 0.7 and 0.8 log CFU/g on peanuts at −24 ± 1 and 4 ± 2°C, respectively, over 365 days (Fig. 5A). The linear rate of decline on peanuts was 0.06 log CFU/g/30 days at −24 ± 1°C ($R^2 = 0.91$) and 4 ± 2°C ($R^2 = 0.75$) ($P < 0.05$; Table 3). On pecans, L. monocytogenes populations remained stable at −24 ± 1°C, with reductions of 0.3 log CFU/g over 365 days (Fig. 5B). At 4 ± 2°C, the populations on pecans declined by 0.6 log CFU/g over 364 days; the linear rate of decline was 0.03 log CFU/g/30 days ($R^2 = 0.06$) (Table 3).

At 22 ± 1°C, L. monocytogenes declined by 3.4 log CFU/g over the first 181 days and by 3.8 log CFU/g over 365 days on peanut kernels (Fig. 5A). According to the best-fit model for the initial slope, the rate of decline was 0.59 log CFU/g/30 days over 181 days ($R^2 = 0.93$; Table 3). L. monocytogenes populations on peanuts fell below the limit of detection on the last sampling day; all samples were positive upon enrichment ($n = 6$) (Fig. 5A). On pecan halves, L. monocytogenes populations declined by 4.9 log CFU/g over the first 150 days (Fig. 5B). L. monocytogenes populations on pecans reached the limit of detection after 150 days of storage at 22 ± 1°C and occasionally fell below the limit of detection during the remaining storage period up to 365 days; upon enrichment, all samples were positive at each time point ($n = 6$) (Fig. 5B). The rate of decline was 1.17 log CFU/g ($R^2 = 0.98$), and the curve was best described by the no-lag model (Table 3). Populations of L. monocytogenes were significantly higher on pecans than on peanuts stored at −24 ± 1 and 4 ± 2°C; at 22 ± 1°C storage, counts obtained from peanuts were significantly higher ($P < 0.05$) than on pecans.

**Differences in pathogen survival during long-term storage.** On peanut kernels at −24 ± 1, 4 ± 2, and 22 ± 1°C, E. coli O157:H7 and L. monocytogenes populations declined significantly ($P < 0.05$), whereas Salmonella populations were stable. There was no significant difference in the decline of populations of E. coli O157:H7 and L. monocytogenes on peanut kernels; and, at 22 ± 1°C, L. monocytogenes declined significantly more than E. coli O157:H7 ($P < 0.05$).

On pecan halves stored at −24 ± 1°C, all pathogen populations were stable for the duration of the study. At 4 ± 2°C, L. monocytogenes declined more than Salmonella on pecans ($P < 0.05$), but no significant differences in the declines of Salmonella and E. coli O157:H7 or E. coli O157:H7 and L. monocytogenes populations were observed ($P > 0.05$). At 22 ± 1°C, L. monocytogenes populations declined significantly more than both E. coli O157:H7 and Salmonella on pecans ($P < 0.05$), and E. coli O157:H7 populations declined significantly more than Salmonella ($P < 0.05$).

All the data in the study were collected in the presence of background microflora, as would be typical under natural contamination events of peanuts or pecans. No population changes in background microflora occurred, and it is unlikely that these organisms affect pathogen survival.

**DISCUSSION**

Peanuts and pecans can become contaminated at any number of points during production or processing. Peanuts grow underground, and at harvest the peanut plants are lifted from the soil and inverted into windrows to allow the nuts to dry (36). The close proximity of peanuts to soil can increase the opportunity for contamination of peanuts (18). At harvest, pecans are shaken from the trees onto the orchard floor, swept up, and then transported to holding locations for drying. Pecans have a thick outer covering, or husk, which is removed prior to shelling (1). Pecans on the ground can absorb moisture from free-standing water during events like rainfall, and the rate of water infiltration into in-shell pecans depends upon the variety of pecan and the degree of shell damage or cracking (3).

Since 1986, peanuts (primarily peanut butter) have been associated with several outbreaks of salmonellosis around the world (19). Pecans have not been associated with recorded outbreaks, but several recalls have been reported in...
the United States since 2001 due to isolation of Salmonella and Listeria during routine sampling (28). The prevalence of Salmonella naturally present on raw shelled peanuts in the United States was 2.3% of 944 peanut samples (375 g each) from three crop years (7), and the corresponding concentration of Salmonella on peanuts as determined by a most-probable-number (MPN) assay was <0.03 to 2.4 MPN/g. Another study of raw shelled peanuts found Salmonella and enterohemorrhagic E. coli in 0.67 and 0.03% of 10,162 peanut samples (350 g each), respectively, averaged over three crop years (27); the calculated Salmonella levels were 0.74 to 5.25 MPN/350 g (0.002 to 0.015 MPN/g). To our knowledge, no published research has reported the natural contamination levels of L. monocytogenes on peanuts and pecans.

Microbiological analysis of previously unopened jars of peanut butter from product involved in several cases of salmonellosis revealed the presence of Salmonella at three cells per gram (31). Salmonella levels recovered from the contaminated peanut butter were well below the inoculum levels commonly used in the long-term survival studies of other nuts (3, 5, 21, 33). However, the short-term survival experiment conducted in the current study confirmed the use of a high-level inoculum (6 log CFU/g) as a suitable predictor of pathogen behavior at low levels, as seen in similar studies on almonds (21, 33), pecans (3), and pistachios (21). Pathogen decline rates were different between the short-term and the long-term studies, likely due to methodology differences in drying the inoculated nuts: ambient conditions for 24 h in the short-term study versus a total of 7 days for the long-term study. Longer drying periods before the beginning of storage bring the moisture content and water activity closer to the original levels, whereas shorter drying periods may not adequately reduce these levels, subsequently affecting the decline rates calculated for pathogens.

In the current study, peanuts and pecans were stored under various conditions typically used by handlers and consumers. In frozen and refrigerated storage, Salmonella populations did not decline significantly with time on peanuts or pecans, E. coli O157:H7 populations declined significantly on peanuts but did not decline significantly on pecans, and L. monocytogenes populations declined significantly with time, except on pecans stored under the freezer conditions. Similarly, no significant decline of Salmonella was observed for over 12 months for almonds or in-shell pistachios inoculated with the same Salmonella cocktail used in the current study (21) or with a single strain of Salmonella (ATCC BAA-1045) (33). In contrast, a previous study conducted on pecan halves documented small declines (0.48 to 0.69 log CFU/g) in Salmonella levels at frozen and refrigerated storage over 9 months (3).

A significant but slow decline of E. coli O157:H7 inoculated on peanuts occurred under refrigeration conditions, which is in agreement with the observations for E. coli O157:H7 inoculated on almonds (21). No significant declines in E. coli O157:H7 populations were observed on pecans in the current study or on in-shell pistachios under either refrigerated or frozen storage (21). The slow declines in L. monocytogenes populations observed on peanuts (in refrigerated and frozen storage) and on pecans (in refrigerated storage, R² = 0.06) were not documented for almonds and pistachios under either condition (21).

Our observations on the survival of Salmonella at ambient conditions are in agreement with similar studies on raw almonds and pistachios (21, 33). The rates of decline of Salmonella at ambient conditions on raw peanuts (0.22 log CFU/g/30 days) and raw pecans (0.15 log CFU/g/30 days) are comparable to the rates of decline reported for almonds (average 0.24 log CFU/g/mo over eight studies) (23) and pistachios (0.15 log CFU/g/mo) (21) but are higher than for walnut kernels (0.05 to 0.1 log CFU/g/mo) (5). The reduction of Salmonella (2.0 log CFU/g) on pecan halves after 365 days of ambient storage, as observed in the current study, is similar to the reduction (2.1 log CFU/g) that was observed on immersion-inoculated pecan halves (3). For immersion inoculation, pecan halves were immersed in Salmonella cocktail for 30 s and then dried in a forced-air oven at 30 °C for 20 to 27 h (3), whereas in the current study, nuts were dried at room temperature for 3 days and then held for another 4 days at ambient conditions. In both studies, Salmonella counts were above the limit of detection throughout the storage period.

The decline rates for E. coli O157:H7 on peanuts (0.37 log CFU/g/30 days) and pecans (0.34 log CFU/g/30 days) were higher than for Salmonella on these nuts, similar to those on pistachios (0.35 log CFU/g/mo) and walnuts (0.21 log CFU/g/mo), and were lower than those previously reported on almonds (0.60 log CFU/g/mo). Decline rates of L. monocytogenes on peanuts (0.59 log CFU/g/30 days), pecans (1.17 log CFU/g/30 days), almonds (0.71 log CFU/g/mo), pistachios (0.86 log CFU/g/mo), and walnuts (1.2 log CFU/g/mo) were the highest among all three pathogens in each of the three studies.

At all storage conditions, Salmonella survived better than E. coli O157:H7 and L. monocytogenes, confirming its use as a representative organism for survival studies on peanuts and pecans. The rates of decline of all pathogens were slower on pecans than on peanuts in most cases in the current study; similarly, in a previous study, slower decline rates were recorded on pistachios than on almonds (21). The distinct tailing of the survival curves for E. coli O157:H7 and L. monocytogenes on peanuts and pecans is similar to that described for E. coli O157:H7 on almonds (21) and for both pathogens on pistachios (21) and walnuts (5). The survival potential also partially explains the reason for the greater number of nut outbreaks associated with Salmonella than with other human pathogens. Studies on the survival of human pathogens on nuts are among the first steps for quantitative microbial risk assessment of pathogens on nuts. The data here contribute to a growing body of knowledge on the survival of foodborne pathogens on nuts. The relatively consistent data trends among nuts suggest that, collectively, these data may have utility for other nuts in the absence of nut-specific studies.
ACKNOWLEDGMENTS

This research was supported by the U.S. Department of Agriculture CSREES NIFS 2009-51110-20146. We are grateful for the technical assistance of Gwen Lundy and Luis Martinez and the editorial assistance of Sylvia Yada.

REFERENCES


