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Research Paper

Salmonella Survival Kinetics on Pecans, Hazelnuts, and Pine Nuts at Various Water Activities and Temperatures

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ABSTRACT

The impact of temperature, water activity (a_w), and nut composition on *Salmonella* survival on tree nuts has not been thoroughly examined. The aim of this study was to determine the effect of temperature, a_w, and nut composition on the survival of *Salmonella* on tree nuts and develop predictive models. Pecans, hazelnuts, and pine nuts were chosen based on differences in their typical fat content. Nuts were inoculated with a cocktail of five *Salmonella* serotypes (11 log CFU/mL) and then were dried and stored at 4, 10, and 25°C at 0.41 ± 0.06 and 0.60 ± 0.05 a_w for 1 year. Ten-gram quantities were removed at different intervals up to 364 days to test for surviving *Salmonella* populations (plating on selective and nonselective media) and a_w. Experiments were carried out in triplicate. *Salmonella* populations were relatively stable over a year at 4 and 10°C at both a_w levels with <1.5-log CFU/g decline. The best predictive model to describe *Salmonella* survival at 4 and 10°C was a log-linear model with a *D*-value for each tree nut and a_w combination. Significant declines in *Salmonella* levels were observed at 25°C, where the best fit was a Weibull model with a fixed ρ for all tree nuts (ρ = 0.86), a δ value for each tree nut and a_w combination, and a random factor to account for variability among replicates. The time for the first log reduction at 25°C and 0.37 ± 0.009 a_w was estimated at 24 ± 2 weeks for hazelnuts, 34 ± 3 weeks for pecans, and 52 ± 7 weeks for pine nuts. At the same temperature, but with 0.54 ± 0.009 a_w, the mean estimated time for the first log reduction decreased to 9 ± 1 weeks for hazelnuts, 10 ± 1 weeks for pecans, and 16 ± 1 weeks for pine nuts. Tree nut, a_w, and temperature were shown to have a statistically significant effect on survival ($P < 0.05$). No apparent influence of fat content on survival was observed. The results of this study can be used to predict changes in *Salmonella* levels on pecans, hazelnuts, and pine nuts after storage at the different temperatures and a_w values.

Key words: Fat; Log linear; Low moisture; Protein; Tree nuts; Weibull

Salmonella survival data on tree nuts at different temperatures (<50°C) are available for almonds *(1, 10, 19, 26)*, pecans *(3, 5, 6, 12)*, pistachios *(19)*, and walnuts *(8–11, 15)*. The kinetics represented by these data are generally characterized by a relatively fast initial population decline of *Salmonella* during the first few weeks of storage, followed by long-term persistence with slow, or no, decline over time. A mathematical model to predict survival of *Salmonella* on almonds, pecans, pistachios, and walnuts at typical ambient storage temperatures (20 to 25°C), incorporating variability and uncertainty separately, was recently developed *(24)*. This model is a Weibull survival model that includes a fixed and random variation of δ per tree nut (δ is the model parameter that represents the time it takes for the first log reduction) and a fixed variation of ρ per tree nut (ρ is the model parameter that defines the shape of the curve).

Temperature, water activity (a_w), and food composition can influence *Salmonella* survival kinetics in low-a_w environments *(4, 14, 22)*. *Salmonella* shows increasing persistence at decreasing a_w *(7, 13, 16, 18, 20)*, and the presence of fat may offer protection *(13, 22, 25)*. *Salmonella* survival data on tree nuts of different fat content at various temperatures and a_ws in controlled relative humidity (RH) environments are not available in the peer-reviewed literature. The aim of this study is to evaluate the influence of nut composition, temperature, and a_w on the survival of *Salmonella* on pecans, hazelnuts, and pine nuts at 4, 10, and 25°C and 0.41 ± 0.06 and 0.60 ± 0.05 a_w and to develop mathematical models that predict the behavior of *Salmonella* under these conditions.

MATERIALS AND METHODS

Microorganisms and cultivation. Stock cultures of *Salmonella* serotypes Anatum strain 6802, Enteritidis strain ATCC BAA-1045, Oranienburg strain 1839, Sundsvall strain 1659, and Tennessee strain K4643, all originally isolated from peanuts or tree nuts or nut-related outbreaks, were obtained from Dr. Larry Beuchat (University of Georgia, Griffin) and were maintained at –20°C. Working stocks were maintained on Trypticase soy agar supplemented with 0.6% yeast extract (TSAYE; BD, Franklin Lakes, NJ) at 4°C and were transferred on a monthly basis. The

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inoculum was prepared as described in Keller et al. *(18)*. Briefly, a single colony from each working stock serotype was first transferred to Trypticase soy broth supplemented with 0.6% yeast extract (TSBYE; BD). After incubation at 37°C for 24 h, 100 μL was spread onto TSAYE plates. TSAYE plates were incubated at 37°C for 24 h and then were harvested by first adding 1.0 mL of sodium phosphate buffer (0.5 M, pH 7.0; Fisher Scientific, Fair Lawn, NJ) to the surface and gently scraping the surface with a sterile L-shaped plate spreader. Each plate yielded approximately 0.5 mL of culture at approximately 11 log CFU/mL. Harvested serotypes were mixed in equal volumes for the inoculum. Inoculum was enumerated by appropriate dilution in buffered peptone water (BPW; BD) followed by spread plating 100-μL quantities of duplicate plates of TSAYE and TSAYE supplemented with nalidixic acid (50 mg/mL; TSAYEN), similar to the procedure of Beuchat and Mann *(5)*, to limit growth of any background microflora.

***Salmonella* survival on tree nuts.** Nutmeats of three types of tree nuts were used in this study, selected in part because of their varied fat content, as reported in Beuchat *(2)*. The types selected include hazelnuts (62.3% fat), pecans (70.3% fat), and pine nuts (48.1% fat) *(2)*. Pecan halves of the desirable variety (*Carya illinoinensis*) and raw, unbleached hazelnuts were obtained from commercial shellers in Georgia. Pine nuts (shelled) were purchased from Sincerely Nuts (Brooklyn, NY). Samples of each type of nut were sent to Silliker Inc. (Crete, IL) to obtain percentage of total fat, ash, carbohydrate, protein, and moisture content (in triplicate). Background microbial populations were determined prior to inoculation by plating on TSAYE. Nutmeats were inoculated by submersion into a high-population suspension of five mixed *Salmonella* serotypes. The high-population suspension for inoculation of dried nuts was made following the protocol of Beuchat and Mann *(5)*. The population of the suspension was determined directly prior to use by plating in duplicate on both TSAYE and TSAYEN. After submersion in the high-population suspension, excess liquid was drained and the nuts were spread in a monolayer on aluminum foil sheets in a biological cabinet and were allowed to dry for 24 h at temperatures from 20 to 25°C. The a_w was determined before and after inoculation, using an AquaLab series 4TEV meter (Decagon Devices, Pullman, WA) that was calibrated against salt standards prior to use. After drying, the inoculated nuts were weighed (10 ± 0.5 g) onto plastic trays for storage under three different temperatures (4, 10, and 25°C) and two different RH levels (34 and 57%), which were selected to represent a low and high a_w within the range of a_w levels in low-a_w foods ($a_w < 0.7$). The a_w of the inoculated and dried nuts was measured on day 1 of the storage period. Conditions were maintained by placing multiple trays into glass or plastic desiccator jars suspended over saturated solutions of either magnesium chloride ($a_w = 0.34$) or sodium bromide ($a_w = 0.57$), by means of a grated shelf. Jars were stored in incubators set at 4 ± 1, 10 ± 1, and 25 ± 1°C, where temperatures were monitored and recorded on a daily basis. A set of three nut samples, one each of pecans, pine nuts, and hazelnuts, was removed at different time intervals, for up to 364 days. Time intervals included 0, 1, 2, 4, 9, 17, 26, 35, 43, 51, and 52 weeks. The time points at which samples were taken at the end of the study (>26 weeks) were dependent on the survival rates observed for each specific condition. For each sample, the a_w was evaluated (as previously described) and *Salmonella* populations were determined. *Salmonella* populations were determined by adding the nut sample (10 ± 0.5 g) to 90 mL of BPW in a Whirl-Pak bag. Whirl-Pak bags were stomached using a Stomacher 400 Circulator (Seward Ltd., Davie, FL) set at 250 rpm for 30 to 60 s. Stomached samples were serially diluted appropriately in BPW, and 100-μL quantities were spread plated in duplicate on both TSAYE and TSAYEN. Initial time intervals were set at 1, 4, 7, 14, and 28 days after storage. Following the first 28 days, time intervals were selected based on the rate of decline in *Salmonella* postinoculation populations. Each experiment was replicated three times, beginning with freshly grown inoculum.

Modeling survival data. The Weibull survival model *(21)* (equation 1) can describe log-linear ($\rho = 1$), concave ($\rho < 1$), and convex ($\rho > 1$) kinetic curves and assumes that the resistance to stress of a population follows a Weibull distribution.

$$\log(N_t) = \log(N_0) - (t/\delta)^{\rho} \quad (1)$$

where N_0 is the concentration at time 0, N_t is the concentration at time t, δ is the time to the first log reduction, and ρ is a fitting parameter that defines the shape of the curve.

The Weibull model has been shown to provide the best fit for survival kinetics of *Salmonella* in low-a_w foods at temperatures from 21 to 80°C and at a_w levels below 0.6 *(23)*. Following the approach presented in Santillana Farakos et al. *(24)*, the best predictive model for *Salmonella* survival on pecans, hazelnuts, and pine nuts at the different a_w levels and temperatures under study was determined. Briefly, the first step was to fit the Weibull fixed-effect models to the data set to characterize variability associated with parameters δ and ρ. Mixed-effect models, including fixed and random factors, were then fitted to the data, in line with the results of the fixed-effect model fitting. Models were compared using an F test ($P < 0.05$). Models and statistical analyses were executed in R (version 3.3.1, R Development Core Team, Vienna, Austria, 2008; http://www.R-project.org). The nls function (package stats) was used to fit the fixed-effect models, and the nlme function (package nlme) was used to fit the mixed-effect models. N_0 was considered a nuisance parameter specific to each fit. The media (TSAYE and TSAYEN) were considered a factor of influence to the fit through N_0 and were captured by the variability in δ for a given substrate.

RESULTS AND DISCUSSION

Fat, protein, and carbohydrate levels. Pine nuts were determined to have the lowest carbohydrate and the highest protein content among all tree nuts under study at the measured moisture level (Table 1). Pecans were found to have the lowest protein and the highest fat content of all three tree nuts (Table 1). Hazelnuts were shown to have the lowest fat and highest carbohydrate content (Table 1). These results are in line with those published by Beuchat *(2)*, who found that, at 0.42 a_w, pecans had the highest measured amount of fat (70.3%) and pine nuts had the highest measured amount of protein (35.5%). However, fat content levels for pine nuts in this study are higher, and protein content levels are lower (Table 1), than those implied by the fat (48.1%) and protein content provided by Beuchat *(2)*. Fat and protein content levels for pecans and hazelnuts in this study (Table 1) are similar to those provided by Beuchat *(2)*, who found that pecans had 70.3% fat and 11.7% protein and that hazelnuts had 62.3% fat and 16.8% protein.

Temperature and a_w during storage. The temperature recorded at the three storage conditions was 4 ±1, 10 ±1, and 25 ±1°C. Average a_w values for the different nutmeats and experimental replicates at the three storage temperatures

TABLE 1. *Composite analysis results for hazelnuts, pecans, and pine nuts*[a]

Composite	% content (mean ± SD)			Reference method
	Hazelnut	Pecan	Pine nut	
Ash	2.2 ± 0.04	1.5 ± 0.03	2.8 ± 0.1	AOAC 950.49
Carbohydrate	14.9 ± 0.4	13.4 ± 0.1	10.4 ± 0.7	Calculated[b]
Protein	17.4 ± 0.4	9.9 ± 0.4	18.3 ± 0.2	AOAC 992.23
Fat	62.7 ± 0.1	72.9 ± 0.4	66.0 ± 0.1	AOAC 948.22
Moisture	4.0 ± 0.03	2.8 ± 0.04	3.0 ± 0.1	AOAC 925.40

[a] Values are reported based on the sample(s) as received.
[b] The percentage of carbohydrates was calculated as the percentage of solids that were not protein or fat.

over the entire storage time were measured as 0.44 ± 0.009 at 34% RH and 0.62 ± 0.006 at 57% RH at 4°C; 0.43 ± 0.008 at 34% RH and 0.60 ± 0.007 at 57% RH at 10°C; and 0.37 ± 0.009 at 34% RH and 0.54 ± 0.009 at 57% RH at 25°C (Fig. 1). Fluctuations in a_w were greater than fluctuations in temperature and were similar at the different temperatures. a_w levels were lowest and closest to the target value at 25°C and were highest at 4°C. At the start of the survival studies, the a_w of the nutmeat equilibrated after the inoculation process and, thus, a prominent shift was observed in all a_w levels recorded during the first days of storage, possibly owing to adjustment of a_w levels to target values (Fig. 1). After that initial shift during the first days, a_w levels remained fairly constant throughout the storage period. This explains the low standard deviations of the average measured a_ws (Fig. 1). To our knowledge, this study is the first *Salmonella* survival study on pine nuts and hazelnuts and the first *Salmonella* survival study on tree nuts for which a_w has been controlled.

Survival of *Salmonella* on hazelnuts, pecans, and pine nuts. Data on *Salmonella* survival on pecans, hazelnuts, and pine nuts at 4°C for 364 days of storage (∼1 year) showed significant differences in survival among the different tree nuts ($P < 0.001$) and a_w levels ($P < 0.001$), with little decline over time (Fig. 2). *Salmonella* on pine nuts stands out for its survival at 4°C, with no significant decline over time ($P = 0.97$ at a_w 0.44 ± 0.009, $P = 0.05$ at a_w 0.62 ± 0.006). *Salmonella* was the most resistant on pine nuts, followed in decreasing order by pecans and hazelnuts at 0.44 ± 0.009 a_w (Fig. 2). *Salmonella* on hazelnuts and pecans (in decreasing order of survival) at a_w 0.62 ± 0.006 had the lowest survival (δ in Table 2). The best applicable model to describe *Salmonella* survival at 4°C was a log-linear fixed-effect model (Weibull model with $\rho = 1$ and no random factors). Significantly different *D*-values (because it is a loglinear model, δ values are the equivalent of a *D*-value because $\rho = 1$) were found for each combination of tree nut and a_w, except for pine nuts (for which the decline was not significant). The lowest significant *D*-value found was for pecans at 0.62 ± 0.006 a_w, and that corresponds to a 1-log decline of *Salmonella* in a period of 2 years (±4 months; Table 2). Decreased survival was observed at higher a_w or RH of storage (Fig. 2). *Salmonella* on pecans at 4°C and 0.44 ± 0.009 a_w had an estimated mean *D*-value of 3.7 years (±4 months). These results are in line with previous survival studies of *Salmonella* on pecan nutmeats by Brar et al. *(12),* which showed no significant decrease of *Salmonella* over time at 4°C (94% ± 9% RH during storage, 3% moisture content) when inoculated at a high level (∼5 log CFU/g). In the study by Brar et al. *(12),* a nonsignificant decrease ($P = 0.06$) of 0.1 log CFU/g was seen on pecans stored for 1 year at 4°C. Our results and those presented by Brar et al. *(12)* are similar to the results in the study by Beuchat and Mann *(5),* who reported a nonsignificant (0.42 log CFU/g) decrease in *Salmonella* populations after 52 weeks (∼1 year) of storage for pecan halves inoculated at a high level (6.16 log CFU/g). There is a lack of information with respect to *Salmonella* survival data on hazelnuts and pine nuts at refrigeration temperatures in the peer-reviewed literature.

Similar to the results seen for survival of *Salmonella* at 4°C, *Salmonella* survival on pecans, hazelnuts, and pine nuts at 10°C for 364 days of storage (∼1 year) showed very little or no decline over time (Fig. 2). The best predictive model to describe the data was a fixed-effect log-linear model with a *D*-value for each tree nut and a_w combination. Statistically significant *D*-values for all tree nuts and a_w levels were found, except for pine nuts at the higher a_w (a_w 0.60 ± 0.007), where *Salmonella* showed no significant decline over time ($P = 0.47$; Table 2). Tree nut and a_w were shown to have a statistically significant effect on the estimated *D*-values at 10°C (Table 2 and Fig. 2). Similar to the observations on survival at 4°C, *Salmonella* survival on pine nuts stands out at 10°C compared with the other tree nuts at both a_w levels (Fig. 2). Compared with survival at 4°C, survival at 10°C and 0.60 ± 0.007 a_w resulted in significantly higher survival, with a *D*-value of 19 ± 26 years versus 3 ± 0.5 years at 0.43 ± 0.008 a_w. The lowest survival was observed for *Salmonella* on hazelnuts at 0.60 ± 0.007 a_w, with an estimated *D*-value of 14 ± 1 months (∼1 year) ($P < 0.05$; Table 2). The interaction between the a_w effect and the tree nut effect was significant ($P < 0.05$), indicating that the influence of a_w level depends on the variety of tree nut. Survival of *Salmonella* on hazelnuts has an average estimated *D*-value of 25 ± 3 months at 0.43 ± 0.008 a_w (∼2.1 years; Table 2). Survival of *Salmonella* on pecans at 10°C had a similar estimated *D*-value of 23 ± 3 months at 0.43 ± 0.008 a_w (∼2 years) and at 0.60 ± 0.007 a_w. To the knowledge of the authors, no other data are available in the peer-reviewed literature for survival of *Salmonella* on pecans, hazelnuts, and pine nuts at 10°C.

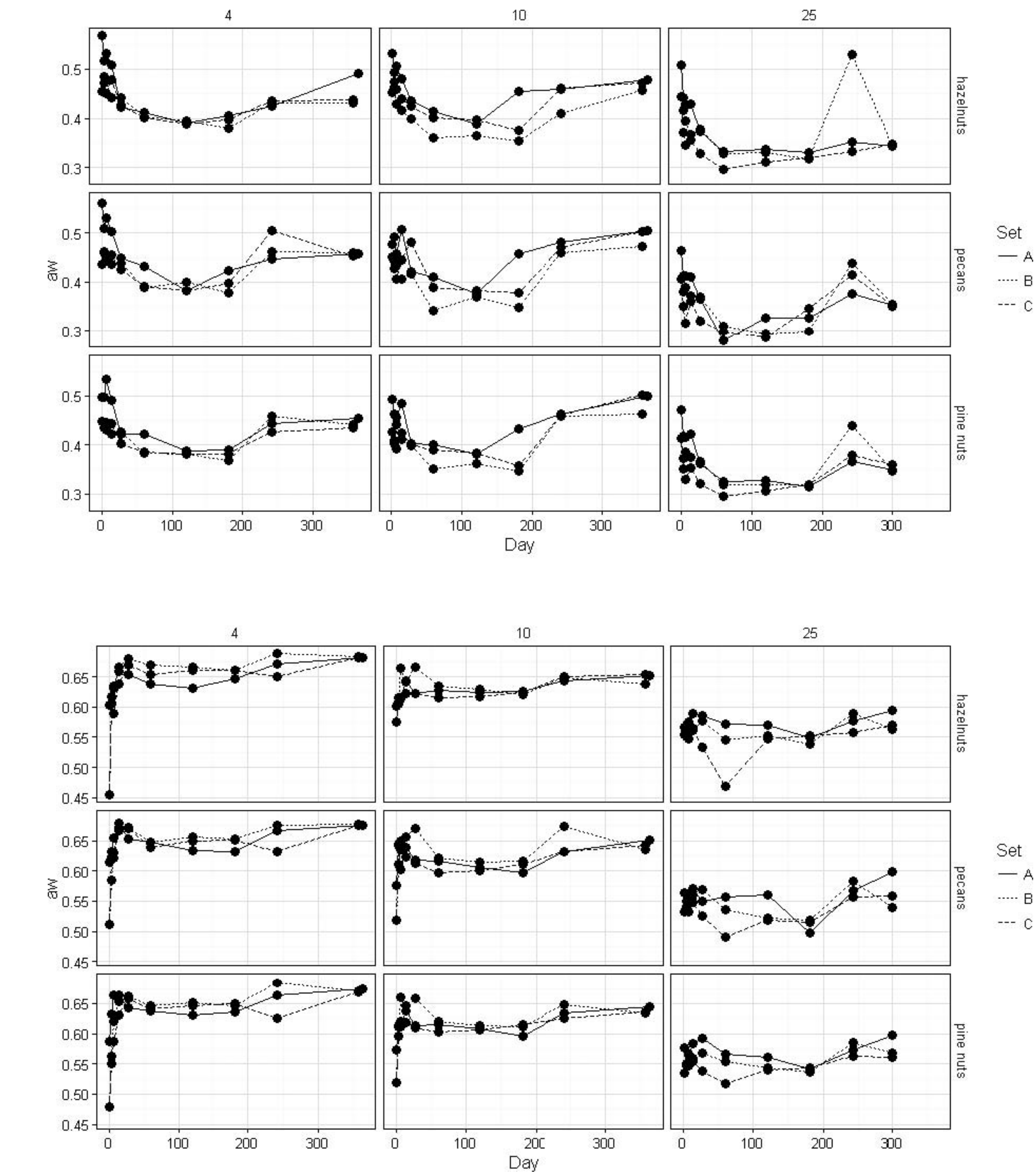

FIGURE 1. *Water activity levels as recorded for three replicate experiments (sets A, B, and C) for* Salmonella *survival on hazelnuts, pecans, and pine nuts at 4 ± 1, 10 ± 1, and 25 ± 1°C at two relative humidity storage levels (34%, top; 57%, bottom) for ≥300 days of storage*.

Decreasing *Salmonella* survival was seen when the temperature was increased from 10 to 25°C. The best applicable model to describe survival was no longer a fixed-effects log-linear model but a mixed-effect Weibull type model with a fixed ρ for all tree nuts (ρ = 0.86) and a δ value for each tree nut and a_w combination. Statistically significant differences in survival were found for *Salmonella* in the three different replicate experiments, and a random factor was added to the model to account for these differences (Fig. 3). No significant differences were found for survival on the different media (TSAYE or TSAYEN) ($P > 0.05$). The time for the first log reduction (δ) at 25°C was estimated at 24 ± 2 weeks for hazelnuts (6 months), 34 ± 3 weeks for pecans (~8 months), and 52 ± 7 weeks for pine nuts (~12 months) at a a_w level of 0.37 ± 0.009 (Table 2). At a higher a_w level (0.54 ± 0.009), the mean estimated time for the first log reduction decreased to 9 ± 1 weeks for hazelnuts, 10 ± 1 weeks for pecans, and 16 ± 1 weeks for pine nuts (Table 2).

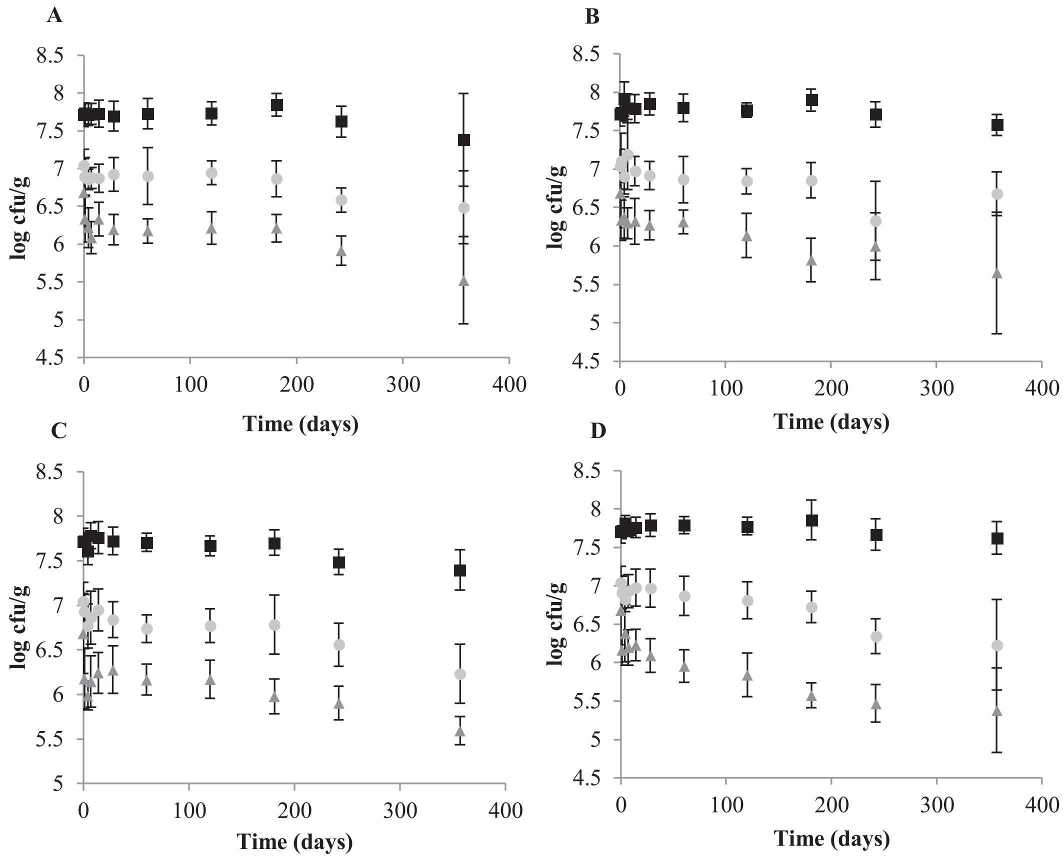

FIGURE 2. *Survival of* Salmonella *at 4°C (top: A and B) and 10°C (bottom: C and D) at 34% (left: A and C) and 57% (right: B and D) relative humidity storage levels on pine nuts (black square), pecans (light gray circle), and hazelnuts (dark gray triangle).*

TABLE 2. *Estimated δ values for survival of* Salmonella *on tree nuts at different temperatures and water activity levels*[a]

Temp (°C)	Tree nut	a_w (±SD)	ρ (SE)	δ (wk)	SE δ (wk)	*P* value
4	Hazelnut	0.44 ± 0.009	Set to 1	144	31	<0.001
		0.62 ± 0.006		111	19	<0.001
	Pecan	0.44 ± 0.009		192	56	<0.001
		0.62 ± 0.006		104	16	<0.001
	Pine nut	0.44 ± 0.009		>1,000	>1,000	0.97
		0.62 ± 0.006		343	178	0.05
10	Hazelnut	0.43 ± 0.008	Set to 1	99	12	<0.001
		0.60 ± 0.007		58	5	<0.001
	Pecan	0.43 ± 0.008		92	11	<0.001
		0.60 ± 0.007		91	12	<0.001
	Pine nut	0.43 ± 0.008		165	34	<0.001
		0.60 ± 0.007		993	>1,000	0.47
25[b]	Hazelnut	0.37 ± 0.009	0.86 (0.045)	24	2	<0.001
		0.54 ± 0.009		9	1	<0.001
	Pecan	0.37 ± 0.009		34	3	<0.001
		0.54 ± 0.009		10	1	<0.001
	Pine nut	0.37 ± 0.009		52	7	<0.001
		0.54 ± 0.009		16	1	<0.001

[a] ρ, parameter defining the shape of the curve (standard error); δ, time (in weeks) to the first log reduction; SE δ, standard error of the estimated time (in weeks) to the first log reduction; *P* value, significance of the null hypothesis that the time to first log reduction was infinite (no decrease with time).

[b] At 25°C, a random effect was included to account for significant differences among the three replicate experiments. The standard deviation of the variability distribution of δ (σ_δ) was 4.33, and the standard error of the residual (σ) was 0.25 (measured in weeks).

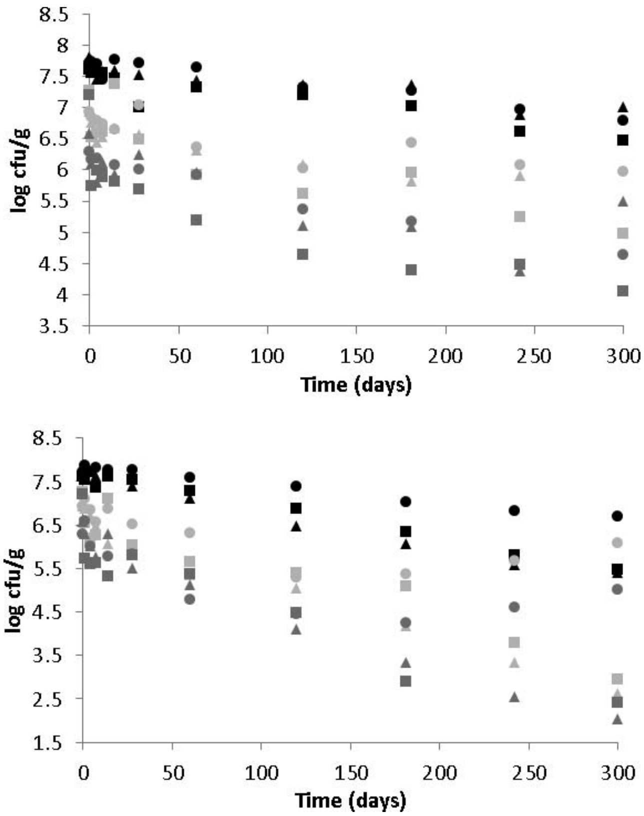

FIGURE 3. *Three replicate experiments (A, circle; B, square; C, triangle) on survival of* Salmonella *at 25°C at 34% relative humidity (top) and 57% relative humidity (bottom) on pine nuts (black), pecans (light gray), and hazelnuts (dark gray).*

Similar to observations at 4 and 10°C, at 25°C, *Salmonella* continued to show the highest survival on pine nuts, followed in decreasing order by pecans and hazelnuts (Fig. 3). Increasing a_w led to decreasing survival for all tree nuts at 25°C (Fig. 3). In the study by Brar et al. *(12),* linear declines of *Salmonella* populations on pecans were estimated at the rate of 0.15 log CFU/g/30 days with an overall 2.0-log CFU/g decline over 365 days for storage at 22 ± 1°C and 2.6% moisture content (56% ± 8% RH during storage). With the current model, a slightly higher initial decrease (1 log in the first 10 weeks versus 0.4 log CFU in 10 weeks using the Brar et al. *(12)* model) is estimated at a similar RH level (57%). However, at longer times, the current best-fit model predicts increasing survival among surviving *Salmonella,* as characterized by the ρ value (<1). Similar to our results in this study, data from the study of Beuchat and Mann *(5)* on survival of *Salmonella* on pecan nutmeats at 21°C at a_ws from 0.51 to 0.63 (moisture content 3.3 to 3.9%, nutmeats with less than 72% oil) showed biphasic survival curves for *Salmonella* on pecan nutmeats *(5)*. Significant decreases in *Salmonella* within 10 weeks of storage, with a reduction of 2.13 log CFU/g after 52 weeks of storage, were observed *(5)*. To the knowledge of the authors, no other data are available in the peer-reviewed literature for survival of *Salmonella* on hazelnuts and pine nuts at 25°C.

Previous research has suggested that fat may contribute to *Salmonella* survival in low-a_w foods *(22, 23)*. However, data from Kataoka et al. *(17)* for *Salmonella* survival at 20 ± 1°C for 12 months in peanut pastes at 47 and 56% fat (a_w 0.3 and 0.6) showed no significant effect of fat on survival. In this study, significant differences in *Salmonella* survival were observed among hazelnuts, pecans, and pine nuts at the various a_w levels and temperatures. Results show that the highest survival for *Salmonella* at 4, 10, and 25°C is on pine nuts. The results of this study suggest that fat content differences among nut types do not influence survival of *Salmonella* on pecans, hazelnuts, and pine nuts during storage at 34 and 57% RH and at temperatures from 4 to 25°C. We note that the fat content varied by less than 10% across the nut types. Survival differences among the different tree nuts cannot be attributed to differences in protein or carbohydrate levels. Based on the results in this study, a_w remains, together with temperature, the main driver influencing survival of *Salmonella* on tree nuts. The reason for the differences in *Salmonella* survival for the different tree nuts is currently unknown. It can be hypothesized that *Salmonella* is in contact with the skin of hazelnuts and pecans. This skin contains polyphenolic compounds known to be antimicrobial. Pine nut kernels, on the other hand, do not have this skin. The naked cotyledon is exposed. The antimicrobials in hazelnut and pecan skins may be responsible, in part, for the higher decrease in *Salmonella* levels (compared with pine nuts).

Kimber et al. *(19)* also observed differences in *Salmonella* survival on almonds and pistachios at temperatures from −19 to 24°C. Santillana Farakos et al. *(24)* observed significant differences in survival among almonds, pecans, pistachios, and walnuts, using data from the peer-reviewed literature. Higher estimated survival rates (δ) were obtained for *Salmonella* on pistachios, followed in decreasing order by pecans, almonds, and walnuts *(24)*. Survival was characterized by a Weibull model with a relatively fast initial population decline during the first few weeks of storage, followed by long-term persistence with slow, or no, decline over time *(24)*. Using the Santillana Farakos et al. *(24)* model for pecans at 21 to 24°C, the δ values were estimated as a mean of 15.6 weeks, with a variability span from 11.4 weeks (2.5% quantile) to 20 weeks (97.5% quantile) *(24)*. The Santillana Farakos et al. *(24)* model did not account for a_w because the studies that were included in the analysis did not control for RH during storage at room temperature. The estimates from the current study show a mean δ value for pecans at 25 ± 1°C of 34 weeks (standard error 3 weeks) at 0.37 ± 0.009 a_w and 10 weeks (standard error 1 week) at 0.54 ± 0.009 a_w. The estimates obtained for storage at 0.54 ± 0.009 a_w are most similar to those found by Santillana Farakos et al. *(24),* whose model was developed using studies of *Salmonella* survival on pecans that reported a_w levels of 0.43 to 0.63 during storage.

In summary, *Salmonella* survival on pecans, hazelnuts, and pine nuts during 1 year of storage is higher at lower a_w (storage at 34 versus 57% RH) and temperature (4, 10, and 25°C). The highest survival was seen on pine nuts, followed in decreasing order by pecans and hazelnuts, regardless of the a_w-temperature combination. Significant differences were seen for *Salmonella* survival on the various tree nuts at the different a_w levels and temperatures, with no apparent influence of fat content of nut varieties on survival. A log-linear model was the best applicable model to describe survival at refrigeration temperatures (4°C) as well as at 10°C at both a_w levels, with no meaningful decline of *Salmonella* over time. As the temperature increased to 25°C, the Weibull model was found to be the best model to describe survival at all a_w values and in all tree nuts under study.

The results of this study can be used to predict changes in *Salmonella* levels after storage at different temperatures and a_w levels.

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Erratum

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A few months ago, a genetic reexamination of all *Salmonella* serotypes used in the authors' past experiments was initiated. Unfortunately, it was discovered that one serotype out of the several used was misidentified. The misidentified serotype was *Salmonella* Enteritidis PT30, which was in fact *Salmonella* Reading.

All of the studies in question were challenge studies in design, where the survival of *Salmonella* was assessed under different environmental conditions (temperature and RH) in different food materials (nuts or spices). The National Advisory Committee on Microbiological Criteria for Foods (NACMCF. 2010. Parameters for determining inoculated pack/challenge study protocols. *J. Food Prot*. 73:140–202) recommends use of a cocktail of three to five *Salmonella* serovars chosen from among those isolated from food sources similar to foods under examination or showing desired characteristics, or from related outbreaks for such studies.

There are literally thousands of *Salmonella* serovars to choose from for such studies. The U.S. Food and Drug Administration draft spice risk assessment (2013) lists approximately 150 serovars associated with spices alone. In this case, four such serovars were chosen, based on their presence in low-moisture foods. Of the four that were chosen, one serovar was misidentified in the culture collection. The misidentified serovar was *Salmonella* Reading, which has been found in spices (cumin) and is desiccation resistant. It was not a different genus, only a different serovar. It was misidentified as *Salmonella* Enteritidis from almonds. Upon testing, it was found that the other serovars used in these studies were correctly identified.

Each serovar was grown individually and then mixed with the others in equal amounts prior to use in any experiment. The reasoning behind this type of usage is that it is not known, nor can it be predicted, which of the serovars will be most resistant. As there is no way to test hundreds of serovars to determine the most resistant, the NACMCF recommendation to use a multi-strain cocktail was followed. All experiments described in the challenge studies published by the research group involved the use of a multi-strain cocktail. Consequently, the misidentification of one of the four *Salmonella* serovars used should have had no impact on the results.

Conclusions made in all articles did not read "for *Salmonella* Enteritidis . . ."; conclusions were simply "for *Salmonella*." Although all the serotypes used are resistant to desiccation and have been found in low-water-activity products, more testing would have been required to compare their individual survival dynamics under dry conditions. Even with the additional testing, there would be no guarantee that the most resistant serovar of *Salmonella* was chosen out of the hundreds that are available. The authors were analyzing samples from a 2-year survival study, which allowed this error to be discovered. The old samples were reexamined in order to identify which of the four *Salmonella* serovars used in the cocktail survived the longest. The misidentified serovar, *Salmonella* Reading, was among the most plentiful present, followed by Tennessee and Anatum. *Salmonella* Oranienberg, the fourth serovar used, originally isolated from raw pecans, was not re-isolated. This last finding simply reinforces NACMCF's recommendation that multiple serovars should be used in challenge studies, and that results and conclusions from the authors' studies are correctly noted. In truth, *Salmonella* Reading could have legitimately been chosen for these tests rather than *Salmonella* Enteritidis, or only three different serovars could have been used from the onset, which would have resulted in the same results and conclusions.

In summary, the described error does not affect the results and conclusions noted in the articles published in the *Journal of Food Protection*. Nonetheless, a correction is required for the articles in which this serotype was listed. The affected articles are as follows:

Keller, S. E., C. N. Stam, D. R. Gradl, Z. Chen, E. L. Larkin, S. R. Pickens, and S. J. Chirtel. 2015. Survival of *Salmonella* on chamomile, peppermint, and green tea during storage and subsequent survival or growth following tea brewing. *J. Food Prot*. 78:661–667. https://doi.org/10.4315/0362-028X.JFP-14-508.

Gradl, D. R., L. Sun, E. L. Larkin, S. J. Chirtel, and S. E. Keller. 2015. Survival of *Salmonella* during drying of fresh ginger root (*Zingiber officinale*) and storage of ground ginger. *J. Food Prot*. 78:1954–1959. https://doi.org/10.4315/0362-028X.JFP-15-153.

Hildebrandt, I. M., C. Hu, E. M. Grasso-Kelly, P. Ye, N. M. Anderson, and S. E. Keller. 2017. Dry transfer inoculation of low-moisture spices containing antimicrobial compounds. *J. Food Prot*. 80:338–344. https://doi.org/10.4315/0362-028X.JFP-16-279.

Santillana Farakos, S. M., R. Pouillot, and S. E. Keller. 2017. Salmonella survival kinetics on pecans, hazelnuts, and pine nuts at various water activities and temperatures. *J. Food Prot*. 80:879–885. https://doi.org/10.4315/0362-028X.JFP-16-392.

The correction should read:

> In this article, *Salmonella enterica* Reading was misidentified as *Salmonella* Enteritidis PT30. Other serovars used are correct as indicated. As a mixture (cocktail) of multiple serovars was used in this study, the misidentification does not impact results or conclusions.