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Research Paper

Evaluating the Risk of Salmonellosis from Dry Roasted Sunflower Seeds

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ABSTRACT

Outbreaks and recalls related to nuts and seeds in the United States have increased recently, and 80% of these recalls are due to *Salmonella*. The U.S. Food and Drug Administration's Food Safety Modernization Act requires food manufacturers to implement risk-based preventive controls based on scientific and technical evidence. Data are limited on the inactivation of *Salmonella* during processing of saltwater brined in-shell sunflower seeds. The goal of this research was to validate the adequacy of roasting in controlling *Salmonella* during the production of sunflower seeds and to assess the resulting risk. Four *Salmonella* strains were inoculated onto sunflower seeds and processed to simulate commercial manufacturing. Seeds were tumbled and roasted at 225°F (107.2°C) and 275°F (135°C) for roasting times from 5 to 45 min. Regression models for *Salmonella* inactivation and water activity change were developed. The inactivation model predicted a 5-log reduction in *Salmonella* when sunflower seeds were roasted at 135°C for 19.2 min, with a corresponding water activity of ~0.61. Roasted sunflower seeds are typically not saleable at water activities >0.6 due to quality issues. Saleable water activities (0.03 to 0.04) were only achieved when the sunflower seeds were roasted for 45 min at 135°C, which resulted in a >7-log reduction in *Salmonella*. A quantitative microbial risk assessment based on literature values, expert opinion, and the above-mentioned models was used to predict risk of salmonellosis from sunflower seeds. The quantitative microbial risk assessment model predicted an arithmetic mean probability of illness of 1.45E–07 per 28-g serving based on roasting at 135°C for 20 min and an arithmetic mean probability of illness of 5.46E–10 per serving based on roasting at 135°C for >45 min (i.e., saleable product process parameters). This study demonstrates that sunflower seeds roasted to saleable parameters should not represent a public health risk from potential presence of *Salmonella*.

Key words: Risk assessment; Roasting; *Salmonella*; Thermal inactivation; Validation

The popularity of snacking in the United States has increased over the past several years. Nearly all (94%) Americans snack at least once a day, and data show that 37% of the time a snack replaces one of the three key meals of the day *(15)*. Consumers often seek out healthy, plant-based proteins and convenient snacking options *(4)*. Sunflower seeds meet this need because they are nutritionally rich and portable (i.e., shelf stable due to their low moisture).

Outbreaks and recalls related to low-moisture foods, particularly nuts and seeds, have increased over recent years *(10–12)*. Approximately 10% of all food recalls in the United States between 2004 and 2013 were attributed to nuts, seeds, and nut products, with 80% of these recalls due to *Salmonella* contamination *(46)*. Low-moisture foods accounted for 19% (13 of 70) of multistate foodborne illness outbreaks investigated by the Centers for Disease Control and Prevention between 2014 and 2018 *(13)*. These outbreaks and recalls are often large, affecting many consumers and multiple food manufacturers. More than 1 million lb (453,592 kg) of roasted pistachios was recalled due to *Salmonella* contamination in March to April 2009 *(10)*. These recalls and outbreaks have heightened awareness of the importance of *Salmonella* control in low-moisture foods.

Salmonella has become the main pathogen of concern in low-moisture foods *(32)* and poses an important food safety challenge for several reasons. *Salmonella* can survive long periods in low-moisture foods including tree nuts *(7)* and wheat flour *(16)* and in the environment *(44)*. Illness has been associated with levels as low as 1 CFU/g *(22),* and a *Salmonella* outbreak caused by paprika and potato chips seasoned with contaminated paprika had *Salmonella* concentrations as low as 0.04 to 0.05 CFU/g *(29)*. Willis et al. *(52)* reported levels of *Salmonella* in the range of 0.1 and 0.2 most probable number (MPN) per g (2 of 6 samples) and <0.1 MPN/g (4 of 6 samples) in a variety of seeds,

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including alfalfa, flax (linseed), melon, sesame, sunflower, and mixed seeds. The heat resistance of *Salmonella* greatly increases with a decrease in water activity (a_w) *(22, 33)* but can differ based on strain and serotype, food matrix, and recovery methodology. Ma et al. *(31)* reported that peanut butter required heating for 42 ± 8 min at 90°C to achieve a 5-log reduction of *Salmonella* Tennessee, an outbreak-associated strain.

The U.S. Food and Drug Administration's (FDA's) Food Safety Modernization Act requires food manufacturers to establish and implement risk-based preventive controls. A key component in the design of food safety limits for preventive controls is validation *(25)*. Validation of preventive controls must include "obtaining and evaluating scientific and technical evidence (or, when such evidence is not available or is inadequate, conducting studies) to determine whether the preventive controls, when properly implemented, will effectively control the hazards" *(49)*. There is guidance available on how to demonstrate pathogen inactivation generally and more specifically on low-moisture foods *(3, 34)*. Some guidance (4-log reduction for treated almonds and 5-log reduction for pasteurized almonds) on performance standards is available for specific low-moisture foods such as almonds *(2, 14, 45)*; however, there is limited guidance on establishing performance standards for low-moisture foods *(17, 39)*.

In-shell sunflower seeds are considered low-moisture foods, but they undergo a unique process relative to other nuts. Sunflower seeds are brined in a saltwater solution, roasted, and then packaged. There is limited published data on the inactivation of pathogens (including *Salmonella*) during processing of in-shell sunflower seeds. This could have negative repercussions for sunflower seed processors, should a source of raw sunflower seeds be implicated in an outbreak or recall, as history has shown. A 2018 recall of vegetable products resulted in several manufacturers recalling additional product due to limited understanding of thermal processing of specific products in which the vegetable products were used as an ingredient *(51)*.

The overall goal of this research was to validate the adequacy of the roasting process in controlling *Salmonella* during the production of roasted sunflower seeds. We also (i) establish critical roasting parameters required to achieve 4- and 5-log reductions in *Salmonella,* (ii) identify the minimum food safety verification parameters required to achieve adequate lethality of *Salmonella* during roasting of sunflower seeds, and (iii) assess the risk of salmonellosis associated with the consumption of dry roasted sunflower seeds via laboratory data in a quantitative microbial risk assessment (QMRA) model framework.

MATERIALS AND METHODS

Bacterial strains and culture preparation. *Salmonella enterica* serovars Typhimurium (ATCC 27198, a commonly used laboratory type strain *(43)*), Newport (BAC 251, food plant isolate), Enteritidis (ATCC BAA 1045, phage type 30, isolated from raw almonds), and Tennessee (isolated from raw sunflower seeds) were obtained from Conagra Brands Omaha Microbiology Laboratory culture collection (Omaha, NE). The stock cultures were kept frozen at −80°C in 0.7 mL of tryptic soy broth (Acumedia, Lansing, MI) and 0.3 mL of 50% glycerol. The four *Salmonella* strains were grown separately in brain heart infusion broth (Acumedia) and incubated (model NSR1301SSS/8, Nor-Lake Scientific, Hudson, WI) at 35 ± 1°C. Each strain was subcultured twice for 22 ± 2 h at 35 ± 1°C after which 1 mL of culture was surface plated onto tryptic soy agar (TSA) plates (150 by 15 mm; Acumedia). Cells were harvested following overnight incubation (20 ± 4 h at 35 ± 1°C) by depositing 5 to 6 mL of sterile 0.1% peptone water on the lawn that had developed on each plate. Cells were suspended with a plastic sterile L-shaped cell spreader (Argos Technologies, Vernon Hills, IL) and collected in sterile 50-mL conical tubes (Fisher Brands, Pittsburgh, PA). Equal volumes of each strain were pooled to form a cocktail. The purpose of modeling in this study was to make a fail-safe conservative model; hence, we inoculated the matrix with a cocktail of multiple *Salmonella* strains and then modeled the survivors. This gave us a conservative model useful for informed decision making. The cultures were centrifuged (model Sorvall Legend XTR, Thermo Fisher Scientific, Waltham, MA) at 6,000 × *g* for 10 min and washed in 10 mL of sterile 0.1% buffered peptone water (BPW; Acumedia). The cells were resuspended in 40 mL of sterile 0.1% BPW and kept at 25°C for immediate use. Cell concentrations in the cocktail on each day were determined and standardized using methods described below.

Products and inoculation procedure. Preparation of sunflower seeds for the study was designed to simulate commercial manufacturing conditions. Sunflower seeds were obtained from Conagra Brands manufacturing facility located on the U.S. West Coast and stored at room temperature for up to 1 month until further analysis. Seeds in batches of 400 g were transferred to a sterile Whirl-Pak bag (9.75 by 19 in. [25 by 48 cm]; Nasco, Fort Atkinson, WI), and 25 mL of inoculum was added. The target level of inoculation was 10^8 to 10^9 CFU/g for *Salmonella*. The actual inoculation level achieved over all replicates was 8.18 ± 0.07 log CFU/g. Uninoculated control samples were also prepared from the same batch of sunflower seed samples, under similar conditions, by inoculating with 25 mL of sterile 0.1% BPW. Bags containing inoculated and uninoculated samples were agitated by hand for 5 min to ensure even coverage of inoculum. Samples were then allowed to equilibrate under ambient conditions for a minimum of 60 min. Next, seeds were separated into two equal batches of 212.5 g to be brined. Each batch was brined separately with 400 mL of brine (118 g of NaCl [Macron Fine Chemicals, Avantor Inc., Radnor, PA] mixed into 400 mL of deionized water) in a 1-L glass beaker inside an Oxoid anaerobe polycarbonate 3.5-L jar (Thermo Fisher Scientific) fitted with vacuum gauge and air valve. A mesh metal plate was placed over the seeds to ensure that they were submerged in the brine. One vacuum pulse of 20 to 25 in. (50 to 64 cm) of Hg was generated for 35 s to simulate brining process under commercial conditions. Brine was drained from seeds with a strainer, and seeds were transferred to sterile wire mesh baskets and then dried for 40 min in an ESPEC environmental chamber (ESPEC North America Inc., Hudsonville, MI) at 25°C and 16% relative humidity. All dried seeds to be used for one roasting repetition were combined into a large bag. The bag was agitated for 1 to 2 min, and samples of 250 g were weighed into separate sterile Whirl-Pak bags for each time point to be tested at each temperature. The water activity of the brined seeds in this study was essentially equivalent to the water activity of the brined seeds under commercial conditions ($a_w = 0.742 \pm 0.004$).

Experimental design. Inoculated and uninoculated sunflower seeds were roasted separately under similar conditions. Sunflower seeds were transferred to a preheated cylindrical roasting screen, placed in a preheated oven (GE Profile model JT915S0F2SS, GE Appliance, Louisville, KY), tumbled, and roasted at oven settings of 225°F (107.2°C) and 275°F (135°C). Oven (hot air) and seed temperatures of uninoculated sunflower seeds were monitored and recorded in 10-s increments with a 20-gauge copper-constantan wire hypodermic needle probe (Omega, Norwalk, CT) and TEF-22-S stranded conductor 22-gauge copper-constantan thermocouple wire with a C-10 male locking connector and subminiature male connector (Ecklund-Harrison Technologies Inc., Fort Myers, FL), all connected to a CalPlex Data logger box (TechniCAL, Metairie, LA). Roasting times of 5, 10, 15, 20, 25, 30, and 45 min were tested in duplicate. Sunflower seeds were cooled immediately after roasting by transferring from the roasting screen onto refrigerated trays covered with sterile foil. Samples were then transferred to sterile Whirl-Pak bags and placed in an ice bath following respective treatments; cool-down times were not considered part of roasting time. The time and temperature parameters used here are less than those typically used commercially and so represent worst-case roasting processes in these products. The roasting parameters used in manufacturing facilities are designed to deliver saleable product, with roasting temperatures higher, and roasting times longer, than those used in this study. Experiments at both temperatures were each replicated three times.

Sample analysis. Sunflower seeds samples (2 by 40 g) were transferred to sterile Whirl-Pak bags from the refrigerated trays and placed in an ice bath until they were diluted (1:10) with sterile BPW and mechanically homogenized (model Smasher, AES Laboratoire, bioMérieux, Marcy-l'Étoile, France) for 2 min. Inoculated, brined, and dried sunflower seeds, considered as time zero, were also weighed out into sterile Whirl-Pak bags, diluted (1:10) with sterile BPW, and mechanically homogenized to determine the initial inoculation level of the sunflower seeds. Sample preparation for microbiological analysis was performed in compliance with FDA-approved methods *(50)*. Serial dilutions were performed in sterile 0.1% BPW and 1 mL or 100 μL of appropriate diluents, and *Salmonella*-inoculated sunflower seed samples were analyzed by pour plate method using TSA with xylose lysine desoxycholate (Acumedia) overlay to aid in recovery of heat-injured or stressed cells while facilitating better identification of target pathogens *(23, 24, 54)*. Duplicate samples were plated at each time point for each replicate. The results from the duplicates were averaged for each replicate. Water activity analysis for uninoculated samples was performed using a digital water activity meter with a chilled-mirror dewpoint technique (model AquaLab 34TEV, Decagon Devices, Inc., Pullman, WA) at ambient temperature. The digital water activity meter was calibrated with four different water activity salt solutions: 13.41 mol/kg LiCl (0.250 ± 0.003 a_w at 25°C), 8.57 mol/kg LiCl (0.500 ± 0.003 a_w at 25°C), 6.00 mol/kg NaCl (0.760 ± 0.003 a_w at 25°C), and 0.50 mol/kg KCl (0.984 ± 0.003 a_w at 25°C).

Statistical analysis. A negative binomial nonlinear exponential decay model of the following form was fit for *Salmonella,* using PROC NLMIXED in SAS software (SAS *(38)*):

$$\log(\mu) = \beta_0 + \beta_1 \times \exp(-\beta_2 \times \text{time}) + u$$

where the negative binomial has the parameter μ equal to the mean of *Salmonella* counts, which is based on three replicates at each time point. Each replicate was based on average of duplicate samples. The β values represent fixed regression constants. The random replicate effect, *u,* is assumed to be normally distributed with mean = 0 and variance = σ_u^2. Generalized linear mixed models have been previously described in the literature for microbiological data *(26, 55)*. The nonlinear model better captures the exponential nature of decline in microbial counts. In this study, a negative binomial model was used to estimate roasting times to achieve a 4- and 5-log reduction in *Salmonella,* along with 95% inverse prediction intervals. The fit of this exponential decay model (full model) was compared with an intercept model (reduced model) using a likelihood ratio test. The ratio of the log likelihood was used as the test statistic for a chi-square test to compare the fit of the full model and the reduced model. Contrary to commonly used ordinary least-squares methods that minimize variance, generalized nonlinear mixed model parameters are estimated using an iterative maximum likelihood method. A traditional R^2 statistic does not exist when analyzing data using generalized nonlinear mixed models, but numerous pseudo-R^2 calculations have been developed to evaluate goodness of fit. The method that we used in this study is Efron's psuedo-R^2 *(53)*:

$$R^2 = \frac{\sum_{i=1}^{N} (y_i - \hat{\pi}_i)^2}{\sum_{i=1}^{N} (y_i - \bar{y})^2}$$

where $\hat{\pi}_i$ is model predicted count.

The model was used to generate a best estimate of roasting times to achieve a 4- and 5-log reduction in the target pathogens, with 95% inverse prediction intervals. These inverse prediction intervals are illustrating the time bounds (minimum and maximum) required to deliver a 4- or 5-log reduction.

A four-parameter logistic regression model of the following form was fit for water activity, using PROC NLIN in SAS software *(38)*:

$$\text{Water activity} = d + \frac{a - d}{1 + \left(\frac{\text{time}}{c}\right)^b}$$

where a is the upper asymptote, b is the maximum slope, c is the inflection point, and d is the lower asymptote. A linear regression model of the following form was fit for product temperature, using PROC MIXED in SAS software *(38)*:

$$\text{Product temperature} = \beta_0 + \beta_1 \times \log(\text{time})$$

The β values represent fixed regression constants. Only effects that were significant at $\alpha = 0.05$ were retained in the final model. A one-way analysis of variance was conducted using the PROC GLM procedure in SAS software to evaluate the impact of roasting time on naturally occurring microflora (aerobic plate and *Enterobacteriaceae* counts) at different temperatures. Treatments were deemed significant at $\alpha \leq 0.05$.

Overview of risk assessment. An overview of the simulation variables and their respective distributions for the quantitative microbial risk assessment model is provided in Table 1. Values for prevalence and concentration of *Salmonella* in sunflower seeds were either taken from literature values or assumed based on expert opinion *(52)*. A distribution of log-reduction values during processing was based on results from previously described roasting experiments. The estimated number of illnesses per 1 million servings was determined using a previously published and widely used dose-response model *(21)*. Results are calculated and reported on a basis of 1 million servings.

Baseline risk scenario. *Salmonella* prevalence and concentration were assumed based on expert opinion and estimated based

TABLE 1. *Overview of baseline risk assessment variables and calculations*

Variable	Description	Reference/source
Salmonella prevalence	*Salmonella* prevalence for raw (untreated) sunflower seeds per 28-g serving; 1 (0.1%) of 976 servings	*52*
No. of contaminated servings	Total no. of 28-g servings (per 1 million servings) contaminated with *Salmonella*; 1 of 976 × 1,000,000 = 1,024 contaminated samples	Calculation
Salmonella concn	*Salmonella* concn in raw (untreated) sunflower seeds based on prevalence; triangular (0, 0.1, 0.2) CFU/g	*52*
Log reduction	Log reduction of *Salmonella* applied during dry roasting at 135°C for 20 min; normal distribution with $\mu = 5.07$ and $\sigma = 0.57$ log reductions	Figure 2 and negative binomial model per Table 3
Concn after processing	*Salmonella* concn in sunflower seeds after dry roasting treatment (*Salmonella* concn/$10^{\log \text{ reduction}}$)	Calculation
Probability of illness	Dose-response model for *Salmonella* (β-Poisson distribution) used to predict probability of illness per serving of dry roasted sunflower seeds $\{1 - [1 + (\text{concn per serving after processing})/51.45]^{-0.1324}\}$	*21*
No. of expected illnesses	No. of illnesses expected per 1 million servings based on the no. of contaminated servings present and probability of illness (probability of illness × no. of contaminated servings)	Calculation

on previously published work on prevalence in sunflower seeds. Prevalence was estimated as 1 (0.1%) in 976 servings being positive for *Salmonella (52)*. Prevalence was represented by a triangular distribution, with minimum value of 0 CFU/g, most likely value of 0.1 CFU/g, and maximum value of 0.2 CFU/g *(52)*. The number of contaminated servings was obtained by multiplying the prevalence by 1 million servings.

Log reductions were obtained experimentally as described above. A heat treatment of 135°C for 20 min was used in the risk assessment baseline scenario because a ~5-log reduction was estimated when sunflower seeds were roasted at 135°C for 19.2 min. The log reductions achieved during the 20-min roasting process at 135°C were assumed to be normally distributed, with mean of 5.07 log reductions and standard deviation of 0.57 log reductions, based on experimental data. The average *Salmonella* concentration per gram of sunflower seeds after applying the log-reduction steps was determined by calculating 10 to the power of the simulated log reduction and then dividing the concentration by this value. The concentration per serving was determined by multiplying the resulting concentration by 28 g (thus, a serving was assumed to be 28 g). This study used a dose-response model for salmonellosis risk associated with eggs and broiler chickens developed by the Food and Agricultural Organization of the United Nations *(21)*. This dose-response model has been used previously for *Salmonella* risk assessments in foods similar to sunflower seeds, such as almonds *(28),* pistachios *(27),* and peanuts (*8*). The probability of illness per serving was calculated from this model, with $\alpha = 0.1324$ and $\beta = 51.45$. The number of expected illnesses was calculated by multiplying the probability of illness per serving by the number of contaminated servings.

Risk scenario analysis. Three scenarios were evaluated against the baseline described above to determine the impact of different log reductions on risk. One simulation evaluated the impact of adding a higher concentration sample on risk. Two different prevalence levels were used, with "normal concentration" prevalence represented by 1 (0.1%) in 976 100-g samples being contaminated *(52)* as in the baseline scenario and "high concentration" prevalence as ~1 order of magnitude less (1 in 10,000 samples is contaminated [0.01%]). Two *Salmonella* concentrations were assumed, with a normal concentration represented by a triangular distribution with minimum value of 0 CFU/g, most likely value of 0.1 CFU/g, and maximum value of 0.2 CFU/g *(52),* and high concentration an order of magnitude higher, represented by a uniform distribution with a minimum value of 1 CFU/g and a maximum value of 2 CFU/g. A previous risk assessment for peanuts showed the difference between low and high concentration of *Salmonella* was ~1 order of magnitude *(8)*. In the model, a discrete distribution was used to select the input concentration for each prevalence level, with a 0.1% chance of selecting a normal concentration and a 0.01% chance of selecting a high concentration.

Two simulations were run related to log reductions achieved during roasting. A simulation with no roasting treatment applied (i.e., 0 log reductions) was run to gain an understanding of the effectiveness of the roasting treatment in reducing risk. We also simulated the effect of a 7.28 ± 0.37-log reduction treatment because sunflower seeds typically undergo a 45-min roasting treatment (with a >7-log reduction expected) to reach a saleable moisture content. This distribution of log reductions was obtained from experimental data.

RESULTS

Effect of roasting on *Salmonella*. Figures 1 and 2 show the inactivation of *Salmonella* during the roasting of sunflower seeds at 107.2 and 135°C, respectively. *Salmonella* counts decreased with increase in roasting time, regardless of temperatures used. Raw data are overlaid on the fitted negative binomial model. Colored error bars represent inverse prediction intervals (95%) for baking times that deliver 4- and 5-log reductions. The overall predictions during the roasting process were consistent with the observed *Salmonella* counts when roasted at 107.2 and 135°C, as shown in Figures 1 and 2, respectively. Table 2 illustrates model fit statistics for the negative binomial model. The full model was used to assess the relationship between *Salmonella* counts and roasting times at the respective temperatures. The reduced model is described by the null hypothesis that β values (excluding the intercept) are equal to zero. Therefore, the reduced model uses only the intercept. The –2-log likelihood is used to compare the fit of the full model and the reduced model. The *P* value of

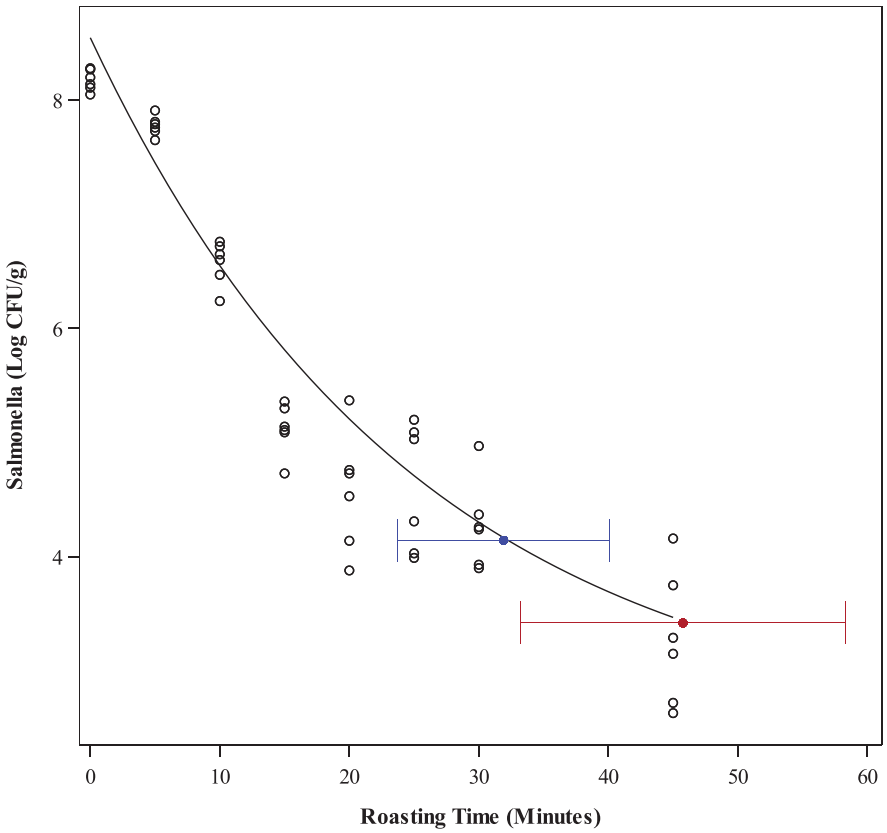

FIGURE 1. *Inactivation of* Salmonella *in sunflower seeds at 107.2°C. Raw data (○) is overlayed on the fitted regression model (solid line). Interval lines represent inverse prediction intervals (95%) for baking times that deliver 4-log (blue) and 5-log (red) reductions.*

the comparison is <0.001, indicating that the full model fits the model significantly better than the reduced model. Model parameters for the negative binomial are given in Table 3.

Product temperatures and water activity when seeds were roasted at 135°C are illustrated in Figure 3. Product temperatures increased with increase in roasting time and water activity decreased with increase in roasting time. This

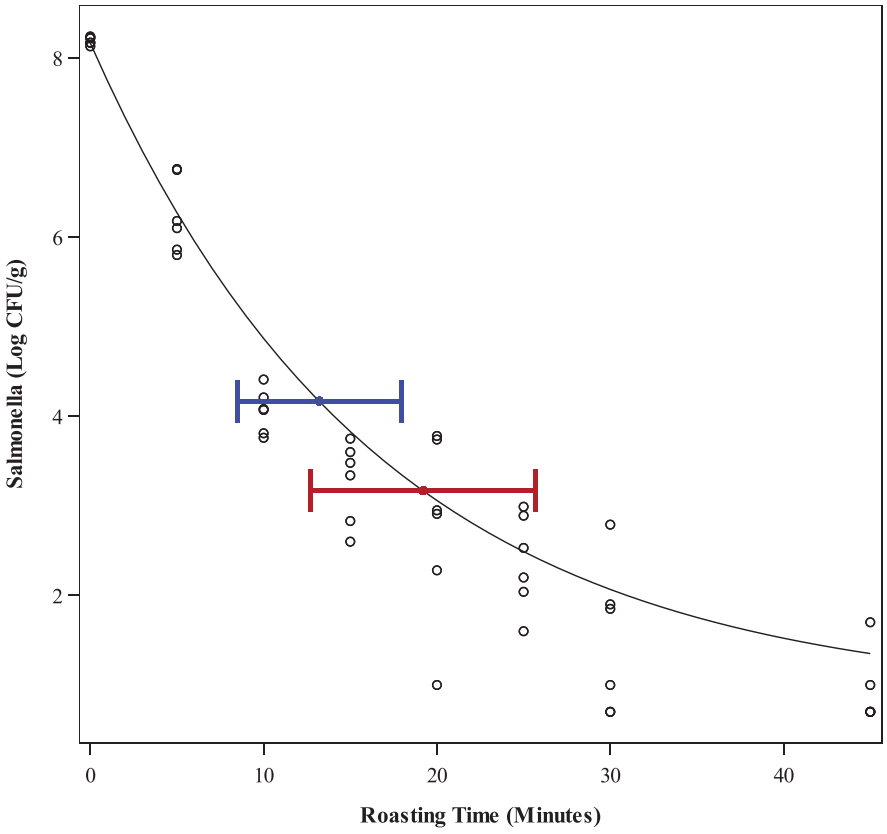

FIGURE 2. *Inactivation of* Salmonella *in sunflower seeds at 135°C. Raw data (○) is overlayed on the fitted regression model (solid line). Interval lines represent inverse prediction intervals (95%) for baking times that deliver 4-log (blue) and 5-log (red) reductions.*

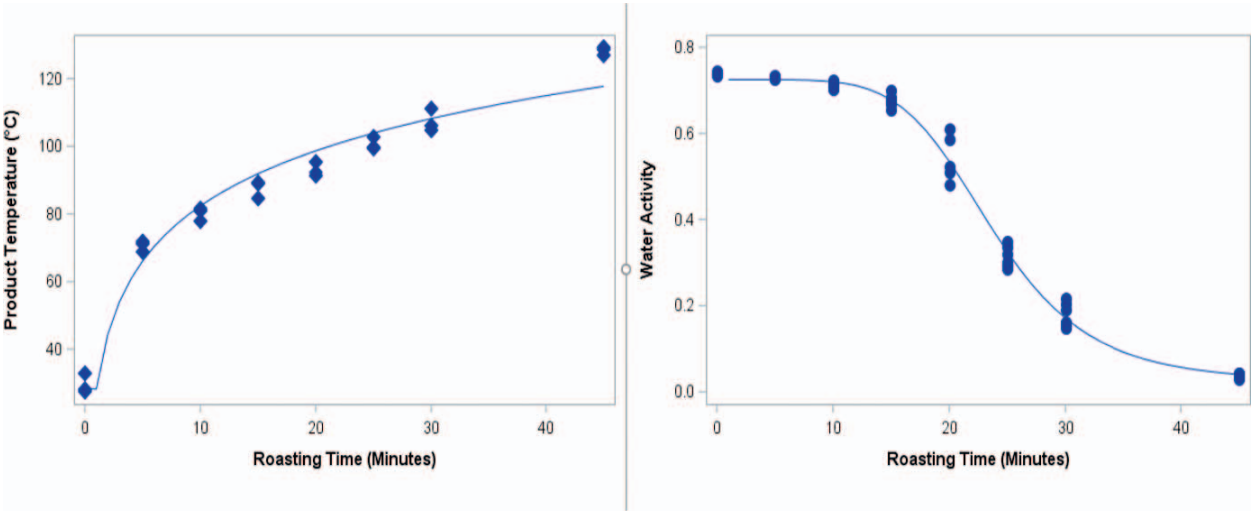

FIGURE 3. *Internal product temperature (left) and water activity (right) during the roasting of sunflower seeds at 135°C. Raw product temperature (◆) and water activity (●) data are overlayed with the fitted regression models.*

inverse relationship between product temperature and water activity during roasting is expected because a significant increase in product temperatures is often accompanied by moisture loss due to increased rate of evaporation. Water activity and product temperatures prediction model parameters are listed in Tables 2 and 3.

The roasting times for specific log reductions in *Salmonella* estimated by the negative binomial model can be used to estimate the minimum food safety verification parameters using the 95% confidence interval estimates from the product temperature and a_w models (Tables 4 and 5). The negative binomial model estimated a 4- and 5-log reduction in *Salmonella* when sunflower seeds were roasted at 135°C for 13.2 and 19.2 min, respectively. Based on calculation of 95% confidence intervals (Tables 4 and 5), the minimum product temperature and water activity corresponding to a roasting time of 13.2 min at 135°C was estimated to be 77.8°C and 0.724, respectively. Similarly, the minimum product temperature and water activity corresponding to a roasting time of 19.2 min at 135°C was estimated (Tables 4 and 5) to be 85.8°C and 0.614, respectively.

Baseline risk assessment scenario. The simulation predicted an arithmetic mean probability of illness of 1.45×10^{-7} per serving and geometric mean probability of illness of 5.50×10^{-8} per serving (Table 6), with the given assumptions about sunflower seeds processing. Those specific assumptions were as follows: a process of 20 min (5-log reduction); a process of 45 min, resulting in a saleable product (>7-log reduction); and no treatment (0-log reduction). Figure 4 shows the logarithm of the geometric mean expected cases per 1 million servings, where the median chance of one case is ~1 in 20,000 (ca. –4.3 log) given 1 million servings. The bars shaded in black (Fig. 4) represent a 1.25% probability of mean expected cases of more than one case per 1 billion servings.

Risk assessment scenario analysis. The salmonellosis risk roughly doubled when a high concentration sample scenario was considered. The arithmetic mean probability of illness increased to 3.20×10^{-7} per serving and the geometric mean probability of illness increased to 6.29×10^{-8} per serving (Table 6). The arithmetic mean predicted number of illnesses per 1 million servings increased to 3.60×10^{-5} and the geometric mean number of predicted illnesses per 1 million servings increased to 7.76×10^{-5} for the high concentration sample scenario (Table 6). The model estimated a 6.9% chance of more than one illness per 1 billion servings in this scenario.

The salmonellosis risk decreased when log reductions were assumed to be at the level achieved when the product reaches a saleable moisture content, or a >7-log reduction after a 45-min roast at 135°C. The arithmetic and geometric mean probability of illness was 5.46×10^{-10} and 3.39×10^{-10}, respectively, per serving (Table 6). The

TABLE 2. *Model fit statistics for negative binomial model used to estimate* Salmonella *counts during roasting of sunflower seeds at 107.2 and 135°C*

Temp		Full	Reduced	Log-likelihood	Degrees		Efron's
°C	°F	(−2-log likelihood)	(−2-log likelihood)	ratio (full − reduced)[a]	of freedom	*P*	pseudo-R^2
107.2	225	1,372.6	1,505.3	132.63	2	<0.001	0.90
135.0	275	980.4	1,138.5	158.05	2	<0.001	0.91

[a] The ratio of the log likelihood was used as the test statistic for a chi-square test to compare the fit of the full model and the reduced model. If the *P* value of the comparison is <0.05, the full model is deemed significantly a better fit than the reduced model.

TABLE 3. *Parameter estimates for negative binomial model used to estimate* Salmonella *counts during roasting of sunflower seeds at 107.2 and 135°C*[a]

Temp					
°C	°F	β_0	β_1	β_2	k
107.2	225	2.44	6.11	0.04	0.87
135.0	275	0.86	7.31	0.06	1.33

[a] β values represent fixed regression constants and k is the dispersion parameter.

estimated arithmetic mean number of illnesses was 5.59×10^{-7} per 1 million servings, and the geometric mean number of illnesses was 3.47×10^{-7} per 1 million servings (Table 6). The model estimated a 14.4% chance of more than one illness per 1 trillion servings.

Salmonellosis risk increased dramatically if no reduction in *Salmonella* concentration was assumed. The arithmetic mean probability of illness was 6.94×10^{-3} per serving, and the geometric mean probability of illness was 6.17×10^{-3} per serving (Table 6). The estimated arithmetic mean and geometric mean of illnesses per 1 million servings was 7.11 and 6.38, respectively (Table 6). The model predicted a 99.1, 17.2, and 0% chance of greater than 1, 10, or 100 illnesses per year, respectively, without any treatment to reduce *Salmonella* on the seeds.

DISCUSSION

There is limited literature available discussing the inactivation kinetics of *Salmonella* during the roasting of sunflower seeds, but results are available for products that undergo similar processes, such as brined pistachios. Casulli et al. *(9)* reported that pistachios presoaked with 27% NaCl had an initial a_w of 0.77 before roasting. Although our experimental conditions and matrix preclude us from making direct comparisons with those of Casulli et al. *(9),* we also observed that presoaking sunflower seeds with brine decreased the heat resistance of *Salmonella*. Our study shows a longer time (45.8 min) was needed to give a 5-log reduction in *Salmonella* when sunflower seeds were roasted at 107.2°C relative to Casulli et al. *(9),* who report a 5-log reduction in *Salmonella* in pistachios subjected to hot-air treatment of 104.4°C for 15 min, which may be due to interactions among strains and the matrix used in these studies. Furthermore, in pistachios, *Salmonella* contamination is more of a surface phenomenon, whereas in sunflower seeds, the meat of the sunflower seed is located in an intact shell containing small crevices; hence, as the seeds were inoculated, inoculum may have penetrated the seed, and therefore longer exposure times were needed to inactivate *Salmonella* in our study compared with the study of Casulli et al. *(9)*.

TABLE 4. *Parameter estimates for four-parameter logistic regression model ($R^2 = 0.999$) used for estimating water activity during roasting of sunflower seeds at 135°C*

Parameter[a]	Estimate	SE	Approximate 95% confidence limits		P
a	0.72	0.007	0.710	0.739	<0.001
b	5.69	0.332	5.017	6.365	<0.0001
c	23.81	0.266	23.269	24.348	<0.001
d	0.02	0.013	−0.004	0.047	0.088

[a] a, upper asymptote; b, maximum slope; c, inflection point; d, lower asymptote.

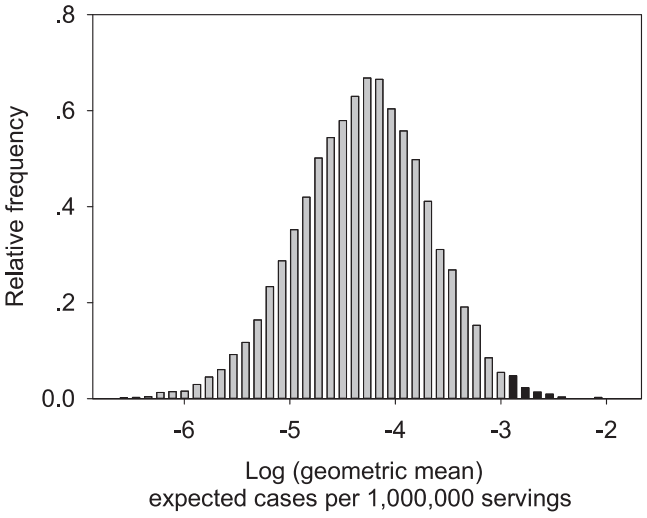

FIGURE 4. *Logarithm of the geometric mean expected salmonellosis cases per 1 million servings of sunflower seeds roasted as per the baseline scenario. The bars shaded in black represent a 1.25% probability of mean expected cases of more than one case per 1 billion servings.*

Beuchat and Mann *(6)* evaluated the effect of hot-air roasting on the inactivation of *Salmonella* in pecan nut meats. They reported a 5-log reduction in *Salmonella* when nut meats were subjected to hot-air treatments under the following conditions: 140°C for 20 min or 150°C for 15 min or 170°C for 10 min. Archer et al. *(5)* reported *D*-values between 40 and 45 min when wheat flour was treated with hot air at 167.0 to 170.6°C. The initial water activities of the flour ranged between 0.2 to 0.6. We observed relatively lower heat resistance of *Salmonella* in sunflower seeds compared with that of Archer et al. *(5)* and Beuchat and Mann *(6)*. Beuchat and Mann *(6)* and Archer et al. *(5)* exposed dry pecan meats and flour, respectively, to heat in a forced-air oven, whereas we exposed brined sunflower seeds to hot air. Hence, the differences in the heat resistance in our study compared with that of Beuchat and Mann *(6)* and Archer et al. *(5)* could be attributed to the initial water activity of the sunflower seeds before roasting. Differences in heat resistance in low-moisture foods may also be

TABLE 5. *Parameter estimates for regression model ($R^2 = 0.962$) used for estimating product temperatures during roasting of sunflower seeds at 135°C*

Parameter	Estimate	SE	t	Pr > |t|	95% confidence limits	
Intercept	28.25	2.75	10.26	<0.001	22.54	33.96
Log (time)	23.50	1.00	23.49	<0.001	21.42	25.57

TABLE 6. *Probability of illness per serving, number of expected illnesses, and number of reported illnesses per 1 million servings of contaminated sunflower seeds consumed given baseline and baseline plus some highly contaminated samples and processing for 20 min (5-log reduction), 45 min (saleable product, >7-log reduction), or untreated product*

		Process of 20 min, 5-log reduction		Process of 45 min, saleable product, >7-log reduction		No treatment (0 min)	
Risk measure	Contamination level	Arithmetic mean	Geometric mean	Arithmetic mean	Geometric mean	Arithmetic mean	Geometric mean
Probability of illness per serving	Baseline	1.45E−07	5.50E−08	5.46E−10	3.39E−10	6.94E−03	6.17E−03
	Baseline + high	3.20E−07	6.92E−08	1.20E−09	4.27E−10	1.31E−02	7.76E−03
No. of expected illnesses per 1 million servings	Baseline	1.49E−04	5.62E−05	5.59E−07	3.47E−07	7.11E+00	6.38E+00
	Baseline + high	3.60E−04	7.76E−05	1.35E−06	4.79E−07	1.48E+01	8.77E+00

attributed to the process temperatures, matrixes, and strains used *(34)*.

Sanders and Calhoun *(37)* noted that dry roasting peanuts at 154°C for 10 and 15 min resulted in a 4.6- to 4.7-log and a 5.4-log reduction of *Salmonella,* respectively. The Almond Board of California guidelines for validation of dry roasting process recommends that almonds be roasted at 137.8°C for 23 min to achieve a 4-log reduction in *Salmonella* Enteritidis phage type 30 *(2)*. In our study, when sunflower seeds were roasted at 135°C, a 4- and 5-log reduction in *Salmonella* was estimated when seed temperatures of sunflower seeds reached 77.8 and 85.8°C, respectively. Water activity of the product is a critical factor in determining an organism's heat resistance *(3, 9, 22, 35)*. Sanders and Calhoun *(37)* used dry peanuts, and Almond Board of California *(2)* recommendations are for dry almonds. In our study, sunflower seeds had an initial a_w of 0.54 that increased to 0.74 after brining in water containing 26% NaCl. As brined sunflower seeds are subjected to hot-air roasting, heat transfer occurs through conduction (from the drum roaster) and convection from outer surface of the sunflower seeds; as a result, the core temperature of each seed tends to increase with an increase in time. This process is also accompanied by rapid moisture loss and generation of latent heat *(41)*. This phenomenon could cause a rapid decline in *Salmonella* counts and hence explain the lower heat resistance in sunflower seeds that we observed.

Poirier et al. *(36)* reported a 5-log reduction in *Enterococcus faecium* (a surrogate of *Salmonella*) when peanuts were dry roasted at 149, 163, and 177°C for 21, 15, and 11 min, respectively. *E. faecium* NRRL B-2354 has been commonly used as a surrogate organism for *Salmonella* Enteritidis phage type 30 for performing validation of thermal treatments for almonds *(35)*. Although performing a study with a surrogate such as *E. faecium* is outside the scope of our research, it is worth discussing the findings of Poirier et al. *(36)* because the initial water activity of peanuts before roasting was similar to that of sunflower seeds in our study. Poirier et al. *(36)* noted the water activity of the peanuts reduced from 0.73 a_w to 0.33 to 0.13 a_w, whereas the moisture content of the peanuts decreased from 6.1 to 1.7%. Unpublished Conagra Brands studies indicated there is a loss of 31% moisture when brined sunflower seeds are hot-air roasted at 135°C for 20 min. Although the water activity of peanuts before roasting in Poirier et al. *(36)* is comparable to our brined sunflower seeds, the differences in moisture loss between both studies (4.4 versus 31%) could account for the observed differences in heat resistance of *Salmonella*. Our estimated product temperature corresponding to a minimum of a 4- and 5-log reduction in *Salmonella* was lower (77.8 and 85.8°C, respectively) compared with that of Poirier et al. *(36)*. This disagreement can be attributed to both the drum roasting process we used and the increased water activity due to brining of sunflower seeds before roasting. Experimental design protocols in previously published studies with roasted peanuts and almonds *(2, 36, 37)* primarily mimic the conditions in continuous-bed roasters that are typically used to produce roasted peanuts or almonds. Because we used the typical drum roasting process for sunflower seeds, thermodynamics of heat and mass transfer during the drum roasting process may be significantly different compared with conventional dry roasting processes used for peanuts and almonds *(2, 36, 37)*.

Drum roasting has been studied in detail with respect to coffee beans. Fadai et al. *(18)* reported that roasting of coffee beans is accompanied with significant moisture loss and generation of latent heat within the coffee bean. Vapor migration and heat transfer cause the rupturing of the cell walls, causing expansion of the bean *(18)*. Sunflower seeds crack and break in the roaster because they have a thinner shell wall compared with that of coffee beans. As a result, we expect the same mechanism reported in coffee bean would be seen in sunflower seeds as much of the heat transfer takes place via convection. Generation of latent heat within the sunflower seeds during the roasting process may also be responsible for the dramatic reduction in *Salmonella* reported herein.

Shah et al. *(40)* determined the efficacy of vacuum steam pasteurization for inactivation of several pathogens in a variety of matrices and reported a >5-log reduction in *Salmonella* when sunflower kernels were treated at 85°C for 3 min. In our study, a 4- and 5-log reduction in *Salmonella* was estimated when seed temperatures of sunflower seeds reached 77.8 and 85.8°C, respectively. Studies by Lucore *(30)* and Mattick et al. *(33)* indicated that the heat resistance of *Salmonella* is relatively lower at higher water activities and gradually increases with significant decrease in water activity. We observed higher heat resistance of *Salmonella* in sunflower seeds than that previously reported in high-

water-activity foods *(47),* although we did not calculate *D*-values. We observed a lower heat resistance of *Salmonella* in sunflower seeds compared with that of Archer et al. *(5)* and Ma et al. *(31)*.

The QMRA model presented herein predicted an arithmetic mean of 1.49×10^{-4} illnesses per million sunflower seed servings (geometric mean of 5.62×10^{-5} illnesses per million servings) for the baseline scenario and 3.60×10^{-4} illnesses per million sunflower seed servings (geometric mean of 7.76×10^{-5} illnesses per million servings) when including a highly contaminated sample. In risk assessments for pistachios and peanuts where storage steps are not included in the risk calculations, the risk of illness from sunflower seeds is lower than that of both pistachios and peanuts. The arithmetic mean risk of illness from pistachios was $\sim 1.4 \times 10^{-3}$ illnesses per million servings (geometric mean of 1.55×10^{-4} illnesses per million servings) for all pistachios (inshell and kernels) roasted to a 4 ± 0-log reduction with no storage steps contributing to log reductions *(27)*. The arithmetic mean risk of illness from peanuts when considering a baseline scenario with no storage steps was $\sim 4.21 \times 10^{-2}$ illnesses per million servings (geometric mean of 1.64×10^{-4} illnesses per million servings).

Calculating the risk at other prevalence levels may be useful when comparing these results with those of other commodities. The number of predicted illnesses will change approximately in proportion to the increase or decrease in prevalence. If other products have reported prevalence levels of ~1% (~1 order of magnitude higher than our baseline scenario prevalence of ~0.1%), this would correspond to an order of magnitude increase in predicted illnesses, resulting in an arithmetic mean of 1.49×10^{-3} illnesses per billion servings or a geometric mean of 5.62×10^{-4} illnesses per billion servings.

In published risk assessments of other low-moisture products that considered storage steps, the risk decreased below that of sunflower seeds as presented in this risk assessment. The arithmetic mean risk of illness from pistachios in a similar scenario as mentioned above, but including storage steps, was reduced to 1.2×10^{-4} illnesses per million servings or a geometric mean of 5.83×10^{-6} illnesses per million servings *(27)*. The arithmetic mean risk of illness from almonds (including storage steps) was 8.4×10^{-6} illnesses per million servings, or a geometric mean of 2.42×10^{-8} *(28)*. The arithmetic mean number of illnesses estimated for peanuts including storage steps was 3.91×10^{-3} cases per million servings, or a geometric mean of 3.30×10^{-6} illnesses per million servings *(8)*. The results of this sunflower seed QMRA are likely conservative for the baseline scenario because the water activity log-reduction values that meet the food safety standard were significantly higher than those needed to produce a saleable product. The arithmetic mean number of illnesses per million servings was 5.59×10^{-7} (geometric mean of 3.47×10^{-7} illnesses per million servings) for the saleable product scenario, a value that was more in line with what was reported for similar products including storage steps. Based on this information, if storage steps were included in this risk assessment, the risk would likely be further reduced. Almonds, pistachios, and peanut butter have all been implicated in salmonellosis outbreaks, so it may be of greater interest to compare sunflower seeds to nut products that have also not been implicated in salmonellosis outbreaks, such as pecans and walnuts. Risk for both pecans and walnuts may be lower than that of sunflower seeds, particularly when considering heating steps applied in the home *(19, 20)*. Because sunflower seeds are seldom used in home baking, they would be less likely to go through further heating steps during home cooking or baking than pecans or walnuts.

The QMRA model included assumptions about *Salmonella* prevalence and concentration, based on a single survey of edible dried seeds at retail in the United Kingdom that was published in 2009 *(52)*. Additional survey data for prevalence and concentration would be beneficial to provide a more representative estimate of risk. Data on storage times and temperatures represent significant data gaps. Storage steps provided a significant reduction in risk in previous risk assessments *(8, 27)*; however, this information was excluded from this study (due to a lack of information on both *Salmonella* death kinetics during sunflower seed storage as well as storage times and temperatures during production and distribution), resulting in a potentially conservative estimate of risk. Adding storage steps decreased arithmetic mean risk ~10-fold (and geometric mean risk ~100-fold) for both pistachios *(27)* and peanuts *(8)*.

There is no specific guidance or performance standard for roasted sunflower seeds. Indeed, limited data are available for most low-moisture products, making it difficult to establish specific log-reduction targets for many of these foods *(39)*. The default for the food industry is often a 5-log reduction *(39),* which is generally considered acceptable by the FDA in the absence of adequate information to establish a data-driven performance standard *(3, 47, 48)*. The 5-log reduction standard has been adopted by a few flour manufacturers *(1, 42),* suggested as a performance target for "adequately reducing *Salmonella*" in peanut-derived ingredients *(48),* and used as an index for "pasteurization" of almonds *(2)*. After a risk assessment was performed in almonds, risk mangers concluded that a 4-log reduction in *Salmonella* would provide adequate public health protection for that commodity *(14, 45)*. This is consistent with the National Advisory Committee on Microbiological Criteria for Foods recommendations that the process delivering reduction to the most resistant pathogen must be "to a level that is not likely to present a public health risk under normal conditions of distribution and storage" *(34)*. We generated best estimates for roasting times required to achieve a minimum of a 4- and a 5-log reduction in target pathogens (Fig. 3) and have demonstrated that the roasting process is an adequate preventive control for protection of public health during the production of roasted sunflower seeds. The experimental protocols designed for this study were based on sunflower seeds and a drum roasting process. Researchers are cautioned not to extrapolate the results reported in this study to those of other products or processes without appropriate scientific justification that may include validation studies (e.g., due to differences in product composition, water activity of the seeds before roasting, mode of heat transfer from the drum roaster to the product) in the specific matrices.

We estimated a 5-log reduction in *Salmonella* when sunflower seeds were roasted at 135°C for 19.2 min. The corresponding a_w value when sunflower seeds were roasted at 135°C for 19.2 min was ~0.61. We conclude that critical processing parameters for sunflower seeds are roasting air temperature and residence time. Correspondingly, the critical roasting verification parameters were found to be water activity and product temperature. Note that roasted (edible) sunflower seeds are typically not saleable at a_w values $\geq$0.6 due to mold growth or other quality issues and that saleable a_w values for roasted sunflower seeds typically range from 0.03 to 0.04. We have shown that saleable water activities were only achieved when the sunflower seeds were roasted for 45 min at 135°C, which resulted in a >7-log reduction in *Salmonella*. QMRA analysis based on critical food safety parameters of 135°C for 20 min as well as saleable product process parameters of 135°C for $\geq$45 min indicated extremely low risk (~0%) of salmonellosis with the consumption of roasted sunflower seeds. These products, when roasted to saleable quality and manufactured under hygienic manufacturing conditions, should not present a public health risk due to the potential presence of *Salmonella*.

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