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Modeling the survival kinetics of *Salmonella* in tree nuts for use in risk assessment

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ABSTRACT

Salmonella has been shown to survive in tree nuts over long periods of time. This survival capacity and its variability are key elements for risk assessment of *Salmonella* in tree nuts. The aim of this study was to develop a mathematical model to predict survival of *Salmonella* in tree nuts at ambient storage temperatures that considers variability and uncertainty separately and can easily be incorporated into a risk assessment model. Data on *Salmonella* survival on raw almonds, pecans, pistachios and walnuts were collected from the peer reviewed literature. The Weibull model was chosen as the baseline model and various fixed effect and mixed effect models were fit to the data. The best model identified through statistical analysis testing was then used to develop a hierarchical Bayesian model. *Salmonella* in tree nuts showed slow declines at temperatures ranging from 21 °C to 24 °C. A high degree of variability in survival was observed across tree nut studies reported in the literature. Statistical analysis results indicated that the best applicable model was a mixed effect model that included a fixed and random variation of δ per tree nut (which is the time it takes for the first $\log_{10}$ reduction) and a fixed variation of ρ per tree nut (parameter which defines the shape of the curve). Higher estimated survival rates (δ) were obtained for *Salmonella* on pistachios, followed in decreasing order by pecans, almonds and walnuts. The posterior distributions obtained from Bayesian inference were used to estimate the variability in the $\log_{10}$ decrease levels in survival for each tree nut, and the uncertainty of these estimates. These modeled uncertainty and variability distributions of the estimates can be used to obtain a complete exposure assessment of *Salmonella* in tree nuts when including time–temperature parameters for storage and consumption data. The statistical approach presented in this study may be applied to any studies that aim to develop predictive models to be implemented in a probabilistic exposure assessment or a quantitative microbial risk assessment.

1. Introduction

Tree nuts have been recognized for their potential contribution to foodborne illnesses (Frelka and Harris, 2014). *Salmonella* in particular, appears to be of concern in tree nuts, as it has been associated with foodborne outbreaks and recalls. From 2010 to January 2015, there have been four outbreaks linked to *Salmonella* in tree nuts in the U.S., one in 2014 involving *S.* Braenderup in almond butter, two in 2013: *S.* Stanley in raw cashew "cheese" and *S.* Senftenberg in roasted pistachios, and one in 2011 involving *S.* Enteritidis in pine nuts (Harris et al., 2015). There have also been numerous recalls indicating reoccurring microbial food safety issues due to *Salmonella* presence in walnuts, macadamia nuts, pecans, cashews, almonds, pistachios, pine nuts and hazelnuts (Palumbo et al., 2015). To assist development of a quantitative assessment of the risk of human salmonellosis associated with the consumption of tree nuts, the U.S. Food and Drug Administration requested data and models on survival of *Salmonella* in tree nuts through Federal Register Notice FDA-2013-N-0747-0001. Available and published *Salmonella* survival data were submitted in response to this notice. This includes raw data from the studies of Abd et al. (2012) (almonds), Beuchat and Mann (2010) (pecans), Blessington et al. (2012) (walnuts), Blessington et al. (2013a) (almonds and walnuts), Blessington et al. (2013b) (walnuts), Brar et al. (2015) (pecans), Kimber et al. (2012) (almonds and pistachios), and Uesugi et al. (2006) (almonds) (which was submitted via personal communication with the authors). In all of these studies, *Salmonella* was shown to be able to survive for weeks, months and even years. Survival curves tend to show a relatively rapid initial decline, followed by slow or no measurable decline over a much longer period of time, i.e., long term persistence (Frelka and Harris, 2014). However, differences in the shape of the survival curves have been observed depending on the study, the substrate, and/or the environmental conditions under which the experiments take place. Several

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mathematical models, including the log-linear model (Bigelow and Esty, 1920), the Geeraerd-tail model (Geeraerd et al., 2000), the Weibull model (Mafart et al., 2002; Peleg and Cole, 1998) and the biphasic linear model (Cerf, 1977) have been shown to appropriately describe the survival kinetics of *Salmonella* in low-a_w foods (Santillana Farakos et al., 2013). Of the aforementioned models, the Weibull model (Mafart et al., 2002; Peleg and Cole, 1998) has been shown to provide the best description of *Salmonella* survival kinetics in low a_w foods (Abd et al., 2012; Ma et al., 2009; Mattick et al., 2001; Santillana Farakos et al., 2013).

Survival kinetics of *Salmonella* in low-a_w foods should be considered in quantitative risk assessment. Two survival models have been developed for use in a quantitative risk assessment of *Salmonella* in almonds (Danyluk et al., 2006; Lambertini et al., 2012). Both of these models assume log-linear declines of *Salmonella* in almonds and do not incorporate variability and uncertainty. Survival curves of *Salmonella* in tree nuts have been shown to be non-linear (Abd et al., 2012; Beuchat and Mann, 2010; Blessington et al., 2012; Blessington et al., 2013a; Brar et al., 2015; Kimber et al., 2012; Uesugi et al., 2006). Moreover, the precision level of a quantitative risk assessment of *Salmonella* in tree nuts may be defined by the ability of the risk model to reflect and evaluate variability and uncertainty separately (Delignette-Muller et al., 2006; Nauta, 2000; Pouillot et al., 2003). Uncertainty represents the lack of perfect knowledge of the system, notably of the parameter value, while variability represents heterogeneity in the population exposure or response (Nauta, 2000). Additional *Salmonella* survival data would be helpful for a variety of tree nuts (e.g. pistachios where the data are available only from a single reference) and data are lacking for several others (e.g. chestnuts, cashews, macadamia nuts, pine nuts and hazelnuts). There is a great variability in the published literature of *Salmonella* survival for the same tree nut tested at similar temperatures. Survival of *Salmonella* in low-a_w foods has been shown to be affected by many factors including temperature, a_w, food composition, strain, and the experimental conditions of the study (Santillana Farakos et al., 2014). Models able to predict the dynamics of *Salmonella* survival in tree nuts that consider variability and uncertainty will be useful in assessing the risk of human salmonellosis from consumption of these products.

The objective of the current study was to develop a mathematical model to predict survival of *Salmonella* in tree nuts at typical storage temperatures (21–24 °C) that incorporated variability and uncertainty separately and could be readily incorporated into a quantitative risk assessment model. In this manuscript, we specifically focus on presenting the methodology to derive such a model.

2. Materials and methods

2.1. Data selection

The peer reviewed literature was searched for data on *Salmonella* survival in tree nuts using search engines PubMed, Web of Science and Google Scholar with keywords such as water activity, low moisture, temperature, tree nuts, nuts, almonds, pecans, walnuts, pistachios, modeling, kinetics, inactivation, and survival. The studies to be included in the analysis were restricted to those in which *Salmonella* survival was determined at ambient lab temperature (20 to 25 °C) which is a common storage temperature for tree nuts. Within these studies, data points with contamination levels lower than 1 cfu/unit were discarded (assuming the minimum amount of *Salmonella* per unit to be 1 cell). Curves showing increased cell population numbers with time were not included because, as no growth is expected at the usual a_w of these nuts, these curves represent specific conditions that are not expected to fall in the process modeled here. These need/will be included in a follow-up risk assessment however. Independent Weibull models were fit to each replicate and those representing outliers presumably the result of experimental error were not included. Out of the eight studies for which we received data, seven studies contained data that met the inclusion criteria: Abd et al. (2012); Beuchat and Mann (2010); Blessington et al. (2013a,b), Brar et al. (2015); Kimber et al. (2012) and Uesugi et al. (2006). Substrates included in the analysis comprise almonds, pecans, pistachios and walnuts. The recovery medium in the selected studies was either tryptic soy or bismuth sulfite based agar with different supplements. For the purpose of this analysis, the recovery medium was classified as either TSA if it was tryptic soy based or BSA if it was bismuth sulfite based, without taking into account the supplements added to the agar base. Each survival curve obtained from the seven studies is represented as a unique replicate for our analysis. A total of 111 replicates were included. The largest number of replicates (74 replicates) were available for *Salmonella* survival in almonds, followed in decreasing order by walnuts (24 replicates), pecans (7 replicates) and pistachios (6 replicates) (see supplementary data).

2.2. Modeling strategy

The classic approach (frequentist) to model selection is a tradeoff between the measure of model fit (e.g. deviance statistic) and the measure of complexity (e.g. number of parameters) (Spiegelhalter et al., 2002). The use of empirical posterior distributions obtained from a Monte-Carlo Markov Chain (MCMC) within a Bayesian inference framework has been previously described as being the most appropriate approach to develop mathematical models that incorporate variability and uncertainty separately (Delignette-Muller et al., 2006; Pouillot et al., 2003; Rigaux et al., 2013; Teunis et al., 2010). However, model selection using a Bayesian inference framework is complex and time consuming. In this study, we developed a strategy for modeling survival of *Salmonella* in tree nuts that combines the strengths of the frequentist and the Bayesian frameworks. We propose using a frequentist framework (testing both fixed and mixed effect models) to find the best applicable model to describe the kinetics of *Salmonella* survival in tree nuts and using a Bayesian framework to estimate the distribution of the survival parameters of the model to be implemented in a risk assessment.

2.3. Choosing the best applicable model

In the extensive data collection and analysis of *Salmonella* survival in low-a_w foods by Santillana Farakos et al. (2013), the Weibull survival model (Peleg and Cole, 1998) was shown to be the best applicable model to describe survival kinetics at temperatures ranging from 21 to 80 °C and a_w levels below 0.6. *Salmonella* in low-a_w foods typically show survival curves with a fast initial linear decline followed by slow decline with a non-zero asymptotic tail. The model is written as:

$$\log_{10}(N_t) = \log_{10}(N_0) - (t/\delta)^{\rho} \tag{1}$$

where N_0 is the concentration at time 0, N_t is the concentration at time t, δ is the time to the first $\log_{10}$ reduction, and ρ is a fitting parameter that defines the shape of the curve. The Weibull model can describe $\log_{10}$ linear ($\rho = 1$), concave ($\rho < 1$) and convex ($\rho > 1$) curves and assumes the resistance to stress of the population follows a Weibull distribution (Coroller et al., 2006; Peleg and Cole, 1998). Although the Weibull model is of an empirical nature, a link can be made with physiological effects: $\rho < 1$ indicates that the remaining cells have the ability to adapt to the applied stress, whereas $\rho > 1$ indicates that the remaining cells become increasingly damaged (Peleg and Cole, 1998; van Boekel, 2002).

The Weibull survival model (Peleg and Cole, 1998) was thus chosen for further analyses.

2.3.1. Fixed effect models

Using classical nonlinear least squares regression, fixed effect models were used to determine the level of variability in the parameters δ and ρ of the Weibull model. The parameters δ and ρ can be the same