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Modeling the influence of temperature, water activity and water mobility on the persistence of *Salmonella* in low-moisture foods ☆

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ABSTRACT

Salmonella can survive in low-moisture foods for long periods of time. Reduced microbial inactivation during heating is believed to be due to the interaction of cells and water, and is thought to be related to water activity (a_w). Little is known about the role of water mobility in influencing the survival of *Salmonella* in low-moisture foods. The aim of this study was to determine how the physical state of water in low-moisture foods influences the survival of *Salmonella* and to use this information to develop mathematical models that predict the behavior of *Salmonella* in these foods. Whey protein powder of differing water mobilities was produced by pH adjustment and heat denaturation, and then equilibrated to a_w levels between 0.19 ± 0.03 and 0.54 ± 0.02. Water mobility was determined by wide-line proton-NMR. Powders were inoculated with a four-strain cocktail of *Salmonella*, vacuum-sealed and stored at 21, 36, 50, 60, 70 and 80 °C. Survival data was fitted to the log-linear, the Geeraerd-tail, the Weibull, the biphasic-linear and the Baranyi models. The model with the best ability to describe the data over all temperatures, water activities and water mobilities ($f_{test} < F_{table}$) was selected for secondary modeling. The Weibull model provided the best description of survival kinetics for *Salmonella*. The influence of temperature, a_w and water mobility on the survival of *Salmonella* was evaluated using multiple linear regression. Secondary models were developed and then validated in dry non-fat dairy and grain, and low-fat peanut and cocoa products within the range of the modeled data. Water activity significantly influenced the survival of *Salmonella* at all temperatures, survival increasing with decreasing a_w. Water mobility did not significantly influence survival independent of a_w. Secondary models were useful in predicting the survival of *Salmonella* in various low-moisture foods providing a correlation of $R = 0.94$ and an acceptable prediction performance of 81%. The % bias and % discrepancy results showed that the models were more accurate in predicting survival in non-fat food systems as compared to foods containing low-fat levels (12% fat). The models developed in this study represent the first predictive models for survival of *Salmonella* in low-moisture foods. These models provide baseline information to be used for research on risk mitigation strategies for low-moisture foods.

1. Introduction

Low-moisture foods are those with water activity (a_w) levels lower than 0.70 (Blessington et al., 2012). Such foods include products which have undergone a lethality step, those that are not subjected to an inactivation step, and those in which ingredients are added after an inactivation step. A review of recall records of low-moisture foods on the Centers for Disease Control and Prevention (CDC) website showed that in the U.S., from 2007 to 2012, there were 119 recalls (5010 entries) involving pet food, powdered infant formula, peanut butter, spices, dry nuts, dry milk, seeds, etc. (CDC, 2012). From 2007 to 2012, 22 reported *Salmonella* outbreaks caused by low-moisture foods occurred globally, resulting in 2293 cases of infection and 9 deaths (CDC, 2012; EFSA, 2009; EFSA, 2010; Rodriguez-Urrego et al, 2010; SFI, 2012). The consumption of only one *Salmonella* cell in a food product may be sufficient to cause illness (D'Aoust and Maurer, 2007), and most low-moisture food products require no further cooking and have a long shelf life. Hence, the presence of *Salmonella* in low-moisture foods can cause extended outbreaks which impact large numbers of people.

Salmonella is able to survive in low-moisture foods for long periods of time. Increased heat resistance in low-moisture foods is believed to be the result of the interaction of *Salmonella* cells with food components (Podolak et al., 2010). Water, as a component of food, is considered a key factor in microbial inactivation (Podolak et al., 2010). The interaction of cells with water is often related to a_w, as it reflects the intensity with which water associates with non-aqueous components at a macroscopic level. Several studies have shown that reduced a_w protects against the inactivation of *Salmonella* in low-moisture foods (Beuchat and Scouten, 2002; Doyle and Mazzotta, 2000; Archer et al, 1998).

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However, different *D*- and *z*- values have been observed for different products under similar moisture conditions (Podolak et al., 2010). Water mobility is a measure of the translocation of water molecules in the food, with the possibility of determining the ability at which water molecules interact with the bacterial cell at a molecular level. At present, little is known about the role of water mobility in influencing the survival of *Salmonella* in low-moisture foods. The aim of this study was to determine how the physical state of water in low-moisture foods influenced the survival of *Salmonella*, and to use this information to develop mathematical models that predict the behavior of *Salmonella* in these foods.

2. Material and methods

2.1. Preparation of modified whey protein powder

The ability of whey protein (beta-lactoglobulin) to immobilize water was modified by changing the secondary and tertiary structure of the protein through pH adjustment and heat. Whey protein powder (95% protein) was obtained from Davisco Foods International (Le Sueur, MN). The pH of three 1.5 liter solutions of 40 g/l whey protein was adjusted to 2, 5 and 7, with 36.5% HCl (J.T. Baker, Phillipsburg, NJ). The protein was then denatured by heating the solution to 80 °C. The solutions were rapidly cooled under cold water and refrigerated overnight. This process stabilized the modified protein structures, but the resulting product contained sufficient bacterial spores to interfere with *Salmonella* analysis. Therefore, the protein suspensions were further pasteurized at 80 °C for 30 min after adjusting pH levels to 2.0. After cooling to room temperature, the pH of all the solutions was re-adjusted to 7 by using 10 N NaOH (J.T. Baker, Phillipsburg, NJ). The solutions were then poured into sterile aluminum pans and frozen to −40 °C overnight in a freeze drier (Freezemobile 25SL Unitop 600 l, Virtis Company, Gardiner, NY). The vacuum of the freeze drier was started once the samples reached −40 °C, and the temperature of the freeze drier was gradually increased from −40 °C to 0 °C every 24 h for a total of 96 h (−20, −10, 0). Once freeze dried, the modified whey protein powder of each structure type (denatured at pH 2, 5 and 7) was broken down to homogenous particles by crushing it with a rolling pin. The powders were stored in the dark under N_2 atmosphere with silica gel packets to avoid oxidation and moisture absorption. Protein powders denatured at pH 2, 5 and 7 are referred to as protein configuration 1, 2 and 3, respectively.

2.2. Water activity equilibration of protein powders

Protein powders were adjusted to the various a_w values in vacuum desiccators by absorption at 21 °C. Target a_w levels were: 0.11 (Lithium Chloride, Fisher scientific, Pittsburgh, PA), 0.23 (Potassium Acetate, Sigma Aldrich, St. Louis, MO), 0.33 (Magnesium Chloride Hexahydrate, Fisher scientific, Pittsburgh, PA), 0.43 (Potassium Carbonate, Anhydrous, Granular, J.T. Baker, Phillipsburg, NJ) and 0.58 (Sodium Bromide Crystal, J.T. Baker, Phillipsburg, NJ). Water activity was determined using a bench top water activity meter (AquaLab Series 4TEV, Decagon Devices Inc., Pullman, WA) of ±0.003 precision.

2.3. Water mobility determination

A Varian Inova 500 MHz spectrometer (Complex Carbohydrate Research Center, The University of Georgia, Athens, GA) was used to obtain the wide line H-NMR spectra for protein powders. Approximately 200 g of sample was packed into a 5 mm ASTM Type 1 Class B glass NMR tube (Norrell Inc., Landisville, NJ). All measurements were obtained in triplicate at 25 °C. The spectral width used was 300 kHz. The methodology used was based on that of Kou et al., 2000.

2.3.1. Effective spin-spin relaxation time (T_2^*)

A 90° 1H pulse with a pre-acquisition delay time of 2.5 s was used to obtain the H-NMR spectra of each a_w equilibrated sample. These spectra have a broad component of the peak corresponding to the immobile protons and a narrow component of the peak corresponding to the mobile protons. The spectrum of each sample was decomposed into broad and narrow components, each fitted to a Lorentzian line shape using MestRenova 7 software (Mestrelab Research, S.L., Santiago de Compostela, Spain). The areas of the broad and narrow components and the line width at half-height of each component were measured by using MestRenova 7. Effective spin–spin relaxation time (T_2^*) values were obtained using Eq. (1).

$$T_2^*\,(\mathrm{s}) = \frac{1}{\pi} \times \nu_{1/2}\ \ (\mathrm{Hz}) \tag{1}$$

where T_2^* represents the effective spin–spin relaxation time and $\nu_{1/2}$ represents the line width at half-height.

Significant differences in water mobility (T_2^*) at different water activities and for different protein configurations were analyzed by ANOVA using the General Linear Model procedure with Tukey's test at $p < 0.05$ (IBM SPSS Statistics for Windows, Version 21.0, IBM Corp. Armonk, NY). Water mobility has units of milliseconds (ms).

2.4. Sample inoculation and packaging for survival experiments

Four *Salmonella* serovars previously involved in outbreaks in dry foods were used in this study: *Salmonella* Typhimurium (peanut), *Salmonella* Tennessee (peanut), *Salmonella* Agona (dry cereal) and *Salmonella* Montevideo (pistachios and others). The cultures were stored in cryovials containing beads suspended in phosphate buffered saline, glycerol and peptone (Cryobank, Copan Diagnostics Inc., CA) and kept at −80 °C. They were prepared for use by consecutive culturing in 9 ml of Tryptic Soy Broth (TSB, Becton, Dickinson and Company, Sparks, MD) at 37 °C for 24 h. Following the second culture, a final transfer of 3 ml to 225 ml of TSB was made, followed by incubation for 24 h at 37 °C. Cells from the final culture were collected by centrifugation (3363 *g*, 30 min), the supernatant fluid was removed, and the pellet was re-suspended in 2 ml of 1% bacto-peptone (Becton, Dickinson and Company, Sparks, MD). The cell suspension was then dried in a vacuum desiccator over anhydrous calcium sulfate for a minimum of three days to obtain a_w levels below 0.1. The dried cells were pooled and manually crushed into a powder. The dried inoculum (0.05 g) was mixed with 0.95 g of moisture equilibrated test protein powder to provide a 1 g sample. This inoculation method led to starting concentrations of 10^9 CFU/g. Re-equilibration of samples to the target a_w was not necessary when using this procedure. Inoculated and control samples were packaged in retort pouches under vacuum to minimize moisture transfer to head space during survival studies. Samples were placed into standard retort pouches (Stock America, Inc., Grafton, WI). Retort pouches were then placed in FoodSaver Quart Bags, and the FoodSaver equipment (FoodSaver Silver, model FSGSSL0300-000, Sunbeam Products, Inc., Boca Raton, FL) was used for pulling a vacuum and sealing. After initial sealing of the FoodSaver bag, a second seal was applied to the retort pouch using an impulse sealer. The vacuum-sealed inoculated samples were stored at different temperatures (21 ± 0.6 °C, 36 ± 0.3 °C, 50 ± 0.5 °C, 60 ± 0.5 °C, 70 ± 0.5 °C and 80 ± 0.5 °C). For the six-month storage experiments (21 °C and 36 °C), the retort pouches were stored in desiccators at their corresponding relative humidity in controlled temperature incubators. Samples were stored in a circulating water bath (Lauda, Lauda-Konigshofen, Germany) for the high temperature experiments (50 °C, 60 °C, 70 °C and 80 °C). The water bath was equipped with custom-designed racks that kept the samples submerged and allowed for water circulation between pouches.