Mathematical modeling and validation of growth of *Salmonella* Enteritidis and background microorganisms in potato salad — One-step kinetic analysis and model development*

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**Abstract**

This study was conducted to examine the growth of *Salmonella* Enteritidis (SE) in potato salad caused by cross-contamination and temperature abuse, and develop mathematical models to predict its growth. The growth of SE was investigated under constant temperature conditions (8, 10, 15, 20, 25, 30, and 37°C) to evaluate the effect of temperature on growth rates and lag times. Duplicated experiments were conducted. The data set from one replicate was used to develop kinetic models and determine kinetic parameters. The data from the other replicate served as an independent data set for model validation. The growth of background microorganism (BK) was also examined. One-step kinetic analysis method was used to directly construct both primary (Huang) and secondary (Ratkowsky square-root) models. Nonlinear regression was used to minimize the global residual sum of squares (RSS) for SE and BK. The results showed that both primary and secondary models can be used to analyze the growth curves, with the kinetic parameters closely matching the characteristics of SE and BK. The validation results showed that the root-mean-square error (RMSE) was only 0.40 Log CFU/g for SE and 0.66 Log CFU/g for BK, with the residual errors of predictions following Laplace and logistic distributions, respectively. This study showed that one-step kinetic analysis is a useful and efficient method for analyzing the entire data set to directly construct primary and secondary growth models and determine kinetic parameters. Since the models are validated, they can be used to predict the growth of SE and conduct risk assessment, and to predict the microbiological shelf-life of potato salad.

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**1. Introduction**

According to a study reported by the Centers for Diseases Control and Prevention (CDC), non-typhoidal *Salmonella* is responsible for approximately 1.2 million cases of foodborne infections and 450 deaths each year in the United States (Scallan et al. 2011). Among more than 2500 different serotypes, *Salmonella* serotype Enteritidis (SE), *Salmonella* serotype Typhimurium, and *Salmonella* serotype Newport are three of the most common serotypes causing foodborne infections in the United States (CDC, 2014). Among laboratory-confirmed cases of *Salmonella* infections caused by top 20 serotypes, SE appears to be the top frequently reported serotype (CDC, 2013, 2014). Most SE infections in the United State are acquired from domestic sources (Chai et al., 2012). Chicken and eggs are no doubt the major sources and vehicles for the transmission of SE (Braden, 2006; Chai et al., 2012; Guard-Petter, 2001).

Chicken eggs are widely used as an ingredient in making a variety of salads, both at home and in commercial settings. Cross-contamination can occur when processing surfaces, kitchen utensils, and cutting boards contaminated with SE are in contact with various materials used to make salads. Therefore, opportunities exist that salads may be contaminated with SE through contaminated ingredients or contact surfaces. Once contaminated, salads may become a health hazard to consumers. A study reported by Alali, Mann, and Beuchat (2012) showed that *Salmonella* survived in highest numbers in potato salad and hummus. Between 1988 and 1992, at least one *Salmonella* outbreak was related to potato salad (CDC, 1996). In 2005, cross-contamination of SE between eggs and potato caused salmonellosis in 85 out of 115 people attending a local traditional fair in an Austrian village after eating mixed salad...
containing potato (Schmid et al., 2006). Potato salad was also identified as the source in another SE outbreak in Austria in 2011 (Hrivniakolak et al., 2011). In 2010, Safeway Inc. voluntarily recalled potato salad from 10 states over the concern of Salmonella contamination.

The objective of this research was to study the growth kinetics of SE in potato salad exposed to cross-contamination during preparation, and to evaluate the effect of temperature abuse on growth rate and lag time under different storage temperatures. The growth of background microorganisms (BK) in potato salad was also evaluated. The goal of this research was to develop mathematical models to describe the growth of SE and BK in potato salad. The models may be used for conducting risk assessments of SE in potato salad and determining microbial shelf-life during storage.

2. Materials and methods

2.1. SE strains and preparation of bacterial cultures

Five outbreak SE strains, obtained from the culture collection of the Eastern Regional Research Center (ERRC) of the USDA Agricultural Research Service (ARS) located in Wyndmoor, PA, were used in this study. The SE strains included FSIS OB030832, FSIS OB040159, FSIS OB050042, FSIS OB060003, and SE Phage Type (PT) 8 strain C405. The working cultures of the bacterial strains were prepared by inoculating each individual strain to brain heart infusion (BHI) broth (BD/Difco Laboratories, Sparks, MD) and incubated at 37 °C overnight with mild agitation (~100 rpm). Each strain obtained from the working culture was induced to resist 100 mg/L rifampicin (rif, Sigma, R 3501-5G, Sigma Aldrich Co., MO) using the induction of the rif-resistance in preliminary experiments. To prepare the bacterial cultures for inoculation, a loopful of each SE-rif strain was transferred to 10 ml BHI broth containing 100 mg/L rif and incubated overnight (24 h) at 37 °C overnight. Since the TSA agar plates were supplemented with rif, all background microorganisms were suppressed. The advantage of using SE-rif in growth studies was that it simplified the microbiological procedures during isolation and enumeration of SE from the samples that contained a significant amount of background microorganisms by suppressing their growth. To maintain the viability of the cells and stability of the antibiotic resistance, the SE-rif cultures were plated onto Tryptic Soy agar (TSA, BD/Difco Laboratories) plates containing 100 mg/L rif (TSA-rif) and stored in a refrigerator (6–8 °C). The SE-rif cultures were regularly propagated, maintained on TSA-rif agar plates, and refrigerated. The growth behaviors of SE were not affected by the induction of the rif-resistance in preliminary experiments.

To prepare the bacterial cultures for inoculation, a loopful of each SE-rif strain was transferred to 10 ml BHI broth containing 100 mg/L rif and incubated overnight (22–24 h) at 37 °C with mild shaking (~100 rpm). The overnight cultures were harvested by centrifugation (2400 g, 15 min, and 4 °C), washed once with 0.1% sterile peptone water (PW, BD/Difco Laboratories). Each bacterial culture was re-centrifuged, re-suspended in 5 ml PW, and then combined to form a 25 ml bacterial cocktail, which contained ~10⁸ colony forming units (CFU) per ml. The bacterial cocktail was properly diluted for use in inoculation of samples. Fresh SE-rif cultures were prepared for each growth study.

2.2. Sample preparation, inoculation, and incubation

Frozen potato cubes (Southern Style Hash Browns) were purchased from a local grocery store. The potato cubes were kept in a freezer and remained frozen (~18 °C) until ready for use in growth studies. To prepare for samples for use in the growth studies, the frozen potato cubes (~250 g) were placed into a plastic bag, and cooked in a microwave oven (1300 W) for 2 min on high power, according to the manufacturer’s suggestion. After microwave cooking, the samples were cooled in a refrigerator (~4 °C) for at least 30 min.

After cooling, the potato samples were transferred to a sterile beaker containing 297 ml PW. The SE-rif culture (3 ml) was inoculated to the beaker and gently stirred for 2 min to allow uniform distribution of the bacteria. The liquid portion was drained after mixing. The inoculated potato cubes (125 g) were mixed with 33 g Hellmann’s Real Mayonnaise (Unilever US, Inc., Englewood Cliffs, NJ). According to the manufacturer’s label, the ingredients in the mayonnaise included soybean oil, water, whole eggs, egg yolks, vinegar, salt, sugar, lemon juice, EDTA, and natural flavors. The pH of the mayonnaise was measured as 3.7 using a Corning pH meter (Model 430, Corning Inc., New York). This value was identical to the value reported in Hwang and Tamplin (2005), in which Hellmann’s Real Mayonnaise was also used. The mayonnaise contained 90 mg of salt per serving (13 g), or which is equivalent to 0.7% of salt in the product. The water activity of the mayonnaise was 0.95 (Hwang & Tamplin, 2005). After mixing, the potato salad (10 g) was weighed into filter stomacher bags (Whirl-Pak®, 7 oz., 95 mm x 180 mm x 0.08 mm, NASCO - Fort Atkinson, Fort Atkinson, WI). The inoculated samples of potato salad were kept in a refrigerator (~4 °C) overnight and used for growth studies the next day.

2.3. Growth studies

The second day after sample inoculation, the inoculated samples were retrieved from the refrigerator and immediately transferred to incubators maintained at constant temperatures under suboptimal conditions between 8 and 37 °C. The incubation temperatures in this study included 8, 10, 15, 20, 25, 30, and 37 °C. A thermocouple was inserted to the center of a sample package to monitor the change in the temperature during incubation in each incubator. The temperature probe was also used to determine the time that the samples needed to increase from the refrigerated temperature to the incubation temperature in each incubator. This time is normally known as the come-up time (CUT) in heat transfer. The CUT was generally less than 30 min. For incubation temperatures below 15 °C, the CUT was negligible and ignored since the incubation time was very long. For incubation temperatures above 15 °C, the incubation time was adjusted from the initial CUT to allow the samples to equilibrate to the incubation temperature in each incubator. The samples were incubated for different durations of time, depending on the incubation temperature, and periodically retrieved to enumerate SE and BK. The experiments were duplicated for each growth temperature.

2.4. Enumeration of microorganisms

After the samples were retrieved from the incubators, PW (10 ml) was added to each sample bag. The samples were pulsed for 2 min on each side at the maximum speed in a stomacher (Model BagMixer® 100W, Interscience Co., France). A small volume of the liquid portion of the stomached samples was plated, directly or after serial dilution, onto TSA-rif agar plates. The plates were incubated at 37 °C overnight. Since the TSA agar plates were supplemented with rif, all background microorganisms were suppressed. Only SE-rif grew into colonies on TSA-rif agar plates. The colonies were counted and converted to logarithms (base 10) of CFU/g. The samples were also plated onto TSA plates without rif to recover total aerobic microorganisms. The TSA plates were also incubated at 37 °C overnight. The total plate counts were determined from the colonies. The total colony counts were subtracted from the counts of SE-rif, and the difference was treated as the counts of background microorganisms (BK). The BK colony counts