IMPROVING PATHOGEN-REDUCTION VALIDATION METHODS FOR PISTACHIO PROCESSING

By

Kaitlyn Eileen Casulli

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Biosystems Engineering – Master of Science

2016

ABSTRACT

IMPROVING PATHOGEN-REDUCTION VALIDATION METHODS
FOR PISTACHIO PROCESSING

By

Kaitlyn Eileen Casulli

The 2015 FDA Preventive Controls for Human Foods Rule requires firms to validate pathogen-reduction steps. Some thermal processes, such as pistachio roasting, are not yet well-characterized with respect to the impact of product and process variables on *Salmonella* lethality. The objective was to quantify and model the effects of product and process factors on *Salmonella* lethality for in-shell pistachios. In-shell pistachios were inoculated with *Salmonella* Enteritidis PT30 (~8.5 log CFU/g), and thermally treated at various levels of temperature, process humidity, and product moisture, under dry and presoaked conditions. *Salmonella* survivors, moisture content, and a_w were quantified at six time points during each treatment, targeting a cumulative lethality of ~3-5 log. Increasing product temperature or process dew point increased *Salmonella* inactivation rates ($P < 0.05$). For dry and presoaked treatments, analyzed separately, initial product water activity did not affect inactivation rates ($P > 0.05$). However, when comparing dry against presoaked treatments, inactivation rates were greater ($P < 0.05$) for the presoaked pistachios. Models were developed to quantify *Salmonella* inactivation in pistachios as a function of product temperature, moisture, and process humidity. These models successfully described the effect of these variables on *Salmonella* inactivation and are the first published attempts to model pathogen reduction in low-moisture products as a function of multiple dynamic variables. Based on these findings, the impact of product and process factors on *Salmonella* inactivation in pistachios must be considered when designing and validating industrial thermal processes for pathogen reduction.

To my brother,

Matthew,

for teaching me

how to ask “why?”

ACKNOWLEDGMENTS

A small section of acknowledgments can hardly do justice to the support I've received during this voyage. Don't let the sole authorship fool you, this ship didn't steer itself.

Dr. Bradley Marks and Dr. Kirk Dolan, my co-advisors, for not only the financial support for two years but also for believing in a "wannabe engineer," who was a food scientist by training. I knew from the time I met both of them that Michigan State was the place for me to get my graduate degree, and I am glad they were willing to be my co-advisors, because there was no way I was going to be able to choose just one of them. They know how to go above and beyond the role of advisor, by inviting me for a Thanksgiving meal, or treating me to a hot beverage. I may have taken the road less traveled in becoming an engineer, but neither of them doubted my abilities, and I've learned and done things I never imagined. They put their faith in me, even when there were times when grad school tried to trip me up and wear me down. I couldn't have asked for better role models, both professionally and personally. No matter what I threw at them in my two years here, they've kept me around, and (somehow!) managed to convince me that I should stick around for a Ph.D.

Dr. Elliot Ryser, the third member of my committee, for the insightful intellectual conversations and good humor along the way. His expertise in microbiology and reputation for asking difficult questions has pushed me to not just learn things, but to learn them well. There are also several food safety experts I've had the chance to get to know through him, which has been incredibly rewarding.

Francisco Garces-Vega, a fellow lab-mate and great friend, for always being willing to lend a hand when needed, and for his friendship, often shared over a cold beer or Google doc. I must owe him about a thousand hours of experimental help, and a million more of moral

support! Our collaboration has been a tremendous asset to my intellectual growth, and I look forward to what we will do for many years to come (but he's in charge of the stats).

Mike James and Nicole Hall, our lab managers, for their long hours and commitment to making our lab the best it could be. Their willingness to help me get the materials and labor I needed made it much easier for me to keep this project on track. I was never without the perfect zip ties! Our lab runs like a well-oiled machine because of them.

The undergrads of the lab (too many to name!), simply for being amazing, motivated individuals who never complained about the workload, and kept us well-stocked despite endless numbers of samples coming their way. Their dedication to making their experiences in the lab more than just a line on their resumes ensured we had top-notch functionality at all times. In addition, their undying passion for Crunchy's and HopCat cannot go without mention.

The remainder of the Department of Biosystems and Agricultural Engineering, for being my second family for two years. I've learned so much from all of you, and I feel at home every time I walk through the doors of Farrall Hall. It's really been an honor this past year serving as the chair of the Graduate Student Advisory group, because I've gotten to know all of you better.

Dr. Brian Farkas, my undergraduate advisor, for being a friend and mentor, and for being my motivation to be here in the first place. My research career started under his guidance (or lack thereof?!). He didn't scare me off from research, and I frequently find myself "channeling my inner Farkas." I am forever grateful that he helped me find my joy for engineering. He held me to high standards and gave me a lot of freedom, and it has made doing research one of the easiest parts of graduate school. It always helped to have someone to talk to outside my project and lab who has been through this before, who could help me with challenges and celebrate successes.

As much as I thought I wanted to work with him for grad school, kicking me out was the best thing he could have done for me.

All the new friends I've made in Michigan, for listening to me prattle on about modeling how *Salmonella* dies. Despite the fact that their eyes began to glaze over with boredom about 17 seconds into me explaining what I do (my apologies), they continued to listen, and sometimes even asked follow-up questions. Our shared interests kept things fun for me. Live SBG!

Beer, for helping my creative juices to flow so I could always get that first draft off to a good start, and for helping me troubleshoot many, many MATLAB codes.

My dog, Porter, for his two years of snuggles, licks, and having a bladder of steel on days where I had to work long hours. I owe him a lot of Milkbones. He was also an awesome conference travel companion! Some days, seeing his wagging tail was all I needed to keep going.

And last, but certainly not least, my family, for not thinking I was crazy (well, not TOO crazy, anyway) for embarking on this journey. I think everyone (myself included!) questioned my decision to move from warm, sunny North Carolina to cold, gray Michigan. I really knew I was going off the deep end when I decided to buy a house and continue into a Ph.D., but they all still backed me 100%! My parents' financial support for my undergraduate education and the lifelong values they've instilled in me during my 24+ years of existence made the transition into being a "big kid" a whole lot easier. There are not enough words to express how much they've given me over the years, in terms of emotional and professional support in helping me make my way through life. They were also always willing to smuggle Bojangles' biscuits and cases of Cheerwine across the border to make me feel more at home in Michigan. Even as I close out this chapter of my life and prepare to enter a new one as a Ph.D. student, I know I'll have my family's continued, unwavering support (particularly in the snowy months).

This research was partially supported by the Center for Produce Safety and the California Pistachio Board.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO SYMBOLS

$a_w(t)$	water activity at t
$a_{w,e}$	equilibrium water activity (0.025)
$a_{w,i}$	initial water activity
$a_{w,ref}$	reference water activity
D	time required for a 90% reduction in microorganisms at a given condition (min)
D_{ref}	D-value at the specified reference conditions (min)
δ	time to first 90% reduction of microorganisms (min)
k	rate constant for moisture loss
K	number of parameters minus one
MC(t)	dry basis moisture content at t
MC_e	equilibrium dry basis moisture content (0.05% MC, db)
MC_i	initial dry basis moisture content
MC_{ref}	reference dry basis moisture content
N(t)	number of microorganisms (CFU/g) at t
N_0	number of microorganisms (CFU/g) present initially (t = 0)
n	shape factor (linear if n = 1, concave down when n > 1, and concave up when n < 1), or number of data
p	number of model parameters
SS	sum of squared residuals
t	treatment time (min)
T(t)	pistachio surface temperature at t (°C)
T_d	process dew point (°C)

$T_{d,ref}$ reference dew point (°C)

T_{ref} reference temperature (°C)

z_{aw} change in pistachio water activity required for a 10-fold change in D-value

z_M change in process dew point required for a 10-fold change in D-value (°C)

z_{MC} change in dry basis pistachio moisture required for a 10-fold change in D-value (%MC, db)

z_T change in pistachio surface temperature required for a 10-fold change in D-value (°C)

1. INTRODUCTION

1.1 Problem Statement

Reduction of pathogens in food is an important step for most food processors (Beuchat et al., 2013). Currently, food commodities such as seafood, meat, and juice fall under the Hazard Analysis and Critical Control Points (HACCP) system, which is a preventative approach to food safety. Under HACCP, food processors must have written documentation of all hazards in a food, control point(s) intended either to eliminate or reduce the hazard to an acceptable level. This method is generally effective at reducing and preventing foodborne illness. As an example, since the juice HACCP rule was implemented, there have been no reported outbreaks linked to *Escherichia coli* O157:H7 in pasteurized juice, the target microorganism for thermal processing of juice (Schaffner et al., 2013). However, because HACCP does not cover all foods, other outbreaks of foodborne illness can still occur.

Recent foodborne illness outbreaks prompted the passing of the Food Safety Modernization Act (FSMA) in 2011. Within FSMA, the Preventative Controls Rule for Human Food is similar to HACCP, because it requires all food processing facilities to have a written validation plan that documents prevention and control of all hazards, which include pathogenic microorganisms such as *Salmonella*, through hazard analyses and establishment and monitoring of preventive controls (Anderson & Lucore, 2012; FDA, 2015). This rule differs from HACCP, because it attempts to mitigate food safety risks from the time of production of raw materials through packaging and distribution.

Foods having a water activity (a_w) below 0.85 are regarded as low-moisture foods and include powders, flour, spices, honey, dry pasta, nut butters, nuts, and cereals, to name a few (Beuchat et al., 2013). Until recently, it typically was presumed that process validations were not

needed for low-moisture foods, because pathogens cannot grow under these conditions, and it was assumed that pathogen contamination was insignificant (Anderson & Lucore, 2012; GMA, 2009). Foodborne illness outbreaks related to low-moisture foods and the passing of FSMA now show that pathogen contamination can occur and be at high enough levels to cause illness. *Salmonella* is one such pathogen that has been implicated in several foodborne illness outbreaks involving low-moisture foods (Beuchat et al., 2013).

Because of its high resistance to thermal treatment in low-moisture foods and low infectious dose, it is important to prevent *Salmonella* from entering the food at any point in production (Beuchat et al., 2013). Efforts in place to reduce potential cross-contamination include using Good Manufacturing Practices (GMPs), appropriate plant design, and proper storage of raw goods (Podolak, Enache, Stone, Black, & Elliott, 2010). However, these efforts are often not fail-safe, and a kill step is necessary to reduce the probability of a food contaminated with a pathogen from reaching a consumer. It is well-known that a product's a_w impacts *Salmonella* thermal resistance, but the impact is product and process specific (Podolak et al., 2010). Thus, there is a critical need for quantifying microbial inactivation kinetics for use in industrial process validation studies. In addition to inactivation kinetics, it is also important to quantify the dynamics of the industrial process and the variability inherent in the process. With this knowledge, food processors can set appropriate critical limits for their pathogen-reduction step, which will assist in documenting a validated process.

The pistachio industry has a need for quantifying inactivation kinetics for *Salmonella* in in-shell pistachios during a traditional roasting process. This project is part of an overall effort to help meet that need. Laboratory-scale experiments, with conditions representative of industrial

operations, were conducted to elucidate the effect of product and process factors on *Salmonella* inactivation kinetics in pistachios.

The model created in this study was unique in its attempt to model *Salmonella* inactivation kinetics as a function of multiple dynamic variables (product temperature and moisture). Most models are developed using data generated from laboratory-scale experiments under static environmental conditions (e.g., fixed temperature, water activity (a_w), pH, salt content, etc.). Despite not being created under industrial conditions, these models are used to validate industrial processes, but are often not validated using data outside of the data used to create the model. In addition, assumptions are often made that conditions are static, when in reality, a variable may be changing. This can be particularly dangerous in the case of declining a_w, because *Salmonella* thermal resistance is known to increase with decreasing a_w (Archer, Jervis, Bird, & Gaze, 1998). The lack of published kinetic data makes it difficult to validate a model under conditions other than those used to create the model (GMA, 2009). This project attempted to bridge the gap between laboratory-scale inactivation studies and industrial application.

1.2 Goal and Objectives

The goal of this project was to gain an understanding of *Salmonella* inactivation kinetics in pistachios as a function of product and process conditions, such that a model can be developed and validated for commercial use. Specifically, the objectives were to:

1. Apply a statistical model to determine the effects of processing conditions on thermal inactivation of *Salmonella* Enteritidis PT30 in pistachios.
2. Select and parameterize an appropriate secondary model to quantify the effect of product temperature, moisture, and process humidity on thermal inactivation of *Salmonella* Enteritidis PT30 in pistachios.

2. LITERATURE REVIEW

2.1 Pistachios

2.1.1 Production and processing in the United States

Pistachio production in the United States (US) is primarily concentrated in California, which contains approximately 98% of the nation's total pistachio harvest, with Arizona and New Mexico accounting for most of the remaining 2%. Pistachios are a biennial crop, meaning the harvest alternates between heavy and light, so harvest amounts vary from year to year. Harvest in the US peaked in 2012, with over 550 million tons of pistachios produced (ACP, 2015). The majority of the crop (~65%) is exported to other countries, and around 90% of the crop is sold as a roasted and salted product (ACP, 2014).

2.1.2 Use as a food commodity

Pistachios can be sold as a ready-to-eat (RTE) shelled or in-shell product, and are often used as ingredients in candies, flavorings, and desserts (Chen, 1990). Pistachios appeal to health-conscious consumers due to their high fiber, protein, and vitamin content, as well as their low saturated fat (APG, 2015).

2.1.3 State-of-the-art in pistachio safety research

Most studies involving pistachios have focused on optimization of drying (Aktas & Polat, 2007; Chen, 1990; Kashaninejad, Mortazavi, Safekordi, & Tabil, 2007; Maskan & Karatag, 2007; Omid, Baharlooei, & Ahmadi, 2009) or roasting (Kahyaoglu, 2008) to achieve certain quality standards for color or for decreasing moisture to prevent mold growth. Numerous studies have also modeled the physical properties of pistachios as functions of moisture content, temperature, or variety, to name a few (Hsu, Mannapperuma, & Singh, 1991; Kashaninejad, Mortazavi, Safekordi, & Tabil, 2006; Peyman, Mahmoudi, & Ghaffari, 2013; Polat, Aydin, &

Erol Ak, 2007; Razavi, Emadzadeh, Rafe, & Amini, 2007; Razavi & Taghizadeh, 2007; Tavakolipour & Kalbasi-Ashtari, 2008).

Few published studies have addressed pathogens in pistachios. Because pistachios are a low-moisture food, pathogen growth has not been a concern; however, it has been recently shown that pathogens can contaminate and survive in pistachios. Contamination may occur through transportation and hulling, which are both processes that involve relatively high levels of moisture and temperature, and a reasonable possibility of cross-contamination. This is a particular concern in large harvest seasons, because the time between harvest and drying is longer, allowing pathogen growth. Pistachios may also be contaminated in the dry state from product dust (Kimber, Kaur, Wang, & Danyluk, 2012). In response to recent *Salmonella* outbreaks in pistachios and pistachio-containing products, researchers have documented *Salmonella* presence (Harris et al., 2016; Little, Jemmott, Hucklesby, & De Pinna, 2009) and survival of *Salmonella*, *Escherichia coli*, and *Listeria monocytogenes* in pistachios during cold storage (Kimber et al., 2012).

Regarding inactivation of pathogens in pistachios, to the author's knowledge, there is no published work that evaluates pathogen reduction in pistachios during a typical commercial roasting process. The use of ozone for *Escherichia coli* and *Bacillus cereus* inactivation in pistachios has been evaluated (Akbas & Ozdemir, 2006), as well as feasibility of superheated steam treatments for inactivating *E. coli*, *Salmonella*, and *L. monocytogenes* in pistachios (Ban & Kang, 2016). (These studies will be discussed in greater detail in subsequent sections.) Despite the presence of many other pathogens, *Salmonella* is the most thermally resistant, and thus, is the target pathogen for validating microbial reduction processes (Harris, 2015).

2.2 *Salmonella* in Low-Moisture Foods

2.2.1 Salmonella *and salmonellosis*

Salmonella is a gram-negative, rod-shaped, motile pathogenic microorganism that is ubiquitous in the environment (WHO, 2013). In developed countries, *Salmonella* causes more foodborne illness, known as salmonellosis, than any other bacterium (Bell & Kyriakides, 2002). Since it is most prevalently found in the digestive tracts of vertebrates, it typically infects humans through the fecal-oral route. *Salmonella* can be present in water or on food processing surfaces, and it can then be transferred to the food. *Salmonella* can contaminate any food or processing surface during all stages of production, from the field to the processing facility (WHO, 2013). Cross-contamination can also occur after the thermal processing step, thereby necessitating GMPs through the entire production system (Bell & Kyriakides, 2002).

A person can contract salmonellosis by consuming food with as few as one *Salmonella* cell (GMA, 2009). The symptoms of salmonellosis include diarrhea, fever, and abdominal cramps and may last up to a week (CDC, 2012; WHO, 2013). In certain populations with reduced immunity, such as children and the elderly, salmonellosis can be severe, and often leads to hospitalization and even death. There are approximately 42,000 reported cases of salmonellosis each year in the United States (CDC, 2012). However, because this illness can be mild, and is hence often unreported, the actual number of cases is estimated to be over one million per year in the United States alone (CDC, 2012; WHO, 2013). It is estimated that about 400 people per year die from salmonellosis in the United States (CDC, 2012). Worldwide, there are tens of millions of cases of salmonellosis per year, resulting in over one hundred thousand deaths (WHO, 2013).

Reducing the cases of salmonellosis requires the food industry and regulatory agencies to better understand the growth and survival kinetics of *Salmonella* in various food matrices. Recent research has been focused on addressing the challenges related to understanding survival and inactivation of *Salmonella* in low-moisture foods and elimination of post-process contamination (GMA, 2009).

2.2.2 Outbreaks and recalls

According to the CDC, from 2012 to 2016 (to date), low-moisture foods have been implicated in just over 30% of salmonellosis outbreaks (CDC, 2016). The most recent outbreak was linked to pistachios (Table 1). Other products included powders and butters.

There have been eight product recalls related to *Salmonella* in pistachios in 2016 alone (FDA, 2016) (Table 2). One of these recalls was related to an outbreak, in which eleven people in nine states became ill from *Salmonella* Montevideo or *Salmonella* Senftenberg, linked to consumption of contaminated pistachios. This outbreak resulted in two hospitalizations and no deaths (CDC, 2016).

Table 1. Centers for Disease Control and Prevention outbreak data for *Salmonella* in low-moisture food products from 2012 to 2016 (to date) (CDC, 2016).

Product	**Implicated *Salmonella* Serovar(s)**	**Year**
Pistachios	Montevideo Senftenberg	2016
Organic shake and meal products	Virchow	2016
Raw sprouted nut butter spreads	Paratyphi B variant L(+) tartrate(+)	2015
Nut butter	Braenderup	2014
Organic sprouted chia powder	Newport Hartford Oranienburg	2014
Raw cashew cheese	Stanley	2014
Tahini sesame paste	Montevideo Mbandaka	2013
Peanut butter	Bredeney	2012

Table 2. Food and Drug Administration recall data for *Salmonella* in pistachio products for 2016 (to date) (FDA, 2016).

Product	Date
Raw pistachios	06/16/2016
Roasted and salted pistachios	03/24/2016
Pistachios and mixes containing pistachios	03/15/2016
Pistachios	03/11/2016
Natural Pistachio Kernels	03/10/2016
In-shell and shelled pistachios	03/09/2016
Pistachios	02/12/2016
Pistachios	02/01/2016

2.2.3 Survival during storage in low-moisture foods

Even though low-moisture foods do not support pathogen growth, some pathogens are able to survive desiccation and can subsequently cause illness upon consumption (Booth, 1998). *Salmonella*, a pathogenic microorganism known for its ability to adapt to extreme conditions in food matrices, may be present in raw materials that enter a food processing facility. *Salmonella* is able to survive drying and can tolerate the low-moisture environment of the dried food matrix (Beuchat et al., 2013; Podolak et al., 2010). Once desiccated, *Salmonella* can remain in a dormant state for extended periods, from months to years, and can become viable upon favorable environmental changes, such as hydration. Once hydrated, if stored at temperatures favorable to growth, *Salmonella* may rapidly multiply (Beuchat et al., 2013).

Product storage conditions can affect *Salmonella* behavior in a food matrix. *Salmonella* has been shown to survive better than both *E. coli* and *L. monocytogenes* in inoculated pistachios and almonds stored at room temperature (Kimber et al., 2012). During cold storage (-19°C and 4°C), survival of the three microorganisms was equivalent. A slower decline was recorded for pistachios as compared to almonds. After 350 days, only a 2 log decrease in *Salmonella* populations was observed in pistachios.

2.2.4 Product and process factors affecting thermal resistance

The intrinsic properties of a food matrix and product storage conditions have an impact on *Salmonella* thermal resistance. It is well-known that raising temperature will increase inactivation rates of microorganisms. Aqueous food matrices tend to present little to no problem with thermal inactivation of *Salmonella*, unlike low-moisture foods.

The time it takes to achieve a ten-fold reduction in bacterial population at a constant temperature (D-value) significantly increases in low-moisture foods. In general, trends have shown that decreased a_w will increase the time required to inactivate *Salmonella*. There are some early accounts of *Salmonella* surviving in a desiccated state on the surfaces of cooked beef, while *Salmonella* in the moist center was destroyed more rapidly (Goodfellow & Brown, 1978). Additionally, the temperature increase needed for a ten-fold reduction in D-value (z-value) also is increased (Archer et al., 1998; Harris, Uesugi, Abd, & McCarthy, 2012; He, Guo, Yang, Tortorello, & Zhang, 2011; Ma et al., 2009; Peñaloza Izurieta & Komitopoulou, 2012). These microbial inactivation studies will be addressed in greater detail in subsequent sections.

Despite the importance of product moisture on *Salmonella* resistance, other factors can influence the rate of *Salmonella* inactivation; for example, prior sublethal stress may lead to increased thermal resistance. In ground turkey, *Salmonella* showed path-dependent inactivation kinetics, exhibiting greater thermal resistance when subjected to sublethal heating (Stasiewicz, Marks, & Smith, 2008).

Desiccation also causes increased stress on *Salmonella* cells, with *Salmonella* becoming more resistant during storage and processing when stressed (He et al., 2011). He *et al.* (2011) concluded that *Salmonella* resistance depended not only on matrix a_w, but also stress history. Thermal resistance was increased after stressing cells by drying and storing at room temperature.

Peanut butter inoculated with healthy cells showed significantly faster inactivation than peanut butter inoculated with stressed cells when treated at the same a_w and temperature. At 90°C, up to 8.84 min were required for a 1 log *Salmonella* reduction in samples with stressed cells, whereas only up to 2.33 min were required for a 1 log reduction in samples inoculated with a healthy culture (He et al., 2011).

In contrast, when rapidly desiccating or hydrating a food, there may be no impact on *Salmonella* thermal resistance (Smith & Marks, 2015). *Salmonella*-inoculated wheat flour equilibrated to 0.3 a_w was rapidly hydrated to 0.6 a_w and thermally treated along with wheat flour equilibrated and held at 0.6 a_w. No differences were observed in inactivation curves generated. In addition, wheat flour equilibrated to 0.6 a_w was rapidly desiccated to 0.3 a_w and thermally treated along with wheat flour equilibrated and held at 0.3 a_w. Similarly, no differences were observed, indicating that moisture history in a product, when changed rapidly, does not impact *Salmonella* thermal resistance.

Storage conditions also impact subsequent *Salmonella* inactivation during thermal treatment. For example, when comparing *Salmonella* resistance in almonds previously stored at 4 and 25°C, *Salmonella* on almonds stored at 4°C showed greater resistance during oil roasting (Abd, McCarthy, & Harris, 2012). These studies indicate a greater need for understanding the cellular mechanisms behind stress and their effects on thermal resistance.

Salmonella thermal resistance also varies depending on other characteristics of the food matrix (He et al., 2011; Ma et al., 2009; Mattick, Legan, Humphrey, & Peleg, 2001). *Salmonella* in carbohydrate-based solutions (sucrose and glucose-fructose) displayed greater resistance to the same thermal treatment than *Salmonella* in NaCl solutions at the same a_w (Mattick et al., 2001). Low-fat peanut butter samples at the same a_w as their full-fat counterparts also showed increased

Salmonella thermal resistance, likely due to their higher carbohydrate contents. Carbohydrates are able to bind free water in a food matrix, thereby promoting slight desiccation of *Salmonella* cells (He et al., 2011; Ma et al., 2009).

Unfortunately, many studies are of limited use, because they are conducted under assumed static environmental conditions (isothermal, iso-moisture, etc.), even though conditions were changing. Changing environmental conditions can have a significant impact on the inactivation kinetics calculated from the data, which can be seen in a study conducted on *Salmonella* inactivation in wheat flour. Previously, *Salmonella* inactivation in wheat flour showed strong tailing, potentially due to a rapid decrease in moisture, and thus an increase in thermal resistance, in the start of heating due to non-iso-moisture conditions (Archer et al., 1998). Despite acknowledgment of the nonlinear behavior of the inactivation data, a linear fit was applied to the data, and a D-value of ~70 min was reported for flour heated at 70°C and initial a_w of 0.4. A new D-value of ~11 min can be estimated that only accounts for the first three data points in the steepest decline. A more recent isothermal/iso-moisture study showed that *Salmonella* has a D-value of ~10 min at 75°C and 0.4 a_w, which closely matches the results of the recalculated D-value from Archer et al.'s (1998) study (Smith, Hildebrandt, Casulli, Dolan, & Marks, 2016). Slight differences in the strain of *Salmonella* or the composition of the wheat flour could have contributed to differences in these two studies; however, it is not expected that a D-value of 70 min could be attributed to these minor differences.

Dynamic processes clearly impact inactivation kinetics, and the phenomena that impact *Salmonella* inactivation should be accounted for as accurately as possible. Assumptions made under static environmental conditions may not always be accurate, as demonstrated by comparing the studies of Archer et al. (1998) and Smith et al. (2016). Jeong et al. (2009)

modeled *Salmonella* inactivation in almonds under dynamic surface temperature and wetness conditions, using a modified model that accounts for surface wetness as a function of process dew point, which is related to process humidity. A traditional D- and z-value Bigelow-type model did not explain *Salmonella* inactivation under moist-air heating conditions. It was found that increasing humidity levels increased *Salmonella* inactivation due to condensation at the surface, but increasing process temperature at a constant process humidity did not impact *Salmonella* inactivation. In most products and processes, it is assumed that process temperature will always have a significant impact on inactivation rates; however, despite a 121°C spread in process between the lowest and highest temperatures evaluated, no significant difference in *Salmonella* reduction was seen at high humidities. The effects of product a_w, assumed to be decreasing during the heating process, were not accounted for in this study, but this may have also influenced *Salmonella* inactivation.

Initial product a_w may not have an impact on *Salmonella* lethality during high-velocity, hot air roasting (Casulli, Garces-Vega, Limcharoenchat, & Marks, 2016). For both almonds and in-shell pistachios heated under dynamic surface temperature conditions at a commercially-relevant air velocity of 1.3 m/s, initial a_w had no impact on *Salmonella* lethality outcomes. There was a slight systematic difference observed in *Salmonella* lethality for almonds, but not for pistachios, potentially because the shells of the pistachios acted as a barrier to heat and mass transfer. Similar trends of increased pathogen resistance in pistachios as compared to almonds have been observed in other studies (Ban & Kang, 2016; Kimber et al., 2012).

2.3 Modeling Microbial Inactivation in Low-Moisture Foods

2.3.1 Primary models

Modeling *Salmonella* inactivation kinetics in low-moisture foods presents a particular challenge, because thermal resistance varies so widely between different food matrices and processing conditions. Despite a few published attempts to model *Salmonella* inactivation in low-moisture foods, proper selection criteria and statistical analyses are lacking for many studies; thus, it is unclear what the best model is for *Salmonella* thermal inactivation in low-moisture foods.

Microbial inactivation is modeled using microbial survivor data. Typically, viable cells are treated with an increasingly severe treatment leading to inactivation. The number of viable cells remaining after the treatments is plotted as a function of treatment severity to obtain a survivor curve (Peleg & Cole, 1998). The relationship between these two variables is determined with a mathematical model. The first, and most commonly used, primary model in the food industry is the log-linear model that describes a first-order reaction rate (Peleg & Cole, 1998). The equation is as follows:

$$\log \frac{N(t)}{N_0} = -\frac{t}{D} \quad (1)$$

In some studies, *Salmonella* inactivation kinetics in low-moisture foods was non-linear when plotting the log survivors as a function of time, which adds additional error in using log-linear inactivation kinetics (D- and z-values) (Marks, 2008). If the reaction kinetics are non-linear, meaning n is significantly different than 1, the Weibull model may be used (Peleg & Cole, 1998). The equation is as follows:

$$\log\frac{N(t)}{N_0} = -\left(\frac{t}{\delta}\right)^n \quad (2)$$

2.3.2 Secondary models

Secondary models can be used to model the impact of environmental and product factors on parameters of the primary model. Some examples of the most commonly used secondary models for microbial inactivation include polynomial, Arrhenius-type, and Bigelow models. This is where external effects can be incorporated into a model, such as temperature, a_w, or pH, to name a few (Valdramidis et al., 2006) Published secondary models will be reviewed in greater depth in subsequent sections.

2.3.3 Model selection for microbial inactivation

There are several published studies that use only primary models to model microbial inactivation processes. Of these studies, selection criteria for using a specific model are often lacking, and either the log-linear model (Ban & Kang, 2016; Goepfert, Iskander, & Amundson, 1970; He et al., 2011; Lee et al., 2006) or the Weibull model (Abd et al., 2012; Du, Abd, McCarthy, & Harris, 2010; Santillana Farakos, Hicks, & Frank, 2014; Shachar & Yaron, 2006) is chosen arbitrarily based on how the microbial data appear when plotted as a function of time.

Few studies fit both log-linear and Weibull primary models and make statistical comparisons between the two fits (Ma et al., 2009; Santillana Farakos, Frank, & Schaffner, 2013; Villa-Rojas et al., 2013). When comparing the log-linear and Weibull models for microbial inactivation, some researchers will choose the Weibull model as the superior model (sometimes incorrectly), simply because a higher R^2 value was obtained (Ma et al., 2009; Villa-Rojas et al., 2013). The problem with simply using R^2 as a measure of how well a model fits the data is that R^2 only quantifies the fraction of variance in the dependent variable that is described by the model and does not give sufficient insight into how well a model actually describes the

functional relationship between independent and dependent variables. In addition, R^2 will always get closer to 1 when the number of model parameters is increased, as is the case when comparing the log-linear model to the Weibull model, because the log-linear model is a special case of the Weibull model, where n=1.

It is generally recommended that when comparing multiple models, statistical measures are used for appropriate discrimination between models, such as residual analysis, root mean squared error (RMSE), and corrected Akaike information criterion (AIC_c), a relationship of the mean squared errors and the number of parameters (Dolan, Valdramidis, & Mishra, 2013). In studies that compare multiple primary (Santillana Farakos et al., 2013) or secondary (Smith et al., 2016; Valdramidis et al., 2006) models for inactivation, it is particularly important to use model discrimination to select the model that is more likely to be correct.

Santillana Farakos et al. (2013) modeled *Salmonella* inactivation in protein powder. They tested multiple primary models, including the log-linear and Weibull models. The other models evaluated were the Geeraerd-tail model, the biphasic linear model, and the Baranyi model; however, these models are rarely used to describe bacterial inactivation, so they will not be discussed at great length. They ultimately selected the Weibull model as the correct primary model based first on the highest adjusted R^2 and lowest RMSE, and then, if these measures for two models were equivalent, the number of parameters and their biological meaning was considered.

Smith et al. (2016) considered three secondary models that described D-values as a function of product a_w and temperature—a response-surface model, a log-linear relationship of both temperature and a_w with the D-value, and a log-linear relationship of temperature and linear relationship of a_w with the D-value. To select the best model, they used AIC_c. The lowest AIC_c

value indicated that the log-linear relationship of both temperature and a_w with the D-value was more likely to be correct. They also used the AIC_c to discriminate between the log-linear model and Weibull model and chose the log-linear model as the best primary model.

2.4 Process Validation for Inactivation Models

2.4.1 Validated models

Under FSMA, the Preventive Controls Rule for Human Foods requires food processors to have a validated kill-step for their products (FDA, 2015). Mathematical models can reduce the financial and time burden imposed by conducting validation studies. While there are few published models for *Salmonella* inactivation in low-moisture foods, there are even fewer that have been validated (Jeong, Marks, & James, 2016; Jeong, Marks, & Orta-Ramirez, 2009; Santillana Farakos, Schaffner, & Frank, 2014). Santillana Farakos et al. (2013) developed a model that accounted for temperature and a_w effects on *Salmonella* inactivation using protein powder as a model system, and then validated it with several low-moisture foods (Santillana Farakos, Schaffner, et al., 2014). They found that their model explained inactivation well for certain foods under specific conditions; however, the model was developed and validated under isothermal and iso-moisture conditions. This model may not be valid for all food products and processing conditions, because dynamically changing systems may behave differently than static systems (Dolan & Mishra, 2013).

As discussed previously, a model accounting for dynamically changing product temperature and surface moisture (quantified as a function of surface temperature and dew point) was developed at the laboratory scale (Jeong et al., 2009) and validated at the pilot scale (Jeong et al., 2016). The model, based on laboratory data collected under dynamically changing product conditions, predicted lethality at the pilot scale well, and, when used in a validation, it actually

had better repeatability than a biological validation using either *Salmonella* or *Enterococcus faecium*. This study emphasized the impact of accurate modeling for microbial inactivation processes, because when a system is well-characterized, a well-tested model can be used for process validation, potentially saving processors time and money.

2.4.2 Limitations to validation

Process validation can be expensive and time-consuming, and requires a high level of expertise (Anderson & Lucore, 2012). Food companies are unable to conduct on-site process validations by introducing a pathogen into their facilities. Instead, process validation studies are typically conducted in off-site laboratories. Many of the laboratory-scale studies are run under static conditions (i.e., fixed temperature, moisture, or humidity), even though the products experience several dynamic conditions during processing (Dolan & Mishra, 2013). Static experiments can be useful for determining the parameters of a proposed model, but the model must be validated under dynamic processing conditions (Van Impe, Nicolaï, Schellekens, Martens, & De Baerdemaeker, 1995). While data from studies conducted under static conditions are a good first step in quantifying microbial inactivation kinetics, they are quite limited when it comes to modeling an industrial-scale process.

Limitations to conducting process validation studies under static laboratory conditions include introduction of equipment-specific variability in processing conditions and variability in the resulting log reductions achieved. Because of these limitations, reliance on modeling industrial processes based on laboratory data can lead to poor quality (overprocessed) or unsafe (underprocessed) food (Anderson & Lucore, 2012). Any use of a model built on laboratory-scale data must be validated separately from the laboratory experiments. Thus, it is desirable to conduct process validation studies under the actual conditions of an industrial process, which can

be dynamic for some variables. To improve process validation methods, kinetic data and accurate mathematical models that account for scale-up process variability and limits are needed.

Current efforts to validate models are sparse and often fail to account for important product and process effects. For example, the model developed and validated by Santillana Farakos et al. (2013) was developed and validated under isothermal and iso-moisture conditions, which is not a valid assumption when considering a real-world process, as shown by Jeong et al. (2009) and Jeong et al. (2016). Also, it was shown in Jeong et al. (2016) that initial product a_w may influence lethality outcomes when considering inactivation of *Salmonella* and *E. faecium* in almonds. Currently, no published model has accounted for dynamically changing a_w.

2.5 Conclusions

While there is much literature that suggests a_w is an important factor to consider in microbial inactivation processes, none of these studies has developed and validated a model that can account for dynamically changing product moisture. In addition, very few models have been rigorously tested for goodness-of-fit and applicability to a real-world process in multiple food commodities. Addressing these literature gaps is critical to ensuring safe, quality foods for consumers.

3. MATERIALS AND METHODS

A schematic overview of the materials and methods is provided in Appendix A.

3.1 Pistachios

Raw, in-shell pistachios (*Pistacia vera*, 21/25 US#1) used in this study were obtained from a commercial processor and stored at 4°C for up to 3 months. The pistachios were raw, untreated product, not intended for human consumption. Before use in experiments, the pistachios were hand-sorted to remove poor-quality (e.g., damaged by insects, discolored from mold) pistachios, in order to reduce biological variability.

Salmonella presence were tested by plating twelve, 12±2 g samples on tryptic soy agar (Difco, BD, Franklin Lakes, NJ) additionally supplemented with 0.6% (wt/vol) yeast extract, 0.05% ammonium ferric citrate and 0.03% sodium thiosulfate (Sigma-Aldrich, St. Louis, MO) (mTSA), resulting in a mean of <44 CFU/g presumptive *Salmonella*. Colonies were identified on this differential, non-selective media by their characteristic black centers; however, further testing was not done to confirm that these colonies were, in fact, *Salmonella*. At these levels, the impact on the subsequent high level of inoculation (8.5±0.5 log CFU/g) was deemed insignificant.

3.2 Bacterial Strain and Inoculation

Pistachios were inoculated with *Salmonella* Enteritidis phage type 30, obtained from Dr. Linda Harris (University of California, Davis). This particular *Salmonella* serovar has been shown to be thermally resistant in almond products and has similar thermal resistance to serovars found naturally in pistachios, such as *Salmonella* Montevideo (Harris, 2015). The culture was stored at -80°C in tryptic soy broth supplemented with 0.6% (wt/vol) yeast extract (TSBYE) and 20% (vol/vol) glycerol until use.

Pistachios were inoculated according to previously published methods, with minor modifications (Danyluk, Uesugi, & Harris, 2005). For each inoculation conducted, a new culture was started from a frozen stock. In preparation for inoculation, two consecutive transfers (24 h each at 37°C) were conducted in TSBYE. The second transfer was spread on three tryptic soy agar plates (150 by 15 mm) supplemented with 0.6% (wt/vol) yeast extract (TSAYE) and incubated for 24 h at 37°C. The three resulting lawn cultures were each harvested with 10 ml sterile 0.1% peptone water (Difco, BD), and transferred to a sterile container, for a final volume of 25 ml inoculum. The inoculum was added to 400 g batches of in-shell pistachios in a large plastic bag that was sealed, and the resulting mixture was mixed thoroughly by hand on the counter. The inoculated pistachios were spread on a tray covered with filter paper and dried overnight in a biosafety cabinet (25°C). Inoculum homogeneity was confirmed through plating six subsamples from a 400 g batch of inoculated pistachios (Appendix B).

3.3 Equilibration

The inoculated pistachios were stored in a a_w conditioning system to equilibrate the samples to target a_w levels of 0.45 and 0.65. The conditioning system consisted of an equilibration chamber (69 cm x 51 cm x 51 cm) and a custom control system, comprised of relative humidity sensors inside the equilibration chamber, a desiccation column, a hydration column, solenoid valves, air pumps, and a computer-based control system that monitored the chamber relative humidity within ±2% (Smith & Marks, 2015). Prior to thermal treatment, samples were conditioned for 2-3 days at the target humidity (45±3 or 65±3% relative humidity) to allow the entire sample to equilibrate to the target a_w (0.45±0.02, corresponding to 6.0±0.2% moisture content, dry basis, or 0.65±0.02, corresponding to 8.9±0.2% moisture content, dry basis). Equilibration targets were confirmed immediately before thermal treatment with a a_w

meter (AquaLab 3TE, Decagon Devices, Pullman, WA) (n = 2) for each target a_w level. Samples were used within 14 days of inoculation. *Salmonella* decline was insignificant during this time (Appendix C); however, samples corresponding to time zero in each microbial inactivation time series (described below) were always enumerated for each experiment performed, and the initial *Salmonella* populations across all inoculated batches were 8.5±0.5 log CFU/g.

3.4 Presoak Treatment

For samples that received a presoak treatment, inoculated pistachios were immersed in either deionized water (resulting in an initial a_w of 0.94±0.02, or 21.3±1.8% moisture content, dry basis) or a 27% NaCl solution (resulting in an initial a_w of 0.77±0.02, or 17.4±3.1% moisture content, dry basis) for 30 s, drained of excess liquid, then immediately thermally processed. This process, for the NaCl solution, closely matches a typical commercial brining process.

3.5 Thermal Treatment

Thermal treatments were conducted in a custom-made, laboratory-scale moist-air convection oven system (Appendix D, Jeong et al., 2009). The sample chamber (10 cm x 10 cm x 10 cm) was equipped with a two-tiered wire rack that allowed for two 20-g samples to be run simultaneously. Treatments were divided into two categories, dry and pre-soaked, with a total of six oven conditions per category, run in duplicate. Samples were treated at two process temperatures (nominally 104.4 and 118.3°C, with an acceptable operational tolerance of ±2°C), three process humidities (~3%, 15%, and 30% moisture by volume, corresponding to nominally dew points of 24.4, 54.4, and 69.4°C, respectively, with an acceptable operational tolerance of ±4°C) and one commercially-relevant air velocity (1.3±0.2 m/s) for a total of six treatments in duplicate for each of the two treatment categories. The absolute humidity scale (e.g., moisture by volume or dew point) was used because air temperature was over 100°C. Humidity in the air was

reported in terms of the dew point for this study and was measured with a dew point sensor (DMP246, Vaisala, Woburn, MA). Air velocity was measured using a hot-wire anemometer (Model 407123, Extech Instruments, Nashua, NH). For treatments that were dry (i.e., no presoak), samples for both initial a_w levels (0.45 and 0.65) were treated, and each a_w was randomly assigned to one of the two racks. Presoaked treatments included either a pure water soak or a 27% NaCl solution soak. For the presoaked treatments, preliminary trials showed that initial a_w equilibration before presoaking had no effect on *Salmonella* reduction (Appendix E), so all samples were processed using pistachios equilibrated to a_w of 0.45±0.05. Each of the presoak treatments was randomly assigned to one of the two sample racks. Treatments were randomized within each replication.

Product surface temperature, product a_w, product moisture content, process temperature, and process dew point were monitored throughout the process (moisture measurement methods detailed in subsequent section). Pistachio surface temperature was measured at a frequency of 0.5 Hz by inserting a thin-wire (0.13 mm diameter) thermocouple (5TC-TT-K-36-36, OMEGA Engineering Inc., Stanford, CT) between the shell and the nut at the base of the crack. For each treatment, six randomly ordered time points were run independently of each other. Total treatment time corresponded to the time required to achieve a 3 to 5 log reduction of *Salmonella*. Samples (19.3±0.6 g) were removed from the oven at appropriate intervals, with approximately 15.6±0.6 g submitted for microbial analysis, and the remaining 3.7±0.6 g subsample used for both a_w measurements and moisture content analyses. For data sets that did not result in four or more data points in the time series, an additional replicate was processed.

3.6 Enumeration of Survivors

To enumerate survivors, 15.6±0.6 g of the 19.3±0.6 g of thermally treated pistachios were immersed in chilled sterile 0.1% peptone water immediately after thermal treatment, resulting in a nominal 1:1 dilution. Samples were stored in a cooler on ice (0°C) until enumeration. Each sample was shaken by hand for 30 s, massaged by hand for 30 s, and shaken again by hand for 30 s (Kimber et al., 2012). For *Salmonella* enumeration, appropriate ten-fold serial dilutions were plated in duplicate on mTSA. After 48±4 h of incubation at 37±2°C for mTSA all black colonies were counted as *Salmonella* and populations converted to log CFU/g. Log reductions were calculated by subtracting survivor counts from the initial population prior to heating (t = 0).

3.7 Moisture Measurements

From each 19.3±0.6 g sample processed, 3.7±0.6 g was used for moisture analysis. The sample was placed in an airtight container (AquaLab sample cup with lid, 3.89 cm diameter, 1.14 cm height, 0.0686 cm wall thickness, Decagon Devices, Pullman, WA) and allowed to cool to room temperature before measuring a_w, as previously described.

After recording a_w measurements, the sample was subsequently analyzed gravimetrically for moisture content (USDA-FSIS, 2009). Samples were dried in a laboratory convection oven at 102±2°C for 18±2 h (DX400 Drying Oven, Yamato, Santa Clara, CA). Percent moisture content for each sample was calculated on a dry basis.

Page's equation was a suitable model for describing moisture loss during drying for in-shell pistachios (Chen, 1990; Kashaninejad et al., 2007). Percent moisture content data were linearized, and a linear regression was performed (Microsoft Excel 2013, Redmond, WA) to obtain estimates for k and n in Page's equation for drying:

$$\frac{MC(t) - MC_e}{MC_i - MC_e} = e^{-kt^n} \quad (3)$$

The a_w decline during heating was also linearized and modeled using a modified version of Page's equation:

$$\frac{a_w(t) - a_{w,e}}{a_{w,i} - a_{w,e}} = e^{-kt^n} \quad (4)$$

The resulting moisture profiles were used in subsequent modeling analyses. Equilibrium values were set just below the lowest value obtained for a_w and moisture content measurements (0.025 a_w and 0.5% MC dry basis, respectively).

3.8 Data Analysis

3.8.1 Analysis of variance

Salmonella log CFU/g survivor counts were analyzed for product and process effects using *anovan*, an n-way analysis of variance (ANOVA) algorithm in MATLAB (2014a, Natick, MA). The variables evaluated were time, process temperature, process humidity (measured as the dew point temperature), and initial product a_w (measured at room temperature). Only main effects and two-way interactions of the four variables were considered in the analysis. All independent variables were considered to be continuous.

Two additional two-way ANOVA tests were conducted to determine the impact of initial a_w within dry and presoaked groups. It was hypothesized that presoaking acted as a variable distinct from the measured a_w. One ANOVA considered *Salmonella* population data only from the dry (not presoaked) samples at 0.45 and 0.65 initial a_w, and a second ANOVA considered data only from presoaked samples (pure water and 27% NaCl solution). For these ANOVAs, the only variables evaluated were time and initial a_w.

3.8.2 Inactivation model fitting and selection

Each model was either used directly or adapted from previously published attempts to model *Salmonella* D-values as a function of temperature, combined with either process humidity, product moisture, or both. Product moisture was quantified as either a_w or percent moisture content (dry basis). For the model fittings, each secondary model was integrated into the log-linear primary model (Equation 1). Attempts were made to fit models to the entire data set; however, the model parameters did not converge to reasonable values (parameter estimates were several orders of magnitude greater than published values), so based on the results of the ANOVA, the models were fit to two subsets of data, according to whether the pistachios were dry (0.45 or 0.65 initial a_w) or pre-soaked (0.77 or 0.94 initial a_w).

Reference conditions for each model were set to conditions believed to be optimal based on the work of Dolan et al. (2013). At optimal reference conditions, the error for the D_{ref} parameter is at its lowest. The reference condition approximation was obtained by perturbing the reference condition above or below an initial guess, and if a significant lowering of the D_{ref} error was seen, the initial guess was changed, until less than a 1% difference in errors was observed.

Parameters were subsequently estimated globally using *nlinfit*, an ordinary least squares nonlinear regression algorithm in MATLAB (2014a). The five secondary models that were evaluated were the modified Michigan State University (MSU) model (Jeong et al., 2009):

$$D_{T,T_d}(t) = D_{ref} \times 10^{\frac{T_{ref}-T(t)}{z_T}+\frac{(T_{d,ref}-T_d)-(T_{ref}-T(t))}{z_M}} \quad (5)$$

a traditional log-linear temperature model that incorporates log-linear a_w effects (Smith et al., 2016):

$$D_{T,a_w}(t) = D_{ref} \times 10^{\frac{T_{ref}-T(t)}{z_T}+\frac{a_{w,ref}-a_w(t)}{z_{a_w}}} \quad (6)$$

equation 6, replacing a_w with moisture content (MC):

$$D_{T,MC}(t) = D_{ref} \times 10^{\frac{T_{ref}-T(t)}{z_T}+\frac{MC_{ref}-MC(t)}{z_{MC}}} \quad (7)$$

the modified MSU model, incorporating the log-linear form for a_w:

$$D_{T,T_d,a_w}(t) = D_{ref} \times 10^{\frac{T_{ref}-T(t)}{z_T}+\frac{(T_{d,ref}-T_d)-(T_{ref}-T(t))}{z_M}+\frac{a_{w,ref}-a_w(t)}{z_{a_w}}} \quad (8)$$

and equation 8, replacing a_w with MC:

$$D_{T,T_d,MC}(t) = D_{ref} \times 10^{\frac{T_{ref}-T_s(t)}{z_T}+\frac{(T_{d,ref}-T_d)-(T_{ref}-T_s(t))}{z_M}+\frac{MC_{ref}-MC(t)}{z_{MC}}} \quad (9)$$

For each parameter, the 95% confidence interval and percent relative error were calculated. The highest acceptable relative error is generally no more than 15% in biological systems.

Upon estimating parameters, statistical criteria for each model were also examined. To examine the goodness-of-fit for each model, the root mean squared error (RMSE) was calculated:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}\left(\log\left({}^{N(t)}/_{N_0}\right)_{data,i} - \log\left({}^{N(t)}/_{N_0}\right)_{model,i}\right)^2}{n-p}} \quad (10)$$

A low RMSE indicates that the model fits the data well. Generally, a RMSE around 1.0 log CFU/g is considered to be acceptable in predictive microbiology (S M Santillana Farakos, Schaffner, et al., 2014).

To statistically compare models with different numbers of parameters, the corrected Akaike Information Criterion (AIC_c) for each model was calculated (Motulsky & Christopoulos, 2004):

$$AIC_c = n\ln\left(\frac{SS}{n}\right) + 2K + \frac{2K(K+1)}{n-K-1} \quad (11)$$

When comparing multiple candidate models, a lower AIC_c indicates that the model is more likely the correct model, because it relates the RMSE obtained to the number of parameters in the

model. Ideally, the best model will have a low RMSE and a relatively low number of parameters. This was used as the selection criterion for determining the best model to describe *Salmonella* inactivation in pistachios.

Additionally, the selected model was examined for parameter identifiability through calculating scaled sensitivity coefficients (SSCs) (Beck & Arnold, 1977), using parameter estimates, representative dynamic temperature and moisture curves, and a fixed process dew point. For a model $\eta(x, t, \beta)$, where x and t are the independent variables and β is the parameter vector, the i^{th} sensitivity coefficient is calculated as follows:

$$X_i = \frac{\partial \eta}{\partial \beta_i} \tag{12}$$

Equation 12 is then multiplied by its parameter, β_i, to scale it, resulting in the SSC:

$$X_i' = \beta_i \frac{\partial \eta}{\partial \beta_i} \tag{13}$$

SSCs were examined for size and correlation. The SSC indicates the sensitivity of the model to each parameter. Because each SSC has the same units as the dependent variable, the sizes of each SSC can be compared directly.

Once the best model was chosen based on AIC_c, the residuals of the model were also examined for bias, measured as a mean of the residuals, with respect to each processing variable (initial a_w = 0.45, 0.65, 0.77, or 0.94, T = 104.4 or 118.3°C, T_d = 24.4, 54.4, or 69.4°C).

4. IMPACT OF PROCESS TEMPERATURE, HUMIDITY, AND INITIAL PRODUCT MOISTURE ON THERMAL INACTIVATION OF *SALMONELLA* ENTERITIDIS PT30 IN PISTACHIOS DURING HOT-AIR HEATING

4.1 Results

A four-way ANOVA was conducted on the survivor data (Appendix F-G) to determine the impact of time, process temperature, humidity, and initial product moisture on thermal inactivation of *Salmonella* in pistachios. (MATLAB code is in Appendix H.) It should be noted that product temperature increased and product moisture decreased during the heating process, so this was not a static system (Figure 1).

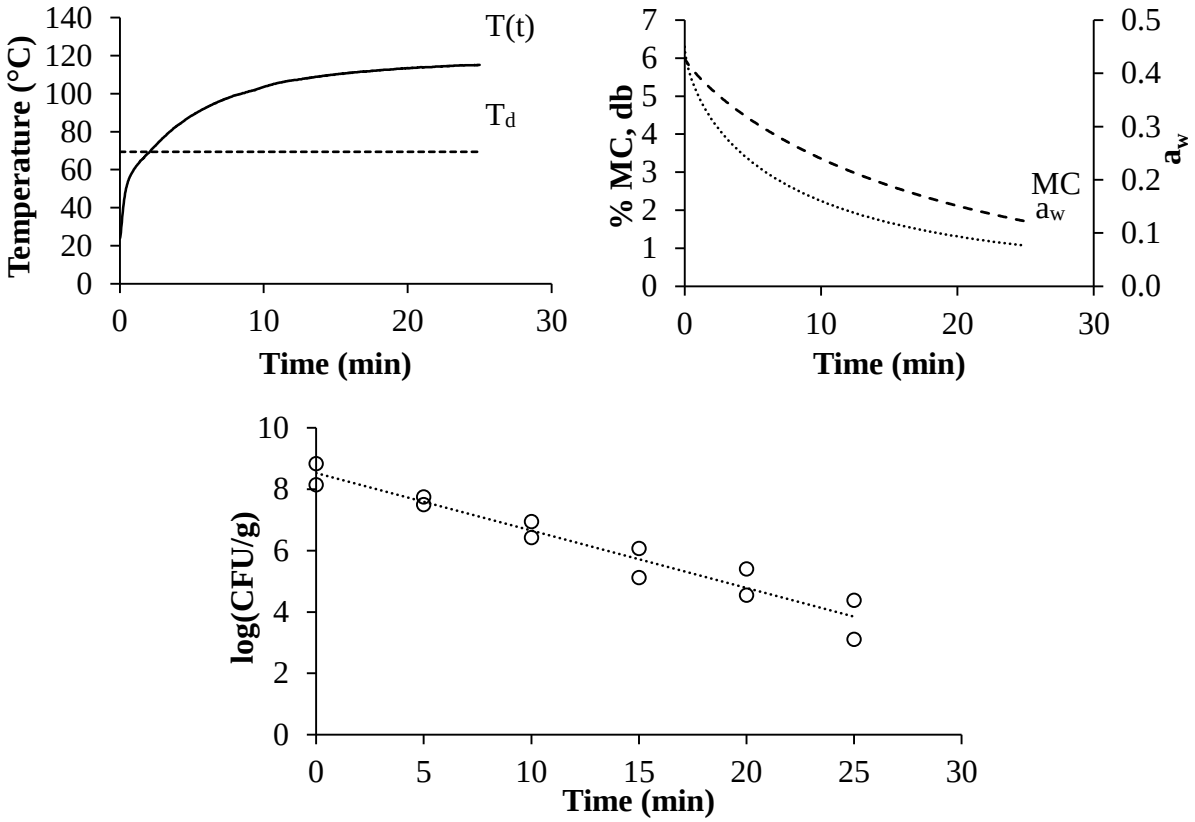

Figure 1. An example of temperature and moisture profiles and the corresponding *Salmonella* inactivation curve for pistachios heated in a lab-scale, moist-air convection oven at T = 118°C, T_d = 69.4°C, and initial a_w = 0.45.

Product temperature increased to just below the processing temperature. For initial a_w values of 0.45 (6% MC, dry basis) and 0.65 (9% MC, dry basis), the moisture declined to a minimum of 0.03 a_w (1% MC, dry basis). For the presoaked pistachios with initial a_w values of 0.77 (17% MC, dry basis) and 0.94 (21% MC, dry basis), the moisture declined to a minimum of 0.1 a_w (2% MC, dry basis). Despite multiple variables dynamically changing, increasing temperature and decreasing moisture interacted with the process humidity in a way that resulted in nearly linear *Salmonella* survivor curves (Figure 1).

Based on the four-way ANOVA performed, interactions of temperature, a_w, and humidity with time, all affected *Salmonella* inactivation ($P < 0.05$) (Table 3, Figure 2). No other interactions were significant. Graphical results indicated that initial a_w within similar groups (i.e., dry and presoaked) did not impact lethality (Figure 2), so two additional ANOVA tests were conducted to test these effects.

Table 3. ANOVA table for treatment effects on *Salmonella* populations in pistachios ($\alpha = 0.05$).

Source	Sum Sq.	d.f.	Mean Sq.	F	Prob > F
T	0.03	1	0.03	0.03	0.86
T_d	0.39	1	0.39	0.40	0.53
a_w	1.56	1	1.56	1.59	0.21
t	60.16	1	60.16	61.60	0.00
$T \times T_d$	0.93	1	0.93	0.95	0.33
$T \times a_w$	0.85	1	0.85	0.87	0.35
$T \times t$	44.10	1	44.10	45.16	0.00
$T_d \times a_w$	0.00	1	0.00	0.00	1.00
$T_d \times t$	85.42	1	85.42	87.47	0.00
$a_w \times t$	18.30	1	18.30	18.74	0.00
Error	278.34	285	0.98		
Total	628.05	295			

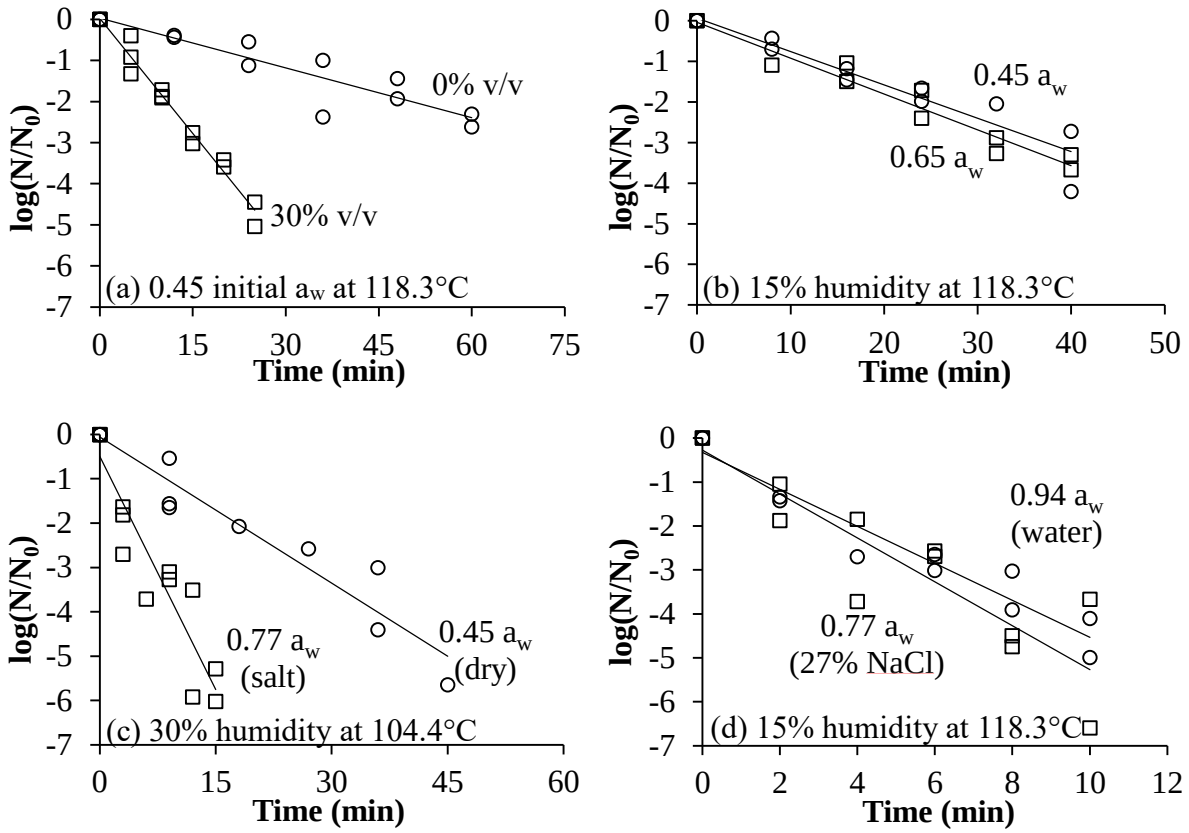

Figure 2. *Salmonella* inactivation curves comparing: (a) process humidity, (b) initial a_w for dry pistachios, (c) presoak treatment, (d) addition of NaCl to the presoaking treatment. Experimental data and linear trendline shown.

Two additional two-way ANOVA tests that tested the effects of time, initial a_w, and the interaction of time with initial a_w revealed no differences ($P > 0.05$) between the initial a_w levels within similar groups, for both dry (0.45 and 0.65 initial a_w) and presoaked (0.77 and 0.94 a_w).

Table 4. ANOVA table for initial a_w effects on *Salmonella* populations in dry pistachios subjected to all thermal treatments ($\alpha = 0.05$).

Source	Sum Sq.	d.f.	Mean Sq.	F	Prob>F
a_w	0.19	1	0.19	0.09	0.76
t	1.06	1	1.06	0.51	0.48
$a_w \times t$	0.049	1	0.049	0.02	0.88
Error	284.1	137	0.74		
Total	304.9	140			

Table 5. ANOVA table for initial a_w effects on *Salmonella* populations in presoaked pistachios subjected to all thermal treatments ($\alpha = 0.05$).

Source	Sum Sq.	d.f.	Mean Sq.	F	Prob>F
a_w	0.18	1	0.18	0.11	0.74
t	0.60	1	0.60	0.36	0.55
$a_w \times t$	0.014	1	0.014	0.01	0.93
Error	251.0	151	1.66		
Total	309.5	154			

4.2 Discussion

4.2.1 Main effects and interactions

Within the range of conditions evaluated in hot-air heating of pistachios, graphical results indicated that increasing temperature, humidity, and initial product a_w increased *Salmonella* inactivation (Figure 2), and the significance of these effects was confirmed through an ANOVA test (Table 3). It was previously reported that increasing process humidity increases thermal inactivation of *Salmonella* on almonds (Jeong et al., 2009). Increased inactivation with increasing humidity was a result of condensation on the almond surface that occurred when its temperature was below the process dew point. During this phase of heating, the condensation process released latent heat that contributed to *Salmonella* lethality. With higher humidity, the dew point is higher, thus, the condensing stage lasts longer. Even after the surface temperature of the pistachio reached the dew point temperature, inactivation was still enhanced by increasing humidity.

While increasing humidity has an enhancing effect on *Salmonella* inactivation on pistachios and almonds, previous work showed that increasing process temperature within a humidity level did not increase inactivation rates when processing almonds (Jeong et al., 2009). This finding was inconsistent with the results of the current study, but the prior study evaluated this effect graphically, and not statistically through ANOVA. There was also no discussion of

whether this effect was observed across all humidity levels, or if the reduced sensitivity to process temperature was due to variance in the data. However, this could be a product-specific effect.

4.2.2 Initial product a_w

Of particular interest to low-moisture food safety is the impact of product moisture, quantified as a_w, on *Salmonella* inactivation. It is usually not possible to remove samples during a process to measure in-process a_w, so initial product a_w is typically used as a control measure. For the ANOVA, product a_w was only considered at initial levels, not as a dynamically changing variable. Within the four levels of initial a_w, two of the levels were considered dry (0.45 and 0.65 a_w), and two of the levels were the result of a presoaking treatment in either a 27% NaCl brine or water (0.77 and 0.94 a_w, respectively). Taken as a whole, ranging from 0.45 to 0.94 initial a_w, increasing initial a_w enhanced *Salmonella* inactivation; however, when making comparisons within the two groups, dry (0.45 and 0.65 a_w) and pre-soaked (0.77 and 0.94 a_w), there was no significant difference between either 0.45 and 0.65 a_w or 0.77 and 0.94 a_w pistachios ($P > 0.05$) (Table 4-5). The difference observed between the dry and presoaked *Salmonella* populations indicates that product moisture, not just product a_w, may be an important variable to consider in products subjected to presoaking treatments, because the product's free water clearly has a significant effect on *Salmonella* inactivation. Measuring a_w, in this case, might not have been a representative measure of the amount of water available to contribute to lethality, because it is not a measurement of the quantity of water available to contribute to lethality, as a_w is an intrinsic property. For example, a pistachio presoaked in a 27% NaCl brine solution and a pistachio equilibrated to 0.77 a_w will have the same a_w, but different moisture contents, and

potentially different *Salmonella* lethality outcomes resulting from the different moisture contents.

Salt has been shown to not have any additional protective effect on *Salmonella* lethality in protein powder, beyond what could be expected based on a_w decrease (Santillana Farakos, Hicks, et al., 2014). While the conditions in the present study are not directly comparable to those of Santillana Farakos et al. (2014), salt did not seem to have any additional protective effect on *Salmonella* in presoaked pistachios, despite the difference in initial a_w levels.

4.3 Conclusions

Process temperature, process humidity, and initial product a_w impacted *Salmonella* populations in pistachios. Increasing dew point from 24.4 to 69.4°C reduced time needed to achieve a 4-log reduction by 50-80%. Further, when examining initial a_w effects on *Salmonella* reductions within the two groups of treatments (dry and presoaked pistachios), no differences were observed ($P > 0.05$). However, when comparing dry pistachios to presoaked pistachios, the *Salmonella* populations in presoaked pistachios declined 55-85% faster ($P < 0.05$), suggesting that presoaking should be considered as a distinct variable, separate from a_w. It is likely that this treatment difference was the driving force behind the significance of the interaction between time and initial a_w. These results indicate a need to use microbial inactivation models to further quantify the effects of product moisture, in addition to temperature and humidity, on *Salmonella* lethality in pistachios.

5. MODELING THE EFFECT OF PRODUCT TEMPERATURE, MOISTURE, AND PROCESS HUMIDITY ON THERMAL INACTIVATION OF *SALMONELLA* ENTERITIDIS PT30 IN PISTACHIOS DURING HOT-AIR HEATING

5.1 Results

5.1.1 Physical results

During the heating process, pistachio surface temperature increased, a_w and moisture content decreased, and *Salmonella* populations decreased (Figure 1). Greater variability was seen in temperature curves for presoaked pistachios as compared to dry pistachios (Figure 3). To model moisture decline, Page's equation for drying was fit to data for moisture content (Equation 3) and a_w (Equation 4) at each of the twenty-four process conditions (Table 6).

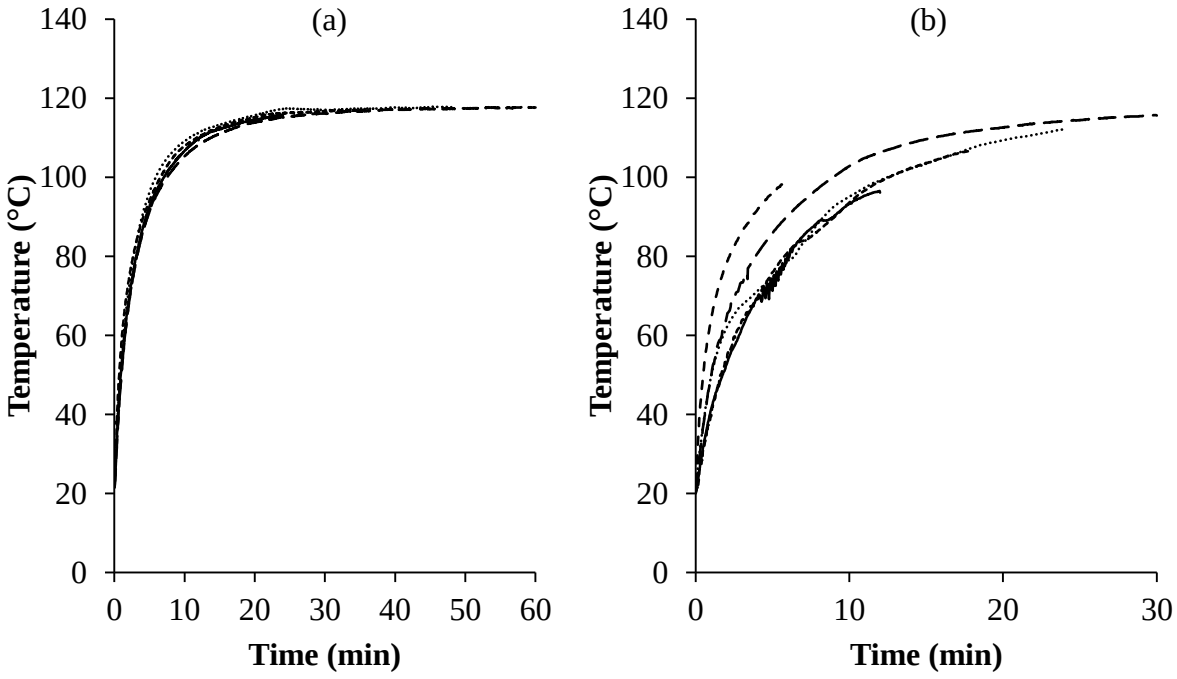

Figure 3. Pistachio surface temperature curves for: (a) dry (initial a_w = 0.45) and (b) presoaked pistachios (initial a_w = 0.77) at T = 118.3°C and T_d = 24.4°C oven condition. Each curve is an average of the temperature data from the two replicates.

Table 6. Values for k and n in Page's equation fitted to dynamic percent moisture content (dry basis) and a_w data at each oven condition.

Initial a_w	Process Temp. (°C)	Dew Point Temp. (°C)	%MC				a_w			
			k	n	R^2	RMSE	k	n	R^2	RMSE
0.45	104.4	24.4	0.12	0.74	0.98	0.06	0.93	0.28	0.68	0.10
0.65	104.4	24.4	0.26	0.54	0.99	0.03	0.63	0.42	0.84	0.10
0.45	104.4	54.4	0.07	0.71	0.62	0.30	0.31	0.43	0.63	0.05
0.65	104.4	54.4	0.10	0.73	0.93	0.41	0.30	0.53	0.96	0.04
0.45	104.4	69.4	0.07	0.71	0.94	0.04	0.31	0.43	0.49	0.10
0.65	104.4	69.4	0.08	0.74	0.90	0.06	0.25	0.54	0.72	0.08
0.45	118.3	24.4	0.11	0.88	0.86	0.08	0.44	0.57	0.44	0.15
0.65	118.3	24.4	0.29	0.67	0.73	0.09	0.52	0.61	0.51	0.13
0.45	118.3	54.4	0.17	0.71	0.92	0.05	0.62	0.40	0.64	0.07
0.65	118.3	54.4	0.17	0.75	0.88	0.06	0.55	0.46	0.66	0.07
0.45	118.3	69.4	0.08	0.85	0.96	0.04	0.24	0.67	0.98	0.02
0.65	118.3	69.4	0.03	1.20	0.92	0.46	0.11	0.96	0.92	0.07
0.77	104.4	24.4	0.27	0.55	0.74	0.23	0.03	1.17	0.72	0.18
0.94	104.4	24.4	0.24	0.65	0.86	0.06	0.24	0.67	0.87	0.07
0.77	104.4	54.4	0.04	1.00	0.91	0.41	0.00	2.01	0.89	0.18
0.94	104.4	54.4	0.10	0.86	0.74	0.11	0.03	1.28	0.93	0.08
0.77	104.4	69.4	0.03	1.32	0.72	0.17	0.01	1.28	0.46	0.29
0.94	104.4	69.4	0.27	0.33	0.14	0.18	0.06	0.79	0.42	0.21
0.77	118.3	24.4	0.01	1.62	0.56	0.32	0.02	1.36	0.61	0.25
0.94	118.3	24.4	0.11	0.98	0.80	0.11	0.07	1.07	0.77	0.13
0.77	118.3	54.4	0.08	1.01	0.80	0.11	0.01	1.86	0.80	0.21
0.94	118.3	54.4	0.04	1.21	0.55	0.23	0.01	1.87	0.79	0.22
0.77	118.3	69.4	0.16	0.77	0.36	0.13	0.00	2.13	0.93	0.22
0.94	118.3	69.4	0.15	0.72	0.44	0.14	0.07	1.07	0.86	0.17

5.1.2 Model evaluation and selection

Reference conditions for each of the models were fixed to specified values before fitting each model. The reference conditions are always going to be statistically correlated to the D_{ref} parameter, because as they change, D_{ref} will change in response, making their simultaneous estimation difficult. For this reason, they were not estimated as part of the model but were fixed at levels that allowed D_{ref} to be estimated with minimal error. Reference conditions are typically in the upper range of the conditions evaluated (Datta, 1993; Dolan et al., 2013), and in this case, the optimal reference conditions followed this trend. Reference conditions were the same within each subset of data (dry and presoaked) used for model fittings.

The primary model used in this study was the log-linear model (Equation 1). Both the Weibull model (Equation 2) and the log-linear model were evaluated by integrating equation 5 into each, replacing the D_{ref} parameter with δ_{ref} for the Weibull model. However, upon further analysis, the Weibull model was removed from further consideration, because tailing effects from dynamically declining moisture may be obscured by adding the shape parameter (n), such that, for example, z_{MC} and n could not be uniquely estimated. For these reasons, the log-linear model was the primary model used for the remainder of the study (Equation 1).

The most appropriate secondary model for each set of data, dry or presoaked, was chosen from a selection of five models, which quantify the D-value for the log-linear primary model (Equation 1) as a function of temperature combined with process humidity, product moisture (a_w or percent moisture content, dry basis), or both (Equations 5-9).

A single model could not be fit for both dry and presoaked pistachios as a whole dataset; rather, the data were partitioned into dry and presoaked, and models were fit to each of those categories separately (Table 7-8). (MATLAB code is in Appendix I.) The differences observed

in the ANOVA test likely explain why a single model was not able to fit all of the data, because one system involved “native” moisture, while the other system involved free water.

Table 7. Parameter estimates for *Salmonella* inactivation data from dry pistachios using secondary models integrated into the log-linear primary model.

Equation	Reference Conditions	Parameter	Estimate	Percent Rel. Error	RMSE log(CFU/g)	Mean of Residuals log(CFU/g)	Maximum Correlation	AIC_c
(5)	T_{ref}=105°C $T_{d,ref}$=65°C	D_{ref} (min)	8.32	4.10	0.86	-0.36	0.73 (z_T and z_M)	-14.2
		z_T (°C)	19.6	8.73				
		z_M (°C)	38.2	7.99				
(6)	T_{ref}=105°C $a_{w,ref}$=0.3	D_{ref} (min)	4.12	8.64	0.95	-0.17	0.83 (D_{ref} and z_{aw})	-8.0
		z_T (°C)	37.1	14.0				
		z_{aw}	0.26	6.59				
(7)	T_{ref}=105°C MC_{ref}=5% MC	D_{ref} (min)	5.60	6.19	0.97	-0.17	0.70 (D_{ref} and z_{MC})	-3.7
		z_T (°C)	31.7	11.9				
		z_{MC} (%MC)	4.64	6.77				
(8)	T_{ref}=105°C $T_{d,ref}$=65°C $a_{w,ref}$=0.3	D_{ref} (min)	4.35	8.13	0.82	-0.22	0.88 (D_{ref} and z_{aw})	-45.9
		z_T (°C)	22.1	8.37				
		z_M (°C)	70.2	13.9				
		z_{aw}	0.41	13.1				
(9)	T_{ref}=105°C $T_{d,ref}$=65°C MC_{ref}=5% MC	D_{ref} (min)	5.78	5.94	0.83	-0.23	0.77 (D_{ref} and z_{MC})	-41.9
		z_T (°C)	22.0	8.13				
		z_M (°C)	67.6	14.0				
		z_{MC} (%MC)	8.54	16.1				

Table 8. Parameter estimates for *Salmonella* inactivation data from presoaked pistachios using secondary models integrated into the log-linear primary model.

Model	Reference Conditions	Parameter	Estimate	Percent Rel. Error	RMSE	Mean of Residuals	Maximum Correlation	AIC_c
(5)	T_{ref}=80°C $T_{d,ref}$=65°C	D_{ref} (min)	3.42	4.31	1.02	-0.22	0.63 (z_T and z_M)	11.6
		z_T (°C)	92.2	26.3				
		z_M (°C)	55.8	6.22				
(6)	T_{ref}=80°C $a_{w,ref}$=0.7	D_{ref} (min)	4.31	3.99	1.06	-0.13	0.34 (z_{aw} and z_T)	23.0
		z_T (°C)	181	42.4				
		z_{aw}	0.50	7.22				
(7)	T_{ref}=80°C MC_{ref}=10	D_{ref} (min)	NO RESULTS					
		z_T (°C)						
		z_{MC} (%MC)						
(8)	T_{ref}=80°C $T_{d,ref}$=65°C $a_{w,ref}$=0.7	D_{ref} (min)	3.43	3.97	0.93	-0.13	-0.78 (z_M and z_{aw})	-14.9
		z_T (°C)	98.0	23.1				
		z_M (°C)	99.8	15.4				
		z_{aw}	0.96	18.4				
(9)	T_{ref}=80°C $T_{d,ref}$=65°C MC_{ref}=10	D_{ref} (min)	4.42	5.39	0.89	-0.11	-0.72 (D_{ref} and z_{MC})	-25.6
		z_T (°C)	77.9	17.9				
		z_M (°C)	74.3	7.02				
		z_{MC} (%MC)	24.0	13.2				

Based on the model fitting results, the most appropriate model to describe thermal inactivation of *Salmonella* in pistachios was equation 9, which incorporated an additional log-linear term that accounted for product moisture. The addition of a parameter to the modified MSU model from Jeong et al. (2009) was justified, because the AIC_c was reduced from -14.2 to -41.9 in dry pistachios and from 11.6 to -25.6 in presoaked pistachios (Table 7-8). In dry pistachios, the AIC_c values for equation 8 (with a a_w term) and equation 9 (with a moisture content term) were similar; however, in presoaked pistachios, the AIC was lower for equation 8 than equation 9, suggesting that moisture content is a better metric for describing *Salmonella* inactivation in this type of system. For these reasons, equation 9 is more likely to be correct.

For the selected secondary model, parameter identifiability was determined through SSC calculation, using parameter estimates and independent data inputs (Figure 4). Because the SSCs for each parameter were uncorrelated, each parameter could be uniquely identified. The SSCs were also all relatively large (>5% minimum threshold) as compared to the predicted log reduction output, which confirmed the low relative errors for parameter estimates (Figure 4).

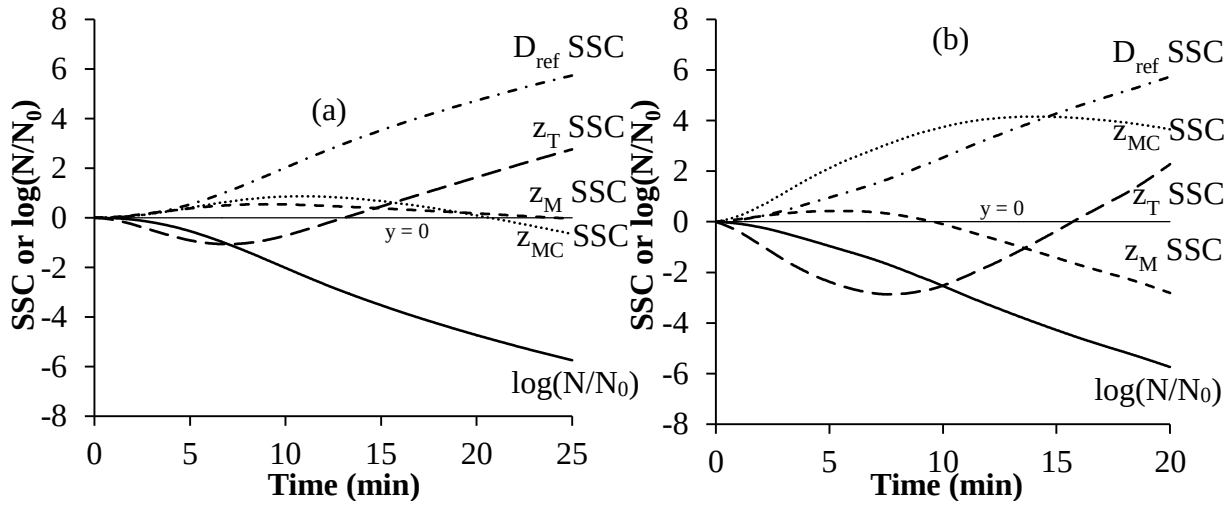

Figure 4. Scaled sensitivity coefficients and predicted log reductions for Equation 9 for: (a) dry pistachio data for T = 118.3°C, T_d = 69.4°C, and initial a_w = 0.65 and (b) presoaked pistachio data for T = 118.3°C, T_d = 69.4°C, and initial a_w = 0.77, calculated using independent data from each condition and final parameter outputs (Table 7-8).

5.2 Discussion

The trends observed when comparing model parameters between the dry and presoaked pistachio datasets were similar for all models, with the presoaked data parameters having larger parameter errors and RMSE, which indicates that this may be an inherent property of the data (Table 8). Overall, the z_T parameter showed the highest relative error in presoaked pistachios. This was likely due to variability in the temperature data. As shown in the SSC plots, z_T was the smallest for presoaked pistachios (Figure 4b). The temperature curves were likely highly dependent on the amount of free water in the system, which was variable. Pistachio nuts (in-shell) vary in the amount of "free space" water can occupy, with some having large, open cracks, allowing more water to become trapped between the shell and nut, and some having smaller, tighter cracks, allowing less water to enter. This variability was minimized to the greatest extent possible through hand-selection of nuts that were probed with thermocouples; however, natural variability could not be eliminated. In contrast, temperature curves for dry pistachios were more consistent and uniform (Figure 3). The parameters estimated for dry pistachios also had lower relative errors and 95% confidence intervals, as compared to presoaked pistachios (Table 7-8). This further supports that free water in the system caused higher temperature variability.

In previous work with almonds, there was a notable improvement from the standard thermal inactivation model that accounts only for product temperature effects to using a model that accounts for both product temperature and process humidity during moist-air heating (Jeong et al., 2009); therefore, that enhanced model (modified MSU model, equation 5) was considered for pistachios. The modified MSU model fit both the dry and presoaked datasets well, as expected, based on the results of the ANOVA that indicated process humidity affected lethality. In dry pistachios, parameter errors were low (ranged from 4.10 to 8.73%) and RMSE was low

(0.86 log CFU/g) (Table 7). By comparison, the RMSE obtained when this model was fit to almond data was 1.40 log CFU/g (Jeong et al., 2009). When fitting the modified MSU model to the dry pistachio data, the parameter estimates for z_T and z_M for the present study, 19.6°C and 38.2°C, respectively (Table 8), were within an expected order of magnitude as compared to reported values (27.9°C and 34.2°C, respectively) in Jeong et al. (2009) for the low humidity model (30-50% moisture by volume). The estimates in the present study for z_T and z_M were also within an expected order of magnitude as compared to the values reported in Jeong et al. (2009) (31.95°C and 40.73°C, respectively) for their full humidity model (5-90% moisture by volume). Surprisingly, although near to what was expected, the values were lower for pistachios, suggesting *Salmonella* inactivation rates show a greater sensitivity to changes in temperature and humidity in pistachios than in almonds. The larger RMSE in Jeong et al. (2009) may have contributed to this contradictory finding, due to the parameter estimates not being as reliable as those in the present study.

There are no literature findings that can be compared to presoaked pistachio parameter estimates. The model also fit the presoaked pistachio dataset reasonably well, although the parameter errors were higher (ranged from 4.31 to 26.26%) and the RMSE was higher (1.02 log CFU/g) but still within reasonable tolerance (~1 log CFU/g) (Table 8). The parameter estimates for z_T and z_M were also higher, 92.2°C and 55.8°C, respectively. Because the presoaked pistachios contained free water, the energy required to increase the temperature of the pistachio goes up, and the temperature of the nut remained at the dew point much longer, instead of heating up quickly. The addition of thermal energy may have contributed to lethality while not affecting surface temperature as much as for the dry samples, resulting in a larger estimate of z_T.

Water activity was an important variable in modeling *Salmonella* inactivation in low-moisture products, such as wheat flour (Smith et al., 2016) or protein powder (Sofía M Santillana Farakos et al., 2013); however, no published model has directly accounted for dynamic product moisture. Tailing in studies that are not iso-moisture indicate that a reduction in product moisture causes an increase in the D-value (Archer et al., 1998). To determine if it would be feasible to incorporate a moisture term in a thermal inactivation model with dynamically changing product moisture as a variable, equations 6 and 7 were fit to each set of data, dry and presoaked pistachios. Quantifying a D-value as a function of moisture content, rather than a_w measurements taken at room temperature, is a novel approach. Using moisture content can be as good as, or better, than using a_w as a metric for thermal inactivation of *Salmonella* in low-moisture foods (Garces-Vega & Marks, 2016), which could be particularly relevant in the case of presoaked pistachios that contain free moisture.

For dry pistachios, both equations 6 and 7 were able to be fit to the data; however, for presoaked pistachios, only equation 6 could be fit to the data, as equation 7 did not converge to reasonable parameter values. For presoaked pistachio data, the parameter error for z_T was high enough to suggest that this model, for either a_w or moisture content, was not suitable to describe *Salmonella* inactivation in pistachios. The relative errors for parameters of the a_w model (Equation 6) fit to the dry pistachio data ranged from 6.59 to 14.0% and the RMSE was 0.95 log CFU/g (Table 7). By comparison, the relative errors of the parameters for the a_w model (Equation 6) fit to the presoaked pistachios were much higher, ranging from 3.99 to 42.4% and the RMSE was 1.06 log CFU/g (Table 8). Using the percent moisture content model (Equation 7) for dry pistachios resulted in parameter relative errors of 6.19 to 11.9%, which are lower than those of the a_w model; however, the RMSE increased slightly to 0.97 CFU/g (Table 7). The z_{aw} parameter

was larger in presoaked pistachios as compared to dry pistachios, which meant that *Salmonella* in presoaked pistachios was less sensitive to a_w changes (Table 7-8). This was likely an artifact related to the additional free moisture present in the system. As discussed in the ANOVA results (Chapter 4), a_w measurement does not always account for the amount of water in the system, and moisture content could be a more suitable metric to use; however, for these data, equation 7 did not converge, potentially due to the large variance in the temperature data resulting in large parameter errors.

Unfortunately, no published data exist to directly compare the results of these model fittings, because inactivation kinetics modeled with respect to dynamically changing product moisture have not been reported. However, the z_T values reported in this study were much larger than the z_T value (15.2 min) for wheat flour reported in Smith et al. (2016) (Table 7-8). This is expected, as many studies reported that higher fat products similar to pistachios, such as almonds or peanut butter, result in greater *Salmonella* heat resistance, due to protective effects of fat (Du et al., 2010; He, Li, Salazar, Yang, & Tortorello, 2013; Ma et al., 2009). However, the z_{aw} value (0.39) reported by Smith et al. (2016) was higher than the z_{aw} reported in the present study for dry pistachios (0.26) and lower for presoaked pistachios (0.50) (Table 7-8). Again, product differences may have contributed to these estimates being different, with these findings further suggesting that the influence of changing product moisture is different in low- and high-moisture systems. Other z_{aw} values reported in the literature include 0.164 a_w units for *B. cereus* spore inactivation in glucose solutions with a_w ranging from 0.8-1.0 (Gaillard, Leguerinel, & Mafart, 1998) and a value of 0.23 a_w units for *L. monocytogenes* inactivation in potato slices with a_w ranging from 0.71-0.99 (Valdramidis et al., 2006). The estimates for z_{aw} in equations 6 and 7 were higher in this study for both dry and presoaked pistachios, indicating that *Salmonella*

thermal resistance in pistachios is greater than other microorganisms in food products with naturally high moisture.

Neither of these models (Equation 6 or 7) showed improvement over the modified MSU model (Equation 5), with respect to parameter errors, RMSE, or AIC_c. In both cases, AIC_c was lowest for the modified MSU model, indicating that this model was more likely the correct model (Table 7-8). Despite not showing improvement over the modified MSU model, it was confirmed that equations 6 and 7 can be used to model the effect of dynamically changing product moisture on *Salmonella* lethality. Therefore, a_w and MC were added to the modified MSU model, resulting in two four-parameter models (Equations 8 and 9) that quantify the D-value of *Salmonella* as a function of product temperature, process humidity, and product moisture (either a_w or MC).

Adding a term to the modified MSU model that accounts for product moisture yielded improved model fits from the previously discussed three-parameter models, as indicated by the lower AIC_c (Table 7-8). Therefore, the additional parameter was statistically justified. Like in previous models, the z_{MC} and z_{aw} parameters were larger for presoaked pistachios, indicating less sensitivity to changes in product moisture (Table 7-8). Incorporating these terms into the modified MSU model for dry pistachios reduced the sensitivity of both temperature and humidity on lethality, by increasing parameter estimates for z_T and z_M, respectively. Adding a moisture term to the modified MSU model for presoaked pistachios increased sensitivity to temperature by reducing z_T, and decreased sensitivity to humidity by increasing z_M, indicating that moisture in the two systems affected the lethality differently. As discussed in previous sections, the amount of free moisture in presoaked pistachios is not properly accounted for in a a_w

measurement, so in such cases, moisture content was the better metric to use in thermal inactivation models.

Overall, moisture content better explained *Salmonella* inactivation in brined pistachios, but was roughly equivalent to a_w in dry pistachios, with a_w showing a marginally better fit (Table 7-8). However, for simplicity and clarity in selecting a model for process validation, it would be desirable to select only one model that is able to describe both product conditions (dry and presoaked). Because both equations 8 and 9 were comparable for dry pistachios, but the AIC_c decreased from -14.9 to -25.6 when comparing equations 8 and 9, respectively, for presoaked pistachios, the modified MSU model with the moisture content term (Equation 9) was selected as the best model to describe thermal inactivation of *Salmonella* in pistachios. Despite having one acceptable model form, parameters should still be estimated independently for dry and presoaked pistachios.

5.2.1 Residual analysis

The modified MSU model with an additional moisture term was examined for residual bias with respect to each of the process conditions (T = 104.4 or 118.3°C, T_d = 24.4, 54.4, or 69.4°C) or initial a_w condition (dry, 0.45 or 0.65 a_w; or presoak, 0.77 or 0.94 a_w). In general, for both the dry and presoak conditions, the model provided a conservative estimate of lethality, as shown by its negative average residual (Table 7-8). However, when considering individual process conditions, underpredictions of lethality were seen for both conditions at a process temperature of 104.4°C and a process dew point of 54.4°C (Table 9). Additionally, a slight underprediction was seen at 0.65 a_w. While the model did show systematic bias for certain conditions, the bias was always less than 0.8 log CFU/g above or 0.3 log CFU/g below and often fell quite close to zero. The bias was always less than the uncertainty of the model, so it is

possible that the bias observed was simply due to this uncertainty, as shown by the confidence intervals (Figure 5-6).

Table 9. Model bias for each processing variable (T = 104.4 or 118.3°C, T_d = 24.4, 54.4, or 69.4°C) or initial a_w condition (dry, 0.45 or 0.65 a_w; or presoak, 0.77 or 0.94 a_w). Bias is the mean of the residuals, with negative values indicating a conservative model prediction (i.e., underpredicting actual lethality).

		Bias (log(CFU/g))	
Variable		**Dry**	**Presoak**
T (°C)	104.4	0.01*	0.17
	118.3	-0.34	-0.43
T_d (°C)	24.4	-0.08*	-0.18
	54.4	0.30	0.22
	69.4	-0.79	-0.31
Initial a_w	0.45	-0.37	--
	0.65	0.03*	--
	0.77	--	-0.09*
	0.94	--	-0.12*

*Not statistically different from 0 ($\alpha = 0.05$).

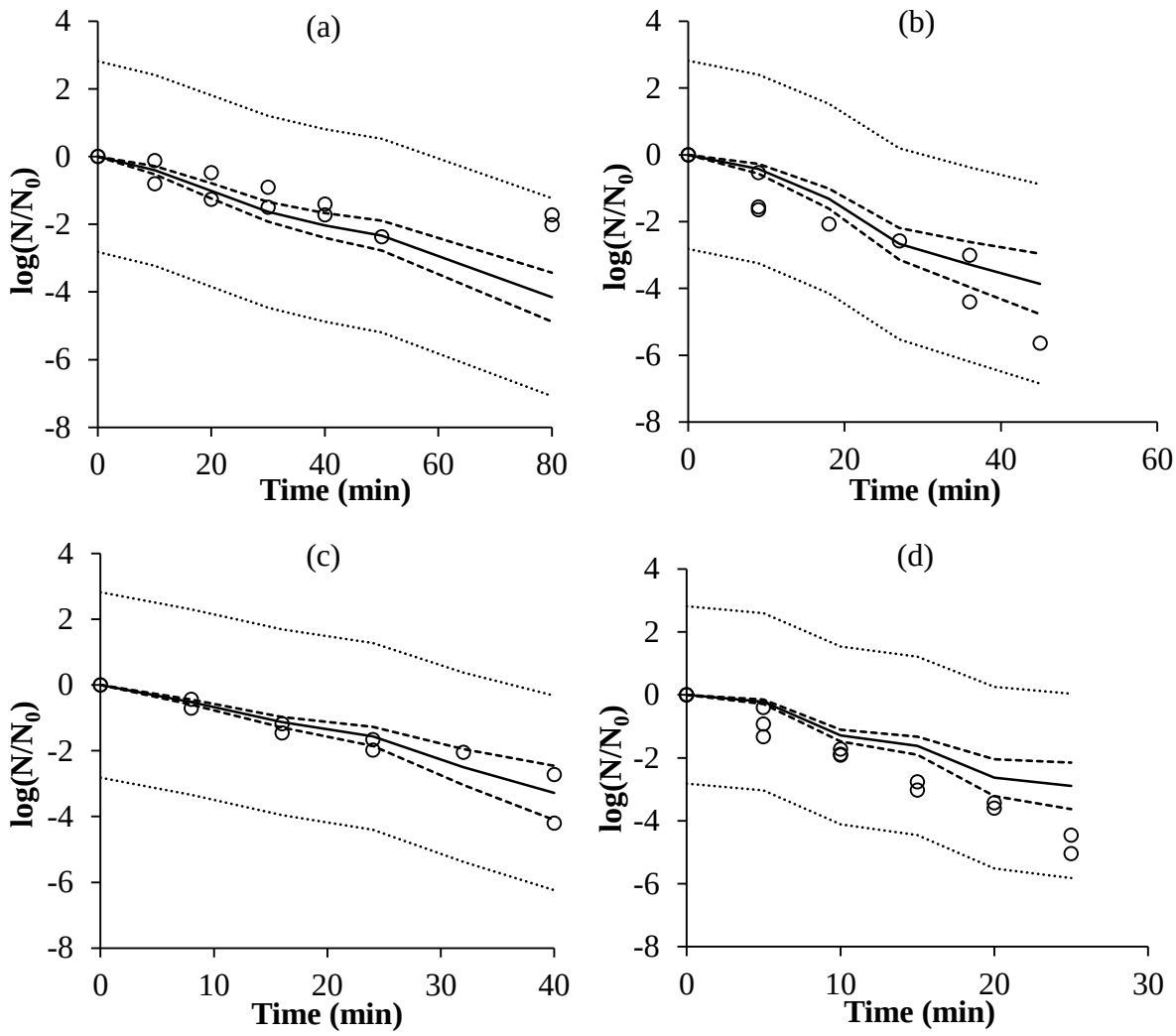

Figure 5. Raw microbial data (markers) and model prediction (solid line) (±95% confidence interval, dashed line, and ±95% prediction interval, dotted line) for dry pistachios with initial a_w = 0.45 heated at: (a) T = 104.4°C and T_d = 54.4°C, (b) T = 104.4°C and T_d = 69.4°C, (c) T = 118.3°C and T_d = 54.4°C, and (d) T = 118.3°C and T_d = 69.4°C. The parameters used for the predictions are those for equation 9 (Table 7).

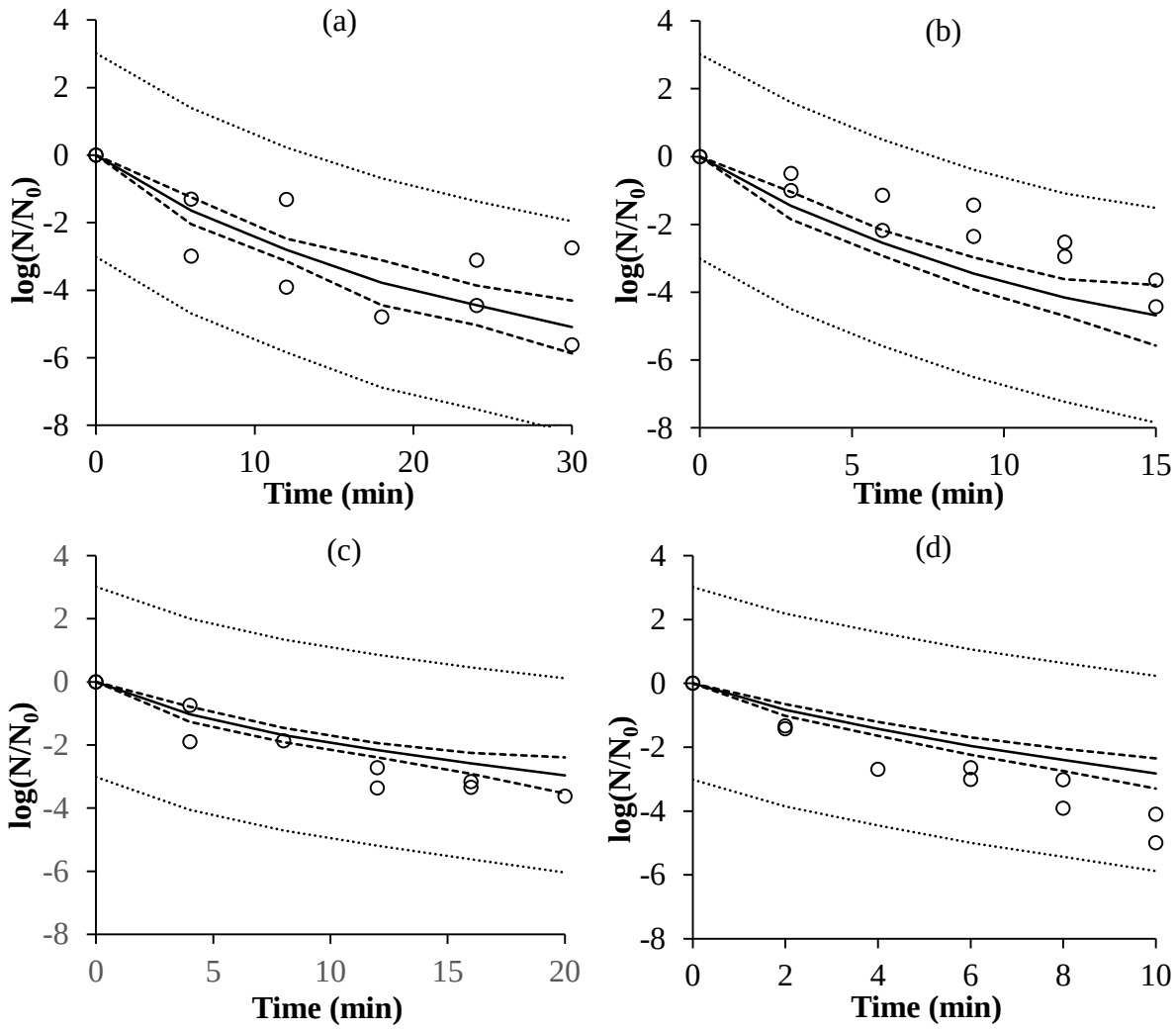

Figure 6. Raw microbial data (markers) and model prediction (solid line) (±95% confidence interval, dashed line, and ±95% prediction interval, dotted line) for presoaked pistachios with initial a_w = 0.77 heated at: (a) T = 104.4°C and T_d = 54.4°C, (b) T = 104.4°C and T_d = 69.4°C, (c) T = 118.3°C and T_d = 54.4°C, and (d) T = 118.3°C and T_d = 69.4°C. The parameters used for the predictions are those for equation 9 (Table 8).

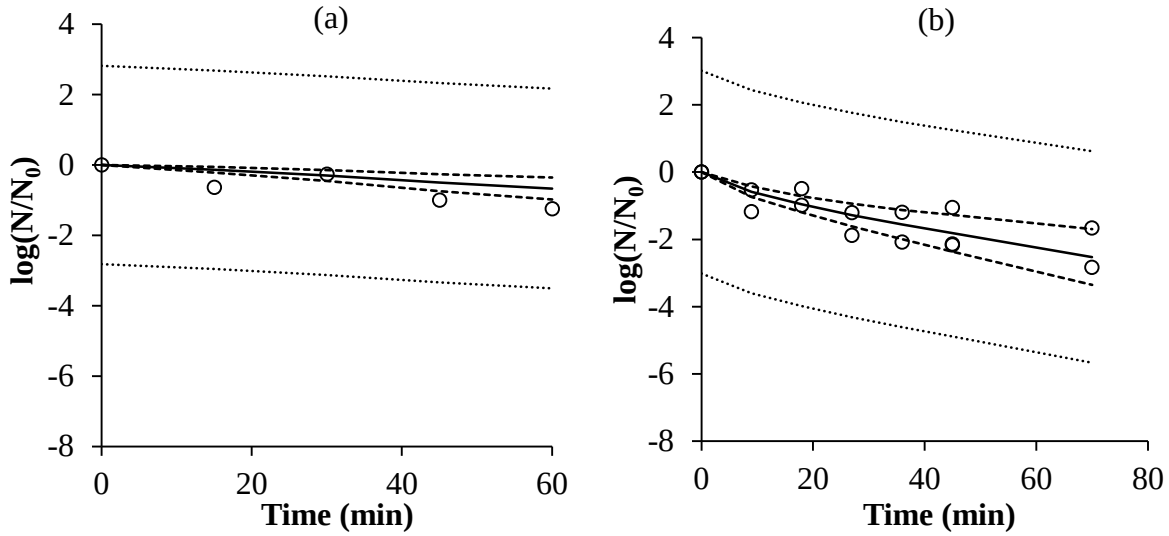

Figure 7. Raw microbial data (markers) and model prediction (solid line) (±95% confidence interval, dashed line, and ±95% prediction interval, dotted line) for T = 104.4°C and T_d = 24.4°C, for: (a) dry (initial a_w = 0.45) and (b) presoaked pistachios (initial a_w = 0.77), showing tailing. The parameters used for the predictions are those for equation 9 (Table 7-8).

5.2.2 Treatments with tailing

Some treatment conditions resulted in severe tailing (Figure 7). For example, when treating dry and presoaked pistachios at a process temperature of T = 104.4°C and T_d = 24.4°C, achieving a 5-log reduction was not feasible. Under these conditions, the highly desiccated state of *Salmonella* resulted in a large increase in the D-value that remained constant after equilibrium conditions were reached. For example, the D-value at the 60 min mark for the conditions in Figure 7a was calculated to be 91.2 min. At this point, temperature is at a maximum (~104.4°C), dew point is constant, and the moisture content is at 0.05%, dry basis. A decline in moisture content down to a theoretical value of 0.001% increases the D-value only slightly, to 92.4 min. Because product moisture was at equilibrium and the surface temperature was above the condensing stage at this point, temperature was the driving force behind inactivation, but the D-value was sufficiently large that a 5-log reduction would require > 7.6 h at this condition.

It is also possible that the *Salmonella* cells experienced some degree of sublethal stress prior to processing under these conditions, resulting in some cells that showed greater resistance than others. However, because tailing was only seen at certain conditions when the air was dry (T_d = 24.4°C), increased resistance due to sublethal stress is not believed to be the sole reason for the increased resistance observed for these treatments.

5.3 Conclusions

Models were developed for describing the effects of product temperature, moisture, and process humidity on *Salmonella* inactivation in pistachios under dynamic processing conditions. These models are the first published attempt to model *Salmonella* inactivation in a low-moisture food as a function of two dynamic variables (temperature and moisture). In addition, due to the nature of the high-moisture presoak condition, a_w was not a suitable metric for modeling *Salmonella* lethality. Within the range of conditions evaluated, the model parameters generated fell within reasonable expectations based on published data. Unfortunately, due to the highly variable nature of the data gathered for presoaked pistachios, the parameter relative errors were relatively large (>15%), with caution necessary if this model is to be used for process validations. In general, the models developed were conservative in their lethality predictions; however, some systematic process effects were observed.

6. OVERALL CONCLUSIONS AND RECOMMENDATIONS

6.1 Process effects on *Salmonella* inactivation

For the range of conditions evaluated, process temperature, humidity, and initial product moisture did not impact *Salmonella* lethality outcomes ($P > 0.05$). However, when considering the interactions of these variables with time, all were significant. Significant differences in lethality outcomes were not observed between the two initial a_w values for dry (0.45 and 0.65) and presoaked (0.77 and 0.94) pistachios. These results suggest a need for a model to quantify the impact of these product and process variables on *Salmonella* inactivation in pistachios.

6.2 Modeling the effect of product temperature, moisture, and process humidity

The most suitable model to describe *Salmonella* thermal resistance as a function of product temperature, moisture, and process humidity was the model presented by Jeong et al. (2009) with an additional log-linear term that accounted for product moisture content (Equation 9). A single model could not be fit to both dry and presoaked lethality data as a whole, rather, separate fits were conducted to obtain two sets of model parameters. Because of the larger variation in temperature profiles in presoaked pistachios, caution must be used, because the error for the z_T parameter was high. While most of the parameter relative errors were within an acceptable range (<15%), in some cases, the errors exceeded these values, indicating a need for further optimization of the reference conditions to obtain the lowest possible error for D_{ref}. Extra caution should be exercised by processors who use this information to validate an industrial process, not only because of the large errors in some parameters, but because this model has not yet been evaluated for scale-up suitability.

6.3 Future Work

6.3.1 Pilot-scale validation trials

To date, the model developed has not yet been evaluated at the pilot scale. A wide range of commercially relevant conditions and several replicates (n = 6) will be tested in a pilot-scale flat-bed roaster to determine the scale-up suitability of the selected model. From these tests, process variability will be quantified, so processors will have guidelines for setting appropriate processing limits that will achieve sufficient *Salmonella* lethality outcomes. Within process variability, both temperature and moisture variability will be determined. For treatments that do not achieve rapid destruction of *Salmonella,* a steam pre-treatment will be evaluated to determine its ability to enhance *Salmonella* lethality.

6.3.2 Feasibility of using E. faecium *as a surrogate*

For almonds, it has been determined that *Enterococcus faecium* NRRL B-2354 is a suitable nonpathogenic surrogate for *Salmonella*. However, *E .faecium* has not yet been evaluated for pistachios. It is important to determine how *E. faecium* behaves under various processing conditions (for example, different levels of temperature, humidity, moisture, or salt) as compared to *Salmonella*. It has been suggested in previous work that its sensitivity to thermal treatments varies with varying external conditions (Jeong et al., 2016).

6.3.3 Commercial-scale validation

To the author's knowledge, no work has been done to quantify variability in a commercial-scale nut roasting process or to determine the impact of this variability on lethality outcomes; however, variability in product and process conditions at the industrial scale can impact lethality outcomes achieved. Limited work has been done in quantifying variability during almond roasting in a pilot-scale flat-bed roaster (Jeong et al., 2016). Commercial-scale

validations using *E. faecium* should take place in various roasters (for example, flat-bed, rotary, etc.) from different manufacturers to gain a better understanding of equipment-specific variability such that appropriate limits may be set by processors.

6.3.4 Other recommendations

Currently, no published risk assessment exists for pistachios, so an appropriate lethality target has not yet been defined. Reductions of five logs are generally accepted, but it is preferable to make data-driven decisions for specific commodities (Schaffner et al., 2013)

This project did not specifically evaluate quality attributes associated with pistachios after roasting, such as color, texture, or nutrient retention. However, upon observation after roasting, no extreme changes in color were noted. Once appropriate commercial roasting conditions are selected, which will achieve adequate food safety outcomes as determined by risk assessment, further analyses will be needed to quantify the impact of roasting conditions on pistachio quality.

APPENDICES

Appendix A – Experimental Flow Chart for Laboratory-Scale Heating Experiments

This appendix contains the flow chart for the experimental design for laboratory-scale heating experiments.

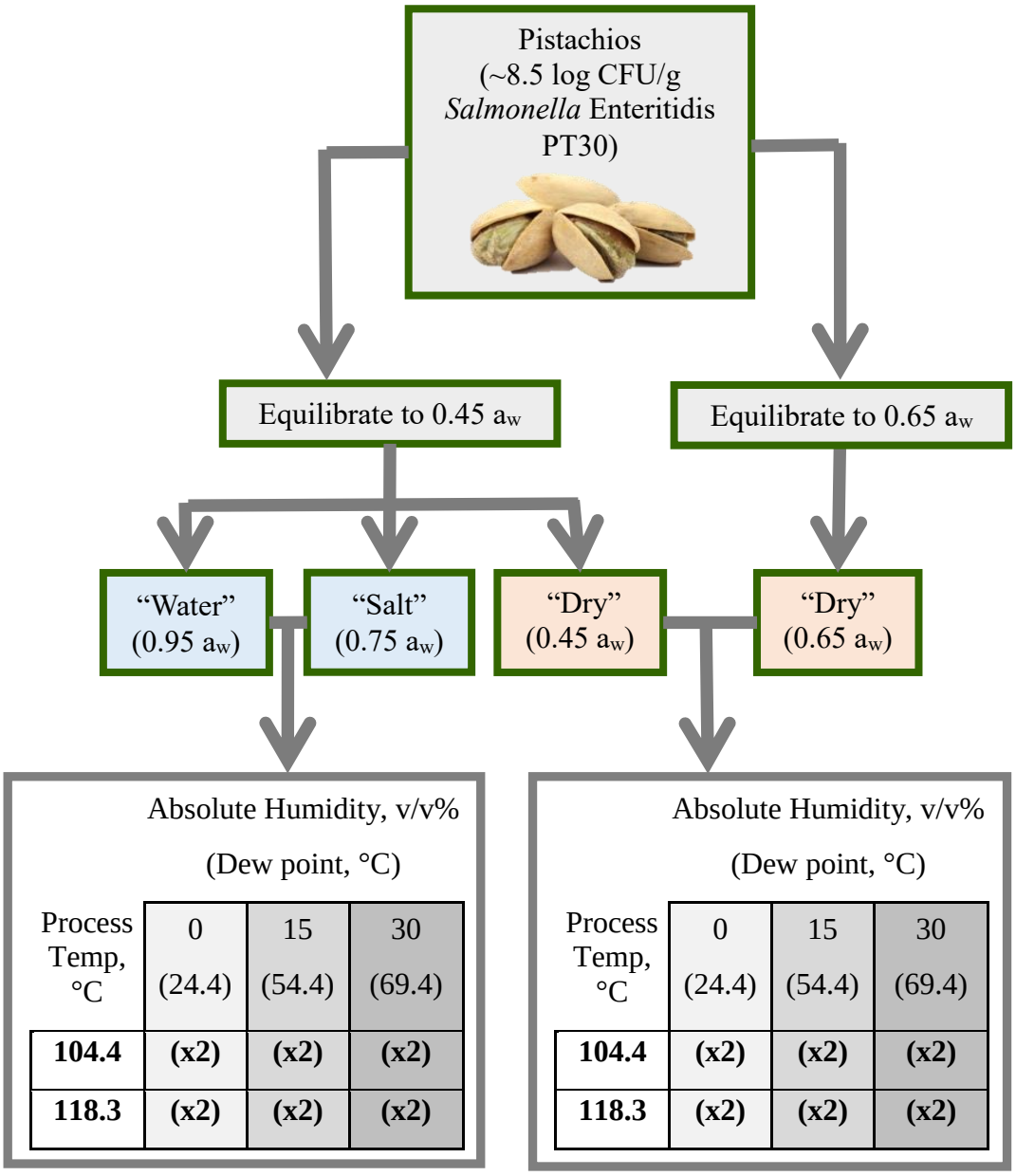

Figure 8. Experimental flow chart for laboratory-scale oven experiments.

Appendix B – Homogeneity of Inoculation

To test the homogeneity of the inoculation, six subsamples (~15 g) were randomly pulled from a 400 g sample of pistachios inoculated with *Salmonella* Enteritidis PT30. This test confirmed a reasonably homogeneous inoculation in the entire sample.

Table 10. Homogeneity results for pistachio inoculation with *Salmonella* Enteritidis PT30.

Sample	Log(CFU/g)
1	8.72
2	8.94
3	9.08
4	9.10
5	9.09
6	9.08
Average	9.00
Std. Deviation	0.14

Appendix C – Inoculum Stability Over Time

The stability of the *Salmonella* inoculum on the pistachios over the storage time (14 days) was evaluated and results are shown below, with three samples evaluated at each day.

Table 11. *Salmonella* inoculum stability on pistachios over storage time.

	***Salmonella* population**	
Sample	**Log(CFU/g)**	**Std. Dev.**
Inoculum	10.54	0.05
Day 0	9.00	0.14
Day 4	9.11	0.04
Day 7	8.86	0.06
Day 10	8.81	0.03
Day 14	8.78	0.07

Appendix D – Photographs of Experimental Setup

The following photos are of the setup from the laboratory-scale oven heating experiments.

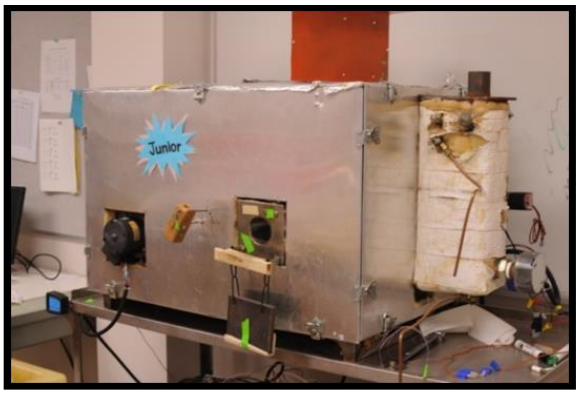

Figure 9. Photo of the laboratory-scale, moist-air convection oven.

Figure 10. Photo of the pistachios in the sample rack in the heating chamber. Arrow indicates direction of flow.

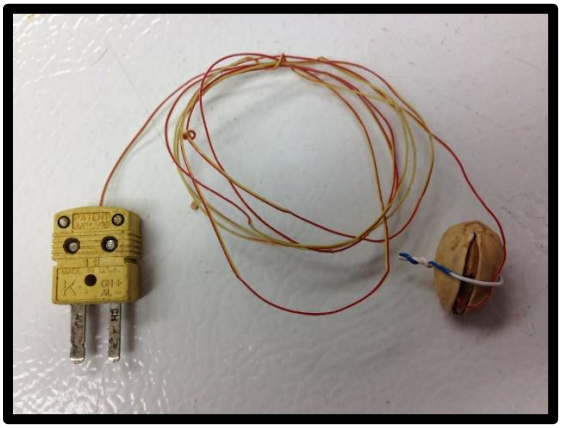

Figure 11. Photo of a pistachio instrumented with a thermocouple. The tip of the thermocouple is placed on the kernel surface at the base of the crack inside the shell, on the end where the nut attaches to the pistachio tree.

Appendix E – Water Activity Equilibration Effect for Presoaked Pistachios

To determine if equilibrated a_w before presoaking had an impact on *Salmonella* lethality, pistachios equilibrated to two initial a_w (0.45 and 0.65) were presoaked and thermally treated at 118.3°C for 30 min. These experiments were replicated three times *Salmonella* survivors were enumerated after treatment and log reductions were calculated. A t-test ($\alpha = 0.05$) was used to statistically compare treatment groups, and it was determined that the equilibrated a_w prior to presoaking had no impact on *Salmonella* log reductions achieved.

Table 12. Water activity equilibration effect for presoaked pistachios.

Presoak treatment	**Equilibrated a_w**	**Log Reductions after 30 min (±SD)**
water	0.45	3.62^{A}±0.38
	0.65	2.98^{A}±0.34
27% NaCl	0.45	3.65^{A}±0.21
	0.65	3.90^{A}±0.10

Appendix F – Survivor Data for Dry Lab-Scale Experiments

The raw *Salmonella* survivor data for the dry (0.45 and 0.65 initial a_w) experiments were as follows, with process temperature and process dew point corresponding to the nominal set points for the experiment.

Table 13. Raw *Salmonella* survivor data for dry (0.45 and 0.65 initial a_w) experiments.

Time (min)	Process Temp. (°C)	Process Dew Point (°C)	Rep	Log(CFU/g)	Log(N/N0)	a_w	%MC, db
0	104.4	24.4	1	9.04	0.00	0.43	5.76
15	104.4	24.4	1	8.40	-0.64	0.08	2.19
30	104.4	24.4	1	8.78	-0.26	0.08	1.22
45	104.4	24.4	1	8.04	-1.00	0.04	0.66
60	104.4	24.4	1	7.80	-1.24	0.05	0.69
0	104.4	24.4	1	9.04	0.00	0.65	9.12
15	104.4	24.4	1	8.00	-1.04	0.13	3.16
30	104.4	24.4	1	8.53	-0.51	0.06	1.59
45	104.4	24.4	1	8.13	-0.91	0.04	1.17
60	104.4	24.4	1	7.84	-1.20	0.06	0.78
0	104.4	54.4	1	8.69	0.00	0.46	6.16
0	104.4	54.4	2	8.83	0.00	0.46	5.91
10	104.4	54.4	1	7.88	-0.81	0.13	2.79
10	104.4	54.4	2	8.70	-0.12	0.20	4.62
20	104.4	54.4	1	7.43	-1.26	N/A	N/A
20	104.4	54.4	2	8.35	-0.48	0.12	2.92
30	104.4	54.4	1	7.19	-1.50	N/A	N/A
30	104.4	54.4	2	7.92	-0.91	0.10	2.47
40	104.4	54.4	1	6.97	-1.73	0.10	2.00
40	104.4	54.4	2	7.42	-1.40	0.09	2.24
50	104.4	54.4	1	6.32	-2.37	0.11	1.41
50	104.4	54.4	2	N/A	N/A	0.10	2.10
80	104.4	54.4	1	6.63	-1.72	N/A	N/A
80	104.4	54.4	2	6.81	-2.01	0.08	2.36
0	104.4	54.4	1	8.53	0.00	0.66	8.84
0	104.4	54.4	2	9.27	0.00	0.65	10.03
10	104.4	54.4	1	7.79	-0.74	0.23	4.74
10	104.4	54.4	2	8.30	-0.97	0.26	6.19
20	104.4	54.4	1	8.40	-0.12	N/A	N/A
20	104.4	54.4	2	8.06	-1.21	0.18	4.45

Table 13. (cont'd)

Time (min)	Process Temp. (°C)	Process Dew Point (°C)	Rep	Log(CFU/g)	Log(N/N$_0$)	a_w	%MC, db
30	104.4	54.4	1	6.73	-1.80	N/A	N/A
30	104.4	54.4	2	7.89	-1.38	0.11	N/A
40	104.4	54.4	1	6.95	-1.58	0.10	1.58
40	104.4	54.4	2	7.42	-1.85	0.09	2.99
50	104.4	54.4	1	6.26	-2.27	0.08	1.47
50	104.4	54.4	2	7.23	-2.03	0.09	N/A
80	104.4	54.4	1	4.80	-1.92	N/A	N/A
80	104.4	54.4	2	6.67	-2.60	0.08	1.89
0	104.4	69.4	1	8.33	0.00	0.45	5.99
0	104.4	69.4	2	8.36	0.00	0.47	5.94
0	104.4	69.4	3	8.79	0.00	N/A	N/A
9	104.4	69.4	1	6.68	-1.64	0.24	4.52
9	104.4	69.4	2	6.80	-1.56	0.26	4.18
9	104.4	69.4	3	8.25	-0.54	N/A	N/A
18	104.4	69.4	1	6.25	-2.07	0.09	3.27
18	104.4	69.4	2	N/A	N/A	0.17	3.21
18	104.4	69.4	3	N/A	N/A	N/A	N/A
27	104.4	69.4	1	N/A	N/A	0.17	2.60
27	104.4	69.4	2	N/A	N/A	0.18	2.65
27	104.4	69.4	3	6.21	-2.58	N/A	N/A
36	104.4	69.4	1	N/A	N/A	0.11	2.47
36	104.4	69.4	2	3.96	-4.40	0.15	2.37
36	104.4	69.4	3	5.78	-3.01	N/A	N/A
45	104.4	69.4	1	N/A	N/A	0.09	2.30
45	104.4	69.4	2	2.72	-5.64	0.17	2.27
45	104.4	69.4	3	N/A	N/A	N/A	N/A
0	104.4	69.4	1	8.60	0.00	0.65	8.82
0	104.4	69.4	2	9.12	0.00	0.64	8.67
0	104.4	69.4	3	9.05	0.00	N/A	N/A
9	104.4	69.4	1	N/A	N/A	0.33	6.36
9	104.4	69.4	2	7.97	-1.15	0.31	5.69
9	104.4	69.4	3	7.25	-1.79	N/A	N/A
18	104.4	69.4	1	N/A	N/A	0.12	3.86
18	104.4	69.4	2	5.96	-3.16	0.23	3.83
18	104.4	69.4	3	6.07	-2.97	N/A	N/A
27	104.4	69.4	1	5.30	-3.30	0.14	2.80
27	104.4	69.4	2	4.60	-4.52	0.17	3.32
27	104.4	69.4	3	5.26	-3.79	N/A	N/A

Table 13. (cont'd)

Time (min)	Process Temp. (°C)	Process Dew Point (°C)	Rep	Log(CFU/g)	Log(N/N$_0$)	a_w	%MC, db
36	104.4	69.4	1	N/A	N/A	0.13	2.90
36	104.4	69.4	2	4.25	-4.86	0.15	2.50
36	104.4	69.4	3	4.76	-4.28	N/A	N/A
45	104.4	69.4	1	N/A	N/A	0.09	2.66
45	104.4	69.4	2	5.08	-4.03	0.17	2.63
45	104.4	69.4	3	4.07	-4.97	N/A	N/A
0	118.3	24.4	1	8.14	0.00	0.46	6.37
0	118.3	24.4	2	8.78	0.00	0.44	5.91
12	118.3	24.4	1	7.74	-0.39	0.12	2.28
12	118.3	24.4	2	8.35	-0.44	0.09	1.96
24	118.3	24.4	1	7.59	-0.55	0.04	0.70
24	118.3	24.4	2	7.66	-1.12	0.07	1.25
36	118.3	24.4	1	7.14	-1.00	0.03	1.04
36	118.3	24.4	2	6.41	-2.37	0.07	0.78
48	118.3	24.4	1	6.70	-1.44	0.03	0.18
48	118.3	24.4	2	6.85	-1.94	0.06	0.13
60	118.3	24.4	1	5.83	-2.31	0.03	0.09
60	118.3	24.4	2	6.17	-2.62	0.05	0.19
0	118.3	24.4	1	8.38	0.00	0.65	8.78
0	118.3	24.4	2	9.37	0.00	0.65	8.73
12	118.3	24.4	1	7.84	-0.54	0.08	2.02
12	118.3	24.4	2	7.63	-1.73	0.14	2.84
24	118.3	24.4	1	7.19	-1.19	0.05	1.12
24	118.3	24.4	2	6.93	-2.44	0.11	1.15
36	118.3	24.4	1	7.06	-1.32	0.03	0.41
36	118.3	24.4	2	6.03	-3.34	0.07	1.10
48	118.3	24.4	1	6.58	-1.80	0.03	0.45
48	118.3	24.4	2	6.91	-2.46	0.03	0.84
60	118.3	24.4	1	6.35	-2.03	0.03	0.06
60	118.3	24.4	2	6.00	-3.37	0.06	0.45
0	118.3	54.4	1	8.21	0.00	0.46	5.73
0	118.3	54.4	2	8.34	0.00	0.47	6.42
8	118.3	54.4	1	7.77	-0.43	0.12	3.16
8	118.3	54.4	2	7.64	-0.70	0.17	3.12
16	118.3	54.4	1	7.03	-1.18	0.06	1.42
16	118.3	54.4	2	6.88	-1.46	0.09	1.54
24	118.3	54.4	1	6.55	-1.66	0.06	1.00
24	118.3	54.4	2	6.35	-1.98	0.10	1.31

Table 13. (cont'd)

Time (min)	Process Temp. (°C)	Process Dew Point (°C)	Rep	Log(CFU/g)	Log(N/N$_0$)	a_w	%MC, db
32	118.3	54.4	1	N/A	N/A	0.05	1.05
32	118.3	54.4	2	6.29	-2.05	0.08	0.97
40	118.3	54.4	1	5.48	-2.73	0.05	0.69
40	118.3	54.4	2	4.13	-4.20	0.08	0.75
0	118.3	54.4	1	8.65	0.00	0.66	9.16
0	118.3	54.4	2	8.35	0.00	0.64	8.43
8	118.3	54.4	1	7.56	-1.09	0.17	3.85
8	118.3	54.4	2	N/A	N/A	0.22	4.39
16	118.3	54.4	1	7.15	-1.50	0.10	2.38
16	118.3	54.4	2	7.31	-1.04	0.10	1.86
24	118.3	54.4	1	6.24	-2.41	0.06	1.54
24	118.3	54.4	2	6.63	-1.72	0.10	1.87
32	118.3	54.4	1	5.77	-2.88	0.05	1.71
32	118.3	54.4	2	5.08	-3.27	0.07	0.54
40	118.3	54.4	1	4.98	-3.67	0.05	0.96
40	118.3	54.4	2	5.05	-3.30	0.11	0.46
0	118.3	69.4	1	8.26	0.00	0.46	6.18
0	118.3	69.4	2	8.15	0.00	0.44	6.18
0	118.3	69.4	3	8.83	0.00	N/A	N/A
5	118.3	69.4	1	7.34	-0.92	0.25	4.44
5	118.3	69.4	2	7.75	-0.40	0.23	4.61
5	118.3	69.4	3	7.50	-1.33	N/A	N/A
10	118.3	69.4	1	6.35	-1.91	0.16	3.64
10	118.3	69.4	2	6.43	-1.72	0.14	3.20
10	118.3	69.4	3	6.95	-1.88	N/A	N/A
15	118.3	69.4	1	N/A	N/A	0.11	2.66
15	118.3	69.4	2	5.13	-3.02	0.12	2.49
15	118.3	69.4	3	6.07	-2.76	N/A	N/A
20	118.3	69.4	1	N/A	N/A	0.09	1.97
20	118.3	69.4	2	4.55	-3.59	0.10	2.19
20	118.3	69.4	3	5.41	-3.42	N/A	N/A
25	118.3	69.4	1	N/A	N/A	0.08	2.15
25	118.3	69.4	2	3.11	-5.04	0.08	1.78
25	118.3	69.4	3	4.38	-4.45	N/A	N/A
0	118.3	69.4	1	8.59	0.00	0.65	8.51
0	118.3	69.4	2	8.76	0.00	0.65	8.96
5	118.3	69.4	1	6.88	-1.71	0.35	6.12
5	118.3	69.4	2	7.16	-1.60	0.45	7.66

Table 13. (cont'd)

Time (min)	Process Temp. (°C)	Process Dew Point (°C)	Rep	Log(CFU/g)	Log(N/N_0)	a_w	%MC, db
10	118.3	69.4	1	5.48	-3.11	0.25	4.92
10	118.3	69.4	2	5.72	-3.04	0.23	5.53
15	118.3	69.4	1	N/A	N/A	0.14	3.47
15	118.3	69.4	2	5.13	-3.62	0.13	3.09
20	118.3	69.4	1	4.35	-4.25	0.10	2.36
20	118.3	69.4	2	4.00	-4.75	N/A	2.47
25	118.3	69.4	1	N/A	N/A	0.08	1.74
25	118.3	69.4	2	4.40	-4.36	0.11	2.43

Appendix G – Survivor Data for Presoaked Lab-Scale Experiments

The raw *Salmonella* survivor data for the dry (0.77 and 0.94 initial a_w, corresponding to a 27% NaCl solution and pure water presoak, respectively) experiments were as follows, with process temperature and process dew point corresponding to the nominal set points for the experiment:

Table 14. Raw *Salmonella* survivor data for presoaked (0.77 and 0.94 initial a_w, corresponding to a 27% NaCl solution and pure water presoak, respectively) experiments.

Time (min)	Process Temp. (°C)	Process Dew Point (°C)	Rep	Log(CFU/g)	Log(N/N0)	a_w	%MC, db
0	104.4	24.4	1	7.72	0.00	0.77	15.69
0	104.4	24.4	2	7.97	0.00	0.75	14.84
9	104.4	24.4	1	7.19	-0.53	0.43	6.23
9	104.4	24.4	2	6.79	-1.18	0.59	6.18
18	104.4	24.4	1	7.23	-0.49	0.50	6.64
18	104.4	24.4	2	6.99	-0.98	0.14	2.62
27	104.4	24.4	1	6.51	-1.21	0.10	2.20
27	104.4	24.4	2	6.09	-1.88	0.19	2.72
36	104.4	24.4	1	6.52	-1.19	0.11	2.08
36	104.4	24.4	2	5.89	-2.08	0.23	3.25
45	104.4	24.4	1	6.66	-1.05	0.12	2.40
45	104.4	24.4	2	6.51	-2.14	0.08	1.75
70	104.4	24.4	1	6.51	-2.83	N/A	N/A
70	104.4	24.4	2	6.09	-1.66	N/A	N/A
0	104.4	24.4	1	8.46	0.00	0.92	22.03
0	104.4	24.4	2	8.18	0.00	0.97	20.75
9	104.4	24.4	1	7.57	-0.89	0.37	6.23
9	104.4	24.4	2	7.96	-0.22	0.47	10.19
18	104.4	24.4	1	7.96	-0.50	0.27	4.77
18	104.4	24.4	2	7.57	-0.61	0.14	3.72
27	104.4	24.4	1	6.03	-2.43	0.10	2.27
27	104.4	24.4	2	6.39	-1.79	0.07	2.43
36	104.4	24.4	1	7.09	-1.37	0.10	2.49
36	104.4	24.4	2	5.88	-2.30	0.07	1.63
45	104.4	24.4	1	5.30	-3.16	0.07	1.66
45	104.4	24.4	2	5.59	-2.59	0.06	1.28
70	104.4	24.4	1	6.03	-2.13	N/A	N/A
70	104.4	24.4	2	6.39	-2.49	N/A	N/A
0	104.4	54.4	1	8.48	0.00	0.78	20.61

Table 14. (cont'd)

Time (min)	Process Temp. (°C)	Process Dew Point (°C)	Rep	Log(CFU/g)	Log(N/N_0)	a_w	%MC, db
0	104.4	54.4	2	10.16	0.00	0.78	11.37
6	104.4	54.4	1	7.18	-1.30	0.71	13.70
6	104.4	54.4	2	7.17	-2.99	0.72	14.64
12	104.4	54.4	1	7.17	-1.31	0.69	11.18
12	104.4	54.4	2	6.25	-3.91	0.45	6.63
18	104.4	54.4	1	N/A	N/A	0.36	5.03
18	104.4	54.4	2	5.37	-4.79	0.24	4.14
24	104.4	54.4	1	5.37	-3.11	0.28	4.82
24	104.4	54.4	2	5.71	-4.46	0.51	5.99
30	104.4	54.4	1	5.74	-2.75	0.13	3.08
30	104.4	54.4	2	4.54	-5.62	0.12	2.33
0	104.4	54.4	1	8.15	0.00	0.95	23.72
0	104.4	54.4	2	8.17	0.00	1.00	16.22
6	104.4	54.4	1	6.62	-1.53	0.76	11.93
6	104.4	54.4	2	6.83	-1.34	0.75	12.38
12	104.4	54.4	1	6.45	-1.70	0.34	6.02
12	104.4	54.4	2	N/A	N/A	0.55	8.59
18	104.4	54.4	1	N/A	N/A	0.39	7.43
18	104.4	54.4	2	5.28	-2.89	0.22	4.36
24	104.4	54.4	1	6.63	-1.51	0.20	4.85
24	104.4	54.4	2	5.34	-2.83	0.20	3.96
30	104.4	54.4	1	5.32	-2.82	0.11	3.43
30	104.4	54.4	2	4.32	-3.85	0.16	3.55
0	104.4	69.4	1	7.77	0.00	0.76	17.89
0	104.4	69.4	2	8.83	0.00	0.77	17.49
3	104.4	69.4	1	7.27	-0.50	0.77	16.53
3	104.4	69.4	2	7.82	-1.00	0.73	9.84
6	104.4	69.4	1	6.63	-1.14	0.71	12.17
6	104.4	69.4	2	6.65	-2.18	0.71	13.29
9	104.4	69.4	1	6.34	-1.43	0.64	8.87
9	104.4	69.4	2	6.47	-2.36	0.44	6.89
12	104.4	69.4	1	5.24	-2.53	0.68	10.56
12	104.4	69.4	2	5.88	-2.94	0.30	5.14
15	104.4	69.4	1	4.13	-3.64	0.56	8.50
15	104.4	69.4	2	4.39	-4.43	0.60	9.80
0	104.4	69.4	1	8.45	0.00	0.95	19.88
0	104.4	69.4	2	9.54	0.00	0.95	19.27
0	104.4	69.4	3	8.56	0.00	N/A	N/A

Table 14. (cont'd)

Time (min)	Process Temp. (°C)	Process Dew Point (°C)	Rep	Log(CFU/g)	Log(N/N_0)	a_w	%MC, db
3	104.4	69.4	1	6.82	-1.63	0.83	13.10
3	104.4	69.4	2	6.84	-2.70	0.79	11.26
3	104.4	69.4	3	6.74	-1.81	N/A	N/A
6	104.4	69.4	1	N/A	N/A	0.82	15.85
6	104.4	69.4	2	5.83	-3.71	0.79	11.85
6	104.4	69.4	3	N/A	N/A	N/A	N/A
9	104.4	69.4	1	5.18	-3.27	0.79	12.43
9	104.4	69.4	2	N/A	N/A	0.43	7.19
9	104.4	69.4	3	5.46	-3.10	N/A	N/A
12	104.4	69.4	1	N/A	N/A	0.61	9.50
12	104.4	69.4	2	3.62	-5.92	0.82	15.03
12	104.4	69.4	3	5.05	-3.51	N/A	N/A
15	104.4	69.4	1	N/A	N/A	0.52	8.11
15	104.4	69.4	2	3.52	-6.02	0.46	7.58
15	104.4	69.4	3	3.27	-5.28	N/A	N/A
0	118.3	24.4	1	8.34	0.00	0.77	11.68
0	118.3	24.4	2	8.36	0.00	0.74	13.27
6	118.3	24.4	1	6.92	-1.42	0.69	11.34
6	118.3	24.4	2	7.07	-1.29	0.31	4.79
12	118.3	24.4	1	N/A	N/A	0.57	7.16
12	118.3	24.4	2	8.10	-0.26	0.49	7.36
18	118.3	24.4	1	6.03	-2.31	0.32	4.00
18	118.3	24.4	2	6.57	-1.79	0.11	2.50
24	118.3	24.4	1	5.32	-3.02	0.18	2.77
24	118.3	24.4	2	6.38	-1.97	0.10	2.41
30	118.3	24.4	1	4.65	-3.68	0.20	1.75
30	118.3	24.4	2	5.22	-3.13	0.05	0.94
0	118.3	24.4	1	8.85	0.00	0.97	23.01
0	118.3	24.4	2	8.13	0.00	0.97	16.26
6	118.3	24.4	1	7.68	-1.17	0.67	9.58
6	118.3	24.4	2	7.40	-0.73	0.71	11.59
12	118.3	24.4	1	6.46	-2.39	0.22	3.81
12	118.3	24.4	2	N/A	N/A	0.31	4.11
18	118.3	24.4	1	6.09	-2.76	0.16	3.42
18	118.3	24.4	2	6.01	-2.12	0.13	2.92
24	118.3	24.4	1	5.65	-3.20	0.12	1.60
24	118.3	24.4	2	5.72	-2.41	0.11	2.84
30	118.3	24.4	1	4.99	-3.86	0.13	1.06

Table 14. (cont'd)

Time (min)	Process Temp. (°C)	Process Dew Point (°C)	Rep	Log(CFU/g)	Log(N/N$_0$)	a_w	%MC, db
30	118.3	24.4	2	5.32	-2.81	0.27	1.09
0	118.3	54.4	1	8.74	0.00	0.77	20.89
0	118.3	54.4	2	8.55	0.00	0.79	13.75
4	118.3	54.4	1	6.85	-1.90	0.71	13.50
4	118.3	54.4	2	7.80	-0.74	0.70	9.63
8	118.3	54.4	1	N/A	N/A	0.60	8.81
8	118.3	54.4	2	6.68	-1.87	0.67	10.04
12	118.3	54.4	1	5.38	-3.36	0.63	8.14
12	118.3	54.4	2	5.82	-2.72	0.21	3.83
16	118.3	54.4	1	5.58	-3.17	0.35	4.99
16	118.3	54.4	2	5.20	-3.35	0.18	3.40
20	118.3	54.4	1	5.12	-3.62	0.18	2.67
20	118.3	54.4	2	N/A	N/A	0.09	2.58
0	118.3	54.4	1	8.57	0.00	0.92	19.93
0	118.3	54.4	2	8.61	0.00	0.85	13.24
4	118.3	54.4	1	7.03	-1.55	0.79	13.59
4	118.3	54.4	2	7.47	-1.14	0.78	18.11
8	118.3	54.4	1	6.40	-2.17	0.85	17.26
8	118.3	54.4	2	7.00	-1.61	0.62	9.09
12	118.3	54.4	1	5.55	-3.02	0.24	3.95
12	118.3	54.4	2	6.88	-1.73	0.47	7.14
16	118.3	54.4	1	N/A	N/A	0.25	3.99
16	118.3	54.4	2	N/A	N/A	0.20	4.40
20	118.3	54.4	1	4.42	-4.16	0.30	5.36
20	118.3	54.4	2	4.78	-3.83	0.07	2.83
0	118.3	69.4	1	9.01	0.00	0.77	17.35
0	118.3	69.4	2	8.62	0.00	0.76	18.26
2	118.3	69.4	1	7.67	-1.34	0.75	18.49
2	118.3	69.4	2	7.20	-1.42	0.74	10.79
4	118.3	69.4	1	6.31	-2.70	0.73	15.00
4	118.3	69.4	2	N/A	N/A	0.70	10.49
6	118.3	69.4	1	6.36	-2.64	0.70	12.19
6	118.3	69.4	2	5.61	-3.01	0.64	9.64
8	118.3	69.4	1	5.98	-3.02	0.54	7.52
8	118.3	69.4	2	4.71	-3.91	0.55	7.61
10	118.3	69.4	1	4.90	-4.10	0.33	5.05
10	118.3	69.4	2	3.63	-4.99	0.34	5.15
0	118.3	69.4	1	8.90	0.00	0.91	19.02

Table 14. (cont'd)

Time (min)	Process Temp. (°C)	Process Dew Point (°C)	Rep	Log(CFU/g)	Log(N/N_0)	a_w	%MC, db
0	118.3	69.4	2	8.62	0.00	0.93	16.00
2	118.3	69.4	1	7.02	-1.87	0.88	16.04
2	118.3	69.4	2	7.58	-1.04	0.88	18.19
4	118.3	69.4	1	5.18	-3.71	0.76	10.40
4	118.3	69.4	2	6.78	-1.85	0.71	8.56
6	118.3	69.4	1	6.20	-2.69	0.66	8.32
6	118.3	69.4	2	6.05	-2.57	0.75	12.35
8	118.3	69.4	1	4.15	-4.74	0.43	6.78
8	118.3	69.4	2	4.13	-4.49	0.60	8.30
10	118.3	69.4	1	2.30	-6.60	0.62	10.58
10	118.3	69.4	2	4.96	-3.67	0.57	7.71

Appendix H – MATLAB Code for ANOVA

```
%% Pistachio ANOVA

%This code runs the ANOVA to determine treatment effects on
Salmonella
%inactivation in pistachios

%% Housekeeping

clear all
clc
format compact

%% Import data, format for 4-way ANOVA

data=xlsread('glmanalysis.xlsx','All');
Temp=data(:,1);
DewPt=data(:,2);
aw=data(:,3);
time=data(:,4);
LogN=data(:,6);

%% 4-way ANOVA

varnames = {'Temp';'DewPt';'aw';'time'};
group={Temp DewPt aw time};
[p,tbl2,stats,terms]=anovan(LogN,group,'varnames',varnames,'mode
l','interaction','continuous',[1, 2, 3, 4])

%% Import data, format for 2-way ANOVA for dry pistachio data

data=xlsread('glmanalysis.xlsx','Dry');
Temp=data(:,1);
DewPt=data(:,2);
aw=data(:,3);
time=data(:,4);
LogN=data(:,6);

%% 2-way ANOVA for dry pistachio data

varnames = {'aw';'time'};
group={aw time};
[p,tbl2,stats,terms]=anovan(LogN,group,'varnames',varnames,'mode
l','interaction','continuous',[1, 2])
```

```
%% Import data, format for 2-way ANOVA for presoaked pistachio
data

data=xlsread('glmanalysis.xlsx','Wet');
Temp=data(:,1);
DewPt=data(:,2);
aw=data(:,3);
time=data(:,4);
LogN=data(:,6);

%% 2-way ANOVA for presoaked pistachio data

varnames = {'aw';'time'};
group={aw time};
[p,tbl2,stats,terms]=anovan(LogN,group,'varnames',varnames,'mode
l','interaction','continuous',[1, 2])
```

Appendix I – MATLAB Code for Model Fitting

```
%% MS thesis code-Dry data with DMM_modbigelow_MC

%This code uses OLS to fit the raw inactivation data,
temperature curves,
%moisture curves, and dew point data to the modified MSU model
(Jeong et al,
%2009) with the addition of a log-linear term to account for
product
%moisture

%% Housekeeping

clear  %clear all variables
close all
format compact

%% Experimental data

load('Pistachio_Dry');

%% Initial parameter guesses

%Based on published values
%initial guesses
beta0(1)=11; %Dref
beta0(2)=20; %zT
beta0(3)=36; %zm
beta0(4)=5; %zMC

beta=beta0';
p=length(beta);

%% Set model and reference conditions

func=@DMM_modbigelow_MC;

global Tref Tdref MCref
Tref=105;
Tdref=65;
MCref=5;

%% nlinfit runs inverse problem

[beta,resids,J,COVB,mse]=nlinfit(x_avg, yobs, func, beta);
```

```
%% Outputs and statistics

%Statistics
ypred=func(beta,x_avg); %predicted y in log reductions, assuming
y=0 at t=0
logN_pred=logN_obs+ypred; %predicted logN
rmse=sqrt(mse) %mean square error = SS/(n-p)
beta
condX=cond(J)
detXTX=det(J'*J)
pct_rmse=(rmse/(max(ypred)-min(ypred)))*100
SSr=resids'*resids;

% R is the correlation matrix for the parameters, sigma is the
standard error vector
[R,sigma]=corrcov(COVB)
relerr=sigma./beta

% confidence intervals for parameters
ci=nlparci(beta,resids,J)

% mean of the residuals
meanr=mean(resids)

% Residual histogram
[n1, xout] = hist(resids,10);
figure
hold on
set(gca, 'fontsize',14,'fontweight','bold');
bar(xout, n1) % plots the histogram
xlabel('Observed y/\sigma - Predicted
y/\sigma','fontsize',14,'fontweight','bold')
ylabel('Frequency','fontsize',14,'fontweight','bold')

%% CBs and PBs

[ypred,delta] =
nlpredci(func,x_avg,beta,resids,J,0.05,'on','curve');
%confidence band for regression line
CBu=ypred+delta;
CBl=ypred-delta;

[ypred,deltaob] =
nlpredci(func,x_avg,beta,resids,J,0.05,'on','observation');%pred
iction band for individual points
PBu=ypred+deltaob;
PBl=ypred-deltaob;
```

REFERENCES

REFERENCES

Abd, S. J., McCarthy, K. L., & Harris, L. J. (2012). Impact of Storage Time and Temperature on Thermal Inactivation of *Salmonella* Enteritidis PT 30 on Oil-Roasted Almonds. *Journal of Food Protection, 71*(1), 42–47.

Administrative Committee for Pistachios (ACP). (2014). Commodity Fact Sheet--Pistachios. Retrieved August 8, 2016, from www.learnaboutag.org/resources/fact/pistachios.pdf

Administrative Committee for Pistachios (ACP). (2015). 2015 Pistachio Bearing Acreage, Production and Yield Per Acre. Retrieved August 8, 2016, from www.acpistachios.org/pdf/2015Statistics.pdf

Akbas, M. Y., & Ozdemir, M. (2006). Effectiveness of Ozone for Inactivation of *Escherichia coli* and *Bacillus cereus* in pistachios. *International Journal of Food Science and Technology, 41*, 513–519.

Aktas, T., & Polat, R. (2007). Changes in the Drying Characteristics and Water Activity Values of Selected Pistachio Cultivars During Hot Air Drying. *Journal of Food Process Engineering, 30*, 607–624.

American Pistachio Growers (APG). (2015). The Power of Pistachios. Retrieved August 8, 2016, from www.americanpistachios.org/power-of-pistachios

Anderson, D., & Lucore, L. A. (2012). *Validating the Reduction of* Salmonella *and Other Pathogens in Heat Processed Low-Moisture Foods.*

Archer, J., Jervis, E. T., Bird, J., & Gaze, J. E. (1998). Heat Resistance of *Salmonella weltevreden* in Low-Moisture Environments. *Journal of Food Protection, 61*(8), 969–973.

Ban, G.-H., & Kang, D.-H. (2016). Effectiveness of Superheated Steam for Inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Salmonella* Enteritidis Phage Type 30, and *Listeria monocytogenes* on Almonds and Pistachios. *International Journal of Food Microbiology, 220*, 19–25.

Beck, J. V., & Arnold, K. J. (1977). *Parameter Estimation in Engineering and Science*. New York, New York: Wiley.

Bell, C., & Kyriakides, A. (2002). Salmonella--*A Practical Approach to the Organism and its Control in Foods*. Malden, MA: Wiley-Blackwell.

Beuchat, L. R., Komitopoulou, E., Beckers, H., Betts, R. P., Bourdichon, F., Fanning, S., … Ter Kuile, B. H. (2013). Low-Water Activity Foods: Increased Concern as Vehicles of Foodborne Pathogens. *Journal of Food Protection, 76*(1), 150–172.

Booth, I. R. (1998). Bacterial Responses to Osmotic Stress: Diverse Mechanisms to Achieve a Common Goal. In D. Reid (Ed.), *The Properties of Water in Foods ISOPOW 6* (1st edition,

pp. 456–485). London, UK: Thomson Science.

Casulli, K. E., Garces-Vega, F. J., Limcharoenchat, P., & Marks, B. P. (2016). Initial Water Activity of Nuts May Not Affect *Salmonella* Lethality during High-Velocity, Hot-Air Heating. *Journal of Food Protection*, In preparation.

CDC. (2012). *Salmonella* Homepage. Retrieved from https://www.cdc.gov/salmonella/general/index.html

CDC. (2016). List of Selected Multistate Foodborne Outbreak Investigations. Retrieved from http://www.cdc.gov/foodsafety/outbreaks/multistate-outbreaks/outbreaks-list.html#salmonella

Chen, H. (1990). *Simulation of Two-Stage Drying of In-Shell Pistachio Nuts*. Univeristy of California, Davis.

Danyluk, M. D., Uesugi, A. R., & Harris, L. J. (2005). Survival of *Salmonella* Enteritidis PT 30 on Inoculated Almonds after Commercial Fumigation with Propylene Oxide. *Journal of Food Protection*, *68*(8), 1613–1622.

Datta, A. K. (1993). Error Estimates for Approximate Kinetic Parameters Used in Food Literature. *Journal of Food Engineering*, *18*, 181–199.

Dolan, K. D., & Mishra, D. K. (2013). Parameter Estimation in Food Science. *Annual Reviews in Food Science and Technology*, *4*, 401–422. https://doi.org/10.1146/annurev-food-022811-101247

Dolan, K. D., Valdramidis, V. P., & Mishra, D. K. (2013). Parameter Estimation for Dynamic Microbial Inactivation: Which Model , Which Precision? *Food Control*, *29*(2), 401–408.

Du, W., Abd, S. J., McCarthy, K. L., & Harris, L. J. (2010). Reduction of *Salmonella* on Inoculated Almonds Exposed to Hot Oil. *Journal of Food Protection*, *73*(7), 1238–1246.

FDA. (2015). Background on the FDA Food Safety Modernization Act (FSMA). Retrieved January 1, 2016, from http://www.fda.gov/NewsEvents/PublicHealthFocus/ucm239907.htm

FDA. (2016). Archive for Recalls, Market Withdrawals & Safety Alerts. Retrieved from http://www.fda.gov/Safety/Recalls/ArchiveRecalls/default.htm

Gaillard, S., Leguerinel, I., & Mafart, P. (1998). Model for Combined Effects of Temperature, pH and Water Activity on Thermal Inactivation of Bacillus cereus Spores. *Journal of Food Science*, *63*(5), 887–889.

Garces-Vega, F. J., & Marks, B. P. (2016). Evaluation of Water Content as a Convenient Metric in Thermal Inactivation Modeling for Low-Moisture Food Products. St. Louis, MO: International Association for Food Protection.

GMA. (2009). *Annex to control of* Salmonella *in Low-Moisture Foods*.

Goepfert, J. M., Iskander, I. K., & Amundson, C. H. (1970). Relation of the Heat Resistance of Salmonellae to the Water Activity of the Environment. *Applied Microbiology*, *19*(3), 429–33.

Goodfellow, S. J., & Brown, W. L. (1978). Fate of *Salmonella* Inoculated into Beef for Cooking. *Journal of Food Protection*, *41*(8), 598–605.

Harris, L. J. (2015). Personal Communication.

Harris, L. J., Lieberman, V., Mashiana, R. P., Atwill, E., Yang, M., Chandler, J. C., … Jones, T. (2016). Prevalence and Amounts of *Salmonella* Found on Raw California Inshell Pistachios. *Journal of Food Protection*, *79*(8), 1304–1315.

Harris, L. J., Uesugi, A. R., Abd, S. J., & McCarthy, K. L. (2012). Survival of *Salmonella* Enteritidis PT 30 on Inoculated Almond Kernels in Hot Water Treatments. *Food Research International*, *45*(2), 1093–1098.

He, Y., Guo, D., Yang, J., Tortorello, M. Lou, & Zhang, W. (2011). Survival and Heat Resistance of *Salmonella enterica* and *Escherichia coli* O157:H7 in Peanut Butter. *Applied and Environmental Microbiology*, *77*(23), 8434–8438.

He, Y., Li, Y., Salazar, J. K., Yang, J., & Tortorello, L. (2013). Increased Water Activity Reduces the Thermal Resistance of *Salmonella enterica* in Peanut Butter. *Applied and Environmental Microbiology*, *79*(15), 4763–4767.

Hsu, M.-H., Mannapperuma, J. D., & Singh, R. P. (1991). Physical and Thermal Properties of Pistachios. *Journal of Agricultural Engineering Research*, *49*, 311–321.

Jeong, S., Marks, B. P., & James, M. K. (2016). Comparing Thermal Process Validation Methods for *Salmonella* Inactivation on Almond Kernels. *Journal of Food Protection*, Accepted.

Jeong, S., Marks, B. P., & Orta-Ramirez, A. (2009). Thermal Inactivation Kinetics for *Salmonella* Enteritidis PT30 on Almonds Subjected to Moist-Air Convection Heating. *Journal of Food Protection*, *72*(8), 1602–1609.

Kahyaoglu, T. (2008). Optimization of the Pistachio Nut Roasting Process Using Response Surface Methodology and Gene Expression Programming. *LWT*, *41*, 26–33.

Kashaninejad, M., Mortazavi, A., Safekordi, A., & Tabil, L. G. (2006). Some Physical Properties of Pistachio (*Pistacia vera* L.) Nut and its Kernel. *Journal of Food Engineering*, *72*, 30–38.

Kashaninejad, M., Mortazavi, A., Safekordi, A., & Tabil, L. G. (2007). Thin-Layer Drying Characteristics and Modeling of Pistachio Nuts. *Journal of Food Engineering*, *78*, 98–108.

Kimber, M. A., Kaur, H., Wang, L., & Danyluk, M. D. (2012). Survival of *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on Inoculated Almonds and Pistachios Stored at -19, 4, and 24°C. *Journal of Food Protection*, *75*(8), 1394–1403.

Lee, S., Oh, S., Chung, H., Reyes-de-Corcuera, J. I., Powers, J. R., & Kang, D.-H. (2006). Reduction of *Salmonella enterica* Serovar Enteritidis on the Surface of Raw Shelled Almonds by Exposure to Steam. *Journal of Food Protection*, *69*(3), 591–595.

Little, C. L., Jemmott, W., Hucklesby, L., & De Pinna, E. (2009). Assessment of the Microbiological Safety of Edible Roasted Nut Kernels on Retail Sale in England, with a Focus on *Salmonella*. *Journal of Food Protection*, *72*(4), 853–855.

Ma, L., Zhang, G., Gerner-Smidt, P., Mantripragada, V., Ezeoke, I., & Doyle, M. P. (2009). Thermal Inactivation of *Salmonella* in Peanut Butter. *Journal of Food Protection*, *72*(8), 1596–1601.

Marks, B. P. (2008). Status of Microbial Modeling in Food Process Models. *Comprehensive Reviews in Food Science and Food Safety*, *7*, 137–143.

Maskan, M., & Karatag, S. (2007). Sorption Characteristics of Whole Pistachio Nuts (*Pistacia Vera* L.). *Drying Technology*, *15*(3), 1119–1139.

Mattick, K. L., Legan, J. D., Humphrey, T. J., & Peleg, M. (2001). Calculating *Salmonella* Inactivation in Nonisothermal Heat Treatments from Isothermal Nonlinear Survival Curves. *Journal of Food Protection*, *64*(5), 606–613.

Motulsky, H., & Christopoulos, A. (2004). *Fitting Models to Biological Data Using Linear Regression: A Practical Guide to Curve Fitting*. Oxford, New York: Oxford University Press.

Omid, M., Baharlooei, A., & Ahmadi, H. (2009). Modeling Drying Kinetics of Pistachio Nuts with Multilayer Feed-Forward Neural Network. *Drying Technology*, *27*, 1069–1077.

Peleg, M., & Cole, M. B. (1998). Reinterpretation of microbial survival curves. *Critical Reviews in Food Science and Nutrition*, *38*(5), 353–380.

Peñaloza Izurieta, W., & Komitopoulou, E. (2012). Effect of Moisture on *Salmonella* spp. Heat Resistance in Cocoa and Hazelnut Shells. *Food Research International*, *45*(2), 1087–1092.

Peyman, L., Mahmoudi, A., & Ghaffari, H. (2013). Some Physical Properties of Pistachio Nuts. *International Journal of Agriculture and Crop Sciences*, *5*(7), 704–711.

Podolak, R., Enache, E., Stone, W., Black, D. G., & Elliott, P. H. (2010). Sources and Risk Factors for Contamination, Survival, Persistence, and Heat Resistance of *Salmonella* in Low-Moisture Foods. *Journal of Food Protection*, *73*(10), 1919–1936.

Polat, R., Aydin, C., & Erol Ak, B. (2007). Some Physical and Mechanical Properties of Pistachio Nut. *Bulgarian Journal of Agricultural Science*, *13*, 237–246.

Razavi, S. M. A., Emadzadeh, B., Rafe, A., & Amini, A. M. (2007). The Physical Properties of Pistachio Nut and its Kernel as a Function of Moisture Content and Variety: Part I. Geometrical Properties. *Journal of Food Engineering*, *81*, 209–217.

Razavi, S. M. A., & Taghizadeh, M. (2007). The Specific Heat of Pistachio Nuts as Affected by Moisture Content, Temperature, and Variety. *Journal of Food Engineering*, *79*, 158–167.

Santillana Farakos, S. M., Frank, J. F., & Schaffner, D. W. (2013). Modeling the Influence of Temperature, Water Activity and Water Mobility on the Persistence of *Salmonella* in Low-Moisture Foods. *International Journal of Food Microbiology*, *166*(2), 280–293.

Santillana Farakos, S. M., Hicks, J. W., & Frank, J. F. (2014). Temperature Resistance of *Salmonella* in Low–Water Activity Whey Protein Powder as Influenced by Salt Content. *Journal of Food Protection*, *77*(4), 631–634.

Santillana Farakos, S. M., Schaffner, D. W., & Frank, J. F. (2014). Predicting Survival of *Salmonella* in Low–Water Activity Foods: An Analysis of Literature Data. *Journal of Food Protection*, *77*(9), 1448–1461.

Schaffner, D. W., Buchanan, R. L., Calhoun, S., Danyluk, M. D., Harris, L. J., Djordjevic, D., … Wiedmann, M. (2013). Issues To Consider When Setting Intervention Targets with Limited Data for Low-Moisture Food Commodities: A Peanut Case Study. *Journal of Food Protection*, *76*(2), 360–369.

Shachar, D., & Yaron, S. (2006). Heat Tolerance of *Salmonella enterica* Serovars Agona, Enteritidis, and Typhimurium in Peanut Butter. *Journal of Food Protection*, *69*(11), 2687–2691.

Smith, D. F., Hildebrandt, I. M., Casulli, K. E., Dolan, K. D., & Marks, B. P. (2016). Modeling the Effect of Temperature and Water Activity on the Thermal Resistance of *Salmonella* Enteritidis PT 30 in Wheat Flour. *Journal of Food Protection*, Accepted.

Smith, D. F., & Marks, B. P. (2015). Effect of Rapid Product Desiccation or Hydration on Thermal Resistance of *Salmonella enterica* Serovar Enteritidis PT 30 in Wheat Flour. *Journal of Food Protection*, *78*(2), 281–286.

Stasiewicz, M. J., Marks, B. P., & Smith, D. M. (2008). Modeling the Effect of Prior Sublethal Thermal History on the Thermal Inactivation Rate of *Salmonella* in Ground Turkey. *Journal of Food Protection*, *71*(2), 279–285.

Tavakolipour, H., & Kalbasi-Ashtari, A. (2008). Estimation of Moisture Sorption Isotherms in Kerman Pistachio Nuts. *Journal of Food Process Engineering*, *31*, 564–582.

USDA-FSIS. (2009). Moisture Determination.

Valdramidis, V. P., Geeraerd, A. H., Gaze, J. E., Kondjoyan, A., Boyd, A. R., Shaw, H. L., & Van Impe, J. F. (2006). Quantitative Description of *Listeria monocytogenes* Inactivation Kinetics with Temperature and Water Activity as the Influencing Factors; Model Prediction and Methodological Validation on Dynamic Data. *Journal of Food Engineering*, *76*, 79–88.

Van Impe, J. F., Nicolaï, B. M., Schellekens, M., Martens, T., & De Baerdemaeker, J. (1995). Predictive Microbiology in a Dynamic Environment: A System Theory Approach.

International Journal of Food Microbiology, *25*(3), 227–249.

Villa-Rojas, R., Tang, J., Wang, S., Gao, M., Kang, D.-H., Mah, J.-H., … López-Malo, A. (2013). Thermal Inactivation of *Salmonella* Enteritidis PT 30 in Almond Kernels as Influenced by Water Activity. *Journal of Food Protection*, *76*(1), 26–32.

WHO. (2013). *Salmonella* (Non-Typhoidal) Fact Sheet N139. Retrieved from http://www.who.int/mediacentre/factsheets/fs139/en/