MODELING THE EFFECT OF WATER ACTIVITY ON THERMAL RESISTANCE OF *SALMONELLA* IN WHEAT FLOUR

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ABSTRACT

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Salmonella is able to survive in low-moisture environments, is known to be more heat resistant as product water activity decreases, and recently has been implicated in several outbreaks and/or recalls associated with low-moisture foods. Therefore, the specific objectives were to: (1) Evaluate the effect of rapid product desiccation and hydration on the thermal resistance of *Salmonella* Enteritidis PT 30 in wheat flour, and (2) Test multiple secondary models for the effect of product (wheat flour) water activity on *Salmonella* thermal resistance. A custom-built fluidized-bed drying and hydrating system was used to rapidly (1-4 min) change the sample's water activity (0.6 to 0.3, or 0.3 to 0.6) prior to thermal treatment, and the results were compared with those from samples with longer equilibration times (4-7 days). Desiccation or hydration rate did not affect *Salmonella* thermal resistance; instead, product water activity at the time of thermal treatment controlled *Salmonella* thermal resistance, regardless of the water activity "history". An additional isothermal inactivation study, with three water activities and three temperatures, generated data used to evaluate the effectiveness of three secondary models (response surface, modified Bigelow-type, and a combined-effects model) in accounting for product water activity. The combined-effects model best accounted for water activity when modeling microbial inactivation under low-moisture conditions, when considering goodness-of-fit, phenomenological basis, and model utility.

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INTRODUCTION

1.1 Problem Statement

Recently, low-moisture foods have made headlines because of numerous *Salmonella* outbreaks and recalls (Li et al., 2014). These incidents have directed increased research attention on the safety of low-moisture foods, which had previously not been considered a food safety risk, because their environments do not support bacterial growth. However, from 2007 – 2012, low-moisture foods accounted for ~20% of all *Salmonella* outbreaks (CDC, 2014). Products such as black pepper, wheat flour, peanut butter, and whole almonds all have been involved in *Salmonella* outbreaks (Gieraltowski et al., 2013; Isaacs et al., 2005; Keady et al., 2004; McCallum et al., 2013; Sheth et al., 2011). Because low-moisture foods encompass a variety of products, many of which are ingredients in other ready-to-eat foods, such as nuts, food powders, and pastes (e.g., peanut butter), hundreds of finished products could be contaminated, which increases the potential breadth of outbreaks and recalls.

Outbreaks and/or recalls have a large negative impact on the economy. Each year, it is estimated that there are over one million cases of salmonellosis just in the United States (CDC, 2011; Scallan et al., 2011). Further, there are close to 20,000 hospitalizations, which cause ~$51 billion in annual health related costs (Scharff, 2012). Aside from the cost of illness, outbreaks and recalls can negatively impact the economics of the food industry because of potential lawsuits, expensive product recovery, and possible negative public opinion (Elgazzar & Marth, 1992). There is a need to improve food safety processes and systems because of the substantial societal impact.

The bad publicity and large costs associated with recalls and outbreaks have contributed to the development of new guidelines and regulations. In 2011, the FDA Food Safety Modernization Act (FSMA) was enacted to shift the focus from responding to contamination to actively preventing it (FDA, 2014). In addition to FSMA, the Grocery Manufacturers Association (GMA) developed guidelines for the control of *Salmonella* in low-moisture foods (GMA, 2009b). These guidelines describe common sources of contamination and methods to prevent the spread of *Salmonella.* The Alliance for Innovative and Operational Excellence (AIOE) also published a general thermal process validation guidelines document for low-moisture foods (D. G. Anderson & Lucore, 2012). These guidelines are standards the industry may use to validate their low-moisture process and ensure that the process is meeting expected federal rules for preventive controls.

However, simply creating guidelines does not automatically ensure food safety, without the knowledge, data, and tools needed to enable implementation and validation of appropriate processing technologies. For example, modeling microbial inactivation has proven to be quite challenging, because the response of *Salmonella* is heavily influenced by many product and processing factors. Improved models are needed to sufficiently account for these factors and thereby enable more accurate and robust calculations of process lethality. To achieve this overall goal, some key existing knowledge gaps need to be addressed, regarding the effect of changing water activity on *Salmonella* thermal resistance.

1.2 Goals and Objectives

Therefore, the overall goal of this thesis project was to better quantify *Salmonella* thermal resistance in low-moisture powders and to use that knowledge to develop improved inactivation models. The specific objectives were to:

1. Evaluate the effect of rapid product desiccation or hydration on the thermal resistance of *Salmonella* Enteritidis PT 30 in wheat flour.
2. Test multiple secondary models for the effect of product (wheat flour) water activity on *Salmonella* thermal resistance.

LITERATURE REVIEW

2.1 Introduction

Salmonella is a gram-negative pathogen that is the leading bacterial cause of food poisoning cases in the United States (CDC, 2011). The organism is widely distributed throughout nature, but its primary reservoir is the gastrointestinal tract of humans and animals. Once a sufficient dose of *Salmonella* has been ingested, salmonellosis can occur in patients, which can cause nausea, vomiting, diarrhea, cramps, and fever (FDA, 2012).

Unfortunately, *Salmonella* can survive for long periods in low-moisture environments. Recent recalls of low-moisture foods, such as peanut butter (Medus et al., 2009), wheat flour (McCallum, et al., 2013), and whole almonds (CDC, 2004), have prompted researchers to study the behavior of *Salmonella* in low-moisture foods, which had previously been considered safe with little to no validation. Because low-moisture foods encompass a variety of products, the potential pool of foods that could be contaminated is considerable. This large group of products, many of which are used as ingredients in hundreds of ready-to-eat products, greatly expands the types of products that can be implicated in outbreaks and recalls.

Outbreaks and recalls negatively impact the economy because of the rising cost of illness and labor lost as a result of workers taking sick days to recover (Scharff, 2012). In addition, potential lawsuits, lost market share, cost of product return, and negative public opinion are all possible negative outcomes that can be avoided by making low-moisture products safer.

The FDA Food Safety Modernization Act (FSMA) was enacted in 2011 to shift from the culture of being reactive to outbreaks to a culture of actively preventing contamination from occurring by establishing preventative controls (FDA, 2014). By establishing guidelines for the

control of *Salmonella* in low-moisture foods, the industry and regulators can better prevent contamination and protect the general public.

2.2 *Salmonella* in Low-Moisture Foods

2.2.1 Disease caused by Salmonella

Approximately one in every six Americans gets sick, and 3,000 die each year from foodborne diseases (CDC, 2011). Of these 3,000 deaths, approximately 35% can be attributed to *Salmonella*, which makes it an important issue to resolve (He, Guo, Yang, Tortorello, & Zhang, 2011). Foodborne gastroenteritis from *Salmonella* causes nausea, vomiting, diarrhea, cramps, and fever (FDA, 2012). Symptoms typically last a couple of days and taper off within a week. For those who possess healthy immune systems, the disease is usually very mild; however, it can be deadly for those with compromised immune systems, such as infants and the elderly.

2.2.2 Outbreaks

Low-moisture foods (those with a water activity, $a_{w,}$ less than 0.60) have recently been implicated in multiple outbreaks and recalls, which have, in turn, focused more research and regulatory attention on the behavior of *Salmonella* in low-moisture foods. Various product types (e.g., pastes, large particulates, and powders) across the range of low-moisture foods have all been implicated.

Pastes (e.g., peanut butter) have been responsible for several high profile *Salmonella* outbreaks. One of the first reported outbreaks from contaminated peanut butter occurred in Australia in 1996 (Burnett, Gehm, Weissinger, & Beuchat, 2000). Various outbreaks and recalls have occurred since then, but the most prominent occurred in 2008 and 2009. A multistate

outbreak of salmonellosis from peanut butter occurred in the United States (Medus, et al., 2009). Ultimately, 529 cases from 43 states were linked to contaminated peanut butter from a single source.

Low-moisture particulates (e.g., nuts) were previously considered a raw product, meaning there was no required pasteurization of the product before distribution. Almonds were implicated in two outbreaks in 2000 and 2003, which resulted in the recall of almost six million kilograms of raw almonds (CDC, 2004; Isaacs, et al., 2005; Keady, et al., 2004).

Traditionally, food powders were not considered a high risk product in terms of *Salmonella* outbreaks, because they typically are ingredients for formulated products that receive appropriate thermal kill steps before consumption (Sperber & North American, 2007). However, food powders (e.g., spices and flavor coatings) have increasingly been added to ready-to-eat foods, meaning there is no subsequent pasteurization process to ensure product safety. In 1993, a nationwide outbreak of salmonellosis in Germany was linked to contaminated paprika and paprika-powdered potato chips (Lehmacher, Bockemuhl, & Aleksic, 1995). This outbreak confirmed that even extremely low numbers of the organism adapted to the low-moisture state were able to cause illness. In 1996, there was an outbreak of foodborne salmonellosis due to powdered infant formula that was contaminated. There were forty-eight cases involving children over a span of six months before health officials in Spain were able to detect the source and recall the formula (Usera et al., 1996). In 2010, over 150 items containing a hydrolyzed vegetable protein (HVP) product were recalled due to the presence of *Salmonella* (FDA, 2010). This product was used as an ingredient in a wide variety of items, such as seasoning mix packets, soup mixes, and ready-to-eat meal products. Most recently, there was an outbreak in New Zealand due to *Salmonella*-contaminated wheat flour (McCallum, et al., 2013). The increasing

number of outbreaks linked to food powders has increased the research attention on the search for more effective intervention methods.

2.2.3 Growth and survival in low-moisture foods

Salmonella has certain requirements in order to grow in an environment, but this project focuses mainly on temperature and a_w. *Salmonella* can grow between 5 and 45°C; with a typical optimal growth temperature of 35°C (Doyle & Mazzotta, 2000). Water activity is one of the major limiting factors to growth. *Salmonella* is unable to grow when the a_w level is below 0.94 (Bell & Kyriakides, 2002). However, even as the a_w of a food product drops below 0.94, *Salmonella* is still able to survive. Recent studies have shown that while *Salmonella* is unable to proliferate in low-moisture environments, it is capable of surviving (Hiramatsu, Matsumoto, Sakae, & Miyazaki, 2005; Janning, Tveld, Notermans, & Kramer, 1994; Juven et al., 1984; Podolak, Enache, Stone, Black, & Elliott, 2010). Janning et al. (1994) studied the survival of 18 pathogenic bacteria, including *Salmonella*, in broth media at 0.20 a_w. After an initial decrease in colonies, *Salmonella* stabilized and survived for very long periods (i.e., 248 to 1,351 days with $\leq$ 1 log reduction). It also was observed that *Salmonella* was more resistant to desiccation than other bacteria.

Salmonella also is able to survive for extended periods in low-moisture pastes (Burnett, et al., 2000; He, et al., 2011; Park, Oh, & Kang, 2008; Shachar & Yaron, 2006). Burnett et al. (2000) reported that the populations of *Salmonella* decreased 2.86 to 4.82 log and 4.14 to 4.50 log in different peanut butter and peanut butter spreads during 24 weeks of storage at 5 and 21°C, respectively. Park et al. (2008) reported that *Salmonella* populations decreased only 0.15 to 0.65 log and 0.34 to 1.29 log in five commercial peanut butters over a 14 day incubation period at 4

and 22°C, respectively. While microbial populations decreased during storage, prolonged storage is insufficient to ensure product safety.

In addition, *Salmonella* is able to survive in large particulates (e.g., almonds) and food powders (Keller, VanDoren, Grasso, & Halik, 2013; Kimber, Kaur, Wang, Danyluk, & Harris, 2012; Uesugi, Danyluk, & Harris, 2006) Uesugi et al. (2006) studied the ability of *Salmonella* to survive on raw almond surfaces during prolonged storage. They reported no significant reductions during 550 days of storage at -20°C and 4°C. Kimber et al. (2012) confirmed these results when they reported no reduction in populations of *Salmonella* on almonds stored at -19°C or 4°C. Keller et al. (2013) reported *Salmonella* survival in black pepper during up to 8 months of typical storage conditions. Obviously, extended storage alone is insufficient to achieve reduced risk of *Salmonella* survival; a more direct intervention method is needed.

2.2.4 Desiccation stress mechanisms

Relatively little is known about physiological responses to desiccation stress in *Salmonella*, but Gruzdev et al. (2011) reported that there is significant overlap with other stress response networks, meaning desiccation stress may activate the thermal stress network. However, the cellular mechanisms behind the response of *Salmonella* to desiccation stress are not well known. Based on other stress network responses, Spector and Kenyon (2012) conjectured possible extracellular and intracellular defenses against desiccation stress, but until further studies are completed, these are merely hypotheses.

2.3 Increased Thermal Resistance at Low Water Activity

Low a_w is a barrier to growth for many pathogens, including *Salmonella* (GMA, 2009a). In addition to surviving desiccation stress, *Salmonella* exhibits significantly enhanced thermal resistance at low a_w (Archer, Jervis, Bird, & Gaze, 1998; Beuchat & Scouten, 2002; Doyle & Mazzotta, 2000). Goepfert et al. (1970) reported increased heat resistance as media a_w decreased. This study was one of the first to make the connection between a_w and heat resistance of *Salmonella*, but the a_w range examined (0.87 – 0.99) did not include a_w values corresponding to low-moisture foods. Also, the experiments were conducted in a broth culture, which is a different environment than would be found in a typical low-moisture food.

Other studies have been performed in systems more closely resembling those found in low-moisture foods (Archer, et al., 1998; Farakos, Hicks, & Frank, 2014; Jeong, Marks, & Orta-Ramirez, 2009; Ma et al., 2009; Villa-Rojas et al., 2013). Villa-Rojas et al. (2013) studied the effect of a_w on the thermal inactivation of *Salmonella* in almond kernel flour. Their research showed that increasing product a_w decreased *Salmonella* thermal resistance; however, their study included a fairly high a_w range (0.601 – 0.946), which included samples above the low-moisture range. Also, for each a_w, the ranges of temperatures tested were different, making it difficult to directly compare the results.

Salmonella thermal resistance in low-moisture powders is still relatively understudied. Vancauwenberge et al. (1981) inoculated eight *Salmonella* serotypes onto corn flour containing 10 to 15% moisture and reported longer thermal decimal reduction times (D-values) at lower moisture contents. Farakos et al. (2014) studied the effect of varying NaCl concentrations on the survival kinetics of *Salmonella* in low a_w (0.23 – 0.58) whey protein powder during heating. Their findings showed that decreasing product a_w increased thermal resistance. Archer et al.

(1998) studied the effect of initial sample a_w on the thermal decimal reduction times of *Salmonella* in wheat flour. D values increased as the a_w of the flour decreased over the range of 0.20 to 0.60. However, because the samples were heated in containers with loose lids in a dry oven, sample a_w would have changed during heating, but that information was not reported. The relatively low temperatures (57 to 75°C) studied generated very large thermal D values (~800 min), so there remains a need for additional data at higher temperatures related to many typical commercial processes.

2.4 Water Activity Equilibration and Control

When studying a_w effects on thermal resistance, prior studies commonly held samples for some period after inoculation (Archer, et al., 1998; Farakos, Hicks, et al., 2014; Jeong, Marks, Ryser, & Harte, 2012); however, this practice is not standardized and varies widely. Given that equilibration and a_w control during the thermal treatment of samples likely impacts the results of such studies, then controlling, measuring, and reporting sample a_w is critically important to the interpretation and utility of the resulting inactivation data and models.

Villa-Rojas et al. (2013) did not equilibrate their samples between inoculation and their thermal treatment; however, sample moisture content was assumed constant, because samples were held in sealed containers during heating. By not equilibrating samples, the true effect of a_w on *Salmonella* thermal resistance in low-moisture might not be sufficiently understood.

Archer et al. (1998) inoculated flour samples and then held them in a sealed container at 37°C for 2-3 h, along with silica gel and a saturated solution of lithium chloride, to generate samples with varying a_w. They then applied their thermal treatments and grouped data based on

sample a_w. In other words, sample a_w was variable and measured, but not controlled to specific, repeatable target levels.

Farakos et al. (2014) allowed protein powder samples to equilibrate to target a_w values in vacuum desiccators before inoculation; however, they did not report the equilibration period or allow an equilibration period after inoculation. They did maintain constant product a_w during thermal treatments by vacuum packaging samples before thermal treatment.

Jeong et al. (2012) allowed almond samples a much longer (6-7 days) equilibration period. Samples were placed in a humidity-controlled chamber during this period so that all samples would have uniform a_w. In order to fully understand the effect of a_w on *Salmonella* resistance to lethal treatments in low-moisture foods, it is necessary to equilibrate samples before treatments and control a_w during the treatment. Additionally, no prior study is known to have reported the effect of equilibration time, or rate of moisture change, on the thermal resistance of *Salmonella* in low-moisture foods.

2.5 Modeling Microbial Inactivation in Low-Moisture Foods

2.5.1 Primary models

Modeling the inactivation of *Salmonella* in low-moisture foods is one tool used to predict the outcomes of thermal processes. Models are an excellent way to optimize existing process parameters, or suggest new ones, and they support risk management options (McKellar & Lu, 2004).

It is well known that microbial inactivation or survival can be modeled using primary models based on first-order kinetics (Peleg, 2006). First-order kinetics are typically used when survival data appear to be log-linear, a isothermal lethal temperature is used, and the number of

surviving colonies decreases as a function of time. The following equation is used to represent first-order kinetics, or the log-linear model:

$$\log\left({}^{N(t)}/_{N_0}\right) = -{}^{t}/_{D} \tag{1}$$

where $N(t)$ and N_0 are the populations (CFU/g) at times t and 0, respectively; t is the time of the isothermal treatment (min); and D is the time (min) required to reduce the microbial population by 90% at a specified temperature (°C) (Peleg, 2006). When the logarithm of the D value is plotted against the corresponding temperatures, the z value, which is the temperature difference needed to decrease (or increase) the D value by a factor of 10, is the negative inverse of the slope (McKellar & Lu, 2004; Peleg, 2006), which can be calculated as follows:

$$\log D = \log D_{ref} - \frac{T - T_{ref}}{z} \tag{2}$$

However, survival curves are not always log-linear. One approach when this occurs is to use alternative models for the distributions of thermal resistance. The Weibull model is the most widely used and follows the form:

$$\log\left({}^{N(t)}/_{N_0}\right) = -\left({}^{t}/_{\delta}\right)^{n} \tag{3}$$

where $N(t)$ and N_0 are the populations (CFU/g) at times t and 0, respectively; t is the time of the isothermal treatment (min); δ refers to the overall steepness of the survival curve (min); and n describes the general shape of the curve and whether it is linear ($n = 1$) or nonlinear ($n \neq 1$) and has an upward concavity ($n < 1$) or a downward concavity ($n > 1$) (Peleg, 2006).

Other primary models have been utilized (Baranyi & Roberts, 1994; Cerf, 1977; Geeraerd, Herremans, & Van Impe, 2000), but none are as widely used as the log-linear model

and the Weibull model. Regardless of the model choice, primary models alone do not account for environmental (product and process) factors, such as product a_w.

2.5.2 Secondary models

Secondary models incorporate the response of one or more parameters of a primary model to changes in temperature (T), pH, a_w, or other factors (Valdramidis et al., 2006). Models can be built in many ways, but, in theory, the strongest are mathematical expressions built from theoretical bases (McKellar & Lu, 2004). These models can then be extrapolated and adapted to a wide variety of processes, assuming the theory is solid.

There are many approaches to secondary modeling. Common examples include response surface-type, Arrhenius-type, and Bigelow-type models (Valdramidis, et al., 2006). The response surface secondary model is a polynomial model with interaction terms, such as:

$$D(T, a_w) = \beta_0 + \beta_1 \cdot T + \beta_2 \cdot T^2 + \beta_3 \cdot a_w + \beta_4 \cdot {a_w}^2 + \beta_5 \cdot T \cdot a_w \tag{4}$$

where the significance of the linear model parameters (β_0 ... β_5) are tested by a partial F-test (Neter, Kutner, Wasserman, & Nachtsheim, 1996).

An Arrhenius-type model structure for the effect of a_w on thermal inactivation is based on the model of Cerf et al. (1996), in which the effect of a_w, T, and pH on the inactivation rate of *Escherichia coli* was studied. Because pH will not be considered in the present study, that model can be reduced to the following:

$$\ln k(T, a_w) = C_0 + \frac{C_1}{T} + C_2 \cdot {a_w}^2 \tag{5}$$

where k is the first-order reaction rate ($k = \ln 10/D$), and C_0, C_1, and C_2 are regression parameters.

The final common secondary model incorporating a_w is a Bigelow-type structure. This structure is based on the model structure of Gaillard et al. (1998) for describing the combined effects of temperature, pH (omitted here), and a_w on the thermal resistance of *Bacillus cereus* spores, as follows:

$$\log D(T, a_w) = \log D_{ref} - \left(\frac{T - T_{ref}}{z_T}\right) - \left(\frac{a_w - 1}{z_{a_w}}\right) \quad (6)$$

where D_{ref} is the time (min) needed to achieve a 1 log reduction in the population at $T = T_{ref}$ and $a_w = 1$; T is the temperature (°C); T_{ref} is the optimized reference temperature (°C); z_{a_w} is the a_w increment needed to change the D-value by 10-fold at T_{ref}; and z_T is the temperature increment (°C) needed to change the D-value by 10-fold at the reference a_w (which is set at 1.0 in the above equation).

2.5.3 Existing models for water activity effects

As noted above, several secondary models have been reported in studies attempting to incorporate a_w into thermal inactivation models (Cerf, et al., 1996; Farakos, Frank, & Schaffner, 2013; Mattick et al., 2001; Neter, et al., 1996; Valdramidis, et al., 2006; Villa-Rojas, et al., 2013). Such secondary models tend to be specific to the processing parameters described in the study, which can make it difficult to extrapolate or extend that model to different processing conditions. In addition, very few published models are valid for the range of water activities typically present in low-moisture foods. For example, Cerf et al. (1996) used a modified Arrhenius equation to incorporate a_w into the model, but only at levels above 0.95.

Mattick et al. (2001) was one of the first to address the influence of a_w on inactivation of *Salmonella* at lower a_w (0.65 to 0.90) in broths. They reported that a Weibull model with a polynomial secondary model best fit their data generated from broth solutions. However, when

they compared the times their model generated with data from actual food products, the model failed to extrapolate accurately, which suggests that product composition and/or structure is an important consideration when modeling bacterial inactivation in low-moisture foods.

Jeong et al. (2009) modified a traditional thermal inactivation model to account for process conditions in moist-air convection non-isothermal heating of almonds. This was accomplished by adding a term to simulate "surface wetness" in a traditional Bigelow-type model based on D values. The model was successful, but did not directly account for product a_w.

Studies by Valdramidis et al. (2006), Villa-Rojas et al. (2013), and Farakos et al. (2013) specifically addressed a_w influence on inactivation, and used environments more typical to low-moisture foods. Valdramidis et al. (2006) examined three model structures that incorporated temperature and water activity into the secondary model – response surface, Arrhenius, and Bigelow-type models using data from potato samples (a_w = 0.71 – 0.99). The Bigelow-type model fit their data better (R^2 = 0.998) than the polynomial and Arrhenius models (R^2 = 0.935 and R^2 = 0.955, respectively).

Villa-Rojas et al. (2013) examined two primary models and two secondary models – a polynomial relationship and a Bigelow-type relationship, based on data from ground almonds (a_w = 0.601 – 0.946). The Weibull model, with a polynomial secondary model, best fit their data, which was selected based on the coefficient of determination, or R^2 value.

Farakos et al. (2013) examined a_w influence on inactivation of *Salmonella* based on experiments from whey powder (a_w = 0.19 – 0.54). They developed a polynomial model that best fit their data; however, that was the only secondary model studied. They performed a robust validation procedure with previously published inactivation data from different food powders (wheat flour, low-fat peanut meal, non-fat dry milk, whey protein, and low-fat cocoa power).

The reported secondary model's predicted values had a high correlation (R = 0.94) to the observed values from inactivation studies, which supported the validity of their model.

2.5.4 Model selection and validation

The effectiveness of models can be evaluated in a variety of ways. Probably the most common method utilized is the coefficient of determination (R^2):

$$R^2 = \frac{\sum(\log N_{model} - \overline{\log N_{data}})^2}{\sum(\log N_{model} - \overline{\log N_{data}})^2 + \sum(\log N_{model} - \log N_{data})^2} \quad (7)$$

which indicates how well a model fits the observed data points, but which is insufficient as a sole measure for model selection, especially for nonlinear models, because it may overestimate the success of the model (McKellar & Lu, 2004).

In order to better analyze and compare model quality and utility, the root mean squared error (RMSE) and the Corrected Aikake Information Criterion (AIC_c) are used because they provide additional and important information (Motulsky & Christopoulos, 2004). Models can be examined based on their RMSE, which quantifies accuracy, or goodness-of-fit, of the model predictions:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}\left(N_{data,i} - N_{model,i}\right)^2}{n-p}} \quad (8)$$

where N_{data} is the measured log reduction (log CFU/g); $N_{model}$is the predicted log reduction from the model (log CFU/g); n is the total number of observations, and p is the number of model parameters.

Additionally, the AIC_c can be calculated, as shown (Motulsky & Christopoulos, 2004):

$$AIC_c = n\ln\left(\frac{SS}{n}\right) + 2K + \frac{2K(K+1)}{n-K-1} \quad (9)$$

where, *n* is the total number of data points, *SS* is the sum of squares of the residuals, and *K* is the number of parameters being estimated plus 1. AIC_c evaluates whether the decrease in the sums of squares of the residuals justifies the addition of parameters to a model. Lower AIC_c values indicate the more likely correct model for the data (Motulsky & Christopoulos, 2004).

Model validation is rarely reported, but it is critically important to determine whether a model is valid outside of the data used to estimate the model parameters. One approach to validating a model is to use only 2/3 of collected data, selected randomly, to "train" the model, or estimate the parameters. The remaining 1/3 of data is then used to "test" the model to quantitatively analyze the efficacy of the model.

A more common approach is to generate truly independent data from a different product and/or laboratory to quantify the effectiveness of a model. Farakos et al. (2014) validated secondary models by testing a model developed earlier (Farakos, et al., 2013) with *Salmonella* survival data previously reported for whole wheat flour, peanut meal, dry milk, whey protein, and cocoa powder. Residuals were obtained by subtracting the predicted value from the observed value. The percentage of residuals within the acceptable residual zone (arbitrarily set as -1 log to 0.5 log) was used to evaluate of the model. However, that study only evaluated and validated one secondary model, which was a very basic response surface model. They did not evaluate multiple secondary models to determine which would better predict the effect of a_w on microbial inactivation.

2.6 Conclusions

Some key knowledge gaps exist concerning the effect of a_w on *Salmonella* thermal resistance, and the objectives addressed in this work are aimed at those better understanding the

effect of moisture and moisture change rate. Better understanding of the effect of a_w status and "history" will assist with future process validations. It will additionally assist in evaluating secondary models to account for product a_w in low-moisture foods. There are existing models; however, no prior studies have systematically compared secondary models used to incorporate the effect of a_w and temperature, and selected the most likely correct model to be used for validations across many processing platforms and product types.

EFFECT OF RAPID PRODUCT DESICCATION OR HYDRATION ON THERMAL RESISTANCE OF *SALMONELLA* ENTERITIDIS PT30 IN WHEAT FLOUR

3.1 Objective

The objective of this study was to evaluate the effect of rapid product desiccation or hydration on the thermal resistance of *Salmonella* Enteritidis PT 30 in wheat flour. Experiments were designed to specifically evaluate the impact of rapid desiccation or hydration on *Salmonella* thermal resistance by equilibrating samples after inoculation and controlling water activity, a_w, during thermal treatments.

3.2 Materials and Methods

3.2.1 Wheat flour

Soft winter wheat organic white flour was obtained in a single batch from Eden Foods (Clinton Township, MI). Initial a_w was measured via an a_w meter (Model 3TE, Decagon Devices, Pullman, WA). Average particle size was determined by performing a particle distribution study, in which Taylor series sieves (#30 through #200) were stacked initially with 100 g of wheat flour. The stack was shaken for 30 min with a motorized sieve shaker (H-4325, Humboldt Manufacturing, Elgin, IL). The weights of flour remaining in each sieve after the shaking period were then used to calculate the geometric mean particle diameter of the wheat flour.

Negligible *Salmonella* population in the source wheat flour was confirmed by collecting ten subsamples from the source flour, diluting 10:1 with 0.1% peptone water (BD, Franklin Lakes, NJ), and plating serial dilutions onto tryptic soy agar (BD) supplemented with 0.6% (w/v) yeast extract (BD) (TSAYE) supplemented with ammonium ferric citrate (0.05%) (Sigma Aldrich, St. Louis, MO) and sodium thiosulfate (0.03%) (Sigma Aldrich) (modified TSAYE),

which differentiated *Salmonella* from other contaminating microorganisms by the characteristic black precipitate in the center of the colonies. The plates were incubated at 37°C for 48 h. No colonies detected indicated that if *Salmonella* were present in the source wheat flour, the counts were too low influence the project results and analysis.

3.2.2 Bacterial strain and inoculation

Salmonella enterica servovar Enteritidis phage type 30 (SE PT30) previously was obtained from Dr. Linda Harris (University of California, Davis), and was used in this study because it previously was shown to be thermally resistant in several low a_w materials (Anderson et al., 2013; Jeong, et al., 2012; Villa-Rojas, et al., 2013). The culture was maintained at -80°C in tryptic soy broth (BD) supplemented with 20% (vol/vol) glycerol.

Before use, the culture was subjected to two consecutive transfers (24 h each at 37°C) in tryptic soy broth (BD) supplemented with 0.6% (w/v) yeast extract (BD) (TSBYE) and then streaked to plates (150 by 15 mm) of TSAYE to obtain uniform lawns for each of the four groups. After 24 h of incubation at 37°C, the bacterial lawn was harvested in 20 ml of sterile 0.1% peptone water (BD) per plate. The suspension was centrifuged for 15 min at 3,795 × *g*. The supernatant was discarded, and the remaining pellet was hand mixed into wheat flour (150 g per group) in a sterile plastic bag until pellet was visibly mixed. After hand mixing, the inoculated wheat flour was stomached (Masticator Basic, Neu-Tec Group Inc., Farmingdale, NY) for 3 min to ensure an even distribution. Uniformity of the inoculum distribution was confirmed by plating six subsamples (~1 g) from a 150 g sample after mixing. Target inoculation level was 10^8 CFU/g.

Because of the risk of small, *Salmonella*-inoculated flour particles becoming airborne during sample inoculation and preparation, increased safety protocols were necessary. Lab coats, gloves, safety goggles, and face masks were all required personal protective equipment used over the duration of this experiment. Additionally, sample desiccation and hydration treatments (described below) were conducted in a biosafety cabinet.

3.2.3 Sample preparation.

The inoculated wheat flour (150 g per group) was transferred to an a_w conditioning system to adjust the samples to the target a_w (Appendix D). 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 (AM2303, Aosong Electronics Co., Ltd, Guangzhou, China) inside the equilibration chamber, a desiccation column (filled with silica gel), a hydration column (filled with water), solenoid valves, air pumps (Fusion Air Pump 400, JW Pet, Arlington, TX), and a computer-based control system that monitored and controlled the chamber relative humidity within ~2-5%. Samples were conditioned for 4-6 days to allow the entire sample to equilibrate to the target a_w, which was confirmed via the a_w meter.

The overall treatment design consisted of four groups of samples. Groups were named using the initially equilibrated a_w and the a_w at the time of the thermal treatment. For example, group I60/F60 was initially equilibrated to 0.60 a_w, which was not altered prior to the thermal treatment, and group I60/F30 was initially equilibrated to 0.60 a_w, but was rapidly desiccated (described below) to a final 0.30 a_w immediately before thermal treatment. Groups I60/F60, I30/F30, and I60/F30 were completed from the same inoculation. Group I30/F60 was run at a

later date (from the same original batch of flour), but one additional replication of group I60/F60 was run alongside to confirm statistical equivalency to previously run replications.

3.2.4 Rapid desiccation treatment.

After initial equilibration to 0.60 a_w, rapidly desiccated samples (I60/F30) were created using a custom-built fluidized-bed drying system (Figure 1) (Appendix D). All treatments were performed in a biosafety cabinet. An air pump (Gast, 1/8 hp, Benton Harbor, MI) drew ambient air (~20°C) through two sequential desiccation columns (L = 38.1 cm; D = 3.81 cm) containing silica gel desiccant (Dry Pak, Encino, CA). Filters with 83.8 micron opening (180 x 180 woven stainless steel mesh, McMaster-Carr, Elmhurst, IL) were installed immediately before and after the sample chamber. Additionally, a 5 µm line filter was installed immediately after the sample chamber, followed by a high-efficiency particulate absorption (HEPA) filter (Filtrete 64703B, 3M, St. Paul, MN) to further prevent the release of *Salmonella* with the exit air. Desiccated air (~20% relative humidity) was pumped through the sample chamber (3.0 m/s, measured with a hot-wire anemometer (Model 407123, Extech, Waltham, MA)) to create a fluidized-bed drying system. The system was calibrated to rapidly desiccate flour samples (~1 g) from nominal a_w values of 0.60 to 0.30 in ≤ 4 min, confirmed by desiccating six samples and measuring the final a_w.

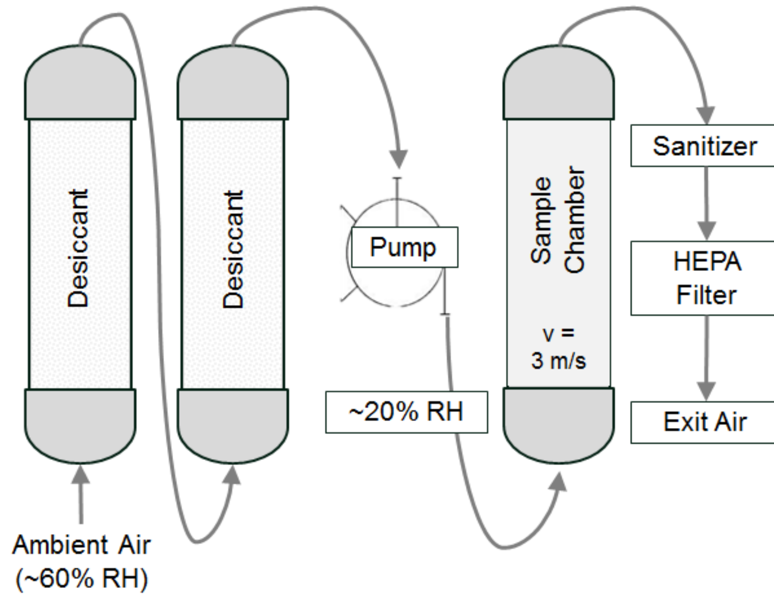

Figure 1. Schematic of the custom-build fluidized-bed drying system for rapid desiccation

The filtration system was tested for efficacy to ensure that it was working as designed in terms of bio-containment. Six plates of modified (differential) TSAYE were placed in the biosafety hood, and six inoculated samples were run through the system. After the six samples were completed, the six plates were incubated at 37°C for 48 h. No *Salmonella* growth on the plates confirmed the efficacy of bio-containment for the desiccation/hydration system.

3.2.5 Additional hold time experiment.

In order to further evaluate the relatively high variability observed in lethality after the rapid desiccation treatment (described later), the desiccation treatment was repeated as previously described (three replicates), but with one modification. Instead of immediately performing the thermal treatments after desiccation, a 1 h hold time was added between the desiccation treatment and the thermal treatment to evaluate the impact of the additional time on thermal resistance and repeatability (I60/F30/1H).

3.2.6 Rapid hydration treatment.

All samples were treated in a custom-built fluidized-bed hydration system, similar to the desiccation system described above (Figure 2) (Appendix D). All treatments were performed in a biosafety cabinet. Ambient air was pumped (Gast, 1/8 hp, Benton Harbor, MI) through a 7.5 liter glass container filled 2/3 with water. Airstones (porous stone, Petco, San Diego, CA) dispersed the air through the water, thus increasing the humidity of the air, measured to be ~80% relative humidity. The humidified air was pumped (4.5 m/s) through the sample chamber to create a fluidized bed hydration system. The same filtration system was used as in the desiccation treatments to prevent release of *Salmonella* in the exit air. The system was calibrated to rapidly hydrate flour samples (~1 g) from nominal a_w values of 0.30 to 0.60 in 2.5 min, confirmed by hydrating six samples and measuring the final a_w.

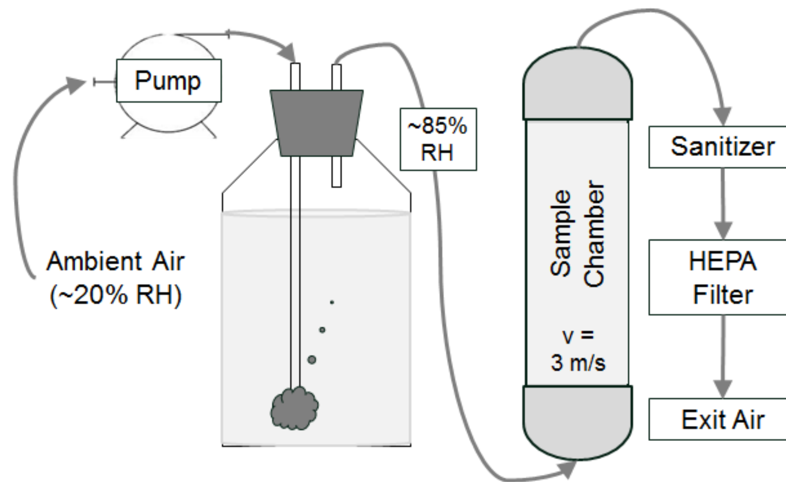

Figure 2. Schematic of the custom-build fluidized-bed hydrating system for rapid hydration

3.2.7 Thermal treatment.

To obtain thermal death curves for *Salmonella,* the moisture-equilibrated wheat flour samples were immediately (within ~1 min of the desiccation or hydration treatments) subjected

to an isothermal (80°C) inactivation treatment. Aluminum test cells (Chung, Birla, & Tang, 2008) were filled with inoculated and equilibrated flour (0.5 to 0.8 g, 4 mm thick) and immersed in a water bath (Neslab GP-400, Newington, NH) at 80°C (Appendix D). Come-up time was verified using a non-inoculated sample inside a test cell with a K-type thermocouple (20 gauge, Omega, Stamford, CT) permanently fixed at the center of the sample in the test cell. The come-up time (~68 s) for the sample core to reach within 0.5°C of 80°C was used as time zero for the isothermal treatment. Samples subsequently were removed at equally spaced time intervals, starting with the time zero samples. Once removed from the water bath, the test cells were immediately placed in an ice-water bath to stop the thermal inactivation (T < 40°C in ~20 s).

3.2.8 Recovery and enumeration.

To enumerate *Salmonella* survivors, thermally treated wheat flour samples were removed from the test cells into sterile plastic bags and diluted 10:1 with 0.1% peptone water (BD). Appropriate serial dilutions were then plated (0.1 mL) in duplicate onto modified TSAYE. The plates were incubated at 37°C for 48 h. *Salmonella* counts were converted to log CFU/g. Log reductions were calculated by subtracting survivor counts (log) from the populations (log) at time zero for the respective replicate, which was measured after the come-up time. The limit of detection was 100 CFU/g.

3.2.9 Statistical analysis.

Each treatment group was replicated three times with duplicate samples in each replicate treatment. Linear regression of the log N/N_0 vs time data was conducted, and the resulting slopes (-1/D) of the data were compared via analyses of variance (ANOVA), with α = 0.05.

3.3 Results and Discussion

3.3.1 Wheat flour

Initial a_w was 0.462, and geometric mean particle size was 144 ± 60 µm. No *Salmonella* colonies were detected in the source flour after diluting and plating on modified TSAYE.

3.3.2 Initial inoculation.

The average initial *Salmonella* population in the inoculated samples across all tests was 8.20 ± 0.19 log CFU/g. The standard deviation of the inoculation across six subsamples (~1 g) randomly pulled from a single inoculated sample (150 g) was 0.17 log CFU/g, which confirmed the uniformity of inoculum mixing into the samples (Appendix A). The average population after the thermal come-up time was 6.88 ± 0.77 log CFU/g.

3.3.3 Product water activity

The a_w of samples in treatment groups I60/F60 and I30/F30 were measured on the day of the thermal treatment. If the sample was within ± 0.05 of the target water activity, the sample was treated. Any flour samples with a_w outside of this tolerance were not used. On the day of thermal treatment, I60/F60 a_w was measured (n = 3) to be 0.583 ± 0.003, and I30/F30 a_w was measured (n = 3) to be 0.306 ± 0.004.

In preliminary tests, the uniformity and consistency of the desiccation and hydration treatments were confirmed before running any inoculated samples, by testing the a_w of six different samples subjected to each of the treatments (Appendix E). These samples were not used for inactivation data, but rather to confirm the repeatability of the desiccation/hydration system. For I60/F30, samples began at 0.648 ± 0.004 a_w and after the desiccation treatment were 0.317 ±

0.012 a_w. For I30/F60, samples began at 0.306 ± 0.002 a_w and after the hydration treatment were 0.602 ± 0.018 a_w. The means of the sample a_w at the time of thermal treatment for groups I60/F60 and I30/F60 (0.583 ± 0.003 and 0.602 ± 0.018, respectively) were not significantly different (as shown by a t-test), nor were the means of the a_w at the time of thermal treatment for groups I30/F30 and I60/F30 (0.306 ± 0.004 and 0.317 ± 0.012, respectively). Sample a_w within ± 0.05 of the target was assumed to have a negligible effect on the thermal resistance (given the final results described below), so that $D_{aw=0.306}$ would be expected to be $\approx D_{aw=0.317}$, and $D_{aw=0.583}$ would be expected to be $\approx D_{aw=0.602}$.

3.3.4 Thermal inactivation and $D_{80°C}$ values

Survivor curves (Figure 3) were calculated using all of the data from the three replicates for each sample (although the figure shows only the means of the three replicates).

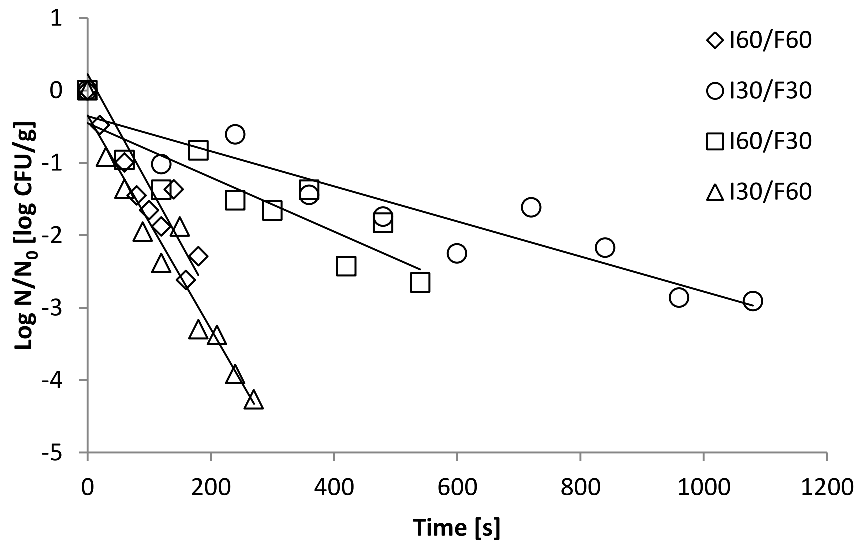

Figure 3. Survivor curves for *Salmonella* Enteritidis PT 30 inoculated into wheat flour during isothermal heating at 80°C. The means of the three replicates for each treatment are shown.

$D_{80°C}$ values were calculated by taking the inverse of the slopes of each replicate, which were generated from the thermal inactivation treatments of the four groups, then averaging the slopes of the three replicates of one treatment to generate one $D_{80°C}$ value per treatment ± standard deviation, (Table 1) (raw data in Appendix B). The slopes (-1/D) for treatments I60/F60 and I30/F60 were statistically equivalent ($P > 0.05$), and the slopes for treatments I30/F30 and I60/F30 were statistically equivalent ($P > 0.05$); however, the $D_{80°C}$ value for I60/F30 was approximately five times larger than the $D_{80°C}$ value for I30/F60 (Appendix C). The common factor within the groups that were not significantly different from each other was a_w at the time of thermal treatment, regardless of the initially equilibrated a_w prior to desiccation or hydration. This result suggests that the a_w state at the time of treatment controls the thermal inactivation of *Salmonella*, regardless of the moisture "history" prior to thermal treatment. In prior work on the heat resistance of *Salmonella* Enteritidis PT30 in almond kernel flour, Villa-Rojas et al. (2013) reported a $D_{80°C}$ value of 1.63 min for 0.601 a_w, which is very similar to the value found in this study. However, they did not examine a_w values below 0.60.

Table 1. Decimal reduction values ($D_{80°C}$) for all treatments based on linear regression of the survivor curves (Figure 3) from the thermal inactivation treatments.

Treatment Group	**$D_{80°C}$ value [min] (mean ± SD)[a]**	**R^2**
I60/F60	1.33 ± 0.15 [A]	0.496
I30/F30	7.32 ± 2.07 [B]	0.719
I60/F30	5.73 ± 3.10 [B]	0.405
I30/F60	1.15 ± 0.09 [A]	0.726
I60/F30/1H	5.98 ± 2.34 [B]	0.568

[a] Different letters within the column indicate that means are significantly different ($P < 0.05$).

Figure 4 shows the treatment groups with stationary water activities (I60/F60 and I30/F30) compared to the treatment groups that were initially equilibrated to the same a_w. Group

I60/F60 and I60/F30 (Fig 4a) both were initially equilibrated to 0.6 a_w and are significantly different ($P < 0.05$). Group I30/F30 and I30/F60, which were initially equilibrated to 0.3 a_w, were significantly different ($P < 0.05$). Figures 4a and 4b show the significantly different slopes between the groups. These data suggest that the treatment had a significant effect on the thermal resistance of *Salmonella*.

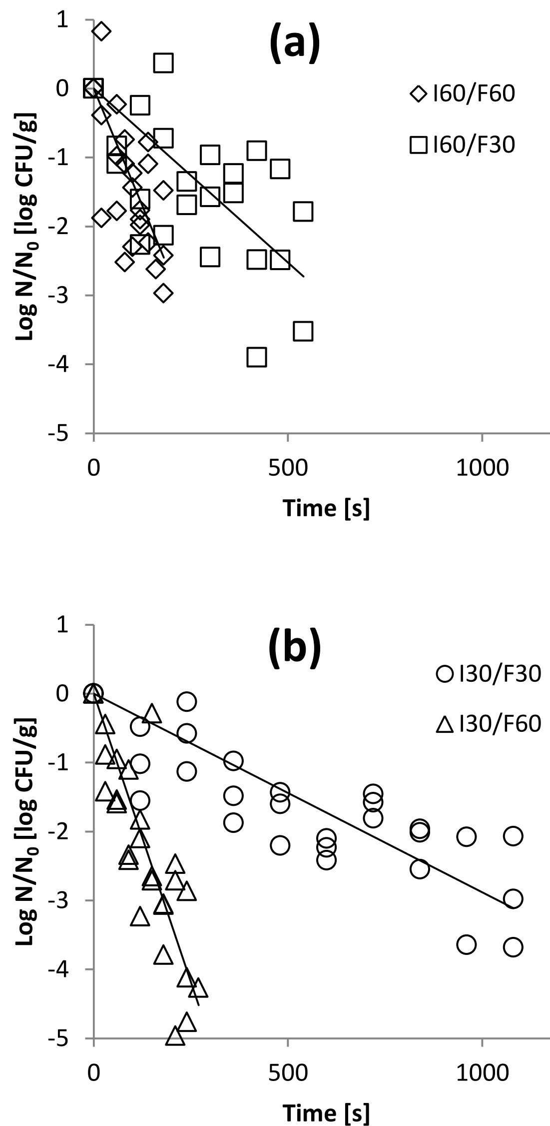

Figure 4. Thermal inactivation kinetics (80°C) of Salmonella Enteritidis PT 30 inoculated in wheat flour, comparing groups that initially were equilibrated to: (a) 0.60 aw (I60) or (b) 0.30 aw (I30).

Figure 5 shows the treatment groups with stationary water activities compared to the treatment groups with similar water activities at the time of thermal treatment. Group I60/F60 and I30/F60 (Fig 5a) were not significantly different ($P > 0.05$). Similarly, Group I30/F30 and I60/F30 (Fig 5b) were not significantly different ($P > 0.05$). These results also can be seen visually as their slopes are very close together. They also suggest that a_w "history" does not affect *Salmonella* thermal resistance, which is controlled by the a_w at the time of thermal treatment. It also suggests that the time required for *Salmonella* thermal resistance to respond to new water activities is effectively negligible.

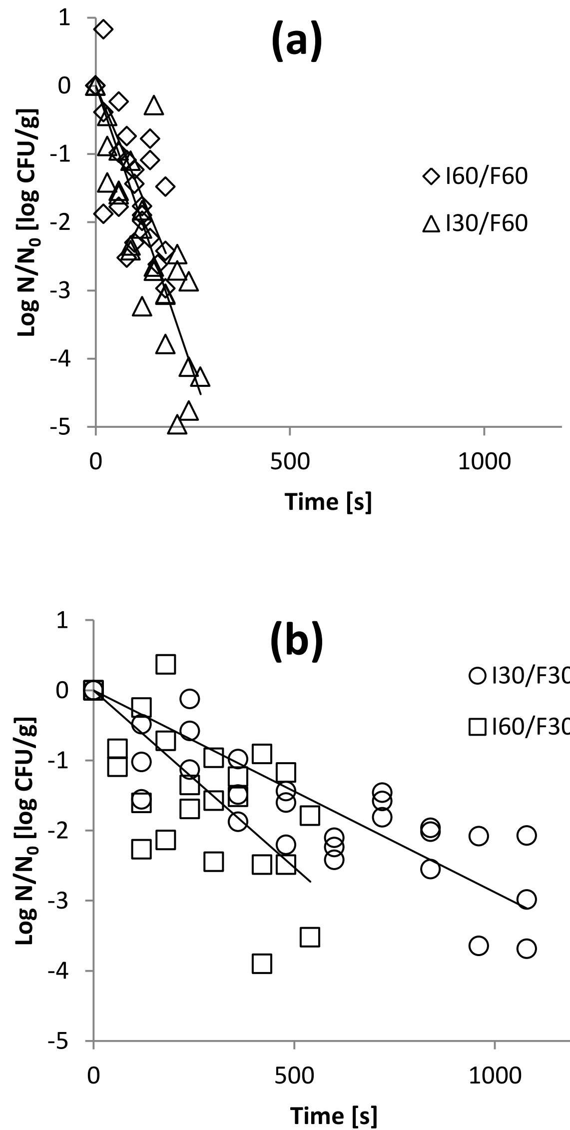

Figure 5. Thermal inactivation kinetics (80°C) of *Salmonella* Enteritidis PT 30 inoculated in wheat flour, comparing groups with: (a) 0.60 a_w (F60) or (b) 0.30 a_w (F30) at the time of isothermal heat treatment.

3.3.4 Variability of rapidly desiccated group

Some of the treatment groups had relatively high variability among replicates in terms of log reductions, with treatment I60/F30 (rapid desiccation) showing the highest variability. Little is known about the physiological response to desiccation stress, but this variability could be partially due to cell components within *Salmonella* that protect against desiccation, and

population variability in this response depending on the individual cell (Spector & Kenyon, 2012). However, because the focus of this study was to quantify the effects of rapid a_w change, the specific cellular mechanisms involved were not examined.

As described in the methods, an additional treatment with a 1 h hold time between the desiccation treatment and the thermal treatment was run to observe any impacts on the response period or variability in the results (raw data in Appendix F). No significant difference was observed in the slope between I60/F30 and I60/F30/1H, but the standard deviation within the replication decreased from 1.20 (immediate) to 0.45 (1 h wait). Therefore, variability among replicates was reduced by the 1 h hold, but the mean result was unchanged. This may suggest "equilibration" of the response across the population, but there was no net effect on the thermal resistance.

3.4 Conclusions

These results reinforce the premise that *Salmonella* thermal resistance increases with decreasing a_w at the time of thermal treatment, supporting findings from previous studies (Archer, et al., 1998; Janning, et al., 1994). However, prior to this study, the response period was not tested or reported, and the present study is the first known to demonstrate the effect of moisture change rate on that resistance. Knowledge regarding the effect of both the extent and rate of material desiccation or hydration on *Salmonella* thermal resistance in low-moisture foods in critically important in validating pasteurization processes or any "kill step" in commercial processes. Future secondary inactivation models, and their application to process validation, clearly need to account for dynamic moisture during processing.

MODELING THE EFFECT OF TEMPERATURE AND WATER ACTIVITY ON THE THERMAL RESISTANCE OF *SALMONELLA* ENTERITIDIS PT 30 IN WHEAT FLOUR

4.1 Objective

The objective of this study was to test multiple secondary models for the effect of product (wheat flour) water activity, a_w, on *Salmonella* thermal resistance. Two primary models and three secondary models were evaluated to determine which best described the inactivation of *Salmonella* in wheat flour during isothermal treatment.

4.2 Methods and Materials

4.2.1 Wheat flour

Soft winter wheat organic white flour was obtained in a single batch from Eden Foods (Clinton Township, MI). Initial a_w was measured via an a_w meter (Model 3TE, Decagon Devices, Pullman, WA). Average particle size was determined by performing a particle distribution study, in which Taylor series sieves (#30 through #200) were stacked initially with 100 g of wheat flour. The stack was shaken for 30 min with a motorized sieve shaker (H-4325, Humboldt Manufacturing, Elgin, IL). The weights of flour remaining in each sieve after the shaking period were then used to calculate the geometric mean of the wheat flour.

Negligible *Salmonella* population in the source wheat flour was confirmed by collecting ten subsamples from the source flour, diluting 10:1 with 0.1% peptone water (BD, Franklin Lakes, NJ), and plating serial dilutions onto tryptic soy agar (BD) supplemented with 0.6% (w/v) yeast extract (BD) (TSAYE) supplemented with ammonium ferric citrate (0.05%) (Sigma Aldrich, St. Louis, MO) and sodium thiosulfate (0.03%) (Sigma Aldrich) (modified TSAYE), which differentiated *Salmonella* from other contaminating microorganisms by the characteristic

black precipitate in the center of the colonies. The plates were incubated at 37°C for 48 h. No colonies detected indicated that if *Salmonella* were present in the source wheat flour, the counts were too low to affect the project results and analyses.

4.2.2 Bacterial strain and inoculation

Salmonella enterica servovar Enteritidis phage type 30 (SE PT30)previously was obtained from Dr. Linda Harris (University of California, Davis), and was used in this study because it previously was shown to be thermally resistant in several low a_w materials (Anderson, et al., 2013; Jeong, et al., 2012; Villa-Rojas, et al., 2013). The culture was maintained at -80°C in tryptic soy broth (BD) supplemented with 20% (vol/vol) glycerol.

Before use, the culture was subjected to two consecutive transfers (24 h each at 37°C) in tryptic soy broth (BD) supplemented with 0.6% (w/v) yeast extract (BD) (TSBYE) and then streaked to plates (150 by 15 mm) of TSAYE to obtain uniform lawns for each replication. After incubation (24 h, 37°C), the bacterial lawn was harvested in 20 ml of sterile 0.1% peptone water (BD) per plate. The suspension was centrifuged for 15 min at 2,988 × g. The supernatant was discarded, and the remaining pellet was hand mixed into wheat flour (25 g per replication) in a sterile plastic bag until the pellet was visibly mixed. After hand mixing, the inoculated wheat flour was stomached (Masticator Basic, Neu-Tec Group Inc., Farmingdale, NY) for 3 min to ensure an even distribution. Uniformity of the inoculum distribution was confirmed by plating six random subsamples (~1 g) from a 25 g sample after mixing. Target inoculation level was 10^9 CFU/g.

Because of the risk of small, *Salmonella*-inoculated flour particles becoming airborne during sample inoculation and preparation, increased safety protocols were necessary. Lab

coats, gloves, safety goggles, and face masks were all required personal protective equipment used for the duration of this experiment.

4.2.3 Sample preparation

The inoculated wheat flour (25 g per replication) was transferred to an a_w conditioning system to adjust the samples to target a_w levels (Appendix D). 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 (AM2303, Aosong Electronics Co., Ltd, Guangzhou, China) inside the equilibration chamber, a desiccation column (filled with silica gel), a hydration column (filled with water), solenoid valves, air pumps (Fusion Air Pump 400, JW Pet, Arlington, TX), and a computer-based control system that monitored and controlled the chamber relative humidity within ~2-5%. Prior to thermal treatment, samples were conditioned for 4-6 days at the target humidity (30, 45, or 60% relative humidity) to allow the entire sample to equilibrate to the target a_w, which was subsequently confirmed via the a_w meter for each target a_w.

4.2.4 Thermal treatment

A full factorial experiment was used with three constant inactivation temperatures (75, 80, and 85°C) and three constant water activities (nominally 0.30, 0.45, and 0.60), with all experiments conducted in triplicate.

To obtain thermal death curves for *Salmonella,* the moisture-equilibrated wheat flour samples were subjected to an isothermal inactivation treatment. Aluminum test cells (Chung, et al., 2008) were filled with inoculated and equilibrated flour (0.5 to 0.8 g, 4 mm thick) and immersed in a water bath (Neslab GP-400, Newington, NH) at 75, 80, or 85°C (Appendix D).

Come-up time was verified using a non-inoculated sample inside a test cell with a K-type thermocouple (20 gauge, Omega, Stamford, CT) permanently fixed at the geometric center of the flour sample in the test cell. The come-up time (64-70 s) for the sample core to reach within 0.5°C of the target temperature was used as time zero for the isothermal treatment. Samples were subsequently removed at uniform time intervals, starting with the time zero samples. Once removed from the water bath, the test cells were immediately placed in an ice-water bath to stop the thermal inactivation (T < 40°C in ~20 s).

4.2.5 Recovery and enumeration

To enumerate *Salmonella* survivors, thermally treated wheat flour samples were removed from the test cells into sterile plastic bags and diluted 10:1 with 0.1% peptone water (BD). Appropriate serial dilutions then were plated (0.1 mL) in duplicate onto modified TSAYE. The plates were incubated (37°C for 48 h), and *Salmonella* colonies were enumerated and populations converted to log CFU per gram. Log reductions were calculated by subtracting survivor counts (log) from the population (log) at time zero for the respective replicate, which was measured after the come-up time. The limit of detection was 100 CFU/g.

4.2.6 Primary models

The two most widely used primary models in bacterial inactivation are the first-order kinetic model and the Weibull-type model. The first-order kinetic, or log-linear, model (Peleg, 2006) was:

$$\log\left({}^{N}/_{N_0}\right) = -{}^{t}/_{D} \tag{10}$$

where N and N_0 are the populations (CFU/g) at times t and 0 respectively, t is the time of the isothermal treatment (min) after come-up, and D is the time (min) required to reduce the microbial population by 10-fold at a specified temperature (°C).

The Weibull model (Peleg, 2006) was:

$$\log\left({}^{N}/_{N_0}\right) = -\left({}^{t}/_{\delta}\right)^{n} \tag{11}$$

where δ refers to the overall steepness of the survival curve (min), and n describes the general shape of the curve and describes whether it is linear ($n = 1$) or nonlinear ($n \neq 1$) with a decreasing ($n < 1$) or increasing ($n > 1$) inactivation rate with time.

Parameters were estimated for each of the nine data sets separately using ordinary least squares (OLS) minimization in MATLAB (version 2014). The parameters were estimated for each of the nine data sets by performing OLS with nlinfit (nonlinear regression routine in the statistical toolbox) (Appendices H and I).

4.2.7 Model selection and evaluation

Models were fit to the data, and the goodness-of-fit for each candidate model was quantified by the root mean square error (RMSE) (Motulsky & Christopoulos, 2004):

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^{n}\left(N_{data,i} - N_{model,i}\right)^2}{n - p}} \tag{12}$$

where N_{data} is the measured log reduction (log CFU/g), N_{model} is the predicted log reduction from the model (log CFU/g), n is the total number of observations, and p is the number of model parameters.

Additionally, the Corrected Aikake Information Criterion (AIC_c) was calculated for each model (Motulsky & Christopoulos, 2004):

$$AIC_c = n \ln\left(\frac{SS}{n}\right) + 2K + \frac{2K(K+1)}{n-K-1} \quad (13)$$

where, *n* is the total number of data points, *SS* is the sum of squares of the residuals, and *K* is the number of parameters being estimated plus 1. AIC_c evaluates whether the decrease in the sum of squares of the residuals justifies the addition of parameters to a model. Lower AIC_c values indicate the model more likely to be correct for the data.

Other statistical indices used to compare the models were the relative error of each parameter, confidence intervals, correlations among the parameters, and the residuals.

4.2.8 Secondary models

Based on the AIC_c (results shown below), the log-linear model was the more likely correct primary model. Therefore, three secondary models were evaluated for the influence of a_w and temperature on D values in the log-linear model. The first secondary model was a second-order response surface model.

$$D(T, a_w) = \beta_0 + \beta_1 \cdot T + \beta_2 \cdot T^2 + \beta_3 \cdot a_w + \beta_4 \cdot {a_w}^2 + \beta_5 \cdot T \cdot a_w \quad (14)$$

The statistical significance of each linear model parameter (β_0 ... β_5) was tested by a partial F-test (Neter, et al., 1996; Valdramidis, et al., 2006).

The second secondary model evaluated was a modified version of a Bigelow-type relationship (Gaillard, et al., 1998).

$$\log D = \log D_{ref} - \frac{T - T_{ref}}{z_T} - \frac{a_w - 1}{z_{a_w}} \quad (15)$$

where D_{ref} is the time (min) needed to achieve a 1 log reduction in the population at T_{ref} and a_w = 1, *T* is the temperature (°C), T_{ref} is the optimized reference temperature (°C), z_{a_w} is the a_w

increment needed to change the D-value by 10-fold at the T_{ref}, and z_T is the temperature increment needed to change the D-value by 10-fold (°C) at the reference a_w.

The final secondary model was a combined-effects model that was built from assumptions based on preliminary data analyses. Preliminary data suggested that the effect of water activity on the D value is linear, while the effect of temperature is non-linear. With these observations, a modified model was created by adding a term to account for the linear effect of water activity to the Bigelow model (Bigelow & Esty, 1920), yielding the following model:

$$D(T, a_w) = \left[D_{ref} + R_{a_w}(0.60 - a_w)\right] \cdot 10^{\frac{T_{ref} - T}{z_T}} \quad (16)$$

where D_{ref} is the time (min) needed to achieve a 1 log reduction in the population at T_{ref} and $a_w =$ 0.60, which was chosen because it is the upper limit of the definition of low-moisture foods, R_{a_w} is the resistance added due to a change in a_w, T is the temperature (°C);, T_{ref} is the reference temperature (°C), and z_T is the temperature increment needed to reduce the D-value by 10-fold (°C).

Dolan et al. (2013) reported that the optimal reference temperature should be found for each data set in order to minimize the correlation between parameters. Additionally, relative error is minimized by minimizing the correlation coefficient. The optimal reference temperature was found for each secondary model by varying T_{ref} over a range of temperatures when estimating the parameters, and plotting the temperature range vs. the correlation coefficient. The temperature at which the correlation coefficient is ~0 is the model's optimal reference temperature.

All parameters for each secondary model were estimated globally using ordinary least squares (OLS) minimization with nlinfit (nonlinear regression routine in the statistical toolbox)

in MATLAB (version 2014) for the combined model of equation 10 with either equation 14, 15, or 16 (Appendix J).

4.3 Results and Discussion

4.3.1 Wheat flour

Initial a_w was 0.462, and geometric mean particle size was 144 ± 60 µm. No *Salmonella* was detected in the source flour.

4.3.2 Initial inoculation

The standard deviation of the inoculation across six subsamples (~1 g) randomly pulled from a single inoculated sample (25 g) was 0.17 log CFU/g, which confirmed the uniformity of the inoculum mixing into the samples (Appendix A). The mean initial *Salmonella* population ± std. deviation across all inoculated samples tests was 9.26 ± 0.25 log CFU/g. The mean *Salmonella* population ± std. deviation of all inoculated samples after the thermal come-up time was 8.75 ± 0.60 log CFU/g, which, within each replication, was treated as the time zero population. The difference between the initial inoculation population and the time zero population was significantly different ($P < 0.05$), which is why it was important to use the time zero population in the subsequent analyses of the isothermal inactivation curves.

4.3.3 Comparison of primary models

Both primary models, the log-linear model and Weibull model, were fit to each of the nine data sets (T, a_w) according to equations 10 and 11 (Table 2) (data shown in Appendix G). The Weibull model showed very slight tailing behavior; however, the log-linear model was the

more likely correct model for all of the cases, based on the lower AIC_c values. Villa-Rojas et al. (2013) reported that the Weibull model was the better predictive model for isothermal inactivation of *Salmonella* in ground almonds; however, that statement was based solely on the R^2 values.

For the log-linear model, Villa-Rojas et al. (2013) reported a $D_{80°C}$ value of 1.63 min for 0.601 a_w, which is very similar to the $D_{80°C}$ value of 1.27 min for 0.60 a_w found in this study. However, they did not examine a_w values below 0.60. Farakos et al. (2014) studied *Salmonella* in protein powders at lower a_w, but they did not report D values, as they utilized a Weibull model. However, their results indicated a time to 1 log reduction of 6.1 min for 0.58 a_w and 80°C, which is larger than the comparable value from this study at 0.6 a_w. The differences might be attributed to compositional differences and the fairly large standard errors in both studies.

Table 2. Parameter estimates (± standard error) for the primary models, as well as the root mean square error (RMSE), and the corrected Aikake Information Criterion (AIC_c) value. Parameters were estimated separately for each data set.

		Log-linear model			Weibull Model			
a_w	T [°C]	D value [min]	RMSE [log CFU/g]	AIC_c [a]	δ [min]	n	RMSE [log CFU/g]	AIC_c [a]
0.310	75	14.53 ± 0.68	0.3594	-47.65	13.37 ± 1.58	0.89 ± 0.13	0.3619	-45.76
± 0.005	80	10.27 ± 0.65	0.6090	-23.30	8.62 ± 1.88	0.83 ± 0.17	0.6106	-21.68
	85	5.05 ± 0.18	0.3563	-52.24	4.58 ± 0.53	0.90 ± 0.10	0.3573	-50.62
0.427	75	9.97 ± 0.46	0.8091	-7.96	8.08 ± 2.00	0.87 ± 0.13	0.8108	-6.36
± 0.003	80	5.51 ± 0.22	0.5431	-29.48	4.97 ± 0.84	0.92 ± 0.12	0.5497	-27.34
	85	2.11 ± 0.09	0.6652	-16.02	1.59 ± 0.34	0.83 ± 0.11	0.6451	-15.93
0.582	75	2.76 ± 0.19	0.9972	3.62	2.25 ± 0.74	0.88 ± 0.16	1.0107	5.89
± 0.003	80	1.27 ± 0.06	0.4305	-42.03	1.10 ± 0.16	0.86 ± 0.12	0.4296	-40.66
	85	0.65 ± 0.04	0.7035	-15.51	0.63 ± 0.13	0.97 ± 0.17	0.7170	-13.00

[a] The lower AIC_c value indicates the model more likely to be correct. Comparisons can only be made horizontally.

4.3.4 Estimation of parameters for secondary models

The parameters of each secondary model (equations 14-16) were estimated globally for all a_w and temperatures simultaneously (Table 3-5). The optimal reference temperatures were estimated for the modified Bigelow-type model and the combined-effects model per the method described in Dolan et al. (2013). However, to more easily compare the models, all parameters were estimated with the reference temperature set to 80°C, which was within 4°C of the optimal values, and was the midpoint of the test range.

The response surface model had a relatively high RMSE value (1.59 log CFU/g), and the relative errors for the parameters were extremely high, ranging from 0.71% to 183% (Table 3). In addition, the confidence intervals indicated that only three of the six parameters were significant. Further, no parameters containing temperature were significant, which indicates an inappropriate model, because temperature is known to affect *Salmonella* thermal resistance. There also were high correlations among the parameters (6 out of 15 greater than 0.95), and the residuals contained additive errors and were not normally distributed. These results indicate that the response surface model was not a good secondary model for this application.

The modified Bigelow-type had a lower RMSE (0.745 log CFU/g), and the parameter relative errors were much lower, ranging from 3.15 to 12.1% (Table 4). Examination of the confidence intervals showed that all parameters were significant; however, D_{ref} and z_{aw} were correlated, which could affect the ability to accurately estimate the parameters. The residuals did not contain additive errors and were normally distributed; however, the mean of the residuals was not zero (i.e., -0.14 log CFU/g).

The combined-effects model had the lowest RMSE (0.668), and the parameter relative errors were the lowest for all models, ranging from 2.93 to 3.05% (Table 5). Examination of the

confidence intervals showed that all parameters were significant. Further, none of the parameters were correlated, meaning the parameters could be accurately estimated. The residuals did not contain additive errors, were normally distributed, and the mean of the residuals was approximately zero (i.e., -0.05 log CFU/g). The residuals did not appear to be correlated; however, upon testing, they were found to be slightly correlated. These results indicate the combined-effects model performed best of the three secondary models in this application.

Table 3. Statistical ordinary least squares (OLS) parameter estimates for the response surface model (equation 14).

T_{ref} [°C]					N/A
SSE					566
MSE					2.53
RMSE					1.59
Parameter	Estimate[a]	Std. Error	95% Lower CI	95% Upper CI	Relative Error
β_0 [min]	-815 *	212	-1570	-64.3	-26.0%
β_1 [min/°C]	-4.26	5.08	-42.3	33.8	-119%
β_2 [min/(°C)^2]	0.02	0.03	-0.19	0.23	183%
β_3 [min]	5760 *	100	1260	10300	1.74%
β_4 [min]	-7900 *	56.4	-15300	-495	-0.71%
β_5 [min/°C]	1.73	1.67	-12.6	16.1	96.2%

[a]Asterisks indicate which parameters were significant ($P < 0.05$). Parameter estimates were generated assuming all parameters were significant. Parameters were not re-estimated using only significant parameters.

Table 4. Statistical ordinary least squares (OLS) parameter estimates for the modified Bigelow-type model (equation 15).

T_{ref} [°C]					80.0
SSE					126
MSE					0.556
RMSE					0.745
Parameter	Estimate	Std. Error	95% Lower CI	95% Upper CI	Relative Error
D_{ref} [min]	0.08	0.01	0.06	0.10	12.1%
z_T [°C]	15.2	0.48	14.3	16.2	3.17%
z_{a_w}	0.32	0.01	0.30	0.34	3.15%

Table 5. Statistical ordinary least squares (OLS) parameter estimates for the combined-effects model (equation 16).

T_{ref} [°C]					80.0
SSE					101
MSE					0.446
RMSE					0.668
Parameter	Estimate	Std. Error	95% Lower CI	95% Upper CI	Relative Error
D_{ref} [s]	1.32	0.04	1.24	1.40	3.05%
R_{a_w} [s]	25.3	0.74	23.8	26.7	2.93%
z_T [°C]	16.3	0.49	15.3	17.2	3.00%

To further evaluate the combined-effects model, the parameter estimates were used to generate values for the range of a_w and temperatures used in this study. The predicted D values were then plotted with respect to a_w and temperature (Figure 6) (Appendix K).

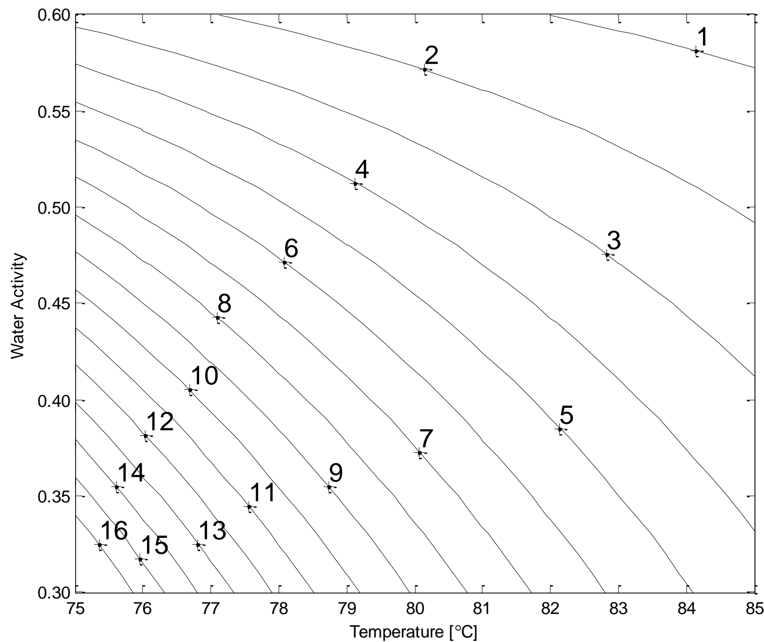

Figure 6. *Salmonella* D values (min) as a function of wheat flour a_w and temperature, predicted by the combined-effects model (equation 16).

4.3.5 Comparison of secondary models

Typically, the best model is selected based on the lowest AIC_c value; therefore, the combined-effects model was selected as the most effective secondary model (Table 6). The surface response model was extremely data and application-intensive because of the number of parameters in the model, and could not be used effectively outside of the range of a_w put into the model when estimating the parameters. The modified Bigelow-type model, which is a widely accepted secondary model, predicted the inactivation relatively well (RMSE = 0.745); however, the combined-effects model had a better goodness-of-fit (RMSE = 0.668), and was more likely to be correct based on lower AIC_c. Further, the parameters of the combined-effects model have a degree of phenomenological meaning to the data set and are not simply coefficients to a model.

Table 6. Statistical ordinary least squares (OLS) results for the secondary models (n = 230). Parameters were globally estimated.

Model	# Parameters	SSE [(log CFU/g)^2]	RMSE [log CFU/g]	Optimum T_{ref}[a] [°C]	AIC_c
Response Surface	6	566	1.59	N/A	222
Modified Bigelow	3	126	0.745	77.4	-130
Combined-Effects	3	101	0.668	76.5	-181

[a]Optimum T_{ref} was set to 80°C for parameter estimation, but these values are the optimal T_{ref} for each model. The Response surface model did not explicitly have a T_{ref}, as it was accounted for in the parameter β_0.

The combined-effects model was selected as the most comprehensive secondary model because it effectively accounted for temperature and a_w. At a specific a_w, the combined-effects model accurately discriminated between differing temperature (Figure 7), and at a set temperature, the combined-effects model accurately discriminated between differing a_w (Figure 8).

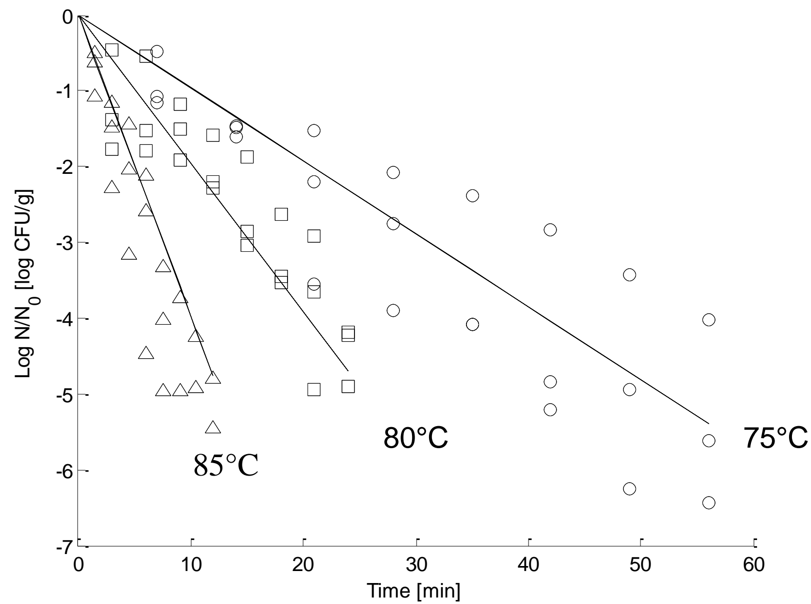

Figure 7. Thermal inactivation of *Salmonella* inoculated into wheat flour equilibrated to 0.45 a_w and treated at 75°C (○), 80°C (□), or 85°C (△). Lines are combined-effects model predictions from the global fit of equation 16 integrated into equation 10.

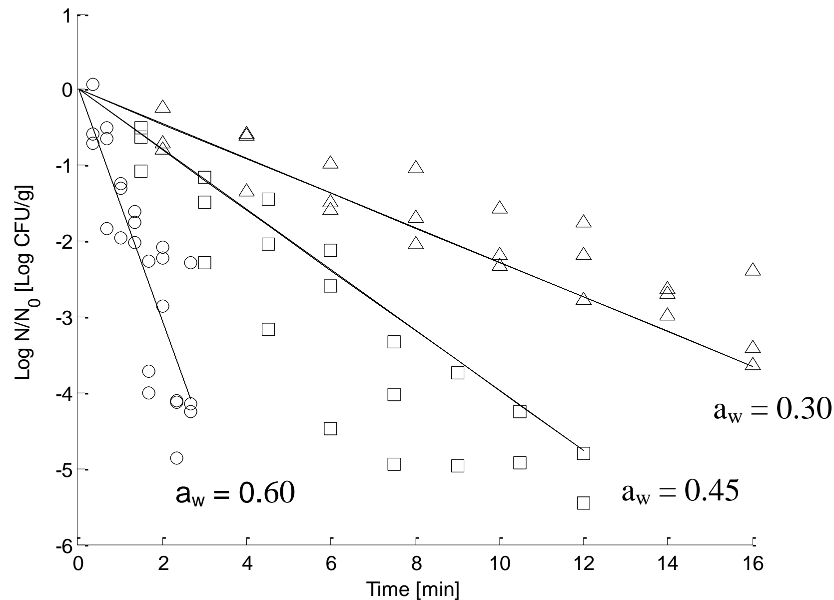

Figure 8. Thermal inactivation of *Salmonella* inoculated into wheat flour at 0.60 (○), 0.45 (□), or 0.30 (△) a_w when treated at 85°C. Lines are combined-effects model predictions from the global fit of equation 16 integrated into equation 10.

Valdramidis et al. (2006) determined that the Bigelow-type model better fit inactivation data generated from *Listeria monocytogenes* in potato slices (a_w = 0.71 to 0.99) when compared with a polynomial and Arrhenius structure, based on R^2 values; however, we cannot directly compare these results, because the product, organism, and environment were quite different. Nevertheless, the results from this study support those from Valdramidis et al. indicating that the Bigelow-type model is more likely correct than the response surface model. Farakos et al. (2013) evaluated primary and secondary models based on survival data generated from *Salmonella* in protein powder (a_w = 0.19 to 0.54). They determined that the Weibull model better described the survival data, when compared to the log-linear model, based on adjusted R^2 and RMSE. They then evaluated a polynomial secondary model integrated into the Weibull model. They subsequently validated that model against independent data (Farakos, Schaffner, et al., 2014), and indicated that their model was fail-safe, with 55% of the prediction residuals being within a

pre-defined acceptable range (-1 to 0.5 log). However, this result was only slightly better than the standard Bigelow model (Bigelow & Esty, 1920), which was fail-safe with 52% of the residuals.

4.4 Conclusions

For the data in this study, the combined-effects model performed better than the modified Bigelow-type model in predicting thermal inactivation as a function of a_w and temperature. The combined-effects secondary model also yielded improved outcomes by accounting for a_w when modeling microbial inactivation, as compared to a traditional Bigelow model (Bigelow & Esty, 1920). The standard Bigelow model (equation 2) integrated into the log-linear model (equation 10) yielded a RMSE of 1.45 log CFU/g. When the combined-effects model (equation 16), which considers temperature and a_w, was integrated into the log-linear model (equation 10), the RMSE was reduced to 0.668 log CFU/g.

In future studies, this model form should be validated with data from other products and treatments; however, it appears to have shown promise for wheat flour. One limitation of the combined-effects model, as presented, is that extrapolation must be avoided, because the model (with the reported parameters) predicts D values less than zero for a_w greater than ~0.65, which is a severe limitation to broader applications. Current tests are extending the data set to higher a_w values to estimate updated model parameters that will enable more robust application to a broader range of conditions. Clearly, a_w is an important factor affecting thermal inactivation of *Salmonella* in low-moisture products, and should be appropriately included in thermal inactivation models for these types of systems.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

5.1 Effect of Desiccation or Hydration on Thermal Resistance

The mechanisms behind desiccation stress and the impact on subsequent thermal resistance are not well known (Spector & Kenyon, 2012). Despite not understanding the cellular mechanisms, it is clear that water activity "history" seems to have little effect on *Salmonella* thermal resistance.

The effect of desiccation or hydration rate was determined to have little effect on thermal resistance; instead, the water activity, a_w, at the time of thermal treatment was determined to control *Salmonella* thermal resistance, regardless of the prior water activity history. These results reinforce findings from previous studies (Archer, et al., 1998; Janning, et al., 1994), in which decreased a_w increased *Salmonella* thermal resistance. However, the impact of moisture change rate on the resistance had not been previously examined. Understanding both the extent and rate of material desiccation or hydration on *Salmonella* thermal resistance in low-moisture foods is critically important in validating pasteurization processes or any "kill step" in commercial processes, which typically consist of both non-isothermal and non-iso-moisture conditions (e.g., drying, baking, toasting, or roasting).

During many commercial processes, product water activity fluctuates with changing production environments. Process validations must consider the dynamic nature of product water activity at the time of thermal processing in order to accurately estimate the process lethality.

5.2 Modeling the Effects of Temperature and Water Activity on Thermal Resistance

The modeling study examined the effectiveness of two primary models. After both the log-linear and Weibull models were fit to the data, AIC_c values showed that the log-linear model better represented the data than did the Weibull model. The apparent linearity of the data also was an indication that the data would be better represented with the log-linear model. For these reasons, along with the inherent simplicity of the model, all secondary models were developed as extensions of the log-linear primary model.

For the data in this study, the combined-effects model performed better than the modified Bigelow-type model in predicting thermal inactivation as a function of a_w and temperature. The combined-effects secondary model was a significant improvement with respect to accounting for a_w when modeling microbial inactivation. When the standard Bigelow model (equation 2) was integrated into the log-linear model, the RMSE was 1.45. When the combined-effects model (equation 16), which accounts for a_w, was integrated into the log-linear model, the RMSE decreased to 0.668. Further, the parameters of the combined effects model have phenomenological meaning and are not simply coefficients to a model. Clearly, a_w is an important factor affecting thermal inactivation of *Salmonella* in low-moisture products, and should be appropriately included in thermal inactivation models for these types of systems.

Although these results are slightly different from previous studies (Valdramidis, et al., 2006; Villa-Rojas, et al., 2013), these prior studies based model selection almost exclusively on the coefficient of determination, or the R^2 value, which is an insufficient sole measure for evaluating nonlinear models, such as the Weibull model (McKellar & Lu, 2004). More robust selection statistics, such as the root mean square error, and/or the corrected Aikake Information Criterion should be used.

Accounting for the product environment is an important part of modeling microbial inactivation. The response surface model and the modified Bigelow-type models studied in this work have previously been applied (Farakos, et al., 2013; Valdramidis, et al., 2006); however, the combined-effects model is a novel effort to better account for the effect of water activity when modeling microbial inactivation for low-moisture conditions.

5.3 Future Work

5.3.1 Cellular mechanisms behind desiccation stress

There is a need for further understanding the cellular mechanisms behind desiccation stress. Deep understanding of these mechanisms was not the purpose of this study, but based on the general responses reported in the present work, future efforts could focus on elucidating the underlying mechanisms, which would in turn help improve future models.

5.3.2 Standardized model selection practices

There is a need for standardized model validation practices when modeling microbial inactivation. Many studies use only the coefficient of determination (R^2) as a selection criterion when comparing linear and nonlinear models, which may overestimate the success of the model, leading to poor model selection (McKellar & Lu, 2004).

5.3.3 Further validation of the combined-effects model

The combined-effects model shows promise in the endeavor to incorporate the effect of water activity into microbial inactivation models. However, the model was only confirmed for the process and product conditions in which the data were generated. A future study should

validate the combined-effects model using different low-moisture products (e.g., pastes, particulates, powders), different product compositions (e.g., protein, fat), different *Salmonella* strains, and, most importantly, non-isothermal and non-iso-moisture conditions.

APPENDICES

Appendix A – Homogeneity of Inoculation

In order to ensure the homogeneity of the inoculation methods, six samples (~1 g) were randomly pulled from an inoculated 25 g sample. This test confirmed the uniformity of the inoculum mixing into the flour samples.

Table 7. Homogeneity test results

Water Activity	Sample	Log CFU/g
0.6	1	8.79
	2	8.79
	3	8.79
	4	8.85
	5	9.19
	6	9.06
	Average	**8.91**
	Std. Deviation	**0.17**

Appendix B – Survivor Data for Rapid Desiccation and Hydration Study

This appendix includes the raw *Salmonella* survivor data generated in the rapid desiccation and hydration study (Chapter 3). N/A indicates data for that point was unavailable due to experimental errors.

Table 8. Raw *Salmonella* survival data I60/F60

Rep	Sample	Time (s)	Log CFU/g	Log N/N_0
REP 1	1	0	7.99	0.00
	2	20	6.11	-1.88
	3	40	3.30	N/A
	4	60	6.21	-1.78
	5	80	5.46	-2.52
	6	100	5.69	-2.30
	7	120	6.22	-1.77
	8	140	5.75	-2.24
	9	160	5.86	N/A
	10	180	5.02	-2.97
REP 2	1	0	7.11	0.00
	2	20	6.72	-0.39
	3	40	5.76	N/A
	4	60	6.12	-0.99
	5	80	6.01	-1.09
	6	100	5.67	-1.44
	7	120	5.12	-1.98
	8	140	6.01	-1.09
	9	160	4.48	-2.62
	10	180	4.68	-2.42
REP 3	1	0	5.69	0.00
	2	20	6.51	0.83
	3	40	6.80	1.11
	4	60	5.46	-0.23
	5	80	4.94	-0.74
	6	100	4.46	-1.23
	7	120	3.79	-1.90
	8	140	4.91	-0.78
	9	160	3.98	N/A
	10	180	4.20	-1.48

Table 9. Raw *Salmonella* survivor data I30/F30

Rep	Sample	Time (s)	Log CFU/g	Log N/N$_0$
REP 1	1	0	7.68	0.00
	2	120	6.66	-1.02
	3	240	7.56	-0.12
	4	360	5.80	-1.87
	5	480	6.24	-1.43
	6	600	5.26	-2.42
	7	720	6.10	-1.58
	8	840	5.66	-2.01
	9	960	5.60	-2.08
	10	1080	5.61	-2.07
REP 2	1	0	8.11	0.00
	2	120	6.56	-1.56
	3	240	6.98	-1.13
	4	360	6.63	-1.48
	5	480	5.91	-2.20
	6	600	5.88	-2.23
	7	720	6.30	-1.81
	8	840	5.56	-2.55
	9	960	N/A	N/A
	10	1080	5.13	-2.98
REP 3	1	0	7.72	0.00
	2	120	7.24	-0.48
	3	240	7.15	-0.58
	4	360	6.74	-0.98
	5	480	6.13	-1.60
	6	600	5.62	-2.10
	7	720	6.27	-1.46
	8	840	5.76	-1.96
	9	960	4.08	-3.65
	10	1080	4.04	-3.68

Table 10. Raw *Salmonella* survivor data I60/F30

Rep	Sample	Time (s)	Log CFU/g	Log N/N_0
REP 1	1	0	7.71	0.00
	2	60	6.88	-0.83
	3	120	5.45	-2.26
	4	180	5.58	-2.13
	5	240	6.02	-1.69
	6	300	5.27	-2.44
	7	360	5.90	N/A
	8	420	5.23	-2.48
	9	480	5.34	N/A
	10	540	5.93	-1.78
REP 2	1	0	6.86	0.00
	2	60	7.46	N/A
	3	120	5.26	-1.61
	4	180	7.23	0.37
	5	240	5.57	N/A
	6	300	5.90	-0.96
	7	360	5.63	-1.23
	8	420	5.95	-0.91
	9	480	5.69	-1.17
	10	540	4.59	N/A
REP 3	1	0	7.54	0.00
	2	60	6.45	-1.09
	3	120	7.30	-0.24
	4	180	6.82	-0.72
	5	240	6.19	-1.35
	6	300	5.97	-1.57
	7	360	6.03	-1.52
	8	420	3.64	-3.90
	9	480	5.06	-2.49
	10	540	4.02	-3.52

Table 11. Raw *Salmonella* survivor data I30/F60

Rep	Sample	Time (s)	Log CFU/g	Log N/N_0
REP 1	1	0	6.66	0.00
	2	30	5.24	-1.42
	3	60	5.12	-1.54
	4	90	4.32	-2.34
	5	120	3.43	-3.23
	6	150	6.38	-0.28
	7	180	2.88	-3.79
	8	210	1.70	-4.96
	9	240	2.54	-4.12
	10	270	2.40	-4.26
REP 2	1	0	6.76	0.00
	2	30	5.88	-0.89
	3	60	5.17	-1.59
	4	90	4.35	-2.41
	5	120	4.67	-2.10
	6	150	4.05	-2.71
	7	180	3.71	-3.05
	8	210	4.29	-2.47
	9	240	2.00	-4.76
	10	270		N/A
REP 3	1	0	6.04	0.00
	2	30	5.60	-0.44
	3	60	5.09	-0.95
	4	90	4.94	-1.10
	5	120	4.21	-1.83
	6	150	3.39	-2.65
	7	180	2.98	-3.06
	8	210	3.33	-2.71
	9	240	3.18	-2.87
	10	270	N/A	N/A

Appendix C – Results of ANOVA

This appendix contains the results of ANOVA performed on the rapid desiccation or hydration study (Appendix B).

Table 12. ANOVA comparison of I60/F60 & I30/F30

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
I60/F60	3	-0.0378	-0.0126	1.75E-06
I30/F30	3	-0.00724	-0.00241	5.52E-07

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.000156	1	0.000156	135.2178	0.000313	7.708647
Within Groups	4.6E-06	4	1.15E-06			
Total	0.00016	5				

Table 13. ANOVA comparison of I60/F60 & I60/F30

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
I60/F60	3	-0.0378	-0.0126	1.75E-06
I60/F30	3	-0.0111	-0.0037	5.33E-06

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.000119	1	0.000119	33.54651	0.004417	7.708647
Within Groups	1.42E-05	4	3.54E-06			
Total	0.000133	5				

Table 14. ANOVA comparison of I60/F60 & I30/F60

SUMMARY

Groups	Count	Sum	Average	Variance
I60/F60	3	-0.0378	-0.0126	1.75E-06
I30/F60	3	-0.04368	-0.01456	1.12E-06

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	5.75E-06	1	5.75E-06	4.011324	0.115742	7.708647
Within Groups	5.74E-06	4	1.43E-06			
Total	1.15E-05	5				

Table 15. ANOVA comparison of I30/F30 & I60/F30

SUMMARY

Groups	Count	Sum	Average	Variance
I30/F30	3	-0.00724	-0.00241	5.52E-07
I60/F30	3	-0.0111	-0.0037	5.33E-06

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	2.48E-06	1	2.48E-06	0.844432	0.410131	7.708647
Within Groups	1.18E-05	4	2.94E-06			
Total	1.42E-05	5				

Table 16. ANOVA comparison of I30/F30 & I30/F60

SUMMARY

Groups	Count	Sum	Average	Variance
I30/F30	3	-0.00724	-0.00241	5.52E-07
I30/F60	3	-0.04368	-0.01456	1.12E-06

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.000221	1	0.000221	264.8682	8.34E-05	7.708647
Within Groups	3.34E-06	4	8.35E-07			
Total	0.000225	5				

Table 17. ANOVA comparison of I60/F30 & I30/F60

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
C	3	-0.0111	-0.0037	5.33E-06
D	3	-0.04368	-0.01456	1.12E-06

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.000177	1	0.000177	54.82662	0.001775	7.708647
Within Groups	1.29E-05	4	3.23E-06			
Total	0.00019	5				

Appendix D – Photographs of Experimental Apparatus

This appendix includes pictures of systems utilized in this study.

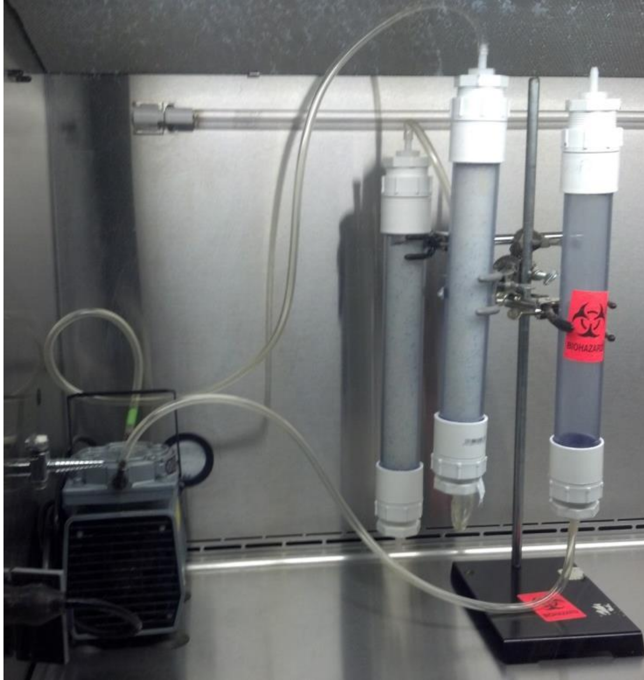

Figure 9. Rapid desiccation system

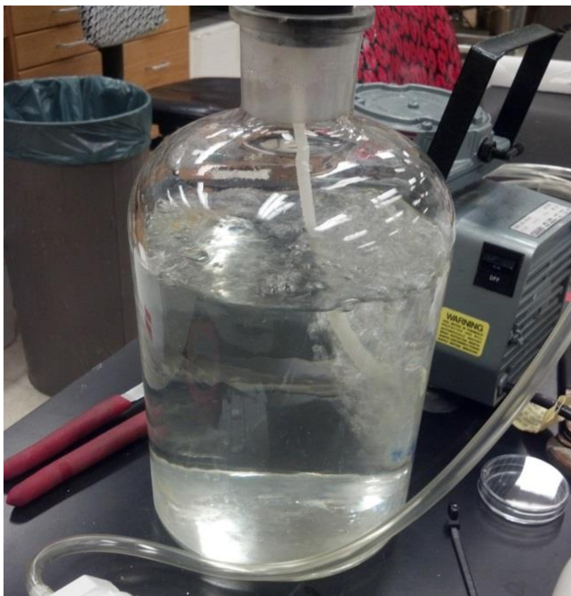

Figure 10. Saturation chamber in rapid hydration system

Figure 11. Test cell filled with wheat flour that was used in thermal inactivation studies.

Figure 12. Equilibration chamber of the conditioning system

Appendix E – Rapid Desiccation and Hydration System Calibration Data

This appendix contains the data that were generated when the desiccation and hydration systems were calibrated, before inoculated trials were completed.

Table 18. Desiccation system calibration data

Sample	Initial	Final	Difference
1	0.654	0.314	0.340
2	0.648	0.313	0.335
3	0.647	0.321	0.326
4	0.644	0.297	0.347
5	0.646	0.323	0.323
6	0.651	0.331	0.320
mean	**0.648**	**0.317**	**0.332**
std dev	**0.004**	**0.012**	**0.011**

Table 19. Hydration system calibration data

Sample	Initial	Final	Difference
1	0.302	0.623	0.321
2	0.306	0.610	0.304
3	0.307	0.591	0.284
4	0.306	0.612	0.306
5	0.306	0.572	0.266
6	0.306	0.602	0.296
mean	**0.306**	**0.602**	**0.296**
std dev	**0.002**	**0.018**	**0.019**

Appendix F – Additional One Hour Hold Data

This appendix contains the raw survival count data from the additional one-hour hold experiment completed in the rapid desiccation/hydration study.

Table 20. One hour hold data (I60/F30/1H)

Rep	Sample	Time (s)	Log CFU/g	Log N/N_0
Rep 1	1	0	8.55	0.00
	2	60	8.70	0.15
	3	120	7.54	-1.01
	4	180	7.81	-0.74
	5	240	7.31	-1.24
	6	300	7.47	-1.08
	7	360	7.25	-1.30
	8	420	7.60	-0.95
	9	480	6.01	-2.54
	10	540	5.19	-3.36
Rep 2	1	0	8.94	0.00
	2	60	8.41	-0.53
	3	120	7.34	-1.60
	4	180	7.39	-1.55
	5	240	7.46	-1.48
	6	300	7.47	-1.47
	7	360	7.46	-1.48
	8	420	7.33	-1.61
	9	480	7.23	-1.71
	10	540	7.28	-1.66
Rep 3	1	0	8.69	0.00
	2	60	8.59	-0.10
	3	120	7.29	-1.40
	4	180	7.17	-1.52
	5	240	7.32	-1.37
	6	300	7.51	-1.17
	7	360	7.17	-1.51
	8	420	7.38	-1.30
	9	480	7.22	-1.46
	10	540	7.22	-1.46

Appendix G – Survivor Data for Factorial Modeling Study

This appendix contains raw survival count data generated from a full factorial modeling study encompassing thermal inactivation of *Salmonella* Enteritidis PT30 in wheat flour at three water activities (0.30, 0.45, 0.60) and three temperatures (75°C, 80°C, 85°C). N/A indicates data for that point was unavailable due to experimental problems/failures.

Table 21. *Salmonella* survival in wheat flour (a_w = 0.30).

Water Activity	Temperature	Sample	Time (s)	Rep 1	Rep 2	Rep 3	Average
0.3	75	0	0	0.00	0.00	0.00	0.00
		1	300	-0.67	-0.34	-0.31	-0.44
		2	600	-0.99	-0.91	-0.36	-0.75
		3	900	-1.45	-1.37	-0.88	-1.23
		4	1200	-1.66	-1.65	-0.84	-1.39
		5	1500	-2.08	-2.03	-1.09	-1.74
		6	1800	-2.16	-2.19	-1.02	-1.79
		7	2100	-2.74	-2.67	N/A	-2.70
		8	2400	-2.77	-2.48	N/A	-2.62
0.3	80	0	0	0.00	0.00	0.00	0.00
		1	240	-0.56	-0.96	-0.01	-0.51
		2	480	-0.91	-1.51	-0.65	-1.02
		3	720	-1.29	-1.85	-0.70	-1.28
		4	960	-1.54	-2.74	-0.95	-1.74
		5	1200	-1.65	-2.74	-1.70	-2.03
		6	1440	-2.35	-2.74	-1.61	-2.23
		7	1680	-2.48	-3.25	-1.69	-2.47
		8	1920	-2.74	-4.59	-2.15	-3.16
0.3	85	0	0	0.00	0.00	0.00	0.00
		1	120	-0.23	-0.78	-0.70	-0.57
		2	240	-0.61	-0.59	-1.35	-0.85
		3	360	-0.98	-1.48	-1.58	-1.35
		4	480	-1.05	-1.69	-2.03	-1.59
		5	600	-1.58	-2.18	-2.32	-2.03
		6	720	-1.75	-2.18	-2.78	-2.24
		7	840	-2.69	-2.98	-2.63	-2.77
		8	960	-2.40	-3.42	-3.63	-3.15

Table 22. *Salmonella* survival in wheat flour (a_w = 0.45).

Water Activity	Temperature	Sample	Time (s)	Rep 1	Rep 2	Rep 3	Average
0.45	75	0	0	0.00	0.00	0.00	0.00
		1	420	-0.47	-1.07	-1.16	-0.90
		2	840	-1.47	-1.60	-1.49	-1.52
		3	1260	-1.53	-3.56	-2.21	-2.43
		4	1680	-2.08	-3.89	-2.76	-2.91
		5	2100	-2.38	-4.08	-4.07	-3.51
		6	2520	-2.83	-4.85	-5.21	-4.30
		7	2940	-3.43	-4.94	-6.25	-4.87
		8	3360	-4.03	-6.45	-5.61	-5.36
0.45	80	0	0	0.00	0.00	0.00	0.00
		1	180	-1.38	-0.47	-1.78	-1.21
		2	360	-1.79	-0.55	-1.53	-1.29
		3	540	-1.92	-1.18	-1.51	-1.54
		4	720	-2.29	-1.58	-2.20	-2.02
		5	900	-2.86	-3.05	-1.88	-2.59
		6	1080	-3.53	-3.45	-2.63	-3.20
		7	1260	-4.93	-3.65	-2.91	-3.83
		8	1440	-4.90	-4.18	-4.23	-4.44
0.45	85	0	0	0.00	0.00	0.00	0.00
		1	90	-0.50	-0.63	-1.08	-0.74
		2	180	-1.16	-1.49	-2.28	-1.64
		3	270	-1.45	-2.03	-3.17	-2.22
		4	360	-2.12	-2.60	-4.48	-3.06
		5	450	-3.32	-4.02	-4.95	-4.10
		6	540	-3.74	-4.97	N/A	-4.36
		7	630	-4.26	-4.92	N/A	-4.59
		8	720	-4.80	-5.46	N/A	-5.13

Table 23. *Salmonella* survival in wheat flour (a_w = 0.60).

Water Activity	Temperature	Sample	Time (s)	Rep 1	Rep 2	Rep 3	Average
0.6	75	0	0	0.00	0.00	0.00	0.00
		1	150	-0.56	-1.01	-0.74	-0.77
		2	300	-1.48	-1.41	-1.28	-1.39
		3	450	-3.55	-5.46	-2.02	-3.68
		4	600	N/A	-5.69	-2.78	-4.23
		5	750	N/A	-4.83	N/A	-4.83
		6	900	N/A	-4.31	-4.65	-4.48
		7	1050	N/A	-7.16	-5.42	-6.29
		8	1200	N/A	N/A	N/A	N/A
0.6	80	0	0	0.00	0.00	0.00	0.00
		1	30	-0.52	-0.65	-1.23	-0.80
		2	60	-0.81	-1.00	-1.33	-1.04
		3	90	-0.93	-1.20	-0.77	-0.97
		4	120	-1.07	-1.68	-2.22	-1.66
		5	150	-1.19	-2.27	-2.46	-1.98
		6	180	-2.58	-2.46	-2.89	-2.64
		7	210	-1.76	-2.57	-2.34	-2.22
		8	240	-2.96	-3.48	-3.53	-3.32
0.6	85	0	0	0.00	0.00	0.00	0.00
		1	20	0.07	-0.59	-0.71	-0.41
		2	40	-1.84	-0.65	-0.50	-1.00
		3	60	-1.97	-1.29	-1.23	-1.50
		4	80	-2.02	-1.60	-1.76	-1.79
		5	100	-4.00	-2.27	-3.71	-3.33
		6	120	-2.22	-2.07	-2.86	-2.38
		7	140	-4.12	-4.87	-4.11	-4.37
		8	160	-2.28	-4.14	-4.25	-3.56

Appendix H – MATLAB Code for Log-Linear Model

This appendix contains the MATLAB code used to fit the log-linear primary model to the raw data from the modeling study.

```
%% Import Data
data = xlsread('modelingdata.xlsx');
T1_60 = data(1:19,1:2);   T2_60 = data(:,3:4);   T3_60 = data(:,5:6);
T1_45 = data(:,7:8);      T2_45 = data(:,9:10);  T3_45 = data(1:24,11:12);
T1_30 = data(1:25,13:14); T2_30 = data(:,15:16); T3_30 = data(:,17:18);

% Sequentially arrange data
T1_60=sortrows(T1_60,-2);T2_60=sortrows(T2_60,-2);T3_60=sortrows(T3_60,-2);
T1_45=sortrows(T1_45,-2);T2_45=sortrows(T2_45,-2);T3_45=sortrows(T3_45,-2);
T1_30=sortrows(T1_30,-2);T2_30=sortrows(T2_30,-2);T3_30=sortrows(T3_30,-2);

%% Estimate Betas
M{1} = T1_60; M{2}= T2_60; M{3} = T3_60;
M{4} = T1_45; M{5}= T2_45; M{6} = T3_45;
M{7} = T1_30; M{8}= T2_30; M{9} = T3_30;
k = length(M);

for q = 1:k
  D = 1; beta0(1) = D; beta=beta0;
  t=M{1,q}(:,1);
  y=M{1,q}(:,2);

  % Estimate Parameters
  [beta,R,J,COVB,mse] = nlinfit(t,y,@lin,beta0);
  D = beta(1);

  % Statistics
  CI = nlparci(beta,R,'jacobian',J);      % 95% CI
  [Rcorr,sigma] = corrcov(COVB);
  se = sigma;                             % Parameter Standard Error
  relerr = sigma./beta';                  % Parameter Relative Error
  CorrCoef = Rcorr;                       % Correlation matrix
  condX = cond(J);
  rmse = sqrt(mse);
  res(q,:)=[q D CI(1,1) CI(1,2) se relerr rmse];
end
disp('         Model #      D                   CI            Std. Error
Rel. Error       RMSE');
disp(res);

%% D value estimates
D = res(:,2);
Dmat = [D(1) D(2) D(3);
     D(4) D(5) D(6);
     D(7) D(8) D(9)];
```

```
%% Model Selection Data

for m = 1:k
    p=1;
    t=M{1,m}(:,1);
    y=M{1,m}(:,2);
    n=length(t);
    beta = D(m);
    ynew = -t./beta;
    e = y-ynew;
    sum = e'*e;
    R = y.'*y;
    DR = R - sum;
    s2 = sum/(n-p);
    F = DR/s2;
    Fcritical=finv(0.95,1,n-p);
    K=p+1;
    AICc=n*log(sum/n)+2*K+2*K*(K+1)/(n-K-1);
    B(m,:)=[m p n-p sum s2 DR F Fcritical AICc];
end
disp('         Model #         p       deg. of fr.    R         Mean sq.
Delta R        F         Fcritical     AICc');
disp(B);



function y = linear(beta,t)
%first-order reaction equation, explicit form
%beta are the parameters, and t are the independent variables values
y=t/-beta(1);
end
```

Appendix I – MATLAB Code for Weibull Model

This appendix contains the MATLAB code used to fit the Weibull primary model to the raw data from the modeling study.

```
%% Import Data
data = xlsread('modelingdata.xlsx');
T1_60 = data(1:19,1:2);   T2_60 = data(:,3:4);   T3_60 = data(:,5:6);
T1_45 = data(:,7:8);      T2_45 = data(:,9:10);  T3_45 = data(1:24,11:12);
T1_30 = data(1:25,13:14); T2_30 = data(:,15:16); T3_30 = data(:,17:18);

% Sequentially arrange data
T1_60=sortrows(T1_60,-2);T2_60=sortrows(T2_60,-2);T3_60=sortrows(T3_60,-2);
T1_45=sortrows(T1_45,-2);T2_45=sortrows(T2_45,-2);T3_45=sortrows(T3_45,-2);
T1_30=sortrows(T1_30,-2);T2_30=sortrows(T2_30,-2);T3_30=sortrows(T3_30,-2);

%% Estimate D value
M{1} = T1_60; M{2}= T2_60; M{3} = T3_60;
M{4} = T1_45; M{5}= T2_45; M{6} = T3_45;
M{7} = T1_30; M{8}= T2_30; M{9} = T3_30;
k = length(M);

for q = 1:k

  D = 0.1; n =0.1;
  beta0(1)=D; beta0(2)=n;
  beta=beta0;
  t=M{1,q}(:,1);
  y=M{1,q}(:,2);

  % Estimate Parameters
  [beta,R,J,COVB,mse] = nlinfit(t,y,@wbl,beta0);
  D = beta(1);
  n = beta(2);

  % Statistics
  CI = nlparci(beta,R,'jacobian',J);     % 95% CI
  [Rcorr,sigma] = corrcov(COVB);
  se = sigma;                            % Parameter Standard Error
  relerr = sigma./beta';                 % Parameter Relative Error
  CorrCoef = Rcorr;                      % Correlation matrix
  condX = cond(J);
  rmse = sqrt(mse);
  res(q,:)=[q beta(1) se(1) beta(2) se(2) rmse];
end
disp('         Model #    Delta     Std. Error        n       Std. Error
RMSE');
disp(res);
beta1=res(:,2); beta2=res(:,4); beta = [beta1 beta2];
```

```
%% Model Selection Data

for m = 1:k
    p=2;
    t=M{1,m}(:,1);
    y=M{1,m}(:,2);
    n=length(t);
    beta1 = beta(m,1);
    beta2 = beta(m,2);
    ynew = -1.*(t./beta1).^beta2;
    e = y-ynew;
    sum = e'*e;
    R = y.'*y;
    DR = R - sum;
    s2 = sum/(n-p);
    F = DR/s2;
    Fcritical=finv(0.95,1,n-p);
    K=p+1;
    AICc=n*log(sum/n)+2*K+2*K*(K+1)/(n-K-1);
    B(m,:)=[m p n-p sum s2 DR F Fcritical AICc];
end
disp('         Model #         p       deg. of fr.    R         Mean sq.
Delta R        F         Fcritical     AICc');
disp(B);



function y = weibull(beta,t)
%first-order reaction equation, explicit form
%beta are the parameters, and t are the independent variables values
y=-1.*(t./beta(1)).^beta(2);
%y=(t/-beta(1))
end
```

Appendix J – MATLAB Code for Secondary Models

This appendix contains the MATLAB code used to fit the secondary models to the raw data from the modeling study.

```
%% Import data
data = xlsread('modelingdata2.xlsx');
t = data(:,1); yobs = data(:,2); T = data(:,3); aw = data(:,4);
%t = t*60;
n=length(t);
aw_60 = data(1:73,:); aw_45 = data(74:151,:); aw_30 = data(152:230,:);
T_75 = [data(1:19,:); data(74:100,:); data(152:176,:)];
T_80 = [data(20:46,:); data(101:127,:); data(177:203,:)];
T_85 = [data(47:73,:); data(128:151,:); data(204:230,:)];
%T=T-80;
X = [t T aw];

%% SURFACE RESPONSE
% Initial parameter guesses
Tr = 80;
beta0(1)= 800;       % constant term
beta0(2)= 4;       % Temperature
beta0(3)= 0.001;       % Temperature^2
beta0(4)= 1000;       % Water Activity
beta0(5)= 1000;       % Water Activity^2
beta0(6)= 1;       % Interaction Term
beta = beta0;
p=length(beta0);

% Estimate Parameters
[beta,resids,J,COVB,mse] = nlinfit(X,yobs,@surfresp,beta0);
BSR = beta
RMSE_SR = sqrt(mse)
CI = nlparci(beta,resids,'jacobian',J)     % 95% CI
[Rcorr,sigma] = corrcov(COVB);
se = sigma                               % Parameter Standard Error
relerr = sigma./beta'                    % Parameter Relative Error
CorrCoef = Rcorr;                        % Correlation matrix
condX = cond(J);

% Use Estimated Parameters For Model Error
D =
beta(1)+(beta(2).*T)+(beta(3).*(T.^2))+(beta(4).*aw)+(beta(5).*(aw.^2))+(beta
(6).*T.*aw);
y = -t./D;

% AIC
p = 6;
e = yobs-y;
sum = e'*e;
R = y.'*y;
DR = R - sum;
s2 = sum/(n-p);
```

```
F = DR/s2;
Fcritical = finv(0.95,1,n-p);
K = p+1;
AICc = n*log(sum/n)+(2*K)+(2*K*(K+1))/(n-K-1)

%% MAFART MODEL
clear beta0
% Initial parameter guesses
Tr = 80;
beta0(1)= 10;       % Dref
beta0(2)= 20;       % zT
beta0(3)= 5;        % zAw
beta = beta0;
p=length(beta0);

% Estimate Parameters
[beta,resids,J,COVB,mse] = nlinfit(X,yobs,@modmafart,beta0);
BM = beta
RMSE_M = sqrt(mse)
CI = nlparci(beta,resids,'jacobian',J);     % 95% CI
[Rcorr,sigma] = corrcov(COVB);
se = sigma;                              % Parameter Standard Error
relerr = sigma./beta';                   % Parameter Relative Error
CorrCoef = Rcorr;                        % Correlation matrix
condX = cond(J);

% Use Estimated Parameters For Model Error
D1 = log10(beta(1))-((T-Tr)./beta(2))-((aw-1)./beta(3));
D = 10.^(D1);
y = -t./D;

% AIC
p = 3;
e = yobs-y;
sum = e'*e;
R = y.'*y;
DR = R - sum;
s2 = sum/(n-p);
F = DR/s2;
Fcritical = finv(0.95,1,n-p);
K = p+1;
AICc = n*log(sum/n)+2*K+2*K*(K+1)/(n-K-1)

% %% COMBINED EFFECTS
clear beta0
% Initial parameter guesses
Tr = 80;
beta0(1)= 450;       % Dref
beta0(2)= 200;       % Raw
beta0(3)= 5;         % zT
beta = beta0;
p=length(beta0);

% Estimate Parameters
[beta,resids,J,COVB,mse] = nlinfit(X,yobs,@combeffects,beta0);
b = beta
```

```
RMSE_CE = sqrt(mse)
CI = nlparci(beta,resids,'jacobian',J);     % 95% CI
[Rcorr,sigma] = corrcov(COVB);
se = sigma;                                % Parameter Standard Error
relerr = sigma./beta';                     % Parameter Relative Error
CorrCoef = Rcorr;                          % Correlation matrix
condX = cond(J);

% Use Estimated Parameters For Model Error
D = (beta(1)+beta(2).*(0.6-aw)).*(10.^((Tr-T)/beta(3)));
y = -t./D;

% AIC
p =3;
e = yobs-y;
sum = e'*e;
R = y.'*y;
DR = R - sum;
s2 = sum/(n-p);
F = DR/s2;
Fcritical = finv(0.95,1,n-p);
K = p+1;
AICc = n*log(sum/n)+2*K+2*K*(K+1)/(n-K-1)



function y = surfresp(beta,X)
% This function represents the secondary surface response model

global Tr
t = X(:,1);
T = X(:,2);
aw = X(:,3);

D =
beta(1)+(beta(2).*T)+(beta(3).*(T.^2))+(beta(4).*aw)+(beta(5).*(aw.^2))+(beta
(6).*T.*aw);
y = -t./D;



function y = modmafart(beta,X)
% This function represents the secondary modified Mafart model

global Tr
t = X(:,1);
T = X(:,2);
aw = X(:,3);

D1 = log10(beta(1))-((T-Tr)./beta(2))-((aw-1)./beta(3));
D = 10.^(D1);
y = -t./D;
```

```
function y = combeffects(beta,X)
% This function represents the secondary combined effects model

global Tr
t = X(:,1);
T = X(:,2);
aw = X(:,3);

D = (beta(1)+beta(2).*(0.6-aw)).*(10.^((Tr-T)/beta(3)));
y = -t./D;
```

Appendix K – Surface Plot of the Combined-Effects Model Response

This appendix contains the surface plot of the response of the D values when the combined-effects model is integrated with the log-linear model.

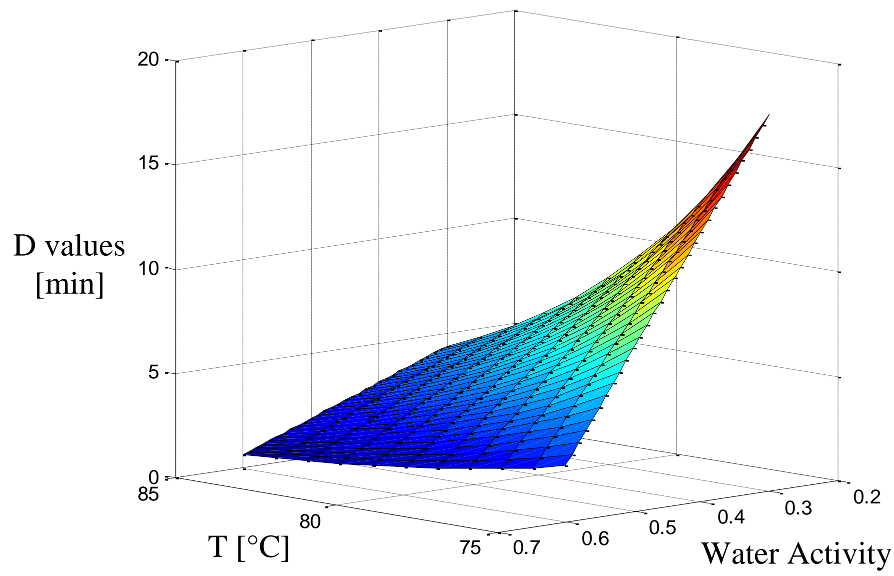

Figure 13. Surface response of the D values (min) with respect to a_w and temperature (3D).

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